Food and Agriculture Organization
of the United Nations

VOLUME TWO

THE POLLINATION OF CULTIVATED PLANTS

A COMPENDIUM FOR PRACTITIONERS

POLLINATION SERVICES FOR SUSTAINABLE AGRICULTURE
EXTENSION OF KNOWLEDGE BASE

THE POLLINATION OF CULTIVATED PLANTS

A COMPENDIUM FOR PRACTITIONERS

Volume 2

Edited by
David Ward Roubik
Smithsonian Tropical Research Institute,
Balboa, Ancon, Republic of Panama

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS
ROME 2018

The text was prepared as part of the Global Environment Fund (GEF) supported project 'Conservation and management of pollinators for sustainable agriculture, through an ecosystem approach' implemented in seven countries – Brazil, Ghana, India, Kenya, Nepal, Pakistan and South Africa.

The project was coordinated by the Food and Agriculture Organization of the United Nations (FAO) with implementation support from the United Nations Environment Programme (UN Environment).

First edition: 1995

Second edition: 2018

ISBN 978-92-5-130506-5

Cover photo
© D. J. Martins

Back cover photos
left to right:
© D. W. Roubik, © Eric Tourneret, © Eric Tourneret

CONTENTS

ANNEXES

TABLES

FIGURES

COMPENDIUM CONTENTS

This publication is comprised of two volumes. The table of contents of the entire, two-volume compendium is provided here. Both can be accessed on the FAO pollination website, http://www.fao.org/pollination/resources/en/

Volume 1

Volume 2

CONTRIBUTORS

M.L. Adriano-Anaya

Universidad Autonoma de Chiapas, Centro de Biociencias, Carretera a Puerto Madero Km 2.0, Tapachula, 30700, Chiapas, Mexico

M.A. Aizen

Laboratorio Ecotono, Centro Regional Universitario Bariloche (CRUB), Universidad Nacional del Comahue and Instituto de Investigaciones en Biodiversidad y Medioambiente (INIBIOMA), CP 8400, San Carlos de Bariloche, Río Negro, Argentina

L. Bergamini

Universidade Federal de Goiás-UFG, Departamento de Botânica, Goiânia, GO, Brasil

D. J. Biddinger

Pennsylvania State University Fruit Research and Extension Center, Entomology, 290 University Drive, Biglerville, PA 17307, Pennsylvania State University, Department of Entomology, 501 ASI Building, University Park, PA 16801, USA

B. Blochtein

Pontificia Universidade Católica do Rio Grande do Sul, Av. Ipiranga, 6681 – 90619900, Porto Alegre, RS, Brasil

M. Brand

Natural History Department, Entomology, Iziko Museums of South Africa; 25 Queen Victoria Street, P.O. Box 61, Cape Town, 8000, South Africa

S.L. Buchmann

Departments of Entomology and of Ecology and Evolutionary Biology, University of Arizona, USA

D.M. Burgett

Oregon State University, Corvallis, Oregon 97350, USA

L.A. de O. Campos

Universidade Federal de Viçosa, Departamento de Biologia Animal, Viçosa, MG, Brasil

M.J. de O. Campos

Universidade Estadual Paulista-UNESP, Departamento de Ecologia, Rio Claro, SP, Brasil

J.H. Cane

USDA, Bee Biology and Systematics Lab, Utah State University, Logan, Utah 84322-5310, USA

M.C. Cavalcante

Universidade Federal Rural de Pernambuco, Serra Talhada, PE, Brasil

C.R. Cervancia

Institute of Biological Sciences, University of the Philippines, Los Baños, Philippines

S.A. Cunningham

CSIRO Ecosystem Sciences, Box 1700, Canberra, ACT, Australia

A.R. Davis

Department of Biology, University of Saskatchewan, Saskatoon, Saskatchewan S7N 0W0, Canada

M.S. Deprá

Universidade Estadual do Norte Fluminense Darcy Ribeiro-UENF, Laboratório de Ciências Ambientais, Campos dos Goytacazes, RJ, Brasil

A. van Doorn

BumbleConsult, the Netherlands

M.A. da S. Elias

Universidade Federal de Goiás-UFG, Departamento de Botânica, Goiânia, GO, Brasil

A.C. Fajardo, Jr.

Institute of Biological Sciences, University of the Philippines, Los Baños, Philippines

E.V. Franceschinelli

Universidade Federal de Goiás-UFG, Departamento de Botânica, Goiânia, GO, Brasil

B.M. Freitas

Universidade Federal do Ceará – UFC, Departamento de Zootecnia – CCA, Campus Universitário do Pici, Bloco 808, CEP 60.356-000 Fortaleza – CE, Brasil

L. Freitas

Jardim Botânico do Rio de Janeiro-JBRJ, Rio de Janeiro, RJ

M.C. Gaglianone

Universidade Estadual do Norte Fluminense Darcy Ribeiro-UENF, Laboratório de Ciências Ambientais, Campos dos Goytacazes, RJ, Brasil

L.A. Garibaldi

Sede Andina, Universidad Nacional de Río Negro (UNRN) and Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Mitre 630, CP 8400, San Carlos de Bariloche, Río Negro, Argentina

J. Grajales-Conesa

Universidad Autonoma de Chiapas, Centro de Biociencias, Carretera a Puerto Madero Km 2.0, Tapachula, 30700, Chiapas, Mexico

L. de Guzman

USDA-ARS Honey Bee Breeding, Genetics, and Physiology Lab, 1157 Ben Hur Rd., Baton Rouge, LA, 70820-0000, USA

M. Guzmán-Diaz

Universidad Autonoma de Chiapas. Centro de Biociencias, Carretera a Puerto Madero Km 2.0, Tapachula, 30700, Chiapas, Mexico

L.D. Harder

Department of Biological Sciences, University of Calgary, Calgary, Alberta T2N 1N4, Canada

A. Hassan Jalil

Koperasi Meiponi K.L. Bhd., Kuala Lumpur, Malaysia

T.A. Heard

Honorary Associate, Social Insects Lab, School of Biological Sciences, Macleay Building A12, University of Sydney, NSW 2006, Australia

M.M. Henao

Laboratorio de investigaciones en Abejas (LABUN), Departamento de Biología, Universidad Nacional de Colombia, Sede Bogotá, Colombia

J. Hipólito

Laboratório de Biologia e Ecologia de Abelhas; Instituto de Biologia – Departamento de Zoologia, Universidade Federal da Bahia (UFBA); Rua Barão de Geremoabo, S/N, Campus de Ondina; CEP 40.170-110 Salvador, BA, Brasil

D.W. Inouye

Department of Biology, University of Maryland, College Park, MD 20742-4415, USA

J. Jaramillo

Laboratorio de investigaciones en Abejas (LABUN), Departamento de Biología, Universidad Nacional de Colombia, Sede Bogotá, Colombia

N.K. Joshi

Pennsylvania State University Fruit Research and Extension Center, Entomology, 290 University Drive, Biglerville, PA 17307, Pennsylvania State University, Department of Entomology, 501 ASI Building, University Park, PA 16801

M. Kasina

Kenya Agricultural Research Institute, NARL, P.O. Box 14733-00800 Nairobi, Kenya

P.G. Kevan

Alexander Building, School of Environmental Sciences, University of Guelph, Guelph, Ontario N1G 2W1, Canada

L.H.P Kiill

Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA) Semiárido, Petrolina PE, Brasil

R. Krell

FAO, Rome, Italy

C. Krug

Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA), Ocidental, Manaus, AM, Brasil

P. Kwapong

Department of Conservation Biology and Entomology (CBE), School of Biological Sciences Sciences, University Post Office, University of Cape Coast, Cape Coast, Ghana

R.P. Macfarlane

Buzzuniversal, 33 Woodside Common; Christchurch, New Zealand

D.J. Martins

Turkana Basin Institute – Stony Brook University, N507 Social & Behavioural Sciences Stony Brook NY 11794 USA, Insect Committee of Nature Kenya, National Museums of Kenya, Museum Hill, Nairobi, P.O. Box 44486 Nairobi GPO 00100, Kenya

M. Maués

Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA) Amazônia Oriental, Belém, PA, Brasil

J.K.S. Mbaya

National beekeeping station, P.O. Box 34188, Nairobi, Kenya

B.G. Meyrelles

Universidade Federal de Viçosa, Departamento de Biologia Animal, Viçosa, MG, Brasil

P.C. Montagnana

Universidade Estadual Paulista-UNESP, Departamento de Ecologia, Rio Claro, SP, Brasil

J.E. Moreno

Smithsonian Tropical Research Institute, Balboa, Republic of Panama

G. Nates-Parra

Laboratorio de investigaciones en Abejas (LABUN). Departamento de Biología, Universidad Nacional de Colombia, Sede Bogotá, Colombia

C.M. da S. Neto

Universidade Federal de Goiás-UFG, Departamento de Botânica, Goiânia, GO

P. Nunes-Silva

Pontificia Universidade Católica do Rio Grande do Sul, Av. Ipiranga, 6681 – 90619900, Porto Alegre, RS, Brasil

R. Ospina Torres

Laboratorio de investigaciones en Abejas (LABUN), Departamento de Biología. Universidad Nacional de Colombia, Sede Bogotá, Colombia

I. Ovando-Medina

Universidad Autonoma de Chiapas. Centro de Biociencias. Carretera a Puerto Madero Km 2.0, Tapachula, 30700, Chiapas, Mexico

L. Packer

Department of Biology, York University, 4700 Keele Street, Toronto, Ontario M3J 1P3, Canada

G.P. Patricio

Universidade Estadual Paulista-UNESP, Departamento de Ecologia, Rio Claro, SP, Brasil

C. Pigozzo

Instituto de Biologia, Universidade Federal da Bahia – Campus de Ondina, Rua Barão de Geremoabo s/n, 40170-210 Salvador, BA, Brasil

C.S.S. Pires

Embrapa Recursos Genéticos e Biotecnologia, Cx. Postal 02372 – Brasília – DF, CEP 70.849-970, Brasil

V.C. Pires

Instituto do Meio Ambiente e Recursos Hídricos da Bahia, Rua Viena, nº. 425, Bairro Dinnah Borges – Eunálopis – BA, CEP 45.820-970, Brasil

E. J. Rajotte

Pennsylvania State University, Department of Entomology, 501 ASI Building, University Park, PA 16801, USA

M.F. Ribeiro

Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA) Semiárido, Petrolina PE, Brasil

M. Rincon-Rabanales

Universidad Autonoma de Chiapas, Centro de Biociencias, Carretera a Puerto Madero Km 2.0, Tapachula, 30700, Chiapas, Mexico

A. Rodriguez-C.

Laboratorio de investigaciones en Abejas (LABUN), Departamento de Biología, Universidad Nacional de Colombia, Sede Bogotá, Colombia

D.W. Roubik

Smithsonian Tropical Research Institute, Balboa, Republic of Panama

M. Salvador-Figueroa

Universidad Autonoma de Chiapas, Centro de Biociencias, Carretera a Puerto Madero Km 2.0, Tapachula, 30700, Chiapas, Mexico

D. Sammataro

USDA-ARS Carl Hayden Honey Bee Research Center, 2000 E. Allen Road, Tucson, AZ 85719-1596, USA

A.C. dos Santos

Embrapa Amazônia Oriental, Belém, PA, Brasil

R.C. Sihag

Department of Zoology, CCS Haryana Agricultural University, Hisar 125004, India

E.M.S. Silva

UNIVASF, Brasil

P.N. Silva

Universidade Federal de Viçosa, Departamento de Biologia Animal, Viçosa, MG, Brasil

C.I. da Silva

Universidade Federal do Ceará – UFC, Departamento de Zootecnia – CCA, Campus Universitário do Pici, Bloco 808, CEP 60.356-000 Fortaleza – CE, Brasil

K.M.M. Siqueira

Universidade do Estado da Bahia, Campus III DTCS, Juazeiro. Av. Egard Chastinet s/n São Geraldo 48905-680 – Juazeiro, BA, Brasil

E.R. Sujii

Embrapa Recursos Genéticos e Biotecnologia, Cx. Postal 02372 – Brasília – DF, CEP 70.849-970, Brasil

H. Taki

Department of Forest Entomology, Forestry and Forest Products Research Institute, 1 Matsunosato, Tsukuba, Ibaraki 305-8687, Japan

L.I. Vargas-Lopez

Universidad Autonoma de Chiapas, Centro de Biociencias, Carretera a Puerto Madero Km 2.0, Tapachula, 30700, Chiapas, Mexico

J.A. Vazquez-Ovando

Universidad Autonoma de Chiapas. Centro de Biociencias. Carretera a Puerto Madero Km 2.0, Tapachula, 30700, Chiapas, Mexico

B.F. Viana

Instituto de Biologia, Universidade Federal da Bahia – Campus de Ondina, Rua Barão de Geremoabo s/n, 40170-210 Salvador, BA, Brasil

S. Witter

Fundação ZooBotânica do Rio Grande do Sul, Salvador França, 1427 – 90690 –000, Porto Alegre, RS, Brasil

© E. Tourneret

PREFACE
TO THE SECOND EDITION

Pollinators such as bees have been declining in diversity, if not abundance, ever since people began to replace their habitats with those suited for human use. Humans are largely responsible for this problem and, thus, might also be expected to remedy it. How to achieve this, however, is not yet exactly clear. Furthermore, there is now increasing awareness that an intact ecosystem has values determined by social, political, economic and a host of other human devices, which are often in conflict with ecological processes that form and maintain ecosystems and the services they provide to humanity [1–3].

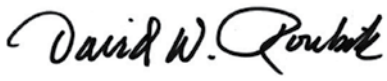
Many advocate the use of "sustainable" approaches in crop pollination. However, it is prudent to draw on the knowledge of experts in related fields. One such group is the sustainable forestry cadre, which encompasses both the so-called developed and developing worlds. In their words, [4] sustainable forestry is not the same as sustainable forests. In the present context, sustainable pollination is not the same as sustainable pollinators. Which pollinators are to be sustained, how and for whom?

There are obvious trade-offs. In the case of agriculture, managed pollinators are brought in when local pollinator numbers are too low in the surrounding environment to pollinate crops at an acceptable level. However, when the environment itself is the source of pollinators, and property boundaries are already set, some difficult decisions are required. How much land or habitat should remain underutilized by agriculture or other activities to sustain pollinators? In other words, how many crops or other materials can be voluntarily sacrificed for the sake of producing fruit and seeds that are only obtained from pollination by wild animals? In larger farms or monocultures, the question is more complex, but similar. If fewer pollinators result in a smaller yield, is it less costly to increase planting density or area, to hire a pollinator service provider (PSP) or to sacrifice arable land for "pollinator reserves" [5]? Finally, biocides almost invariably reduce pollinator populations [6, 7]. Is the cost of such chemical input compensated by the increased saleable produce and the profit margin, compared to lost production due to a pollination/pollinator deficit?

As if this were not already complicated enough, bee keepers are hard pressed to maintain their profit margins, which seem to hover at a level of net profit being just shy of 10 percent of the gross profit [8]. In other words, no one is getting rich, but commercial beekeeping is sustainable – meaning that it can continue and is not going "into the red". The fact that nature is deemed sustainable only when such a decline is avoided is a sure sign of trouble. Nature must not only continue, but advance

by a process known as natural selection, to keep pace with the mounting challenges posed both by environmental change and human impact. Without the appropriate habitat and populations it supports, that cannot occur.

The present compendium for practitioners shows the reader how to strive to maintain important checks and balances, taking into consideration pollinators in croplands, both large and small, and within the world's temperate and tropical realms. While it describes a range of methods and goals, it does not advocate any particular product or copyrighted item. Thanks are due to FAO for its service in furthering applied pollination science, and to B. Gemmill-Herren, who managed to initiate the Global Pollination Project and provide FAO with professional expertise, thus continuing to support this work.



David W. Roubik
Smithsonian Tropical Research Institute,
Balboa, Ancon, Republic of Panama

REFERENCES

[1] Foley, J.A., DeFries, R., Asner, G.P., Barford, C., Bonan, C., Carpenter, S.R., Chapin, F.S., Coe, M.T., Daily, G.C., Gibbs, H.K., Helkowski, J.H., Holloway, T., Howard, E.A., Kucharik, C.J., Monfreda, C., Patz, J.A., Prentice, I.C., Ramankutty, N. & Snyder, P.K. 2005. Global consequences of land use. *Science*, 309: 570–574.

[2] Breeze, T.D., Vaissière, B.E., Bommarco, R., Petanidou, T., Seraphides, N., Kozák, L., Scheper, J., Biesmeijer, J.C., Kleijn, D., Gyldenkærne, S., Moretti, M., Holzschuh, A., Steffan-Dewenter, I., Stout, J.C., Pärtel, M., Zobel, M. & Potts, S.G. 2014. Agricultural policies exacerbate honeybee pollination service supply-demand mismatches across Europe. *PLoS One*, 9: e82996.

[3] Pimm, S.I., Jenkins, C.N., Abell, R., Brooks, T.M., Gittleman, J.L., Joppa, L.N., Raven, P.H., Roberts, C.M & Sexton, J.O. 2014. The biodiversity of species and their rates of extinction, distribution, and protection. *Science*, 344, doi: 10.1126/science.1246752.

[4] Aplet, G.H., Johnson, N., Olson, J.T. & Sample, V.A. (eds.). 1993. *Defining sustainable forestry*. Washington, DC, Island Press.

[5] Roubik, D.W. 1996. Diversity in the real world: tropical forests as pollinator reserves. In M. Mardan, A. Sipat, K.M. Yusoff, H.M.S.R. Kiew & M.M. Abdullah, eds. *Proceedings of the International Conference on Tropical Bees and the Environment*, pp. 25–33. Penang, Malaysia, Beenet Asia and Southbound.

[6] van der Valk, H. & Koomen, I., eds. 2013. Aspects determining the risk of pesticides to wild bees: risk profiles for focal crops on three continents. Pollination Services for Sustainable Agriculture, Field Manuals. Rome, FAO.

[7] Roubik, D.W., ed. 2014. *Pollinator safety in agriculture*. Rome, FAO.

[8] Spivak, M. 2010. The business of pollination. In E. Mader, M. Spivak & E. Evans, eds. *Managing alternative pollinators: a handbook for beekeepers, growers, and conservationists*, pp. 1–14. Ithaca, NY, SARE Handbook 11, NRAES-186.

A NOTE ON REFERENCES

Several of the chapters included in this publication appeared in earlier forms in the previous edition of this Compendium: *Pollination of Cultivated Plants in the Tropics* (1995). The presentation of the references in these chapters has remained the same, with the inclusion of newer publications where these are mentioned in the text. New chapters and sections use a numbered reference system. All chapters have been revised and updated for this second edition.

ACKNOWLEDGEMENTS

Funding for **Chapter 4** (D.J. Biddinger, E.G. Rajotte and N.K. Joshi) was provided by a State Horticultural Association of Pennsylvania grant, an USDA-SCRI Research and Extension grant (PEN04398) on native pollinators, an USDA-NRCS Conservation Innovation grant with the Xerces Society for Invertebrate Conservation, and a current regional USDA-SCRI Coordinated Agricultural Project grant (MICL05063) on integrated crop pollination. The authors thank Dr. Sheena Sidhu, Department of Entomology, University of California, Riverside, CA for reviewing a previous draft of this chapter.

The work for **Chapter 7.1** (*M. Kasina*) was carried out within the Kenya Pollination Project (KPP), which forms part of the Global Pollination Project, and is financially supported by GEF and implemented in seven countries, including Kenya, by UNEP through FAO Rome. The author thanks the team involved in project implementation at Kakamega, Mt Kenya and Kilimambogo, as well as the national project coordination team, and the farmers who agreed to participate in the project.

The study that comprises **Chapter 7.3** (*D. Martins*) was conducted as part of an analysis of insect diversity and plant-pollinator interactions in the Turkana Basin at the Turkana Basin Institute. The following student volunteers from Hillcrest Secondary School assisted with fieldwork: N. Morey, E. Stephanou and T. Maseland and P. Lomosingo. The Nachekichok Women's Group are also thanked for allowing the project team to work on their farms. Assistance, useful comments and various insights were provided by S.C. Collins, R. Leakey, M. Leakey, L. Leakey, I. Angelei, A. Powys, M. Kasina, M.N. Mutiso, W. Kinuthia, J. Mamlin and S.E. Mamlin, and the scientists and staff of the East African Herbarium, National Museums of Kenya and Nature Kenya.

Chapter 9.3.1, the study on Brazil nut (*Bertholletia*) in the Amazon (*M. Maués, M.C. Cavalcante, A.C. dos Santos and C. Krug*), was made possible by support from Embrapa, CNPq – Process n° 556406/2009-05, and the Global Pollinator Project GEF/UNEP/FAO/MMA, FUNBIO.

The authors of **Chapter 9.3.4** (*R. Ospina-Torres, J. Jaramillo, A. Rodriguez-C., M.M. Henao and G. Nates-Parra*), a study of passion fruit on Colombia, would like to offer their thanks to the Universidad Nacional de Colombia (DIB and Departamento de Biología) and COLCIENCIAS for funding the study (DIB 8003251; COL 1101-52128758) and to GNP and ROT and the Young Researcher (JJ). They also thank the team of the Bee Research Laboratory (LABUN) for their support. They are grateful to Scott Bridges for his translation and editorial assistance, and to David Roubik for his invitation to participate in this publication and for his editorial comments. The owners of the crops are also thanked for their collaboration.

Chapter 9.3.4 (*M.C. Gaglianone, E.V. Franceschinelli, M.J.O. Campos, L. Freitas, C.M. Silva Neto, M.S. Deprá, M.A.S. Elias, L. Bergamini, P. Netto, B.G. Meyrelles, P.C. Montagnana, G.P. Patricio and L.A.O. Campos*) resulted from the CNPq project (556057/2009-0) and the Tomato Pollinators Network Research in Brazil, as part of the Project "Conservation and Management of Pollinators for Sustainable Agriculture, through an Ecosystem Approach", supported by the Global Environmental Facility Bank (GEF), coordinated by FAO with implementation support from UNEP, and supported in Brazil by the Ministry of Environment (MMA) and the Brazilian Biodiversity Fund (Funbio). Thanks are due to G.C.G. Delaqua, M.F. Marques, A.P. Hautequestt, M.C.M. Morais, L.F. Ferreira, I.R. Stanciola, G. Paiva, B.B. Grisolia, F.G. Brocanelli and M.A. Pizano for their assistance in the field and laboratory, and to the farmers that allowed the study on their proprieties. Grateful thanks are also due to G.A.R. Melo, F.A. Silveira, S.R.M. Pedro and F.F. Oliveira for taxonomic identification. EMATER and RioRural/SEAPPA/RJ and FAPERJ (MCG: E-26/112.652/2012) are thanked for logistical and financial support. M.C.Gaglianone, L. Freitas and L.A.O. Campos are grateful to CNPq for the PQ productivity scholarships.

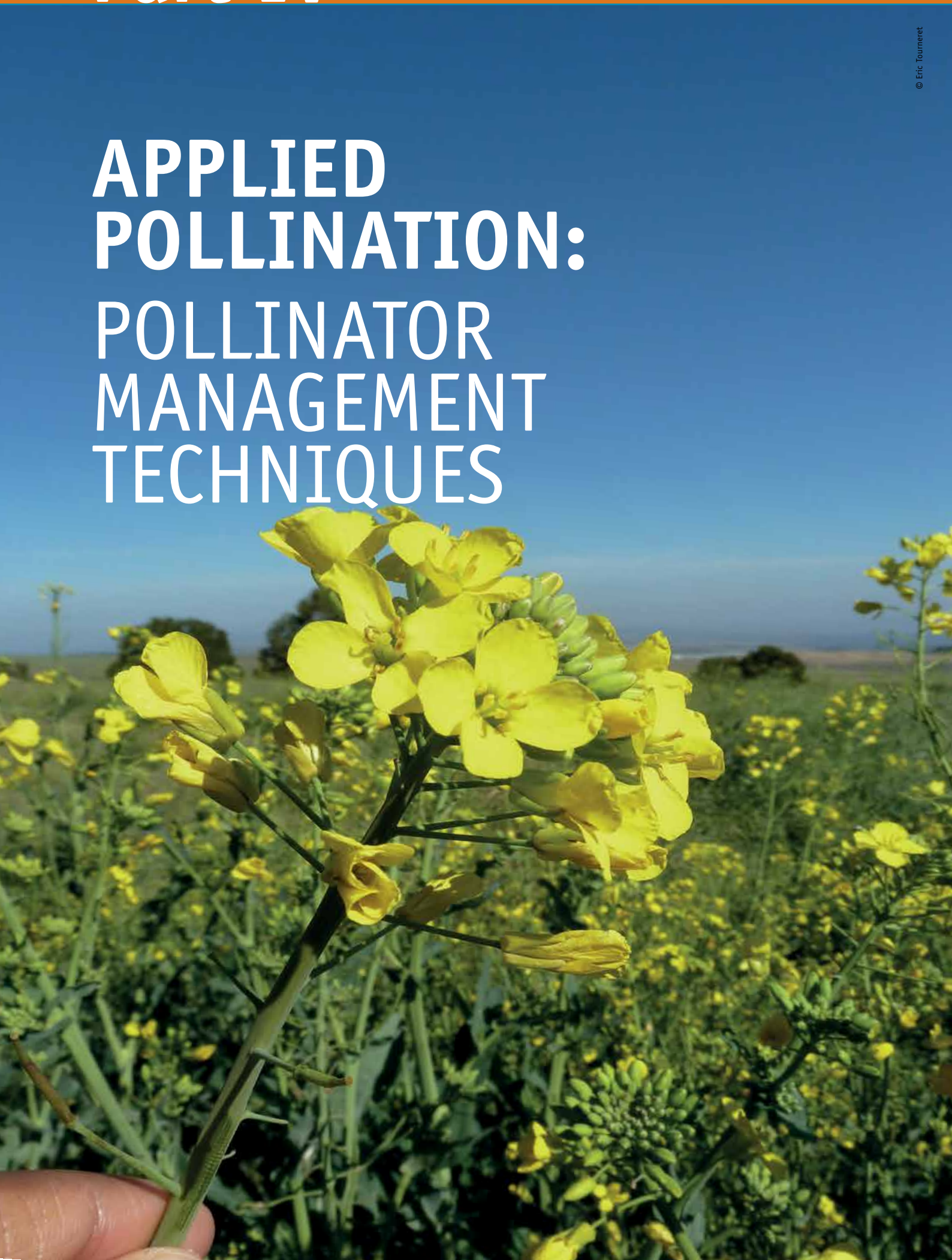
Chapter 9.3.11 contains information gathered over 20 years of research on cashew pollination through projects supported by different institutions and in partnership with academics, researchers, students and cashew growers to whom the author (B.M. Freitas) is indebted. Part of the content presented here resulted from the CNPq-Brasilia/Brazil projects 521946/96-4, 305062/2007 and 7556042/2009-3, and the Cashew Pollinators Network Research in Brazil, as part of the Project "Conservation and Management of Pollinators for Sustainable Agriculture, through an Ecosystem Approach", which is supported by the Global Environmental Facility Bank (GEF), and coordinated by FAO with implementation support from the United Nations Environment Programme (UNEP) and supported in Brazil by the Ministry of Environment (MMA) and the Brazilian Biodiversity Fund (Funbio). The author thanks the Brazilian National Research Centre for Tropical Agro-Industry (EMBRAPA/CNPAT) for use of its experimental station to conduct field research and the supportive help of its staff over many years. The author is also grateful to CNPq-Brasilia/Brazil and CAPES-Brasilia/Brazil for M.Sc. and Ph.D. sponsorship for the students involved in the projects and the British Council (NE Brazil) and CNPq-Brasilia/Brazil for travel grants.

The author of **Chapter 11** (*A. vanDoorn*) offers his thanks to H.H.W. Velthuis for critically reading the manuscript.

The authors of **Chapter 15** (*C.I. da Silva and B.M. Freitas*) would like to thank many people and institutions involved with their studies of carpenter bees and their role as pollinators over the last two decades. B.M. Freitas would like to thank his former and present students for their dedication, passion fruit growers F. Ferreira Neto, J.B.C. Nunes and V.C. Aquino for their interest and collaborative work in the field trial, and CNPq–Brasilia/Brazil for supporting project # 467275/2000-9 and CNPq–Brasilia/Brazil, CAPES–Brasilia/Brazil and FUNCAP–Ceará/Brazil for the M.Sc. and Ph.D. sponsorship of students involved in the projects. C.I. da Silva would like to thank FAPEMIG–MG/Brazil for its financial support.

The Editor wishes to express a particular acknowledgment to Dr. Barbara Gemmill-Herren for devoting much effort and input to these works, both while at the FAO and in her continuing dedication to the global pollination field.

Part IV



APPLIED POLLINATION: POLLINATOR MANAGEMENT TECHNIQUES

Chapter 10

SUCCESSFUL POLLINATION WITH ENHANCED POLLINATOR ABUNDANCE

R. Krell

The term "enhanced pollinator abundance" is used here in reference to populations that are artificially manipulated or managed for the purpose of crop pollination (see Chapters 3 and 5–7 for details of wild pollinator populations that have not been manipulated directly). A variety of conditions must be fulfilled to use enhanced pollinator populations for agricultural crop pollination. The local crop requirements must be understood and the environmental, economic, infrastructural and social conditions necessary to ensure access to pollinator populations must exist. This chapter presents a broad overview of the conditions needed to ensure successful pollination services.

Exceptional advances made with one or two temperate races of *Apis mellifera* (usually *ligustica*) have resulted in observations such as the following: "When ... a crop is difficult to pollinate, usually because it is a species relatively unattractive to honey bees, more than 2.5 colonies (and often 3 to 6) per hectare are sometimes recommended. In contrast, when a crop is attractive, but its flowers are comparatively sparse, or can be self-pollinated, fewer than 2.5 colonies per hectare are suggested (e.g. *Cucumis melo*, *Trifolium repens*)" (Free, 1993, p. 52). Unfortunately, no such generalized figures exist for other pollinators, although some recommendations were recently given for the "stocking density" of *Apis cerana* in crop fields. Those seem similar to the recommended densities for the western hive bee, because although the Eastern hive bees (*Apis cerana* and *A. indica*) maintain much smaller colonies than those of *A. mellifera*, they do not forage as far from the hive. As a consequence, higher numbers visit the target crop. No studies for the many other tropical pollinators are sufficiently complete to permit basic estimates of pollinator abundance requirements in croplands or other "managed systems".

Before a crop is selected for cultivation in any environment, its pollination needs should ideally be evaluated. However, the present lack of information results in ignorance of the pollination requirements of many plants cultivated in the tropics. In the case of commercially grown crops, it is known that abundant pollinators will increase their fruit or seed production and quality. What is not known for most local conditions is whether the pollination needs can be taken care of by local or wild pollinator populations. The question becomes more difficult when little or no pollination research has been conducted with a particular crop.

As a guideline, it may be assumed that any crop requiring pollination will be less productive without extra pollinators if planted in fields wider than 50–100 m – even if the fields border areas with abundant pollinators. Pollination success of course also

depends on many other conditions, as emphasized throughout this book. In the case of crops, it is important to consider:

- the species of natural pollinators and their foraging ranges
- the attractiveness of the crop flowers
- the use of pesticides in the area
- the total size of the field
- the distance to the natural habitat of wild pollinators.

For example, natural pollinators of a Macadamia nut plantation in Malawi (mostly *Apis mellifera scutellata*) are effective only for the first few rows of trees, despite abundant honey bee colonies in forested slopes not more than 100 m from the edge of the plantation.

Distribution studies of honey bee foragers in field crops highlight the importance of saturation of the area with bee colonies and the resulting irregular distribution with lower densities. Depending on the crop's flowering characteristics, lower pollinator populations may be sufficient. A general "rule of thumb" for the density of honey bee colonies needed for successful pollination is that approximately 2.5 colonies per hectare (one colony per acre) should be distributed evenly throughout the crop. This number increases with unattractive crops, hybrid seed production or in the presence of significant competition from other floral resources.

10.1 SELECTION OF CROPS AND CULTIVATION METHODS

In many countries, food self-sufficiency at any cost, often referred to as the Green Revolution, has brought about significant environmental destruction. Many of the resulting agricultural systems are not self-sustaining or efficient, because they depend to increasing degrees on outside inputs of imported pesticides, fertilizers, machines and fuel. Large-scale extensive cultivation practices have demonstrated their destructive nature under most tropical climates, soil conditions and management. However, they are continued in many regions because of the expected high, short-term yields, which are often not sustained. This is a familiar scenario for many enterprises in East Asia and South and Central America. Meanwhile, non-destructive agricultural methods have been shown to be equally as profitable as high-technology, high-input agriculture.

Regardless of region, selection of the right crop or crop variety is of great importance, as this ultimately determines whether cultivation will be profitable. Local indigenous varieties are usually highly resistant to diseases common in their area, but do not have the high productivity of hybrids grown with all the requisite chemical support. In addition, native crops often do not respond to fertilizers as dramatically as hybrids. However, when both are grown without all the refined techniques and inputs, indigenous crops in most cases out-compete hybrids. Many local crops could be improved for yield, particularly in conjunction with less chemically enforced cultivation methods.

Net benefits from managed pollination (in the form of extra fruit or seed set and better quality) will occur only when necessary cultivation practices are already in place. Given limited investment capital, the efficiency of crop production could be improved through better cultivation methods, storage, processing and shipping, or even advertising. Pollination is just one of the several avenues available to growers seeking to increase profits. Crops grown under marginal conditions cannot be expected to improve significantly through pollination management unless they depend almost 100 percent on pollinators. Growing conditions must be optimal to benefit most from pollination enhancement. Normally, there is no need for manipulated pollination services for exotic crops that do not require highly specialized pollinators. In such cases, the soil, water and weather conditions mostly limit fruit set. Unless the environment has been badly damaged, a relatively diverse pollinator fauna should still exist that can fulfill basic pollination requirements (see Part II). Such conditions are necessary for experiments to demonstrate the benefit of additional pollination and/or improved cultivation methods.

10.2 SELECTION OF POLLINATORS

Pollinator selection depends not only on crop requirements, but also on technical, practical and economic considerations such as basic infrastructure, including reliable transport and good road conditions. Under most circumstances, pollination with honey bees implies a capability for migratory beekeeping, which in turn requires advanced frame-hive equipment and knowledgeable beekeepers.

The type of honey bees present in most parts of Africa and South and Central America are overly sensitive to disturbance and often react with highly defensive behaviour. Such bees cannot be situated on the edge of fields where tractors or other vehicles pass (or where people work), as the engine vibrations upset the colonies. Similarly, workers in Argentina often wear bee suits and veils while weeding and irrigating alfalfa fields, as they are surrounded by Africanized honey bee colonies used for pollination.

Maintaining the less defensive European honey bees is often very difficult, with losses resulting from diseases, queen replacement and low productivity. Importing less defensive queens is relatively expensive and presents a great risk, as most bee diseases are spread through such importations. Even if the diseases are already present, new stock may be less resistant to certain forms of disease present in the area. Serious diseases not only kill colonies but also drastically reduce the number of active beekeepers and wild colonies. Colonies with imported queens often fail to produce a honey crop for beekeepers. This may be because imported bees are not adapted to the new area or to competition with local bees. As a result, the costs of importation programmes can add up, beyond the point where they are justified by the economic benefits of better pollination or the monetary price of the service.

Where European bees can be bred in isolation and the colonies maintained at least part of the year in colder areas, such as mountains or other latitudes, the cost and feasibility of maintaining European colonies in zones of Africanized or African bees (*Apis mellifera scutellata*) may be acceptable. In other areas, the selection of less defensive local strains may be possible but requires time and a well-organized breeding programme, as well as regular re-queening by beekeepers and a sufficient market for such queens.

Colonies of *Apis cerana* in most tropical environments have relatively small forager populations and react readily to disturbances by absconding. However, programmes in southwestern India and southern China have demonstrated that large-scale honey production and stable management using modern hives is possible. Well-organized breeding programmes combined with improved management may improve the characteristics of *A. cerana* throughout Asia. Indeed, limited migratory beekeeping with this species is possible. The other species of potential hive bee – the Malesian honey bee *Apis koschevnikovi* – produces little honey and very readily absconds (D. Roubik, personal communication). No breeding or management programmes seem useful for this species.

The benefits and limitations of non-*Apis* pollinators are discussed in other parts of this book. Due to their greater degree of adjustment to specific environmental conditions, they may perform better in areas where honey bees have difficulty surviving. As many of them are solitary, short-lived species, their life cycles have to be well synchronized with the flowering of the crop (or their diapause and emergence times must be controlled). Meliponine (stingless bee) colonies may provide an alternative due to their usually docile nature and the domestic market for their honey (see Chapters 13 and 14).

Instead of selecting a specific pollinator species and supplying the additional training and infrastructure required, it may be preferable to preserve suitable pollinator habitats and adapt existing cultivation practices (see section 3.2 for more details).

10.3 MIGRATORY BEEKEEPING

Migratory beekeeping offers an opportunity for much higher honey yields, despite the higher cost and need for reliable transport. Many migratory beekeepers rely upon

the income from rental of their colonies for pollination. Most also benefit from installing their colonies at sites that are very productive, even if only temporarily.

The procedure for hiring commercial pollination services requires agreements in the form of contracts. The most advantageous form of contract and legal structure may be differ from country to country, but a few key items should be contained in any verbal or written agreement (see Annex 1 for a sample contract for use by growers and beekeepers).

Once pollinators have been chosen, they must be transported to the fields where their services are required. In the case of insects kept in containers this is relatively easy, but where specific nesting sites or domiciles are used, mobility is often more limited. The importance of mobility increases with the frequency of pesticide use. Perennial pollinators, which live in colonies or established aggregations, must be protected from poisoning. The future abundance of annual populations is less susceptible to spraying as long as their broods are provisioned with food and protected from exposure.

When the target crops are not in bloom but abundant floral resources are present, permanent colonies may be kept, and the pollinators prevented from flying during application of pesticides to crops. Once crop flowering has ceased, the prevention of spray drift and use of less toxic insecticides in lower quantities (less frequent application during the evening or at night) will considerably reduce the risk to managed pollinators.

Where permanently established or immobile colonies are used for pollination, these usually cannot be kept within crop fields and many foragers may visit non-crop flowers. Thus, larger numbers of colonies may be required, compared to the usual guidelines mentioned above. If the natural flora does not support such large numbers of colonies, then additional colonies must be brought in when pollination services are needed. Habitat improvement would diminish the need for migratory beekeeping.

Migratory beekeeping with stingless bees may be possible under conditions similar to honey bees (Chapter 14). Some non-bee species such as flies may be reared in large numbers and simply released where needed, when the adult insects emerge (Chapter 12).

Several phases of development apply to migratory beekeeping. These are outlined and discussed in the remainder of this section.

Equipment. The pollination services of honey bees require colonies domiciled in advanced hives that can be used to provide colonies of the right strength at the right time. Hives can be constructed using top-bar or frame hive equipment, but for migratory beekeeping the frames must be wired to support combs during transport, and screening material must be used to allow adequate ventilation (Chapter 16).

Top-bar hive beekeeping, as widely practised in most tropical climates, is ill suited to migratory beekeeping. Comb breakage occurs during transport, with subsequent loss of the colony. Extremely careful transport of top-bar hives can nonetheless be attempted, but only when combs contain little or no honey and sealed brood, and when roads are smooth and colonies well ventilated. However, relatively high losses are likely.

Alternatively, smaller colonies or "nuclei" may be used. These can be transported more easily and do not have combs or frames. A larger number of colonies would be needed, however. The use of small, "disposable" honey bee colonies or "pollination units" was piloted in the United States and may be feasible elsewhere, where large numbers of swarms can be easily captured during the time of year when pollinators are needed. This could be particularly useful in Latin America, where very large numbers of colonies can be obtained during the swarming season of the African honey bee. These pollinators can be maintained temporarily in almost any container and place. However, absconding by the queen and colony occurs frequently after transport. Two means are available to reduce this tendency: the queen is either confined in a small "queen cage" prior to transport, or "queen excluder" material is placed over the entrance. Despite its relative ease, the latter method has the drawback that a queen temporarily starved by the workers may still pass through the excluder, and the colony can

escape. Furthermore, African honey bees (*A. mellifera scutellata*) frequently abandon their own queen in such situations, somehow joining other colonies.

Stingless bees (meliponines) and bumblebees require hives or (sometimes in the case of the former) log hives of suitable size and design to accommodate their colonies, and to enable easy access by the beekeeper. They also need to be transportable in large numbers to sites where they can serve as pollinators. Carpenter bees and solitary bees require specially prepared nesting sites. The bees are often kept in holding for several months as immatures, and their use therefore requires advanced planning. Some of these domiciles may not be movable. More research is therefore required to develop appropriate containers for bee species and other pollinators.

Infrastructure. Appropriate access to the field is necessary for any supply of enhanced or increased pollinator populations. This usually means road access, and not just any road or trail, but reasonably smooth road surfaces to avoid the destruction of hives, combs and therefore whole colonies. This is less important where colonies can be left in place permanently. Waterways may be used in some countries for transport to the point that whole apiaries may be installed on barges or boats. Such river transport disturbs the bees less than most forms of road transport. Regardless, the mode of transport must be safe and dependable. Breakdowns may create a hazard to other people or result in the death of the colonies due to overheating. Road and vehicle conditions should therefore be highly reliable, as transportation usually takes place at night.

Legal regulations should facilitate transport licencing or transit permits, and also regulate sanitary inspection and certification.

Communication (between both contract partners) must be reliable in order to coordinate and time the delivery of pollinators correctly, during the right phase of flowering, so to avoid conflicts with pesticide applications.

Research. Nectar secretion depends highly on environmental factors and soil conditions. Because flower attractiveness is key for pollination success, and since attractiveness depends on the amount of nectar or pollen made available, pollination success depends strongly on soil conditions and weather prior to and during flowering. Difference between local conditions may mean that research results are not applicable from one area to another. Local populations of pollinators may also be sufficiently different to change comparative results on the pollination of open-pollinated flowers. In other words, while additional pollinators in one area may increase production, the natural pollinator population in other areas may be sufficiently large to achieve the same production level without pollinator management. In summary, different crop varieties, or even the same ones, should be tested under local conditions and with different pollinators to determine attractiveness and pollination (see Chapter 17).

With a few exceptions, little is known about non-*Apis* pollinators in tropical climates, and less about wild pollinator populations or their management and effectiveness in agricultural settings. Many possibilities remain for research and, as a consequence, many new practical applications. Pollination using honey bees, among the races of *Apis mellifera* and to an increasing extent *Apis cerana*, is still the easiest and quickest solution when the crop in question does not demand the use of other pollinators.

The life history and behaviour of many bee species and other possible pollinators has to be studied locally, as is increasingly done in many countries. According to their behaviour, some species can be selected for studies on their manageability, the possibility of increasing populations and their suitability for various crops. Management skills will have to be suitable for local farming communities or be economically feasible for specialists.

Simultaneously, research and application trials must be conducted on different cultivation methods. Better choice of crops including more traditional, indigenous species and varieties also plays an important role. In addition, cultivation methods that are less toxic and destructive, and which actually improve or at least maintain soil quality, would result in benefits far exceeding the provision of extra pollinators.

This kind of research is likely to receive more attention and funding, since it not only improves food production, but also enhances the quality of food and the environment. Amid these anticipated changes, it is important not to lose sight of pollinator populations and their maintenance. In particular, supporting technology for pollinator management is underdeveloped and more research is essential.

Training, skills and technical assistance. Beekeeping with honey bees, contrary to the belief of many laymen, politicians, agricultural officers and development planners alike, cannot be "learned in a day". Years of training and experience are needed to ensure that beekeeping is cost-efficient and applicable to migratory practices, or adequate for the management of population sizes required for crop pollination. While many countries now have trained and often experienced beekeeping technicians, beekeeping practices in most village environments do not meet the necessary standards. This may be due to equipment choices, numbers of colonies managed or lack of management skills.

More intensive technical assistance is required to improve the situation. Due to economic difficulties in most tropical countries, however, such technical assistance is often lacking. Even if sufficient numbers of technicians are available, they may not have access to transport to the villages, or receive travel allowances for their extra expenses; they maybe overloaded with other extension responsibilities, and lack practical training and experience, or any combination of the above. In addition, beekeeping products need access to an attractive and reliable market, in order to entice farmers to invest time and money in more costly management and equipment. The management of non-*Apis* pollinators will require completely new skills, which are not part of traditional practise, except in a few instances with meliponine bees (see Chapters 13 and 14). However, lack of tradition practice can make the introduction of new skills easier. Regardless, specialized service and training will be essential.

Prior to introducing crops that require pollination services, it is important to conduct an economic feasibility study, as well as a realistic survey of the possibility of transfering the required skills (i.e. frame-hive beekeeping, management of other pollinator species or different cultivation methods). Most of all, there must be serious long-term commitment at various levels.

Economic feasibility. Increased production or the higher quality of the crop is essential to justify the additional expense for pollination services. The additional cost first has to be determined. The following must be included:

- the cost of improved crop cultivation practices (mechanization, fertilizers, pesticides, etc.)
- frame hive beekeeping or management of other pollinators
- alternative planting schemes
- extra transportation requirements
- costs to society or government for skills acquisition, technical assistance and research into local requirements.

Alternative improvements always merit consideration, such as soil fertility, varietal selection, irrigation, pest damage and post harvest losses. These alternatives may be less expensive to improve and may also result in additional benefits aside from improved pollination. An in-depth feasibility study is needed to establish the weight of these factors and priorities.

Adequate cultivation practices are essential with enhanced pollination; otherwise, increased production increase will be sacrificed. (Plants that are starved for nutrients do not produce more seeds and fruit after receiving better pollinator service.) This, too, may mean higher investments by the farmer and more material or management inputs, unless cultivation methods are changed. The cost to society consequently increases. Additional income from pollination fees, as well as from higher honey yields, are essential to justify the higher investment in beekeeping equipment. However, less costly beekeeping methods are usually adequate for local beekeeping conditions.

Investment capital is an important limitation, as it is unlikely that the beekeeper has the required funds. Bank loans are usually very expensive and difficult to obtain. Banks in general are not willing to accept beekeeping equipment as collateral and are usually unwilling to finance beekeepers, unless the beekeeper can offer more standard forms of collateral. Even with sufficient finances available, the establishment of beekeeping operations capable of providing considerable numbers of bee colonies will take time – at best a few seasons, even with highly experienced beekeepers and managers. Consequently, there is a difficult gap to bridge. While honey production alone seldom justifies improvement in beekeeping material and techniques, pollination of crops will not be feasible until this more expensive form of beekeeping is widely practised and cultivation practices are adequate. It will prove difficult and uneconomical to introduce crops requiring pollination, unless they can be grown profitably without pollination services from the beginning (until beekeepers and growers have learned and put into practice the mutual benefits).

The prospects for managing non-*Apis* pollinators appear to be even less promising because of their limited use. However, it is exactly this higher degree of specialization or adaptation to more extreme conditions that makes them valuable. More specialized skills will therefore be necessary for their management. This again increases the overall cost, however the specialized applications may render this profitable, not just from an economic perspective, but also in terms of the choice of crop.

To summarize, it is important to ask the following questions:

- Are there other crops or varieties that produce slightly lower yields, but necessitate lower investment and pollination requirements?
- Is the new crop worth the extra cost of additional training, technical assistance and research?
- Would the costs involved (particularly the "hidden" or "secondary costs" of training, research, etc.) not be better spent on improving environmental conditions?

The market must acknowledge the improved quality of the crops by attributing higher prices, or compensate storage losses. It also follows that consumer demand must be equal to the increased production. If these conditions are not met, the additional costs will not be economically sound and cannot be recommended.

Social acceptance. To ensure new methods are put into practice, those involved need to accept them. Will a beekeeper want to move his bees or hire them out? More importantly, will a farmer be willing to pay for pollination services? Will both sides trust the other to comply with a contract or agreement? In some countries, it has taken a long time to convince farmers of the value of hiring additional pollination services for particular crops. For example, in one Latin American country, several years passed after the introduction of sunflowers as a crop, before farmers were willing to pay for honey bee colonies in their fields. Initially, beekeepers were content to have their colonies in or near sunflower fields because of the high honey yields. However, larger numbers of colonies in sunflowers – necessary for increased seed production – led to lower honey production per hive. The concept of paying for pollination services evolved slowly as a means to satisfy both sides. It is apparently difficult to convince farmers that naturally available pollinators, or those of the few beekeepers nearby, are not sufficient to increase seed or fruit yields significantly.

10.4 ENVIRONMENTAL IMPACT

Manipulating any population of plant, insect or other animal will have an impact on the environment. Increasing honey bee populations will displace native nectar and pollen-feeding insects to other flowering species, which may displace other species, and so on. The same will be true for agricultural systems that have sustained large human disturbances. Lack of natural pollinators under such circumstances is due primarily to the negative impact of large monocultures. Adding pollinators to such a system should lead to an

improvement in environmental conditions. In border environments near natural forests, the displacement effect, outlined above, may be minimal because the depth of such an effect is likely to be very limited. In contrast, small plantations or hedge communities might experience greater impacts.

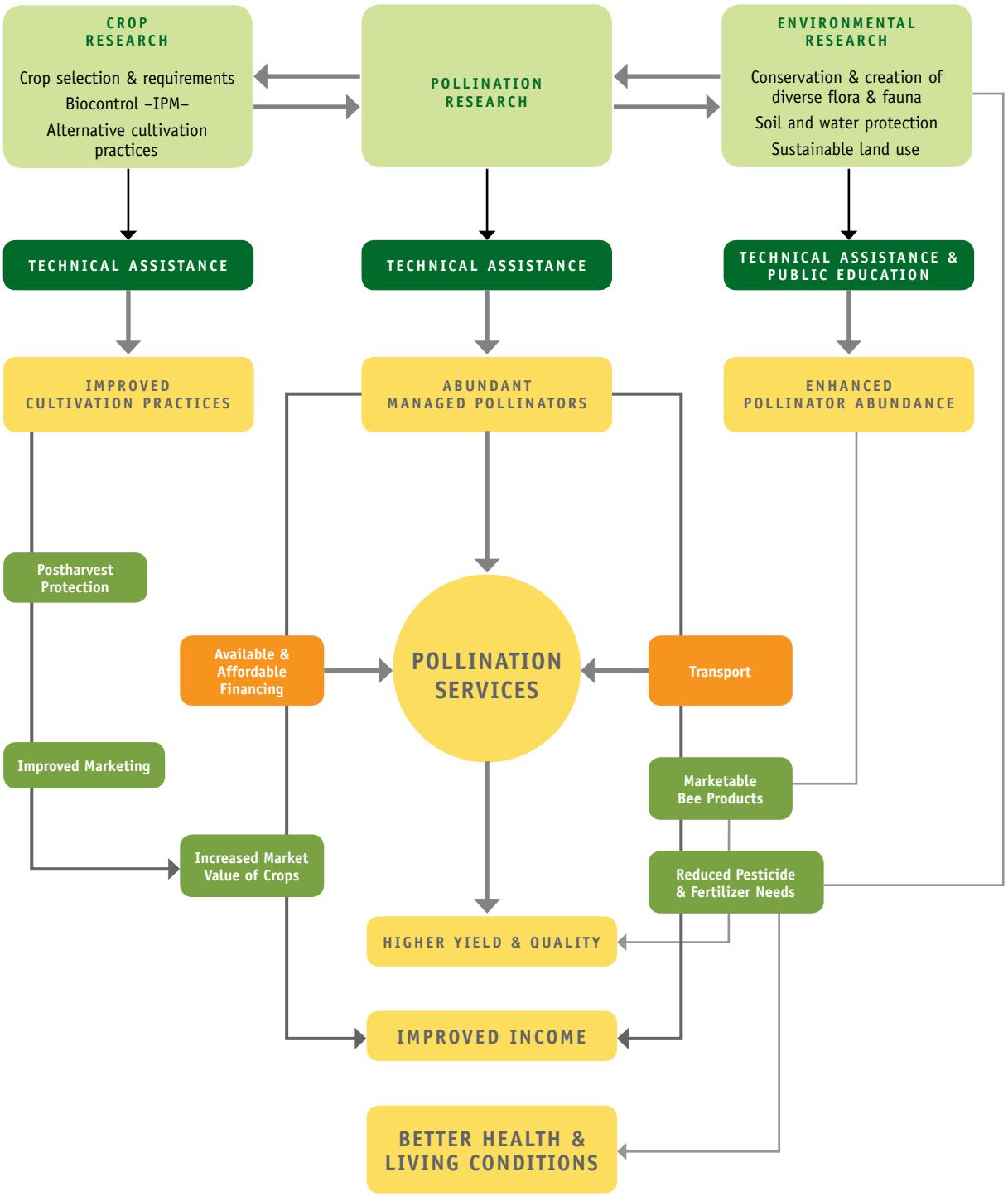
The general fact that bees or other pollinators are beneficial for the environment may be correct, but the assumption that the introduction of additional pollinators is always beneficial is not justified. In areas where manipulated pollinators are native, increasing their populations will probably have local and temporary effects. For exotic species, the impact may range from the displacement of local species to their extinction in certain habitats, as well as favouring the spread of certain plants (including weeds or diseases.

Improved habitat diversity, which promotes the creation of numerous microhabitats such as hedges and planted forest patches, seems to produce the least negative impact. This approach and less destructive cultivation practices generally improve habitat conditions and should contribute eventually to higher production for all crops, whether they require pollination or not.

10.5 CONCLUSIONS

This chapter highlighted some of the essential requirements for improving crop yield and quality through pollination. The basic mechanisms and requirements are known and have been tested under various conditions. Less information is available on the individual situation in each country. In industrialized countries the basic infrastructure, including beekeepers, is already present, having been built up over the last century or so. However, beekeepers have only recently become essential for agricultural production, as a consequence of increased environmental degradation and larger monocultures. Creating the required infrastructure in a short period of time is both difficult and expensive. This eventuality can be avoided through the practice of environmental conservation, small-scale less destructive farming, better planning, logical choice of crops and other practices outlined above. The end result is less expensive and more practical, both in the short and long term.

Figure 10.1
POLLINATION SERVICES



Source: R. Krell [present study]

© D. W. Roubik

Chapter 11

BUMBLEBEES (*BOMBUS* SPP.) IN MANAGED POLLINATION

A. vanDoorn

11.1 SUMMARY

Bumblebees have been propagated commercially since 1987. Five species are now distributed for crop pollination: *Bombus terrestris* in Europe, *B. impatiens* in North America, *B. ephippiatus* in Central America, *B. atratus* in South America and *B. ignitus* in Asia. The colonies are used mainly for greenhouse crops, notably tomato, but their application in open field crops is increasing, partly the result of decreasing honey bee availability. In the greenhouse and the open field, optimal pollination performance requires a number of management considerations. Good sanitary practices are needed, not only to improve the rearing process, but also to eliminate the risk of pathogen spillover in the environment – probably the most important threat emerging from commercially produced bumblebees. Through proper management the risks can be restricted but not eliminated. At the same time, tomato and other crop growers gain large advantages by using bumblebees to increase crop quality and quantity. In those countries where reliable alternative pollinators are often unavailable (e.g. Australia), tomato growers may even seek imported bumblebees, but this practice is not a recommended. A careful balance between environmental risks and economic importance is therefore required.

11.2 THE HISTORY OF COMMERCIAL BUMBLEBEE REARING

Bumblebee importance for crop pollination was recognized in the late nineteenth century [1]. Initial applications to improve the seed set of red clover involved bumblebee queens, caught in the United Kingdom and then introduced to New Zealand. Following that phase, more researchers progressively investigated bumblebee behaviour and physiology. Some researchers also attempted to rear bumblebees in captivity and increase their abundance, either for laboratory studies or for pollination trials. Knowledge concerning variables such as the appropriate temperature regime in the rearing chamber and during hibernation, the means to circumvent or break diapause, or to stimulate colony initiation, and regarding mating and food storage, increased steadily. They culminated in the foundation of Biobest, the first company devoted to bumblebee rearing, in Belgium in 1987.

During the foregoing years, the founder of Biobest, Dr. de Jonghe, a veterinarian and amateur bumblebee researcher, discovered that bumblebees were reliable pollinators of tomato crops in greenhouses. Prior to this discovery, tomato flowers in Western European greenhouses were pollinated mechanically by vibrating the flowers three times a week (tomato flowers are self-fertile, but cannot be pollinated without "buzzing" or "sonication" – vibration that

ejects pollen from the tubular anthers). The labour costs were high, as much as €10 000 per ha per year, and the labour itself was unattractive, resulting in a search for alternatives. This search included the application of honey bees, however this proved unsuccessful, as honey bees are only a suitable alternative during winter (see [1] for references).

Within a few years, other companies were rearing bumblebees, not only in Europe but also in North America, New Zealand, the Middle East (Israel) and East Asia (Japan). The application of bumblebees for tomato pollination increased very rapidly as a consequence. In some countries or regions, tomato crops had not previously been pollinated mechanically, instead being treated with plant growth hormones that induce fruit development. However, bumblebees were cheaper and produced higher fruit quality and yield. Furthernore, farmers can easily monitor bee flower visitation using "bite marks" left on the anther cone by bees buzzing the anthers for pollen. Farmers also were motivated not to use chemical pesticides, which killed bumblebees in greenhouses, and switched to biocontrol as an alternative [1].

Since the early years of commercial bumblebee rearing, the market has been dominated by two companies: Biobest of Belgium and Koppert of the Netherlands. Those companies soon incorporated smaller companies in Western Europe and elsewhere, or founded subsidiaries, depending upon the legalities of importing or domesticating bees. Nevertheless, a number of smaller bumblebee rearing companies continue to sell their colonies on the local market, and sometimes predominate (e.g. Agrobio in Spain).

11.3 FUNDAMENTALS OF BUMBLEBEE REARING

In order to set up a bumblebee-rearing programme, queens are first collected from natural populations, preferably those that have just emerged from their hibernation sites. Within a few years, however, a bumblebee producer will rely solely on managed queen production. All bumblebee producers today have developed their own rearing systems, which are largely kept secret. The general procedure has been described previously [1–3] and is as follows. Each week, young hibernated queens are taken from the stock, their number depending on the production plan related to the sales forecast (i.e. about eight weeks later). The length of queen hibernation is timed to fulfill demand during peaks in bee colony sales. The queens may receive a CO_2 narcosis [1]. They are then installed in small starter boxes in a climate room (28 °C or lower, depending on the system, with relative humidity (RH) around 60 percent). Different methods are used to stimulate colony initiation in the rearing industry. These include the use of bumblebee or honey bee workers, sometimes in combination with male cocoons or artificial cocoons. Artificial cocoons are molded out of Styrofoam™ or plastic and may be used in combination with a heating device to raise their temperature (using a hot water system, light bulbs or electric heating). At the same time or after the first workers emerge, the colonies are transferred to a larger nest box (usually a click-in system). This permits full colony development once the nest box is placed in the greenhouse or the field. The colonies are fed sugar syrup (approx. 50 percent sugar content, wt/wt) and pollen pellets from *Apis mellifera* (purchased from beekeepers) while in the rearing facilities. The sugar contains a preservative and the sugar composition is balanced to prevent crystallization.

Colonies are typically selected at a size of around 50 workers. The nest box usually consists of a plastic inner box and a cardboard outer box. The bees have access to sugar solution underneath the inner box (usually around 2 L, ca. 65 percent sugar content, wt/wt). This amount is needed because the flowers of the main target crop, tomatoes, do not produce nectar. Colonies meant for most other greenhouse crops, as well as for outdoor crops, are given less (or no) sugar syrup. The amount of syrup is sufficient for the entire lifespan of the colony, which is typically between 8 and 12 weeks. The adult worker population of a colony typically increases to a peak of around 200 about three to five weeks after its introduction. The colony then starts producing hundreds of males and some queens.

A small proportion of colonies are set apart for the rearing of reproductives (queens and males). By monitoring those colonies, a parasite-free queen stock is formed. A parasite-free status is not only important for bumblebee rearing, but also for exporting colonies to other countries. Rearing facilities are checked by the national veterinary services, and veterinary certificates are issued when needed. If managed properly, the reproductive colonies produce, on average, more than 200 queens each. Males are usually produced in abundance by the same colonies and do not need to be reared separately. It is important to prevent brother-sister mating, because inbreeding has immediate deleterious effects. After mating with a brother, half of the queen's diploid eggs, which normally produce females, develop into males [4]. As a consequence, the colony produces only half the normal quantity of workers and thus will remain rather small in size.

Mating queens are either collected directly from the mating cages or allowed to dig themselves into heaps of soil or peat. In the former case, the queens undergo pre-treatment before they are stored at 5 °C. In the latter, they are dug out after some time, transferred to smaller containers and then stored at 5 °C.

Currently, five species of bumblebees are reared commercially. The main species is the Eurasian *Bombus terrestris*, commercially reared since 1987. It has a wide natural distribution encompassing all of Europe, coastal North Africa, and West and Central Asia [5]. The second species (with respect to the number of reared colonies) is the North American *B. impatiens,* commercially reared since 1990. Another North American species, *B. occidentalis*, has been reared from 1991 to 1996, but was later discontinued commercially because it suffered heavy infestation from the protozoan *Nosema bombi* (see also Chapter 16). Other commercially reared species are *B. ignitus* for the East Asian market (China, Japan and Korea – reared since 1999), *B. ephippiatus* in Central America (Mexico – reared since 2008) and *B. atratus* in Argentina, reared since 2012, as well as in Uruguay [6] and Colombia [7] where breeding experiments with the species are ongoing. In China, *B. ignitus* and *B. lucorum* [8], as well as *B. lantschouensis* and *B. patagiatus* (previously called *B. hypocrita* [9]), are being evaluated for commercial rearing (Li, personal communication).

Interestingly, *B. atratus* belongs to the group of so-called pocket-makers, bumblebees that, because of the way they feed their larvae, are deemed less suitable for commercial rearing (being more human labour-intensive) [1]. However, the lack of an eligible species belonging to the other group – pollen-storers – forces producers to choose the pocket-maker *B. atratus* and to develop appropriate rearing techniques [6].

11.4 BUMBLEBEE PATHOGENS AND PARASITES

Bumblebee breeders make significant efforts to maintain parasite-free operations, by using an eradication procedure, since there are no effective biological agents against the most common pathogens. The major producers may appear successful in pathogen control [1], but recent findings [10] question this conclusion.

Parasites and pathogens that are known to occur in bumblebee-rearing facilities include the tracheal mite *Locustacarus buchneri*, the microsporidian *Nosema bombi*, the trypanosome *Crithidia bombi* and the neogragarine *Apicystis bombi*.

Large numbers of the tracheal mite cause diarrhea [11], but the impact on colony development is limited [12–15]. However, a tracheal mite infection may have a negative impact on worker lifespan [11, 16] and thus on the pollination performance of a colony, even though infected workers visit flowers as rapidly as uninfected workers [17].

An infection with *Nosema bombi* may not initially be harmful to a colony [18–22]. However, it will eventually weaken infected bees and cause their early death [23–26]. Since there is no effective biocontrol for *Nosema bombi* [21], an infection with this pathogen is catastrophic and has led to the breakdown of *Bombus occidentalis* rearing in Western North America [1].

An infection with *Crithidia bombi* does not usually have a strong impact on colony development or worker lifespan [20], but does negatively affect worker foraging behaviour [17, 26–28].

The impact of infection by *Apicystis bombi* on colony development or worker performance remains unknown, but the pathogen is capable of completely destroying the fat body [29] and thus preventing queens from establishing a colony [30].

Other potential threats to indoor rearing are the wasp brood parasite *Melittobia acasta* [12, 31, 32] and the Indian meal moth *Plodia interpunctella*, known to feed on pollen, but also capable of feeding on bumblebee pupae and thus affecting brood development [33].

11.5 CROPS POLLINATED BY COMMERCIALLY REARED BUMBLEBEES

The main agricultural crop pollinated by bumblebees is the greenhouse tomato (*Solanum lycopersicum* = *Lycopersicon esculentum*). Worldwide, this crop accounts for about 95 percent of all commercial bumblebees and comprises a total of over 40 000 ha of greenhouse culture. The colony density needed for tomato and other crops depends upon factors such as flower density and attractiveness [34]. A "cherry tomato" crop, for instance, requires at least twice as many colonies/ha as a "beef tomato" crop, because it contains so many flowers. The growing season of tomato plants in greenhouse cultures typically lasts between 7 and 11 months, depending upon climatic conditions in the area. After a first introduction of three to five colonies/ha (depending upon the type of crop), new colonies are added every second week to achieve a stable bumblebee population inside the greenhouse. Up to 50 bumblebee colonies/ha are used during the growing season. In 2006, the value of those bumblebee pollinated tomato crops was estimated to be €12 000 million [1], and is probably significantly more at present.

Bumblebee colonies are increasingly used for the pollination of outdoor crops (e.g. almond, apple, pear, cherry, blueberry and cranberry). For this purpose, bumblebee producers have developed special, insulated and rainproof hive bodies, each containing three or four bumblebee colonies. Table 11.1 lists the crops pollinated by bumblebees.

Table 11.1
CROPS COMMERCIALLY POLLINATED BY BUMBLEBEES

CROP	LATIN NAME	REFERENCES
Tomato	*Solanum lycopersicum*	ref. in 1; also 35 (Korea, using *B. ignitus*), 36 (Colombia, using *B. atratus*), 37, 38 (Mexico, using *B. ephippiatus*)
Pepper (sweet, hot)	*Capsicum annuum*	ref. in 1; also 39, 78
Eggplant	*Solanum melongena*	40, 41
Melon	*Cucumis melo*	42
Watermelon	*Citrillus lanatus*	43, 44, 45, 46
Cucumber	*Cucumis sativa*	43, 45, 46
Courgette (zucchini)	*Cucurbita pepo*	47
Strawberry	*Fragaria x ananassa*	48, 49, 50
Raspberry	*Rubus idaeus*	51, 52
Blackberry	*Rubus fructicosus*	
Blackcurrant	*Ribes nigrum*	53, 54, 55
Redcurrant	*Ribes sativum*	
Cranberry	*Vaccinium macrocarpon*	56, 57, 58
Blueberry (highbush, lowbush, rabbiteye)	*Vaccinium corymbosum, V. angustifolium, V. ashei*	58, 59, 60, 61, 62, 63
Apple	*Malus domestica*	64, 65, 66
Pear	*Pyrus communis*	67, 68
Cherry	*Prunus cerasus, P. avium*	
Kiwifruit	*Actinidia deliciosa*	69
Peach	*Prunus persica*	70, 71, 72
Apricot	*Prunus armeniaca*	
Plum	*Prunus domestica*	73
Almond	*Prunus dulcis*	65, 74
Sunflower	*Helianthus annuus*	75

Source: A. vanDoorn [present study]

As mentioned above, bumblebees release pollen from tomato flowers by sonication. To do this, they grasp the anther cone with their mandibles, which leaves brown bite marks on the flowers. This behaviour can damage the receptacle if the bee-flower ratio is too high. Bees may visit individual flowers over and

over, trying to release the pollen, and their bites can damage the tissue, which causes malformation of the fruit. This phenomenon is called **over-pollination** and may occur not only in tomatoes, but also in sweet peppers and strawberries (see [1] for references). When this phenomenon is observed, the grower must either temporarily close the hives or remove some of them. Tomato varieties with relatively small flowers, such as cherry tomatoes, are more vulnerable to over-pollination than other varieties.

Honey bees can also pollinate most of the crops listed in Table 11.1, but bumblebees are preferable when the temperature and/or light intensity are low, both in the greenhouse and in the open field. Honey bees do not usually forage at an air temperature below 16 °C, whereas bumblebee workers are still active at temperatures as low as 10 °C [76, 77]. Conversely, bumblebees stop foraging when the temperature rises above 32 °C [78]. Bumblebees tend to visit more flowers per minute than honey bees, and honey bees usually treat flowers more tenderly than bumblebees (they exhibit no buzz-collecting behaviour, thus do not bite the anthers or vibrate them), and present no risk of over-pollination. They are known, however, to cause damage to the incipient fruits of strawberry plants [1].

Sometimes, it is preferable to use a group of bees instead of an entire colony. Small packages containing only a dozen or so bumblebee males are used for seed production in onion (*Allium cepa*), cabbage (*Brassica* spp.) [79] and leek (*Allium ampeloprasum*). These bees can be used only in completely closed environments since, without a true colony with a queen and a home base, they would otherwise leave. The pollination potential of bumblebee males was recently confirmed [54, 80].

11.6 MANAGEMENT CONSIDERATIONS

Bumblebees feed on nectar and pollen. The pollen of plant species differs with respect to content of important ingredients such as protein, amino acids, lipids, sterols and vitamins [81]. In nature, most bees, including bumblebees, use multifloral pollen. In the greenhouse, however, the pollen source is usually a single species. Nevertheless, bumblebee colonies in a greenhouse, where they only have access to a single pollen source, develop normally (except for the number of new queens), as demonstrated by supplemental feeding results with diverse pollen to *Bombus occidentalis* in a tomato greenhouse [82]. During the summer season, bumblebee workers may leave the greenhouse and collect some pollen outside [83]. The proportion of this pollen differs strongly between locations, and the development of commercial bumblebee colonies in the greenhouse is indeed influenced by several factors [84].

11.6.1 Climatic conditions

Adverse climatic conditions (low light level, temperature below 10 °C or above 40 °C) affect the viability, quantity and floral release of pollen, as demonstrated for tomato, pepper and eggplant [85, 86]. Such conditions may lead to pollen shortages for bumblebee colonies, underfeeding and eventually the mortality of larvae. Small workers (resulting from underfed larvae) usually stay inside the nest and perform nest duties such as nursing [87], but may contribute to pollen foraging and pollination if there is a shortage of larger workers [88].

Additionally, worker activity is affected by adverse climatic conditions. Bumblebee workers usually do not forage at temperatures below 10 °C [77] or above 32 °C [78]. They are able to fly at temperatures up to 35 °C, but instead stay at the nest to ventilate the brood. Moreover, above 32 °C they stop feeding the larvae [77, 89]. Bumblebee larvae can endure up to a few days of starvation [90], but their development is then delayed [91]. Bumblebee workers stop fanning their wings to cause nest temperature regulation at an ambient temperature of 40 °C, to prevent overheating of their body. They die at 44 °C [77].

11.6.2 Placement inside the greenhouse

During the crop-growing season, climatic conditions in the greenhouse, notably the temperature, may change considerably. In temperate climates, solar radiation usually is limited during winter, and has a positive effect on colony activity. At this time, the risk of

overheating is low and an exposed position is preferred for the hives. However, when radiant intensity leads to hive overheating, colonies must be moved to a shaded place. When the temperature rises to extremes of 40 °C or more, colonies should be removed from the greenhouse and stored at a cooler place. If this happens often, the nest box might be placed in a hole in the ground or inside a cooling box or refrigerator (thus keeping it inside the greenhouse) [84].

As mentioned, hives must be placed in the shade during the hot season (e.g. along sidewalks, etc.), instead of beneath or between the plants. That is because bumblebee workers tend to drift from nest boxes placed among crop plants to hives placed in a more exposed position. Drifting bees are readily accepted by the recipient colonies, especially when they carry pollen, and will thus contribute to the development of those colonies. Conversely, if the worker population of the "delivering" colonies decreases too much, they are negatively affected. Such colonies will decline and pollinate less [84].

Drifting also occurs when hives are placed in groups: within horizontal groups, workers from "central" colonies tend to drift to the outer boxes, whereas within vertical groups (stacks) workers tend to drift from top boxes to lower positions. In fact, they are inclined to enter the nest box they encounter first. Usually, horizontal groups are placed beneath and in line with the plants; in such cases, the bumblebees follow the foraging paths between plants to reach their hive. Vertical stacks are placed between plants or along the open aisles; in both cases, the bees usually approach the stack from below [84]. Interestingly, vertical stacks also give rise to drifting from below to above [92, 93]. It remains unclear what causes such differing behaviour; however the different positions of the vertical stacks may be a factor (e.g. above the leaf canopy [92] or outdoors [93]). Bees probably approach the stacks from above more often than below. Alternating the hive entrances of neighbouring colonies (facing in opposite directions) in both horizontal groups and vertical stacks diminishes drifting to some extent, but does not prevent it [84, 92]. Placement of different patterns on the entrance side and on the top of nest boxes, or large landmarks inside the greenhouse, has no effect on drifting [92]. Drifting workers may have, or attain, different roles in the host colony; usually they work as foragers, but some may possess developed ovaries, stay in the nest and even lay eggs [94, 95].

In modern greenhouses, the CO_2 level is artificially increased from approximately 400 ppm to 1 000 ppm to stimulate plant growth [96]. In this setting, the CO_2 level close to the tube outlets can reach 10 000 ppm. Such a high level has a negative impact on colony foraging activity. Therefore, hives must be kept away from CO_2 tubes, or all outlets in the vicinity of nest boxes must be closed [84]. Experiments in which colonies are kept inside CO_2-controlled containers (during two days) show increasing levels of larval and adult mortality ≥ 5 000 ppm, resulting in colony death above 15 000 ppm [84]. Under normal conditions, such values are reached within bumblebee nests [97], and range between 1 000 ppm and 12 000 ppm, depending on colony size and ventilation. Naturally, bumblebee workers respond to an increased CO_2 level by fanning their wings, in an attempt to lower the concentration. If environmental CO_2 reaches 10 000 ppm, the bees cannot adequately reduce it and colony damage occurs.

11.6.3 Greenhouse coverings

In recent years, modifications to greenhouse coverings have been made. The most important of these is partial or complete absorption of UV (ultraviolet) radiation to protect crops from insects and insect-borne viral diseases, and to suppress proliferation of foliar diseases [98, 99]. Although one laboratory study [100] indicates that UV radiation is not essential for efficient foraging, there is good evidence that, in greenhouses covered with uv-blocking plastics, bumblebee foraging activity is strongly reduced due to orientation problems and the bees' efforts to leave the greenhouse [84, 96, 101, 102]. A negative impact on bumblebee performance has also been observed in greenhouses covered with UV-blocking polycarbonate, both single plane and double layered [103].

11.6.4 Supplemental lighting

In temperate climates, supplemental lighting is increasingly added above tomato (and other) greenhouse crops. Growers hope to increase yield, especially during the winter out-of-season period. With year-round production the greenhouse attains maximum use and labour input is consistent. The visual spectrum of supplemental lighting suits plant development, but lacks a UV component and has a negative impact on pest and disease control, and also bumblebee performance [104, 105]. Bumblebee colonies suffer heavy losses due to navigation problems and attraction to heat lamps. Bees are frequently found starving or dead along sidewalls and elsewhere in the greenhouse [84].

To tackle this problem, bee activity is curtailed during artificial lighting. For this purpose, nest boxes are equipped with electronic doors, which are opened when no artificial lighting is applied, usually between 10:00 hours and 14:00 hours [106].

11.6.5 Pesticide use

An important factor in all agricultural settings is the use of pesticides, notably insecticides for crop protection. Fortunately, the increasing use of bumblebees for pollination in greenhouses diminishes pesticide use [1]. At the same time, it stimulates the search for compatible insecticides, non-toxic to humans, to be applied with bumblebees and biocontrol agents present. A group of insecticides, called neonicotinoids and presently controversial (e.g. imidacloprid, thiamethoxam, clothianidin and dinotefuran), was developed with such qualities in mind. These systemic insecticides – water soluble and readily absorbed by plants via roots or leaves – are transported throughout plant tissues. Pollinators were thought not to come into contact with such insecticides. However, neonicotinoids are not as harmless to pollinators and other organisms as promised [review in 107]. To obtain accurate information about the use of a pesticide or insecticide in combination with pollinators or biocontrol agents, it is important to always consider the negative effects noted by bumblebee producers, retailers and field advisers [84].

11.6.6 Natural enemies of bumblebees

Under natural conditions, a wide range of predators, parasites and parasitoids attack bumblebees [108]. Those in bumblebee-rearing facilities are mentioned in section 11.4. Both inside and outside the greenhouse, reared bumblebees can be attacked by a number of other enemies. Avian predators such as flycatchers and bee-eaters, and predatory robber flies (*Asilus* spp.) may kill workers or queens, while the wax moth *Aphomia sociella*, a brood parasite, may enter bumblebee nest boxes and destroy colonies [84].

In addition, bumblebees, both inside and outside the greenhouse, are affected by honey bee pathogens such as "*Deformed wing virus*" *DWV* and "*Black queen cell virus*" [109–111], and the microsporidian *Nosema ceranae*, an emerging honey bee (*Apis mellifera*) pathogen from Asia [112]. Transmission of such pathogens may occur through direct contact or via flowers (pollen). Both DWV and *Nosema ceranae* have recently [113] infected bumblebees. DWV infection results in reduced longevity and non-viable offspring, whereas *Nosema ceranae* has few symptoms. A small "hive beetle" *Aethina tumida*, native to sub-Saharan Africa, is invasive in North America and thrives in bumblebee (*B. impatiens*) colonies [114–116]. Effective control measures against those enemies and pathogens are not widely available (but see Chapter 16).

Figure 11.1
A COLONY OF *BOMBUS HUNTII*

11.6.7 Plant virus transmission

Bumblebees spread plant viruses inside greenhouses, such as "*Pepino mosaic virus*" *PMV*, [11, 118, 119], "*Tobacco mosaic virus*" *TMV* [120] and "*Tomato chloric dwarf viroid*" *TCDVd*, [121]. Nevertheless, pollination by bumblebees is still recommended because the risk of contamination by mechanical pollination is greater. Strict sanitation and elimination of infected plants are needed to keep the viruses under control.

11.6.8 Dispensing microbiological control agents and pollen

Like honey bees, bumblebees can be used to deliver microbiological control agents to infected plants [122–126]. Moreover, bumblebees are nowadays also used to deliver selected pollen to fruit crops, by means of a pollen dispenser connected to the nest box [186].

11.7 ENVIRONMENTAL CONCERNS

During the early 1990s, bumblebee producers reared few species (*Bombus terrestris* in Western Europe and *B. impatiens* in North America, after the collapse of *B. occidentalis* rearing), and also began to use bees outside their natural range, including *B. terrestris* colonies imported to Chile, China, Japan and Korea, and *B. impatiens* in Mexico and western North America [1]. Such practices have several problems.

11.7.1 Hybridization between subspecies

Laboratory experiments demonstrate hybridization between subspecies of *Bombus terrestris* [references in 1; 127]. During the early 1990s, the subspecies *B. terrestris sassaricus* (endemic to Sardinia) was used in Western European greenhouses (Belgium, France and the Netherlands) and colonized areas outside greenhouses [1], which permitted hybridization. Moreover, *B. terrestris* subspecies differ in foraging performance (largely based upon differences in forager size), and native subspecies may be displaced [128]. Use of *B. t. sassaricus* in Western European greenhouses was terminated after a few years [1]. Recently, *B. terrestris* in France revealed no supporting data for the presence of exotic genetic material [129]. However, a recent study in Poland indeed found a strong genetic representation of commercially reared *B. terrestris* in native bumblebees [130].

There was concern over the exotic subspecies *B. terrestris dalmatinus* in the United Kingdom, where *B. terrestris audax* is endemic [131]. Legislation now restricts its use and only the native subspecies is allowed in outdoor crops [132]. Since 2010, bumblebee breeders therefore offer *B. t. audax* colonies.

11.7.2 Establishment and competition with native bees

Queens of four UK bumblebee species, among them *Bombus terrestris*, were introduced into New Zealand in 1885 and 1906, and subsequently naturalized there. The purpose of those introductions was to improve seed-set of red clover, an important fodder for cattle and horses [133]. From New Zealand, *B. terrestris* traveled to Tasmania in 1992 [see references in 1]. Because there are no native bumblebees in New Zealand or Tasmania, competition for nest sites with other bumblebees was not a problem. However, a number of studies have shown competition for food with native bees [summarized in references in 1]. However, the impact at a population or community level (both of bees and plants) is still unclear. Comparably, queens of European *B. ruderatus* were introduced into South America (Chile) in 1982, also for the pollination of red clover [134]. From Chile, *B. ruderatus* traveled to Argentina in 1993 [135].

Since commercial bumblebee rearing began in 1987, reared colonies of *B. terrestris* introduced into Japan have become established there [136, 137], as well as in Chile [138]. Subsequently, *B. terrestris* travelled from Chile to Argentina [139, 140]. In Japan, there is some evidence of competition for nest sites and/or food sources with native *Bombus* [see e.g. 137, 141–145], the evidence is inconclusive and the impact appears quite limited. The same applies to impact in Argentina and Chile [138, 146–148].

Up to now, it is still unclear whether *B. impatiens* has become established in western North America, but individuals have been observed up to 5 km from greenhouses [149].

11.7.3 Hybridization between closely related species

In Japan, *B. terrestris* breeds with native *B. hypocrita* (subsp. *sapporoensis* on the island of Hokkaido and subsp. *hypocrita* on the main island of Honshu) in the field [150, 151]. Laboratory tests reveal that hybrid eggs are not viable [150], while studies in Korea reveal interspecific mating of introduced *B. terrestris* and native *B. ignitus* [152]. In that case, some viable offspring were produced, but only workers and males, no queens.

11.7.4 Spillover of pathogens

Pathogen spillover was first reported from Japan with regard to the tracheal mite *Locustacarus buchneri* [153]. Spillover, or in this case the transfer of associated organisms from an exotic species to a native species, probably occurred inside bumblebee-rearing facilities in Europe [153]. Genetic characteristics of the European strain were later found in natural populations of Japanese bumblebees [154]. Nowadays, imported commercial bumblebee colonies are probably free of tracheal mites [see 154, 155].

Pathogen spillover from commercial colonies to wild bumblebees (e.g. the microsporidian *Nosema bombi*) has been hypothesized to be the cause for the recent decline of some western North American bumblebee species (notably *B. occidentalis*) [156, 157] but, up to now, solid proof is lacking and other causes for the declines, such as reduced genetic diversity, agricultural intensification and pesticide use, seem just as likely [158–165].

It may be true that commercial bumblebee colonies are now free of the tracheal mite, but lesser pathogens such as *Crithidia bombi*, *Nosema bombi* and *Apicystis bombi* [for Europe see 155, but also 166; for North America see 17, 157, 167; for South America see 140, 168–170] are not to be discounted.

In Argentina, *Apicystis bombi* was recently detected not only among invasive *B. terrestris*, but also in workers of the (introduced) honey bee, *Apis mellifera* [168, 169, 171]. The pathogen was not detected in five native bumblebee species [168]. It is questionable whether this represents an example of pathogen spillover from bumblebees to honey bees. Genetic analysis indicates that pathogens found in the European honey bee and European *B. terrestris* share a common origin, or that both *Apis mellifera* and *B. terrestris* already harboured *Apicystis bombi* when they arrived in Argentina [171].

The spillover of pathogens from honey bees to bumblebees has been described in section 11.6. In Argentina, the emerging honey bee pathogen *Nosema ceranae* probably spilled over from honey bees to bumblebees [112]. Recently, *N. ceranae* spores were detected in honey bee-collected pollen fed to commercially produced bumblebee colonies. It is therefore recommended to sterilize the pollen used in commercial bumblebee rearing [10].

Unfortunately, there is little knowledge about the occurrence of most parasites and pathogens in natural bee populations across the world, including their possible transmission between bee genera or species. Commercial use of bumblebees has promoted research, and continues to do so. The lack of historical data on parasites or pathogens means that spillovers from commercially produced to natural bumblebee or other bee populations cannot be readily identified.

11.8 FINAL REMARKS

Increasing awareness of environmental problems related to non-native bumblebee introduction has led to regulations and restrictions on bumblebee importation and use. For instance, in Japan, the *Invasive Alien Species Act* (2005) allows exotic *Bombus terrestris* only within screened structures, whereas the Ministry recommends native *B. ignitus*, supplied by some bumblebee producers. However, *B. ignitus* is native in only part of Japan, not on the island of Hokkaido, an important tomato-growing area [172]. To address the issue, another native species, such as *B. hypocrita sapporoensis* (native to Hokkaido), would be necessary. Unfortunately, that subspecies only produces rather small colonies and is therefore unattractive for commercial use. Likewise, some western North American states (California and Washington) allow non-native *B. impatiens* only in

screened structures [173]. Since 2013, the United Kingdom requires endemic *B. terrestris audax* for field crops [132].

Restrictive legislation has led to the domestication of Asian *B. ignitus*, Central American *B. ephippiatus* and South American *B. atratus,* and will possibly foster domestication of other species. However, such developments are only possible in countries where bumblebees occur naturally. In countries where bumblebees are lacking (e.g. Australia and in Africa), tomato growers are forced to continue using traditional methods of artificial pollination until an alternative – a natural pollination system – is developed. This can be a long and difficult road, as shown in Australia, where after many years of study with regard to some promising genera, *Xylocopa* and *Amegilla* (initiated in 1996), and the stingless bee *Tetragonula* (formerly called *Trigona*) *carbonaria*, a breeding programme for reliable industrial supply of those bees could not be developed [174–180]. Thus, Australian tomato growers are still hoping for a permit to import bumblebees. In South Africa, breeding programmes to study native alternatives are not yet forthcoming, thus South African tomato growers continue to seek importation permits.

Finally, in the United Kingdom, a bumblebee species has recently been introduced for nature conservation purposes. *Bombus subterraneus*, which was declared extinct in 2000, was reintroduced in nature. The first attempts were made in 2009–2010 to repatriate the species from New Zealand, where it was introduced from England at the end of the nineteenth century [133], together with *B. terrestris* and other species (see section 11.2). Those attempts failed, due to limited rearing success and high levels of inbreeding [181, 182] (they were reared in captivity in New Zealand). Thereafter, queens were collected in Sweden in 2011–2013 and, after a period of quarantine, released in selected areas (nature reserves) [183, 184]. In July 2013 the first *B. subterraneus* workers were recorded within 5 km of the release zone, indicating that the queens had successfully established colonies [185].

REFERENCES

[1] Velthuis, H.H.W. & van Doorn, A. 2006. A century of advances in bumblebee domestication and the economic and environmental aspects of its commercialization for pollination. *Apidologie*, 37: 421–451.

[2] Hudges M.J. 1996. Commercial rearing of bumble bees. In A. Matheson, ed., *Bumble bees for pleasure and profit,* pp. 40–47. Cardiff: IBRA.

[3] Yoon, H.J., Lee, K.Y., Kim, M. E. & Park, I.G. 2011. Agricultural utilization and year-round rearing techniques of bumblebees in Korea. *International Journal of Industrial Entomology*, 22: 29–37.

[4] Duchateau, M.J., Hoshiba, H. & Velthuis, H.H.W. 1994. Diploid males in the bumble bee *Bombus terrestris*. *Entomologia Experimentalis et Applicata*, 71: 263–269.

[5] Rasmont, P., Coppée, A., Michez, D. & De Meulemeester, T. 2008. An overview of the *Bombus terrestris* (L. 1758) subspecies (Hymenoptera: Apidae). *Annales de la Société entomologique de France* (n.s.), 44: 243–250.

[6] Salvarrey, S., Arbulo, N., Santos, E. & Invernizzi, C. 2013. Cría artificial de abejorros nativos *Bombus atratus* y *Bombus bellicosus* (Hymenoptera, Apidae) [Artificial breeding of native bumble bees *Bombus atratus* and *Bombus bellicosus* (Hymenoptera, Apidae)]. *Agrociencia Uruguay*, 17: 75–82.

[7] Bichopolis & BioBee. 2014. Abejorros para polinización natural: *Bombus terrestris* [Bumble bees *for natural pollination*: *Bombus terrestris*] (available at http://biobee.co/2014/01/abejorros–para–polinizacion–natural–bombus–terrestris).

[8] Li, J., Wu, J., Cai, W., Peng, W., An, J. & Huang, J. 2008. Comparison of the colony development of two native bumblebee species *Bombus ignitus* and *Bombus lucorum* as candidates for commercial pollination in China. *Journal of Apicultural Research and Bee World*, 47: 22–26.

[9] Williams, P.H., An, J., Brown, M.J.F., Carolan, J.C., Goulson, D., Huang, J. & Ito, M. 2012. Cryptic bumblebee species: consequences for conservation and the trade in greenhouse pollinators. *PLoS ONE*, 7: e32992.

[10] Graystock, P., Yates, K., Evison, S.E.F., Darvill, B., Goulson, D. & Hughes, W.O.H. 2013. The Trojan hives: pollinator pathogens, imported and distributed in bumblebee colonies. *Journal of Applied Ecology*, 50: 1207–1215.

[11] Husband, R.W. & Sinha, R.N. 1970. A revision of genus *Locustacarus* with a key to genera of family Podapolipidae (Acarina). *Annals of the Entomological Society of America*, 63: 1152–1162.

[12] de Wael, L., de Greef, M. & van Laere, O. 1993. *Melittobia acasta* and *Bombacarus buchneri*, dangerous parasites in the *in vitro* rearing of bumblebees. *Apiacta*, 28: 93–101.

[13] van den Eijnde, J. & de Ruijter, A. 1998. *Locustacarus buchneri* (Acarina: Podapolipidae) infection of bumblebee colonies (*Bombus terrestris* L.). *Proceedings of the Section Experimental and Applied Entomology, N.E.V., Amsterdam*, 9: 177–178.

[14] Yoneda, M., Furuta, H., Tsuchida, K., Okabe, K. & Goka, K. 2008. Commercial colonies of *Bombus terrestris* (Hymenoptera: Apidae) are reservoirs of the tracheal mite *Locustacarus buchneri* (Acari: Podapolipidae). *Applied Entomology and Zoology*, 43: 73–76.

[15] Yoneda, M., Furuta, H., Kanbe, Y., Tsuchida, K., Okabe, K. & Goka, K. 2008. Reproduction and transmission within a colony of bumblebee tracheal mite *Locustacarus buchneri* (Acari: Podapolipidae) in *Bombus terrestris* (Hymenoptera: Apidae). *Applied Entomology and Zoology*, 43: 391–395.

[16] Otterstatter, M.C. & Whidden, T.L. 2004. Patterns of parasitism by tracheal mites (*Locustacarus buchneri*) in natural bumble bee populations. *Apidologie*, 35: 351–357.

[17] Otterstatter, M.C., Gegear, R.J., Colla, S.R. & Thomson, J.D. 2005. Effects of parasitic mites and protozoa on the flower constancy and foraging rate of bumble bees. *Behavioral Ecology and Sociobiology*, 58: 383–389.

[18] Fisher, R.M. & Pomeroy, N. 1989. Incipient colony manipulation, *Nosema* incidence and colony productivity of the bumble bee *Bombus terrestris* Hymenoptera: Apidae). *Journal of the Kansas Entomological Society*, 62: 581–589.

[19] McIvor, C. & Malone, L.A. 1995. *Nosema bombi*, a microsporidian pathogen of the bumble bee *Bombus terrestris* L. *New Zealand Journal of Zoology*, 22: 25–31.

[20] Imhoof, B. & Schmid-Hempel, P. 1999. Colony success of the bumble bee, *Bombus terrestris*, in relation to infections by two protozoan parasites, *Crithidia bombi* and *Nosema bombi*. *Insectes Sociaux*, 46: 233–238.

[21] Whittington, R. & Winston, M.L. 2003. Effects of *Nosema bombi* and its treatment fumagillin on bumble bee (*Bombus occidentalis*) colonies. *Journal of Invertebrate Pathology*, 84: 54–58.

[22] van der Steen, J.J.M. 2008. Infection and transmission of *Nosema bombi* in *Bombus terrestris* colonies and its effect on hibernation, mating and colony founding. *Apidologie*, 39: 273–282.

[23] de Jonghe, R. 1986. Crossing experiments with *Bombus terrestris terrestris* (Linnaeus, 1758) and *Bombus terrestris xanthopus* Kriechbaumer, 1870 and some notes on diapause and nosemose (Hymenoptera: Apidae). *Phegea*, 14: 19–23.

[24] Otti, O. & Schmid-Hempel, P. 2007. *Nosema bombi*: A pollinator parasite with detrimental fitness effects. *Journal of Invertebrate Pathology*, 96: 118–124.

[25] Otti, O. & Schmid-Hempel, P. 2008. A field experiment on the effect of *Nosema bombi* in colonies of the bumblebee *Bombus terrestris*. *Ecological Entomology*, 33: 577–582.

[26] Rutrecht, S.T. & Brown, M.J.F. 2009. Differential virulence in a multiple-host parasite of bumble bees: resolving the paradox of parasite survival? *Oikos*, 118: 941–949.

[27] Gegear, R.J., Otterstatter, M.C. & Thomson, J.D. 2005. Does parasitic infection impair the ability of bumblebees to learn flower-handling techniques? *Animal Behaviour*, 70: 209–215.

[28] Gegear, R.J., Otterstatter, M.C. & Thomson, J.D. 2006. Bumble-bee foragers infected by a gut parasite have an impaired ability to utilize floral information. *Proceedings of the Royal Society B*, 273: 1073–1078.

[29] Schmid-Hempel, P. 2001. On the evolutionary ecology of host-parasite interactions: addressing the question with regard to bumblebees and their parasites. *Naturwissenschaften*, 88: 147–158.

[30] Rutrecht, S.T. & Brown, M.J.F. 2008. The life-history impact and implications of multiple parasites for bumble bee queens. *International Journal for Parasitology*, 38: 799–808.

[31] de Wael, L., de Greef, M. & van Laere, O. 1995. Biology and control of *Melittobia acasta*. *Bee World*, 76: 72–76.

[32] Kwon, Y.J., Amin, M.R. & Inn, N.S.L. 2012. Parasitism and oviposition of *Melittobia acasta* on *Bombus terrestris*: impact of larval overwintering and host status on pupation and adult emergence. *Entomological Research*, 42: 94–98.

[33] Kwon, Y.J., Saeed, S. & Duchateau, M.J. 2003. Control of *Plodia interpunctella* (Lepidoptera: Pyralidae), a pest in *Bombus terrestris* (Hymenoptera: Apidae) colonies. *Canadian Entomologist*, 135: 893–902.

[34] Griffiths, D. & Robberts, E.J. 1996. Bumble bees as pollinators of glasshouse crops. In A. Matheson, ed. *Bumble bees for pleasure and profit*, pp. 33–39. IBRA, Cardiff.

[35] Lee, S.B., Bae, T.W., Kim, S.E. & Yoon, H.J. 2003. The influence of over-foraging, and pollinating activities on tomato fruits by a Korean native bumblebee, *Bombus ignitus* S. (Hymenoptera: Apidae) in cherry-tomato houses. *Korean Journal of Applied Entomology*, 42: 293–300 (in Korean with English summary and tables).

[36] Aldana, J., Cure, J.R., Almanza, M.T., Vecil, D. & Rodríguez, D. 2007. Efecto de *Bombus atratus* (Hymenoptera: Apidae) sobre la productividad de tomate (*Lycopersicon esculentum* Mill.) bajo invernadero en la Sabana de Bogotá, Colombia [Effect of *Bombus atratus* (Hymenoptera: Apidae) on tomato productivity (*Lycopersicon esculentum* Mill.) under greenhouses in the Savannah of Bogotá, Colombia]. *Agronomía Colombiana*, 25: 62–72.

[37] Vergara, C.H. & Fonseca-Buendía, P. 2012. Pollination of greenhouse tomatoes by the Mexican bumblebee *Bombus ephippiatus* (Hymenoptera: Apidae). *Journal of Pollination Ecology*, 7: 27–30.

[38] Torres-Ruiz, A. & Jones, R.W. 2012. Comparison of the efficiency of the bumble bees *Bombus impatiens* and *Bombus ephippiatus* (Hymenoptera: Apidae) as pollinators of tomato in greenhouses. *Journal of Economic Entomology*, 105: 1871–1877.

[39] Lee, S.B., Ha, N.G., Yoon, H.J., Park, I.G., Lee, K.Y., Gang, H.S. & Hwang, S.J. 2008. Effect on the pollinating activities of honeybee, *Apis mellifera* L. (Hymenoptera: Apidae) and bumblebee, *Bombus terrestris* L. in paprika vinyl-houses. *Korean Journal of Applied Entomology*, 47: 293–298 (in Korean with English summary and tables).

[40] Abak, K., Sari, N. & Paksoy, M. 1995. Efficiency of bumble bees on the yield and quality of eggplant and tomato grown in unheated glasshouses. *Acta Horticulturae*, 412: 268–274.

[41] Kowalska, G. 2006. Eggplant (*Solanum melongena* L.) flowering and fruiting dynamics depending on pistil type as well as way of pollination and flower hormonization. *Folia Horticulturae*, 18(1): 17–29.

[42] Fisher, R.M. & Pomeroy, N. 1989. Pollination of greenhouse muskmelons by bumble bees (Hymenoptera: Apidae). *Journal of Economic Entomology*, 82: 1061–1066.

[43] Stanghellini, M.S., Ambrose, J.T. & Schultheis, J.R. 1997. The effects of honey bee and bumble bee pollination on fruit set and abortion of cucumber and watermelon. *American Bee Journal*, 137: 386–391.

[44] Stanghellini, M.S., Ambrose, J.T. & Schultheis, J.R. 1998. Seed production in watermelon: a comparison between two commercially available pollinators. *HortScience*, 33: 28–30.

[45] Stanghellini, M.S., Ambrose, J.T. & Schultheis, J.R. 1998. Using commercial bumble bee colonies as backup pollinators for honey bees to produce cucumbers and watermelon. *HortTechnology*, 8: 590–594.

[46] Stanghellini, M.S., Ambrose, J.T. & Schultheis, J.R. 2002. Diurnal activity, floral visitation and pollen deposition by honey bees and bumble bees on field-grown cucumber and watermelon. *Journal of Apicultural Research*, 41: 27–34.

[47] Artz, D.R. & Nault, B.A. 2011. Performance of *Apis mellifera*, *Bombus impatiens*, and *Peponapis pruinosa* (Hymenoptera: Apidae) as pollinators of pumpkin. *Journal of Economic Entomology*, 104: 1153–1161.

[48] Paydas, S., Eti, S., Kaftanoglu, O., Yasa, E. & Derin, K. 2000. Effects of pollination of strawberries grown in plastic greenhouses by honeybees and bumblebees on the yield and quality of the fruits. *Acta Horticulturae*, 513: 443–451.

[49] Paydas, S., Eti, S., Sevinc, S., Yasa, E., Derin, K., Kaska, N. & Kaftanoglu, O. 2000. Effects of different pollinators to the yield and quality of strawberries. *Acta Horticulturae*, 522: 209–215.

[50] Dimou, M., Taraza, S., Thrasyvoulou, A. & Vasilakakis, M. 2008. Effect of bumble bee pollination on greenhouse strawberry production. *Journal of Apicultural Research and Bee World*, 47: 99–101.

[51] Willmer, P.G., Bataw, A.A.M. & Hughes, J.P. 1994. The superiority of bumblebees to honeybees as pollinators: insect visits to raspberry flowers. *Ecological Entomology*, 19: 271–284.

[52] Lye, G.C., Jennings, S.N., Osborne, J.L. & Goulson, D. 2011. Impacts of the use of nonnative commercial bumble bees for pollinator supplementation in raspberry. *Journal of Economic Entomology*, 104: 107–114.

[53] Koltowski, Z., Pluta, S., Jablonski, B. & Szklanowska, K. 1999. Pollination requirements of eight cultivars of black currant (*Ribes nigrum* L.). *Journal of Horticultural Science & Biotechnology*, 74: 472–474.

[54] Fliszkiewicz, M., Giejdasz, K. & Wilkaniec, Z. 2011. The importance of male red mason bee (*Osmia rufa* L.) and male bufftailed bumblebee (*Bombus terrestris* L.) pollination in blackcurrant (*Ribes nigrum* L.). *Journal of Horticultural Science & Biotechnology*, 86: 457–460.

[55] Fountain, M., Dean, R. & Cross, J. 2011. The effect of pollinating insects on blackcurrant fruitset, yield and quality. *IOBC–WPRS Bulletin*, 70: 151–157.

[56] MacFarlane, R.P., Patten, K.D., Mayer, D.F. & Shanks, C.H. 1994. Evaluation of commercial bumble bee colonies for cranberry pollination. *Melanderia*, 50: 13–19.

[57] MacKenzie, K.E. 1994. The foraging behaviour of honey bees (*Apis mellifera* L.) and bumble bees (*Bombus* spp.) on cranberry (*Vaccinium macrocarpon* Ait). *Apidologie*, 25: 375–383.

[58] Ratti, C.M., Higo, H.A., Griswold, T.L. & Winston, M.L. 2008. Bumble bees influence berry size in commercial *Vaccinium* spp. cultivation in British Columbia. *Canadian Entomologist*, 140: 348–363.

[59] Whidden, T.L. 1996. The fidelity of commercially reared colonies of *Bombus impatiens* Cresson (Hymenoptera: Apidae) to lowbush blueberry in Southern New Brunswick. *Canadian Entomologist*, 128: 957–958.

[60] Stubbs, C.S. & Drummond, F.A. 2001. *Bombus impatiens* (Hymenoptera: Apidae): an alternative to *Apis mellifera* (Hymenoptera: Apidae) for lowbush blueberry pollination. *Journal of Economic Entomology*, 94: 609–616.

[61] Sampson, B.J. & Spiers, J.M. 2002. Evaluating bumblebees as pollinators of "Misty" southern highbush blueberry growing inside plastic tunnels. *Acta Horticulturae*, 574: 53–61.

[62] Javorek, S.K., MacKenzie, K.E. & Vander Kloet, S.P. 2002. Comparative pollination effectiveness among bees (Hymenoptera: Apidae) on lowbush blueberry (Ericaceae: *Vaccinium angustifolium*). *Annals of the Entomological Society of America*, 95: 345–351.

[63] Desjardins, È-C. & de Oliveira, D. 2006. Commercial bumble bee *Bombus impatiens* (Hymenoptera: Apidae) as a pollinator in lowbush blueberry (Ericale: Ericaceae) fields. *Journal of Economic Entomology*, 99: 443–449.

[64] Goodell, K. & Thomson, J.D. 1997. Comparisons of pollen removal and deposition by honeybees and bumblebees visiting apple. *Acta Horticulturae*, 437: 103–107.

[65] Thomson, J.D. & Goodell, K. 2001. Pollen removal and deposition by honeybee and bumblebee visitors to apple and almond flowers. *Journal of Applied Ecology*, 38: 1032–1044.

[66] Elias, E. 2005. Einsatz einheimischer Hummeln für den Obstbau [Use of local bumblebees for orchards]. *Obstbau-Zeitschrift*, 1: 37–38.

[67] Jacquemart, A-L., Michotte-van der Aa, A. & Raspé, O. 2006. Compatibility and pollinator efficiency tests on *Pyrus communis* L. cv. "Conference". *Journal of Horticultural Science & Biotechnology*, 81: 827–830.

[68] Zisovich, A.H., Goldway, M., Schneider, D., Steinberg, S., Stern, E. & Stern, R.A. 2012. Adding bumblebees (*Bombus terrestris* L., Hymenoptera: Apidae) to pear orchards increases seed number per fruit, fruit set, fruit size and yield. *Journal of Horticultural Science & Biotechnology*, 87: 353–359.

[69] Pomeroy, N. & Fisher, R.M. 2002. Pollination of kiwifruit (*Actinidia deliciosa*) by bumble bees (*Bombus terrestris*): effects of bee density and patterns of flower visitation. *New Zealand Entomologist*, 25: 41–49.

[70] An, J-D., Wu, J., Peng, W-J., Tong, Y-M., Guo, Z-B. & Li, J-L. 2007. Foraging behaviour and pollination ecology of *Bombus lucorum* L. and *Apis mellifera* L. in greenhouse peach garden. *Chinese Journal of Applied Ecology*, 18: 1071–1076 (in Chinese with English summary and tables).

[71] Lee, K-Y., Lee, S-B., Ahn, K-S., Park, S-K. & Yoon, H-J. 2007. Characteristics on the pollinating activity of Korean native bumblebee, *Bombus ignitus* Smith (Hymenoptera: Apidae) in the peach house. *Korean Journal of Applied Entomology*, 46: 287–293 (in Korean with English summary and tables).

[72] Zhao, Y-Z., An, J-D., Zhou, Z-Y., Dong, J., Xing, Y-H. & Qin, J-J. 2011. Pollination behaviour of *Apis mellifera ligustica* and *Bombus hypocrita* (Hymenoptera, Apidae) and the influencing factors in peach greenhouse. *Acta Entomologica Sinica*, 54: 89–96 (in Chinese with English summary and tables).

[73] Calzoni, G.L. & Speranza, A. 1996. Pear and plum pollination: honey bees and bumble bees or both? *Acta Horticulturae*, 423: 83–90.

[74] Dag, A., Zipori, I. & Pleser, Y. 2006. Using bumblebees to improve almond pollination by honeybees. *Journal of Apicultural Research*, 45: 215–216.

[75] Aslan, M.M. & Yavuksuz, C. 2010. Effect of honey bee (*Apis mellifera* L.) and bumblebee (*Bombus terrestris* L.) pollinators on yield and yield factors in sunflower (*Helianthus annuus* L.) production areas. *Journal of Animal and Veterinary Advances*, 9: 332–335.

[76] Paarmann, W. 1977. Untersuchungen zur Bedeutung von Hummeln (*Bombus* spp.) für die Bestäubung blühender Obstbäume [Investigations into the importance of bumblebees (*Bombus* spp.) for the pollination of flowering fruit trees]. *Zeitschrift für angewandte Entomologie*, 84: 164–178.

[77] Heinrich, B. 1979. *Bumblebee economics*. Harvard University Press, Cambridge (MA)/London.

[78] Kwon, Y.J. & Saeed, S. 2003. Effect of temperature on the foraging activity of *Bombus terrestris* L. (Hymenoptera: Apidae) on greenhouse hot pepper (*Capsicum annuum* L.). *Applied Entomology and Zoology*, 38: 275–280.

[79] Ostevik, K.L., Manson, J.S. & Thomson, J.D. 2010. Pollination potential of male bumble bees (*Bombus impatiens*): movement patterns and pollen-transfer efficiency. *Journal of Pollination Ecology*, 2: 21–26.

[80] Wolf, S. & Moritz, R.F.A. 2014. The pollination potential of free-foraging bumblebee (*Bombus* spp.) males (Hymenoptera: Apidae). *Apidologie*, 45: 440–450.

[81] Roulston, T.H. & Cane, J.H. 2000. Pollen nutritional content and digestibility for animals. In A. Dafni, M. Hesse, & E. Pacini, eds. *Pollen and pollination*, pp. 187–209. Wein, Springer-Verlag.

[82] Whittington, R. & Winston, M.L. 2003. Are bumble bee colonies in tomato greenhouses obtaining adequate nutrition? *Canadian Entomologist*, 135: 883–892.

[83] Whittington, R., Winston, M.L., Tucker, C. & Parachnowitsch, A.L. 2004. Plant-species identity of pollen collected by bumblebees placed in greenhouses for tomato pollination. *Canadian Journal of Plant Science*, 84: 599–602.

[84] van Doorn, A. 2006. Factors influencing the performance of bumblebee colonies in the greenhouse. In J. M. Guerra-Sanz, J.M., Roldán Serrano, A. & Mena Granero, A., eds. *Second short course on pollination of horticultural plants*, pp. 172–183. La Mojonera, Almería, Spain, CIFA La Mojonera-La Cañada IFAPA.

[85] Picken, A.J.F. 1984. A review of pollination and fruit set in the tomato (*Lycopersicon esculentum* Mill.). *Journal of Horticultural Sciences*, 59: 1–13.

[86] Abak, K. & Dasgan, H.Y. 2005. *Efficiency of bumblebees as pollinators in unheated or antifrost heated greenhouses*. In A. Guerra-Sanz, A. Roldán Serrano & A. Mena Granero, eds. *First short course on pollination of horticulture plants*, pp. 19–29. La Mojonera, Almería, Spain, CIFA.

[87] Free, J.B. 1955. The division of labour within bumblebee colonies. *Insectes Sociaux*, 2: 195–212.

[88] Goulson, D., Peat, J., Stout, J.C., Tucker, J., Darvill, B., Derwent, L.C. & Hughes, W.O.H. 2002. Can alloethism in workers of the bumblebee, *Bombus terrestris*, be explained in terms of foraging efficiency? *Animal Behaviour*, 64: 123–130.

[89] Vogt, F.D. 1986. Thermoregulation in bumblebee colonies. 1. Thermoregulatory versus brood-maintenance behaviors during acute changes in ambient temperature. *Physiological Zoology*, 59: 55–59.

[90] Plowright, R.C. & Pendrel, B.A. 1977. Larval growth in bumble bees (Hymenoptera: Apidae). *Canadian Entomologist*, 109: 967–973.

[91] Sutcliffe, G.H. & Plowright, R.C. 1990. The effects of pollen availability on development time in the bumble bee *Bombus terricola* K. (Hymenoptera: Apidae). *Canadian Journal of Zoology*, 68: 1120–1123.

[92] Birmingham, A.L. & Winston, M.L. 2004. Orientation and drifting behaviour of bumblebees (Hymenoptera: Apidae) in commercial tomato greenhouses. *Canadian Journal of Zoology*, 82: 52–59.

[93] Lefebvre, D. & Pierre, J. 2007. Demographic consequences of drift in contiguous hives of *Bombus terrestris*. *Journal of Economic Entomology*, 100: 1756–1763.

[94] Birmingham, A.L., Hoover, S.E., Winston, M.L. & Ydenberg, R.C. 2004. Drifting bumble bee (Hymenoptera: Apidae) workers in commercial greenhouses may be social parasites. *Canadian Journal of Zoology*, 82: 1843–1853.

[95] Lopez-Vaamonde, C., Koning, J.W., Brown, R.M., Jordan, W.C. & Bourke, A.F.G. 2004. Social parasitism by male-producing reproductive workers in a eusocial insect. *Nature,* 430: 557–560.

[96] Dag, A. 2008. Bee pollination of crop plants under environmental conditions unique to enclosures. *Journal of Apicultural Research and Bee World*, 47: 162–165.

[97] Weidenmüller, A., Kleineidam, C. & Tautz, J. 2002. Collective control of nest climate parameters in bumblebee colonies. *Animal Behaviour*, 63: 1065–1071.

[98] Antignus, Y., Mor, N., ben Joseph, R., Lapidot, M. & Cohen, S. 1996. Ultraviolet-absorbing plastic sheets protect crops from insect pests and from virus diseases vectored by insects. *Environmental Entomology*, 25: 919–924.

[99] Costa, H.S., Robb, K.L. & Wilen, C.A. 2002. Field trials measuring the effects of ultraviolet-absorbing greenhouse plastic films on insect populations. *Journal of Economic Entomology*, 95: 113–120.

[100] Dyer, A.G. & Chittka, L. 2004. Bumblebee search time without ultraviolet light. *Journal of Experimental Biology*, 207: 1683–1688.

[101] Morandin, L.A., Laverty, T.M., Kevan, P.G., Khosla, S. & Shipp, L. 2001. Bumble bee (Hymenoptera: Apidae) activity and loss in commercial tomato greenhouses. *Canadian Entomologist*, 133: 883–893.

[102] Morandin, L.A., Laverty, T.M., Gegear, R.J. & Kevan, P.G. 2002. Effect of greenhouse polyethylene covering on activity level and photo-response of bumble bees. *Canadian Entomologist*, 134: 539–549.

[103] Blacquière, T., van der Aa-Furnée, J., Cornelissen, B. & Donders, J. 2006. Behaviour of honey bees and bumble bees beneath three different greenhouse claddings. *Proceedings of the Netherlands Entomological Society Meeting*, 17: 93–102.

[104] Armstrong, H. 2004. Effects of lights on insects and predators. *Fruit and Vegetable Technology*, 4: 14–15.

[105] Blacquière, T., Cornelissen, B. & Donders, J. 2007. Bumble bee colony decline in greenhouses with supplementary lighting. *Proceedings of the Netherlands Entomological Society Meeting*, 18: 71–77.

[106] Visser, P. 2006. Belichting tomaat leeft. *Groenten & Fruit*, 5: 16–17.

[107] Goulson, D. 2013. An overview of the environmental risks posed by neonicotinoid insecticides. *Journal of Applied Ecology*, 50: 977–987.

[108] Alford, D.V. 1975. *Bumblebees*. London, Davis-Poynter.

[109] Genersch, E., Yue, C., Fries, I. & de Miranda, J.R. 2006. Detection of *Deformed wing virus*, a honey bee viral pathogen, in bumble bees (*Bombus terrestris* and *Bombus pascuorum*) with wing deformities. *Journal of Invertebrate Pathology*, 91: 61–63.

[110] Singh, R., Levitt, A.L., Rajotte, E.G. Holmes, E.C., Ostiguy, N., van Engelsdorp, D., Lipkin, W.I., de Pamphilis, C.W., Toth, A.L. & Cox-Foster, D.L. 2010. RNA viruses in hymenopteran pollinators: evidence of inter-taxa virus transmission via pollen and potential impact on non-*Apis* hymenopteran species. *PLoS ONE*, 5: e14357.

[111] Peng, W., Li, J., Boncristiani, H., Strange, J.P., Hamilton, M. & Chen, Y. 2011. Host range expansion of honey bee Black Queen Cell Virus in the bumble bee, *Bombus huntii*. *Apidologie*, 42: 650–658.

[112] Plischuk, S., Martín-Hernandez, R., Prieto, L., Luciá, M., Botías, C., Meana, A., Abrahamovich, A.H., Lange, C. & Higes, M. 2009. South American native bumblebees (Hymenoptera: Apidae) infected by *Nosema ceranae* (Microsporidia), an emerging pathogen of honeybees (*Apis mellifera*). *Environmental Microbiology Reports*, 1: 131–135.

[113] Fürst, M.A., McMahon, D.P., Osborne, J.L., Paxton, R.J. & Brown, M.J.F. 2014. Disease associations between honeybees and bumblebees as a threat to wild pollinators. *Nature*, 506: 364–373.

[114] Spiewok, S. & Neumann, P. 2006. Infestation of commercial bumblebee (*Bombus impatiens*) field colonies by small hive beetles (*Aethina tumida*). *Ecological Entomology*, 31: 623–628.

[115] Hoffmann, D., Pettis, J.S. & Neumann, P. 2008. Potential host shift of the small hive beetle (*Aethina tumida*) to bumblebee colonies (*Bombus impatiens*). *Insectes Sociaux*, 55: 153–162.

[116] Graham, J.R., Ellis, J.D., Carroll, M.J. & Teal, P.E.A. 2011. *Aethina tumida* (Coleoptera: Nutidulidae) attraction to volatiles produced by *Apis mellifera* (Hymenoptera: Apidae) and *Bombus impatiens* (Hymenoptera: Apidae) colonies. *Apidologie*, 42: 326–336.

[117] van der Vlugt, R.A.A., Stijger, C.C.M.M., Verhoeven, J.Th.J. & Lesemann, D-E. 2000. First report of *Pepino Mosaic Virus* in tomato. *Plant Disease*, 84: 103.

[118] Lacasa, A., Guerrero, M.M., Hita, I., Martínez, M.A., Jordá, C., Bielza, P., Contreras, J., Alcázar, A. & Cano, A. 2003. Implicaciones de los abejorros (*Bombus* spp.) en la dispersión del virus del mosaico del pepino dulce (*Pepino Mosaic Virus*) en cultivos de tomate [Implications of bumble bees (*Bombus* spp.) on the dispersion of the sweet cucumber mosaic virus (*Pepino Mosaic Virus*) in tomato crops]. *Boletín de Sanidad Vegetal Plagas*, 29: 393–403.

[119] Shipp, J.L., Buitenhuis, R., Stobbs, L., Wang, K., Kim, W.S. & Ferguson, G. 2008. Vectoring of *Pepino mosaic virus* by bumble-bees in tomato greenhouses. *Annals of Applied Biology*, 153: 149–155.

[120] Okada, K., Kusakari, S-I., Kawaratani, M., Negoro, J-I., Ohki, S.T. & Osaki, T. 2000. *Tobacco mosaic virus* is transmissible from tomato to tomato by pollinating bumblebees. *Journal of General Plant Pathology*, 66: 71–74.

[121] Matsuura, S., Matsushita, Y., Kozuka, R., Shimizu, S. & Tsuda, S. 2010. Transmission of *Tomato chlorotic dwarf viroid* by bumblebees (*Bombus ignitus*) in tomato plants. *European Journal of Plant Pathology*, 126: 111–115.

[122] Yu, H. & Sutton, J.C. 1997. Effectiveness of bumblebees and honeybees for delivering inoculum of *Gliocladium roseum* to raspberry flowers to control *Botrytis cinerea*. *Biological Control*, 10: 113–122.

[123] Kovach, J., Petzoldt, R. & Harman, G.E. 2000. Use of honey bees and bumble bees to disseminate *Trichoderma harzianum* 1295–22 to strawberries for *Botrytis* control. *Biological Control*, 18: 235–242.

[124] Al-mazra'awi, M.S., Shipp, L., Broadbent, B. & Kevan, P. 2006. Biological control of *Lygus lineolaris* (Hemiptera: Miridae) and *Frankliniella occidentalis* (Thysanoptera: Thripidae) by *Bombus impatiens* (Hymenoptera: Apidae) vectored *Beauveria bassiana* in greenhouse sweet pepper. *Biological Control*, 37: 89–97.

[125] Kapongo, J.P., Shipp, L., Kevan, P. & Broadbent, B. 2008. Optimal concentration of *Beauveria bassiana* vectored by bumble bees in relation to pest and bee mortality in greenhouse tomato and sweet pepper. *BioControl*, 53: 797–812.

[126] Mommaerts, V., Put, K., Vandeven, J., Jans, K., Sterk, G., Hoffmann, L. & Smagghe, G. 2010. Development of a new dispenser for microbiological control agents and evaluation of dissemination by bumblebees in greenhouse strawberries. *Pest Management Science*, 66: 1199–1207.

[127] Ings, T.C., Raine, N.E. & Chittka, L. 2005. Mating preference in the commercially imported bumblebee species *Bombus terrestris* in Britain (Hymenoptera: Apidae). *Entomologia Generalis*, 28: 233–238.

[128] Ings, T.C., Schikora, J. & Chittka, L. 2005. Bumblebees, humble pollinators or assiduous invaders? A population comparison of foraging performance in *Bombus terrestris*. *Oecologia*, 144: 508–516.

[129] Ings, T.C., Ings, N.L., Chittka, L. & Rasmont, P. 2010. A failed invasion? Commercially introduced pollinators in southern France. *Apidologie*, 41: 1–13.

[130] Kraus, F.B., Szentgyörgyi, H., Rozej, E., Rhode, M., Moron, D., Woyciechowski, M. & Moritz, R.F.A. 2011. Greenhouse bumblebees (*Bombus terrestris*) spread their genes into the wild. *Conservation Genetics*, 12: 187–192.

[131] Ings, T.C., Ward, N.L. & Chittka, L. 2006. Can commercially imported bumble bees out-compete their native conspecifics? *Journal of Applied Ecology*, 43: 940–948.

[132] Natural England. 2013. Consultation on improving honey bee health – proposed changes to managing and controlling pests and diseases (available at www.naturalengland.org.uk/Images/12–13–080_Improving_Honey_Bee_Health_tcm6–36187.pdf).

[133] Hopkins, I. 1914. History of the humble-bee in New Zealand: its introduction and results. *New Zealand Department of Agriculture and Industry Comments*, 46: 1–29.

[134] Arretz, P.V. & MacFarlane, R.P. 1986. The introduction of *Bombus ruderatus* to Chile for red clover pollination. *Bee World*, 67: 15–22.

[135] Roig Alsina, A. & Aizen, M.A. 1996. *Bombus ruderatus* Fabricius, un nuevo *Bombus* para la Argentina (Hymenoptera: Apidae) [*Bombus ruderatus* Fabricius, a new *Bombus* for Argentina (Hymenoptera: Apidae)]. *Physis (Buenos Aires), secc. C,* 51: 49–50.

[136] Ono, M. 1997. Ecological implications of introduced *Bombus terrestris*, and significance of domestication of Japanese native species (*Bombus* spp.). *Proceedings of the International Workshop on Biological Invasions of Ecosystem by Pests and Beneficial Organisms*. *Tsukuba, Japan*, pp. 244–252.

[137] Yokoyama, J. & Inoue, M.N. 2010. Status of the invasion and range expansion of an introduced bumblebee, *Bombus terrestris* (L.), in Japan. *Applied Entomology and Zoology*, 45: 21–27.

[138] Ruz, L. & Herrera, R. 2001. Preliminary observations on foraging activites of *Bombus dahlbomii* and *Bombus terrestris* (Hym.: Apidae) on native and non-native vegetation in Chile. *Acta Horticulturae*, 561: 165–169.

[139] Torretta, J.P., Medan, D. & Abrahamovich, A.H. 2006. First record of the invasive bumblebee *Bombus terrestris* (L.) (Hymenoptera, Apidae) in Argentina. *Transactions of the American Entomological Society*, 132: 285–289.

[140] Schmid-Hempel, R., Eckhardt, M., Goulson, D., Heinzmann, D., Lange, C., Plischuk, S., Escudero, L.R., Salathé, R., Scriven, J.J. & Schmid-Hempel, P. 2014. The invasion of southern South America by imported bumblebees and associated parasites. *Journal of Animal Ecology*, 83: 823–837.

[141] Nagamitsu, T., Kenta, T., Inari, N., Kato, E. & Hiura, T. 2007. Abundance, body size, and morphology of bumblebees in an area where an exotic species, *Bombus terrestris*, has colonized in Japan. *Ecological Research*, 22: 331–341.

[142] Inoue, M.N., Yokoyama, J. & Washitani, I. 2008. Displacement of Japanese native bumblebees by the recently introduced *Bombus terrestris* (L.) (Hymenoptera: Apidae). *Journal of Insect Conservation*, 12: 135–146.

[143] Nagamitsu, T., Yamagishi, H., Kenta, T., Inari, N. & Kato, E. 2010. Competitive effects of the exotic *Bombus terrestris* on native bumble bees revealed by a field removal experiment. *Population Ecology*, 52: 123–136.

[144] Inoue, M.N., Makino, T.T., Yokoyama, J. & Sakai, S. 2010. Is *Bombus terrestris* (Hymenoptera: Apidae) a stronger competitor against native Japanese species? A comparison of foraging efficiency. *Applied Entomology and Zoology*, 45: 71–75.

[145] Inoue, M.N. & Yokoyama, J. 2010. Competition for flower resources and nest sites between *Bombus terrestris* (L.) and Japanese native bumblebees. *Applied Entomology and Zoology*, 45: 29–35.

[146] Morales, C.L. 2007. Introducción de abejorros (*Bombus*) no nativos: causas, consecuencias ecológicas y perspectivas [Introduction of non-native bumblebees: causes, ecological consequences and perspectives]. *Ecología Austral*, 17: 51–65.

[147] Madjidian, J.A., Morales, C.L. & Smith, H.G. 2008. Displacement of a native by an alien bumblebee: lower pollinator efficiency overcome by overwhelmingly higher visitation frequency. *Oecologia*, 156: 835–845.

[148] Aizen, M.A., Lozada, M. & Morales, C.L. 2011. Comparative nectar-foraging behaviors and efficiencies of an alien and a native bumble bee. *Biological Invasions*, 13: 2901–2909.

[149] Ratti, C.M. & Colla, S.R. 2010. Discussion of the presence of an eastern bumble bee species (*Bombus impatiens* Cresson) in western Canada. *Pan-Pacific Entomologist*, 86: 29–31.

[150] Kanbe, Y., Okada, I., Yoneda, M., Goka, K. & Tsuchida, K. 2008. Interspecific mating of the introduced bumblebee *Bombus terrestris* and the native Japanese bumblebee *Bombus hypocrita sapporoensis* results in inviable hybrids. *Naturwissenschaften*, 95: 1003–1008.

[151] Kondo, N.I., Yamanaka, D., Kanbe, Y., Kunitake, Y.K., Yoneda, M., Tsuchida, K. & Goka, K. 2009. Reproductive disturbance of Japanese bumblebees by the introduced European bumblebee *Bombus terrestris*. *Naturwissenschaften*, 96: 467–475.

[152] Yoon, H.J., Kim, S.Y., Lee, K.Y., Lee, S.B., Park, I.G. & Kim, I. 2009. Interspecific hybridization of the bumblebees *Bombus ignitus* and *B. terrestris*. *International Journal of Industrial Entomology*, 18: 41–48.

[153] Goka, K., Okabe, K., Yoneda, M. & Niwa, S. 2001. Bumblebee commercialization will cause worldwide migration of parasitic mites. *Molecular Ecology*, 10: 2095–2099.

[154] Goka, K., Okabe, K. & Yoneda, M. 2006. Worldwide migration of parasitic mites as a result of bumblebee commercialization. *Population Ecology*, 48: 285–291.

[155] Murray, T.E., Coffey, M.F., Kehoe, E. & Horgan, F.G. 2013. Pathogen prevalence in commercially reared bumble bees and evidence of spillover in conspecific populations. *Biological Conservation*, 159: 269–276.

[156] Thorp, R.W., Schroeder, P.C. & Ferguson, C.S. 2002. Bumble bees: boisterous pollinators of native California flowers. *Fremontia*, 30(3–4): 26–31.

[157] Colla, S.R., Otterstatter, M.C., Gegear, R.J. & Thomson, J.D. 2006. Plight of the bumble bee: Pathogen spillover from commercial to wild populations. *Biological Conservation*, 129: 461–467.

[158] Cameron, S.A., Lozier, J.D., Strange, J.P., Koch, J.B., Cordes, N., Solter, L.F. & Griswold, T.L. 2011. Patterns of widespread decline in North American bumble bees. *Proceedings of the National Academy of Sciences, USA*, 108: 662–667.

[159] Grixti, J.C., Wong, L.T. Cameron, S.A. & Favret, C. 2009. Decline of bumbe bees (*Bombus*) in the North American Midwest. *Biological Conservation*, 142: 75–84.

[160] Brown, M.J.F. 2011. The trouble with bumblebees. *Nature*, 469: 169–170.

[161] Meeus, I., Brown, M.J.F., de Graaf, D.C. & Smagghe, G. 2011. Effects of invasive parasites on bumble bee declines. *Conservation Biology*, 25: 662–671.

[162] Bushmann, S.L., Drummond, F.A., Beers, L.A. & Groden, E. 2012. Wild bumblebee (*Bombus*) diversity and *Nosema* (Microsporidia: Nosematidae) infection levels associated with lowbush blueberry (*Vaccinium angustifolium*) production and commercial bumblebee pollinators. *Psyche*, 2012: article ID 429398.

[163] Cordes, N., Huang, W-F., Strange, J.P., Cameron, S.A., Griswold, T.L., Lozier, J.D. & Solter, L.F. 2012. Interspecific geographic distribution and variation of the pathogens *Nosema bombi* and *Crithidia* species in United States bumble bee populations. *Journal of Invertebrate Pathology*, 109: 209–216.

[164] Koch, J.B. & Strange, J.P. 2012. The status of *Bombus occidentalis* and *B. moderatus* in Alaska with special focus on *Nosema bombi* incidence. *Northwest Science*, 86: 212–220.

[165] Szabo, N.D., Colla, S.R., Wagner, D.L., Gall, L.F. & Kerr, J.T. 2012. Do pathogen spillover, pesticide use, or habitat loss explain recent North American bumblebee declines? *Conservation Letters*, 5: 232–239.

[166] Baron, G.L., Raine, N.E. & Brown, M.J.F. 2014. Impact of chronic exposure to a pyrethroid pesticide on bumblebees and interactions with a trypanosome parasite. *Journal of Applied Ecology*, 51: 460–469.

[167] Otterstatter, M.C. & Thomson, J.D. 2008. Does pathogen spillover from commercially reared bumble bees threaten wild pollinators? *PLoS ONE*, 3: e2771.

[168] Plischuk, S. & Lange, C.E. 2009. Invasive *Bombus terrestris* (Hymenoptera: Apidae) parasitized by a flagellate (Euglenozoa: Kinetoplastea) and a neogregarine (Apicomplexe: Neogregarinorida). *Journal of Invertebrate Pathology*, 102: 263–265.

[169] Plischuk, S., Meeus, I., Smagghe, G. & Lange, C.E. 2011. *Apicystis bombi* (Apicomplexa: Neogregarinorida) parasitizing *Apis mellifera* and *Bombus terrestris* (Hymenoptera: Apidae) in Argentina. *Environmental Microbiology Reports*, 3: 565–568.

[170] Arbetman, M.P., Meeus, I., Morales, C.L., Aizen, M.A. & Smagghe, G. 2013. Alien parasite hitchhikes to Patagonia on invasive bumblebee. *Biological Invasions*, 15: 489–494.

[171] Maharromov, J., Meeus, I., Maebe, K., Arbetman, M., Morales, C., Graystock, P., Hughes, W.O.H., Plischuk, S., Lange, C.E., de Graaf, D.C., Zapata, N., Perez de la Rosa, J.J., Murray, T.E., Brown, M.J.F. & Smagghe, G. 2013. Genetic variability of the neogregarine *Apicystis bombi*, an etiological agent of an emergent bumblebee disease. *PLoS ONE,* 8: e81475.

[172] Goka, K. 2010. Introduction to the special feature for ecological risk assessment of introduced bumblebees: status of the European bumblebee, *Bombus terrestris*, in Japan as a beneficial pollinator and an invasive alien species. *Applied Entomology and Zoology*, 45: 1–6.

[173] Green Methods. nd. Bumblebees for pollination (available at https://greenmethods.com/bumblebees–for–pollination.

[174] Hogendoorn, K., Steen, Z. & Schwarz, M.P. 2000. Native Australian Carpenter bees as a potential alternative to introducing bumble bees for tomato pollination in greenhouses. *Journal of Apicultural Research*, 39: 67–74.

[175] Bell, M.C., Spooner-Hart, R.N. & Haigh, A.M. 2006. Pollination of greenhouse tomatoes by the Australian bluebanded bee *Amegilla* (*Zonamegilla*) *holmesi* (Hymenoptera: Apidae). *Journal of Economic Entomology*, 99: 437–442.

[176] Hogendoorn, K., Gross, C.L., Sedgley, M. & Keller, M.A. 2006. Increased tomato yield through pollination by native Australian *Amegilla chlorocyanea* (Hymenoptera: Anthophoridae). *Journal of Economic Entomology*, 99: 828–833.

[177] Hogendoorn, K., Coventry, S. & Keller, M.A. 2007. Foraging behaviour of a blue banded bee, *Amegilla chlorocyanea* in greenhouses: implications for use as tomato pollinators. *Apidologie*, 38: 86–92.

[178] Hogendoorn, K., Bartholomaeus, F. & Keller, M.A. 2010. Chemical and sensory comparison of tomatoes pollinated by bees and by a pollination wand. *Journal of Economic Entomology*, 103: 1286–1292.

[179] Greco, M.K., Spooner-Hart, R.N., Beattie, G.A., Barchia, I. & Holford, P. 2011. Australian stingless bees improve greenhouse *Capsicum* production. *Journal of Apicultural Research*, 50: 102–115.

[180] Greco, M.K., Spooner-Hart, R.N., Beattie, G.A., Barchia, I. & Holford, P. 2011. Stocking rates of *Trigona carbonaria* for the pollination of greenhouse capsicums. *Journal of Apicultural Research*, 50: 299–305.

[181] Lye, G.C. 2009. *Nesting ecology, management and population genetics of bumblebees: an integrated approach to the conservation of an endangered pollinator taxon*. Stirling, UK, University of Stirling (PhD dissertation).

[182] Gammans, N. 2011. *The short-haired bumblebee reintroduction project report 2009–2011*. Stirling, UK, Bumblebee Conservation Trust/Natural England.

[183] Gammans, N. 2012. Bombus subterraneus *reintroduction project report April 2011–March 2012*. Stirling, UK, Bumblebee Conservation Trust/Natural England.

[184] Gammans, N. 2013. *The short-haired bumblebee reintroduction project progress report April 2012–March 2013*. Stirling, UK, Bumblebee Conservation Trust/Natural England.

[185] Bumblebee Conservation Trust. nd. The short-haired bumblebee reintroduction. Stirling, UK, Bumblebee Conservation Trust (available at http://bumblebeeconservation.org/about-us/case-study/short-haired-bumblebee-reintroduction).

[186] Biobest. 2014. Pear: higher yield and better fruit caliber with bumblebee pollination (available at www.biobest.be/nieuws/308/3/0).

© D. W. Roubik

Chapter 12

DIPTERA AS CROP POLLINATORS

D.W. Inouye

12.1 THE IMPORTANCE OF FLIES

Pollination is an important ecosystem service in both natural and managed ecosystems. Bees, butterflies, moths, birds and even bats are relatively well known as providers of this service. Diptera (flies) are also common flower visitors, but are relatively understudied and under-appreciated. For example, McGregor's USDA publication *Insect Pollination of Cultivated Crop Plants* [1] devotes about about the same amount of space to flies as to snails and slugs, although he does acknowledge their importance for a few crops. The diversity of flowers they visit is great, encompassing close to 600 species recorded in the pollination literature [2, with more recent updates [66]].

In tropical areas of the world, the diversity of Diptera (measured as families recorded as flower visitors) can rival or exceed that of bees. For example, 4 856 species of Diptera are recorded from Australasia from flower-visiting families, compared with approximately 2 570 bees (superfamily Apoidea), while for the Neotropics the estimates are > 2 940 species for Diptera and 5 630 species for bees [3].

The great diversity among flowering-visiting flies encompasses a variety of anatomical, behavioural and physiological adaptations for collecting nectar and/or pollen, reviewed recently by Woodcock *et al.* [4]. The long evolutionary history between flies and plants [5–8] has likely led to adaptations for flower visitation arising independently in different lines, and a variety of reasons for flies to visit flowers. In addition to the nutritional rewards provided by nectar and pollen, flowers may provide protection from predators or weather, brood places for larvae, or even warmth for increasing a pollinator species' metabolic rate [4]. Correspondingly, flowers have evolved a variety of visual, chemical and tactile traits to attract dipteran pollinators, sometimes employing mimicry or sexual deception [4]. Goodrich *et al.* [9] have analysed the chemistry of yeasty floral scent in pawpaw flowers (*Asimina triloba*; Annonaceae). Mature flowers emit a variety of fermentation volatiles, and male flowers also produce additional nitrogenous compounds, which may help to attract fly pollinators. Similarly, saprophagous flies that breed in decomposing flowers are pollinators of *Aristolochia* spp. (Aristolochiaceae); apparently they are deceived into visiting the flowers because of the floral odours [10].

Figure 12.1
***HELLIANTHELLA* BEING VISITED BY TWO FLIES**

© D. W. Inouye

Figure 12.2
***MERIDON*, A FLY MIMIC OF *BOMBUS* ADULTS**

© D. W. Roubik

Species from at least 86 families of Diptera visit flowers, and over 1 100 species of plants from 172 families are visited by flies [11, D.W. Inouye, unpubl.].[1] Several families are commonly mentioned in the literature as flower visitors, including Syrphidae [12], Anthomyiidae [13], Empididae [14], Calliphoridae [15, 16], Bibionidae [17, 18], Ceratopogonidae [19, 20], Tachinidae [21] and Bombyliidae [21, 22]. In spite of this, only two recent review papers address the contribution Diptera make to plant diversity and agricultural production [23, 66]. These point out that flies are used in greenhouses, in agriculture and for the production of seeds for seed banks, and that large gaps in our knowledge remain on their roles in biocontrol and pollination networks.

Crops for which Diptera are known as pollinators include onions [15, 24–26], ginseng [27], cashew [22], mango [28, 29], cocoa [19, 20, 30, 31], apples [13, 32], sweet pepper [33, 34], oilseed rape [18, 35–37], strawberry [21], Brussels sprout [16], cashew [22], tea [38], apples [13] and pawpaw [9, 39]. Wang and Li [40] found that a syrphid fly (*Sphaerophoria scripta*) accounts for 20 percent of visits to buckwheat flowers, although they do not report how much pollination is effected by them. Wickramaratne and Vitarana [38] report that Diptera are the most numerous visitors to flowers in a tea seed garden, where at least seven families are represented, with individual flies carrying up to 500 pollen grains. Roubik [3] lists pollinators of 785 species of cultivated plants in the tropics, 26–31 of which are apparently pollinated only by flies, 32–33 by flies as the primarily pollinators, and 87–101 more by flies as secondary pollinators.

Flies are also described as the best solution for pollination of carrots [41] and onions [42] in plant-breeding research, and are also recommended for Brussels sprout [16]. The tiny size of flowers of those two species makes experimental pollination by hand difficult, and flies a good alternative. Although this works well in enclosures, it may not always work in the field. Parker *et al.* report an unpublished study where large numbers of *Eristalis tenax* were raised for pollination of commercial seed onions, but upon release they avoided the onions and instead flew to nearby carrot fields [43].

[1] From a database with 10 900 entries culled from the literature on fly visitation of flowers, available from the author or from http://drum.lib.umd.edu

12.2 LIFE HISTORY ECOLOGY

Larval flies (maggots) have a distinctly different lifestyle and diet from adult flies, and the larvae can either benefit agriculture or be detrimental. For example, some syrphid larvae are predators on aphids, and are beneficial as biocontrol agents for aphid pests of crops [12]. But some bibionid larvae can be herbivores on grass and cereals, attacking a wide range of crops [17], and as such, are more likely to pose a problem for stressed plants. Their populations are likely to be higher when there is more organic material present on the ground at the time of oviposition.

Diptera do not typically have specialized co-evolved relationships with flowers, although the long-tongued Nemestrinidae in South Africa and the flowers they visit with long corolla tubes are an exception [44–46], as is a case of joint pollination by hummingbirds and species of the tangle-wing flies in Argentina [47]. Some Tabanidae are also involved in pollination of flowers with long corollas [48]. Although the relationships may not involve specialist Diptera, there are many non-crop plants that seem to be specialized for pollination by flies, including the largest flowers known – *Rafflesia* in Southeast Asia [49]. *Amorphophallus titanum*, the Titan arum, also known as the "corpse flower" for its unpleasant odour, has an extremely large inflorescence that is pollinated by flies. Some of the Neotropical *Dracula* orchids are specialized for pollination by flies attracted by morphological and scent cues that mimic the mushrooms they usually use for oviposition [50, 51]. Temperate orchids that seem specialized for fly pollination include some pollinated by mosquitoes [52], which also visit a variety of other flowers for nectar [53].

Most Diptera have relatively short proboscides, and are thus restricted to visiting open flowers or those with very short corolla tubes. At present, there is no single source of information on proboscis lengths, despite its potential usefulness for the pollination literature. In addition to collecting nectar, adult Diptera may also eat pollen [12]. The abdomens of syrphid flies are often yellow when observed from below, due to the pollen they contain. Presumably this resource provides a source of protein for them, as it does for other pollinators.

As generalists, it is not surprising that the same species of fly visits a large variety of flowers. This lack of flower constancy, as well as the fact that the flies only collect nectar and pollen to feed themselves, rather than colonies (as in social bees) or their own offspring (as in solitary bees), explains why Diptera are typically considered poor pollinators. In direct comparisons with bees, the number of pollen grains carried or deposited on stigmas indicates that flies typically carry or deposit substantially fewer per visit. However, under some circumstances their abundance may be significantly greater than bees, such that overall, they account for the majority of pollination for particular plants [e.g. 54].

The generalist nature of their behaviour and the fact that they are not tied spatially to a nest, as are bees, means that Diptera have the potential to move genes from GMO crops to nearby plants that could include wild relatives or organic crops where GMO genes are undesirable. Although most studies reveal that pollen is typically moved on a scale of a few metres, it is known that flies can travel long distances. Marked flies (although probably not pollinators) reappeared eight miles away in one study [55]. In wildflower meadows in Colorado, the flight distances of bombyliid flies vary seasonally with flower density [56]. However, such mark-recapture studies are typically hampered by low recapture rates [57]. Chifflet *et al.* [58] considered the spatial scale of insect-mediated pollen dispersal in oilseed rape (*Brassica napus*) in an open agricultural landscape, and found that Diptera are among the flower visitors that can transfer pollen between plants over a considerable distance. Syrphidae disperse as far as 20 m in agricultural fields [59], but also travel distances of hundreds of kilometres (60, cited in [12]). Thus, land managers should bear in mind the potential for flies to disperse genes via pollen from GMO plants to wild and cultivated relatives.

12.3 CONSERVATION OF DIPTERA

The importance of Diptera as pollinators should raise the same concerns about their conservation that have been raised for pollinators in general [60–62], and for pollinators of crops in particular [63]. One species of Diptera (Delhi Sands flower-loving fly; *Rhaphiomidas terminatus abdominalis*), although not an important crop pollinator, is listed under the Endangered Species Act in the United States, and there is also concern about the conservation status of some Syrphidae in the United Kingdom [64]. Although their study was not conducted with pollinating Diptera in mind, Goulson *et al.* [65] highlight the potential for climate change to influence fly populations in the future. Certain management practices could be used to increase populations of some flies in agricultural situations. For example, beef lungs have been used as a substrate for calliphorid fly reproduction for studies of flies as pollinators of onions [42], and breeding sites have been made available for calliphorids near mango cultivation (Marden, in Roubik, ed. 1995). In many cases the information about fly life histories is incomplete or unknown, making management options uncertain. In addition, fly species may have dual roles in pollination and disease transmission, so their propagation en masse should be considered in the context of local human and domesticated animal health and hygiene. Thus, it seems that much remains to be learned about the significance of Diptera as pollinators, both in natural and agricultural environments, as was pointed out in 1987, when Parker *et al.* wrote "their value as crop pollinators has the potential to exceed that currently realized from their moderate use as pollinators of selected crops in enclosures"[43].

REFERENCES

[1] McGregor, S.E. 1976. *Insect pollination of cultivated crop plants*. Agriculture Handbook No. 496. Washington, DC, Agricultural Research Service, United States Department of Agriculture.

[2] Larson, B.M.H., Kevan, P.G. & Inouye, D.W. 2001. Flies and flowers: taxonomic diversity of anthophiles and pollinators. *Canadian Entomologist*, 133: 439–465.

[3] Roubik, D.W., ed. 1995. *Pollination of cultivated plants in the tropics*. Agricultural Services Bulletin No. 118. Rome, FAO.

[4] Woodcock, T.S., Larson, B.M.H., Kevan, P.G., Inouye, D.W. & Lunau, K. 2014. Flies and flowers II: floral rewards and attractants. *Journal of Pollination Ecology*, 12: 63–94.

[5] Gottsberger, G. 1974. The structure and function of the primitive angiosperm flower – a discussion. *Acta Botanica Neerlandica*, 23: 461–471.

[6] Crepet, W.L. 1979. Insect pollination: a paleontological perspective. *BioScience*, 29: 102–108.

[7] Bernhardt, P. & Thien, L.B. 1987. Self-isolation and insect pollination in the primitive angiosperms: new evaluations of older hypotheses. *Plant Systematics and Evolution*, 156: 159–176.

[8] Labandeira, C.C., Dilcher, D.L., Davis, D.R. & Wagner, D.L. 1994. Ninety-seven million years of angiosperm-insect association: paleobiological insights into the meaning of coevolution. *Proceedings of the National Academy of Sciences, USA,* 91: 12278–12282.

[9] Goodrich, K.R., Zjhra, M.L., Ley, C.A. & Raguso, R.A. 2006. When flowers smell fermented: the chemistry and ontogeny of yeasty floral scent in pawpaw (*Asimina triloba*: Annonaceae). *International Journal of Plant Sciences*, 167: 33–46.

[10] Sakai, S. 2002. *Aristolochia* spp. (Aristolochiaceae) pollinated by flies breeding on decomposing flowers in Panama. *American Journal of Botany*, 89: 527–534.

[11] Kearns, C.A. 2002. Flies and flowers: an enduring partnership. *Wings*, 25: 3–8.

[12] Rotheray, G.E. & Gilbert, F. 2011. *The natural history of hoverflies*. Ceredigion, UK, Forrest Text.

[13] Boyle, R.M.D. & Philogène, B.J.R. 1983. The native pollinators of an apple orchard: variations and significance. *Journal of Horticultural Science*, 58: 355–363.

[14] Dzhambazov, B.M. & Teneva, I.I. 2000. Observations on the flower visiting of diptera of the Superfamily Empidoidea (Hybotidae, Empididae). *Studia Dipterologica*, 7: 553–557.

[15] Currah, L. & Ockendon, D.J. 1984. Pollination activity by blowflies and honeybees on onions in breeders' cages. *Annals of Applied Biology*, 105: 67–176.

[16] Smith, B.M. & Mee, C. 1984. Seed multiplication of brussels sprout inbred lines. *Zeitschrift für Pflanzenzüchtg*, 92: 259–262.

[17] D'Arcy-Burt, S. & Blackshaw, R.P. 1991. Bibionids (Diptera: Bibionidae) in agricultural land: a review of damage, benefits, natural enemies and control. *Annals of Applied Biology*, 118: 695–708.

[18] Rader, R., Howlett, B.G., Cunningham, S.A., Westcott, D.A., Newstrom-Lloyd, L.E., Walker, M.K., Teulon, D.A.J. & Edwards, W. 2009. Alternative pollinator taxa are equally efficient but not as effective as the honeybee in a mass flowering crop. *Journal of Applied Ecology*, 46: 1080–1087.

[19] Kaufmann, T. 1974. Behavioral biology of a cocoa pollinator, *Forcipomyia inornatipennis* (Diptera: Ceratopogonidae) in Ghana. *Journal of the Kansas Entomological Society*, 47: 541–548.

[20] Kaufmann, T. 1975. Cocoa pollination by males of *Forcipomyia squamipennis* (Diptera: Ceratopogonidae) in Ghana. *Tropical Agriculture*, 52: 71–74.

[21] Nye, W.P. & Anderson, J.L. 1974. Insect pollinators frequenting strawberry blossoms and the effect of honey bees on yield and fruit quality. *Journal of the American Society of Horticultural Science,* 99: 40–44.

[22] Heard, T.A., Vithanage, V. & Chacko, E.K. 1990. Pollination biology of cashew in the Northern Territory of Australia. *Australian Journal of Agricultural Research*, 41: 1101–1114.

[23] Ssymank, A., Kearns, C.A., Pape, T. & Thompson, F.C. 2008. Pollinating flies (Diptera): a major contribution to plant diversity and agricultural production. *Biodiversity Issues*, 9: 86–89.

[24] Green, J.H. 1973. Note on the use of flies for onion pollination under arid conditions in Nigeria. *Tropical Agriculture*, 50: 165–167.

[25] Kumar, J., Mishra, R.C. & Gupta, J.K. 1985. The effect of mode of pollination on *Allium* species with observation on insects as pollinators. *Journal of Apicultural Research*, 24: 62–66.

[26] Saeed, S., Sajjad, A., Kwon, O. & Kwon, Y.J. 2008. Fidelity of Hymenoptera and Diptera pollinators in onion (*Allium cepa* L.) pollination. *Entomological Research*, 38: 276–280.

[27] Catling, P.M. & Spicer, K.W. 1995. Pollen vectors in an American ginseng (*Panax quinquefolius*) crop. *Economic Botany*, 49: 99–102.

[28] Anderson, D., Sedgley, M., Short, J. & Allwood, A. 1982. Insect pollination of mango in northern Australia. *Australian Journal of Agricultural Research*, 33: 541–548.

[29] Jiron, L.F. & Hedstrom. 1985. Pollination ecology of mango (*Mangifera indica* L.) (Anacardiaceae) in the Neotropic region. *Turrialba*, 35: 269–277.

[30] Winder, J.A. 1977. Field observations on Ceratopogonidae and other Diptera: Nematocera associated with cocoa flowers in Brazil. *Bulletin of Entomological Research*, 67: 57–63.

[31] Winder, J.A. 1978. Cocoa flower Diptera; their identity, pollinating ability and breeding sites. *Pans*, 24: 5–18.

[32] Kendall, D.E. & Solomon, M.E. 1971. Testing the syrphid fly *Eristalis tenax* as a pollinator of apple. Long Ashton Research Station Report for 1971.

[33] Jarlan, A., De Oliveira, D. & Gingras, J. 1997. Pollination by *Eristalis tenax* (Diptera: Syrphidae) and seed set of greenhouse sweet pepper. *Journal of Economic Entomology*, 90: 1646–1649.

[34] Jarlan, A., deOliveira, D. & Gingras, J. 1997. Pollination of sweet pepper (*Capsicum annuum* L.) in green-house by the syrphid fly *Eristalis tenax*. *Acta Horticulturae*, 437: 335–339.

[35] Jauker, F. & Wolters, V. 2008. Hover flies are efficient pollinators of oilseed rape. *Oecologia*, 156: 819–823.

[36] Langridge, D.F. & Goodman, R.D. 1975. A study on pollination of oilseed rape (*Brassica campestris*). *Australian Journal of Experimental Animal Husbandry*, 15: 285–288.

[37] Ohsawa, R. & Namai, H. 1988. Cross-pollination efficiency of insect pollinators (Shimahanaabu, *Eristalis cerealis*) in rapeseed, *Brassica napus* L. Japan. *Journal of Breeding*, 38: 91–102.

[38] Wickramaratne, M.R.T. & Vitarana, S.I. 1985. Insect pollination of tea (*Camellia sinensis* L.) in Sri Lanka. *Tropical Agriculture*, 62: 243–247.

[39] Willson, M.F. & Schemske, D.W. 1980. Pollinator limitation, fruit production, and floral display in pawpaw (*Asimina triloba*). *Bulletin of the Torrey Botanical Club*, 107: 401–408.

[40] Wang, R. & Li, C. 1998. Insect pollinators and yield of common buckwheat. In C. Campbell & R. Przybylski R, eds. *Proceedings of the 7th International Symposium on Buckwheat, Advances in Buckwheat Research*, 12–14 Aug, pp. 25–28. Winnipeg, Manitoba, Canada.

[41] Borthwick, H.A. & Emsweller, S.L. 1934. Carrot breeding experiments. *Proceedings of the American Society for Horticultural Science*, 30: 531–533.

[42] Jones, H.A. & Emsweller, S.L. 1934. The use of flies as onion pollinators. *American Society of Horticultrue Scientific Proceedings*, 31: 161–164.

[43] Parker, F.D., Batra, S.W.T. & Tepedino, V.J. 1987. New pollinators for our crops. *Agricultural and Zoological Review*, 2: 279–304.

[44] Goldblatt, P. & Manning, J.C. 2007. Pollination of *Romulea syringodeoflora* (Iridaceae: Crocoideae) by a long-proboscid fly, *Prosoeca* sp. (Diptera: Nemestrinidae). *South African Journal of Botany,* 73: 56–59.

[45] Anderson, B. & Johnson, S.D. 2009. Geographical covariation and local convergence of flower depth in a guild of fly-pollinated plants. *New Phytologist*, 182: 533–540.

[46] Johnson, S.D., Hollens, H. & Kuhlmann, M. 2012. Competition versus facilitation: conspecific effects on pollinator visitation and seed set in the iris *Lapeirousia oreogena*. *Oikos*, 121: 545–550.

[47] Devoto, M., Montaldo, N.H. & Medan, D. 2006. Mixed hummingbird: long-proboscid-fly pollination in "ornithophilous" *Embothrium coccineum* (Proteaceae) along a rainfall gradient in Patagonia, Argentina. *Austral Ecology*, 31: 512–519.

[48] Potgieter, C.J., Edwards, T.J., Miller, R.M. & van Staden, J. 1999. Pollination of seven *Plectranthus* spp. (Lamiaceae) in southern Natal, South Africa. *Plant Systematics and Evolution*, 218: 99–112.

[49] Beaman, R.S., Decker, P.J. & Beaman, J.H. 1988. Pollination of *Rafflesia* (Rafflesiaceae). *American Journal of Botany*, 75: 1148–1162.

[50] Dentinger, B.T.M. & Roy, B.A. 2010. A mushroom by any other name would smell as sweet: *Dracula* orchids. *McIlvainea*, 19: 1–13.

[51] Endara, L., Grimaldi, D.A. & Roy, B.A. 2010. Lord of the flies: pollination of *Dracula* orchids. *Lankesteriana*, 10: 1–11.

[52] Thien, L.B. 1969. Mosquitoes and *Habenaria obtusata* (Orchidaceae). *Mosquito News*, 29: 252–255.

[53] Grimstad, P.R. & DeFoliart, G.R. 1974. Nectar sources of Wisconsin mosquitoes. *Journal of Medical Entomology*, 11: 331–341.

[54] Kearns, C.A. & Inouye, D.W. 1994. Fly pollination of *Linum lewisii* (Linaceae). *American Journal of Botany*, 81: 1091–1095.

[55] Bishopp, F.C. & Laake, E.W. 1921. Dispersion of flies by flight. *Journal of Agricultural Research*, 21: 729–766.

[56] Schmitt, J. 1983. Density-dependent pollinator foraging, flowering phenology, and temporal pollen dispersal patterns in *Linanthus bicolor*. *Evolution*, 37: 1247–1257.

[57] Holloway, G.J. & McCaffery, A.R. 1990. Habitat utilization and dispersion in *Eristalis pertinax* (Diptera: Syrphidae). *Entomology*, 109: 116–124.

[58] Chifflet, R., Klein, E.K., Lavigne, C., Le Féon, V., Ricroch, A.E., Lecomte, J. & Vaissière, B.E. 2011. Spatial scale of insect-mediated pollen dispersal in oilseed rape in an open agricultural landscape. *Journal of Applied Ecology*, 48: 689–696.

[59] Lövei, G.L., Macleod, A. & Hickman, J.M. 1998. Dispersal and effects of barriers on the movement of the New Zealand hover fly *Melanostoma fasciatum* (Dipt., Syrphidae) on cultivated land. *Journal of Applied Entomology*, 122: 115–120.

[60] Kearns, C.A. & Inouye, D.W. 1997. Pollinators, flowering plants, and conservation biology. *BioScience*, 47: 297–307.

[61] Kearns, C.A., Inouye, D.W. & Waser, N.M. 1998. Endangered mutualisms: the conservation of plant-pollinator interactions. *Annual Review of Ecology and Systematics*, 29: 83–112.

[62] Kearns, C.A. 2001. North American dipteran pollinators: assessing their value and conservation status. *Conservation Ecology*, 5(1): 5.

[63] Allen-Wardell, G., Bernhardt, P., Bitner, R., Burquez, A., Buchmann, S., Cane, J., Cox, P.A., Dalton, V., Feinsinger, P., Ingram, M., Inouye, D., Jones, C.E., Kennedy, K., Kevan, P., Koopowitz, H., Medellin, R., Medellin-Morales, S., Nabhan, G.P., Pavlik, B., Tepedino, V., Torchio, P. & Walker, S. 1998. The potential consequences of pollinator declines on the conservation of biodiversity and stability of food crop yields. *Conservation Biology*, 12: 8–17.

[64] Rotheray, G.E. & MacGowan, I. 2000. Status and breeding sites of three presumed endangered Scottish saproxylic syrphids (Diptera, Syrphidae). *Journal of Insect Conservation*, 4: 215–223.

[65] Goulson, D., Derwent, L.C., Hanley, M.E., Dunn, D.W. & Abolins, S.R. 2005. Predicting calyptrate fly populations from weather; likely consequences of climate change. *Journal of Applied Ecology*, 42: 795–804.

[66] Rader, R., Bartomeus, I., Garibaldi, L.A., Garratt, M.P.D, Howlett, B.G., Winfree, R., Cunningham, S.A., Mayfield, M.M., Arthur, A.D., Andersson, G.K.S., Bommarco, R., Brittain, C., Carvalheiro, L.G., Chacoff, N.P., Entling, M.H., Foully, B., Freitas, B.M., Gemmill-Herren, B., Ghazoul, J., Griffin, S.R., Gross, C.L., Herbertsson, L., Herzog, F., Hipólito, J., Jaggar, S., Jauker, F., Klein, A-M., Kleijn, D., Krishnan, S., Lemos, C.Q., Lindström, S.A.M., Mandelik, Y., Monteiro, V.M., Nelson, W., Nilsson, L., Pattemore, D.E., Pereira, N.d.O., Pisanty, G., Potts, S.G., Reemer, M., Rundlöf, M., Sheffield, C.S., Scheper, J., Schüepp, C., Smith, H.G., Stanley, D.A., Stout, J.C., Szentgyörgyi, H., Taki, H., Vergara, C.H., Viana, B.F. 1 Woyciechowski M. 2015. Non-bee insects are important contributors to global crop pollination. *Proc. Nat. Acad. Sci*, November 2015. doi: 10.1073/pnas.1517092112.

© D. W. Roubik

Chapter 13

STINGLESS BEE COLONIES AND POLLINATION

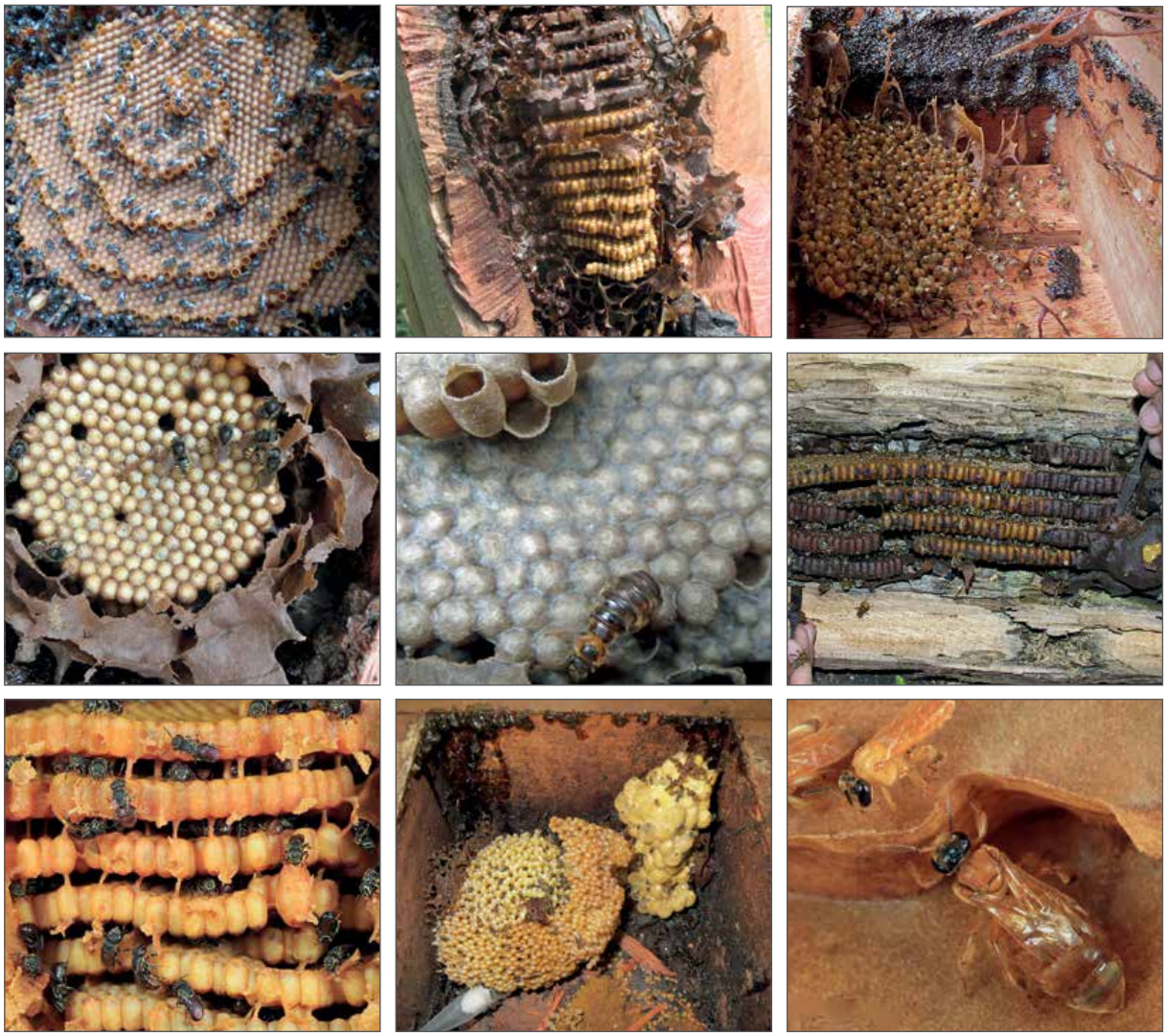
D.W. Roubik, T.A. Heard and P. Kwapong

13.1 CONSERVATION AND UTILIZATION OF STINGLESS BEES

Most crop plants depend on pollination for fruit or seed set and in many cases are pollinated by insects. A wide variety of bees function as effective pollinators because they collect pollen to feed their brood. The variety of conservation and development schemes involving bees and other pollinators is expanding at an impressive rate, with "productive conservation" looking closely at pollinators and their profitability [1–8]. Stingless bees – the apine tribe Meliponini – are major candidates for pollination management in open cropland and greenhouse settings, and many produce commercially viable honey. In all tropical environments, stingless bees (also called stingless honey bees) are kept for their honey, pollen, resin and cerumen (building material made of bee wax mixed with collected resin) see also [127, 128].

Apis mellifera is not considered the ideal candidate for management worldwide, evidenced by traditional and continuing use of other *Apis* across most of Asia. The use of this Western hive bee, *Apis mellifera*, as a pollinator also presents economic disadvantages, linked to increasingly costly maintenance and pest or pesticide problems (see Chapters 3 and 16, and section 13.2 for a detailed comparison of the two apine honey bee groups). Careful analysis and policies are needed to define and organize sustainable pollinators in agriculture, such as justification of commercial honey standards to acknowledge the higher water content of stingless bee honey [4, 6–12]. In much of the forested or humid tropics experts expect to see progressively more beekeeping with Meliponini. The use of such stingless bees as crop pollinators is plausible (see Chapter 14, and sections 9.3.5 and 9.3.9), but caution and real discipline must be exercised to prevent over-exploitation [13–15, 21, 22], as the misuse of bees can occur to the point of extinction. Meliponine husbandry – the human-guided reproduction of bee colonies – is seldom practised in the most intensive and traditional stingless beekeeping area known in the world (the Yucatán Peninsula, Mexico) [17, 18]. However, in other places it functions as a mainstay of modern stingless beekeeping [16–36].

Figure 13.1
STINGLESS BEES FOR BEEKEEPING: NATURAL NESTS AND BEES, BROOD, RESIN DEPOSITS, HONEY AND POLLEN STORAGE POTS



From left to right, by row: *Tetragonula carbonaria* (Australia – note queen cell at lower left); *Axestotrigona ferruginea* (Ghana); *Frieseomelitta* sp. (Brazil) – note black resin deposited along hive edge; *Melipona anthidioides* (Brazil); *M. anthidioides*, queen; *M. beecheii* (Mexico); *Paratrigona opaca* (Costa Rica); *Plebeia* sp. (Brazil) and *Tetragona ziegleri* (Panama)

photos by DW Roubik (rows 1-3), E. Tourneret (row 3, 1 and 3) and P. Kwapong (row 1, photo 2).

13.1.1 Rational stingless beekeeping

There is some debate as to what constitutes a "rational" relationship between humans and wild organisms. Proponents of meliponiculture or apiculture in general often claim that beekeeping advances both bee conservation and the pollination of wildland plants as well as crops. However, this begs the question – which wildlands, plants and pollinators, and for whom?

The integration of bees and habitats, and the implied processes, may take place by propagating colonies or maintaining them in an appropriate setting. At its worst, stingless beekeeping relies on the theft of colonies from the forest (killing the nest trees in the process), to be used elsewhere without replacement or husbandry, until such colonies die. Approximately 33 percent of colonies may die in the Neotropics from phorid

fly attack during transfer, establishment or division (E. Stierlin and D.W. Roubik, pers. obs.). Sustainable approaches, such as the replacement of native colonies removed from the forest, after their initial establishment in hives (artificial housing for bees), are unknown. Furthermore, humans use only a minority of stingless bees (Tables 13.1 and 13.2). In addition, increased stingless beekeeping has the potential to alter drastically the stingless bee component of both agricultural and natural communities. That is, certain species may be favoured at the expense of others, thereby altering the balance of different species living in natural or agricultural communities.

Table 13.1
THE REALIZED EXPLOITATION OF STINGLESS BEE POTENTIAL IN DIFFERENT TROPICAL ZONES

COUNTRY	% USED	TOTAL SPECIES
Mexico	41	46
Costa Rica	10	58
Panama	12	65
Colombia	21	120
Peru	12	>> 100
Brazil	13	250
Ghana	40	10
Malaysia	20	38
S. China	30	6
Australia	28	14

Sources: 16, 34, 58 and D.W. Roubik, pers. obs.

At its best, stingless beekeeping removes small numbers of wild colonies, which become part of a colony multiplication programme. This allows residents of the forest frontier or rural areas to improve their economies, and also empowers beekeepers through direct involvement either in home or gardens, or in the business of pollination. As a further benefit, the bees are often valued as a source of medication (honey or collected nest resin), primarily for skin and alimentary ailments [4, 6, 37–40]. Colonies in hives also provide environmental education tools [e.g. 41, 42], as they offer varied building and art materials [2, 3, 19, 37, 42]. On a larger scale, meliponiculture produces boutique honey or mobile hived colonies for pollination purposes [6, 7, 16, 28, 42–51, and see Chapter 14].

Table 13.2
STINGLESS BEE SPECIES COMMONLY KEPT IN BRAZIL AND THEIR SCIENTIFIC NAMES

BRAZILIAN BEES UTILIZED	
Species	Genus
angustula	*Tetragonisca*
anthidioides	*Melipona*
asilvai	*Melipona*
bicolor	*Melipona*
bipunctata	*Melipona*
clavipes	*Tetragona*
compressipes	*Melipona*
crinita	*Melipona*
fasciculata	*Melipona*
feibrigi	*Tetragonisca*
flavolineata	*Melipona*
fuscopilosa	*Melipona*
interrupta	*Melipona*
mandacaia	*Melipona*
melanoventer	*Melipona*
merrilae	*Melipona*
mondury	*Melipona*
obscurior	*Melipona*
nigriceps	*Plebeia*
paraensis	*Melipona*
pernigra	*Melipona*
quadrangula	*Tetragona*
quadrifasciata	*Melipona*
rufiventris	*Melipona*
Scaptotrigona sp.	*Scaptotrigona*
schencki	*Melipona*
scutellaris	*Melipona*
subnitida	*Melipona*
testaceicornis	*Nannotrigona*
varia	*Frieseomelitta*
weyrauchii	*Tetragonisca*
xanthotricha	*Scaptotrigona*

Note: All listed species have one or more local names, where they occur [see Table 13.1].

Source: D.W. Roubik

Two particular errors are common among new stingless beekeepers: colonies are removed from their natural nest and brought into a bee yard without due planning; and are treated as though they were *Apis*, and expected to quickly produce new colonies and honey crops. In short, colonies are treated as "microlivestock". However, unlike livestock, colonies are wild animals; they are rooted in their place within wildlands; they are not often renewed and their habitat is decreasing. Often beekeepers lack real knowledge of the necessary requirements to maintain or propagate a colony. Indigenous people with a history of stingless beekeeping, however, still reside in largely natural habitats, where the bees forage and mate as they always have [37–40, 52, 53, 61–64]. Under current environmental circumstances in the tropics and elsewhere, about one-third of honey bee species are now sustained by people and have been integrated into rural or farming communities. Use of the different geographic races (those with a given sub-species name) of honey bee is by no means equal, however. Only a few of the nearly 30 races of *A. mellifera* are used extensively by beekeepers. This raises the question as to whether many of the estimated 600 species and untallied varieties of Meliponini (see Table 13.1 and section 13.2) will have equal appeal. Although it is highly unlikely that many species will be truly kept and propagated, much new activity is talking place around the age-old custom of exploiting local bees for honey. Newer techniques for hive development and propagation are now leading to stingless bee management for pollination and other purposes (see below).

An ideal source of bees for stingless beekeeping is colonies rescued from land cleared for other purposes. Such colonies form the basis of colony increase programmes. If the market gives colonies a high value, an incentive exists to save them from legal and illegal land-clearing operations. In Central America, "barter" or the exchange of goods and services, rather than money, is the best means of saving colonies on smallholder plots or in unprotected forests colonized by squatters that are being cut and burned. Intact bee colonies can be saved most effectively by keeping and transporting them in their original logs, and then later moving them to hives.

13.2 BIOLOGY

13.2.1 Meliponines in the present and future

The diversity and abundance of stingless bees is notable (see section 13.2.3) where great primary forests are present and at least somewhat intact. Honey from stingless bees or honey bees, as well as other colony products, therefore fits the category of non-timber forest products. With the exception of sharp-eyed lumber mill operators and experienced subsistence farmers or honey hunters – all of whom have the opportunity to find and exploit stingless bee nests – fewer and fewer people will encounter natural stingless bee nests, aside from those found in parks, gardens or buildings. The species found in those disturbed habitats, interestingly, include many species now under some form of management. However, the potential management innovations for stingless bees have not been exhausted, and information regarding which bees are adapted only to survive in forests, or are flexible, remains uncertain. Colonies used at present consist of those recently taken "out of the forest". They are fairly common in fabricated chambers and buildings – notably *Hypotrigona* in Africa, *Tetragonula* in Asia and Australia, and certain *Melipona* and *Tetragonisca* in tropical America. Those particular stingless bees have adapted relatively well to drier and certain degraded habitats, open and sunny environments (but not permanently arid deserts or extremely hot areas), and are able to forage an adequate distance. They procure food, nesting sites and then reproduce in diverse yet little studied or understood ways, and often accomplish this outside of their original forest or wildlands. In contrast, preferred Brazilian species are of the genus *Melipona* (Table 13.2). This large genus comprises some 80 species of large bees, but only a few are not restricted to forest [52, 53]. Few meliponines, as a whole, can be expected to live outside their native forests, as indicated by studies

of their number in the agroecoscape compared to nearby forest [e.g. 53–58].

The meliponines, like honey bees, are nonetheless often used to represent the preservation of forests and local or indigenous traditions (a point not unnoticed by those seeking to reinforce support for forest conservation) [1, 2, 58]. However, the primary home of stingless bees is the tropical wildlands and not cultivated areas, although this issue may be of lesser importance. For the sake of simple management prescriptions, the conservation of natural areas adjacent to cultivated lands has been recommended to foster stingless bee services for agriculture (Table 13.1). The main stingless bees used in honey production and/or pollination – several *Melipona* and some *Scaptotrigona, Nannotrigona, Paratrigona, Tetragonisca, Plebeia, Tetragonula, Austroplebeia* and *Meliponula* – are successfully propagated by serious practitioners and are kept in home gardens as well as agricultural areas. These bees now in use are species pre-adapted to survive in disturbed human landscapes. As such, they have become the current choice largely by default, not by design (see Chapter 14.3).

The world's two major honey bee groups (meliponines and apines) differ in their biology, but each has a queen, workers and drones. The workers perform multiple tasks for the queen, including building her a nest and feeding her. These permanent colonies thus consist of a "swarm" of workers surrounding their queen, as opposed to colonies initiated by a single fecund female, or a group of fecund females (like most social insects). Unlike honey bees, however, the laying queens of meliponines cannot fly, and a swarm (colony in transit) cannot disperse freely. If the nest is disrupted and the colony left exposed for even a short time, it will almost certainly be exterminated by natural enemies. The colony is not mobile or free-swarming like *Apis*. Stingless bees are limited in terms of their capacity to escape adversity and start a nest and colony. Further aspects of comparative biology are outlined below, with an emphasis on traits that influence the success or failure of beekeeping efforts using stingless bees.

13.2.2 Stingless bees compared with other honey bees: food and resources

Honey bee development schemes only rarely subject the local ecology to explicit study or make use of field data; instead, they largely take assumptions for granted (e.g. "stingless bees are generalists that pollinate the forest plants and also crops"). Stingless beekeeping, particularly for honey, is viewed as taking advantage of a "subsidy from nature", as was the case for Africanized honey bees in the Neotropics and honey bees in general from the 1970s to 1990s [1]. This belief encourages the exploitation of colonies, both for honey and pollination, however the assumption that colonies are free and their support within their habitat is guaranteed seems shortsighted. In some cases (D.W. Roubik, pers. obs.), the "carrying capacity" for beekeeping is considered the calculated sum of all pollen and nectar produced yearly by the local flora – implicitly excluding all other flower visitors.

More and better biological knowledge would be useful in guiding stingless beekeeping, habitat restoration or pollination service development. Accurate reports on the recent traditional uses of stingless bees by forest inhabitants are available [36–39, 58, 60–64]. However, controlled species or those that are kept by virtue of productive conservation are relatively few in number (an outcome that is not accidental). The potential contribution to biodiversity from all bees and other pollinators is much greater than those of a few managed species, but most of these animals will not necessarily pollinate crops where and when they are needed, and are scarcely amenable to current maintenance schemes. The majority may truly be, as portrayed in the book by G.P. Nabhan and S.L. Buchmann, "*Forgotten Pollinators*".

Although commercial honey production and potential bee resources – nest trees and nesting sites, nectar, resin and pollen – are increasingly documented [65–74] and yield scientific knowledge that underpins productive conservation, a number of important qualifiers must be understood. There are numerous distinctions between stingless honey bees (Meliponini) and common honey bees (Apini), as indicated above. The concept of improving bee forage for stingless bees

is valid (see Chapter 14) and provides yet another advantage over honey bees, whose normal maximum flight range of 8 km means they may readily make use of 20 000 ha within their foraging range. The normal foraging range of a stingless bee colony (1 or 2 km from their home base in a nest) may encompass several hundred hectares [65, 75 and D.W. Roubik, pers. obs.] Therefore, efforts to plant and maintain stingless bee gardens are logistically more likely to bear fruit than those targeted at honey bees.

The distinctions between the two tribes of Apinae are less apparent when emphasizing honey production. Both kinds of bees harvest floral resources from "weeds" and rapidly growing flowers of secondary vegetation including trees, herbs, small shrubs and vines (many Neotropical and several now Cosmopolitan) that grow in open areas or along edges, such as *Cecropia*, *Mimosa* spp., *Piper, Muntingia, Mikania, Ageratum, Pluchea, Vernonia, Miconia* and many more. In forests, highly social apines, stingless honey bees and *Apis* – the honey bees – concentrate on large trees with bountiful resources, often taking much of their food from nectar and pollen left over in flowers pollinated by large nocturnal animals [65, 70, 71]. The differences among bee species may determine which survive and reproduce in altered and degraded habitats, or those of low successional stages (e.g. areas recovering after fire, clearing or other changes – see Part I). The numerous plant families and genera in tropical wildlands seem to allow generalist foragers to choose among alternative resources at a given time. Bee species avoid severe competition by partitioning their resources. In croplands or mixed agricultural habitats, prominent resource plants for stingless bees can be expected to include native and exotic and younger and older successional species. A variety of species and especially those providing large amounts of resources within a small area are desirable for stingless bees (see Chapter 14). Furthermore, some flowers such as those that are smaller and have little nectar are often used by stingless bees, but are rarely visited by honey bees (e.g. Chapter 9.3.5).

Given that the adult bee population in a colony of *Apis mellifera* is much larger than that of almost any stingless bee colony, the amount of stored honey (compared to the number of worker bees) runs contrary to many expectations [61]. The larger stingless bees such as *Melipona, Cephalotrigona, Scaptotrigona* or *Meliponula,* and even relatively small *Tetragonula*, appear much more efficient as honey producers, based on the available stores (pollen and honey) in the nest, and the number of worker bees in the colony. Biologically speaking, beekeepers using *Apis* know that the addition of more space to hived colonies may result in greater honey production, unless the bees use the space to produce more brood and daughter colonies. Among stingless bees, while there is very little potential for the production of daughter colonies through swarming, although males or roving queens may also achieve colony reproductive fitness, space in the nest normally leads only to potential honey storage (see below). A plausible reason for the basic difference in nest space use in natural nests of stingless bees and honey bees is that much of what is harvested by honey bees is turned into brood, wax and devoted to swarming (reproduction, see also below). Beekeepers of honey bees prevent swarming by eliminating queen cells. In marked contrast, stingless bees store food to survive dearth seasons, but very seldom reproduce, simply due to a lack of suitable nesting sites with minimal competition from other colonies [54, 57, 65, 67, 75–77]. There is, however, evidence given by Portugal-Araújo [78] that *Meliponula bocandei*, the largest African meliponine, can greatly expand both its colony size and its honey stores when given a large hive by a beekeeper.

13.2.3 Stingless bees compared with other honey bees: colony organization and foraging

The fact that Meliponini have no functional sting seemingly implies they are unable to defend the nest or pose defensive problems for beekeepers. This is not true among the meliponine builders of exposed nests, which include most *Trigona*, *Partamona* and some *Paratrigona* and *Plebeia*, as well as African *Dactylurina* and some Asian *Tetragonula*. In addition, *Oxytrigona*, some *Trigona*, *Scaptotrigona*, some *Melipona* and Asian

Lophotrigona canifrons, all of which nest in protected cavities, are fiercely defensive.

The notion that stingless bees are all good pollinators is also contradicted by the performance of many American tropical *Trigona*, which damage orchards and flowers, stems or leaves, and do not pollinate the flowers, or *Scaura* and *Partamona*, which scavenge pollen fallen on leaves and petals so have little contact with flower reproductive structures. Several genera that consist of tiny bees (2–4 mm in length) have a low probability of providing outcrossing services due to their restricted flight ranges (see also below), while others derive all their food from non-floral sources [53, 65, 75, 76].

The size of meliponines (2–13 mm) is more varied than that of the apines (6–19 mm), with the largest worker *Melipona* equal to the largest worker *Apis* in mass. The body length among stingless bees varies by 6x, but differs by only 3x among *Apis* (D.W. Roubik, pers. obs.).

The lifespan of an apine or meliponine colony may reach several years to decades. The workers each live a few to several weeks, and a replacement queen is produced each year or two. A meliponine queen normally mates with a single drone, unlike the multiple matings that occur over a few days in the life of a honey bee queen. This important difference leads to a greater range of biological traits and flexibility within a colony of honey bees, compared to those of stingless bees, because of the potentially larger genetic variety and its expression in both drones and workers.

Colonies organize foraging workers to massively exploit available resources [65, 77, 79, 80]. Stingless bee colonies are rather small – the whole colony would fit into a small bag (depending on the species), with only a few hundred to a few thousand adult bees in most cases. Is this an advantage or a disadvantage for pollination purposes? Flight ranges of > 2 km are easily possible for large species, such as *Melipona* or *Geniotrigona thoracica*, but even *Trigona fulviventris*, a medium-sized species, and *Cephalotrigona zexmeniae*, a rather large bee, fly up to 2 km from their nest in forests [65, and D.W. Roubik, pers. obs.]. A recent study of pollen species literally forming a nest documents foraging at trees > 0.8 km distant from the bee nest by a medium-sized bee, *Trigona corvina* [69]. Another method, baiting with sugar resources to entice bees to fly farther and farther, has resulted in records of > 2 km for *Melipona* [75]. In conclusion, the flight ranges reported in the literature seem too conservative, and often focus on mean but not maximum ranges, or are presented after short-term studies during favourable resource conditions. *Tetragonula carbonaria* in subtropical Australia will fly up to 1 km to harvest highly preferred resin from *Corymbia torreliana* (T.A. Heard, pers. obs.).

Stingless bee colonies within their nests are stationary and unable to relocate, and thus become finely integrated within the local biota. Their physical positions and use of particular resources, as well as pollination interactions, are maintained for decades or for the life of their nest substrate. Nest-hollow ownership may occasionally change between colonies of different species [54]. Besides producing a new queen and having her mated, a colony may occasionally replace its queen with a foreign queen that enters the nest when the original queen has been lost. The commonness or rarity of this peculiar act remains to be determined [81–85]. Moreover, a newly mated usurping queen may have mated with a male of the original colony that occupied the nesting site [see 82].

Foragers locate and potentially follow or avoid scents deposited on foliage and resources by competing colonies [80, 86, 87]. Certain species forage aggressively in large to small groups and dominate resource patches, while others wait until most foraging species have departed, or forage unmolested by the presence of other species. Different species that share the same area may display greatly diverse foraging breadth, preference and strategies [65, 87].

Potential colony nesting sites among different species in the natural environment are diverse, yet limited and highly conserved within species. Some facultatively parasitic species, such as *Tetragonisca angustula*, attack and kill conspecific colonies or those of *Lestrimelitta* (an obligate parasite) that attack them [88]. Large colonies such as *Trigona fulviventris*

sometimes attack and kill nearby competing colonies. When nesting colonies die in a particular spot, and the site remains unoccupied for months or years, it may be reoccupied by a new colony of the same species (D.W. Roubik, unpublished data). Colonies of *Tetragonula carbonaria* and *T. hockingsi* attack each other and after a period of fighting, often successfully usurp each other's nests, both inter and intra-specifically [89–91].

All of the above point to the underlying interactions and organization of stingless bee communities. While not all of their mechanics or elements have been deciphered, it is possible to make certain inferences that can guide beekeeping and conservation efforts. Having many hives of a given species within a small area is not a natural situation. Nonetheless, large trees or suitable substrate may have several colonies of a given species, and more than one species. Neotropical *Partamona*, *Trigonisca* and some exposed-nesting *Plebeia,* Asian *Tetragonula* and *Pariotrigona* or African *Hypotrigona* may occupy a natural or artificial substrate by the dozens or even hundreds of colonies, with more than one species. An area of approximately 2 ha of eucalypt forest is home to more than 100 colonies of *Tetragonula carbonaria* in Australia (T. Heard, pers. obs.). *Melipona* colonies may number a few dozen in a large wall [21] and a single large tree of *Enterolobium* in Panama may have up to 40 nests of several species (D.W. Roubik, pers. obs.). On the Pacific shore of the same country, the cliffsides have large aggregations of intensely defensive *Partamona peckolti* surrounding a few colonies of wholly unaggressive *Melipona favosa*. Such associations appear adaptive for mild mannered species like *M. favosa*. Finally, in Ghana, many colonies of *Hypotrigona* have been observed occupying several internodes of a single bamboo tree. It is also common to find *Hypotrigona* sharing a hollow tree with *Meliponula boccandei* (P. Kwapong, pers. obs.).

13.2.4 Estimated species number

Individual species, their hybrids and even regional varieties are liable to become more familiar within the science of stingless beekeeping. However, for now, insights obtained by beekeepers and other biologists are still contributing to the available information on living species. The best available data, analysed by Rasmussen and Gonzalez [92], agree with the estimate given by Michener [93] that there are between 500 and 600 species of Meliponini. That number may nevertheless be conservative. African species alone may number 27 according to Michener [94] or 45 (V. Portugal-Araújo, personal communication to D.W. Roubik, 1976). The number of valid species names (i.e. not synonyms, and by no means a maximum number of species to be found) given by Rasmussen and Gonzalez [92] is 526. Of these, 411 are Neotropical and 115 occur in the Old World. The academic work of verifying and testing species status is still ongoing, and corrections are inevitable [95]. Large genera such as *Melipona* and *Trigona*, both of the New World, were previously extended to include Old World species that have now been placed in other genera, particularly *Tetragonula* (e.g. *T. carbonaria, T. iridipennis, T. fuscobalteata* and *T. laeviceps*), or *Lepidotrigona* (e.g. *L. ventralis* and *L. terminata*). The generic status of Asian meliponines needs reworking, but several genera in addition to the distinctive and tiny *Pariotrigona* and *Lisotrigona*, the distinctive and large *Homotrigona* and *Geniotrigona*, and *Lepidotrigona* and *Tetragonula*, will probably be recognized. In the Neotropics and Africa, in contrast, most subgenera in older accounts are now viewed, with some morphological and molecular justification, as separate genera. The total number of extant genera in the Meliponini is about 60, but when genera are compounded, and subgenera are included in the classification, the number drops to 32 [93]. There are even "new" genera still being discovered and described in the Neotropics and Paleotropics [127, 128].

13.3 BEE COLONIES FOR POLLINATION

13.3.1 General considerations

The idea of maintaining colonies of stingless bees for pollination outdoors is relatively new [13.5, 14]. Moreover, removing any colony and transporting it to a foreign or highly modified environment, such

as cropland or countryside populated by humans and their livestock, not to mention the cultivated and exotic plants, can be considered an experiment. The related question of whether such colonies could fare better in their natural environment needs some clarification. Modified and agricultural environments may have the advantage of containing few potential competitors, species with the same ecological niche, or (if colony density is low) natural enemies such as parasites and predators, as well as basic limiting factors (e.g. potential nesting sites). If food and nesting resources are available, a novel environment may greatly favour a given species of stingless bee. However, it should be noted that the single study of stingless beekeeping over decades provides evidence that too many hives in one place can diminish colony survival [18]. The prudent approach, therefore, may be to situate only a few colonies in one place, as recommended for *Apis cerana* in Asia [59]. A previous synthesis of stingless beekeeping in Brazil [16, 19, 21, 22, 96, 97] does recommend a minimum of four colonies and an ideal number of 44 colonies per meliponary. The former pertains to colonies moved outside of their native distribution, while the latter derives from findings that inbreeding eventually leads to the production of many sterile diploid males. When no other colonies of a species are located in the area, the recommendation would serve to maintain the breeding population free of inbreeding. However, if there are more colonies living in the wild, this number seems too large to avoid serious food competition or occasional nest usurpation or fighting. Finally, because almost no stingless bee builds nests in such high densities and a distance of 100 m or more is usually found between conspecific nests, large colony numbers will require supplemental feeding or unusually abundant floral resources. Feeding of either honey or sugar syrup is sometimes recommended, or even the addition of multiple vitamins [21]. Artificial sources of protein derived, for example, from soy milk powder, may provide a temporary pollen substitute.

Perhaps validating much of the above, in Australia, high colony densities of native *Tetragonula carbonaria* and *Tetragonula hockingsi* in anthropic ecosystems, both agricultural and urban, show that colonies may thrive under such conditions and have low mortality rates (< 5 percent per annum), while regularly "splitting" to create new colonies.

13.3.2 Identifying colonies for potential utilization

In rural tropical regions, meliponines can be used to provide managed pollination in mobile, permanent or greenhouse pollination [48, 98]. Good hive management is likely to result in increased crop production. Conversely, less well-regarded kinds of stingless bees function as attackers and can even be destructive to cultivated plants [65, 99]. They overwhelm the observer; fly into the mouth, nose and ears; bite the skin, eyebrows and eyelids; and produce rather foul smelling and tasting honey in small quantities [77]. Their toothed mandibles are employed to extract sap, resin or open a flower for direct access to nectar or pollen – all at a cost to the plants. Their foraging groups sometimes attack or discourage legitimate flower visitors and potential pollinators. Furthermore, their husbandry – or colony multiplication – is seldom easy. Informed choice and consideration of obstacles are therefore important when selecting colonies.

Bee behaviour at a nest entrance, even to the novice observer, is often sufficient to ascertain whether or not the colony would serve in a hive or bee yard. A colony with many returning foragers and with no immediate defensive response to an observer close by indicates a potential crop pollinator. However, sometimes a smaller colony or bee would be preferable, depending on the crop and setting. When chosing a colony, many beekeepers are impressed by the presence of abundant honey, produced by larger bees or those with larger colonies. In general, farmers are not knowledgeable about pollination benefits, as little or no information is available, either traditional or scientific. However, there is some knowledge relating to potential risks. Farmers eliminate large colonies of certain Neotropical *Trigona* near orchards, because they know that the the bees cut stems or fruit and destroy flower buds, and also attack people when molested. In fact, some of those bee species prefer to nest upon *Citrus* trees, whose

spines give additional protection from enemies – and the bees also remove leaves and girdle the stems, so may be seen as destructive. However, the same bees may deter certain pests of the *Citrus* leaves or fruit.

Not all (and certainly not a majority of) stingless bees exhibit a ready potential for beekeeping or honey production [e.g. 30, 45, 88, 100, 101] (Tables 13.1 and 13.2). In general, bees must be carefully identified in the field and, often, permission received from a landowner or resident for the removal of a colony from its nesting site.

In Colombia, for example, the list of stingless bee species numbers over 120 species, around 20 percent of which are utilized in some form. Meanwhile, Brazil can claim over 250 species, and those deemed to have apicultural potential (their honey is sold, and they have common names and thus are used) number around 32. Mexico has considerably fewer species, but over 40 percent are reportedly utilized in some form. As a result of the still selective exploitation of stingless bee species, source wild colonies need to be located, identified and conserved. Non-destructive methods for colony propagation come from Australia [20, 31, 34]. A novel method for splitting or dividing a colony is to trap an emerging virgin queen and some workers in a nest box placed in the exit tube of an existing colony.

Most species are found exclusively within hollow cavities of both living and, occasionally, dead hardwood trees or lianas, or in cavities under the bark. A few, such as *Nogueirapis mirandula, Axestotrigona ferruginea* and *Meliponula bocandei*, nest both in the ground – always in a cavity not made by the bees – and in tree hollows. Quite a few species, worldwide, nest solely underground, while many Neotropical and a few African and Asian species nest in termite or ant nests, either occupied or abandoned, and both on trees or woody substrates, or in the ground. A few stingless bees nest in rock hollows, such as *Trigona, Cephalotrigona, Hypotrigona, Lophotrigona, Pariotrigona* and *Lisotrigona* [77 and D.W. Roubik pers. obs.]. They may also build aerial or exposed nests using resins, their own pollen feces, soil (mud) and chewed leaves [22, 33, 69]. Still other meliponines build nests in abandoned bird nests or within rootlet bundles of epiphytic plants. For some of the relatively small bees, pipes, window and door frames, and keyholes within abandoned buildings, also provide suitable nesting spaces. In summary, stingless bees accept many different nesting sites, unless located in direct sunlight, high winds or periodically flooded, although each species has a very restricted preference for only a few kinds and volumes of sites. The stingless bee-keeper must experiment and observe the results, and also seek valid information from experienced individuals.

13.3.3 Colony re-location

If bees are found in a particular climatic and geographic habitat, it is better to work with them within the same conditions. This will enable them to settle quickly in a new setting and habit – presumably because they are able to locate the resources they need and avoid stressors. Various hive designs can be constructed for the bees and tested, taking into consideration volume, separable honey "supers" or brood chambers, security from pests and predators, rain, extreme temperatures or high humidity. Adequate hive size and shape are usually estimated by carefully observing and measuring natural nests in their cavities [102, 103] or opening them, ordinarily with an axe, shovel or chainsaw.

During the process of carrying colonies in logs to the stingless bee meliponary, care should be taken to avoid changing the original orientation (such as inverting the log). The nest itself also must be completely sealed to keep the adult bees in and the parasites out. Thin plastic cling wrap works very well to temporarily seal logs. For a more permanent seal, a staple gun to attach a tarp, cardboard or cloth is an option, or even a fast drying wood filler.

In remote sites or when the natural nests are damaged or destroyed, the colony can survive by transfer to a temporary domicile. Workers need to be conserved and collected, if possible using an aspirator and at night, when parasite attack is minimal. Workers are more effectively attracted to the temporary domicile if a ring of cerumen (a waxy material made

by stingless bees in their nest, from a mixture of plant resin and beeswax) is placed around the new entrance, or an intact original nest entrance.

Hives that are receiving a new colony are sometimes fitted with artificial nest entrances, modelled in plastic clay, crushed and then molded batumen, or a plaster substance that resembles the original nest entrance. These may help bee orientation and also deter the entrance of parasitic beetles or flies. Several recommendations given below for colony propagation or splitting also apply to procedures for colony transfer from a natural nesting site to a hive, and eventual relocation.

13.4 MANAGEMENT BASICS IN THE STINGLESS BEE FARM

13.4.1 Hive design

Hives come in a wide variety of shapes and sizes. As such, this section does not devote much space to depicting or explaining their various structures and advantages or disadvantages. The following summary of hive requirements provides some key concepts and guidelines:

- A good hive is made of a material that is strong and durable but light, and provides good thermal insulation. Painting the exterior with non-toxic paint should be considered.
- It should be portable and easily managed by one person.
- It should ideally give bees the freedom of circulating air through the nest entrance (their basic means of heat or moisture regulation), and thus might be given one or more screened holes for air to enter and then circulate out through the normal entrance.
- It should afford a quick and minimally destructive means of colony division for multiplication.
- It should have separate chambers for brood and honey, so that the latter are easy to remove without damage and spillage in the nest, and may also have a bottom chamber for liquid, debris and fecal deposits, which can be cleaned out.
- It should be affordable.

The above list is drawn from several sources, but is by no means exhaustive.[2]

Considerable variety in material exists among hives. They can be made from handcrafted hardwood, metal containers such as vegetable oil tins, plastic bottles, coconut or gourd shells, cardboard, cement, adobe, fired clay or Styrofoam. All may function adequately, but some have a shorter life and strength than others; some are heavy, and others are expensive. Those with poor insulating properties – a criterion important for species kept in areas where there are temperature extremes – are not recommended.

Moisture in the hive is a significant consideration and is often remedied by providing a screened hole, or two holes, on a side of the hive well separated from the entrance. In addition, a hive base comprising a narrow compartment for the deposit of liquid or colony trash is a means of separating the brood, bees and stored food from accumulated liquid. A softwood or particle board hive will rot within a few years or sooner if there is no drainage or adequate ventilation. Bees sometimes provide this function themselves; for example, workers of *Melipona* and *Tetragonula* remove liquid from the nest and regurgitate it at the nest entrance. Such liquid comes primarily from the evaporation of water from nectar to make honey. Nectar contains approximately 60 percent water, while honey contains 30 percent to 15 percent among stingless bees. Bees make honey only within their nest, where water is removed by bees fanning their wings, while exposing a small droplet of ripening nectar to the air, on their mouthparts. Hive designs have increasingly incorporated small portals – usually screened with fine mesh to prevent parasite entry – to allow water to escape as vapour. However, stingless bees normally have only a single, small nest entrance that leads to their nesting chamber. This restricted tube or entrance is where they regulate colony

[2] Hive designs and management studies are available at the following addresses: www.cpatu.embrapa.br/paginas/meliponicultura.htm, www.sugarbag.net and www.aussiebee.com.au, among others.

conditions including carbon dioxide, moisture and oxygen. Colonies that nest in the ground, and some in tree cavities, have a drainage hole at the base of the nest. When such colonies are given a hive wall with a hole in it, they rapidly cover it with resin or building material. Thus, the intended opening may not function as a vent for moisture, the intake for oxygen or the exit for carbon dioxide.

In all areas of the world, beekeeping is shaped by the source materials – both the choice of bees and their fabricated hives. For example, the forested Yucatan peninsula of Mexico has three widely kept species among the 14 Meliponini that reside there: *Melipona beecheii, Scaptotrigona pectoralis* and *Cephalotrigona zexmeniae* [64, and D.W. Roubik, pers. obs.]. The first of these produces ample virgin queens throughout the year, is among the largest species in Mexico and is relatively docile; the second is moderately aggressive and produces less honey; and the third has relatively small colonies, but the bees are large and docile, and store substantial honey. All three are kept in log hives made of native hardwood hollow tree trunks (e.g. the hardwood chicle tree, *Manilkara*, locally called "silicote") and remain largely protected from predator attack by the placement by beekeepers of limestone plugs at each end, cemented in place with moistened soil or mud, and later by the bees using resin. As limestone rock constitutes the substrate in the lowland forest of the flat and seasonally dry peninsula, limestone nest plugs of the right size and shape can be located near hives and used in their formation. The hive volume is often determined by experiments or direct measurement of natural cavity size [e.g. 102, 103].

Elsewhere in Mexico and the Neotropics, hives are made of clay pots or with dry, hollow gourds (*Lagenaria* or *Crescentia*). In the Old World tropics, from Africa to China and Borneo or Indonesia, the original log nests are often used, along with a variety of box hives – usually with no separable honey storage or brood sections (D.W. Roubik, pers. obs.). As in the New World, such rustic hives may be adequate for protecting the colonies and obtaining honey, but the honey harvest invariably spills honey within the nest, damages its contents, and the large hives are awkward to handle or keep clean. Colony division for multiplication ("splitting" or husbandry) is particularly difficult and seldom practised (but see the following sections).

13.4.2 Management against natural enemies

Observations show that colony transfer is most successful when bees have access to abundant forage in the environment. In such cases, bees settle quickly, even when newly transferred colonies are denied their stored products – pollen and honey. In fact, the practice of separating food stores from the brood and bees placed in a new hive may encourage foragers to quickly stock the nest with basic food for survival. If stored hive products are included with brood, they often become a source of infection or cause for attack of the new colony. Other bees, parasitic flies, beetles or larger predators are readily attracted to the odour of spilled honey, pollen and brood provisions. This debilitating attraction is particularly strong and evident in the Neotropics, where phorid flies, *Pseudohypocera kerteszi* locate the acetic acid smell of pollen in a matter of minutes, then quickly arrive and mate, laying eggs deep within the nest if possible. The employment of various vinegar traps or supposed repellants is not entirely effective, and the number of flies is very large. In Africa, the native small hive beetle, *Aethina tumida* seeks and enters bee nests if attracted by honey or pollen and brood odours, and becomes a serious pest that can destroy a colony [104, 105]. The same is now true in Australia, where this and another smaller "hive beetle" of the family Nitidulidae, *Brachypepalus*, also invades some stingless beehives [34]. Certain larger phileurine dynastine beetles invade stingless bee nests in Neotropical forests, as do cetoniine scarab beetles in Africa and occasionally in the Neotropics; these are covered with resin by the defending bees (D.W. Roubik, pers. obs.). This behaviour has also been documented for *Austroplebeia* in Australia against *Aethina tumida* [105]. The stratiomyid fly *Hermetia illucens* is a persistent attacker of damaged stingless bee nests in the

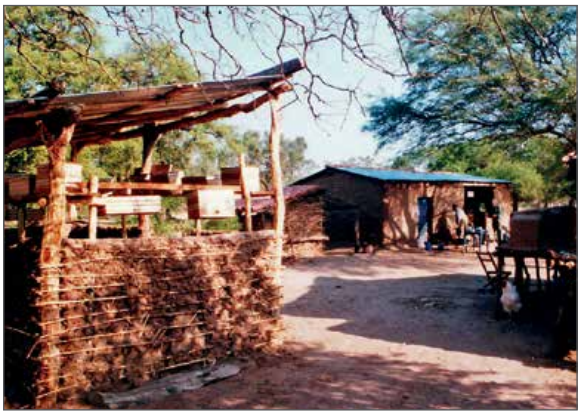
Neotropics, and its large, armoured larvae invade the hive and destroy pollen and brood. The neuropteran *Plega hagenella* is now a pest of stingless bees in South America [107]. In Australia, the syrphid fly, *Ceriana ornata* constitutes a serious pest to colonies with damaged food stores [34]. Chemical management of pest insects, such as the small hive beetle which breeds in the soil near bee hives, is sometimes applied for honey bees (Chapter 16) and might be used for stingless beekeeping, but other options are available that avoid introducing pesticide in any form to the meliponary. Thus far, nothing comparable to these natural enemies seems to attack colonies in Asia, aside from ants such as *Oecophylla* and large vertebrate predators like the sun bear.

The smaller predators of stingless bee colonies consume foragers at the nest entrance. Such animals are persistent and can seriously damage the colony. They include the cane toad, *Rhinella marina* [formerly *Bufo marinus*], lizards (*Hemidactilis, Tropiduras*), birds, ants (*Eciton, Dorylus, Oecophylla*), flies (Phoridae, Stratiomyidae), wasps (*Bembix*) and macropredators. Those predators are discussed below. The combined action of the two natural enemy groups, vertebrates and insects, is relentless.

The meliponary (or apiary – bees in general) will need to be secured in the following ways against these enemies:

- *Ants* attack frequently at night and attempt to overcome the defenses of guard bees at the nest entrance, or find unprotected openings through which they can enter. Water and oil in a container will keep ants from crawling up a hive stand. Suspending the hive by wire or rope from a roof can also prevent ants from gaining access to the bee nest.
- *Phorid*, *stratiomyid* and *syrphid* flies include species that seek damaged stingless bee nests into which they lay their eggs. In the Neotropics, the phorids mount a pheromone-guided attack and deposit eggs throughout the nest, always laying in areas too small to be reached by the bees. Within a few hours or overnight, a nest that has any openings, including the entrance, will be thoroughly parasitized, and the fly larvae will kill the colony within a few days. The flies find the nests by smell, in particular the acetic acid of fermenting pollen and the smell of honey. Plugging all the holes with cotton and keeping all edges sealed with tape will temporarily help to keep flies out. The best defenses are intact nests and nest contents (which the bees repair in a few days after a major disturbance, such as moving the nest). The other important defense is a restricted nest entrance of the proper size for the bees in a natural nest. A gaping hole for a nest entrance is an invitation for disaster. Stingless bees usually build entrance tubes for defense. An artificial entrance tube, usually placed internally may help the bees to defend their nest particularly in the early stages of establishment. Further innovations, such as a plastic or metal funnel placed around the entrance hole, may also be used to keep bee predators away from the hive entrance (see below).
- *Termites* constantly attack the wood of hives and logs. The bees can defend themselves by applying resin to the inside of the cavity, which will sometimes hold the hive together even if the wood is consumed by termites. Termites also enter the nest entrance of stingless bees, building their galleries through the bees' only exit, which may result in the death of the colony. It is likely that some termites will also attack bees and their food stores. Chemical deterrence of termites is

Figure 13.2
MELIPONARY WITH STINGLESS HONEY BEES PLACED IN HIVES (BOLIVIA)



inadvisable. Such insecticides are extremely toxic and, even if applied outside the hive and only to one extreme, such as the base, the bee colony will usually succumb to the poison. The same methods used to prevent ants from reaching the nest will protect against termites.

- *Parasitic stingless bees* (*robber bees*) are ordinarily the only bees that can overcome stingless bee defenses and enter their nests. Although their attacks do not usually result in the death of the host colony, it is debilitated and will be attacked again. Because the robbers, the Neotropical *Lestrimelitta* and the African *Cleptotrigona*, are small bees measuring only < 7 mm in length, constriction of the host nest entrance to prevent entry would be impractical. The only permanent solution to protect stingless bee nests from the robbing parasites is to locate and destroy the robber colony.

The main predators and parasites that invade hives and consume the larvae, brood provisions and stored food are insects or large mammals. The former consist of a suite of stingless bees that are obligate parasites of other stingless bees. In the Neotropics these include some 20 species of robber bees (*Lestrimelitta*), as well as a number of species that pilfer food and occasionally usurp host nests, including *Melipona*, *Tetragonisca*, *Oxytrigona* and *Trichotrigona*. In Africa, the meliponine honey bee *Cleptotrigona* has a single species, which also steals brood provisions, honey and building material. In contrast, *Lestrimelitta* attacks, or rather, systematically parasitizes (without killing the host) stingless bees of many medium to large species. Last but not least, the mustelids (*Eira*, *Mellivora* – tayra and honey badger), tropical bears (*Tremarctos, Helarctos* – sun bear and spectacled bear) and certain anteaters, civets and the aardvark, are known stingless bee colony predators. Another African parasite is the Hive Beetle, *Aethina* – a small nitidulid beetle previously known to attack weak stingless bee colonies recently transferred to hives [78]. It has become a serious pest. In Australia, the hive beetle is a minor pest but can occasionally destroy colonies. Stingless bees possess a strong natural defense against this invader and normally repel attacks by applying resin on the beetles to immobilize them. This behaviour has been observed for species of both *Tetragonula* and *Austroplebeia* [105, 107].

Prevention of nest entry or access via the nest entrance tube, both by lizards in Brazil and hive beetles in Africa, is reduced by the placement of a funnel-shaped plastic or metal object (Figure 13.3), with the narrow end placed against the hive wall around the entrance.

The final and perhaps most devastating predator of stingless bee colonies is the Neotropical phorid fly *Pseudohypocera*. This fly quickly locates the acetic acid smell of opened hives and their pollen and brood provisions, or the smell of honey. It also seems able to identify the mating pheromones of stingless bee males and queens (D.W. Roubik, pers. obs.). The phorids then mate outside the nest entrance, hovering briefly while in copula, and females quickly invade the hive to lay eggs. Those eggs hatch immediately and thousands of fly larvae may overwhelm the colony and consume all the brood and pollen, accomplishing their life cycle to continue the process in little more than a week. In such cases, the entire colony is consumed before the bees have a chance to find another nesting site or escape. However, in Panama, a swarm of *Melipona fallax* was observed escaping to a nearby tree trunk,

Figure 13.3
METAL FUNNEL PLACED OVER HIVE ENTRANCE OF *MELIPONA*, AS A MEANS TO PREVENT PREDATION BY LIZARDS (BRAZIL)

abandoning its queen without leaving any workers behind (D.W. Roubik, pers. obs.). A virgin queen and some workers may conceivably escape such attacks, but this is undocumented and highly speculative. The Australian phorid-fly predator, *Dohrniphora trigonae*, is not as damaging as the Neotropical species, but can also cause the loss of colony, especially at the vulnerable stages following transfer into a hive, or after splitting [105].

13.4.3 Propagating colonies

Humans have tried three basic methods to procure colonies for hives. These differ substantially in their probable success rates. The first is the use of a swarm box to capture a colony during the nesting process through the use of an artificial domicile. One in 40 swarm boxes produces a viable colony. The second is colony eduction, which relies on a new queen coming into a new hive, physically connected to the mother hive. One in 20 boxes connected to a normal colony for eduction produce a viable colony. The third is "splitting" or the removal of part of the brood from the mother colony, which later obtains a fertilized queen. Two out of three transfers from a natural nesting cavity produce a viable colony.

Furthermore, the need for abundant new colonies has led researchers in Brazil, since 1972, to find ways to artificially inseminate virgin queens, mate virgin queens in small boxes with males, or rear queens from larvae in-vitro [16, 30, 39]. In-vitro or laboratory queen production, done by manipulating the amount of food given to a larva grown in an incubator, seems most profitable and has been performed for *Plebeia, Scaptotrigona, Frieseomelitta* and *Tetragonisca*. However, in general, colony reproduction – the heart of the matter for any sustainable approach – is difficult for meliponine bees. Beekeepers replace this natural process by dividing a colony artificially. Most stingless bees, with exceptions among the obligate necrophage *Trigona* species, certain Old World *Tetragonula* and the Neotropical genus *Melipona*, produce very few virgin queens. The process whereby the beekeeper divides the brood and places one part in a new nest box, to form an independent new colony with a soon-mated queen, must therefore be carefully judged and planned. The key is the presence of new brood cells containing virgin queens. Fortunately, such cells are on or near the margin of the cell comb stack, and are twice the size of a normal, worker or male, brood cell. The ability to produce emergency queen cells, in the absence of a reigning queen by extending a worker cell, has been observed recently in *Tetragonula carbonaria* [108] and may be more common than previously thought. This is significant for the propagation of hives by colony division, as it means that queenless colonies can receive new queens, even if queen cells or virgin queens are not present. A large number of queen cells normally exist only in the genus *Melipona*, averaging around 10 percent of the brood [81, 84].

In order to reproduce, stingless bee colonies *in nature* first locate a new nesting site and make part of a new nest. This is structured around the appropriate nest entrance, often with an internal and/or external tube, and completed with stored honey and pollen. After a period of several days to several months, adult bees from the mother nest including the new virgin queen occupy the new nest. This event does not take place on a regular basis; it is neither seasonal nor annual, as without the new nesting site there is no colony reproduction. The artificial division of colonies – the practice of animal husbandry applied to a permanently social bee with a number of workers and their queen – is essential to increase the colonies available for management. It may utilize a variety of strategies and devices, which are outlined below.

Colony division should be undertaken with great care and follow a relatively simple procedure:

- Division should be made when the weather and bee forage are optimal.
- Carefully remove half of the brood combs (both mature pupae and young brood), along with workers on the combs, and some of the stored pollen and honey (taking care to avoid damage or spillage, as this attracts phorids and hive beetles). Place them in a new hive box. Ensure that the laying queen is not removed. Alternatively, the colony can be

divided by separating the two boxes that constitute the hive and replacing each half with a new empty half box [20, 23]. This method is similar to that of G. Venturieri and his hive design [32], and has the advantage of causing fewer disturbances to the structure of the nest.

- Place the mother and daughter hives next to each other, allowing foraging bees to enter each hive. Alternately, remove the hive with the mated queen to another locality, thus making the returning foragers enter the new hive.
- That night, place the new hive with its half nest in the position formerly occupied by the mother colony. Move the hive of the mother colony with its queen to a new location, at least > 200 m, depending on the species. Seal each nest with cotton or other material that will allow air but not parasites to pass through.
- The next day and thereafter, let the bees forage freely. Do not otherwise open the hives unless absolutely necessary. Some of the foragers from the mother colony will return to their former site, thereby strengthening the new colony. Many, however, will remain in the nest at its new position. A relative advantage of the mother colony is the intact nest and likely more young bees. The relative advantage to the new colony is the presence of many of the older bees, which will forage and work to defend the new nest.

Within a few days of this operation, the new nest, which lacked a laying queen, should have male (drone) bees flying and landing near the nest entrance. They quickly find sites with virgin queens. If no males are present, the area may not have enough conspecific colonies, or a virgin queen may not be present. In one to two weeks, depending on the species, a mated queen should be laying eggs in both the new and the old hive. Both colonies should be actively foraging and collecting pollen, unless they lack queens. As of the time of writing, the processes by which a stingless bee queen successfully mates are still not understood. The queen mates only once with a single drone during her lifetime – usually one to two years. What has been clearly established is that queens very seldom mate with brothers or closely related drones. To do so is detrimental to the welfare of the colony [82, 109]. Successful mating ordinarily requires that at least several colonies of the same species be located within 100 m of the nest having a virgin queen. Several to many virgin queens may leave the nest and not return, having been caught by an enemy or prevented from re-entering by worker bees in the hive. Alternatively, a queen that has recently mated can seek a foreign colony and be accepted as the laying queen, as studied in *Melipona scutellaris* in Brazilian meliponaries [75]. Finally, one queen mates and then returns to the nest to lay eggs. A few stingless bee species will reproduce in order to utilize suitable nest sites located close to their hives. This is especially true in orchards or habitats that have many flowers and available resources for the bees (largely habitats where forest bee species are unable to forage). Colony numbers may increase slightly through the natural occupancy of domiciles left in the vicinity of meliponaries, but this is not an effective management strategy in most situations.

13.4.4 Soft-splitting colonies

A novel method for splitting or dividing a colony is to trap an emerging virgin queen and some workers in a nest box placed at the exit tube of an existing colony [24, 34]. This method is termed "soft splitting", the gradual "eduction" of one colony from another, but is not a type of "budding", as this refers to clonal propagation. The incipient colony in the connected nest box acquires a queen, likely a virgin queen from the colony to which it is attached [see alternative possibilities [82–84], and the newly mated queen then begins to lay eggs and form a complete colony.

13.5 CROP POLLINATION

Stingless bees are common visitors to flowering plants in the tropics. Yet, evidence for their importance and effectiveness as crop pollinators is lacking for most plant species, and few species are used [43, 48]. They were documented by 1998 [44] visiting the

flowers of approximately 90 crop species, whereas confirmation of their effectiveness in perhaps a dozen species has been established. Information probably exists in many tropical countries where stingless bees are recognized as pollinators, but published studies are lacking. In Ghana, stingless bees have been observed and collected on important crops such as tomato, chili pepper, mango, cashew, coconut, citrus, avocado, pears, shea, several ornamental plants and uncultivated natural vegetation (P. Kwapong, pers. obs.). The many possibilities and claims that stingless bees pollinate – rather than merely visit (see Part V) a considerable proportion of tropical flowering plants – remain to be adequately documented or tested in farm environments.

Roubik [110, 111] found that in Panama bee pollination by stingless bees and honey bees results in more rapid development, higher fruit set and heavier mature fruits compared to bagged branches from which pollinators are excluded. In two separate studies he concludes that bees, including stingless bees and Africanized honey bees, consistently control approximately 36 percent of total coffee production. Klein *et al.* [112] found that coffee fruit set in Indonesia is higher in areas with a high bee diversity (approximately 90 percent fruit set), many of which are stingless bees, compared to areas with a low diversity (approximately 60 percent fruit set), and conclude overall that bee diversity, not abundance, is important for pollination success. Using bagging experiments, they found that 15 bee species, including four *Tetragonula* and *Lepidotrigona* species, contribute to pollination of that shrub. However, pollination efficiency (fruit set after a single flower visit) varies among them, with *Lepidotrigona terminata* producing the observed maximum 80 percent fruit set.

13.5.1 Pollination in the greenhouse

Colonies are sometimes imported from the tropics and kept indoors in the temperate zone. They have overwintered successfully (e.g. in Europe and Japan [113-121]), where small colonies of *Melipona favosa*, *Plebeia, Scaptotrigona* and *Nannotrigona* temporarily survive in the temperate zone. Some Asian *Tetragonula* have also been used for pollination in glasshouses or greenhouses. South American subtropical microclimates contain several potentially cool-adaptable *Paratrigona, Melipona, Tetragonisca, Parapartamona* and others [77]. One species in Australia lives in the warm temperate zone to 36.5° S [24]; and various *Tetragonula* and *Lepidotrigona* live in seasonally cool, highland subtropical Asian climates. In fact, meliponines inhabit virtually all of the tropics and subtropics, but not all of the nearby islands or much of the desert regions. To date, there have been few translocations by people of stingless bees to meliponaries distant from native ranges, including on islands. Within Brazil, *Melipona* colonies have been transported for study and utilization outside their native regions and climates [16]. It is uncertain whether this will continue, due to the advent of prohibitive laws, although transport for research purposes had bee permitted.

Stingless bees are undeniably efficient pollinators of crops in greenhouses, as documented. The details of success in similar bumblebee greenhouse utilization are discussed by van Doorn in Chapter 11 and likely apply to stingless bees in many instances. Persistent challenges to the commercial use of stingless bees in growing houses relate to the lack of technique for mass rearing, and their conservation status is also not clearly known. In Brazil, legislation aimed at protecting the environment prohibits the sale of hives and limits commercial use. Some workers suggest that stingless bees are dependent on tropical forests and vice versa. In Ghana, where forests have been degraded, stingless bees have been observed inhabiting subterranean habitats such as termite mounds and underground nests (A. Tornyie and P. Kwapong, in press).

In practice, *Melipona* are the only stingless bees generally known to buzz pollinate Solanaceae (e.g. tomato) flowers [114, 115, 118], and because of well-developed techniques for rearing and multiplying their colonies are ideal for greenhouse pollination. However, other genera such as *Nannotrigona* or *Hypotrigona* (P. Kwapong, pers. obs.), which do not loudly sonicate the anthers, still may effectively pollinate solanaceous flowers [117, 119].

13.5.2 Effectiveness of stingless bees as crop pollinators

Stingless bees possess a number of traits that make them good pollinators [123–126]. However, not all species of stingless bees demonstrate the same biology and so not all attributes apply to all species. The following generalizations therefore need to be verified for each particular species. It should be noted that most of the following traits are common for honey bees, but differ from those of primitively eusocial bumblebees and solitary bees.

In summary, from articles mentioned in this chapter and various observations undertaken by the authors in the field, most species of stingless bees for which information is available appear to be opportunistic, and therefore able to exploit the pollen and nectar of many plants. They readily adapt to exotic plant species, including crops, introduced into their geographic range. Generalization for food at the colony level is balanced by floral constancy at the level of the individual bee: a worker on a foraging trip usually only visits one flower species.

Colonies can be housed in hives where they are easily subject to management such as inspection, propagation, feeding, re-queening, pest management, protection from extreme weather and transportation. Colonies of stingless bees are perennial, allowing workers to forage continuously despite constraints imposed by temperature and light [126]. There is no need to breed new colonies each year, as is the case for bumblebees.

Reserves of pollen and nectar are stored in the nests, which has the obvious benefit of allowing colonies to survive long periods of low food availability. Additionally, it means that workers will collect floral resources beyond their immediate needs, which usually results in intensive visitation of preferred flowers – provided there is storage room in the hive. Workers of some species intensively recruit nest mates to rewarding floral resources and provide information on the position of those floral resources, which allows the rapid deployment of many foragers.

The advantages of using stingless bees over honey bees as crop pollinators are as follows:

- Stingless bees are generally harmless to humans and domesticated animals. Less defensive strains of honey bees are now available, but problems still occur particularly to allergic humans and where defensive honey bees occur. An additional advantage is that minimal equipment is needed in meliponiculture compared to apiculture.
- Colonies are unable to abscond because the queen isunable to fly, due to her weight and small wings. Some honey bees regularly leave their nest and move to another location. The queen of a stingless bee colony is unable to fly again after her mating flight, and so the colony is fixed in its location, although a few exceptions are known (i.e. *T. minangkabau* studied by T. Inoue and S.F. Sakagami).
- Stingless bees are resistant to the diseases and parasites of honey bees. Grave concerns about natural enemies of honey bees dominate discussions on the use of this species. Stingless bees have their own natural enemies, although curiously they are not known to incur pathological diseases. (Some 5 percent of 500 hived *Melipona scutellaris*, however [21] demonstrate symptoms of "black larva" or trembling adults, thought to result from pathogens.) Although some natural enemies can be serious, they differ from those of honey bees. Honey bees and stingless bees are thus complementary in this regard. One exception is the small hive beetle, *Aethina tumida*, a nest parasite of honey bees that also attacks stingless bees. The authors observed this on farms where honey bees are infested with the small hive beetle.
- Stingless bees have short flight ranges relative to honey bees. A common range of approximately 500 m is typical for small Australian species. This is short compared to the common range of 5 km or more of honey bees. That can work to the favour of farmers. While honey bees can leave the farm and forage alternative plant species, stingless bees are fixed within the shorter flight range, which is more

likely to keep them within the target crop. The larger area and potentially greater number of plant species within the flight range of honey bees may act to attract a greater proportion to non-target species.

- All members of the Neotropical stingless bee genus *Melipona* are able to buzz pollinate flowers. This makes them useful for the pollination of crops that require sonication to release pollen [35, 46]. For example, blueberry and members of the Solanaceae family, such as tomato, chile and eggplant, require buzz pollination for efficient fertilization. Old World stingless bees, such as honey bees, cannot buzz pollinate or are not known to do so effectively, which may limit their range of target crop species.
- Colonies of stingless bees can provide another use on farms. Foraging bees may constitute a constant supply of prey for predators. Such predators may then help to control crop pests. Although empirical evidence is lacking, a macadamia grower in Australia claims that birds, frogs, lizards and beneficial insect predators increase as a result of introducing stingless bees. He is happy to lose a portion of his bees to predators, in exchange for the pressure they place on pest insects (F. Adcock, personal communication).

The disadvantages of using stingless bees over honey bees as crop pollinators are as follows:

- Stingless bees have decidedly limited eco-climatic tolerance, and their innate behaviour or limitations may not suit humans and their modified environment. (On the other hand, their inability to freely swarm makes stingless bees highly unlikely to become invasive, where introduced into cooler climates, outside their normal range limits or the temperate zone in greenhouse settings.)
- Similarly, some species that occur naturally in tropical forests cannot be moved to dry or even moist cropping zones. Some species have particular habitat requirements and do not thrive when out of their preferred habitat. For example, the Australian species *Austroplebeia australis* is easily kept in drier areas of inland Australia, but when moved outside its preferred habitat the colonies lose weight and often die. Other species have particular nesting requirements. Species that naturally nest underground will die when moved to hives that cannot offer the required stable thermal or moisture characteristics. Even species that thrive in human-disturbed ecosystems may have slow colony growth rates, especially when compared to honey bees.
- Colonies of some species interact negatively with each other. They may attempt to parasitize, usurp or rob other colonies, particularly when placed in the close proximity required for pollination. Some species damage plants to produce resin flows for their collection. Some species rob flowers by extracting the pollen or nectar resources and damaging the flowers without effectively pollinating them.
- Other limitations of stingless bees are the result of human behaviour. Large numbers of hives are not yet available, due to the slow albeit continuing growth of meliponiculture around the world [127, 128]. This is currently being addressed in some areas for certain species. There is a poor understanding of the rearing requirements for most species, which limits the ability to multiply these species. In some areas, laws may impede progress. For example, in Brazil, legislation aimed at protecting the environment prohibits the sale of hives, even those reared in captivity, and limits commercial use. At the same time, the uncontrolled exploitation of stingless bee colonies is a real and persistent danger in all tropical and subtropical frontiers.

REFERENCES

[1] Brown J.C. 2006. Productive conservation and its representation: the case of beekeeping in the Brazilian Amazon. In K.S. Zimmer, ed. *Globalization and new geographies of conservation*, pp. 92–116. Chicago, University of Chicago Press.

[2] Bradbear, N. 2009. *Bees and their role in forest livelihoods: a guide to the services provided by bees and the sustainable harvesting, processing and marketing of their products.* Non-Wood Forest Products, No. 18. Rome, FAO.

[3] Crane, E. 1990. *Bees and beekeeping; science, practice and world resources.* Ithaca, NY, Comstock Publishing.

[4] Crane, E. 1992. The past and present status of beekeeping with stingless bees. *Bee World*, 73: 29–42.

[5] Vaughan, M., Vaissière, B.E., Maynard, G., Kasina, M., Nocelli, R.C.F., Scott-Dupree, C., Johansen, E., Brittain, C., Coulson, M. & Dinter, A. 2014. Overview of non-Apis bees. In D. Fischer & T. Moriarty, eds. *Pesticide risk assessment for pollinators*. Hoboken, NJ, John Wiley & Sons. doi:10.1002/9781118852408.ch3.

[6] Vit, P., Pedro, S.R.M. & Roubik, D.W., eds. 2013. *Pot-honey: a legacy of stingless bees*. New York, Springer Science + Business Media.

[7] Vit, P. & Roubik, D.W., eds. 2013. *Stingless bees process honey and pollen in cerumen pots*. Facultad de Farmacia y Bioanálisis, Universidad de Los Andes, Mérida, Venezuela (available at www.saber.ula.ve/handle/123456789/35292).

[8] Carvalho, C.A.L., Souza, B.A., Alves, R.M.O. & Justina G.D. 2005. *Custo de produção de mel: uma proposta para abelhas Africanizadas e Meliponíneos* [Honey production cost: a proposal for Africanized and Meliponine bees]. Serie Meliponicultura, 02. Salvador, Bahia, SEAGRI/DDP.

[9] Carvalho, C.A.L., Souza, B.A., Silva, C.G.N. da, Marchini, L.C. & Alves, R.M.O. 2005. *Mel de abelhas sem ferrão: Contribuição para a caracterização fisico-quimica* [Honey from stingless bees: contribution to physical-chemical characterization]. Serie Meliponicultura, 04. Salvador, Bahia, SEAGRI/DDP.

[10] Souza, B.A., Roubik, D.W., Barth, O.M, Heard, T.A., Enriquez, E., Carvalho, C., Jerônimo Villas-Bôas, J., Marchini, L, Locatelli, J., Persano-Oddo, L., Almeida-Muradian, L., Bogdanov, S. & Vit, P. 2006. Composition of stingless bee honey: setting quality standards. Interciencia, 31: 857–875.

[11] Venturieri, G.C., Sertão, P. de O., Vasconcelos, M.A.M. & Mattietto, R. de A. 2007. *caracterização, colheita, conservação e embalagem de meis de abelhas indígenas sem ferrão*. [Characterization, harvesting, conservation and packing of indigenous stingless honeybees]. Belem, PA, EMBRAPA.

[12] Carvalho, C.A.L. Alves, R.M.O., Souza, B.A., Véras, S.O., Alves, E.M.& Sodré. G.S. 2013. Proposta de regulamento técnico de qualidade fisico-química do mel floral processado produzido por abelhas do gênero *Melipona*. [Proposal of a technical regulation of physico-chemical quality of processed floral honey produced by bees of the genus Melipona]. In P. Vit P & D.W. Roubik, eds. *Stingless bees process honey and pollen in cerumen pots*, pp. 1–9. Mérida, Venezuela, Facultad de Farmacia y Bioanálisis, Universidad de Los Andes (available at www.saber.ula.ve/handle/123456789/35292).

[13] Alves, J.E., Freitas, B.M., Lima-Verde, L.W. & Ribeiro, F.R. 2006. A uruçu do chão (*Melipona quinquefasciata*) no nordeste: extrativismo de mel e esforços para a preservação da espécie [Uruçu de chão (*Melipona quinquefasciata*) in the northeast: the extraction of honey and efforts for the preservation of the species]. Mensagen Doce 85 (available at www.apacame.org.br).

[14] Souza, L., Nascimento, A.S., Silva, S.P.C., Santos, P.C., Santana, A.L.A., Vieira, J.F., Jesus, J.N., Oliveira, D.J, Andrade, J.P & Costa, S.N. 2009. Preservação e manejo sustentável de abelhas sem ferrão do estado da Bahia: capacitação para a sustentabilidade [Preservation and sustainable management of stingless bees in the state of Bahia: capacity building for sustainability]. *Revista Brasileira de Agroecologia,* 4: 4114–4117.

[15] Freitas, B.M., Imperatriz-Fonseca, V.L., Medina, L.M., Peixoto Kleinert, A.M., Galetto, L., Nates-Parra, G. & Quezada-Euán, J.J.G. 2009. Diversity, threats and conservation of native bees in the Neotropics. *Apidologie*, 40: 332–346.

[16] Venturieri, G.C., Alves, D. de A., Villa-Bôas, J.K., Caravalho, C.A.L., Menezes, C., Vollet-Neto, A., Leon Contrera, F.A., Cortopassi-Laurino, M., Nogueira-Neto, P., Imperatriz-Fonseca, V.L. 2012. Meliponicultura no Brasil: situação atual e perspectivas futuras para o uso na polinização agrícola [Meliponicultura in Brazil: current situation and future prospects for use in agricultural pollination]. In V.L. Imperatriz-Fonseca, D.A.L. Canhos, D. de Arajujo Alves & A.M. Saraiva,

eds. *Polinizadores no Brasil. Contribuição e perspectivas para a biodivesidade, uso sustentável, conservação e serviços ambientais* [Contributions and prospects for biodiversity, sustainable use, conservation and environmental services], pp. 213–236. São Paulo, University of São Paulo Press.

[17] Villanueva-G.R., Roubik, D.W. & Colli-Ucán, W. 2006. Extinction of *Melipona beecheii* and traditional beekeeping in the Yucatán Peninsula. *Bee World*, 86: 35–41.

[18] Villanueva-G.R., Roubik, D.W., Colli-Ucán, W., Güemez-Ricalde, F.J. & Buchmann, S.L. 2013. A critical view of colony losses in managed Mayan honey-making bees (Apidae: Meliponini) in the heart of zona Maya. *Journal of the Kansas Entomological Society*, 86: 352–362.

[19] Nogueira-Neto, P. 1970. *A criação de abelhas indígenas sem ferrão* [The creation of stingless indigenous bees]. 2nd edn. São Paulo, Chácaras e Quintais.

[20] Heard, T.A. 1988. Propagation of hives of *Trigona carbonaria*. *Journal of the Australian Entomological Society,* 27: 303–304.

[21] Kerr, W.E., Caravalho, G.A. & Nascimento, V.A. 1996. *Abelha uruçu: biologia, manejo e conservação* [The uruçu bee: biology, management and conservation]. Belo Horizonte, Brazil, Fundação Acangaú.

[22] Nogueira-Neto, P. 1997. *Vida e criação de abelhas indígenas sem ferrão* [Life and rearing of stingless indigenous bees]. São Paulo, Editora Tecnapis.

[23] Dollin, A. & Heard, T.A. 1997. *Beekeeping with native stingless bees in Australia*. Native Bees of Australia Series, Booklet 5. Sydney, Australian Native Bee Research Centre.

[24] Heard, T.A. & Dollin, A. 2000. Stingless beekeeping in Australia, snapshot of an infant industry. *Bee World,* 82: 116–125.

[25] Carvalho, C.A.L., Alves, R.M.O. & Souza, B.A. 2003. *Criação de abelhas sem ferrão: aspectos prácticos* [Beekeeping without stings: practical aspects]. Serie Meliponicultura, 01. Salvador, Bahia, SEAGRI/DDP.

[26] Stierlin, E. & Szabo, H. 2004. *Manual de manejo de abejas nativas: suro y obobosí,* Scaptotrigona *spp* [*Manual of native bee management: southwest and obobosí,* Scaptotrigona *spp*]. Santa Cruz de la Sierra, Bolivia, Aguarague.

[27] Carvalho-Zilse, G.A., Silva, C.G.N. da, Zilse, N., Vilas Boas, H.C., da Silva, A.C, Laray, J.P., Freire, D.C.B. & Kerr, W.E. 2005. *Criação de abelhas sem ferrão* [Breeding stingless bees]. Manaus, AM, INPA.

[28] Quezada–Euán, J.J.G. 2005. *Biología y uso de las abejas sin aguijón de la pennsula de Yucatán, México (Hymenoptera: Meliponini)* [Biology and use of stingless bees of the Yucatan pennsula, Mexico (Hymenoptera: Meliponini)]. Mérida, YUC, Ediciones de la Universidad Autónoma de Yucatán.

[29] Villanueva-G., R., Buchmann, S., Donovan, A.J., & Roubik, D. 2005. *Crianza y manejo de la abeja Xunancab en la peninsula de Yucatán* [Breeding and management of the Xunancab bee in the Yucatan peninsula]. Spanish/Mayan edition. Tucson, AZ and Chetumal, QR, Mexico, the Bee Works/ECOSUR.

[30] Cortopassi-Laurino, M., Imperatriz-Fonseca, V., Roubik, D.W., Dollin, A., Heard, T., Aguilar, I., Venturieri, G.C., Eardley, C. & Nogueira-Neto, P. 2006. Global meliponiculture: challenges and opportunities. *Apidologie*, 37: 275–292.

[31] Klumpp, J. 2007. *Australian stingless bees. A guide to sugarbag beekeeping*. West End, QLD, Earthling Enterprises.

[32] Venturieri, G.C. 2008. *Criação de abelhas indígenas sem ferrão* [Breeding of stingless indigenous bees]. 2nd edn. Belém, PA, Brazil, EMBRAPA Amazônica.

[33] Kwapong, P., Aidoo, K., Combey, R. & Karikari, A. 2010. *Stingless bees – importance, management and utilization: a training manual for stingless beekeeping*. Accra, Ghana, Unimax Macmillan Ltd.

[34] Halcroft, M., Spooner-Hart, R. & Dollin, L.A. 2013. Australian stingless bees. In P. Vit, S.R.M. Pedro & D.W. Roubik, eds. *Pot honey: a legacy of stingless bees*, pp. 35–72. New York, Springer Science + Business Media.

[35] Alves, R.M.O., Carvalho, Souza, C.A.L., Souza, B.A. & Justina, G.D. 2005. *Sistema de produção para abelhas sem ferrão: uma proposta para o Estado da Bahia* [Production system for stingless bees: a proposal for the State of Bahia]. Serie Meliponicultura, 03. Salvador, Bahia: SEAGRI/DDP.

[36] Carvalho, F.C., Carvalho, S., Cortopassi Laurino, M. & Neto, T.A. 2012. *Iniciação à criação de uruçu de chão* [Introduction to the rearing of uruçu de chão]. Chapada do Araripe, Brazil, Refúgio das abelhas "Paulo Nogueira-Neto".

[37] Stearman, A.M., Stierlin, E., Sigman, M.E., Roubik, D.W. & Dorrien, D. 2008. Stradivarius in the jungle: traditional knowledge and use of "black beeswax" among the Yuquí of the Bolivian Amazon. *Human Ecology*, 36: 149–159.

[38] Carvalho, R.M.A. de, Martins, C.F. & da Silva Mourão, J. 2014. Meliponiculture in Quilombola communities of Ipiranga and Gurugi, Paraíba state, Brazil: an ethnoecological approach. *Journal of Ethnobiology and Ethnomedicine,* 10:3. doi:10.1186/1746-4269-10-3.

[39] Dos Santos, G.M. & Antonini, Y. 2008. The traditional knowledge on stingless bees (Apidae: Meliponina) used by the Enawene-Nawe tribe in western Brazil. *Journal of Ethnobiology and Ethnomedicine*, 4:19. doi:10.1186/1746-4269-4-19.

[40] Jara, F. 1995. Bees and wasps: ethno-entomological notions and myths among the Andoke of the Caquetá River in the Colombian Amazon. *Latin American Indian Literatures Journal,* 11: 148–165.

[41] Maginnity, S. 2013. Using stingless bees as an educational tool in Australian schools. In P. Vit & D.W. Roubik, eds. *Stingless bees process honey and pollen in cerumen pots*, pp. 1–4. Mérida, Venezuela, Facultad de Farmacia y Bioanálisis, Universidad de Los Andes (available at www.saber.ula.ve/handle/123456789/35292).

[42] Nates-Parra, G. 2001. *Abeja Angelita o Virginita,* Tetragonisca angustula *Illiger* [The *Angelita or Virginita bee*, Tetragonisca angustula Illiger]. Bogotá, DC, Convenio Andrés Bello.

[43] Roubik, D.W. 1995. Stingless bee colonies for pollination. In D.W. Roubik, ed. *Pollination of cultivated plants in the tropics*, pp. 150–154. Agricultural Services Bulletin 118, Rome, FAO.

[44] Heard, T. 1999. The role of stingless bees in crop pollination. *Annual Review of Entomology*, 44: 183–206.

[45] Amano, K., Nemoto, T. & Heard, T. 2000. What are stingless bees and why and how to use them as crop pollinators? A review. *Japanese Agricultural Research,* 34:183–190.

[46] Carvalho, C.A.L., Souza, B.A., Alves, R.M.O. & Justina, G.D. 2005. Custo de produção de mel: uma proposta para abelhas africanizadas e meliponíneos [Honey production cost: a proposal for Africanized and meliponine bees]. Serie Meliponicultura, 02. Salvador, Bahia, SEAGRI/DDP.

[47] Fonseca, A.A.O., Sodré, G.S., Carvalho, C.A.L., Alves, R.M., Souza, B.A., da Silva, S.M.P.C., de Olivera, G.A., Machado, C.S. & Clarton, L. 2006. *Qualidade do mel de abelhas sem ferrao: uma proposta para boas praticas de fabricação* [Honey quality without fermentation: a proposal for good manufacturing practices]. Serie Meliponicultura, 05. Salvador, Bahia, SEAGRI/DDP.

[48] Slaa, E.J., Sánchez-Chaves, L.A., Malagodi-Braga, K.S. & Hofstede, F.E. 2006. Stingless bees in applied pollination: practice and perspectives. *Apidologie*, 37: 293–315.

[49] Quezada-Euán, J. 2009. Potencial de las abejas nativas en la polinización de cultivos [Potential of native bees in crop pollination]. *Acta Biológica Colombiana,* 14: 169–172.

[50] Naju, M.A., Mituri, F.A. & Mpuya, P.M. 2010. Options for stingless honey-beekeeping around Udzungwa Mountains National Park, Tanzania, and importance for biodiversity management. *International Journal of Biodiversity Science, Ecosystem Service and Management*, 6: 89–95.

[51] Torres Ruiz, A., Jones, R.W. & Ayala-Barajas, R. 2013. Present and potential use of bees as managed pollinators in Mexico. *Southwestern Entomologist*, 38: 133–148.

[52] Brown, J.C. & Albrecht, C. 2001. The effect of tropical deforestation on stingless bees of the genus *Melipona* (Insecta: Hymenoptera: Apidae: Meliponini) in central Rondônia, Brazil. *Journal of Biogeography,* 28: 623–634.

[53] Camargo, J.M.F. 2013. Historical biogeography of the Meliponini (Hymenoptera, Apidae, Apinae) of the Neotropical region. In P. Vit, S.R.M. Pedro & D.W. Roubik, eds. *Pot honey: a legacy of stingless bees*, pp. 19–34. New York, Springer Science + Business Media.

[54] Roubik, D.W. 1983. Nest and colony characteristics of stingless bees from Panama. *Journal of the Kansas Entomological Society*, 56: 327–355.

[55] Gruber, M.H., Jarau, S., Morawetz, L., Reichle, C., Albert, W., Huber, W. & Weissonhofer, A. 2009. *Corbiculate bees of the Golfo Dulce Region, Costa Rica*. Vienne, Verein zur Förderung der Tropenstation La Gamba.

[56] Brosi, B.J., Daily, G.C., Shih, T.M., Oviedo, F. & Durán, G. 2008. The effects of forest fragmentation on bee communities in tropical countryside. *Journal of Applied Ecology*, 45: 773–783.

[57] Fierro, M.M., Cruz-López, L., Sánchez, D., Villanueva-Gutiérrez, R. & Vandame, R. 2012. Effect of biotic factors on the spatial distribution of stingless bees (Hymenoptera: Apidae, Meliponini) in fragmented neotropical habitats. *Neotropical Entomology*, 41: 95–104.

[58] Nates-Parra, G. & Rosso-Londoño, J.M. 2013. Diversidad de abejas sin aguijón (Hymenoptera: Meliponini) utilizadas en meliponicultura en Colombia [Diversity of stingless bees (Hymenoptera: Meliponini) used in meliponicultura in Colombia]. *Acta Biológica Colombiana*, 18: 415–426.

[59] Kevan, P.G., ed. 1995. *The Asiatic hive bee: apiculture, biology, and role in sustainable development in tropical and subtropical Asia*. Cambridge, Ontario, Canada, Enviroquest, Ltd.

[60] Roubik, D.W. 2000. Pollination system stability in tropical America. *Conservation Biology*, 14: 1235–1236. doi:10.1046/j.1523-1739.2000.00016.x.

[61] Oliveira, F.F. de, Trautman-Richers, B.T, Rodrigues da Silva, J., Cunha Farias, R. & Matos, T.A. de Lima. 2013. *Guia ilustrado das abelhas "sem ferrão" das Reservas Amanã e Mamirauá, Amazonas, Brasil (Hymenoptra, Apidae, Meliponini)* [Illustrated guide of "stingless" bees from the Amanã and Mamirauá Reserves, Amazonas, Brazil (Hymenoptra, Apidae, Meliponini)]. Tefé, AM, Instituto de Desenvolvimento Sustantável Mamirauá.

[62] Macharia J.K., Raina S.K & Muli, E.M. 2007. Stingless bees in Kenya. *Agricultural Innovations for Sustainable Development*, 1: 97–103.

[63] Njoroge, G.N. & Gikungu, M.W. 2007. Traditional knowledge of stingless bees in Mwingi District, Eastern Province, Kenya. *Discovery and Innovation* (RPSUD Special Edition), 19 (4).

[64] Ayala, R., Herrera, Gonzalez, V.H. & Engel, M.S. 2013. Mexican stingless bees (Hymenoptera: Apidae): diversity, distribution, and indigenous knowledge. In P. Vit, S.R.M. Pedro & D.W. Roubik, eds. *Pot honey: a legacy of stingless bees*, pp. 135–152. New York, Springer Science + Business Media.

[65] Roubik, D.W. 1989. *Ecology and natural history of tropical bees*. New York, Cambridge University Press.

[66] Martins. C., Courtopassi-Laurino, M., Koedam, D. & Imperatriz-Fonseca, V.L. 2004. Tree species used for nidification by stingless bees in the Brazilian caatinga (Seridó, PB; João Câmara, RN). *Biotica Neotropica* (available at www.biotaneotropica.org.br/v4n2/en).

[67] Slaa, E.J. 2006. Population dynamics of a stingless bee community in the seasonal dry lowlands of Costa Rica. *Insectes Sociaux*, 53: 70–79.

[68] Kajobe, R. 2013. Important bee plants for African and other stingless bees. In P. Vit., S.R.M. Pedro & D.W. Roubik, eds. *Pot honey: a legacy of stingless bees*, pp. 315–335. New York, Springer Science + Media.

[69] Roubik, D.W. & Moreno, J.E. 2009. *Trigona corvina*: an ecological study based on unusual nest structure and pollen analysis. *Psyche*. doi:10.1155/2009/268756.

[70] Roubik, D.W. & Moreno, J.E. 2013. How to be a bee-botanist using pollen spectra. In P. Vit, S.R.M. Pedro & D.W. Roubik, eds. *Pot honey: a legacy of stingless bees*, pp. 295–314. New York, Springer Science + Business Media.

[71] Wilms, W., Imperatriz-Fonseca, V. & Engels, W. 1996. Resource partitioning between highly eusocial bees and possible impact of the introduced Africanized honey bee on native stingless bees in the Brazilian Atlantic rainforest. *Studies of Neotropical Fauna & Environment,* 31: 137–151.

[72] Ramalho, M., Silva, M.D. & Carvalho, C.A.L. 2007. Dinâmica de uso de fonts de pólen por *Melipona scutellaris* Latrielle (Hymenoptera: Apidae): uma análise comparativa com *Apis mellifera* L. (Apidae), no domínio tropical atlântico [Dynamics of pollen source use by *Melipona scutellaris* Latrielle (Hymenoptera: Apidae): a comparative analysis with *Apis mellifera* L. (Apidae) in the Atlantic tropical domain]. *Neotropical Entomology,* 36: 38–45.

[73] Bänziger, H., Boongird, S., Sukumalanand, P. & Bänziger, S. 2009. Bees (Hymenoptera: Apidae) that drink human tears. *Journal of the Kansas Entomological Society*, 82: 135–150.

[74] Bänziger, H., Supalak, P & Kanok-orn, S. 2011. The remarkable nest entrance of tear-drinking *Pariotrigona klossi* and other stingless bees nesting in limestone cavities (Hymenoptera: Apidae). *Journal of the Kansas Entomological Society*, 84: 22–35.

[75] Kuhn-Neto, B., Contrera, F.A.L., Castro, M.S. & Nieh, J.L. 2009. Long distance foraging and recruitment by a stingless bee, *Melipona mandaçaia*. *Apidologie*, 40: 472–480.

[76] Kajobe, R. & Roubik, D.W. 2006. Honey-making bee colony abundance and predation by apes and humans in a Uganda forest reserve. *Biotropica*, 38: 1–9.

[77] Roubik, D.W. 2006. Stingless bee nesting biology. *Apidologie*, 37: 124–143.

[78] Portugal-Araújo, V. de. 1958. Notas sôbre colônias de meliponineos de Angola-Africa [Notes on colonies of meliponineos in Angola-Africa]. *Dusenia*, 6: 97–114.

[79] Eckles, M.A, Roubik, D.W. & Nieh, J.H. 2012. A stingless bee can use odometry to estimate both height and distance. *Journal of Experimental Biology*, 215: 3155–3160.

[80] Lichtenberg, E.M., Imperatriz-Fonseca, V.L. & Nieh, J.C. 2010. Behavioral suites mediate group-level foraging dynamics in communities of tropical stingless bees. *Insectes Sociaux*, 57: 105–113.

[81] Santos-Filho, P.S., Alves, D.A., Eterovic, A., Imperatriz-Fonseca, V.L. & Peixoto, A.M. 2006. Numerical investment in sex and caste by stingless bees (Apidae: Meliponini): a comparative analysis. *Apidologie,* 37: 207–221.

[82] Wenseleers, T., Alves, D. A., Francoy, T. M., Billen, J. & Imperatriz-Fonseca, V.L., 2011. Intraspecific queen parasitism in a highly eusocial bee. *Biology Letters*, 7: 173–176. doi:10.1098/rsbl.2010.0819.

[83] Wenseleers, T., Helantera, H., Alves, D.A., Dueñez-Guzmán & Pamilo, P. 2013. Towards greater realism in inclusive fitness models: the case of worker reproduction in insect societies. *Biology Letters,* 9: 20130334 (available at http://dx.doi.org/10.1098/rsbl.2013.0334).

[84] Van Oystaeyen, A., Alves, D.A., Oliveira, R.C., Nascimento, D.L., Nascimento, F.S., Billen, J. & Wenseleers, T. 2013. Sneaky queens in *Melipona* bees selectively detect and infiltrate queenless colonies. *Animal Behaviour*, 86: 603–609.

[85] Nunes-Silva, P., Alves, D.A., Hilário, S.D., Santos-Filho, P. de S. & Imperatriz-Fonseca, V.L. 2014. A scientific note on the founding and the early growth of new nests of the stingless bee *Plebeia remota*. *Apidologie*. doi:10.1007/s13592-014-0290-5.

[86] Biesmeijer, J.C. & Slaa, E.J. 2006. The structure of eusocial bee assemblages in Brazil. *Apidologie*, 37: 240–258.

[87] Roubik, D.W. 2012. *Ecology and social organization of bees.* Wiley Library of Science. eLS, DOI:10.1002/9780470015902.a0023596.

[88] Sakagami, S.F., Roubik, D.W. & Zucchi, R. 1993. Ethology of the robber bee, *Lestrimelitta limao*. *Sociobiology*, 21: 237–277.

[89] Gloag, R.S., Heard, T.A., Beekman, M.B. & Oldroyd, B.P. 2008. Nest defence in a stingless bee: what causes fighting swarms in *Trigona carbonaria* (Hymenoptera: Meliponini)? *Insectes Sociaux*, 55: 387–391.

[90] Cunningham, J.P., Hereward, J.P., Heard, T.A., De Barro, P.J. & West, S.A. 2014. Bee wars: interspecific battles and nest usurpation in stingless bees. *American Naturalist,* 184(6): 777–786.

[91] Halcroft, M., Spooner-Hart, R., Haigh, A.M., Heard, A. & Dollin, A., 2013. The Australian stingless bee industry: a follow-up survey, one decade on. *Journal of Apicultural Research*, 52: 1-7.

[92] Rasmussen, C. & Gonzalez, V.H. 2013. Prologue. Stingless bees now and in the future. In P. Vit & D.W. Roubik, eds. *Stingless bees produce honey and pollen in cerumen pots*, pp. vi–ix. Mérida, Venezuela, Facultad de Farmacia y Bioanálisis, Universidad de Los Andes (available at www.saber.ula.ve/handle/123456789/35292).

[93] Michener, C.D. 2013. The Meliponini. In P. Vit, S.R.M. Pedro & D.W. Roubik, eds. *Pot honey: a legacy of stingless bees*, pp. 261–268. New York, Springer Science + Business Media.

[94] Eardley, C. & Kwapong, P. 2013. Taxonomy as a tool for conservation of African stingless bees and their honey. In P. Vit, S.R.M. Pedro & D.W. Roubik, eds. *Pot honey: a legacy of stingless bees*, pp. 3–17. New York, Springer Science + Business Media.

[95] Roubik, D.W. 2013. Why they keep changing the names of our stingless bees. In P. Vit & D.W. Roubik, eds. *Stingless bees process honey and pollen in cerumen pots*, pp. 1–7. Mérida, Venezuela, Facultad de Farmacia y Bioanálisis, Universidad de Los Andes; www.saber.ula.ve/handle/123456789/35292.

[96] Nogueira-Neto, P. 2002. Inbreeding and building up small populations of stingless bees. *Revista Brasileira de Zoologia*, 19: 1181–1214.

[97] Alves, D.A., Imperatriz-Fonseca, V.L., Francoy, T.M., Santos-Filjo, P. S., Billen, J. & Wenseleers, T. 2010. Successful maintenance of a stingless bee population despite a severe genetic bottleneck. *Conservation Genetics*, DOI:10.1007/s10592-010-0171–z.

[98] Malagodi-Braga, K.S., Kleinert, A.M.P. & Imperatriz-Fonseca, V.L. 2000. Stingless bees: greenhouse pollination and meliponiculture. In *Proceedings, 4th Encontro Sobre Abelhas,* pp. 145–150. Ribeirão Preto, SP, FFCLRP-USP.

[99] Rasmussen, C., Orihuela-Pasquel, P. & Sánchez-Bocanegra, V. 2009. Abejas del género *Trigona* Jurine, 1807 (Hymenoptera: Apidae) como plaga del piñón blanco [Bees of the genus *Trigona* Jurine, 1807 (Hymenoptera: Apidae) as a pest of the white pinyon]. *Entomotropica*, 24: 31–34.

[100] Malagoldi-Braga, K.S. & Kleinert, A.M.P. 2004. Could *Tetragonisca angustula* Latreille (Apidae: Meliponini) be effective as strawberry pollinator in greenhouses? *Australian Journal of Agricultural Research,* 55: 771–773.

[101] Leonhardt S.D., Heard T.A., & Wallace, H. 2014. Differences in the resource intake of two sympatric Australian stingless bee species. *Apidologie*, 45: 514–527. DOI:10.1007/s13592-013-0266-x.

[102] Seeley, T.D. 2010. *Honeybee democracy*. Princeton, NJ, Princeton University Press.

[103] Kerr, W.E., Petrere, M. & Diniz Filho, J.A.F. 2001. Informações biológicas e estimativa do tamanho ideal da colmeia para a abelha tiúba do Maranhão (*Melipona compressipes fasciculata* Smith-Hymenoptera, Apidae) [Biological information and estimation of the ideal size of the beehive for the Maranhão bee (*Melipona compressipes fasciculata* Smith-Hymenoptera, Apidae)]. *Revista Brasileira de Zoologia*, 18: 45–52.

[104] Hood, W.M. 2004. The small hive beetle, *Aethina tumida*: a review. *Bee World*, 85: 51–59.

[105] Greco, M.K., Hoffmann, D., Dollin, A., Duncan, M., Spooner-Hart, R. & Neumann, P. 2010. The alternative Pharaoh approach: stingless bees mummify beetle parasites alive. *Naturwissenschaften,* 97: 319–323.

[106] Machado, R.J.P. & Imperatriz-Fonseca, V.L. 2013. Out with the garbage: the parasitic strategy of the mantisfly *Plega hagenella* mass-infesting colonies of the eusocial bee *Melipona subnitida* in northeastern Brazil. *Naturwissenschaften*, 100: 101–105.

[107] Dollin, A.E., Dollin, L.J. & Sakagami, S.F. 1997. Australian stingless bees of the genus *Trigona* (Hymenoptera: Apidae). *Invertebrate Taxonomy*, 11: 861–896.

[108] Nunes, T.M., Heard, T.A., Venturieri, G.C. & Oldroyd, B.P. 2014. Emergency queens in *Tetragonula carbonaria* (Smith, 1854) (Hymenoptera: Apidae: Meliponini). *Austral Entomology*, DOI:10.1111/aen.12104.

[109] Paxton, R.J., Bego, L., Shah, M.M. & Mateus, S. 2003. Low mating frequency of queens in the stingless bee *Scaptotrigona postica* and worker maternity of males. *Behavioral Ecology and Sociobiology*, 53: 174–181.

[110] Roubik, D.W. 2002. The value of bees to the coffee harvest. *Nature*, 417: 708.

[111] Roubik, D.W. 2002. Feral African bees augment Neotropical coffee yield. In P.G. Kevan & V.L. Imperatriz-Fonseca, eds. *Pollinating bees: the conservation link between agriculture and nature*, pp. 255–266. Brasilia, Ministério do Meio Ambiente.

[112] Klein, A.M., Steffan-Dewenter, I. & Tscharntke, T. 2003. Pollination of *Coffea canephora* in relation to local and regional agroforestry management. *Journal of Applied Ecology,* 40: 837–845.

[113] Maeta, Y., Tezuka, T., Nadano, H. & Suzuki, K. 1992. Utilization of the Brazilian stingless bee, *Nannotrigona testaceicornis* as a pollinator of strawberries. *Honeybee Science*, 13: 71–80.

[114] Kakutani, T., Inoue, T., Tezuka, T. & Maeta, Y. 1993. Pollination of strawberry by the stingless bee, *Trigona minangkabau*, and the honey bee, *Apis mellifera*: an experimental study of fertilization efficiency. *Researches in Population Ecology*, 35: 95–111.

[115] Amano, K. 1998. Attempts to introduce stingless bees for the pollination of crops under greenhouse conditions in Japan, pp. 1–9. Taipei, Food and Fertilizer Technology Center.

[116] Amano, K. & Boongird, S. 1997. Keeping of the stingless bee, *Trigona fuscobalteata* (Hymenoptera: Apidae) in an environmentally enclosed chamber. *Annual Report Prot. North Japan*, 48: 210–212.

[117] Del Sarto, M.C., Peruquetti, R.C. & Campos, L.A.O. 2005. Evaluation of the Neotropical stingless bee *Melipona quadrifasciata* (Hymenoptera: Apidae) as pollinator of greenhouse tomatoes. *Journal of Economic Entomology,* 98: 260–266. DOI:http://dx.doi.org/10.1603/0022-0493-98.2.260.

[118] Cruz, D., Freitas, B.M., Silva, L., Silva, E., & Bomfim, I. 2005. Pollination efficiency of the stingless bee *Melipona subnitida* on greenhouse sweet pepper. *Pesquisa Agropecuária Brasiliera*, 40: 1197–1201.

[119] Cauich, O., Quezada-Euán, J.J.Q., Melendez-Ramirez, V., Valdovinos-Nuñez, G.R. & Moo-Valle, H. 2006. Pollination of habanero pepper (*Capsicum chinense*) and production in enclosures using stingless bee *Nannotrigona perilampoides*. *Journal of Applied Ecology,* 43: 18–27.

[120] Palma, G., Quezada-Euán, J.J.G., Reyes-Oregel, V., Melendez, V. & Moo-Valle, H. 2008. Production of greenhouse tomatoes (*Lycopericon esculentum*) using *Nannotrigona perilampoides*, *Bombus impatiens* and mechanical vibration (Hymenoptera: Apoidea). *Journal of Applied Entomology,* 132: 79–85.

[121] Nunes-Silva, P., Hrncir, M., Inês Silva, C., Roldão, Y.S. & Imperatriz-Fonseca, V.L. 2013. Stingless bees, *Melipona fasciculata*, as efficient pollinators of eggplant (*Solanum melongena*) in greenhouses. *Apidologie,* 44: 537–546.

[122] Kiatoko, N., Raina, S. K., Muli, E. & Mueke, J. 2014. Enhancement of fruit quality in *Capsicum annuum* through pollination by *Hypotrigona gribodoi* in Kakamega, Western Kenya. *Entomological Science*, 17: 106–110.

[123] Can-Alonzo, Quezada-Euán, J.J.G., Xiu-Ancona, P., Moo-Valle, H., Valdovinos-Nuñez, R. & Medina-Peralta, S. 2005. Pollination of "criollo" avocados (*Persea americana*) and the behaviour of associated bees in subtropical Mexico. *Journal of Apicultural Research*, 44: 3–8.

[124] Reyes-Novelo, E., Ramírez, V.M., González, V.H. & Ayala, R. 2009. Abejas silvestres (Hymenoptera: Apoidea) como bioindicadores en el neotrópico [Wild bees (Hymenoptera: Apoidea) as bioindicators in the neotropics]. *Tropical and Subtropical Agroecosystems*, 10: 1–13.

[125] McKey, D., Elias, M., Pujol, B & Deputié, A. 2012. The evolutionary ecology of clonally propagated domesticated plants. *New Phytologist*, 186: 318–332.

[126] Heard, T.A. & Hendrikz, J.K. 1993. Factors influencing flight activity of colonies of the stingless bee, *Trigona carbonaria* (Hymenoptera: Apidae). *Australian Journal of Zoology,* 41: 343–353.

[127] Heard, T.A. 2016. *The Australian native bee book: keeping stingless bee hives for pets, pollination and sugarbag honey*. Brisbane, Sugarbag Bees.

[128] Hassan Jalil, A. & Roubik, D.W., eds. 2017. *Handbook of meliponiculture: Indo Malayan stingless bee clade*. Kuala Lumpur.

Chapter 14

APPLIED STINGLESS BEEKEEPING

14.1 STINGLESS BEES AND CROP POLLINATION IN THE PHILIPPINES

C.R. Cervancia and A.C. Fajardo, Jr.

14.1.1 Pollination potential

In the Philippines, stingless bees are successfully mass-produced in coconut shells [1] and wooden hives [1, 2]. They are used for large-scale mango pollination, and may be more effective pollinators than honey bees, syrphids or blowflies in mango orchards [2]. In mangrove ecosystems, honey bees and stingless bees co-exist, but the latter visit more plant species [3, 4]. As an alternative species for glasshouse pollination, stingless bees are effective pollinators of tomatoes [5, 6], sweet pepper [7] and eggplant [8], and also pollinate *Macadamia* flowers (see Chapter 14.2) [9]. One study undertaken in an open farm [10] presents a model using stingless bees to help beekeepers select the best hive location site. The model is based on the "fuzzy preference" of the beekeeper, potential numbers of available colonies, the carrying capacity of the plant assemblage and the spatial orientation of the meliponary. This section focuses on the conservation and utilization of stingless bees for pollination in the Philippines. About 42 meliponines occur in Asia and Indonesia west of the Wallace-Weber line, and 20 in Australia, New Guinea and the Solomon Islands [11, 12]. Eusocial bees in the Old World are generally less species-rich than in the New World Tropics. In the Philippines, most Meliponini belong to the taxonomically difficult group *Tetragonula* [13]. A further Palawan stingless bee is *Lepidotrigona palawanica* [14].

14.1.2 Nesting behaviour

Stingless bees establish colonies in hollow tree trunks and branches, and among the roots or older leaves of the epiphytic fern *Drynaria* sp., attached to a host tree. Nests are also found in unoccupied termite chambers, in spaces within the walls of buildings, and in natural crevices in rocks and many suitable sized cavities (Figure 14.1) Several stingless bee colonies are also found sharing nesting chambers with active ant and termite colonies, separated only by the batumen lining or sheet [15].

Figure 14.1
STINGLESS BEE COLONY NEST ENTRANCE IN HOLLOW BUOY (PHILIPPINES)

Stingless bees build nests in a wide array of plants (Table 14.1). They prefer natural hollows usually resulting from termite and borer (beetle or moth) infestation, fungus-induced rots and also abandoned woodpecker nests. Stingless bees line or separate their nest chamber with batumen or resin laminate with a smooth inner part and a coarse outer layer. In *Tetragonula biroi*, the batumen covering is almost non-existent, but the inner wall of the nesting chamber is pockmarked with an incomplete, smooth waxy layer [16] described as the batumen lining. It is followed by a latticework of cerumen pillars and sheets that support pollen and honey pots. A series of soft cerumen sheets two to three layers deep constitute the involucrum that surrounds the brood in the centre of the nest (Figure 14.2). *Tetragonula clypearis* and *T. sapiens* that occupy hollow logs have batumen walls below and above the nest chamber and lack a distinct involucrum. The brood is arranged in a cluster with pollen and honey pots below it. A similar trait occurs in *T. fuscobalteata* that occupy bamboo internodes (Figure 14.3).

Figure 14.2
OPEN HIVE AND EXPOSED NEST OF *T. BIROI*, SHOWING QUEEN ON NEW BROOD CELLS IN CENTRE, AND OPEN SHEATH OF INVOLUCRUM

Table 14.1
SOME PLANTS IN THE PHILIPPINES AND ASIA USED FOR NESTING BY *TETRAGONULA* SPP. (MELIPONINI)

DESCRIPTION	COMMON NAME	SCIENTIFIC NAME
Inside trunk, abandoned termite gallery	Mango	*Mangifera indica* L.
Inside trunk, abandoned termite gallery	Himbabao, paper mulberry	*Alleaenthus luzonicus* (Bl.) Vill.
Between entwined trunks	Coffee	*Coffea arabica* L.
Near the ground between exposed roots, inside larger branches	Madre de cacao	*Gliricidia sepium* (Jacq.) Kunth ex Walp
Hollow trunk, beetle infested	Coconut	*Cocos nucifera* L.
Internodes	Bamboo	*Bambusa* sp.
Rot-hollowed trunk and branches alongside active termite galleries	Lauan	*Parashorea* sp.
Rot or borer-hollowed trunk and branches alongside active termite galleries	Bintang/Midnight blue tree; Duhat/ Philippine plum; Lomboi/Brush Berries	*Syzygium* sp.
Rot-hollowed trunk and branches or near termite colonies, but in abandoned galleries	Round leaf acacia, rain tree	*Albizia saman* F. Muell. *(Samanea saman)*
Spaces between roots and old leaves	Staghorn fern	*Drynaria* sp.

Source: Cervancia and Fajardo [present study]

Figure 14.3
NESTING COLONY OF *TETRAGONULA FUSCOBALTEATA* IN BAMBOO

14.1.3 Nest entrance architecture

The species of stingless bees inhabiting the Philippines can be identified by nest design or architecture. Nest entrances are characterized by openings of various appearances. A typical nest entrance usually has a circular tube 0.5–1.0 cm protruding from the surface of the substrate, constructed from a mixture of resin, wax, plant materials or earth. Depending on the species, simple or ornate extensions of various shapes and sizes are added (Figure 14.4). While this may help in identifying colonies in the field, scholars caution its use for systematic studies.

Figure 14.4
STINGLESS BEE NEST ENTRANCE MODIFIED AROUND GRASS STEM

14.1.4 Mass-rearing, applied pollination and the coconut shell method

Natural nests may be transferred or manipulated on-site to facilitate honey and pollen harvest. The materials used may vary between localities or countries, but the basic techniques remain the same, providing room for colony expansion. Coconut shells are used to facilitate honey harvest (Figure 14.5). The usual way of harvesting honey from a wild nest of stingless bees disturbs the colony because it destroys a large portion of the nest, and requires time and energy for the bees to mend the damage. This problem is minimized with the coconut method, described below.

Halved coconut shells are cleaned thoroughly to prevent ant infestation. Brief flaming with a propane torch is used, but alternative sterilization with a chlorine solution is also recommended. A diameter hole of 1 cm is made at the top of the shell to serve as an entrance for the bees. Several smaller holes are drilled around the rim to accommodate supporting wires. Next, enlarge the nest entrance to expose brood and food stores, a place shell on the opening and secure with wires or twines, and seal other openings with cerumen and pieces of involucrum.

The shells are fastened with wires to tree trunks or branches. Leaves of the fern *Drynaria* sp. are used to cover gaps between the shell and tree. Stingless bee colonies then transfer easily to the improvised nest. The shells may be primed with melted resin to prevent the invasion of ants.

New shells should be added one at a time, since unoccupied shells may harbour ants, beetles and other pests. A good indication that bees use the added chamber is the resin lining added around edges of the shell. The bees keep their brood near the base of the nest and utilize the upper portion of the shells as food chambers. During intensive nectar and pollen foraging, the coconut shells may be removed and replaced with larger ones, and most leaves piled along the sides – where colonies expand their brood. As colonies expand, more shells are added on top of the existing shells, and are placed in front of several nest entrances (Figure 14.5).

Figure 14.5
NEST PORTIONS OF *TETRAGONULA BIROI* IN THREE COCONUT HALF SHELLS, UNITED TO FORM A HIVE, REMOVED HERE TO REVEAL BROOD AND FOOD STORES IN THE CENTRE (ORIGINAL) SHELL

Strong colonies may be divided when pollen and nectar sources are abundant. This can be accomplished by lifting the top coconut shell, removing a portion of the brood containing the queen cell from the colonies, and placing brood and accompanying bees on a platform 1 or 2 m from the parent colony. A new shell or a food chamber may be added. The new colonies are then allowed to rear their own queen. Two starter colonies can be made from a single ten-shell colony.

This method can also be used to harvest honey and pollen from colonies established in crevices. The topmost shell is lifted at harvest time, leaving the brood intact. The technique allows for a sufficient honey and pollen harvest with minimal disturbance to the colony. This helps to conserve these bees that are vital for the pollination of wild plants or crops. Colonies can be reared without transferring or relocating existing nests from the wild.

Use of wooden pollination hives. The wooden hive was designed to facilitate colony transport either by land, sea or air for mango pollination [10]. The hive consists of two chambers, separated by a division board. The lower chamber has a single entrance and an elevated platform at the bottom made from a piece of 6.5 cm x 6.5 cm ⅛ inch plywood glued to four 1.0 cm x 1.0 cm x 1.0 cm studs, and provided with ventilation holes. Four 1.0 cm x 1.0 cm x 3.0 cm studs are placed on each corner as stoppers to hold the upper chamber during transport. The upper chamber has two entrances, one in front and one in back. The division board is provided with access points. The hive has a telescoping top cover and a removable bottom board (Figure 14.6).

Transporting colonies for pollination. Only established colonies are used in pollination. These have around five to eight layers of brood in the lower chamber, and pollen and honey stores in the upper chamber. This is to ensure continuous food supply during transport. A competent authority should certify the colonies to ensure they are free from pests and diseases. The latter include possible plant diseases transported by the bees, as stingless bees have no known diseases (see Chapter 13). Colonies may be bundled on pallets in sets of three or four, depending on the requirement of the carrier. These are secured with plastic straps, and the hive sections stapled to each other. Entrances are sealed prior to loading. The hives are then covered with fine mesh netting to prevent the escape of bees.

Colony inspection. Entrance holes of good colonies should be about 1 to 2 cm wide. The entrance tube should be moist and pliable, and protrude about 5 cm from the entrance hole. Very dry and hard or reduced entrances indicate a weak colony, not recommended for use.

Figure 14.6
HIVED COLONIES OF *TETRAGONULA BIROI* BEING TRANSPORTED FOR MANGO POLLINATION, AND HIVE ON STAND WITH PROTECTIVE ROPE ATTACHED FOR SECURITY IN MANGO ORCHARD

Source: Photos by A.C. Fajardo

To inspect the colony, open the hive and a portion of the surrounding cerumen. Check the brood size and number of brood combs. Brood combs should be about 10 cm tall and have at least four or five layers. Brood should be fresh, moist and free from debris. All the brood stages should be present in similar proportion. The colonies should have a ring of pollen and honey pots around the brood area, about 5 cm in all directions.

Weak colonies are prone to infestation by *histerid beetles* and *carpoglyphid pollen mites*. Adult beetles and larvae crawl on the top side of the division board, between cerumen sheets and the brood area, and feed on the pollen and honey. The presence of beetles and mites is indicated by tiny holes on the cerumen and powdery debris on the pollen and honey pots. During the rainy season the larvae of a soldier fly (Stratiomyidae: *Hermetia illucens*) infest the nest, burrowing through wet batumen and laying eggs that turn into large larvae that feed on wet pollen and honey. Heavy pest infestation results in colony loss or nest abandonment.

Utilization of stingless bees for mango pollination. The introduction of managed stingless bee colonies increases the total number of fruit that will form by as much as 49 percent [1]. An added bonus is the honey that can be harvested from pollinating colonies. Optimal pollination using stingless bees may be attained in a mango orchard when there is 1:2 ratio of colonies to trees. Colonies used for pollination are prepared at least one week prior to the expected blooming period. Only strong colonies are used, placed on stations 24 to 25 days after flower induction, when 25 percent of mango flowers are open. Colonies introduced in this way have a better chance of visiting mango flowers. The relatively large number of flowers prevents them from seeking other forage.

14.2 BEESCAPE FOR A MALAYSIAN MELIPONARY (STINGLESS BEE FARM)

A. Hassan Jalil

14.2.1 Meliponines and the meliponary

Stingless bees of the Indo-Malayan clade are among the smallest of all bees, some measuring little more than 2 mm long; however, a few (in the *Geniotrigona*) are quite large and measure nearly 9 mm in length. Their sting is vestigial and useless as a weapon of defense. Instead, they swarm out of their nest if disturbed and buzz about in front of the intruder's face, settling in the hair, eyebrows and forehead, crawling into eyes, mouth and nose, and on some occasions biting freely. They nest in hollow tree trunks and in holes in walls or rocks. The ground-nesting species also use readymade cavities. Some species use the nests of ants or termites as a nesting site (see section 13.1).

Stingless bees need resin and other plant substances to help build and defend their nests. The nesting material is called cerumen, and is usually dark in colour. It is a mixture of light wax and large amounts of resin gathered by the bees. Stingless bees produce the wax between the dorsal segments of the abdomen, unlike the honey bee which produces it from the underside. The cerumen is used to block up cracks and crevices, and also in the construction of the spout or funnel that often marks the entrance to the nest. The sticky funnel at the entrance also functions to prevents ants and other unwanted visitors from entering. Sentries remain on guard at the entrance during the day, but at night some species of stingless bees close up the funnel with a temporary plug of cerumen and resin.

The interior of the stingless bee nest consists of a brood section and a section for the storage of food. In some nests, the brood portion consists of irregular clusters of oval-shaped cells and cocoons, supported by slender pillars of cerumen. The cells, vertical and open at the top, are first filled with food and then closed after an egg has been laid erect on top of the food supply in each one. As such, these little bees, although social, rear their young in very much the same way as solitary bees. The large storage pots, which contain

pollen and honey, are up to 7 mm in diameter, and made from soft cerumen, yellowish to brownish in colour. Both the honey and the brood of stingless bees are relished all over the world. The honey is usually somewhat sour and thin compared to that of honey bees, but can be just as tasty, depending on the species of stingless bee and the source of the nectar. On rare occasions they may make honey from Euphorbia flowers, and since the plant is known to be poisonous, the honey can also be regarded as toxic for consumption.

Personal taste dictates expectations for stingless bee honey. The sweetest is far more delicate and appetizing than any honey from Apis, while the sourest is almost rancid. Both are perceived as delicacies. In Panama for example, the indigenous people love to eat the brood and spit out the cerumen. However, the honey still seems to be the most prized part of the nest for indigenous human consumption. [17].

The inner walls of the nest, which are lined with a mixture of cerumen and mud (referred to as the batumen), are connected to the outside by an entrance tube. A second passage similar to a pipe, which simply ends in the ground, is occasionally found at the bottom of the nest. This may act as a drain for any water that reaches the nest. Within the nest cavity the brood area is enclosed in laminated sheets of cerumen, separate from the storage pots. The brood cells in some nests are arranged in layers, forming a spiral, however most species build cells that are arranged in a cluster or sheets, not a spiral.

The bees store sticky cerumen in the nest chamber near the entrance tunnel. This is applied by defending bees to any insect that attempts to invade the nest, and puts them out of action. When disturbed, the bees attack in large numbers (congregating as a result of alarm pheromones), carrying lumps of very sticky resin (as a defense mechanism), which they glue onto their foe, while simultaneously biting.

14.2.2 Meliponary landscape management

A meliponary should be carefully planned to ensure that bees are able to make optimum use of the surrounding area, not have to fly too far for their food and nesting material, and not compete too intensely with one another. Planning for maximum capacity of colonies or hives can be based on a minimum distance of 2 m between each one. A single colony therefore has 4 m^2 to exist comfortably and peacefully. Working on a 1:4 ratio, the allocated planting area should be 16 m^2 for one hive. For one acre (4 000 m^2), a maximum of 200 hives can be housed in 3 200 m^2 of crops or flowerbeds. If the desired planting area is not achievable then the beekeeper should resort to locations near forest fringes or forest parks, shaded from direct sunlight. Otherwise, battling among colonies generally results when forage sources are scarce, or colonies overheat or spend too much time gathering water for cooling or honey dilution.

In anticipation of the multiplication of hives or division of colonies, every acre will house an initial 100 hives that will double, if all goes well, within one year. If palms such as coconuts or betel nuts and salaccas are present, an arrangement of hives on stands can be increased to two or three cascading levels. It is imperative to also identify the bee species being kept, and whether they are dependent on dipterocarp resin and other resources (see below), or otherwise.

When stocking hives for commercial pollination services, sheds can be used with "A" frames to place the box hives on stepped shelves. This can be tricky because the necessary planting must have a high density, because many colonies are needed to perform their function over a small area. An assortment of at least 50 different species of flowers including ground cover, shrubs and resinous tree saplings (plants that exude resin in bark cracks as they grow or trees that produce milky sap when the leaves or shoots are pricked) will be required. Integration with other farm stock may be considered for the tear-drinking habits of some stingless bees [18]. The bees also forage sweat for their salt and mineral needs. Furthermore, these stingless bees visit dried salted fish in villages. It would be prudent to have such "salt and mineral" supplements incorporated for appropriate landscape planning, to prepare for the future expansion of commercial meliponaries.

Landscape management for the purposes of establishing a meliponary includes strategic planting (Figure 14.7).

Figure 14.7
BEESCAPE IMPRESSIONS (MALAYSIA)

Source: A. Hassan Jalil [present study]

Strategic planting is a means by which humans tell bees what and where to eat in comfort and safety, and when (by way of arranging plants by criteria of anthesis – flower opening and presentation of pollen and nectar) [19]. There are several available means to achieve this goal:

- Flower boosters, basal fertilizers and soil amendments at appropriate seasons, times and schedules should be used to ensure optimum benefits for the bees. Beekeepers need to know some basic horticulture to employ these methods.
- Hives should be positioned, oriented and protected to avoid predators and ensure survival in the meliponary. A study (pers. obs.) of predators shows that the main culprits in a Malaysian meliponary are frogs and lizards.
- Water bodies should be prepared for the necessary humidity and moisture requirements, especially during droughts and dry periods. Meliponines regularly visit water bodies, especially on hot dry days.
- Hives and foraging areas should be sheltered from extreme weather and seasonal change, and hives should have appropriate roofing or shade to protect from sun exposure (in nature the nests are protected by forest canopies).
- Species peculiarities such as resin dependencies on dipterocarp saplings and trees should be recognized. Specific flowers should be provided for species with selective habits and resin or sap for those that have an affinity for such plants.
- Plots of forest simulacra should be provided to create an almost natural environment (Figure 14.8).

Interactions and possibly signaling and triggers exist in plants by way of damage-associated molecular pattern molecules (DAMPs) (Figure 14.9).

Oligogalacturonides are plant damage-associated molecules, and regulators of growth and development. According to Ferrari *et al.* [20], oligogalacturonides are well-characterized elicitors of plant defense and are capable of protecting plants against diseases. Their involvement in the local wound response is another interesting feature of oligogalacturonides. Possibly, these elicitors have a general function of "priming" plant defenses upon cell wall damage in the early stages of a microbial invasion or during insect attack [21]. DAMPs are molecules that can initiate and perpetuate immune responses in the non-infectious inflammatory response [22]. This inflammatory response in plants also oozes phloem taken up by bees for nest construction or used to adsorb alarm molecules into a medium for discharge of the alarm pheromones when required (Figure 14.10b).

Figure 14.8
EXAMPLE OF MELIPONARY FOREST SIMULACRA

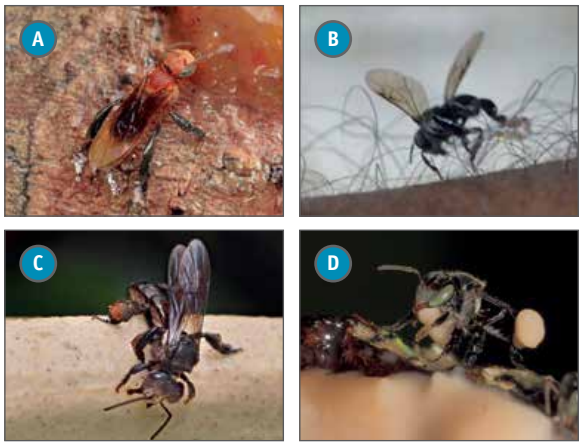
(a) *Acacia mangium* plot, (b) *Melaleuca cajuputi* plot, (c) *Syzygium* sp. plot, and (d) Bamboo grove. See Jalil, A.H. & Shuib, I. 2014.

Source: A. Hassan Jalil [present study]

Figure 14.9
MANGIFERA FOETIDA LEAF DAMAGE CAUSED BY MELIPONINES NIBBLING AT APICAL SHOOTS

Source: A. Hassan Jalil [present study]

Figure 14.10
(A) *TETRAGONILLA ATRIPES* COLLECTING RESIN, (B) DROPLET OF STICKY RESIN AS A FIRST LINE OF DEFENCE, (C) STINGLESS BEE IMBIBING WATER, AND (D) STINGLESS BEE COLLECTING MANGO TREE SAP



Source: A. Hassan Jalil [present study]

Most of the plants containing oligogalacturonides have red-to-maroon-coloured young shoots with which the bees seem to have an affinity (Figure 14.11a). As the apical buds are damaged (Figure 14.9), the plant growth hormones start to concentrate at the petiole/node intersections and push out more lateral buds and shoots. The deciduous plant affected by this actually becomes bushier, with more apical shoots sprouting out with flowering buds and subsequently more nutrition available for the pollinators. The increase in abundance of flowers improves the chance of fruit set and may result in higher crop yields.

Figure 14.11
(A) *HETERITRIGONA ITAMA* NIBBLING *MANGIFERA FOETIDA* SHOOTS, (B) STINGLESS BEE FORAGING SAP OR RESIN THROUGH BARK OPENING, (C) STINGLESS BEES FORAGING RIND SAP IN BRAZIL, AND (D) *LEPIDOTRIGONA TERMINATA* FORAGING ON *EUPATORIUM MIKANOI* FLOWER HEAD

Source: A. Hassan Jalil [present study]

14.2.3 Flowers and resin

It has been estimated that a honey bee needs to make 1 million visits to flowers within their reach to produce 100 g of honey. Meliponines may differ, yet every single forage flight covers at least half a dozen flowers before getting their fill and returning home to their colony. The farther the forage destination, the more flight energy is consumed. This spent energy will subsequently be replenished by consuming the stored honey. To conserve this energy, the beekeeper needs to provide a forage source as close to the hives as possible. The appropriate type of flowers and plants chosen for the meliponary and its surroundings can thus conserve energy. A plant with an abundant flowering habit reduces efforts by the bees to accumulate their fill on every flight. Palms are among the best options, as they produce many flowers in a small area. The bee therefore only requires crawling energy to move from one flower to another. Observations of coconut palm flowers show that all sizes and species of stingless bees crowd the spadixes from dawn to dusk, unselfishly and peacefully. In flowering shrubs, the bee still requires flight energy to "hop" from flower to flower to gain food. Large inflorescences (e.g. the bottle brush and melaleucas) or flower heads (angelonias and ocimums) with many small flowers, such as palms, are also good choices for bee forage. The foraging bee need visit only one or two flower heads or inflorescences to make its quota on a forage flight.

The spadix of a palm inflorescence provides among the most abundant flowers of any plant. The *Corypha* species have the largest inflorescence, reaching up to 7.5 m tall, and containing millions of small flowers. Palms are the best option for a meliponary landscape effort. They not only provide food for the bees [23], but also provide fruit for the planters. With the exception of oil palms that have allergenic pollen [24, 25], many palms can be beneficial for stingless bees and humans alike (Figure 14.12 and 14.13).

Figure 14.12
LOG HIVE SHADED BY PALM LEAVES

Source: A. Hassan Jalil [present study]

Figure 14.13
BUILDING STEPS FOR STATION STRUCTURES IN A MELIPONARY IN NORTHERN PENINSULAR MALAYSIA

Source: A. Hassan Jalil [present study]

Selected palm genera. Palms contain many genera and species. Not all flowers are visited by bees and many produce little food or present only female (and therefore pollenless) flowers at one time. The following list highlights those palms that appear to be preferred by stingless and other bees.

- ***Archontophoenix*** – Bangalow palm (native to New South Wales and Queensland, E. Australia)
- ***Areca*** – Betelnut palm (Indonesian origin)
- ***Bactris*** – Pupunha (genus of spiny palms native to Mexico, South and Central America, and the Caribbean)
- ***Beccariophoenix*** – B. alfredii (High Plateau Coconut Palm, endemic to Madagascar)
- ***Bismarckia*** – Bismarck palm (*B. nobilis* is endemic to NW. Madagascar)
- ***Borassus*** – Palmyra palm, sugar palm, toddy palm (predominantly Southeast Asian)
- ***Calamus*** – Rattan palm (Paleotropical distribution)
- ***Cocos*** – Coconut (Indo-Pacific origin)
- ***Copernicia*** – Carnauba wax palm (native to South America and the Caribbean)
- ***Corypha*** – Gebang palm, Buri palm or Talipot palm (native to India, Malaysia, Indonesia, the Philippines, New Guinea and northeastern Australia)
- ***Elaeis*** – Oil palm (native to west and southwest Africa)
- ***Euterpe*** – Cabbage heart palm, açaí palm (*E. oleracea*, native to Central and South America, mainly in swamps and floodplains)
- ***Hyphaene*** – Doum palm (native to Tropical Africa)
- ***Jubaea*** – Chilean wine palm, Coquito palm (endemic to a small area of central Chile)
- ***Latania*** – Latan palm (native to islands in the western Indian Ocean)
- ***Livistona*** – Cabbage palm (native to southern and southeastern Asia, Australasia and the Horn of Africa)
- ***Mauritia*** – Moriche palm (native to northern South America and Trinidad)
- ***Metroxylon*** – Sago palm (native to Western Samoa, New Guinea, the Solomon Islands, the Moluccas, the Carolines and Fiji in a variety of habitats, and cultivated westward to Malayaand Thailand)
- ***Nypa*** – Nipa palm (native to the coastlines and estuarine habitats of the Indian and Pacific Oceans)
- ***Parajubaea*** – Bolivian coconut palms (native to the northern Andes)
- ***Phoenix*** – Date palm (native to the Canary Islands east across northern and central Africa, the extreme southeast of Europe (Crete), and South Asia from Turkey to Malaysia.)
- ***Phoenix sylvestris*** – Wild date palm (native to South Pakistan, most of India, Sri Lanka, Nepal, Bhutan, Myanmar and Bangladesh, naturalized in Mauritius, the Chagos Archipelago, Puerto Rico and the Leeward Islands)
- ***Raphia*** – Raffia palm (native to tropical Africa, and especially Madagascar)
- ***Roystonea*** – Royal palm (native to Caribbean Islands, Florida in the United States, and Central and South America)
- ***Sabal*** – Palmettos (native to American tropics and subtropics)
- ***Salacca*** – Salak (native to Indonesia and Malaysia)
- ***Syagrus*** – Queen palm (native to South America)
- ***Trachycarpus*** – Windmill palm, Kumaon palm (native to Asia, from the east Himalayas to eastern China)
- ***Veitchia*** (syn. *Adonidia*) – Manila palm, Joannis palm Christmas palm, Kerpis Palm (native to Philippines and the Pacific islands (Fiji, Vanuatu, Tonga and the Solomon Islands)
- ***Washingtonia*** – Fan palm (native to the southwestern United States and northwest Mexico)

The genera that are specifically dependent on Dipterocarpaceae are *Homotrigona, Tetrigona, Tetragonilla, Lophotrigona* and *Odontotrigona*. To identify this group, it is necessary examine the nest entrances. They have a hard and brittle resinous composition, and the bees are generally of medium to large body size. It is necessary to ensure that dipterocarp trees are available well within their flight range (preferably not more than 800 m). The flowering is very irregular, with some tree species flowering only once every two years or even ten years. However, the main forage source for stingless bees is not necessarily the flowers, but more importantly the resin (Figures 14.10a, 14.11b and 14.14).

14.2.4 Dipterocarpaceae and bee resources

Dipterocarp trees often emerge from the forest canopy (the top layer of a rainforest) and can reach up to 60 m when mature. These trees thrive on well-drained lands and can usually be found up to an altitude of around 1 000 m. Their fruits consist of a hard and oily seed with one or two "wings", which lends this family its name (from the Greek di = two; ptero = wing; carpos = seed). A phenomenon known as General Flowering sometimes occurs, usually at the same time as for other dipterocarp trees in the vicinity [26]. The underlying cause is the El Niño Southern Oscillation (ENSO) – a natural climatic event that begins in the western Pacific Ocean.

The dipterocarp species readily available in plant nurseries and landscaping outlets in Thailand, Malaysian Peninsula, northern Sumatra and Borneo are listed in Table 14.2 by their wood name and commercial name.

Figure 14.14
EFFECTS OF DIPTEROCARP RESIN SHORTAGE

(a) natural entrance tube of *Tetrigona melanoleuca* on limestone rock face in full sun, (b) thinning of tube wall, (c) distorted apex of tube (likely due to incompatible resin), and (d) melting nest entrance (likely due to poor substitute type of resin used)

Source: A. Hassan Jalil [present study]

Table 14.2
DIPTEROCARPACEAE GENUS, SPECIES AND CORRESPONDING COMMERCIAL WOOD NAMES

GENUS AND SECTION	SPECIES	WOOD NAME
Anisoptera	*A. cochinchinensis, A. marginata, A. scaphula, A. thurifera* and about 10 other species	Mersawa
Cotylelobium	*C. burckii, C. lanceolatum,*	Resak
Cotylelobium	*C. leucocarpum Syn. C. melanoxylon, Sunaptea sp., Vatica sp.*	Resak Batu
Dipterocarpus	*D. alatus, D. baudii, D. basilanicus, D. borneensis, D. caudiferus, D. grandiflorus, D. kerrii, D. tonkinensis, D. verrucosus* and about 60 other species	Keruing
Dipterocarpus	*D. costulatus*	Keruing Kepas
Dipterocarpus	*D. palembanicus*	Keruing Ternek
Dipterocarpus	*D. retusus*	Hollong (Eng)
Dipterocarpus	*D. turbinatus*	Gurjan (Eng)
Dipterocarpus	*D. warburgii*	Keruing Borneo
Dipterocarpus	*D. zeylanicus*	Hora (Eng)
Dryobalanops	*D. aromatica, D. beccarii, D. fusca, D. keithii, D. lanceolata, D. oblongifolia, D. rappa*	Kapur, Kapor, Camphor (Eng)

→

GENUS AND SECTION	SPECIES	WOOD NAME
Hopea	*H. acuminata, H. beccariana, H. dryobalanoides, H. mengarawan, H. nervosa, H. odorata, H. sangal* and other species	Merawan
Hopea	*H. ferrea, H. forbesii, H. helferi, H. nutans, H. semicuneata* and other species	Giam
Hopea	*H. tinctoria*	Sweetleaf (Eng)
Neobalanocarpus	*N. heimii*	Cengal
Parashorea	*P. aptera, P. buchananii, P. chinensis, P. parvifolia, P. smythiesii, P. tomentella*	Gerutu
Parashorea	*P. densiflora, P. globosa, P. stellata*	White Seraya
Parashorea	*Parashorea plicata*	Bagtikan
Parashorea	*P. lucida*	White Meranti
Parashorea	*P. macrophylla, P. malaanonan*	White Lauan
Shorea (Pentacme)	*S. contorta, S. minandensis*	White Lauan
Shorea sect. Shorea	*S. atrinervosa, S. exelliptica, S. falciferoides*	Selangan Batu
Shorea sect. Shorea	*S. brunnescens, S. crassa, S. foxworthyi, S. glauca, S. laevis, S. havilandii, S. leptoderma, S. materialis, S. maxwelliana, S. seminis, S. submontana, S. sumatrana, S. superba*	Balau
Shorea	*S. beccariana, S. fallax, S. ferruginea, S. johorensis, S. macroptera,S. parviflor, S. smithiana, S. waltonii*	Red Seraya
Shorea	*S. mecistopterix*	Kawang
Shorea	*S. multiflora*	Banjutan
Shorea	*S. pauciflora*	Oba suluk
Shorea sect. Almon	*S. almon, S. contorta, S. leprosula, S. leptoclados, S. smithiana*	Almon
Shorea sect. Anthoshorea	*S. assamica, S. bracteolata, S. dealbata, S. hypochra*	White Meranti
	S. javanica, S. lamellata, S. maranti	
Shorea sect. Richetia	*S. acuminatissima, S. faguetiana, S. gibbosa, S. hopeifolia*	Yellow Meranti Yellow Seraya
	S. multiflora	
Shorea sect. Rubroshorea	*S. curtisii, S. hemsleyana, S. macrantha, S. pauciflora*	Dark red Meranti (Meranti bukit)
	S. platyclados, S. rugosa, S. singkawang and four other spp.	
	S. acuminata, S. dasyphylla, S. johorensis, S. lepidota	Light red Meranti
	S. parvifolia	
	S. balangeran, S. collina, S. guiso, S. kunstleri	Red Balau
	S. ochrophloia, S. plagata	
Shorea	*S. macroptera*	Melantai
Shorea	*S. negrosensis*	Red Lauan
Shorea	*S. ovata*	Tianong
Shorea	*S. polysperma*	Tanguile
Shorea	*S. robusta*	Sal
Shorea	*S. palosapis*	Mayapis
Shorea	*S. uliginosa*	Meranti Bakau

See Jalil, A.H. & Shuib, I. 2014.

The enthusiasm to provide flowering plants should also take into account that some plants may not be desirable because of their toxicity. The poisonous *Nerium oleander* and *Spathodea campunulata* or African Tulip tree, along with those that contain grayanotoxins, are among such plants. Grayanotoxins are a group of toxins found in rhododendrons and other plants of the family Ericaceae.

14.3 CROP POLLINATION USING STINGLESS BEES IN AUSTRALIA

T.A. Heard

Global food production is currently facing risks of low abundance and diversity of crop pollinators and over-reliance on a single species, the Western honey bee *Apis mellifera*. The need to develop alternative pollinators is compelling when the significant threats facing those Western honey bees are taken into consideration [27]. Some bee species are amenable to development as alternative pollinators. In the case of northern Australia, as with many warmer parts of the world, stingless bees (Meliponini) constitute a group of potentially viable alternative pollinators, especially for horticultural crops [28].

Over the last three decades, progress in the research and practise of bee husbandry and management has reached a critical point. The employment of bees in farm management on a large scale is now a real possibility. This section discusses current practise and the steps that have led to this point, and looks at which bee species are most used and the reasons why. It also reviews the management of both natural populations and hived colonies of stingless bees for pollination. It outlines the growth in supply and demand of hives of stingless bees, and research undertaken towards this goal. This is followed by a list of crop species that appear to be suitable candidates for managed pollination by stingless bees. The review closes with some recommendations for future research.

14.3.1 Selection of bee species

To a large extent, the species used for pollination were not deliberately selected but were instead self-selecting. Most Australian species were moved into hives, and those that performed best became the most common. Their strengths and weaknesses for pollination were then tested.

The most commonly kept stingless bee species in Australia are *Tetragonula carbonaria*, *Tetragonula hockingsi* and *Austroplebeia australis*. Of these, *T. carbonaria* (61.5 percent of colonies) is the most popular, with *A. australis* (22.9 percent) the second most popular, followed by *T. hockingsi* (8.8 percent) [29]. The three species are also possibly the most common in nature (T.A. Heard, pers. obs.).

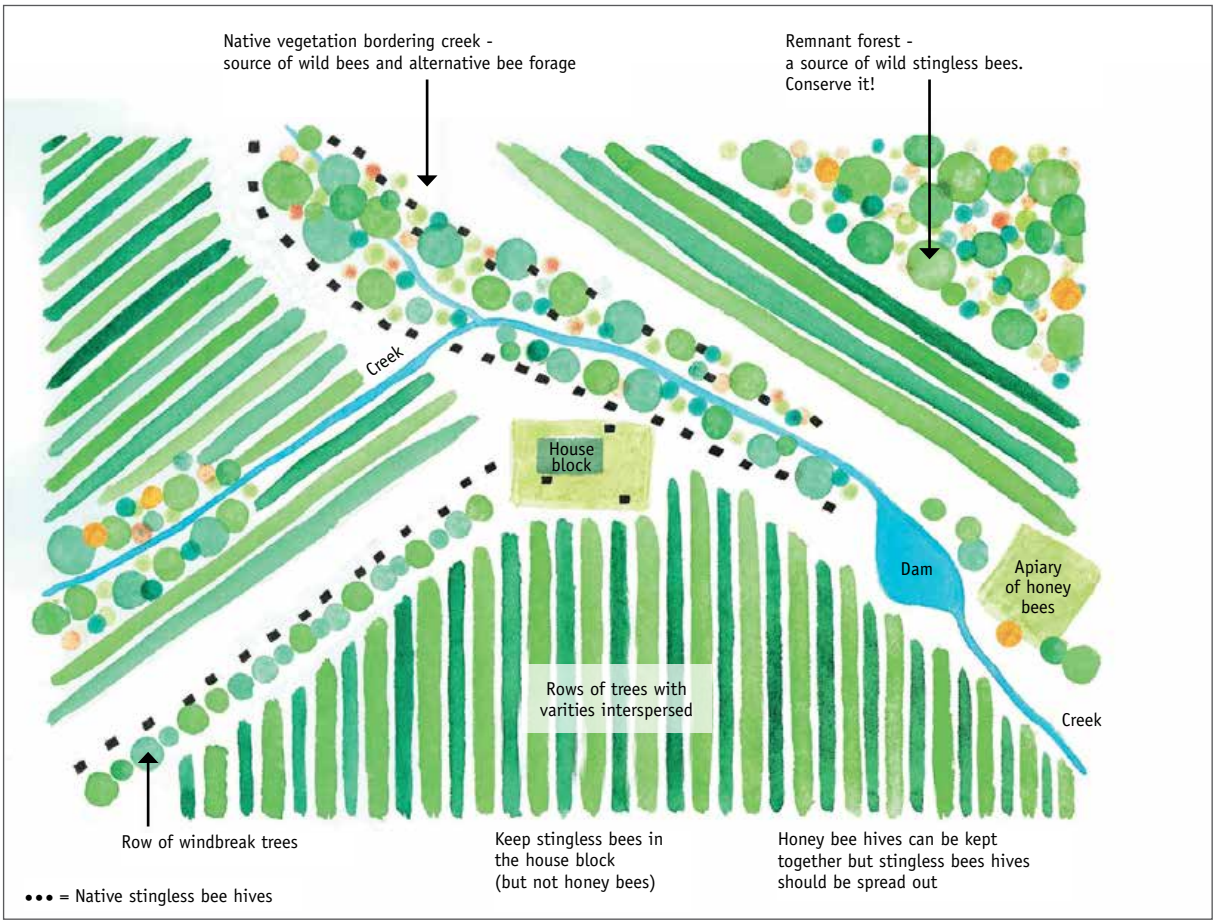
The two *Tetragonula* species naturally occur on the tropical and subtropical east coast of Australia [30] and co-exist with major areas of horticultural production (T.A. Heard, pers. obs.). *Tetragonula carbonaria* has a predominantly subtropical distribution and *T. hockingsi* is more tropical [30]. The two species are excellent candidates for stingless beekeeping and use for pollination. They form large colonies of approximately 10 000 adult bees that are active throughout the year in their natural geographic range, although at the southern end of the range, their activity in cooler weather is restricted. They thrive in disturbed habitats, and survive translocation from natural sites into hives. They are also forgiving of hive shape and construction material. In addition, their colony mortality rates are low and they are easy to propagate by a number of methods including colony division or "eduction", also called "soft splitting" (see Chapter 13.4.4). Both species produce abundant virgin queens throughout the year, such that colonies normally replace the queen naturally and independently following colony division or splitting. They are not defensive toward humans, although they bite moderately when hives are opened. The bees are also able to defend themselves effectively against natural enemies. Perhaps most importantly, *T. hockingsi* and *T. carbonaria* recruit nest mates to rich floral resources. A weakness of the two species is that they can expend significant energy in fighting activity associated with attempts to usurp the nests of other colonies.

Austroplebeia australis is the second most commonly kept species in Australia [29]. It has moderate potential as a crop pollinator and occurs naturally in the less humid inland parts of Australia, where it thrives in artificial hives. The bee could be developed and used for crops such as sunflower and canola, both cultivated in the area. It also has strengths as a glasshouse pollinator, but is limited by its smaller colony size. The slower colony reproduction rates, higher temperature threshold for foraging, unsuitability for humid areas where the higher value crops are grown, narrower diet range, and weaker foraging activity also limit this bee's potential application [30].

14.3.2 Management of wild stingless bee populations

In addition to direct management of stingless bees on farms, it is possible to manage wild populations. In such cases, it is important to protect the vegetation that these colonies depend upon for forage and nesting needs. Remnant vegetation within or near farms should not be cleared without consideration of the consequences for pollinators (Figure 14.15). On one coffee farm in Costa Rica, for example, the small amount of remnant vegetation generated more income each year, by providing pollinators for coffee that increase yields, than any competing land use at that time [31].

Figure 14.15
HIVES OF STINGLESS BEES (SHOWN ON THIS MAP BY THE BLACK RECTANGLES) SHOULD BE SPREAD OUT AROUND THE FARM AS SHOWN HERE, IF THEY ARE KNOWN TO FIGHT



Source: T. A. Heard [present study]

Fire can be a real hazard for bee colonies in natural areas. Unlike honey bees, stingless bees cannot respond to heat and smoke by rapidly abandoning or leaving the threatened location, because the mated queen cannot fly. The natural hollows in logs vary in their capacity to protect the nest from a fire. It is probable that a significant proportion of hived colonies are lost due to fire. Protection of natural locations from fire will also protect the wild colonies there.

14.3.3 Development of meliponiculture

Colonies of stingless bees nest naturally in hollow trees, but can be transferred to wooden hive boxes for easier management [32]. There has been a wave of interest in stingless beekeeping in Australia over the last two decades [29, 34, 35]. This interest has resulted in a rapid increase in the supply of artificial hives containing stingless bees. Demand for the hives has also strengthened, driven by interest from various sectors including city dwellers, who enjoy stingless bees as pets and as garden pollinators, and for producers of coveted "sugarbag" honey. The high demand has kept the price high, which may, however, inhibit adoption by farmers to improve pollination and crop yield. The price is expected to drop in the future as supply increases exponentially, and farmers will take note accordingly.

A critical element for using stingless bees in crop pollination is a ready supply of "pollination units" that can be cheaply and sustainably produced. A crucial development in Australia was the advent of a hive design that protects the bees and allows colony propagation [33]. This was followed by a diverse array of hive designs. The common elements to all good designs are as follows. They must provide correct internal volume, allow safe and reliable propagation by colony division, ensure good insulation but also good ventilation, be cheap and easy to make, and durable in outdoor conditions but small and light for transportation.

Stingless bees reproduce naturally by colony fission. Attempts to trap naturally reproducing colonies by providing empty nest boxes (i.e. "swarm boxes") have not yet achieved much success (see Chapter 13). Trials are continuing with this technique in situations where an attacking colony is attempting to usurp the nest of an existing colony. The defending colony can be moved and a box containing some nest provisions can be added in its place. Once the attacking colony has initiated usurpation with a nest at a particular position, it may establish in an empty box located there.

14.3.4 Management of stingless bees in hives

In northern Australia and many warmer parts of the world, stingless bees occur in varying numbers in the mosaic of agricultural ecosystems. Where extensive clearing removes the natural vegetation, stingless bees may be lost from the ecosystem. In those situations, there are opportunities to improve pollination by introducing colonies in hives.

Several strategies for managing hived colonies on farms are evolving. They may include temporary or permanent movement of hives to farms, with the hives managed by the farmer or beekeepers.

In the case of temporary movement of hives to farms, the beekeeper usually makes an agreement with the farmer to move the hives to the farm for a specified period while flowering occurs (see sections 9.3.5 and 14.1, and Chapter 20). The advantage of this strategy is that the hives can be moved to more favourable locations when the crop is not in flower. This strategy is especially effective where the flowering period is short. Farms may be hostile environments for bees if insecticides are used heavily and if little plant variety exists. Furthermore, homogeneous monocultures provide few alternative food sources for bees or other wildlife.

Beekeepers may be able to increase their opportunities by moving hives onto alternative crops, in succession. A disadvantage of temporary placement is the high cost of moving hives. This is especially the case with stingless bees that fight, because they need to be spaced more evenly than colonies of certain honey bees. Diseases and natural enemies also spread more easily between colonies when many hives are located close together (see Chapter 20).

Most hives temporarily relocated into farms will be from local sources. Nonetheless, opportunities may arise to move colonies larger distances. The movement

of hives from subtropical Australia to temperate regions may be worthwhile when a temperate crop flowers at a time of year when the bees will be active. Potential invasiveness or infectious microbes spread by colonies moved outside their normal range should result in restrictions, or inspection and quarantine, as they do for *Apis mellifera* (Chapter 16), but no known instances currently justify this approach. The full range and opportunities for sustainable migratory beekeeping have not yet been identified. A shortage of bees to pollinate the increasing almond crops in temperate Australia, for example, cannot be met with stingless bees, because the almonds flower when the temperature is too low for stingless bee activity.

Permanent placement of stingless beehives near crops is gaining popularity in some situations in Australia. In such cases, the farmer usually buys the hives and may care for them or may make an agreement with a beekeeper for management (see Annex 1). A permanent colony residence works particularly well in more heterogeneous environments that provide resources year round. An agricultural ecosystem that consists of a mix of multiple crop species, natural bushland and ornamental garden plant species (e.g. Malaysian palm and dipterocarp species, see Chapter 14.2) is particularly favourable for social bees.

The introduction of species of stingless bees to areas outside their native range is not recommended, however. This can lead to negative ecological consequences, as has been the case for *Bombus terrestris* when moved to other continents, largely for pollination of glasshouse tomatoes [36] (see

Figure 14.16
HIVES WITH VARIOUS COLOURS AND SHAPES PAINTED ON THE ROOFS TO ALLOW RECOGNITION AND AVOID DRIFT OF FORAGERS TO THE WRONG HIVE

Chapter 11). Instead, the selection and development of local species is encouraged.

14.3.5 Location of stingless bee hives on farms

Hives of stingless bees may be placed evenly across the farm or concentrated in particular sites. A site of multiple honey bee hives is called an apiary. In the Americas, the corresponding name for a collection of stingless bee hives is "meliponary". This term has not been adopted in Australia and an appropriate term is not generally applied. Here, the term "stingless bee farm" is used, an alternative term being "stingless bee yard".

There are logistical advantages to concentrating hives at one site, as all hives can be placed at one or a few points that are easily accessible. However, in apiary sites foraging bees may return to the wrong hive, a phenomen known as "drift". Drift can cause strong defensive reactions, such as a large number of bees flying close together in a circular motion or hovering near the hive entrance. When a colony engages in this defensive activity, normal foraging activity stops and the colony becomes ineffective for pollination. The defensive reaction may result in fighting between bees and result in large losses among workers. Hives need to be placed in locations to avoid drift. The locations should allow the foraging bees to easily recognize the hive of their origin and return to it. Hives can be marked with various shapes and colours to allow bees to easily recognize them (Figure 14.16). Where hives are placed permanently on farms, they should be well separated to avoid drift and fighting (Figure 14.17). Among other factors of note, stingless bees do not need access to water and it is safe to keep hives close to the house.

Figure 14.17
HIVES OF *TETRAGONULA* STINGLESS BEES IN MACADAMIA ORCHARD DURING FLOWERING

Hives are located in outside rows for solar warming (*Macadamia* flowers in subtropical Australia during a cool time of year) and are separated to avoid fighting among colonies

Hives need to be securely mounted and protected (e.g. Figures 14.16 and 14.17). If they are mounted at least 1 m above the ground, the hive boxes obtain protection from decay associated with high humidity and also gain some protection from termites. The height may also give foragers an advantage when leaving the hive in cooler months, as they do not fall to the ground where they are susceptible to predators. Hives may be mounted individually or collectively in a common shelter. The boxes also benefit from a roof that gives protection from rain and sun (Figure 14.17). The roof may be small and specific to each hive or large enough to cover the entire shelter.

Hives need to be placed in situations that provide the best microclimate. This may be a shaded or sunny position depending on the circumstances. On one strawberry farm in subtropical Australia, the farmer moves the hives into a sunny position for the full flowering period – an extended period of approximately six months in the coolest part of the year. Those positions are a poor choice in summer because they allow hive overheating in the full sun. Thus, for the non-flowering hotter part of the year, hives are moved to another location and placed in the shade.

14.3.6 Providing resources for stingless bees

Providing alternative food and resin resources may be a useful strategy for strengthening both wild and managed colonies of stingless bees (see also section 14.2). Planting of vegetation on farms may provide alternative forage for bees. For maximum benefit in the shortest time, early successional plant species will be most useful because they grow rapidly and often flower over extended periods. Replanting of natural vegetation will grant benefits in the longer term and also provide conservation gains and other farm management benefits, such as the conservation of natural enemies of pests [37]. Replanting is often done on farms for various reasons, such as windbreaks, fences or prevention of erosion in watercourses. When planning such plantings, preference should be given to plant species that provide resources for bees. Choose from local or introduced species that grow well and have flowers that are good bee forage. This represents yet another advantage over honey bees, whose normal maximum flight range of 8 km means they cover 20 000 ha. Far fewer hectares are used in the normal foraging range of a stingless bee colony, making efforts to plant and maintain stingless bee gardens logistically and economically feasible.

In addition to pollen and nectar resources, plants provide resinous material for stingless bees. Such resources have multiple benefits in the nest including building material, protection against pathogens, social defences against predators and nest entrance repellents. A diversity of resins is beneficial. Some natural plant resins are particularly repellent against hive beetles, while others are more effective against certain pathogens [38]. Therefore, in addition to a diversity of floral resources, a diversity of resin sources is needed. It is important to make a list of local plant species that produce resins attractive to bees and plant those species on the farm.

14.3.7 Protecting stingless bees from pesticides

Farm management practises that minimize the impact of insecticides and other pesticides need to be practised. The number of sprays should be reduced though careful monitoring of crop pests and beneficial natural enemies of the pests, along with related factors such as weather conditions (Chapter 2.1). When insecticide applications are truly required, they should be performed at night or when wind speeds and insect activity are lowest. Insecticides should be selected that have minimal impact on bees. The farmer should do everything possible to decrease the drift of insecticides onto sites where wild or managed colonies are located. The hives should be placed outside the crop rows to minimize exposure to insecticide sprays (Figure 14.17). Windbreaks consisting of rows of vegetation between crops and natural vegetation or bee yards can be useful to reduce pesticide drift (Figure 14.15). Hives may need to be moved, covered or closed during periods of pesticide application. Closing the entrance will prevent the bees leaving and making contact with insecticides. Covering the hives

will prevent application of insecticide to the outside of the box, where the bees have a high probability of exposure. Moving the hives can be used to reduce exposure. The hive needs to be closed at night, with a screen or a suitable closure that does not prevent air exchange, and moved to a cool dark place protected from insecticide drift.

14.3.8 Selection of candidate targets for pollination

The following six crops are the most promising candidates for managed pollination by stingless bees in Australia: *macadamia, mango, avocado, lychee, blueberry* and *strawberry*. Other possibilities exist but are either minor crops in Australia (e.g. coffee, rambutan, coconut, guava and longan), have uncertain pollinator requirements (e.g. citrus) or cover a broad acerage and require large numbers of hives in areas where colony populations are low (e.g. sunflower, canola).

In order to use resources in an optimal way, it is important to select from among those crops grown locally, and then decide which are most benefitted by enhancing pollination by bees. Such selection is based on the following criteria:

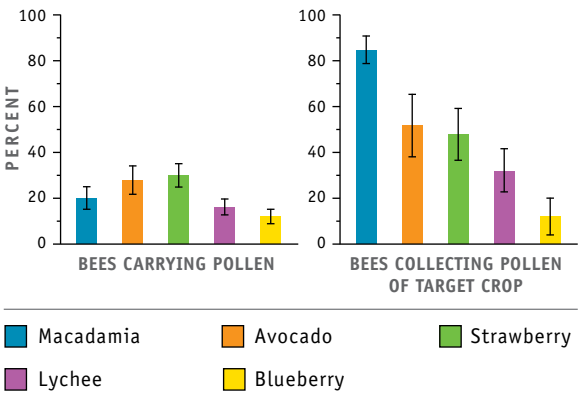
- *Yield and/or quality of the crop benefit from pollination*. Pollinator requirements of the crop species are not well known and differ between varieties. However, evidence exists that yields are increased by insect visits to flowers. For example, nut set in macadamia increases with increasing insect visits [39].
- *Stingless bees effectively pollinate the crop flowers*. Similarly, more research is needed to determine the efficiency of stingless bees in effectively moving pollen from where it is produced to where it is needed. Stingless bees are efficient pollinators of macadamia [40], mango [41], avocado [42] and strawberries [43, 44]. No studies could be found that show how efficiently they pollinate lychee or blueberry.
- *Stingless bees are naturally attracted to the crop*. In the present case, stingless bees are consistently seen on the flowers of these crops when grown in areas where bee populations occur naturally.
- *The crop blooms at a time of year when the bees are active*.
- *Ideally, the crops show a succession of flowering*. When crops do not flower synchronously, hives can be moved from one crop to the next. This is not generally the case with the six selected crops.

14.3.9 Crop fidelity

Usually an individual bee shows fidelity to one plant species within a foraging flight [45]. However, the numerous individuals that make up a colony will normally forage on a variety of plant species, thus displaying "polylecty". Polylecty is important for the colonies to gain a broad and balanced diet, but in crop pollination, fidelity to the crop is desirable. Crop fidelity is the proportion of bees from a hive that forage only on the target crop into which the hive is placed.

The foraging activity of colonies of two stingless bee species was recently determibed for five crops: macadamia, avocado, lychee, blueberry and strawberry (T.A. Heard, G. Venturieri and C. Fuller, unpublished data). On average, 52 percent of pollen foragers from introduced hives of these two species of stingless bees will visit the target crops into which they are placed. This varies between species, being highest on macadamia (85 percent) and lowest on blueberry (12 percent) (Figure 14.18).

Figure 14.18
THE PROPORTION OF BEES OBSERVED FORAGING FOR POLLEN AND COLLECTING POLLEN OF THE TARGET CROP FOR FIVE CROP SPECIES IN AUSTRALIA



Source: T. A. Heard

14.3.10 Future research needs

An atlas and calendar of pollinator needs can help farmers and beekeepers to move hives to where they are most needed. Determining the crops that benefit from pollination can help develop the atlas. Those visited and potentially pollinated by stingless bees can then be listed by locality and flowering (or maximum flowering) periods. A stingless bee pollination calendar can then be prepared (see also Chapter 13.5.1).

Research is needed to determine the potential economic value of stingless bees for the pollination and yield increase of target crops. Such research will need to account for plant varietal differences.

Fighting behaviour among colonies both within species [46] and between species [47] remains a challenge for the use of stingless bee in crop pollination. Research is needed to reduce this behaviour and mitigate its consequences.

An experimental approach is needed to study factors crucial to management of stingless bees in crops. Detailed behavioural studies to examine the flight range of stingless bees in orchards and the benefits of artificial feeding would permit better management of these important pollinating agents.

REFERENCES

[1] Fajardo, A.C., Jr., Medina, J.R., Opina, O.S. & Cervancia, C.R. 2008. Insect pollinators and floral visitors of mango (*Mangifera indica* L. cv Carabao). *Philippine Agricultural Scientist*, 91: 372–382.

[2] Heard, T.A. 1988. Propagation of hives of *Trigona carbonaria* Smith (Hymenoptera: Apidae). *Journal of the Australian Entomological Society*, 27: 303–304.

[3] Fajardo, A.C. Jr. & Cervancia, C.R. 2003. Simple ways to manage stingless bees. *Bees for Development*, 67: 3–5.

[4] Almazol A.E. & Cervancia, C.R. 2014. Pollen sources of bees in Pagbilao Mangrove Ecosystem. *Phillipine Entomologist* (in press).

[5] Bispo dos Santos, R.A.C., Hrncir, M. & Bego, L.R. 2009. Pollination of tomatoes by stingless bee *Melipona quadrifasciata* and honey bee *Apis mellifera* (Hymenoptera, Apidae). *Genetics and Molecular Research*, 8: 751–757.

[6] Cauich, O., Quezada-Euán, J.G., Macias-Macias, J.O., Reyes-Oregel, V., Medina-Peralta, S. & Parra-Tabla, V. 2004. Behavior and pollination efficiency of *Nannotrigona perilampoides* (Hymenoptera: Meliponini) on greenhouse tomatoes (Lycopersicon *esculentum*) in subtropical Mexico. *Journal of Economic Entomology*, 97: 475–481.

[7] Cruz, D., Freitas, B.M., Silva, L., Silva, E. & Bomfim, I. 2005. Pollination efficiency of the stingless bee *Melipona subnitida* on greenhouse sweet pepper. *Pesquisa Agropecuária Brasiliera*, 40: 1197–1201. http://dx.doi.org/10.1590/S0100-204X2005001200006.

[8] Nunes-Silva, P., Hrncir, M. & da Silva, C.I. 2013. Stingless bees, *Melipona fasciculata*, as efficient pollinators of eggplant (*Solanum melongena*) in greenhouses. *Apidologie*, 44: 537–546.

[9] Heard, T.A. 1994. Behaviour and pollinator efficiency of stingless bees and honey bees on macadamia flowers. *Journal of Apicultural Research*, 33: 191–198.

[10] Gavina, M.K.A., Rabajante, J.F. & Cervancia, C.R. 2014. Mathematical programming for determining the optimal location of beehives. *Bulletin of Mathematical Biology*. DOI:10.1007/s11538-014-9943-9.

[11] Rasmussen, C. & Cameron, S.A. 2010. Global stingless bee phylogeny supports ancient divergence, vicariance, and long distance dispersal. *Biological Journal of the Linnaean Society*, 99: 206–232.

[12] Kerr, W.E. & Maule, V. 1964. Geographic distribution of stingless bees and its implications (Hymenoptera: Apidae). *Journal of the New York Entomological Society,* 1: 2–18. www.jstor.org/stable/25005888.

[13] Schwarz, H.F. 1939. The Indo-Malayan species of *Trigona*. *Bulletin of the American Museum of Natural History*, 76: 83–141

[14] Rasmussen, C. 2008. Catalog of the Indo-Malayan/Australasian stingless bees (Hymenoptera: Apidae: Meliponini). *Zootaxa*, 1935: 1–80.

[15] Roubik, D.W. 2006. Stingless bee nesting biology. *Apidologie*, 37: 124–143.

[16] Wille, A. & Michener, C.D. 1973. The nest architecture of stingless bees with special reference to those of Costa Rica. *Revista de Biología Tropical*, Special Supplement 1. 21: 1–278.

[17] Roubik, D.W. 2014. Personal communication, May 2014.

[18] Bänziger, H. & Bänziger, S. 2010. Mammals, birds and reptiles as hosts of *Lisotrigona* bees, the tear drinkers with the broadest host range (Hymenoptera, Apidae). *Bulletin de la Société Entomologique Suisse*, 83: 271–282.

[19] Jalil, A.H. & Shuib, I. 2014. *Beescape for Meliponines – conservation of Indo-Malayan stingless bees.* Singapore, Partridge Publishing.

[20] Ferrari, S., Savatin, D.V., Sicilia, F., Gramegna, G., Cervone, F. and De Lorenzo, G. 2013. Oligogalacturonides: plant damage-associated molecular patterns and regulators of growth and development. *Front Plant Sci*., 2013(4): 49. DOI:10.3389/fpls.2013.00049.

[21] Steinman, H. 2012. Oil palm, *Thermo Scientific,* www.phadia.com/fr/5/Produits/ImmunoCAP-Allergens/Tree-Pollens/Allergens/Oil-Palm- (accessed July 2014).

[22] Cherney, K. 2013. Palm tree allergy. *Livestrong*, www.livestrong.com/article/526957-palm-tree-allergy/ (accessed July, 2014).

[23] Janeway, C. 1989. "Immunogenicity signals 1,2,3 ... and 0". *Immunology Today,* 10: 283–286. DOI:10.1016/0167-5699(89)90081-9 (accessed July 2014).

[24] Roubik, D.W. & Moreno, J.E. 1990. Social bees and palm trees: what do pollen diets tell us? *In* G.K. Veeresh, B. Mallik & C.A. Viraktamath, eds. *Social insects and the enrivonment*, pp. 427-428. New Delhi, Oxford and IBH Publishing Co.

[25] Eltz, T., Brühl, C.A., Imiyabir, Z. & Linsenmair, K.E. 2001. Nesting and nest trees in Sabah lowland dipterocarp forest of Malaysia, with implications for forest management. *Forest Ecology and Management,* 172: 301–313.

[26] Appanah, S. 1992. Mass flowering of Dipterocarp forests in the aseasonal tropics. *Journal of Bioscience,* 18: 457–474.

[27] Cunningham, S.A., Fitzgibbon, F. & Heard, T.A. 2002. The future of pollination in Australia. *Australian Journal of Agricultural Research,* 53: 893–900.

[28] Heard, T.A. 1999. The role of stingless bees in crop pollination. *Annual Review of Entomology,* 44: 183–206.

[29] Halcroft, M., Spooner-Hart, R., Haigh, A.M., Heard, A. & Dollin, A. 2013. The Australian stingless bee industry: a follow-up survey, one decade on. *Journal of Apicultural Research,* 52: 1–7.

[30] Dollin, A.E., Dollin, L.J. & Sakagami, S.F. 1997. Australian stingless bees of the genus *Trigona* (Hymenoptera: Apidae). *Invertebrate Taxonomy,* 11: 861–896.

[31] Leonhardt, S.D., Heard, T.A. & Wallace, H. 2014. Differences in the resource intake of two sympatric Australian stingless bee species. *Apidologie,* 45: 514–527. DOI:10.1007/s13592-013-0266-x.

[32] Ricketts, T.H., Daily, G.C., Ehrlich, P.R. & Michener, C.D. 2004. Economic value of tropical forest to coffee production. *Proceedings of the National Academy of Sciences, USA,* 101: 12579–12582.

[33] Heard, T.A. 1988. Propagation of hives of *Trigona carbonaria*. *Journal of the Australian Entomological Society,* 27: 303–304.

[34] Heard, T.A. & Dollin, A. 2000. Stingless beekeeping in Australia, snapshot of an infant industry. *Bee World,* 82: 116–125.

[35] Halcroft, M., Spooner-Hart, R. & Dollin, L.A. 2013. Australian stingless bees. In P. Vit, S.R.M. Pedro & D.W. Roubik, eds. *Pot honey: a legacy of stingless bees*, pp. 35–72. New York, Springer Science + Business Media.

[36] Slaa, E.J., Sánchez-Chaves, L.A., Malagodi-Bragac, K.S. & Hofstede, F.E. 2006. Stingless bees in applied pollination: practice and perspectives. *Apidologie*, 37: 293–315.

[37] Wratten, S.D, Gillespie, M., Decourtye, A., Mader, E. & Desneux, N. 2012. Pollinator habitat enhancement: benefits to other ecosystem services. *Agriculture, Ecosystems & Environment,* 159: 112–122.

[38] Drescher, N., Wallace, H.M, Katouli, M., Massaro, C.F. & Leonhardt, S.D. 2014. Diversity matters: how bees benefit from different resin sources. *Oecologia* (in press).

[39] Heard, T.A. 1993. Pollinator requirements and flowering patterns of *Macadamia integrifolia*. *Australian Journal of Botany,* 41: 491–497.

[40] Heard, T.A. 1994. Behaviour and pollinator efficiency of stingless bees and honey bees on macadamia flowers. *Journal of Apicultural Research,* 33: 191–198.

[41] Anderson, D.L., Sedgley, M., Short, J.R.T. & Allwood, A.J. 1982. Insect pollination of mango in Northern Australia. *Australian Journal of Agricultural Research*, 33: 541–48.

[42] Wysoki, M., van den Berg, M.A., Ish-Am, G., Gazit, S., Pena, J.E. & Waite, G.K. 2002. Pests and pollinators of avocado. In J.E. Pena, J.L. Sharp & M. Wysoki, eds. *Tropical fruit pests and pollinators: biology, economic importance, natural enemies, and control*, pp. 223–293. Wallingford, CABI.

[43] Kakutani, T., Inoue, T., Tezuka, T. & Maeta, Y. 1993. Pollination of strawberry by the stingless bee, *Trigona minangkabau*, and the honey bee, *Apis mellifera*: an experimental study of fertilization efficiency. *Researches in Population Ecology*, 35: 95–111.

[44] Malagodi-Braga, K.S. & Peixoto Kleinert, A.M. 2004. Could *Tetragonisca angustula* Latreille (Apinae, Meliponini) be effective as strawberry pollinator in greenhouses? *Australian Journal of Agricultural Research,* 55: 771–773.

[45] White, D., Cribb, B.W. & Heard, T.A. 2001. Flower constancy of the stingless bee, *Trigona carbonaria* Smith (Hymenoptera: Apidae: Meliponini). *Australian Journal of Entomology,* 40: 61–64.

[46] Gloag, R.S., Heard, T.A., Beekman, M.B. & Oldroyd, B.P. 2008. Nest defence in a stingless bee: what causes fighting swarms in *Trigona carbonaria* (Hymenoptera: Meliponini)? *Insectes Sociaux,* 55: 387–391.

[47] Cunningham, J.P., Hereward, J.P., Heard, T.A., De Barro, P.J. & West, S.A. 2014. Bee wars: interspecific battles and nest usurpation in stingless bees. *American Naturalist* (in press).

Chapter 15

REARING CARPENTER BEES (*XYLOCOPA* SPP.) FOR CROP POLLINATION: A CASE STUDY WITH PASSION FRUIT (*PASSIFLORA EDULIS*)

C.I. da Silva and B.M. Freitas

15.1 INTRODUCTION

Bee rearing is usually associated with managed social bees destined for honey production. These comprise honey bees and a variety of stingless bee species, and more recently *Bombus* and a few solitary bees species for pollination purposes [1]. Carpenter bees, however, are considered a pest in many regions because of their habit of boring dead wood to build their nests, and their painful sting. Only few attempts have been made to rear the bees commercially [2–3].

Despite their poor reputation, however, carpenter bees are docile and important pollinators of wild and cultivated plant species. In agriculture, they pollinate various food and fodder crops such as cowpea (*Vigna unguiculata*), canavalia (*Canavalia ensiformis*), Brazil nut (*Bertholletia excelsa*), pumpkin (*Cucurbita pepo*), squash (*C. moschata*), gourd (*C. maxima*), chayote (*Sechium edule*), guava (*Psidium guajava*), watermelon (*Citrullus lanatus*), tomato (*Solanum lycopersicum*) and passion fruit (*Passiflora* spp.) to mention only a few [4–7] (Figure 15.1a and b).

In the particular case of yellow passion fruit (*Passiflora edulis flavicarpa*), intensification and expansion of the crop makes the carpenter bee, as its main pollinator, an indispensable asset for profitable yields (Figure 15.1b). The importance of carpenter bees for passion fruit production is such that their population size in plantings drives crop profitability, because unpollinated flowers do not set fruit. Farmers are forced to hire people to hand pollinate flowers, increasing costs by up to 30 percent and reducing profit margins [8–9]. However, factors such as deforestation near agricultural settings, increased pesticide application on crops, replacement of wooden posts with those made from cement in the passion fruit plantation, and lack of suitable nesting sites close to or inside the commercial plantings greatly reduce wild carpenter bee numbers in passion fruit areas [10–11].

Rearing carpenter bees for pollination of passion fruit and other crops could be an alternative to low bee abundance in plantations. However, unlike most social and solitary bees already reared in hives and trap nests for use in crop pollination, carpenter bees do not nest in pre-existing cavities and need to excavate their own nests in a wooden substrate. This trait hinders the development of artificial nests for carpenter bees [2–3]. This chapter discusses relevant aspects of the carpenter bee life cycle that must be understood to rear carpenter bees in artificial nests. It presents a management proposal for increasing the number of pollinating carpenter bees to mitigate a pollination deficit and augment yield.

Figure 15.1
CARPENTER BEES (*XYLOCOPA* SPP.) ARE IMPORTANT POLLINATORS OF WILD AND CULTIVATED PLANT SPECIES AND EFFORTS ARE MADE TO REAR THESE BEES FOR CROP POLLINATION

(a) female *X. cearensis* pollinating a cowpea (*Vigna unguiculata*) flower; (b) male *Xylocopa frontalis* pollinating passion fruit (*Passiflora edulis*); (c) natural nests of carpenter bees excavated in a dead tree trunk; (d) trap nests made of *Eucalyptus* posts hanging in a shelter; (e) trap nests made of bamboo canes arranged in construction bricks; (f) female *X. cearensis* dehydrating nectar at the entrance of its nest built in a bamboo trap-nest; (g) wood trunk sliced in many sections to force carpenter bees to nest in a bi-dimensional plane within each wood section; (h) nest formed by wood blocks, which can be kept side by side to form a battery of nests [31]; (i) drawing of the *Xylocopa* "rational rearing" box proposed by [10]; (j) *Xylocopa frontalis* female returning to its nest in a rational rearing box; (k) manipulating a *X. frontalis* nest in rational rearing box containing seven adult bees; (l) adult and larva developing in a nest built inside a *Xylocopa* rational rearing box; (m) a new design of a *Xylocopa* rational rearing box presently being tested in the Universidade Federal do Ceará (UFC); and (n) *Xylocopa* rational rearing box placed in a passion fruit orchard for pollination purposes

Source: da Silva and Freitas [present study]

15.2 THE CARPENTER BEES

The common name "carpenter bee" is attributed to a variety of wood-boring adult bees, such as the small carpenter bees *Lithurgus* and *Trichothurgus* (family Megachilidae), the dwarf carpenter bees *Manuelia, Allodapula, Pithitis* and *Ceratina* (family Apidae), and the carpenter bees *Lestis* and *Proxylocopa* (family Apidae) [10, 12–15]. This chapter focuses on the large carpenter bees of the genus *Xylocopa* (family Apidae) because they are widely recognized as important pollinators of a range of wild plants and crop species, and have greater rearing potential and success.

There are more than 730 species of carpenter bees, *Xylocopa* spp. They are robust and large, among the largest bee species known, with some reaching 4.5 cm in length [12, 14–15]. The females are usually black or shiny blue, and sometimes give off greenish or purplish reflections, depending on the angle of light on their bodies. Many species display sexual dimorphism, with the male covered in yellow-orange hair. Patterns of black, white, yellow or light powder blue stripes are also found among females, while the nocturnal species are generally light brown (subgenus *Nyctomelitta*) [15, 16].

Most *Xylocopa* species are tropical or subtropical. However, some maintain their body temperature even when the air is cool [12, 15–17]. A major characteristic of carpenter bees is that females excavate their nests in dead, dry, sometimes rotten wood, instead of using pre-existing cavities like many other bees and insects (Figure 15.1c). Certain species, however, build nests in live plants, while some use the dead parts of fistulated plants such as bamboo (91 genera in the tribe Bambuseae), and others use the live tissues of those plant species [12, 15, 17].

15.3 NESTING BEHAVIOUR

The nesting behaviour of carpenter bees is the source of their popular name. The behaviour seems closely related to their reproduction, as females only excavate their own nests after being mated. Even when reusing nests from the previous generations, the

female carpenter bee usually builds her own cells and galleries, although she takes advantage of the nest entrance left by the previous owners [10].

Young females searching for suitable nest sites inspect many potential sites before choosing one. Females usually search for dead dry wood soft enough to be excavated, but with no cracks or fissures. They prefer tree branches or trunks, although they also nest in wooden fences, posts, poles and almost any built wooden structure. Although less common, a few species nest in dead portions of live plants or the live parts of vegetation [15, 17–19].

Once a satisfactory substrate is located, the female excavates a tunnel first, usually in an ascendant direction following the wood fibres, vertically from the nest entrance. The first tunnel is normally excavated immediately below the trunk bark or the wood surface. There, the female builds one or two cells to rear its offspring. Only after finishing building a cell, provisioning it with a ball made of enough pollen and nectar to feed the larva from egg to adulthood, laying an egg on the top of the food ball and closing the cell, does the female start building the next cell in the same tunnel. The cells are always constructed from the end of the tunnel towards the entrance [18, 19]. Later, the female excavates other galleries deeper in the wood, but always following the pattern described above. The new tunnels all originate near the nest entrance, where the female builds a wide entry chamber.

The new female frequently reuses the nest left by her mother, due to death or abandonment, or by any other conspecific female. In the latter case, the female begins by cleaning up all remnants from the previous occupants, such as remains of cells, pollen, and dead larvae or adults. Then she may excavate new galleries either from existing ones or from the chamber near the nest entrance. Alternatively, she simply modifies existing galleries and cells by excavating them slightly further [10, 20].

Although carpenter bees are basically solitary bees, some species are social and form associations between mother and daughter or between sisters, displaying behaviour and social interactions that change as the bees mature physiologically. When that happens, both the daughter in relation to its mother, or the young sister(s) in relation to its (their) older sister, may initially help with collecting food to provision a brood cell, but egg laying is performed by the mother (first case) or the eldest sister (second case). When the daughter or young daughters reach sexual maturity and mate, they may continue the association, but now also lay eggs. As a consequence, nests with two or more females collecting food and laying eggs produce a greater number of brood and adults than nests headed by a single female [16, 21].

If the substrate allows, the same nest can be reused year after year and additional new nest galleries are made in the same wood structure. As with most wood-nesting bees, "philopatry" occurs, and females nest close to the site where they were born [22]. Therefore, when looking for a suitable place to excavate their nests they first inspect the surroundings of the mother nest and may build their own nests in the same wooden structure. With time, a carpenter bee population grows inside the wood and the neighbouring suitable nesting sites (Figure 15.1c).

15.4 EGG LAYING AND LARVAL DEVELOPMENT

Once a cell is built, the female carpenter bee begins collecting pollen and nectar for provisioning. When the bee arrives, bringing food from the field, it enters the nest and goes immediately to the cell it is working on. There, the bee removes pollen from the scopae (hair brushes) of the rear legs, grooms itself and adds pollen to the bottom of the cell. If the nectar it brings from the crop has an adequate concentration, the bee regurgitates it in small droplets on the pollen mass, while manipulating it with the mandibles. If the nectar is too watery, the bee goes to the nest entrance where it exposes a nectar droplet on its protruding head, between the galea and tongue, making repeated movements with its mouthparts to dehydrate the nectar (Figure 15.1f). Once the bee has concentrated the sugar in the nectar droplet, it returns

to the cell, places the droplet on the pollen mass, and returns immediately to the nest entrance to repeat the process with a new droplet. It may continue until all the nectar brought from the field has been added to the pollen mass of the brood cell. The procedure is repeated after each field trip, until the amount of pollen and nectar deposited in the cell is sufficient to feed its future offspring through the entire larval phase. Then, the female uses the mandibles, legs and abdomen to manipulate the mass of pollen and nectar, shaping it into an oval ball with two protuberances, one on each side, that it fixes to the cell wall. After that, the bee moves backwards towards the food mass and lays a large egg on the top [10, 20].

After laying its egg, the female closes the cell with wood scrapings it chews from the inner surface of the gallery. The wood is combined with saliva to create a partition between the finished cell and the next cell to be built. Once closed, the bee does not reopen the cell. The larva hatched from the egg depends exclusively on the pollen and nectar ball left by its mother inside that cell. Because the egg is laid on top of the food ball, the larva is born laying on its food supply, where it remains and consumes the food until it reaches the prepupal stage, when it stops feeding and goes through metamorphosis to become an adult bee [10, 16]. The cycle from egg to adult is usually quite long and varies among species, ranging from 35 to 40 days for *X. fenestrata* in India [23]; 45 to 65 days for *X. frontalis*, *X. grisescens* and *X. suspecta* in Brazil [19, 24]; and 83 to 90 days for *X. caffra* in South Africa [16].

The biological cycle of carpenter bees and the environmental conditions of the region they inhabit determine the average number of siblings produced by each female and the number of generations per year. Depending on the species, a female carpenter bee produces from five to 15 new adults and has only one, two, four or more, generations per year. In the same way, adult longevity can vary from a few months to two to three years [20, 25].

15.5 THE ADULT LIFE OF CARPENTER BEES

Carpenter bees, unlike most solitary bees, do not leave the nest as soon as they become adults. In many species, newly emerged bees encounter the mother, an aunt or other adult relative from the previous generation still active in the nest. The relatives bring food from the field to the new bees, while the newly emerged adults guard the nest against enemies or other carpenter bees that may try to use the nest. During this phase of their lives, males and females cohabit in the nest until they are capable of flying to obtain food for themselves [16]. In species such as *X. frontalis*, *X. grisescens* and *X. suspecta*, the new bees start to leave the maternal nest for the first time around 30 days after emerging from their cells as adults [10, 18].

The period in the nest without external flights is normally associated with sexual maturation, as both male and female carpenter bees need time before they are physiologically capable of reproducing, and must consume some pollen. The bees only mate after making a few initial flights outside the nest.

The first flights are usually short and take place around the nests, serving as orientation flights. As the bee becomes acquainted with the nest location, she makes longer flights, reaching greater distances and takes more time, but always returns to the nest. During those external excursions, both males and females learn to locate food sources and exploit them correctly. Soon afterwards, the males leave their maternal nest or are expelled by their sisters [10]. They then look for wood crevices where they can find shelter and establish territories near plants where females arrive to search for food and mating opportunities. Within these mating territories, males exhibit territorial behaviour, chasing any male that approaches and even fighting for territories or females [25]. This behaviour continues until the end of their life, during which time they mate several times, if possible.

After a male leaves the nest, the mother carpenter bee becomes intolerant toward her daughters and

forces them to leave as well and build their own nests. Sometimes, the elder daughter expels her mother and sisters, before taking over the nest alone. In other cases, associations between the mother and daughters or between sisters are made, as already discussed. In each case, the reproductive cycle is then reinitiated [16, 20].

15.6 REARING CARPENTER BEES

The need for carpenter bees as pollinators of certain crops, primarily passion fruit, has created interest in keeping bees among crops to ensure adequate pollination levels and fruit set. Usually, growers try to increase the carpenter bee population in their plantations by introducing logs of dead wood [26–27]. The logs can be either uninhabited, with the hope of attracting females to nest, or already inhabited to encourage the presence of carpenter bees in the crop area and visitation of flowers (Figure 15.1c).

Such attempts are usually successful in increasing the number of carpenter bees visiting the crop, especially in agricultural settings where the natural vegetation with suitable nesting sites is scarce due to deforestation. However, these strategies are not always entirely successful, because the use of logs as attractants for nesting female carpenter bees depends on the previous presence of bees in the area. Existing nests are very important because most new nests are founded by young females when they leave their mother nest and search nearby. Thus, colonization of logs is normally low (5–10 percent). The introduction of inhabited logs may produce satisfactory results, depending on the number of nests distributed across the plantation. In Brazil, 25 nests of *Xylocopa frontalis* per hectare are used to attain the ideal 25 percent setting for passion fruit flowers [3, 14]. The main problem lies in finding sufficient inhabited logs to establish the desired carpenter bee density in the plantations. If natural nesting sites are being removed by deforestation, problems arise similar to those encountered when establishing meliponaries (see Chapters 13 and 14). If there are no bees in the surrounding forest or fragments of natural vegetation, either successful husbandry or "predatory beekeeping" of some form is pursued [28]. Even if they are found, the problem will persist because bees will not remain in the area after crop flowering, and may abandon their nesting logs to search for other places to nest near better food sources. They may also die from pesticides applied during or soon after blooming. In most cases, carpenter bees are forgotten as soon as the crop finishes flowering, and they receive little or no attention from growers. When the time for next blooming arrives, the bees are no longer nesting in the improvised nest logs. As a result, the number of nests that can be harvested from sites where previous ones were obtained decreases, year after year [10].

Even if the grower decides to take care of carpenter bee nests in the plantation, he or she will not be able to provide much help, because the nests are excavated inside wood. It is therefore impossible to ascertain the developmental stage of the nest, how many adults, larvae or pupae are alive or dead inside (due to pesticides, pests or parasites), and whether there is a food shortage for cell provisioning. Therefore, it is preferable to rear carpenter bees in other structures that facilitate nest observation, manipulation and caring for the bees [2, 3, 10, 29–31].

15.6.1 Rearing carpenter bees in trap nests

This technique has been used to mitigate problems related to the introduction and maintenance of carpenter bees in plantations [11]. The technique consists of using small logs with ideal conditions for carpenter bee nesting (i.e. dead, dried [around 40 percent moisture] and "soft" wood of species known to be favoured in that region by carpenter bees to build their nests [26–27, 29]). The wood hardness is important and the use of a densiometer will be necessary to determine it in the field. The logs must be 40 to 50 cm long, have no cracks that could allow water to penetrate, and be protected from the sun and rain, or hung from posts. The logs should have a number of holes of the diameter of natural nests found in the region, drilled sloping upwards.

These aim to shelter and provide a starting point for females searching for new sites to establish nests [31]. An alternative is to make trap nests with *Pinus* or *Eucalyptus* posts measuring 10 x 10 x 50 cm [32] (Figure 15.1d).

Such trap nests also work well for the introduction to crop areas of large numbers of laboratory-reared carpenter bees. After emergence, the carpenter bees can be placed in these nests and taken to the field, where up to 67 percent colonize the trap nests [30].

Another possibility is to make trap nests using bamboo canes [33–35]. The bamboo cane is sawed into pieces measuring between 17 and 25 cm long and 1.8 to 2.0 cm in diameter. The bamboo cane walls should be between 3.6 and 4.0 mm thick, because the female needs to chew them for material to build cells, in particular the cell partitions. If the walls are not sufficiently thick, the carpenter bee will reject the trap nest. When making the trap nest from bamboo, the cane should always be cut near its node in order fix the bottom of the nest, leaving the other extremity open to function as the entrance [35–37]. The bamboo canes should then be placed horizontally in a covered shelter near the plantation. In Brazil, holed construction bricks are used to secure the bamboo trap nests (Figure 15.1e).

However, unsuccessful attempts in using bamboo canes as trap nests have also been reported. In India, for example, observations over a three-year period showed that *X. fenestrata* did not nest in bamboo trap nests, though it colonized 57–75 percent of *Arundo* sp. and 25–38 percent of castor oil (*Ricinus communis*) branches [23]. The diversity of *Xylocopa* and bamboo species and the fabrication and management of the trap nests may explain such disparate results.

Despite their relative success, trap nests present the same problem as logs and posts introduced in plantations for carpenter bee colonization – the lack of female carpenter bees available to colonize the *Pinus*, *Eucalyptus* or bamboo substrate in numbers large enough to mitigate the crop's pollination deficit. Nevertheless, trap nests have some advantages when compared to logs collected in the forests or nearby. They are smaller and much lighter, and can be better arranged in the plantation to increase the population and distribution of foraging females. They also allow the introduction of new females reared in trap nests from other places (husbandry), avoiding their removal from the native habitat. Furthermore, trap nests can be removed from the plantation when the crop is not in bloom. The carpenter bees can then be reared in other areas, and bee poisoning with pesticides and accidents with labourers in the field can be avoided.

However, despite the aforementioned advantages, trap nests do not allow for the rational rearing of carpenter bees. The interior of the nests cannot be inspected to follow brood development, prevent pest and disease attacks, or quickly identify the developmental stage of the nest. This information is vital in order to transport females demanding more pollen or nectar to plantations or elsewhere.

15.6.2 Rearing carpenter bees in rational nesting boxes

The main difficulty with rearing and managing carpenter bees is the fact that the bees never accept boxes or other hollow wood structures, unlike many honey bees and stingless bees. The idea of a rational hive for carpenter bees occurred when tree trunks were sliced in vertical sections, in a manner that forced the bees to nest in multidimensional planes within a wooden block. The sections, however, remained together, despite their appearance to an external observer (Figure 15.1g).

The nesting sections then progressed to rectangular wooden slabs, in experiments with *X. latipes* in Malaysia [2]. The nesting slabs were kept side by side, separated by 0.5 cm, to prevent females from excavating into a neighbouring slab (Figure 15.1h).

The first major progress in rationally rearing carpenter bees occurred with a plain piece of wood. This was mounted in the same manner as the frames of honey bee hives, inside rectangular boxes, allowing the removal and inspection of each frame [31]. This pioneering idea was later improved upon, and a rational rearing box was successfully developed [3, 10, 24].

Despite the obvious differences between the nesting biology of honey bees and carpenter bees, the rational rearing box is based on honey bee hives, with a similar nesting box, frames and a cover (Figure 15.1i). The major difference between the two hives resides in the use of frames equipped with wires and embossed beeswax for honey bees, while for *Xylocopa* a solid wood sheet replaces the beeswax comb or starter strip. The width of the wood sheet should match that of the natural nests. The tunnels excavated by the bee should open at each side, allowing the beekeeper to see somewhat inside the nest. A glass or acrylic sheet covers both sides of the frame, allowing visibility and sealing the lateral sides of the tunnels, and also preventing the female from abandoning the nest. However, despite a certain similarity with a honey bee hive, each frame in the carpenter bee hive functions as a nest in itself. The set of frames placed inside the box is in reality a small community of carpenter bee nests [3, 10, 31].

Rational rearing boxes have occupation rates ranging from 18 percent to 52 percent, representing 3.0 to 4.7 of nests founded among the nine potential nests per box [3]. The boxes or hives allow for management of carpenter bee nests and their population in the crops, which have reached increments of up to 505 percent in the number of carpenter bees visiting passion fruit flowers and 92.3 percent of fruit set after the bees are introduced [3]. Because the frame-like nests are movable, it is possible to follow females in their external and internal activities, inspect nest development and offspring production, control pest attacks, and move hives in and out of plantations as needed [10, 24] (Figure 15.1j, k, l and n). However, the current rational nesting box model used for this case study is big and heavy, making it difficult to handle and transport. Because of this drawback, smaller and lighter designs following the same basic principles are currently being tested at the Universidade Federal do Ceará (Figure 15.1m).

15.7 CARPENTER BEES FOR POLLINATION

Although carpenter bees are good pollinators of diverse wild and cultivated plant species, they are most sought for passion fruit pollination. Passion fruit is the common name given to many species of *Passiflora*, including those that are cultivated. This genus comprises more than 530 known species, all of them Pantropical in distribution [38] (see also Chapter 9.3.2).

Over 90 percent of *Passiflora* species are found in South America, with the greatest species diversity found in the Andean region. However, they are also found in southern Asia, Australia, China, New Guinea and the United States, and as an endemic species in New Zealand [38]. Two *Passiflora* species, in particular, stand out in the global market: *Passiflora edulis* f. *flavicarpa* (yellow passion fruit) and *Passiflora alata* (fragrant granadilla, winged stem passion flower).

Millions of tonnes of passion fruit are currently produced and commercialized around the world. Brazil is the greatest producer, but most of its production concentrates on yellow passion fruit for the internal market. Other producing countries such as Colombia, Ecuador, Indonesia and Peru export to Europe [40–42]. Most passion fruit production comes from small growers; however, yields vary due to production costs such as pesticides and pollination services [43].

The natural pollination of passion fruit is carried out by large bees capable of touching the reproductive structures of its large flowers while collecting nectar. This is the reason carpenter bees are the primary pollinators. Many growers complain of low yield due to loss of diversity and drops in the population size of bees in cultivated areas, which forces them to pay for hand pollination services, increasing their production costs. In Brazil, estimates of pollination costs approximate US$2 500/ha per year – a considerable cost to small growers in any developing country [9]. However, natural pollination, when available, produces more and better-formed fruits with more seeds and pulp, and greater market value [9, 44].

15.8 PASSION FRUIT POLLINATION REQUIREMENTS

Passion fruit flowers are hermaphrodite, bearing five stamens and three fertile pistils. The yellow passion fruit flowers open at noon and depend on cross-pollination to set and produce fruits. The species have three distinct mechanisms to prevent self-pollination: (i) *hercogamy*, or spatial separation of the reproductive organs, where the passion fruit pistils are located above stamens; (ii) *protandry*, where the male organ (stamens) mature before the female organs (pistil); and (iii) a self-incompability system that prevents pollen from the same plant from germinating in any of its own flowers [45–46].

Flowers produce an average 462 ovules and about 140 000 pollen grains, while fruits set an average of 180 seeds/fruit, representing 60 percent of ovules fertilized [47–48]. In addition to pollen, passion fruit flowers also produce nectar, which is the main attractant for carpenter bees and other floral visitors because of the large quantities and high caloric value. The average nectar production per flower ranges from 75 to 100 µl, while the sugar concentration varies from 42 percent to 50 percent, although quantity and quality change throughout the day [48–50].

Because of their morphology, passion fruit flowers require visits by large bees such as those of the genera *Bombus*, *Centris* (subgenera *Ptilotopus* and *Melacentris*), *Epicharis, Eulaema* and *Xylocopa*, to achieve proper pollination, although some medium-sized bees can act as occasional pollinators, due to their foraging behaviour or variations in the height of the androgynophore [45, 50–51]. Among all potential pollinators, carpenter bees (*Xylocopa* spp.) are indicated as the main pollinators of passion fruit due to their abundance, frequency of visits and, principally, their large body size, which guarantees flower manipulation and contact with the anthers and stigmas [48, 50–51]. A single carpenter bee visit may deposit 1 624 pollen grains on a flower's stigma, enough to set the fruit [48]. However, an even pollen distribution among the three stigmas is necessary for the development of a well-shaped fruit.

15.9 RECOMMENDED BEST PRACTICES

Lack of carpenter bees in passion fruit plantations or or an inadequate population size can be responsible for severe pollination deficits, which make the crop unprofitable or increase production costs, as people must replace the bees. Growers must therefore ensure the presence of adequate populations of carpenter bees to pollinate the flowers.

However, passion fruit plants bloom only a few months of the year, while carpenter bees are active all year round. The passion fruit flowers produce both pollen and nectar, however only nectar, used in the diet of adults and immatures, is attractive to carpenter bees [52]. The pollen functions as the protein source for new brood, and provides nutrition for some adult bees [53]. Thus, it is important to know which plant sources are selected by carpenter bees when passion fruit flowers are not available, and which other sources are used when the plants are blooming – since the pollen in *Passiflora* is not collected or eaten – to complement their diet.

It is therefore desirable to maintain or reintroduce natural sources of pollen for bees in the neighbourhood of the passion fruit farms. Plant species with poricidal anthers are among the most used by carpenter bees as pollen sources, and are thus recommended. In Brazil, these include *Senna* and *Chamaecrista* (Caesalpinioideae), *Solanum* (Solanaceae), *Ouratea* (Ochnaceae), *Cochlospermum* (Bixaceae or Cochlospermaceae), *Tibouchina* and *Miconia* (Melastomataceae), along with plants with bowl-shaped flowers such as *Kielmeyera* (Clusiaceae) and *Eriotheca* (Malvaceae), among others [52].

Nectar flowers are also essential, especially those that bloom outside the passion fruit blooming season, such as species of *Crotalaria* (Leguminosae), *Thunbergia* (Acanthaceae) and *Libidibia* (Fabaceae), among others. Those nectar sources help to maintain carpenter bees in or around the passion fruit growing area between consecutive bloomings of the crop. When the passion fruit comes into bloom again, the pollinators then come immediately to the flowers [52].

A study carried out in Brazil that sampled pollen grains from the bodies of adult bees and feces from brood cells showed that carpenter bees use 122 native plant species in their diet, out of which 26 are prominent in the diets of adults and immature bees [53]. Those plant species, however, are considered undesirable invaders by growers and are constantly removed from the passion fruit plantations and their surroundings. This lowers the amount and quality of food available to the bees, especially protein, because many of the plant species function as principal pollen sources [53].

The maintenance or re-establishment of natural vegetation around passion fruit plantations seems to be of paramount importance to restore not only an adequate diet for the carpenter bees, but also their natural nesting sites. Both are essential to ensure the development of large bee populations in the vicinity of growing areas. In addition, the adoption of pollinator-friendly measures, in association with the use of inhabited rational rearing boxes, can increase the number of carpenter bees visiting passion fruit flowers. Such measures include the use of wooden fence posts for passion fruit posts or trellises where bees can nest, instead of concrete ones; the introduction of uninhabited trap nests as potential nesting sites; the reduction or at least more rational use of biocides; and avoidance of systemic pesticides and spraying during blooming or, if not possible, spraying at night when bees are not active in the field. These combined efforts will mitigate pollination deficit and, consequently, augment yield, reduce production costs and increase profit, while resulting in a more sustainable and ecologically sound form of agriculture.

The development of conservation and management plans for plantations is essential to ensure the presence of carpenter bees and adequate passion fruit pollination. This is especially appropriate due to the large size of the pollinators, which forage over several hundred square kilometres if given adequate habitat. Recently, management plans for passion fruit cultivation in the Cerrado region of Brazil [54] have extended aid to growers and may influence their future thinking, not only within the borders of their plantations, but also beyond them. Similar studies can be carried out in other passion fruit areas around the world.

15.10 CONCLUSIONS

A series of factors limit the number of bees in agroecosystems, but in the particular case of carpenter bees, deforestation near cultivated areas has drastically reduced potential bee nesting and pollen or nectar resources. It is therefore recommended to preserve or restore natural vegetation surrounding cultivated areas to attract and keep the bees in or near those areas. Such measures may ensure that bees are available to visit the flowers whenever needed. Adoption of pollinator-friendly practices within the cultivated area, such as increasing suitable nesting sites, also helps to ensure proper pollination. Finally, for areas where such measures are not sufficient to reduce real pollination deficits, the introduction of managed carpenter bees is recommended. A better understanding of carpenter bee nesting biology and behaviour and a rational rearing box, where management and reproduction techniques can be applied, may quickly increase the number of nests for pollination purposes [55].

REFERENCES

[1] Magalhães, C.B. & Freitas, B.M. 2013. Introducing nests of the oil-collecting bee *Centris analis* (Hymenoptera: Apidae: Centridini) for pollination of acerola (*Malpighia emarginata*) increases yield. *Apidologie*, 44: 234–239.

[2] Mardan, M., Rahman, H.A. & Wong, M.L. 1994. A new technique for mass rearing carpenter bees on dry wood logs. In *International Conference On Apiculture In Tropical Climates*, *Proceedings,* 5, pp. 149–155. Port of Spain, Trinidad, International Bee Research Association.

[3] Freitas, B. & Oliveira-Filho, J. 2003. Ninhos racionais para mamangava (*Xylocopa frontalis*) na polinização do maracujá-amarelo (*Passiflora edulis*) [Rational nests for mamangava (*Xylocopa frontalis*) for pollination of yellow passion fruit (*Passiflora edulis*)]. *Ciência Rural*, 33: 1135–1139.

[4] Corbet, S.A. & Willmer, P.G. 1980. Pollination of the yellow passionfruit: nectar, pollen and carpenter bees. *Journal of Agricultural Science,* 95: 655–666.

[5] Free, J.B. 1993. *Insect pollination of crops* (2nd edn). Cardiff, UK, University Press.

[6] Alves, J.E. 2000. *Eficiência de cinco espécies de abelhas na polinização da goiabeira (Psidium guajava)* [Efficiency of five bee species for pollination of guava (Psidium guajava)]. Fortaleza, Universidade Federal do Ceará (MA thesis).

[7] Cavalcante, M.C., Oliveira, F.F., Maués, M.M. & Freitas, B.M. 2012. Pollination requirements and the foraging behavior of potential pollinators of cultivated Brazil nut (*Bertholletia excelsa* Bonpl.) trees in Central Amazon Rainforest. *Psyche*, article ID 978019. doi:10.1155/2012/978019.

[8] Ruggiero, C. 2000. Situação da cultura do maracujazeiro no Brasil [Situation of passion fruit culture in Brazil]. *Informe Agropecuário,* 21: 5–9.

[9] Pereira-Vieira, P.F.S.P., Cruz, D.O., Gomes, M.F.M., Campos, L.A.O. & Lima, J.E. 2010. Valor econômico da polinização por abelhas mamangavas no cultivo do maracujá-amarelo [Economic value of pollination by mammoth bees in the cultivation of yellow passion fruit]. *Revista Iberoamericana de Economía Ecológica*, 15: 43–53.

[10] Freitas, B.M. & Oliveira-Filho, J.H. 2001. *Criação racional de mamangavas para polinização em áreas agrícolas* [Rational breeding of mammals for pollination in agricultural areas]. Fortaleza, Banco do Nordeste.

[11] Silva, C.I., Oliveira, P.E.A.M. & Garófalo, C.A. 2012. Manejo e conservação de polinizadores do maracujazeiro amarelo (*Passiflora edulis* f. *flavicarpa*) [Management and conservation of yellow passion fruit pollinators (*Passiflora edulis* f. *flavicarpa*)]. *Documentos (Embrapa Semi-Árido. Online),* 249: 163–178.

[12] Hurd, P.D. 1978. *An annotated catalog of the carpenter bees (genus Xylocopa Latreille) of the Western Hemisphere (Hymenoptera, Anthophoridae)*. Washington, DC, Smithsonian Institution Press.

[13] Flores-Prado, L., Flores, S.V. & McAllister, B.F. 2010. Phylogenetic relationships among tribes in Xylocopinae (Apidae) and implications on nest structure evolution. *Molecular Phylogenetics and Evolution*, 57: 237–244.

[14] Camillo, E. 1996. Utilização de espécies de *Xylocopa* (Hymenoptera, Anthophoridae) na polinização do maracujá amarelo [Utilization of *Xylocopa* species (Hymenoptera, Anthophoridae) in the pollination of yellow passion fruit]. In *Anais do Encontro Sobre Abelhas*, 2. Ribeirão Preto, pp. 141–146. Ribeirão Preto, Universidade de São Paulo, Faculdade de Filosofia Ciências e Letras.

[15] Martins, D.J. 2014. *Our friends the pollinators: a handbook of pollinator diversity and conservation in East Africa*. Nairobi, Nature Kenya – the East Africa Natural History Society.

[16] O'Toole, C. & Raw, A. 1991. *Bees of the world*. London, Blanford.

[17] Sakagami, S.F. & Laroca, S. 1971. Observations on the bionomics of some neotropical Xylocopinae bees, with comparative and biofaunistic notes (Hymenoptera, Anthophoridae). *Journal of the Faculty of Science of Hokkaido,* 18: 57–127.

[18] Camillo, E. & Garófalo, C.A. 1982. On the bionomics of *Xylocopa frontalis* (Olivier) and *Xylocopa grisescens* (Lepeletier) in southern Brazil. I. Nest construction and biological cycle. *Revista Brasileira de Biologia*, 42: 571–582.

[19] Camillo, E., Garófalo, C.A. & Muccillo, G. 1986. On the bionomics of *Xylocopa suspecta* (Moure) in southern Brazil: nest construction and biological cycle (Hymenoptera, Anthophoridae). *Revista Brasileira de Biologia*, 46: 383–393.

[20] Camillo, E. 1979. Aspectos ecológicos e evolutivos de abelhas do gênero *Xylocopa* (Hymenoptera, Anthophoridae) [Ecological and evolutionary aspects of bees of the genus *Xylocopa* (Hymenoptera, Anthophoridae)]. São Carlos, Universidade Federal de São Carlos (MA thesis).

[21] Camillo, E. & Garófalo, C.A. 1989. Social organization in reactivated nests of three species of *Xylocopa* (Hymenoptera, Anthophoridae) in southeastern Brazil. *Insectes Sociaux,* 36: 92–105.

[22] Roulston, T.H. & Goodell, K. 2011. The role of resources and risks in regulating wild bee populations. *Annual Review of Entomology*, 56: 293–312.

[23] Sihag, R.C. 1993. Behaviour and ecology of the subtropical carpenter bee, *Xylocopa fenestrata*, nest preferences and response to nest translocation. *Journal of Apicultural Research*, 32: 102–108.

[24] Oliveira-Filho, J.H. & Freitas, B.M. 2003. Colonização e biologia reprodutiva de mamangavas (*Xylocopa frontalis*) em um modelo de ninho racional [Colonization and reproductive biology of mammals (*Xylocopa frontalis*) in a rational nest model]. *Ciência Rural,* 33: 693–697.

[25] Sihag, R.C. 1993. Behaviour and ecology of the subtropical carpenter bee, *Xylocopa fenestrata* F.8. Life cycle, seasonal mortality, parasites and sex ratio. *Journal of Apicultural Research*, 32: 109–114.

[26] Nishida, T. 1963. *Ecology of the pollinators of passion fruit*. Honolulu. Technical Bulletin of the University of Hawaii, Honolulu.

[27] Kapil, R.P. & Dhaliwal, J.S. 1969. Biology of *Xylocopa* species. II. Field activities, flight range and trials on transportation of nests. *Journal of Research, Punjab Agricultural College*, 6: 262–271.

[28] Roubik, D.W. & Gemmill-Herren, B. 2016. Developing pollination management plans across agricultural landscapes: quo vadis, sustainable crop pollination? In B. Gemmill-Herren, ed. *Pollination services to agriculture: sustaining and enhancing a key ecosystem service*, pp. 131–152. London and New York, Earthscan/Routledge.

[29] Camillo, E. 2003. *Polinização do maracujá* [Pollination of passion fruit]. Ribeirão Preto, Holos Editora.

[30] Camillo, E. 1978. Polinização do maracujazeiro [Pollination of passion fruit]. In *Anais do simpósio sobre a cultura do maracujazeiro*, 2, pp. 32–39. Jaboticabal, UNESP.

[31] Mardan, M. 1995. Varied pollinators for Southeast Asian crops. In D.W. Roubik, ed. *Pollination of cultivated plants in the tropics*, pp. 142–149. Agricultural Services Bulletin 118. Rome, FAO.

[32] Camillo, E. 2000. Polinização do maracujazeiro: mamangavas x africanizadas [Pollination of passion fruit: mamanavas x Africanized]. In *Anais do Congresso Brasileiro de Apicultura*, 13. Florianópolis, Confederação Brasileira de Apicultura.

[33] Camillo, E. 1998. Polinização: abelhas solitárias [Pollination: solitary bees]. In *Anais do Congresso Brasileiro de Apicultura*, 12, pp. 107–112. Salvador, BA, Confederação Brasileira de Apicultura.

[34] Marchi, P. & Melo, G.A.R. 2010. Biologia de nidificação de *Xylocopa* (*Neoxylocopa*) *frontalis* (Olivier) (Hymenoptera, Apidae, Xylocopini) em Morretes [Nesting biology of *Xylocopa* (*Neoxylocopa*) *frontalis* (Olivier) (Hymenoptera, Apidae, Xylocopini) in Morretes]. *Oecologia Australis*, 14: 210–231.

[35] Pereira, M. & Garófalo, C. A. 2010. Biologia da nidificação de *Xylocopa frontalis* e *Xylocopa grisescens* (Hymenoptera, Apidae, Xylocopini) em ninhos-armadilha [Biology of nesting of *Xylocopa frontalis* and *Xylocopa grisescens* (Hymenoptera, Apidae, Xylocopini) in trap nests]. *Oecologia Australis*, 14: 193–209.

[36] Camillo, E. 1998. Estudos sobre o incremento dos polinizadores (Hymenoptera, Apidae, Xylocopini) do maracujá amarelo *(Passiflora edulis* f. *flavicarpa* Deg.) [Studies on the increase of the yellow passion fruit (*Passiflora edulis* f. *flavicarpa* Deg.*)* Pollinators (Hymenoptera, Apidae, Xylocopini)]. In *Anais do Congresso Brasileiro de Apicultura*, 12, pp. 134–136. Salvador, BA, Confederação Brasileira de Apicultura.

[37] Marchi, P. & Alves-dos-Santos, I. 2013. As abelhas do gênero *Xylocopa* Latreille (Xylocopini, Apidae) do Estado de São Paulo, Brasil [Bees of the genus *Xylocopa* Latreille (Xylocopini, Apidae) from the State of São Paulo, Brazil]. *Biota Neotropica*, 13: 249–269.

[38] Ulmer, T. & MacDougal, J.M. 2004. *Passiflora: passion flowers of the world*. Portland, OR, Timber Press.

[39] Deginani, N.B. 2001. Las especies argentinas del género *Passiflora* (Passifloraceae) [The Argentine species of the genus *Passiflora* (Passifloraceae)]. *Darwiniana*, 39: 43–129.

[40] Meletti, L.M.M. 1998. *Caracterização agronômica de progênies de maracujá-amarelo (*Passiflora edulis *f.* flavicarpa O.Deg.*)* [Agronomic characterization of yellow passion fruit progenies (*Passiflora edulis* f. *Flavicarpa* O.Deg.)]. Piracicaba, SP, Tese (Doutorado) – Escola Superior de Agricultura "Luiz de Queiros".

[41] Meletti, L.M.M. & Brückner, C.H. 2001. Melhoramento genético [Genetic enhancement]. In C.H. Brückner & M.C. Picanço, eds. *Maracujá: tecnologia de produção, pós-colheita, agroindústria, mercado* [Passion fruit: production technology, post-harvest, agro-industry, market], pp. 345–385. Porto Alegre, Cinco Continentes.

[42] FAO. 2011. *Tropical Fruits Compendium*. Committee on Commodity Problems, Intergovernmental Group on Bananas and Tropical Fruits, 5th Session, Yaoundé, Cameroon, 3–5 May 2011.

[43] Furlaneto, F.P.B., Martins, A.N., Esperancini, M.S.T., Vidal, A.A. & Okamoto, F. 2011. Custo de produção do maracujá-amarelo (*Passiflora edulis*) [Production cost of yellow passion fruit (*Passiflora edulis*)]. *Revista Brasileira de Fruticultura*, 33: 441–446.

[44] Roubik, D.W., ed. 1995. *Pollination of cultivated plants in the tropics*. Agricultural Services Bulletin 118. Rome, FAO.

[45] Silva, C.I., Marchi, P., Aleixo, K.P., Silva, B.N., Freitas, B.M., Garófalo, C.A., Imperatriz-Fonseca, V.L., Oliveira, P.E.A.M. & Alves-dos-Santos, I. 2014. *Manejo dos polinizadores e polinização de flores do maracujazeiro* [Management of pollinators and pollination of passion fruit flowers]. São Paulo, Brazil, Institute of Advanced Studies of the University of São Paulo and Ministry of the Environment.

[46] Bruckner, C.H., Casali, V.W.D., Moraes, C.F., Regazzi, A.J. & Silva, E.A.M. 1995. Self-incompatibility in passion fruit (*Passiflora edulis* Sims). *Acta Horticulturae*, 370: 45–57.

[47] Gilmartin, A.L. 1959. Post-fertilization seed and ovary development in *Passiflora edulis* Sims. *Tropical Agriculture*, 35: 41–58.

[48] Siqueira, K.M.M. de, Kill, L.H.P., Martins, C.F., Lemos, I.B., Monteiro, S.P. & Feitoza, E.A. 2009. Ecologia da polinização do maracujá-amarelo, na região do Vale do Submédio São Francisco [Ecology of pollination of yellow passion fruit in the region of the Submédio São Francisco Valley]. *Revista Brasileira de Fruticultura*, 1: 1–12.

[49] Hammer, I.H. 1987. The pollinators of yellow passion fruit – do they limit the success of *Passiflora edulis f. flavicarpa* as a tropical crop? *Proceedings of the Florida State Horticultural Society*, 100: 283–287.

[50] Benevides, C.R., Gaglianone, M.C. & Hoffmann, M. 2009. Visitantes florais do maracujá-amarelo (*Passiflora edulis* f. *flavicarpa* Deg. Passifloraceae) em áreas de cultivo com diferentes proximidades a fragmentos florestais na região Norte Fluminense, RJ [Passionflower (*Passiflora edulis* f. *Flavicarpa* Deg. Passifloraceae) flower visitors in cultivated areas with different forest fragments in the North Fluminense region, RJ]. *Revista Brasileira de Entomologia*, 53: 415–421.

[51] Yamamoto, M., Silva, C.I., Augusto, S.C., Barbosa, A.A.A. & Oliveira, P.E.A.M. 2012. The role of bee diversity in pollination and fruit set of yellow passion fruit (*Passiflora edulis f. flavicarpa*, Passifloraceae) crop in Central Brazil. *Apidologie*, 43: 51–62.

[52] Silva, C.I., Ballesteros, P.L.O., Palmero, M.A., Bauermann, S.G., Evaldit, A.C. & Oliveira, P.E.A.M. 2010. *Catálogo Polínico – palinologia aplicada em estudos de conservação de abelhas do g*ênero Xylocopa [Polynesian Catalogue – applied palynology in conservation studies of bees of the genus Xylocopa]. Uberlândia, EDUFU.

[53] Silva, C.I. 2009. *Distribuição espaço-temporal de recursos florais utilizados por* Xylocopa *spp. e interação com plantas de Cerrado sentido restrito no Triângulo Mineiro* [Spatial distribution of floral resources used by *Xylocopa* spp. and interactions with Cerrado sentido restrito in the Triângulo Mineiro]. 287 f. Tese (Doutorado). Uberlândia, MG, Universidade Federal de Uberlândia.

[54] Giannini, T.C., Acosta, A.L., Silva, C.I., Oliveira, P.E.A.M, Imperatriz-Fonseca, V.L. & Saraiva, A.M. 2013. Identifying the areas to preserve passion fruit pollination service in Brazilian tropical savannas under climate change. *Agriculture, Ecosystems & Environment,* 171: 39–46.

[55] Freitas, B.M., Silva, C.I. & Bezerra, A. D. M. 2017. The illustrated natural history of a pollinator: the Carpenter bee *Xylocopa frontalis*. São Paulo: A.B.E.L.H.A.

Chapter 16

MANAGING HONEY BEE COLONIES AGAINST PARASITIC MITES, PESTS, PATHOGENS AND PESTICIDES

D. Sammataro and L. de Guzman

The performance and productivity of honey bee colonies depends greatly on their health. In the United States, devastating parasites, pests and diseases have recently been introduced, significantly decreasing beekeepers' profit. Honey bee colonies are also being transported over long distances to meet the pollination needs of different crops. Some crops that need pollination are very poor sources of nectar and pollen, adding more strain to already troubled colonies. In addition to the chemicals applied inside the hives to control parasites, pests and diseases, the crops are also sprayed with pesticides that are highly toxic to bees [1–17].

16.1 PARASITIC MITES

16.1.1 *Varroa* destructor

Infestation by *Varroa* mites remains the number one enemy of *A. mellifera* in much of the world, except Asia where a new, emerging mite pest of the bees, *Tropilaelaps*, is found [18] (Figure 16.1). Both mites now constitute a major problem for the European honey bee (*A. mellifera*), although different stocks of bees respond differently to parasitic mites, pests and diseases. The problem arises when bee colonies used for pollination need to build their populations rapidly, which makes them more susceptible to mite infestation and the honey bee viruses they can vector.

Figure 16.1
(A) VENTRAL AND (B) DORSAL VIEWS OF VARROA DESTRUCTOR, (C) *TROPILAELAPS* SP.

Source: Photos by D. Sammataro (a and c) and S. Bauer (b).

Varroa destructor draws its nourishment from developing hosts and results in wing deformation, reduced longevity, reduced weight and adult size, and sometimes death of young brood [19–22]. The feeding of *Varroa* mites generally leads to a complex of symptoms known as Parasitic Mite Syndrome (PMS) [23]. A reduction in the number of capped brood and the bee population is evident in spring and summer when colonies have high *Varroa* infestations in the Mediterranean region [24]. *Varroa* mites also reduce the weight and survival of drones, presenting a serious problem for queen breeders who need to maintain large numbers of drones for successful mating [25, 26]. Honey bee queens mate with 10 to 30 drones [27] and the limited availability of mature drones during mating indirectly contributes to colony loss. Queens are less likely to survive if they are mated with fewer drones [28], which reduces the genetic diversity among workers. Genetically diverse colonies are more resilient to various pathogens and other types of stressors [29, 30] and have higher foraging rates [31].

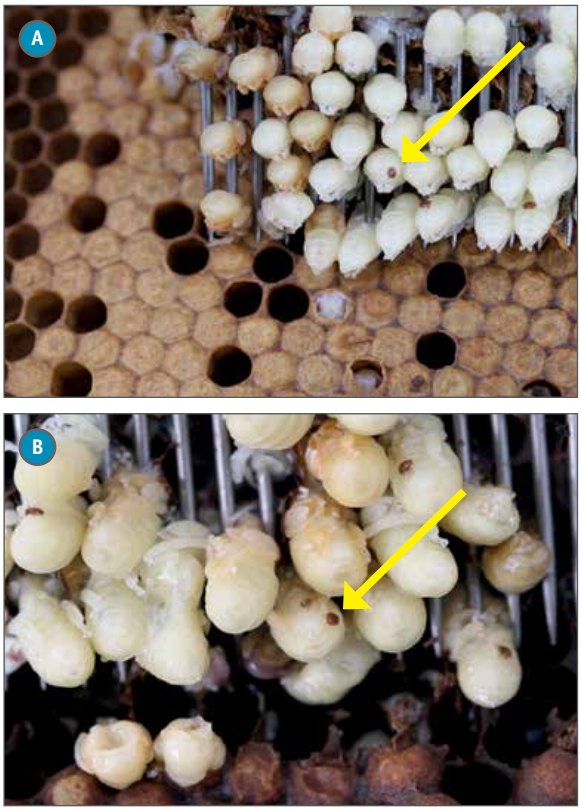
The varied and often overwhelming stresses may negatively affect the behaviour of infested bees. For example, bees infested with *Varroa* mites (or bees experimentally infested with *Varroa* later as adults) lose their ability to effectively forage, or their homing ability may be compromised [32]. High *Varroa* infestation can also compromise the recruitment of foragers to resources [33].

16.1.2 *Varroa* detection

It is important to monitor honey bee colonies for *Varroa* to establish economic threshold levels (parasite densities that call for control measures) and to determine the efficacy of control measures. A complete guide to detecting and sampling for *Varroa* mites has been published [34].

Examining the brood cells. Worker brood is examined by removing about 100 dark-eyed pupae using forceps. A "cappings-scratcher" can also be used to remove pupae in groups, especially those that are close to the bottom of a frame (Figure 16.2).

Figure 16.2
(A) WORKER PUPAE AND (B) DRONE PUPAE PULLED UP, WITH A CAPPINGS-SCRATCHER WITH *VARROA* MITES ATTACHED TO THE BROOD (ARROWS)

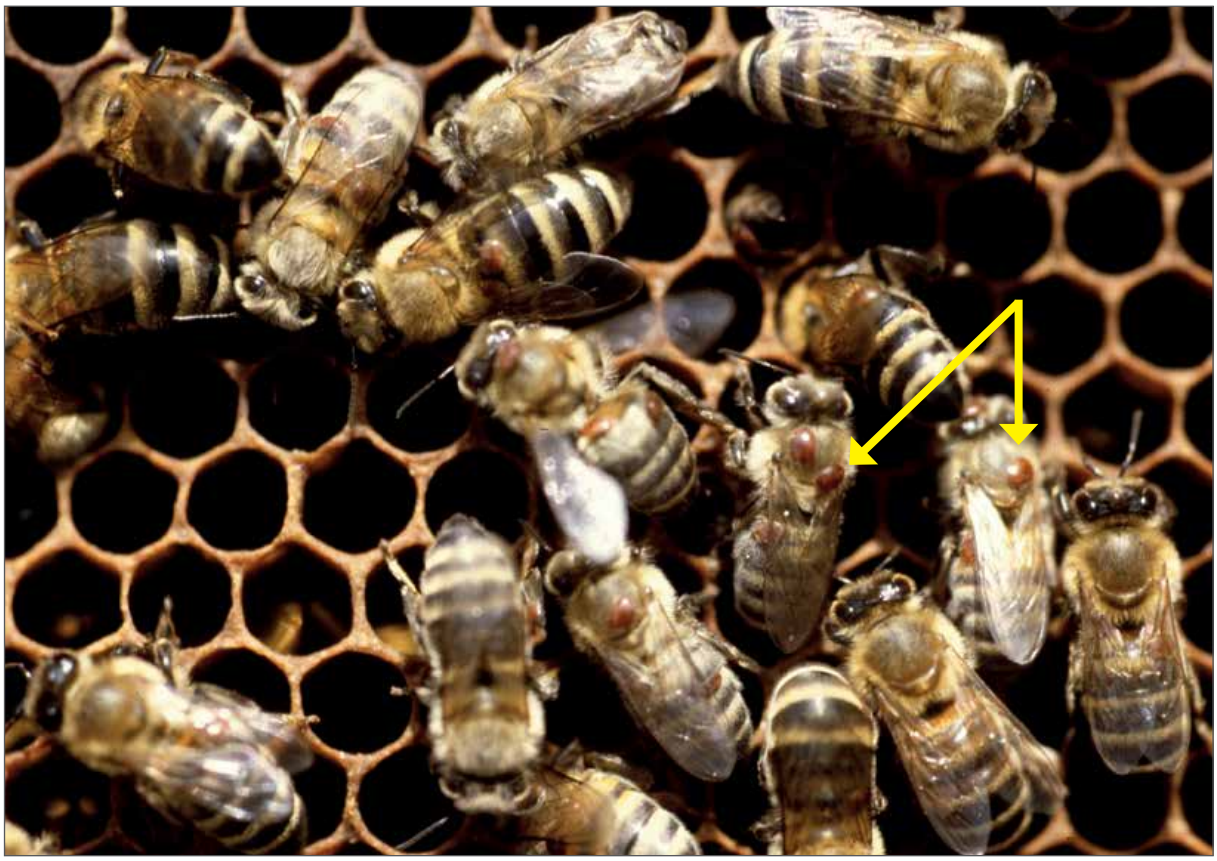


Source: Photos by A. Frake.

Adult *Varroa* mites are brown in colour and can easily be seen adhering to the bodies of bee pupae. Sometimes young mites and feces are also visible. The presence of feces or white spots on the cell walls can also indicate mite presence when none are seen on pupae. An infestation level < 5 percent of pupae indicates low mite numbers, while ≥ 25 percent indicates a severe infestation (L. de Guzman, pers. obs.). This method is more easily perfomed with drone brood because the brood has larger, raised cells and is easier to pull up.

Examining adult bees. If there is no brood, adult bees can also be examined for phoretic *Varroa* mites (or mites attached to adult bees; see Figure 16.3 using three different methods:

Figure 16.3
ADULT WORKERS WITH *VARROA* MITES ADHERING TO THEIR BODIES (ARROWS)



Source: Photo by L. de Guzman.

- **Powdered sugar method**. Collect about 300 bees (less than one cup or 100 mL) from the brood chamber by shaking the bees from at least two brood frames into a nuc-box, plastic tub or bucket. Find the queen first, before shaking the bees off the frames. Using a wide-mouthed jar (e.g. Mason jar) add bees to the jar, then sprinkle in two tablespoons (25 g or 1 oz.) of powdered sugar or flour, close the jar with a lid and shake the bees for 1 to 2 minutes to evenly coat the bees and dislodge the mites (Figure 16.4). Replace the lid with an 8-mesh wire screen (8 divisions per inch, approximately 3 mm between each mesh) and invert the jar while shaking the bees over a tray or a sheet of white paper, to remove the mites and powdered sugar. Spread the sugar thinly to facilitate mite detection. Repeat if necessary by adding more powdered sugar to the jar. Return the sugar-coated bees to the colonies (Figure 16.4b, c) when done. To determine the level of infestation, count the numbers of *Varroa* mites and bees. A mite count of 0–15 (per 300 bees) is low enough to wait before treatment, while a count of over 30 (10 percent) indicates a need for intervention by the beekeeper to address the infestation [35]. This is calculated by dividing the number of mites counted by three (mites per 100 bees) and multiplying by a correction factor of two (mites in brood) [35]. It is best to sample at least eight colonies in each apiary to obtain a clearer picture of mite loads.

Figure 16.4
(A) BEES SHAKEN IN A JAR WITH POWDERED SUGAR TO DISLODGE THE MITES, (B) BEES BEING RETURNED TO THE COLONY, AND (C) A MIST OF WATER BEING SPRAYED TO EXPOSE MITES COVERED WITH POWDERED SUGAR

Source: Photos by A. Frake (a) and D. Sammataro (b and c).

- **Soapy water wash method.** Collect about 300 adult bees in a jar or a plastic container, as described above, and add water and some liquid dishwashing detergent (Figure 16.5). Shake the bees vigorously and pour them through a strainer using a screen mesh large enough to allow mites to pass through, but small enough to catch the bees. As above, an infestation rate of over 10 percent is a treatable number [35].
- **Monitoring natural mite-drop using sticky board.** Commercial sticky boards can be obtained at bee supply companies, either with a sticky substance and marked with blackened areas (for easy counting) or with no glue applied. Non-commercial boards can be made using plastic signboards cut to fit the bottom of the hive. Apply a mixture of petroleum jelly and vegetable oil (1:1) to trap mites. However, in especially hot climates, the oil/jelly mixture often melts and is ineffective (use a sticky insect trap glue, such as Tanglefoot™) [36] (Figure 16.6). Place 8-mesh screen (8 squares to the inch) on top of each board to prevent bees from cleaning the traps. Insert the traps for at least 24 hours or up to three days, and then count the mites that drop. *Varroa*

Figure 16.5
(A) COLLECTING BEES FOR THE WASH METHOD OF COUNTING MITES, AND (B) USING A RULER TO MARK WITH TAPE AT THE 300-BEE-MARK (AROUND 2 INCHES IN A QUART JAR)

Source: Photos by A. Frake (a) and D. Sammataro (b).

mites are oval and brown in colour and should not be confused with wax scales or pollen pellets of bee origin. When in doubt, probe any dark object that might be a mite; pollen and wax scales will easily break apart when probed, whereas mites are hard to the touch and do not fall apart. Count the number of *Varroa* mites (to determine the level of infestation) that drop naturally on a sticky board, left in for 24 hours. An overnight mite-drop of < 20 mites does not require treatment [37].

It is important message that mites are monitored on a regular basis to determine the change in mite population over time. If mite infestation increases significantly, beekeepers should intervene with a treatment.

16.1.3 Control of *Varroa*

A number of acaricides are available for used to control parasitic mites. It is important that beekeepers refer to product labels for instructions on specific products. Wearing gloves or safety gear is essential. It is critical to remember that **no acaricide or antibiotic** should be administered to honey bee colonies during a honeyflow, as honey is a food product that can be contaminated.

Cultural controls, such as the use of screened bottom boards, help to reduce mite populations by preventing re-entry of *Varroa* mites that have dropped onto the bottom board. Also, the value of using *Varroa*-resistant stocks to mitigate mite problems has been well established [9]. Two USDA-developed stocks (Russian honey bees [RHB] and *Varroa* Sensitive Hygienic [VSH] bees) have economically useful levels of resistance to *V. destructor* [9]. The use of resistant bee stock is the best way of maintaining colony health, and involves little use of chemicals. Botanical oils and organic acids are sometimes used to control *Varroa*, with mixed success.

Figure 16.6
A) BOARDS INSERTED INTO COLONIES; TWO TYPES OF BOARDS ARE SHOWN, B) THE BOARD WITH THE BLACKENED AREAS SHOULD BE INSERTED FOR THREE DAYS

Source: Photos by A. Frake (a) and D. Sammataro (b).

Figure 16.7
(A) SIZE COMPARISON OF *VARROA* (LEFT) AND *TROPILAELAPS* (RIGHT) MITES, (B) MALE *TROPILAELAPS*, AND (C) FEMALE *TROPILAELAPS*

Source: Photos by K. Khongphinitbunjong.

16.1.4 *Tropilaelaps* mites

The mite genus *Tropilaelaps* has recently been reviewed and there are now four recognized species. *Tropilaelaps clareae* occurs in Asia, where it is a parasite of the native honey bee *Apis breviligula*. It also parasitizes the introduced Western hive bee *A. mellifera* in the Philippines and the native honey bee *A. binghami* on Sulawesi Island, Indonesia. *T. mercedesae* (often mistaken for *T. clareae*), together with *T. koenigerum*, parasitizes the native *A. dorsata* in mainland Asia and Indonesia (except Sulawesi Island). *Tropilaelaps mercedesae* also parasitizes the introduced *A. mellifera* in those and surrounding regions, while *T. thaii* parasitizes *A. laboriosa* in the mountainous Himalayan region [8].

Life cycle. The foundress *Tropilaelaps* lays from one to four eggs on mature bee larvae shortly before the brood cell is capped (it should be noted that as many as a dozen foundresses may infest one individual brood cell). As with *Varroa*, drone brood is preferred and may be 100 percent parasitized [7]. The mite progeny, usually one male (Figure 16.7b) and several females (Figure 16.7c), feed on and seriously damage the brood. Development of the mite requires about one week. The young adults, including the foundress female, then emerge with the adult bee and begin to search for new hosts. The short life cycle, as well as a very brief stay on adult bees, explains why populations of *Tropilaelaps* increase faster than those of *Varroa* mites. When both *T. clareae* and *V. destructor* infest the same colony, the former may out compete the latter [7, 38]. When both mite species are present in the same cell, their reproduction declines [39].

Phoretic survival of the mites on adult bees lasts only one to three days because *Tropilaelaps* cannot feed on adult bees, being unable to pierce the integument [40, 41]. The phoretic stage plays a major part in the life cycle and usually ranges from 5 to 10 days [42, 43]. Gravid female mites die within two days unless they deposit their eggs [44].

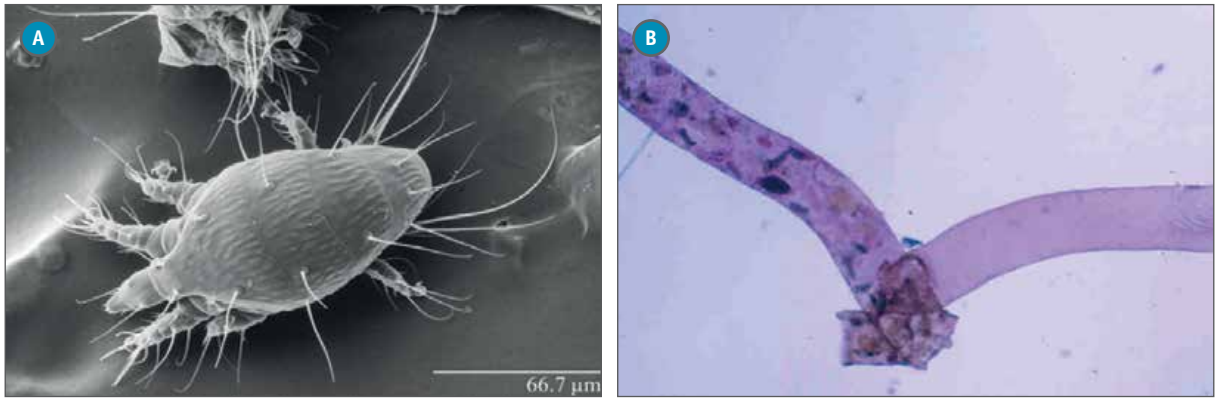
Much like *Varroa*, infestation by *Tropilaelaps* causes the death of many bee larvae (up to 50 percent), resulting in an irregular brood pattern. Many malformed bees are evident, with distorted abdomens and deformed wings or legs. Affected bees can be seen crawling at the hive entrance [45]. In addition, opened brood areas are also present, as a result of sanitation activities by worker bees, which remove the infested bee pupae or young adults. Some infested colonies abscond, carrying the mites to a new location.

Identification and detection. The first sign of *Tropilaelaps* infestation is often the presence of small, red-brown, elongate mites on the combs or on adult bees. *T. clareae* (< 1 mm in length) and *T. mercedesae* (< 0.9 mm in length) differ in size but are otherwise similar in appearance; *T. koenigerum* is slightly smaller at about 0.7 mm in length [46]. The females also differ in the structure of their ventral anal plate and subapical tooth of the chelicerae (mouthparts) [8]. The mite is easily differentiated from *Varroa* using a 10× magnifying glass. *Varroa* is wider than its length and moves slowly; *Tropilaelaps* is elongate, and moves quickly [47] (Figure 16.7a). Methods of detecting and collecting *Tropilaelaps* are discussed in [47]. Pulling up brood with a cappings-scratcher, as for *Varroa,* is the easiest method to assess mite presence, while the "bump test" is used to monitor *Tropilaelaps* infestation [48]. Because *Tropilaelaps* are quick to exit when a brood cell is opened, the best method to assess mite infestation is to cut and freeze a brood frame or section.

16.1.5 The *Acarapis* complex

Three *Acarapis* species parasitize *A. mellifera*. Two species, *Acarapis dorsalis* and *A. externus*, parasitize the host externally, while one, *A. woodi* (Rennie), lives and reproduces inside the tracheae of honey bees (Figure 16.8). The three species can co-exist on a single colony [49, 50]; however, concurrent infestation by the three species on a single bee is rarely seen. Although all species feed on haemolymph, the two external *Acarapis* are normally harmless to honey bees. However, their impact on bee colonies may be greater than generally perceived. Colonies highly infested with *A. externus* in Oregon apparently die from such infestation [49, 51]. It is not known if the mites transmit or carry honey bee viruses.

Figure 16.8
(A) SEM IMAGE OF FEMALE TRACHEAL MITE, *ACAPARIS WOODI*, AND (B) STAINED TRACHEAL TUBES SHOWING INFESTED (LEFT) AND CLEAN (RIGHT) TUBES



Source: Photos by D. Sammataro.

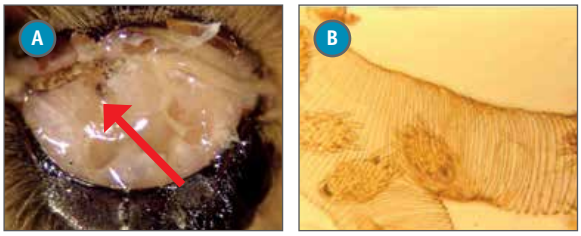
Acarapis woodi has presented a serious problem in the United States, making it an intensely studied species. The damage to bee colonies includes a shortened lifespan of adult bees [52]. Significant winter losses are also associated with high levels of infestation [53]. With the introduction of *Varroa* and its control, the mite has almost disappeared in some areas.

Detection of *Acarapis woodi*. Several methods can be used to detect tracheal mites [54, 55]. Thoracic dissection is the easiest and most reliable method (Figure 16.9), and should be undertaken as follows:

- Dissect about 30 worker bees per colony.
- Collect bees from under the hive covers or from outer frames to obtain older bees. Drones can also be sampled.
- Count the number of infested bees and examined bees to determine the levels of infestation. If tracheal mite infestation occurs in less than 15 percent of the sample, no treatment is required [53].

Control of *Acarapis*. Where *Varroa* mites are controlled with acaricides, tracheal mites are seldom found. When detected, those mites are controlled with menthol crystals as well as vegetable shortening/sugar patties [55, 56]. *Varroa*-resistant bee lines, RHB and VSH, are also resistant to *A. woodi*. The use of resistant bee stock is the best way of maintaining colony health with minimal pesticide use.

Figure 16.9
(A) EXPOSED PROTHORACIC TRACHEAE SHOWING INFESTATION (ARROW), AND (B) TRACHEAL TUBE SHOWING SILHOUETTES OF DIFFERENT STAGES OF TRACHEAL MITES



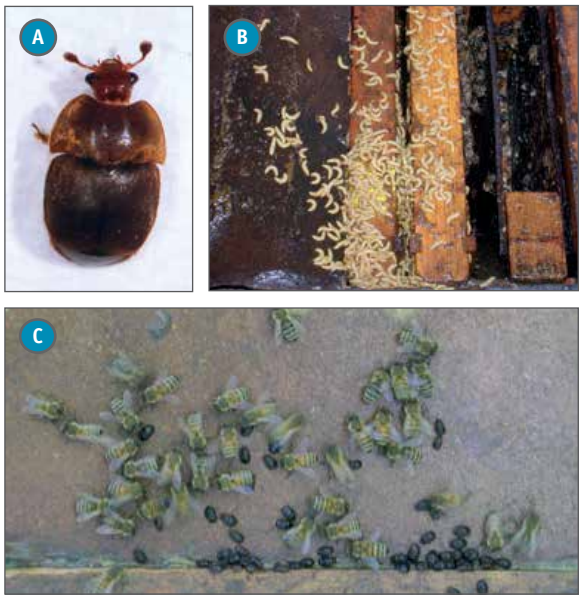
Source: Photos by L. de Guzman.

16.2 INSECT PESTS OF HONEY BEES

16.2.1 The small hive beetle (SHB)

Aethina tumida (the small hive beetle [SHB], family Nitidulidae) presents a serious emerging threat to beekeepers in the southern United States, and also among stingless honey bees (Meliponini in Africa) (Figure 16.10) (see Chapter 13). SHB has also been recorded at damaging densities and is an important scavenger of honey bee colonies in Australia and the United States [57, 58]. Both adults and larvae of SHB feed on brood, honey, pollen and wax comb. The beetles also defecate on combs, which can cause pollen and honey to ferment via yeast identified as *Kodamaea ohmeri* [59]. Destruction of a colony's food supply also results in the loss of bee equipment, which cannot be sterilized after heavy infestation.

Figure 16.10
(A) ADULT SMALL HIVE BEETLE (SHB), (B) LARVAE OF SHB ON THE TOP BARS OF A MATING NUCLEUS COLONY, AND (C) ADULT SHBS AND WORKER BEES ON AN INNER COVER OF A HIVE



Source: Photos by A. Frake (a) and L. de Guzman (b and c).

Figure 16.11
THE LARVA OF A SHB (LEFT) AND A WAX MOTH (RIGHT)

Source: Photos by A. Frake.

Detection. Detection of SHB is relatively easy [60]. The mature adult SHB is dark brown to black in colour and about 5–7 mm long, or two-thirds the size of a worker bee (Figure 16.10c). SHB adults can easily be seen scurrying on inner covers, top bars, edges of frames, hive walls and bottom boards when infested hives are opened, especially in small, weak colonies. They destroy weak colonies very quickly in warmer regions.

SHB and wax moth (*Galleria mellonella*) can infest the same colony, and SHB larvae are often confused with wax moth larvae. They can be distinguished by the two rows of dorsal spines on the beetle larva. In addition, the head capsule of *Aethina* is smaller and has well-defined segments. Wax moth larvae do not have spines and are longer than SHB larvae (Figure 16.11). SHB larvae have tougher skins, and thus do not burst open readily when squeezed.

Control. The following are the ten best management practices for SHB control [60]:

- Remove dead colonies from apiaries as soon as possible. One dead colony produces thousands of beetle larvae, which then pupate in the soil. Prompt action prevents larvae from reaching the adult stage (wandering phase).
- Keep colonies strong. Do not stack infested supers onto strong colonies. Freeze lightly infested combs before re-using, and burn heavily infested ones.
- Maintain queenright colonies only. Do not allow queenless colonies to become weak or to turn into drone layers. Queenlessness attracts beetles, and small amounts of brood or pollen encourage beetles to reproduce.
- Do not add supers or put brood on top of supers if bees cannot take care of them. Similarly, avoid putting brood frames against the inside hive wall, and squeezing brood against the wall of the box or adjacent frames. Such spaces or empty frames act as hiding places for SHB, while damaged brood attracts beetles and stimulates reproduction.
- Remove (excess) burr combs and propolis (corals), as these are used as hiding places by adult beetles. Bees remove exposed SHBs.

- Place colonies in the sun. Beetle traps positioned in shaded apiaries trap more than those in the sun.
- When feeding colonies with pollen supplement, provide just enough patties to be consumed in two days. An excess of protein supplements supports SHB reproduction.
- Keep in-hive feeders and bottom boards clean. Dead bees in feeders, and pollen and dead bees on bottom boards are a protein source, which stimulates SHB reproduction.
- Keep honey houses neat and clean. Store frames with pollen and brood in a freezer (such frames are the main source of protein for stimulating beetle reproduction).
- Extract honey two or three days before massive hatching of SHB eggs occurs. Return wet supers to hives immediately. If done within four days, allow wet supers to be cleaned out by bees before returning them to colonies; store them in a cooler to reduce beetle attraction. Melt all wax cappings as soon as possible.

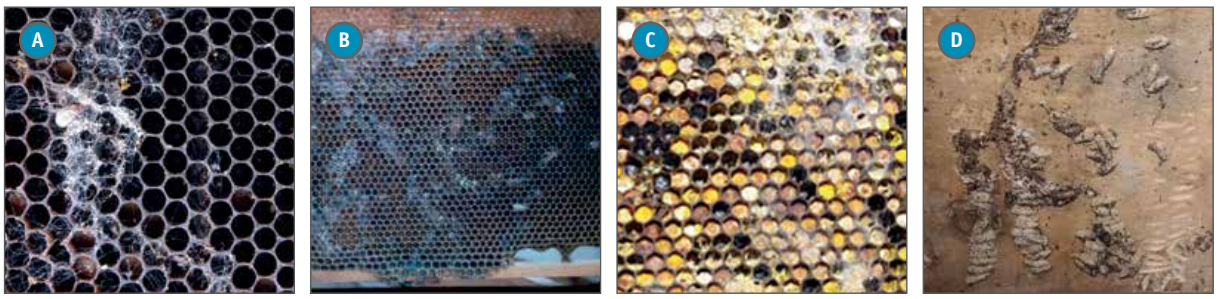
There are chemicals on the market that are used to control SHB. Those containing coumaphos (at 10 percent) are used to regulate SHB populations inside hives by stapling strips onto a piece of cardboard or corrugated plastic. Because adult beetles frequently hide, plastic corrugated cardboard (with popsicle sticks stapled on both ends) should be placed towards the rear of the bottom board. Do not treat hives during honey flow. Soil-drenches can kill pupae developing in the soil. Both chemicals should be used appropriately and according to label directions. Misuse of any chemical may result in the contamination of honey, pollen and beeswax.

16.2.2 Wax moths

The greater wax moth, *Galleria mellonella*, and the lesser wax moth, *Achroia grisella* (family Pyralidae), are serious pests of stored wax combs, especially in warmer regions. Like SHB, they are opportunistic scavengers and constitute a problem for colonies weakened by mite infestation or other stressors [61]. Wax moths are most destructive at the larval stage. Larvae make silk tunnels throughout the comb as they burrow to feed on wax, honey, pollen or brood (Figure 16.12). Tunnels provide protection from bees that would otherwise remove or kill larvae. Damage to combs and beekeeping equipment caused by wax moths is more pronounced when combs are stored in dark, warm and poorly ventilated areas. Larvae also can damage wood by chewing and burrowing while spinning their cocoons (Figure 16.12d).

Control. Worker honey bees invest significant energy in making wax and drawing combs. Thus, wax comb frames should be protected properly when not in use. Management practices for SHB, mentioned above, are largely applicable for wax moth control. In general, worker bees suppress populations if the colony is strong. It is therefore important not to add supers when the bees cannot take care of them. Keep bottom boards clean from debris, and maintain cleanliness in the honey house. Allow all wet supers to be cleaned by bees first before storing them. Do not store combs

Figure 16.12
(A–B) WAX MOTH LARVAE AND SILK TUNNELS, (C) SILK TUNNELS ON A FRAME OF POLLEN, AND (D) COCOONS OF THE WAX MOTH SHOWING DAMAGE TO THE WOOD CAUSED BY PUPATING LARVAE



Source: Photos by D. Sammataro (b) and L. de Guzman (a, c and d).

with brood or pollen. For lightly infested combs, freeze or clean out infested comb areas to kill larvae and eggs. Burn seriously damaged combs.

Paradichlorobenzene (PDB) crystals are used in the United States to protect stored combs from wax moth damage. Combs should be free of honey prior to treatment with PDB. Allow bees to clean wet supers before treatment. Do not use PDB to control wax moth in active honey bee colonies. Since PDB is toxic to honey bees, allow treated combs to be aired out (for at least seven days) before using them again. A formulated *Bacillus thuringiensis* (bacterial) biocide product can also be sprayed on stored combs to kill larvae. At present, this is available for use in Canada and some European countries. It is important to read the label for instructions. Other controls include ozone chambers and gamma ray irradiation, and newer techniques are being developed. However, the best way to keep wax moths from harming colonies is to maintain strong bee populations [61].

16.2.3 Other pests

While not a problem in temperate climates, ants (Hymenoptera: Formicidae) can be a serious pest in subtropical areas, attacking brood and bees as they forage, or raiding inside the hives. So-called carpenter ants (*Camponotus* spp.) and predacious wasps (Vespidae) can also present a problem in certain areas. Termites (Isoptera) can destroy wooden hive equipment.

Mammals and reptiles can also be a problem. Lizards often eat bees on the ground, and skunks (family Mustelidae), weasels, badgers, raccoons (family Procyonidae), and also mice (*Mus, Micromys, Clethrionomys, Peromyscus*) are serious pests to bees. Skunks and raccoons often visit hives in the early evening as well as during the day [17], eating bees and disturbing the colony. Bears (Ursidae) can eat brood and honey, and cause extensive damage to equipment. Electric fencing usually keeps these pests away from bee colonies. Placing hives on stands off the ground will keep smaller pests out. Certain birds can also be a nuisance, feeding on bees at the hive entrance (D. Sammataro, pers. obs).

16.3 PATHOGENS

Honey bees face challenged from many bacterial, fungal and viral pathogens. Correct diagnoses of such diseases help to determine which control measures to apply.

16.3.1 Viruses

Several viruses are associated with honey bees [62]. Deformed Wing Virus (DWV) is commonly associated with parasitism by *Varroa* [63] and *Tropilaelaps mercedesae* [64, 65]. Both present symptoms similar to PMS [23] (Figure 16.13). Interaction between mite parasitism and viral levels magnifies their negative impact on honey bee health. Resistance to *Varroa* mites is known to vary among stocks [66–68]. However, it is still not known whether *Varroa* resistant stocks are also resistant to virus infection. At present, there are no available control measures against viral infection. Hence, beekeepers try to regulate viral infection by suppressing the *Varroa* population in bee colonies.

16.3.2 *Nosema*

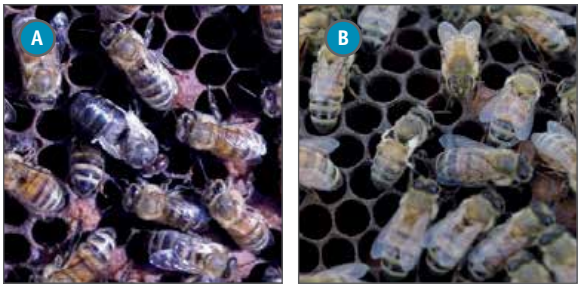
The two species of *Nosema* that currently infect honey bees are *N. apis* and *N. ceranae*. The latter is now ubiquitous in nature and its presence is presumed harmful to infected bees or the colony as a whole. For *N. apis*, typical symptoms of infection are bee defecation by the hive entrance during winter, and a milky coloured midgut. Infection by *N. ceranae* does not cause the same symptoms. Bees infected with *N. apis* decrease pollen foraging [69]. In Spain, *N. ceranae* is the primary cause of colony mortality [70] and suppresses immune response [71]. However, no correlation indicates *Nosema* causes decreased colony size. Perhaps there is a threshold level that bees can tolerate, but that has not yet been established. Worker bees infected with *N. ceranae* reportedly have their foraging and homing ability affected [72]. The bees are likely to become precocious foragers and live shorter lives (possibly due to mortality from predators), compared to uninfected bees [73, 74].

Detection. The standard laboratory technique for detecting and measuring *Nosema* infection is as follows [75]:

- Collect at least 30 worker bees per colony.
- Collect older bees from the hive entrance, under the hive cover or on the edges of the cluster. Older bees will have higher levels of *Nosema* infection.
- Remove the abdomens of the bees and place them in 30 ml distilled water (1 ml per bee).
- Grind the abdomens using a mortar and pestle to release the spores from the tissue.
- Using a small eyedropper, place a single drop of the liquid onto a microscope slide and cover with a cover slip. To determine the degree of *Nosema* infection, use a haemocytometer. Follow instructions that come with the haemocytometer for determining counts or density.
- Examine under a compound microscope (400**x** magnification) – *Nosema ceranae* is smaller and more almond-shaped than *N. apis* (Figure 16.14).

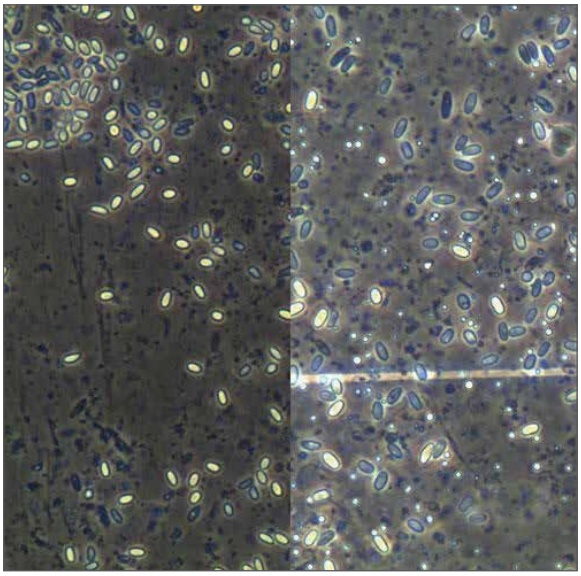
Control. Colonies infected with either *N. apis* or *N. ceranae* can be treated with products based on the fungus *Aspergillus fumigatus*. Read and follow the label directions carefully.

Figure 16.13
(A) A DRONE (LEFT), AND (B) A WORKER BEE WITH DEFORMED WINGS, A SYMPTOM OF DEFORMED WING VIRUS (DWV) INFECTION



Source: Photos by K. Khongphinitbunjong.

Figure 16.14
NOSEMA CERANAE (LEFT) AND *N. APIS* (RIGHT) SPORES VIEWED UNDER THE COMPOUND MICROSCOPE (400X)

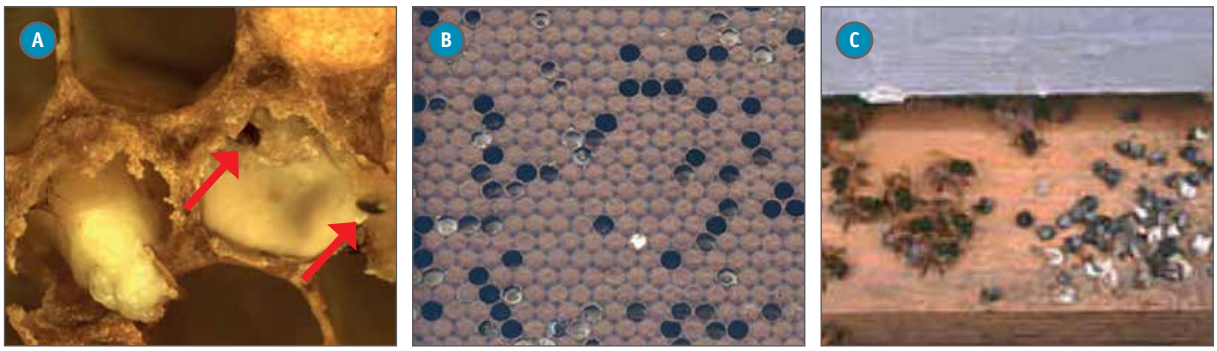


Source: Photos by Z. Huang.

16.3.3 Chalkbrood

Chalkbrood is a honey bee disease caused by the fungus *Ascosphaera apis*. It is easily identified from the white cotton-like mycelia growing on infected larvae (Figure 16.15). At high levels of infection, mummies

Figure 16.15
(A) BEE LARVAE INFECTED WITH CHALKBROOD (NOTE THE PRESENCE OF *VARROA* [ARROWS]), (B) BROOD COMB SHOWING HIGH CHALKBROOD INFECTION, AND (C) CHALKBROOD MUMMIES AT HIVE ENTRANCE



Source: Photos by D. Sammataro (a) and L. de Guzman (b) and USDA–ARS (c).

(hardened infected larvae) are found at the hive entrance or bottom. *Varroa* mites can be found even in mummified larvae (see Figure 16.15a). If chalkbrood is a recurring problem, re-queening colonies often rids them of the disease. There is no known treatment for the fungus, but increasing hive ventilation and replacing old combs is suggested [76]. Chalkbrood may also result from an inherent vulnerability of certain bee stock.

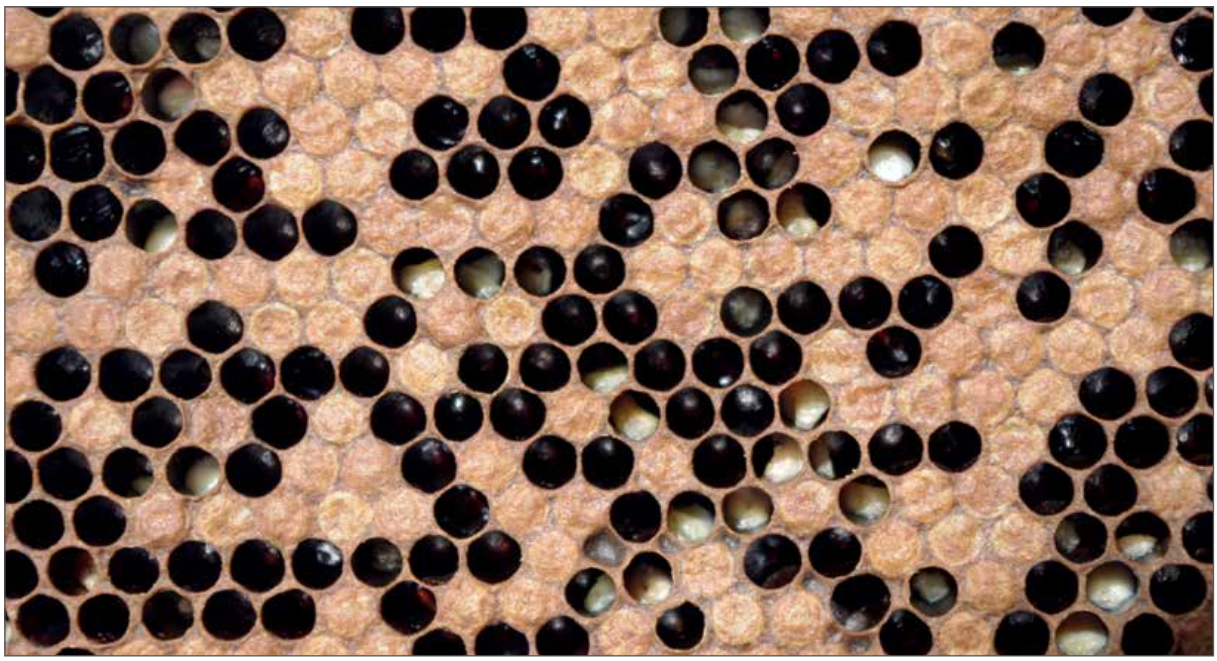
16.3.4 American foulbrood (AFB) disease

American foulbrood disease (AFB) is caused by the bacterium *Paenibacillus larvae*. The infection is characterized by discoloured sunken or punctured brood cappings, a ropey consistency of dead brood (Figure 16.16) and a foul smell. AFB spores are also found in hive products such as honey, pollen and beeswax. However, spore presence can only be demonstrated by their growth in standard culture media. Because the AFB spores persist in combs and hive products for many years, hive equipment from infected colonies are usually burned [77]. **Never use honey from infected colonies to feed other colonies**.

Figure 16.16
FIELD TEST FOR AMERICAN FOULBROOD (EFB); DEAD LARVAE HAVE A ROPEY CONSISTENCY

Source: Photo by V. Williams.

Figure 16.17
DEAD LARVAE INFECTED WITH EUROPEAN FOULBROOD (EFB), SHOWING A SPOTTY BROOD PATTERN ON THE COMB



Source: Photo by L. de Guzman.

16.3.5 European foulbrood (EFB) disease

European foulbrood disease (EFB) is caused by the bacterium *Melissococcus pluton*. Infected larvae are off-white to brown in colour (Figure 16.17). Unlike AFB, death of brood usually occurs before larvae are capped. No odour is present and they lack a ropey consistency. Infected colonies have a spotty brood pattern.

Control. Colonies infected with EFB can be treated with antibiotic oxytetracycline in the United States. Read the labels for instructions. Colonies with AFB should have all infected combs and woodenware burned, and colonies should be re-queened with more resistant lines.

16.4 PESTICIDES

Honey bee colonies are increasingly exposed to a multitude of pesticides and biocides in general (chemicals or biological agents). Such chemicals are suspected to be a major contributor to honey bee colony decline [78] (see also Chapter 2.1). Many examples, aside from those used in agriculture and silviculture, or in fumigation of human disease agents such as mosquitoes, are used to protect the bees themselves. Bees can evolve a resistance to parasites, pests and diseases, but the beekeeper and the agricultural client of managed colonies prefer to use rapid control methods, which are readily available.

Nowadays, acaricides and antibiotics are applied in colonies more than three times a year. The organophosphate coumaphos and the pyrethroid tau-fluvalinate are compounds that can be absorbed and accumulated in the beeswax. Chemical residues can also accumulate in the pollen and honey [79]. In fact, more than 120 pesticides occur in some honey bee colonies [80]. Modernization of the agricultural system relies heavily on pesticide use (see Chapters 1 and 4), with insecticides and fungicides applied to pollinated crops to control various insect pests and plant diseases. Some growers also use chemical attractants to increase bee foraging, particularly with crops not especially attractive to bees. These chemicals usually contain the Nasanov or queen pheromone. Nonetheless, such attractants do not necessarily increase pollination or seed set and can be washed off by rainfall. In addition, herbicides are used to keep orchards free of weeds. All these chemicals are potential contaminants to water sources for honey bees, which need water to maintain colony temperature and to dilute honey to feed the larvae. If no water source is provided, bees collect water from puddles around the orchard, especially when it rains.

Fluvalinate and coumaphos strips are used worldwide to suppress *Varroa* mites. Coumaphos is also administered in the form of strips stapled in corrugated plastic to control SHB within colonies. However, acaricides have negative effects on the reproductive performance of drones and queens. Fluvalinate reduces weight and causes early death of drones [25]. Nonetheless, drones that survive fluvalinate treatment mate and produce offspring [81]. Coumaphos lowers sperm viability in drones exposed to the chemical [82]. Both chemicals affect queens. Developing queens exposed to fluvalinate have a high mortality, while coumaphos-treated adult queens are lighter and smaller [83]. Colony acceptance and the performance of queens raised in beeswax queen cups impregnated with coumaphos are also negatively affected [84]. Plant-based acaricides and organic acids are generally safe for human consumption. However, thymol (the main component of several acaricides) induces brood removal [85]. The use of menthol (and thymol) to control tracheal mites may drive worker bees and queens out of the hive, especially during hot weather. Although formic acid occurs naturally in honey, this chemical can reduce production and the life span of drones [26], as well as those of workers [86]. Oxalic acid (registered in Canada and Europe, and recently in the United States) reduces queen longevity and also brood production [87].

Foragers are often exposed to various agrochemicals as they visit treated flowers. Within their nests, nurse bees and young bees feed on contaminated bee bread and nectar. Some fungicides applied to crops are harmful to bees and to beneficial molds or fungi living with the colony [88]. For example, imidacloprid, which

is blamed for causing widespread colony loss in France, impairs olfactory learning and the immune system of honey bees [89, 90]. The use of acaricides in hives can also suppress the honey bee immune system [91]. Flower and fruit "thinners" applied to crops are also toxic to bees (see also Chapter 4).

The increased use of chemicals to save colonies from various forms of colony disorder is alarming. The additive, synergistic or antagonistic effects of the numerous pesticides and their potential contribution to colony loss are often overlooked (Chapter 4). Oldroyd [92] suspects that some insecticide-related phenomena manifest as CCD. Interaction between compounds can occur when they accumulate in the wax, as in the case of coumaphos and fluvalinate. Johnson et al. [93] document an increase in toxicity of acaricide in three-day-old bees previously treated with another biocide. The same synergistic effect can also be illustrated using *Varroa* mites. When combs drawn from coumaphos and fluvalinate-laden wax foundation are used, increased mortality of foundress *Varroa* is observed [94]. After one generation of brood, however, the negative effect diminishes. The cocoon may serve as barrier between the chemicals in the wax and the mite. Furthermore, injudicious use of acaricides leads to the development of *Varroa* populations resistant to these pesticides [95–97]. Acaricide rotation is therefore recommended to minimize the development of resistance among mites [98]. Nonetheless, the effect of pesticides on bee susceptibility to various bee pathogens is also a pressing concern. Bees treated with imidacloprid are more likely to suffer *Nosema* infection than are untreated bees [99].

The exposure of bees to agricultural pesticides during pollination should be avoided (see Chapter 4). If application is inevitable, careful management practice is required to minimize bee loss. Less toxic chemicals should be used when bees are foraging, and a liquid formulation of pesticides is less dangerous to bees than powder. If areas are sprayed with highly toxic compounds, colonies should be removed immediately. If colonies cannot be removed, the hive entrances should be closed with screen to keep the confined colonies from overheating, and water should be given in internal feeders.

REFERENCES

[1] Morse, R.A. & Calderone, N.W. 2000. The value of honeybees as pollinators of U.S. crops in 2000. *Bee Culture*, 128: 1–15.

[2] Carreck, N. & Williams, I. 1998. The economic value of bees in the UK. *Bee World*, 79: 115–123.

[3] Delaplane, K.S. & Mayer, D.F. 2000. *Crop pollination by bees*. New York, CABI Publishing.

[4] McGregor, S.E. 1976. *Insect pollination of cultivated crop plants*. Agriculture Handbook 496. Washington, DC, USDA-ARS.

[5] vanEngelsdorp, D., Hayes, J., Underwood, R.M. & Pettis, J. 2008. A survey of honeybee colony losses in the U.S., fall 2007 to spring 2008. *PLoS ONE*, 3(12): e4071. doi:10.1371/journal.pone.0004071.

[6] Cox-Foster, D.L., Conlan, S., Holmes, E.C., Palacios, G., Evans, J.D., Moran, N.A., Phenix-Lan Quan, Briese, T., Hornig, M., Geiser, D.M., Martinson, V., vanEngelsdorp, D., Kalkstein, A.L., Drysdale, A., Hui, J., Zhai, J., Cui, L., Hutchison, S.K., Simons, J.F., Egholm, M., Pettis, J.S. & Lipkin, W.I. 2007. A metagenomic survey of microbes in honeybee colony collapse disorder. *Science*, 318: 283–287.

[7] Burgett, M., Akratanakul, P. & Morse, R.A. 1983. Tropilaelaps clareae: a parasite of honeybees in Southeast Asia. *Bee World*, 64: 25–28.

[8] Anderson, D. & Morgan, M.J. 2007. Genetic and morphological variation of bee-parasitic *Tropilaelaps* (Acari: Laelapidae) new and re-defined species. *Experimental and Applied Acarology,* 43: 1–24. http://dx.doi.org/10.1007/s10493-007-9103-0.

[9] Danka, R.G., de Guzman, L.I., Rinderer, T.E., Sylvester, H.A., Wagener, C. M., Bourgeois, A.L., Harris, J.W. & Villa, J.D. 2012. Functionality of varroa-resistant honeybees (Hymenoptera: Apidae) when used in migratory beekeeping for crop pollination. *Journal of Economic Entomology,* 105: 313–321

[10] Scott-Dupree, C., Winston, M., Hergert, G., McKenzie, K., Murrell, D., Dixon, M., Dogterom, R. Currie, R., Gates, J., Corner, J. & Jay, S.C. 1995. *A guide to managing bees for crop pollination*. Ontario, Canadian Association of Professional Apiculturists.

[11] Mussen, E.C., Foote, L., Gary, N.E., Laidlaw, H.H., Thorp, T.W. & Watkins, L.H. 1987. *Beekeeping in California*, Publication 21422, Cooperative Extension, Univ. of CA.

[12] Delaplane, K.S., van der Steen, J. & Guzman, E. 2012. Standard methods for estimating strength parameters of *Apis mellifera* colonies. In V. Dietemann, J.D. Ellis & P. Neumann, eds. *The COLOSS beebook, Volume I: standard methods for Apis mellifera research*. *Journal of Apicultural Research*, 51(5).

[13] Goodwin, R.M. 1997. Feeding sugar syrup to honeybee colonies to improve pollination: a review. *Bee World,* 78: 56–62.

[14] Collison, C.H. 2000. *Beekeeping basics.* Pennsylvania State University.

[15] Free, J.B. & Williams, I.H. 1971. The effect of giving pollen and pollen supplements to honeybee colonies on the amount of food collected. *Journal of Apicultural Research,* 10: 87–90.

[16] Camazine, S. 1993. The regulation of pollen foraging by honeybees: how foragers assess the colony's need for pollen. *Behavioral Ecology and Sociobiology,* 32: 265–272.

[17] Sammataro, D. & Avitabile, A. 2012. *The beekeepers handbook* (4th edn), Ithaca, NY, Cornell University Press.

[18] Baker, R.A., Hick, A. & Chmielewski, W. 2005. Aspects of the history and biogeography of the bee mites *Tropilaelaps clareae* and *T. koenigerum*. *Journal of Apicultural Science,* 49: 13–19.

[19] Akratanakul, P. & Burgett M. 1975. *Varroa jacobsoni*: a prospective pest of honeybees in many parts of the world. *Bee World,* 56: 119–121.

[20] De Jong, D., Morse, R.A. & Eickwort, G.C. 1982. Mite pests of honeybees. *Annual Review of Entomology,* 27: 229–252.

[21] Engels, W. & Schatton, K. 1986. Changes in hemolymph and weight loss in worker bees due to varroa parasitization. *Varroa* workshop feldafing, 5.

[22] Ellis, A. & Delaplane, K.S. 2009. Individual forager profits in *Apis mellifera* unaffected by a range of colony *Varroa* destructor densities. *Insectes Sociaux*. doi:10:1007/s00040-009-0040-2.

[23] Shimanuki, H., Calderone, N.W. & Knox, D.A. 1994. Parasitic mite syndrome: the symptoms. *American Bee Journal,* 134: 827–828.

[24] Murilhas, A.M. 2002. Varroa destructor infestation impact on *Apis mellifera* carnica capped brood, production, bee population and honey storage in a Mediterranean climate. *Apidologie*, 33: 271–281.

[25] Rinderer, T.E., de Guzman, L.I., Lancaster, V.A., Delatte, G.T. & Stelzer, J.A. 1999. *Varroa* in the mating yard: I. The effects of *Varroa jacobsoni* and Apistan® on drones. *American Bee Journal,* 139: 134–138.

[26] de Guzman, L.I., Rinderer, T.E., Lancaster, V.A., Delatte, G.T. & Stelzer, A. 1999. *Varroa* in the mating yard: III. Effects of formic acid gel formulation on drone production. *American Bee Journal*, 139: 304–307.

[27] Palmer, K.A., & Oldroyd, B.P. 2000. Evolution of multiple mating in the genus *Apis*. *Apidologie,* 31: 235–248.

[28] Tarpy, D.R, vanEngelsdorp, D. & Pettis, J.S. 2013. Genetic diversity affects colony survivorship in commercial honeybee colonies. *Naturwissenschaften*, doi: 10.1007/s00114-013-1065-y.

[29] Oldroyd, B.P. 2007. What's killing American honeybees? *PLoS Biology,* 5: e168.

[30] Tarpy, D. 2003. Genetic diversity within honeybee colonies prevents severe infections and promotes colony growth. *Proc. R. Soc. Biol. Sci.*, 270: 99–103.

[31] Mantilla, H.R. & Seeley, T.D. 2007. Genetic diversity in honeybee colonies enhances productivity and fitness. *Science,* 317: 362–364.

[32] Kralj, J. & Fuchs, S. 2006. Parasitic *Varroa destructor* mites influence flight duration and homing ability of infested *Apis mellifera* foragers. *Apidologie,* 37: 577–587.

[33] Janmaat, A.F., Winston, M.L. & Ydenberg, R.C. 2000. Condition-dependent response to changes in pollen stores by honeybee (*Apis mellifera*) colonies with different parasitic loads. *Behavioral Ecology and Sociobiology,* 47: 171–179.

[34] Dietemann, V., Nazzi, F., Martin, S.J., Anderson, D.L., Locke, B., Delaplane, K.S. Wauquiez, Q., Tannahill, C., Frey, E., Ziegelmann, B., Rosenkranz, P. & Ellis, J.D. 2013 Standard methods for varroa research. *Journal of Apicultural Research,* 52. doi:10.3896/IBRA.1.52.1.09.

[35] Lee, K.V., Moon, R.D., Burknes, E.C., Hutchison, W.D. & Spivak, M. 2010. Practical sampling plans for *Varroa destructor* in *Apis mellifera* colonies and apiaries. *Journal of Economic Entomology,* 103: 1039–1050. doi: 10.1603/EC10037.

[36] Ostiguy, N. & Sammataro, D. 1999. A simplified technique for counting *Varroa* on sticky boards. *Apidologie*, 31: 707–16.

[37] Delaplane, K.S., Berry, J.A., Skinner, J.A., Parkman, J.P. & Hood, W.M. 2005. Integrated pest management against *Varroa destructor* reduces colony mite levels and delays treatment threshold. *Journal of Apicultural Research,* 44: 157–162.

[38] Ritter, W. & Schneider-Ritter, U. 1988. Differences in biology and means of controlling *Varroa jacobsoni* and *Tropilaelaps clareae*, two novel parasitic mites of *Apis mellifera*. In G.R. Needham, R.E. Page, Jr., M. Delfinado-Baker & C.E. Bowman, eds. *Africanized honeybees and bee mites*, pp. 387–395. Chichester, UK, Ellis Horwood.

[39] Rath, W., Boecking, O. & Drescher, W. 1995. The phenomena of simultaneous infestation of *Apis meliferea* in Asia with the parasitic mites *Varroa jacobsoni* Oud. and *Tropilaelaps clareae* Delfinado and Barker. *American Bee Journal,* 135: 125–127.

[40] Koeniger, N. & Musaffar N. 1988. Lifespan of the parasitic honeybee mite, Tropilaelaps clareae, on Apis cerana, dorsata and mellifera. *Journal of Apicultural Research*, 27: 2017–212.

[41] Rinderer, T.E., Oldroyd, B.P., Lekprayoon, C., Wongsiri, S. & Thapa, R. 1994. Extended survival of the parasitic mite *Tropilaelaps clareae* on adult workers of *Apis mellifera* and *Apis dorsata*. *Journal of Apicultural Research,* 33: 171–174.

[42] Wilde, J. 2000a. How long can *Tropilaelaps clareae* survive on adult honeybee workers? In *Proceedings of the Euroconference on Molecular Mechanisms of Disease Tolerance in Honeybees*, held in Kralupy near Prague, Czech Republic, 17–19 October 2000. Bee Research Institute, Dol, Czech Republic, pp. 217–221.

[43] Wilde, J. 2000b. *Varroa destructor* and *Tropilaelaps clareae* in *Apis melliferea* colonies in Nepal. In *Proceedings of the Euroconference on Molecular Mechanisms of Disease Tolerance in Honeybees*, held in Kralupy near Prague, Czech Republic, 17–19 October 2000. Bee Research Institute, Dol, Czech Republic, pp. 223–238.

[44] Woyke, J. 1987. Length of stay of the parasitic mite *Tropilaelaps clareae* outside sealed honeybee brood cells as a basis for its effective control. *Journal of Apicultural Research,* 26: 104–109.

[45] Atwal, A.S. & Goyal, N.P. 1971. Infestations of honeybee colonies with *Tropilaelaps*, and its control. *Journal of Apicultural Research,* 10: 137–142.

[46] Delfinado-Baker, M. & Baker, E.W. 1982. A new species of *Tropilaelaps* parasitic on honeybees. *American Bee Journal,* 122: 416–417.

[47] Anderson, D.L. & Roberts, J.M.K. 2013. Standard Methods for *Tropilaelaps* Mite Research. *Journal of Apicultural Research,* 52 (and Coloss Bee Book, Volume II available at www.coloss.org/beebook/II/tropilaelaps/2/2). doi.10.3896/IBRA.1.52.4.21.

[48] Pettis, J.S., Rose, R., Lichtenberg, E.M., Chantawannakul, P., Buawangpong, N., Somana, W., Sukumalanand, P. & Dennis Vanengelsdorp, D. 2013. A rapid survey technique for *Tropilaelaps* mite (Mesostigmata: Laelapidae) detection. *Journal of Economic Entomology,* 106: 1535–1544. doi: http://dx.doi.org/10.1603/EC12339.

[49] de Guzman, L.I., Burgett, D.M. & Rinderer, T.E. 2001. Biology and life history of *Acarapis dorsalis* and *Acarapis externus*. In T.C. Webster & K.S. Delaplane, eds. *Mites of the honeybees*, pp. 17–27. Illinois, Dadant and Sons, Inc.

[50] Ibay, L.I. 1989. Biology of the two external *Acarapis* species of honeybees: *Acarapis dorsalis* Morganthaler and *Acarapis externus* Morganthaler [Acari: Tarsonemidae]. Oregon State University (MS thesis).

[51] de Guzman, L.I. & Burgett, D.M. 1991. Seasonal abundance of the *A. mellifera* L. ectoparasites *Acarapis dorsalis* Morgenthaler and *Acarapis externus* Morgenthaler (Acari: Tarsonemidae) in Oregon. *BeeScience*, 1: 219–224.

[52] Bailey, L. 1958. The epidemiology of the infestation of the honeybee, *Apis mellifera* L., by the mite *Acarapis woodi* Rennie and the mortality of infested bees. *Parasitology,* 48: 493–506.

[53] Otis, G.W. & Scott-Dupree, C.D. 1992. Effects of *Acarapis woodi* on overwintered colonies of honeybees (Hymenoptera: Apidae) in New York. *Journal of Economic Entomology,* 85: 40–48.

[54] Sammataro, D. 2006. An easy dissection technique for finding tracheal mites (Acari: Tarsonemidae) in honeybees (with Video link). *International Journal of Acarology,* 32: 339–343.

[55] Sammataro, D., de Guzman, L., George, S., Ochoa, R. & Otis, G. 2013. Standard methods for tracheal mite research. *Journal of Apicultural Research,* 52. doi:10.3896/IBRA.1.52.4.20.

[56] Sammataro, D., Cobey, S., Smith, B.H. & Needham, G.R. 1994. Controlling tracheal mites (Acari: Tarsonemidae) in honeybees (Hymenoptera: Apidae) with vegetable oil. *Journal of Economic Entomology,* 57: 910–916.

[57] Neumann, P. & Elzen, P.J. 2004. The biology of the small hive beetle (*Aethina tumida*, Coleoptera: Nitidulidae): Gaps in our knowledge of an invasive species. *Apidologie,* 35: 229–247.

[58] Neumann, P., Evans, J.D., Pettis, J.S., Pirk, C.W.W., Schäfer, M.O., Tanner, G. & Ellis, J.D. 2013. Standard methods for small hive beetle research. *Journal of Apicultural Research*, 52. doi:10.3896/IBRA.1.52.4.19.

[59] Torto, B., Boucias, D.G., Arbogast, R.T., Tumlinson, J.H., & Teal, P.E.A. 2007. Multitrophic interaction facilitates parasite-host relationship between an invasive beetle and the honeybee. *Proceedings of the National Academy of Sciences, USA,* 104: 8374–8378.

[60] de Guzman, L.I., Frake, A.M., Rinderer, T.E. & Pollett, D.K. 2009. Small hive beetles. Louisiana Agricultural Experiment Station, Publication 3135.

[61] Ellis, J.D., Graham, J.R. & Mortensen, A. 2013. Standard methods for wax moth research. *Journal of Apicultural Research,* 52. doi:10.3896/IBRA.1.52.1.10.

[62] De Miranda, J.R., Gauthier, L., Ribiére, M. & Chen, Y.P. 2012. Honeybee viruses and their effect on bee and colony health. In D. Sammataro & J.A. Yoder, eds. *Honeybee colony health: challenges and sustainable solutions*. New York, CRC Press, Taylor and Francis Group.

[63] Chen, Y.P. & Siede, R. 2007. Honeybee viruses. In *Advances in virus research* (Vol. 70), pp. 33–80. New York, Academic Press.

[64] Dainat, B., Ken, T., Berthoud, H. & Neumann, P. 2009. The ectoparasitic mite *Tropilaelaps mercedesae* (Acari, Laelapidae) as a vector of honeybee viruses. *Insectes Sociaux,* 56: 40–43. doi:10.1007/s00040-008-1030-5.

[65] Forsgren, E., de Miranda, J., Isaksson, M., Wei, S. & Fries, I. 2009. Deformed wing virus associated with Tropilaelaps mercedesae infesting European honeybees (*Apis mellifera*). *Experimental and Applied Acarology*, 47: 87–97.

[66] de Guzman, L.I., Rinderer, T.E. & Frake, A.M. 2007. Growth of *Varroa destructor* Anderson and Trueman (Acari: Varroidae) populations in Russian honeybee (*Apis mellifera* L.) (Hymenoptera: Apidae) colonies. *Annals of the Entomological Society of America,* 100: 187–195.

[67] Rinderer, T.E., Harris, J.W., Hunt, G. & de Guzman, L.I. 2010. Breeding for resistance to *Varroa destructor* in North America. *Apidologie,* 41: 409–424.

[68] Büchler, R., Berg, S. & Le Conte, Y. 2010. Breeding for resistance to *Varroa destructor* in Europe. *Apidologie,* 41: 393–408.

[69] Anderson, D.L. & Giacon, H. 1992. Reduced pollen collection by honeybee (Hymenoptera: Apidae) colonies infected with *Nosema apis* and sacbrood virus. *Journal of Economic Entomology,* 85: 47–51.

[70] Higes, M., Martín-Hernández, R., Botías, C., Bailón, E.G., González-Porto, A.V., Barrios, L., Jesús del Nozal, M., Bernal, J.L., Jiménez, J.J., Garcia Palenica, P. & Meana, A. 2008. How natural infection by *Nosema ceranae* causes honeybee colony collapse. *Environmental Microbiology,* 10: 2659–2669.

[71] Chaimanee, V., Chantawannakul, P., Chen, Y., Evans, J.D. & Pettis, J.S. 2012. Differential expression of immune genes of adult honeybee (*Apis mellifera*) after inoculated by *Nosema ceranae*. *Journal of Insect Physiology,* 58: 1090–1095.

[72] Kralj, J. & Fuchs, S. 2010. *Nosema* sp. influences flight behavior of infected honeybee (*Apis mellifera*) foragers. *Apidologie,* 41: 21–28.

[73] Dussaubat, C., Sagastume, S., Gómez-Moracho, T., Botıas, C., Garcıa-Palencia, P., Martín-Hernandez, R., Le Conte, Y. & Higes, M. 2013. Comparative study of *Nosema ceranae* (Microsporidia) isolates from two different geographic origins. *Veterinary Microbiology,* 162: 670–678.

[74] Goblirsch, M., Huang, Z.Y. & Spivak, M. 2013. Physiological and behavioral changes in honeybees (*Apis mellifera*) induced by *Nosema ceranae* infection. *PLoS ONE,* 8(3): e58165. doi:10.1371/journal.pone.0058165.

[75] Fries, I., Chauzat, M.P., Chen, Y.P., Doublet, V., Genersch, E., Gisder, S., Higes, M., Mcmahon, D.P, Martín-Hernández, R., Natsopoulou, M., Paxton, R., Retschnig, G., Webster, T.C. & Williams, G.R. 2013. Standard methods for *Nosema* research. *Journal of Apicultural Research*, 52(1). doi:10.3896/IBRA.1.52.1.14.

[76] Jensen, A.B., Aronstein, K., Flores, J.M., Vojvodic, S., Palacio, M.A. & Spivak, M. 2013. Standard methods for fungal brood disease research. *Journal of Apicultural Research,* 52(1). doi:10.3896/IBRA.1.52.1.13.

[77] de Graaf, D.C., Alippi, A.M., Antúnez, K., Aronstein, K.A., Budge, G., De Koker, D., De Smet, L., Dingman, D.W., Evans, J.D., Foster, L.J., Fünfhaus, A., Garcia-Gonzalez, E., Aleš Gregorc, A., Human, H., Murray, K.D. Bach Kim Nguyen, Poppinga, L., Spivak, M., vanEngelsdorp, D., Wilkins, S. & Elke Genersch, E. 2013. Standard methods for American foulbrood research. *Journal of Apicultural Research*, 52(1). doi:10.3896/IBRA.1.52.1.11.

[78] Le Conte, Y., Brunet, J., McDonnell, C., Dussaubat, C. & Alaux, C. 2012. Interactions between risk factors in honeybees. In D. Sammataro & J.A. Yoder, eds. *Honeybee colony health: challenges and sustainable solutions*, pp. 215–222. New York, CRC Press, Taylor and Francis Group.

[79] Lodesani, M., Pellacani, A., Bergomi, S., Carpana, E., Rabitti, T. & Lasagni, P. 1992. Residue determination for some products used against *Varroa* infestations in bees. *Apidologie,* 23: 257–272.

[80] Mullin, C.A., Frazier, M., Frazier, J.L., Ashcraft, S., Simonds, R., vanEngelsdorp, D. & Pettis, J.S. 2010. High levels of miticides and agrochemicals in North American apiaries: implications for honeybee health. *PLoS ONE*, 5: e9754.

[81] Sylvester, H.A., Watts, R., de Guzman, L.I., Stelzer, J.A. & Rinderer, T.E. 1999. *Varroa* in the mating yard: II. The effects of *Varroa jacobsoni* and Apistan on the mating ability of drones. *American Bee Journal,* 139: 225–227.

[82] Burley, L.M., Fell, R.D. & Saacke, R.G. 2008. Survival of honeybee (Hymenoptera: Apidae) spermatozoa incubated at room temperature from drones exposed to miticides. *Journal of Economic Entomology,* 101: 1081–1087.

[83] Haarmann, T., Spivak, M., Weaver, D., Weaver, B. & Glenn, T. 2002. Effects of fluvalinate and coumaphos on queen honeybees (Hymenoptera: Apidae) in two commercial queen rearing operations. *Journal of Economic Entomology,* 95: 28–95.

[84] Collins, A.M., Pettis, J.S., Wilbanks, R. & Feldlaufer, M.F. 2004. Performance of honeybee (*Apis mellifera*) queens reared in beeswax cells impregnated with coumaphos. *Journal of Apicultural Research,* 43: 128–134.

[85] Floris, I., Satta, A., Cabras, P., Garau, V.L. & Angioni, A. 2004. Comparison between two thymol formulations in the control of *Varroa destructor*: effectiveness, persistence, and residues. *Journal of Economic Entomology,* 97: 187–191.

[86] Underwood, R. & Currie, R. 2003. The effects of temperature and dose of formic acid on treatment efficacy against *Varroa destructor* (Acari: Varroidae), a parasite of *Apis mellifera* (Hymenoptera: Apidae). *Experimental and Applied Acarology,* 29: 303–313.

[87] Higes, M., Meana, A., Suarez, M. & Llorente, J. 1999. Negative long-term effects on bee colonies treated with oxalic acid against *Varroa jacobsoni* Oud. *Apidologie,* 30: 289–292.

[88] Yoder, J.A., Heydinger, D.J., Hedges, B.Z., Sammataro, D., Finley, J., DeGrandi-Hoffman, G., Croxall, T.J. & Christensen, B.S. 2012. Fungicides reduce symbiotic fungi in bee bread and the beneficial fungi in colonies. In D. Sammataro & J.A. Yoder, eds., *Honeybee colony health: challenges and sustainable solutions*, pp 193–214. New York, CRC Press, Taylor and Francis Group.

[89] Decourtye, A., Armengaud, C., Renou, M., Devillers, J., Cluzeau, S., Gauthier, M. & Pham-Delègue, M-H. 2004. Imidacloprid impairs memory and brain metabolism in the honeybee (*Apis mellifera* L.). *Pesticide Biochemistry and Physiology,* 78: 83–92.

[90] Desneux, N., Decourtye, A. & Delpuech, J. 2007. The sublethal effects of pesticides on beneficial arthropods. *Annual Review of Entomology,* 52: 81–106.

[91] Boncristiani, H., Underwood, R., Schwarz, R., Evans, J.D., Pettis, J. & vanEngelsdorp, D. 2012. Direct effect of acaricides on pathogen loads and gene expression levels in honeybees, *Apis mellifera*. *Journal of Insect Physiology,* 58: 613–620.

[92] Oldroyd, B. & Wongsiri, S. 2006. *Asian honeybees: biology, conservation, and human interactions*. Cambridge, MA, Harvard University Press.

[93] Johnson, R.M., Pollock, H., & Berembaum, M.R. 2009. Synergistic interaction between in-hive miticides in *Apis mellifera*. *Journal of Economic Entomology,* 102: 474–479.

[94] Fries, I., Wallner, K. & Rosenkranz, P. 1998. Effects on *Varroa jacobsoni* from acaricides in beeswax. *Journal of Apicultural Research,* 37: 85–90.

[95] Elzen, P.J., Baxter, J.R., Spivak, M. & Wilson, W.T. 1999. Amitraz resistance in *Varroa*: a new discovery in North America. *American Bee Journal,* 139: 362.

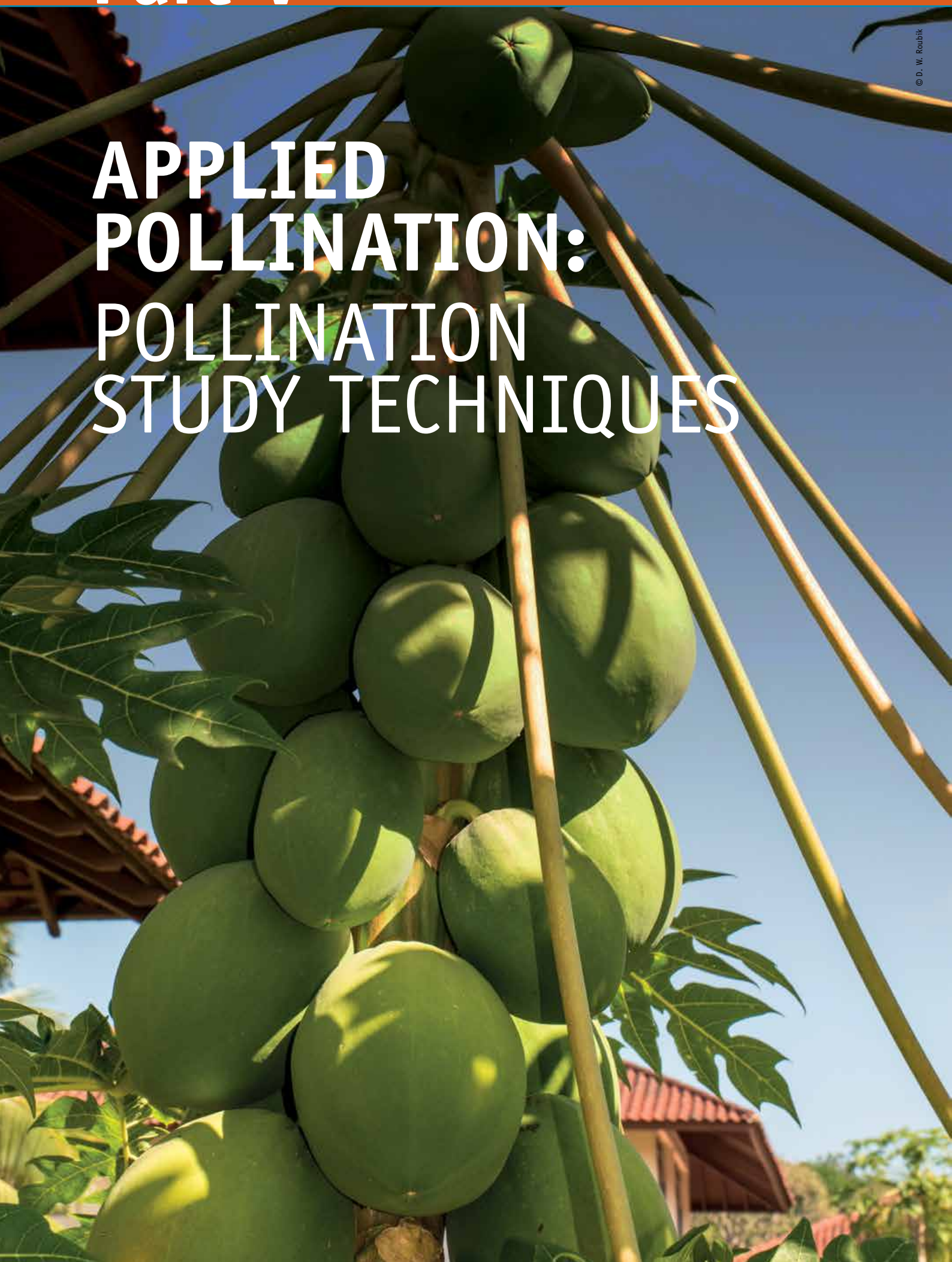
[96] Pettis, J.S., Collins, A.M., Wilbanks, R. & Feldlaufer, M.F. 2004. Effects of coumaphos on queen rearing in the honeybee, *Apis mellifera*. *Apidologie,* 35: 605–610.

[97] Sammataro, D., Untalan, P., Guerrero, F. & Finley, J. 2005. The resistance of *Varroa* mites (Acari: Varroidae) to acaricides and the presence of esterase. *International Journal of Acarology,* 31: 67–74.

[98] Elzen, P.J., Baxter, J.R., Westervelt. D., Causey, D., Randall, C., Cutts, L. & Wilson, W.T. 2001. Acaricide rotation plan for control of *Varroa*. *American Bee Journal,* 141: 412.

[99] Pettis, J.S., vanEngelsdorp, D., Johnson, J. & Dively, G. 2011. Pesticide exposure in honeybees results in increased levels of the gut pathogen *Nosema*. *Naturwissenschaften*. doi:10.1007/s00114-011-0881-1.

Part V



© D. W. Roubik

APPLIED POLLINATION: POLLINATION STUDY TECHNIQUES

Chapter 17

POLLINATOR BEHAVIOUR AND PLANT PHENOLOGY

P.G. Kevan

17.1 BASIC FORAGING ECOLOGY

The behaviour of potential pollinators on flowers represents a crucial step in the transfer of pollen from the anthers to the stigma of flowers of different plants, between different flowers of the same plant or within the same flower (see Chapters 2 and 6). Many writers refer to flower visitors as pollinators without knowing for certain whether or not the visitors actually effect pollination. Numerous examples of this type of error occur in the literature on crop pollination.

The behaviour of flower visitors can be broken down into activities taking place at different scales. The movement of flower visitors among plants or between areas under crop production occurs at a larger scale, while the movement of flower visitors between plants in patches or within areas under crop production takes place at a slightly smaller scale. The movement of flower visitors within a particular plant takes place at a much finer scale. The actual dimensions of these scales vary and depend on the distances between areas and the spacing and size of plants. For example, the spacing of small herbaceous plants contrasts with that of large orchard trees.

The most effective pollinators are those that move between plants and flowers transferring pollen in a manner that satisfies the breeding system of the plant. From the viewpoint of agriculture and the production of crops requiring pollination by insects, honey bees are the pollinators of choice. They are well understood biologically and can be managed for crop pollination. Thus, honey bee behaviour in relation to crop pollination is discussed first, before other pollinators are introduced and the roles of floral attractants and morphology in pollinator behaviour are considered.

All species of honey bees have a well-developed form of dance language through which they communicate information to other bees in the nest or hive regarding the location (distance and direction) of resources, usually floral nectar and pollen. When that information is exchanged in the colony, the richness of the resource is conveyed along with chemical information, for example, through floral scents carried on the bee's body.

In general, this dance language takes the form of honey bee foragers returning to their colonies and performing a dance on the wax comb of the nest. If resources are located a particular distance from the nest, the dance consists of two loops to the left and the right, with a central run during which the bee wags its abdomen. The directional component of the dance is indicated by the orientation of the central run with respect to the sun, while the distance is indicated by the tempo of the dance. Inside the darkness of the hive (for *Apis mellifera*, *A. cerana*, *A. indica*, *A. nigrocincta*, *A. nuluensis*

and *A. koschevnikovi*), vertical movement represents the position of the sun. Thus, if a forager dances its central run vertically, foraging recruits interpret this as an instruction to fly in the direction of the sun. If the dancer orients its vertical run 60° to the left, the recruits interpret this as an instruction to fly horizontally 60° to the left of the direction to the sun. The distance component of the instructions is transmitted through the tempo of the dance, with closer distances indicated by faster movements. If the resources are nearby, a round dance is substituted for the wagging dance. Details of this dance language can be found in most beekeeping and bee biology books.

The dance language of honey bees varies from species to species, and between races within species. Knowledge of the dance language is of major practical importance for understanding the general foraging ranges expected from a particular species or race of honey bee. Furthermore, the information is significant for apiary planning in terms of the number of hives that can be supported for honey production or in pollination activity on crops. If the foraging range is small, large numbers of honey bees will deplete the available resources and soon compete with each other. This will detract from honey production and can cause competing colonies to decline in population as the total population of honey bees exceeds the carrying capacity of the region (i.e. the number of honey bees the area can support) within their foraging range.

The tempos of the dance language vary among species and races of honey bees. The differences relate approximately to the sizes of the bees: the smaller the bee, the faster the change in dance tempo with distance, and the shorter the distance at which the wagging dance becomes operational.

The general distances over which different species and races of honey bee normally forage have been studied for many crop situations, and demonstrate significant variability both in terms of natural environment and agricultural area. Honey bees may forage distances of less than 1 km or up to several kilometres from their nests, or even further. Their foraging range can therefore be enormous, and may exceed a few hundred square kilometres.[1] Knowledge of the production of nectar or pollen, or both, in this area would thus enable estimates of the density of honey bees that could be supported in the habitat (not necessarily pertaining to a specific apiary). In the case of crop pollination, the foraging range also dictates the expected effectiveness of honey bees.

Given that most cropping systems make use of areas smaller than the foraging areas of *Apis mellifera* and *A. cerana*, the issue is not that serious. However, overcrowding of crops with bees can quickly cause a decline in the pollinator force, as they move off to other forage or even debilitate each other's colonies by depleting resources.

An adequate distribution of pollinators on a crop is important both for the needs of the crop and the needs of the pollinators. In the case of *A. mellifera*, pollinating foragers have been observed to spread out widely over a given crop. Although more activity might be expected closer to the hive, the bees are often more or less evenly dispersed within an orchard or field. Foragers usually occur in greater density near the hive, but the areas covered are far greater at increasing distance from the hive. Thus, according to the results of field experiments on oil-seed rape and in orchards, it is *not* necessary to place colonies of pollinating honey bees in small groups (e.g. four) throughout the crop. Larger groupings of hives seem to have the same effect and ease the job of the beekeepers providing pollination services.

[1] It is interesting to note that in both the crop and bee literature, the foraging range of the honey bee is often given as 1 or 2 km, and there is serious discussion of enhancing bee forage with plantings over a small area. As a colony can cover 20 000 ha in its foraging area and may readily travel 8 km in foraging distance, the practical value of such observations is doubtful [Ed.].

17.2 PATTERNS OF MOVEMENT BETWEEN PLANTS

Knowledge rearding optimal foraging behaviour – by which animals expend as little energy as possible to obtain resources (food) that provide them with the greatest amount of energy in a given period of time – is also being applied to pollinator foraging. The majority of research focuses on bumblebees, however the same patterns have been noted for honey bees.

In general, bees foraging in a patch of flowers rich in nectar or pollen tend to visit many plants in the patch, skipping only a few and visiting neighbouring plants. They also tend to change direction between one plant and the next, moving forward in a tight zig-zag and often crossing their own path. If a patch of flowers provides few rewards, they tend to skip over plants as they sample, and move forward in a shallower zig-zag, or almost a straight line. The result of this behaviour is that a pollinator on a rich patch will quickly accomplish more floral visits per unit area, and presumably effect more pollination, than one on a poor patch. If the patch is poorer, the mobile pollinator tends to progress through it relatively directly and quickly.[2]

The clues a forager uses to decide to change patches are not fully understood. The relative richness of rewards in various patches is presumably compared in some way, enabling the pollinating forager to track the sources of the richest returns. However, some experiments on honey bees strongly suggest that this is not the case, with individuals instead remaining constant to relatively poor or rich sources, and not switching as long as some reward is available. This sort of behaviour may be linked to the sociality of honey bees and the overall foraging behaviour of the colony to maximize resource accumulation. It does not seem to apply to other bees or pollinators.

[2] This behaviour, although seemingly increasing desirable outcrossing, can make bees eventually abandon the flower patch. If many different species continue to visit the patch and sample it at an intensity suitable to their needs, then local resource depletion may increase outcrossing and/or fruit and seed set and yield (see Chapter 3) [Ed.]

17.3 MOVEMENTS ON INDIVIDUAL PLANTS

Tree crops, which are particularly important in the tropics, present pollinators with a vast array of flowers and copious quantities of resources when in bloom. However, the pollination mechanisms for blooming trees are poorly understood. In general, flower-visiting insects on a given tree tend to work their way downwards over a day or half day. The reason for this behaviour presumably relates to the sequence of the opening of flowers and the way in which rewards are distributed among the flowers over the crown of the tree. Individual bees generally enter a flowering tree at a higher level than they leave. This is attributed to the dynamics of flight while carrying the foraging load, with more energy conserved by starting "high and light" and then descending while nectar or pollen is accumulated. Movements onto the next tree have not been studied. However, research in Malaysia show that *Apis koschevnikovi* and *A. dorsata* readily move between large trees, while remaining at about the same level above the ground.

The pattern of movement of bees, moths and birds on smaller plants with inflorescences is generally similar. The forager ascends the plant, visiting the flowers as it does so. Several reasons are given for this behaviour and relate to the presentation of flowers, the amount of resources they contain and the energy used for flight versus crawling. The flowers are usually fairly close together in inflorescences, often with their openings or petal access platforms pitched slightly downwards. Thus, approaching from below is easier. Furthermore, upward flight and crawling in combination is more easily controlled than downward motion by either, while crawling expends significantly less energy than flight. Often, the volume of nectar in flowers found lower on the plant is greater than in those found higher up, although the solution of sugars is less concentrated. The possibility of taste satiation (by moving from relatively insipid to relatively strong) does not therefore occur, enabling the forager to recognize the quality of reward as it progressed. From the standpoint of the plants, it is important to note

that many plants with inflorescences present flowers during the male phase that produce pollen, and then during the female phase produce flowers with receptive stigmas. As the youngest flowers are located at the top of inflorescences, pollen is removed from male phase flowers of a given plant first, when there are no female flowers on that particular plant. Self-pollination is thereby discouraged.

17.4 BEHAVIOUR ON FLOWERS

It is crucial for the plants that pollinators operate in such a way as to cause the transfer of pollen. Some flower visitors that fail to do this may not be pollinators at all, or may be inefficient to a greater or lesser extent. The least efficient pollinators are nectar or pollen robbers. These visitors cause damage to the flowers they visit, remove nectar or pollen, and do not pollinate the plants. They also discourage visits by legitimate pollinators, either by attacking them or by making flowers less rewarding. Some carpenter bees and bumblebees are notorious for making punctures in the base of flowers and removing nectar. Several *Trigona* (a Neotropical genus) and *Tetragonula*, or other Old World bee groups, are also nectar and pollen robbers, and spend many minutes chewing holes in anthers or corollas. Such bees never enter the flower to touch the stigma or anthers.

Other visitors may just be thieves. They do no physical damage to the flower, but remove pollen or nectar without bringing about pollination. Small bees, particularly Meliponini and small Halictidae in the tropics, visit a wide diversity of flowers, removing pollen from the anthers or nectar from the nectaries of large flowers without touching the stigmas. Even honey bees can be nectar thieves. Although often recorded as visitors to passion fruit flowers, from which they remove nectar, they very rarely touch the anthers or stigmas because of the large size of the flowers (see Chapters 6.4.2, 9.3.2). Honey bees also are known for "side-working" flowers – removing nectar by inserting their proboscides between the floral parts from the side. This behaviour is a common problem with certain varieties of apple, some legumes and other crops.

Such "floral larceny" detracts from the activities of legitimate pollinators by depleting the resources they seek as they visit flowers and touch both anthers and stigmas. Careful attention to flower-visiting behaviour enables observers to discriminate between effective pollination and mere floral visitation. Unfortunately, many published accounts on "pollination" do not include this important detail.

Many crop plants have relatively simple flowers from the standpoint of a pollinator seeking to obtain a particular reward. Open bowl-shaped flowers and the complex but uniform inflorescences of the sunflower (Asteraceae) and carrot (Apiaceae) families pose little challenge to flower visitors extracting resources (see Figure 17.1.). However, complex flowers that contain hidden rewards, such as nectar in corolla tubes of flowers of the mint family (Lamiaceae) and legumes (Fabaceae), or the hidden pollen of the blueberry family (Ericaceae) or tomato family (Solanaceae), require special skills on the part of the pollinator. Researchers have found that pollinators (e.g. bumblebees, honey bees and leafcutter bees) must invest time in learning how to manipulate such complex flowers, in order to extract the reward they seek quickly and efficiently and, coincidentally, causing pollination.

Watching naïve bees fumble with complex flowers during their first few encounters with them can be quite amusing. It is worth keeping in mind that the more complex flowers usually produce rewards of a higher quality, obtainable only by a restricted number of pollinators.

17.5 MEMORY AND CONSTANCY

As noted above, bees and other pollinators in general are capable of learning to recognize various cues and to perform various tasks. Although the dance language is of prime importance in establishing orientation and transferring information among honey bees, individual foragers are able to memorize the landscape over which they forage. They develop "cognitive maps" which use visual and olfactory landmarks to enable orientation independent of the position of the sun,

Figure 17.1
PRINCIPAL FLOWER FEATURES AND CORRESPONDING FLOWER VISITORS

								
	BOWL	**BELL**	**BRUSH**	**FLAG**	**GULLET**	**TRUMPET**	**TUBE**	**TRAP**
Advertisement								
visual & olfactory	corolla	corolla	stamens	standard petal	labella	limb & tube	corolla	corolla, labella
Pollinator Aids								
landing surface	whole flower	flower margins	any part	kell	lower lip	margin	margin	margin
guiding marks				lateral petals (foothold)	nectar guides	nectar guide		color & pattern
guiding structures				symmetry & marks on standard whole flower	none or hairs whole flower	surface structures	narrow tube scales, hairs	hairs
Pollen Cache	exposed	partly hidden centralized	exposed	hidden	hidden	somewhat hidden	hidden	hidden
Nectar Cache	localized		diffuse	centralized (if present)	centralized	centralized	centralized	
Reward Type and Main Pollinator								
Pollen	***	*	*-	*	*	*-	*-	
Nectar	*-	*	*	*-	*	**	***	
	beetle, unspecialized bee, fly, Lepidoptera		bee, butterfly, beetle, mammal, bird		long-tongued bee, hawkmoth, bird, butterfly		hovering & perching moth, butterfly, bird, long-proboscis fly	
		short-tongued bee, wasp, fly, settling moth, bird, bat		long-tongued bee, bird		butterfly, hawkmoth, bird		carrion fly, beetle, microdiptera, bee

Source: D. W. Roubik

and also provide supplementary information. Other pollinators also rely on familiarity with the landscape in their foraging range for orientation purposes.

Such behaviour greatly enhances accuracy in homing for bees and other pollinators, as they return to their nests or roosts, and also works on outbound foraging flights. Another consequence is that individuals have a tendency to return to the same foraging sites, especially if the resources there are plentiful. However, site constancy can detract from pollination efficiency, especially if the site happens to be a single large tree that requires cross-pollination.

The importance of floral constancy by pollinators is clear for plants: a pollinator that visits the flowers of only one species becomes more efficient at transferring pollen. From the standpoint of the pollinator, familiarity with the flowers of a given species, and knowledge of how to most efficiently obtain the resources sought, is enhanced by learning and floral constancy.

17.6 FLORAL PHENOLOGY

Floral phenology refers to the blooming sequence of flowers and their development over time. Both concepts are important to pollination and its management. In a given location, plants flower in more or less the same sequence from year to year. How this sequence is governed by nature is not fully understood. Some plants bloom with the stimulus of lengthening days (as in the early part of the year in the northern hemisphere) or declining day length (as in the latter part of the year in the northern hemisphere). Others are unaffected by day length and bloom once the plant reaches a certain size and are daylength-neutral. However, the pantropical phenomenon of extensive flowering during special years or periods, called "masting", "general flowering" or sometimes "mass flowering" seems to be regulated by drought and sometimes an unusual temperature drop for a few days, which synchronizes the flowering of many plant species.

The rate at which plants come into bloom is also affected by the amount of heat that has accumulated during the growing season. This is often measured in "Growing Degree Days" (GDDs) above a certain threshold temperature. The details of how to accomplish those measurements and establish the threshold temperatures are well known, but beyond the scope of pollination *per se*. For some crops such as maize, "Maize Heat Units" (which are GDDs) are well known for many varieties and useful for predicting flowering and ear-ripening dates. The flowering sequence of apple varieties can also be predicted by GDDs, and the sequence, asynchronicity and synchronicity of bloom between varieties are important considerations in orchard planning for interplantings of pollinizer varieties for the main crop. One variety of pollinizer potentially may be able to fertilize another variety, but if crop varieties bloom at different times separated by as little as a week or so, cross-pollination by insects cannot occur. In many parts of the world, especially in the seasonal tropics (having wet and dry seasons), the presence or absence of rainfall may override the effect of day length or temperature, or both. Thus, growers and pollination managers must be able to "read" the seasons, and keep in close contact and otherwise coordinate their activities to ensure pollination reaches its maximum potential.

Beekeepers and pollinator managers must be conscious that their wards require food at all times, not just when the crop to be pollinated is in bloom. A thorough knowledge of flowering phenology in a region is therefore important. Good pollen and nectar availability stimulates active foraging and brood rearing. Thus, to provide a strong pollinator force for a crop, pollinator managers should keep their animals in resource-rich areas for some time before moving them to the crop. Furthermore, managers must be aware of the timing and duration of any periods of dearth or diminished availability of resources. This will allow them to calculate supplementary feeding or know whether migratory practices are required (see Chapter 20). Pollinator managers may also be able to provide services to a sequence of different crops. Clearly, they need to know when each plant blooms, for how long and what types (nectar or pollen or both), quality and quantity of resources the crop offers to the pollinators.

Once pollination services are complete, honey bee colonies (or populations of other managed pollinators) may be weakened. The aim of providing pollinators is often to saturate the blooming crop, to ensure maximum pollination. This creates intense competition among flower visitors, with the result that their populations exceed local (year long) carrying capacity and may weaken. A weakened

pollinator population, such as honey bees, should be moved to a resource-rich environment to recover. This sort of intervention is often necessary with pollination in greenhouses.[3]

The cases described above vary from place to place and crop to crop. Thus, it is difficult to arrive at any but the most general of recommendations. Nevertheless, the value of a "floral calender" can be appreciated. A floral calender is a list describing which flowers bloom, when and for how long, coupled (for the sake of practicality) with their abundance and value to pollinators. Floral calenders must be specifically prepared for particular regions (see the examples in Chapter 14.3 and Chapter 18), as information gathered for one locality may not apply in another, even if quite nearby.

At a finer level, it is important to consider the developmental sequence of flowering on individual plants. Some plants are "indeterminate bloomers", while others are "determinate". Indeterminate means that plants continue to produce flowers over rather ill-defined periods. Some *continue to produce flowers especially if pollination has been lacking*. Most insect-pollinated annual crops (except cereals) fit this category. For such plants, the timing of pollination is less critical because the plants generally compensate for un-pollinated flowers by producing greater quantities. In the tropics, some perennial crops are indeterminate bloomers. For these, the pollination season is long. In contrast, determinate bloomers are best illustrated by perennial crops, especially fruits. Pome and stone fruits are good examples because they have a fixed number of flowers that will open over a relatively short period. In the wet tropics, determinate bloomers may produce inflorescences that are determinate, but the plant may continue to bloom almost or completely year round. Oil palm is an excellent example (see Chapter 9.3.10). However, at all latitudes, the period for pollination is often short, and is critical to obtaining good yields. With such crops, grower and "PSP" (pollination service provider) efforts must be closely coordinated to achieve the best results (e.g. see Annex 1. Pollination Contract).

At an even finer level, there remains the issue of phenology within individual flowers. It is important to understand the stages in flower maturation, especially for pesticide applications (see Chapter 4). For example, insecticides and miticides used to control leaf rollers, leaf miners, fruitworm and red mites on pome fruits are applied up until the first flowers come into "full pink bud". Once the flowers are open, the risk of poisoning pollinators is great. Even after petal fall or the calyx stage, when spraying against other insect and mite pests is recommended, pollinator populations (honey bees or other removable bees) should be taken away.

This section presents the stages adopted for pome fruits. However, some hermaphroditic flowers shed pollen before the stigma is receptive. These are termed "protandrous". In others, the stigmas mature before the anthers dehisce. These are termed "protogynous". Lastly, some flowers have only very slight differences in maturation, or have mature stigma and anthers simultaneously. These are termed "homogamous". Most crop plants produce homogamous flowers, while protogyny is quite uncommon. The importance of understanding such fine differences lies in understanding the activities of the pollinators. Pollen-collecting bees quickly learn to avoid flowers lacking in pollen (i.e. flowers in the female stage). Fortunately, for crop pollination, dichogamy (protandry or protogyny) is generally weak or absent in domesticated plants. However, much remains to be learned about the floral biology of tropical perennial crops.

[3] It is also often necessary during crop rotation or following disturbances of the bee or pollinator habitat or nesting area, resulting in an "heirloom pollination" situation (see Chapter 5 and section 7.3) [Ed.].

© D. W. Roubik

Chapter 18

EVALUATING POLLINATORS

R.C. Macfarlane, A.R. Davis and D.W. Roubik

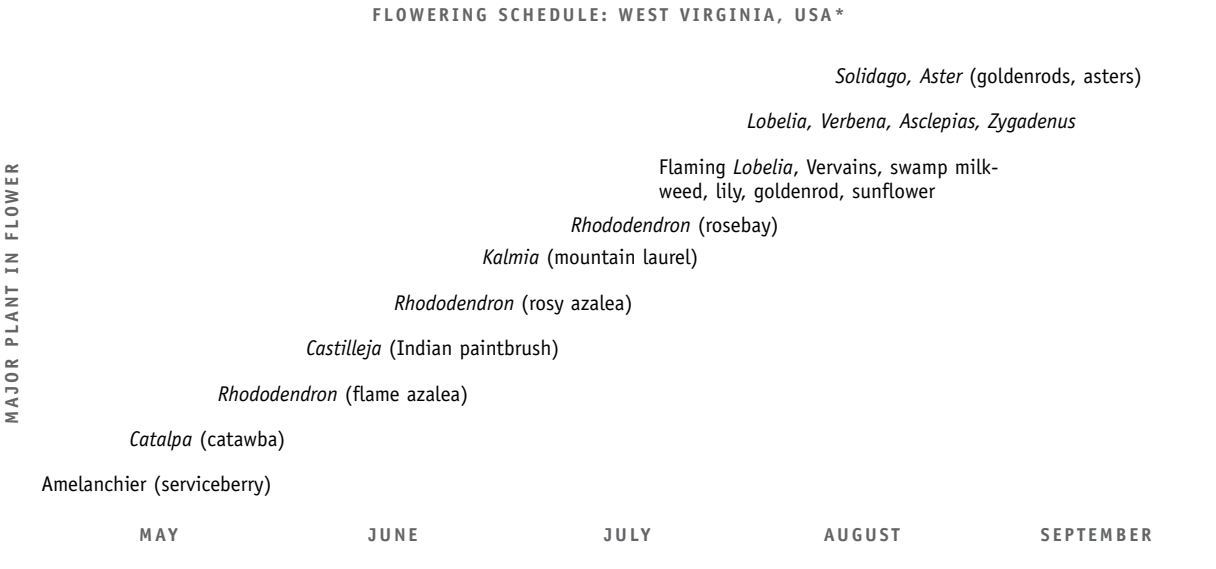
The factors influencing the effectiveness of pollinator species must be considered when studying the pollination of any plant. Flower structure provides the framework in which biotic and abiotic pollination agents operate and gives clues as to which species will likely make effective pollinating agents. However, those considerations alone are not sufficient – studies of visitor behaviour and critical field assays are essential, as emphasized elsewhere in this book (see in particular Chapter 19 and 20).[4] Studies should also focus on the availability of pollinating agents to determine the most important and manageable species in the particular habitat and circumstances. A fundamental consideration is the period in which pollinators, especially in the temperate zone, are active as adults – when searching for protein and energy at flowers. A "schedule" for the different crops as they bloom (Figure 18.1) is the first step in planning. The next step involves knowing the seasonal and everyday biology of the pollinators, as emphasized throughout this book (see especially in Chapter 9 and Part IV).

18.1 PARAMETERS FOR MEASURING POLLINATION

Several measurements, both direct and indirect, can be taken to assess pollination. However, such measurements differ greatly in their dependability as indices of pollination. In the past, fruit or seed yields (e.g. weight, number and size) were emphasized. Unfortunately, these can be poor estimators of pollination for several reasons. First of all, fruit production – a measure of flower fertilization – can only be an indirect measure of pollination or the placement of pollen grains on the stigma. The problem with counting fruit is that the investigator is at the mercy of maternal influences in plants that occur after pollination. That is, pollination can be grossly underestimated in plants that undergo abortion of developing fruits (fruit drop). Such is the case when relatively low nutritive resources are available to the plant. Although pollination occurs, the event is not indicated by the presence of fruit.

[4] See also FAO's pollination publications: www.fao.org/pollination/en.

Figure 18.1
FLOWERING SCHEDULE



*U.S. Forest Service; "A Blooming Schedule", Maurice Brooks

Another hindrance to determining pollination from fruit yield can occur in plant species that have a low ovule number per flower relative to the number of pollen grains placed on the stigma. In such cases, the potential number of ovules fertilized cannot be known from fruit counts, and hence pollination will again be underestimated. Yet another problem is that some plant species can develop fruit parthenocarpically (see Parts I and III) in the complete absence of pollination. In such cases, pollination would be overestimated if it were measured by fruit count. It is also important to determine whether or not a plant species is self-compatible. A zero fruit count may not truly indicate lack of pollination when incompatibility systems are present – the example of apples that are interfertile but sterile within a single clone comes to mind. For many crops, without pollinizers – the individual male flower-bearing individuals of a compatible genetic stock – no fruit can be set. And as stated elsewhere, 100 percent fruit set from flowers fertilized is extremely rare, even in optimum conditions. Moreover, many orchardists routinely reduce their fruit crop by thinning at various times before fruit is mature – particularly in varieties grown for the table and ripened to perfection, and to please the eye.

A much more direct method to quantify pollination is to actually count the pollen grains placed on the stigma. This can be accomplished by various methods, the suitability of which depends largely on flower size and morphology of the stigma and pollen (see the following sections). One method involves the mounting of the upper part of the pistil (gynoecium) in acid fuchsin/gelatin on microscope slides in the field, and then counting the pollen grains with a light microscope when back at the laboratory. Certain species may have arrangements of stigmatic papillae, or copious quantities of stigmatic exudate, which make this practice difficult. There is also a chance of counting pollen grains on the stigma that do not belong to the species represented by the pistil. In addition, it may not be possible to observe the entire stigma when mounted on a microscope slide. Scanning electron microscopy may help to visualize

the entire stigmatic surface, and may even allow a true assessment of pollen germination and pollen tube growth, but involves a more expensive and time-consuming process.

The counting of pollen tubes at the style base in plant species that are self-compatible is another technique used to measure pollination directly and accurately. Each viable pollen grain, properly placed on a receptive stigma of the same species, sends down one pollen tube to deliver the sperm cell nuclei necessary for fertilization to occur. Pollen tubes within the centre of the style are relatively protected, and are therefore not nearly as susceptible to loss from the style as pollen grains might be from the stigma, during tissue processing.

Various techniques are used for pollen tube detection in the style. The most common involves the fluorescence of callose, a major component of pollen tubes, after staining of styles with a fluorochrome called aniline blue. One such procedure involves the following steps:

- Fix styles in the field in separate, labelled vials containing 1:3 acetic acid:ethanol for about 4 hours, before replacing the fixative with 70 percent ethanol.
- In the laboratory, soften the styles in 10 percent NaSO3 (sodium sulphite) or other strong base for several hours at 60 °C in an oven.
- Immerse the styles in 0.1 percent aniline blue in 0.1M K3PO4, overnight.
- Remove the styles, mount in a drop of aniline blue on a microscope slide, and gently squash under a coverslip.
- Examine the style bases for pollen tubes using a fluorescence microscope with an exciter filter of 450–490 nm. Callose stains yellow-green. Count the number of pollen tubes.

Although an excellent technique for pollen tube detection, the necessity of a fluorescence microscope may limit the practicality of this technique. Other methods that require only an ordinary light microscope for staining pollen tubes at the style base may be helpful (see References and Chapter 17).

18.2 ASSESSMENT OF FLOWER VISITORS AS POLLINATORS

Very useful information about pollination can be obtained from experiments that exclude visitors to flowers of the plant species under investigation. Typically, prevention of floral visitation is achieved in the field by bagging or caging. This practice serves as a valuable control to determine at the outset whether insects or other vectors are even required for pollination (is the plant parthenocarpic or autogamous?). Mesh bags can be sewn easily by hand or machine, and are often made from curtain lining, cheesecloth, bridal veil or other porous materials. The mesh size of the material used can be of utmost importance, and can provide very useful information. For example, in a study of *Echium plantagineum* in Australia, which excluded the large insects, adult thrips still traversed the bagging material used. Observed low levels of pollination in emasculated, bagged flowers of this species is attributed to their activity.

Emasculation, if it can be performed, is an outstanding technique when used in conjunction with bagging experiments, because any pollination of emasculated flowers must occur through the introduction of pollen from elsewhere. The feasibility of this technique depends much on the floral morphology. In addition, anther or stamen removal should be conducted with extreme care, in order to prevent selfing during the process. However, if available, it allows the investigator to get a quantitative handle on how much pollen foreign to the flower insect species "X" can introduce from its body during each visit. Such information is very pertinent from the standpoint of assessing predominant pollinators, and also with regard to managing and cultivating such particularly useful species. Emasculated, new (or "virgin") flowers that are bagged and belong to plant species pollinated by airborne grains are also easily assessed for outcrossing by such abiotic means.

Comparison of exclusion via bagging to normal biotic visitation at flowers is a simple and extremely useful technique. However, more intricate bagging

experiments can disclose further valuable information. A number of uncomplicated, additional steps can help determine which insects (and other animals) are responsible for the majority of crop pollination in a region. For instance, allowing single visits to virgin flowers allows actual pollinators to be distinguished from mere flower visitors, and also has the advantage of ranking various insect species according to their efficacy as pollinators of the crop in question.[5] Virgin flowers are blossoms initially bagged as unopened, mature buds to exclude any visitors. Then, after the buds have opened within the bags, they are carefully unbagged, labelled and then continuously watched for the first insect visitor. This insect should be captured and later identified by a specialist, if need be. As soon as that initial visitor departs, the flower is carefully rebagged, where it remains for the rest of its flowering lifetime. For comparison, other flowers are bagged for their entire lifetime or left open to multiple visits. After the necessary, predetermined time period for pollen tubes to reach the style base has elapsed, styles are harvested and processed for pollen tube counts (see above).

By measuring characteristics such as whether the insect was seeking pollen, nectar, both (or other), the length of the visit or whether the insect carried free pollen during single visits, the investigator can build an even stronger picture of the pollination story. This method even allows pollination efficacy to be discriminated within an insect species: queen vs. worker, male vs. female. Another advantage of this method is that it allows the investigator to determine the number of visits by insect species "X", on average, necessary to achieve adequate pollination of the crop.

18.3 HALLMARKS OF PLANT-POLLINATOR ASSOCIATIONS

For wind, bat, bird and to some extent large bee-pollinated flowers, the concept that flowers have marked features associated with their major pollinators has some utility.[6] However, less efficient pollinators also visit flowers better suited to other animals. Very different kinds of pollinators and flower visitors do in fact occur at each species of flowering plant. For instance, bumblebees and carpenter bees visit so-called "bird flowers", and long-tongued bumblebees visit flowers suited to butterflies. In other cases, a mixture of wind and large bee pollination may occur. Flowers pollinated by a guild of generalist insect pollinators, such as honey bees, stingless bees and flies, have less predictable features. Even so, Apiaceae flowers, for example, have a characteristically high concentration of flies and short-tongued bees, compared to legume or passion fruit flowers. Subtle floral features, such as the chemical composition of pollen and nectar, or floral odours and even microbes in nectar, may have a significant influence on flower-visiting animals.

A number of floral traits are associated with certain visitor types, or "pollination syndromes". Floral features that can strongly indicate dominant pollinators or mechanisms are as follows:

- **Wind**. The flowers are inconspicuous, often green or white, with little or no aroma and no nectar. They have a high pollen-to-ovule ratio. Pollen is abundant, light and dry, and lacking in pollenkitt (see section 19.2). The multi-branched stigma and mass flowering suit both wind dispersal and collection by some insects – grasses, *Casuarina* and *Plantago* are examples. Honey bees, stingless bees and bumblebees avidly collect pollen from crops such as maize and weeds like *Plantago*. The collection of grass pollen is common in the tropics, particularly for halictids, meliponines and honey bees.
- **Bats**. The flowers open at dusk or night and have a strong, musty smell. They are generally large in size, dangle on long-hanging stalks, or are arranged as pincushion or pagoda structures, with large

[5] The procedure is outlined in the original 1995 book (see reference at the end of Chapter 18).

[6] But see Ollerton *et al*. 2009 [Ed.]

mouthed flowers or strong brush inflorescences. Pollen grains are often "gemmate" (R. Palacios, pers. comm. to D.W. Roubik). The flowers are usually whitish, creamy, drab greenish or purple and rarely pink, with large quantities of nectar that can be dilute (10–30 percent sugar) and pollen. Examples include some bananas (*Musa*) and *Agave* species.

- **Moths**. The flowers have a heavy, sweet perfume and open at night, often closing by the next day. They are horizontal or pendant, with white or faint colours, or drab red. Nectar is deeply hidden in long, narrow tubes or spurs.
- **Birds**. The flowers open during the day and lack a scent. They are deep, tubular or spurred, with hard walls, stiff unit filaments and a well protected ovary – some examples are Malvaceae, Acanthaceae, Helioniaceae, Myrtaceae and some Rubiaceae. Brush flowers, (e.g. *Eucalyptus*), gullet or legume flowers (e.g. firebush *Erythrina*) can be pollinated by birds. Flowers are often scarlet or have contrasting bright colours, and a lip or margin is absent or curved back. Nectar guides may be conspicuous or absent, but nectar is abundant and well protected.
- **Bumblebees and carpenter bees**. The flowers are open during the day and give a fresh but generally not strong smell. Some pollen-producing flowers may smell more strongly (roses, poppies). Gullet (*Impatiens*), smaller tube (*Fuschia*, *Digitalis*) legume, bowl (Rosaceae, Malvaceae) and even large pendulous flowers (*Actinidia, Passiflora*, Solanaceae) seem preferred. Flowers are often pollen-only species, lacking nectar and nectaries. Flowers are arranged on a conspicuous rounded flowerhead or spike, so they are more readily seen at a distance (*Digitalis, Echium*). A nectar guide is usually present. Nectar of over 30 percent sugar is preferred, and is hidden at least 4 to 10 mm within the flower at times in a narrow tube. Flowers with structural depth and an uneven outline are preferred (e.g Lamiaceae, Scrophulariaceae, legumes). At times, the flowers have a "resting platform" for visitors. Colours are lively blue to yellow, pink or white, but nothing as inconspicuous as green. Nectar guides, which indicate the position of a nectar source to potential pollinators, are much less conspicuous to humans than to the bees.
- **Honey bees and short-tongued bees**. The floral traits are similar to those described above for the larger bees, although with less tendency to seek an uneven flower outline. Flowers with conspicuous spikes, and smaller flowers with shorter tubes or green colour and without much petal area, are satisfactory. The spherical flowerheads of *Acacia* and *Mimosa* are often attractive to smaller bees.
- **Butterflies and hawkmoths**. The flowers are open either during the day or night and have a weak, agreeable smell. They present a long, narrow tube or spur, ample nectar, an undissected rim with vivid red or purple colours, and a simple nectar guide or groove. *Buddleia davidii* and *Lantana* illustrate the type of flower, but other *Buddleia* species are among those favoured by bumblebees. Honeysuckle partially fits the criteria for butterfly flowers, but is among the preferred flowers for long-tongued bumblebees.
- **Flies, wasps**. Unspecialized flies and wasps can be prominent visitors of flowers with a platform and regular, simple flowers without much depth and light, dull colours, such as *Cissus* (Vitaceae), Apiaceae, ivy (*Hedera*) – many bees visit this flower in Europe,[7] holly (*Ilex*) and some golden rods (*Solidago*). Other flies with long, thin tongues such as some Bombyliidae and Conopidae tend to act like small bumblebees or butterflies, often visiting Lauraceae, Asteraceae and some Rubiaceae.
- **Beetles, some flies**. Certain flies (e.g. Sarcophagidae, Calliphoridae and Drosophilidae) and beetles (e.g. Staphylinidae, Cetoninae, Scarabaeidae and Nitidulidae) are attracted to carrion or fermenting fruit. These insects tend to seek strong smelling flowers, often presenting the resource in a spadix, a long, cylindrical floral organ holding many minute flowers. As among Araceae, the odour is dispersed by active heat production within the inflorescence (see Chapter 12).

[7] For further information see Garbuzov and Ratnieks. 2014. [Ed.]

18.4 POLLINATOR EFFICACY AND THE CROP

The effectiveness of a pollination agent depends on several factors:

- **Frequency and kind of stigmatic contact**. This can be a major factor in pollination of large flowers or flowers with extrafloral nectaries. Some flowers need at least ten stigmas to be contacted for a bee to transfer enough pollen to form a full-sized fruit with one visit. A few bees (*Bombus*, subgenera *Bombus* and *Mendacibombus*) and *Xylocopa* or wasps (*Ropalidia*) make a hole or a slit at the base of flowers of red clover, field beans and blueberries, which is then used by *Apis mellifera*. Failure to pollinate also results from probing through the side of the flower, for example, in crucifers or almonds, or from failure to "trip" flowers such as alfalfa. This can occur consistently with nectar-collecting *A. mellifera* and *A. florea*. On small and open flowers such as Apiaceae, Asteraceae and many less specialized flower families, stigmatic contact is achieved easily by most visitors.
- **Amount and availability of pollen carried per pollinator**. On large and relatively unspecialized flowers, bumblebees, honey bees and solitary bees can average 20–90 000 (max. 0.5 million) pollen grains on their body, compared to only a few on thrips. Other bee species, *Hylaeus* and other Hylaeinae, carry pollen internally, as they are relatively small and hairless. On kiwi fruit, the pollen-carrying ability of *Hylaeus*, at 100–500 pollen grains each, was similar to that of smaller hover flies (Syrphidae) (see also Chapter 12). Some pollinator species frequently carry pollen on part of the head, claws or body that closely contacts the stigma. Flower structure and the grooming habits of the pollinator usually combine to make some part of the body carry pollen available for pollination (other pollen is lost during grooming and transport to brood cells). The number of pollen grains that can make contact with a stigma is usually much less than the total carried on the body.
- **Quality of pollen transferred**. Bees, honey-making wasps such as *Brachygastra* and *Nectarina*, and masarid wasps collect pollen or nectar from flowers to feed their larvae as well as themselves, while other insects sustain only themselves at flowers. Thus female bees, needing a relatively large quantity of pollen to supply their broods, are likely to visit different plants to collect the food, which enhances their cross-pollinating activity far beyond that of all other flower visitors including masarid wasps. Frequent movement between flowers on separate plants is important for plants that need cross-pollination or that have distinct male and female individuals. In fruit orchards, honey bees mainly forage along rows, which restricts their effectiveness in cross-pollination unless suitable "pollinizers" are grown in the same row. When there are low nectar levels, bees on red clover and fruit blossoms move more often from flowerhead (or tree) to flowerhead, and over larger distances between flowerheads. With relatively high levels of nectar more flowers are visited on a flowerhead, and movements between flowerheads are less frequent and occur over shorter distances. High nectar production will support greater densities of foraging bees, but in principle cross-pollination may be somewhat reduced, compared to crops secreting less nectar. Differences in this foraging behaviour can be used in the field to assess whether limited nectar is restricting the populations of bees on a crop or encouraging cross-pollination.
- **Rate of flower visitation and length of "workday" (number of flowers visited)**. Honey bee foraging usually starts at 10–15 °C, with a lower threshold in spring than in summer. Flight activity rapidly increases at up to 20–25 °C. Wind above 10–20 kph curtails foraging. Thus, honey bee flight varies more within the day and with temperature variation than for many other bees and flies that visit flowers. Variation in the average hours worked per day has seldom been determined with much rigour. Bernd Heinrich's 1977 text on bumblebees

elaborates on the energy economics of foraging and their consequences on flower visitation at different temperatures. Among different bee species, differences in the rate of floral visitation (e.g. flowers visited per minute) quite commonly are on the order of 200–400 percent. Other flower visitors may be much slower. Insects that walk on the platform of Asteraceae and Apiaceae flower heads are able to economize greatly on energy expenditure. This allows them to continue collecting small amounts of nectar at flowers (when more active pollinators would shun them), and their small rate of return in cooler weather is still sufficient to offset the costs (if any) of warming up. The pollinating rate of visitors that walk on flowers may be only 33 percent when pollinators readily fly between flowers. However, greater time spent on individual flowers, as shown recently in studies of flies, can greatly improve effective pollination.

- **Preference for crop or competing flowers.** Concurrent blooms within the field or adjacent to the crop likely cause competition for pollinators, especially for generalized pollinators such as honey bees. However, under certain conditions those "competing" flowers also complement pollination. This is often the case for nectar-collecting workers when the effective pollinating bees collect only pollen. An individual bee may then collect both nectar and pollen on one foraging trip, and the general level of colony foraging is augmented through stimulus from available food. Again, the pollen-only crops, such as Solanaceae and Mimosoideae, among others, benefit from flowers near the crop used for nectar. In addition, flowers used to supply critical nectar sources can support the growth or slow the decline in honey bee colonies, which favours continuation of pollen collection for brood rearing. For example, a four-year trial with supplementary sugar feeding of bee colonies boosted kiwi fruit pollen collection 300 percent throughout flowering, with daily increases over 40 times the amount observed before feeding.
- If colonies fed with sugar perform comparably to those given additional, external nectar sources, it should be clear that some competing blooms do not detract from pollinator service but actually augment it. Hence, studies of competing blooms should focus more critically on the intensity of both pollen and nectar usage by pollinators before concluding that adjacent flowers are significant competitors (i.e. they have a net negative effect). This can be done through direct observation of pollen on foraging bees. A comparison of the ratio of honey bees to other bees on the crop and adjacent flowers will allow alternative flower sources to be ranked according to their relative attractiveness for the main pollinators. Investigation of the other pollen species present on bees foraging on the crop (see Annex 2) can provide further evidence to help identify the most important competing flowers.
- **Pollinator availability.** Honey bees are often the commonest flower visitors to crops because hives are introduced to the site for pollination or are kept there for honey production. This effect becomes more pronounced as the crops become larger, beyond 2 to 5 ha, because feral populations of other bees and insects may not forage throughout the field, and populations are simply not large enough locally to compare with honey bees. In Japan and the United States, populations of 600–1 000 solitary bees/ha (0.6–1.0/10 m^2) are enough to pollinate apple, almond, blueberry and sunflower crops effectively. In New Zealand, an estimated peak population of 2 000–4 000 pollinating bumblebees/ha will fully pollinate red clover crops with 36–72 million flowers/ha opening per day. Thus, low populations of alternative bee species can serve some selected crops if they are efficient in pollination (see Chapter 3). An assessment of the six above-mentioned factors should allow studies to focus on more detailed aspects regarding the impact of selected pollinator species, or the use of mechanical pollinators on seed formation or fruit production.

18.5 ASSESSMENT OF REQUIRED POLLINATOR NUMBERS

Four distinct methods can be used to assess the impact of a pollinator on crop yields and the number of pollinators or beehives needed for optimum production. Too few pollinators can lead to low crop yields, delays in harvesting and poorer quality fruit. Too many pollinators will result in excessive pollination expenses and over-investment in bee equipment. Real damage, as a result of breaking of branches through overbearing, shortened life expectancy of orchard trees, or reduced size and quality of individual fruit, may result. In certain crops, these factors prompt growers to thin a fruit crop during ripening. High populations will virtually force honey bees to seek other food sources more assiduously beyond the crop and possibly cause periods of pollen depletion, less than optimal honey production, and increased exposure to losses of bees from insecticides and natural enemies. It is therefore desirable to test the effect of alternative native pollinators as soon as adequate pollinators can be managed. Trials on yield responses aid the selection of better species to develop for management. Done carefully, they also allow direct checks to be made on the needed quantity of pollinators.

Approaches to determine the needed numbers of pollinators are based on field trials. Following this phase, the information gained can be used in various ways. The basic methods are as follows:

- **Yields in cages with bees enclosed or excluded, compared to open fields**. Cages that exclude insects and compare yields with open fields enable investigators to appreciate effective responses to pollination. However, the insights gained from such cages is limited – evident from the application of this approach too many crops. The enclosure of bees has mainly involved small honey bee colonies. The apparent merit of caging honey bees with a crop is to determine the approximate upper response in plant production of seed or fruit. At times, however, excessive bees in cages have resulted in poorer production, perhaps because of rapid pollen depletion, or shading that may affect both foraging behaviour and plant resources. Shading reduces nectar production and hours of bee flight. Results with caged honey bees have quite often produced yields that are difficult to interpret. Claims have even been made that honey bees rob (perforate) flowers in such situations, which is unknown in nature and suggests that other organisms in the cages (e.g. short-tongued bumblebees) have performed the actual robbing. Shading and fine mesh cut wind velocity within the cage and reduce wind flow and its effect on various crops.
- Caging bees, especially honey bees, also has the inherent weakness of eliminating competing blooms, which are quickly located by the colony (in contrast to many bees). Thus, consistent or high yields may not be obtainable under field condltions. Megachilid bees, carpenter bees or bumblebees forage more comfortably in restricted cages. On fruit and tree crops, this may be equal to only a part of the vine or tree. A cage can conceivably disturb parasite populations by excluding or concentrating them, or increasing the humidity, thereby affecting plant diseases that may influence yields. Initial testing of alternative pollinators is usually restricted by the limited supply of bees in hives, nesting material, flies, butterflies or other pollinators. A range of stocking rates can be achieved for small numbers of alternative pollinators in a cage to provide an initial estimate of the numbers of pollinators needed for application to commercial crops. An alternative is to use small plots in open fields while carefully monitoring the visitors.
- **Comparison of yields on large crop fields**. The judicious use of large and small crop areas can allow contrasting populations of the pollinating guild to be studied, where competition occurs in a commercial setting (unlike cage studies). Feral populations of alternative bee pollinators are usually limited, so that in a large crop of at least 15 ha, the effect of feral bees is often minimal compared to pollination from crops stocked with honey bees. (Exactly the opposite, however, occurs where there

are large numbers of wild honey bees, or any other kind of pollinator). The introduced pollinators cannot find unused flower patches and must cease to forage or otherwise seek resources elsewhere.

- Honey bees fly up to 10 km or more to forage, but in so doing gain few resources for the colony. Honey bees prefer to forage near their hives and the majority may forage within 400 to 500 m, provided that the crop is attractive, while a minority may freely fly to alternative flowers within 1.5 to 4.0 km. With adverse weather (12–15 °C) and winds, activity may concentrate within 200–300 m of hives, as foragers normally found on more distant flowers remain inside. Studies of production on strips of apple, longan and crops at varying distances from honey bee hives find differential pollination by honey bees, but the possible confounding effect of other pollinators is seldom clearly demonstrated.
- Large, active carpenter bees apparently forage up to 1.5 km from their nests according to a Malaysian study on passion fruit pollination. Most bumblebees forage within 400 to 800 m of the nest, based on marked bumblebees from feral colonies in Nebraska, United States, and a study in New Zealand that moved nests and recorded the distances from which displaced bees returned to their former nest sites. In the former Czechoslovakia, lucerne yields were found to be lower beyond 40 m of nests of the halictid bee *Rhophites canus*. Other small solitary bees are unlikely to fly customarily farther from their nests than bumblebees or carpenter bees. In New Zealand, seed yields of lucerne almost doubled within 20 m of hives in a field with megachilid bee numbers inadequate to permit maximum seed yields in alfalfa.
- **Plots of small crops – tests of different stocking rates**. Replicated small plots can provide unequivocal evidence of the floral preferences of bees, as shown for legumes, cruciferous crops and borage, and particularly for bumblebee species in Canada, Denmark and New Zealand. Plots of 0.1 to about 2 ha can be valuable for testing the actual impact of alternative pollinators, provided the species has a preference for the crop being studied. Several small plots spaced at least 1 km apart (beyond the usual foraging distance for most bees and blowflies) allow stocking rates at 12.5 percent, 25 percent, 50 percent, 75 percent and 100 percent at peak flowering. The rates tested will depend on the available supply of pollinators and the expected response in yield. A detailed study of a plot can illustrate the optimum pollinator abundance. In addition, the validity of yields and other parameters used for predictive modeling may be checked under field conditions. Small plots can also attract modest honey bee numbers, especially where other attractive crops or weeds are present in the vicinity.
- **Estimating required pollinator number and the use of predictive models**. A predictive model that makes few assumptions can yield estimates of required pollinator numbers. The model would ideally ensure that the value of each main pollinator species is adequately assessed. Once pollinator populations have been measured in the field, and their efficacy verified, a national assessment can be made without unduly complicated mathematical formulae or computer simulation. For instance, in New Zealand typical pollinator populations on red clover crops were only about one-third of those needed for full pollination. Smaller plantings of < 3 ha yield up to twice that of plantings > 5 ha. The available pollinators are better able to service smaller patches. This illustrates why some of the large-scale predictive models might fail – as with all models, they are only as accurate as the figures and variables they employ. Variables that are lengthy or costly to measure, such as the number of flowers opening per day, might still produce no reliable predictions if the model does not fit the setting, or if other measurements are not taken.[8]

[8] For further information see Degen and Roubik. 2004. [Ed.]

Two formulas are needed to assess crop (or grower) demand for pollination and the capacity of pollinators (beekeeper or supplier) to provide the required pollination. The first is straightforward:

> Flower pollination requirement (or grower demand) = Number of open flowers (standing flower crop) + new flowers opening (per day)

Some flux in daily needs for pollination must occur. Cooler weather, for example, may prolong stigmatic receptivity, as sometimes will fair weather. The response of a crop to pollination will always be below 100 percent and may depend on weather and growth during the flowering period. It will also be affected by fertilizer or pesticide application. Further responses in yield will not be possible above a certain level, which will vary from year to year and from crop to crop. Nonetheless, a second equation may be given to show the relation between pollination supply and demand:

- Daily pollination delivery (by supplier) = average number of pollinators per unit area (ha, tree, flower group, etc.)
- Flowers visited per pollinator per hour
- Hours of foraging per day

Combining and rearranging the two foregoing equations, the number of needed pollinators can be computed in a slightly expanded form, as follows:

> N pollinators needed = N flowers open x N visits for full seed set/N visits per hour x N hours daily foraging.

A predictive model can provide at least an order-of-magnitude estimate (e.g. whether 1 000 or 10 000 are appropriate numbers) of the required pollinators, so long as accurate figures are incorporated. This particular model would require knowledge of the numbers of visits needed from each pollinator species to produce full seed set, as well as reliable counts of the other three variables.

For apples and almonds, interactive computer-based models have been developed in the United States to predict fruit and nut set from variables including weather, flowering of the crop and honey bee foraging behaviour. That in turn permits colonies to be moved from the orchard as soon as possible to allow pesticide spraying. Such computer models are valuable, but have limited value in places with minimal access to computers.[9]

[9] Additional references and figures for this chapter can be found in Roubik, D.W., ed. 1995. Pollination of cultivated plants in the tropics. Agricultural Services Bulletin No. 118. Rome, FAO, available online: http://books.google.com/books?isbn=9251036594

REFERENCES

Beattie, A.J. 1971. A technique for the study of insect-borne pollen. *Pan-Pacific Entomologist*, 47(1): 82.

Davis, A.R. 1992. Evaluating honeybees as pollinators of virgin flowers of *Echium plantagineum* L. (Boraginaceae) by pollen-tube florescence. *Journal of Apicultural Research*, 31(2): 83–95.

Degen, B. & Roubik, D.W. 2004. Effects of animal pollination on pollen dispersal, self-pollination and effective reproductive population size of tropical trees: a simulation study. *Biotropica*, 36: 165–179. doi: 10.111/j.1744-7429.2004.tb00309.x.

Garbuzov, M. & Ratnieks, F.L.W. 2014. Ivy: an underappreciated key resource to flower-visiting insects in autumn. *Insect Conservation and Diversity*, 7(1): 91–102. doi: 10.1111/icad.12033.

Heinrich, B. 1979. *Bumblebee economics*. Cambridge. MA, USA, Harvard Univ.

Ollerton, J., Alarcón, R., Waser, N.M., Price, M.V., Watts, S., Cranmer, L., Hingston, A., Peter, C.I. & Rotenberry, J. 2009. A global test of the pollination syndrome hypothesis. *Annals of Botany*, 103: 1471–1480. doi:10.1093/aob/mcp031.

Chapter 19

FLORAL BIOLOGY AND RESEARCH TECHNIQUES

19.1 NECTAR AND NECTARIES

A.R. Davis

Although it usually contains a number of minor chemical constituents, nectar is composed largely of water and sugars produced by the photosynthetic machinery in plant leaves. This sweet exudate is secreted from glands called nectaries.

19.1.1 Nectaries and where to find them

Nectaries can be found on many plant parts – cotyledons, stems, leaves, stipules, flowers and fruits – and are sometimes located in more than one place. For example, two nectaries of chickpea *(Vigna unguiculata*) are present on stipules and the inflorescence stalk between flower bases instead of on floral parts, while a third nectary found in the flower is associated with the ovary base. In exceptional cases, such as *Campsis radicans* (Bignoniaceae), they can be found in five separate locations with four of these located on floral parts (on sepal and petal lobes, surrounding the base of the ovary and on the surface of developing fruits). A distinction is drawn between nectaries according to their location. *Extra-reproductive* nectaries are located on vegetative parts and, as such, are *extrafloral* nectaries. *Reproductive* nectaries include glands on the inflorescence, flower stem and bracts, as well as those found on floral parts of the plant. Extra-reproductive nectaries frequently provide mutual benefits for both plant and insect, particularly in the tropics. For example, certain tropical ants that forage nectar at "extrafloral nectaries" defend plants by attacking herbivores. However, it is the reproductive nectaries that are most significant for pollination. Some nectaries are situated on the lower surfaces of outer floral parts, and because they may secrete nectar even before the flower opens, they are not directly involved in attracting potential pollinators. Glands that encourage pollination by flower visitors are those that actively secrete nectar in open flowers and are usually located near the base of the flower's reproductive structures – the stamens and/or the ovaries – or positioned elsewhere on the flower (e.g. the spurs of the corolla). Their location and the timing of nectar secretion ensure pollinator contact with the anthers and stigma when the gland is probed by a visitor of the appropriate size. Thus, of the four nectaries situated on floral parts in *Campsis,* it is the nectary gland below the ovary that is associated with pollination.

Within taxonomic groups, the position of nectarial tissue is usually consistent. In monocotyledons, nectaries are situated in septae of the flower's ovary. In the mustard family (Brassicaceae), the glandular tissue is always located at the base of stamens. In *Eucalyptus* (Myrtaceae), nectar exudes around the floral cup, in which it accumulates beneath the

ring of stamens. The location of nectar itself is a way to detect nectaries, but there are species that under appropriate environmental conditions produce copious nectar- which runs from the flower, thus making detection of the glands more difficult. If nectar secretion has not yet begun or nectar resorption has occurred, nectaries are difficult to find. However, a difference in colour between the nectarial tissue and that of adjacent floral parts can assist with detection. Several species of the mint family (Lamiaceae) display bright yellow glandular tissue below the four green ovules or patches of dark purple. Furthermore, pigmentation of some extrafloral nectaries makes them visible from a distance.

The morphology of nectarial tissue is another feature used to detect nectar glands, although higher magnification is sometimes required. Many nectaries surrounding the base of ovaries take the form of multicellular protuberances or outgrowths. In *Ajuga reptans* (Lamiaceae), nectarial tissue forms a swelling on the lower side of the flower.

Echium plantagineum (Boraginaceae) has a disk-shaped nectary that encompasses the bases of the four developing nutlets. The discoid nectary of *Vicia faba* (Leguminosae) bears a long protuberance below the developing bean. In each of these examples, scanning electron microscopy reveals pores of modified stomata within the nectary surfaces through which nectar can escape. However, some nectar glands are composed of aggregated secretory hairs *(trichomes)*, also usually distinguishable only by microscopy. In *Hibiscus* (Malvaceae), the inner surface of each sepal bears thousands of multicellular trichomes, while the extra-reproductive nectaries on the stipules of *Vicia faba* are also composed of hairs. Many nectaries do not protrude and are instead contiguous with the surfaces of adjacent floral petals, normally consisting of relatively few layers of cells. These types of nectaries are often only recognizable by the presence of nectar itself. In the case of *Medicago sativa* (Leguminosae) and *Eucalyptus leucoxylon*, for example, the surface under the exudate bears stomatal pores. However, other *non-structural* nectaries, such as that on the outer calyx surfaces of *Paeonia* buds (Ranunculaceae), contain no specialized glandular tissue and are only identifiable by the nectar droplets above them.

19.1.2 Nectar volume, concentration and influencing factors

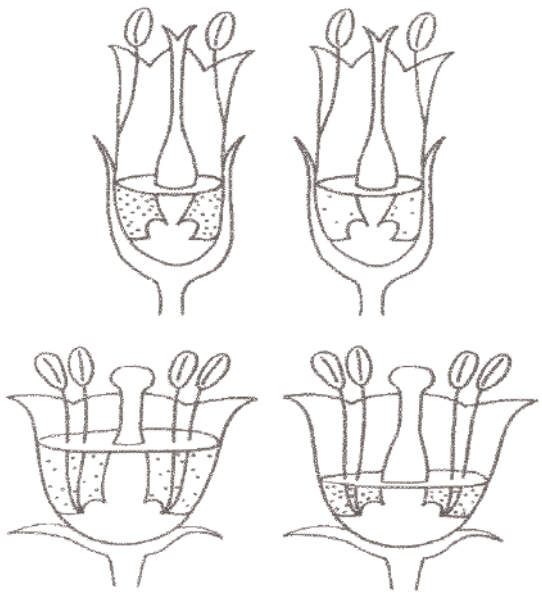
Nectar standing in a flower undergoes continual changes, influenced by the forces acting on it. While secreted by nectaries, nectar can also in many cases be reclaimed or reabsorbed by the gland. In this way, plants have the ability to conserve the constituents of nectar and utilize them elsewhere. Nectar volume decreases as a result of resorption or embedment. However, it may also decrease as a result of evaporation. Water evaporation from floral nectar is greater when nectar is relatively exposed to the atmosphere, instead of protected at the base of a long corolla tube. When evaporation is intense, nectar volume can decrease despite continuing secretion. Similarly, increases in floral nectar volume can be brought about by raindrops that fall or run into the flower and mix with standing nectar. In addition, standing nectar with a relatively high sugar concentration in humid air can obtain water vapour from the atmosphere. Nectar accumulates whenever net secretion rate exceeds the combined losses of reabsorption, embedment by visitors and evaporation.

Sugar concentration changes as water evaporates from sugar solution: volume *decreases* as the concentration of sugar *increases*. This relationship between volume and concentration must be emphasized in nectar analysis because the *quantity of nectar sugar* in the sample is of primary importance. The flowers shown in Figure 19.1 (top) have identical nectar volumes, but unless nectar samples are checked for sugar concentration, one might assume, erroneously, that each flower's nectar contains the same sugar. Estimates of a flower's nectar sugar are calculated by multiplying nectar volume by sugar concentration. For example, suppose that two flowers contain 3.5 µL (microlitres, or 1/1 000 000th of a litre) of nectar. In one the nectar contains 36 percent sugar

(i.e. 36 g of sugar per 100 ml of water), while the other has a concentration of 9 percent. Although the two flowers hold equal volumes of nectar, one contains 1.26 mg sugar while the other contains only 0.315 mg. These quantities were calculated by multiplying nectar volume by sugar concentration (e.g. 3.5 µL x 36 g sugar/100 ml water = 1.26 mg sugar).

The same approach can be used when volumes of nectar in two flowers differ. In Figure 19.1, the flower on the left holds 5.0 µL containing 12 percent sugar. The flower on the right holds 2.0 µL containing 30 percent sugar. Both flowers have exactly the same sugar content in their nectars (5.0 µL x 12 g sugar/100 ml water = 0.60 mg sugar = 2.0 µL x 30 g sugar/100 ml water), even though the flower on the left has a nectar volume 2.5 times higher. Therefore, it follows that measuring two of the three nectar characteristics (volume, sugar concentration, quantity of sugar) will provide an estimate of the unmeasured third characteristic.

Figure 19.1
SCHEMATIC FLORAL COROLLAS SHOWING NECTAR CONTENT AND SUGAR CONCENTRATION, INDICATED BY STIPPLING. THE SAME AMOUNT OF NECTAR CAN HOLD DIFFERING AMOUNTS OF SUGAR AND ENERGY, TAKEN BY FLOWER VISITING ANIMALS



Source: A. R. Davis [present study]

19.1.3 Collection and measurement of nectar samples

A number of techniques are used to collect floral nectar. As well as depending on the equipment available, the methods used vary according to the morphology and size of the flower. For instance, only minute quantities of nectar may be present in very small flowers. In addition, if nectar is released from a nectary located at the flower base and is relatively inaccessible, nectar collection is possible only after careful dissection of the flower.

Nectar volume. Several techniques available for nectar collection can also yield estimates of nectar volume, without too much difficulty. Accuracy depends of course on the method used. One relatively simple technique does not involve nectar collection – visual observation is coupled with a scale measurement. This technique can be performed most accurately for tubular corollas where the accumulated nectar can be measured within the cylindrical tube. However, any meaningful analysis of nectar will require measurement of nectar sugar – either in terms of sugar concentration (percentage) or sugar content (weight). This involves direct sampling of nectar.

A popular technique to collect nectar and measure its volume involves glass microcapillary tubes (many types are commercially available). One end of the capillary is inserted into the nectar droplet, and the nectar is drawn into it through capillary action. Using capillaries of a specified inside diameter, marked to show the exact volume (also called calibrated microcapillary tubes), allows the length of the sample column to be expressed as a fraction of total microcapillary capacity. Capillary tubes work best when the viscosity of the nectar is not too high. Capillary movement of the nectar is low when nectar concentration exceeds 60 percent. In certain cases, it is best to remove all standing nectar, such as when comparisons of absolute nectar sugar content are being made between cultivars of a crop species. However, with many flower and nectary morphologies, capillaries may fall short of removing all accumulated nectar.

A calibrated syringe or pipette can also be used to collect nectar directly. This approach is used most when nectar volumes are very large (e.g. 50–100 µL or more) and collection using several micro-capillaries would be too time consuming. Centrifugation is another method to simultaneously collect nectar and measure its volume. This technique has been used to collectively sample the nectar of a compound inflorescence of several small florets. An inflorescence is inverted into the top of a specially designed glass centrifuge tube and secured with a stopper. Nectar spun out of the florets during centrifugation flows to the bottom into a calibrated channel, where volume can be assessed. However, a recent study utilizing this system for *Brassica napus* gave a lower estimate of nectar sugar, compared to that sampled directly by a capillary. Nectar volume during centrifugation may therefore have been increased artificially, as a result of water condensation inside the centrifuge tubes entering the spun nectar. If this system is used extensively to measure nectar volume, it is recommended to first determine the time required to extract the nectar from the flowers under investigation and to compare the nectar sugar concentration of spun nectar with nectar withdrawn by capillary. Ensuring that centrifugation duration times are identical between samples will also help to minimize variation due to technique.

Filter-paper wicks are becoming increasingly popular as tools to collect floral nectar. Small pieces of filter paper can be held at one end with forceps or impaled on pins, and their edges dipped into the nectar. The wicks are then air-dried and stored, preferably in a desiccator that will prevent growth of micro-organisms on the enriched wicks. When analysis is performed, the nectar sugar is eluted from the wicks by submerging in a known volume of water and shaken vigorously for several minutes. Although the use of wicks alone does not permit direct measurement of nectar volume, there are instances where volumes of nectar are too low to be determined by the other methods (capillaries, syringes, centrifugation, see below). Other researchers have added known amounts of distilled water from a pipette dispenser, like an Eppendorf, as discussed below.

Rinsing flowers in a known volume of water is another means to collect nectar. The flower must be shaken vigorously to ensure wetting of nectaries, and then allowed to soak before it is removed and the sugar-containing rinsate is available for assay. This method is best when nectar is scarce or viscous, and acquisition of at least some data is preferable to none at all. The rinsate is then analysed for its sugar (see below). One potential drawback of soaking the flower to collect nectar is that other sources of floral sugar (e.g. leakage from damaged flower parts, or leaching of the sugar from the stigmatic exudate in the case of wet stigmas) may confound the true sugar concentration of the nectar.

A related technique to rinsing that does not involve submersion of the entire flower in water is the controlled addition of a measured small volume of water onto the nectar droplet. This technique again is useful for small nectar quantities or when nectar is too viscous to be otherwise collected. Repeated and careful uptake and expulsion of this water using a micropipette will mix it with the residual nectar, thus providing another way to recover nectar sugar. Again, with this technique, nectar volume is not measured directly.

Occasionally, a combination of these techniques may prove useful. For instance, when it is desirable to obtain all available nectar from the base of a flower, such as one possessing a protruding nectary, the task may be difficult to perform with only a microcapillary. It is advisable to first use the microcapillary to draw some nectar from the edge of the nectar droplet (without probing the nectary, which may be damaged by the end of the glass capillary). Nectar volume and concentration (see below) are determined from this subsample. The nectary is then gently swabbed to gather residual nectar at the flower base using a filter-paper wick. Subsequent determination of the sugar content from wick and capillary allows total nectar volume to be calculated, assuming the nectar is a homogeneous solution. *Such a combined method of nectar collection is recommended where repeated sampling from the same nectary is desired.* Experimental studies of the effect of nectar removal on total nectar production, or of resorption, should utilize a wick and

thereby avoid inflicting physical damage on the nectary during the previous collection period by contact of the gland with the probing end of a glass capillary.

Nectar sugar concentration. A simple taste of a nectar sample often allows distinction between weak and strongly concentrated nectars, but this cannot provide sufficient accuracy. Refractometry is the common and most accurate method of measuring solute concentration of a nectar sample. Because sugars make up almost all of the dry weight of nectar, it is generally a safe assumption that solute concentration represents a close estimate of sugar concentration. Amino acids, as secondary constituents of nectar, have been shown in certain species to make up 10 percent of the nectar by dry weight. Amino acids are difficult to quantify chemically. When comparisons are made between nectar samples of the same species, refractometry can be depended upon to provide a close, relative estimate of sugar concentration.

In the past, nectar concentration was determined in the laboratory using Abbe refractometers. However, modern, lightweight, hand-held refactometers can be carried and utilized easily in the field. For capillary and syringe collection, this flexibility allows nectar concentrations to be determined outside, immediately after volume measurements are taken. Refactometers can be obtained that give concentration readings across various ranges. Concentration measurements for samples are indicated on the refractometer usually in the range of 0–50 percent or 40–80 percent, or in some cases, 0–90 percent. If the study is limited to nectar at the base of long-tubed corollas, the smaller refractometer may be sufficient, but if the intent is to study nectar from the relatively smaller flowers visited by bees, it will often be necessary to measure nectar concentrations greater than 50 percent. To obtain a concentration reading, the minimum volume of nectar required depends on the type of refractometer, but can be as low as 1/50th of a µL in refractometers specially modified by the manufacturer to accommodate small samples (e.g. Bellingham Stanley from Turnbridge Wells). Such a refractometer is preferable, because it allows the measurement of nectar solute concentration on a small nectar sample available per flower, and thus conserves the variation between individual flowers that is lost when pooling nectar from several flowers.

The procedure to measure nectar sugar by refractometry is quite simple. The sample is expelled onto one of the two facing prisms in the refractometer. The sample area of the refractometer is then closed, which spreads the sample into a very fine layer, through which light passes. The more concentrated the nectar solution, the greater its refractive index. Refractometers vary by manufacturer, but some available models give readings in percent directly on a sucrose sugar scale. It is important to note that the refractive index of the nectar sample is dependent on temperature, and therefore a record of the ambient temperature during nectar handling is necessary in order to correct the true concentration (usually compared to 20.0 °C). The data tables necessary to allow such temperature conversions are usually supplied by the manufacturer. Some refractometers give concentration readings after automatically compensating for temperature.

Another conversion needs to be performed on the refractometer reading of percent concentration before the true solute concentration of the nectar is known. This conversion is necessary because the refractometer reading is expressed as the gram weight of sucrose (or its equivalent as glucose and fructose) in a 100 g solution, instead of the volume measurement of 100 ml of solution. Fortunately, a simple quadratic equation can be used for the conversion. The equation is:

$$\mathbf{NCV} = \mathbf{NCW}\ (9.224 \times 1(10\text{-}3) + \mathbf{NCW}^2\ (59.6 \times 10\text{-}6) + 7.08 \times 10\text{-}3$$

where **NCV** is the percent nectar concentration based on volume, and NCW is the refractometer reading of percent nectar concentration based on weight, after correction for temperature.

As an example, take a nectar sample that gives a refractometer reading of 56.5 percent when measured in the field at 29.0 °C. At this concentration, 0.73 percent must be added to the reading for each degree in temperature above 20.0 °C. Therefore, the

temperature-corrected reading is 56.5 + (9)(0.73) = 56.5 + 6.57 = 63.07 percent. This is the **NCW**. By substituting this value into the equation above, the nectar sample has an **NCV** (sugar concentration based on volume of solution) of 82.6 g per 100 ml solution (i.e. **NCV** = (63.07)(9.224 x 10^{-3}) + $(63.07)^2$ (59.6 x 10^{-3}) + 7.08 x 10^{-3} = 0.82592, or 82.6 percent). This value of **NCV** (82.6 g/100 ml) can now be multiplied by nectar volume to determine the nectar sugar content (see Figure 19.1).

If refractometers are not available, another (less accurate) indirect method can be used to obtain estimates of nectar concentration. The technique is based on the degree of spreading of a nectar droplet, as pioneered by Irene Baker. Using identical volumes, highly concentrated droplets of sugar solution spread less than those that are very diluted when dabbed on filter paper from capillaries. The maximum diameter of the faint outline of the nectar spot is measured immediately with digital calipers, say three times per spot, and the average diameter is calculated. In the laboratory, a series of standard curves can be constructed from which nectar concentrations can be extrapolated later. The curves are created by using freshly prepared sugar solutions of known concentrations (e.g. 5 percent increments). The use of different nectar volumes (µL) (e.g. 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, etc.) of each concentration allows a series of diameters to be plotted against nectar volume for each concentration. Then, using the known volume of the nectar sample, it is possible to estimate its sugar concentration from the standard curves.

Sugar content. Analyses of nectars have identified a number of sugars including some apparently found only in nectar. The three most common sugars in nectar are the disaccharide sucrose and the monosaccharides glucose and fructose. Each sucrose molecule consists of one glucose and one fructose molecule joined together. Generally, flowers with relatively long corollas and a deeply concealed nectaries produce sucrose-dominant nectar, whereas flowers that are relatively "open" in form produce nectar richer in glucose and fructose. There are many exceptions to this rule, however, and not all nectars contain all three sugars – the so-called "big three". The ratios of the various sugars vary in standing crop nectar found in plant species that produce an enzyme called invertase with their nectar. Invertase assists the conversion of sucrose into its separate building blocks: glucose and fructose. Invertase is also added by honey bees to floral nectar when they are ripening it into glucose-rich honey.

As stated earlier, the sugar content of a nectar sample is of particular interest, because it remains constant during any change in nectar volume or concentration. An acceptable estimate of nectar sugar content (e.g. percentTDS, total dissolved solids) can usually be obtained indirectly from the product of volume and concentration. When the nectar collection method (wicks, rinsing, etc.) makes it impossible to measure accurately either volume or concentration – or the necessary equipment to do so is unavailable – direct measurement of the quantity of nectar sugar in the sample is required.

Unlike measurements of volume or concentration, which can normally be performed in the field, procedures to determine the total nectar sugar content of a nectar sample can only be conducted in the laboratory. The various chemical methods used to analyse nectar sugar content are based either on *chromatography* or *spectrophotometry* (colourimetry), which detect changes in the intensity of colour of a sample or subsample of nectar. This colour change is a result of a chemical reaction that involves conversion of the nectar's sugar to other compounds, which are intensely coloured.

In addition to spectrophotometric and paper-chromatographic techniques (detailed below), there are several other methods including enzymatic analysis, gas chromatography and high-pressure liquid chromatography (HPLC). Because of the specialized instrumentation required, those three methods are much more expensive and are not presented here.[10]

[10] For further information about these methods see Dafni, Kevan and Husband (2005), and Kearns and Inouye (1993). Nuclear Magnetic Resonance, or NMR methods, are also used for fine resolution of chemical contents [Ed].

Spectrophotometric techniques differ in the information they provide. Some can determine quantitatively the relative amounts of sugar components of a nectar sample. Three spectrophotometric methods for nectar analysis are given below. The first two are the simplest and only provide the total quantity of nectar sugar, whereas the third is more complex and can yield additional information about the relative abundance of dominant sugars in nectar. One of the two simplest methods is usually sufficient. An advantage of the third protocol is that the relative quantities of sugars may be subjected to artificial selection in plant breeding programmes.

Nectar sugar content can be determined by spectrophotometry using either freshly collected samples or a large number of stored samples. Small wicks on which nectar has been previously gathered and allowed to air dry can be stored at room temperature for long periods in glassine envelopes or inside aluminum foil packets in a desiccator. This affords greater ease than using the rinsates of diluted nectar recovered from shaking and rinsing flowers in water. The latter must be stored under refrigeration or, better yet, frozen in vials that can withstand freezing temperatures, to ensure the prevention of microbial growth which normally consumes sugar.

The appropriate volume of water to be used for soaking each flower or eluting the dried sugar from each wick will vary with flower size and nectar production of the species under investigation, but commonly falls in the range of 1 to 5 ml. The exact water volume required will be determined by prior experimentation. Only the minimum volume necessary should be used, as it is better to dilute the rinsate with additional water, than produce a sugar concentration that is too weak to analyse.

- Using a pipette, transfer a known volume of rinsate to a cuvette (the glass container in which the chemical reaction will occur). Place the cuvette in the light/detection chamber of the spectrophotometer.
- Light of a specified wavelength is passed through the cuvette and the absorbance of the solution is measured automatically.
- Each cuvette must be clean and free from scratches. Each nectar sample should be analysed at least twice, preferably three times, and the mean absorbance then calculated.
- Greatest accuracy is achieved if the absorbance reading is kept within the range of 0.10 to 0.90. Prior experimentation involving changes in the total volume in the cuvette, the amount of rinsate placed into the cuvette, the minimum volume required in the cuvette to achieve absorbance readings, etc. is necessary to register routine absorbance in this range.
- After the initial analysis, if the rinsate is found to be too concentrated (i.e. absorbance > 0.90), it must be diluted in order to obtain a reading. Remember to include this dilution factor and the initial volume of rinsate used when working back from the standard curve to ascertain how much nectar sugar was present in the flower.

Phenol-sulphuric acic test for total sugar. WARNING: *Both phenol (carbolic acid) and sulphuric acid H2S04) at high concentrations can be extremely caustic to the skin, and must only be handled with extreme care by authorized laboratory personnel, wearing adequate eye and cloth protection. In case of skin contact, rinse the area repeatedly with water.*

Prepare the nectar sample:

- Filter paper wick: Soak the wick in a known volume of distilled water in a test tube or vial, with periodic and vigorous shaking, over a 5-minute period. Because this test is strongly exothennic and non-selective, even sugar in the form of lint or plant tissue, such as pollen grains, or fibres loosened from the wicks during shaking, can be broken down and measured erroneously as nectar sugar. Therefore, it is strongly recommended after shaking to centrifuge the vials or tubes containing the wick rinsate, to cause any such particles of potential contamination to settle at the bottom, and then draw off the supernatant aliquot to be assayed for nectar sugar from the top portion of the centrifuge.
- Nectar collection by rinsing: Shake the flower vigorously in a known volume of distilled water in a vial, and let it soak for 45-60 minutes. Transfer

the rinsate to a tube, and centrifuge, to displace particulate matter to the bottom. If the rinsate was previously collected and stored frozen, thaw it now.

- Using a pipette, transfer a known volume of the rinsate to a glass cuvette. For spectrophotometers requiring a minimum volume of 3 ml in the cuvette, 0.5 ml of rinsate works well.
- Add 0.5 ml of 5 percent phenol to the rinsate in the cuvette.
- To the cuvette, now add 2.5 ml of concentrated sulphuric acid, being careful of splattering. In this example, the final assay volume is 3.5 ml.
- Mix this hot solution thoroughly (e.g. using a clean glass rod, taking care not to scratch the cuvette) and allow 45 minutes for a yellowish-orange colour to develop at room temperature. Once developed, the colour is stable for up to two hours.
- Measure the absorbance at 490 nm wavelength compared to a blank cuvette containing the same volumes of phenol and sulphuric acid, but with water instead of rinsate and calculate sugar content from a linear standard curve. The standard curve is created by plotting the absorbances obtained against the quantity of sugar analysed after assaying standard sugar solutions (i.e. solutions carefully prepared using known sugar concentrations). For instance, take 0.5 ml of a 10 µm sucrose solution (prepared by diluting 1.0 ml of a 10 mm sucrose solution [3.42 g sucrose dissolved in water in a 1 000 ml volumetric flask] in 999 ml of water). Add 0.5 ml of 5 percent phenol, 2.5 ml of H_2SO_4 and then measure absorbance. For final assay volumes of 3.5 ml, the functional range of this test is 0.1 to 1.5 µg sugar.

When preparing the standard sugar solutions, the ratio of sucrose/glucose/fructose used in the standards should ideally match the ratio of these three sugars in the nectar species being analysed. A semi-quantitative method to determine the approximate ratios of these three sugars is given below.

Anthrone reagent test for total sugar content. The anthrone reagent test is less sensitive than the phenol-sulphuric acid test, and hence is useful for relatively large amounts of nectar sugar. However, it can fall short of detecting sugar with sufficient accuracy in nectars low in volume or sugar concentration. Like the phenol-sulphuric acid test, the anthrone reagent reacts similarly with all carbohydrates: mono, di and polysaccharides, dextrins, dextrans, starches, gums and glucosides. Because the saccharides are by far the predominant carbohydrates in nectar, total carbohydrate content is assumed to represent total sugar content.

- *Prepare* the nectar sample in water, as in the phenol-sulphuric *acid* test. Centrifuge the rinsates to avoid any *foreign* debris in *the* analysis.
- Transfer a known volume of the rinsate to test tubes: 2 ml of rinsate works well per test tube. Tubes with crew caps are ideal for this test, because at some stage the tubes will be subjected to a boiling water bath.
- To each test tube add 4.0 ml of fresh (0.2 percent) anthrone agent. This reagent is prepared by dissolving 0.4 g of anthrone in 200 ml of concentrated sulphuric acid. Storage of the reagent in the refrigerator extends its usable life to one or two weeks. However, as the reagent darkens with storage, it is imperative that sugar standards are always analysed as well.
- Heat the test tubes for 10 mins at 100 °C (a boiling water bath). A blue colour will develop.
- After the test tubes have cooled to room temperature, transfer the contents to cuvettes for spectrophotometry.
- Measure the absorbance at 620 nm wavelength, after zeroing the spectrophotometer against a blank cuvetter containing only water and anthrone reagent. The sugar content of the nectar is calculated from a plot of absorbance versus sugar prepared from standard solutions.

Acid hydrolysis test: reducing sugars and sucrose content. This method determines the amount of reducing sugars (mostly glucose and fructose, although maltose is found in some nectars) both before and after subsamples are subjected to acid hydrolysis. The apparent sucrose content of the nectar is calculated as the difference in sugar quantity between the two assays.

- Prepare the nectar sample in water, as in the phenol-sulphuric acid test (using at least 2.0 ml water), then centrifuge the sample.
- Prepare the reagent, a solution of 3.5-dinitrosalicylic acid: At room temperature, dissolve 1 g of the acid in 20 ml of 2 N sodium hydroxide (NaOH) and 50 ml of distilled water. Then dissolve 30 g of Rochelle salt (the sodium-potassium salt of d-tartaric acid, $NaKC_4H_4O_6$, $4H_2O$), and add distilled water up to a final volume of 100 ml. Cover the solution to protect it from carbon dioxide present in the air.

Reducing sugars assay:

- Add 1.5 ml of the reagent to 1.0 ml of the rinsate and mix well in a test tube. Incubate the mixture for 5 minutes at 100 °C (boiling water bath). A brown colour will develop. After cooling to room temperature, transfer the solution to a cuvette, add 2.0 ml of distilled water and measure spectrophotometrically the absorbance at 540 nm (green filter) against a blank cuvette consisting of water instead of rinsate. The content of reducing sugars in the rinsate is determined from a standard curve prepared using equimolar solutions of the reducing sugars glucose and fructose.

Total sugars assay:

- Add 1.0 ml of 2 N hydrochloric acid (HCl) to 1.0 ml of the rinsate in a test tube and incubate for 5 minutes at 100 °C. This procedure converts any sucrose present into its monosaccharide components, glucose and fructose. Then add 1.0 ml of 2N NaOH and 1.5 ml of the 3.5-dinitrosalicylic acid reagent, to achieve the final volume of 4.5 ml. This solution is then heated for another 5 minutes in the boiling water bath. After cooling, the solution is transferred to a cuvette and the absorbance read at 540 nm wavelength.
- Calculate the amount of sucrose in the nectar sample by subtracting the content of reducing sugars from the assay of total sugars, above. The method cannot distinguish fructose from glucose. In most nectars analysed to date, however, the ratio of these two reducing sugars is usually at or near 1.

Paper chromatography is an excellent technique that enables the separation and identification of components of a mixture in a solution. It has long been used to detect the various sugars present in a nectar sample. Unlike the acid hydrolysis test, this technique is not able to yield accurate quantitative data (such as the percent sucrose in the nectar), but can provide an indication of whether the nectar is dominated by sucrose. The test is also used to determine if the glucose and fructose proportions are equal.

The method involves "spotting" using capillaries containing small amounts of nectar. Nectar samples and standard sugar solutions (sucrose, glucose, fructose and maltose, if available) are "spotted onto a large sheet (e.g. 30 cm x 45 cm) of chromatography or filter paper, along a labelled baseline. 10 µg of sugar, or 1 µL of a 1 percent solution, is sufficient for standards. Once the spots have air dried, the sheet is rolled into a cylinder and the bottom edge placed into a solvent inside a large glass vessel. Prior experimentation is necessary to ensure that solvent height in the vessel is well below the cylinder's baseline bearing the spotted nectar and standards.

The paper cylinder is sealed on top with a glass plate and plasticine or vaseline, and incubates inside the chamber for 24 hours at 25 to 30 °C. The solvent front gradually rises and carries sugars with it. The distance travelled up the cylinder by the sugars, and the degree of separation of the nectar's sugars, depend on the height of the paper cylinder and the type of solvent used.

Satisfactory separation of the sucrose, glucose and fructose of nectars has been achieved using the following solvent systems: (i) butanol/ethanol/water in a ratio of 4:2:1, by volume, and (ii) n-propanol/ethyl acetate/water in a ratio of 7:1:2, by volume.

After the solvent front has reached the top of the chromatogram (paper cylinder), the cylinder can be removed from the sealed chamber, stood upright and allowed to air dry for 48 hours at room temperature. Thereafter, the chromatogram is mist-sprayed with a reagent consisting of a 1 percent solution of p-anisidine hydrochloride in absolute ethanol (e.g. 0.5 g p-anisidine hydrochloride dissolved in

50 ml ethanol). The chromatogram is then heated in a 75 °C oven for 30 minutes.

The sugars are identified by the distances they have travelled from the baseline, assisted by the characteristic colour reactions with the reagent. Sucrose, which travels least, and fructose, which migrates farther than the glucose standard, both stain yellow. In contrast, glucose stains dark brown.

If the spots stain weakly, it may be possible to better elucidate the chromatogram by exposing it to the bulb of an ultraviolet lamp, under which the spots will fluoresce. *To prevent eye damage, wear a recommended safety shield or UV-protective eye goggles, and take care not to stare at the bulb.*

19.1.4 Measuring rates of nectar secretion and resorption

Nectars taken by honey bee foragers at flowers usually contain between 15 percent and 60 percent sugar. Plant species can vary tremendously in flower size and morphology, and in the location and anatomy of their floral nectaries. Thus, it is not surprising that characteristic properties of floral nectar, such as volume and sugar concentration, can also differ dramatically. Even the relative quantities of vascular tissue (phloem, less often accompanied by xylem) that enter the glands of different species, can influence the sugar concentration of freshly secreted nectar. Each species has its own *initial nectar sugar concentration,* commonly of the order of 5 percent to 20 percent. Besides the properties inherent to individual species, many environmental factors influence nectar sugar production and eventual concentration, including sunlight, temperature, humidity and plant nutrition.

To understand more about nectar production, it is worth investigating other aspects of flower "behaviour". Answers to the following questions can provide meaningful details about patterns of nectar production in the species under investigation: When does nectar secretion commence? Is secretion continuous or does it stop? Are there differences in the rate of nectar secretion or reabsorption during the life of a flower?

The question of changes in rates of floral nectar secretion and reabsorption has been addressed in several flowering plant species. The research strategy used to investigate these rates under field conditions (Corbet, 2003) involves two systems utilized in tandem. The systems are similar in that they both involve the exclusion of flower visitors to avoid unwanted nectar removal. However, they differ in that one of the systems requires careful, repeated sampling of the nectary of an individual flower – a practice that may result in injury to the nectar gland, especially in the case of nectaries situated out of sight at the flower base.

The *first system* involves labelling or tagging many flowers of a young and identical stage or age from several plants (with pieces of drinking straw, coloured tape, string, etc.) for caging or bagging in the early morning, before flower visitors arrive. For plants with flowers that open and attract visitors at night, caging is performed before nightfall. After the initial caging, nectar is collected from eight to ten previously undisturbed tagged flowers from the cage at each of several sampling intervals (e.g. every 2 hours) throughout the flowering lifetime.

The *second system* involves the sampling of a fresh batch of eight to ten flowers from previously undisturbed plants in the field. The timing of the flower sampling stage should closely match that of the first system. After all nectar is removed, the flowers are tagged and covered (bagged), before they are sampled once again, about 20 to 90 minutes later. The volume and concentration of nectar initially gathered to empty these flowers yields an estimate of the total standing crop of nectar.

Three components require measurement for this technique:

- The standing crop of nectar in flowers exposed to visitors. Changes in this component at different times of the day or night reflect the relative rates of gain by secretion and loss by reabsorption and/or removal by visitors.
- The rate of nectar secretion in flowers of the *second system*. This measurement is likely to be an underestimate if some nectar is reabsorbed during the brief bagging period, or the nectary is mechanically injured during initial gathering of nectar.

- The rate of change in the quantity of nectar, and therefore the net rate of secretion and/or resorption, in flowers of the *first system*. Since each flower was sampled for nectar only once, there are no injury effects here.

Various physiological studies involving the application of radio-labelled sugar to nectaries or to nectar in situ have given direct confirmation that reabsorption of sugars by glands can take place. However, in some species, reclamation of all uncollected nectar sugar may not occur. This can happen when the corolla of an old flower finally falls off and carries with it the residual nectar that no longer contacts the flower's persistent nectary. Other constituents besides the sugars of nectar can be reabsorbed from standing nectar. Radio-labelled forms of some amino acids, for example, have been shown to re-enter the flower after being experimentally added to floral nectar. However, low quantities of amino acids in nectar may not always be the result of reabsorption – highly developed nectaries, consisting of aggregations of trichomes, tend to produce nectars low in amino acid content.

19.1.5 Selection and breeding for high nectar sugar production

Varieties or lines of crop plants can differ markedly in many traits, including several aspects of flowering. Selection of varieties or lines that yield high amounts of floral nectar can be useful for increasing food production. A higher quantity of nectar sugar can dramatically increase attractiveness to honey bees, native bees and other pollinators, resulting in enhanced floral visitation and subsequent pollination, along with potential increases in fruit and seed set. Honey production, honey bee colony strength and pollinator populations also benefit from higher quality floral resources.

When making nectar comparisons between plant varieties or selections, some emphasize the need to grow the plants (preferably simultaneously) under controlled conditions. This helps to eliminate variation due to environmental factors. Also, it is essential to standardize procedures of nectar collection and measurement. Flowers should be sampled for nectar at the same time each day and must be of the same age, to ensure unbiased comparisons.

To avoid errors in measurement of nectar yields, which may result from previous nectar withdrawal in open flowers by insects, plants used for such comparisons are often grown in greenhouses or bagged in the field. The highest yields may be lower here than under field conditions, but relative performance between variants is usually maintained. Alternative methods to exclude nectar collection by flower visitors include the caging of several plants in the field or plot, and even the bagging of many flowers on inflorescences or as individuals. Because each exclusion technique introduces unique variables in *macro* and *microclimate,* comparisons are meaningful only if the same procedure is utilized for all selections. For comparative tests, nectar yields are usually based on nectar sugar accumulated over a certain period (generally 24 hours). The choice of duration will vary with species and depends on factors like floral longevity and nectar sugar reabsorption.

Although there is still great potential in this field of research, significant discoveries have already been made. More applications for *nectar breeding* (artificial selection for higher nectar yields or more sugar) will surface once knowledge of the process of nectar secretion is more complete. Most attempts at selection for nectar sugar production have been made empirically, including assessments of the number of flowers per plant, the number of flowering periods per year, flower colour, aroma, size, receptacle diameter, volume of functional phloem in the flower stalk (pedicel) and number of nectary stomata. Species of Leguminosae (Fabaceae) are among those studied most extensively.

Regarding flower colour, honey bees were found to prefer clones of *Medicago sativa* that had purplish flowers rather than yellowish-green ones (the latter tend to yield lower amounts of nectar). Florets with yellow-green petals were also found to have a musty smell, which may account for their relative unattractiveness to *Apis mellifera*. In addition, weakly scented flowers of *Lotus corniculatus* produce relatively little nectar, and can be rejected early in the artificial selection process for improved nectar sugar production.

One very practical method of selection for nectar production, *based on flower size,* may be suitable for a wide variety of species. By inserting the bases of flowers into a template (previously drilled to provide holes of increasing diameter), it is possible to rapidly screen lines with the largest flowers. Flowers of *Lotus corniculatus* that produced the most nectar had the largest diameter. Alfalfa clones with the greatest receptacle diameter also yielded the most nectar.

The final two parameters, nectary stomata and phloem in the flower stalk, require microscopy and hence are not as practical, but are still potentially very useful. In selections of birdsfoot trefoil, a strong relationship was demonstrated between nectar yield and the quantity of phloem in cross-sections of the flower stalk. In other words, flowers producing the most nectar were those that could apparently receive the greatest quantities of photosynthate.

Investigations of the relationship between the number of nectary stomata and floral nectar production have now been conducted in three legume species. Results obtained with *Lotus corniculatus* and *Medicago sativa* were inconclusive. In *Vicia faba*, however, an inverse relationship was detected –plants with the *largest number* of nectary stomata per gland produced the *lowest* quantities of nectar sugar. Therefore, for breeding purposes, plants whose floral nectaries bear relatively large numbers of stomata should not be selected. High stomatal numbers per unit area might best be avoided, if the results for the fava (broad) bean are applicable elsewhere.

As well as selection for total nectar sugar production, similar efforts can be directed at selecting for desirable sugar types in nectar. For instance, sugar solutions (mimicking nectar) with very high proportions of glucose and fructose, but very low in sucrose, have tended to be less attractive to honey bees (*Apis mellifera*) in feeding tests. Plants within a species might have greater appeal to these insects if their nectar were not so rich in hexose sugars. Furthermore, nectar of Brassicaceae is notorious for its high glucose content. Honey produced by *A. mellifera* from such nectars crystallizes (granulates) quickly, a physical property that can be a nuisance for honey extraction from the comb, and also causes a reduced shelf life for honey in the liquid state.

FURTHER READING

Bentley, B. & Elias, T. 1983. *The biology of nectaries*. New York, Columbia University Press.

Brown, M., Downs, C.T. & Johnson, S.D. 2008. Sugar preferences of nectar feeding birds: a comparison of experimental techniques. *Journal of Avian Biology*, 39: 479–483.

Corbet, S. 2003. Nectar sugar content: estimating standing crop and secretion rate in the field. *Apidologie*, 34: 1-10.

Cruden, R. M. & Hermann, S.M. 1983. Studying nectar? Some observations on the art. In B.J. Bentley, ed. *The biology of nectaries*, pp. 223-240. New York, Academic Press.

Dafni, A. 1993. *Pollination ecology: a practical approach*. Oxford, Oxford University Press.

Dafni, A., Kevan, P.G. & Husband, B.C. 2005. *Practical pollination biology*. Cambridge, ON, Environquest, Ltd.

Heil, M. 2015. Extrafloral nectar at the plant-insect interface: a spotlight on chemical ecology, phenotypic plasticity, and food webs. *Annual Review of Entomology*, 60: 213–232. DOI:10.1146/annurev-ento-010814-020753.

Kearns, C.A. & Inouye, D.W. 1993. *Techniques for pollination biologists*. Boulder, CO, University Press of Colorado.

Shivanna, K.R. & Tandon, R. 2014. *Reproductive ecology of flowering plants: a manual*. New Delhi, Springer.

Vannette, R.L., Gauthier, M-P.L. & Fukami, T. 2013. Nectar bacteria, but not yeast, weaken a plant-pollinator mutualism. *Proceedings of the Royal Society B*, 280, 20122601.

Wyatt, R., Broyles, S.B. & Derda, G.S. 1992. Environmental influences on nectar production in milkweeds (*Asclepias syriaca* and *A. exaltata*). *American Journal of Botany*, 79: 636–642.

19.2 POLLEN, ANTHERS AND DEHISCENCE

S.L. Buchmann

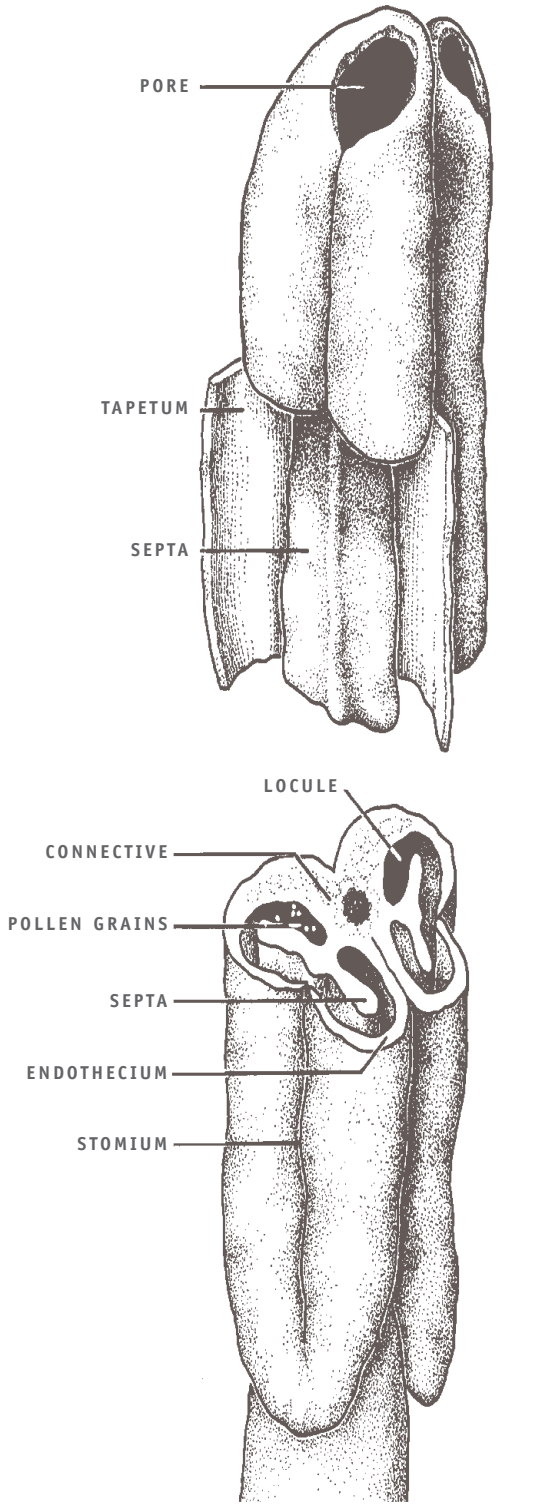
Anthers are the floral organs that produce, then house, the pollen grains that contain male gametophytes or nuclei. Although it may be technically incorrect, the pollen grains are roughly the plant equivalent to animal spermatozoa. Microsporogenesis from pollen mother cells occurs within the young anther locules at an early stage within the floral buds. The pollen grains are also nourished by the anthers. At this time, the pollen grains acquire their final structural details and a lipoidal/carotenoid coating, known as the surface pollenkitt. Pollenkitt lipids are chemically diverse; they protect the nuclear material within from the sun's ultraviolet-damaging rays, and keep the grains somewhat moist. The pollenkitt lipids are also a primary source of food eaten by bees and their larvae after the pollen is ingested. Anthers may be relatively sessile (basifixed) or attached to long thin filaments that project them beyond the floral perianth. They almost always consist of two hollow chambers, the locules, inside which the pollen grains are produced. From the locules the grains are released to be carried away by vectors such as wind and water, or animals including bees, wasps, flies, butterflies, beetles, birds or bats.

19.2.1 Pollen release

Pollen is released by a process known as *dehiscence*: the anthers split, opening along a pre-determined longitudinal line of weakness, the stomium. The locule walls peel back, exposing the pollen to insects and other floral visitors. The pollen grains are then shed into the environment.

Anther dehiscence is largely mediated by environmental humidity levels and water relations within the parent plant. It often takes place during the driest times of the day. A little-understood and equally important factor that controls anther dehiscence is the amount of water (turgor pressure) mediated by the supporting anther filaments (see Schmid and Alpert, 1977 for an explanation of this process, called Burck's hypothesis for anther dehiscence [1]).

Figure 19.2
DIAGRAM OF ANTHER ON A "BUZZ POLLINATED" PLANT



Source: Original drawing by M. Buchmann.

Anther dehiscence only rarely departs from the typical full-length stomial rupture pattern. Some flowering plants have anthers that dehisce by means of valves or flaps of tissue (such as in Berberidaceae or some tropical Araceae). The only other major form of anther dehiscence considered here is the widespread type known as poricidal dehiscence, which occurs in many crop plants. As much as 6 percent of the world's flowering plants have highly specialized anthers that do not dehisce along their entire length. Instead, these anthers dehisce by means of two small pores, the only openings through which pollen is released and available for harvesting by foraging female bees. The foraging females use floral sonication to collect pollen [2]. Tropical and temperate blossoms (exemplified by the genera *Apeiba*, *Bixa, Cassia, Cyphomandra, Dillenia, Hibbertia, Melastoma, Miconia, Senna, Solanum* and *Vaccinium*) are usually "pollen-only" flowers that give no floral nectar but have abundant and protein-rich small, "dry" (pollenkitt poor) pollen grains.

Pollinating bees, mainly belonging to the Apidae (including many temperate genera like *Anthophora and Xylocopa*, as well as tropical *Bombus, Melipona, Euglossa, Eulaema, and Eufriesea, Amegilla*, some colletids, the *Oxaea*, and many halictids, for example *Neocorynura, Augochloropsis, Augochlora, Augochlorella* and *Pseudaugochloropsis*) are the usual visitors of flowers bearing poricidal anthers. For as yet unknown reasons the honey bee genus *Apis* and most stingless bees (except *Melipona*) do not use this form of harvesting and instead collect pollen by scrabbling over the anthers using their legs to pick up pollen. Sonicating bees use their indirect flight muscles as if they were living tuning forks. Thus, the small, dry pollen is forcibly ejected or "sonicated" out of the poricidal anthers, in an efficient manner by bees which can accumulate large scopal loads of pollen very quickly. Pollen from *Solanum*, the deadly nightshades, has the highest protein level (48 percent to 56 percent) of any angiosperm pollen analysed to date. When they sonicate the blossoms, bees utilize strong vibrations which produce loud sounds, hence the name "buzz pollination".

The anthers of buzz-pollinated plants are large and may also form a cone or brush around the pistil. An example of this "shaving brush" style of androecial presentation is found in the genus *Bixa*. The anthers of buzzed flowers are often bright yellow, contrased with a blue or white perianth. This mechanism probably attracts pollinators, since the enlarged anthers give the appearance of abundant yellow pollen, even when none is present. Examples of *Solanum*-type anther presentation include the flowers of tomato or eggplant. Both crops are visited by bees, especially bumblebees, carpenter bees, *Amegilla* and others.

When working on crop plants such as blueberry, cranberry, eggplant, kiwi fruit, tree tomatoes or regular tomatoes, pollen for crosses can easily be obtained by using an inexpensive tuning fork (in the keys of A or C, 440 Hz or 512 Hz). Strike the tuning fork against a piece of rubber and touch it to the anther cone of a tomato or eggplant blossom. The flower should be held over a piece of thick flat glass, resting on a piece of black paper or cloth, so as to better see the ejected pollen. The pollen can then be easily scraped up with a knife edge or razor blade, and stored in a tight-fitting glass vial in the freezer.

Pollen is not always released from anthers as single grains or monads; instead, it may be shed as a unit – a clump of adhering grains (e.g. the polyads in the genus *Acacia*) or in even more elaborate "shrink-wrapped" packages called "pollinia" found in orchids and milkweeds. Such pollen is not used by bees or other pollinators as food, but the pollinia are transferred by bees, wasps and butterflies as they fly between flowers. The study of pollen dispersal and receipt are greatly facilitated in angiosperm taxa bearing pollinia, often identifiable to species even without the aid of a microscope. Roubik and Hanson provide assistance with identifying the kinds of pollinia from various orchid genera [3].

Similarly, the science of palynology – the study of the structural features of pollen grains – can assist with the identification of pollen grains recovered from insects, birds or bats visiting a crop or wild plant. The greatest single contribution to Neotropical palynology was Roubik and Moreno's

profusely illustrated regional pollen catalog, produced for Panamanian plants [4]. The keys and plates of photomicrographs are particularly useful for linking plants to family and often genus in other tropical regions of the world. A freeware tool was introduced in 2007 that offers a multi-access key and searchable database for neotropical pollen types [5]. This resource is especially useful as the Roubik and Moreno (1991) keys are out-of-print or may be difficult to access.

19.2.2 Pollen viability and germination

Many floral biologists assume that pollen is always viable, with each grain having the same chances of germinating on a stigma, sending down a pollen tube and fertilizing ovules. That is often not the case. Pollen grains can have widely varying levels of germinability, with many grains either infertile or dead. Workers studying pollination systems should test this for themselves to determine the length of time pollen grains remain viable within anthers and while residing on various animal pollen vectors. When pollen leaves anthers following dehiscence, the individual grains, polyads or pollinia begin a journey that may last hours or many days. The usual sequence of events for pollen grains is as follows:

- Pollen arrives at a stigma via abiotic or biotic vectors.
- Attachment and adhesion of grains to the stigmatic surface is controlled by the chemical composition of both stigmatic pappilae and the pollen grains.
- Hydration of pollen grains occurs and stigmatic papillae recognize the grain as either genetically compatible or totally foreign.
- The germinal pores of the pollen (usually three apertures per grain) open and full hydration takes place. A pollen tube emerges from one of the pores and begins growing inside and down the stylar tissue (if no incompatibility mechanisms prevent growth).
- The growing pollen tube reaches the ovary, where nuclei are delivered to the ovules to produce a fertilized zygote that will become an embryo, then a seed within a fruit.

It is not possible to determine whether pollen grains are viable or capable of germinating upon a stigma and effecting fertilization just from observation. In most cases, grains will not appear to be shrunken or misshapen when viewed under a microscope. During their long journey from the protective anthers to a receptive stigma, the pollen grains are exposed to hostile external environments that dry or wet the grain, along with intense UV radiation. Pollen grains that land upon a moist stigma rehydrate and usually send down healthy pollen tubes and nuceli. In most cases, the key to understanding whether a grain can successfully fertilize an ovule is *pollen viability* or *germinability*. Various *in vivo* germination assays and *in vitro* chemical tests have been developed to gauge such aspects of pollen vitality. The use of aniline blue in lactophenol [6] is commonly used as a pollen viability test. However, the most obvious and probably the most accurate method is simply to perform hand pollination by placing fresh pollen onto receptive stigmas for stigmatic receptivity tests (see Annex 2). It is only necessary to know that the stigmas are indeed receptive, and at what point during floral ontogeny. Several hours to many days later, depending upon the plant species, pollen tubes can be seen within the stylar tissues and counted. Alternatively, following hand pollination, the resulting fruit can be weighed or counted, as well as the seeds within.

An alternative method is to bag a cohort of flower buds and the next morning selectively open the new flowers individually, allowing a pollinator to visit the virgin flower just once. The bagged flower is then closed up and labelled with a small paper ID tag. One-third of the floral cohorts are left bagged (to check for apomictic fruit development), and another third are labelled but left open for a full day to control for open pollination. For the final set of bud treatment, the bag is opened and the flower exposed just once, long enough to observe a visit from a single pollinator. If multiple species of floral visitors are present, an attempt is made to obtain single visits for several individuals. No other actions need be performed for a while. Once the fruits have matured but have not split open, they are harvested and their seeds counted. A

ratio is established between the seeds per fruit in the open-pollinated controls and the single visit trials. The only drawback with this approach is that a single visit from a pollinator must deliver enough pollen grains to set a fruit. This ingenious technique (Spears Pollination Efficiency Index) for comparing pollinator visitation/pollination efficiencies was developed and published by Eugene Spears [7]. The Spears PEI is elegant and simple, yielding an index from 0 (least) to 1 (most efficient). Calculating the reciprocal of those indices yields a measure of the minimum number of trips necessary by a particular pollinator to set a fruit. When this method works, it eliminates a lot of the tedium caused by counting pollen grains on stigmas.

19.2.3 Minimum pollen grains required to set a fruit

Another assessment approach – one that is rarely used but can nonetheless prove valuable – involves determining the minimum number of pollen grains per stigma that will result in setting a fruit. This requires a microscope that will allow the user to count pollen grains and determine the number of grains needed to produce a marketable-sized fruit. The process is as follows:

- Using a fine paint brush or toothpick, add various amounts (judged by eye) of pollen to receptive stigmas on flowers within bags, which are then re-bagged to exclude pollinators.
- Wait until the flower begins to wilt and then harvest these stigmas.
- The stigmas can be acetolyzed or simply placed on a microscope slide with an appropriate pollen stain, squashed and the stigmatic pollen load quantified.
- The fruit resulting from the same flower is left in place and labelled, in order to compare the determined pollen grain load and quality of the fruit produced by this amount of pollen.

A "stigmatic loading curve" may be useful to assess the minimum number of grains needed to effect fertilization or to produce a fruit or seeds of the desired size. Such information is still very scarce for many crops. However, a number of free software aids are available that automatically count large numbers of stigmatic pollen grains in digital images (assuming it is possible to photograph the grains). Pollen images and grain count advice are found at the National Institute of Health, USA.[11]

Traditionally, floral biologists seldom perform hand pollinations,[12] which provide a way to assess not only pollen function but also stigmatic receptivity. All too often, *in vitro* chemical assays of pollen germinability (stainability) are relied upon, often with mixed results. Pollen germination also can be studied by placing pollen samples to be assayed on various agar-based culture media or 5-60 percent sucrose solutions. These tests are relatively elaborate and are not discussed in this chapter. For many years one chemical assay was widely used to determine pollen viability. This is the so-called "Cotton Blue" (aniline blue in lactophenol) test. This chemical assay supposedly stains viable pollen a bright blue, based on staining the callose layer, while inviable grains either do not stain or take up the dye only very slightly. Under close scrutiny, this test fails to yield accurate results for many flowering plants, and its general use is not recommended.

The above and other chemical tests may exhibit a low correlation with real pollen germinability *in vivo*. Furthermore, they completely miss the important role of the pistillate flower (i.e. female choice) in determining whether or not pollen germination and fertilization occur. In summary, the use of various vital dyes (e.g. 1 percent methylene blue, neutral red or aniline blue) should be used with the utmost caution, since pollen stainability depends not on the true viability but on the grain content. In some cases, even long-dead pollen from herbarium specimens produce reactions with certain dyes.

Several additional chemical tests have been used to measure pollen viability/germinability with better

[11] Available online at: http://rsbweb.nih.gov/ij/.

[12] See section 2.3.4 in Roubik, D.W., ed. 1995. *Pollination of cultivated plants in the tropics*. Agricultural Services Bulletin No. 118. Rome, FAO, available online: http://books.google.com.pa/books?id=A1O8Ow6wDDUC&printsec=frontcover&source=gbs_ge_summary_r&cad=0#v=onepage&q&f=false.

results. One chemical pollen assay, *Alexander's stain*, distinguishes aborted from non-aborted pollen grains (only the non-aborted grains have the chance to fertilize ovules). A recipe for the *modified Alexander's stain* is given here:

- 10 ml 35 percent ethanol
- 10 ml Malachite Green (1 ml of 1 percent solution in 95 percent ethanol)
- 50 ml distilled water
- 5 ml Glycerine (glycerol)
- 5 g Phenol (*use caution as phenol can cause severe skin burns)
- 5 g Chloral Hydrate (a controlled chemical substance in some countries)
- 50 ml Fuchsin (5 ml of 1 percent aqueous solution)
- 5 ml Orange G (0.5 ml of 1 percent aqueous solution)
- 1-4 ml Glacial Acetic Acid

The modified procedure for Alexander's stain is as follows:

- Mix all of the above ingredients in the order given and store the resulting mixture in a brown or aluminum foil-wrapped bottle at room temperature. The stain solution should last about one month at room temperature, or longer if refrigerated. It has been suggested that more glacial acetic acid should be used depending upon the thickness of the pollen grain walls, up to 4 ml for thick-walled pollen types.
- Pollen grains to be tested are placed on a microscope slide to which is added a drop of stain. A coverslip is added and the slide may be gently warmed over a small flame from an alcohol lamp. Non-abortive grains should stain red while aborted grains, lacking functional cytoplasm, will appear greenish.
- At this time, the grains should be examined under a compound microscope at about 100 to 400X. If no colour differentiation is apparent, then the slides should be kept at 50 °C for 24 hours and re-examined later. This chemical test often gives highly positive results for germinability, which occurs because cytoplasm within grains does not guarantee that the grains are viable and would germinate produce pollen tubes penetrating a stigma.

Recently, many floral biologists have used a newer chemical test, the *Fluorochromatic Reaction (FCR test)* [8], to assess pollen viability. The materials needed for the FCR test are:

- 20 mg of fluorescein diacetate in 10 ml acetone
- 15 percent sucrose solution

The method used to carry out the FCR test is as follows:

- The reagent is prepared by placing 10 ml of the freshly made sucrose solution in a clear glass vial. The fluorescein diacetate in acetone is added (about 1–3 drops) until the solution turns a light milky or greyish colour.
- Pollen grains for tests must be stored for 10 to 30 minutes in a chamber with high relative humidity. This enables the membranes in the dehydrated grains to recover and prevents false-negative results in the following FCR test procedure.
- If pollen bursts in the 15 percent sucrose solution, try using a 20 percent to 30 percent solution. The pollen sample is dispersed in a drop of fluorescein diacetate on a microscope slide. Place the slide in a Petri dish atop wet filter paper for 10 min and then cover with a coverslip.
- Examine the pollen under a compound microscope equipped with the appropriate microscope fluorescence filters and illumination. A violet exciter filter should be used. Pollen grains that fluoresce a bright golden-yellow should be scored as viable. Empty, undeveloped grains will not fluoresce at all. Grains that have lost their viability will show only a pale yellow fluorescence. Observations and counts should be completed on the grains no more than 10 to 15 minutes after the coverslip is added. The test solution must be fresh each time.

The FCR test seems to be the most reliable and accurate of all the chemical assays. However, while testing for active esterases and plasmalemma integrity of the vegetative cell, it is still not an absolute test of pollen viability. Dehydrated grains can give false positive results and should be rehydrated in a small chamber prior to testing. An obvious disadvantage of this otherwise excellent technique is the requirement for the assay to be conducted in a well-equipped

laboratory, using an expensive fluorescence microscope and the appropriate filters – equipment that may not be readily available in some countries, and certainly not in most remote biological field stations.

19.2.4 Pollen chemistry

As a floral reward, pollen is far richer in nutritive components than sugar-containing nectars. Pollen is an essential source of protein, amino acids, anti-oxidants and lipids for the majority of the worlds' bee pollinators, flower flies (Syrphidae) and their offspring, along with some flower-feeding bats. A few heliconiine butterflies and some masarid wasps also feed on pollen. Bees are the chief arthropod consumers of floral pollen in all tropical and temperate environments. All bees achieve high assimilation efficiencies as they rapidly turn pollen nitrogen into body nitrogenous compounds. This is especially true for tropical stingless bees living in large colonies. Most of the nutritive-deriving substances within pollen grains are contained inside the pollen walls known as the *exine* and *intine*. The intine inner walls are cellulosic, but the outer walls, the exine, comprise a tough biopolymer (called sporopollenin) made up of carotenoid pigments. The exine is essentially indigestible for most organisms, except for a few collembolans and some bacteria. Pollen exines pass untouched through the intestines, maintaining a recognizable sculptured exine shell in the feces, which can be acetolyzed and identified to determine plant taxa in pollinator diets (see Annex 2). Thus, feces recovered from solitary or social bee nests, are excellent first-hand clues to the pollen diet breadth of bees. The exine is an incredibly resistant biological natural product, very similar in composition to the outer shell of insects and other arthropods. Fossilized pollen grains 100 million years old have been recovered from sediments, with little degradation.

Inside the exine pollen walls, the cytoplasm contains rich protein, lipoidal and carbohydrate sugar/starch energy reserves for the developing pollen tube, later supplemented by the stylar tissue. Bees and other pollinators need not "crack" or digest the tough pollen grain walls, as incorrectly suggested in the literature, to release the nutrients within. Pollen within animal guts is exposed to high osmotic pressures, acids and various degradative enzymes. Thus, the pollen grains swell, then "pseudo-germinate", making thin membranes extrude from germinal apertures. Osmotic shock and the bees' normal digestive biochemistry do the rest. Surface oils, including colourful pollenkitt, which are nutritionally very important, notably in taxa with oily pollen such as the Asteraceae, are also efficiently removed within pollinator digestive tracts. In temperate regions, a flush of autumn composite flowers often function as the final pollen nutritive input needed by bumblebee reproductives, and also by overwintering honey bee colonies.

The diversity of nutritious, and sometimes non-nutritive, chemicals within pollen is staggering. The complex array of substances present within and on the surface of pollen grains and their relative importance for pollinator diets has only just begun to be understood. A summary of commonly reported values for the chemical composition of pollen harvested by honey bees is given in Table 19.1. Pollen typically contains from 20 percent to 50 percent water and the indigestible exine comprises about 8 percent to 35 percent of the pollen dry weight. Protein levels for pollen harvested by bees typically range from 7 percent to 35 percent (see Table 19.1) but the levels are much lower (e.g. from 5 percent to 20 percent) in pollen from anemophilous plant species. The highest yet recorded nitrogen (7 percent to 9 percent) and protein levels (48 percent to 56 percent) in any type of pollen were found in pollen from buzz-pollinated *Solanum* species, related in an article by Buchmann [9].

As is apparent from Table 19.1, while pollen may be a perfect food for certain pollinators, such as bees, it is far from perfect as a diet for humans, as is sometimes claimed in the modern health food literature and especially online. Pollen contains none of the important lipid-soluble vitamins (A, E and K). The impact of such nutritional information for pollinators is largely unknown, but remains a fertile area for new research. With a mean value of almost 25 percent, protein is a major component of pollen, and is clearly the most important for

Table 19.1
COMMONLY REPORTED VALUES FOR THE CHEMICAL COMPOSITION OF POLLEN HARVESTED BY HONEY BEES

COMPONENT	NO. SPP. ANALYZED	TYPICAL LEVEL	RANGE
Protein	277	24%	7.5-35%
Lipids	52	5%	1-15%
Carbohydrates	47	27%	15-45%
Phosphorous	54	0,50%	0.1-0.6%
Ash	60	3,10%	1-5%
Potassium	56	0,60%	0.2-1.1%
Calcium	60	0,20%	0.1-0.5%
Magnesium	60	0,20%	0.1-0.4%
Sodium	30	0,04	0.15-0.8%
Iron	51	140µg/g	wide
Manganese	28	100µg/g	wide
Zinc	21	78µg/g	wide
Copper	27	14µg/g	6-25µg/g
Nickel	23	5µg/g	0-?µg/g
Boron	?	trace	?
Iodine	?	?	4-10µg/g
Thiamin	8	9µg/g	4-22µg/g
Niacin	6	157µg/g	130-210µg/g
Riboflavin	8	19µg/g	?
Pyridoxine	2	9µg/g	?
Pantothenic acid	33	28µg/g	5-50µg/g
Folic acid	8	5µg/g	?
Biotin	4	0.3µg/g	0.2-0.6µg/g
Vitamin C	7	350µg/g	0-740µg/g
Vitamin A	?	0	0
Carotenes	4	95µg/g	50-150µg/g
Vitamin D	4	0	0
Vitamin E	4	14µg/g	?
Vitamin K	4	0	0

Source: S. L. Buchmann {present study]

pollinator nutrition. Honey bees may also collect large amounts of pollen from highly allergenic pollen taxa (e.g. ragweeds, olive, mulberry and African sumac). If ingested, or rubbed into eye or nasal mucosa, such pollen sometimes produces potentially life-threatening allergic responses in humans. Thus, caution should be used when eating bee-collected pollen, especially if the pollen comes from wind-pollinated plants.

Pollen protein levels are especially high in pollen from bee-pollinated plants (25 percent to 56 percent protein) and those pollinated by bats (40 percent to 44 percent protein) – likely due to the fact that these plants are entirely dependent upon the pollinators in question and have "upped the stakes" by providing very rich protein. All bees, even those that are parasites and collect no pollen themselves, and many flower-visiting bats, are dependent on nitrogen and protein in pollen for amino acids and proteins. Certain aminoid acids (e.g. proline and tyrosine) are very rich in pollen from bat-pollinated tropical plants and have been hypothesized to be important in forming strong wing membranes. Roles for specific amino acids for invertebrates like bees are unknown. Most nutritional studies have revealed that bees, and most other insects, require the same so-called "mouse 10 essential amino acids" as humans do.

19.2.5 Determining protein levels in pollen

Reliable and accurate nitrogen, protein and amino acid determinations can only be produced with the aid of a well-equipped chemical laboratory, often containing expensive and specialized modern equipment. Nitrogen determinations are usually performed on pollen, or food or soils using a traditional technique known as the *micro-Kjeldahl reaction*. This method requires high temperature cooking, a mercury catalyst and a specialized apparatus. Recently, progress has been made in combusting small amounts of a sample in a furnace and then examining the pyrrolysis products for nitrogen. Very expensive, dedicated amino acid analysers are also available (for both hydrolysates and native proteins/peptides – the so-called physiological "free" amino acids) for the quantification of amino acids in pollen. These are usually found only in the most modern biochemistry department laboratories.

Alternatively, amino acids can be qualitatively, and somewhat quantitatively, determined from pollen and nectar samples using a miniaturized *two-dimensional TLC (thin layer chromatography)* methodology perfected by I. Baker, a pioneer in experimental

pollination biology (see account on pp. 156-159 in [10]). Readers are directed to the nectar analysis section in this publication for a brief account of semi-quantitative amino acid concentration technique, called the histidine scale (also developed by Baker and Baker) using ninhydrin reagent and sucrose solutions.

Another option is to actually disrupt pollen grains using aluminum powder in a mortar and pestle, and then put the pollen into a solution that leaches out the amino acids and proteins, which are then analysed in a spectrophotometer. This technique has been pioneered for pollen N and protein analyses by Roulston and Cane [11] who extensively survey nitrogen levels in pollen.

19.2.6 Assays for starch in pollen

Pollen often contains large amounts of starch in the form of microscopic starch granules (amyloplasts) similar to those found in some plant stems and root storage tissues. As an energy storage reserve substrate for developing pollen tubes, starch contains far less energy (15 562 to 16 638 joules/g) than pollenkitt lipids (38 883 to 42 248 joules/g), as determined by bomb calorimetry (S.L. Buchmann, unpubl.). Starchy pollen is largely absent in flowers pollinated by animals, with a few tropical plants as exceptions. Pollen with high levels of starch is representative of wind-pollinated plant taxa.

A very old chemical test that is still used is the iodine reaction given by starch granules. The test has been used by floral biologists to assess whether starch is present or absent from pollen grains. The materials for the *Iodine Pollen Starch Test (IKI)* are as follows:

- 0.2 g Potassium iodide
- 1 g iodine crystals (use caution as they stain everything and the fumes are toxic)
- 100 ml distilled water

The following procedure for the IKI test should be conducted under a chemical fume hood. The potassium iodide is dissolved in a small amount of water, then iodine crystals are added while stirring. Once dissolved, the remainder of the water should be added. The resulting solution should be kept in a tightly stoppered brown or aluminum-wrapped glass bottle. The solution will last for several months. Pollen grains thought to be starchy are placed on a microscope slide to which one or two drops of the IKI test solution are added. Wait 3 to 5 minutes for the grains to stain, then add a coverslip and examine under the microscope. Starch-containing pollen grains look black because the starch amyloplasts stain blue-black or occasionally red to purple.

More elaborate spectrophotometric quantitative assays (e.g. using o-toluidine) for starches and other complex carbohydrates are available but are beyond the needs of most floral biologists. The IKI test is best used with mature pollen grains freshly removed from dehisced anthers. Normally starchless mature pollen grains may contain some starch when they are immature. Even old, dried pollen removed from herbarium sheets after many years storage give reliable data for the presence of starch. Baker and Baker [13] show that starchless pollen grains (normally containing lots of lipids – another energy storage reserve) are typical of bee-pollinated angiosperms, especially in *pollen-only* flowers, and in some fly-pollinated flowers. Starch grains invariably contain some lipids and are typical of species that are autogamous, anemophilous or pollinated by certain butterflies and birds.

19.2.7 Lipids in pollen grains

Most, if not all, pollen grains have diverse lipids of various classes and in widely varying amounts. A simple test for lipid presence in pollen is to place some pollen or honey bee-collected corbicular loads on a piece of brown absorbent paper, such as a brown paper bag. After several hours, a noticeable greasy dark spot will appear. A simple staining technique for the presence of lipids, especially in abundant surface pollenkitt, uses Sudan IV (see the following method). The stain solution should be made up fresh for each application. A red colour indicates the presence of lipids. The pollen grains should be examined microscopically within 3 to 4 minutes after the stain has been applied, as the red colour can disappears in as little as 10 minutes. Details of other lipid assays are given in section 19.2.9 on floral lipids (produced by specialized floral glands as rewards for pollinating

bees). Various extraction schemes have been devised for extracting, isolating and identifying the various lipids found in pollen and other botanical sources. Most require extraction with a non-polar organic solvent such as chloroform or methanol at reduced pressure and with gentle heating (e.g. Sohxlet apparatus). Lipid classes may be identified using relatively simple *Silica gel TLC plates and reagent sprays*. Exact identification of lipids, especially their degree of unsaturation, is best done in collaboration with a lipid chemist. The methods involved are beyond the scope of this book.

19.2.8 Collecting and identifying pollen

Pollen collection. Pollen for chemical assay is best when harvested fresh from flowers then used immediately. If it cannot be used right away, then it should be frozen at -20 °C in a deep freeze, or lyophilized frozen at -70 °C in an ultra-cold freezer as found in biochemistry labs, for archival storage. For feeding/nutritional tests with bees and other insects, pollen should be used fresh or frozen/lyophilized for later use. Pollen stored at room temperatures rapidly loses its full nutritional value and may spoil (the lipids become rancid).

Fresh flowers are brought into the laboratory and the anthers separated from the rest of the perianth parts by stripping them off by hand or with forceps. The anthers plus pollen should be placed upon a brass screen (such as a standardized soil sieve) that can then be vibrated on a shaker table; alternatively, the anthers can be gently stirred with a camel's hair or nylon bristle paintbrush. Debris from the pollen can cleaned up using screens (the finest have a mesh of only 100 to 300 microns) and then stored in individual glass or plastic vials in the freezer. Often, it is easier to dislodge pollen, even large oily grains, from flowers using a trick practised by bees: floral sonication. A poricidal blossom of the "shaving brush" type, with dozens or hundreds of stamens, is held in one hand or in a clamp a few centimetres above a glass plate resting upon a black or white piece of paper (depending upon the pollen colour). The anthers are then touched with a vibrating tuning fork (512 Hz, or middle "C", works nicely). Usually, a large cloud of pollen results, which after several dozen flowers have been vibrated, can be scraped into a pile using a clean razor blade or glass microscope slide. This method also allows hidden pollen feeders (notably thrips) to be ejected along with the pollen and removed. The procedure can even be by automated using a battery-powered vibratile device, such as an electric toothbrush.

Pollen harvested by hand using the above methods is very clean and can be used for many different assays. In addition, this method allows large amounts of clean pollen to be obtained for studies including palynological microscope slide vouchers, simple staining or for acetolysis.

Pollen can also be collected from honey bees, bumblebees, stingless bees or solitary bees after they have already performed the harvesting work. That can be extremely helpful in the case of plants with very small flowers or those with a miniscule amount of pollen. Most native ground-nesting bees (e.g. *Anthophora* or the leafcutter bee family Megachilidae) carry their pollen dry – unwetted with nectar – in specialized bands of hair known as scopae. Bees can be captured live and transferred into vials, or taken from a net into a killing tube (using ethyl acetate or sodium or potassium cyanide). If a pollen reference collection is being gathered, pollen can be removed directly from flowers or indirectly after harvesting by bees. If bee scopal or corbicular loads are used as pollen vouchers, every effort should be made to collect bees that have pure loads from a single plant species. Use a visual check to look for bicoloured pellets, which are sometimes produced by honey bees.

Large amounts of pollen (kg) can be easily collected with the use of pollen traps installed on honey bee colonies housed in Langstroth frames and supers. The details of constructing and using such traps have been reported elsewhere [15]. Of particular note is the bottom-type trap known as the modified "*Ontario Agricultural College*" *(O.A.C.) pollen trap*. This trap removes about 60-65 percent of incoming corbicular pollen pellets, which the colonies compensate for by

sending out more pollen foragers. The drawer can be opened and pollen removed at hourly, daily or weekly intervals to suit the needs of the researcher. Such traps are excellent devices not only for delving into the dietary specifics of honey bees living in various ecosystems, but also for determining phenological patterns in the local plant community. During one year, a single honey bee colony forages for pollen and nectar over an area of 80 to 100 square kilometres or more. Pollen traps have also been used to study African honey bee diet breadth in the tropics [14] and European honey bees in the Sonoran Desert, and for studies of competition between honey bees and native bees [16]. Standardized analytical techniques for counting and working with bee-collected pollen grains have been presented by O'Rourke and Buchmann [17] and Faegri, Kaland and Krywinski [18].

Pollen identification (palynology). Pollen identification (see Annex 2) is not easy process, but the necessary techniques can be learned by floral biologists with access to a laboratory and a good compound microscope, who are willing to take the time to study the methods necessary to prepare, slide-mount and identify the different pollen grains.[13]

Pollen can be prepared for light microscopy and easily studied with only the aid of a good compound microscope (capable of magnifications of 400-1 000 X) and a few common laboratory chemicals. Airborne pollen is often collected by aerobiologists on sticky tapes or on a glass "gravity slide" with petroleum jelly or silicone oil. Medical allergists routinely examine and identify this "raw" pollen without staining or chemical preparation. *This method should not be used by bee or floral biologists, however, since the surface pollenkitt oils and cytoplasm obscure important taxonomic details necessary to identify the family, genus or species of pollen grains*. Pollen grains, whether fresh from flowers, present on bees or from larval provisions or feces, should be minimally de-greased and treated with a common pollen stain (e.g. Safranin-O, Basic Fuchsin and similar red stains) to improve the resolution of the morphological surface features. One of the simplest techniques for staining pollen is to use glycerine jelly, which can be prepared ahead of time, stored and even carried into the field (see the recipe and procedure below). A semi-permanent slide mount of pollen samples can be made by adding a coverslip and ringing the edges with beeswax plus resin or one of the commercially available "metal flake" clear fingernail polishes. Ideally, access to a chemical laboratory or fume hood is required, where pollen samples can be treated with solvents and acids. Potassium hydroxide (KOH), which is used by most entomologists to clear insect genitalia, is also used to treat modern pollen grains to help distinguish morphological features. No matter the pollen preparation technique employed, collection of pollen directly from plants also requires taking a pressed *plant voucher specimen*, identified by an herbarium botanist, which can be deposited in a public herbarium along with a specimen number that can be referenced in subsequent publications.

Glycerine jelly mounting medium/stain

Materials:

- 7 g gelatin
- 42 ml cold distilled water, in which gelatine is mixed and warmed gently with stirring
- 50 ml glycerine and 0.5 g Phenol (or 10 drops of 80 percent phenol solution)

Procedure:

To prepare the glycerine jelly slide-mounting medium and stain, dissolve 0.1 g of Basic Fuchsin stain in 10 ml ethanol. Take one-third of the glycerine jelly, and slowly add the stain, drop by drop, until a clear pink colour is produced (the powder represents approximately 1/10 000 of the total solution). The stained glycerine jelly can now be stored in dark glass vials or jars at room temperature for many months until needed. It is easy to produce jelly of

[13] Tropical researchers should refer to the comprehensive pollen catalogs (pollen floras) prepared by Roubik and Moreno [4] for Barro Colorado Island and other areas in Panama. The pollen flora in this region serves to identify many types of tropical pollen from southernmost Mexico, through Central America and into northern Colombia, Venezuela and Brazil.

various colour intensities. Some experimentation on pollen may be necessary to achieve the desired staining results.

Small globules of stained glycerine jelly are sticky and can be picked up on the tip of an insect pin or dissecting probe, then applied to different body areas of pollinators. This is an excellent way to selectively sample the adhering pollen grains. The pollen-containing blob is simply placed on a microscope slide then heated over a small flame and a coverslip added. This technique is easy and inexpensive.

Another simple technique involves the collection of *undehisced anthers* from large floral buds and subsequent softening of tissues in lactic acid for several hours. These are then transferred without the acid to a test tube containing 10 percent KOH at room temperature for 24 hours. Alternatively, the pollen can be boiled for 5 to 10 minutes in KOH. This results in hydrolyzation of cellulose and lysing of protoplast. The resulting pollen grain walls (exines) are now free of obscuring pollenkitt lipids, but are practically colourless and must be stained prior to microscopy. Caution should be used with pollen grains and KOH because pollen is rapidly destroyed by strong bases (such as KOH), although it can withstand immersion in very strong acid. Any of the red pollen stains will work here.

Pollen obtained from bee nest samples or bees. In this instance, the collected material can go be placed directly into the KOH solution. Very detailed recommendations and methods (standardized quantitative analytical techniques for bee-collected pollen samples, minimum sample sizes and appropriate statistical procedures) are given by O'Rourke and Buchmann [17]. This study also emphasizes the importance of not neglecting disparate pollen volumes when considering the nutritional value to pollinators from pollen of various plant taxa, and presents formulae for calculating volumes for pollen grains of different shapes. These are among the subjects of *melissopalynology*, a discipline that is starting to receive increasing critical scrutiny within the study of bees, pollen and pollination.

The standard pollen treatment technique used by professional palynologists worldwide is the traditional *Acetolysis technique* [5]. This technique should be applied with caution since acetolysis involves caustic acids in a highly exothermic chemical reaction. It should always be performed in an approved chemical fume hood while wearing a protective laboratory coat, gloves and safety glasses or face shield (see the recipe and procedure for Acetolysis reagent below). The chemical reaction progresses rapidly and the pollen samples must be free of all traces of water, as water in the samples can produce a violent explosion with boiling acid venting from the open test tubes. Floral tissue or bee materials can be placed directly into concentrated glacial acetic acid as a pre-treatment dehydration step, prior to acetolysis.

Acetolyzing pollen grains (from pure pollen, anthers, flowers, bee materials, etc.) involves gently boiling the materials in a mixture of nine parts acetic anhydride (anhydrous, concentrated acetic acid) and one part concentrated sulfuric acid for several minutes. The caustic reagent dissolves away the interior grain cytoplasm and the surface oils leaving the pollen in a "fossilized" state. The sporopollenin polymer constituting the pollen grain exine walls survives unchanged (for exceptions in tropical pollen, such as Musaceae, Lauraceae and others, see [4]), but does take on a darker or brownish-black colour. Such acetolyzed pollen is thus ideal for light microscopy and usually requires no additional staining. Grains that become too dark can easily be bleached using sodium hypochlorite (laundry bleach). Acetolyzed pollen is washed and then stored in glycerin or silicone oil. For reference slides the best technique is to remove anthers directly from flowers on dried herbarium voucher specimens collected for this purpose, which correspond to field notes and specimen numbers and have been identified by a trained plant taxonomist.

Acetolysis method:

Materials:

- Concentrated Sulfuric Acid (>98 percent)
- Acetic Anydride (anhydrous)

Procedure:

WARNING: No water must be present inside the reaction test tube. Wear a lab coat, gloves and protective eyewear at all times.

- After the KOH treatment (or without that step), the pollen residue inside a centrifuge tube is dehydrated with glacial acetic acid, centrifuged and the supernatant discarded.
- Treat the sample with a fresh (made daily) mixture of nine parts acetic anhydride (concentrated anhydrous acetic acid) and one part concentrated sulfuric acid. This reaction is highly exothermic (HOT) so mixing should be slow and stirred constantly. The tube can be cooled by partial immersion in an external beaker of cold water. Heat the tube gently in the same test tube in a beaker partly full of boiling water on a hot plate under the fume hood. Heat to the boiling point (e.g. by immersion for 3–5 minutes in the boiling water bath). Allow the acetolysis solution, now black, to cool. Centrifuge for 3–5 minutes (at least 3–5 000 rpm) then decant the supernatant and discard. The pollen residue will be a blackened mass in the bottom of the sample tube.
- Rinse the pollen residue one to two times with glacial acetic acid, centrifuge and decant the supernatant.
- Rinse one to two times with water, centrifuge and decant. At this time, it is suggested to put the sample, with traces of water, into a desiccant environment or vacuum oven to remove any water before further processing.
- Store the acetolyzed pollen residue in glycerine (glycerol) or mount in glycerine on microscope slides under a coverslip. Silicone oil can also be used and requires additional steps.
- Glycerol slide mounts should be water-free and then sealed with fingernail polish to make semi-permanent mounts. The slides will last for many years, stored *horizontally* in a *darkened* microscope slide cabinet. In the case of glycerine or Silicone Oil slide mounts, the pollen grains can be rotated while viewed at high magnification simply by gently taping on the coverslip with the point of a lead pencil.

A *fume hood and strict adherence to safety precautions* are essential for any floral biologist using this technique. Important morphological details necessary for accurate identifications can only be seen (often at 1000 X magnification) using acetolyzed pollen grains. Acetolysis also allows so-called LO analysis (focus changed slightly from the surface to the interior of the grains) allowing the microscopist to discern structural wall elements within the exine, which are important diagnostic tools.

Counting pollen grains. Diverse methods exist to count grains in anthers, sediments or on bees to obtain *relative pollen frequencies*. A recent palynology textbook is a good way to become acquainted with these methods and statistical analysis. Access to sensitive electronic microbalances (Cahn-type electrobalances) will allow the use of gravimetric methods for estimating pollen grain number. One of the oldest and least expensive, but accurate, techniques is a combination of volumetric dilution and counting with the aid or a *haemocytometer* (blood cell counting reticle microscope slide). With this technique, an unknown number of pollen grains, for example from a honey bee corbicular load, are dispersed into a known volume of water or ethanol (often with the aid of a surfactant and ultrasonic dispersing probe). Then a subsample, perhaps 10 µL, of the swirled and mixed solution plus pollen grains (not allowing them to settle) is placed on the haemocytometer counting grid. Counts are made according to standardized procedures used by clinical laboratory technicians for blood cells.

If no haemocytometer counting slide is available, another simple counting method can be substituted. First, the area of the microscopic visual field is calculated using about 400 X magnification. The area is then divided into the area of the coverslip, thus providing a number of the order of 4 000. This number will remain a constant for future use with the same microscope objective and ocular lenses. Count the pollen grains (usually a count of 300 to 500, even up to 700, is necessary) in ten randomly selected visual fields, and then compute the mean. Multiply the mean by the constant determined above, and since

the pollen "strew" does not cover the entire area under the cover glass, also multiply by an estimate of the fraction of the area covered.

Palynologists often use exotic pollen or spores (usually from *Eucalyptus, Zea* or *Lycopodium*) as an internal calibration method. The calibration technique also allows the summation of results from different microscope slides, prepared from the same pollen mixture. Techniques such as the *Lycopodium Method* are preferable for achieving repeatable quantitative results. Floral biologists can use this relatively simple and inexpensive technique to good advantage in their own studies. The technique is based upon an original method developed by Stockmarr [19]. One or more *Lycopodium clavatum* spore tablets[14] are used in each microsample. A tablet contains approximately 10 000 acetolyzed spores. The number per tablet is calculated based upon a referenced batch number and accompanying calibration sheet, and stated to an accuracy of 3.3 percent.[15] The user selects how many tablets to use and first dissolves these completely in 10 percent HCL. The sample is centrifuged and the supernatant decanted. The unknown test sample is then added to the tube with the internal spore standard. The sample is thoroughly mixed, acetolyzed or otherwise processed carrying the spores throughout. A microscopic count (usually 500 to 1 000 grains) is made and the *number of pollen grains and spores* is tabulated. This functions like a "mark-release/recapture" population estimate in animal studies. It gives an accurate estimate of the original number of pollen grains present in a mixture, will eliminating the tedium of counting them all. When used to equate pollen type counts on several slides, the spores counted on each slide provide a weighting factor, by which the number of each pollen type can be altered to represent the portion of the entire pollen mixture that it actually represents.

[14] These are available commercially from Lund University, Department of Quaternary Geology, Tornavagen 13, S-223 63, Sweden.

[15] The price is about US$ 10.00 per bottle of 500 tablets; they also come in larger tablets with more spores per tablet.

For example, if two kinds of pollen are found on two slides prepared from a single mixture, and the number of spores counted on slide A is twice that on slide B, then the two pollen types on slide B comprise two-thirds the sample represented by the two slides. (If the spore counts were identical on both slides, then the numbers of pollen grains on each slide would each represent half of the total sample.) Statistical reliability demands that the number of spores used not be less than 10-20 percent of the expected total pollen grains present. However, this can prove impractical when dealing with giant pollen grains or polyads, because of the large difference in size from the *Lycopodium* spores. Nonetheless, because the numerical relationship between the pollen and spore internal standard is constant throughout the procedure, the total number of pollen grains present in the original sample is given by the following formula:

$$\text{Total pollen grains} = \frac{\text{Grains counted} \times \text{total } \textit{Lycopodium} \text{ spores added}}{\text{Number of spores counted in subsample}}$$

In recent decades, automated electronic counters have been developed to deal with particle counts in the range of thousands to hundreds of thousands. One such counter, the *Coulter Counter*, has recently been used by some floral biologists. One drawback of the Coulter Counter, however, is the requirement that pollen grains be dispersed and counted in particle-free expensive electrolytic (salty) aqueous solvents. Other particle counters measure the pollen grains by the shadows they cast on a sensor/photomultiplier, and samples can be run in pure water or ethanol. Such instruments are expensive but have revolutionized the counting of pollen grains.

19.2.9 Oils produced as floral rewards by flowers

In the late 1960s, S. Vogel discovered that many tropical plants, especially Malpighiaceae, produce specialized calycine glands (*elaiophores*) that secrete novel free fatty acids as floral rewards for their specialized bee pollinators. Some tropical

plants bearing such elaiophores include the nance (*Byrsonima crassifolia*), acerola and Barbados cherry (*Malpighia glabra, M. punicifolia*) in the Neotropics. In the tropical Malpighiaceae, oil glands are large oil-filled "blisters" on the calyx. In other flowers, such as some tropical cucurbits (e.g. *Momordica* and *Thladiantha* spp.) they form dense mats of oil-secreting trichomes. Elaiophore floral oils are energetically rich foodstuffs (about 3 300 j/g. Such floral products are usually substituted for nectar in the provision masses of bees, such as *Centris* and *Ephicharis*. Some of those floral lipids are somehow altered by the bees into waterproof cell linings. Many other details are reviewed elsewhere [20].

Chemical testing for floral oils. Suspected oil glands (trichome or epithelial elaiophores) on flowers can be tested by simple chemical means to determine if true floral lipids are present. One such test, proposed by Vogel, is the use of *Sudan IV stain* (used for pollenkitt lipids). This detects the essential oils (e.g. terpenes and other floral scent components) produced by specialized areas of the perianth, known as osmophores, by soaking fresh flowers, often with a surfactant and under reduced atmospheric pressure, in a 1:10 000 solution of Neutral Red dye to distilled water. The author used the Neutral Red test on regular elaiophores, whose lipids also stain red. Others suggest the use of 4 percent osmium tetroxide, which stains the oils and elaiophore tissues black almost immediately. However, osmium is a dangerous chemical and should be used only under a fume hood wearing "water type" safety goggles. Its use for detecting floral oils is therefore not recommended. Lastly, TLC and GC/mass spectral techniques can also be used on derivatized floral oils to identify oily molecules.

19.3 PLANT BREEDING SYSTEMS AND POLLINATION

P.G. Kevan

19.3.1 Introduction

Pollination is the first step in the process of sexual reproduction in plants. The pollination requirements of plants are varied. Knowing how these are met and how the process of sexual reproduction functions requires an understanding of plant breeding systems and reproductive strategies. Tropical crops represent the gamut of possibilities known to science. Thus, effective application of pollination technology in tropical crop production necessitates a strong understanding of plant reproductive systems. Unfortunately, this subject involves a suite of technical terms (see Glossary) unfamiliar to many people involved in pollination and agriculture. Plant breeding systems are covered extensively in a book by A. Richards [21].

As flowers develop, the sexual organs or sporophylls undergo genetic changes in some of their cells. In the anthers, special cells called "pollen mother cells" are formed (see section 19.1). These go through the process of meiosis, during which the number of chromosomes is halved. From each pollen mother cell, four pollen grains are generated, each with half the number of chromosomes (i.e. haploid) of the parent plant (which is diploid). In the ovary, similar but more complex events take place which give rise to a multi-celled ovule in which each cell is haploid.

After pollination has taken place, the pollen grains on the receptive stigma germinate. They produce a pollen tube which grows down through the style and into the plant's ovary. While the tube is growing, it contains two nuclei: the one at the tip of the pollen tube is the *tube nucleus* and the one located behind it is the *generative nucleus*. As growth of the tube proceeds, the generative nucleus divides into two *sperm nuclei*. These two nuclei are liberated into the ovary to fertilize a single ovule. Each ovule in the ovary comprises several cells, one of which is the egg nucleus. One of the sperm nuclei unites with the egg nucleus eventually to give rise to the embryo, while the other sperm nucleus unites

with one or more of the other nuclei in the ovule to give rise eventually to the endosperm. This is referred to as "double fertilization" and is peculiar to flowering plants (Angiospermae), to which nearly all crop plants belong. Through this process, the embryo becomes diploid and is ready to grow into another plant. The endosperm is often rich in stored resources from which the growing embryo can draw during its growth. However, the relative importance of the endosperm and other tissues in providing nutrients to the growing embryo and seedling varies greatly between different plants.

After fertilization, and in some plants even before, a fruit begins to develop. There are numerous kinds of fruits (e.g. pomes, drupes, berries, siliques, follicles) depending on how they are formed and which of the parent plant's tissues expand to produce the seed-containing fruit. Further aspects of the mechanics of pollination are reviewed in section 2.1.

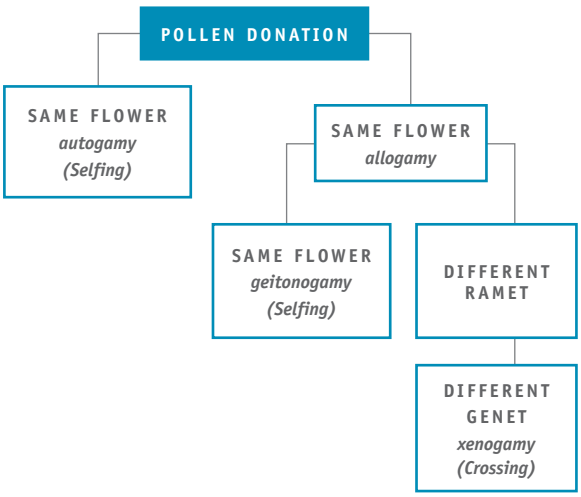
19.3.2 Sexuality in plants

Most crop plants are *hermaphroditic* – both male and female reproductive organs are functional and present on every plant, but not necessarily at the same time. When the male and female flowers are separated, the plants are termed "monoecious". Some monoecious, hermaphroditic plants self-pollinate readily, and some of these may be self-fertile. Some monoecious plants have pollen-producing flowers on one part of the plant, but ovary-bearing flowers on other parts. In some cases, there may be differences in the timing of maturation of the two kinds of flowers. For hermaphroditic plants with hermaphroditic (so-called "perfect") flowers, there may be spatial or temporal separation of the sexual function as well.

Some plants, however, are *unisexual*. These plants bear flowers that produce only pollen, with others producing flowers that bear the ovaries, and eventually the fruits and seeds. Clearly, *self-pollination* or *self-fertilization* is impossible in these cases.

It is possible to summarize the spectrum of possibilities by considering the pathway taken by pollen during the natural process of pollination and noting whether or not pollination results in fertilization and seed set [21]. Figure 19.3 helps to introduce some of the technical terms used in pollination biology.

Figure 19.3
GENERAL BREEDING SYSTEM OF PLANTS, AS INDICATED BY THE PATH OF FERTILIZING POLLEN



General breeding systems of plants – as indicated by the path of fertilizing pollen

Source: P. G. Kevan [present study]

Autogamy or *self-pollination* results from pollen fertilizing ovules of the same flower. The opposite of this process is *cross-pollination* or *outcrossing* (technically, xenogamy). Many crop plants are autogamous including tomatoes, some citrus, peanuts and many other cultivated Fabaceae.[16] Plant breeders have selected for autogamy in some plants that are naturally outcrossing. Nevertheless, it is worth remembering that many self-compatible crop plants may still produce a higher quality crop if cross-pollination takes place (sunflowers and oil-seed varieties of *Brassica* are examples). Autogamy and xenogamy are shown in Figure 19.3.

[16] See Appendix I in Roubik, D.W., ed. 1995. *Pollination of cultivated plants in the tropics*. Agricultural Services Bulletin No. 118. Rome, FAO, available online: http://books.google.com/books?isbn=9251036594

Geitonogamy refers to *self-fertilization*. Here, the pollen fertilizing the ovules originates from the same parent plant or from a genetically identical clone (called a *ramet*). Geitonogamy and autogamy are genetically, or almost, equivalent in that both result in self-fertilization and *inbreeding*. However, it is known that minor genetic differences can occur between flowers of the same plant or clone. Geitonogamy is common in self-fertile plants with flowers that are of different sexes, or that have flowers in which the anthers and stigmata mature at different times.

Xenogamy takes place when pollen from one plant (or *genet*) results in the fertilization of the ovules of a different plant (another genet). Of course, most plants that are self-fruitful can be also be xenogamous. These may be referred to as facultative inbreeders or facultative out-crossers. In fact, in many self-fertile plants, xenogamy is encouraged by differences in the timing of maturation of the anthers and stigmas, or by the structure of the flowers. Furthermore, as noted above, the quality of the crop may be higher if natural *selfers* are *outcrossed*.

However, there are many crop plants that require exchange of pollen with other plants of the same species to set fruit. These are referred to as *obligate out-crossers*. Obligate out-crossers have various mechanisms by which self-fertilization is prevented:

- In some monoecious plants, the staminate and pistillate flowers may be produced at different times (e.g. in oil palm, *Elaeis*).
- In others, the flowers are produced at about the same time but mature separately (e.g. in coconut).
- In some, staminate flowers and pistillate flowers are produced at different places and times on the plant (e.g. squashes, gourds, pumpkins, melons, cucumbers).

In plants with hermaphroditic flowers, self-pollination may be prevented by spatial separation of the anthers and stigmas (this is called *herkogamy*), or temporal separation of maturation (called *dichogamy*). In *dichogamous* plants, the anthers mature first, a condition referred to as *protandry*. If the stigmas and pistils mature first, the condition is referred to as *protogyny*.

A particular form of herkogamy is found in starfruit and bilimbi. In these species, the flowers of one plant have long styles and short stamens, while the flowers of another plant have short styles and long stamens – a condition termed *heterostyly*. The sporophylls on different plants of the same species are of different sizes, and the pollen from the long stamens is larger than that from the short stamens. This is perhaps a reflection of the length of the style through which the tubes of each must grow.

In many monoecious plants that produce hermaphroditic flowers, dichogamy and herkogamy may be poorly developed. These plants rely on incompatibility mechanisms (which often also operate in other obligately xenogamous plants) to prevent self-fertilization. Plants with self-incompatibility cannot self-fertilize. If self-pollination does take place, as is often the case, seed-set will not eventuate. Self-incompatibility is quite complex. Gametophytic, multiallelic self-incompatibility is the most common form. This sort of incompatibility is genetically controlled by two or more alleles (called S alleles) and is affected by the genetic make-up of the pollen, the tubes of which grow into the stylar tissue but fail to penetrate the ovary. Sporophytic, multiallelic self-incompatibility is also controlled by two or more alleles, but is affected by the genetic make-up of the anther and the failure of the pollen to germinate on the stigma. This form of self-incompatibility is best known in the Brassicaceae or cabbage family. Other incompatibility mechanisms involve gametophytic or sporophytic interactions, but with single or double allelic genetic control. Breeders have bred lines of self-incompatible plants as a means to produce hybrid seeds in plants that are normally self-compatible.

The concept of plants of different sexes seems to have been introduced through heterostyly and self-incompatibility. But even in such plants, each breeding type has both male (pollen donor) and female (seed production) functions. In some plants, however, the sexes are separated to a greater or lesser extent. These different breeding systems are referred to as forms of *dicliny*.

Dioecy is the most marked form of sexual separation within dicliny. Some plants are pollen donors only (i.e. males) while others are pollen recipients (seed producers) only (females). There are few dioecious and widely domesticated crop plants, but some grapes (*Vitis*), jojoba (*Simmondsia*), asparagus (*Asparagus*) and spinach (*Spinacia oleracea*) serve as examples. To harvest the seeds or fruit of such plants, a minimum number of male pollinizer plants are needed to allow for pollination and to maximize crop production. For crops such as asparagus, male plants tend to grow more vigorously and are, thus, the plants of choice for crop production. The seed is needed only for breeding or re-planting.

There are examples of plants that are incompletely dioecious, but still diclinous. In species of *gynodioecious* plants, some individuals are male sterile (i.e. female), while others are hermaphroditic. The combination of female-sterility and hermaphroditism (*androdioecy*) is almost unknown in nature. Some species show various intergradations of sexuality and are variously referred to as polygamous, polygamo-dioecious, subgynoecious and so on. Gynodioecy has been bred into some crops (e.g. cotton and oil seed rape) to produce hybrid seed that is harvested from the male sterile variety planted with hermaphroditic pollen donors.

19.3.3 Apomixis and parthenocarpy

Apomixis refers to asexual reproduction. This category of reproduction includes *agamospermy* and vegetative reproduction.

Agamospermy is the process by which a plant's ovules develop directly into seeds without fertilization. Pollination is not required, even though flowers (including very vibrant and colourful ones) are produced. Agamospermy is rare among crop plants, but is represented in *Citrus* (oranges, grapefruit, lemons, etc.), mango, mangosteen, some bramble berries (*Rubus*), and certain cereals and grasses. Seedless clementines are in great demand in California, and beekeepers are warned by growers to avoid their orchards, so as to ensure that seeded fruits are not produced, which would destroy the value of their crop.

A number of crops are propagated vegetatively; for these pollination is never, or rarely, a problem. Tropical crops that are propagated vegetatively include some of the important tubers, such as potatoes, yams, manihot, bananas and sugar cane. Many trees and shrubs can be propagated vegetatively from cuttings. Seeds are not used except in the case of plant breeding for new varieties.

Some plants that produce harvested fruits (as fruits or vegetables) do not require pollination by any means. The fruits develop without fertilization of the ovuls taking place. This process is called *parthenocarpy*. Examples include normal seedless bananas (although wild bananas are pollinated by birds and bats), pineapples and some varieties of citrus (seedless varieties like Clementines) and seedless cucumbers (which if pollinated become misshapen and bitter).

19.3.4 Methods for determining pollination requirements

The methods for determining pollination requirements of plants are not complex. The following is a step-by-step guide:

- Examine the flowers of a number of plants to determine the structure of the flower and the relative positions of all the floral parts. Note especially if various parts are reduced, absent, whether they mature at different times or change their positions as they mature. Determine how the nectar and pollen are presented to pollinators, and how the floral mechanism works. Keep careful notes.
- Make observations on the kinds of pollinators that seem to be effective at pollen transfer (e.g. wind, insects [and kind], birds and bats).
- If the flowers are perfect, testing for self-fertilization or agamospermy can be done by bagging. The exclusion bags should be pollinator proof. Also, they should not create an overly humid or hot microclimate within. Cheesecloth works well, but keep in mind that wind-borne pollen grains can easily blow through the mesh. The bags should be anchored firmly to the plant's stem and not be

allowed to touch the flowers. White paper bags are suitable for use on wind-pollinated plants. If the bagged flowers fail to set seed, it is reasonably certain that pollination is required. However, the need for cross-pollination may not have been proven if the flowers are dichogamous.

- To determine if a plant is out-crossing or selfing, controlled pollination must be attempted. Bagged flowers must be cross-pollinated with pollen from: (a) other plants and (b) within the same plant. If seeds are set by treatment (b), it can be concluded that the plant was geitonogamous. But if seeds were set only from treatment (a), obligate out-crossing would be the explanation. Pollen can be transferred by plucking stamens and touching the anthers to stigmas, brushing pollen from anthers onto an artist's paint brush and then brushing against flora stigmas, or by using a freshly killed bee (impaled on a toothpick or insect pin) as the paint brush. For smaller flowers, sometimes it is necessary to use the whole flower instead of the stamens.
- If the bagged flowers set seed, three explanations can be invoked: (a) the flowers are self-fertile, (b) they are agamospermous, or (c) pollen entered the bag and confounded the results.
- To test for agamospermy, the immature stigmata of the flowers can be clipped or, if large enough, covered with aluminum foil. If clipping is used, the wound should be dressed with a little soft wax (molten bees' wax works well) to prevent drying and infection. If the flowers set seed, agamospermy would be the explanation. If they do not, self-fertility would be invoked. If both treatments result in seed-set, facultative agamospermy would be presumed, but this is rare, and therefore highly unlikely.
- To test for self-fertility and be certain of the result, make controlled self and cross-pollinations in flowers within bags.
- For all the treatments noted above, remember to have open pollinated controls, or check flowers on the experimental plants and on others nearby. In general, at least ten plants should be sampled for each treatment and several flowers on each plant. Depending on the size of the plant, one to several treatments can be made on a single plant. Large plants, such as shrubs and trees, can accommodate several treatments. Small, herbaceous plants may have to be bagged in their entirety to accommodate a single treatment. The actual experimental design has to be tailored to the plant, the flowers and the suspected pollination mechanism.

19.3.5 Conclusions

From the viewpoint of crop pollination biology, the details of breeding systems are important, although the actual mechanisms of self-incompatibility need not be of much concern to most pollination ecologists and crop biologists. Artificial crop selection for breeding systems, however, necessitates more detailed knowledge and training. Understanding breeding systems helps investigators or the pollination ecologist to determine the pollination requirements of the plants of interest, and to develop appropriate and informed recommendations. *The published literature on pollination contains many errors and partially correct generalizations*. Much apicultural literature, which encompasses a great deal of pollination, fails to recognize the complexities of plant breeding systems, as is the case with much the plant science literature. In particular, the latter tends to over-simplify zoological and physical issues in pollination.

Such understanding is especially important given the increasing complexity of pollination requirements in crop plants, as breeders manipulate plant sexualities to maximize growth or productivity, or to preserve desirable plant characteristics. To date, these include artificial selection:

- to bring about apomixis or self-compatibility and self pollination, and to circumvent insect pollination;
- to introduce self-compatibility and dicliny to allow for the production of hybrid seed;
- to develop parthenocarpic varieties to assure fruit set and seedlessness.

In general, it is advisable to experimentally investigate the pollination requirements of crop plants, variety by variety in trial plots and under local growing conditions, and with localized pollinator guilds.

Many exciting new developments have occurred in the field of pollination ecology and crop biology in the intervening years since the first edition of this volume was published by the Food and Agriculture Organization of the United Nations. The authors of this chapter are especially excited to recommend several excellent books dedicated to the topics of methodology in botany, entomology, crop science and pollination ecology by some of the pioneers in the field [10, 22 and 23].[17]

[17] Additional figures for this chapter can be found in Roubik, D.W., ed. 1995. Pollination of cultivated plants in the tropics. Agricultural Services Bulletin No. 118. Rome, FAO, available online: http://books.google.com/books?isbn=9251036594

REFERENCES

[1] Schmid, R. & Alpert, P.H. 1977. A test of Burck's hypothesis relating anther dehiscence to nectar secretion. *New Phytologist,* 78: 487–498.

[2] Buchmann, S.L. 1976. Buzz pollination in angiosperms. In C.E. Jones & R.J. Little, eds. *Handbook of experimental pollination biology*, pp. 73–113. New York, Van Nostrand-Reinhold, Inc.

[3] Roubik, D.W. & Hanson, P.E. 2004. Abejas de orquideas de la América tropical: biologia y guia de campo/orchid bees of tropical America: biology and field guide. Santo Domingo de Heredia, Costa Rica, INBio Press.

[4] Roubik, D.W. & Moreno, J.E. 1991. *Pollen and spores of Barro Colorado Island. Monographs in Systematic Botany*, 36. St. Louis, Missouri Botanical Garden.

[5] Bush, M.B. & Weng, C. 2007. Introducing a new (freeware) tool for palynology. *Journal of Biogeography*, 34: 377–380.

[6] Rodriguez-Riano, T. & Dafni, A. 2000. A new procedure to assess pollen viability. *Sex Plant Reproduction*, 12: 241–144.

[7] Spears, E.E. 1983. A direct measure of pollinator effectiveness. *Oecologia,* 57: 196–199.

[8] Pinillos, V. & Cuevas, J. 2008. Standardization of the fluorochromatic reaction test to assess pollen viability. *Biotechnic & Histochemistry*, 83: 15–21.

[9] Buchmann, S.L. 1986. Vibratile pollination in *Solanum* and *Lycopersicon*: a look at pollen chemistry. In W.G. D'Arcy, ed. *Solanaceae*: *biology and systematics*, pp. 237–252. New York, Columbia University Press.

[10] Dafni, A. 1993. *Pollination ecology: a practical approach*. Oxford, Oxford University Press.

[11] Roulston, T. & Cane, J.H. 2000. Pollen nutritional content and digestibility for animals. *Plant Systematics and Evolution*, 222: 187–209.

[12] Baker, H.G. & Baker, I. 1986. The occurrence and significance of amino acids in floral nectar. *Plant Systematics and Evolution,* 151: 175–186.

[13] Baker, H.G. & Baker, I. 1979. Starch in angiosperm pollen grains and its evolutionary significance. *American Journal of Botany*, 66: 591–600.

[14] Roubik, D.W., Moreno, J.E., Vergara, C. & Wittmann, D. 1986. Sporadic food competition with the African honeybee: projected impact on neotropical social bees. *Journal of Tropical Ecology*, 2: 97–111.

[15] Waller, G.D. 1980. A modification of the OAC (Ontario Agricultural College) pollen trap. *American Bee Journal*, 120: 119–121.

[16] Buchmann, S.L. 1996. Competition between honeybees and native bees in the Sonoran Desert and global bee conservation issues. In A. Matheson, S. Buchmann, C. O'Toole, C. Westrich, P. Williams & I. Williams, eds. *The conservation of bees*, pp. 125–142. London, Academic Press.

[17] O'Rourke, M.K. & Buchmann, S.L. 1991. Standardized analytical techniques for bee-collected pollen. *Environmental Entomology*, 20: 507–513.

[18] Faegri, K., Kaland, P.E. & Krywinski, K. 1992. *Textbook of pollen analysis*. New York, John Wiley and Sons, Ltd.

[19] Stockmarr, J. 1971. Tablets with spores used in absolute pollen analysis. *Pollen et Spores*, 13: 615–621.

[20] Buchmann, S.L. 1987. The ecology of oil flowers and their bees. *Annual Review of Ecology and Systematics*, 18: 343–369.

[21] Richards, A.J. 1997. *Plant breeding systems*. London, Chapman & Hall

[22] Dafni, A., Kevan, P.G. & Husband. B.C. 2005. *Practical pollination biology*. Cambridge, ON, Enviroquest.

[23] Kearns, C.A. & Inouye, D.W. 1993. *Techniques for pollination biologists*. Boulder, University Press of Colorado.

ADDITIONAL REFERENCES

Block, R.J., Durrum, E.L. & Zweig, C. 1958. *A manual of paper chromatography and paper electrophoresis*. New York, Academic Press.

Buchmann, S.L. 1985. Bees use vibration to aid pollen collection from non-poricidal flowers. *J. Kansas Entomol. Soc.*, 58: 517–525.

Buchmann, S.L. & Buchmann, M.D. 1981. Anthecology of *Mouiiri myrtilloides* Melastomataceae: Memecyleae) an oil flower in Panama. *Biotropica*, 13: 7-24, special supplement on reproductive botany.

Buchmann, S.L. & Cane. J.H. 1989. Bees assess pollen returns while sonicating Solanum flowers. *Oecologia*, 81: 289–294.

Buchmann, S.L. & O'Rourke, M.K. 1991. Importance of pollen grain volumes for calculating bee diets. *Grana*, 30: 591–595.

Buchmann, S.L. O'Rourke, M.K., Shipman, C.W., Thoenes, S.C. & Schmidt, J.O. 1992. Pollen harvest by honeybees in Saguaro National Monument potential effects on plant reproduction. In C.P. Stone & E.S. Bellantoni, eds. *Proceedings of the symposium on research in Saguaro National Monument, Tucson, AZ*, pp. 149–156.

Burquez, A. & Corbet, S.A. 1991. Do flowers reabsorb nectar? *Functional Ecology*, 5: 369–379.

Campbell, N.J. & Ayers, G.S. 1989. Selecting for high carbohydrate production in birdsfoot trefoil (*Lotus corniculatus* L.) through direct and indirect evaluation. *Journal of Apicultural Research*, 28(3): 121–125.

Corbet, S.A. & Delfosse, E.S. 1984. Honeybees and the nectar of *Echium planta-gineum* in southeastern Australia. *Australian Journal of Ecology*, 9: 125–139.

Cruden, R.W. & Hermann, S.M. 1983. Studying nectar? Some observations on the art. In B. Bentley and T. Elias, eds. *The biology of nectaries*, pp. 223–241. New York, Columbia University Press.

Davis, M.B. 1966. Determination of absolute pollen frequency. *Ecology*, 47: 310–311.

Davis, A.R. & Gunning, B.E.S. 1991. The modified stomata of the floral nectary of *Vicia faba* L. 2. Stomatal number and distribution as selection criteria for breeding for increased nectar sugar production. *Acta Horticulturae*, 288: 329–334.

Elias, T.S. & Celhand, H. 1976. Morphology and anatomy of floral and extrafloral nectaries in Campsis (Bignoniaceae). *American Journal of Botany*, 63: 1349–1353.

Faegri, K. & Iversen, J. 1989. *Textbook of pollen analysis.* (4 edn), New York, Wiley.

Frey-Wyssling, A. & Häusermann, E. 1960. Deutung der gestaltIosen Nektarien. Ber. schweiz. *Bot. Gesell*, 70: 150–162.

Hesse, M. & Waha, M. 1989. A new look at the acetolysis method. *Pl. Syst. Evol.*, 163: 147–152.

Howell, D.J. 1972. Physiological adaptations in the syndrome of chiropterophily with emphasis on the bat *Leptonycteris* Lydekker. Unpubl. Ph.D. dissertation. Tucson, Dept. Biol. Sci., Univ. Arizona.

Inouye, D.W, Favre, N.D., Lanum, J.A., Levine, D.M., Meyers, J.B., Roberts, F.C., Tsao, F.C. & Wang, Y. 1980. The effects of nonsugar nectar constituents on nectar energy content. *Ecology*, 61: 992–996.

Käpylä, M. 1978. Amount and type of nectar sugar in some wild flowers in Finland. *Annales Botanici Fennici*, 15: 85–88.

Kevan, P.G., Lee, H. & Shuel, R.W. 1991. Sugar ratios in nectars of varieties of canoIa (*Brassica napus*). *Journal of Apicultural Research*, 30: 99–102.

Kronestedt-Robards, E.C., Greger, M. & Robards, A.W. 1989. The nectar of the Strelitzia reginae flower. *Physiologia Plantarum*, 77: 341–346.

Kuo, J. & Pate, J.S. 1985. The extrafloral nectaries of cowpea (*Vigna unguiculata* (L) Walp.): I. Morphology, anatomy and fine structure. *Planta*, 166: 15–27.

Lüttge, U. & Schnepf, E. 1976. 5.3. Organic substances. In U. Lüttge & M.G. Pitman, eds. *Encyclopedia of plant physiology. Transport in plants II. B. Tissues and organs*, pp. 244–277. New York, Springer-Verlag.

McKenna, M. & Thomson, J.D. 1988. A technique for sampling and measuring small amounts of floral nectar. *Ecology*, 69: 1306–1307.

Mesquida, J., Marilleau, R., Pham-Delegue, M-H. & Renard, M. 1988. A study of rapeseed (*Brassica napus* L. var. *oleifera* Metzger) flower nectar secretions. *Apidologie*, 193: 307–318.

Olesen, J.M. & Warncke, E. 1989. Flowering and seasonal changes in flower sex ratio and frequency of flower visitors in a population of *Saxifraga hirculus*. *Holarctic Ecology*, 12: 21–30.

O'Rourke, M.K. & Buchmann, S.L. 1991. Standardized pollen analytical techniques used for various bee-collected samples. *Environ. Entomol*, 20: 508–528.

Ordway, E., Buchmann, S.L., Kuehl, R.O. & Shipman, C.W. 1987. Pollen dispersal in *Cucurbita foetidissima* HBK (Cucur-bitaceae) by bees of the genera *Apis*, *Peponapis* and *Xenoglossa*. *J. Kansas. Entomol. Soc.*, 60: 489–503.

Punchihewa, R.W.K. 1984. Anthophilous insects and the poflination ecology of *Asclepias syriaca* L. and *Asclepias incarnata* L. in southern Ontario. M.Sc. Thesis, University of Guelph, Guelph, Canada.

Roberts, R.B. 1979. Spectrophotometric analysis of sugars produced by plants and harvested by insects. *Journal of Apicultural Research*, 18(3): 191–195.

Sclnnid, R. 1976. Filament histology and anther dehiscence. *Bot. J. Linnean Soc.*, 75: 303–315.

Schmid, R. 1988. Reproductive versus extra-reproductive nectaries – historical perspective and terminological recommendations. *Botanical Review*, 54: 180–232.

Severson, D.W & Erickson, E.H. 1984. High performance liquid chromatography of carbohydrates in cucumber nectar. *Journal of Apicultural Research*, 22: 158–162.

Schmidt, J.O. & Buchmann, S.L. 1992. Other products of the hive. In J. Graham, ed. *The hive and the honeybee*, pp. 927–988. Hamilton, IL, Dadant and Sons, Inc.

Schuman, G.E., Stanley M.A. & Knudsen, D. 1973. Automated total nitrogen analysis of soil and plant samples. *Soil Science Society of America Proc.*, 37: 480–431.

Shuel, R.W. 1989. Improving honey production through plant breeding. *Bee World*, 70: 36–45.

Shuel, R.W. 1992. The production of nectar and pollen. In J. Graham, ed. *The hive and the honeybee*, pp. 401–436. Hamilton, IL, Dadant and Sons, Inc.

Stanley, R.G. & Linskens, H.E. 1974. *Pollen: biology, biochemistry, management*. Berlin, Springer-Verlag.

TeubeI, L.R. & Barnes, D.K. 1979. Breeding alfalfa for increased nectar production. ln *Proceedings of the 4th International Symposium on Pollination*, Maryland Agricultural Experimental Station, Special Miscellaneous Publication 1: 109–116.

Teuber, L.R., Albertsen, M.C., Barnes, D.K. & Heichel, G.H. 1980. Structure of floral nectaries of alfalfa (*Medicago sativa* L.) in relation to nectar production. *American Journal of Botany*, 67: 433–439.

Vogel, S. 1969. Flowers offering fatty oil instead of nectar. XI Proc. Intl. Bot. Congr., Seattle, p. 229.

Vogel, S. 1974. Olblumen und olsammelnde Bienen. Akad. Wissenschaften u. Literature Mainz. Wiesbaden, Franz Steiner.

Wykes, G.R. 1952. The preferences of honeybees for solutions of various sugars which occur in nectar. *Journal of Experimental Biology*, 51: 294–300.

Chapter 20

HONEY BEE MANAGEMENT IN THE UNITED STATES

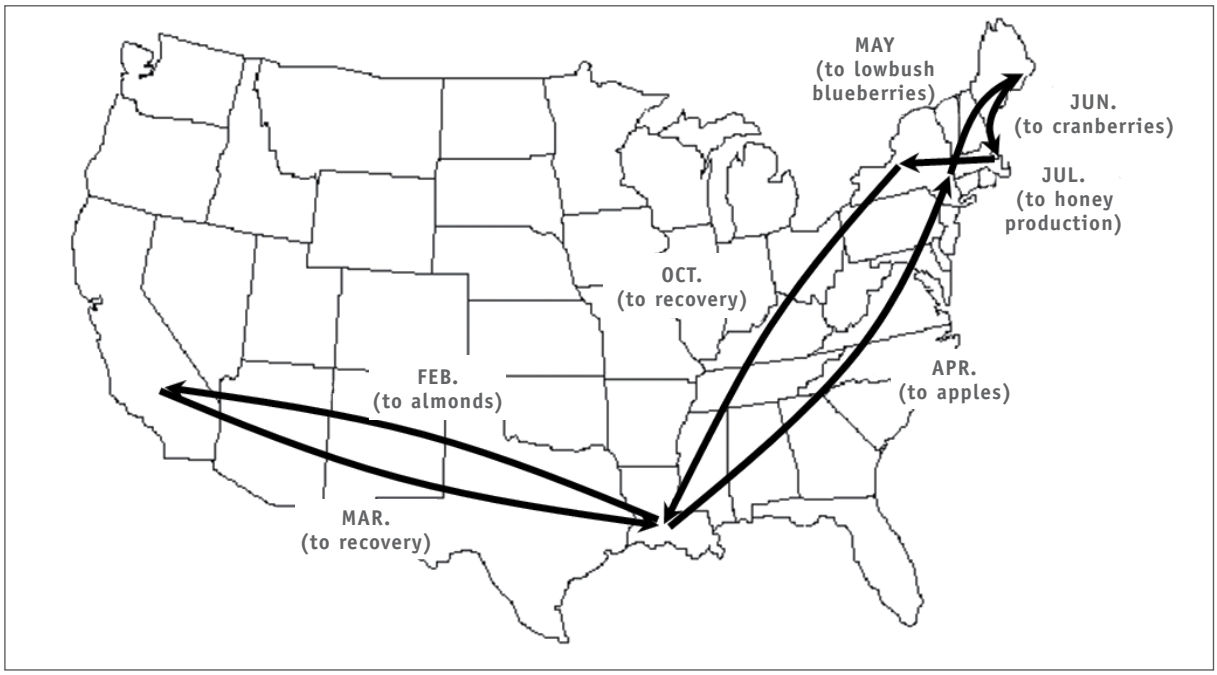
D. Sammataro and L. de Guzman

Honey bees are the most important pollinators of agricultural crops. In the United States, the value of crop pollination services was estimated at US$14.6 million at the beginning of this century [1], a figure that has likely more than doubled in the intervening years. Bee colonies are transported across the country to meet the pollination needs of many orchardists and growers (Figure 20.1) where particular crops are grown and the natural habitat contains limited native or feral bee populations. European honey bees (*Apis mellifera*) are responsible for about 80 percent of pollination services [2–5], mainly because they are readily available and can be transported from field to field when crops come into bloom. However, the demand for pollination services threatens to exceed the availability of colonies because of increased colony losses in the past decade. Deaths of colonies are reported to be a result of myriad factors including Colony Collapse Disorder (CCD) and high levels of parasitic mite infestations [7–12]. Although the presence of pathogens such as viruses and fungi are reported to be good indicators of CCD, *Varroa destructor* remains the most serious parasite of *A. mellifera* not only in the United States but across much of the world. However, in Asia beekeepers consider *Tropilaelaps* a more serious problem for *A. mellifera* colonies than *Varroa* [13, 14].

20.1 MEETING RENTAL REQUIREMENTS

Colonies rented for pollination services must meet population size requirements to ensure there are enough bees to enhance crop productivity. Hence, colonies need to be managed properly in order to produce large bee populations before the crops to be pollinated start to bloom. An ideal pollinating colony should be strong, consisting of five to six Langstroth brood frames (or over 3 870 cm^2), and seven to nine frames of bees, or 20 000 adults [16, 17]. Stimulative feeding with proteins and carbohydrates helps colonies to produce large bee populations to meet colony size requirements. It is important to feed colonies, especially if there is no natural forage available, as this will keep bees from starving. This will enable colonies to remain healthy during dearth periods and raise brood, in order to be ready for the pollination season.

Figure 20.1
MAP SHOWING THE ROUTES AND TIMING OF SHIPMENTS OF HONEY BEE COLONIES FOR ONE COMMERCIAL MIGRATORY BEEKEEPER IN LOUISIANA, USA



Activities include winter management in Louisiana, followed by a move to almond orchards in California (February). In March, bees are moved back to Louisiana to recover and for general spring management work. In April, bees are transported to New York for apple pollination, then moved to lowbush blueberries in Maine (May) and cranberries in Massachusetts (June). In July, the bees are taken back to New York for recovery and honey production, and before winter they are transported to Louisiana to recover and prepare for overwintering in October [see 25]

Source: Sammataro and de Guzman [present study]

20.1.1 Feeding with sugar

Outside feeding: Feeders can be set up outside to stimulate bees all at once, using dry sugar or sugar syrup. Food should be sheltered from the weather to keep it dry, and located centrally among all the bee colonies. However, open feeding is generally not recommended, as this can lead to robbing between colonies as well as spread diseases and pests. It will also attract other animals that could then prey on the bee colonies. Feeding inside each hive is therefore encouraged.

In-hive feeding: Feeding liquid sugar syrup inside the hive will increase pollen collection [13] as well as stimulate brood production. Sugars can be fed as syrup, dry or mixed as a patty. The easiest way to feed bees is with cane or beet sugar (white sugar only). One gallon (3.8 L) of sugar syrup (2:1 sugar: water) will increase the food reserves of a colony by about 7 lb. (3.2 kg). However, a 1:1 sugar: water (by weight or by volume) mix is usually the most acceptable to bees and the easiest to make. Higher sugar proportions are better late in the year. Use white, granulated sugar only; brown or raw sugar, molasses or sorghum contain impurities and can cause dysentery in bees. Mix granulated sugar and hot water together and stir adequately until all the sugar is dissolved.

Syrup feeders: The easiest way to feed individual bee colonies [18] is to use a glass jar with a few small holes punched into the metal lid; the cardboard liner under the lid must be removed. The jar is then turned upside down and placed on the hive top bars or over the oval hole of the inner cover. Some beekeepers cut

a hole in the outer cover for a glass jar, especially for five-frame nucleus colonies. Plastic containers can be used, but often will collapse and can leak.

Other feeders are available at bee supply companies; most have a wooden rim surrounding a container for liquid. They hold various amounts of syrup and are placed directly on the top bars near the cluster then covered with the outer cover (see Figure 20.2). Instead of a feeder, plastic bags can be used; a gallon zip bag is half filled with syrup and pricked with a few pinholes to allow the liquid to ooze out slowly. Care must be taken to not overfill the bags, otherwise the outer covers will not fit snugly and could allow robbing bees to enter. Remove and discard the plastic bags when empty.

There are several types of in-hive syrup feeders available. Some are the size of a frame and fit inside the hive, while others slide into the hive entrance.

Non-syrup food: Dry, white, granulated sugar is often used as an emergency food as long as bees have access to water. The sugar is carefully spread on the top bars, on the inner cover or on a single sheet of newspaper over half the top bars of the frames. Only strong colonies will benefit from the feeding of dry sugar.

Figure 20.2
MILLER-TYPE FEEDERS ARE PLACED ON TOP OF THE HIVE BODY. THE OUTER COVER THEN GOES ON TOP.

Bees climb up through the centre slot (a) and feed on syrup from floating devices (arrow) designed to keep bees from drowning. Plastic feeders direct the bees to the ends where openings allow bees to move up and feed (b)

Source: Sammataro and de Guzman [present study]

A "mock" candy can be made by mixing honey (clean, disease-free honey) or thick sugar syrup with confectioners' or powdered sugar. Knead it to form a stiff paste, flatten with a roller, then wrap in plastic and freeze until needed. To feed, thaw a piece of candy and place it on the top bars. Cooked fondant candy can also be used. Use a basic fondant or sugar candy recipe (see [19]):

- 15 lb. white sugar (6.80 kg)
- 3 lb. corn syrup (light) (1.36 kg)
- 1/2 teaspoon cream of tartar (tartaric acid)
- 4 cups boiling water (0.95 L)

Combine and heat the ingredients, stirring until the sugar dissolves. Heat without stirring to 238 °F (115 °C, or up to the medium ball on a candy thermometer), pour out onto a bowl and cool until warm to the touch. Beat the syrup until it turns white in colour, and pour into molds or shallow dishes.

Several combs full of honey or several frames of honey placed next to the broodnest is the best form of food to feed colonies. The honey must be clean, not from diseased colonies, and not fermented or crystallized. Weak colonies fed with this will often be robbed by larger, stronger colonies, so all of the colonies should be fed at the same time, and the entrances should be reduced in size. Store-bought honey should **not** be used to feed bees.

20.1.2 Pollen supplements and substitutes

Pollen is the principal source of protein for honey bees; the protein content ranges from 8 percent to 40 percent. Nurse bees feed on bee bread – pollen fermented and stored in colonies (see Figure 20.3). This activates their food glands (hypopharyngeal glands) which then secrete the protein rich food that is fed to the bee larvae and queen. *Supplements* consist of pollen and other substances nutritious to bees, whereas *substitute* feed contains no pollen.

It is important to note here that pollen feeding by worker bees decreases colony pollen collection activity [20] because foragers respond to the pollen patty by switching to nectar foraging, or by not foraging at all; however, this can depend on the particular crops being pollinated [21]. Hence, feeding colonies with

pollen patties should be stopped before the target crop begins to bloom. Feeding can occur prior to moving bees into the pollination fields or before the bloom has begun, to stimulate brood rearing.

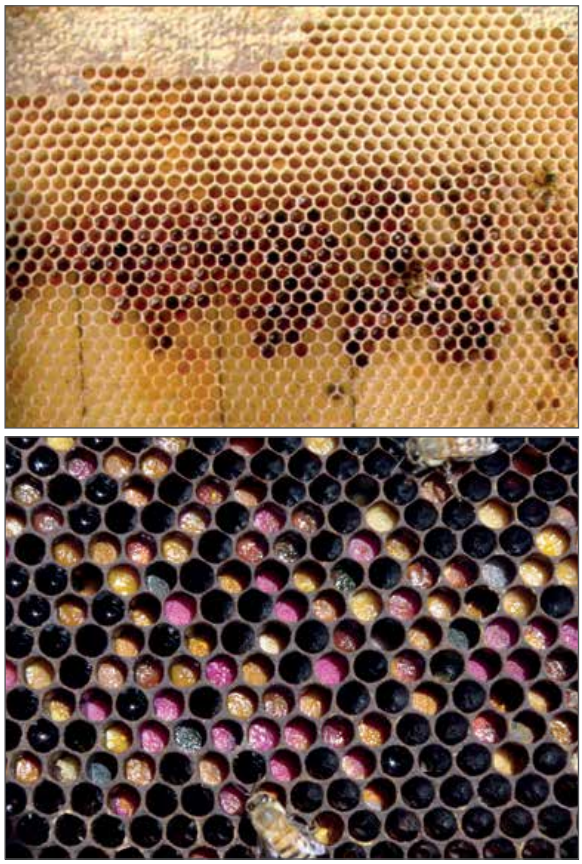
Pollen, pollen supplements and pollen substitutes are commercially available from bee supply companies. Pollen is often trapped using various devices that collect the pollen pellets carried into the hive by foraging bees. However, there is some danger that collected pollen may be contaminated with pesticides, or contain chalkbrood, American or European foulbrood spores. Care must therefore be taken to ensure that any pollen purchased is obtained from pesticide and disease-free colonies. Diseased pollen can be sterilized with gamma irradiation and the pollen then tested for contamination before use. It is necessary to preserve and store the collected pollen quickly to prevent spoilage. Pellets should be collected daily, cleaned of debris, and dried or frozen for future use (Figure 20.4). To feed pollen to colonies, sprinkle the pellets into an empty comb. Dried pollen pellets also can be ground into a powder, mixed with sugar and sprinkled, dry, onto an empty comb (see Figure 20.4b).

Collected pollen can also be made into patties by first soaking the pellets in water or syrup for an hour. The patties are made by kneading softened pellets with clean honey or heavy sugar syrup that has been heated before use; the dough should be stiff, not runny. Sandwich the patties between two pieces of *waxed paper*, not plastic wrap, to keep them moist, then store them in the freezer inside a plastic bag to keep them from drying out. One formula is: 4 parts hot water to 1 part pollen to 8 parts sugar. Use a commercial bread mixer or similar appliance if making a large quantity of patties, and always store the wrapped patties in a freezer.

If pollen is not available or comes from a questionable source, use supplements or substitutes from commercial bee supply companies. To make several patties using pollen, pollen supplements or substitutes, follow this method:

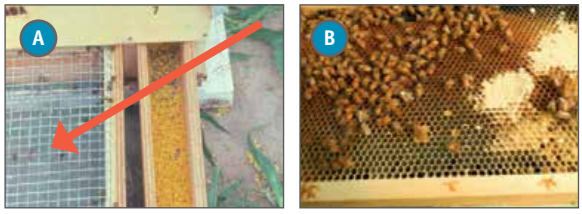
- Take one pound (0.45 kg) of the dry material.
- Take four cups (0.95 L) of 2:1 (sugar: water) syrup.
- Mix together to form a soft patty that is thick enough not to drip.
- Some beekeepers add anise oil, fennel oil, mint or chamomile oil to make the material more attractive to bees.

Figure 20.3
BEE BREAD STORED IN COMBS



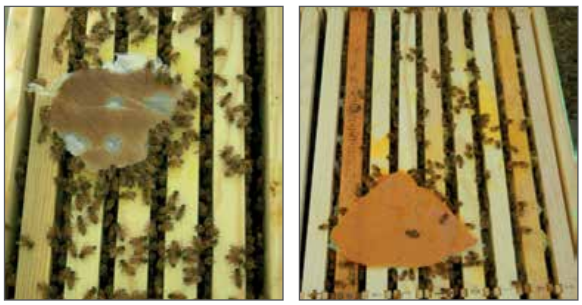
Source: Sammataro and de Guzman [present study]

Figure 20.4
(A) TRAPPED POLLEN (ARROW) CAN BE COLLECTED EITHER FOR FEEDING BACK TO BEES, OR TO DIMINISH THE EFFECTS FROM A CROP AND ITS POLLEN SPRAYED WITH PESTICIDES. (B) DRIED POLLEN SUBSTITUTE CAN BE FED BY POURING THE MIXTURE INTO AN EMPTY COMB



Source: Sammataro and de Guzman [present study]

Figure 20.5
BEES FEEDING ON A PROTEIN PATTY OR SUBSTITUTE DIET (LEFT) AND POLLEN PATTY (RIGHT)



Source: Sammataro and de Guzman [present study]

Figure 20.6
BEES PLACED IN CALIFORNIA ALMOND ORCHARDS. BEEKEEPER CONTACT INFORMATION IS PRINTED ON THE HIVES (LEFT). NOTE THE LACK OF OTHER FORAGE AND HOW THE COLONIES ARE PLACED ON THE PALLETS

Source: Sammataro and de Guzman [present study]

- Sandwich the mixture between two pieces of waxed paper (not plastic wrap) to keep it moist and then store it in the freezer.

Feed the patties to the bees by placing them on top of the frames above the brood nest (see Figure 20.5). If a patty starts to become moldy or dry, remove it. Remove the patty if it is not consumed within ten days to keep out other pests, such as wax moths. In areas with small hive beetles (SHB), provide just enough patties to be consumed within two days to prevent the development and maturity of SHB larvae.

20.1.3 Moving the colonies

General considerations: The best time to move bee colonies for pollination is at night and when the air temperature is low (50 °F or 10 °C), as this prevents bees from flying out. Before moving bees, make sure the grower knows when they will arrive and where they will be placed; these arrangements must be made prior to moving any colonies. Contracts are often made between the growers and beekeepers for this purpose (see an example in Annex 1).

The hive entrances can be closed with screens so that the bees do not suffocate, before the colonies are loaded onto a truck or trailer. Close the entrances after dark to ensure that all the bees are in the hive. Up to six colonies can be strapped together onto a pallet and loaded using a mechanical forklift, then covered with netting (Figure 20.6). It is important that colonies are placed in the fields when the crop is in flower. If they are installed before bloom, bees will find other plants (weeds and other non-target plants) on which to forage. The timing of colony introduction according to bloom varies with the crop.

Number of colonies needed: Each crop has its own requirements as to the number of bee colonies needed per acre (or hectare) of blooming plants. If there are areas of blooming, non-target plants (weeds, etc.), or if the crop is not particularly attractive to bees, or the weather is inclement, more colonies may be required to set seed or fruit. In general, one colony per acre is the starting point. Check individual crops for their specific needs (see references).[18]

20.2 APIARY SETUP

20.2.1 Choosing a site

Apiary sites, even temporary ones, should take into account the following recommendations to help ensure apiary success [18]. These are especially important if the apiary is permanent. Even if the location is temporary, each recommendation should be carefully considered to ensure that the bees do not become a nuisance (disturb people or animals) or are exposed to danger (toxins or other hazards).

[18] For more information see www.ent.uga.edu/bees/pollination/crop-pollination.html.

- Locate bees close to fresh water (e.g. a stream, pond or lake). If water is not readily available within flight range, bees will visit swimming pools, bird baths or animal troughs for water and could become a nuisance. If this is the case, or if water sources are contaminated, provide water. Syrup feeders can be filled with water, if needed.
- Make sure that there is easy vehicle access, especially to unload colonies. Avoid heavy traffic or populated areas.
- Install bees within flight range of the crops to be pollinated.
- Locate the hives near a windbreak to keep strong winds from cooling the colonies (or for winter protection).
- Provide shade to keep the hives cool in hot weather.
- Fence the apiary to discourage vandals/thieves and animals (e.g. bears or cattle).
- Separate the hives by at least 4 miles (6.4 km) from other bee yards to diminish the spread of diseases and pests.
- Protect the colonies from exposure to pesticide applications. Move colonies, if necessary, at least 4 miles (6.4 km) from any potential exposure.

Colonies should not be placed at the following types of sites:

- bottom lands where moist air tends to stagnate,
- fire-prone regions or flood areas (check soils or land use maps),
- areas where vandalism and thievery may be a problem,
- areas where bears or other colony predators are prevalent.

Some growers use chemical attractants to increase bee foraging, especially to crops not especially attractive to bees. These usually contain the Nasanov or queen pheromone. These attractants do not necessarily increase pollination or seed set and can be washed off by rain.

20.2.2 Contact information

The name and address of the beekeeper should be posted at each location; individual colonies can also be identified (see Figure 20.6). This will allow neighbours to reach the beekeeper in the event of swarms, animals destroying colonies, or if colonies are missing or overturned. It is advisable to have written agreements between the beekeeper and the property owner that clearly state who owns the bees in the event of an emergency (see Annex 1). The property owner may also wish to be protected from liability and request that the beekeeper have insurance.

To remain on good terms with neighbours, land owners, the grower and other people likely to come in contact with bee colonies:

- Make sure it is legal to keep bee hives at a particular location.
- Keep hives out of sight by placing them behind tall shrubs or fencing, which forces bees to fly higher, over people or animals.
- Provide water if bees have easy access to swimming pools or animal water troughs.

20.2.3 Hive arrangement

In most apiaries, hives are placed in rows or are paired on hive stands or pallets in rows. If hives are going to be opened and the colonies worked, they should be 6 to 8 inches apart (15 to 20 cm); colonies on pallets can be spaced 5 to 8 ft. (1.5 to 2.4 m) apart. This minimizes vibrations and jostling while working a colony, but keeps the hives close enough together to make working more efficient.

When the hives are placed in long rows, there is a tendency for some bees to drift to the hives at the end of the row, probably due to prevailing winds. Drifting can lead to the spread of diseases, mites and also to robbing (and killing) of weaker colonies.

To reduce bee drift, place beehives in a horseshoe configuration (entrances facing in or out), or shorten or stagger the rows. Entrances can also be alternated front and back along the row. Another method is to paint the hives different colours or patterns, or to have landmarks nearby, such as rocks, fencing or bushes. In areas where there is a flat horizon or no vertical landmarks, bees may be unable to find their way back to their colony. Consider erecting a snow fence or other object to help foragers achieve orientation. Do not install more bee colonies than the site will support.

© E. Tourneret

20.2.4 Colony placement

Foraging bees readily fly 2 to 4 miles (3.2 to 6.4 km) in any direction from their hive; therefore, bees should be clustered at 500 ft. intervals (160 m) throughout the crop, and not just placed at the field edges.

Colonies should be removed as soon as the crop is finished blooming. This allows the grower to spray for pests and diseases, and otherwise treat the orchard with chemicals that could have an adverse effect on bee health [22] (see also Chapter 4). Colonies can then be moved into recovery fields to rest, replenish stores, and be treated for pests or diseases.

REFERENCES

[1] Morse, R.A. & Calderone, N.W. 2000. *The value of honey bees as pollinators of U.S. crops in 2000*. Bee Culture, 28(3): 1–15.

[2] Carreck, N.L. & Williams, I.H. 1998. The economic value of bees in the UK. *Bee World*, 79: 115–123.

[3] Delaplane, K. S. & Mayer, D.F. 2000. *Crop Pollination by Bees*. New York, CABI Publishing Co.

[4] Delaplane, K.S. 1998. *Bee conservation in the southeast*. Update of circular 1164. College of Agricultural & Environmental Sciences (online).

[5] McGregor, S.E. 1976. *Insect pollination of cultivated crop plants*. Agriculture Handbook 496. Washington, DC, USDA-ARS.

[6] vanEngelsdorp, D., Hayes, J. Jr., Underwood, R.M. & Pettis, J.S. 2008. A survey of honey bee colony losses in the U.S., fall 2007 to spring. 2008. *PLoS ONE*, 3(12): e4071. doi:10.1371/journal.pone.0004071.

[7] vanEngelsdorp, D., Hayes, J. Jr., Underwood, R.M & Pettis, J.S. 2010. A survey of honey bee colony losses in the US, Fall 2008 to Spring. 2009. *Journal of Apicultural Research,* 49: 7–14. doi: 10.3896/IBRA.1.49.1.03.

[8] Delaplane, K.S. 2011. Understanding the impact of honey bee disorders on crop pollination. In D. Sammataro & J.A. Yoder, eds. *Honey bee colony health: challenges and sustainable solutions*, pp. 223–228. Boca Raton, FL, CRC Press.

[9] vanEngelsdorp, D., Steinhauer, N., Rennich, K., Wilson, M., Baylis, K., Caron, D.M., Delaplane, K.S., Ellis, J., Lee, K., Lengerich, E.J., Pettis, J., Rose, R., Sagili, R., Skinner, J., Spleen, A.M., Tarpy, D.R., Travis, D. & Wilkes, J.T. 2014. *Colony loss 2013–2014. Preliminary results: honey bee colony losses in the United States, 2013–2014*. Bee Informed Partnership, http://beeinformed.org.

[10] Cox-Foster, D.L., Conlan, S., Holmes, E.C., Palacios, G., Evans, J.D., Moran, N.A, Quan, P-L., Briese, T., Hornig, M., Geiser, D.M., Martinson, V., vanEngelsdorp, D., Kalkstein, A.L., Drysdale, A., Hui, J., Zhai, J., Cui, L., Hutchinson, S.K., Simons, J.F., Egholm, M., Pettis, J.S. & Lipkin, W.I. 2007. A metagenomic survey of microbes in honey bee colony collapse disorder. *Science*, 318: 283–287. doi: 10.1126/science.1146498.

[11] van der Sluijs, J.P., Simon-Delso, N., Goulson, D., Maxim, L., Bonmatin, J-M. & Belzunces, L.P. 2013. Neonicotinoids, bee disorders and the sustainability of pollinator services. *Current Opinion in Environmental Sustainability*, 5: 293–305. doi:10.1016/j.cosust.2013.05.007.

[12] Ellis, J.D., Evans, J.D. & Pettis, J. 2010. Colony losses, managed colony population decline, and colony collapse cisorder in the United States. *Journal of Apicultural Research*, 49: 134–136. doi: 10.3896/IBRA.1.49.1.30.

[13] Anderson, D. & Morgan, M.J. 2007. Genetic and morphological variation of bee-parasitic *Tropilaelaps* (Acari: Laelapidae) new and re-defined species. *Experimental and Applied Acarology*, 43(1): 1–24.

[14] Burgett, M., Akratanakul, P. & Morse, R.A. 1983. *Tropilaelaps clareae*: a parasite of honeybees in Southeast Asia. *Bee World*, 64, 25–28.

[15] Danka, R.G., de Guzman, L.I., Rinderer, T.E., Sylvester, H.A., Wagener, C.M., Bourgeois, A.L., Harris, J.W. & Villa, J.D. 2012. Functionality of varroa-resistant honey bees (Hymenoptera: Apidae) when used in migratory beekeeping for crop pollination. *Journal of Economic Entomology,* 105: 313–321.

[16] Delaplane, K.S., Van Der Steen, J. & Guzman, E. 2013. Standard methods for estimating strength parameters of *Apis mellifera* colonies. In V. Dietemann, J.D. Ellis & P. Neumann, eds. *The COLOSS beebook. Volume I: standard methods for* Apis mellifera *research*. Bern, Switzerland, COLOSS.

[17] Scott-Dupree, C., Winston, M., Hergert, G., Jay, S.C., Nelson, D., Gates, J., Termeer, B. & Otis, G., eds. 1995. *A guide to managing bees for crop pollination*. Guelph, ON, Canadian Association of Professional Apiculturists.

[18] Sammataro, D. & Avitabile, A. 2011. *Beekeepers handbook* (4th edn). Ithaca, NY, Cornell University Press.

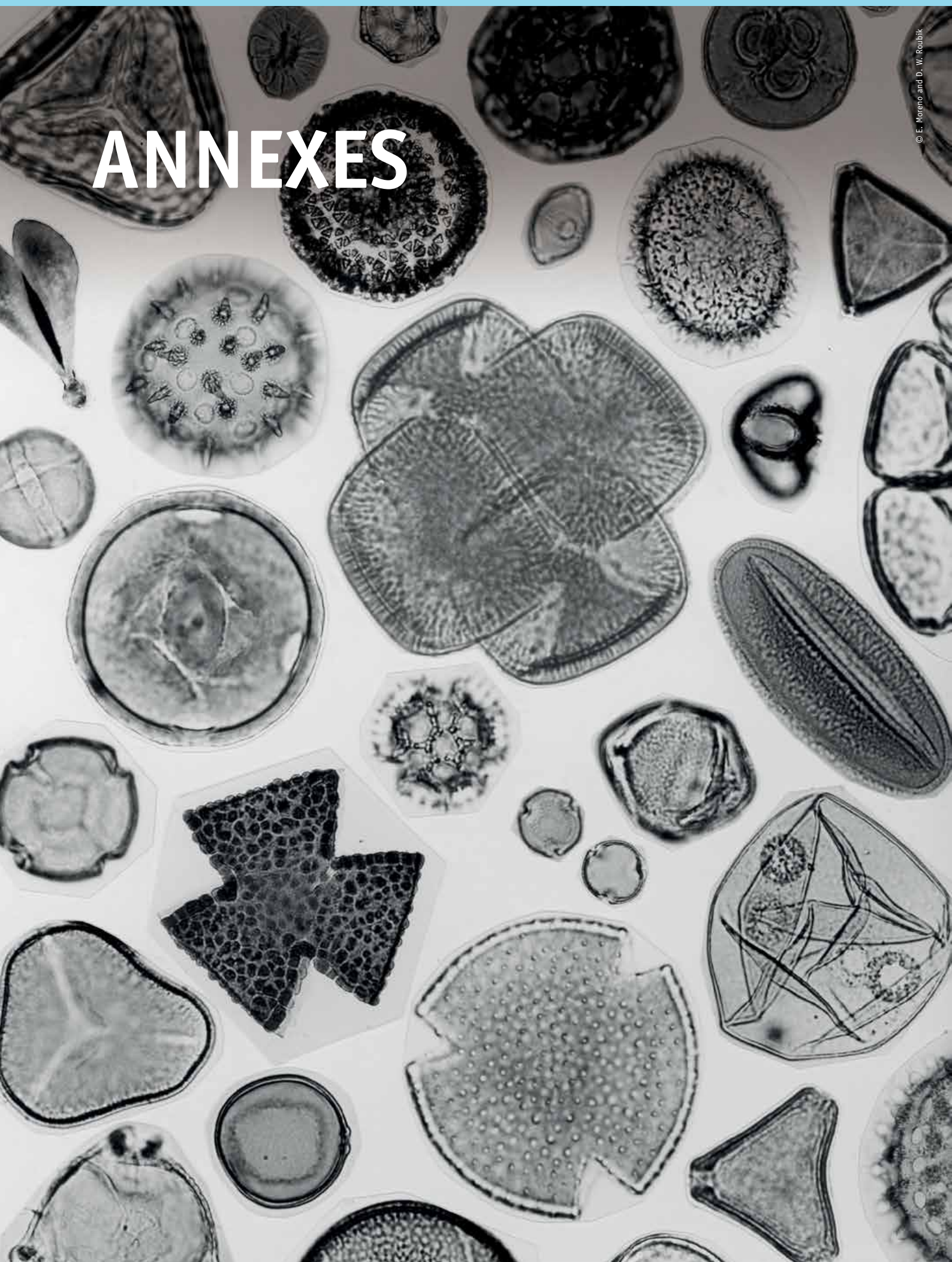
[19] Collison, C. 2000. *Fundamentals of beekeeping*. State College, PA, Pennsylvania State University.

[20] Free, J.B. & Williams, H.I. 1971. The effect of giving pollen and pollen supplements to honey bee colonies on the amount of pollen collected. *Journal of Apicultural Research*, 10: 87–90.

[21] Goodwin, R.M., Houten, T.E.N., & Perry, J.H. 1994. Effect of feeding pollen substitutes to honey-bee colonies used for kiwifruit pollination and honey production. *New Zealand Journal of Crop and Horticultural Science*, 22: 459–462.

[22] Yoder, J.A., Heydinger, D.J., Hedges, B.Z., Sammataro, D., Finley, J., DeGrandi-Hoffman, G., Croxall, T.J. & Christensen, B.S. 2012. Fungicides reduce symbiotic fungi in bee bread and the beneficial fungi in colonies. In D. Sammataro & J.A. Yoder, eds. pp. 193–214. *Honey bee colony health: challenges and sustainable solutions.* Boca Raton, FL, CRC Press.

ANNEXES



Annex 1

POLLINATION CONTRACT

This AGREEMENT, made on the date ..... ,
between .....
of .....(**GROWER** of the CROP to be POLLINATED)
and .....
(**POLLINATION SERVICE PROVIDER**, **PSP**), of ..... ,

AGREE TO THE FOLLOWING TERMS:

A. The SUM of ..... will be paid for POLLINATION SERVICES of the CROP ..... ,

B. LOCATED at ..... & including an AREA of ..... ,

C. to BEGIN on ..... & END on ..... .

D. PAYMENT is to be made ..... and the POLLINATORS ..... ,

E. STANDARD SIZE or STRENGTH of ..... , will be PAID FOR at the UNIT PRICE (per UNIT or per DAY or TOTAL HOURS) of ..... .

F. MOVING & MAINTENANCE of POLLINATORS, in addition to LIVESTOCK AND HUMAN SAFETY, are RESPONSIBILITIES of BOTH the **PSP** and **GROWER**. POLLINATION UNITS will CARRY IDENTIFICATION and CONTACT NUMBERS, and WARNINGS or BARRIERS IF SAFETY HAZARDS EXIST.

G. POLLINATION UNIT PLACEMENT will be AGREED UPON, IN ADVANCE, by the **PSP** and **GROWER**.

H. THE **GROWER** will NOTIFY the **PSP** or their AGENT, 48 HOURS in ADVANCE, if BIOCIDE is to be APPLIED, within a DISTANCE of ..... from the CROP.

I. MOVEMENT and MAINTENANCE COSTS of POLLINATORS due to BIOCIDE APPLICATION, or DAMAGE OR LOSS DUE TO BIOCIDE, ACCIDENTS OR THEFT, WILL BE COMPENSATED BY THE **GROWER** to the **POLLINATION SERVICE PROVIDER**, on a BASIS of ..... per UNIT.

J. The **GROWER** or the P**OLLINATION SERVICE PROVIDER** or their AGENT will make a COUNT ..... of POLLINATION UNITS and VERIFY their PERFORMANCE within ..... DAYS of RENTAL.

ADDITIONAL AGREEMENTS INCLUDE .....

SIGNED, SEALED & DELIVERED

Annex 2

A POLLEN ATLAS OF CULTIVATED PLANTS

D.W. Roubik and J.E. Moreno

1 ALAN GRAHAM'S CONTRIBUTION AT STRI

The pollen and spore collection of Dr Alan Graham was donated to the Smithsonian Institution at the Tropical Research Institute in 2008, and integrated there with existing collections of D.W. Roubik, P. Colinvaux, D. Piperno and C. Jaramillo. It now contains approximately 30 000 vouchered reference slides [1].

2 MORPHOLOGY AS A BACKUP FOR MODERN MOLECULAR METHODS

Modern "barcoding" techniques are not perfect and the "next generation data firehose" requires more personnel and computing facilities than are readily available. Accordingly, pollen morphology-light microscope examination of prepared pollen structures remains a highly valuable research tool. The work in this chapter emphasizes those pollen traits and provides formal descriptions of the world's cultivated plants. While not all cultivated plants are treated and the descriptions lack many varieties or cultivars, an attempt is made to organize and compare material across plant orders from different collections and slide preparations.

2.1 LABORATORY METHODS

Fresh and dried flowers and/or buds are chemically processed in order to clean the pollen grains and eliminate the larger fragments of plant tissue. Permanent pollen slides of abundant grains can then be used for microscopic examination and description. The basic protocol steps are as follows:

- The samples are placed in distilled water.
- The samples are concentrated at 2 700 rpm for 5 minutes and the supernatant is discarded.
- The residues are dried with glacial acetic acid.
- The samples are concentrated at 2 700 rpm for 5 minutes and the supernatant is discarded.
- Acetolysis solution is added (nine parts anhydric acetic acid and one part concentrated sulfuric acid) and boiled in a water bath for 5 min to destroy all cellulose content and to clean pollen.
- The samples are concentrated at 2 700 rpm for 5 minutes and the supernatant is discarded. The samples are washed with distilled water and the residues are concentrated.

- Ethanol is used as dessicant and the samples are concentrated at 2 700 rpm for 5 minutes.
- The alcohol is discarded and a few drops of glycerol are added.
- Permanent microscope preparations are made using glycerin jelly as a mounting medium and paraffin as a sealant.

2.2 Microscopic photography and description

Pollen descriptions are given including the following general to species-level palynological characters: group, symmetry, pollen class, sculpture, exine characteristics, apertures, form, size and particular details, following established terminology [2, 3]. Botanical names are confirmed [4, 5] and when possible, common names. Vouchers and collection sites are listed in Annex 2. Electronic black and white micro-photographs were taken at 100x magnification using a Pixera Camera System/Olympus BH-2 binocular scope. Photographs are presented alphabetically by botanical order, family, genus and species. The image scale is 1 cm = 10 microns, as indicated on each plate. Exceptions for the largest or smallest grains, also indicated on each plate, are: *= 400x, += 200x and enlargement reductions as follows: 1/2 = 50%, 1/3 = 66.6%, 1/4 = 75%, 1/5 = 80%, 1/6 = 83.5%, 1/8 = 85.5%, 1/12 = 91.5%. Some cases of pollen dimorphism are indicated directly on plates using the letters a, b or c.

3 CROP POLLEN SPECIES DESCRIPTIONS AND VOUCHERS ARRANGED BY PLANT ORDER

The remainder of this Annex presents the species descriptions and associated vouchers by plant order.

The following abbreviations are used in the species descriptions:	
µm	micron
ca.	approximately
ea.	each
ev	equatorial view
pv	polar view
dv	distal view
PA	polar area distance between adjacent apertures

The following abbreviations are used in the species vouchers:	
Loc	Locality
Coll	Collector
AGPC	Alan Graham Pollen Collection
CIQRO	Centro de Investigacion Quintana Roo
COL	Herbario Nacional de Colombia
CR	Herbario Nacional de Costa Rica
DU	Duke University
FLAS	Florida Herbarium
FMNH	Field Museum of Natural History
HIFP	Institut Francais Pondichery
MNS	National Museum of Canada
MO	Missouri Botanical Garden
NY	New York Botanical Garden
STRI	Smithsonian Tropical Research Institute
USGS	United States Geological Survey
USNH	United States National Herbarium

3.1 ALISMATALES

Araceae

Alocasia longiloba Miq. (= Alocasia lowii Hook. f.) Plate 1:1

Grain monad, apolar, radial, inaperturate; granulate; exine tectate, 1.5 µm thick, stratification undifferentiated, columellae inconspicuous; grain outline circular, 47.0 µm in size (range 40.0 to 54.0) (striped elephant ear).

⊙ Loc: USA, Orlando, Orange Co. Coll: H.N. Miller, E. West and R. McColley, 17 December 1955. Herbarium: FLAS # 69407. Process: Acetolysis, May 2014. Mounting medium: glycerin jelly.

Colocasia esculenta (L.) Schott. Plate 1:2

Grain monad, heteropolar-bilateral, inaperturate; psilate; exine tectate, 1.2 µm thick, columellae inconspicuous, very short; grain outline circular, 42.0 µm in size (taro).

⊙ AGPC 6522. Loc: Argentina, Prov. of Tucuman. Coll.: Venturi # 483-L. Herbarium: USNH. Process: KO-Ac, 9-67. Mounting medium: Canada balsam.

3.2 APIALES

Apiaceae

Coriandrum sativum L. Plate 1:3

Grain monad, radial, isopolar, tricolporate, planaperturate; reticulate, homobrochate, brochi fine, lumina < 1.0 µm wide, muri thin, simplicolumellate; exine tectate, variable in size, 3.0 µm thick at polar area and 2.5 µm thick at equatorial area; colpus very thin, as long as grain, having costae endocolpi, ends acute; pore endexinic, lalongate-oval 2.5 µm long x 5.5 µm wide; equatorial outline resembling a "number eight", depressed at centre, polar shape triangular; grains prolate, 39.0 µm long (range 35.0 to 44.0) x 18.0 µm wide (range 16.0 to 19.0) (coriander).

⊙ AGPC 23385. Loc: Mexico. Coll.: Cavazos # 47. Herbarium: DU 176793. Process: KOH – Acet., DL 1973. Mounting medium: glycerin jelly.

Daucus carota L. Plate 1:4

Grain monad, radial, isopolar, tricolporate, planaperturate; reticulate, homobrochate, brochi fine, lumina < 1.0 µm wide, muri thin, simplicolumellate; exine tectate, variable in size, 2.5 µm thick at polar area and 3.0 µm thick at equatorial area; colpus very thin, three-quarters as long as grain, having costae endocolpi, ends acute; pore endexinic, lalongate-square, 3.0 µm long x 5.0 µm wide; polar shape triangular; grains prolate, 28.0 µm long (range 26.5 to 29.0) x 19.0 µm wide (range 16.5 to 21.0) (carrot).

⊙ Loc: USA, Florida, Gainesville, Alachua Co., U. of Florida. Coll: J.R. Abbott, 10 May 2008. Herbarium: FLAS # 227057. Process: Acetolysis, May 2014. Mounting medium: glycerin

3.3 ARECALES

Arecaceae

Acrocomia aculeata (Jacq.) Lodd. Ex Mart. (= *Cocos aculeata* Jacq.) Plate 1:5

Grain monad, heteropolar-radiosymmetric, trichotomosulcate; scabrate-perforate, scabrae thin, < 1.0 µm long; exine semitectate, 4.5 µm thick, tectum irregular, undulant, columellae thin, straight; three-slit sulcus on distal face, as long as face x 4.0 µm wide, apparently, marginate, margines irregular, ends rounded; polar shape triangular, equatorial outline plano-convex, grains oblate, 42.0 µm long x 60.0 µm wide (macaw palm).

⊙ Lodd. Ex Mart. (= *Cocos aculeata* Jacq.). AGPC 8196. Mounting medium: glycerin jelly.

Areca catechu L. Plate 1:6

Grain monad, heteropolar-bilateral, monosulcate; reticulate, heterobrochate, brochi diminishing toward apertures from 2.0 to < 0.5 µm wide, lumina irregular, muri thin, < 1 µm wide, simplicolumellate, columellae baculae shaped; exine tectate, 1.8 µm thick; sulcus as long as grain x 4.0 µm wide, ends rounded; grain outline rounded to irregular trapezoid, 29.0 µm in size (distal view) (dv range 27.0 to 31.0) (betel nut palm, areca nut palm).

⊙ AGPC 14334. Coll.: Hainan # 130. Herbarium: MNS (Nat. Mus. Can.), Paleobotany Section, MBG 1109851, Jarzen exchange. Mounting medium: Canada balsam, stained.

Bactris gasipaes Kunth. Plate 1:7

Grain monad, heteropolar-radiosymmetric, trichotomosulcate; reticulate, homobrochate, brochi fine, thin, < 1.0 µm wide, muri simplicolumellate; exine tectate, 2.5 µm; three-slit opening on distal face, as long as face x 3.0 µm wide, ends rounded, margins irregular; polar shape triangular, equatorial outline oval, grains oblate, 35.0 µm long x 50.0 µm wide (peach palm).

⊙ AGPC 18553. Loc: Pananma. Coll.: T.B. Croat # 14479. Mounting medium: glycerin jelly.

Cocos nucifera L. Plate 1:8

Grain monad, heteropolar-bilateral, monosulcate; psilate to slightly scabrate; exine tectate, slightly undulating, 3.0 µm thick, columellae thin; sulcus as long as grain, sinuous, marginate, ends rounded; grain outline ellipsoidal, 91.5 µm long x 54.0 µm wide (distal view) (dv range 87.0 to 95.0) (coconut).

⊙ AGPC 18870. Loc: Panama, Balboa. Coll.: R.J. Schmalzel # 909. Herbarium: STRI exchange. Mounting medium: glycerin jelly.

Elaeis guineensis Jacq. Plate 1:9

Grain monad, heteropolar-radiosymmetric and bilateral, dimporphic, displaying trichotomosulcate and monosulcate conditions; baculate, baculae ca. 1.0 µm long; exine intectate, 2.0 µm thick, columellae dense; trichotomosulcate grains displaying three-slit sulcus on distal face, as long as face, wide, marginate, irregular, ends rounded; polar shape triangular-concave, equatorial outline plano-convex, grain oblate 32.0 µm long x 56.5 µm wide (range 48.0 to 56.5); monosulcate grains sulcus as long as grain, wide, slightly constricted at centre, ends rounded; grain outline trapezoid-rounded, 30.5 µm long x 24.0 µm wide (distal view) (dv range 28.0 to 33.0) (African oil palm).

⊙ AGPC 7677. Loc: Brazil. Herbarium: Shell exchange & AGPC 9465. Herbarium: van Der Hammen exchange. Mounting medium: glycerin jelly.

Euterpe edulis Mart. Plate 1:10

Grain monad, heteropolar-bilateral, monosulcate; reticulate, homobrochate, brochi very fine, < 1.0 µm wide, muri simplicolumellate, columella baculae shaped; exine tectate, 1.2 µm thick, sulcus as long as grain, constricted at centre, ends rounded; grain outline ellipsoidal, 45.0 µm long x 34.0 µm wide (distal view) (dv range 43.0 to 47.0) (assai palm, açaí).

⊙ AGPC 4929. Loc: Brazil. Coll.: P. Dusen # n.a. Herbarium: HU 6093. Process: A. barlett, 7-64. Mounting medium: Canada balsam.

Phoenix dactylifera L. Plate 1:11

Grain monad, heteropolar-bilateral, monosulcate; reticulate, homobrochate, brochi fine, < 1.0 µm wide, muri simplicolumellate, columella baculae shaped, very thin; exine tectate, 1.0 µm thick; sulcus as long as grain, 3.0 µm wide, ends rounded; grain outline ellipsoidal, 27.0 µm long x 21.0 µm wide (distal view) (dv range 26.5.0 to 29.0) (date palm).

⊙ AGPC 19602. Loc: USA, CA. Info: Cult. Coll.: Bailey & Bailey. Herbarium: MNS (Nat. Mus. Can.), Paleobotany Section; Jarzen exchange. Mounting medium: Canada balsam.

3.4 ASPARAGALES

Amaryllidaceae

Allium schoenoprasum var. *sibiricum* (L.) Garcke Plate 1:12

Grain monad, heteropolar-bilateral, monosulcate; reticulate, homobrochate, brochi < 1.0 µm wide, muri thin, simplicolumellate, columellae baculae shaped; exine tectate, 1.0 µm thick; sulcus as long as grain, ends rounded; grain outline ellipsoidal, 35.0 µm long x 23.0 µm wide (distal view) (chive).

⊙ AGPC 1186. Loc: n.a. Restigauche and Patapedia rivers. Coll.: J. Rousseau. Herbarium: Harvard, T.H. Bonin #32148; D. Livingston exchange. Process: KO-AC, Safra. Mounting medium: Canada balsam.

Asparagaceae

Agave sisalana Perrine ex Engelm Plate 2:13

Grain monad, heteropolar-bilateral, monosulcate; reticulate, heterobrochate, per-reticulate, brochi rounded, irregular, 3.5 to 24.0 µm wide, muri coarse, undulating, 3.0 to 7.0 µm thick, pluricolumellate, columella baculae shaped, coarse; exine tectate, 6.5 µm thick; sulcus as long as grain; outline ellipsoidal resembling bivalve condition, 122.0 µm in size (sisal).

⊙ AGPC 1100. Coll.: UM Bot. Gard. 15/xi/57. Herbarium: Univ. Mich., Pollen Lab., Dept. Bot. Process: KOH-AC clrear, 11-57; Acetol. 58.5.6.5. Mounting medium: Canada balsam.

Orchidaceae

Vanilla planifolia Andrews Plate 2:14

Massulae (fragments) containing many grains from 15 to hundreds. Isolated grains monad, asymmetric, anisopolar, apparently inaperturate; baculate, resembling regulate pattern, baculae short, thin; exine intectate, 1.8 µm thick; outline square to rounded, irregular; 52.0 µm in size; massulae (fragment) outline elongate, irregular, 207.0 µm in size (vanilla).

⊙ AGPC 9586. Coll.: W.D. Stoutamire material, U. of Mich. Bot. Gardens, Ann Arbor, Mich., June 1969. Info: Cultivated. Herbarium: AU. Mounting medium: Canada balsam.

Xanthorrhoeaceae

Aloe africana Miller Plate 2:15

Grain monad, heteropolar, bilateral, monosulcate; reticulate, slightly heterobrochate, brochi fine, < 1.0 µm, diminishing toward aperture, lumina rounded, resembling foveolate condition, muri simplicolumellate, columellae thin, baculae shaped; exine tectate, thin, 1.2 µm thick; sulcus three-quarters as long as grain, 42.0 µm long x 6.0 µm wide, ends rounded; grain outline elongate, 58.0 µm long x 42.0 µm wide (distal view) (dv range 52.0 to 61.0) (African aloe).

⊙ AGPC 19344. Loc: S. Africa. Herbarium: MNS, palynology.

3.5 ASTERALES

Asteraceae-Helianthae

Helianthus annuus L. Plate 2:16

Grain monad, radial, isopolar, tricolporate, planaperturate; echinate, echini acute; exine tectate, 9.5 µm thick including sculptural elements, densely columellate, columellae pilum-shaped; colpus one-half as long as grain x 3.0 µm wide, ends acute; pore endexinic, lalongate-linear 1.5 µm long x 14.0 µm wide; polar shape circular; grains spheroidal, 56.0 µm in size (sunflower).

⊙ L. AGPC 3768. Herbarium: Harvard. Mounting medium: Canada balsam.

Parthenium argentatum Gray, A. Plate 2:17

Grain monad, radial, isopolar, tricolporate and stephanocolporate (4 aperturate), planaperturate; echinate, echini conical, conus type; exine tectate, tectum caveate, 7.0 to 8.0 µm thick including sculptural elements, densely columellate, columellae pilum-shaped; colpus two-thirds as long as grain, ends acute, PA 12.0 µm; pore endexinic, lalongate, ends acute, 2.5 µm long x 12.0 µm wide; polar shape circular when tricolporate and square when stephanocolporate; grains spheroidal, 37.0 µm in size (range 35.0 to 39.0) (guayule).

⊙ AGPC 3773. Herbarium: Harvard. Mounting medium: Canada balsam.

3.6 BRASSICALES

Brassicaceae

Brassica rapa L. (= Brassica campestris L.) Plate 2:18

Grain monad, radial, isopolar, tricolporate, planaperturate; reticulate, homobrochate, brochi coarse, lumina ca. 1.0 µm wide, muri very thin, simplicolumellate, columellae baculae shaped; exine tectate, 3.0 µm; colpus as long as grain, wide, displaying thin and inconspicuous costae endocolpi, PA 6.0 µm; polar shape circular; grains spheroidal to slightly suboblate, 23.0 µm long x 24.0 µm wide (ev range 22.0 to 24.0) (field mustard, Chinese cabbage).

⊙ AGPC 3793. Herbarium: Harvard. Mounting medium: Canada balsam.

Caricaceae

Carica papaya L. Plate 3:19

Grain monad, radial, isopolar, tricolporate, planaperturate; reticulate, homobrochate, lumina 1.0 µm wide, muri thin, simplicolumellate; exine tectate, 1.2 µm thick; colpus three-quarters as long as grain 35.0 µm long x 2.0 µm wide, equatorially constricted, having costae endocolpi, ends rounded; pore inconspicuous, lalongate 3.5 µm long x 9.0 µm wide; PA 12.0 µm; polar shape circular; grains subprolate, equatorial length 45.5 µm (range 43.0-46.0), equatorial width 40.0 µm (ev range 39.0 to 40.5) (papaya).

⊙ AGPC 18274. Loc: Panama, Maden Dam road. Coll.: Hansen # 2993. Herbarium: MO. Mounting medium: Canada balsam.

Jacaratia dolichaula (Donn. Sm.) Woodson Plate 3:20

Grain monad, radial, isopolar, tricolporate, planaperturate; foveolate, resembling reticulate pattern; foveolae irregular ca. 1.0 µm wide, having free elements within; exine semitectate, 2.5 µm thick; colpus short ca. 13.0 µm long x 1.0 µm wide, PA 22.0 µm; polar shape circular; grains spheroidal to prolate spheroidal, 24.0 µm long x 26.0 µm wide (ev range 27.0 to 32.5) (wild papaya).

⊙ Woodson. AGPC 8276. Loc: Panama. Mounting medium: Canada balsam.

Moringaceae

Moringa oleifera Lam. Plate 3:21

Grain monad, radial, isopolar, tricolporate, planaperturate; scabrate, scabrae thin; exine tectate, 1.2 µm thick, densely columellate, columellae very thin; colpus as long as grain, thin, sometimes appearing joined at polar areas, having costae endocolpi, margo 3.5 µm thick, ends acute; PA 12.0 µm; pores endexinic, circular, ca. 9.5 µm in diameter; polar shape circular; grains subprolate, 52.0 µm long x 45.0 µm wide (benzoil tree).

⊙ AGPC 3061. Mounting medium: Canada balsam.

3.7 CARYOPHYLLALES

Amaranthaceae

Amaranthus caudatus L. Plate 3:22

Grain monad, radially symmetric, apolar, periporate, > 40 pores/grain; scabrate, scabrae coarse; exine tectate, 2.5 µm thick; pore circular 1.5 µm in diameter, subtly annulate, annulus thin, < 1.0 µm thick, interpore distance 2.5 µm; grain outline circular; grains spheroidal, 24.0 to 27.0 µm in size (foxtail amaranth).

⊙ AGPC 6097. Loc: Palestine. Coll.: Dinsmore # 4853, University of Minnesota, Pollen Laboratory, Limnological Research Center No. 968. Process: KOH – Acet., Saf. Mounting medium: Silicone, stained

Cactaceae

Hylocereus polyrhizus (F.A.C. Weber) Britton & Rose Plate 3:23

Grain monad, radially symmetric, isopolar, syncolpate (triaperturate), echinate-baculate; echini short 1.0 to 2.0µm long, conical, ends acute; baculae appearing as free colummellae, short, dense; exine intectate, 3.5 µm thick; colpus joined at polar areas, wide; polar shape circular; grains irregular, probably oblate, 115.0 µm in size (pv range 108.5 to 125.0) (dragonfruit).

⊙ AGPC 6724. Loc: Ecuador. Coll.: Rose # 23396. Herbarium: NY. Process: KOH-Acet., PC-ES 1967. Mounting medium: glycerin jelly.

Opuntia erinacea Engelm. & Bigelow, J.M. Plate 3:24

Grain monad, radially symmetric, apolar, periporate-lophate, ca. 20 fenestrae/grain; reticulate, heterobrochate, brochi variable, per-reticulate, muri (bridges) 13.0 µm wide, undulating, pluricolumellate, columellae baculae shaped, irregular, 2.5 µm thick; exine semitectate, 13.0 µm thick; fenestrae elongated, irregular, 44.0 µm x 25.0 µm in size, having free baculae; grains spheroidal, 159.0 µm in size (range 137.0 to 166.0) (Mojave prickly pear).

⊙ AGPC 9902. Loc: Nevada, Goldfield. Coll.: I. Tridestrom # 9786, 6/1919. Herbarium: USGS P631, Denver exchange, USNH. Mounting medium: Canada balsam.

Polygonaceae

Fagopyrum esculentum Moench Plate 3:25

Grain monad, radial, isopolar, tricolporate, planaperturate; baculate, baculae coarse, 1.0 to 2.0 µm wide; exine intectate, 4.5 µm thick; colpus as long as grain, thin, ends acute, exhibiting costae endocolpi 2.0 µm thick; pore lalongate, depressed, inconspicuous, 6.0 µm long x 9.5 µm wide; polar shape circular lobate; grains subprolate, 54.0 µm long x 34.0 µm wide (ev range 49.0 to 56.0) (buckwheat).

⊙ AGPC 22266. Loc: Belgium, Theux. Herbarium: HM # 297; Leroy (Belgium) exchange. Mounting medium: glycerin jelly.

Simmondsiaceae

Simmondsia chinensis Nutt. Plate 4:26

Grain monad, radial, isopolar, apparently tricolporate, resembling tricolpate condition, planaperturate; baculate; exine intectate, 1.8 µm thick, columellae baculae shaped; colpus elongate, resembling large pore, 24.0 µm long x 10.0 µm wide; pore if present, endexinic, inconspicuous; polar shape circular trilobate; grains subprolate, 44.0 µm long x 34.0 µm wide (ev range 41.0 to 50.0) (jojoba, quinine nut).

⊙ AGPC 3432. Herbarium: Harvard. Mounting medium: Canada balsam.

3.8 CUCURBITALES

Cucurbitaceae

Citrullus lanatus (Thunb.) Matsum. & Nakai Plate 4:27

Grain monad, radial, isopolar, tricolporate, planaperturate; reticulate, heterobrochate, brochi irregular, 1.0 to 6.0 µm wide, muri very thin, < 0.5 µm wide, undulating, simplicolumellate, columellae baculae shaped; exine tectate, 3.0 µm thick; colpus inconspicuous, short, wide, ends rounded; pore circular 12.0 µm in diameter, annulate, annulus diffuse; polar shape circular; grains spheroidal, 76.5 µm in size (range 73.0 to 80.0) (watermelon).

⊙ Loc: Mexico, Reserva de Sian Ka'an, km. 3 carretera Vigia Chico a Felipe carrillo Puerto. Info: hierba rastrera, floracion Julio, cultivada. Coll.: Villanueva # 374. Herbarium: CIQRO. Mounting medium: Canada balsam.

Cucumis dipsaceus Ehrenb. Plate 4:28

Grain monad, radial, isopolar, triporate, planaperturate; reticulate, homobrochate, brochi regular, < 1.0 µm wide, muri thin, simplicolumellate, columellae conspicuous, baculae shaped; exine tectate ca. 1.0 µm thick; pore circular, 12.0 µm in diameter; polar shape circular; grains spheroidal, 60.0 µm in size (range 59.0 to 63.0) (chuzzle cucumber).

⊙ AGPC 9508. Info: Garden, cultivated. Herbarium: U. Michigan. Mounting medium: Canada balsam.

Cucumis melo L. Plate 4:29

Grain monad, radial, isopolar, triporate, planaperturate; reticulate, homobrochate, brochi fine, < 1.0 µm wide, muri thin, simplicolumellate, columellae small, thin; exine tectate 2.5 µm thick; pore circular, 9.5 µm in diameter, annulate, annulus 2.5 µm wide; polar shape circular; grains suboblate, 46.0 µm long x 53.0 µm wide (pv range 53.0 to 59.0) (musk melon).

⊙ Loc: USA, Maryland, Worcester Co. Coll: H. L. Moldenke # 3115, 25 September 1926. Herbarium: FLAS # 55396. Process: Acetolysis, May 2014. Mounting medium: glycerin jelly.

Cucurbita maxima Duchesne Plate 4:30

Grain monad, radially symmetric, apolar, periporate; echinate-echinulate, exhibiting two types: echini conical, acute, 12.0 µm long and echinulae, 1.5 µm long; exine tectate, 3.5 µm thick (excluding sculptural elements); six pores/grain, pore circular to oval 40.0 µm in diameter, annulate, annulus diffuse; polar shape circular; grains spheroidal, 212.5 µm in size (range 208.0 to 215.0) (pumpkin, winter squash).

⊙ Loc: Colombia, Cundinamarca, Arbelaez, Santa Barbara km. 3 via Saragoza, 1750 msnm. Info: *ahuyama*, hierba rastrera, cultivada, bosque humedo premontano. Coll.: Moreno & Devia # 114, 30 May 1980. Herbario: COL # 206982. Mounting medium: glycerin jelly.

Cucurbita pepo Duchesne Plate 4:31

Grain monad, radially symmetric, apolar, periporate; echinate-echinulate, exhibiting two types: echini conical, acute, 12.0 µm long and echinulae, 2.5 µm long, resembling small thin baculae; exine tectate, 3.0 µm thick (excluding sculptural elements); eight pores/grain, pore circular 25.0 µm in diameter; polar shape circular irregular; grains spheroidal, 200.0 µm in size (range 180.0 to 220.0) (summer squash).

⊙ AGPC 8385. Loc: El Salvador. Coll.: Standley # 22554. Herbarium: US, OSU exchange. Process: KOH - Acet., PC-ES 1968. Mounting medium: glycerin jelly.

Lagenaria siceraria (Molina) Standley Plate 4:32

Grain monad, radial, isopolar, tricolporate, planaperturate; reticulate, homobrochate, brochi fine ca. 1.0 µm wide, muri very thin, < 0.5 µm wide, simplicolumellate, columellae baculae shaped; exine tectate, 1.8 µm thick; colpus inconspicuous, as long as grain; pore circular 13.0 µm in diameter, annulate; polar shape circular; grains suboblate, 67.0 µm long x 73.0 µm wide (pv range 67.0 to 80.0) (bottle gourd).

⊙ Loc: Mexico. Coll.: Villanueva # 585. Herbarium: CIQRO. Mounting medium: Canada balsam.

Momordica charantia L. Plate 4:33

Grain monad, radial, isopolar, tricolporate, planaperturate; reticulate, heterobrochate, brochi irregular, slightly diminishing toward apertures, 1.0 to 2.5 µm wide, polygonal, muri thin, < 1.0 µm wide, simplicolumellate, columellae baculae shaped, long, head rounded; exine tectate, 3.0 µm thick; colpus as long as grain, wide, ends acute, margines not well defined; pore circular 13.0 to 15.0 µm in diameter; polar shape circular; grains spheroidal, 77.0 µm in size (range 73.0 to 80.0) (bitter melon).

⊙ AGPC 17757. Loc: Panama, Canal Zone, vicinity of Miraflores Locks. Coll.: Stern et al. # 64. Herbarium: MO. Mounting medium: Canada balsam.

Sechium edule (Jacq.) Sw. Plate 5:34

Grain monad, radial, isopolar, stephanocolpate, nine to ten colpi per grain; echinate baculate, echinae, conical, acute 5.0 µm long x 3.0 µm wide; baculae present on surface between echini, small, ca. 1.0 µm thick; exine intectate, 7.0 µm thick including sculptural elements; colpus as long as grain almost joined at polar area, covered by fine ectexinic membrane, tortuous, margines diffuse, PA 14.0 µm; grains subprolate, 114.0 µm long x 92.0 µm wide (ev range 114.0 to 125.0) (chayote, guatila).

⊙ AGPC 9497. Loc: Mexico, Jalisco. Coll.: JVA Dieterle # 3508, 21 September 1969. Herbarium: UM. Mounting medium: Canada balsam.

3.9 DIOSCOREALES

Dioscoreaceae

Dioscorea salvadorensis Standl. Plate 5:35

Grain monad, bilateral, isopolar, dicolpate; reticulate, homobrochate, brochi fine, ca. 1.0 µm wide, muri thin, simplicolumellate, columellae baculae shaped, baculae wide; exine tectate, 2.0 µm thick; colpus ¾ as long as grain, thin; grains prolate, 27.5 µm long x 20.0 µm wide (ev range 26.0 to 29.5) (ñame, yam).

⊙ AGPC 6477. Mounting medium: Canada balsam.

Dioscorea trifida L. f. Plate 5:36

Grain monad, bilateral, isopolar, dicolpate; reticulate, homobrochate, brochi fine, ca. 1.0 µm wide, muri thin, simplicolumellate, columellae baculae shaped; exine tectate, 1.8 µm thick; colpus as long as grain, thin, irregular; grains prolate, 33.0 µm long x 23.0 µm wide (ev range 29.5 to 36.5) (Indian yam).

⊙ AGPC 8223. Herbarium: HNB 6065. Mounting medium: glycerin jelly.

3.10 ERICALES

Actinidiaceae

Actinidia kolomikta (Maxim. & Rupr.) Maxim. Plate 5:37

Grain monad, radial, isopolar, tricolpate, planaperturate; psilate to slightly scabrate; exine tectate, 1.2 µm thick, columellae thin, dense, conspicuous; colpus as long as grain, thin, marginate, ends acute; polar shape circular; grains subprolate, 28.5 µm long x 18.50 µm wide (ev range 28.0 to 29.0) (kiwi fruit).

⊙ AGPC 4647. Mounting medium: Canada balsam.

Lecythidaceae

Bertholletia excelsa Bonpl. Plate 5:38

Grain monad, radial, isopolar, tricolporate, planaperturate; reticulate, homobrochate, brochi fine < 1.0 µm wide, muri simplicolumellate, columellae baculae shaped; exine intectate, 2.5 µm thick; colpus as long as grain, displaying equatorial constriction and costae colpi, margo conspicuous, colpi displaying ends acute; pore inconspicuous, apparently circular; PA 6.0 µm; polar shape circular; grains subprolate, 37.5 µm long x 32.0 µm wide (ev range 36.0 to 39.0) (Brazil nut).

⊙ Loc: Brazil, Rondonia, basin Rio Madeira, trail to Fortaleza, Rio Abuna 20 km, capoeira. Info: Tree 12 m. Herbarium: FMNH # 1713487. Mounting medium: glycerin jelly.

Couroupita guianensis var. *surinamensis* Aubl. Plate 5:39

Pollen dimorphic, crossed tetrads and monads. Tetrads having grains radial, isopolar, apparently tricolporate, apertures irregular and not well defined; verrucate-baculate, verrucae variable in size from 1.0 to 4.0 µm; exine semitectate, 2.5 µm thick, densely columellate, columellae thin, straight; colpus as long as grain, marginate, irregular; equatorial outline resembling rounded trapezoidal shaped; tetrads 73.0 to 75.0 µm in size; grains subprolate 35.0 µm long x 31.0 µm wide. Monads: radial, isopolar, tricolporate, planaperturate; apparently reticulate, homobrochate, brochi fine < 0.5 µm wide, muri simplicolumellate, columellae baculae shaped, very thin; grains sometimes resembling microbaculate pattern; exine tectate, densely columellate, 2.5 µm thick; colpus as long as grain, displaying equatorial constriction and costae colpi, margo conspicuous ca. 1.8 µm thick, colpi exhibiting square ends; pore inconspicuous, apparently circular, ca. 10.0 µm in diameter; PA 7.0 µm; polar shape circular; grains subprolate, 36.5 µm long x 29.5 µm wide (cannonball fruit).

⊙ AGPC 20494. Loc: Brazil. Coll.: Capucho # 490. Herbarium: MNS, Palynology/Paleobotany, F 669935. Mounting medium: Canada balsam.

Lecythis pisonis Cambess. Plate 5:40

Pollen dimorphic. Grains monad, radial, isopolar, tricolporate and tricolpate, planaperturate; reticulate, slightly heterobrochate, brochi fine ca. 1.0 µm wide, subtly diminishing toward apertures, muri simplicolumellate, columellae baculae shaped, thin when tricolporate and coarse when tricolpate conditions; exine tectate, 2.0 to 2.5 µm thick; colpus as long as grain almost joining at polar areas, displaying equatorial constriction, having costae colpi, margo conspicuous ca. 1.5 µm thick, ends acute; pore inconspicuous, masked by equatorial bridge, apparently lalongate linear; PA 2.5 µm; polar shape circular; grains subprolate when tricolpate, 33.0 µm long x 27.0 µm wide and oblate-spheroidal to spheroidal when tricolporate, 25.0 µm long x 26.0 µm wide (cream nut).

⊙ Loc: Panama, Summit Gardens. Coll.: D.W. Roubik # n.a., Feb. 28, 2014. Mounting medium: glycerin jelly.

Sapotaceae

Chrysophyllum cainito L. Plate 5:41

Grain monad, radial, isopolar, tricolporate, planaperturate; psilate; exine tectate, <1.0 µm thick; colpus thin, as long as grain, having costae endocolpi 1.0 µm thick, exhibiting exitus digitatus; pore small, lalongate 1.0 µm long x 4.0 µm wide, slightly protruding; polar shape circular; grains subprolate, 23.5 µm long x 13.5 µm wide (ev range 21.0 to 32.5) (star apple).

⊙ AGPC 9872. Loc: Nicaragua. Coll.: D. Chaves # 91, 10/1924. Herbarium: USNH, USGS P-601, Denver exchange. Mounting medium: Canada balsam.

Madhuca malaccensis (C.B. Clarke) H.J. Lam Plate 6:42

Grain monad, radial, isopolar, stephanocolporate (4 aperturate), less frequent tricolporate, planaperturate; psilate to slightly scabrate; exine tectate 1.5 µm thick, columellae very thin; colpus thin, as long as grain, having thin costae endocolpi 1.5 µm thick; pore endexinic, lalongate 5 µm long x 9.5 µm wide, slightly protruding; polar shape probably circular; grains subprolate, 58.0 µm long x 48.0 µm wide (ev range 52.0–64.0) (bitis, batu).

⊙ AGPC 19912. Loc: Malay Peninsula. Coll.: A. Koch 10/3/1959. Herbarium: MNS, Paleobotany Section, Jarzen exchange. Mounting medium: Canada balsam.

Manilkara zapotilla (Jacq.) Gilly Plate 6:43

Grain monad, radial, isopolar, stephanocolporate (4 aperturate, less frequently tricolporate), planaperturate; scabrate; exine tectate 3.0 µm thick; colpus as long as grain, having costae ectocolpi 2.0 µm thick; pore endexinic, lalongate 5.0 µm long x 9.5 µm wide, protruding, resembling vestibulate condition; polar shape circular or square; grains spheroidal ranging from prolate-spheroidal to oblate-spheroidal, 44.0 µm long x 43.0 µm wide (pv range 39.0 to 46.0; ev range 41.0 to 47.0) (sapodilla).

⊙ AGPC 13656. Coll.: C.F. Baker # 66, 1942. Herbarium: NYBG. Process: Acetolysis. Mounting medium: Canada balsam.

Pouteria caimito var. *laurifolia* (Gomes) Baehni Plate 6:44

Grain monad, radial, isopolar, tricolporate; planaperturate; psilate; exine tectate 3.5 µm thick; colpus inconspicuous, very thin, as long as grain, having costae ectocolpi 6.0 µm thick; pore endexinic, lalongate 5.0 µm long x 9.5 µm wide, protruding, appearing vestibulate; polar shape probably circular; grains subprolate, 57.5 µm long x 44.5 µm wide (ev range 52.0 to 64.0) (abiu, egg fruit).

⊙ AGPC 7511. Loc: Brazil. Herbarium: Shell exchange. Mounting medium: glycerin jelly.

Pouteria campechiana (Kunth) Baehni Plate 6:45

Grain monad, radial, isopolar, stephanocolporate (4 aperturate), planaperturate; verrucate, verrucae fine, flat; exine tectate 2.5 µm thick; colpus thin, 24.0 µm long x 1.0 µm wide, having costae endocolpi 2.5 µm thick, ends acute, PA 20.0 µm; pore endexinic, lalongate 5.0 µm long x 12.0 µm wide; polar shape square; grains spheroidal, slightly prolate-spheroidal, 47.0 µm long x 46.0 µm wide (ev range 42.5 to 52.0) (canistel).

⊙ AGPC 5462. Loc: Honduras. Coll.: Molina R. # 783, 1948. Herbarium: HU # 8100, Bartlett, 8-64. Mounting medium: Canada balsam.

Theaceae

Camellia sp. Plate 6:46

Grain monad, radial, isopolar, tricolporate, planaperturate; reticulate, hombrochate, brochi very fine, muri simplicolumellate, columellae thin, dense, baculae shaped; exine tectate, 1.8 µm thick; colpus as long as grain, ends acute, almost joined at apices, PA 7.0 µm; pore inconspicuous, apparently lalongate, slightly protruding; polar shape triangular; grains suboblate, 34.0 µm long x 38.5 µm wide (pv range 38.0 to 39.0) (tea).

⊙ AGPC 10519. Loc: Hawai. Coll.: W.H. Elder, 1/5/57. Herbarium: USGS P-1230. Mounting medium: Canada balsam.

3.11 FABALES

Fabaceae-Caesalpinioideae

Tamarindus indica L. Plate 6:47

Grain monad, radial, isopolar, tricolporate, less frequent stephanocolporate (4 aperturate), planaperturate; striate-reticulate, brochi very fine, striae dense, irregular, short; exine tectate, 1.2 µm thick; colpus as long as grain, thin, wider equatorially, ends acute, slightly covered by fine ectexinic membrane; pore inconspicuous, circular, apparently having a diffuse operculum; polar shape circular; grains subprolate, 47.0 µm long x 41.0 µm wide (ev range 43.0 to 51.0) (tamarind).

⊙ AGPC 5042. Loc: Guatemala. Coll.: Lundell # 3303, 1933. Herbarium: HU 7030, Process: Barlett, 7-64. Mounting medium: Canada balsam.

Fabaceae-Faboideae

Arachis hypogaea L. Plate 7:48

Grain monad, radial, isopolar, tricolporate, planaperturate, less frequent pericolporate; reticulate, homobrochate, brochi very fine, < 1.0 µm wide, lumina rounded, muri simplicolumellate; exine tectate, 1.5 µm thick; colpus as long as grain, wide, slightly covered by fine ectexinic membrane, opposite colpus crossed when pericolporate; pore inconspicuous, apparently lolongate and having a diffuse operculum; polar shape circular; grains subprolate, 41.0 µm long x 30.0 µm wide (ev range 38.0 to 43.0) (peanut, groundnut).

⊙ AGPC 13664. Loc: Paraguay. Coll.: Hassler # 231. Process: Acetolysis, 7-72. Herbarium: NYBG. Mounting medium: Canada balsam.

Cajanus cajan (L.) Huth Plate 7:49

Grain monad, radial, isopolar, tricolporate, planaperturate; reticulate, heterobrochate, brochi variable 1.0 to 3.5 µm wide, lumina having free elements, muri simplicolumellate, 1.0 µm thick; exine semitectate, 2.0 µm thick; colpus short, one-half as long as grain, wide, ends acute, marginate, margo not well defined; pore inconspicuous, circular, 13.0 µm in diameter; polar shape circular to triangular; grains subprolate, 57.0 µm long x 45.0 µm wide (ev range 55.0 to 61.0) (pigeon pea).

⊙ AGPC 22692. Loc: Panama. Coll.: Greenman # 5194. Herbarium: MO. Mounting medium: Canada balsam.

Crotalaria juncea L. Plate 7:50

Grain monad, radial, isopolar, tricolporate, planaperturate; reticulate, homobrochate, brochi ca. 1.0 µm wide, lumina rounded, muri simplicolumellate; exine tectate, 1.0 µm thick; colpus straight, as long as grain, 1.0 µm wide, ends acute, displaying exitus digitatus; pore circular to slightly oval, 5.0 µm in diameter; polar shape circular trilobate; grains subprolate, 30.0 µm long x 16.5 µm wide (sunhemp).

⊙ Loc: USA, Gainesville, Alachua Co., U. of Florida, horticultural grounds. Coll: R. Lange # 625, 2 November 2010. Herbarium: FLAS # 228625. Process: Acetolysis, May 2014. Mounting medium: glycerin jelly.

Derris ferruginea Benth. Plate 7:51

Grain monad, radial, isopolar, tricolporate, planaperturate; psilate to slightly scabrate; exine tectate, 1.2 µm thick; colpus thin, straight, as long as grain, becoming almost to join at apices, ends acute, having costae endocolpi, margo conspicuous; pore inconspicuous, probably circular ca. 8.0 µm in diameter; polar shape circular; grains subprolate, 38.0 µm long x 32.5 µm wide (tuba root, Indian tuber root).

⊙ AGPC 4764. Mounting medium: Canada balsam.

Glycine max (L.) Merr. Plate 7:52

Grain monad, radial, isopolar, tricolporate, planaperturate; reticulate, heterobrochate, brochi variable fine, inconspicuous, < 1.0 to 2.0 µm wide, diminishing toward apertures, muri very thin, simplicolumellate; exine tectate, 1.8 µm thick; colpus subtle, one-half as long as grain, wide, ends acute; pore oval, 4.0 µm long x 6 µm wide, slightly protruding; polar shape circular; grains suboblate to oblate spheroidal, 20.0 µm long x 21.5 µm wide (ev range 19.0 to 22.5) (soybean).

⊙ Info: cultivated, "soja", "soya". Loc: Mexico, Quintana Roo, Chetumal, Coll.: R. Villanueva # n.a. Herbarium: El Colegio de la Frontera Sur. Mounting medium: glycerin jelly.

Myroxylon balsamum (L.) Harms Plate 7:53

Grain monad, radial, isopolar, tricolporate, planaperturate; psilate; exine tectate, 1.2 µm thick; colpus ¾ as long as grain, wide, ends acute, equatorially constricted; pore inconspicuous, apparently lalongate, 5.0 µm wide, masked by bridge of colpus; polar shape circular trilobate; grains subprolate, 20.5 µm long x 13.5 µm wide (ev range 19.0 to 22.5) (balsam).

⊙ AGPC 10192. Loc: Dominican Republic, Prov. La Vega. Coll.: H.N. Whitferd & S. Piazon # 9, 1917. Herbarium: USNH # 119722059, USGS P855; Denver exchange. Mounting medium: Canada balsam.

Phaseolus vulgaris L. Plate 7:54

Grain monad, radial, isopolar, triporate, planaperturate; reticulate, resembling rugulate condition; heterobrochate, brochi variable 1.0 to 2.0 µm wide, muri simplicolumellate, 1.0 µm thick; exine semitectate, 3.5 µm thick; pore circular, 10.0 µm in diameter, annulate, annulus 3.5 µm thick, slightly protruding; polar shape triangular; grains suboblate, 43.0 µm long x 49.0 µm wide (pv range 47.0 to 49.5) (common bean).

⊙ Loc: Colombia, Cundinamarca, Arbelaez, Santa Barbara via a la Hoya, 1700 msnm. Info: habichuela, enredadera, cultivada, bosque humedo premontano. Coll.: Moreno & Devia # 077, 28 May 1980. Herbario: COL # 206976. Mounting medium: glycerin jelly.

Vicia faba L. Plate 7:55

Grain monad, radial, isopolar, tricolporate, planaperturate; rugulate at equator to psilate at polar areas, rugulae 2.5 µm wide, irregular, transversally oriented; exine tectate, 1.5 µm thick; colpi as long as grain, thin, straight, ca. 47.0 µm long, having costae endocolpi 3.5 µm thick; pore circular to slightly lalongate, 8.0 µm in diameter; polar shape probably circular; grains prolate, 61.5 µm long x 32.0 µm wide (ev range 59.0 to 65.0) (broadbean).

⊙ Loc: USA, Florida, Gainesville, Alachua Co., Kanapoha Botanical Garden. Coll: S.B. Davis # 1316, 6 March 2006. Herbarium: FLAS # 218363. Process: Acetolysis, May 2014. Mounting medium: glycerin jelly.

Fabaceae-Mimosoideae

Acacia farnesiana (L.) Willd. Plate 7:56

Polyad, 16 cells. Isolated grains monad, asymmetric, anisopolar, apparently inaperturate; partially reticulate, heterobrochate on borders of polar area, irregular, muri simplicolumellate, columellae baculae shaped; exine semitectate, variable, 0.5 µm thick at polar area and 2.0 µm at lateral side; pore if present, inconspicuous; polar shape square, lateral outline trapezoid; grains suboblate, 19.0 µm long x 20.0 µm wide; polyad regular, grains symmetrically arranged, outline circular to square; polyad 55.0 µm long x 38.0 µm wide (cassie, fragrant acacia).

⊙ AGPC 16754. Herbarium: MNS, Paleobotany Section, # 396174; Jarzen exchange. Mounting medium: Canada balsam.

Adenanthera pavonina L. Plate 7:57

Polyad, 16 cells. Isolated grains monad, asymmetric, anisopolar, periporate; psilate to slightly scabrate; exine tectate, 2.0 µm thick; four pores/grain, pore inconspicuous, circular, annulate, vestibulate, irregular; polar shape square, lateral outline trapezoid; grains suboblate, 15.0 µm long x 18.0 µm wide; polyad regular, grains symmetrically arranged, outline ellipsoidal; polyad 50.0 to 54.0 µm in size (red bead tree).

⊙ AGPC 5731. Loc: Philipines. Coll.: A.S.G.J. M1102. Herbarium: FM # 188800; exchange Pan American. Mounting medium: glycerin jelly.

Leucaena esculenta (Moçiño & Sessé ex DC) Benth. Plate 8:58

Grain monad, radial, isopolar, tricolporate, planaperturate; baculate; exine intectate, 3.5 µm thick; densely baculate, baculae coarse, ca. 1.0 µm long; colpus as long as grain, ends acute, having costae endocolpi; pore inconspicuous, apparently lolongate, 6.5 µm long x 4.0 µm wide; PA 7.0 µm; polar shape circular; grains subprolate, 44.0 µm long x 38.0 µm wide (ev range 43.0 to 47.0) (guaje).

⊙ AGPC 8474. Loc: Mexico, Oaxaca. Herbarium: ASU 382; Schoenwetter exchange, USNH. Process: 11-68. Mounting medium: glycerin jelly.

Leucaena leucocephala L. Plate 8:59

Grain monad, radial, isopolar, tricolporate, planaperturate; baculate; exine intectate, 4.0 µm thick, densely baculate, baculae thin; colpus as long as grain, wide, ends acute, having costae endocolpi 4.0 µm thick; pore inconspicuous, apparently lolongate to circular, 9.5 µm wide; PA 9.0 µm; polar shape circular; grains subprolate, 53.0 µm long x 39.0 µm wide (ev range 52.5 to 54.0) (guaje).

⊙ Loc: Mexico, Reserva de Sian Ka'an, km. 282 carretera Carrillo Puerto a Cancún. Info: arbol, floracion Febrero, vegetacion secundaria, selva subcaducifolia. Coll.: Duran & Olmsted # 450. Herbarium: CIQRO. Mounting medium: Canada balsam.

3.12 GENTIANALES

Apocynaceae

Willughbeia luzoniensis Merr. Plate 8:60

Tetrahedral tetrad. Isolated grains radial, isopolar, tricolporate, planaperturate; psilate to slightly scabrate; exine tectate, fine, very thin, < 1.0 µm thick; colpus very thin, as long as grain, inconspicuous; pores lalongate 4.0 µm long x 10.0 µm wide, polar shape circular. Tetrads 67.5 µm in size (range 65.0 to 72.0); isolated grain apparently suboblate, 37.0 µm long x 44.0 µm wide.

⊙ AGPC 7062. Mounting medium: Canada balsam.

Rubiaceae

Cinchona calisaya Wedd. Plate 8:61

Grain monad, radial, isopolar, tricolporate, planaperturate; reticulate, brochi < 1.0 µm wide; exine tectate, 1.5 µm thick, slightly ticker at polar areas, columellae baculae shaped, head rounded; colpus as long as grain, irregular, ends rounded, marginate, exhibiting costae endocolpi 2.5 µm thick; pore inconspicuous, lalongate elongate almost joining at apices, 3.5 µm long x 7.0 to 10.0 µm wide; grains subprolate, 25.0 µm long x 17.0 µm wide (ev range 24.0 to 29.0) (quinine).

⊙ AGPC 13667. Loc: Bolivia. Coll.: J.I. Rusby. Herbarium: NYBG. Process: Acetolysis, 7-72. Mounting medium: Canada balsam.

Coffea arabica L. Plate 8:62

Grain monad, radial, isopolar, tricolporate and stephanocolporate (4 aperturate), planaperturate; reticulate, brochi < 1.0 µm wide, muri simplicolumellate, columellae thin, baculae shaped; exine tectate, 2.0 µm thick; colpus irregular, two-thirds as long as grain, ends acute, PA 6.0 µm; pore circular irregular; polar shape circular; grains subprolate, 36.5 µm long x 31.0 µm wide (ev range 34.0 to 39.0) (coffee).

⊙ BCI. Loc: Panama, Chiriqui, Boquete. Info: cultivated. Coll.: R.J. Schmalzel s.n., 24 April 1982. Mounting medium: glycerin jelly.

Morinda citrifolia L. Plate 8:63

Grain monad, radial, isopolar, tricolporate, planaperturate; reticulate, heterobrochate, brochi irregular, coarse, ca. 1.0 to 2.0 µm, muri ca. 1.0 µm thick, simplicolumellate, columellae baculae shaped, dense, ends rounded, exine tectate 3.5 to 4.0 µm thick; apertures masked by sculptural elements, colpus as long as grain, thin, ends acute, borders not well defined, PA 20.0 µm; pore apparently circular, sometimes resembling lalongate condition, 12.0 µm in diameter, annulate, annulus 4.0 µm thick; polar shape circular; grains suboblate, 56.0 µm long x 66.0 µm wide (ev range 55.0 to 62.0) (noni).

⊙ Loc: Panama, Gamboa, garden. Coll.: E. Moreno # n.a., 25 February 2014. Mounting medium: glycerin jelly.

3.13 LAMIALES

Acanthaceae

Adhatoda vasica Nees Plate 8:64

Grain monad, bilateral, isopolar, dicolporate, angulaperturate; reticulate, heterobrochate, brochi from 1.0 µm wide to < 0.5 µm, from centre of mesocolpium (peripheral area condition) toward apertures where special area (trema condition) is present; trema area displaying between six to eight small patches of condensed columellae (areolae) longitudinally oriented on either side of aperture; exine tectate, variable from 4.0 up to 6.0 µm thick; slightly thicker at poles; colp(or)us simple, short 23.0 to 26.0 µm long, thin, 1.0 to 2.0 µm wide, displaying ends acute; pore endexinic, lalongate, 2.5 to 4.5 µm long x 4.0 to 7.0 µm wide; polar shape apparently circular; grains prolate, 76.0 µm long x 50.0 µm wide (ev range 61.5 to 80.0) (Malabar nut).

⊙ AGPC 13295. Herbarium: Guinet exchange. Mounting medium: glycerin jelly.

Pedaliaceae

Sesamum indicum L. Plate 9:65

Grain monad, radial, isopolar, stephanocolpate, planaperturate; baculate, baculae variable < 1.0 to 1.0 µm wide; exine intectate, 3.5 µm thick; 12 colpus/grain, colpus as long as grain, wide, deep, PA 12.0 µm; polar shape circular lobate; grains suboblate, 67.0 µm long x 85.0 µm wide (pv range 63.0 to 97.0) (sesame).

⊙ AGPC 22668. Loc: Mexico, Michoacan. Coll.: Leavenworth # 464. Herbarium: MO. Mounting medium: Canada balsam.

3.14 LAURALES

Lauraceae

Cinnamomum bodinieri H. Lév. Plate 9:66

Grains sometimes grouped as irregular massulae. Isolated grain radial, apolar, inaperturate; echinate, echini short, conical, small, < 0.5 µm long; exine intectate; outline circular; massulae 175.0 x 115.0 µm, having > 30 grains; grains spheroidal, 30.0 µm in size (range 28.0 to 31.0) (cinnamom).

⊙ AGPC 10083. Loc: Ichang, W. China. Coll.: E.H. Wilson # 464, 5/1900. Herbarium: USNH # 596460, USGS P413. Mounting medium: Canada balsam.

Persea americana Mill. Plate 9:67

Grain monad, radial, apolar, inaperturate; echinate, echini short, conical, small, < 1.0 µm long; exine intectate ca. 1.0 µm thick; outline circular; grains spheroidal, 47.0 µm in size (range 46.0 to 47.5) (avocado).

⊙ BCI. Loc: Panama, BCI, near dinning hall. Coll.: T.B. Croat # 4162, 9 December 1967. Herbarium: MO. Mounting medium: glycerin jelly, fresh material.

3.15 MAGNOLIALES

Annonaceae

Annona cherimola Mill. Plate 9:68

Square tetrad, sometimes joined in linear packages, up to 14 tetrads, ca. 450.0 µm/package. Grain asymmetric, apolar, inaperturate; baculate, baculae dense, coarse, ca. 1.5 µm wide, end rounded; exine intectate, 2.0 µm thick; tetrads ranging from 95.0 to 112.0 µm long; outline of isolated grain displaying irregular trapezoid shape, 35.5 x 59.0 µm in size (custard apple).

⊙ Loc: USA, Gainesville, Alachua Co., U. of Fla., Clearwater, cultivated. Coll: P. H. Rolfs, 14 July 1843. Herbarium: FLAS # 3028. Process: Acetolysis, May 2014. Mounting medium: glycerin jelly.

Annona muricata L. Plate 9:69

Square tetrad. Grain asymmetric, apolar, inaperturate; reticulate, heterobrochate, lumina rounded, variable 1.0 to 7.0 µm wide, muri 1.5 µm thick, simplicolumellate, columellae baculae shaped, head rounded, 1.0 to 3.5 µm wide; exine tectate, coarse, not well defined, 7.0 µm thick; undulating (per-reticulate condition); tetrads square, 233.0 µm in size (range 230.0 to 241.0); isolated grain displaying trapezoid condition in lateral view, 100.0 x 145.0 µm in size (soursop).

⊙ BCI. Loc: Honduras, Gracias a Dios. Info: Cultivated. Coll.: A. Clewell & Cruz # 4128. Herbarium: MO. Mounting medium: glycerin jelly.

Annona neosalicifolia H. Rainer (= *Rollinia salicifolia* Schltdl.) Plate 9:70

Grain monad, asymmetric, apolar, inaperturate; reticulate, homobrochate, brochi fine ca. 1.0 wide, lumina irregular, muri thin, < 1.0 µm thick, simplicolumellate, columellae baculae shaped, head rounded, coarse; exine tectate, 2.5 µm thick; outline circular; grains spheroidal, 43.0 µm in size (range 38.0 to 48.0) (anonillo).

⊙ AGPC 7056. Mounting medium: Canada balsam.

Annona reticulata L. Plate 9:71

Square tetrad, sometimes joined in linear packages up to 12 tetrads, ca. 600.0 µm/package. Grain asymmetric, apolar, inaperturate; reticulate, homobrochate, brochi fine ca. 1.0 wide, muri thin, simplicolumellate, columellae baculae shaped, head rounded, thin; exine tectate, 2.5 µm thick; tetrads square, 53.0 µm in size (range 41.0 to 57.0); isolated grain displaying trapezoid condition in lateral view, 21.0 x 33.0 µm in size (wild sweetsop).

⊙ AGPC 9398. Herbarium: van Der Hammen exchange. Mounting medium: glycerin jelly.

Annona squamosa L. Plate 10:72

Square tetrad. Grain asymmetric, apolar, inaperturate; reticulate, homobrochate, brochi fine ca. 1.0 wide, lumina rounded, muri thin, simplicolumellate, columellae baculae shaped, head rounded, thin; exine tectate, 2.5 µm thick; tetrads square, 115.0 µm in size (range 111.0 to 119.0); isolated grain displaying trapezoid condition in lateral view, 55.0 x 67.0 µm in size (sweetsop).

⊙ AGPC 13643. Loc: Mexico, Morelos, Palo Bolero, al N del balneario. Coll.: R.Palacios, 15-IX-65. Herbarium: ENCB, Rzedowski exchange. Mounting medium: Canada balsam.

Myristicaceae

Myristica fragans Houtt. Plate 10:73

Grain monad, asymmetric, apolar, inaperturate, sometimes resembling three inconspicuous colpus, other times a simple diffuse colpus; reticulate, heterobrochate, per-reticulate, brochi coarse 1.0 to 1.5 µm wide, lumina having free columellae, muri very thin, < 1.0 µm thick, undulating, simplicolumellate, columellae baculae shaped; exine semitectate, 4.5 µm thick; outline circular; grains spheroidal, 48.0 µm in size (range 47.0 to 55.0) (nutmeg).

⊙ AGPC 8306. Loc: Venezuela. Coll.: Pittier # 6463, 15 July 1913. Herbarium: USNH. Process: 11-68. Mounting medium: Canada balsam.

3.16 MALPIGHIALES

Chrysobalanaceae

Chrysobalanus icaco L. Plate 10:74

Grain monad, radial, isopolar, tricolporate, planaperturate; striate, striae dense, thin, longitudinally oriented; exine tectate, 1.8 µm thick; colpus as long as grain, wide, marginate, margo conspicuous, colpus equatorially constricted displaying an irregular bridge, ends rounded; pore lalongate 5.0 µm long x 24.0 µm wide, slightly protruding, ends acute; polar shape triangular rounded; grains spheroidal, 41.0 µm in size (range 39.5 to 42.0) (cocoplum).

⊙ AGPC 4383. Herbarium: Harvard. Mounting medium: Canada balsam.

Clusiaceae

Garcinia binucao (Blanco) Choisy Plate 10:75

Grain monad, radial, isopolar, tricolporate and stephanocolporate (4 aperturate), planaperturate; baculate, baculae coarse, short, head rounded; exine intectate, 1.5 µm thick; colpus inconspicuous, short; pore circular, having costae endopori resembling a caverna condition, 7.0 µm in diameter; polar shape circular; grains suboblate, 25.0 µm long x 28.0 µm wide (mangostan, binukaw).

⊙ AGPC 22251. Loc: Ceylan. Herbarium: BR 121. Mounting medium: glycerin jelly.

Euphorbiaceae

Hevea brasiliensis (Willd. ex Juss., A.) Müll. Arg. Plate 10:76

Grain monad, radial, isopolar, tricolporate, planaperturate; reticulate, homobrochate, brochi fine, circular, < 1.0 µm wide, muri very thin, simplicolumellate, columellae baculae shaped; exine tectate, 1.8 µm thick; colpus as long as grain, wide, operculate, operculum 18.0 µm long x 5.0 µm wide; pore masked by operculum, PA 16.0 µm; polar shape circular; grains subprolate, 47.0 µm long x 40.0 µm wide (ev range 47.0 to 52.0) (rubber tree).

⊙ AGPC 9775. Loc: Dominican Republic, Prov. La Vega. Coll.: H.A. Allard # 15724, 9/1947. Herbarium: USNH # 1959559, USGS P501; Denver exchange. Mounting medium: Canada balsam.

Jatropha curcas L. Plate 10:77

Grain monad, radially symmetric, apolar, inaperturate, clavate- baculate, clavae hexagonal, 6.0 µm long x 3.5 µm wide, grouped in "rosettes" having six clavae ea., rosettes symmetrically arranged, small baculae present on surface; exine intectate, 6.0 µm thick, nexine masked by sculptural elements; outline circular; grains spheroidal, 88.0 µm in size (range 71.0 to 105 µm), less frequent grains smaller ca. 54.0 µm (range 49.0 to 59.0 µm) (Barbados nut).

⊙ Loc: Panama. Coll.: Burch et al. # 1258. Herbarium: MO. Mounting medium: Canada balsam.

Manihot esculenta Crantz Plate 11:78

Grain monad, radially symmetric, apolar, periporate, clavate, displaying "croton type" pattern, clavae triangular, head 12.0 µm wide x 13.0 µm long, grouped in "rosettes" having five to seven clavae ea., rosettes arranged symmetrically, having inconspicuous and circular pore ea., ca. 15.0 µm in diameter; exine semitectate, 7.0 µm thick (excluding clavae), densely columellate, columellae not well defined, resembling small scabrae; pore circular 25.0 µm in diameter; outline circular; grains spheroidal, 295.0 to 300.0 µm in size (bitter cassava).

⊙ AGPC 18404. Loc: Panama, San Blas. Coll.: D'Arcy # 9485. Herbarium: MO. Mounting medium: Canada balsam.

Ricinus communis L. Plate 11:79

Grain monad, radial, isopolar, tricolporate, planaperturate; reticulate, homobrochate, brochi fine, < 1.0 µm wide, muri very thin, simplicolumellate, columellae baculae shaped, thin; exine tectate, 1.8 µm thick; colpus as long as grain, thin, slightly constricted equtorially, ends acute, having costae endocolpi, margo 2.5 µm thick, PA 11.0 µm; pore lalongate, almost linear, 2.5 µm long x 20.0 µm wide; polar shape circular; grains spheroidal, 39.5 µm in size (range 39.0 to 40.0) (castorbean).

⊙ AGPC 896. Loc: India, Delhi, University campus. Coll.: G.S. Grow, 2 September 1955. Process: KO-AC, clear, 7-58. Mounting medium: Canada balsam.

Malpighiaceae

Bunchosia palmeri S. Watsonn Plate 11:80

Grain monad, asymmetric, apolar, periporate; psilate to slightly scabrate; exine tectate, 3.5 µm thick; six to eight pores/grain, pore circular, 6.0 to 8.0 µm in diameter, annulate, annulus dim, 2.5 µm wide, interpore distance 25.0 to 27.0 µm; outline circular; grains spheroidal, 59.0 µm in size (range 50.0 to 65.0) (butter fruit, nanchi de perro).

⊙ AGPC 8793. Loc: Mexico. Mounting medium: Canada balsam.

Byrsonima crassifolia (L.) Kunth Plate 11:81

Grain monad, radial, isopolar, tricolporate, planaperturate; reticulate, homobrochate, brochi fine < 1.0 µm wide, muri simplicolumellate, columellae baculae shaped; exine intectate, 1.0 µm thick; colpus thin, two-thirds as long as grain, marginate; pore lalongate, 2.5 µm long x 6.0 µm wide; PA 6.0 µm; polar shape circular; grains spheroidal to slightly prolate-spheroidal, 16.5 µm long x 16.0 µm wide (ev range 16.0 to 17.0) (murici, nance, yoco).

⊙ Loc: Panama, BCI. Coll.: R.J. Schmalzel # 647. Herbarium: MO. Mounting medium: glycerin jelly.

Malpighia emarginata DC. Plate 11:82

Grain monad, asymmetric, apolar, periporate; sculpture not well defined, sometimes resembling scabrate condition other times verrucate condition; exine semitectate, 3.0 µm thick; ca. ten pores/grain, pore circular, 4.0 µm in diameter, interpore distance 14.0 to 16.5 µm; pores apparently connected by irregular pseudocolpi; outline circular; grains spheroidal, 41.0 µm in size (range 39.0 to 45.0) (acerola, Barbados cherry).

⊙ Loc: Mexico, Puerto Morelos, jardin Botanico CIQRO. Info: arbol, floracion Abril, vegetacion selva mediumna subperennifolia. Coll.: Escalante # 19. Herbarium: CIQRO. Mounting medium: Canada balsam.

Passifloraceae

Passiflora edulis Sims Plate 11:83

Grain monad, radial, isopolar, structure complex; grains described according to different criteria: (1) tricolpate having three large opercula, and (2) stephanocolpate (6-aperturate) exhibiting pairs of colpi joined at polar area separately. Grains planaperturate; reticulate, heterobrochate, per-reticulate, brochi irregular, lumina 5.0 to 10.0 µm wide, having free columellae, muri undulating, pluricolumellate, 2.0 µm thick, columellae baculae shaped, 5.0 µm long x 2.5 µm wide; exine semitectate, 7.0 µm thick; colpus thin; polar area always displaying tri-radial structure supporting three large opercula; isolated operculum 58.0 µm long x 33.0 µm wide; polar shape circular-hexalobate; grains spheroidal, 115.0 µm (range 105.0 to 131.0) (passionfruit).

⊙ Loc: Colombia, Cundinamarca, Arbelaez, Santa Barbara, via La Hoya, 1700 msnm. Info: maracuya, enredadera, cultivado, frutal, bosque humedo premontano. Coll.: Moreno & Devia # 067, 28 May 1980. Herbarium: COL # 206971. Mounting medium: glycerin jelly.

Phyllanthaceae

Antidesma bunius (L.) Sprengel Plate 11:84

Grain monad, radial, isopolar, tricolporate, planaperturate; reticulate resembling striate-reticulate condition; brochi fine, < 1.0 µm wide, longitudinally oriented, muri simplicolumellate, columella very thin; exine tectate, 2.0 µm thick; colpus as long as grain, ends rounded, apparently marginate; pore lalongate, almost joning at ends, 2.5µm long; polar shape circular lobate; grains prolate, 42.0 µm long x 21.5 µm wide (ev range 40.0 to 44.0) (salamander tree, largenay).

⊙ AGPC 17564. Loc: Costa Rica, Prov. Alajuela. Coll.: Poveda # 1580. Herbarium: CR. Mounting medium: Canada balsam.

Baccaurea bracteata Müll. Arg. Plate 11:85

Grain monad, radial, isopolar, tricolporate, planaperturate; reticulate, homobrochate, brochi fine resembling subtle striate pattern, lumina < 1.0 µm wide, muri thin, simplicolumellate, columellae baculae shaped, very thin; exine tectate 1.5 µm thick; colpus three-quarters as long as grain, having thick costae endocolpi ca. 1.5 µm; pore lalongate to linear, 1.0 µm long x 5.0 µm wide; polar shape circular trilobate; grains subprolate, 20.0 µm long x 14.5 µm wide (tampoi paya).

⊙ AGPC 7357. Mounting medium: glycerin jelly.

3.17 MALVALES

Bixaceae

Bixa orellana L. Plate 12:86

Grain monad, radial, isopolar, tricolporate, planaperturate; reticulate, homobrochate, brochi fine, lumina < 1.0 µm wide, muri thin, simplicolumellate; exine tectate, 2.0 µm thick; colpus as long as grain, covered by fine ectexinic membrane, displaying thin costae endocolpi, ends acute; pore lalongate 6.0 µm long x 13.0 µm wide, ends rounded, PA 13.0 µm; polar shape circular to triangular; grains spheroidal, 41.0 µm in size (range 39.5 to 43.0) (annatto).

⊙ AGPC 18595. Loc: Panama. Coll.: E.L. Tyson et al. # 3109. Herbarium: NM exchange. Mounting medium: glycerin jelly.

Malvaceae-Bombacoideae

Adansonia digitata L. Plate 12:87

Grain monad, radial, isopolar, triporate, planaperturate; baculate-gemmate; exine intectate, 2.0 µm thick, columellae baculae shaped, thin, short; gemmae rounded, variable in size, ca. 3.0 to 4.0 µm in diameter, resembling oil drops; pore circular, irregular, 26.0 to 30.0 µm in diameter, subtly annulate, annulus fine; polar shape circular; grains spheroidal, 124.0 µm in size (range 119.0 to 125.0) (baobab).

⊙ AGPC 16478. Loc: Africa. Info: Cult. Herbarium: MNS Paleobotany Section, MO # 2034444; Jarzen exchange. Mounting medium: Canada balsam.

Ceiba pentandra (L.) Gaertn. Plate 12:88

Grain monad, radial, isopolar, tricolporate, planaperturate; reticulate, heterobrochate, per-reticulate, brochi wide, lumina 7.0 to 8.0 µm wide, hexagonal, having free columellae within, muri undulating, pluricolumellate, thin, columellae baculae shaped; exine semitectate, 1.5 µm thick; colpus short, 28.0 µm long x 5.0 to 11.0 µm wide, marginate, ends acute; pore lolongate, inconspicuous, apparently annulate, slightly protruding; PA 35.0 µm; polar shape circular; grains spheroidal, variable in size 68.5 µm (range 61.0–72.0) and 47.0 µm (range 46.0 to 47.5) (kapok, cotton silk tree).

⊙ AGPC 16490. Loc: Mexico. Herbarium: MNS Paleobotany Section, MO # 1733103; Jarzen exchange. Mounting medium: Canada balsam.

Malvaceae-Grewioideae

Cola acuminata (Beauv., P.) Schott & Endl. Plate 12:89

Grain monad, radial, isopolar, tricolporate sporadically stephanocolporate (4 aperturate), planaperturate; reticulate, heterobrochate, brochi fine < 1.0 to 1.0 µm wide, lumina apparently having free columellae, muri simplicolumellate, thin, columellae baculae shaped; exine semitectate, variable in size, 2.5 µm thick at polar area and 1.5 µm thick at equator; colpus as long as grain, marginate (costae endocolpi), ends rounded, exhibiting equatorial constriction, resembling exitus digitatus; pore circular 6.0 µm in diameter; polar shape circular; grains subprolate, 43.5 µm long x 30.5 µm wide (ev range 39.0 to 48.0) (cola nut, kola).

⊙ AGPC 21180. Loc: Jamaica, Mt. Dakin. Coll.: Harris # 5647. Herbarium: MNS, Palynology/Paleobotany, F # 145372; Jarzen exchange. Mounting medium: Canada balsam.

Theobroma cacao L. Plate 12:90

Grain monad, radial, isopolar, tricolporate, planaperturate; reticulate, heterobrochate, brochi irregular, 1.0 to 1.5 µm wide, lumina apparently having free columellae, muri very thin, simplicolumellate, columellae baculae shaped; exine semitectate, variable in size, 1.0 µm thick; colpus inconspicuous, very short, ends acute; pore circular; polar shape circular; grains suboblate, 19.0 µm long x 21.0 µm wide (ev range 19.0 to 22.0) (chocolate, cacao).

⊙ AGPC 21226. Loc: Costa Rica, Limon. Coll.: Burger et al. # 10967. Herbarium: MNS, Palynology/Paleobotany, F # 1859769; Jarzen exchange. Mounting medium: Canada balsam.

Malvaceae-Helicterioideae

Durio zibethinus Rumph. ex. Murray Plate 12:91

Grain monad, radial, isopolar, tricolporate, planaperturate; psilate to slightly scabrate; exine tectate, 2.0 µm thick; colpus very short, ends rounded, 18.0 µm long x 5.0 µm wide, marginate, margo 6.0 µm wide x 7.5 µm thick; pore circular to slightly lolongate, 10.0 µm long x 9.0 µm wide; PA 35.0 µm; polar shape circular sometimes resembling triangular condition; grains suboblate, variable in size, 82.5 to 103.0 µm and 67.0 to 71.0 µm (durian).

⊙ AGPC 16494. Loc: Honduras. Herbarium: MNS, Paleobotany Section, MO # 2144317; Jarzen exchange. Mounting medium: Canada balsam.

Malvaceae-Malvoideae

Abelmoschus esculentus (L.) Moench Plate 12:92

Grain monad, radially symmetric, apolar, periporate; baculate-echinate; baculae variable < 1.0 to 1.5 µm long, echinae conical, 32.0 µm long x 11.0 µm wide; exine semitectate, 40.0 µm thick (including sculptural elements); > 20 pores/grain, pore circular to irregular, 15.0 µm in diameter; outline circular; grains spheroidal, 275.0 µm (range 254.0 to 281.0) (okra).

⊙ AGPC 17309. Loc: Panama. Coll.: Dwyer, 1979. Herbarium: MO. Mounting medium: Canada balsam.

Abutilon angulatum (Guill. & Perr.) Mast. Plate 13:93

Grain monad, radially symmetric, isopolar, tricolporate; echinate; echini conical, 8.0 µm high x 4.0 µm wide; exine semitectate, 7.5 µm thick (excluding sculptural elements); bacuae shaped, ends rounded; colpus short, irregular, thin, inconspicuous; pore apparently circular, 6.0 µm in diameter; polar shape circular; grains suboblate, 125.0 µm long x 146.0 µm wide (size reaching up to 162 µm) (elephant's ear).

⊙ AGPC 16627. Loc: Zambia. Coll.: Mitchell # 2868. Herbarium: MNS, Paleobotany Section. Mounting medium: Canada balsam.

Gossypium barbadense L. Plate 13:94

Grain monad, radially symmetric, apolar, periporate; baculate-echinate; baculae variable, echinae acute, 7.0 µm high x 8.0 µm wide; exine semitectate, 11.0 µm thick (including sculptural elements); > 20 pores/grain, pore circular, 7.0 µm in diameter; outline circular; grains spheroidal, 139.0 µm (range 122.0 to 156.0) (cotton, Egyptian cotton).

⊙ AGPC 21098. Loc: Costa Rica, parc de observation a San Jose. Coll.: Tonduz # 7299. Herbarium: CR. Mounting medium: Canada balsam.

Hibiscus ovalifolius (Forssk.) Vahl (= *Hibiscus fuscus* Garcke) Plate 13:95

Grain monad, radially symmetric, apolar, periporate; baculate-echinate; baculae variable, echinae conical, bottle-shaped, 18.0µm long x 9.0 µm wide; exine intectate, 3.5 µm thick (excluding sculptural elements); columellae baculae shaped; grains having > 0 pores, pore circular, 8.5 µm in diameter, distance between adjacent pores 16.5 µm; outline circular; grains spheroidal, 174.0 µm (range 169.0 to 178.0) (oorikai).

⊙ AGPC 4527. Mounting medium: Canada balsam.

Malvaceae-Sterculioideae

Herrania purpurea (Pittier) R.E. Schult. Plate 13:96

Grain monad, radial, isopolar, tricolporate, planaperturate; reticulate, heterobrochate, brochi ample, irregular 1.0 to 3.0 µm wide, muri very thin, simplicolumellate, columellae baculae shaped; exine tectate, 1.2 µm thick; colpus as long as grain, having costae endocolpi 1.8 µm thick, ends acute, interpore distance 5.0 µm; pore lalongate, 2.0 µm long x 5 µm wide; polar shape circular; grains subprolate, 27.0 µm long x 22.5 µm wide (monkey cacao, cacao de ardilla).

⊙ Loc: Panama, BCI. Coll.: T.B. Croat # 13798. Herbarium: MO. Mounting medium: glycerin jelly.

3.18 MYRTALES

Myrtaceae

Acca sellowiana (O. Berg) Burret. Plate 13:97

Grain monad, radial, isopolar, syncolporate (triaperturate), planaperturate; scabrate; exine tectate, 1.5 µm thick; colpus thin, joined at polar area forming a small triangle (parasyncolporate condition); pore lalongate linear, thin, ca. 1.0 µm long x 6.0 µm wide; polar shape triangular; grains oblate, 12.0 µm long x 24.0 µm wide (feijoa).

⊙ Loc: USA, Florida, Gainesville, Alachua Co., U. of Florida, campus. Coll: J. Richard Abbott # 24596, 10 May 2008. Herbarium: FLAS # 227644. Process: Acetolysis, May 2014. Mounting medium: glycerin jelly.

Psidium guajava L. Plate 13:98

Grain monad, radial, isopolar, syncolporate (triaperturate), planaperturate; scabrate; exine tectate, 1.5 µm thick; colpus thin, as long as grain, joined at polar area; pore lalongate, ends acute, slightly vestibulate, 2.5 µm long x 6.0 µm wide; polar shape triangular; grains suboblate, 14.0 µm long x 20.0 µm wide (common guava).

⊙ Loc: Colombia, Cundinamarca, Arbelaez, Santa Barbara, finca Cootransfusa, 1760 msnm. Info: guayaba, arbol 3 m., cultivado, frutal, bosque humedo premontano. Coll.: Moreno & Devia # 019, Mayo 25, 1980. Herbarium: COL # 206780. Mounting medium: glycerin jelly.

Syzygium aromaticum (L.) Merr. & L.M. Perry Plate 13:99

Grain monad, radial, isopolar, syncolporate (triaperturate), planaperturate; psilate; exine tectate, 1.8 µm thick, columellae thin; colpus thin, joined at polar area forming a triangle (parasyncolporate condition); pore lalongate, protruding, vestibulate, irregular; polar shape triangular; grains oblate, 12.5 µm long x 20.0 µm wide (pv range 11.0 to 14.0) (clove).

⊙ Loc: USA, Florida, Miami, Dade Co. Coll: Kristen Porter-Utley # 95-255, Dec. 1995. Herbarium: FLAS # 215526. Process: Acetolysis, May 2014. Mounting medium: glycerin jelly.

Syzygium jambos (L.) Alston Plate 13:100

Grain monad, radial, isopolar, syncolporate (triaperturate, sporadically exhibiting 4 aperturate condition), planaperturate; psilate to slightly scabrate; exine tectate, 1.2 µm thick; colpus thin, as long as grain joined at polar area forming a triangle (parasyncolporate condition); pore inconspicuous, apparently lalongate, vestibulate, costae pori 2.0 µm thick; grains not seen in equatorial view; polar shape triangular; grains probably oblate, 29.0 µm wide (pv range 26.5 to 31.0) (rose apple, Malabar plum).

⊙ AGPC 18941. Loc: Panama, Barro Colorado Island. Coll.: R. Schmalzel # 950. Herbarium: MO. Mounting medium: glycerin jelly.

3.19 OXALIDALES

Oxalidaceae

Averrhoa carambola L. Plate 13:101

Grain monad, radial, isopolar, tricolpate, planaperturate; baculate, sometimes resembling reticulate condition; exine intectate, 2.0 µm thick; columellae baculae shaped; colpus 22.0 µm long x 3.5 µm wide, ends rounded; PA 6.0 µm; polar shape circular; grains subprolate, 28.0 µm long x 22.5 µm wide (ev range 27.0 to 32.0) (starfruit).

⊙ Loc: Panama, BCI, along stairs to ZMA house. Coll.: T.B. Croat # 9195, 30 March, 1970. Herbarium: MO. Mounting medium: glycerin jelly.

3.20 PIPERALES

Piperaceae

Piper cf. *hispidum* M. Martens & Galeotti Plate 13:102

Grain monad, heteropolar-bilateral, asymmetric, monosulcate; scabrate to granular; exine tectate ca. 1.0 µm thick, stratification undifferentiated; colpus as long as grain, inconspicuous, irregular, masked by larger sacbrae becoming granules; grain outline elongated, 9.0 µm in size (range 47.0 to 53.0) (black pepper).

⊙ Loc: Panama, Gamboa, Pipeline Road. Coll.: R.J. Schmalzel # 762. Herbarium: MO. Mounting medium: glycerin jelly.

3.21 POALES

Bromeliaceae

Ananas comosus (L.) Merr. Plate 13:103

Grain monad, heteropolar-bilateral, monosulcate; reticulate, heterobrochate, brochi variable, < 1.0 up to 2.0 µm wide, lumina rounded, resembling foveolate condition, muri simplicolumellate; exine tectate, thin, 1.0 µm thick; sulcus as long as grain, inconspicuous, irregular, ends rounded; grain outline circular, irregular, 49.0 µm in size (range 47.0 to 53.0) (pineapple).

- AGPC 19148. Loc: Panama, Balboa. Coll.: R.J. Schmalzel # 187. Herbarium: STRI exchange. Process: Acetolyzed. Mounting medium: glycerin jelly.

Poaceae

Bambusa arundinacea (Retz.) Willd. Plate 13:104

Grain monad, heteropolar-bilateral, monoporate; scabrate, scabrae ca. 1.0 µm in size; exine tectate, < 1.0 µm thick; pore circular, 5.0 µm in diameter, annulate, annulus 3.5 µm thick; outline circular, irregular, folded; grains spheroidal, 57.0 to 65.0 µm in size (bamboo).

- Loc: Panama, BCI, near Lab. dormitory. Info: Cultivated. Coll.: T.B. Croat # 11746, 15 April 1976. Herbarium: MO. Mounting medium: glycerin jelly.

Digitaria ciliaris (Retz.) Koeler. Plate 13:105

Grain monad, heteropolar-bilateral, monoporate; scabrate, scabrae conspicuous; exine tectate, 1.2 µm thick; columellae thin; pore circular, 3.5 µm in diameter, annulate, annulus 3.5 µm thick; outline circular, irregular; grains spheroidal, 45.0 to 47.0 µm in size (southern crabgrass).

- Loc: India, Kempuhole. Coll.: C.J. Saldanha # 12958, 12 March 1969. Herbarium: Institut Francais Pondichery, HIFP # 15430. Mounting medium: glycerin jelly.

Eleusine indica (L.) Gaertn. Plate 13:106

Grain monad, heteropolar-bilateral, monoporate; scabrate, scabrae conspicuous; exine tectate, < 1.0 µm thick; pore circular, 3.5 µm in diameter, annulate, annulus 2.0 µm thick; outline circular, irregular, folded; grains spheroidal, 43.0 to 47.0 µm in size (Indian goosegrass).

- Loc: Panama, BCI, laboratory clearing. Coll.: T.B. Croat # 9241, 1 April 1970. Herbarium: MO. Mounting medium: glycerin jelly.

Eragrostis prolifera (Sw.) Steudel. Plate 13:107

Grain monad, heteropolar-bilateral, monoporate; scabrate, scabrae fine; exine tectate, 1.2 µm thick; pore circular, 6.0 µm in diameter, annulate, annulus, 3.0 µm thick; outline circular, irregular; grains spheroidal, 46.0 to 47.0 µm in size (Dominican lovegrass).

- Loc: Mexico, Municipio Cozumel, Reserva de Sian Ka'an, km. 50 Tuluan a Punta Allen. Info: hierba, floracion Octubre, vegetacion duna costera. Coll.: Duran & Espejel # 541. Herbarium: CIQRO. Mounting medium: Canada balsam.

Isachne conferta Merr. (= *Setaria geniculata* Beauv., P.) Plate 14:108

Grain monad, heteropolar-bilateral, monoporate; scabrate, scabrae conspicuous, dense; exine tectate, 1.0 µm thick; columellae thin; pore circular, 3.0 µm in diameter, annulate, annulus 2.5 µm thick; outline circular, irregular; grains spheroidal, 54.0 µm in size (roughish witch grass).

- Loc: Panama, BCI, clearing N of dock. Coll.: T.B. Croat # 6811. Herbarium: MO. Mounting medium: glycerin jelly.

Panicum virgatum L. Plate 14:109

Grain monad, heteropolar-bilateral, monoporate; scabrate to granulate, scabrae coarse becoming to be granulae; exine tectate, 2.0 µm thick; pore circular, 3.0 µm in diameter, annulate, annulus thin, 1.5 µm thick; outline circular; grains spheroidal, 32.0 to 35.0 µm in size (old switch panic grass).

⊙ Loc: Mexico, Reserva de Sian Ka'an, km. 9 al sureste del crucero Chumpon. Info: hierba, floracion Agosto, vegetacion selva baja indundable. Coll.: Duran & Olmsted # 1039. Herbarium: CIQRO. Mounting medium: Canada balsam.

Paspalum paniculatum L. Plate 14:110

Grain monad, heteropolar-bilateral, monoporate; scabrate, scabrae fine; exine tectate, 1.5 µm thick; pore circular, 3.0 µm in diameter, annulate, annulus 2.0 µm thick; outline circular, irregular; grains spheroidal, 37.0 to 43.0 µm in size (Russell river grass, angel grass, arrocillo).

⊙ Loc: Panama. Coll.: E.L. Tyson # 5441. Herbarium: MO. Mounting medium: glycerin jelly.

Pennisetum purpureum Schum. Plate 14:111

Grain monad, heteropolar-bilateral, monoporate; scabrate; exine tectate, 2.5 µm thick; pore circular, 3.0 µm in diameter, annulate, annulus 2.5 µm thick; outline circular, irregular; grains spheroidal, 45.0 µm in size (elephant grass, Napier grass).

⊙ Loc: Mexico, Municipio de Felie Carrillo Puerto, rancho "La Gracia de Dios". Info: hierba, floracion Agosto, vegetacion secundaria. Coll.: Duran & Olmsted # 839. Herbarium: CIQRO. Mounting medium: Canada balsam.

Saccharum spontaneum L. Plate 14:112

Grain monad, heteropolar-bilateral, monoporate; scabrate; exine tectate, 1.2 µm thick; pore circular, 3.0 µm in diameter, annulate, annulus 2.5 µm thick; outline circular; grains spheroidal, 48.0 µm in size (wild sugar cane).

⊙ Loc: Panama. Coll.: E.L. Tyson # 4459. Herbarium: MO. Mounting medium: glycerin jelly.

Zea mays L. Plate 14:113

Grain monad, heteropolar-bilateral, monoporate; scabrate; exine tectate, 1.5 µm thick; columellae thin; pore circular, 8.0 µm in diameter, annulate, annulus 5.0 µm thick; outline circular, folded; grains spheroidal, 114.0 to 151.0 µm in size (maize, corn).

⊙ Loc: Colombia, Mpio. Arbelaez, Sta. Barbara, 1700 msnm. Info: cultivated. Coll.: Moreno & Devia # 071, 28 May 1980. Herbarium: COL # 207014. Mounting medium: glycerin jelly.

3.22 PROTEALES

Proteaceae

Grevillea robusta A. Cunn. ex R. Br. Plate 14:114

Grain monad, radial, isopolar, triporate, planaperturate; rugulate; exine tectate, 3.0 µm thick; pore circular, 13.0 µm in diameter, covered by fine ectexinic membrane; polar shape triangular; grains suboblate, 57.0 µm in size (pv range 56.0 to 59.0) (silk oak, silver oak).

⊙ AGPC 5682. Loc: California. Coll.: A.S.G.J. M863. Herbarium: Ore. # 75588; exchange Pan American. Mounting medium: Canada balsam, stained.

Macadamia integrifolia Maiden & Betche Plate 14:115

Grain monad, radial, isopolar, triporate, planaperturate; granulate; exine tectate, 2.5 µm thick, columellae very thin; pore circular, 4.0 µm in diameter, annulate, annulus 1.8 µm thick; polar shape triangular; grains oblate, 21.0 µm long x 36.5 µm wide (pv range 34.0 to 39.5) (Macadamia nut).

⊙ Loc: USA, Florida, Miami, Dade Co., homestead. Coll: S.W. Lynch, 12 April 1943. Herbarium: FLAS # 40777. Process: Acetolysis, May 2014. Mounting medium: glycerin jelly.

Macadamia tetraphylla Johnson, L. Plate 15:116

Grain monad, radial, isopolar, triporate, planaperturate; baculate, resembling micro-reticulate pattern; baculae very thin, head rounded, dense; exine intectate, 1.8 µm thick; pore circular, 5.0 µm in diameter, annulate; polar shape triangular; grains suboblate, 24.0 µm long x 39.0 µm wide (pv range 39.0 to 40.0) (rough shell Queensland nut).

⊙ AGPC 7464. Loc: Australia, Queensland. Coll.: L. Johnson. Herbarium: Shell exchange. Mounting medium: glycerin jelly.

3.23 ROSALES

Cannabaceae

Cannabis sativa L. Plate 15:117

Grain monad, radial, isopolar, triporate; scabrate; exine tectate, 1.0 µm thick, columellae very thin; pore circular, 2.5 µm in diameter, subtly protruding, annulate, annulus 1.0 µm thick; polar shape circular; grains suboblate, 23.5 µm long x 26.5 µm (ev range 22.0 to 25.0) (hemp, marijuana).

⊙ Loc: USA, Florida, Gainesville, Alachua Co., greenhouse. Coll: ex. D. Burch, 18 October 1965. Herbarium: FLAS # 92546. Process: Acetolysis, May 2014. Mounting medium: glycerin jelly.

Moraceae

Artocarpus altilis (Parkinson) Fosb. Plate 15:118

Grain monad, radial, isopolar, diporate, less frequent triporate; psilate; exine tectate, 1.0 µm thick; pore circular, 2.0 µm in diameter, subtly protruding; polar shape circular to ellipsoidal; grains suboblate, 14.0 µm long x 16.0 µm wide (breadfruit).

⊙ Loc: Panama, BCI. Coll.: R.J. Schmalzel # 937. Herbarium: MO. Mounting medium: glycerin jelly, fresh material.

Rhamnaceae

Ziziphus jujuba Mill. Plate 15:119

Grain monad, radial, isopolar, tricolporate, planaperturate; reticulate, heterobrochate, brochi fine, subtle, < 1.0 µm wide, slightly variable, diminishing toward apertures, muri simplicolumellate; exine tectate 2.0 µm thick; colpus as long as grain, thin, marginate, ends acute, PA 5.0 µm; pore vestibulate, lalongate 3.5 µm long x 7.0 µm wide; polar shape triangular-concave; grains oblate, 21.0 µm long x 27 µm wide (pv range 25.0 to 29.0) (jujube).

⊙ Loc: USA, Florida, Gainesville, Alachua Co., U. of Fla. Horticultural grounds. Coll: H. H. Hume, 30 April 1936. Herbarium: FLAS # 12288. Process: Acetolysis, May 2014. Mounting medium: glycerin jelly.

Rosaceae

Eriobotrya japonica (Thunb.) Lindl. Plate 15:120

Grain monad, radial, isopolar, tricolporate, planaperturate; apparently psilate, sometimes resembling fine and subtle striate pattern; exine tectate 3.0 µm thick, columellae thin, straight; colpus as long as grain, thin, having costae endocolpi, displaying equatorial constriction, ends acute; pore endexinic, inconspicuous, irregular; polar shape circular; grains subprolate, 41.5 µm long x 34.0 µm wide (ev range 38.0 to 44.0) (loquat, Japanese medlar).

⊙ Loc: Mexico, Morelos, Ocotepec. Coll.: R. Palacios, 13/VIII/65. Mounting medium: Canada balsam.

Fragaria chiloensis (L.) Mill. Plate 15:121

Grain monad, radial, isopolar, tricolporate, planaperturate; striate, striae long, thin, longitudinally oriented; exine tectate, 1.8 µm thick, columellae thin, short; colpus as long as grain, end roundeds, marginate, costae endocolpi subtle, PA 5.0 µm; pore endecxinic, inconspicuous, ca. 5.0 µm long x 8.5 µm wide, masked by ornamentation, resembling the "H" pattern, protruding, vestibulate, apparently operculate; polar shape circular; grains subprolate, 28.0 µm long x 24.0 µm wide (ev range 26.0 to 29.0) (beach strawberry).

⊙ Loc: USA, Calif, Crescent City, sand dunes. Coll: S. C. Hood # 906, 26 May 1948. Herbarium: FLAS # 53354. Process: Acetolysis, May 2014. Mounting medium: glycerin jelly.

Fragaria sp. Plate 15:122

Grain monad, radial, isopolar, tricolporate, planaperturate; striate, striae long, irregular, ca. 1.0 µm wide, longitudinally oriented; exine tectate, 3.0 µm thick, columellae thin, short, duplicolumellate; colpus as long as grain, end roundeds, marginate, costae endocolpi subtle, displaying exitus digitatus; pore endecxinic, apparently lalongate to circular 8.0 µm in diameter, masked by ornamentation, protruding, vestibulate, having subtle operculae; polar shape circular; grains subprolate, 36.5 µm long x 25.0 µm wide (ev range 33.5 to 41.0) (cultivated strawberry).

⊙ Loc: USA, Florida, Gainesville, Alachua Co., Dudley Historic farm site. Coll: Irma E. Riley # 042, 4 May 2010. Herbarium: FLAS # 228903. Process: Acetolysis, May 2014. Mounting medium: glycerin jelly.

Malus bracteata Sarg. Plate 15:123

Grain monad, radial, isopolar, tricolporate, planaperturate; reticulate resembling striate-reticulate condition; brochi fine, < 1.0 µm wide, longitudinally oriented, muri simplicolumellate, columellae thin, abundant; exine tectate, 2.5 µm thick; colpus as long as grain, ends acute, displaying equatorial constriction; pore inconspicuous, lalongate, resembling the "H" pattern; polar shape circular; grains subprolate, 38.0 µm long x 36.5 µm wide (apple).

⊙ AGPC 4144. Herbarium: Harvard. Mounting medium: Canada balsam.

Prunus dulcis (Mill.) D.A. Webb (= *Prunus amygdalus* Bastch) Plate 15:124

Grain monad, radial, isopolar, tricolporate, planaperturate; striate-reticulate, striae fine, longitudinally oriented, brochi < 1.0 µm wide; exine tectate, 2.5 µm thick, columellae baculae shaped; colpus as long as grain, marginate, exhibiting costae endocolpi and exitus digitatus; pore lalongate, oblongate, 14.0 µm long x 19.0 µm wide; grains prolate, 68.0 µm long x 45.5 µm wide (ev range 66.0 to 70.0) (sweet almond).

⊙ AGPC 2317. Loc: Iraq, Bagdad. Coll.: A.S.G.J. M369. Herbarium: FM # 772245. Mounting medium: Canada balsam.

Rubus hispidus L. Plate 15:125

Grain monad, radial, isopolar, tricolporate, planaperturate; striate-reticulate, striae fine, thin, subtle, longitudinally oriented, brochi very fine; exine tectate, 2.5 µm thick; colpus as long as grain, marginate, ends rounded, displaying equatorial constriction; pore lalongate; grains spheroidal, 24.0 µm in size (running blackberry, swamp dewberry).

⊙ AGPC 4150. Herbarium: Harvard. Mounting medium: Canada balsam.

3.24 SAPINDALES

Anacardiaceae

Anacardium occidentale L. Plate 16:126

Grain monad, radial, isopolar, tricolporate, planaperturate; striate-reticulate, striae longitudinally oriented, homobrochate, brochi 1.0 µm wide, irregular, muri thin, simplicolumellate; exine tectate 3.5 µm thick; colpus 12.0 µm long x 1.0 µm wide, having costae endocolpi 1.0 µm thick, exhibiting exitus digitatus, ends rounded; pore endexinic, lalongate 6.0 µm long x 14.0 µm wide, slightly protruding; polar shape circular; grains subprolate, 46.5 µm long (range 45.0 to 48.0) x 42.0 µm wide (range 41.0 to 42.5) (cashew, marañon)

⊙ AGPC 18351. Loc: Panama, 1 mi. E. Tocumen airport, 1965. Coll.: Blum & Tyson # 1964. Mounting medium: Canada balsam.

Mangifera indica L. Plate 16:127

Grain monad, radial, isopolar, tricolporate, planaperturate; striate-reticulate, striae longitudinally oriented, lumina homobrochate, < 1.0 µm wide, muri thin, simplicolumellate; exine tectate 2.0 µm thick; colpus three-quarters as long as grain, ends acute; pore endexinic, inconspicuous, lalongate 1.0 µm long x 7.0 µm wide, slightly marginate; polar shape circular; grains subprolate, 23.5 µm long (range 20.0-23.5) x 22.5 µm wide (range 20.0 to 27.0) (mango)

⊙ AGPC 18953. Loc: Panama, Balboa. Coll.: R.J. Schmalzel # 685. Herbarium: MO, STRI exchange. Mounting medium: glycerin jelly.

Spondias dulcis Parkinson Plate 16:128

Grain monad, radial, isopolar, tricolporate sporadically stephanocolporate (4 aperturate), planaperturate; striate-reticulate, striae dense, longitudinally oriented; homobrochate, brochi < 1.0 µm wide, muri thin, simplicolumellate; exine tectate 2.5 µm thick; colpus as long as grain, ends acute, marginate, margo 2.5 µm thick, opposite colpi appearing crossed when 4 aperturate condition; pore endexinic, apparently lalongate, inconspicuous; polar shape circular; grains subprolate, 50.0 µm long (range 48.0 to 53.0) x 33.0 µm wide (range 28.0 to 38.0) (hog plum).

⊙ Loc: USA, Florida, Gainesville, Alachua Co., U. of Fla. Gardens. Coll: Ronald Lange # 1133, 17 July 2011. Herbarium: FLAS # 232105. Process: Acetolysis, May 2014. Mounting medium: glycerin jelly.

Spondias mombin L. Plate 16:129

Grain monad, radial, isopolar, tricolporate, planaperturate; striate-reticulate, striae dense, longitudinally oriented, homobrochate, 1.0 µm wide, muri thin, simplicolumellate; exine tectate 2.0 µm thick; colpus as long as grain, marginate, ends acute; pore endexinic, slightly lalongate 4.0 µm long x 8.0 µm wide; polar shape circular; grains subprolate, 35.0 µm long (range 33.0 to 35.5) x 31.0 µm wide (range 30.5 to 32.0) (hog plum, java plum).

⊙ AGPC 20833. Loc: Costa Rica, San Ramon. Coll.: Brenes # 20316. Herbarium: CR. Mounting medium: Canada balsam.

Burseraceae

Commiphora baluensis Engl. Plate 16:130

Grain monad, radial, isopolar, tricolporate, planaperturate; echinate-reticuloid, echini acute, wide base resembling a long galea type, ordered as a reticuloid pattern; exine intectate 3.0 µm thick; pore circular 2.5 µm in diameter, annulate, annulus 2.5 µm wide; colpus inconspicuous, very short, thin, ends acute; polar shape circular; grains suboblate, 27.0 µm long x 29.0 µm wide (ev range 26.0 to 28.0) (mirra).

⊙ AGPC 22986. Loc: Kenya. Coll.: F.G. Smith No. n.a. Process: KOH-Acet., DL 1969. Mounting medium: glycerin jelly.

Rutaceae

Citrus aurantiifolia (Christm.) Swingle Plate 16:131

Grain monad, radial, isopolar, stephanocolporate (4 aperturate, less frequently 5 aperturate), planaperturate; reticulate, heterobrochate, brochi variable, diminishing toward apertures, lumina < 1.0 to 1.5 µm wide, muri coarse, simplicolumellate, columellae baculae shaped, head rounded; exine tectate, 2.5 µm thick; colpus as long as grain x 3.0 µm wide, displaying costae endocolpi 2.5 µm thick; pore lalongate 2.5 µm long x 12.0 µm wide; polar shape square when 4 aperturate and circular pentalobate when 5 aperturate; grains subprolate, 34.0 µm long x 31.0 µm wide (ev range 30.0 to 35.0) (lemon)

⊙ Loc: Mexico, Chancah Veracruz, Municipio Felipe Carrillo Puerto. Info: arbol, floracion Marzo, huerto familiar. Coll.: Gutierrez # 463. Herbarium: CIQRO. Mounting medium: Canada balsam.

Citrus reticulata Blanco Plate 17:132

Grain monad, radial, isopolar, stephanocolporate (4 aperturate, less frequently 5 aperturate), planaperturate; reticulate, heterobrochate, brochi slightly variable, lumina rounded, fine, ca. 1.0 µm wide, muri coarse, ca. 1.0 µmn thick, simplicolumellate, columellae baculae shaped; exine tectate, 1.5 µm thick; colpus thin, 26.0 µm long x 1.5 µm wide; pore lalongate 2.5 µm long x 12.0 µm wide; polar shape circular-square; grains spheroidal to slightly subprolate, 41.0 µm long x 40.0 µm wide (tangerine).

⊙ AGPC 18436. Loc: Panama, BCI, Donato trail start. Coll.: T.B. Croat # 14870. Herbarium: MO. Mounting medium: Canada balsam.

Triphasia trifoliata DC. Plate 17:133

Grain monad, radial, isopolar, tricolporate, planaperturate; reticulate, heterobrochate, brochi variable, diminishing toward apertures, lumina < 1.0 to 1.5 µm wide, muri simplicolumellate, columellae baculae shaped; exine tectate, 2.0 µm thick; colpus as long as grain, thin, ends rounded, slightly constricted at equator, displaying costae endocolpi 4.0 µm thick, PA 14.0 µm; pore lalongate 5.0 µm long x 18.0 µm wide; polar shape circular; grains subprolate, 47.5 µm long (range 42.0 to 50.0) x 43.0 µm wide (range 38.0 to 46.0) (limeberry).

⊙ AGPC 4382. Herbarium: Harvard. Mounting medium: Canada balsam.

Sapindaceae

Blighia sapida Koenig, C. Plate 17:134

Grain monad, radial, isopolar, tricolporate, planaperturate; striate-reticulate, homobrochate, brochi fine, subtly ordered longitudinally resembling striate pattern, lumina < 1.0 µm wide, muri simplicolumellate; exine tectate, 1.5 µm thick; colpus as long as grain, PA 6.0 µm; pore inconspicuous, irregular, circular 2.5 µm in diameter, apparently annulate; polar shape circular to trilobate; grains suboblate, 19.0 µm long x 21.0 µm wide (akee).

⊙ AGPC 7214. Herbarium: Lab. Palynol. Museum Paris; Paris exchange. Mounting medium: glycerin jelly.

Melicoccus bijugatus Jacq. Plate 17:135

Grain monad, radial, isopolar, parasyncolporate (triaperturate), planaperturate; reticulate, homobrochate, brochi fine, < 1.0 µm wide, muri simplicolumellate, columellae baculae shaped; exine tectate, 1.2 µm thick; colpus joined at polar area forming a small triangle (parasyncolporate condition); pore lalongate-elongate 3.5 µm long x 6.0 µm wide; polar shape triangular; grains oblate, 20.0 µm long x 27.0 µm wide (Spanish lime).

⊙ AGPC 17980. Loc: Panama. Herbarium: MNS, Paleobotany Section, MO # 2103568; Jarzen exchange. Mounting medium: Canada balsam.

Nephelium mutabile Blume Plate 17:136

Grain monad, radial, isopolar, tricolporate, planaperturate; subtly striate, striae fine, thin, inconspicuous; exine tectate, 1.2 µm thick, slightly thinner at equator, columellae thin, dense; colpus as long as grain, thin, marginate, having costae endocolpi; PA 6.0 µm; pore endexinic, inconspicuous, irregular; polar shape circular; grains subprolate, 25.0 µm long x 23.0 µm wide (ev range 23.5 to 26.5 µm) (pulasan).

⊙ AGPC 21017. Loc: Costa Rica. Mounting medium: Canada balsam.

Talisia nervosa Radlk. Plate 17:137

Grain monad, radial, isopolar, tricolporate, planaperturate; psilate; exine tectate, 1.8 µm thick; slightly thinner at intercolpium, columellae thin; colpus subtle, very short, thin, straight; PA 26.0 µm; pore endexinic, circular, ca. 2.0 µm in diameter, having costae endopori, annulus ca. 2.5 µm thick; polar shape triangular; grains oblate, 30.0 µm long x 40.0 µm wide (pv range 32.0 to 45.0 µm) (mamon).

⊙ AGPC 18336. Loc: Panama, BCI. Coll.: Blum & DWYER #2101. Mounting medium: Canada balsam.

Simaroubaceae

Simarouba glauca DC. Plate 17:138

Grain monad, radial, isopolar, tricolporate, planaperturate; reticulate, homobrochate, brochi fine, < 1.0 µm wide, muri simplicolumellate, columellae baculae shaped; exine tectate, 1.2 µm thick; colpus as long as grain, thin, straight; pore lalongate, almost linear, ca. 2.0 µm long x 6.0 µm wide; polar shape circular trilobate; grains prolate, 27.0 µm long x 15.0 µm wide (ev range 26.0 to 28.0 µm) (bitter damson, paradise tree).

⊙ AGPC 9927. Loc: Guatemala. Coll.: C.L. Lundell # 2185, 1933. Herbarium: USNH # 1685864, USGS P656. Mounting medium: Canada balsam.

3.25 SOLANALES

Convolvulaceae

Ipomoea batatas (L.) Lam. Plate 18:139

Grain monad, radially symmetric, apolar, periporate; echinate-baculate, echinae dense, conical, bottle-shaped 12.0 µm long x 6.5 µm wide at base and 2.5 µm wide at apice; baculae densely present on surface between echini, variable in long and wide, < 1.0 to 2.5 µm wide; exine intectate, 23.0 µm thick including sculptural elements; > 100 pores/grain, pores circular, 9.5 µm in diameter, interpore distance 7.0 µm; grains spheroidal, 119.0 µm in size (range 106.0 to 166.0) (sweet potato).

⊙ AGPC 18176. Loc: Panama, Chiriqui, Volcan. Coll.: Davidson # 1368. Herbarium: MO. Mounting medium: Canada balsam.

Solanaceae

Capsicum annuum L. Plate 18:140

Grain monad, radial, isopolar, tricolporate; planaperturate; psilate to slightly scabrate; exine tectate, variable, 1.8 µm thick at polar area becoming to 7.0 µm thick at pore level; colpus as long as grain, ends rounded, displaying equatorial constriction and exitus digitatus, PA 9.5 µm; pore lalongate, almost reaching at ends, resembling a continuous equatorial ring, protruding, vestibulate; polar shape circular; grains subprolate, 30.0 µm long x 28.0 µm wide (chili pepper, Cayenne pepper).

⊙ Loc: Mexico, Reserva de Sian Ka'an, km. 3 carretera Vigia Chico a Felipe Carrillo Puerto. Info: arbusto, floracion Enero, cultivada. Coll.: Villanueva # 591. Herbarium: CIQRO. Mounting medium: Canada balsam.

Nicotiana tabacum L. Plate 18:141

Grain monad, radial, isopolar, tricolporate and stephanocolporate (4 aperturate); planaperturate; striate, striae coarse when triaperturate, ca. 1.2 µm wide and thin when 4 aperturate, < 1.0 µm thick, striae longitudinally oriented; exine tectate 2.5 µm; colpus as long as grain, displaying subtle equatorial constriction and costae endocolpi, PA 9.5 µm when tricolporate and 12.0 µm when stephanocolporate; pore lalongate 4.0 µm long x 8.0 µm wide; polar shape circular; grains subprolate, 39.0 to 40.0 µm long x 30.0 to 31.0 µm wide (tobacco).

⊙ Loc: Mexico, Reserva de Sian Ka'an, km. 18 km. al sureste del crucero Chumpon, Municipio Felipe Carrillo Puerto. Info: hierba, floracion Junio, vegetacion secundaria de selva baja subcaducifolia. Coll.: Duran & Olmsted # 1003. Herbarium: CIQRO. Mounting medium: Canada balsam.

Solanum lycopersicum L. (= *Lycopersicon esculentum* Mill.) Plate 18:142

Grain monad, radial, isopolar, tricolporate; planaperturate; psilate to slightly scabrate; exine tectate 1.5 µm thick; columellae baculae shaped; colpus as long as grain, ends acute, displaying equatorial constriction, PA 5.0 µm; pore lalongate to linear 1.5 µm long x 8.0 µm wide, slightly protruding; polar shape circular; grains subprolate, 24.0 µm long x 21.0 µm wide (tomato, garden tomato).

⊙ AGPC 13673. Loc: Hawaii, Oahu. Coll.: Degener & Parks # 9957, 1935. Herbarium: NYBG. Process: Acetolysis, 7-12. Mounting medium: Canada balsam.

Solanum quitoense Lam. Plate 18:143

Grain monad, radial, isopolar, tricolporate; planaperturate; psilate to slightly scabrate; exine tectate 1.5 µm thick; columellae baculae shaped, thin; colpus as long as grain x 2.5 µm wide, ends acute, PA 6.0 µm; pore irregular, lalongate-rectangular, almost joined at apices, 7.0 µm long x 18.0 µm wide, resembling a continuous equatorial ring, protruding slightly; polar shape circular; grains subprolate, 32.5 µm long x 29.0 µm wide (Quito orange, naranjilla, lulo).

⊙ AGPC 18550. Loc: Panama. Coll.: K.E. Blum et al. # 2569. Herbarium: NM exchange. Mounting medium: glycerin jelly.

3.26 VITALES

Vitaceae

Vitis rotundifolia Michaux Plate 18:144

Grain monad, radial, apolar, apparently inaperturate but probably tricolporate; reticulate, brochi < 1.0 µ wide, muri very thin, simplicolumellate, columellae short; exine tectate 2.5 µm thick; if apertures present, inconspicuous; outline circular; grains subprolate, 28.0 µm in size (muscadine).

⊙ AGPC 4214. Herbarium: Harvard. Mounting medium: Canada balsam.

3.27 ZINGIBERALES

Zingiberaceae

Alpinia purpurata (Vieill.) Schum. Plate 18:145

Grain monad, apolar, radial, inaperturate, psilate, translucent; exine stratification indistinct, 5.0 µm thick; outline circular; grains spheroidal, 69.0 to 73.0 µm in size; grains susceptible to damage by chemical treatment (red ginger).

⊙ Loc: Panama, Gamboa, garden. Coll.: E. Moreno # n.a., 25 February 2014. Mounting medium: glycerin jelly, fresh material.

REFERENCES

[1] Moreno, J.E., Vergara, D. & Jaramillo, C. 2014. Las colecciones palinológicas del Instituto Smithsonian de Investigaciones Tropicales (STRI), Panamá [The palynological collections of the Smithsonian Tropical Research Institute (STRI), Panama]. *Boletín de la Asociación Latinoamericana de Paleobotánica y Palinología (ALPP)*, 14: 207–222.

[2] Punt, W., Hoen, P.P., Blackmore, S., Nilsson, S. & Le Thomas, A. 2007. Glossary of pollen and spore terminology. *Review of Paleobotany and Palynology*, 143: 1–81.

[3] Roubik, D.W. & Moreno, J.E. 1991. *Pollen and spores of Barro Colorado Island*. Monographs in Systematic Botany 36. St. Louis, Missouri Botanical Garden.

[4] Mabberley, D.J. 1997. *The plant-book, a portable dictionary of the vascular plants* (2nd edn). Cambridge, Cambridge University Press.

[5] Tropicos.org. Missouri Botanical Garden. May 2014 www.tropicos.org. Accessed May 2014

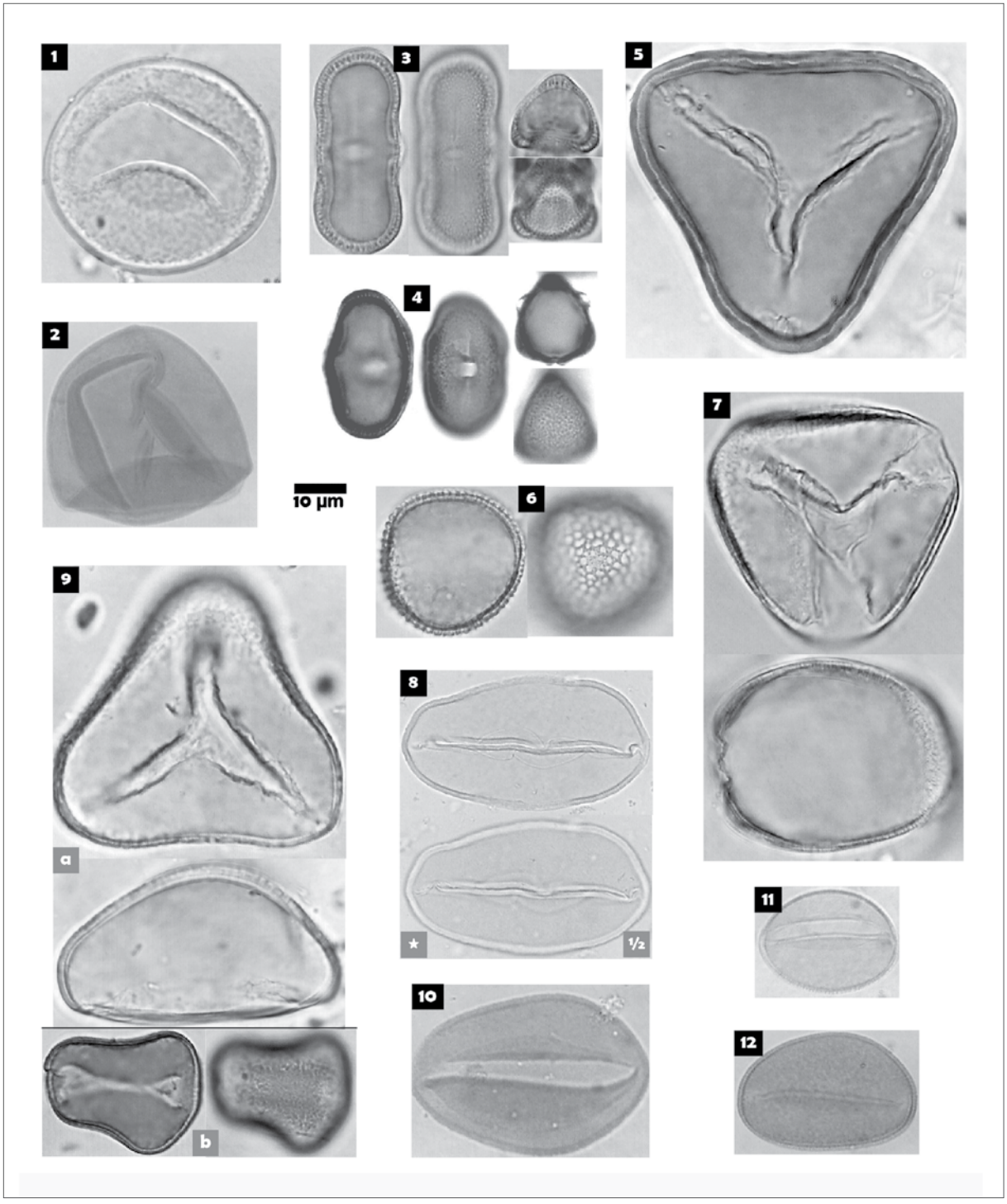


PLATE 1

ALISMATALES. Araceae: 1. *Alocasia longiloba*; **2.** *Colocasia esculenta*. **APIALES. Apiaceae: 3.** *Coriandrum sa-tivum*; **4.** *Daucus carota*. **ARECALES. Arecaceae: 5.** *Acrocomia aculeata*; **6.** *Areca catechu*; **7.** *Bactris gasipaes*; **8.** *Cocos nucifera*; **9.** *Elaeis guineensis* (**a**= trichotomosulcate form, **b**= monosulcate form); **10.** *Euterpe edulis*; **11.** *Phoenix dactili-fera*. **ASPARAGALES. Amaryllidaceae: 12.** *Allium schoenoprasum* var. *sibiricum*. x1000 (*= x400; ½= 50% reduced)

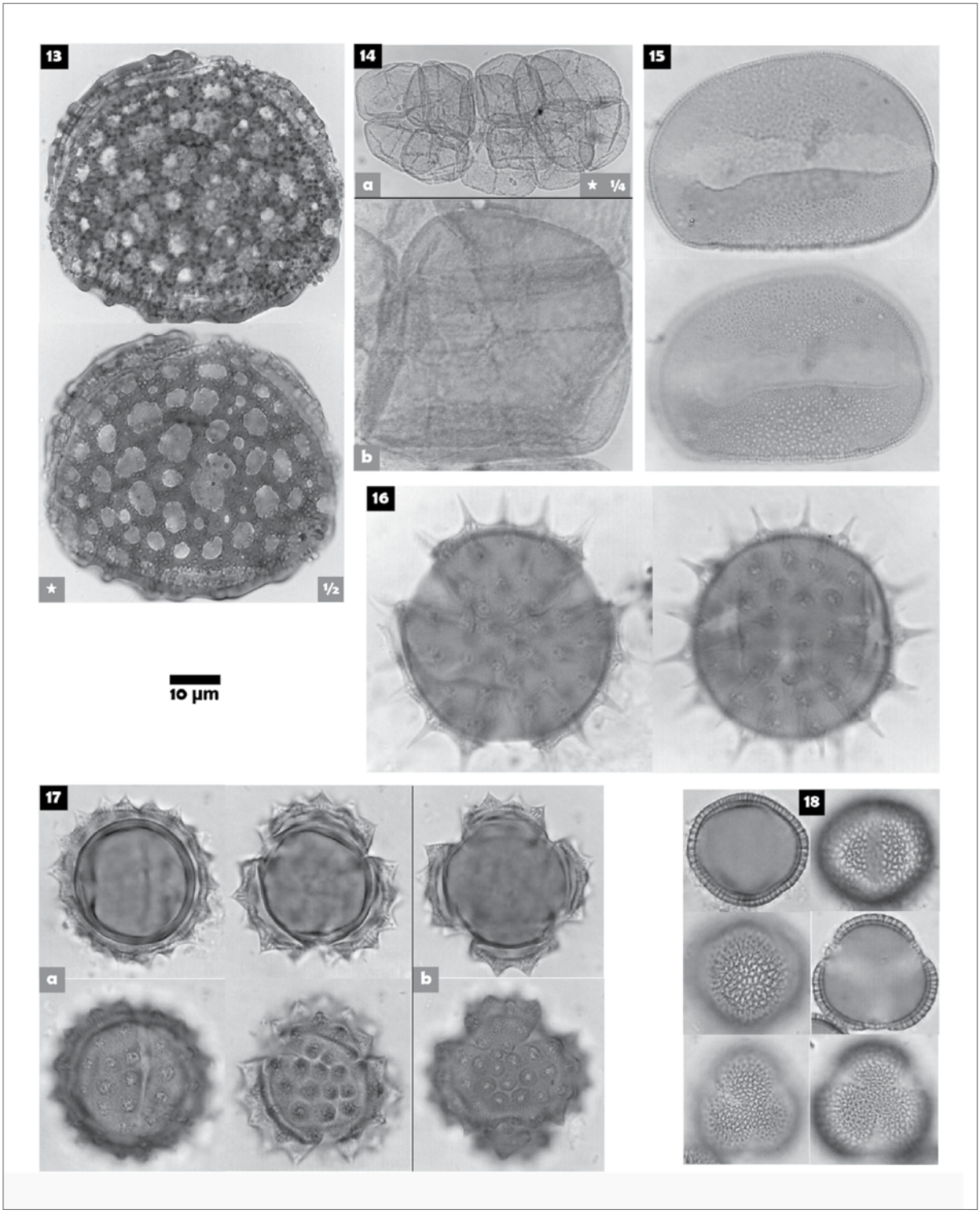


PLATE 2

ASPARAGALES. Asparagaceae: 13. *Agave sisalana*. **Orchidaceae**: **14.** *Vanilla planifolia* (**a**= massulae, **b**= isola-ted grain). **Xanthorrhoeaceae: 15.** *Aloe africana*. **ASTERALES. Asteraceae: 16.** *Helianthus annuus*; **17.** *Parthenium ar-gentatum* (**a**= tricolporate condition, **b**= stephanocolporate condition). **BRASSICALES. Brassicaceae: 18.** *Brassica rapa*. x1000 (*= x400; ½= 50% reduced, ¼= 75% reduced)

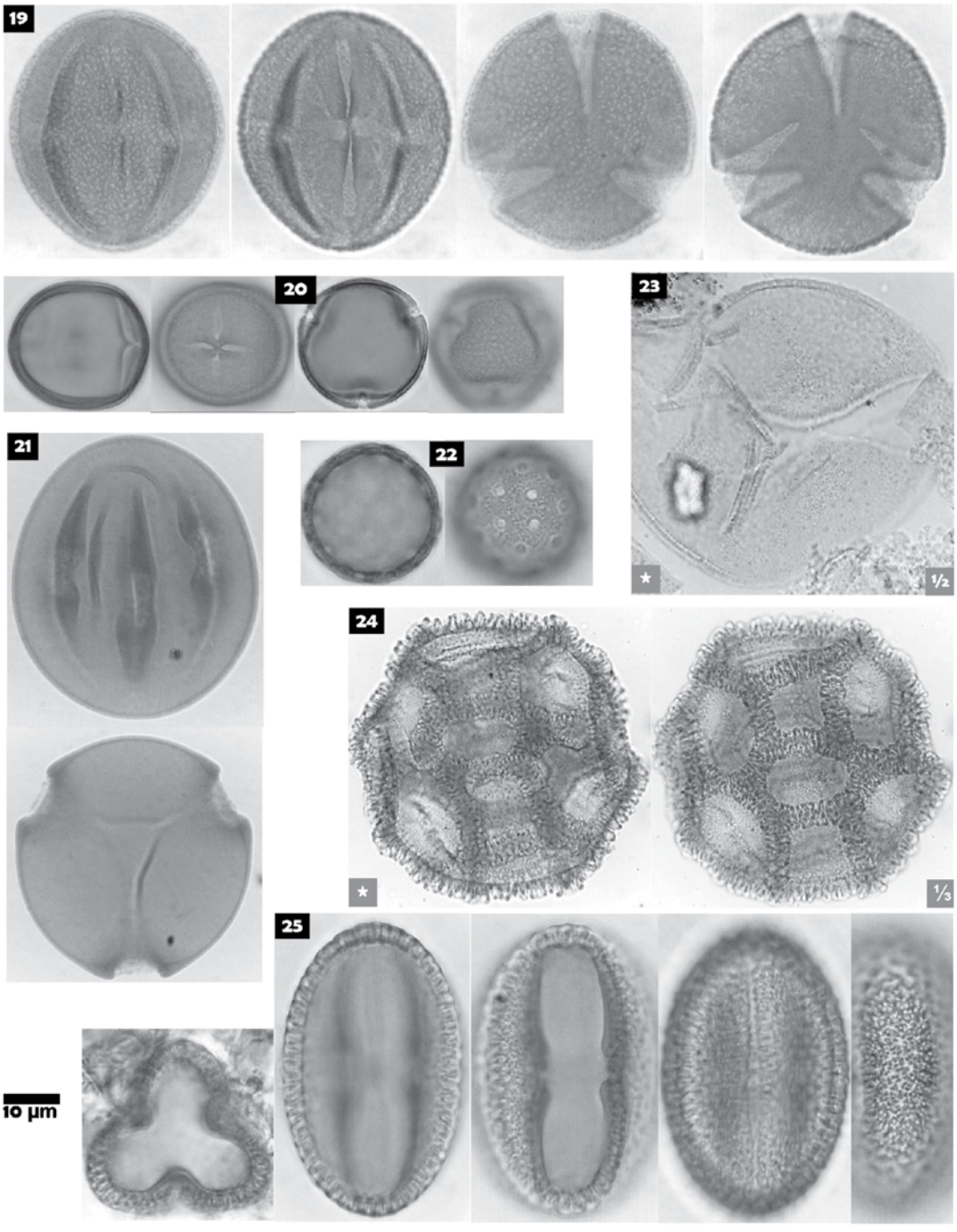


PLATE 3

BRASSICALES. Caricaceae: 19. *Carica papaya*; **20.** *Jacaratia dolichaula*. **Moringaceae: 21.** *Moringa oleifera*. **CARYOPHYLLALES. Amaranthaceae: 22.** *Amaranthus cruentus*. **Cactaceae: 23.** *Hylocereus polyrhizus*; **24.** *Opuntia erina-cea*. **Polygonaceae: 25.** *Fagopyrum esculentum*. x1000 (*= x400; ½= 50% reduced, ⅓= 66.6% reduced)

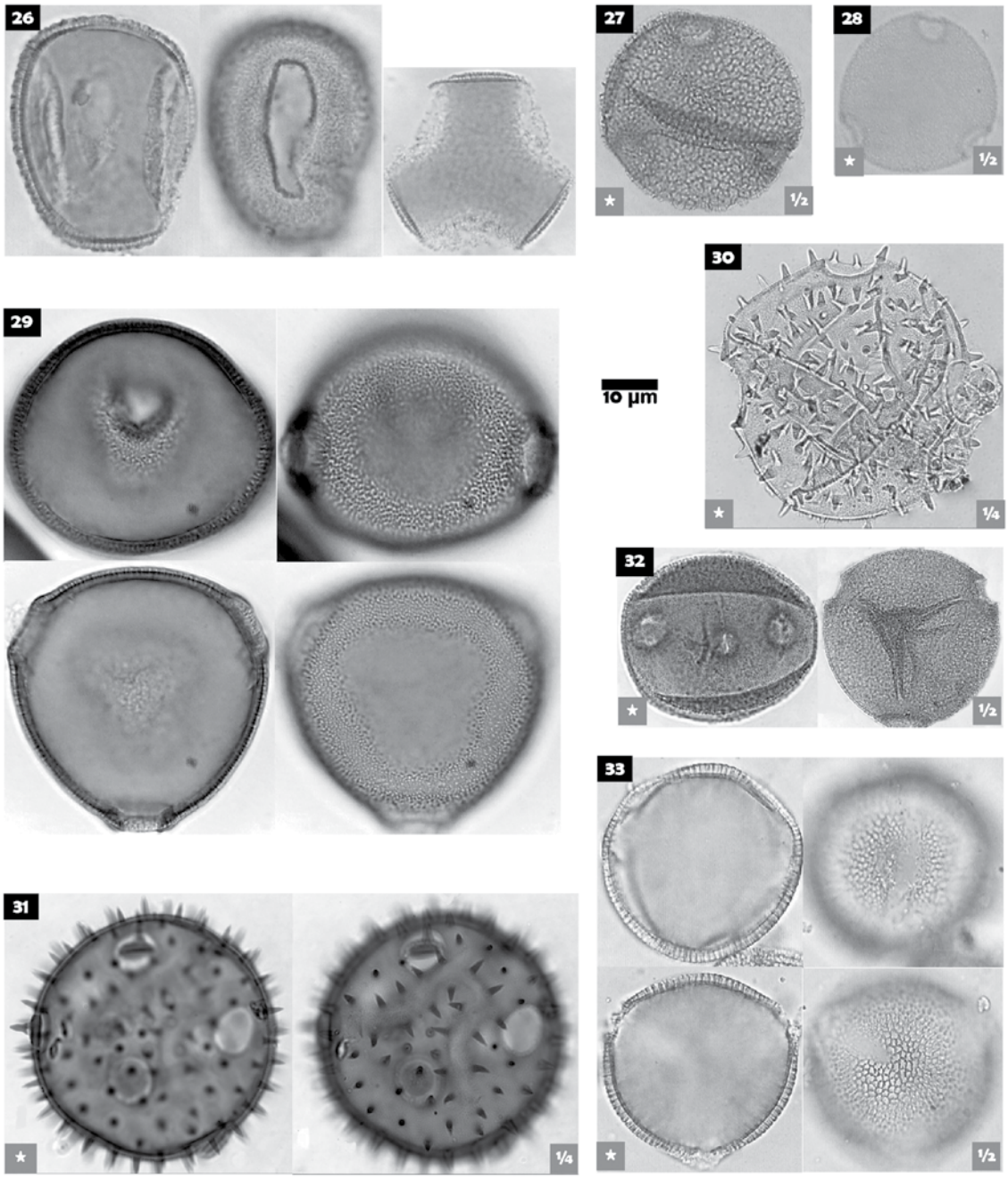


PLATE 4

CARYOPHYLLALES. Simmondsiaceae: 26. *Simmondsia chinensis*. **CUCURBITALES. Cucurbitaceae: 27.** *Citrullus lanatus*; **28.** *Cucumis dipsaceus*; **29.** *C. melo*; **30.** *Cucurbita maxima*; **31.** *C. pepo*; **32.** *Lagenaria siceraria*; **33.** *Momordica charantia*. x1000 (*= x400; ½= 50% reduced, ¼= 75% reduced)

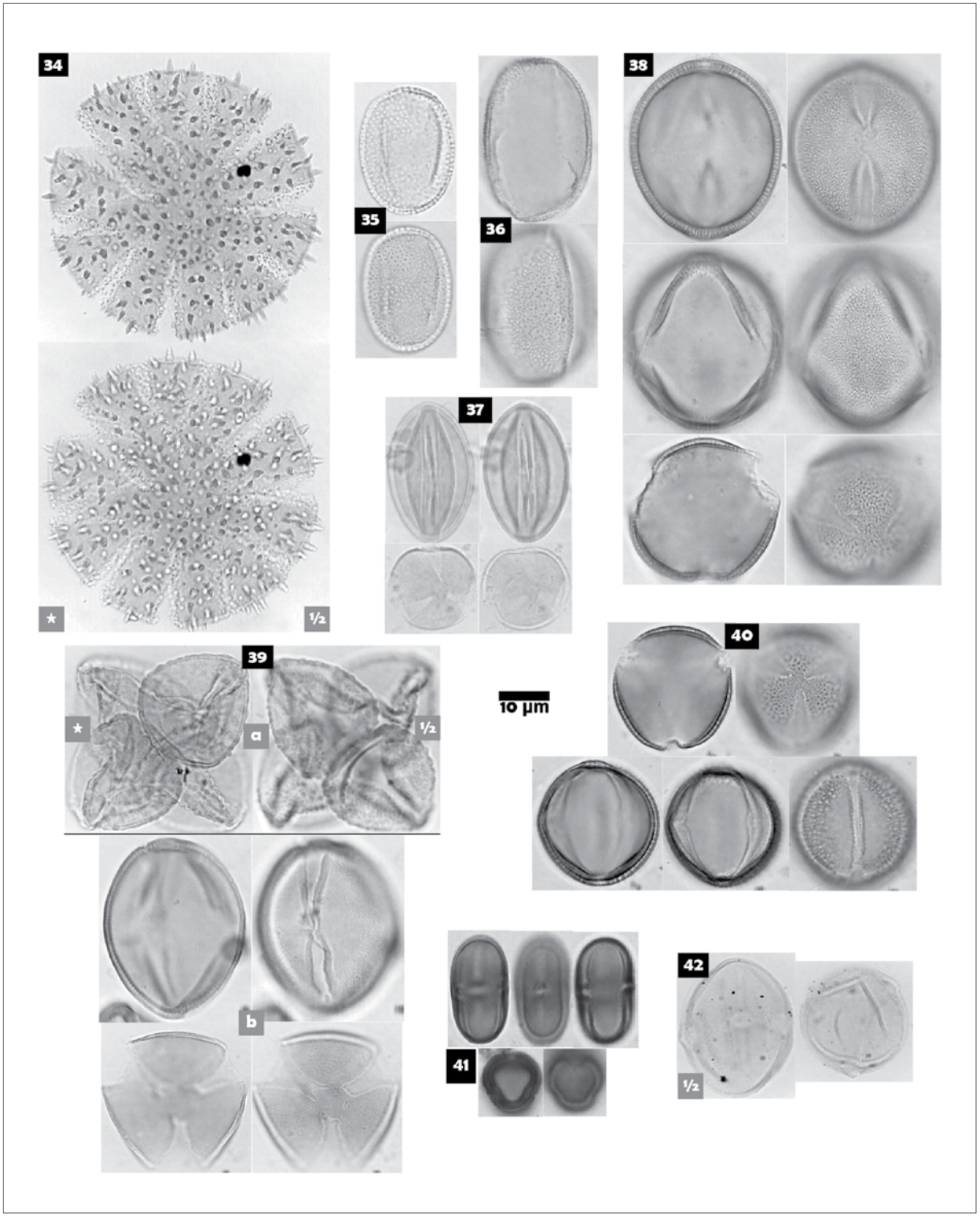


PLATE 5

CUCURBITALES. Cucurbitaceae: 34. *Sechium edule.* **DIOSCOREALES. Dioscoreaceae: 35.** *Dioscorea salvadoren-sis;* **36.** *D. trifida*. **ERICALES. Actinidiaceae: 37.** *Actinidia kolomikta*. **Lecythidaceae: 38.** *Bertholletia excelsa;* **39.** *Couroupita guianensis* (**a**= tetrad condition; **b**= monad condition); **40.** *Lecythis pisonis.* **Sapotaceae: 41.** *Chrysophyllum cainito;* **42.** *Madhuca malaccensis;* x1000 (*= x 400; ½= 50% reduced)

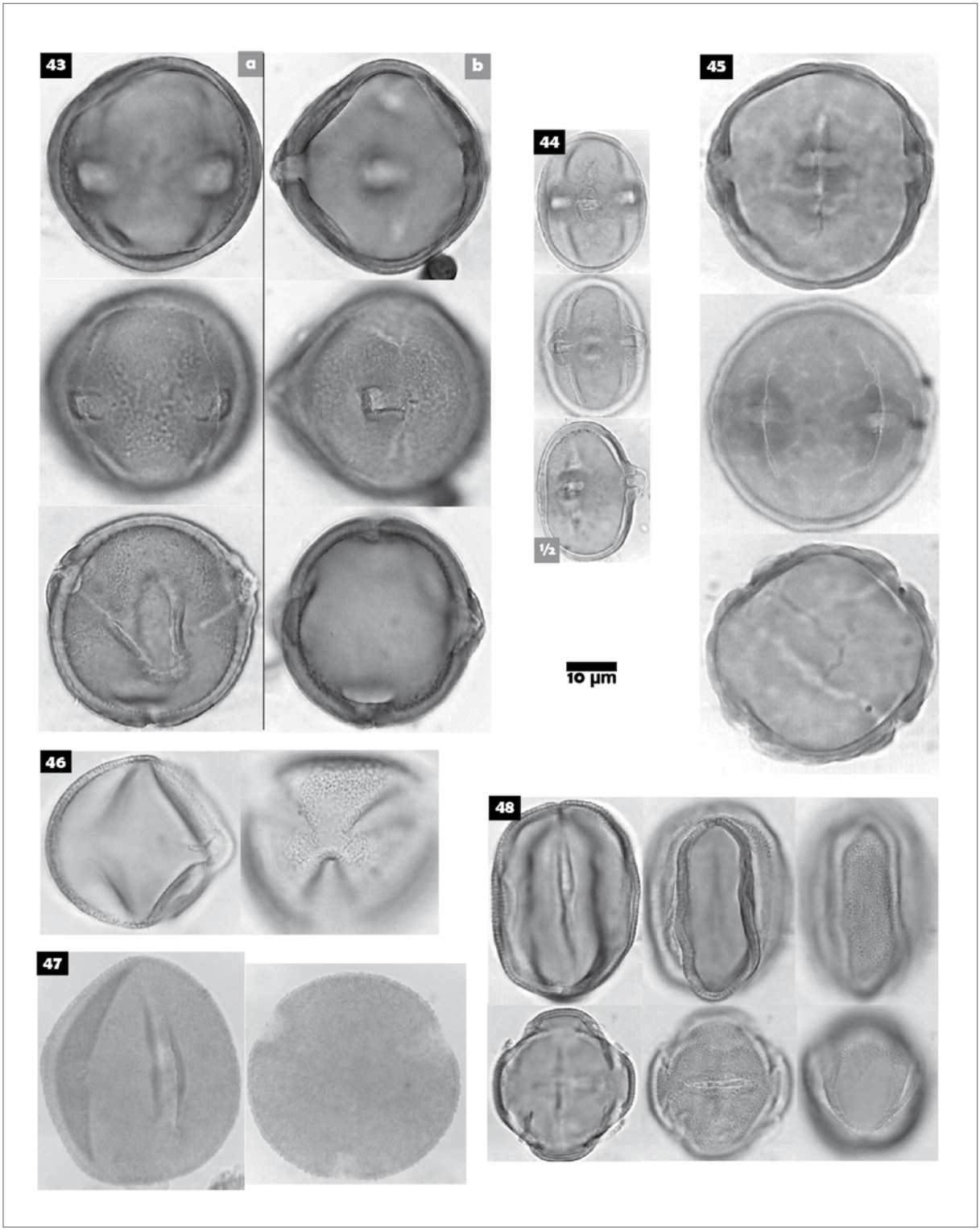


PLATE 6

ERICALES. Lecythidaceae: 43. *Manilkara zapotilla* (**a**= tricolporate condition; **b**= stephanocolporate condition); **44.** *Pouteria caimito* var. *laurifolia*; **45.** *P. campechiana*. **Theaceae: 46.** *Camellia* sp. **FABALES. Fabaceae-Caesalpinioideae: 47.** *Tamarindus indica*. **Fabaceae-Faboideae: 48.** *Arachys hypogaea*. x1000 (**½**= 50% reduced)

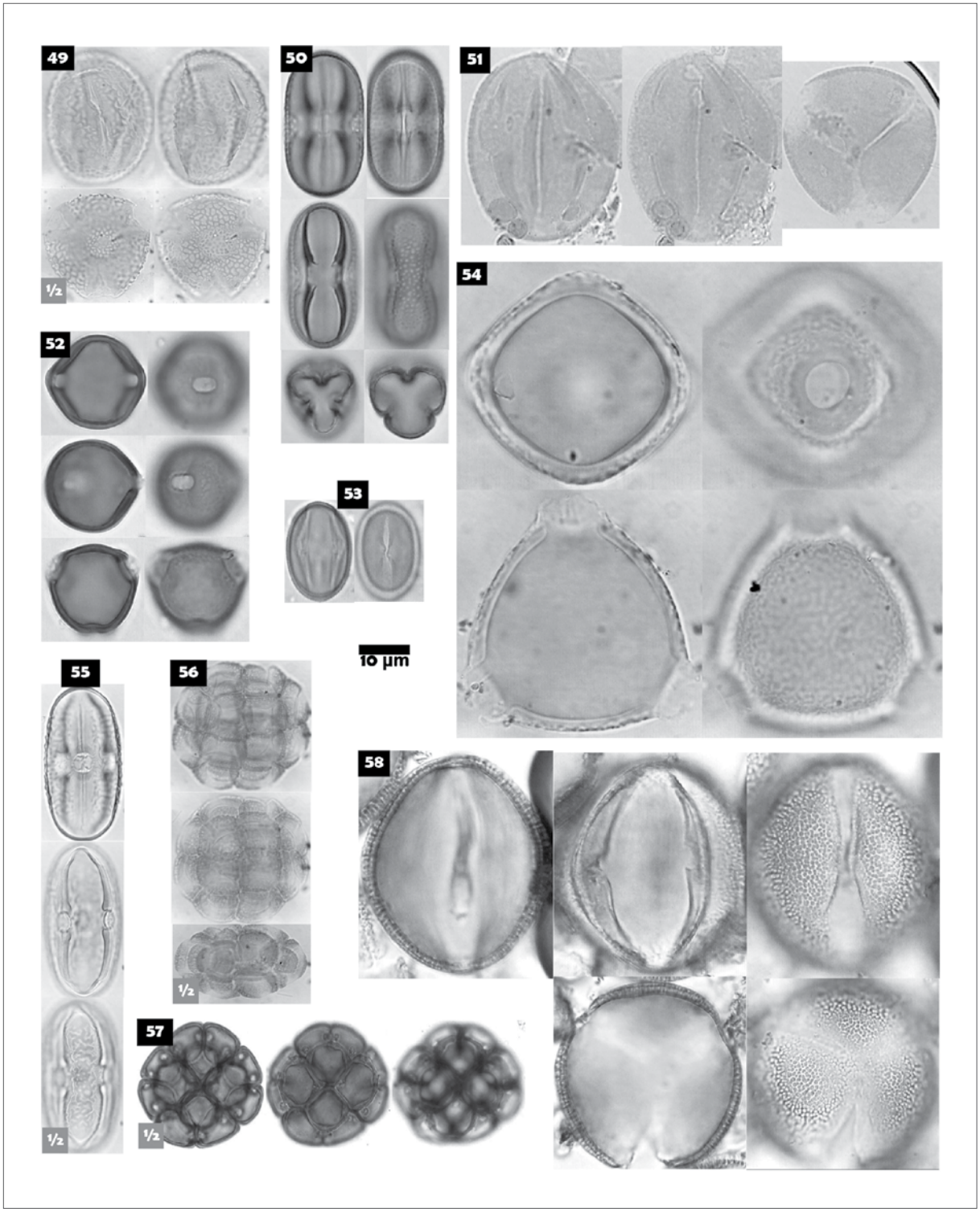


PLATE 7

FABALES. Fabaceae-Faboideae: 49. *Cajanus cajan*; **50.** *Crotalaria juncea*; **51.** *Derris fe-rruginea*; **52.** *Glycine max*; **53.** *Myroxylon balsamum*; **54.** *Phaseolus vulgaris*; **55.** *Vicia faba*. **Fabaceae-Mimosoideae: 56.** *Acacia farnesiana*; **57.** *Adenanthera pavonina*; **58.** *Leucaena esculenta*. x1000 (½= 50% reduced)

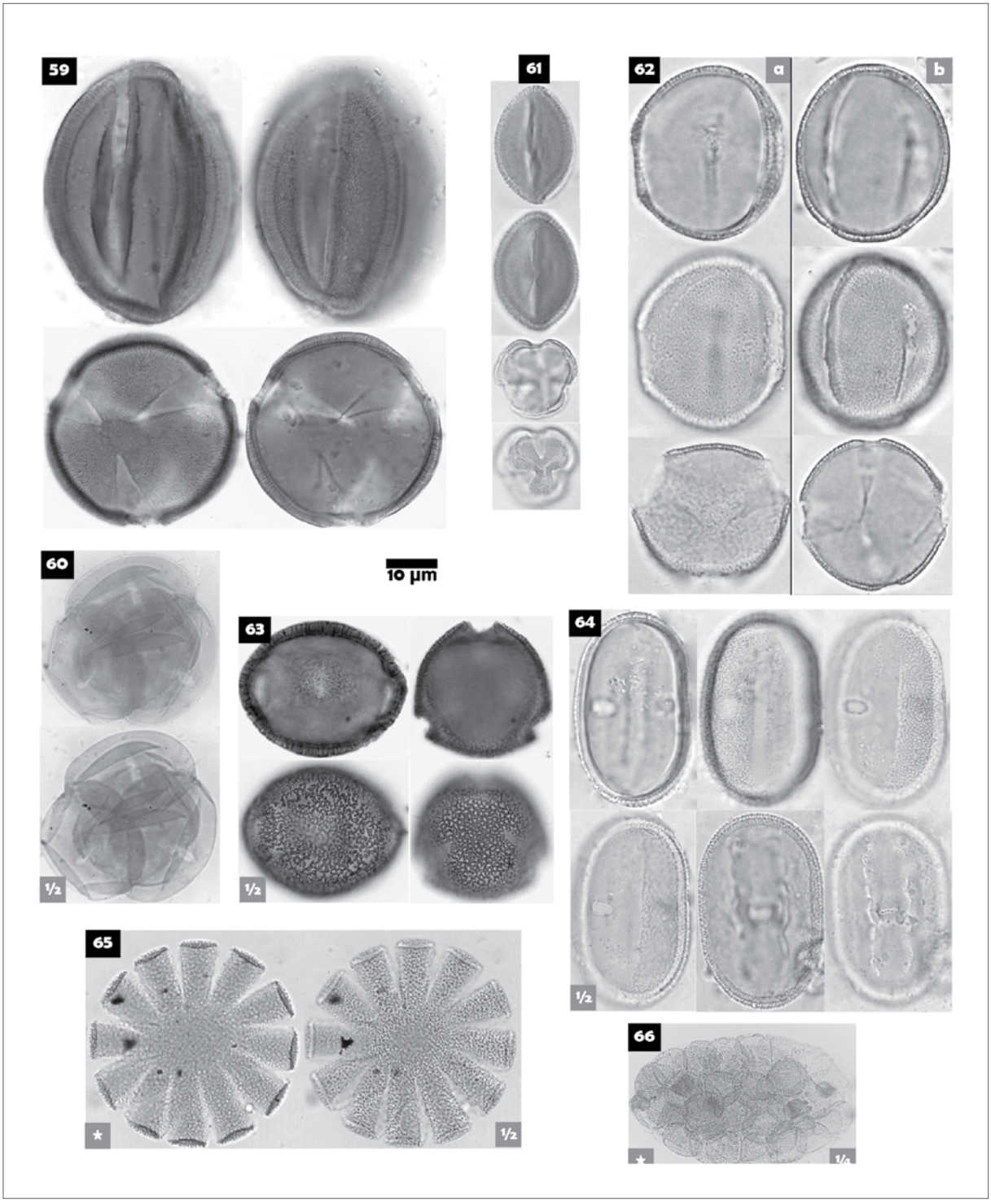


PLATE 8

FABALES. Fabaceae-Mimosoideae: 59. *L. leucocephala*. **GENTIANALES. Apocynaceae: 60.** *Willughbeia lu-zonensis*. **Rubiaceae. 61.** *Cinchona calisaya*; **62.** *Coffea arabica* (**a**= tricolporate condition, **b**= stephanocolporate condi-tion); **63.** *Morinda citrifolia*. **LAMIALES. Acanthaceae: 64.** *Adathoda vasica*. **Pedaliaceae: 65.** *Sesamum indicum*. **LAURA-LES. Lauraceae: 66.** *Cinnamomum bodinieri* (massulae). x1000 (*****= x400, reductions: **½**= 50% **1/4**= 75%)

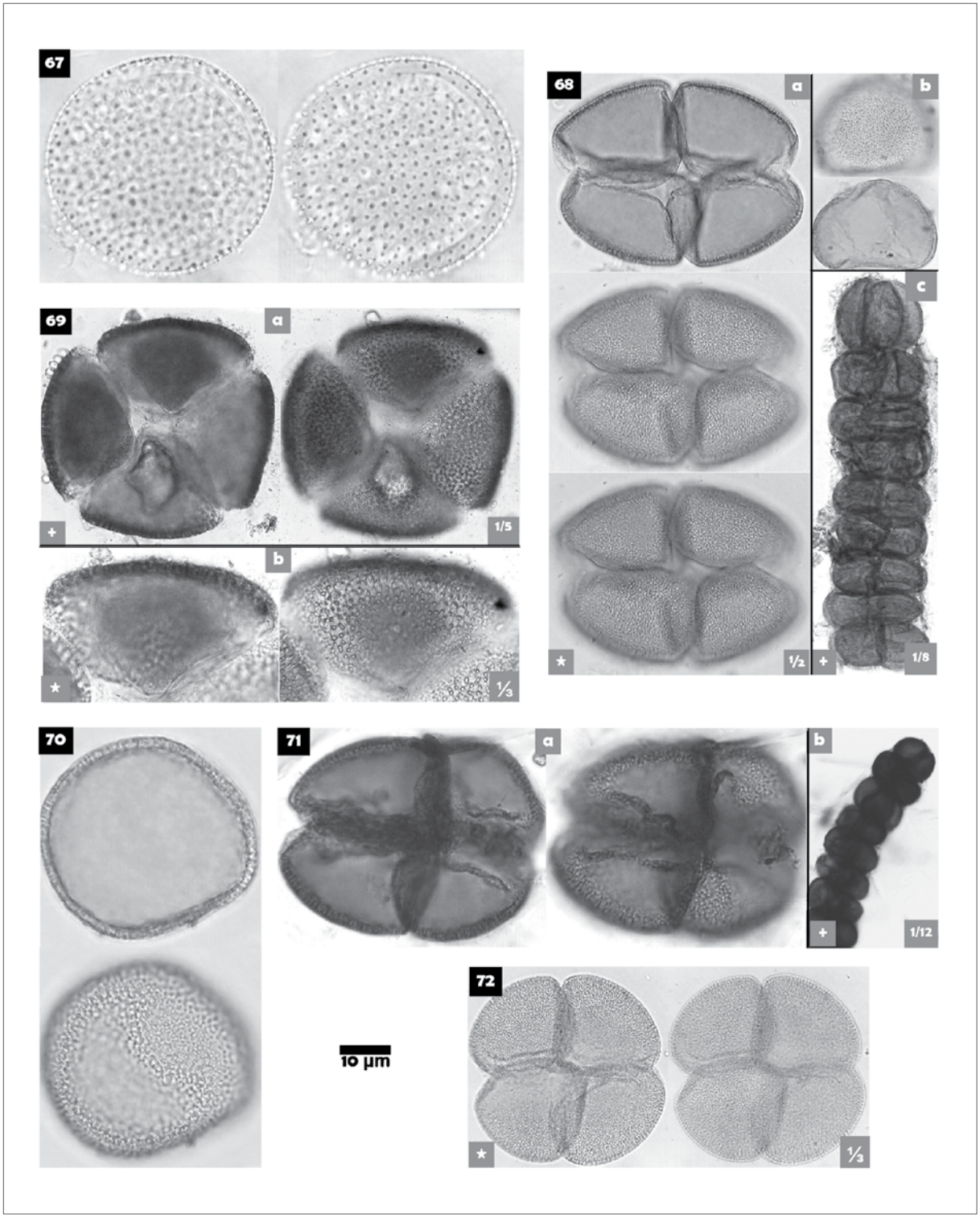


PLATE 9

LAURALES. Lauraceae: 67. *Persea Americana*. **MAGNOLIALES. Annonaceae: 68.** *Annona cherimolia*; **69.** *A. muricata*; **70.** *A. neosalicifolia*; **71.** *A. reticulata*; **72.** *A. squamosa*. x1000 (**a**= tetrad form; **b**= monad form; **c**= massulae; ***=** x400, **+**= x200; reductions: **½**= 50%; **1/3**= 66%; **1/5**= 80%; **1/8**= 85.5%; **1/12**= 91.5%)

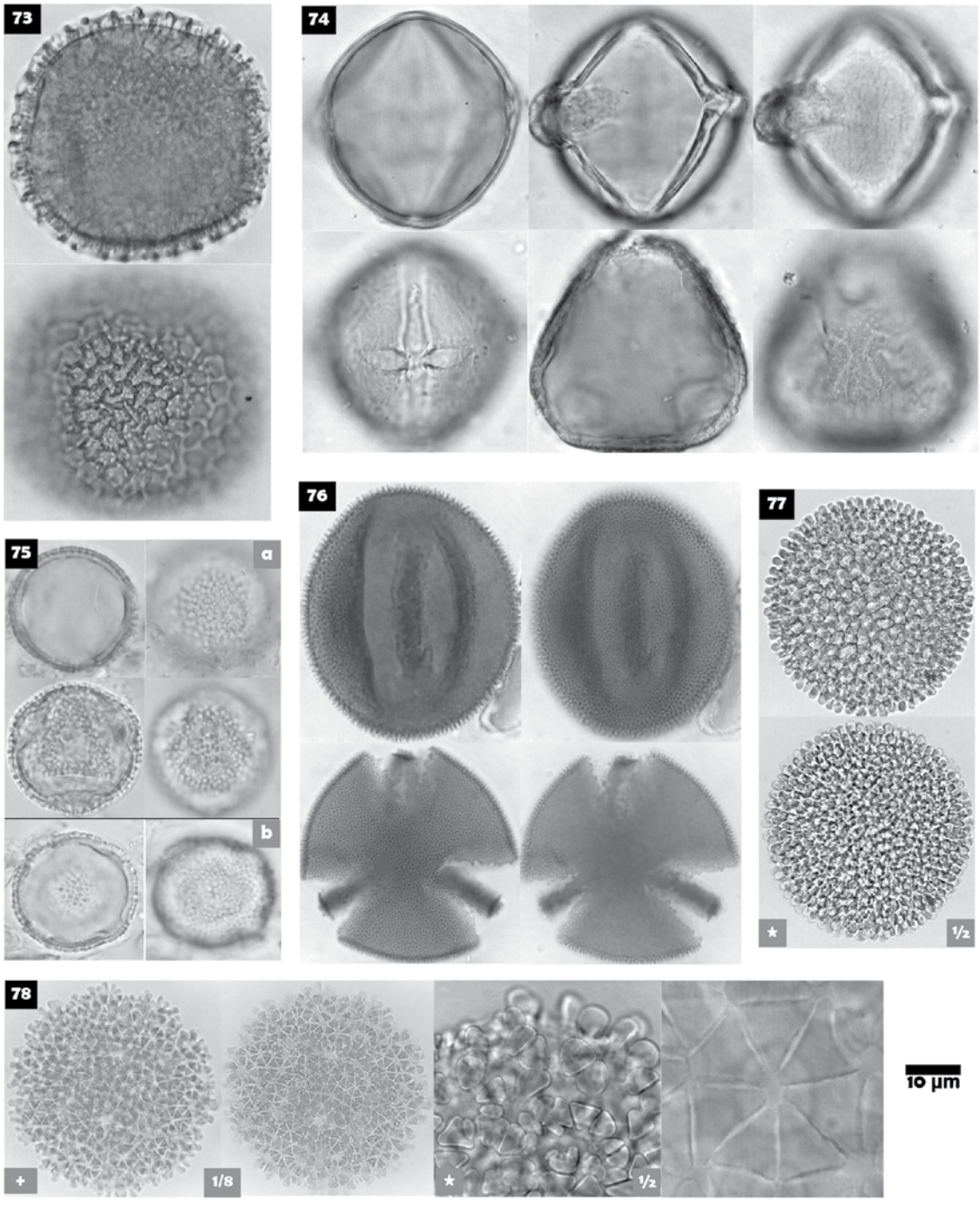


PLATE 10

MAGNOLIALES. Myristicaceae: 73. *Myristica fragans*. **MALPIGHIALES. Chrysobalanaceae: 74.** *Chrysobalanus icaco*. **Clusiaceae: 75.** *Garcinia binucao*. **Euphorbiaceae: 76.** *Hevea brasiliensis*; **77.** *Jatropha curcas*; **78.** *Manihot escu-lenta*. x1000 (**a**= tricolporate condition, **b**= stephanocolporate condition; *****= x400; **+**= x200; reductions: **1/2**= 50%; **1/8**= 85.5%)

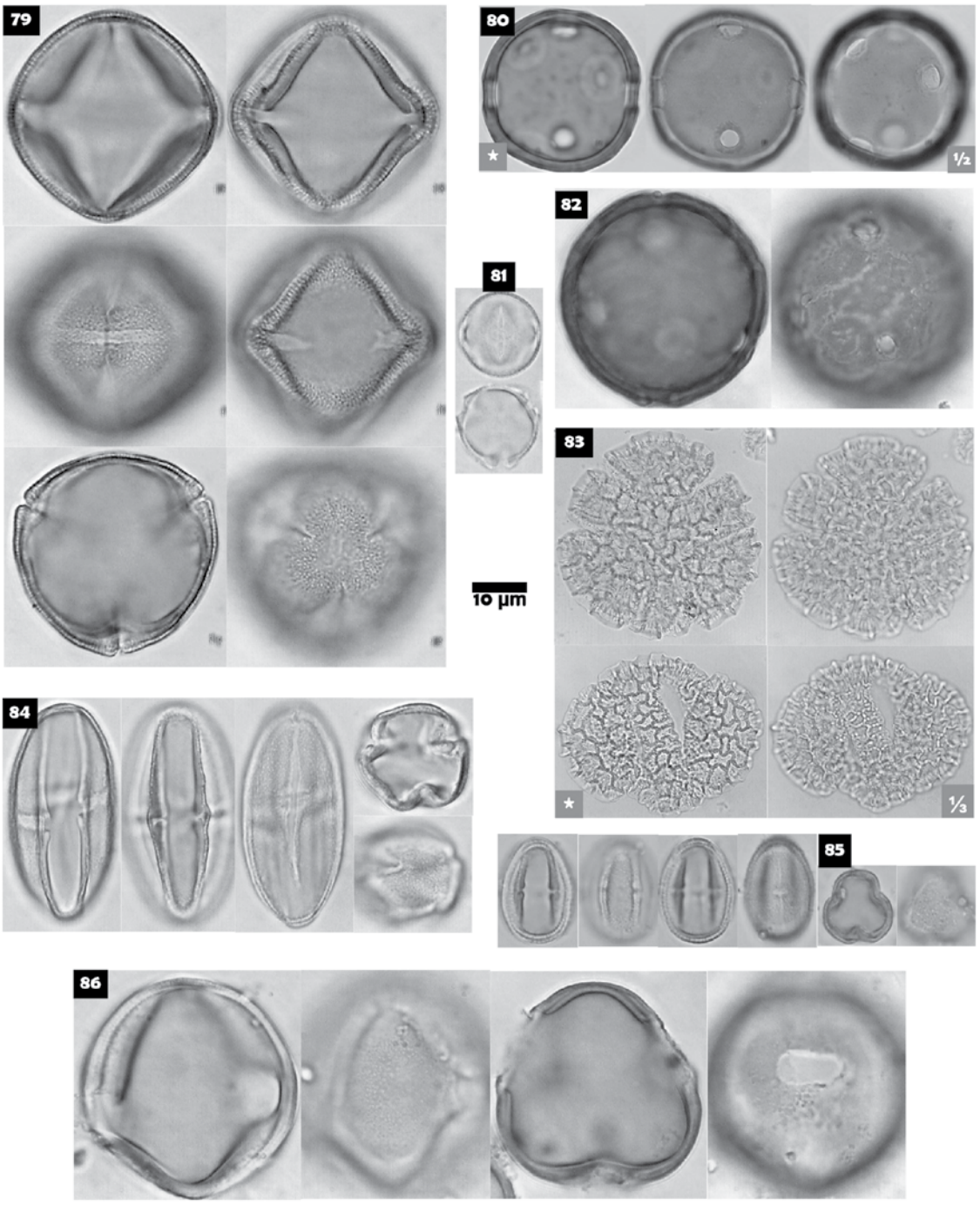


PLATE 11

MALPIGHIALES. Euphorbiaceae: 79. *Ricinus communis*. **Malpighiaceae: 80.** *Bunchosia palmeri*; **81.** *Byrsonima crassifolia*; **82.** *Malpighia emarginata*. **Passifloraceae: 83.** *Passiflora edulis*. **Phyllanthaceae: 84.** *Antidesma bunius*; **85.** *Baccaurea bracteata*. **MALVALES. Bixaceae: 86.** *Bixa orellana*. x1000 (*****= x400; **1/2**= 50%. reduced)

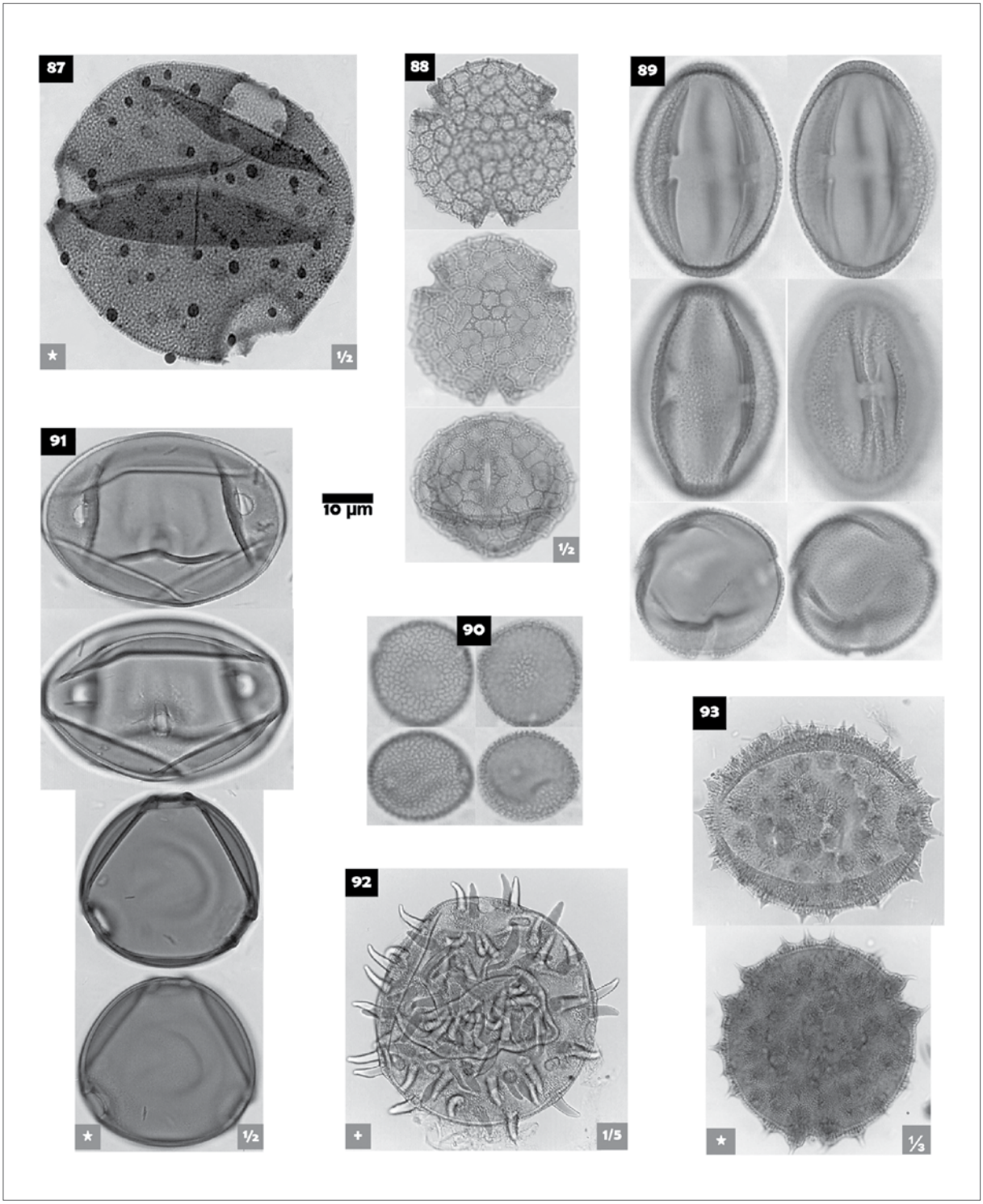


PLATE 12

MALVALES. Malvaceae-Bombacoideae: 87. *Adansonia digitata*; **88.** *Ceiba pentandra*. **Malvaceae-Grewioideae: 89.** *Cola acuminata*; **90.** *Theobroma cacao*. **Malvaceae-Helicterioideae: 91.** *Durio zibethinus*. **Malvaceae-Malvoideae: 92.** *Abelmoschus esculentus*; **93.** *Abutilon angulatum*. x1000 (*****= x400; **+**= x200; reductions: **1/2**= 50%; **1/3**= 66.6%; **1/5**= 80%)

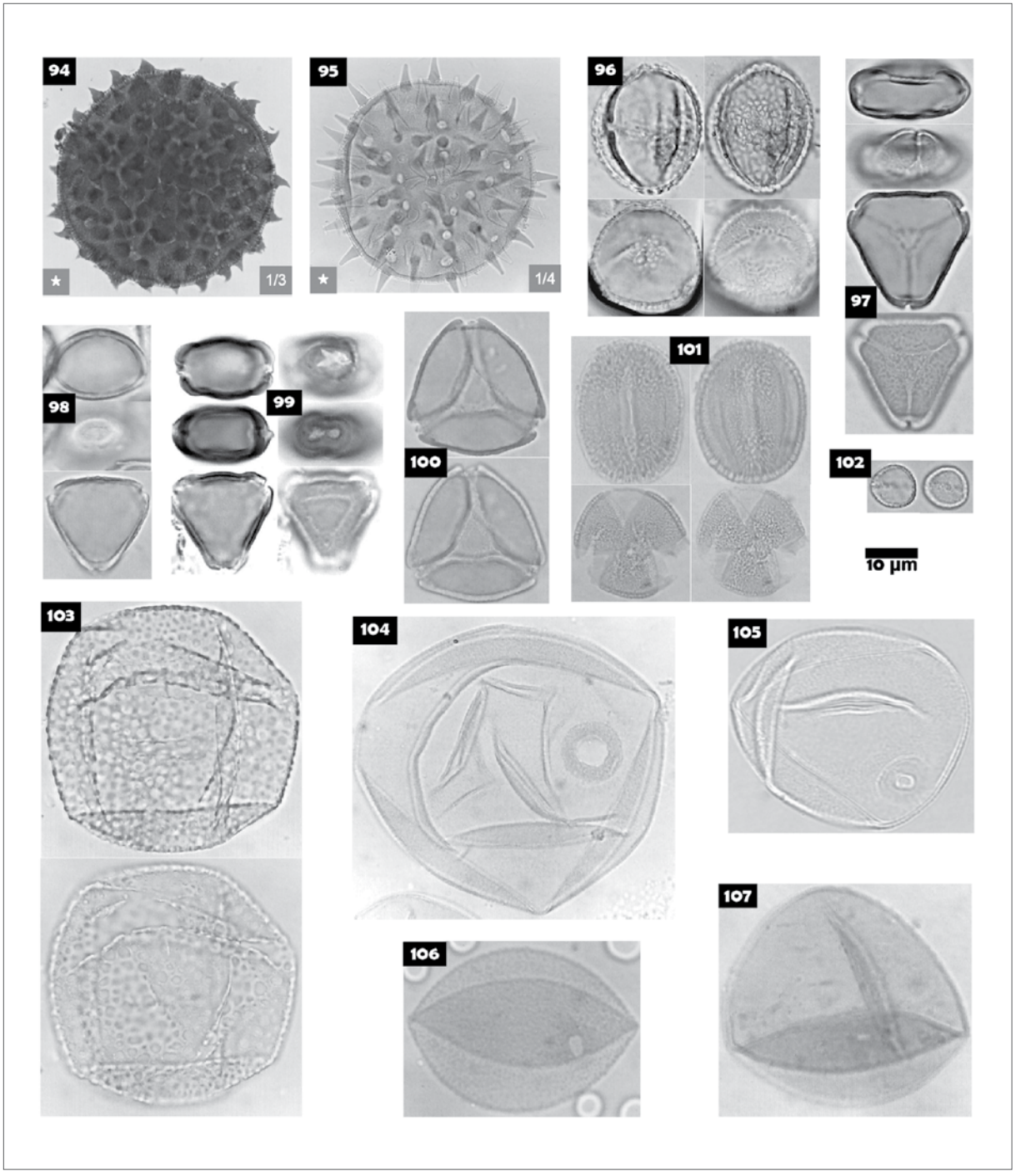


PLATE 13

MALVALES. Malvaceae-Malvoideae: 94. *Gossipium barbadense*; **95.** *Hibiscus ovalifolius*. **Malvaceae-Sterculioideae: 96.** *Herrania purpurea*. **MYRTALES. Myrtaceae: 97.** *Acca sellowiana*; **98.** *Psidium guajava*; **99.** *Syzygium aromaticum*; **100.** *S. jambos.* **OXALIDALES. Oxalidaceae: 101.** *Averrhoa carambola*. **PIPERALES. Piperaceae: 102.** *Piper hispidum*. **POALES. Bromeliaceae: 103.** *Ananas comosus*. **Poaceae: 104.** *Bambusa arundinacea*; **105.** *Digitaria ciliaris*; **106.** *Eleusine indica*; **107.** *Eragrostis prolifera*. X1000 (*****= x400; reductions: **1/3**= 66.6%; **1/4**= 75%)

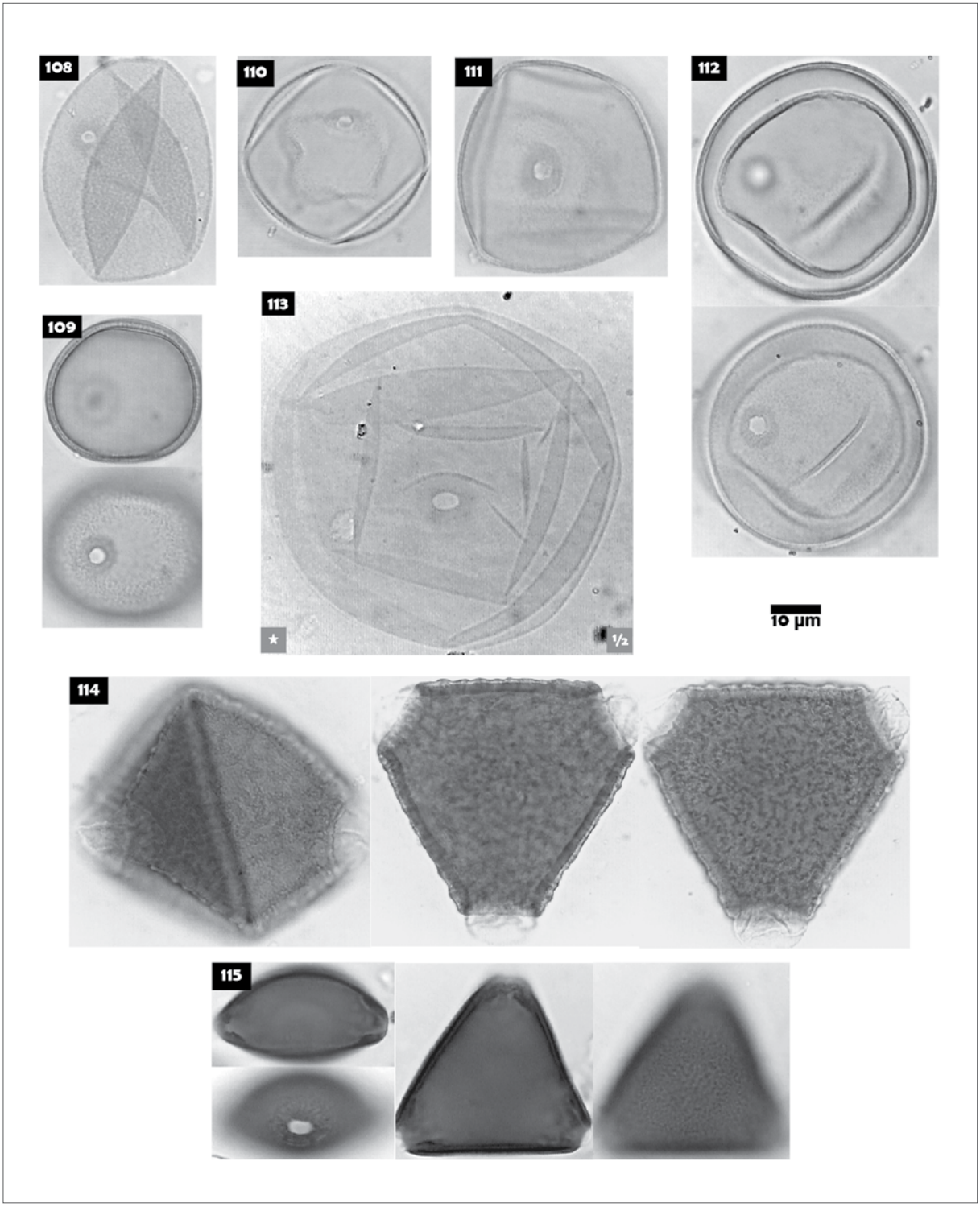


PLATE 14

POALES. Poaceae: 108. *Isachne conferta*; **109.** *Panicum virgatum*; **110.** *Paspalum paniculatum*; **111.** *Pennise-tum purpureum*; **112.** *Saccharum spontaneum*; **113.** *Zea mays*. **PROTEALES. Proteaceae: 115.** *Grevillea robusta*; **116.** *Macadamia integrifolia*. x1000 (*****= x400; **1/2**= 50% reduced)

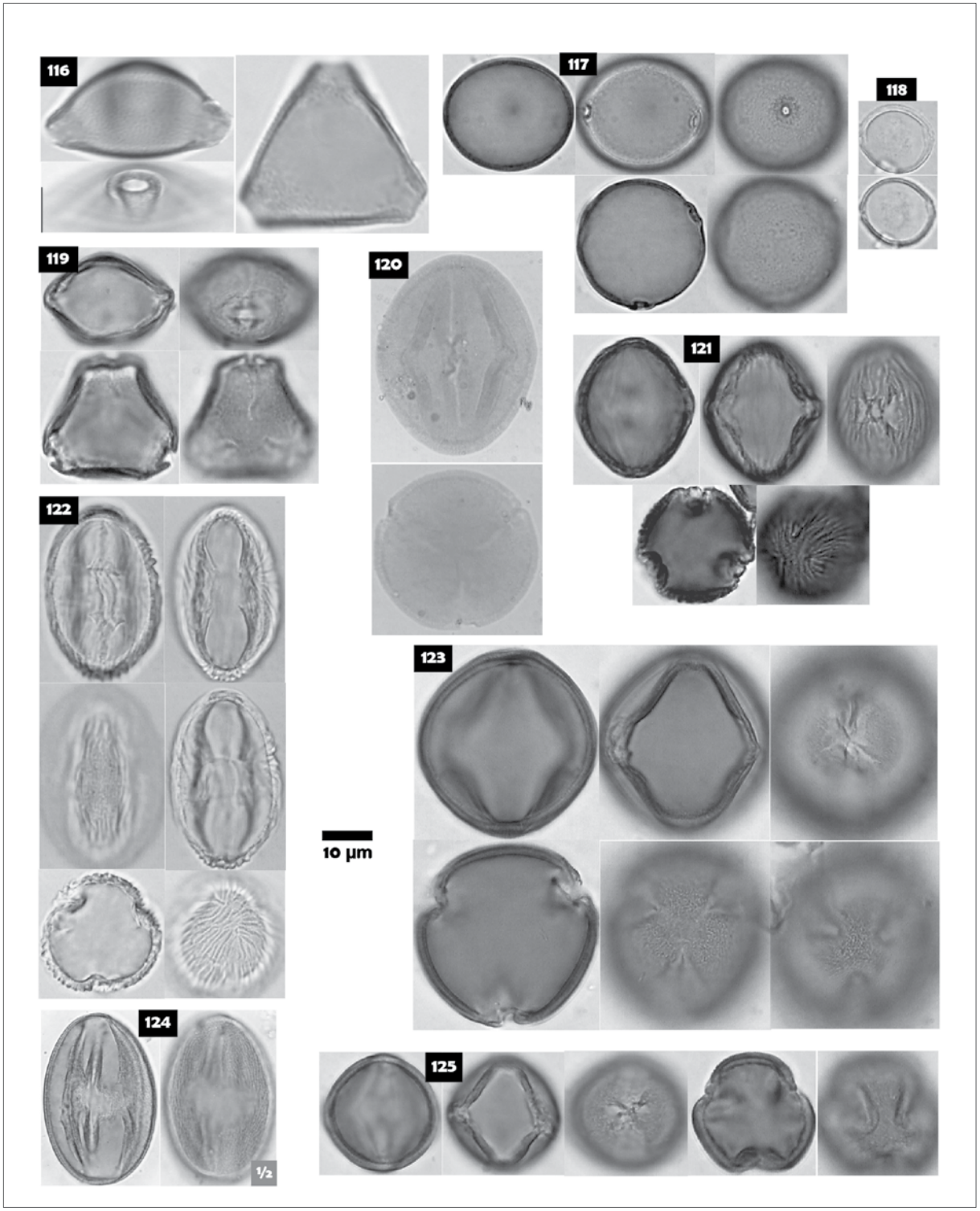


PLATE 15

PROTEALES. Proteaceae: 116. *M. tetraphylla*. **ROSALES. Cannabaceae: 117.** *Cannabis sativa*. **Moraceae: 118.** *Artocarpus altilis*. **Rhamnaceae: 119.** *Ziziphus jujuba*. **Rosaceae: 120.** *Eriobotrya japonica*; **121.** *Fragaria chiloensis*; **122.** *Fragaria* sp. **123.** *Malus bracteata*; **124.** *Prunus dulcis*; **125.** *Rubus hispidus*. x1000 (**1/2**= 50% reduced)

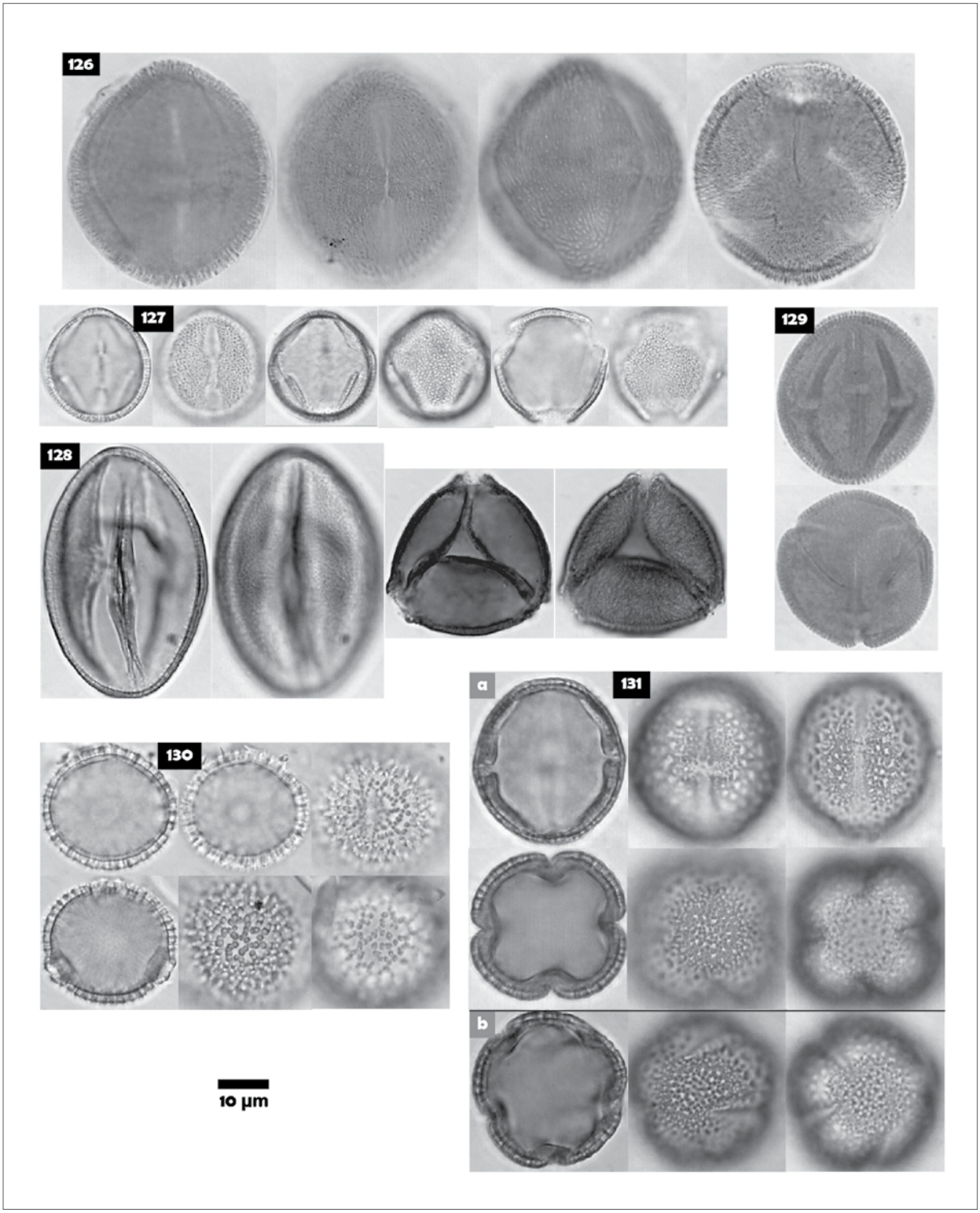


PLATE 16

SAPINDALES. Anacardiaceae: 126. *Anacardium occidentale*; **127.** *Mangifera indica*; **128.** *Spondias dulcis*; **129.** *S. mombin*. **Burseraceae: 130.** *Commiphora baluensis*. **Rutaceae: 131.** *Citrus aurantiifolia* (**a**= 4 aperturate condition, **b**= 5 aperturate condition). x1000

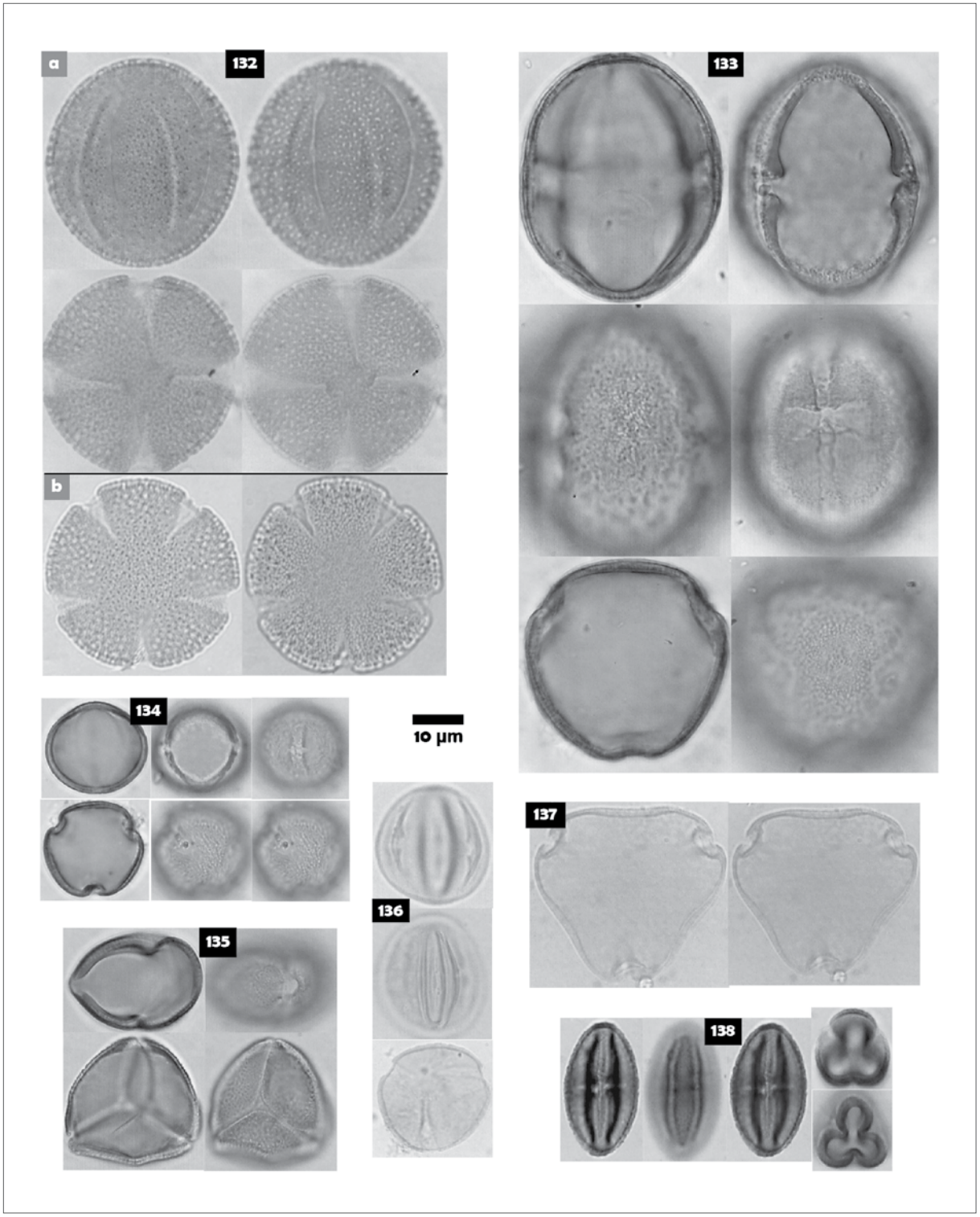


PLATE 17

SAPINDALES. Rutaceae: 132. *C. reticulata* (**a**= 4 aperturate condition, **b**= 5 aperturate condition); **133.** *Tripha-sia trifoliata*. **Sapindaceae: 134.** *Blighia sapida*; **135.** *Melicoccus bijugatus*; **136.** *Nephelium mutabile*; **137.** *Talisia nervo-sa*. **Simaroubaceae: 138.** *Simarouba glauca*. X1000

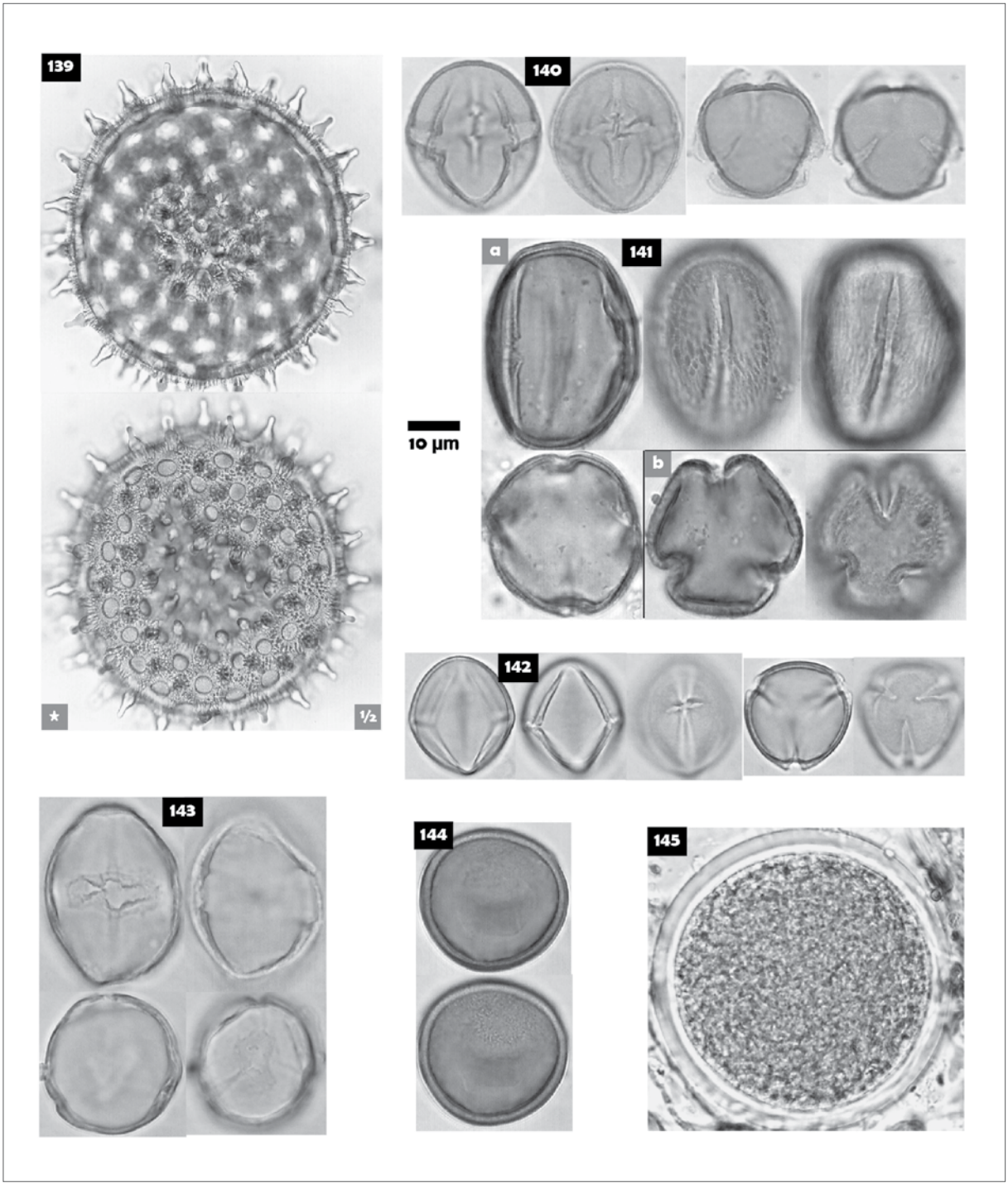


PLATE 18

SOLANALES. Convolvulaceae: 139. *Ipomoea batatas*. **Solanaceae: 140**. *Capsicum annuum*; **141.** *Nicotiana ta-bacum* (**a**= 4 aperturate condition; **b**= tricolporate condition); **142.** *Solanum lycopersicum*; **143.** *S. quitoense*. **VITALES. Vitaceae: 144.** *Vitis rotundifolia*. **ZINGIBERALES. Zingiberaceae. 145.** *Alpinia purpurata*. x1000 (*= x400; **1/2**= 50% reduced)

GLOSSARY

A

Abiotic
not involving living things

Abortion
dropping of fertilized or unfertilized fruit from the mother plant

Achene
small, one-seeded fruit in which the thin walls are dry

Agamospermy
seed formation without sexual reproduction

Aggregate fruit
consisting of the many separate carpels of one flower

Allogamy
fertilization between pollen and ovules of different flowers

Alternate bearing
fruit bearing on a plant, which fluctuates from high to low in successive years (e.g. kiwi fruit and some apples)

Androdioecy
where male and hermaphrodite genets co-exist

Androecium
male unit or stamens, as a unit of flower

Andromonoecy/andromonecious
a hermaphrodite bears male and hermaphrodite flowers

Anemophily/anemophilous
wind-pollinated; plants that normally shed pollen carried by wind

Angiosperm
flowering plant – a major group of seed plants in which seeds develop within a closed ovary

Anther
part of the floral stamen that normally produces pollen

Anthesis
when flower is fully open and functional

Anthophilous
flower-loving – applied to an animal that can be a pollinator

Apogamety
autonomous development of a nucleus, apart from the egg nucleus into an embryo in an agamosperm

Apomixis/apomictic
non-sexual reproduction of a plant, including both forms in which no seeds are produced (vegetative reproduction) and those in which seeds are produced (agamospermy)

Apospory
elimination of spore formation from the life cycle with the formation of the gametophyte from vegetative tissues, usually the nucellus, not from spore

Archesporium
tissue within the nucellus of a young ovule that gives rise to the embryo sac mother cell, female meiosis and the embryo sac

Aril
network or covering of a seed from the point of seed attachment

Autogamy/autogamous
self-fertilizated (within a flower) without the need of a pollinator

Automixis
fusion of nuclei within the embryo sac

B

Berry
fleshy fruit with skin-like covering, having one to many seeds (but no stone), developed from a single pistil

Biocide
chemical that substantially debilitates or interrupts life cycle, reproduction or poisons a fungus, mite, insect, animal, plant or other living organism – includes miticide, acaricide, fungicide, herbicide, insecticide, germicide, bacteriacide, etc.

Bract
a small leaf or scalelike structure near the base of a flower

Brood parasite
organism feeding on and potentially killing a host organism in its egg, larval or pupal stage

Buzz pollination
pollen collection by a bee using bursts of wing muscle vibration while clinging to the anther— ejecting pollen from the anther apical pore, or shedding pollen from a normal anther

C

CCD
colony collapse disorder, reaction in honey bees to sublethal doses of imidacloprid and clothianidin neonicitinoid pesticide, resulting in dwindling colony size and death during winter; also refers to abrupt disappearance of most adult bees from hive, from multiple or unknown causes

Calyx
sepals or outer whorl of the perianth

Cantharophily/cantharophilous
pollination by beetles

Caprification
pollination of figs with certain tiny wasps (Agaonidae)

Capsule
dry, dehiscent seedpod from a flower with a compound pistil

Carpel
unit formed by ovary within a compound pistil

Catkin
spike or pendulous inflorescence made up of flowers of one sex

Certified seed
progeny of foundation, registered or certified seed, for example *Sweet Gold*® (a registered tomato cultivar) approved by official agency (see also "cultivar")

Chalaza
basal part of an ovule where it is attached to a stalk (funiculus)

Chalazogamy
entry of the pollen tube through the chalaza of the ovule

Chalkbrood
fungus *Ascosphaera* that infects and kills immature bees in open or closed brood cells (for honey bees, this is *A. apis*; *A. aggregata* attacks leafcutting bees)

Chasmogamous
having flowers in which pollination occurs while the flower is open

Chiropterophily/chiropterophilous
pollinated by bats

Cleistogamy/cleistogamous
having flowers that are self-fertilized without opening (opposite of chasmogamous)

Clone
one or more individuals obtained from a single parent by vegetative reproduction (i.e. clone plants are ramets that belong to the same genet)

Comb
bee nest component where eggs are hatched and develop into adults within brood cells; also cells where honey and pollen is stored in nests of *Apis* (honey bee species)

Corbicula/corbiculae
portion of the bee hind leg of certain Apidae on which pollen for brood is carried to the nest

Compatible
capable of producing fertile offspring between plants

Corolla
whole sheath of petals of a flower

Cross
union of different cultivars of the same species

Cross compatible
capable of being fertilized with pollen of a different variety

Cross pollination
pollination in which the pollen is transferred to another flower on a different plant of the same species

Crop fidelity
proportion of bees from a hive that forage only on the target crop

Cultivar
(similar to variety) international name for group of cultivated plants which, when reproduced sexually or asexually, retain their distinguishing characteristics (but are not necessarily a distinctive botanical species)

D

Deceit pollination
flowers offering no food or other reward receive pollinator visits by resembling rewarding flowers

Dehisce/dehiscence
opening of a seed pod or anther and release of contents

Dichogamy
floral condition in which male and female parts mature at different times, preventing self-pollination – in synchronous dichogamy the stamens and pistils reach maturity at different times in definite periods, as in the avocado, *Persea*

Dicliny
separation of sexes among flowers; not all genets in a population are hermaphroditic, such that males, females or both occur

Dioecy/dioecious
having separate sexes and two kinds of genets, such that stamens or male parts and pistils or female parts are on different plants.

Diploid
having a double set of chromosomes, usually one set from each parent

Diplospory
development of an apomictic embryo sac by mitosis or modified meioses of the archesporial cells

Drupe
succulent or fleshy fruit having one seed enclosed in stony endocarp

E

Elaiophore
floral oil-secreting gland

Embryo
rudimentary organism

Embryo sac
female gametophyte of flowering plants contained within ovule, developing from the surviving megaspore after female meiosis, and containing eight nuclei

Embryony
development of an embryo

Endocarp
inner layer of the pericarp

Endosperm
food reserve tissue in a seed, triploid in angiosperms, formed from a fertilizing sperm cell combining with the fused polar nuclei

Entomophily/entomophilous
pollination by insects

Ethephon
commercial horticultural product (2-chloroethyl phosphonic acid) that breaks down to release ethylene gas, which promotes uniform ripening in crops such as coffee, apples, figs, cherries, blueberries and pineapple

Ethylene
gas (C2H2) naturally produced by plants but sometimes applied commercially to induce flowering (pineapple) or increase production of female flowers (cucumber, pumpkin)

Exocarp
outermost layer of the fruit wall

Exserted
protruding beyond the margin of envelope or corolla

Exrareproductive nectary
("extrafloral nectary") a nectary on part of the plant outside of the flower

F

Female flower
one with a stigma and that bears fruit

Fertigation
irrigation with water combined with fertilizer

Fertile
capable of bearing fruit

Fertilization
union of male and female gametes to produce a zygote

Filament
hair-like element of stamen supporting the anther

Floral nectary (reproductive nectary)
nectary within a flower

Floret
one small flower

Flower
much shortened axis (the receptacle) bearing whorls of appendages concerned with reproduction – sepals, petals, stamens and carpels

Fruit
mature ovary with all its parts and adherents

G

Gamete
sexual cell

Geitonogamy
fertilization between pollen and ovules of different flowers on the same genet (plant)

Gametophyte
gamete-producing phase of algae, bryophytes and ferns, displaying alternation of generations; composed of haploid cells only

Genet
genetically individual plant, resulting from a single sexual fusion or zygote, and consisting of one to many ramets

Germination
development of plant from seed

Gynodioecy/gynodioecious
where female and hermaphrodite genets co-exist

Gynoecious
producing only or predominantly pistillate flowers

Gynoecium
female parts, or carpels, of a flower

Gynomonoecy/gynomonoecious
hermaphrodite bears both female and hermaphrodite flowers

H

Haploid
having a single set of chromosomes from a single parent; usually refers to a germ cell or gamete

Herkogamy/herkogamous
separation of anthers and stigma within a flower such that autogamy cannot occur in the absence of a pollinator

Hermaphrodite
(see perfect flower)

Heterogamy/heterogamous
a plant having two or more kinds of flowers

Heterostyly/heterostylous
a genetically determined condition in which stamens and styles come in two or three distinctive lengths, and individual flowers have stigmas and styles of different lengths – thus promoting crossing (e.g. distyly and tristyly)

Hive beetle
Aethina tumida (Nitidulidae), pest of stingless bees and honey bees which consumes brood and pollen in bee nests

Homogamy/homogamous
coincidence of anther dehiscence and stigma receptivity within a flower, so that autogamy is possible if herkogamy does not exist

Honey bee
bee that lives in a colony, has a queen, males and workers, and concentrates nectar, by evaporating water content, to make honey – normally 60-80% sugar; honey-making bees include the "honeybee" or honey bee *Apis* spp., stingless honey bees or stingless bees (Meliponini) and bumblebees, *Bombus* spp.

I

Indehiscent
not splitting open by its valves

Indeterminate
continuing to grow after flowering starts

Inflorescence
flower or group of flowers on a stem

Intersterile
failure to set fruit when flowers are crossed with pollen of certain other cultivars of the same species

L

Legume
one-celled fruit (pod) usually dehiscing down both sutures, and having the seed attached along a ventral suture

Locule
compartment or cell of ovary, anther or fruit

M

Male sterile
flowers lack stamens or viable pollen – commercially developed to increase outcrossing or fruit production by removing male function

Megaspore
in plant species producing two kinds of spores, spore-type that gives rise to female gametophyte

Meiosis
the reduction division of chromosomes, giving rise to two gametes, each with half the chromosomes of the parent cell

Melittophily/melittophilous
pollinated by bees

Mesocarp
the middle layer of pericarp or fruit wall

Micropyle
the pore or hole in the end of an egg through which sperm enters to fertilize the egg

Miticide/Acaracide
chemical biocide applied to rid plants or animals of mites (Acari)

Mitosis
the ordinary changes through which a cell nucleus passes during cell multiplication, producing daughter cells of chromosome number equal to the parent cell

Mixed inflorescence
branched inflorescence with both racemose and cymose components, as in grape and mango, in which the main inflorescence axis is racemose, and the small ultimate branches cymose

Monoecy/monoecious
having separate male and female flowers, but on the same plant

Multiple fruit
fruit consisting of the compressed fleshy fruitlets of the many flowers of a compact inflorescence in which the axis also becomes fleshy, as in pineapple, custard apple and *Monstera*

Myophily/myophilous
pollinated by flies

N

Nectar
sweet liquid produced in the nectary of plant, usually within a flower

Nectar guide
certain highly visible (to insects) ultraviolet-absorbing markings on a flower that guide or direct nectar feeders to nectar

Nectary
plant gland that secretes nectar

Neonicitinoid
neuro-active insecticide chemically similar to nicotine (tobacco plant), relatively harmless to mammals, although the most commonly used pesticide contains imidacloprid, which harms bee colonies (see "CCD")

Nosema
fungus parasite *Nosema* spp., with highly resistant spores, kills adult honey bees and other bees

Nucellus
central body of ovule containing the embryo-sac, which acts as a nurse to the archesporium

O

Oosphere
the unfertilized female gamete

Ornithophily/ornithophilous
pollinated by birds

Outcrossing
sexual reproduction between different genets, usually plants that are different individuals

Ovary
seedcase or part of the pistil, bearing ovules that develop into seed or fruit

Overbearing
excessive fruit production that damages the plant (breakage or mortality) or diminishes size and commercial quality of individual fruit

Ovule
structure that contains egg nucleus and develops into seed after fertilization of the egg cell within

P

Panicle
inflorescence with the main axis branched into an open racemose flower cluster

Papilionoceous
butterflylike, pealike flowers, with a large upper petal, two lateral wing petals, and two small united keel petals

Papillae
specialized epidermal cells of the stigma that receive the pollen grains

Parthenogenesis
production of new individuals from unfertilized egg cells

Parthenocarpy/parthenocarpic
development of a fruit without fertilization and therefore without seeds – as in navel orange, some figs, seedless grapes, pineapple and banana

Pedicel
stalk or stem of individual flower of inflorescence

Peduncle
primary flower stalk of an inflorescence

Perfect flower
bisexual or hermaphroditic; a flower having both stamens and pistil

Perianth
entire floral envelope including both corolla and calyx

Pericarp
ovary or fruit wall

Perisperm
storage tissue similar to the endosperm, but formed from the nucellus

Pesticide
chemical product used to remove unwanted plants, animals, fungi or other organisms (see also "biocide")

Petal
leaf or unit of a usually colored corolla or inner floral envelope

Petiole
leaf-stalk

Phorid fly
(Phoridae *Neohypocephala* and other genera) pest of stingless bees and honey bees, larvae consume brood and stored pollen, often killing colony

Pistil
normally central, seed-producing part of flower; usually consisting of ovary, style and stigma

Pistiilate
having pistils but no stamens

Placenta
surface or tissue part of ovary to which ovules become attached

Plant growth hormone
natural plant hormone used commercially to induce or improve seed and fruit production, including cytokinin, gibberellin, ethylene and auxin

Plant growth regulator
synthetic or natural plant hormone or non-nutrient chemical that influences growth and development, including 4-chlorophenoxy-acetic CID (4-CPA), similar to 2,4-D, used commercially to increase flowering and fruit set; another example is synthetic auxin naphthaleneacetic acid, used commercially to prevent fruit drop

Pollen
powdery grains produced by angiosperm anthers or microsporangia of gymnosperms, which contain the nucleus that fertilizes the oosphere to form a seed

Pollen robber
animal that damages the anther or its protective surrounding tissue to remove pollen, and does not contact the stigma

Pollen thief
animal that removes pollen without having contact with the stigma

Pollen tube
thin tubular outgrowth of pollen grain usually upon contact with stigma, and which penetrates style to ovary, permitting sperm nuclei to unite with egg cell

Pollenizer
plant source of compatible pollen for fertilizing receptive stigmas

Pollenkitt
outermost, pigmented oily layer of pollen grain

Pollinarium
in orchids and asclepiads, structure detached from the flower, bearing sacs of pollen grains, united to an adhesive disc that attaches to the pollinator

Pollinating
transferring pollen from anther to stigma

Pollination
placement of pollen on a stigma, the first step in fertilization

Pollination deficit
capacity for greater fruit and seed production (in a single season) with augmented pollination

Pollinator
animal that moves compatible pollen to a receptive stigma of the same plant species, such that fertilization and seed production can occur

Pod
monocarpellary fruit that dehisces down both sutures

Polyembryony
with more than one embryo in a fertilized ovule (e.g. mango)

Polygamy/polygamous
having both perfect (hermaphrodite) flowers and those of one sex (staminate or pistillate)

Polyembryonic
presence of more than one embryo in a fertilized ovule, formed adventitiously from the nucellus, for example mango (*Mangifera*)

Pome
fleshy fruit derived from several carpels, the receptacle and outer pericarp being fleshy, and the inner pericarp, papery

Poricidal
anther dehiscence occurs through an apical pore

Porogamy
entry of the pollen tube into the ovule via the micropyle

Proboscis/proboscides
tube-like or spongy feeding structure used by an insect to imbibe nectar or liquid

Protogynous-dichogamous
condition in which stigma receptivity precedes anther dehiscence within flower

Protandry/protandrous
flower in which anthers mature and release pollen before stigma is receptive

Protogyny/protogynous
flower in which the stigma is receptive before anthers release pollen

PSP
pollination service provider — a technical support service, soon to include more than *Megachile, Osmia, Tetragonula, Plebeia, Scaptotrigona, Apis, Melipona, Tetragonisca, Xylocopa* or *Trigonisca*

Psychophily/psychophilous
pollinated by butterflies and moths

R

Raceme
unbranched inflorescence

Ramet
physiologically independent individual (from one to many may compose a genet)

Receptacle
enlarged end of pedicel to which one or more flowers attached

Recruitment
behavioural process whereby foraging bee alerts/guides other workers of colony to field resource

Reproductives
among bees or social insects – males and queens (see also "workers")

Robbing
in pollination ecology, the destruction of at least part of a flower to obtain nectar, pollen or another resource

Roundup Ready™
glyphosate herbicide tolerant, usually applied to crops modified genetically by inserting a fish gene, making their seed produce plants tolerant of the specific herbicide

S

Scopa/scopae
portion of the body, usually rows of branched hairs on the abdomen or hindlegs, where female bees carry pollen for brood

Seed
fertilized and matured ovule or rudimentary plant, usually with [orchids have none- need symbiotic fungi...] food necessary for its germination

Self-compatible
a plant capable of being fertilized by pollen within its own group of flowers or genet

Self-fertile/self-fertilizing
a flower or floret capable of being fertilized by its own pollen

Selfing
fertilization of an ovule by a pollen grain of the same genet

Self pollinating
(autogamous) capable of placing its own pollen upon its own stigma; sometimes called "spontaneous pollination"

Self sterile
incapable of becoming fertilized by its own pollen

Semi compatibility
where two genets share some but not all gametophytic compatability traits, thus in some crosses some pollen grains can effect fertilization and others cannot

Sepal
the outermost part of flower, the parts of which form the calyx

Sessile
sitting, lacking a stalk or petiole

Sexual reproduction
reproduction through union of male and female gametes (as opposed to vegetative reproduction)

Social bee
a bee having females sharing a nest, either mutually tolerating the others or sharing a nesting site and material, with highly advanced or "eusocial behavior" involving a reproductive division of labour and overlap of generations (mother and daughter in the same nest)

Solitary bee
a bee completing its life cycle by emerging from a cell in a nest, then feeding and mating, after which the female constructs and provisions a new nest and produces brood there

Sonication
(see "buzz pollination")

Spadix
the fleshy axis of certain inflorescences, such as those of arum lilies, bearing the small flowers or florets

Spathe
a large bract enclosing the flower cluster

Spike
an inflorescence with elongated main axis and sessile flowers

Sporangium
a spore-producing organ

Sporophyte
the spore-producing, diploid phase of a species displaying alternation of generations

Stamen
the male part of a flower consisting of a filaments and anthers

Staminate
being entirely male, bearing only stamens

Staminode
an abortive stamen

Standard
a large upper petal of a papilionaceous legume flower

Stem
main axis of a plant

Sterile
barren, unfruitful, incapable of being fertilized

Stigma
receptive portion of the female sexual column

Style
part of the sexual column between the ovary and stigma

Syconium
multiple fruit of a fig in which the edible receptacle (flower axis) is hollow and lined on the inside with numerous fruitlets and seeds

T

Thievery
in pollination ecology, removal of a floral reward by an animal, where pollination does not follow as a result (as opposed to robbing)

Triocey
where co-existing genets are male only, female only, and hermaphrodite (complete polygamy)

Tripping
release of sexual column in legume flowers

Tuber
swollen end of an underground stem containing food reserves

U

Unisexual/imperfect flowers
flowers in which either stamens or pistils are functional, the non-functional member may be completely lacking

V

Varroa
mite genus responsible for parasitism of honey bee brood; genera *Varroa* and *Euvarroa* are similar

Vegetative reproduction
asexual reproduction (see apomixis)

Visitor
all animals visiting a flower, but not necessarily a pollinator which enhances plant reproduction

W

Worker
in social insects, individual that cannot mate or produce diploid (female) offspring

X

Xenogamy
fertilization between pollen and ovules between different genets

Z

Zoophily
pollinated by animals

Zygote
product of the two gametes or germ cells

THE POLLINATION OF CULTIVATED PLANTS

A COMPENDIUM FOR PRACTITIONERS

Volume 2

More than twenty years ago, the Food and Agriculture Organization of the United Nations contributed to the growing recognition of the role of pollination in agricultural production, with the publication of "The Pollination of Cultivated Plants in the Tropics". Since that time, the appreciation of pollinators has grown, alongside the realization that we stand to lose them. But our knowledge and understanding of crop pollination, pollinator biology, and best management practices has also expanded over this time.

This volume is the second of two "compendiums for practitioners", sharing expert knowledge on all dimensions of crop pollination in both temperate and tropical zones. The focus in this second volume is on management, study and research tools and techniques.

GLOBAL ACTION ON **POLLINATION SERVICES**
FOR **SUSTAINABLE AGRICULTURE**

Food and Agriculture Organization of the United Nations
Viale delle Terme di Caracalla,
00153 Rome, Italy

www.fao.org/pollination
www.fao.org/agriculture/crops/agp-home
e-mail: GlobalAction-Pollination@fao.org

ISBN 978-92-5-130506-5

9 789251 305065
I9184EN/1/04.18