Area-wide management

OF FRUIT FLY PESTS

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Area-Wide Management of Fruit Fly Pests

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Dedication

Dedicated to the Memory of Don McInnis, Rubén Leal Mubarqui, and Roger Vargas, leaders in area-wide management of fruit flies, and to Jorge Hendrichs, still a pillar in the fruit fly community

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Contents

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Preface

The *10th International Symposium on Fruit Flies of Economic Importance* was held in Tapachula, Chiapas, Mexico, from April 23 to 27, 2018. It was co-organized by El Colegio de la Frontera Sur (ECOSUR), the Mexican Fruit Fly program of the Servicio Nacional de Sanidad Inocuidad y Calidad Agroalimentaria (SENASICA), the Interamerican Institute for Cooperation in Agriculture (IICA), the Soconusco Association of Fruit Growers and the Joint Food and Agriculture Organization/ International Atomic Energy Agency (FAO/IAEA) Division of Nuclear Techniques in Food and Agriculture.

The symposium was attended by 289 fruit fly researchers, plant protection officials, fruit industry representatives, students, and exhibitors from 56 countries. There were 59 oral presentations and 134 posters. These were organized in 10 sessions: (1) Biology, Ecology, Physiology, and Behavior; (2) Taxonomy and Morphology; (3) Genetics and Biotechnology; (4) Chemical Ecology and Attractants; (5) Risk Assessment, Quarantine, and Post-Harvest; (6) Sterile Insect Technique; (7) Natural Enemies and Biological Control; (8) Other Control Methods and New Developments; (9) Area-Wide Integrated Pest Management (AW-IPM) and Action Programs; and (10) Social, Economic, and Policy Issues of Action Programs. Three field trips took place: (1) moscafrut mass-rearing facility in Metapa, (2) mango exporting process, release of sterile flies, and mango packing export center, and (3) surveillance of Mediterranean fruit fly, field operations, and coffee plantations.

Highlights of the symposium were new knowledge on microbial symbionts associations, the use of models to better understand and predict population dynamics, and new knowledge and developments regarding the chemical ecology of fruit flies that contribute to more specific and efficient control methods. The audience received research on social aspects regarding farmers' perceptions and education on fruit fly problems and management options.

Successful stories on the use of the sterile insect technique (SIT) were shared, including the US–Mexico–Guatemala Medfly program, Mediterranean fruit fly eradication in the Dominican Republic, and the Moscafrut program in Mexico.

A special session was devoted to honoring those that have left their print in the fruit fly community: Serge Quilici, Don McInnis, Rubén Leal-Mubarqui, and Jorge Gutiérrez-Samperio.

The poster sessions, coffee breaks, lunch, welcome cocktail reception, closing dinner, and field trips provided ample opportunity for participants to share their knowledge and experiences informally and establish new friendships and collaboration ties and demonstrated the camaraderie that exists in the fruit fly community, which undoubtedly has contributed to the various success stories.

Two videos were prepared for the symposium, one on the graphic history of the nine previous symposia (previous ISFFEIs) (https://www.youtube.com/watch?v=BJrBkfkDWrg) and another one with pictures from the 10th ISFFEI (10th ISFFEI) (https://www.youtube.com/watch?v=zEpvxK4eVN8).

This book represents the proceedings of the symposium, and continuing a long-lasting tradition, it is the 10th volume. It contains 31 contributions from 126 authors from all over the world. All these papers were peer reviewed. The editorial work was carried out by Diana Pérez-Staples, María Teresa Vera, Francisco Díaz-Fleischer, and Pablo Montoya. I highly appreciate and acknowledge their high level of commitment and the quality of their work. For the first time, this proceedings book will be open access, available online to a large audience through the CRC website. This was possible thanks to the registration fees of all the participants.

A few weeks after the symposium, we received the very sad news that our colleague and appreciated friend, Roger Vargas passed away in an unfortunate accident. We want to remember and honor him here. Roger's impact and influence on the community of fruit fly workers will never be diminished.

Pablo Liedo
Tapachula, Chiapas, Mexico

Acknowledgments

This book is a compilation of the *Proceedings of the 10th International Symposium on Fruit Flies of Economic Importance*, held in Tapachula, Chiapas, Mexico, from April 21 to 27, 2018. We thank Pablo Liedo and Rui Pereira for the invitation and the opportunity to edit the Proceedings.

Thank you Pablo and Rui!

The aim of this book is to highlight research on tephritid flies in different countries, showcasing research that would not necessarily be available in peer-reviewed journals. The book is organized in the following sections: Biology, Ecology, Physiology, and Behavior; Taxonomy and Morphology; Chemical Ecology and Attractants; Risk Assessment, Quarantine, and Post-Harvest; Sterile Insect Technique; Natural Enemies and Biological Control; Area-Wide Integrated Pest Management and Action Programs; and Social, Economic and Policy Issues of Action Programs. It also contains the plenary talk on 'Holistic Pest Management' by Dr. Barrera and reviews on subjects such as long distance flight of *Bactrocera dorsalis*, desiccation resistance, *Anastrepha* immature stage taxonomy, biological control of *Anastrepha*, area-wide management of *Anastrepha grandis* in Brazil, and natural host plants of the *Anastrepha fraterculus* complex, among others.

All chapters were peer reviewed by at least two experts in the field. Reviewers were from the following countries: Argentina, Australia, Austria, Belgium, Brazil, Chile, Czech Republic, France, Greece, Israel, Italy, Kenya, Malaysia, Mexico, Morocco, South Africa, Spain, Suriname, and the United States. As such we are in debt for thorough and insightful reviews by (in alphabetical order):

Isabel Arevalo-Vigne, Abdel Bakri, Ken Bloem, Carlos Cáceres, Jorge Cancino, Dong Cha, Jorge Luis Cladera, Des Conlong, Hugh Conway, Carol Cuashie-Williams, Francisco Devescovi, Maria Luisa Dindo, Bernie Dominiack, Sunday Ekesi, Salvador Flores, Flávio Roberto Mello Garcia, Yoav Gazit, Guy Hallman, Alvin Hee, Jorge Hendrichs, Martha Hendrichs, Michael K. Hennessey, Emilio Hernández, Vicente Hernández-Ortiz, Iara Joachim-Bravo, Nikos Kouloussis, Daniel Frías Lasserre, Aruna Manrakhan, Marc De Meyer, Salvador Meza, David Midgarden, Tahere Moadeli, Laura Moquet, Allies van Sauers Muller, Devaiah A. Muruvanda, Dori E. Nava, Vicente Navarro-Llopis, David Nestel, Allen Norrbom, Dina Orozco-Davila, Andrea Oviedo, Nikos T. Papadopoulos, Beatriz Jordao Parhanos, Jaime Piñero, Polychronis Rempoulakis, Jesús Reyes, Olivia Reynolds, Juan Rull, Mark Schutze, Diego Segura, Todd Shelly, Greg Simmons, Gary Steck, Karl Suiter, Donald Thomas, Jorge Toledo, Lucie Vaníčková, Venancio Vanoye, Marc Vreysen, Roberto Zucchi.

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Cover photographs (left to right): (1) *Ceratitis capitata* (photograph by Katja Schulz, licensed by Attribution [CC BY 2.0]), (2) *Bactrocera tryoni* mating (photograph by Ajay Narendra), (3) *Anastrepha ludens* ovipositing (photograph by Andrés Díaz Cervantes), (4) flies attracted to multi-lure trap (photograph by Pablo Montoya).

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Diana Pérez-Staples is a faculty member at the Universidad Veracruzana in Xalapa, Veracruz, Mexico. Her research is focused on the sexual behavior of tephritid fruit flies and other insect pests and on improving current control methods with more than 50 published papers in scientific journals.

Francisco Díaz-Fleischer is a faculty member at the Universidad Veracruzana in Xalapa, Veracruz, Mexico. He is interested in the relationship between behavior and life history of tephritid fruit flies to improve control method, with more than 70 published papers in scientific journals.

Pablo Montoya is an expert on the use of biological control by augmentation (BCA) and the application of the sterile insect technique (SIT) against fruit fly pests, with more than 80 published papers in scientific journals. He is a researcher and head of the Unit of Methods Development in the Mexican Program against Fruit Flies, SENASICA-SADER.

María Teresa Vera is an expert on fruit fly reproductive biology and the assessment of sexual competitiveness for the implementation of the SIT with more than 50 published papers in scientific journals. Currently she is a researcher at the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) and teaches at the Facultad de Agronomía y Zootecnia, Universidad Nacional de Tucumán, where she is member of the editorial board of the Revista Agronómica del Noroeste Argentino.

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Section I

Biology, Ecology, Physiology, and Behavior

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1 Identification of the Profile of Cuticular Hydrocarbons of *Anastrepha curvicauda* (Diptera: Tephritidae)

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CONTENTS

Abstract Cuticular hydrocarbons (CHCs) are constituents of the epicuticle of insects, which have the function of preventing dehydration and are signs of inter- and intraspecific recognition. Because CHCs vary between species and according to adulthood, sex, and mating status, they have been studied in species of economic importance such as *Ceratitis capitata* Wiedemann, *C. anonae* Graham, *C. rosa* Karsch, and *Anastrepha fraterculus* Wiedemann as an effective means of taxonomic identification. However, there are no studies of its intervention in chemical communication, and they have not been studied in *Anastrepha curvicauda* Gerstaecker, an insect pest of *Carica papaya* Linnaeus. In this work, we studied the CHC profile of virgin males and females of different ages of *A. curvicauda*. The extraction was done with hexane and was injected into a gas chromatograph coupled with a mass spectrometer. The identification of compounds was performed considering retention times, retention index, and spectral evaluation through a comparison with the NIST mass spectra library. The CHC profile of *A. curvicauda* consists of long chains of 20–29 carbons, and four major compounds were identified: 2-methyloctacosane, 1-heptacosanol, (Z)-14-tricosenyl formate, and a (Z)-14-tricosenyl formate isomer. 1-heptacosanol was the main compound in females and (Z)-14-tricosenyl formate in males. 1-heptacosanol in females increased in abundance at 5–7 days, a period that coincides with their sexual maturity. The obtained CHC profile is specific to this species. The compounds are sex-specific, too, and their differences are apparent at 7 days of age when abundance is higher in females than males.

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1.1 INTRODUCTION

The cuticle of insects has several functions, such as protecting against environmental conditions, pathogens, and other insects, as well as supporting the body. The epicuticle is the external layer of the cuticle and consists of two layers: the first one, composed of chitin, and the second one, composed of hydrocarbons, which helps the insect avoid dehydration and damage by ultraviolet (UV) rays (Hadley 1984; Vrkoslav et al. 2010). These hydrocarbons are inter- and intraspecific recognition signals because they vary in species, age, sex, and physiological stage. Nevertheless, cuticular hydrocarbons (CHC) have been studied to taxonomically identify pest insects, such as *Ceratitis capitata, Ceratitis anonae, Ceratitis fasciventris* Bezzi, *Ceratitis rosa*, and *Anastrepha fraterculus* (Vaníčková et al. 2014). However, our interest focuses on inter- and intraspecific recognition (Blomquist 2010). In this work, we studied the CHC profile of virgin males and females of different ages of *Anastrepha curvicauda*, formerly *Toxotrypana* (Diptera: Tephritidae) (Norrbom et al. 2018).

1.2 MATERIALS AND METHODS

Insects were collected from a small plantation at CeProBi, IPN, Yautepec, Morelos, Mexico, between 18° 05′ N latitude and 99° 03′ W longitude. Larvae were collected from infested fruit and deposited in plastic containers with soil from the original collection site for pupation. Once emerged, adults were individually separated according to sex into 9 × 4-cm acrylic containers (NX2185C, Daiger, Vernon Hills, IL) and provided with 10% sugar water as food.

We used virgin flies of 1 (24 hours after the fly emerged), 3, 5, 7, 9, and 11 days of age, and a weight interval of 0.0469 g ± 0.0050 for males and 0.0510 g ± 0.0055 for females. Flies were kept for 30 minutes in a freezer (−20°C) (Norlake Scientific). They were then placed for 15 min in a desiccator with silica gel granules. The extraction was done with 1 mL of hexane in a glass vial, which was concentrated to 150 µL under a nitrogen flow. The extracts were stored at −20°C until chemical analysis. Two µL of extract were injected into a gas chromatograph (GC; HP6890) coupled with a mass spectrometer (MS; HP 5972) (Agilent, USA). The samples were analyzed using a nonpolar column SLB-5ms (30-m long, 250-µm internal diameter, and 0.25-µm film thickness, SUPELCO Analytical). The initial oven temperature was 150°C for 2 min, increasing to 5°C/min until reaching 308°C. The carrier gas was hydrogen at a constant flow of 2 mL/min. The injector temperature was 250°C and the auxiliary was 280°C; the injector functioned in split mode 1:25. The MS functioned by electronic ionization (70 EV) in SCAN mode and at a mass interval of 29 to 400 AMU (modified from Vaníčková et al. 2014).

The identification of compounds was performed considering retention times, retention index (Clarke 1978), and spectral evaluation through a comparison with the NIST mass spectra library (NIST/EPA/NIH 2002).

Linear regressions were performed to determine the influence of the flies' weight on the abundance of CHCs. Results of the regressions showed a relation between these variables. Therefore, CHC abundance was divided by weight for each fly.

Comparisons of CHC abundance between virgin females and males were performed with a *t*-student test; natural logarithm (α), logarithm base 10 (β), square root (σ), and reciprocal (γ) transformations were applied to some data to fulfill the requirements of normality and equality of variance. A Mann–Whitney test was used for data that could not be normalized or homogenized by a transformation. For all cases, mean ± standard error of mean (SEM) is reported, even for nontransformed data. All analyses were performed using SigmaPlot 12.5, and the rejecting error was 0.05.

1.3 RESULTS

Our results show that the profiles for virgin flies included a mix of large-chain hydrocarbons of 20-31 carbons for both sexes. We identified the following CHCs: 2-methylactosane, 1-heptacosanol, (Z)-14-tricosenyl formate, and a (Z)-14-tricosenyl formate isomer.

For females, 1-heptacosanol and (Z)-14-tricosenyl formate were compounds that were highly abundant in all samples. For males, (Z)-14-tricosenyl formate was highly abundant (Table 1.1).

There were no qualitative differences in CHC profiles between virgin males and females. The abundance of 2-methyloctacosane in 5-day-old virgin males was higher than in 5-day-old virgin females ($t = 7.072$, $df = 24$, $P < 0.001$). This CHC also had a higher abundance in 7-day-old virgin females than in virgin males of the same age ($T = 256$, $df = 24$, $P < 0.001$, respectively). There were no significant differences between virgin females and males, which were 1, 3, 9, and 11 days old ($t = 2.014$, $df = 24$, $P = 0.028$; $t = 0.395$, $df = 24$, $P = 0.348$, $t = 0.463$, $df = 24$, $P = 0.324$; $t = 0.598$, $df = 24$, $P = 0.278$, respectively) (Figure 1.1).

The abundance of 1-heptacosanol was higher in virgin males than in virgin females at 5 days of age ($T = 238$; $df = 24$; $P < 0.001$). However, the abundance of this CHC was higher in virgin females than in virgin males of 7 and 11 days of age ($t = 6.732$, $df = 24$, $P < 0.001$; $t = 2.118$, $df = 24$, $P = 0.022$, respectively). There were no differences in this CHC among sexes in flies of 1, 3, and 9 days of age ($T = 153$, $df = 24$, $P = 0.885$; $t = 1.485$, $df = 24$, $P = 0.075$; $t = 1.47$, $df = 24$, $P = 0.324$, respectively) (Figure 1.1).

The abundance of (Z)-14-tricosenyl formate was higher in virgin females than in virgin males of 7 days of age ($t = 3.102$, $df = 24$, $P = 0.002$). However, its abundance was similar between sexes at 1, 3, 5, 9, and 11 days of age ($T = 134$, $df = 24$, $P = 0.470$; $t = 0.330$, $df = 24$, $P = 372$; $t = 0.330$, $df = 24$, $P = 0.372$; $t = 0.835$, $df = 24$, $P = 206$; $t = 1.042$, $df = 24$, $P = 0.154$, respectively) (Figure 1.1).

Virgin females showed a higher abundance of (Z)-14-tricosenyl formate than virgin males at 7 days of age ($t = 2.881$, $df = 24$, $P = 0.004$). There were no differences between sexes in the abundance of this compound for the rest of the evaluated ages.

Ethanol, 2-(Z) (octadecene-9-enoxy) traces were detected in both sexes; thus, no analysis was performed.

TABLE 1.1
Cuticular Hydrocarbon Compounds in Virgin Males and Females of *Anastrepha curvicauda*

Compound[a]	Rt (min)	Formula[b]	MW (g/mol)	CAS	RI	F%	M%
2-methyloctacosane	24.981	$C_{29}H_{60}$	408.4695	1560-98-1	2868	6.84	8.80
1-heptacosanol	27.784	$C_{27}H_{56}O$	396.4331	2004-39-9	3086	**36.17**	**28.93**
(Z)-14-tricosenyl formate	29.960	$C_{24}H_{46}O_2$	366.5776	77899-10-6	3246	**36.14**	**41.04**
A (Z)-14-tricosenyl formate isomer	30.088	—	278.48	—	3255	18.34	18.55
Ethanol, 2-(Z) (octadecene-9-enoxy)	32.310	$C_{20}H_{40}O_2$	312.4928	5353-25-3	3412	2.48	2.67

CAS, Chemical Abstract Service; F%, female percentage; IUPAC, International Union of Pure and Applied Chemistry; M%, male percentage; MW, molecular weight; RI, retention index; Rt, retention time.

[a] IUPAC name

[b] Condensed formula.

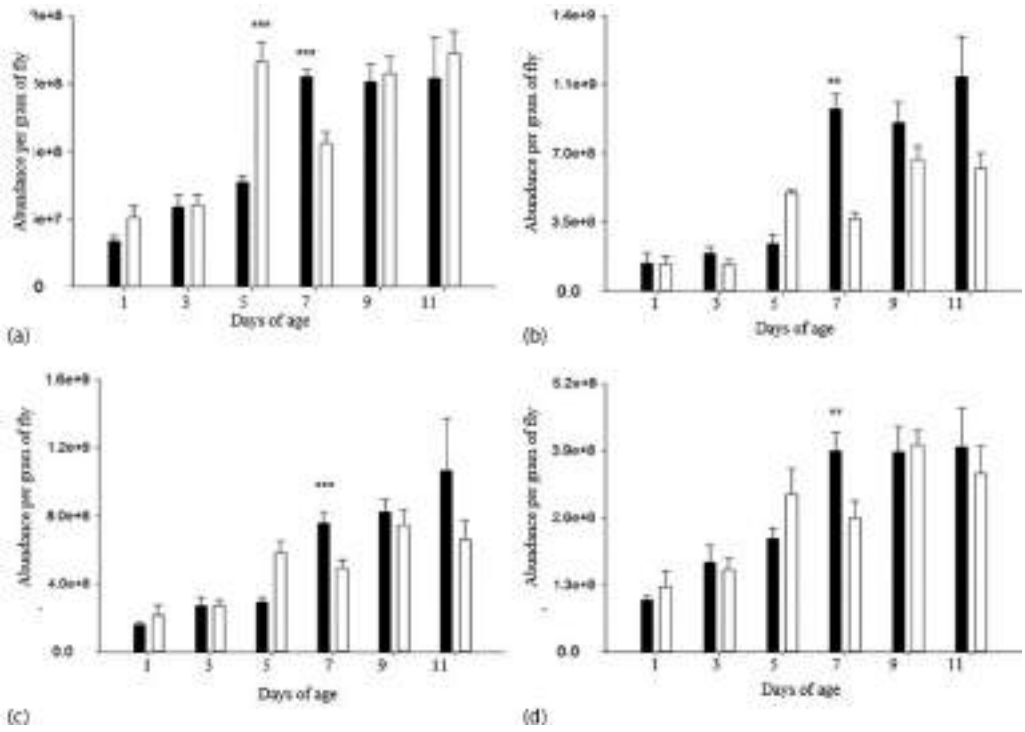

FIGURE 1.1 Abundance (mean + SEM) of (a) 2-methyloctacosane, (b) 1-heptacosanol, (c) (Z)-14-tricosenyl formate, and (d) an isomer of (Z)-14-tricosenyl formate present in virgin females (black bar) and males (white bar) (n = 13). For the 2-methyloctacosane compound analysis, a α data transformation was applied, except for 7 days of age; for the 1-heptacosanol compound, a α transformation was applied for 3, 7, and 11 days of age and no transformation was used for 1, 5, and 9 days of age; for (Z)-14-tricosenyl, a α transformation was applied, except for 1 and 7 days of age; and for the (Z)-14-tricosenyl formate isomer, a α transformation was applied, except for 1 and 3 days of age ($P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$).

1.4 DISCUSSION

In profiles of virgin males and females of *A. curvicauda*, as well as in both sexes of the dipteran species *Drosophila birchii*, Ayala, *Drosophila serrata* Ayala, and *Drosophila suzukii* Matsumura (Howard et al. 2003; Snellings et al. 2018), in species such as *Aldrichina grahami* Aldrich, *Achoetandrus rufifacies* Macquart, *Chrysomya megacephala* Fabricius, and *Lucilia sericata* Meigen (Diptera: Calliphoridae) (Ye et al. 2007), in *A. fraterculus* (Vaníčková et al. 2012), *C. capitata*, *C. anonae*, *C. fascivertis*, and in female *C. rosa* flies 2-methyloctacosane has been detected (Vaníčková et al. 2014, 2015). However, this is the first study that reports 1-heptacosanol, (Z)-14-tricosenyl formate, and a 14-tricosenyl formate isomer as part of CHC profiles. Therefore, the results of this study suggest a profile specificity for *A. curvicauda*.

Compound profiles were the same for both sexes, which is known as a monomorphic profile and has been observed in *D. serrata* (Howard et al. 2003), *D. suzukii* (Snellings et al. 2018), *Drosophila persimilis* Dobzhansky and Epling, *Drosophila pseudobscura* Frolova, and *Drosophila takahashii* Sturtevant, which are cases that reported similar results as this study (Shirangi et al. 2009).

Abundance differences between sexes have been reported in profiles of different species; for example, in *D. suzukii*, 7-tricosene was more abundant in virgin females than in virgin males but only in 1-day-old flies (Shirangi et al. 2009). Abundance of all compounds has been observed to be higher in females than in males in similar species such as *D. birchii*, *D. serrata* (Howard et al. 2003), *A. fraterculus* (Vaníčková et al. 2012), and *C. rosa* (Vaníčková et al. 2014, 2015). Such differences are associated with sex differentiation because they are related to recognition between the sexes (Blomquist 2010).

The difference is observed at 7 days of age, when females showed a high CHC abundance. Substantial differences between sexes of advanced ages were reported for *Anopheles gambiae* Giles (Diptera: Culicidae, Caputo et al. 2005), where the abundance of n-alkanes increased with age.

Quantitative variability of co mpound profiles between sexes is related to a specific role of recognition between sexes that can be involved in selection or discrimination of a fly of the same sex or of the opposite sex (Blomquist 2010). Thus, in studies with *D. serrata* and *Drosophila melanogaster* Meigen, CHCs can be used as a signal for pheromonal communication (Grillet et al. 2006; Thomas and Simmons 2010), or intraspecific recognition (Blomquist 2010). Currently, bioassays on intraspecific recognition are in process, taking as a starting point patterns that occur in agonistic and courtship behavior.

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2 Reported Long-Distance Flight of the Invasive Oriental Fruit Fly and Its Trade Implications

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CONTENTS

Abstract Online biological databases are a popular method of summarizing and storing scientific information. Invasive species databases are used by risk analysts and policy makers from many counties as their main source of scientific data. A majority of the information found in invasive species databases is useful, but data can be oversimplified and errors do exist. For example, the statement "Many *Bactrocera* spp. can fly 50–100 km (Fletcher 1989)" is found in multiple invasive species databases and has been repeated in phytosanitary documents written in different countries. This broad statement has been presented to the United States as evidence that they should extend the radii of quarantine areas placed around new detections of the Oriental fruit fly, *Bactrocera dorsalis* (Hendel), in California from 7.5 to 150 km. In reviewing the available literature, this work finds that the flight distance of 50–100 km for *Bactrocera* spp. as summarized in invasive species databases cannot be attributed to Brian S. Fletcher. A review of 17 publications describing mark-release-recapture studies or field observations on *B. dorsalis* showed that long distance (>20 km) captures of the flies do occur, but such captures are atypical and occur only rarely. Dispersal distances up to 2 km are much more typical and commonly reported. Data summarized in invasive species databases on fruit flies may not be precise. Therefore, consideration

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of biological evidence found in the original source material and other scientific publications is also necessary when developing pest-management strategies or phytosanitary policies. Mark-release-recapture studies clearly show that flight capacity differs among *Bactrocera* species. Although flight capacity is a major factor in determining the size of quarantine areas for fruit flies, host availability, climate suitability, potential pathways, and community demographics are also important risk factors. In California and Florida, *B. dorsalis* have been captured on a number of occasions in their respective state fruit fly detection trapping networks. However, despite these detections, rapidly delimiting an outbreak, establishing quarantine areas, and when needed implementing additional eradication measures have successfully prevented establishment of this invasive pest and has prevented the export of any infested host fruits from quarantine areas to other countries for more than 30 years. Therefore, trading partners should also consider whether rigorous, established trapping programs are in place and proven response protocols exist when determining the radii of required quarantine zones rather than simply setting standards based on the most distant recapture of a fruit fly species.

2.1 INTRODUCTION

Scientists and policy makers routinely refer to pest databases and other scientific literature to gather facts when developing phytosanitary documents, formulating trade policies, and implementing management strategies for harmful exotic pest species. Online biological databases are popular repositories for sharing scientific information. The rising incidence of detection, introduction, and establishment of invasive species has prompted the development of new databases that contain biological information necessary to prevent, detect, manage, and develop policies on invasive species (Katsanevakis and Roy 2015). There are more than 250 comprehensive open-sourced databases that contain information on invasive species that are listed on the Global Invasive Species Information Network (GISIN; http://www.gisin.org).

Overwhelmingly, the scientific information that biological databases provide is valuable and helpful; however, databases are not free of errors. In organizational databases, between 1% and 10% of data items are estimated to be inaccurate (Klein et al. 1997). Errors in invasive species lists have been reported by McGeoch et al. (2012) and Jacobs et al. (2017). Misidentification of a species can lead to incorrect distribution records in databases (Emig et al. 2015).

In addition, information that originates in one reference database is often replicated in related databases with the information spreading throughout both printed and online reference material. An example of a controversial statement that originated in an invasive species database is the conclusion that "Many *Bactrocera* spp. can fly 50–100 km (Fletcher 1989)." This exact statement has been repeated in multiple databases. It has been presented as evidence to implement 150-km pest-free areas beyond the established standard quarantine of 7.2-km (4.5 mile) radius surrounding new detections of *Bactrocera dorsalis* (Hendel) in California (FreshPlaza 2015). *Bactrocera dorsalis* is an invasive species to the continental United States that has been introduced, quarantined, and then eradicated on a number of occasions in California (CDFA 2008) and Florida (Weems et al. 2016).

The flight ability of a fruit fly species to move or disperse is a major factor that influences the size of imposed quarantine areas and boundaries for pest-free zones. Agricultural-based countries spend millions of dollars to detect, delimit, quarantine, and eradicate new fruit fly introductions. The imposition of unreasonably large quarantine areas can result in unnecessary trade restrictions and loss of export markets for growers, as well as unnecessary pesticide applications and use of monetary resources. In this chapter, a review and an analysis of published data indicates that the statement "Many *Bactrocera* spp. can fly 50–100 km" does not reflect the typical dispersal distance and should be replaced with more specific flight details for each *Bactrocera* species. In addition, this review on the flight capability of *B. dorsalis* will provide support for decisions on phytosanitary measures, quarantine restrictions, and integrated pest management (IPM) for this invasive species.

2.2 METHODS

A search of the World Wide Web was performed July 27, 2016 for the statement "Many *Bactrocera* spp. can fly 50–100 km (Fletcher 1989)." Multiple searches were performed while including or excluding the citation "(Fletcher 1989)."

Literature searches for articles by Brian S. Fletcher and other authors on fruit fly biology and movement were performed through scientific search engines available through Academic Search™ Premier (https://www.ebscohost.com/academic/academic-search-premier), Web of Science™ (http:/ clarivate.com/scientific-and-academic-research/research-discovery/web-of-science/), and US Department of Agriculture's National Agricultural Library (https://www.nal.usda.gov/). A generic search of the World Wide Web was also performed. Literature searches for biology and flight data for *Bactrocera dorsalis* were inclusive of all taxonomic synonyms including *Bactrocera papayae*, Drew & Hancock, *Bactrocera invadens*, Drew, Tsuruta & White, and *Bactrocera philippinensis*, Drew & Hancock, which were recently declared synonyms of *B. dorsalis* (Schutze et al. 2015). Titles and abstracts of articles were examined and relevant articles on fruit fly biology and movement were reviewed. References cited within each article were screened and pertinent articles were selected for further review. Thirty-five articles or book chapters by Fletcher focusing on fruit fly biology, population dynamics, and movement were reviewed (Table 2.1). Two books, Shelly et al.

TABLE 2.1
List of Publications by Brian S. Fletcher on *Bactrocera* Ecology or Biology

Bellas, T. E., and B. S. Fletcher. 1979. Identification of the major components in the secretion from the rectal pheromone glands of the Queensland fruit flies *Dacus tryoni* and *Dacus neohumeralis* (Diptera: Tephritidae). *Journal of Chemical Ecology* 5:795–803.

Comins, H. N., and B. S. Fletcher. 1988. Simulation of fruit fly population-dynamics with particular reference to the olive fruit-fly, *Dacus oleae*. *Ecological Modelling* 40:213–231.

Dorji, C., A. R. Clarke, R. A. I. Drew et al. 2006. Seasonal phenology of *Bactrocera minax* (Diptera: Tephritidae) in Western Bhutan. *Bulletin of Entomological Research* 96:531–538.

Fletcher, B. S. 1968. Storage and release of a sex pheromone by Queensland fruit fly *Dacus tryoni* (Diptera: Tephritidae). *Nature* 219:631–632.

Fletcher, B. S. 1969. Structure and function of sex pheromone glands of male Queensland fruit fly, *Dacus tryoni*. *Journal of Insect Physiology* 15:1309.

Fletcher, B. S. 1973. The ecology of a natural population of the Queensland fruit fly, *Dacus tryoni*. IV. The immigration and emigration of adults. *Australian Journal of Zoology* 21:541–556.

Fletcher, B. S. 1974. The ecology of a natural population of the Queensland fruit fly, *Dacus tryoni.* V. The dispersal of adults. *Australian Journal of Zoology* 22:189–202.

Fletcher, B. S. 1974. The ecology of a natural population of the Queensland fruit fly, *Dacus tryoni*. VI. Seasonal changes in fruit fly numbers in the areas surrounding the orchard. *Australian Journal of Zoology* 22:353–363.

Fletcher, B. S. 1975. Temperature-regulated changes in ovaries of overwintering females of Queensland fruit fly, *Dacus tryoni*. *Australian Journal of Zoology* 23:91–102.

Fletcher, B. S. 1979. Overwintering survival of adults of the Queensland Fruit fly, *Dacus tryoni*, under natural conditions. *Australian Journal of Zoology* 27:403–411.

Fletcher, B. S. 1987. The biology of dacine fruit flies. *Annual Reviews of Entomology* 32:115–144.

Fletcher, B. S. 1989. Life history strategies of tephritid fruit flies. In *World Crop Pests. Fruit Flies: Their Biology, Natural Enemies and Their Control*, Vol. 3B, ed. A. S. Robinson and G. Hooper, pp. 195–208. Amsterdam, the Netherlands: Elsevier Science Publishers.

Fletcher, B. S. 1989. Movements of tephritid fruit flies. In *World Crop Pests. Fruit Flies: Their Biology, Natural Enemies and Their Control*, Vol. 3B, ed. A. S. Robinson and G. Hooper, pp. 209–219. Amsterdam, the Netherlands: Elsevier Science Publishers.

Fletcher, B. S. 1998. Dacine fruit flies collected during the dry season in the lowland rainforest of Madang Province, Papua New Guinea (Diptera: Tephritidae). *Australian Journal of Entomology* 37:315–318.

(*Continued*)

TABLE 2.1 (*Continued*)
List of Publications by Brian S. Fletcher on *Bactrocera* Ecology or Biology

Fletcher, B. S., M. A. Bateman, N. K. Hart et al. 1975. Identification of a fruit fly attractant in an Australian plant, *Zieria smithii*, as O-methyl eugenol. *Journal of Economic Entomology* 68:815–816.

Fletcher, B. S., and A. P. Economopoulos. 1976. Dispersal of normal and irradiated laboratory strains and wild strains of the olive fly *Dacus oleae* in an olive grove. *Entomologia Experimentalis et Applicata* 20:183–194.

Fletcher, B. S., and A. Giannakakis. 1973. Factors limiting response of females of Queensland fruit fly, *Dacus tryoni*, to sex pheromone of male. *Journal of Insect Physiology* 19:1147–1155.

Fletcher, B. S., and A. Giannakakis. 1973. Sex pheromone production in irradiated males of *Dacus (Strumeta) tryoni*. *Journal of Economic Entomology* 66:62–64.

Fletcher, B. S., and E. Kapatos. 1981. Dispersal of the olive fly, *Dacus oleae*, during the summer period on Corfu. *Entomologia Experimentalis et Applicata* 29:1–8.

Fletcher, B. S., E. Kapatos, and T. R. E. Southwood. 1981. A modification of the Lincoln index for estimating the population densities of mobile insects. *Ecological Entomology* 6:397–400.

Fletcher, B. S., and E. Kapatos. 1983. The influence of temperature, diet and olive fruits on the maturation rates of female olive flies at different times of the year. *Entomologia Experimentalis et Applicata* 33:244–252.

Fletcher, B. S., S. Pappas, and E. Kapatos. 1978. Changes in ovaries of olive flies *Dacus oleae* (*Gmelin*) during summer, and their relationship to temperature, humidity and fruit availability. *Ecological Entomology* 3:99–107.

Fletcher, B. S., and C. A. Watson. 1974. Ovipositional response of Tephritid fruit fly, *Dacus tryoni*, to 2-Chloro-ethanol in laboratory bioassays. *Annals of the Entomological Society of America* 67:21–23.

Fletcher, B. S., and G. Zervas. 1977. Acclimation of different strains of olive fly, *Dacus oleae*, to low temperatures. *Journal of Insect Physiology* 23:649–653.

Giannakakis, A., and B. S. Fletcher. 1974. Production and release of sex-pheromone in *Dacus tryoni* males sterilized with aziridine derivative HMAC. *Journal of Economic Entomology* 67:3–4.

Giannakakis, A., and B. S. Fletcher. 1978. Improved bioassay technique for sex-pheromone of male *Dacus tryoni* (Diptera: Tephritidae). *Canadian Entomologist* 110:125–129.

Giannakakis, A, and B. S. Fletcher. 1981. Ablation studies related to the location of the sex-pheromone receptors of the Queensland fruit fly, *Dacus tryoni* (Froggatt) (Diptera, Tephritidae). *Journal of the Australian Entomological Society* 20:9–12.

Giannakakis, A., and B. S. Fletcher. 1985. Morphology and distribution of antennal sensilla of *Dacus tryoni* (Froggatt) (Diptera: Tephritidae). *Journal of the Australian Entomological Society* 24:31–35.

Hendrichs, J., B. S. Fletcher., and R. J. Prokopy. 1993. Feeding behavior of *Rhagoletis pomonella* flies (Diptera: Tephritidae): Effect of initial food quantity and quality on food foraging, handling costs, and bubbling. *Journal of Insect Behavior* 6:43–64.

Kapatos, E., and B. S. Fletcher. 1983. Seasonal changes in the efficiency of McPhail traps and a model for estimating olive fly densities from trap catches using temperature data. *Entomologia Experimentalis et Applicata* 33:20–26.

Kapatos, E., B. S. Fletcher, S. Pappas, and Y. Laudeho. 1977. Release of *Opius concolor* and *Opius concolor* var. *siculus* (Hymenoptera: Braconidae) against spring generation of *Dacus oleae* (Diptera: Tephritidae) on Corfu. *Entomophaga* 22:265–270.

Kapatos, E. T., and B. S. Fletcher. 1984. The phenology of the olive fly, *Dacus oleae* (Gmel) (Diptera: Tephritidae), in Corfu. *Zeitschrift Fur Angewandte Entomologie. Journal of Applied Entomology* 97:360–370.

Kapatos, E. T., and B. S. Fletcher. 1986. Mortality factors and life-budgets for immature stages of the olive fly, *Dacus oleae* (Gmel) (Diptera: Tephritidae), in Corfu. *Zeitschrift Fur Angewandte Entomologie.* 102:326–342.

Prokopy, R. J., and B. S. Fletcher. 1987. The role of adult learning in the acceptance of host fruit for egg laying by the Queensland fruit fly, *Dacus tryoni*. *Entomologia Experimentalis et Applicata* 45:259–263.

Tychsen, P. H., and B. S. Fletcher. 1971. Studies on rhythm of mating in Queensland fruit fly, *Dacus tryoni*. *Journal of Insect Physiology* 17:2139–2156.

(2014) and Robinson and Hooper (1989), on fruit flies were searched in their entirety. Flight-distance records for *B. dorsalis* were collected from the scientific articles and tabulated.

The classification of the genus *Bactrocera* Macquart has been revised recently based on phylogenetic studies of the tribe Dacini (Doorenweerd et al. 2018). As a result, several species classified as *Bactrocera* in the searched literature of flight studies are now included in the genus *Zeugodacus*

Hendel. The revised names reported in Doorenweerd et al. (2018) are used here, but the *Bactrocera* and *Zeugodacus* species treated as one group when comparing trends to the older classification of *Bactrocera* in the literature search.

2.3 RESULTS AND DISCUSSION

2.3.1 "Many *Bactrocera* spp. Can Fly 50–100 Km" and Its Frequency on the World Wide Web

A Web search for the statement "Many *Bactrocera* spp. can fly 50–100 km (Fletcher 1989)" returned 66 results, which included links to databases, Websites, and trade-related phytosanitary documents. The results included 20 unique sources, and the remainder were duplicates from databases that had information on numerous species of *Bactrocera*. When the citation, "(Fletcher 1989)," was not included in the search, the results increased to 76.

Databases or Websites that contain the flight distance of 50–100 km and cite Fletcher (1989) include the CABI Invasive Species Compendium, EPPO Global Database, the Pests and Diseases Image Library (PaDIL) Plant Biosecurity Toolbox (http://www.padil.gov.au/), EcoPort (http://epf.ecoport.org/), and DiscoverLife (http://www.discoverlife.org/). The CABI Invasive Species Compendium is a comprehensive database with datasheets on 42 different *Bactrocera* spp., and 10 of these datasheets contained the identical statement "Many *Bactrocera* spp. can fly 50–100 km (Fletcher 1989)." In addition to the online databases, phytosanitary documents from Australia (Plant Health Australia 2010), Iran (Bureau of Plant Pest Surveillance and Pest Risk Analysis 2013), Kenya (KEPHIS n.d.), and Malaysia (CAB International Southeast Asia 2013) were listed in the search results.

2.3.2 Review of Scientific Publications on *Bactrocera* Movement by Brian S. Fletcher

Fletcher's 1989 book chapter "Life History Strategies of Tephritid Fruit Flies" (Fletcher 1989a) is often referenced in databases as the source for the statement "Many *Bactrocera* spp. can fly 50–100 km." However, there is no evidence in "Life History Strategies of Tephritid Fruit Flies" (Fletcher 1989a) that supports the flight range of 50–100 km. The closest reference to the flight distance of *Bactrocera* spp. in this chapter is a general description that polyphagous, multivoltine tephritids have "high mobility" and a "high capacity for dispersal" without any quantification for these descriptors.

Although Fletcher, an expert on fruit fly biology, never implied that *Bactrocera* spp. have a flight range of 50–100 km in any of his 35 articles (Table 2.1), the article "Movement of Tephritid Fruit Flies" (Fletcher 1989b) is most likely the intended citation for the "50–100 km" conclusion found in biological databases for three reasons. First, "Movement of Tephritid Fruit Flies" is found in the same book, *World Crop Pests: Fruit Flies: Their Biology, Natural Enemies and Control* (Robinson and Hooper 1989), as the often miscited chapter "Life History Strategies of Tephritid Fruit Flies." Given that both chapters are in the same book and written by Fletcher, the misidentification of chapter title is plausible. Second, some resources including (EPPO 2018) cite "Movement of Tephritid Fruit Flies" (Fletcher 1989b) as the source for the "50–100 km" flight capability statement. Third, this chapter by Fletcher includes dispersal information for several species of *Bactrocera* along with some quantitative information. Although our reasoning finds the "Movement of Tephritid Fruit Flies" (Fletcher 1989b) as the most logical source in support of the statement "Many *Bactrocera* spp. can fly 50–100 km," we conclude that this chapter lacks sufficient documentation to indicate that in fact "many *Bactrocera* spp. can fly 50–100 km" using the following rationale:

2.3.2.1 Flight Data for Many *Bactrocera* Species Are Not Found in the Chapter

Foremost, the phrase "many *Bactrocera* spp." does not accurately reflect the data presented in the review "Movement of Tephritid Fruit Flies" (Fletcher 1989b). There are 657 described species in

the genera *Bactrocera* and *Zeugodacus* (Doorenweerd et al. 2018), and the book chapter by Fletcher (1989b) provides dispersal information on exactly 7 species and only 3 of the 7 had information indicating that their dispersal distances were 50 km or greater (Table 2.2). Flight data are not available for many species because research is conducted most often on the economically important *Bactrocera* spp. (Aluja 1993). Furthermore, movement by fruit flies is influenced in part by their life history

TABLE 2.2
Movements of *Bactrocera* spp. as Described in "Movements of Tephritid Fruit Flies"

Species Common Name	Movement Recorded	Sex and Numbers Trapped	Citation Used by Fletcher
Z. cucurbitae[1] (Coquillett) Melon fly	Left field; some traveled long distances	Some adults	Nishida and Bess (1957)
	Move from host to surrounding vegetation before nightfall with diurnal pattern of movement	Mature females	
	Up to 65 km in Mariana Islands	Sterile marked	Steiner et al. (1962)
	34–64 km away on adjacent islands	Small number of marked males	Kawai et al. (1978)
	200 km away on Okinoerabi Island	1 sterile male	Miyahara and Kawai (1979)
	Less than 0.2 km on average	Released mature males 2–3 weeks old	Hamada (1980), Nakamori and Soemori (1981) Soemori and Kuba (1983)
Z. diversus[1] (Coquillett) Three striped fruit fly	Seek sheltered refuges – flight distance not provided	Adults	Syed (1968)
B. dorsalis (Hendel) Oriental fruit fly	Up to 65 km in Mariana Islands	Sterile marked	Steiner et al. (1962)
	"Must have flown at least 50 km, mostly over open ocean"	9 marked males	Iwahashi (1972)
	Considerable amount, between islands	Marked males	
	Moved toward host trees	Sterile adults	Yao et al. (1977)
	0.6 km mean 0.33 km	Sterile adults	Chiu (1983)
	2 km mean 0.94 km	Some sterile adults	
	Moved toward host trees	Sterile adults	Yao et al. (1977)
B. oleae (Rossi) Olive fruit fly	0.017–0.018 km, mean dispersal rate	Very few flies left the grove, lab reared, wild males and females	Fletcher and Economopoulos (1976)
	4 km	Small number of males and females	Economopoulos et al. (1978)
	10 km	Small number of released flies	Brnetic (1981)
	Movement increases in the absence of hosts	Adults	Michelakis and Neuenschwander (1981)
	up to 0.02 km	Males and females	Katsoyannos (1983)

(*Continued*)

TABLE 2.2 (*Continued*)
Movements of *Bactrocera* spp. as Described in "Movements of Tephritid Fruit Flies"

Species Common Name	Movement Recorded	Sex and Numbers Trapped	Citation Used by Fletcher
Z. scutellaris[1] (Bezzi) Cucurbit fruit fly	Seek sheltered refuges – flight distance not provided	Adults	Syed (1968)
B. tryoni (Froggatt) Queensland fruit fly	Remain on and around hosts (non-dispersive)	Mature adults	Sonleitner and Bateman (1963), Bateman and Sonleitner (1967)
	Distance not provided	75% of released males emigrated	Fletcher (1973)
	Estimated 3–4 km in 2 to 3 weeks	Male flies	Fletcher (1974)
	12–13 km overall	Male flies	
	24 km	Few marked males	
	"Circumstantial evidence suggested" migration of 25–35 km	Some gravid females	
	Distance not provided	Overwintering adults	Fletcher (1979) and unpublished data
	High rate	Males	Drew and Hooper (1983)
	1.5 km	Most released males	MacFarlane et al. (1987)
	80–94 km	Some males	
B. zonata (Saunders) Peach fruit fly	40 km	Few sterile males	Qureshi et al. (1975)
	Considerable amount of dispersal occurred	Sterile males	

[1] Recent phylogenetic work places this species in the genus *Zeugodacus*. (Doorenweerd et al. 2018)

strategies (univoltine vs. multivoltine, monophagous vs. polyphagous), intrinsic capabilities (flight capacity, polymorphism), physiology (age, nutrition), and sex and body traits (wing shape, size) (Aluja 1993), which makes it difficult to generalize flight capabilities among different species of *Bactrocera*.

2.3.2.2 Case Studies on *Bactrocera* spp. Lack Necessary Quantifiable Dispersal Data

In "Movement of Tephritid Fruit Flies," Fletcher (1989b) includes flight information from field trials, mainly mark-release-recapture experiments of *Bactrocera* species. The pertinent data from these case studies, which include both qualitative and quantitative records, are summarized in Table 2.2. Other genera of fruit flies are discussed in the chapter but are not relevant to this analysis and are therefore not included.

The 22 case studies as described in the chapter include 31 observations (Table 2.2, column 2) on the movements of *Bactrocera* spp. in mark-release-recapture or field studies. Almost 40% of these comments do not provide a calculated distance that the flies traveled; instead, the conclusion are generalizations such as "seeked sheltered refuges," "some adults traveled long distances," or "moved toward host trees." These vague comments emphasize long flight distances over shorter distances and leave the reader to define the term "long."

Quantitative flight distances for *Bactrocera* spp. are provided for 13 case studies (Table 2.2). Five of the 13 studies resulted in recaptures of one to nine flies at distances that ranged from 50 to 200 km. Fletcher does not provide the number of flies that are captured at distances less than

50 km but indicates that more flies are captured at distances less than 4 km by using descriptive terms such as "most males," "male flies," or "very few left the grove." The lack of case study details presented in this chapter have led to different interpretation of the results. For example, when citing "Movement of Tephritid Fruit Flies" (Fletcher 1989b), Peck et al. (2005) came to the conclusion, "Many studies on *Bactrocera* have reported that these flies do not move far," whereas the EPPO Global Database (EPPO 2018) states nearly the opposite: "Many *Bactrocera* spp. can fly 50–100 km." An article published in the EFSA Journal (European Food Safety Authority 2007) addressing the pest risk of *B. zonata* (Saunders) clarifies the findings in the chapter by stating, "Although many *Bactrocera* spp. can fly 50–100 km (Fletcher 1989), the maximum reported for *B. zonata* is 40 km (Qureshi et al. 1975)." Overall, Fletcher (1989b) provides a good historical review of fruit fly movement, but the evidence from the case studies is insufficient to speculate generally about *Bactrocera* spp. flight capacity. Most studies simply do not place traps out more than a few kilometers from the release sites because of increased need for resources and diminished likelihood of captures.

2.3.3 Additional Published Reviews on Fruit Fly Movement

Historically, the majority of scientific publications on the movement of fruit flies focus on the species in the tribe Dacini that are of economic importance, especially *Zeugodacus cucurbitae* (Coquillett), *B. dorsalis*, *Bactrocera oleae* (Rossi), and *Bactrocera tryoni* (Froggatt) (Aluja 1993). Published reviews on tephritid fruit fly biology that, in whole or part, discuss fruit fly movements date back to Christenson and Foote (1960) and include articles by Aluja (1993); Bateman (1972, 1977); Díaz-Fleischer and Aluja (2000); Dominiak (2012); Fletcher (1987, 1989b); Prokopy and Roitberg (1984); and Zwolfer (1983).

Weldon et al. (2014) provide an excellent review of tephritid movement. They redefine types of movement, discuss dispersal and its implications for pest management, and include mark-release-recapture studies, molecular methods, and remote sensing as tools for measuring movement. Key results from published recapture studies on 12 tephritid species are summarized in a table and evaluated in depth. The consolidation of the recapture data required a significant effort by Weldon et al. (2014), and the data are a useful resource when comparing movement among fruit fly species.

Although mark-and-recapture studies have some limitations, they are the most practical means for studying movement, especially the long-distance movement of organisms (Southwood and Henderson 2000). In the future, new tracking technologies could provide more accurate estimates of long-distance dispersal (Nathan et al. 2003). Trap array design and the strength of the trap attractant can influence recapture rates (Weldon et al. 2014). The maximum flight distance studied can be limited by the costs associated with setting up, maintaining, and checking the trap array. Overcrowding because of large numbers of released flies in a small area may result in a higher occurrence of long-distance dispersal. Despite the limitations, the trapping results from 38 recapture studies on tephritid fruit flies that are reviewed by Weldon et al. (2014) indicate that some species are more mobile than others and that long-distance recapture is a rare event. Mean dispersal distance of tephritid flies is usually well below 1 km (Weldon et al. 2014).

The recapture results indicate that the most mobile economically important fruit fly species are in the genus *Bactrocera* (Weldon et al. 2014). Among the *Bactrocera* species studied, *B. oleae*, the olive fruit fly, is the least mobile. *B. oleae* adults from the first two generations emerge during a time when olive fruit is ripe and abundant (Fletcher 1989a). When suitable fruit is available, dispersal is low. In a recapture study with eight replications, *B. oleae* adults moved a distance of only 0.019 to 0.068 km on average (Weldon et al. 2014). Other studies not included in Weldon et al. (2014) also indicate that *B. oleae* does not move far. Laboratory-reared olive fruit flies took 12 to 14 days to travel a mean distance of 0.018 to 0.020 km (Fletcher and Economopoulos 1976). When wild and artificially reared *B. oleae* flies were released outside an olive grove, fewer than 15 flies moved 2 km to reach the grove, and just a few flies were trapped 4 km away (Economopoulos et al. 1978). During

a 4-year study, an average of 99.45% of the recaptured flies were trapped within 1 km and only a few flies were found 10 km away (Brnetic 1981).

In contrast, other studied species have ranges beyond 10 km. *Zeugodacus cucurbitae*, *B. dorsalis*, and *B. tryoni* male adults were captured at 56, 50, and 94 km, respectively, from their release sites (Weldon et al. 2014). It is not possible to tell from these studies how common such dispersal distances were given the fact that relatively few of the total number of released flies were recaptured at any distance (0.3% or less when recorded) and the fact that, by necessity, trap density decreased the further out from the release site. In the study where a single *B. tryoni* fly was captured at 94 km, the mean distance for all captured flies (approximately 800 of the 400,000 released), was 1.1 km (MacFarlane et al. 1987; Weldon et al. 2014). Although most recapture studies do not report mean distance traveled, it is apparent that dispersive movements can vary greatly among species of dacine fruit flies.

2.3.4 Flight Capacity of *Bactrocera dorsalis*

Shortly after *Bactrocera dorsalis* was discovered in Hawaii, research on this invasive pest became a top priority to the Hawaiian islands and to the mainland United States. Research began on biological and chemical control, area-wide control, ecology, biology, and commodity treatments (Carter 1950). Reinfestation of areas by *B. dorsalis* was perceived as a problem; therefore, data were collected on the movements of Oriental fruit flies during area-wide control projects. The published results from these initial studies, which include the earliest knowledge of movements by *B. dorsalis* in Hawaii and subsequent research on the flight capabilities of *B. dorsalis*, are summarized in Table 2.3. There are 17 primary source publications (Table 2.3). Published flight distances from field studies on *B. dorsalis* can be grouped into two broad categories:

1. Observations reported without experimental methods
2. Experimental results, including scientific methods

Seven out of the 17 publications listed in Table 2.3 do not provide methodology to demonstrate how the flight data were collected. If materials and methods are furnished in a listed publication, details for the release sites are given. In addition, environmental factors that are considered to affect flight, including topography, host availability, and wind currents, are noted.

From their inception, the reported results on the flight of *B. dorsalis* emphasized the movement of longer distances by a few flies. For example, Carter (1950) does not include experimental methods for the ecology and control studies in Hawaii and summarizes the results without population densities and seasonal population trends. In his article, Carter (1950) states,

> Data were therefore obtained on fly movements into and out of the study areas, and from this data detailed studies were made of population densities and seasonal fluctuations. During these studies marked male flies have been recovered 20 miles [32.2 km] from their original point of liberation. Other data on fly movement acquired throughout the investigations have confirmed the migratory habit of the fly or at least its great capacity for dispersal. It has crossed an ocean strait 9 miles [14.5 km] wide; it evidently moves back and forth over each island and possibly over more than one island; and it can be carried on the outside of fast-moving vehicles for long distances.

Four subsequent articles (Christenson and Foote 1960; Porter and Christenson 1960; Steiner 1957; Steiner et al. 1962) also generalize findings on flight capacity of *B. dorsalis* as observed in the Hawaiian islands (Table 2.3). These articles recorded flight distances spanning 6 to 42 km. Two articles include the numbers trapped, with just 1 fly at 38.6 km and 133 flies at 15 to 17 km. Steiner et al. (1962) combine capture data for three fruit fly species and report distances of 19–72 km. Unfortunately, detailed information regarding the total number of flies that were released and recaptured at different distances from the release sites during these studies is not indicated. Certainly, some flies must have been captured closer to the release sites than 19 km, and excluding this data can

TABLE 2.3
Field Research on the Flight Capability of *Bactrocera dorsalis*

Distance (km)	Number Trapped (% trapped)†	Release Area and Trapping Details and Reference
14.5	Not provided	Cited as "It has crossed an ocean strait 9 miles wide; it evidently moves back and forth over each island and possibly over more than one island."
32.1	Marked male flies	Cited as "recovered 20 miles from their original point of liberation." No materials and methods provided for study. Carter (1950)
38.6	1	Cited as "One marked male has been recovered 24 miles from its release point, and many others have been taken in methyl eugenol traps far removed from any known breeding sites-even at an elevation of 7600 feet on Mauna Loa volcano." No materials and methods provided for study. Steiner (1957)
6–24	Not provided	Cited as "The males of *D. dorsalis* have frequently been found to travel 4 to 15 mi. from the point of their release and to cross 9 mi. of open sea between islands. Occasionally this species may fly several miles within a few days (United States Department of Agriculture, unpublished data)." Christenson and Foote (1960)
42	Not provided	Cited as "Marked Oriental fruit flies have been retaken in traps as far as 26 miles from a liberation point, having crossed at least
15 over water	Not provided	9 miles of water in the course of their flight. In one recent
15–17	133	experiment, 133 Oriental fruit flies were recovered in traps located 9 to 11 miles from the release site." Porter and Christenson (1960)
16 (256 km^2)	Not provided	Cited as "Oriental fruit flies from a single release site have spread over 100 square miles of surrounding mountain and coastal areas" (Hawaii–no materials and methods).
40–72 after emergence 19–64 over water	Not provided	Cited as "These flies have moved distances ranging from 25 to 45 miles from their point of emergence and made sustained overwater flights of 12–40 miles" (collectively referring to *B. dorsalis, B. cucurbitae* and *Ceratitis capitata* (Wiedemann). No materials and methods provided.
Not measured		Aerial drop boxes of marked sterile flies ½ mile [0.8 km] apart over length of Rota Island. Releases averaging 6 million Oriental fruit flies weekly. "It is apparent from the results that a predominant downward drift of flies, both sterile and wild, is taking place." Steiner et al. (1962)
2–5	202 (87%)	A total of 4,831 marked males released on 4 different islets in the Ogasawara Islands, Japan. Islets in China Jima group are 2 km
7–8	26 (11%)	apart. All traps set at least 2 km from the release sites. Host availability varied among release sites. Overall recapture rate
15–16	5 (2%)	was 4.8%.
50*	9 (0.3%)	3,000 flies were released on the islet Haha Jima, approximately 50 km from Chichi Jima. *3 months later, 9 flies were caught in 3 traps; flies were apparently aided by prevailing winds. Iwahashi (1972)

(*Continued*)

TABLE 2.3 (*Continued*)
Field Research on the Flight Capability of *Bactrocera dorsalis*

Distance (km)	Number Trapped (% trapped)†	Release Area and Trapping Details and Reference
0.2–2.8	Not provided	Taiwan Yao et al. (1977) [cited in Chiu (1983)]
0.60 avg. 0.33	Not provided	Release site in host plant areas. Traps set in 4 directions at 150, 300, 450 and 600 m. Note: Furthest trap set 0.60 km from release site.
2.0 avg. 0.94		Release site in non-host plant areas 0.5, 1 m, 1.5 and 2 km Note: Furthest trap set 2 km from release site. Chiu (1983)
0.5	16 (4 - N, 12 - S)	Wild flies marked and released in town with hosts plants present. Traps placed to north, south and west of release sites. Traps 1 km from town did not trap any marked flies.
1	None	Tan (1985)
Within villages	Avg of 7.4%–11.9% of released flies	Released total of 6,838 marked wild flies in 2 villages, Batu Uban and Sungei Dua. Both village had host plants, but differed in kind and quantity. Villages were 1 km apart.
Outside villages	1	Tan and Jaal (1986)
Within respective ecosystem	Most marked flies	Three different ecosystems (each 2.25 ha) about 1 km apart. Most wild marked flies stayed within each ecosystem.
1 km	37 (0.20%)	Only 0.20% of flies emigrated (based on 18,624 total released flies). 13 out of the 37 flies moved to forest ecosystem, where host plants were scarce. Tan and Serit (1988)
27 max	Few males	50,000 sterilized males were released in a favorable habitat in western Taiwan. A few flies were captured on Lambay Island. During releases on Lambay Island (6.8 km^2), *B. dorsalis* flies moved to more favorable habitats within the island Chu and Chiu (1989).
13	1 marked	Cited as "observed a marked *B. dorsalis* traverse an upwind distance of 13 km in a single 24-h period in response to a methyl eugenol–baited trap (unpublished data)" Peck et al. (2005)
0.2	Most marked flies	Studied diurnal movement in orchard. Released flies moved from guava orchards to other fruit orchards up to 0.2 km away. Chen et al. (2006)
13–34**	30	All marked flies released from 1 site. Numbers released is not apparent. Set traps at 4 cardinal directions. No marked flies were trapped to the east or west due obstruction by mountain ranges.
63–82**	8	
97**	5	**Under suitable climatic conditions, longer movement inside the Nujiang valley from south to north could be attributed to southern air currents.
8–15 max	17	Captured at southern sites. Chen et al. (2007)

(*Continued*)

TABLE 2.3 (*Continued*)
Field Research on the Flight Capability of *Bactrocera dorsalis*

Distance (km)	Number Trapped (% trapped)†	Release Area and Trapping Details and Reference
<0.5	571 (30%)	In Hawaii, 217,560 total sterile male fliers were released at 4 different sites located 5 km (release 1), 8 km (release 2), 10 km (release 3) and 2 km (release 4) from a 51 km^2 study grid with 2 traps/ km^2. Five days after the 4th release, traps were placed within 0.1 and 0.5 km of the release site. Recapture rates are as follows: 1st release - 0.0005% 2nd release – 0.0% 3rd release – 0.10% 4th release – 0.98% Host fruits were found around all 4 release sites, but were distributed heterogeneously. The study grid contained agricultural crops, secondary vegetation or native/disturbed forests with abundant preferred host plants. Wind speed and direction patterns were consistent. Froerer et al. (2010)
0.5–2	1310 (68%)	
>2–5	6	
> 5–10	7	
>10–11.7	17 (0.8%)	
0.3 or less	1820 (71.1%)	Released 14-day-old sterilized males in 6 grids, each 2.6 km^2 with hosts available. Flies were released in 4 compass directions at 25, 50, 75, 100, 200 and 300 m away from a central trap within the grid. The 6 grids were 3.2 km or more apart in Orange County, CA, and within the *Bactrocera* detection program that spans 6,400 km^2 in the Los Angeles area. Overall 2,558 flies were recaptured (22.9%). Shelly et al. (2010)
Within grid	316 (12.4%)	
Adjoining grid	304 (11.9%)	
1.6–19.2 in detached grids in Orange Co.	96 (3.7%)	
<19 in LA Co. and San Bernadino Co. Exact distances not measured	22 (0.9%)	
>19.2	None	

† Percent tapped = (numbers trapped within specified distance/ total trapped)*100

unintentionally mislead readers in regard to which are the expected dispersal patterns for these species, especially if most of the flies were captured close to release sites. During the 1950s and 1960s, discovery of efficacious control methods was of utmost importance; thus, results from research on flight activity, although directly related to control, were rarely published in their entirety. Thus, specific questions about the flight habits of *B. dorsalis*, such as the timing of captures, the likelihood of multiple flights, and the occurrence of short versus long flights, were not recorded.

Mark-release-recapture studies are labor and financially intensive. Therefore, there are more investigations on movement of fruit flies within a habitat, a grove, or between closely aligned fields than between islands or from urban to rural agricultural production areas that involve monitoring long distances. *Bactrocera dorsalis* has been the subject of multiple experiments monitoring movement in host-rich habitats versus poor host or nonhost environments (See Table 2.3: Chen, et al. 2006; Chiu 1983; Chu and Chiu 1989; Tan 1985; Tan and Jaal 1986; Tan and Serit 1988). Although these studies concluded that released *B. dorsalis* adults stay within host-rich habitats, traps beyond 2 km were not monitored.

Osamu Iwahashi (1972) was the first researcher to design a recapture experiment to specifically measure the long-distance movement (2 km or greater) of *B. dorsalis*. Iwahashi demonstrated that marked males could move from one islet to another in the Ogasawara Islands; each islet is about 2 km apart. A majority, 87%, of the flies were captured 2–5 km away from four release sites

(Table 2.3). Thirteen percent of captured flies were trapped at distances from 7–16 km. Differences were found among recapture rates for the four releases. It was hypothesized that the greatest recapture rates were associated with the lack of suitable hosts at the release sites; however, 43 flies moved from favorable habitat conditions to less-ideal conditions.

A fifth release took place on the distant island of Haha Jima. Nine flies were recaptured three months later on another island 50 km away. This recapture study receives the most attention in the literature. Multiple authors cite Iwahashi's discovery of a 50-km flight but do not mention the possibility of prevailing winds influencing the results (Chen et al. 2006; Fletcher 1989b; MacFarlane et al. 1987; Shelly et al. 2010). Findings from the 1972 study by Iwahashi in the Ogasawara Islands is controversial because the study did not differentiate between active and passive transport (Froerer et al. 2010); however, for the release at Haha Jima, Iwahashi (1972) suggests that prevailing winds were likely a factor in the dispersal of the nine flies that were trapped 50 km away.

In addition to the 1972 paper by Iwahashi, there are three other published articles on recapture studies that investigated the long distance movement of *B. dorsalis* (Chen et al. 2007; Chu and Chiu 1989; Froerer et al. 2010). Research on the capture probability of *B. dorsalis* in the southern California grid system conducted by T. Shelly et al. (2010) provides additional trap data. Summaries for these four publications are found in Table 2.3. All four studies report trap captures at distances greater than 10 km. The four releases occurred in different climatic and geographical environments, but host plants were available at all sites. In two environments, the movement of *B. dorsalis* was likely influenced by wind, as noted by the authors (Chen et al. 2007; Chu and Chiu 1989). In one study, out of 50,000 released flies, a few male flies moved from mainland Taiwan to Lambay Inlet, a maximum of 27 km away (Chu and Chiu 1989). The farthest distance traveled by *B. dorsalis* was recorded in the Nujiang Valley, where five flies were captured 97 km away when aided by southerly winds (Chen et al. 2007). In contrast, in Hawaii and southern California releases when wind was not likely a factor, *B. dorsalis* were trapped at a maximum distance of 11.7 km (Froerer et al. 2010) and 19.2 km, respectively, from their release point (Shelly et al. 2010). Results from these release studies (Chen et al. 2007; Chu and Chiu 1989; Froerer et al. 2010; Iwahashi 1972; Shelly et al. 2010) suggest that wind is a factor in the dispersal of *B. dorsalis* flies and that trap recaptures at distances greater than 20 km are not typical (less than 1% of all flies captured). Dispersion distances of 2 km are common (Froerer et al. 2010).

2.4 CONCLUSIONS

Biological information in invasive species databases provides data for integrated pest management (IPM) and policy decisions for many countries. Data errors associated with an invasive species can cause unnecessary disagreements or confusion among farmers, policy makers, and international trade partners. The statement "Many *Bactrocera* spp. can fly 50–100 km (Fletcher 1989)," which is found in multiple databases and phytosanitary documents, has been presented as evidence to extend the radii of quarantine areas for new detections of *B. dorsalis* in California from 7.5 to 150 km. The tabulated summary of scientific literature on the movements of *Bactrocera* spp. contained herein provides evidence that the statement does not accurately represent the material presented by Fletcher in his 1989 publications (Fletcher 1989a, 1989b) or any of his publications on *Bactrocera* biology or ecology (Table 2.1). Initially, long-distance movement was emphasized in research findings about fruit flies. Generalizations about flight distances from field observations and recapture studies have led readers to conclude that long-distance flights of 50 km or greater are common occurrences. In fact, few *Bactrocera* species have actually been studied regarding their dispersal capabilities and patterns, and for those that have been studied, dispersal distances more than 50 km, although it does occur, is atypical.

Results from recapture studies can support decision makers in area-wide control, survey, and quarantine activities. Interpreting the results from recapture studies can be challenging because of complex interactions of abiotic and biotic forces on fruit fly flight. Most published results from

recapture studies of fruit flies include the maximum dispersal distance, but do not communicate the mean dispersal distance and variance, which could be important in defining quarantine areas (Weldon et al. 2014). Currently, there are no universal standards among trading partners for quarantine distances based solely on the biology or dispersal habits of a fruit fly species. Dominiak (2012) points out that quarantine distances imposed for *B. tryoni* varies among trading countries and a consensus based on scientific principles is needed to harmonize trade.

Like *B. tryoni*, there is not a universal quarantine size area defined for *B. dorsalis*. Quarantine radii up to 150 km have been imposed based on the statement "Many *Bactrocera* spp. can fly 50–100 km (Fletcher 1989a, 1989b)" found in invasive species databases. This review of scientific publications on *B. dorsalis* reported distances of 0.2–97 km; however very few *B. dorsalis* flies moved beyond 20 km during the duration of recapture studies and the majority of released Oriental fruit flies moved 2 km or less. Outbreaks of *B. dorsalis* in California and Florida have been successfully contained with a quarantine radius of 7.4 km for more than 30 years. Therefore, quarantine distances and trade regulations for *B. dorsalis* (and other invasive species) should be based on the overall behavior of the species as well as the effectiveness of the trapping grid that is in place and the level of preparedness a trading partner is to implement eradication tactics. Although, long-distance dispersal for *B. dorsalis* is atypical, the longer it takes to detect an outbreak and initiate control actions, the more likely it may occur.

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3 Desiccation Resistance of Tephritid Flies

Recent Research Results and Future Directions

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CONTENTS

Abstract The ability of organisms to withstand water stress is a fundamental determinant of their abundance and distribution. The ability to survive periods of water loss ("desiccation resistance") is also related to the ability of species to become invasive pests. This is equally true of the true fruit flies (Diptera: Tephritidae), which include highly invasive, damaging pests of fruit and vegetable production. This chapter describes current knowledge of the desiccation resistance of tephritid species. Patterns of whole-organism desiccation resistance are summarized for the egg, larval, pupal, and adult life stages. Associations of desiccation resistance in tephritids with body size, body water content, and lipid content are explained. Artificial selection for desiccation resistance as a means to improve the performance of sterile

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males in sterile insect technique (SIT) programs is examined. With few exceptions, desiccation resistance of the adult and pupal stages of tephritid species is best studied. Adult desiccation resistance is much higher than anticipated. At least in *Ceratitis capitata* (Wiedemann), this may result from the ability to use water released by lipid catabolism. However, there is also considerable evidence of variation in adult desiccation resistance within species in relation to environmental variability, sex, age, and genetic background. As a consequence, there is capacity for adult tephritid desiccation resistance to be improved by artificial selection through water stress in the laboratory. It is necessary to improve taxonomic, life stage, and life history coverage of studies on tephritid desiccation resistance. Building on results available to date will improve our understanding of the genetic and physiological mechanisms conferring desiccation resistance and develop tephritid strains that can tolerate water stress. By doing so, a better understanding of responses by nonpest tephritids to a changing world and an improved management of pest tephritids will be achieved.

3.1 WHAT IS DESICCATION RESISTANCE AND WHY IS IT IMPORTANT?

Water is an important nutrient for the growth and survival of insects, so its regulation is essential. For blood-feeding, xylem-feeding, and freshwater insects, water is in abundance, at least during part of their life cycle. As a result, they face the problem of removing excess water to regulate body mass and solute concentrations in their tissues (Benoit and Denlinger 2010; Le Caherec et al. 1997). However, most insects are small and terrestrial, so they are at risk of losing water ("dehydration") because of their high surface area-to-volume ratio and the relative scarcity of water in their environment. Additionally, insects living in saline waters (Bradley 2008) or feeding on diets with high osmotic pressure (Douglas 2006) are also susceptible to osmotic water loss. The consequences of dehydration for insects include changes in membrane potential and enzyme function as cellular or hemolymph concentrations increase, decreased circulatory transport of nutrients and hormones as hemolymph volume declines, and retarded movement (and ultimately feeding and predator avoidance) in insects with hydrostatic skeletons (Harrison et al. 2012). The length of time that an organism can survive in a dehydrating environment is referred to as "desiccation resistance." This is distinct from "dehydration tolerance," which is the proportion of body water that can be lost before death (Gibbs et al. 1997).

Environmental stress resistance, and the ability of species to adapt to novel or variable environments, is of central interest in biology because it contributes to niche partitioning and biogeographic patterns. For example, across *Drosophila* species, there are clear correlations between average minimum temperature and critical thermal minimum (lower threshold for muscular function) and between annual precipitation and desiccation resistance (Kellermann et al. 2012). Environmental adaptations have particular importance for invasion biology because the ability of invasive species to survive variable environmental conditions has been suggested as a key trait that contributes to their dispersal and potential to invade new habitats (Lee 2002). Invasive species are introduced species that become established, spread, and cause negative impacts on the environment, human activities, or human health (Lee 2002); as such, they are regarded as major global threats and their management is an international research priority. Studies estimate the monetary costs for the control of invasive species to be enormous (Olson 2006). As climate change increases global temperatures, the threat of invasive insect species will increase as tropical and subtropical insects expand their range into temperate areas (Chown et al. 2007). In this context, the desiccation resistance of insects is likely to contribute to their invasive potential because, as they move away from the wetter tropical and subtropical regions, they will be exposed to reduced rainfall and increasingly dry environments (Chown et al. 2007). This has been illustrated in the dengue mosquito, *Aedes aegypti* (Linnaeus in Hasselquist) (Diptera: Culicidae), in which desiccation resistance of the eggs was a key determinant of its potential current and future distribution in Australia (Kearney et al. 2009).

The first hurdle in the success of an insect pest to colonize new environments is its capacity to adapt to stressful abiotic factors, mainly to those considered critical, such as temperature and water deficit, which can both lead to desiccation. Rapid physiological responses through processes of acclimation and acclimatization allow organisms to display reproductive behaviors temporally and spatially and to establish in new areas (Meats 1989a, 1989b, 1989c; Weldon et al. 2016). Acclimation is a rapid and reversible change in phenotype (be it physiological, biochemical, or anatomical) in response to chronic exposure under controlled conditions to a new environmental condition within the lifetime of an individual (Bowler 2005; Woods and Harrison 2002). Acclimatization is similar, except that it occurs under natural conditions (Chown and Terblanche 2006). Both acclimation and acclimatization are examples of phenotypic plasticity, which permits organism performance under varying natural conditions without any change to the genetic architecture of the individual (Arendt 2015). However, if fitness is enhanced by the capacity to exhibit phenotypic plasticity, its expression over many generations may originate differences in life history and basal tolerance among populations (Chown and Terblanche 2006). Both basal adaptations and plastic responses of individuals and populations determine their bioclimatic potential, or ability to colonize and persist in a particular environment (Meats 1989b).

To manage invasive insect pests, ecologically and socially sustainable tactics are continually being developed and optimized. However, the efficacy of tactics relying on living control agents may be reduced if released organisms are sensitive to local environmental conditions (Sørensen et al. 2012). Some evidence exists of a mismatch of environmental stress tolerance between pest insects and parasitoids released as biological control agents (Hance et al. 2007; Mutamiswa et al. 2018), although studies have focused on thermal tolerance rather than on desiccation resistance. This difference in tolerance of pests and their parasitoids is further exacerbated by changes in the tolerance of parasitoids reared under and adapted to controlled environments for classical biological control or augmentative releases (Colinet and Boivin 2011). The same considerations apply to genetic control tactics, such as various forms of strain replacement or the sterile insect technique (SIT), where live insects must be released into the field, disperse, survive, and ultimately mate with their wild counterparts. In the case of sterile insects, the capacity to achieve these goals is often diminished as a consequence of adaptation to mass-rearing conditions, inbreeding depression, direct rearing and handling effects, irradiation, and transport and release methods. However, environmental tolerance traits, including desiccation resistance, of insects reared for SIT programs are relatively understudied. This is concerning because poor survival of sterile insects in the field under current environmental conditions may be expected to worsen as climates change (Chidawanyika et al. 2012). Because of this, steps need to be taken to ensure the continued success of biologically based control tactics that rely on the release of live insects.

Some notable exceptions to the paucity of studies on the environmental tolerance traits of insects used in SIT programs include the Mexican fruit fly, *Anastrepha ludens* (Loew) (Tejeda et al. 2017); Queensland fruit fly, *Bactrocera tryoni* (Froggatt) (Weldon et al. 2013); and the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Nyamukondiwa et al. 2013). These three species are representatives of the true fruit flies (Diptera: Tephritidae), a family with about 4300 species from more than 420 genera (White and Elson-Harris 1992). Nearly all tephritids lay eggs inside of plants and the larvae feed on stems, flowers, or fruit before pupating in the soil or inside galls. However, most agriculturally important tephritids are frugivorous, with larval feeding making fruit unmarketable. Fruit fly pests also pose a significant quarantine risk, which affects international trade (De Meyer and Ekesi 2016). Pest tephritids are found throughout the tropical and temperate regions of the world, and some, including *C. capitata*, have become globally invasive pests (Malacrida et al. 2007). The success of some tephritid flies to invade new regions of the world has been attributed to their wide host range and basal developmental response and tolerance to physical environmental variables (e.g., temperature and water availability) (Hill and Terblanche 2014; Malacrida et al. 2007; Vera et al. 2002). However, it is clear that they are also highly adaptable, with flexible responses to environmental conditions through phenotypic plasticity and genetic adaptation (Diamantidis et al. 2011a, 2011b, 2008, 2009; Malacrida et al.

2007; Weldon et al. 2018). Consequently, it is important to understand the abilities of tephritid species to tolerate the physical environment. This includes determining the genetic and epigenetic architecture, biochemical pathways, and physiological processes that lead to variation in desiccation resistance within and between tephritid species.

This review describes current knowledge of the desiccation resistance of tephritid species. Whole-organism desiccation resistance is summarized in the egg, larval, pupal, and adult life stages before the proposed mechanisms underlying these patterns are explained. Because of limited knowledge of these patterns and processes in tephritids, the review is limited to pest fruit fly species within the genera *Anastrepha*, *Bactrocera*, *Ceratitis*, and *Rhagoletis*, as well as the non-pest, stem-galling genus *Eurosta*. As such, it represents species and populations scattered across Africa, Australia, and Central and North America. Thereafter, recent selection experiments to produce desiccation resistant fruit flies are reviewed. Artificial selection for desiccation resistance may offer a means to improve the performance of sterile males in SIT programs. Finally, future directions for fundamental and applied research on desiccation resistance in tephritids are identified.

3.2 DESICCATION RESISTANCE IN TEPHRITIDS

3.2.1 Eggs and Larvae

It has been proposed that eggs and larvae of frugivorous tephritid flies are usually not exposed to desiccating conditions (Meats 1989c). This is because of eggs and larvae being located inside fruits, in a moist environment. Exceptions may occur when fruits drop from a tree prematurely or become hyperosmotic during dry conditions (Bateman 1968), increasing the potential for eggs and larvae to suffer osmotic stress resulting from high fruit solute concentration. However, we are not aware of any studies that have directly compared the osmolality of the cells and hemolymph or osmotic regulation of frugivorous tephritid larvae in relation to their host fruit.

In contrast to the assumptions made for frugivorous tephritids, larvae of the stem-feeding Goldenrod gall fly, *Eurosta solidaginis* (Coquillett), survive extremely cold and dry conditions. This has led to *E. solidaginis* becoming an important model for understanding programmed responses to the cold in Nearctic region, and the link between cold temperature tolerance and desiccation resistance (Sinclair et al. 2003). Larvae of *E. solidaginis* experience extremely low temperatures and humidity during winter inside galls that they induce in their goldenrod host plants, *Solidago canadensis* L., *Solidago gigantea* Ait., and *Solidago altissima* L. Despite this, they maintain a relatively constant water content throughout the winter (e.g., Nelson and Lee 2004).

The high desiccation resistance of *E. solidaginis* larvae relates to the impermeability of their cuticular lipids to water (Ramløv and Lee 2000). Impermeability of the cuticle is acquired as hydrocarbons, mainly 2-methyltriacontane, are deposited during the third instar from September to January (Nelson and Lee 2004). During this period, total hydrocarbons increase from 122 ng/larva to 4900 ng/larva (Nelson and Lee 2004). The permeability of the cuticular lipids to water remains low up to a temperature of 40°C, at which point permeability abruptly increases (Ramløv and Lee 2000). This temperature-dependent change in cuticular permeability likely represents the melting point of hydrocarbons coating the cuticle (Gibbs 1998). To further illustrate the water-proofing properties of the cuticular lipids deposited during the third instar, studies have shown that larvae treated with a chloroform and methanol solution to remove lipids experience high water loss rates (Nelson and Lee 2004; Ramløv and Lee 2000).

Larvae of *E. solidaginis* also exhibit high levels of dehydration tolerance, which results from the movement of solutes and cryoprotectants (glycerol and sorbitol) from the hemolymph to the cells (Williams and Lee 2011). This reduces the osmotic gradient for water to leave the cells and likely maintains cellular water volume during desiccation (Williams and Lee 2011). Expression of pathways leading to stress resistance in larval *E. solidaginis* are triggered after as few as two hours of desiccation and a loss of less than 1% of fresh mass (Gantz and Lee 2015). Larvae experience these

conditions during the senescence of host plants. Evaporative water loss is also reduced through depressed metabolic rate (Williams and Lee 2005).

3.2.2 Pupae

The effects of relative humidity and immersion in water on the survival of tephritid pupae were determined by Duyck et al. (2006). Pupae of *C. capitata*, *Ceratitis catoirii* Guérin-Mèneville, the Cape fruit fly, *Ceratitis quilicii* De Meyer, Mwatawala & Virgilio (formerly identified as the Natal fly, *Ceratitis rosa* Karsch), and the peach fruit fly, *Bactrocera zonata* (Saunders), exhibited high survival at 100% relative humidity (Duyck et al. 2006). Pupae of *C. catoirii* and *C. quilicii* were the most susceptible to reduced relative humidity. In contrast, *C. capitata* suffered from even short durations of immersion in water, which may be experienced during periods of flooding. *Bactrocera zonata* pupae were the most tolerant to both low humidity and flooding (Duyck et al. 2006). These patterns of pupal desiccation resistance and flooding tolerance were aligned with the identified niche differentiation of these indigenous and invasive tephritids in the island of La Reunion (Duyck et al. 2006).

Exposure to low relative humidity has also been implicated in poor pupal survival in *Rhagoletis* species but is dependent on their origin. Results from snowberry maggot, *Rhagoletis zephyria* Snow, populations sampled from regions along a rainfall gradient in the state of Washington, United States, suggest local adaptation of pupae to dry conditions (Hill 2016). Exposure of *R. zephyria* pupae sampled from a wet, coastal location to 43% relative humidity for 8 days led to a greater than 60% reduction in adult emergence after diapause in comparison with pupae from a drier, inland location. Along the rainfall gradient, from high to low, pupal mass increased and the proportion of body water remaining after desiccation also increased (Hill 2016). Analyses of gene expression found greater differences between populations than among humidity treatments (Kohnert 2017), supporting the role of local adaptation suggested by phenotypic observations. In particular, there was an upregulation of oxidioreductases in a desiccation resistant population of *R. zephyria* (Kohnert 2017), which are important for the production of long-chain cuticular hydrocarbons (Qiu et al. 2012). In the apple and hawthorn host-races of *Rhagoletis pomonella* (Walsh), the proportion of water remaining in pupae after desiccation was higher in the hawthorn host-race (Hill 2016). This was despite pupae from the apple host-race being 33% heavier than those from the hawthorn race. In both *R. pomonella* and *R. zephyria*, pupal diapause does not contribute to improved resistance to desiccation (Kohnert 2017).

3.2.3 Adults

Meats (1989c) noted that no study had been performed to determine how adult tephritids maintain water reserves within viable limits. In the absence of empirical data, he used a physiological model based on the fundamental relationships between temperature, relative humidity, rate of water loss, and an assumption of the water reserve required for survival (i.e., dehydration tolerance) to predict adult tephritid survival. By assuming that most terrestrial insects can only replace lost water by drinking or eating food with adequate water content, Meats (1989c) predicted that a fly weighing 15 mg with 10 μg of water would be able to survive 24 hours when held at 25°C and 10% relative humidity. Declining temperature or increasing relative humidity would lead to lower water loss rates and higher survival time, and vice versa, in the absence of a source of ingestible water. At 100% relative humidity, no evaporative water loss was anticipated.

When desiccation resistance at 25°C and relative humidity below 10% was evaluated in a range of tephritid species, the survival times expected by Meats (1989c) were exceeded by a considerable margin (Figure 3.1). In *B. tryoni*, the species on which the predictions of Meats (1989c) were based, median survival time was longer than 24 hours in most cases (Weldon and Taylor 2010; Weldon et al. 2013). This was regardless of fly origin (wild or mass-reared), sex, or age, which all have significant effects on desiccation resistance in *B. tryoni* (Weldon and Taylor 2010; Weldon et al. 2013). Only in mass-reared *B. tryoni* that were 20 days old did desiccation resistance decline to a median of

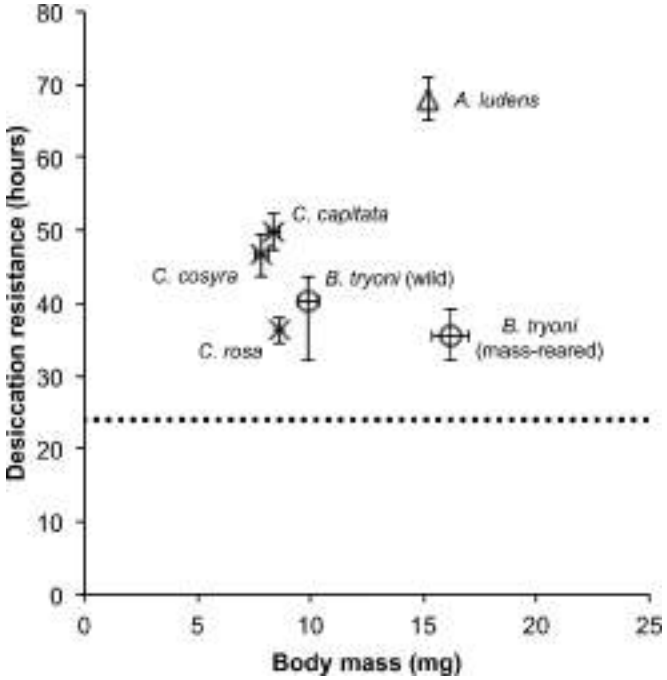

FIGURE 3.1 Relationship between mean body mass and mean desiccation resistance in adult tephritid flies. Different marker styles represent data sources: triangles (Tejeda et al. 2014); crosses (Weldon et al. 2013); circles (Weldon et al. 2016). With the exception of *Anastrepha ludens*, all body mass and desiccation resistance values are for ten-day-old flies. Body mass and desiccation resistance of *A. ludens* were determined on adult emergence. Error bars represent ± 1 standard error except for *Bactrocera tryoni*, where they indicate 95% confidence intervals. The dotted line shows desiccation resistance predicted for *B. tryoni* by Meats (1989c).

25 hours (Weldon et al. 2013). In a comparison of *Ceratitis* species, even the least desiccation resistant species, 10-day-old *C. rosa*, survived an average of 36 hours at 25°C and less than 10% relative humidity. Desiccation resistance of *C. capitata* and marula fruit flies, *Ceratitis cosyra* (Walker), of the same age was even higher, at 50 and 47 hours, respectively (Weldon et al. 2016). This is despite *C. capitata* and *C. cosyra* generally being smaller with less body water than *C. rosa* (Weldon et al. 2016). In *A. ludens*, which are large flies with a body mass within the range of 11–20 mg, mean desiccation resistance was approximately 68 hours at 25°C and 22% relative humidity (Tejeda et al. 2014).

Increasing empirical evidence shows that desiccation resistance of adult tephritid flies varies within species. There are strong effects of age, sex, and their interaction that may relate to differences in life history or senescence. Desiccation resistance of a laboratory-adapted strain of *C. cosyra* tested on the day of adult emergence was higher than when they were 10 days old (Weldon et al. 2019). In a laboratory-adapted strain of *B. tryoni*, desiccation resistance of adults declined in a continuous and regular manner over the first 20 days after adult eclosion (Weldon and Taylor 2010; Weldon et al. 2013). However, in the same species, there was no consistent effect of age on desiccation resistance over 20 days among flies derived from field-collected fruit (loquats, *Eriobotrya japonica* (Thunb.) Lindl.) and their first- and second-generation offspring (Weldon et al. 2013). Desiccation resistance of adult female *B. dorsalis*, *C. capitata*, *C. cosyra*, and *A. ludens* is generally lower than that of males (Weldon et al. 2013, 2016; Weldon and Taylor 2010). This observation correlates with lower dehydration tolerance but higher total body water in females, which suggests that the water contained in eggs represents an inaccessible pool of total body water (Weldon et al. 2013, 2016, 2019; Weldon and Taylor 2010).

Desiccation resistance of tephritid flies may also vary within individuals due to prior experience of various environmental conditions. In *C. capitata*, desiccation resistance varied among individuals based on exposure for 5 days to temperatures of 20°C, 25°C, or 30°C, although the direction of the change in desiccation resistance differed among populations (Weldon et al. 2018). Larval diet can also affect desiccation resistance, with adult *C. cosyra* reared on a standard, high yeast larval diet exhibiting lower desiccation resistance on the day of eclosion when compared to those reared on a low yeast larval diet with a protein content akin to the preferred host (Weldon et al. 2019).

As already mentioned, the origin of adult tephritid fly populations also affects desiccation resistance within a species. In populations of *C. capitata* sampled from parts of southeastern Africa and tested under common conditions (25°C, <10% relative humidity), there are considerable differences in desiccation resistance between populations (Weldon et al. 2018). These differences appear to be associated with the bioclimatic conditions prevailing in the region where the populations were sampled. Relevant to this discussion, desiccation resistance of adult *C. capitata* was weakly but negatively affected by growing degree-days, which may suggest a role of resource availability coupled with plant productivity (Weldon et al. 2018). This was despite evidence from microsatellite markers of little genetic differentiation of populations, which suggests that *C. capitata* has a high potential for evolutionary responses to environmental conditions (Weldon et al. 2018). Rapid adaptation to prevailing conditions among tephritid flies is also evident in the laboratory. Intentional application of water stress has successfully selected for improved desiccation resistance in *A. ludens* (Tejeda et al. 2016). In this case, significantly improved desiccation resistance relative to the parental generation was observed within as little as two generations and was double of that of a control population after 10 generations of selection (112 vs. 56 hours) (Tejeda et al. 2016). Unintentional selection for differences in desiccation resistance also results from mass-rearing, with wild *B. tryoni* exhibiting greater desiccation resistance than their mass-reared counterparts (Weldon et al. 2013). This was particularly evident in older adults, where median desiccation resistance of wild female and male *B. tryoni* was approximately 12 hours longer than mass-reared *B. tryoni* (25 vs. 37 hours) (Weldon et al. 2013). Whether recorded population differences in desiccation resistance in the field and laboratory represent genetic differences or potential heritable epigenetic effects is yet to be determined.

3.3 WHAT ENABLES DESICCATION RESISTANCE?

There are three physiological mechanisms by which insects can reduce the risk of water stress: increased water storage, reduced water loss rates, and enhanced dehydration tolerance (Gibbs et al. 1997). Body water content can be improved by increasing body size and hemolymph volume (Folk and Bradley 2003). Increased body size can reduce the surface area-to-volume ratio and, thereby, reduce evaporative water losses. More importantly, insect hemolymph may often serve as a reservoir that buffers insect tissues during periods of water stress (Gibbs et al. 1997). For example, laboratory-selected, desiccation-resistant *Drosophila* populations exhibited a striking increase in hemolymph volume (~330 nl, a >6-fold increase) (Folk and Bradley 2003). Body water reserves can also be elevated by increased food intake and not only due to the free water content of food but also through the release of metabolic water from the breakdown of ingested nutrients. Metabolic reserves are thought to play a large role in adaptation to desiccation resistance (Djawdan et al. 1998). Oxidative phosphorylation of glucose leads to the production of water and carbon dioxide (CO_2) as by-products, with water retained and CO_2 expired into the environment (Djawdan et al. 1998). Lipids also play a part in desiccation resistance as they represent the main form of energy storage in insects as triglycerides in body fat (Arrese and Soulages 2010) and act as a source of water upon oxidation in some species (Kleynhans and Terblanche 2009; Naidu 2001; Naidu and Hattingh 1988; Nicolson 1980). Reduced water loss rates are achieved by closing spiracles to reduce respiratory water loss, increasing rectal water reabsorption to reduce excretory water loss, or by enhancing the water-proofing properties of the cuticle to reduce cuticular

evaporative water loss (Gibbs 1998; Harrison et al. 2012). Improved dehydration tolerance in insects is largely associated with osmotic properties of cells and the hemolymph. Carbohydrates, particularly trehalose, are known to play a large role in insect osmoregulation by binding water molecules (Djawdan et al. 1998; Gefen et al. 2006). In addition, proteins also help insects to tolerate dry conditions, with heat shock protein expression upregulated, presumably to protect water stressed cells from changes in pH and solute concentrations (Hayward et al. 2004; Tammariello et al. 1999). In addition, expression of late embryogenic abundant (LEA) proteins is also associated with dehydration (Kikawada et al. 2006) and may have a role in DNA protection during desiccation (Ryabova et al. 2016).

In the case of tephritid flies, the mechanisms that confer improved desiccation resistance are yet to be fully explored. However, as will be discussed, results to date suggest some of the mechanisms associated with improved water stress in other insects, particularly body size, water content, and lipid reserves, are also correlated with enhanced desiccation resistance in tephritid species that have been studied.

3.3.1 Body Size

In the model organism *Drosophila melanogaster* Meigen, body size has not been a good indicator of individual desiccation tolerance. Some studies have reported a positive relationship between size and stress resistance (Chippindale et al. 1996; Gibbs and Matzkin 2001; Telonis-Scott et al. 2006), whereas others have not found this relationship (Gibbs et al. 1997; Hoffmann and Harshman 1999). For *B. tryoni*, as in *Drosophila*, mixed patterns have been observed among size, sex, and stress resistance. When wing length was used as a proxy for size, resistance under desiccation was not correlated with size, and both sexes showed similar resistance (Weldon and Taylor 2010). However, further studies demonstrated that body mass correlated positively with desiccation resistance (Weldon et al. 2013). In the case of *A. ludens*, it was observed that desiccation resistant individuals were heavier and exhibited higher lipid and water content than unselected, control flies (Tejeda et al. 2014). Larger pupae of *R. pomonella* and *R. zephyria* also survive better during exposure to dry conditions than smaller ones (Hill 2016). Nevertheless, despite often being smaller than females, males are more desiccation resistant (see above). This pattern was observed in *B. tryoni* and *A. ludens* and may be explained by differences in life history between the sexes. In addition, males have higher lipid and water content than females at emergence (Tejeda et al. 2014; Weldon et al. 2013). Thus, size by itself does not explain improved desiccation resistance in tephritids studied to date.

3.3.2 Lipid and Water Content and Lipid Catabolism

Ambiguous results have been observed among studies on the role of lipid reserves and water content in insects. Higher lipid reserves have correlated with higher desiccation resistance in some *Drosophila* species (Telonis-Scott et al. 2006). However, in studies involving artificial selection for desiccation resistance, invariable (Hoffmann and Parsons 1989) or even reduced (Djawdan et al. 1998) lipid content has been observed. This inconsistency is also observed in the role of water reserves. For example, some studies in *Drosophila* report the canteen strategy, with an increase of body water content and water storage in the hemolymph (Folk et al. 2001; Gibbs et al. 1997). However, more recently, it has been observed that individuals with a high water content presented reduced desiccation resistance if associated with a low level of desaturated cuticular hydrocarbons (CHCs) (Ferveur et al. 2018).

In tephritids, lipid and water contents seem to play a decisive role in the desiccation resistance of some species. Individuals with higher desiccation resistance exhibit higher levels of both lipid and water contents (Tejeda et al. 2014). Moreover, *A. ludens* flies artificially selected for desiccation resistance exhibited higher levels of water and lipids than nonselected flies (Tejeda et al. 2016). Desiccation resistance is greatest among adults with high body water content in *B. tryoni* (Weldon

et al. 2013), *C. capitata*, *C. cosyra*, and *C. rosa* (Weldon et al. 2016). In *C. capitata*, body lipid content was higher than in other tested *Ceratitis* species, and lipids were selectively catabolized during a short period of dehydration (Weldon et al. 2016). However, the use of lipids as a source of metabolic water was not apparent in *C. cosyra* or *C. rosa* (Weldon et al. 2016).

3.3.3 Cuticular Lipids

Transpiration through the cuticle is the main route of water loss in insects (Gibbs and Rajpurohit 2010). For this reason, cuticular water loss by insects in dry environments is minimized through changes in the quantity and composition of water-proofing epicuticular lipids (Gibbs 1998). For example, different desiccation resistant lines of *D. melanogaster* have reduced cuticular water permeability as a consequence of altered lipid composition, in addition to increased hemolymph volume, higher extracellular carbohydrate storage that increases hemolymph osmolality (Gibbs et al. 1997), and elevated tolerance to water loss and lipid storage (Telonis-Scott et al. 2006).

The epicuticular layer, which covers almost the entire surface of an insect, is mainly responsible for protecting insects from desiccation (Downer and Matthews 1976; Drijfthout et al. 2010). A large component of the lipids that comprise the epicuticle are long-chain hydrocarbons, generally known as CHCs that, aside from protection, also play an important role in sexual selection (Chung and Carroll 2015; Ferveur et al. 2018). By using artificial selection in *D. melanogaster*, it was observed that desiccation resistance was positively linked to the proportion of desaturated CHCs, which considerably reduce transpiration (Ferveur et al. 2018). Furthermore, once selected, this proportion is kept even after several generations without the stressor (Ferveur et al. 2018).

In tephritids, CHC profiles have been employed mainly for taxonomic purposes. For example, in "lowland" and "highland" populations of *C. rosa* (now *C. rosa s.s.* and *C. quilicii*, respectively), statistical analyses of CHC composition showed distinct interspecific identities, with several CHC specific to each of the lowland and highland populations (Vaníčková et al. 2015). Whether this difference confers differences in desiccation tolerance is not known, but the two species are associated with different bioclimatic regions (Mwatawala et al. 2015; Tanga et al. 2018). Furthermore, it has been reported that two stenophagous species, *C. cosyra* and *C. rosa*, lost water at significantly higher rates under hot, dry conditions and do not catabolize lipids or other sources of metabolic water during water stress compared to the polyphagous *C. capitata* (Weldon et al. 2018). Thus, it is possible that differences in the CHC profiles of the populations also confer differences in their desiccation resistance. Furthermore, Weldon et al. (2019) postulated that changes in desiccation resistance as adult tephritids age may also relate to abrasion or qualitative changes of the epicuticular lipids.

3.4 DEVELOPMENT OF DESICCATION RESISTANT STRAINS FOR SIT

The development of desiccation resistant strains can be a powerful tool that improves the efficiency of SIT through increased longevity of released males in environments causing water stress. Thus, a promising method to improve SIT is to produce and release strains that are resistant to desiccation. So far, desiccation resistant strains have been produced only in *A. ludens*. These strains, derived from the already adapted mass-rearing strains, would provide SIT with an added advantage over regular bisexual strains in terms of increased longevity of sterile males under environmentally stressful situations. The increased survival of adults from the selected strain has been attributed to the fact that they are heavier and store 20% more water than control strains (Tejeda et al. 2014). Also, they store considerably higher lipid reserves than control strains.

Nevertheless, although these strains have increased longevity for males, they are not without disadvantages because certain trade-offs have been detected. Furthermore, these strains are still at the experimental stage; the added detrimental effect of mass rearing has not been documented, and they have not been tested under field conditions. For example, even though no detrimental effects

are predicted, their ability to detect protein baited traps has not been tested. Thus, we review the effects on fecundity, fertility, and pre- and postmating competitiveness of the desiccation resistant strain of *A. ludens*.

The desiccation resistant strain of *A. ludens* was developed from the standard bisexual mass-rearing strain produced in the Moscafrut facility, Metapa de Dominguez, Chiapas, Mexico. Ten experimental families were separated from the main colony. Five of these were selected for experiments using directional selection for fly longevity under desiccation stress, and the other five families served as unselected, control populations. Each family or population was comprised of 200 males and 200 females and placed in separate plexiglass cages from emergence. Selection for desiccation resistance was carried out by placing each selected population in a cage with three containers of silica gel, covered with mesh to avoid direct contact but no food or water. Cages were then sealed with plastic film. Humidity inside each cage was thus reduced to 20%–30%. When 12% of the population remained alive, the survivors were transferred to another cage with water and food. Control cages were handled in the same way except they were not subjected to low humidity. For each of the 10 populations, 25 fly pairs were used to produce the following generation. This was repeated for 10 generations (Tejeda et al. 2016).

3.4.1 Fecundity/Fertility/Longevity

Changes in the mean survival time of flies was observed as early as the second (F_2) generation (Control: 62.01 ± 1.31 [mean ± s.e.]; Resistant: 74.12 ± 1.34). After only 10 generations, selected populations exposed to low humidity without food or water lived twice as long as unselected populations (Tejeda et al. 2016). Individuals also had a higher life expectancy than control flies. The average number of eggs that females laid was not significantly different between selected and control lines (Control: 56.38 ± 2.69; Resistant: 112.33 ± 5.50) (Tejeda et al. 2016).

However, some interesting trade-offs were observed. For example, the mean age of females at which reproduction started was significantly delayed. Control flies started reproducing (laying eggs) at an average of 25 days of age in comparison with selected females, which started approximately 10 days later. This could have detrimental effects in terms of logistics and costs of mass rearing because flies would need to be kept and fed for longer periods of time before they start producing eggs. Thus, this is something that warrants further research.

Also, another trade-off between longer survival and reproduction was observed, as selected females, on average, had a lower daily egg production compared to control females (34 eggs/female vs. 25 eggs/female). The intervals between generations were shorter for control compared to desiccation-selected lines. Pupal stage duration was approximately 40 hours longer for the selected populations compared to the control (Tejeda et al. 2016). Again, this may imply considerable costs for mass rearing in terms of diet, personnel, and oviposition devices allocated to the colony. For example, the size of the colony would probably need to be increased to meet production standards. However, a longer pupal stage duration could be advantageous if pupae need to be transported between production and prerelease holding facilities or release sites.

3.4.2 Sexual Behavior

Sexual behavior can be grossly divided into precopulatory and postcopulatory behaviors. For the desiccation resistant line of *A. ludens*, male sexual competitiveness was evaluated in field cages, where desiccation resistant and control males competed for matings with wild females. There was no significant difference in the frequency of matings between selected or control males, indicating that selected males were just as competitive as control nonresistant males in obtaining matings with wild females (Tejeda et al. 2016). This is an important result because the main objective of SIT is for males to mate with a wild female and render her infertile. No detrimental effects on mating performance indicate that, at least for this component of behavior, there are no apparent trade-offs between desiccation resistance and sexual performance.

One further aspect of sexual behavior that needed to be evaluated is the effect of irradiation on sexual performance. For this, the resistant and nonresistant strains were sterilized 48 hours before emergence and in hypoxia to the standard sterilization dose of 80 Gy using a ^{60}Co irradiator (model GB-127, Nordion International Inc., Ottawa, ON, Canada). Selected and nonselected males competed for matings with wild females in field cages. Again, as with fertile males, there was no significant difference in mating competitiveness between selected and control lines (Tejeda et al. 2017).

Compatibility tests were carried out also in field cages between sterile males and females of the selected strain against the standard bisexual mass-reared strain of *A. ludens* from Moscafrut and against wild males for matings with wild females. The Relative Sterility Index (RSI) and the Male Relative Performance Index (MRPI) (Cayol et al. 1999) were the same between both the selected and the Moscafrut strain. Wild males were more likely to obtain copulations than males from either of these strains. There was no significant difference in the Index of Sexual Isolation (ISI) between strains, indicating that both strains were compatible with the wild population. The Female Relative Performance Index (FRPI) (Cayol et al. 1999) indicated for both strains a higher participation of sterile females in obtaining matings compared to wild females. Despite slightly higher FRPI values for the Moscafrut strain compared to the resistant selected strain, there was no statistically significant difference between these two strains (Tejeda et al. 2017).

A further test of previously stressed sterile males (no water or food and 30%–40% humidity for 24 hours before observations) compared with nonstressed sterile males (food and water ad libitum at 60%–80% humidity) of both strains (Moscafrut vs. selected) revealed that desiccation-selected males obtained 88% of matings with wild females. When males were reared in the nonstressed environment, both strains had similar RSI, as opposed to when males were reared in the stressful environment, where RSI was significantly different from the expected value, indicating that the selected strain outperformed the Moscafrut strain (Tejeda et al. 2017). This suggests that if the desiccation-selected males were released in a dry environment, their capacity to mate with and induce sterility in wild females would likely be better than the currently used strain.

3.4.3 Postcopulatory Behaviors and Mechanisms

There is now widespread recognition that sexual selection and behavior do not end with mating but, rather, continue during and after copulation. During mating, males transfer sperm and secretions from the male accessory glands. These are products from the male ejaculate and have important effects on female remating (Abraham et al. 2016; Radhakrishnan and Taylor 2007, 2008). Thus, any strain developed for SIT should not only exhibit suitable precopulatory behavior but should also have adequate postcopulatory behaviors, including sperm and accessory gland product transfer.

Recent studies have found that there are also biological trade-offs between resistance to desiccation and the male ejaculate. Control females mating with the selected strain of *A. ludens* had less sperm stored in their spermathecae than control females mating with nonselected males (Pérez-Staples et al. 2017). Also, resistant males were found to have smaller accessory glands and seminal vesicles (organ where males store mature sperm; Martínez and Hernández-Ortiz 1997) than nonselected males (Pérez-Staples et al. 2017). These results suggest that the evolution of desiccation resistance comes at a cost for the male, in this case manifested through smaller accessory glands and lower amount of sperm stored by females, which probably indicates lower sperm transfer as well.

Control females that mated with resistant males also suffered a cost in terms of lower fecundity compared to control females mating with nonresistant males. One of the peptides that are produced in the male accessory glands is ovulin, which in *D. melanogaster* affects female oogenesis, fecundity, and the egg-laying process (Avila et al. 2011). Although the production and function of specific peptides in the ejaculate is unknown in *A. ludens* and, indeed for most tephritids of

economic importance, a lower fecundity for females mating with resistant males suggests that some components of the male ejaculate may be compromised during the evolution of the ability to withstand water stress.

Desiccation-selection did not affect all components of the ejaculate equally. For example, no effect on protein content of the male accessory glands or testes were found, and no detrimental effects were found in the male ability to inhibit female remating. Wild *A. ludens* females were just as likely to remate after mating with a desiccation-selected or control male.

3.5 FUTURE DIRECTIONS

3.5.1 Improved Taxonomic, Life Stage, and Life History Coverage

To date, desiccation resistance data are available only for *A. ludens*, *B. tryoni*, *B. zonata*, *C. capitata*, *C. catoirii*, *C. cosyra*, *C. rosa*, *E. solidaginis*, *R. pomonella*, and *R. zephyria*. Of these, *C. capitata* is the only species with published desiccation resistance data for more than one life stage: pupae (Duyck et al. 2006) and adults (Weldon et al. 2016; Weldon et al. 2018). There is clearly a need to study the water stress experienced and desiccation resistance exhibited by a wider range of tephritid species throughout all life stages. As noted previously, eggs and larvae may experience water stress in some fruits owing to osmotic gradients between the insect and the osmolality of fruits of varying species and conditions. The pupal stage may also be susceptible to dehydration or drowning under some circumstances, and the tolerance of species, and even populations, to these conditions may vary (Duyck et al. 2006; Hill 2016). Development of a wider database of tephritid desiccation resistance will assist with identifying the potential invasiveness of pest species. It will also enable predictions of the effects of global change on the majority of tephritid species that are not of economic concern but are rather important components of ecosystems throughout the world. In relation to this, it is important to obtain environmental tolerance data not only for multivoltine frugivorous pest tephritids but also for the full range of life histories and host use patterns that are encompassed by this diverse insect group. Here we have already discussed how *E. solidaginis*, a univoltine, stem-galling host specialist with no economic importance, has profoundly shaped our understanding of the role of water loss in insect cold tolerance (e.g., Gantz and Lee 2015; Williams et al. 2004). It may be that other understudied species in this group will have a similar contribution.

3.5.2 Understanding Mechanisms of Desiccation Resistance

Most studies indicate that the invasive potential of polyphagous flies, such as *C. capitata* and *A. ludens*, is related to a rapid capacity for adaptation to abiotic stressors. Polyphagous insects have the ability to face heterogeneous environments of host plants and abiotic conditions but keep the reproductive potential that allows them to persist in those variable habitats (Gilchrist et al. 2008; Weldon et al. 2016). Thus, studying complex interactions among different types of trade-offs is necessary for a better understanding of the response to stressors (i.e., specialist–generalist trade-offs, allocation trade-offs, and acquisition trade-offs) (Angilletta et al. 2003). For example, future studies could include the voltinism of species as a character of their life history to determine their bioclimatic potential. Comparative genomic, epigenetic, and transcriptomic data are also required to understand the mechanisms leading to desiccation resistance and any associated trade-offs.

3.5.3 Selection for Desiccation Resistant, Mass-Reared Tephritids

In their recent review, Hoffmann and Ross (2018) showed that, in general, the evolutionary response of laboratory-adapted lines to stress was negative, although studies for Diptera were particularly promising. Thus, it is important to carry out these selection experiments on different tephritid

species. For programs that use SIT and that will release sterile males in dry and arid conditions, it would be desirable to examine how their mass-rearing strains can develop a higher resistance to desiccation. Because laboratory-adapted lines tend to be more sensitive to stress (Hoffmann and Ross 2018), this should be countered in mass-rearing programs. Potentially, all mass-rearing strains that require release in arid environments can be improved in terms of response to water stress.

Further studies are needed on some key issues of desiccation resistant lines. Although field cage studies for *A. ludens* have demonstrated that selected lines exposed to stress before mating perform as well or better than control lines (Tejeda et al. 2017), it would be ideal to test calling and mating activities of these males in arid conditions. As biological trade-offs were detected in certain life history components of the selected line, it is also important to study the effect of resistance to water stress on the age of sexual maturation for males. This can have important consequences for holding males during the prerelease period.

3.6 CONCLUSIONS

Knowledge of the desiccation resistance of tephritid flies is limited, which is surprising considering their importance as economic pests of fruit and vegetable production, as well as biological control agents for invasive plants. The research reviewed here highlights that the adults of some tephritid fly species, particularly those that are widespread pests, are able to tolerate water stress to a greater extent than predicted by physiological models. Furthermore, selection for improved desiccation resistance is possible, which may lead to the development of strains better able to survive when released in SIT programs. However, much still needs to be done to identify the mechanisms underlying the ability of tephritids to tolerate water stress. The in-depth understanding of the ability of the larval stages of *E. solidaginis*, a nonpest, univoltine, host specialist, to tolerate extreme desiccation, illustrates how the study of this trait in a wider range of tephritid species and life stages can contribute to fundamental knowledge of insect physiology. But furthermore, this kind of knowledge can help to predict how species distributions may be affected in a changing world, in particular given the scenarios of climate change that consistently predict higher temperatures and reduced rainfall in many temperate areas. Tephritid species particularly tolerant of desiccation may also pose the next threat to global food security through their effect on fruit and vegetable production.

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4 Mating Compatibility between Two Populations of *Anastrepha fraterculus* (Wiedemann) (Diptera: Tephritidae) from Argentina and Uruguay

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CONTENTS

Abstract *Anastrepha fraterculus* (Wiedemann) has been reported to show extensive morphological variation along its geographic distribution and is currently recognized as a complex of cryptic species composed of at least eight different morphotypes. The Brazilian-1 morphotype includes the Argentinean and southern Brazilian populations. To contribute with basic information on the distribution of *A. fraterculus* morphotypes, the sexual compatibility between a Uruguayan and an Argentinean population was evaluated. Mating compatibility was evaluated in field cages under semi-natural conditions. The Argentinean population was obtained from a colony of the laboratory of the Instituto de Genética "E. A. Favret" (INTA Castelar), Buenos Aires, established in 2007. The Uruguayan population came from infested fruits of *Acca sellowiana* (Berg. 1855) Burret 1941 (Myrtaceae). At the moment of the trials, Argentinean flies were between 11 and 17 days old and Uruguayan flies were between 16 and 26 days old. Sexual compatibility was established using the index of sexual isolation (ISI), the male and female relative performance indices (MRPI, FRPI), and a Kruskal-Wallis one-way analysis of variance with subsequent pairwise comparison tests of the four types of pairs formed according to male and female origin. Latency, mating duration, and location of the couples were also recorded. The ISI value was significantly different from zero because

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of a greater performance of the Argentinean adults. There were no significant differences between the frequency of homotypic Uruguayan couples and heterotypic couples, whereas the frequency of Argentinean homotypic couples was significantly higher than the rest. No significant differences were found for the other evaluated parameters. Results suggest that Uruguayan populations belong to the Brazilian-1 morphotype considering that the greater performance of Argentinean flies is probably because of faster sexual maturation rates and an inherent greater mating propensity rather than to reproductive isolation.

4.1 INTRODUCTION

Anastrepha fraterculus (Wiedemann), the South American fruit fly, is widely distributed from the Rio Grande Valley in northern Mexico to central Argentina (Malavasi et al. 1999). With more than 100 plants reported as hosts (Norrbom 2004), it is a species of major economic importance in many countries in South America, not only because of its destructive potential but also because of quarantine restrictions imposed on fruit export (Steck 1999).

The South American fruit fly has long been reported to show extensive morphological variation along its geographic distribution (Lima 1934, Stone 1942, Steck 1999, Hernández-Ortíz et al. 2004, 2015). Many studies confirm that this morphological variation is associated with differences in host use, the presence and degree of reproductive isolation, karyotypic differences, isozyme divergence, and DNA sequence divergence. The existence of this morphological variation has been revealed by multivariate morphometric analyses (Hernández-Ortiz et al. 2012). An extensive list of bibliography reviewing this research can be consulted in Rull et al. (2013), Cladera et al. (2014), Hernández-Ortíz et al. (2015), and Manni et al. (2015). In consequence, *A. fraterculus* is currently recognized as a complex of cryptic species composed of at least eight different morphotypes clustered into three phenotypic lineages. The Mesoamerican-Caribbean lineage consists of Mexican and Venezuelan morphotypes. The Andean lineage consists of the Andean, the Peruvian, and the Ecuadorian morphotypes. Finally, the Brazilian lineage is composed of three Brazilian morphotypes: Brazilian-1, Brazilian-2, and Brazilian-3 (Hernández-Ortiz et al. 2015).

In the Brazilian lineage, the Brazilian-1 morphotype includes the Argentinean populations (Alberti et al. 2002, Petit-Marty et al. 2004, Vera et al. 2006) and southern Brazilian populations (Smith-Caldas et al. 2001, Alberti et al. 2002, Basso et al. 2003, Hernández-Ortíz et al. 2004, Rull et al. 2013, Vaníčková et al. 2015, Dias et al. 2016). In the state of Sao Paulo, Brazilian-1 overlaps with Brazilian-2, where both morphotypes maintain their genetic integrity despite sympatry and partial reproductive compatibility (Selivon et al. 2005). The Brazilian-3 morphotype was also found in sympatry with Brazilian-1 and Brazilian-2 in the coastal areas of the state of Sao Paulo and in the inland plateau of southeastern and southern Brazil (Selivon et al. 2004).

The main practical reason that makes the delimitation of the *A. fraterculus* morphotypes essential is that it is a basic requirement for the implementation of the sterile insect technique (SIT). SIT is a method of pest control that consists of inundative releases of sterile insects into a wide area to reduce reproduction in a field population of the same species (FAO-IPPC 2016). A great research effort is being made to gather basic knowledge to enable the adjustment of this technique for the control of *A. fraterculus* (Cladera et al. 2014). In addition to the control of the pest, the key benefits of SIT derive mostly from a reduction in pesticide use, minimizing environmental and health costs. Furthermore, SIT is an environmentally friendly strategy for use in area-wide pest management (AWPM) because it can be applied not only in commercial orchards but also in backyards and urban areas not usually protected by insecticides (FAO/IAEA 2005, Dias and García 2014).

Although there is a great deal of research attempting to elucidate how the complex of cryptic species under the name of *A. fraterculus* is composed, none of it includes populations from Uruguay. Rull et al. (2013) suggest a geographical range of the Brazilian-1 morphotype from

Monte Alegre do Sul in southeastern Brazil to Buenos Aires, Argentina; the southern limit of this morphotype has been determined up to the present day. To contribute with basic information on the distribution of *A. fraterculus* morphotypes and to prepare for an eventual application of the SIT in Uruguay, a study on the sexual compatibility between a population from Uruguay and a population from Argentina (Brazilian-1 morphotype) of *A. fraterculus* was carried out.

4.2 MATERIALS AND METHODS

4.2.1 Biological Material

The Argentinean population of *A. fraterculus* was obtained from a laboratory colony. This population was derived from an experimental colony kept at the Estación Experimental Agroindustrial Obispo Colombres (EEAOC), Tucumán, Argentina, which was originally established with pupae recovered from infested guavas (*Psidium guajava* L., Myrtaceae) at the vicinity of Tafi Viejo (Tucuman) in 1997, (Jaldo et al. 2001, Vera et al. 2007).

The Uruguayan population was obtained directly from infested fruits of *Acca sellowiana* (Berg. 1855) Burret 1941 (Myrtaceae) collected from a commercial orchard located in Montevideo (34° 44′S; 56° 16′ W). Fruits were placed in sandboxes covered with voile and checked daily to separate the pupae.

Pupae and adult flies from both origins were maintained under the same controlled conditions (temperature [T]: 23°C, relative humidity [RH]: 60%–70%, photoperiod: 10L–14D) until the day of the trials. Pupae were first placed separately in emergence cages. After emergence, flies were separated and placed in 1-L containers with 25 adult flies each and sorted according to date, sex, and origin. In each container, adult flies were supplied with water and a diet composed of bee honey, hydrolyzed yeast (in a 3:1 ratio), and food dye (Laboratorio Fleibor S.R.L., Buenos Aires, Argentina). The Argentinean population was colored red and the Uruguayan population was colored blue.

4.2.2 Mating Compatibility Test

The test took place in the campus of the Facultad de Agronomía, Universidad de la República, Uruguay. Three field cages made of screen fabric, measuring 3 meters in diameter by 2 meters in height, were set inside a greenhouse to obtain suitable temperature conditions at the moment of release. Six potted plants of *Citrus limon* (Rutaceae) var. “Limon criollo” were placed inside each cage to provide perching places.

Between June 9 and 22, 2018, eight replicates of the trial were carried out: three on June 9, one on June 13, three on June 20, and one on June 22. The dates of the replications depended on the availability of sexually mature adults. At the moment of the trials, Argentinean flies were between 11 and 17 days old and Uruguayan flies were between 16 and 26 days old.

Twenty-five males and 25 females of each origin were released inside of each field cage. Because mating occurs mainly at sunrise (Malavasi et al. 1983, Vera et al. 2006) and given that the experiments were carried out in winter (where the average temperature is 10.4°C), the photoperiod in the rearing room was adjusted so the lights were turned on at 10 am to allow the greenhouse to reach temperatures higher than 18°C at the moment when the flies were released. The maximum temperature recorded during the tests was 32°C and the mean relative humidity (RH) was of 75%.

Most releases started at 9:45 am with males and finished at 10:00 am with females. Only on June 13, due to prevailing low temperatures, fruit flies were kept in the dark and males and females were released at 10:45 am and 11:00 am, respectively. One observer remained inside each cage until the end of the trials, until 2 hours after the last male callings occurred and sexual activity ceased. Each observed mating pair was collected in a 50 mL vial and placed in the shade until the pair disengaged. Male and female color, time of start and end of copulation, and location were recorded for each mating pair. Location was recorded as either net, ground, stem, abaxial-adaxial side of leaf, height on tree, and cardinal point (FAO/IAEA/USDA 2014).

4.2.3 Data Analysis

The percentage of mating inside each cage was calculated to corroborate that all replicates had reached at least 20% of potential couples on the plants (FAO/IAEA/USDA 2014).

Mating compatibility was assessed by means of the Index of Sexual Isolation (ISI). An ISI value of 1 indicates complete assortative mating, an ISI value of −1 indicates complete outbreeding, and an ISI estimate of 0 indicates random mating. Mating competitiveness was assessed by means of the Male and Female Relative Performance Indices (MRPI and FRPI). MRPI varies from 1, when males of one of the tested populations engage in all the copulations, to −1, when males from the other population are present in all copulations. The range for FRPI is the same as that of MRPI. Values close to zero indicate similar participation from males (MRPI) or females (FRPI) independently of the origin of the population (Cayol et al. 1999). To verify random mating, confidence intervals at 95% were estimated to assess departures from zero (Rull et al. 2013). Differences among mating combination frequencies were tested using a Kruskall–Wallis one-way analysis of variance by ranks, followed by pairwise comparisons (Conover 1999). Statistical analyses were performed with InfoStat/Libre (Infostat 2018).

Data recorded for latency to first mating and mating duration were log transformed and compared between mating combinations by means of a one-way ANOVA followed by Tukey multiple comparison tests. Differences between homotypic couples in location of mating on plant height and cardinal point were tested using a Kruskall–Wallis one-way ANOVA by ranks and pairwise comparisons.

4.3 RESULTS

The percentage of mating exceeded 20% in all cages, with a mean value of 28.8% ± 2.59%, indicating that the environment was suitable for mating. The ISI, MRPI, and FRPI were statistically different from zero (Figure 4.1). The sexual isolation index was 0.29 ± 0.13, a positive value that indicates that there were more homotypic than heterotypic mating couples. The mating performance index was 0.23 ± 0.07 for males and 0.18 ± 0.12 for females, which implies that males and females from the Argentinean population were engaged in most of the copulations, which indicates a greater activity of the Argentinean population in both sexes (Figure 4.1). In addition, when we compared the number of couples per type of mating combination, there were no statistically significant differences among the heterotypic mating combinations and the Uruguayan homotypic combination, whereas the frequency of the homotypic combination of the Argentinean population was about twice the frequency of the homotypic combination of the Uruguayan population (Figure 4.2).

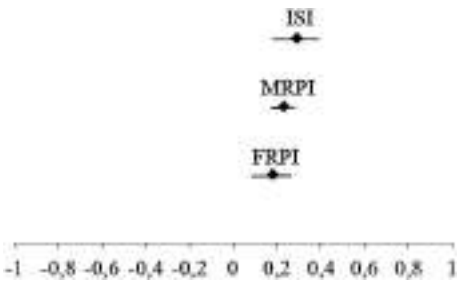

FIGURE 4.1 Index of sexual isolation (ISI) and relative performance indices for males (MRPI) and females (FRPI) with associated 95 Cis.

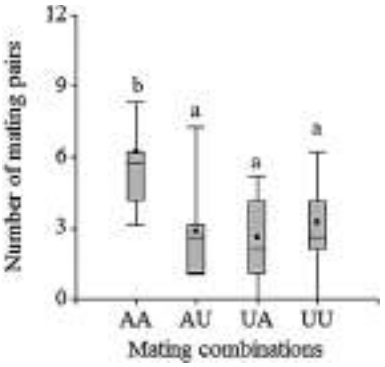

FIGURE 4.2 Mean, median, and quartiles of each mating combination. Capital letters indicate the origin of fruit flies: A refers to fruit flies from Argentina and U refers to fruit flies from Uruguay. The first letter indicates the origin of males and the second letter the origin of females. Data with the same lowercase letter are not significantly different according to the Kruskal–Wallis test (P <0.02) and pairwise comparisons tests (P <0.05). (From Conover, W.J., *Practical Nonparametric Statistics*, John Wiley & Sons, New York, 1999).

TABLE 4.1
Mean ± SE Mating Duration and Latency in Minutes

Mating Combination	Mating Duration	Latency
AA	59.5 ± 6.8	45.9 ± 5.4
AU	55.6 ± 6.5	34.5 ± 6.7
UA	45.1 ± 8.5	51.0 ± 14.4
UU	60.5 ± 6.8	43.6 ± 7.7

n = 8; Data were log transformed prior to the ANOVA and no statistical differences were found for either mating duration (P = 0.23) or latency (P = 0.4). Capital letters indicate the origin of fruit flies: A refers to fruit flies from Argentina and U refers to fruit flies from Uruguay. The first letter indicates the origin of males and the second letter the origin of females.

There were no statistically significant differences (P = 0, 13) in location on plant height, and just 1 out the 113 couples that mated on the leaves did so on the adaxial side. Matings outside the tree involved 17.2% of those recorded, with 9% taking place on the floor of the cage and 8.2% on the net walls and the ceiling. Matings on the ceiling were all homotypic mating couples from Uruguay, whereas matings on the floor were all homotypic mating couples from Argentina. Mating duration and latency were not statistically different among mating combinations (Table 4.1).

All copulations occurred mostly during the first hour after flies were released (Figure 4.3), and no sexual activity was observed after 3 hours.

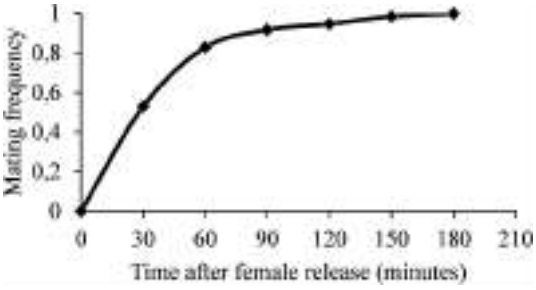

FIGURE 4.3 Accumulated mating frequency as a function of time.

4.4 DISCUSSION

In previous studies where *A. fraterculus* populations from southern South America showed full sexual compatibility, ISI values ranged between −0.01 and −0.14. Pairwise comparisons included populations from Argentina (Petit-Marti et al. 2004), southern Brazil (Dias et al. 2016), and Argentina and southern Brazil (Rull et al. 2012). In comparisons among populations with strong sexual incompatibility, such as the Mexican, Peruvian, and Brazilian-1 linages, ISI values varied between 0.74 and 0.92 (Vera et al. 2006, Cáceres et al. 2009, Rull et al. 2012), whereas populations from southeastern Brazil mating with one Argentinean population and one Peruvian population showed partial incompatibility, with ISI values of 0.43 and 0.55, respectively (Vera et al. 2006, Dias et al. 2016).

The ISI value obtained during this study (0.29) indicates a tendency toward random mating, being closer to 0 than to 1 or −1. Comparisons among different mating combinations showed similar frequencies for the Uruguayan homotypic combination and both heterotypic combinations; thus, the slight deviance from 0 (random mating) could be attributed to a greater propensity to mate of the Argentinean laboratory population. Liedo et al. (2002) observed that laboratory-reared females of *Ceratitis capitata* were more prone to mate than wild females, and that laboratory flies, both females and males, matured much earlier than wild flies. Calkins (1984) stated that it takes a few generations for insects to thrive under artificial rearing conditions and that the rearing regime probably selects for simpler courtship and mating behavior and also for earlier reproduction than in wild populations. That is considering that long and intense courtships have a risk of being interrupted by other males adapted to crowded conditions. In addition, because under artificial conditions all females become sexually mature at about the same time and earlier than wild females, an intense competition between females for fit males is promoted, favoring females that require fewer criteria for male selection (Calkins 1984, Calcagno et al. 1999).

Argentinean males had a better mating performance than Uruguayan males. Although it is expected that males from artificial-rearing conditions are less competitive than wild males when they are competing for wild females (Liedo et al. 2002), the better performance of Argentinean males was as a result of their mating with females coming from the same artificial-rearing conditions. In addition, the evaluated laboratory colony of *A. fraterculus* has shown good competitiveness in previous studies, particularly when given the same adult diet as wild males (Rull et al. 2013, Vera et al. 2007). If we consider only Uruguayan females, Uruguayan and Argentinean males showed a similar performance. It is possible that the Argentinean population had a greater proportion of sexually mature individuals because of artificial selection during rearing and, in some way, it favored a greater mating performance in this population.

Regarding the location on the plant and cardinal point, we did not find significant differences. However, it is important to consider that to interpret the results on location on the plant, we have to take into account that the plants were relatively small, although they provided enough foliage for flies to perch on, and it was not possible to clearly delimit the top, middle, and bottom. A similar situation occurred with the cardinal points.

Most of the copulations occurred during the first 2 hours after artificial sunrise, which agrees with the results of Petit-Marty et al. (2004). Also, there were no differences in latency, indicating that there is no temporal isolation between these populations.

Different studies agree with the fact that the morphotype present in Argentina and southern Brazil is the Brazilian-1 morphotype (Hernández-Ortíz 2012). Petit-Marty et al. (2004) evaluated mating compatibility among four populations from Argentina, including a population from Tucumán, the same region where the Argentinean flies used in the present study came from, and confirmed full compatibility among populations. Rull et al. (2013) evaluated the reproductive compatibility among two strains from southern Brazil (Pelotas and Vacaria) and one strain from Argentina (Tucumán) and also found that they were fully compatible. These results were later confirmed by a study with a multidisciplinary approach that included wild flies of four populations from southern Brazil (Bento Gonçalvez, Pelotas, Vacaria and São Joaquim) (Dias et al. 2016) and by a recent courtship-behavior study including different populations of *A. fraterculus* (Roriz et al. 2019).

The distribution suggested by Rull et al. (2013) puts Montevideo, Uruguay, in the distribution limits of the Brazilian-1 morphotype. The relatively small size and geographical location of Uruguay, with relatively homogeneous climate conditions and a geographical landscape with a topography without great variation in elevation (INIA 2018), suggest that Brazilian-1, reported as the only morphotype currently present in Argentina and also present in the south of Brazil, would be the morphotype that is present in Uruguay. Although our results are not entirely conclusive, if we consider only the ISI value, the fact that the mating combination of flies that deviated from the expected behavior were those coming exclusively from artificial breeding could be indicating that the observed behavioral differences were mainly as a result of breeding conditions and origin rather than to interpopulation differences. In any case, it is important to consider that the three morphotypes of the Brazilian lineage are reported to coexist in Vale do Paraiba in the state of Sao Paulo (Selivon et al. 2004, 2005), and hence, it is still not clear whether the distribution of the Brazilian morphotypes is related to lower and higher altitudes or to a north/south differentiation (Selivon et al. 2005, Vaníčková et al. 2015).

This is the first study intending to begin to clarify the taxonomic situation of *A. fraterculus* in Uruguay. Further studies using molecular and morphometric approaches need to be carried out before a specific morphotype can be conclusively assigned. Comparing populations of wild origin from Argentina, Uruguay, and Brazil would be useful to resolve this issue.

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Section II

Taxonomy and Morphology

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5 Review of *Anastrepha* (Diptera: Tephritidae) Immature Stage Taxonomy

Gary J. Steck[*], *Erick J. Rodriguez, Allen L. Norrbom, Vivian S. Dutra, Beatriz Ronchi-Teles, and Janisete Gomes Silva*

CONTENTS

Abstract Taxonomic study of fruit fly immature stages is important for developing identification keys, especially of pest species, and understanding phylogeny of the Tephritidae. A review of the entomological literature revealed 78 publications describing one or more of the immature stages (egg, larvae, pupa) of the genus *Anastrepha* dating from 1909 to the present. Descriptions of varying quality exist for larvae of 27 species and eggs of 49 species. A table listing 74 species of *Anastrepha* with corresponding publications (or lack of), and annotations on their descriptive content is provided. A diagnosis of *Anastrepha* larvae distinguishes them from other genera. Synapomorphies to distinguish larvae of the species groups of *Anastrepha* have not been found, except for the *curvicauda* group. Taxonomic study of immature stages will advance with new collections, detailed scanning electron microscopic observation, and development of multi-entry keys.

5.1 INTRODUCTION

Invasive fruit fly pests move to new areas primarily through human transport of infested fruits bearing the immature stages. Much of the morphological study of fruit fly immature stages has been driven by the need to identify larvae at all ports of entry where infested fruits are frequently intercepted. In the United States, for example, commodities infested with fruit flies arrive on a daily basis at one or more of the many ports of entry. During the period from 1984 to 2015, more than 122,000 such instances were recorded, and the diversity of host material is very large, comprising at least 328 genera of plants; approximately 97% of the intercepted material arrived as "baggage" (AQAS:PestID 2016). Prior analysis of airline-baggage interception data between 1984 and 1999 suggested that air travel could be an important pathway for fruit fly invasion (Liebhold et al. 2006).

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Although traditional commercial shipments of produce have fewer total numbers of interceptions than passenger baggage, it is important to note that larvae could be present in other forms of transport such as e-commerce (Humair et al. 2015). The chronic invasions of fruit flies into California, Florida, and elsewhere may frequently originate from these various sources. Clearly, there is a need to understand the threat posed by this vast human-mediated movement of economically injurious pests by knowing the identity of the insects in transit. Knowledge of the sources, host plants, seasonality, and pathways of pest species allows development of mitigations to reduce their threat to agriculture.

From the 122,000+ documented interceptions of items infested with flies referenced previously, approximately 82,000 (67%) originated in the Americas (South and Central America, Mexico, and the West Indies) and were identified as genus *Anastrepha*. The genus is indigenous to the Americas, where it is the most speciose of the fruit-infesting tephritids and the most economically important because of its numerous major and minor pests of commercial agricultural and dooryard crops. Only about 300 (0.4%) of the *Anastrepha* interceptions were identified to species level, mostly as *Anastrepha ludens* (Loew), the Mexican fruit fly.

5.2 TAXONOMIC STUDY OF *ANASTREPHA* IMMATURE STAGES

To date, 305 species of *Anastrepha* have been described (Norrbom et al. 2012, 2015, 2018). Of these, larvae of 27 species have been described (9% of the total) with varying levels of detail. Similarly, eggs of 49 species have been described (16% of the total). The history and breadth of descriptive morphological work on *Anastrepha* immature stages can be seen in Table 5.1, which lists the published (and some unpublished) scientific papers and a brief summary of their contents on a species-by-species basis.

Fifty-one species of *Anastrepha* are considered to be of economic interest. Designation as major or minor pests is somewhat arbitrary, but in general, those species that are important to commercial agriculture are considered major pests, and those species that primarily infest edible, noncommercial dooryard or forest hosts are considered minor pests. Most of these species are included in White and Elson-Harris (1992). Larvae have been described for 23 such pest species (43% of total pest species).

Published descriptions vary greatly in detail, quality, and originality. Table 5.1 summarizes this information by noting which developmental stage was described (egg, any of the three instars, pupa) denoted by an *x* in the table. An "original description" included some novel descriptive or measurement data. Numerous publications duplicated or assembled data or figures from previous authors into keys or comparative tables without adding original observations. Descriptions may be supplemented by photomicrographs, drawings, and scanning electron micrographs (SEMs), also denoted by an *x* in the table. In the Comments column, we further characterize the descriptions by their thoroughness. For example, a "thorough description" provided details for all major body features including head (sensory structures and associated lobes, oral ridges, cephalopharyngeal skeleton), anterior and posterior spiracles (internal and external portions), cuticle (spinules and creeping welts), usually including imagery (photomicrographs or SEM) and comparison with other species; a "partial description" lacked details for one or more major body features; a "basic description" lacked comparison to other species; and a "superficial description" had insufficient detail for identification or classification purposes.

The earliest larval descriptions beginning with Froggatt (1909) were of a few major pests, such as *A. ludens*, *Anastrepha fraterculus* (Wiedemann), *Anastrepha serpentina* (Wiedemann), *Anastrepha striata* Schiner, and *Anastrepha curvicauda* (Gerstaecker), with no attempt to describe larvae carefully enough to distinguish them from other pest species. Greene (1929) was the first to describe larvae in a comparative manner and provide an identification key to these same *Anastrepha* species plus those of other major pests: *Ceratitis capitata* (Wiedemann), *Bactrocera oleae* (Rossi), *Rhagoletis cingulata* (Loew), *Rhagoletis pomonella* (Walsh), and *Zeugodacus cucurbitae* (Coquillett). Over time, the number of species described and the general quality of descriptions increased. Notable

TABLE 5.1
Literature Describing Immature Stages of *Anastrepha* Species

Species/Species Group/Pest Status Publication	Original Description	Key	Photo	Drawing	SEM	Egg	1st	2nd	3rd	Pupa	Comments
***Anastrepha acris* Stone/*fraterculus*/minor**											Not described or keyed.
***Anastrepha alveata* Stone/*spatulata*/not a pest**											
Figueiredo et al. (2011, 2017)	Yes		x		x	x					Thorough description, comparisons among 17 *Anastrepha* spp.
***Anastrepha alveatoides* Blanchard/*spatulata*/not a pest**											
Norrbom et al. (1999)	Yes					x					Comparative table of egg dimensions, shape, and sculpture of 26 species.
***Anastrepha amita* Zucchi/*fraterculus*/not a pest**											
Norrbom et al. (1999)	Yes					x					Comparative table of egg dimensions, shape, and sculpture of 26 species.
Figueiredo et al. (2011, 2017)	Yes		x		x	x					Thorough description, comparisons among 17 *Anastrepha* spp.
Dutra et al. (2018b)	Yes		x		x			x	x		Thorough description.
***Anastrepha amplidentata* Norrbom/*fraterculus*/minor**											Not described or keyed.
***Anastrepha annonae* Norrbom/not assigned/minor**											Not described or keyed.
***Anastrepha antunesi* Lima/*fraterculus*/minor**											
Dutra et al. (2011a)	Yes		x		x	x					Thorough description.
***Anastrepha atrox* (Aldrich)/*mucronota*/minor**											
Norrbom (1985)	Yes					x					Comparative table of egg length, width, lobe length of 11 species.
Norrbom et al. (1999)	Yes					x					Comparative table of egg dimensions, shape, and sculpture of 26 species.

(*Continued*)

TABLE 5.1 (*Continued*)
Literature Describing Immature Stages of *Anastrepha* Species

Species/Species Group/Pest Status Publication	Original Description	Key	Photo	Drawing	SEM	Egg	1st	2nd	3rd	Pupa	Comments
***Anastrepha bahiensis* Lima/*fraterculus*/minor**											
Dutra et al. (2011a)	Yes		x		x	x					Thorough description.
Dutra et al. (2012)	Yes		x	x	x			x	x		Thorough description.
***Anastrepha barbiellinii* Lima/not assigned/not a pest**											
Norrbom et al. (1999)	Yes					x					Comparative table of egg dimensions, shape, and sculpture of 26 species.
***Anastrepha bella* Norrbom & Korytkowski/not assigned/not a pest**											
Norrbom and Korytkowski (2009)	Yes					x					Basic description.
***Anastrepha bezzii* Lima/*mucronota*/minor**											Not described or keyed.
***Anastrepha bistrigata* Bezzi/*striata*/minor**											
Steck and Malavasi (1988)	Yes			x		x	x	x	x	x	Thorough description, but lacking imagery. Comparison with larvae of other *Anastrepha* spp. that infest guava.
Steck et al. (1990)	Yes	Yes							x		Key to larvae of 13 *Anastrepha* spp. based on novel data.
White and Elson-Harris (1992)		Yes							x		Description based on Steck and Malavasi (1988).
Norrbom et al. (1999)						x					Comparative table of egg dimensions, shape, and sculpture of 26 species. Data from Steck and Malavasi (1988).
Carroll et al. (2004)		Yes		x							Description and drawings taken from Steck and Malavasi (1988).
Figueiredo et al. (2011, 2017)	Yes		x		x	x					Thorough description, comparisons among 17 *Anastrepha* spp.
***Anastrepha carreroi* Canal/*fraterculus*/minor**											Not described or keyed.
***Anastrepha consobrina* (Loew)/*pseudoparallela*/minor**											

(*Continued*)

TABLE 5.1 (*Continued*)
Literature Describing Immature Stages of *Anastrepha* Species

Species/Species Group/Pest Status Publication	Original Description	Key	Photo	Drawing	SEM	Egg	1st	2nd	3rd	Pupa	Comments
Lima (1930)	Yes		x						x		Superficial description, but clear images of CPS, anterior and posterior spiracles.
Norrbom (1985)									x		Comparative table of various larval characters of 14 species; data from various authors.
Figueiredo et al. (2011, 2017)	Yes		x		x	x					Thorough description, comparisons among 17 *Anastrepha* spp.
***Anastrepha cordata* Aldrich/*cryptostrepha*/not a pest**											
Norrbom (1985)	Yes				x	x					Comparative table of egg length, width, lobe length of 11 species.
Norrbom and Foote (1989)			x			x					Limited comparison with other *Anastrepha* species.
Norrbom et al. (1999)	Yes					x					Comparative table of egg dimensions, shape, and sculpture of 26 species.
Norrbom and Korytkowski (2009)	Yes					x					Basic description.
***Anastrepha coronilli* Carrejo & González/*fraterculus*/not a pest**											
Dutra et al. (2011a)	Yes		x		x	x					Thorough description.
Dutra el al. (2012)	Yes		x	x	x			x	x		Thorough description.
***Anastrepha curitis* Stone/*pseudoparallela*/ minor**											
Dutra et al. (2013)	Yes		x		x	x					Thorough description.
Dutra et al. (2018a)	Yes		x		x			x	x		Thorough description.
***Anastrepha curvicauda* (Gerstaecker)/*curvicauda*/major**											
Knab and Yothers (1914)				x		x			x	x	As *Toxotrypana curvicauda*. Partial, basic description.
Greene (1929)	Yes	Yes		x					x	x	As *Toxotrypana curvicauda*. Keys to larvae and pupae. Partial description.
Baker et al. (1944)	Yes			x					x		As *Toxotrypana curvicauda*. Partial description (posterior spiracle).

(*Continued*)

TABLE 5.1 (*Continued*)
Literature Describing Immature Stages of *Anastrepha* Species

Species/Species Group/Pest Status Publication	Original Description	Key	Photo	Drawing	SEM	Egg	1st	2nd	3rd	Pupa	Comments
Phillips (1946)	Yes	Yes		x					x		As *Toxotrypana curvicauda*. Thorough description but lacking imagery.
Peterson (1951)	Yes			x					x		As *Toxotrypana curvicauda*. Partial description with good detail.
Deputy (1957)		Yes		x					x		As *Toxotrypana curvicauda*. Key to larvae of pest fruit flies. Superficial drawings modified from Greene (1929).
Berg (1979)		Yes		x					x		As *Toxotrypana curvicauda*. Key to larvae of pest fruit flies. Superficial drawing of caudal segment.
Heppner (1986)	Yes			x					x		As *Toxotrypana curvicauda*. Partial description but good detail and comparisons with other fruit fly larvae.
Foote (1991)									x		As *Toxotrypana curvicauda*. Drawing duplicated from Peterson (1951).
Carroll (1992)	Yes				x				x		As *Toxotrypana curvicauda*. Partial description (lacks CPS, cuticular spinules).
White and Elson-Harris (1992)		Yes							x		As *Toxotrypana curvicauda*. Partial description based on Phillips (1946) and Heppner (1986).
Norrbom et al. (1999)						x					As *Toxotrypana curvicauda*. Comparative table of egg dimensions, shape, and sculpture of 26 species. Data from Knab and Yothers (1914).
Carroll et al. (2004)	Yes	Yes			x				x		As *Toxotrypana curvicauda*. Thorough description based on Heppner (1986), Phillips (1946), unpublished data.
Frías et al. (2006)	Yes	Yes		x	x				x		As *Toxotrypana curvicauda*. Key to genus of pest New World fruit flies. Thorough description (except lacking cuticular spinules).
Frías et al. (2008)		Yes	x	x	x				x		As *Toxotrypana curvicauda*. General comparison with larvae of other fruit flies.
***Anastrepha* sp./*curvicauda*/not a pest**											
Frías et al. (2006)	Yes			x	x				x		As *Toxotrypana* sp. Thorough description (except lacking cuticular spinules).

(*Continued*)

TABLE 5.1 (*Continued*)
Literature Describing Immature Stages of *Anastrepha* Species

Species/Species Group/Pest Status Publication	Original Description	Key	Photo	Drawing	SEM	Egg	1st	2nd	3rd	Pupa	Comments
***Anastrepha daciformis* Bezzi/*daciformis*/not a pest**											Not described or keyed.
***Anastrepha distincta* Greene/*fraterculus*/minor**											
Norrbom (1985)	Yes								x		Comparative table of various larval characters of 14 species; data from various authors.
Steck et al. (1990)	Yes	Yes							x		Key to larvae of 13 *Anastrepha* spp. based on novel data.
Carroll (1992)	Yes				x				x		Partial description (lacks CPS and cuticular spinules).
Carroll et al. (2004)	Yes	Yes			x				x		Thorough description based on Steck et al. (1990), unpublished data.
Dutra et al. (2011a)	Yes		x		x	x					Thorough description.
***Anastrepha ethalea* (Walker)/*pseudoparallela*/minor**											Not described or keyed.
***Anastrepha fraterculus* (Wiedemann)—Andean morphotype/*fraterculus*/major**											
Canal et al. (2015)	Yes		x	x					x		Geometric and linear morphometric comparisons of Andean, Brazilian-1, Ecuadorian, Mexican, and Peruvian morphotypes.
Canal et al. (2018)	Yes					x			x		Morphometric study of adults, larvae, and eggs of nine Colombian populations.
***Anastrepha fraterculus* (Wiedemann)—Brazil-1 morphotype/*fraterculus*/major**											
Pruitt (1953)	Yes								x		As *A. fraterculus* Brazil form. Partial description with good detail (lacks spinules, facial features).
Selivon et al. (1997)	Yes					x					As *A. fraterculus* type I. Egg and embryo characteristics compared with those of *A. fraterculus* type II.
Selivon and Perondini (1998)	Yes		x		x	x					As *A.* sp.1 *aff. fraterculus*. Comparison with eggs of Brazil-1 morphotype (as *A.* sp.2 *aff. fraterculus*).

(*Continued*)

TABLE 5.1 (*Continued*)
Literature Describing Immature Stages of *Anastrepha* Species

Species/Species Group/Pest Status Publication	Original Description	Key	Photo	Drawing	SEM	Egg	1st	2nd	3rd	Pupa	Comments
Perondini and Selivon (1999)	Yes		x		x	x					As *A. fraterculus* sp. 1. Includes transmission electron micrographs and comparison with eggs of other genera.
Norrbom et al. (1999)						x					As *A. fraterculus* II in comparative table of egg dimensions, shape, and sculpture of 26 species. Data from Selivon et al. (1997).
Selivon and Perondini (2000)	Yes		x		x	x					As *A.* sp.1 *aff. fraterculus*. Comparisons with numerous *Anastrepha* spp.
Delprat et al. 2001	Yes		x	x			x	x	x		Thorough description.
Carroll et al. (2004)		Yes	x						x		As *A. fraterculus* (Brazil). Partial description based on Steck et al. (1990) and unpublished data, lacking imagery.
Frías et al. (2006)	Yes			x	x		x	x	x		Presumed Brazil-1 morphotype based on geographical source. Key to genus of pest New World fruit flies.
Frías et al. (2008)									x		Generic comparison with other fruit flies.
Figueiredo et al. (2011, 2017)						x					As *A.* sp.1 *aff. fraterculus*. Thorough description and comparisons among 17 *Anastrepha* spp.
Canal et al. (2015)			x	x					x		Geometric and linear morphometric comparisons of Andean, Brazil-1, Ecuadorian, Mexican, and Peruvian morphotypes.
***Anastrepha fraterculus* (Wiedemann)—Brazil-2 morphotype/*fraterculus*/major**											
Selivon et al. (1997)	Yes					x					As *A. fraterculus* type II. Egg and embryo characteristics compared with those of *A. fraterculus* type I.
Selivon and Perondini (1998)	Yes		x		x	x					As *A.* sp.2 *aff. fraterculus*. Comparison with eggs of Brazil-1 morphotype (as *A.* sp.1 *aff. fraterculus*).
Norrbom et al. (1999)						x					As *A. fraterculus* I in comparative table of egg dimensions, shape, and sculpture of 26 species. Data from Selivon et al. (1997).
Selivon and Perondini (2000)	Yes		x		x	x					As *A.* sp.2 *aff. fraterculus*. Comparisons with numerous *Anastrepha* spp.

(*Continued*)

TABLE 5.1 (*Continued*)
Literature Describing Immature Stages of *Anastrepha* Species

Species/Species Group/Pest Status Publication	Original Description	Key	Photo	Drawing	SEM	Egg	1st	2nd	3rd	Pupa	Comments
Selivon et al. (2003)					x	x					As *Anastrepha* sp. 2 *aff fraterculus*. Compared egg morphology of eggs taken from females preserved in ethanol.
Frías et al. (2008)			x						x		Generic comparison with other fruit flies.
Figueiredo et al. (2011, 2017)					x	x					As *fraterculus* (sp.2), comparisons among 17 *Anastrepha* spp.
***Anastrepha fraterculus* (Wiedemann)—Brazil-3 morphotype/*fraterculus*/major**											
Selivon et al. (2004)	Yes				x	x					As *A.* sp. 3. Comparison with eggs of Brazil-1 and Brazil-2 morphotypes (as *A.* sp. 1 and *A.* sp. 2, respectively).
Figueiredo et al. (2011, 2017)						x					As *A.* sp.3 *aff. fraterculus*. Thorough description and comparisons among 17 *Anastrepha* spp.
Frías et al. (2008)	Yes				x				x		Generic comparison with other fruit flies.
***Anastrepha fraterculus* (Wiedemann)—Ecuadorian morphotype/*fraterculus*/major**											
White and Elson-Harris (1992)	Yes	Yes			x				x		As *A. fraterculus* species complex. Thorough description (but limited detail of CPS) based on specimens from Ecuador: Pichincha: Perucho.
Elson-Harris (1992)	Yes	Yes		x	x				x		As *A. fraterculus*. Specimens from Ecuador: Pichincha: Perucho. Thorough description (but limited detail of CPS).
Carroll et al. (2004)		Yes		x					x		Based on White and Elson-Harris (1992).
Canal et al. (2015)	Yes		x	x					x		Geometric and linear morphometric comparisons of Andean, Brazil-1, Ecuadorian, Mexican, and Peruvian morphotypes.
***Anastrepha fraterculus* (Wiedemann)—Mexican/Mesoamerican morphotype/*fraterculus*/major**											

(*Continued*)

TABLE 5.1 (*Continued*)
Literature Describing Immature Stages of *Anastrepha* Species

Species/Species Group/Pest Status Publication	Original Description	Key	Photo	Drawing	SEM	Egg	1st	2nd	3rd	Pupa	Comments
Greene (1929)	Yes	Yes		x					x	x	Morphotype presumed from geographic origin of specimens. Key to larvae and pupae. Partial description.
Emmart (1933)	Yes			x		x					A mis-identification of *A. obliqua* (see Norrbom & Foote 1989).
Baker et al. (1944)	Yes			x					x		Partial description (posterior spiracles).
Baker (1945)	Yes			x		x	x	x	x		Partial description (mandibles).
Pruitt (1953)	Yes								x		As *A. fraterculus* Mexican form. Partial description with good detail (lacks spinules, facial features).
Weems (1980)				x		x			x		Descriptions and drawings taken from Greene (1929), Emmart (1933), and Baker et al. (1944).
Anonymous 1982	Yes								x		Character table: spiracles, anal lobes, tubercles.
Carroll (1992)	Yes				x				x		Partial description (lacks CPS and distribution of cuticular spinules).
Murillo and Jiron (1994)	Yes		x		x	x					Comparison with egg of *Anastrepha obliqua*.
Norrbom et al. (1999)						x					Comparative table of egg dimensions, shape, and sculpture of 26 species. Data from Murillo and Jiron (1994).
Carroll (2004)	Yes	Yes		x	x				x		Thorough description based on Steck et al. (1990) and unpublished data.
Frías et al. (2008)									x		Generic comparison with other fruit flies.
Canal et al. (2015)			x	x					x		Geometric and linear morphometric comparisons of Andean, Brazil-1, Ecuadorian, Mexican, and Peruvian morphotypes.
***Anastrepha fraterculus* (Wiedemann); Peruvian morphotype/*fraterculus*/ major**											
Selivon et al. (2004)	Yes				x	x					As *A.* sp. 4. Comparison with eggs of Brazil-1 and Brazil-2 morphotypes (as *A.* sp. 1 and *A.* sp. 2, respectively).

(*Continued*)

TABLE 5.1 (*Continued*)
Literature Describing Immature Stages of *Anastrepha* Species

Species/Species Group/Pest Status Publication	Original Description	Key	Photo	Drawing	SEM	Egg	1st	2nd	3rd	Pupa	Comments
Figueiredo et al. (2011, 2017)						x					As *A.* sp.4 *aff. fraterculus*. Thorough description and comparisons among 17 *Anastrepha* spp.
Frías et al. (2008)									x		As *A fraterculus* Ecuador. Presumed Peruvian morphotype based on Guayaquil origin. Generic comparison with other fruit flies.
Canal et al. (2015)			x	x					x		Geometric and linear morphometric comparisons of Andean, Brazil-1, Ecuadorian, Mexican, and Peruvian morphotypes.
***Anastrepha fraterculus* (Wiedemann); Venezuelan morphotype/*fraterculus*/major**											Not described or keyed.
***Anastrepha fraterculus* (Wiedemann)—morphotype not specified/*fraterculus*/major**											
Bezzi (1913)										x	Superficial description.
Deputy (1957)		Yes		x					x		Key to larvae of pest fruit flies. Superficial drawings modified from Greene (1929).
Berg (1979)		Yes							x		Key to larvae of pest fruit flies.
Norrbom (1985)									x		Comparative table of various larval characters of 14 species; data from various authors.
Steck et al. (1990)		Yes			x				x		Novel data taken from larvae representing five different morphotypes. Key to larvae of 13 *Anastrepha* spp.
***Anastrepha grandis* (Macquart)/*grandis*/major**											
Fischer (1932)	Yes			x					x	x	Partial description, detailed drawings.
Pruitt (1953)	Yes								x		Partial description with good detail (lacks spinules, facial features).
Norrbom (1985)									x		Comparative table of various larval characters of 14 species; data from various authors.

(*Continued*)

TABLE 5.1 (*Continued*)
Literature Describing Immature Stages of *Anastrepha* Species

Species/Species Group/Pest Status Publication	Original Description	Key	Photo	Drawing	SEM	Egg	1st	2nd	3rd	Pupa	Comments
Whittle and Norrbom (1987)				x					x	x	Description and drawings taken from Fischer (1932).
Steck and Wharton (1988)	Yes			x		x		x	x	x	Thorough description but lacking imagery.
Norrbom and Foote (1989)									x		Limited comparison with larvae of other *Anastrepha* spp.
Steck et al. (1990)	Yes	Yes							x		Key to larvae of 13 *Anastrepha* spp. based on novel data.
White and Elson-Harris (1992)		Yes							x		Description based on Steck and Wharton (1988).
Norrbom et al. (1999)						x					Comparative table of egg dimensions, shape, and sculpture of 26 species. Data from Steck and Wharton (1988).
Carroll et al. (2004)		Yes		x					x		Description and drawings taken from Steck and Wharton (1988).
Figueiredo et al. (2011, 2017)	Yes		x		x	x					Thorough description, comparisons among 17 *Anastrepha* spp.
***Anastrepha haywardi* Blanchard/*spatulata*/not a pest**											
Norrbom et al. (1999)	Yes					x					Comparative table of egg dimensions, shape, and sculpture of 26 species.
***Anastrepha interrupta* Stone/*spatulata*/ not a pest**											
Norrbom (1985)	Yes			x	x				x		Comparative table of various larval characters of 14 species; data from various authors.
Steck and Wharton (1988)	Yes			x					x		Thorough description but lacking imagery.
Norrbom and Foote (1989)									x		Limited comparison with larvae of other *Anastrepha* species.
Steck et al. (1990)	Yes	Yes							x		Key to larvae of 13 *Anastrepha* spp. based on novel data.
Heppner (1990)	Yes			x					x		Partial description but good detail and comparisons with other *Anastrepha* larvae.
Carroll et al. (2004)		Yes		x					x		Description and drawings taken from Steck and Wharton (1988).

(*Continued*)

TABLE 5.1 (*Continued*)
Literature Describing Immature Stages of *Anastrepha* Species

Species/Species Group/Pest Status Publication	Original Description	Key	Photo	Drawing	SEM	Egg	1st	2nd	3rd	Pupa	Comments
***Anastrepha leptozona* Hendel/*leptozona*/ major**											
Norrbom (1985)	Yes				x	x					Comparative table of egg length, width, lobe length of 11 species.
Norrbom and Foote (1989)			x			x					Limited comparison with eggs of other *Anastrepha* spp.
Steck et al. (1990)	Yes	Yes							x		Key to larvae of 13 *Anastrepha* spp. based on novel data.
Carroll (1992)	Yes				x				x		Partial description (lacks CPS and cuticular spinules).
Norrbom et al. (1999)					x	x					Comparative table of egg dimensions, shape, and sculpture of 26 species. Data and SEM from Norrbom (1985).
Carroll et al. (2004)	Yes	Yes			x				x		Thorough description based on Steck et al. (1990) and unpublished data.
Frías et al. (2008)									x		Generic comparison with other fruit flies.
Frias et al. (2009)	Yes		x		x				x		Thorough description.
Dutra et al. (2013)	Yes		x		x	x					Thorough description.
***Anastrepha limae* Stone/*pseudoparallela*/ minor**											
Norrbom (1985)	Yes			x	x				x		Comparative table of various larval characters of 14 species; data from various authors.
Steck and Wharton (1988)	Yes			x					x	x	Thorough description but lacking imagery.
Norrbom and Foote (1989)									x		Limited comparison with larvae of other *Anastrepha* spp.
Steck et al. (1990)	Yes	Yes			x				x		Key to larvae of 13 *Anastrepha* spp. based on novel data.
Carroll et al. (2004)		Yes		x							Description and drawings taken from Steck and Wharton (1988).
***Anastrepha ludens* (Loew)/*fraterculus*/ major**											
Froggatt (1909)				x					x	x	Superficial drawings duplicated from report of California State Board of Horticulture 1905.

(*Continued*)

TABLE 5.1 (*Continued*)
Literature Describing Immature Stages of *Anastrepha* Species

Species/Species Group/Pest Status Publication	Original Description	Key	Photo	Drawing	SEM	Egg	1st	2nd	3rd	Pupa	Comments
Banks (1912)	Yes			x					x		Superficial description.
Greene (1929)	Yes	Yes		x					x	x	Key to larvae and pupae. Partial description.
Emmart (1933)	Yes			x		x					Detailed drawings and comparison with eggs of other pest species in Mexico.
Baker et al. (1944)	Yes		x	x		x			x		Partial description with good detail and biology. Egg drawings from Emmart (1933).
Phillips (1946)	Yes								x		Probably a misidentification of *A. obliqua* (see Carroll & Wharton 1989).
Peterson (1951)	Yes			x					x		Partial description with good detail.
Pruitt (1953)	Yes								x		Partial description with good detail (lacks most facial features).
Foote (1991)									x		Drawings duplicated from Peterson (1951).
Deputy (1957)		Yes		x					x		Key to larvae of pest fruit flies. Superficial drawings modified from Greene (1929).
Kandybina (1977)	Yes			x					x		Thorough description but lacking imagery.
Berg (1979)		Yes		x					x		Key to larvae of pest fruit flies.
Anonymous (1982)	Yes			x					x		Partial data (oral ridges, anterior spiracle, caudal papillae); superficial drawings.
Heppner (1984)	Yes	Yes		x					x		Partial description, but good detail and comparison with *A. suspensa.*
Norrbom (1985)	Yes				x	x			x		Comparative table of egg dimensions of 11 species and various larval characters of 14 species; data from various authors.
Norrbom and Foote (1989)			x			x					Limited comparison with other *Anastrepha* spp.
Carroll and Wharton (1989)	Yes			x	x	x	x	x	x	x	Thorough description.
Steck et al. (1990)	Yes	Yes							x		Key to larvae of 13 *Anastrepha* spp. based on novel data.
Carroll (1992)	Yes			x	x						Thorough description and discussion of identification vs. other pest fruit fly larvae.

(*Continued*)

TABLE 5.1 (*Continued*)
Literature Describing Immature Stages of *Anastrepha* Species

Species/Species Group/Pest Status Publication	Original Description	Key	Photo	Drawing	SEM	Egg	1st	2nd	3rd	Pupa	Comments
White and Elson-Harris (1992)		Yes							x		Description based on Carroll and Wharton (1989).
Norrbom et al. (1999)	Yes				x	x					Comparative table of egg dimensions, shape, and sculpture of 26 species, including data from previous authors.
Carroll et al. (2004)	Yes	Yes		x	x				x		Thorough description based on Carroll and Wharton (1989) and unpublished data.
Frías et al. (2006)	Yes			x					x		Key to genus of pest New World fruit flies. Partial description.
Frías et al. (2008)	Yes		x						x		Generic comparison with other fruit flies.
***Anastrepha macrura* Hendel/*daciformis*/not a pest**											Not described or keyed.
***Anastrepha manihoti* Lima/*spatulata*/minor**											
Norrbom et al. (1999)	Yes					x					Comparative table of egg dimensions, shape, and sculpture of 26 species.
Dutra et al. (2011b)	Yes				x	x					Thorough description.
***Anastrepha margarita* Caraballo/*panamensis*/minor**											Not described or keyed.
***Anastrepha montei* Lima/*spatulata*/minor**											
Dutra et al. (2011b)	Yes				x	x					Thorough description.
***Anastrepha mucronota* Stone/*mucronota*/minor**											
Steyskal (1977)	Yes			x					x		As *A. nunezae*. Superficial description with drawing of posterior spiracle.
Norrbom (1985)									x		As *A. nunezae*. Comparative table of various larval characters of 14 species; data from various authors.
Carroll et al. (2004)		Yes		x					x		Description and drawing taken from Steyskal (1977).
***Anastrepha nigrifascia* Stone/*robusta*/not a pest**											

(*Continued*)

TABLE 5.1 (*Continued*)
Literature Describing Immature Stages of *Anastrepha* Species

Species/Species Group/Pest Status Publication	Original Description	Key	Photo	Drawing	SEM	Egg	1st	2nd	3rd	Pupa	Comments
Norrbom (1985)	Yes			x		x					Comparative table of egg length, width, lobe length of 11 species.
Norrbom and Foote (1989)						x					Limited comparison with eggs of other *Anastrepha* species.
Norrbom et al. (1999)						x					Comparative table of egg dimensions, shape, and sculpture of 26 species. Data from Norrbom (1985).
Norrbom and Korytkowski (2009)	Yes					x					Basic description.
***Anastrepha nolazcoae* Norrbom & Korytkowski/*mucronota*/minor**											Not described or keyed.
***Anastrepha obliqua* (Macquart)/*fraterculus*/major**											
Sein (1933)	Yes		x			x			x	x	As *A. fraterculus* var. *mombinpraeoptans*. Partial descriptions, detailed figures.
Emmart (1933)	Yes					x					As *Anastrepha fraterculus* (Wiedemann) (misidentification). Detailed drawings and comparison with other pest species in Mexico.
Baker et al. (1944)	Yes			x		x			x		As *Anastrepha mombinpraeoptans* Sein. Partial description (posterior spiracles). Egg drawings from Emmart (1933).
Phillips (1946)	Yes	Yes		x							As *Anastrepha ludens* (Loew), probable misidentification (Carroll & Wharton 1989). Thorough description, but lacking imagery.
Peterson (1951)	Yes			x					x		As *Anastrepha fraterculus* prob. var. *mombinpraeoptans* Sein. Partial description with good detail.
Pruitt (1953)	Yes								x		As *A. mombinpraeoptans* Sein. Partial description with good detail (lacks most facial features).
Deputy (1957)		Yes		x					x		As *A. mombinpraeoptans*. Key to larvae of pest fruit flies. Superficial drawings modified from Greene (1929).

(*Continued*)

TABLE 5.1 (*Continued*)
Literature Describing Immature Stages of *Anastrepha* Species

Species/Species Group/Pest Status Publication	Original Description	Key	Photo	Drawing	SEM	Egg	1st	2nd	3rd	Pupa	Comments
Berg (1979)		Yes		x					x		As *A. mombinpraeoptans*. Key to larvae of pest fruit flies.
Jiron and Zeledon (1979)	Yes			x					x		Drawings only of anterior and posterior spiracles.
Anonymous (1982)	Yes								x		Character table: spiracles, anal lobes, tubercles.
Norrbom (1985)	Yes				x	x			x		Comparative table of egg dimensions of 11 species and various larval characters of 14 species; data from various authors.
Norrbom and Foote (1989)			x			x					Limited comparison with other *Anastrepha* spp.
Steck et al. (1990)	Yes	Yes							x		Key to larvae of 13 *Anastrepha* spp. based on novel data.
Heppner (1991)	Yes								x		Partial description but good detail and comparisons with other *Anastrepha* larvae.
Boleli and Teles (1992)	Yes					x					Egg dimensions.
Carroll (1992)	Yes				x				x		Partial description (lacks CPS, cuticular spinules).
White and Elson-Harris (1992)	Yes	Yes		x	x				x		Thorough description (but limited detail of CPS).
Murillo and Jiron (1994)	Yes		x		x	x					Comparison with egg of *Anastrepha obliqua*.
Norrbom et al. (1999)	Yes				x	x					Comparative table of egg dimensions, shape, and sculpture of 26 species including data from previous authors.
Selivon and Perondini (2000)	Yes		x			x					Comparisons with numerous *Anastrepha* species.
Carroll et al. (2004)	Yes	Yes		x	x				x		Thorough description based on Steck et al. (1990), White and Elson-Harris (1992), and unpublished data.
Frías et al. (2006)	Yes			x	x			x	x		Key to genus of pest New World fruit flies. Thorough description except lacking cuticular spinules.
Figueiredo et al. (2011, 2017)	Yes		x		x	x					Thorough description, comparisons among 17 *Anastrepha* spp.
***Anastrepha ocresia* (Walker)/*serpentina*/ minor**											Not described or keyed.
***Anastrepha ornata* Aldrich/*striata*/minor**											Not described or keyed.
***Anastrepha pallens* Coquillett/*daciformis*/ not a pest**											

(*Continued*)

TABLE 5.1 (*Continued*)
Literature Describing Immature Stages of *Anastrepha* Species

Species/Species Group/Pest Status Publication	Original Description	Key	Photo	Drawing	SEM	Egg	1st	2nd	3rd	Pupa	Comments
Baker et al. (1944)	Yes			x					x		As *Pseudodacus pallens* Coq. Partial description (posterior spiracle).
Phillips (1946)	Yes	Yes		x					x		Thorough description but lacking imagery.
Peterson (1951)	Yes			x					x		As *Pseudodacus pallens*. Partial description.
Norrbom (1985)	Yes			x		x			x		Comparative table of egg dimensions of 11 species and various larval characters of 14 species; data from various authors.
Norrbom and Foote (1989)									x		Limited comparison with larvae of other *Anastrepha* species.
Steck et al. (1990)	Yes	Yes							x		Key to larvae of 13 *Anastrepha* species based on novel data.
Norrbom et al. (1999)						x					Comparative table of egg dimensions, shape, and sculpture of 26 species. Data from Norrbom (1985).
Carroll et al. (2004)		Yes		x					x		Description taken from Steck et al. (1990). Drawings taken from Phillips (1946).
***Anastrepha pallidipennis* Greene/*pseudoparallela*/minor**											Not described or keyed.
***Anastrepha panamensis* Greene/*panamensis*/minor**											Not described or keyed.
***Anastrepha parishi* Stone/not assigned/minor**											Not described or keyed.
***Anastrepha passiflorae* Greene/*pseudoparallela*/minor**											Not described or keyed.
***Anastrepha pastranai* Blanchard/*pseudoparallela*/not a pest**											
Norrbom et al. (1999)	Yes					x					Comparative table of egg dimensions, shape, and sculpture of 26 species.
***Anastrepha perdita* Stone/*fraterculus*/minor**											Not described or keyed.

(*Continued*)

TABLE 5.1 (*Continued*)
Literature Describing Immature Stages of *Anastrepha* Species

Species/Species Group/Pest Status Publication	Original Description	Key	Photo	Drawing	SEM	Egg	1st	2nd	3rd	Pupa	Comments
***Anastrepha pickeli* Lima/*spatulata*/not a pest**											
Norrbom et al. (1999)	Yes					x					Comparative table of egg dimensions, shape, and sculpture of 26 species.
Dutra et al. (2011b)					x	x					Thorough description.
Figueiredo et al. (2011, 2017)	Yes		x		x	x					Thorough description, comparisons among 17 *Anastrepha* spp.
Dutra et al. (2018a)	Yes		x		x				x		Thorough description.
***Anastrepha pittieri* Caraballo/*robusta*/not a pest**											
Norrbom (1985)	Yes				x	x					Comparative table of egg length, width, lobe length of 11 species.
Norrbom and Foote (1989)			x			x					Limited comparison with other *Anastrepha* spp.
Norrbom et al. (1999)					x	x					Comparative table of egg dimensions, shape, and sculpture of 26 species. Data from Norrbom (1985).
Norrbom and Korytkowski (2009)	Yes				x	x					Basic description.
***Anastrepha pseudoparallela* (Loew)/*pseudoparallela*/minor**											
Norrbom et al. (1999)	Yes					x					Comparative table of egg dimensions, shape, and sculpture of 26 species.
Figueiredo et al. (2011, 2017)	Yes		x		x	x					Thorough description, comparisons among 17 *Anastrepha* spp.
***Anastrepha psidivora* Norrbom/not assigned/minor**											Not described or keyed.
***Anastrepha pulchra* Stone/*serpentina*/not a pest**											
Dutra et al. (2018a)	Yes		x		x				x		Thorough description.

(*Continued*)

TABLE 5.1 (*Continued*)
Literature Describing Immature Stages of *Anastrepha* Species

Species/Species Group/Pest Status Publication	Original Description	Key	Photo	Drawing	SEM	Egg	1st	2nd	3rd	Pupa	Comments
***Anastrepha punctata* Hendel/*punctata*/ minor**											Not described or keyed.
***Anastrepha rheediae* Stone/not assigned/ minor**											Not described or keyed.
***Anastrepha sagitatta* (Stone)/*dentata*/ minor**											
Baker et al. (1944)	Yes			x					x		As *Lucumaphila sagitatta* Stone. Partial description (posterior spiracle, CPS).
Norrbom (1985)				x					x		Comparative table of various larval characters of 14 species; data and drawing from various authors.
Norrbom and Foote (1989)									x		Limited comparison with larvae of other *Anastrepha* species.
Carroll et al. (2004)		Yes							x		Description taken from Steck et al. (1990) and unpublished data.
***Anastrepha schultzi* Blanchard/*fraterculus*/minor**											Not described or keyed.
***Anastrepha serpentina* (Wiedemann)/*serpentina*/major**											
Greene (1929)	Yes	Yes		x					x	x	Key to larvae and pupae. Partial description.
Emmart (1933)	Yes			x		x					Detailed drawings and comparison with eggs of other pest species in Mexico.
Baker et al. (1944)	Yes			x		x			x		Partial description (posterior spiracles). Egg drawings from Emmart (1933).
Shaw and Starr (1946)	Yes						x	x	x		Drawings and sizes of mandibles only.
Phillips (1946)	Yes	Yes		x					x		Thorough description but lacking imagery.
Pruitt (1953)	Yes			x					x		Partial description with good detail (lacks most facial features).
Burgers (1953)				x					x		Superficial description. Drawing of lateral view of the larva.

(*Continued*)

TABLE 5.1 (*Continued*)
Literature Describing Immature Stages of *Anastrepha* Species

Species/Species Group/Pest Status Publication	Original Description	Key	Photo	Drawing	SEM	Egg	1st	2nd	3rd	Pupa	Comments
Deputy (1957)		Yes		x					x		Key to larvae of pest fruit flies. Superficial drawings modified from Greene (1929).
Weems (1969)									x		Brief description from Phillips (1946).
Berg (1979)		Yes		x					x		Key to larvae of pest fruit flies.
Jiron and Zeledon (1979)	Yes			x					x		Drawings only of anterior and posterior spiracles.
Anonymous (1982)	Yes								x		Character table: spiracles, anal lobes, tubercles.
Norrbom (1985)						x			x		Comparative table of egg dimensions of 11 species and various larval characters of 14 species; data from various authors.
Steck et al. (1990)	Yes	Yes			x				x		Key to larvae of 13 *Anastrepha* spp. based on novel data.
Carroll (1992)	Yes				x				x		Partial description (lacks CPS and distribution of cuticular spinules).
White and Elson-Harris (1992)	Yes	Yes		x	x				x		Thorough description (but limited detail of CPS).
Selivon and Perondini (1999)	Yes				x	x					Thorough description.
Norrbom et al. (1999						x					Comparative table of egg dimensions, shape, and sculpture of 26 species, data from previous authors.
Selivon and Perondini (2000)	Yes		x			x					Comparisons with numerous *Anastrepha* spp.
Carroll et al. (2004)	Yes	Yes		x	x				x		Thorough description based on Steck et al. (1990), White and Elson-Harris (1992), and unpublished data. Drawings from Phillips (1946) and others.
Figueiredo et al. (2011, 2017)						x					Thorough description, comparisons among 17 *Anastrepha* spp.
***Anastrepha shannoni* Stone/*grandis*/not a pest**											
Norrbom (1991)	Yes			x		x					Comparison with eggs of *A. grandis*.
Norrbom et al. (1999)						x					Comparative table of egg dimensions, shape, and sculpture of 26 species. Data from Norrbom (1991).
***Anastrepha sororcula* Zucchi/*fraterculus*/ minor**											

(*Continued*)

TABLE 5.1 (*Continued*)
Literature Describing Immature Stages of *Anastrepha* Species

Species/Species Group/Pest Status Publication	Original Description	Key	Photo	Drawing	SEM	Egg	1st	2nd	3rd	Pupa	Comments
Selivon and Perondini (1999)	Yes				x	x					Thorough description.
Norrbom et al. (1999)						x					Comparative table of egg dimensions, shape, and sculpture of 26 species. Data from Selivon and Perondini (1999).
Selivon and Perondini (2000)	Yes		x			x					Positions of larvae before eclosion; egg dimensions.
Figueiredo et al. (2011, 2017)						x					Thorough description, comparisons among 17 *Anastrepha* spp.
Dutra et al. (2018b)	Yes		x		x				x		Thorough description.
***Anastrepha speciosa* Stone/*speciosa*/not a pest**											
Norrbom and Korytkowski (2009)	Yes				x	x					Basic description.
***Anastrepha steyskali* Korytkowski/*leptozona*/not a pest**											Not described or keyed.
***Anastrepha striata* Schiner/*striata*/major**											
Keilin and Picado (1920)	Yes			x					x	x	Detailed description and drawings, also internal anatomy.
Picado (1920)	Yes			x					x	x	Superficial description.
Greene (1929)	Yes	Yes		x					x	x	Key to larvae and pupae. Partial description.
Emmart (1933)	Yes			x		x					Detailed drawings and comparison with eggs of other pest species in Mexico.
Baker et al. (1944)	Yes			x		x			x		Partial description (posterior spiracles). Egg drawings from Emmart (1933).
Pruitt (1953)	Yes			x					x		Partial description with good detail (lacks some facial features).
Deputy (1957)		Yes		x					x		Key to larvae of pest fruit flies. Superficial drawings modified from Greene (1929).
Berg (1979)		Yes		x					x		Key to larvae of pest fruit flies.
Jiron and Zeledon (1979)	Yes			x					x		Drawings only of anterior and posterior spiracles.
Anonymous (1982)	Yes								x		Character table: spiracles, anal lobes, tubercles.

(*Continued*)

TABLE 5.1 (*Continued*)
Literature Describing Immature Stages of *Anastrepha* Species

Species/Species Group/Pest Status Publication	Original Description	Key	Photo	Drawing	SEM	Egg	1st	2nd	3rd	Pupa	Comments
Weems 1982				x					x		Description from Pruitt (1953) and others. Drawings from Berg (1979).
Norrbom 1985	Yes					x			x		Comparative table of egg dimensions of 11 species and various larval characters of 14 species; data from various authors.
Jones and Kim (1987)	Yes		x						x		Dimensions of posterior spiracles and abnormality.
Steck et al. (1990)	Yes	Yes							x		Key to larvae of 13 *Anastrepha* spp. based on novel data.
Carroll (1992)	Yes				x				x		Partial description (lacks CPS and distribution of cuticular spinules)
White and Elson-Harris (1992)	Yes	Yes		x	x				x		Thorough description (but limited detail of CPS).
Norrbom et al. (1999)						x					Comparative table of egg dimensions, shape, and sculpture of 26 species. Data from Emmart (1933).
Carroll et al. (2004)	Yes	Yes		x	x				x		Thorough description based on Steck et al. (1990), White and Elson-Harris (1992), and unpublished data.
Figueiredo et al. (2011, 2017)	Yes		x		x	x					Thorough description, comparisons among 17 *Anastrepha* spp.
***Anastrepha suspensa* (Loew)/*fraterculus*/ major**											
Sein (1933)	Yes					x			x	x	As *A. unipuncta*. Partial descriptions, detailed figures.
Pruitt (1953)	Yes			x					x		Partial description with good detail (lacks some facial features).
Lawrence (1979)	Yes			x			x	x	x		Partial descriptions, especially CPS and anterior spiracles.
Berg (1979)		Yes		x					x		Key to larvae of pest fruit flies.
Anonymous (1982)	Yes								x		Character table: spiracles, anal lobes, tubercles.
Heppner (1984)	Yes			x					x		Partial description but good detail and comparison *with A. ludens.*

(*Continued*)

TABLE 5.1 (*Continued*)
Literature Describing Immature Stages of *Anastrepha* Species

Species/Species Group/Pest Status Publication	Original Description	Key	Photo	Drawing	SEM	Egg	1st	2nd	3rd	Pupa	Comments
Norrbom (1985)						x			x		Comparative table of egg dimensions of 11 species and various larval characters of 14 species; data from various authors.
Steck et al. (1990)	Yes	Yes			x				x		Key to larvae of 13 *Anastrepha* spp. based on novel data.
Carroll (1992)	Yes				x				x		Partial description (lacks CPS and distribution of cuticular spinules)
White and Elson-Harris (1992)		Yes		x	x				x		Thorough description (but limited detail of CPS).
Norrbom et al. (1999)						x					Comparative table of egg dimensions, shape, and sculpture of 26 species. Data from previous authors.
Carroll et al. (2004)	Yes	Yes		x	x				x		Thorough description based on Steck et al. (1990), White and Elson-Harris (1992), and unpublished data.
Figueiredo et al. (2011, 2017)	Yes		x		x	x					Thorough description, comparisons among 17 *Anastrepha* spp.
***Anastrepha tehuacana* Norrbom/*tripunctata*/not a pest**											
Norrbom et al. (2014)	Yes		x			x					Compared to egg of *A. cordata.*
***Anastrepha tumida* Stone/not assigned/ not a pest**											
Norrbom et al. (1999)	Yes					x					Comparative table of egg dimensions, shape, and sculpture of 26 species.
***Anastrepha turpiniae* Stone/*fraterculus*/ minor**											
Dutra et al. (2011a)	Yes		x		x	x					Thorough description.
Dutra et al. (2012)	Yes		x	x	x			x	x		Thorough description.
***Anastrepha zenildae* Zucchi/*fraterculus*/ minor**											

(*Continued*)

TABLE 5.1 (*Continued*)
Literature Describing Immature Stages of *Anastrepha* Species

Species/Species Group/Pest Status Publication	Original Description	Key	Photo	Drawing	SEM	Egg	1st	2nd	3rd	Pupa	Comments
Dutra et al. (2011a)	Yes		x		x	x					Thorough description.
Figueiredo et al. (2011, 2017)	Yes		x		x	x					Thorough description, comparisons among 17 *Anastrepha* spp.
Dutra et al. (2018b)	Yes		x		x				x		Thorough description.

X indicates feature is present in cited publication.
CPS, cephalopharyngeal skeleton; SEM, scanning electron micrograph.

contributors to further comparative larval studies of *Anastrepha* pest species were Baker et al. (1944, 1945; seven species), Phillips (1946; four species), Peterson (1951; four species), and Pruitt (1953; eight species). Berg's key (1979) did not show much progress over that of Greene (1929), as it incorporated only two additional *Anastrepha* species (*Anastrepha obliqua* [Macquart] and *Anastrepha suspensa* [Loew]) and did not add any original data. Around that time, it was generally agreed that there was low confidence in the reliability of any of the existing keys to provide accurate identifications of *Anastrepha* larvae to species level (Anonymous 1982).

A new generation of researchers began work on *Anastrepha*, including its immature stages, in the 1980s. Norrbom (1985) compiled existing and new comparative data on larvae of 14 species. Steck and Malavasi (1988) and Steck and Wharton (1988) described larvae of four *Anastrepha* species in excellent detail but without benefit of photomicroscopy. Carroll and Wharton (1989) first used SEMs of *Anastrepha* larvae in their very thorough descriptions of all immature stages of *A. ludens*. Steck et al. (1990) explicitly addressed the question of geographic variation in larval characters by examining specimens from multiple localities when available and created a reliable key to larvae of 13 *Anastrepha* species. Note, however, that some closely related species (*striata/bistrigata*, and *fraterculus/obliqua/suspensa*) could not be separated reliably based on the key character data set, and the concept of *A. fraterculus* at that time did not include the multiple morphotypes/putative species recognized today (Hernández-Ortiz et al. 2004, 2012, 2015). Carroll (1992), Elson-Harris (1992), and White and Elson-Harris (1992) greatly expanded the use of SEM in describing larvae, which revealed the fine detail and utility of facial mask characters such as oral ridges, their margins and accessory plates, and the stomal organ and its associated lobes that help to distinguish among species. Currently the most comprehensive public data set of comparative larval morphological characters is that on which the identification tool of Carroll et al. (2004) is based. It incorporates all of the character data included in Carroll's and Elson-Harris's previous publications with the addition of a large number of novel SEM images. Frías et al. (2006, 2008) provided some novel data on larvae of several *Anastrepha* species with the aim of improving the generic diagnosis of *Anastrepha* and keys to major genera of fruit fly pests. The most recent and highly detailed alpha-taxonomic studies of *Anastrepha* larvae are those of Dutra et al. (2011a, 2011b, 2012, 2013, 2018a, 2018b) and Dutra (2012). Canal et al. (2015, 2018) applied morphometric techniques to the study of larvae of several morphotypes of *A. fraterculus* to augment similar studies done on the adult stages.

Based on cumulative studies to date, *Anastrepha* larvae can be recognized at the genus level and separated from other fruit fly pest genera based on mandible with a single primary tooth (without secondary tooth) and basally truncate (absence of a neck); dental sclerite apparently absent, not visible in lateral view; preoral teeth (stomal guards) lacking; accessory plates to oral ridges present and short; stomal organ at apex of large elongate-rounded primary lobe that lacks secondary lobes; anterior spiracle usually concave centrally; and caudal ridge absent (White and Elson-Harris 1992; Carroll et al. 2004; Frías et al. 2006, 2008; Balmès and Mouttet 2017).

At the intrageneric level, however, potentially diagnostic information is sparse. At present 26 species groups are recognized (Norrbom et al. 2012; Mengual et al. 2017; see Table 5.2). Larvae have been described for at least 1 member of 11 of the species groups, and larvae of 15 species groups are entirely unknown. With the exception of the *fraterculus* species group, in which larvae of 11 species have been described, the overall level of descriptive coverage of *Anastrepha* larvae is very low: 9 of the species groups are represented by only 1 or 2 species with described larvae. Therefore, it is difficult to say whether informative synapomorphies exist to help define species groups. Even with this limited data, however, there appears to be a disappointing lack of larval character states useful in recognizing species groups of *Anastrepha*. For example, even the most character-rich feature of tephritid larvae, the facial mask, tends to be rather uniform across species groups. There is some variation in the appearance of the oral ridges and accessory plates, being either smooth or serrate, but both character states are shared among species groups. The one exception is the *curvicauda* group, which until recently was classified as

TABLE 5.2
***Anastrepha* Species Groups, Numbers of Included Species, and Numbers of Species for Which Larvae Have Been Described**

Species Group	# spp.	No. Species Described to Date
benjamini	10	—
binodosa	3	—
caudata	3	—
cryptostrepha	4	—
curvicauda	7	2
daciformis	14	1
dentata	11	1
doryphoros	4	—
fraterculus	40+[a]	11
grandis	10	1
hastata	3	—
leptozona	6	1
mucronota	52	1
nigrina	2	—
panamenis	3	—
pseudoparallela	25	3
punctata	4	—
ramosa	3	—
raveni	2	—
robusta	15	—
schausi	5	—
serpentina	9	2
spatulata	16	2
striata	3	2
speciosa	3	—
tripunctata	4	—
Unassigned	41	—
TOTAL		27

[a] plus unnamed members of the *A. fraterculus* complex.

a distinct genus, *Toxotrypana* (Norrbom et al. 2018). All of the major keys to fruit fly pest genera clearly distinguish this group from the remainder of other *Anastrepha* larvae based on several characters such as shape of mandible and reduction in tubercles on caudal segment (see especially Frías et al. 2008).

The study of eggs has outpaced that of larvae because fully developed eggs can be extracted from gravid females collected in the field and from museum specimens, and their identity is known from the associated adult specimens. The first comparative study of *Anastrepha* eggs was that of Emmart (1933), who described eggs of four pest species in Mexico. Norrbom (1985) compiled existing and new comparative data on eggs of 11 species and was the first to use SEMs to observe eggs. Norrbom et al. (1999) increased comparative data for *Anastrepha* eggs to 26 species. Selivon and Perondini (1999, 2000) and Selivon et al. (2004) provided detailed descriptions of eggs of several *Anastrepha* species and especially contributed to the growing realization that *A. fraterculus s.l.* comprises numerous cryptic species. Norrbom and Korytkowski (2009), Figueiredo et al. (2011),

and Dutra et al. (2011a, 2011b, 2013) have further expanded the list to 49 *Anastrepha* species with described eggs. As is mostly the case with larvae, the egg stage does not present synapomorphies linking members of any of the proposed species groups together (Figueiredo et al. 2011), although some related species share some derived features.

5.3 FUTURE WORK

Further needs for study of *Anastrepha* immature stages include (1) alpha taxonomy, (2) development of identification tools, and (3) phylogenetic analysis.

The first order of business is to acquire specimens for study. There are no research specimens available for more than half of the recorded *Anastrepha* pest species. Larvae are troublesome because they must be collected from fruits infested in the field and a subset reared to adult stage for positive identification. However, even reared, associated adults do not guarantee a definitive identification of a given larva because field-collected fruits can be multiply infested by two or more different species. Alternatively, a pure laboratory colony can be established as a source of the immature stages.

Good alpha taxonomy is greatly facilitated by high-quality optical microscopes, digital cameras, and a scanning electron microscope. Optical microscopy has limited capability of imaging surface features of larvae and eggs, therefore thorough observation requires scanning electron microscopy, which is not readily available to many researchers and identifiers. However, it should be noted that skilled and observant optical microscopists are capable of detecting fine detail. For example, Keilin and Picado's (1920) drawings show amazing detail of the facial mask, including the stomal organ and lobes of *Anastrepha striata*, and the drawings of Phillips (1946) and Kandybina (1977) are excellent. Good standards are already in place for ongoing comparative morphological studies.

The future of fruit fly larvae identification based on morphology lies in digital multi-entry keys such as that of Carroll et al. (2004). The species-level key of Steck et al. (1990) relies on minor variation in numerous "trivial" characters such as presence or absence of dorsal spinules on various body segments, the number of lobes on the anterior spiracles, numbers of posterior spiracular processes and branches, etc. At present, the paucity of intrageneric synapomorphies makes species-level identification difficult when there are many possible outcomes. For example, all of Dutra et al.'s recent descriptions of *Anastrepha* larvae fail to easily incorporate into the binomial key of Steck et al. (1990). It will be relatively easy to build upon the key of Carroll et al. (2004), which includes 18 *Anastrepha* taxa, by adding new taxa and perhaps additional characters and imagery.

The current phylogeny of Tephritidae is largely based on adult morphology and molecular data, but larval characters provide useful corroboration and additional insight. For example, the affinities of the tribe Gastrozonini were questioned for many years, and the presence of a caudal ridge on their larvae strongly supports its inclusion in the Dacinae (Kovac et al. 2006). We expect that further taxon sampling within *Anastrepha* will reveal useful species group synapomorphies (unpublished data).

Finally, molecular identification tools offer great promise in addressing the problem of identifying fruit fly immature stages. We already know that morphological identification is difficult, and the larvae of closely related species may be effectively indistinguishable, just as is the case for adults (males, cryptic species complexes). Molecular data already are often used to support identifications in difficult cases. However, it should be noted that identifications based on molecular data suffer similar limitations as morphological keys (e.g., missing taxa, limited geographic sampling, lack of validation, and lack of in-house equipment and expertise by identifiers).

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6 A Review of the Natural Host Plants of the *Anastrepha fraterculus* Complex in the Americas

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CONTENTS

Abstract There is now enough support for the hypothesis that nominal *Anastrepha fraterculus* is a complex of cryptic species that are currently recognized, using morphometric procedures, as eight morphotypes that probably correspond to different biological species. In addition to this variability, there is also evidence that this nominal species presents important variation in its range of preferential host use. The aim of this chapter is to provide a comprehensive understanding of the natural host plants used by the nominal *A. fraterculus* under natural field conditions. This was accomplished through a bibliographic examination of information from the original sources of host plants recorded for this fly species. A total of 200 references from all regions of the Americas were examined. Data useful to the analysis were captured in a database incorporating information pertaining to host identity, original source of data, and location of distribution, where available. The list of host plants for the *A. fraterculus* complex comprised 177 species belonging to 40 plant families, which together accounted for 1,622 documented reports. The most highly represented families were Myrtaceae (27.1%), Rosaceae (11.9%), and Rutaceae (8.5%). The Myrtaceae exhibited a high percentage (>90%) of native species in contrast to the higher proportions of exotic species presented in the other families. Guava was the only common host shared by different populations throughout the tropical and subtropical landscapes of the Americas. The highest number of hosts was recorded in Brazil (121), followed by Argentina (40), Ecuador (40), Colombia (38), Venezuela (24), and Mexico (19). The landscapes occupied by different populations of this nominal species presented some preferential patterns in terms

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of resource use. This reinforces the hypothesis of distinct taxonomic entities because most of the plants are present throughout the range but are not found to be common hosts to all of the fly populations. In this context, the potential application of the sterile insect technique (SIT) in certain geographical areas requires knowledge of the particular hosts consumed by the target species.

6.1 INTRODUCTION

The South American fruit fly (SAFF), *Anastrepha fraterculus* (Wiedemann, 1830), is widely distributed throughout the Americas; from the southern United States (Texas), through Mexico, Central America and South America to Argentina (Hernández-Ortiz & Aluja 1993). Based on previous research under different approaches, including the use of karyotypes, molecular DNA sequences, reproductive isolation, pheromone profiles, or even integrative approaches (reviewed in Selivon et al. 2004, 2005a, 2005b, Vera et al. 2006, Cáceres et al. 2009, Vaníčková et al. 2015, Dias et al. 2016, and others), there is now enough support for the hypothesis that this nominal species in fact comprises a complex of cryptic species that are currently recognized using morphometric procedures as eight morphotypes, most likely corresponding to different biological species (Hernández-Ortiz et al. 2004, 2012, 2015).

There is also evidence that some populations exhibit important differences in the host range used, using preferential hosts at the regional scale. Moreover, their pest status can differ depending on the geographical area in which they occur; for instance, Baker et al. (1944) showed that citrus fruits such as oranges were unsuitable hosts for Mexican SAFF females and Aluja et al. (2003a) later confirmed that fruits of *Citrus sinensis* and *Citrus paradisi* in Mexico are not infested, either in the field or under laboratory-induced conditions. In contrast, studies in South America revealed that citrus fruits are common hosts for Brazilian populations of the SAFF (Malavasi et al. 1980, Zucchi 2007, and others). In this regard, there are some highly questionable reports indicating that *A. fraterculus* is able to infest citrus fruits in Guatemala (Eskafi & Cunningham 1987, Eskafi 1990). This variation in the range of host plants consumed in different regions of the neotropics presents a problem in terms of enforcing effective quarantine procedures.

Historical accounts indicate that nearly 150 plant species host larvae feeding on their fruits across the Americas (Norrbom 2004). However, many of these records were derived from observations under laboratory conditions or copied from previous literature reports, and some fail to specify an explicit location, or even country, or there is some uncertainty regarding the fly or host plant identity. Various studies at the regional level provide information on the population dynamics of the SAFF derived from specimens caught in traps baited with food attractants. These traps are usually hung on fruit trees of commercial importance; however, this does not necessarily demonstrate that these fly larvae infest such fruits under natural conditions. This information has led to the identification of three key problems of the phytosanitary measures that must be applied: (1) the presence of erroneous records featuring wrongly identified or unconfirmed hosts; (2) misidentification of the taxonomic species because other wild fly species are also attracted to the food baits; and (3) misinterpretation of distribution patterns based on records with inaccurate locations. As a consequence, implementation of quarantine measures and methods of integrated pest management (IPM) based on such erroneous or ambiguous information can lead to poor planning of control strategies based on concepts of fly-free or low prevalence areas, or even the application of the sterile insect technique (SIT) in specific geographical areas, which requires accurate identification of the hosts consumed by the target species.

This study therefore aims to provide a comprehensive understanding of the host plants used as natural food resources by *A. fraterculus.* The review focuses particularly on records produced under field conditions to conduct an analysis of botanical families and host species using current botanical nomenclature and homogenizing past and current species names, as well as examining their occurrence in different countries using the information available in the literature produced over nearly a century. This is a critical first step to elucidate the host range of the SAFF across several regions of the neotropics.

6.2 METHODS AND ANALYSIS

Information was sought in the original published sources of recorded host plants of the nominal species *A. fraterculus*. The search was largely conducted in articles published in scientific journals, books, and unpublished dissertations available online. The main requirements of credibility for each record were inclusion of the full identity of the host plant, as well as the occurrence of fruit infestation under natural conditions in the field, with no ambiguity regarding the recorded location (to country level at least). We therefore excluded doubtful reports, those obtained under laboratory conditions, and those in which the accurate identity of the fruit fly species could not be verified.

Nearly 170 references as primary sources of information from all regions of the Americas were examined. All information useful for the analysis was captured in a database, including the host identity, botanical family, original source of data, country, state or province, locality, coordinates and collection date, where available. If a single publication reported the same host in several locations, each report was considered a separate record in the database. Furthermore, each host plant was classified as native or exotic based on its origin because many were species that had been introduced to the Americas.

A few records were omitted from the analysis, even though they were obtained under natural conditions. This was the case of two historical records for *Annona cherimola* Mill. in Arica (Chile) because *A. fraterculus* does not occur in that country since its eradication in 1964. In addition, other reports for several countries of *Rubus* spp., as well as *Ravenia wampi* Oliv (Rutaceae) and *Cyphomandra betacea* (Cav.) Stendtn. (Solanaceae) for Brazil were also excluded from the analysis because their status as unresolved names prevented confirmation of their identity and origin.

Because some original names have changed because publication in the primary source, the attached list presents a single identity for each host plant. The nomenclature used for the scientific names of plants was updated following the classification of "*The Plant List*" (2013) to avoid duplication or potential synonymies among species, as well as to facilitate their correct assignment to the corresponding family. The list of host plants of *A. fraterculus* (*sensu lato*) is organized and presented by host family and specific binomial name, with the authorship, origin, key code by host species, total number of records and countries where this fruit fly has been reported, as well as the original source of information (Table 6.1).

To analyze host plant trends, interaction networks depicting the associations of *A. fraterculus* and its host plant species across the Americas were evaluated, considering the origin of the hosts (native or exotic) and their plant families. A cluster analysis was also conducted based on the frequency of records of hosts throughout the countries. All analyses were executed using the "plotweb function" of the "bipartite" package (Dormann & Gruber 2009) in "R software" (R Core Team 2014).

6.3 RESULTS

6.3.1 Diversity of Host Plants

As a result of the examination of natural host plants of *A. fraterculus* throughout the Americas, 177 host plants belonging to 40 plant families were recorded, which together accounted for 1,622 documented reports in different localities. The plant families with highest species richness were Myrtaceae (48), Rosaceae (20), Rutaceae (14), Leguminosae (11), Sapotaceae (10), Anacardiaceae (8), and Annonaceae (7), which together represented nearly 66% of all recorded hosts. For instance, the family Myrtaceae was represented by at least 10 different genera, having the highest species richness. The highest frequency of records was found for *Psidium guajava* (298), *Eugenia uniflora* (48), *Syzygium jambos* (35), *Acca sellowiana* (31), and *Psidium cattleianum* (22). Myrtaceae is therefore the most important food plant family widespread throughout the distribution range of the SAFF.

TABLE 6.1
List of Host Plants for the Nominal Species *Anastrepha fraterculus* Reported in the Literature as Food Plants Infested in Nature

Host Family	Valid Scientific Name	Host Origin	Host-Code	Total records	Countries	References
Actinidiaceae	*Actinidia deliciosa* (A Chev) CS Liang & AR Ferguson	Exotic	*Ac-del*	2	Bra	Hickel and Schuck (1993)
Anacardiaceae	*Anacardium occidentale* L.	Native	*An-occ*	2	Bra, Gua	Eskafi and Cunningham (1987), Jesus-Barros et al. (2012)
Anacardiaceae	*Byrsonima crassifolia* (L.) Kunth	Native	*By-cra*	1	Bra	Jesus-Barros et al. (2012)
Anacardiaceae	*Mangifera indica* L.	Exotic	*Ma-ind*	46	Arg, Bra, Col, Ecu, FrG, Gua, Mex, Per, Ven	Aluja et al. (1987), Alvarenga et al. (2009), Araujo (2015), Boscán de Martínez and Godoy (1996), Campos (1960), Caraballo (1981), Eskafi (1990), Eskafi and Cunningham (1987), Ferreira et al. (2003), Gonzalez Mendoza (1952), Guagliumi (1966), Hernández-Ortiz et al. (2012), Korytkowski and Ojeda Peña (1968), Korytkowski and Ojeda Peña. (1969), Malavasi et al. (1980), Marchiori et al. (2000), Marinho (2004), Molineros et al. (1992), Núñez Bueno (1981), Ovruski et al. (2003), Putruele et al. (1996), Raga et al. (2011), Sá et al. (2008), Silva (1993), Silva et al. (1996), Stone (1942b), Tigrero (2009), Uramoto (2002), Uramoto et al. (2004), Vayssières et al. (2013), Veloso et al. (2000), Zucchi (2000)
Anacardiaceae	*Spondias dulcis* Parkinson	Exotic	*Sp-dul*	5	Bra	Araujo (2015), Souza-Filho et al. (2000), Uramoto (2002), Uramoto et al. (2004)
Anacardiaceae	*Spondias mombin* L.	Native	*Sp-mom*	12	Bra, Col, Mex, Per, Ven	Aguiar-Menezes et al. (2007), Almeida (2016), Boscán de Martínez and Godoy (1996), Gonzalez Mendoza (1952), Jesus-Barros et al. (2012), Korytkowski and Ojeda Peña (1968), Korytkowski and Ojeda Peña (1969), Lemos (2014), Lemos et al. (2017), McPhail and Bliss (1933), Sarmiento et al. (2012)
Anacardiaceae	*Spondias purpurea* L.	Native	*Sp-pur*	23	Bra, Ecu, Pan, Per	Aguiar-Menezes et al. (2001), Aguiar-Menezes et al. (2007), Alvarenga et al. (2000, 2009), Bressan and Da Costa Teles (1991), Campos (1960), Korytkowski and Ojeda Peña (1968), Korytkowski and Ojeda Peña. (1969), Leal et al. (2009), Lemos (2014), Lemos et al. (2017), Malavasi et al. (1980), Pirovani (2011), Pirovani et al. (2010), Raga et al. (2011), Sá et al. (2008), Silva et al. (2010), Souza-Filho et al. (2000), Stone (1942b), Tigrero (2009), Zucchi (2000)

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TABLE 6.1 (*Continued*)
List of Host Plants for the Nominal Species *Anastrepha fraterculus* Reported in the Literature as Food Plants Infested in Nature

Host Family	Valid Scientific Name	Host Origin	Host-Code	Total records	Countries	References
Anacardiaceae	*Spondias* sp.	Native	*Sp-sp*	7	Bra, Col, Ecu, Mex	Aluja (1984), Molineros et al. (1992), Núñez Bueno (1981), Sá et al. (2008), Santos (2003), Silva et al. (2010), Zucchi (2000)
Anacardiaceae	*Spondias tuberosa* Arruda	Native	*Sp-tub*	2	Bra	Alvarenga et al. (2009), Sá et al. (2008)
Annonaceae	*Annona cherimola* Mill.	Native	*An-che*	21	Arg, Bra, Col, Ecu, Per	Gonzalez Mendoza (1952), Greene (1934), Hernández-Ortiz et al. (2012), Korytkowski and Ojeda Peña (1968), Korytkowski and Ojeda Peña. (1969), Molineros et al. (1992), Núñez Bueno (1981), Ovruski et al. (2003), Rust (1918), Stone (1942b), Tigrero (2009), White and Elson-Harris (1992), Wille (1941)
Annonaceae	*Annona crassiflora* Mart.	Native	*An-cra*	2	Bra	Veloso et al. (2000), Zucchi (2000)
Annonaceae	*Annona muricata* L.	Native	*An-mur*	4	Col	Gonzalez Mendoza (1952)
Annonaceae	*Annona rugulosa* (Schltdl.) H. Rainer	Native	*An-rug*	2	Bra	Garcia and Norrbom (2011), Marsaro Júnior (2014)
Annonaceae	*Rollinia emarginata* Schltdl.	Native	*Ro-ema*	2	Bra	Souza-Filho et al. (2000), Zucchi (2000)
Annonaceae	*Rollinia laurifolia* Schltdl.	Native	*Ro-lau*	2	Bra	Uramoto (2007), Uramoto et al. (2008)
Annonaceae	*Rollinia sericea* (R. E. Fr.) R. E. Fr.	Native	*Ro-ser*	3	Bra	Raga et al. (2011), Souza-Filho et al. (2000), Zucchi (2000)
Arecaceae	*Butia eriospatha* (Mart. ex Drude) Becc.	Native	*Bu-eri*	1	Bra	Savaris et al. (2013)
Arecaceae	*Syagrus romanzoffiana* (Cham.) Glassman	Native	*Sy-rom*	2	Bra	Araujo (2015), Uramoto (2002)
Calophyllaceae	*Mammea americana* L.	Exotic	*Ma-ame*	2	Col, Ecu	Campos (1960), Gonzalez Mendoza (1952)
Cannabaceae	*Celtis iguanaea* (Jacq.) Sarg.	Native	*Ce-igu*	1	Bra	Garcia and Norrbom (2011)
Caricaceae	*Carica papaya* L.	Native	*Ca-pap*	2	Arg, Col	Gonzalez Mendoza (1952)

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TABLE 6.1 (*Continued*)
List of Host Plants for the Nominal Species *Anastrepha fraterculus* Reported in the Literature as Food Plants Infested in Nature

Host Family	Valid Scientific Name	Host Origin	Host-Code	Total records	Countries	References
Celastraceae	*Peritassa campestris* (Cambess.) A. C. Sm.	Native	*Pe-cam*	2	Bra	Veloso et al. (2000), Zucchi (2000)
Chrysobalanaceae	*Chrysobalanus icaco* L.	Native	*Ch-ica*	2	Bra, Col	Deus et al. (2013), Gonzalez Mendoza (1952)
Clusiaceae	*Garcinia brasiliensis* Mart.	Native	*Ga-bra*	2	Bra	Pirovani (2011), Raga et al. (2011)
Combretaceae	*Terminalia catappa* L.	Exotic	*Te-cat*	32	Bra, CR, Ecu, Mex, Ven	Aguiar-Menezes et al. (2007), Aluja et al. (1987, 2000), Araujo et al. (2000), Araujo (2012), Boscán de Martínez and Godoy (1996), Boscán de Martínez et al. (1980), Caraballo (1981), Hernández-Ortiz and Morales-Valles (2004), Hernández-Ortiz et al. (2012), Katiyar et al. (1995), Mascarenhas (2007), Raga et al. (2011), Silva et al. (1996), Souza-Filho et al. (2000), Steck (1991), Steck et al. (1990), Tigrero (2009), Zucchi (2000)
Ebenaceae	*Diospyros kaki* L.	Exotic	*Di-kak*	12	Arg, Bra	Garcia and Norrbom (2011), Malavasi et al. (1980), Marsaro Júnior (2014), Nasca et al. (1996), Ovruski et al. (2003), Segura et al. (2004, 2006), Souza-Filho et al. (2000), Zucchi (2000)
Euphorbiaceae	*Alchornea latifolia* Sw.	Native	*Al-lat*	2	Mex	Aluja (1984), Aluja et al. (1987)
Euphorbiaceae	*Manihot esculenta* Crantz	Native	*Mn-esc*	1	Ven	Boscán de Martínez and Godoy (1996)
Juglandaceae	*Juglans australis* Griseb.	Native	*Ju-aus*	6	Arg	Ovruski et al. (2003, 2004)
Juglandaceae	*Juglans neotropica* Diels	Native	*Ju-neo*	4	Ecu, Per	Korytkowski and Ojeda Peña (1969), Molineros et al. (1992), Tigrero (2009)
Lauraceae	*Cryptocarya aschersoniana* Mez	Native	*Cr-asc*	2	Bra	Marinho et al. (2009), Raga et al. (2011)
Lauraceae	*Endlicheria paniculata* (Spreng.) J. F. Macbr.	Native	*En-pan*	1	Bra	Pirovani (2011)
Lauraceae	*Persea americana* Mill.	Native	*Pe-ame*	4	Arg, Bra	Araujo (2015), Putruele et al. (1996), Rust (1918), Uramoto (2002)
Leguminosae	*Andira humilis* Mart. ex Benth.	Native	*An-hum*	2	Bra	Veloso et al. (2000), Zucchi (2000)

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TABLE 6.1 (*Continued*)
List of Host Plants for the Nominal Species *Anastrepha fraterculus* Reported in the Literature as Food Plants Infested in Nature

Host Family	Valid Scientific Name	Host Origin	Host-Code	Total records	Countries	References
Leguminosae	*Inga edulis* Mart.	Native	*In-edu*	8	Bra, Col, Ecu	Deus et al. (2010), Gonzalez Mendoza (1952), Lemos (2014), Lemos et al. (2017), Malavasi et al. (1980), Tigrero (2009), Zucchi (2000)
Leguminosae	*Inga feuilleei* DC.	Native	*In-feu*	1	Ecu	Tigrero (2009)
Leguminosae	*Inga insignis* Kunth	Native	*In-ins*	1	Ecu	Tigrero (2009)
Leguminosae	*Inga micheliana* Harms	Native	*In-mic*	1	Gua	Eskafi and Cunningham (1987)
Leguminosae	*Inga paterna* Harms	Native	*In-pat*	1	Gua	Eskafi and Cunningham (1987)
Leguminosae	*Inga sellowiana* Benth.	Native	*In-sel*	1	Bra	Garcia and Norrbom (2011)
Leguminosae	*Inga semialata* (Vell.) C. Mart.	Native	*In-sem*	1	Arg	Oroño et al. (2005)
Leguminosae	*Inga* sp.	Native	*In-sp*	4	Bra, Ecu, Ven	Briceño Vergara (1975), Campos (1960), Molineros et al. (1992), Raga et al. (2011)
Leguminosae	*Inga spectabilis* (Vahl) Willd.	Native	*In-spe*	2	Ecu	Tigrero (2009)
Leguminosae	*Inga vera* subsp. *spuria* (Willd.) J. Leon	Native	*In-ver*	1	Col	Gonzalez Mendoza (1952)
Lythraceae	*Punica granatum* L.	Exotic	*Pu-gra*	5	Arg, Bra, Ecu	Molineros et al. (1992), Nasca et al. (1996), Putruele et al. (1996), Raga et al. (2011), Tigrero (2009)
Malpighiaceae	*Malpighia emarginata* DC.	Exotic	*Ml-ema*	4	Col, Ecu	Campos (1960), Pirovani (2011), Raga et al. (2011), Sá et al. (2008)
Malpighiaceae	*Malpighia glabra* L.	Native	*Ml-gla*	13	Bra, Col, Ecu	Aguiar-Menezes et al. (2001), Araujo (2015), Gonzalez Mendoza (1952), Leal et al. (2009), Malavasi et al. (1980), Marinho (2004), Souza-Filho et al. (2000), Uramoto (2002), Uramoto et al. (2004), Zucchi (2000)
Malvaceae	*Quararibea cordata* (Bonpl.) Vischer	Native	*Qu-cor*	3	Ecu	Molineros et al. (1992), Tigrero (2009)
Malvaceae	*Theobroma cacao* L.	Native	*Th-cac*	2	Ven	Caraballo (1981), Hernández-Ortiz and Morales-Valles (2004)
Melastomataceae	*Bellucia grossularioides* (L.) Triana	Native	*Be-gro*	1	FrGuy	Vayssières et al. (2013)

(*Continued*)

TABLE 6.1 (*Continued*)
List of Host Plants for the Nominal Species *Anastrepha fraterculus* Reported in the Literature as Food Plants Infested in Nature

Host Family	Valid Scientific Name	Host Origin	Host-Code	Total records	Countries	References
Melastomataceae	*Mouriri acutiflora* Naudin	Native	*Mo-acu*	1	Bra	Jesus-Barros et al. (2012)
Melastomataceae	*Mouriri glazioviana* Cogn.	Native	*Mo-gla*	2	Bra	Uramoto (2007), Uramoto et al. (2008)
Moraceae	*Ficus carica* L.	Exotic	*Fi-car*	16	Arg, Bra, Ecu	Garcia and Norrbom (2011), Molineros et al. (1992), Nasca et al. (1996), Ovruski et al. (2003), Putruele et al. (1996), Rust (1918), Tigrero (2009)
Moraceae	*Helicostylis* sp.	Native	*He-sp*	3	Bra	Bondar (1950), Kovaleski et al. (1999), Zucchi (2000)
Moraceae	*Helicostylis tomentosa* (Poepp. & Endl.) J. F. Macbr.	Native	*He-tom*	1	Bra	Bondar (1950)
Myrtaceae	*Acca sellowiana* (O. Berg) Burret	Native	*Ac-sel*	31	Arg, Bra, Col, Ecu	Alberti et al. (2002), Cruz et al. (2017), Custódio et al. (2017), Garcia and Norrbom (2011), Hernández-Ortiz et al. (2015), Hickel and Ducroquet (1994), Kovaleski et al. (1999, 2000), Marsaro Júnior (2014), Molineros et al. (1992), Nasca et al. (1996), Nunes et al. (2012), Ovruski et al. (2008), Pereira-Rêgo et al. (2011), Rust (1918), Salles (1995), Schliserman et al. (2010), Segura et al. (2004, 2006), Tigrero (2009), Yepes and Vélez (1989), Zucchi (2000)
Myrtaceae	*Calycolpus moritzianus* (O. Berg) Burret	Native	*Ca-mor*	3	Ven	Hernández-Ortiz and Morales-Valles (2004), Hernández-Ortiz et al. (2012), Katiyar et al. (2000)
Myrtaceae	*Campomanesia adamantium* (Cambess.) O. Berg	Native	*Cm-ada*	8	Bra	Malavasi et al. (1980), Selivon (2000), Souza-Filho et al. (2000), Veloso et al. (2000), Zucchi (2000)
Myrtaceae	*Campomanesia espiritosantensis* Landrum	Native	*Cm-esp*	2	Bra	Uramoto (2007), Uramoto et al. (2008)
Myrtaceae	*Campomanesia guaviroba* (DC.) Kiaersk.	Native	*Cm-gua*	1	Bra	Zucchi (2000)

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TABLE 6.1 (*Continued*)
List of Host Plants for the Nominal Species *Anastrepha fraterculus* Reported in the Literature as Food Plants Infested in Nature

Host Family	Valid Scientific Name	Host Origin	Host-Code	Total records	Countries	References
Myrtaceae	*Campomanesia guazumifolia* (Cambess.) O. Berg	Native	*Cm-guz*	2	Bra	Garcia and Norrbom (2011), Marsaro Júnior (2014)
Myrtaceae	*Campomanesia lineatifolia* Ruiz & Pav.	Native	*Cm-lin*	2	Bra	Uramoto (2007), Uramoto et al. (2008)
Myrtaceae	*Campomanesia pubescens* (Mart. ex DC.) O. Berg	Native	*Cm-pub*	2	Bra	Guimarães et al. (1999), Raga et al. (2005)
Myrtaceae	*Campomanesia xanthocarpa* (Mart.) O. Berg	Native	*Cm-xan*	8	Arg, Bra	Garcia and Norrbom (2011), Kovaleski et al. (1999, 2000), Malavasi et al. (1980), Marsaro Júnior (2014), Salles (1995), Schliserman et al. (2010), Zucchi (2000)
Myrtaceae	*Eugenia brasiliensis* Lam.	Native	*Eu-bra*	8	Bra	Aguiar-Menezes et al. (2007), Raga et al. (2005), Steck and Malavasi (1988), Steck et al. (1990), Stone (1942b), Uramoto (2007), Uramoto et al. (2008), Wille (1941)
Myrtaceae	*Eugenia dodonaeifolia* Cambess.	Native	*Eu-dod*	1	Bra	Zucchi (2000)
Myrtaceae	*Eugenia dysenterica* DC.	Native	*Eu-dys*	3	Bra	Silva et al. (2010), Veloso et al. (2000), Zucchi (2000)
Myrtaceae	*Eugenia florida* DC.	Native	*Eu-flo*	1	Pan	Stone (1942a)
Myrtaceae	*Eugenia gemmiflora* O. Berg	Native	*Eu-gem*	2	Bra	Uramoto (2007), Uramoto et al. (2008)
Myrtaceae	*Eugenia involucrata* DC.	Native	*Eu-inv*	15	Bra	Araujo (2015), Garcia and Norrbom (2011), Haji and Da Gama Miranda (2000), Kovaleski et al. (1999, 2000), Marsaro Júnior (2014), Nunes et al. (2012), Raga et al. (2005), Salles (1995), Uramoto (2002, 2007), Uramoto et al. (2004, 2008), Zucchi (2000)
Myrtaceae	*Eugenia lambertiana* DC.	Native	*Eu-lam*	2	Bra	Raga et al. (2005), Zucchi (2000)
Myrtaceae	*Eugenia leitonii* D. Legrand	Native	*Eu-lei*	2	Bra	Raga et al. (2005), Zucchi (2000)

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TABLE 6.1 (*Continued*)
List of Host Plants for the Nominal Species *Anastrepha fraterculus* Reported in the Literature as Food Plants Infested in Nature

Host Family	Valid Scientific Name	Host Origin	Host-Code	Total records	Countries	References
Myrtaceae	*Eugenia myrcianthes* Nied.	Native	*Eu-myr*	2	Arg	Putruele et al. (1996), Segura et al. (2006)
Myrtaceae	*Eugenia platyphylla* O. Berg	Native	*Eu-pla*	3	Bra	Uramoto (2007)
Myrtaceae	*Eugenia platysema* O. Berg	Native	*Eu-plt*	1	Bra	Uramoto et al. (2008)
Myrtaceae	*Eugenia punicifolia* (Kunth) DC.	Native	*Eu-pun*	2	Ven	Fernandez Yepez (1953), Guagliumi (1966)
Myrtaceae	*Eugenia pyriformis* Cambess.	Native	*Eu-pyr*	19	Bra	Araujo (2015), Garcia and Norrbom (2011), Guimarães et al. (1999), Kovaleski et al. (1999, 2000), Malavasi et al. (1980), Marinho (2004), Marsaro Júnior (2014), Nunes et al. (2012), Perre (2016), Raga et al. (2005), Souza-Filho et al. (2000), Uramoto (2002), Uramoto et al. (2004), Zucchi (2000)
Myrtaceae	*Eugenia stipitata* McVaugh	Native	*Eu-sti*	8	Bra, Ecu	Lemos (2014), Lemos et al. (2017), Molineros et al. (1992), Silva et al. (2010), Tigrero (2009), Uramoto (2007), Uramoto et al. (2008)
Myrtaceae	*Eugenia uniflora* L.	Native	*Eu-uni*	48	Arg, Bra, Gua, Mex, Pan	Aguiar-Menezes and Menezes (2000), Aguiar-Menezes et al. (2003), Aguiar-Menezes et al. (2007), Aluja et al. (1987), Alvarenga et al. (2009), Alvarenga et al. (2000), Araujo (2015), Eskafi (1990), Garcia and Norrbom (2011), Guimarães et al. (1999), Hernández-Ortiz et al. (2012), Malavasi et al. (1980), Marchiori et al. (2000), Marinho (2004), Marsaro Júnior (2014), Nunes et al. (2012), Ovruski et al. (2003, 2004), Ovruski et al. (2008), Pirovani (2011), Pirovani et al. (2010), Raga et al. (2005), Salles (1995), Silva et al. (2010, 2011), Souza-Filho et al. (2000), Stone (1942b), Uramoto (2002), Uramoto et al. (2004), Veloso et al. (2000), Zucchi (2000)
Myrtaceae	*Myrceugenia euosma* (O. Berg) D. Legrand	Native	*Me-euo*	3	Bra	Kovaleski et al. (1999, 2000), Zucchi (2000)
Myrtaceae	*Myrcia clausseniana* Berg	Native	*Mc-cla*	2	Bra	Uramoto (2007), Uramoto et al. (2008)
Myrtaceae	*Myrcia popayanensis* Hieron.	Native	*Mc-pop*	1	Col	Olarte Espinosa (1980)

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TABLE 6.1 (*Continued*)
List of Host Plants for the Nominal Species *Anastrepha fraterculus* Reported in the Literature as Food Plants Infested in Nature

Host Family	Valid Scientific Name	Host Origin	Host-Code	Total records	Countries	References
Myrtaceae	*Myrcianthes fragrans* (Sw.) McVaugh	Exotic	*My-fra*	1	Col	Gonzalez Mendoza (1952)
Myrtaceae	*Myrcianthes pungens* (O. Berg) D. Legrand	Native	*My-pun*	11	Arg, Bra	Garcia and Norrbom (2011), Marsaro Júnior (2014), Ovruski et al. (2003, 2004, 2008), Putruele et al. (1996)
Myrtaceae	*Myrciaria dubia* (Kunth) McVaugh	Native	*Mr-dub*	1	Bra	Custódio et al. (2017)
Myrtaceae	*Myrciaria floribunda* (H.West ex Willd.) O. Berg	Native	*Mr-flo*	1	Mex	Aluja et al. (2000)
Myrtaceae	*Myrciaria glazioviana* (Kiaersk.) G. M. Barroso ex Sobral	Native	*Mr-gla*	1	Bra	Pirovani (2011)
Myrtaceae	*Myrciaria glomerata* O. Berg	Native	*Mr-glo*	5	Bra	Araujo (2015), Marinho (2004), Raga et al. (2005), Uramoto (2002), Uramoto et al. (2004)
Myrtaceae	*Myrciaria strigipes* O. Berg	Native	*Mr-str*	2	Bra	Uramoto (2007), Uramoto et al. (2008)
Myrtaceae	*Plinia cauliflora* (Mart.) Kausel	Native	*Pl-cau*	15	Bra	Aguiar-Menezes and Menezes (2000), Aguiar-Menezes et al. (2007), Araujo (2015), Garcia and Norrbom (2011), Marinho (2004), Pirovani (2011), Pirovani et al. (2010), Raga et al. (2005), Salles (1995), Silva et al. (2011), Souza-Filho et al. (2000), Uramoto (2002), Uramoto et al. (2004), Zucchi (2000)
Myrtaceae	*Plinia edulis* (Vell.) Sobral	Native	*Pl-edu*	2	Bra	Raga et al. (2005), Zucchi (2000)
Myrtaceae	*Psidium acutangulum* Mart. ex DC.	Native	*Ps-acu*	1	Col	Hernández-Ortiz et al. (2015)
Myrtaceae	*Psidium cattleianum* Afzel. ex Sabine	Native	*Ps-cat*	22	Arg, Bra, Gua	Aguiar-Menezes et al. (2001), Eskafi and Cunningham (1987), Garcia and Norrbom (2011), Kovaleski et al. (1999, 2000), Leal et al. (2009), Manni et al. (2015), Marinho (2004), Marsaro Júnior (2014), Nunes et al. (2012), Pereira-Rêgo et al. (2011), Raga et al. (2005), Rust (1918), Zucchi (2000)

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TABLE 6.1 (*Continued*)
List of Host Plants for the Nominal Species *Anastrepha fraterculus* Reported in the Literature as Food Plants Infested in Nature

Host Family	Valid Scientific Name	Host Origin	Host-Code	Total records	Countries	References
Myrtaceae	*Psidium guajava* L.	Native	*Ps-gua*	298	Arg, Bol, Bra, Col, CR, Ecu, FrG, Gua, Mex, Pan, Per, Ven	Adaime et al. (2017), Aguiar-Menezes and Menezes (1997, 2000), Aguiar-Menezes et al. (2001, 2007), Alberti et al. (2002), Aluja (1984), Aluja et al. (1987, 2000), Alvarenga et al. (2000. 2009), Araujo et al. (2000), Araujo (2012, 2015), Baker et al. (1944), Barros (2008), Bomfim et al. (2007), Boscán de Martínez and Godoy (1996), Campos (1960), Caraballo (1981), Castañeda et al. (2010), Corsato (2004), Cruz et al. (2017), Deus et al. (2010), Eskafi (1990), Eskafi and Cunningham (1987), Fernandez Yepez (1953), Fischer (1934), Garcia and Norrbom (2011), Gonzalez Mendoza (1952), Guagliumi (1966), Haji and Da Gama Miranda (2000), Hedström (1987), Hernández-Ortiz and Morales-Valles (2004), Hernández-Ortiz and Pérez-Alonso (1993), Hernández-Ortiz et al. (1994, 2004, 2012, 2015), Isaac (1905), Jesus-Barros et al. (2012), Jirón and Hedström (1988), Katiyar et al. (2000), Korytkowski et al. (2001), Korytkowski and Ojeda Peña (1968, 1969),Leal et al. (2009), Lemos (2014), Lemos et al. (2017), Malavasi et al. (1980), Manni et al. (2015), Marchiori et al. (2000), Marsaro Júnior (2014), Mascarenhas (2007), McPhail and Bliss (1933), Molineros et al. (1992), Nunes et al. (2012), Núñez Bueno (1981), Nuñez Bueno et al. (2004), Ovruski (1995), Ovruski et al. (2003, 2004, 2005, 2008, 2009), Pereira-Rêgo et al. (2011), Perre (2016), Pirovani (2011), Pirovani et al. (2010), Putruele et al. (1996), Querino et al. (2014), Raga et al. (2005), Sá et al. (2008), Salles (1995), Sarmiento et al. (2012), Schliserman et al. (2010), Segura et al. (2006), Selivon (2000), Silva et al. (2010, 2011a, 2011b), Silva and Silva (2007), Souza-Filho (2005, 2006), Souza-Filho et al. (2009), Steck (1991), Steck et al. (1990), Stone (1942b), Taira (2012), Taira et al. (2013), Tigrero (1998, 2009), Uchôa-Fernandes and Zucchi (2000), Uchôa-Fernandes et al. (2002), Uramoto (2002), Uramoto et al. (2004), Vayssières et al. (2013), Veloso et al. (2000), Wille (1937), Zucchi (2000)
Myrtaceae	*Psidium guineense* Sw.	Native	*Ps-gui*	17	Bra, Col, Mex, Ven	Aluja et al. (2000), Araujo et al. (2000), Bomfim et al. (2007), Castañeda et al. (2010), Fernandez Yepez (1953), Gonzalez Mendoza (1952), Guagliumi (1966), Hernández-Ortiz et al. (2004), Katiyar et al. (2000), Pirovani (2011), Pirovani et al. (2010), Silva et al. (2010, 2011), Uramoto et al. (2008), Veloso et al. (2000), Zucchi (2000)

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TABLE 6.1 (*Continued*)
List of Host Plants for the Nominal Species *Anastrepha fraterculus* Reported in the Literature as Food Plants Infested in Nature

Host Family	Valid Scientific Name	Host Origin	Host-Code	Total records	Countries	References
Myrtaceae	*Psidium guyanense* Pers.	Native	*Ps-guy*	1	Bra	Uramoto (2007)
Myrtaceae	*Psidium kennedyanum* Morong	Native	*Ps-ken*	1	Bra	Uchôa-Fernandes and Nicácio (2010)
Myrtaceae	*Psidium myrtoides* O. Berg	Native	*Ps-myr*	2	Bra	Uramoto (2007), Uramoto et al. (2008)
Myrtaceae	*Psidium sartorianum* (O. Berg) Nied.	Native	*Ps-sar*	4	Mex	Aluja et al. (2000), Aluja et al. (2003b)
Myrtaceae	*Psidium* sp.	Native	*Ps-sp*	8	Arg, Bra, Ecu	Molineros et al. (1992), Rust (1918), Salles (1995), Tigrero (2009), Uchôa-Fernandes and Zucchi (2000), Uchôa-Fernandes et al. (2002)
Myrtaceae	*Syzygium aqueum* (Burm. f.) Alston	Exotic	*Sy-aqu*	1	Bra	Zucchi (2000)
Myrtaceae	*Syzygium jambos* (L.) Alston	Exotic	*Sy-jam*	35	Bra, Ecu, Mex, Per, Ven	Aluja (1984), Aluja et al. (1987, 2000), Araujo (2015), Baker et al. (1944), Baker (1945), Bomfim et al. (2007), Bush (1962), Campos (1960), Hernández-Ortiz et al. (2004), Korytkowski and Ojeda Peña (1968, 1969), Marinho (2004), Molineros et al. (1992), Perre (2016), Raga et al. (2005), Souza-Filho et al. (2000), Steck (1991), Steck et al. (1990), Tigrero (2009), Uchôa-Fernandes and Nicácio (2010), Uramoto (2002), Uramoto et al. (2004)
Myrtaceae	*Syzygium malaccense* (L.) Merr. & L. M. Perry	Exotic	*Sy-mal*	4	Bra, Pan	Silva et al. (2010, 2011), Stone (1942b)
Oleaceae	*Olea europaea* L.	Exotic	*Ol-eur*	1	Arg	Nasca et al. (1996)
Oxalidaceae	*Averrhoa carambola* L.	Exotic	*Av-car*	18	Bra	Aguiar-Menezes et al. (2001, 2007), Araujo (2015), Feitosa et al. (2007), Garcia and Norrbom (2011), Leal et al. (2009), Malavasi et al. (1980), Marchiori et al. (2000), Raga et al. (2011), Silva et al. (2010), Souza-Filho et al. (2000), Uramoto (2002), Uramoto et al. (2004), Veloso et al. (2000), Zucchi (2000)
Passifloraceae	*Passiflora alata* Curtis	Native	*Pa-ala*	3	Bra	Pirovani (2011), Pirovani et al. (2010), Souza-Filho et al. (2000)
Passifloraceae	*Passiflora caerulea* L.	Native	*Pa-cae*	3	Arg	Ovruski et al. (2003), Putruele et al. (1996)

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TABLE 6.1 (*Continued*)
List of Host Plants for the Nominal Species *Anastrepha fraterculus* Reported in the Literature as Food Plants Infested in Nature

Host Family	Valid Scientific Name	Host Origin	Host-Code	Total records	Countries	References
Passifloraceae	*Passiflora edulis* Sims	Native	*Pa-edu*	4	Bra	Araujo (2015), Souza-Filho et al. (2000), Uramoto (2002), Uramoto et al. (2004)
Passifloraceae	*Passiflora* sp.	Native	*Pa-sp*	3	Bra	Kovaleski et al. (1999, 2000), Zucchi (2000)
Passifloraceae	*Passiflora tripartita* (Juss.) Poir.	Native	*Pa-tri*	1	Col	Castro (2009)
Picramniaceae	*Picramnia* sp.	Native	*Pi-sp*	1	Bra	Raga et al. (2011)
Rhamnaceae	*Ziziphus joazeiro* Mart.	Native	*Zi-joa*	1	Bra	Sá et al. (2008)
Rhamnaceae	*Ziziphus jujuba* Mill.	Native	*Zi-juj*	1	Col	Gonzalez Mendoza (1952)
Rosaceae	*Cydonia oblonga* Mill.	Exotic	*Cy-obl*	6	Arg, Per, Ven	Guagliumi (1966), Korytkowski and Ojeda Peña (1968, 1969), Nasca et al. (1996), Ovruski et al. (2010), Putruele et al. (1996)
Rosaceae	*Eriobotrya japonica* (Thunb.) Lindl.	Exotic	*Er-jap*	75	Arg, Bra, Col, Ecu, Mex, Per, Ven	Aguiar-Menezes and Menezes (2000), Aguiar-Menezes et al. (2007), Alberti et al. (2002), Aluja (1984), Araujo (2015), Briceño Vergara (1975, 1979), Caraballo (1981), Cruz et al. (2017), Fernandez Yepez (1953), Garcia and Norrbom (2011), Gonzalez Mendoza (1952), Guagliumi (1966), Hernández-Ortiz and Morales-Valles (2004), Hernández-Ortiz et al. (2012), Katiyar et al. (1995), Kovaleski et al. (1999, 2000), Malavasi et al. (1980), Marinho (2004), Mascarenhas (2007), Molineros et al. (1992), Nunes et al. (2012), Ovruski et al. (2003), Perre (2016), Pirovani (2011), Pirovani et al. (2010), Putruele et al. (1996), Raga et al. (2011), Salles (1995), Schliserman et al. (2010), Segura et al. (2006), Souza-Filho (2006), Souza-Filho et al. (2000, 2009), Steck (1991), Stone (1942b), Tigrero (2009), Uramoto (2002), Uramoto et al. (2004), Yepes and Vélez (1989), Zucchi (2000)
Rosaceae	*Fragaria* × *ananassa* (Duchesne ex Weston) Duchesne ex Rozier	Exotic	*Fg-ana*	1	Bra	Raga et al. (2011)
Rosaceae	*Fragaria vesca* L.	Exotic	*Fg-ves*	2	Bra	Salles (1995), Zucchi (2000)
Rosaceae	*Malus domestica* Borkh.	Exotic	*Ma-dom*	16	Bra, Col, Ecu, Ven	Ballou (1945), Boscán de Martínez et al. (1980), Campos (1960), Caraballo (1981), Gonzalez Mendoza (1952), Guagliumi (1966), Kovaleski et al. (1999, 2000), Malavasi et al. (1980), Molineros et al. (1992), Perre (2016), Raga et al. (2011), Salles (1995), Tigrero (2009), Zucchi (2000)

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TABLE 6.1 (*Continued*)
List of Host Plants for the Nominal Species *Anastrepha fraterculus* Reported in the Literature as Food Plants Infested in Nature

Host Family	Valid Scientific Name	Host Origin	Host-Code	Total records	Countries	References
Rosaceae	*Prunus armeniaca* L.	Exotic	*Pr-arm*	6	Arg	Nasca et al. (1996), Ovruski et al. (2003, 2004), Putruele et al. (1996), Rust (1918)
Rosaceae	*Prunus avium* (L.) L.	Exotic	*Pr-avi*	1	Bra	Garcia and Norrbom (2011)
Rosaceae	*Prunus domestica* L.	Exotic	*Pr-dom*	17	Arg, Bra, Col, Ecu	Garcia and Norrbom (2011), Guimarães et al. (1999), Kovaleski et al. (1999, 2000), Marin Patiño (2002), Molineros et al. (1992), Nasca et al. (1996), Ovruski et al. (2003, 2004, 2010), Raga et al. (2011), Salles (1995), Tigrero (2009), Zucchi (2000)
Rosaceae	*Prunus domestica* subsp. *insititia* (L.) Bonnier & Layens	Exotic	*Pr-dom*	2	Arg	Putruele et al. (1996), Segura et al. (2006)
Rosaceae	*Prunus dulcis* (Mill.) D. A.Webb	Exotic	*Pr-dul*	1	Arg	Nasca et al. (1996)
Rosaceae	*Prunus mume* (Siebold) Siebold & Zucc.	Exotic	*Pr-mum*	2	Bra	Perre (2016), Raga et al. (2011)
Rosaceae	*Prunus persica* (L.) Batsch	Exotic	*Pr-per*	177	Arg, Bol, Bra, Col, Ecu, Gua, Mex, Per, Ven	Aguiar-Menezes and Menezes (1997), Alberti et al. (2002), Aluja et al. (2000), Araujo (2015), Baker et al. (1944), Boscán de Martínez and Godoy (1996), Boscán de Martínez et al. (1980), Briceño Vergara (1975, 1979), Campos (1960), Caraballo (1981), Cruz et al. (2017), Eskafi and Cunningham (1987), Garcia and Norrbom (2011), Gonzalez Mendoza (1952), Greene (1934), Guagliumi (1966), Hernández-Ortiz and Morales-Valles (2004), Kovaleski et al. (1999, 2000), Malavasi et al. (1980), Marsaro Júnior (2014), Molineros et al. (1992), Nasca et al. (1996), Nunes et al. (2012), Ovruski (1995), Ovruski et al. (2003, 2004, 2009, 2010), Perre (2016), Putruele et al. (1996), Raga et al. (2011), Salles (1995), Schliserman et al. (2010), Segura et al. (2004, 2006), Shaw (1947), Souza-Filho (2006), Souza-Filho et al. (2000, 2009), Steck (1991), Tigrero (2009), Uramoto (2002), Uramoto et al. (2004), Zucchi (2000)
Rosaceae	*Prunus salicina* Lindl.	Exotic	*Pr-sal*	7	Arg, Bra	Alberti et al. (2002), Marinho (2004), Marinho et al. (2009), Perre (2016)
Rosaceae	*Prunus serotina* Ehrh. ssp. *capuli* (Cav.) McVaugh	Native	*Pr-ser*	1	Gua	Eskafi and Cunningham (1987)

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TABLE 6.1 (*Continued*)
List of Host Plants for the Nominal Species *Anastrepha fraterculus* Reported in the Literature as Food Plants Infested in Nature

Host Family	Valid Scientific Name	Host Origin	Host-Code	Total records	Countries	References
Rosaceae	*Prunus* sp.	Exotic	*Pr-sp*	5	Bra, Col	Gonzalez Mendoza (1952), Malavasi et al. (1980), Salles (1995), Zucchi (2000)
Rosaceae	*Pyrus communis* L.	Exotic	*Py-com*	18	Arg, Bra, Ecu, Per	Aguiar-Menezes and Menezes (2000), Campos (1960), Garcia and Norrbom (2011), Korytkowski and Ojeda Peña (1968, 1969), Kovaleski et al. (2000), Molineros et al. (1992), Nasca et al. (1996), Nunes et al. (2015), Raga et al. (2011), Salles (1995), Souza-Filho et al. (2000), Stone (1942b), Tigrero (2009), Zucchi (2000)
Rosaceae	*Rubus eriocarpus* Liebm.	Native	*Ru-eri*	3	Ecu, Ven	Hernández-Ortiz and Morales-Valles (2004), Tigrero (2009)
Rosaceae	*Rubus idaeus* L.	Exotic	*Ru-ida*	1	Arg	Funes et al. (2017)
Rosaceae	*Rubus* sp.	?	*Ru-sp*	26	Arg, Bra, Col, Ecu, Ven	Araujo (2015), Briceño Vergara (1975, 1979), Castañeda et al. (2010), Funes et al. (2017), Hernández-Ortiz et al. (2012), Katiyar et al. (1995), Kovaleski et al. (2000), Manni et al. (2015), Molineros et al. (1992), Núñez Bueno (1981), Salles (1995), Steck (1991), Steck et al. (1990), Uramoto (2002), Uramoto et al. (2004), Zucchi (2000)
Rosaceae	*Rubus ulmifolius* Schott	Exotic	*Ru-ulm*	1	Bra	Raga et al. (2011)
Rubiaceae	*Coffea arabica* L.	Exotic	*Co-ara*	51	Bra, Col, Mex, Ven	Aluja (1984), Aluja et al. (1987), Araujo (2015), Boscán de Martínez et al. (1980), Caraballo (1981), Castañeda et al. (2010), Cruz et al. (2017), Gonzalez Mendoza (1952), Hernández-Ortiz and Morales-Valles (2004), Hernández-Ortiz et al. (2012), Hernández-Ortiz et al. (2015), Katiyar et al. (1995), Malavasi et al. (1980), Núñez Bueno (1981), Nuñez Bueno et al. (2004), Sarmiento et al. (2012), Souza et al. (2005), Steck (1991), Steck et al. (1990), Stone (1942b), Torres (2004), Uramoto (2002), Uramoto et al. (2004), Yepes and Vélez (1989), Zucchi (2000)
Rubiaceae	*Coffea liberica* Hiern	Exotic	*Co-lib*	1	Ven	Guagliumi (1966)
Rutaceae	*Citrus aurantiifolia* (Christm.) Swingle	Exotic	*Ct-arn*	4	Bra	Salles (1995), Zucchi (2000)
Rutaceae	*Citrus japonica* Thunb.	Exotic	*Ct-jap*	5	Arg, Bra, Col	Gonzalez Mendoza (1952), Raga et al. (2004), Rust (1918), Salles (1995), Zucchi (2000)
Rutaceae	*Citrus limon* (L.) Osbeck	Exotic	*Ct-lim*	3	Bra	Salles (1995). Souza-Filho et al. (2000), Zucchi (2000)

(*Continued*)

TABLE 6.1 (*Continued*)
List of Host Plants for the Nominal Species *Anastrepha fraterculus* Reported in the Literature as Food Plants Infested in Nature

Host Family	Valid Scientific Name	Host Origin	Host-Code	Total records	Countries	References
Rutaceae	*Citrus maxima* (Burm.) Merr.	Exotic	*Ct-max*	8	Arg, Bra, Ecu, Per	Korytkowski and Ojeda Peña (1968, 1969), Malavasi et al. (1980), Raga et al. (2004), Rust (1918), Souza-Filho et al. (2000), Tigrero (2009), Zucchi (2000)
Rutaceae	*Citrus medica* L.	Exotic	*Ct-med*	2	Bra, Gua	Eskafi and Cunningham (1987), Raga et al. (2004)
Rutaceae	*Citrus nobilis* Lour.	Exotic	*Ct-nob*	1	Arg	Rust (1918)
Rutaceae	*Citrus paradisi* Macfad.	Exotic	*Ct-par*	32	Arg, Bra, Ecu, Gua, Ven	Caraballo (1981), Eskafi and Cunningham (1987), Molineros et al. (1992), Nasca et al. (1996), Ovruski et al. (2003), Putruele et al. (1996), Schliserman et al. (2010), Segura et al. (2006), Zucchi (2000)
Rutaceae	*Citrus reticulata* Blanco	Exotic	*Ct-ret*	30	Arg, Bra, Col, Ecu	Araujo (2015), Castañeda et al. (2010), Garcia and Norrbom (2011), Molineros et al. (1992), Ovruski et al. (2003), Putruele et al. (1996), Raga et al. (2004), Schliserman et al. (2010), Segura et al. (2004, 2006), Souza-Filho et al. (2000), Tigrero (2009), Uramoto (2002), Uramoto et al. (2004)
Rutaceae	*Citrus sinensis* (L.) Osbeck	Exotic	*Ct-sin*	44	Arg, Bra, Ecu, Gua, Per	Aguiar-Menezes and Menezes (2000), Ballou (1945), Boscán de Martínez et al. (1980), Eskafi (1990), Eskafi and Cunningham (1987), Guagliumi (1966), Guimarães et al. (1999), Korytkowski and Ojeda Peña (1968, 1969), Malavasi et al. (1980), Molineros et al. (1992), Ovruski et al. (2003), Pirovani (2011), Pirovani et al. (2010), Putruele et al. (1996), Raga et al. (2004), Salles (1995), Selivon (2000), Silva et al. (2006), Stone (1942b), Tigrero (2009), Zucchi (2000)
Rutaceae	*Citrus sinensis x C reticulata*	Exotic	*Ct-sxr*	2	Bra	Silva et al. (2006)
Rutaceae	*Citrus* sp.	Exotic	*Ct-sp*	34	Bra, Col, Ecu, Ven	Araujo (2012, 2015), Briceño Vergara (1979), Caraballo (1981), Gonzalez Mendoza (1952), Molineros et al. (1992), Núñez Bueno (1981), Raga et al. (2004), Salles (1995), Uramoto (2002), Uramoto et al. (2004), Zucchi (2000)
Rutaceae	*Citrus trifoliata* L.	Exotic	*Ct-tri*	2	Ecu	Molineros et al. (1992), Tigrero (2009)
Rutaceae	*Citrus x aurantium* L.	Exotic	*Ct-aur*	39	Arg, Bra, Col, Ecu, Mex, Ven	Briceño Vergara (1975), Campos (1960), Gonzalez Mendoza (1952), Hernández-Ortiz et al. (1994, 2006), Malavasi et al. (1980), Marchiori et al. (2000), Ovruski et al. (2003), Putruele et al. (1996), Raga et al. (2004), Schliserman and Ovruski (2004), Schliserman et al. (2010), Segura et al. (2006), Tigrero (1998, 2009), Yepes and Vélez (1989), Zucchi (2000)

(*Continued*)

TABLE 6.1 (*Continued*)
List of Host Plants for the Nominal Species *Anastrepha fraterculus* Reported in the Literature as Food Plants Infested in Nature

Host Family	Valid Scientific Name	Host Origin	Host-Code	Total records	Countries	References
Rutaceae	*Ravenia wampi* Oliv.	?	*unres. name*	1	Bra	Aguiar-Menezes et al. (2001)
Salicaceae	*Dovyalis abyssinica* (A. Rich.) Warb.	Exotic	*Do-aby*	2	Ecu	Molineros et al. (1992), Tigrero (2009)
Salicaceae	*Dovyalis hebecarpa* (Gardner) Warb.	Exotic	*Do-heb*	1	Pan	Stone (1942b)
Salicaceae	*Zuelania guidonia* (Sw.) Britton & Millsp.	Native	*Zu-gui*	1	Mex	García-Ramírez et al. (2010)
Sapindaceae	*Diatenopteryx sorbifolia* Radlk.	Native	*Di-sor*	2	Bra	Salles (1995), Zucchi (2000)
Sapindaceae	*Melicoccus bijugatus* Jacq.	Native	*Me-bij*	1	Bra	Gonzalez Mendoza (1952)
Sapindaceae	*Melicoccus oliviformis* Kunth	Native	*Me-oli*	1	Mex	García-Ramírez et al. (2010)
Sapotaceae	*Chrysophyllum cainito* L.	Native	*Cs-cai*	3	Ecu, Ven	Campos (1960), Hernández-Ortiz and Morales-Valles (2004)
Sapotaceae	*Chrysophyllum gonocarpum* (Mart. & Eichler ex Miq.) Engl.	Native	*Cs-gon*	3	Arg, Bra	Oroño et al. (2005), Salles (1995), Zucchi (2000)
Sapotaceae	*Chrysophyllum mexicanum* Brandegee	Native	*Cs-mex*	1	Bra	Souza-Filho et al. (2000)
Sapotaceae	*Manilkara zapota* (L.) P. Royen	Native	*Ma-zap*	8	Bra, Col, Ecu, Pan	Araujo (2015), Campos (1960), Gonzalez Mendoza (1952), Núñez Bueno (1981), Raga et al. (2011), Stone (1942b), Uramoto (2002), Uramoto et al. (2004)
Sapotaceae	*Planchonella obovata* (R. Br.) Pierre	Native	*Pl-obo*	1	Per	Korytkowski and Ojeda Peña. (1969)
Sapotaceae	*Pouteria caimito* (Ruiz & Pav.) Radlk.	Native	*Po-cai*	10	Bra, Ecu	Aguiar-Menezes et al. (2007), Araujo (2015), Raga et al. (2011), Souza-Filho et al. (2000), Tigrero (2009), Uramoto (2002), Uramoto et al. (2004), Veloso et al. (2000), Zucchi (2000)

(*Continued*)

TABLE 6.1 (*Continued*)
List of Host Plants for the Nominal Species *Anastrepha fraterculus* Reported in the Literature as Food Plants Infested in Nature

Host Family	Valid Scientific Name	Host Origin	Host-Code	Total records	Countries	References
Sapotaceae	*Pouteria gardneriana* (A. DC.) Radlk.	Native	*Po-gar*	3	Bra	Veloso et al. (1996, 2000), Zucchi (2000)
Sapotaceae	*Pouteria ramiflora* (Mart.) Radlk.	Native	*Po-ram*	3	Bra	Veloso et al. (1996, 2000), Zucchi (2000)
Sapotaceae	*Pouteria torta* (Mart.) Radlk.	Native	*Po-tor*	1	Bra	Taira (2012)
Sapotaceae	*Sideroxylon capiri* (A. DC.) Pittier	Native	*Si-cap*	1	Mex	Aluja et al. (1987)
Simaroubaceae	*Simaba guianensis* Aubl.	Native	*Sm-gui*	1	Bra	Deus et al. (2013)
Siparunaceae	*Siparuna guianensis* Aubl.	Native	*Sp-gui*	1	Bra	Pirovani (2011)
Solanaceae	*Capsicum annuum* L.	Native	*Ca-ann*	1	Col	Castañeda et al. (2010)
Solanaceae	*Cyphomandra betacea* (Cav.) Sendtn.	?	*unres. name*	1	Col	Gonzalez Mendoza (1952)
Solanaceae	*Solanum decompositiflorum* Sendtn.	Native	*So-dec*	1	Bra	Pirovani (2011)
Solanaceae	*Solanum nudum* Dunal	Native	*So-nud*	1	Col	Castañeda et al. (2010)
Solanaceae	*Solanum quitoense* Lam.	Native	*So-qui*	1	Col	Núñez Bueno (1981)
Solanaceae	*Solanum triste* Jacq.	Native	*So-tri*	1	Col	Gonzalez Mendoza (1952)
Staphyleaceae	*Turpinia occidentalis* (Sw.) G. Don	Native	*Tu-occ*	1	Pan	Stone (1942b)

(*Continued*)

TABLE 6.1 (*Continued*)
List of Host Plants for the Nominal Species *Anastrepha fraterculus* Reported in the Literature as Food Plants Infested in Nature

Host Family	Valid Scientific Name	Host Origin	Host-Code	Total records	Countries	References
Ulmaceae	*Ampelocera hottlei* (Standl.) Standl.	Native	*Am-hot*	1	Mex	Aluja et al. (2003b)
Urticaceae	*Pourouma* sp.	Native	*Po-sp*	1	Bra	Bondar (1950)
Vitaceae	*Vitis vinifera* L.	Exotic	*Vi-vin*	3	Arg, Col	Gonzalez Mendoza (1952), Nasca et al. (1996), Stone (1942b)

Note: Nomenclatures of scientific names are currently valid according to *The Plant List* (2013).

Abbreviations: Arg, Argentina; Bra, Brazil; Bol, Bolivia; Col, Colombia; CR, Costa Rica; Ecu, Ecuador; FrG, French Guyana; Gua, Guatemala; Mex, Mexico; Pan, Panama; Per, Peru; Ven, Venezuela.

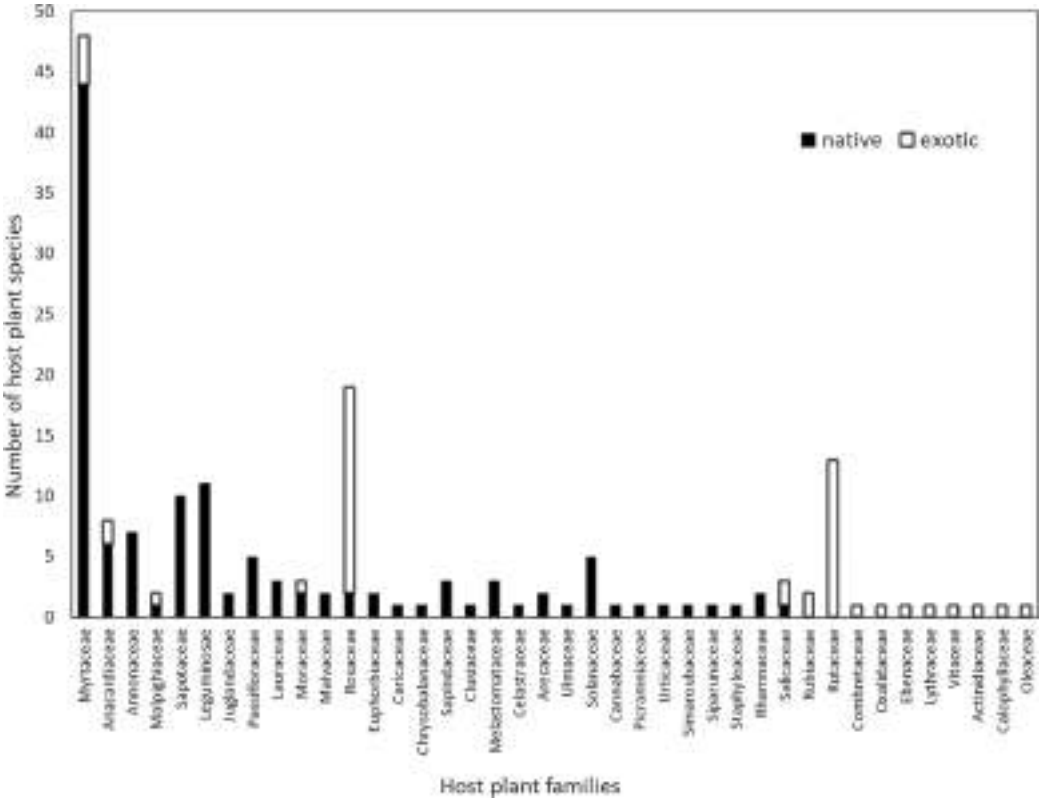

FIGURE 6.1 Host plant families for the *Anastrepha fraterculus* complex recorded in the Americas. Bars indicate the number of plant species; black bars, native species; white bars, exotic species.

In the family Rosaceae, 20 species were found, with most reports featuring *Prunus* spp., *Rubus* spp., and *Eriobotrya japonica*. The family Rutaceae included 14 host species, almost all of which were *Citrus* spp. In the case of the family Combretaceae, all reports were of a single host, the tropical almond *Terminalia cattapa*. A large proportion of at least 18 plant families presented only one to three hosts and a similarly low number of reports; thus, these could be considered rare hosts. These included *Actinidia deliciosa* (Actinidaceae), *Celtis iguanaea* (Cannabaceae), *Endlicheria paniculata* (Lauraceae), *Butia eriospatha* (Arecaceae), and *Simaba guianensis* (Simaroubaceae), among others (Figure 6.1 and Table 6.1).

6.3.2 Host Origin

Examination of the origin of hosts revealed that the SAFF feeds on 50 exotic plant species in the Americas, representing 28.3% of the total number of their host plant species. Five of these, all belonging to different families, accounted for the highest number of records: *Prunus persica* (Rosaceae), *Eriobotrya japonica* (Myrtaceae), *Coffea arabica* (Rubiaceae), *Mangifera indica* (Anacardiaceae), and *Citrus* spp. (Rutaceae). The frequency of records for these species accounted for slightly more than 30% of the total number of reports. The peach is recognized as the exotic host that is most frequently reported throughout the distributional range of the SAFF. However, at a regional level, there is a higher proportion of records for other species, such as coffee, mango, and citrus because each of these have economic significance in specific regions or countries (Figure 6.2). For instance, infestation of *Coffea arabica* coffee cherries was found mainly in

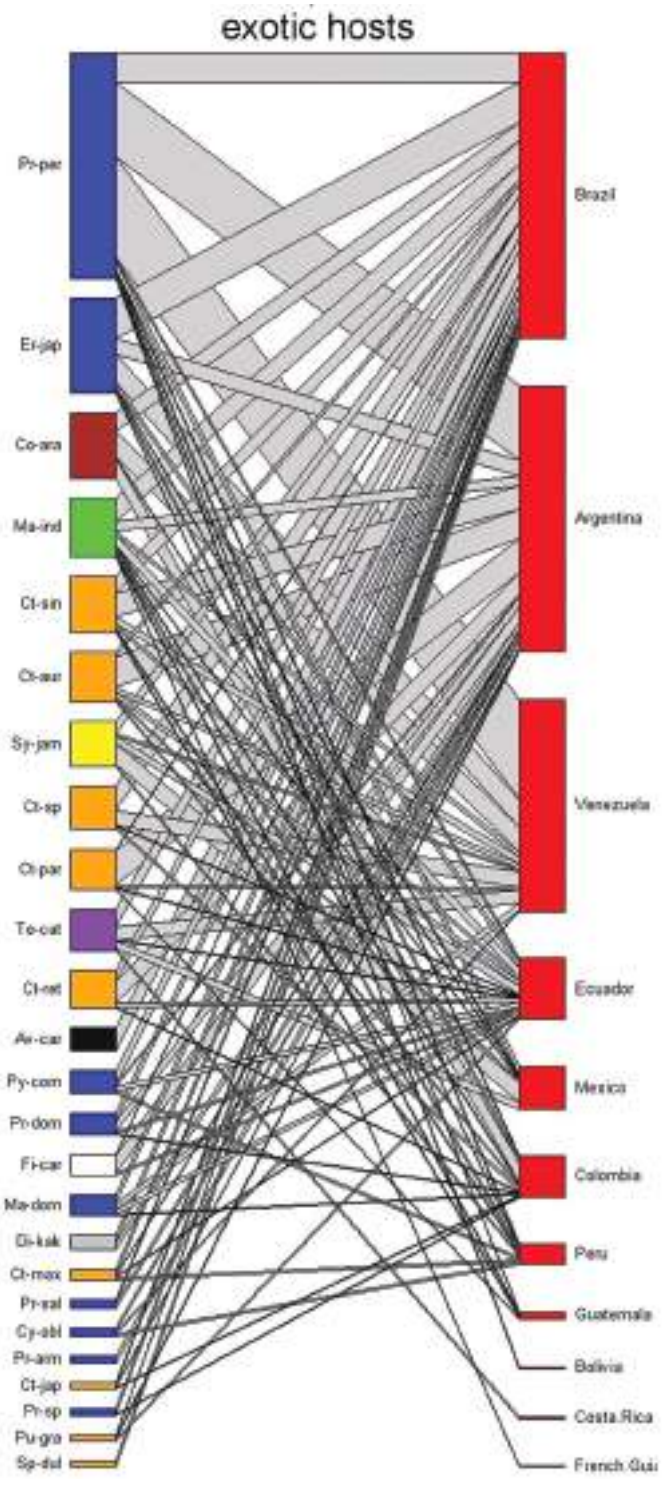

FIGURE 6.2 Trophic network interactions of the *Anastrepha fraterculus* complex with exotic host plants recorded in various countries across the Americas. Plant species with less than five records are omitted. The thickness of the linking gray lines represents the frequency of records. Same color nodes (left) denote that the hosts belong to the same family; the red nodes (right) depict countries of distribution.

the northern region of the Andes in Venezuela and Colombia but also in some Brazilian locations. In *Citrus* spp., almost all of the records were from South America, especially Brazil and Argentina, whereas fruits of *Mangifera indica* have been most commonly reported as hosts in Brazil, Argentina, and Ecuador. These results revealed the occurrence of certain preferences in the use of hosts at a regional level, suggesting the relatively recent adaptation of some fly populations in order to exploit those exotic hosts.

On the other hand, the list of native hosts comprised a total of 124 plant species throughout the American tropics, accounting for 70% of all of the known hosts listed. The highest frequencies of records were found for *Psidium guajava* (298), *Eugenia uniflora* (48), *Acca sellowiana* (31), *Spondias purpurea* (23), *Psidium cattleianum* (22), and *Annona cherimola* (21), among others. It should be noted that 8 of the 10 native species with the highest number of records belonged to the family Myrtaceae, which represented nearly 29% of the total. It is also noteworthy that guava fruits are the only common host shared by different populations throughout the tropical and subtropical regions of the Americas. In contrast, other common host species (e.g., Surinam cherry, feijoa, seriguela, araçá, and chirimoya) are only found infested in certain biogeographical provinces of the neotropical region (Figure 6.3).

6.3.3 Geographical Distribution

Examination of the geographical distribution of host plants used by the SAFF produced records for 12 Latin American countries. The largest numbers of host plants were documented for South America, including Brazil (121), Argentina (40), Ecuador (40), Colombia (38), and Venezuela (24). In Mexico, one of the most well-studied countries in the north of the Americas, the infestation of 19 host plants has been recorded.

The data on host plants recorded for each country allowed us to explore shared resources through a cluster analysis. In this context, Brazil exhibited the highest dissimilarities of hosts shared with all other countries. This is supported by previous studies that highlight the presence of three morphotypes in this territory (Selivon 2004, 2005a, 2005b), which would explain the existence of 120 host species, including many native species. In contrast, Colombia, Ecuador, and Argentina added another subgroup with closer affinities in terms of resource use. These countries shared exotic plants such as *Prunus domestica*, *Punica granatum*, and *Ficus carica*, but also native plants such as *Acca sellowiana*, *Malphigia glabra*, and *Annona cherimola*, among others. Mexico and Venezuela formed a third cluster, sharing hosts such as *Terminalia catappa*, *Syzygium jambos*, and *Psidium guineense* (Figure 6.4).

With respect to taxonomic richness, 30 plant families were found in Brazil, representing nearly twice the number of families found in other countries. This contrasts with the situation in Argentina, where only 16 families were recognized. The Brazilian territory is larger, and three distinct morphotypes have been characterized within the SAFF species complex, whereas just one of them has been reported in Argentina (Hernández-Ortiz et al. 2012). This has led to the hypothesis that the widespread host range found in Brazilian populations could be explained by the presence of different taxa, which requires further investigation.

The richness in the Andean region comprised 10–17 families and 24–40 plant species. Those countries share the common infestation of coffee berries, pineapple guava, custard apple, and berry of the Andes, among others. These are hosts that are rare or absent elsewhere in the Americas. In the entire northern region, from Mesoamerica to Central America, including Mexico, Costa Rica, Guatemala, and Panama, 33 host plant species are recognized. In the northern and central Andean countries, such as Venezuela, Colombia, Ecuador, Peru, and Bolivia, a total of 68 host plants are known. In contrast, in the Amazonian, Atlantic Forest and Chacoan subregions of Brazil and Argentina, a total of 135 plant species are recorded.

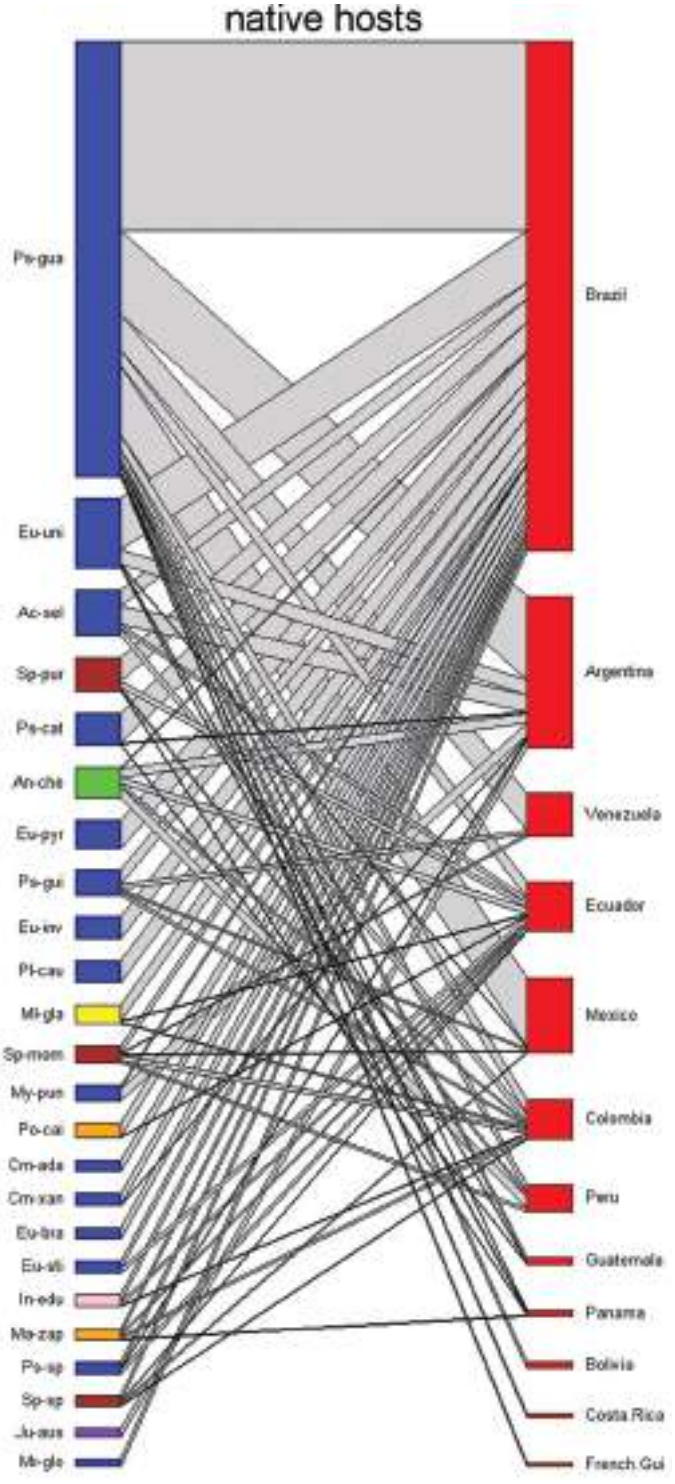

FIGURE 6.3 Network of trophic interactions of the *Anastrepha fraterculus* complex with native host plants recorded in various countries across the Americas. Plant species with less than five records are omitted. The thickness of the linking gray lines represents the frequency of records. Same color nodes (left) denote that the hosts belong to the same family; the red nodes (right) depict countries of distribution.

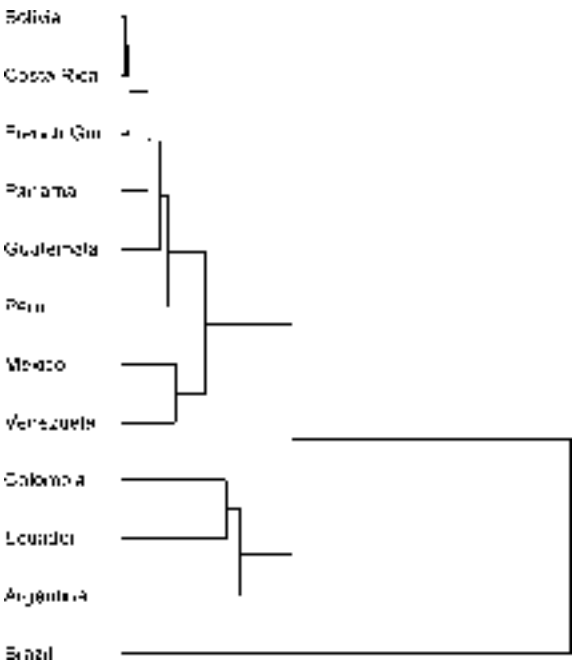

FIGURE 6.4 Dendrogram of dissimilarities (Ward distance: 0–2.33) by countries across the Americas, based on qualitative records (presence–absence) of the host plant species.

6.4 REMARKS AND CONCLUSIONS

The current knowledge of host plants occupied by populations of the nominal species *A. fraterculus* throughout the Americas shows the persistence of gaps in the information. This was evident in nearly all of the countries of Central America and some others from South America (i.e., Bolivia, Peru, Surinam, Paraguay, and Uruguay).

Plant richness showed a great diversification of hosts under natural conditions constituted by 70.1% of native species. Prominent among these are the species of the Myrtaceae family, which accounted for 25% of the total number of host species. These plants could therefore have been the most important influence in the original diversification of the *A. fraterculus* species complex. However, other families, such as the Sapotaceae, Annonaceae, Leguminosae, Anacardiaceae, Passifloraceae, and Solanaceae, might have been used secondarily. The exotic species belonged to 17 botanical families, although most were represented only by a few species. The notable exceptions to this were the two richest families, Rosaceae and Rutaceae, which together accounted for two-thirds of all the exotic species recorded, particularly of plants within the respective genera *Prunus* and *Citrus*.

The high richness of native host plants for the *A. fraterculus* complex denotes an explicit sign of host diversification. However, it should be noted that exotic plants have played a significant role in the divergence of the species complex because some have become almost exclusive hosts of populations in certain biogeographical zones. For example, in the central region of the Andes, *Coffea arabica* is widely used as a preferred host by the SAFF but is virtually an unknown host in other neotropical locations. This suggests that such plants could have played a critical role in the current dispersion of the SAFF following their introduction to the Americas.

The areas occupied by certain populations of this nominal species reflect some preferences in the use of resources. This would reinforce the hypothesis regarding the existence of distinct taxonomic entities because, although these resources exist throughout the range, they are not common hosts

for all fly populations. The fact that there are at least eight morphotypes, presumably corresponding to different species within the SAFF complex, leads us to conclude that knowledge on host use at a regional level is critical. The data analyzed here indicated strong preferences at the regional level for both native and introduced plants, and the latter have served as a scattering factor, thus converting some of these populations into distinct species or hosting races of economic importance.

In this context, the potential application of the SIT in certain geographical areas requires knowledge in terms of the host plants consumed by the target species. A holistic view of the trophic interactions among populations of this complex allows the use of much of the published host information. This could help to outline a confidence range of preferred hosts exploited by each biological species and ultimately determine their pest status in different regions of the Americas.

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7 Preliminary Report of *Anastrepha* Species Associated with "Kaniste" Fruits (*Pouteria campechiana*) (Sapotaceae) in the State of Campeche, Mexico

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CONTENTS

Abstract Campeche is a state located in the southeast of the Gulf of Mexico and has a great variety of microclimates and native vegetation. Backyard orchards in rural communities in this state provide both introduced and native host fruits for *Anastrepha* spp. fruit flies, such as the "Kaniste" fruit (*Pouteria campechiana*) (Sapotacea). In this study, we sampled Kaniste fruits from backyards located in the municipality of Escarcega, Campeche, Mexico. Three species of the genus *Anastrepha* were recorded feeding on Kaniste fruits: *Anastrepha fraterculus*, *Anastrepha serpentina*, and *Anastrepha hamata*. These findings contribute to our knowledge of the hosts and diversity of the genus *Anastrepha* in Campeche, Mexico.

7.1 INTRODUCTION

Fruit flies (Diptera: Tephritidae) are one of the most important agricultural pests in the world because they represent an economic impact on fruit production worldwide (Aluja 1994). Moreover, national and international quarantines are imposed on the mobilization of commercial hosts infected by

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several species of fruit flies. Campeche is a state located in the southeast of Mexico, and it is a region with a high diversity of plants, which provides various habitats for tephritid species such as those of the genus *Anastrepha* (García-Ramírez et al. 2018). *Anastrepha* includes species of economic importance, as well as species with ecological roles that do not require phytosanitary management (Aluja 1994). "Kaniste," *Pouteria campechiana* (HBK) Baehni (Sapotaceae), is a native tree from southern Mexico with a distribution that extends to South America (Martin and Malo 1999). Kaniste fruits are orange-yellow with an ovoid-round shape; they measure around 4 cm in diameter and weigh around 7 g. The fruiting period extends from July to January. This study aimed to determine the occurrence of *Anastrepha* species in fruits of *P. campechiana* in Campeche, Mexico.

7.2 MATERIALS AND METHODS

Fruit sampling was conducted in familiar backyard orchards in the municipality of Escarcega, Campeche, Mexico (18°29′25.1″N and 90°55′30.9″W, altitude of 42 m. a. s. l.). The region is surrounded by evergreen medium elevation forests and is characterized by a subhumid climate with rainfall in the summer. Fruit sampling was carried out in two periods: from August to September 2017 and from November to December 2017. Ripe fruits of *P. campechiana* were collected directly from the ground, they were inspected for the presence of tephritid larvae, and infested fruits were placed in plastic containers (20 × 20 cm). Samples were transported to the Entomology Laboratory of the Escuela Superior de Ciencias Agropecuarias, Universidad Autónoma de Campeche (ESCA-UAC) in Escarcega, Campeche, Mexico.

Infested fruits were placed in individual transparent cylindrical plastic chambers (10 cm in diameter × 20 cm high). In the second period of sampling, larvae were found in the seeds of the fruits; thus, they were placed in different containers. Moist vermiculite was used as a pupation substrate in the chambers described. Pupae were recovered and placed in small plastic containers of 5 × 5 cm and were inspected daily. Emerged adults were identified by Enrique Antonio Hernández and María de Jesús García-Ramírez using the taxonomic keys provided by Korytkowski (2008). The entomological material was deposited in the Entomology Laboratory of the ESCA-UAC.

7.3 RESULTS

Fruit sampling data are summarized in Table 7.1. Overall, three species of the genus *Anastrepha* were found to be associated with fruits of *P. campechiana*. Specimens of *Anastrepha serpentina* larvae (Wiedemann, 1830) were observed feeding on the pulp of fruits collected in both the

TABLE 7.1
***Anastrepha* spp. Recorded in Fruits of *Pouteria campechiana* Collected in Familiar Backyard Orchards in the Municipality of Escarcega, Campeche, Mexico**

				Adults			
Sampling Period	Sample Weight	Infested Fruit Part	No. of Pupae	♀	♂	Date of Emergence	Species
August–September 17	340 g	Mesocarp	77	24	26	August 18, 2017	*Anastrepha serpentina*
November–December 17	400 g	Mesocarp	87	31	28	August 12, 2017	*Anastrepha serpentina*
				—	2	December 2, 2017	*Anastrepha fraterculus*
		Seed	32	8	12	December 25, 2017	*Anastrepha hamata*

first and the second sampling periods. In the second sampling period, *Anastrepha fraterculus* specimens (Wiedemann, 1830) were also observed feeding on the pulp of the fruits, whereas *Anastrepha hamata* specimens (Loew, 1983) were observed feeding on the seeds. It is important to note that in the second sampling period, there were fruit fly larvae feeding on both the pulp and the seeds of the same Kaniste fruit. In some cases, all three *Anastrepha* species emerged from the same fruit.

7.4 DISCUSSION AND CONCLUSION

We report for the first time the occurrence of three species of the genus *Anastrepha* (*A. fraterculus, A. serpentina,* and *A. hamata*) simultaneously infesting fruits of *P. campechiana* in Campeche, Mexico. This is also the first record of *A. fraterculus* infesting fruits of *P. campechiana*. Even though we only obtained two male adults of this species from the infested fruits (Table 7.1), its occurrence is not considered to be accidental because *A. fraterculus* has been reported with complete development in *Pouteria caimito* fruits in the province of Guayas, Ecuador (Tigrero 2009). However, in this study, most of the infestation in fruits of *P. campechiana* was by *A. serpentina*, a multivoltine species commonly associated with plants of the family Sapotaceae in Mexico (Sosa-Armenta et al. 2015).

The simultaneous infestation of *P. campechiana* by three species of *Anastrepha* is an important finding. This could be explained by the inability of these species to recognize the fruit-marking pheromone used by heterospecifics. This recognition has been reported by Aluja et al. (2000) for *Anastrepha bahiensis*, *A. fraterculus*, and *Anastrepha obliqua* infesting *Myrciaria floribunda* (Myrtaceae) in Apazapan, Veracruz, and by Antonio-Hernández (2006) for *A. fraterculus* and *Anastrepha striata* in *Psidium guajava* in Tehuantepec, Oaxaca. Another possible explanation is the low availability of host fruits in the region of Escarcega at the time of the samplings, which could have resulted in more than one species of *Anastrepha* infesting these fruits (Hernandez-Ortiz et al. 2002).

Compared to *A. serpentina* and *A. fraterculus*, *A. hamata* showed a relatively long pupation period, with a time of emergence of 28–34 days. This physiological condition is likely because of the atypical temperatures recorded at the time of the year (22°C–25°C) in the sampling site, which are lower than the temperatures that normally occur in this location (35°C–40°C). It is possible that some *Anastrepha* species in an immature state are able to synchronize their physiological mechanisms according to abiotic factors such as climate and temperature (Aluja et al. 1998), which would allow them to regulate the time of adult emergence. This could be the case in *A. hamata* based on the observations made in this study.

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Section III

Chemical Ecology and Attractants

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8 Bait Stations for Control of Mexican Fruit Flies (*Anastrepha ludens*), First Year

Hugh Conway[*]*, Guadalupe Gracia, Pedro Rendón, and Christopher Vitek*

CONTENTS

Abstract The Mexican fruit fly, *Anastrepha ludens* (Loew) (Mexfly), is a pest of economic importance with the potential to cause millions of dollars in damage to citrus and other fruits. This chapter presents the first-year results from a three-year field study (2014–2017) conducted to evaluate the effectiveness of bait stations with Spinosad embedded in a wax matrix to control Mexfly. Spinosad is produced by a naturally occurring bacteria, *Saccharopolyspora spinosa*, and is considered an organic insecticide acceptable for use by organic growers. The flies feed on the wax matrix, and the Spinosad acts as a stomach poison killing the flies. Each bait station contains a two-component lure consisting of the attractants, putrescine, and ammonium acetate. The study used 500 bait stations strategically placed based on historic wild fly capture data at 12 locations in the Lower Rio Grande Valley in south Texas, United States. Results indicate that a hat or protective covering over the bait station extends the residual killing effect with fly mortality of up to 3 months from aged bait stations taken from the field. In addition, a reduction in wild Mexfly capture was observed in the areas where the bait stations were used. Potential uses would be around wild fly finds, in abandoned or poorly maintained groves, in organic groves, or with permission, in residential citrus plantings. This study indicates that bait stations are another valuable integrated pest management (IPM) tool for program managers in their effort to control Mexfly.

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8.1 INTRODUCTION

Fruit fly managers are investigating unique methods of controlling invasive fruit fly species including the use of attract-and-kill devices. A bait station is a type of attract-and-kill device described as a discreet type of container (with or without a visual component) with attractants and toxins that target specific pests (Piñero et al. 2014). Bait stations may require servicing to remain active during the season, and insects attracted and killed, if retained, should be discarded and not counted (Piñero et al. 2014). Mangan et al. (2006) tested a mixture of GF 120 fruit fly bait with a killing component of Spinosad that had been purified from soil bacteria actinomycete, *Saccharopolyspora spinosa* Mertz. Mangan and Moreno (2007) developed and tested a form of bait station using protein-based baits with a photoactive dye toxicant in small discrete containers resulting in a 70%–90% sterile Mexican fruit fly (Mexfly), *Anastrepha ludens* (Loew), reduction compared to the control but only 22% as effective as spot insecticidal sprays. In 2008, Food and Agricultural Organization/International Atomic Energy Association (FAO/IAEA) initiated the development of bait stations for fruit fly suppression in support of sterile insect technique (SIT) (IAEA 2009). The bait station initiative investigated the type and category of different bait stations, including the attributes and components needed for a successful attract-and-kill device, and the potential application of bait stations (IAEA 2009). Epsky et al. (2012) demonstrated that wax matrix bait stations embedded with Spinosad were effective in controlling the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann). Since 2010, Animal and Plant Health Inspection Service (APHIS) Plant Protection and Quarantine (PPQ) Guatemala has been working on developing a bait station incorporating the wax matrix reported on by Epsky et al. (2012). This bait station would be able to accept a variety of lures allowing its use against a number of different fruit fly species. Important characteristics in the designed bait station, include: easy to deploy, biodegradable, and ability to retrieve (if necessary). The bait station should satisfy organic production concerns and concerns with use in urban settings. Such a bait station was eventually developed, and a US Department of Agriculture patent claim presented and granted in early 2018. The bait station has been tested in several locations including Guatemala, Texas, Florida, and Hawaii to validate the technology. We report here on the findings of its use for Mexfly control in the Lower Rio Grande Valley, Texas.

The fruit fly trapping and surveillance program in the Lower Rio Grande Valley of South Texas includes 13 Multilure traps per km^2 across the 109.3 km^2 citrus growing area employing 2,905 traps. Each trap contains a two-component lure (putrescine and ammonium acetate) and 300 mL of a 10% propylene glycol mixture as a drowning solution and fly preservative. The trapping design provides a means for conducting tests to compare the Multilure traps with the effect of bait stations in controlling Mexfly.

A 3-year study was conducted in the Lower Rio Grande Valley of south Texas evaluating the effectiveness in the field of bait stations. First-year data analysis was used to compare capture of feral and sterile Mexfly at (1) sites with bait stations versus (2) sites without bait stations. Laboratory bioassays on bait stations aged in the field was used to compare fly mortality between (1) bait stations, (2) bait station with protective hat, and (3) no bait station control. Efficacy of aged bait stations from servicing intervals across the year were compared in laboratory bioassays using bait stations with or without hats to a no bait station control. Results from the first year of the study are presented in this chapter.

8.2 METHODS AND MATERIALS

8.2.1 Insects

Anastrepha ludens used in Hidalgo County comparisons were obtained as pupae from colonies maintained at the Mexican fruit fly rearing facility in San Miguel Petapa, Guatemala. The arriving Tapachula-7 strain, also referred to as Black Pupae Strain (BPS), were presorted by brown pupae color, resulting in ~90% male flies. The aerial release rate for BPS flies was 61,775 flies per km^2 (250 flies per acre). Mexican fruit flies used in the majority of Cameron Country (Texas), Willacy

County (Texas), and for laboratory bioassays comparisons were from a colony maintained by the Department of Agriculture (APHIS) at the Moore Air Base Mexican fruit fly mass-rearing facility near Edinburg, Texas. The colony is an isofemale line originating from an outbreak of wild flies captured in Willacy County, Texas, in April 2008. The aerial release rate for Texas reared flies was 123,550 flies per km^2 (500 flies per acre).

8.2.2 Wax-Based Bait Stations

The bait station used in this study was made of a cardboard box approximately 7.6 × 10.2 × 1.3 cm with an opening to slide a cardboard strip with the attractant fruit fly lures using two-component patches (putrescine and ammonium acetate) inside the box (Figure 8.1). The box has 24 holes in the front and back, allowing movement for the odor from the lures. The box is dipped into melted wax that contains Spinosad (kill-component active ingredient) and phagostimulants, including corn syrup and sugar (Epsky et al. 2012). The bait station containing the Spinosad toxicant must attract and then stimulate the flies to feed on the waxy matrix. Spinosad must be ingested to obtain maximum effect (Prokopy et al. 2003). The wax matrix serves to extend the insecticide killing effect or longevity over a longer period of time. Fruit flies attracted by the lure land on the wax bait station and begin feeding. Within 24–48 hours after ingesting the waxy matrix, the fly will die. The second bait station configuration involved the addition of a conical shaped covering (hat) dipped in the wax matrix with Spinosad positioned over and covering the bait station (Figure 8.2). The conical hat was made of a cardboard circle with a diameter of 17.8 cm (7 in), having one cut to the middle of the circle, which allowed the circle to be formed into a cone shape and stapled in place above the bait station. The conical hat protected the bait station from sun and weather (rain) with the aim of extending the efficacy of the bait station.

A total of 500 bait stations (half with and half without protective hats) were placed in the field at each placement period. Often, field placement of bait stations would require up to 1 week in duration to place all of the bait stations into a test comparison site. Bait stations were placed in the field at approximately 4-week intervals on August 25, September 22, and again on October 25, 2014, when the two-component lures were replaced in the test traps. For the rest of the year, bait stations were added to the field when the lures in the traps were replaced at 8-week intervals with bait stations

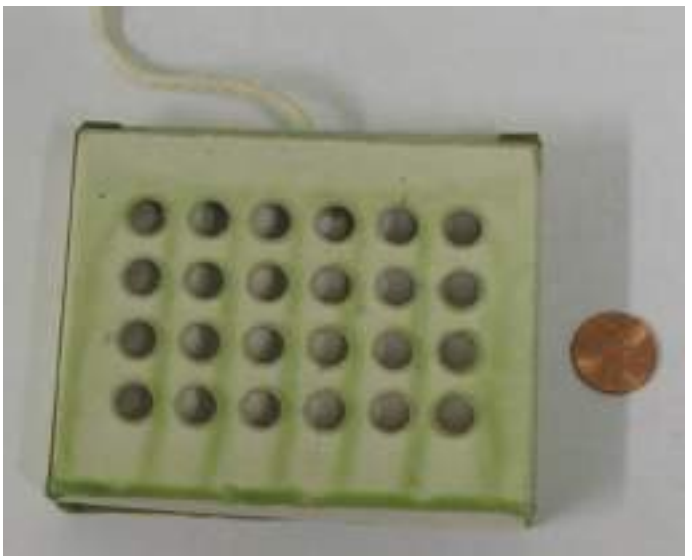

FIGURE 8.1 Bait station covered with waxy matrix mixed with Spinosad (toxin).

FIGURE 8.2 Bait station with conical hat protection.

placement occurring on December 25, 2014, and on February 25, April 25, and June 25, 2015. For each placement period, bait stations were marked with different colored plastic ties with the date the bait station was placed into the field.

8.2.3 Field Tests

Texas was declared eradicated of Mexican fruit fly in the beginning of 2012. Since then, only sporadic and localized wild Mexfly finds have occurred: 38 adults and six larvae were captured in the 2013 citrus-growing season. In this study, sterile Mexfly captures were used for field comparisons because of the low number of wild flies. Previous research by Mangan and Moreno (1995) showed that sterile mass-reared Mexflies are equivalent to fertile flies in attraction and feeding behavior.

Two-piece plastic Multilure traps with two-component lures (putrescine and ammonium acetate) and 300 mL of 10% propylene glycol capture solution were used for fly capture. The field comparison tests used sterile Mexican fruit flies released by the program at 123,550 flies per km^2 (500 flies per acre) weekly in Cameron and Willacy Counties, and 61,775 flies per km^2 (250 flies per acre) in Hidalgo County where the Guatemala male-sorted Tapachula-7 BPS flies were released.

Test comparison sites were based on previous hot-spot locations with historic recurring feral *A. ludens* capture and larva finds (Figure 8.3). Specific comparison metrics were identified at different locations as indicated in Table 8.1. Grove comparisons were made by using two small groves and placing 40 bait stations (20 with hats and 20 without hats) in one orchard and no bait stations in the control orchard. For both grove tests, four Multilure traps were placed in the bait station and control orchards. The grove site comparison in La Feria, Texas, received aerial-released sterile Mexican fruit flies and the south orchard received 40 bait stations (Table 8.1). The grove site comparison in Bayview, Texas, in Cameron County did not receive sterile flies. The northwest grove in Bayview received the 40 bait stations and the southeast grove was the control (Table 8.1). The city of Lyford, Texas, in Willacy County, was used to compare urban areas with primarily residential properties by dividing the town in half using Business 77 and placing 40 bait stations on the east side and no bait stations on the west side. Both sides of the Lyford test received six Multilure traps (Table 8.1). Two similar-sized trailer parks near Donna, Texas, were used for fly capture comparison in a residential park setting with Country Sunshine receiving 50 bait stations and Southern Comfort no bait stations. Both trailer parks received four Multilure traps each (Table 8.1). The next comparison site was the trailer park Ranchero Village with 50 bait stations compared to the Estero Llano Grande

FIGURE 8.3 Site locations for bait stations test marked by dots.

TABLE 8.1
Site Locations and Number of Traps and Bait Stations per Site

Comparison	Site Name	Size m²	Location Center	County	BS	Traps
Field Test Fly Releases	La Feria S	22,674	26°08′17″N 97°49′02″W	Cameron	40	4
	La Feria N	20,738	26°08′25″N 97°48′53″W	Cameron	0	4
Field Test No Fly Release	Bayview N	24,402	26°08′07″N 97°24′12″W	Cameron	40	10
	Bayview S	23,776	26°07′59″N 97°23′46″W	Cameron	0	10
Abandoned Grove	Abandoned	24,965	25°58′32″N 97°27′15″W	Cameron	40	8
	Sol grove	25,900	25°58′45″N 97°27′08″W	Cameron	0	8
Urban Lyford, TX	Bus 77 E	73,996	26°24′42″N 97°47′19″W	Willacy	40	6
	Bus 77 W	80,935	26°24′42″N 97°47′31″W	Willacy	0	6
RV Parks	Country Sunshine	139,211	26°08′35″N 97°58′22″W	Hidalgo	50	4
	Southern Comfort	138,402	26°08′43″N 97°58′17″W	Hidalgo	0	4
RV Park Bird Center	Ranchero	63,523	26°08′15″N 97°58′56″W	Hidalgo	50	4
	Estero Llano	45,052	26°08′28″N 97°57′30″W	Hidalgo	0	4
Test Spots Without Comparison Sites Based on Larvae or Wild Fly Captures in 2013						
Larvae	South Point Brownsville, TX		25°50′23″N 97°23′44″W	Cameron	60	12
Fly Find	Monica Brownsville, TX		25°53′22″N 97°26′02″W	Cameron	40	8
Fly Find	Toronja Brownsville, TX		25°54′39″N 97°24′14″W	Cameron	40	10
Larvae	Nevada St. Weslaco		26°10′06″N 97°58′54″W	Hidalgo	50	6
Fly Find	Illinois St. Weslaco		26°09′11″N 97°59′95″W	Hidalgo	25	4
Fly Find	7th St. Donna		26°09′13″N 97°59′06″W	Hidalgo	25	2

State Park (Weslaco Birding Center) with no bait stations. The Weslaco Birding Center contains the remnants of an old recreational vehicle (RV) Park started in the 1950s with a variety of fruit fly host trees. Both locations received four Multilure traps each with traps in the Birding Center placed on trees in the old RV Park (Table 8.1). Comparisons were made near Robindale Road/Sol in Brownsville using an abandoned citrus grove with 40 bait stations compared to small citrus grove with no bait stations. Both the abandoned and small active grove received eight traps each (Table 8.1). In each site, comparisons were made between bait station and non-bait station based on the numbers of feral and sterile flies captured.

The rest of the bait stations were placed in hot spot locations. A hot spot is a location of recurring wild fly or larva finds in previous years and across numerous years. The hot spots used in Cameron and Hidalgo Counties were in sites of larvae or wild flies captures from the 2013–2014 citrus season. Three hot-spot locations in Cameron County were near the Mexican border in Brownsville, Texas, at South Point (60 bait stations), Monica Street (40 bait stations), and Toronja Street (40 bait stations). The three locations in Hidalgo County were near Weslaco and Donna, Texas, at Nevada Street (50 bait stations), Illinois Street (25 bait stations), and 7th Street (25 bait stations).

8.2.4 Laboratory Bioassay of Bait Station Efficacy in the Field

The length of effectiveness of bait stations was tested across time by taking eight random samples (four bait station with hats and four bait station without hats) from the field at no comparison locations. Bait stations from the initial release date of August 25 were used with collection and comparisons occurring at 2- to 4-week intervals. Laboratory bioassay tests were conducted on mortality inside Plexiglas observation cages (30 cm × 30 cm × 40 cm). Each clear Plexiglas observation cage contained 50 Mexican fruit flies, food, and water ad libitum and one bait station, either with or without hat. Each bait station was suspended downward from the top in the middle of the observation cage. Mortality comparisons were made at 24-hour intervals across 72 hours against two control cages with 50 adult Mexican fruit flies, food, and water ad libitum. Because each cage started with 50 live flies, percentage of mortality per cage was calculated by taking the number of dead flies in an observation cage and multiplying by two.

An additional test was conducted at the end of the year on bait stations with and without a protective hat that had been weathered in the field for 8–52 weeks (Figure 8.4). Twelve random samples (six bait station with hats and six bait station without hats) were taken from the field at comparison locations by time of placement. Half of the tested bait stations were obtained from Hidalgo County and half from Cameron and Willacy Counties. The same procedures described previously were used for the mortality test. Because each cage started with 50 live flies, percentage of mortality per cage was calculated by taking the number of dead flies in an observation cage and multiplying by two.

8.2.5 Data Analysis

In this study, feral Mexican fruit fly capture was small and presented by location and the number of flies captured. For field tests, Mexican fruit fly capture comparisons between bait station and without bait stations for each site were conducted for sterile fly capture using t-tests and means comparisons using ALL-Pairs Tukey's honestly significant difference (HSD) tests ($P = 0.05$) (SAS JMP 13 2017). Data results were presented in table form.

For both the laboratory bioassay tests, fly mortality was analyzed using analyses of variance (ANOVA) with means separation using ALL-PairsTukey's HSD test ($P = 0.05$) by date, comparing percentage of mortality in observation cage from bait station with hat, bait stations without hat, and control (SAS JMP 13 2017). The mean ± standard error (SE) values by treatment were graphed for comparisons of fly mortality using bait station, bait stations with hat, and no bait station control. Only the mortality at 72 hours is presented because Spinosad is a stomach poison that may take up

(a)

(b)

FIGURE 8.4 Photos of weathered bait stations (a) with hats and (b) aged for 8 weeks (bottom right) to 52 weeks (upper left).

to 48 hours to kill (Cisneros et al. 2002); thus, the mean mortality reading at 72 hours provides the most accurate measure of total mortality obtained from the treatments. Mexican fruit fly mortality comparisons were made by length of time bait stations were aged in the field by number of weeks; 8–9, 17–18, 25–26, 33–34, 41–42, 45–46, and 51–52 weeks. Placing bait stations in each test location required more than 1 week, which is indicated by the values spanning 2 weeks. The same procedures as listed previously for laboratory bioassays were used for conducting mortality testing. The mean ± SE values by treatment were graphed for comparisons of fly mortality using bait station, bait stations with hat, and no bait station control by the number of weeks the bait stations had aged in the field.

8.3 RESULTS

8.3.1 Field Tests

In the 2014 growing season, there were 75 feral (wild) adult Mexflies captured in traps and seven larvae found inside fruit in the south of Texas. There were no feral flies captured in traps on the bait stations side in the field in any of the test comparison sites compared to three feral flies captured at two control locations. One feral fly was captured in a trap on the control side in Lyford and two were captured in the control side in the small grove at Sol in Brownsville, Texas. There was one feral fly in a no comparison site at South Point near Brownsville (Table 8.2). Sterile fly capture was variable in the test based on site location. Significantly more sterile Mexflies were captured in the bait stations at Ranchero Way compared to Birding Center, the Brownsville abandoned orchard, and overall comparison of all fly captures in the test (Table 8.2). Similar capture results occurred at the La Feria field test, the Bayview field test, and the Lyford urban test. The control side (non-bait station side) captured significantly more Mexflies at Southern Comfort RV park compared to Country Sunshine RV park. Across the whole year, only one sterile fly was captured on the control side in the Bayview grove comparison where no sterile fly release occurred (Table 8.2).

TABLE 8.2
Wild and Sterile Mexican Fruit Fly Capture ± SE by Location

				Fly Capture					
Comparison	**Site Name**	**BS**	**Trap**	**Wild**	**SIT**	**Mean ± SE**	**DF**	**T-ratio**	**Prob > ltl**
Field test fly releases	**La Feria S**	40	4	0	4420	49.9 ± 7.4	150	−1.747	0.08
	La Feria N	0	4	0	2838	38.2 ± 6.4			
Field test no fly release	**Bayview N**	40	10	0	0	One SIT fly captured			
	Bayview S	0	10	0	1				
Abandoned grove	**Abandoned**	40	8	0	24282	111.5 ± 12.7	340	−2.781	0.006
	Sol grove	0	8	2	11597	66.3 ± 7.4			
Urban Lyford, TX	**Bus 77 E**	40	6	0	9281	75.8 ± 18.9	240	−1.082	0.28
	Bus 77 W	0	6	1	5504	55.1 ± 7.7			
RV Parks	**Country Sunshine**	50	4	0	836	8.7 ± 1.7	190	3.078	0.002
	Southern Comfort	0	4	0	3545	36.4 ± 8.6			
RV Park Bird Center	**Ranchero Way**	50	4	0	1934	18.0 ± 3.7	206	3.870	0.0001
	Estero Llano	0	4	0	368	3.4 ± 0.6			
Capture comparison across all Sites with comparison tests		**Bait Station**		0	40753	61.3 ± 6.0	1130	−3.075	0.002
		Control		3	23853	43.9 ± 3.4			
Test spots without comparison sites based on larvae or wild fly captures in 2013									
Site name		**BS**	**Trap**	**Wild**	**SIT flies captured**	**Mean capture ± SE**			
South Point Brownsville, TX		60	12	1	9835	32.5 ± 4.0			
Monica Brownsville, TX		40	8	0	12073	56.2 ± 6.4			
Toronja Brownsville, TX		40	10	0	10406	53.5 ± 7.7			
Nevada St. Weslaco		50	6	0	1768	17.4 ± 4.0			
Illinois St. Weslaco		25	4	0	1528	14.4 ± 3.5			
7th St. Donna		25	2	0	479	5.8 ± 1.9			

DF, degree of freedom; BS, bait station; SE, standard error; SIT, sterile insect technique.

In the noncomparison sites in the areas of Brownsville, Texas, and the Weslaco/Donna, Texas areas, only 1 feral fly was captured compared to 10 Mexfly adults and larvae in the previous citrus season. The feral fly was captured in a trap near bait stations at South Point located close to Brownsville, Texas.

8.3.2 Laboratory Bioassay of Bait Station Efficacy in the Field

Some of the bait stations were partially covered with mold from rains, and many of the older bait stations lacked much of the original waxy coating (Figure 8.4). The bait stations were effective in controlling and killing adult Mexican fruit flies across 12 weeks under field conditions. After 14 weeks, bait station mortality decreased to ~35% at 72 hours in the laboratory bioassay tests as compared to controls at ~9% (Figure 8.5).

The bait stations with hats were effective for approximately 30 weeks with mortality at or above 80% compared to control Mexfly mortality of 8%–11% (Figure 8.5). At 32 weeks, Mexfly mortality dropped to 60% on the bait station with hats and gradually decreased in each subsequent testing period up to week 52 (Figure 8.5). Mortality in the control cages ranged from 2% to 11% with a mean across time of 6.1% mortality at 72 hours in the cage.

After 8–9 weeks of exposure in the field, the tested bait stations without hats only obtained 38% mortality in the laboratory efficacy trial (Figure 8.6). The bait stations with hats did maintain efficacy having more than 60% mortality for over 41–42 weeks of exposure in the field (Figure 8.6). There was variability between bait stations received from Hidalgo County compared to Cameron County. Cameron County is closer to the Gulf of Mexico and tends to receive much more precipitation than Hidalgo County. The hat protected the bait station and maintained the waxy coating for a longer period of time, especially on the bottom (unexposed) portion of the hat.

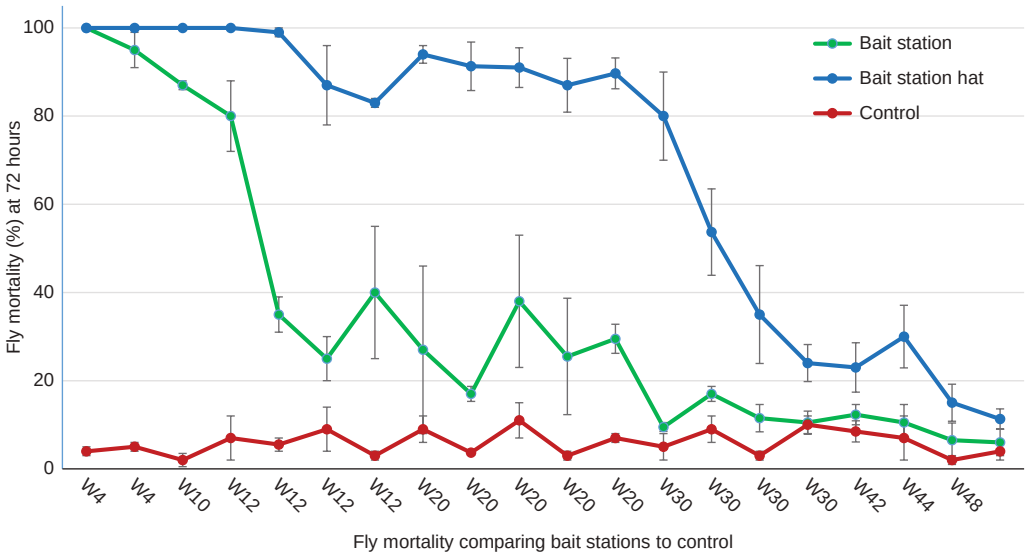

FIGURE 8.5 Mexican fruit fly percentage of mortality at 72 hours in the laboratory bioassay comparing bait stations with and without hat to control at distinct periods across the year.

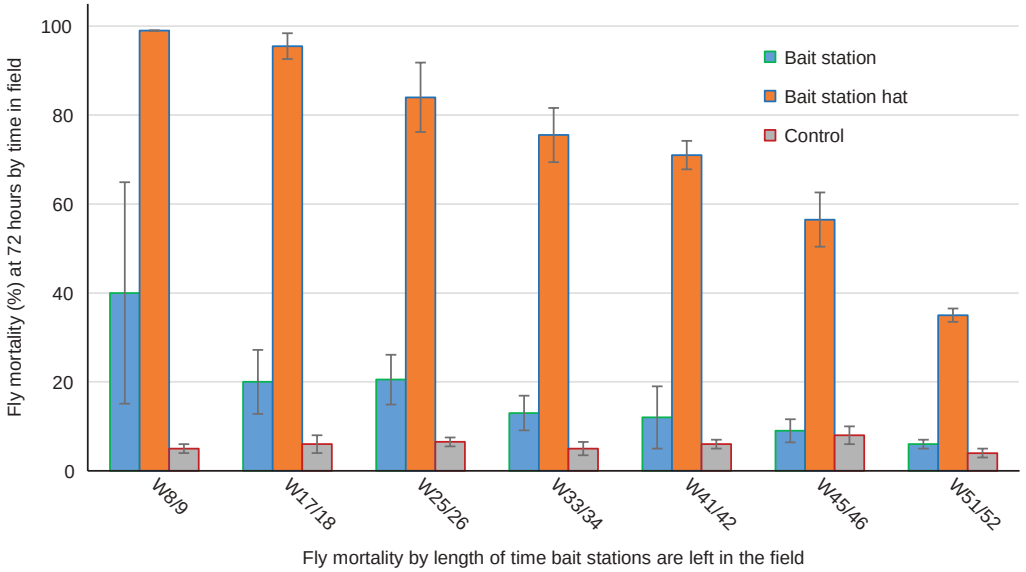

FIGURE 8.6 Mexican fruit fly percentage of mortality at 72 hours based on length of time the bait station was left in the field comparing bait stations with and without hat to control.

8.4 DISCUSSION

In the field study, more flies were captured in traps located in the sites with bait stations except for the RV Park comparison site. There were only three feral Mexflies captured in the comparison tests and all three wild flies were captured in traps on the sites without bait stations. Bait stations with protective hat coverings were significantly more effective in attracting and killing adult Mexican fruit flies in laboratory bioassay tests based on bait station samples taken from fields across time and from tested bait station samples brought in from the field by release dates.

The type of trap and most effective attractant are important factors to consider. Two means of attracting Mexican fruit flies are aqueous slurries often made of torula yeast and two component biolures (putrescine and ammonium acetate) (Conway and Forrester 2007). Thomas et al. (2001) found that open-bottomed, plastic traps baited with a two-component synthetic lure (putrescine and ammonium acetate) captured as many and sometimes more Mexflies than the standard glass McPhail trap baited with aqueous solution of torula yeast. In a citrus orchard in Mexico, more feral Mexflies were captured in traps using two-component biolures (putrescine and ammonium acetate) in Multilure traps with 10% Prestone Low Tox antifreeze than in similar type traps baited with *Anastrepha* fruit fly lure (Robacker and Thomas 2007). Thomas (2008) found that a capture solution of 10% propylene glycol based antifreeze captured significantly more flies than the more toxic automotive antifreeze.

The bait stations function as attract-and-kill stations using the two-component patch (putrescine and ammonium acetate) as the attractant. Flies landing on the surface of the bait station detect the phagostimulant with the fly's taste sensors on the feet (Reinhard 2010), activating a feeding response. The flies feed on the waxy outer covering and also ingest the insecticide Spinosad imbedded in the waxy matrix, killing the flies within 72 hours. Spinosad acts as a stomach poison (Mangan et al. 2006), requiring a period of time to kill. During the time to kill, the flies with poison in their stomach seek a source of water, which often is the capture solution in the Multilure traps. The bait stations side of the test sites have the lures inside the Multilure traps as well as 40–50 additional sets of the attractant lures containing putrescine and ammonium acetate inside the bait station compared to the 4–8 putrescine and ammonium acetate lures inside

the Multilure traps in the control site. Yet, the bait station sites captured more sterile Mexflies in all but one comparison site at an RV Park. Home owners in RV parks are protective of their fruit trees and may have applied pesticides to control other insect pests, which could have negative effects on Mexfly.

Bait stations are a form of attract-and-kill device that have been effective on a number of pestiferous fruit fly species. Díaz-Fleischer et al. (2017) and Flores et al. (2017) used bait stations for control of *Anastrepha* fruit flies in mango orchards. Piñero et al. (2009) helped develop bait stations to control *Bactrocera* species. Epsky et al. (2012) used bait stations to help control Mediterranean fruit flies in Guatemala, and Rahman and Broughton (2016) used them for control of Mediterranean fruit flies in Australia.

Laboratory bioassay tests with observation cages indicate significantly higher mortality from bait stations with hats taken from the field over both control and regular bait stations. The regular bait stations provide control of Mexican fruit flies for up to 12 weeks in the field. The bait stations with hats provided additional protection, resulting in good fly mortality for up to 30 weeks. The addition of the hats increases the efficacy of the bait stations by delaying degradation of the active ingredient (Spinosad) due to protection from exposure to the sun and rain. The hat provides a larger quantity (nearly double the amount found in a standard bait station) of waxy coating with Spinosad for the flies to feed.

This study indicates that bait stations can be another valuable tool for integrated pest management (IPM) for program managers in their effort to control invasive fruit flies. Potential bait station uses would include: around wild fly finds, in specific locations with historic recurrent fly captures, in abandoned or poorly maintained groves, or with permission, in residential backyard plantings of citrus. Discussions are ongoing with Dow Chemical Company and the US Environmental Protection Agency to register commercial use of these bait stations in US fruit fly programs.

DISCLAIMER

The findings and conclusions in this preliminary publication have not been formally disseminated by the US Department of Agriculture and should not be construed to represent any agency determination or policy.

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9 Assessment of Modified Waste Brewery Yeast as an Attractant for Fruit Flies of Economic Importance in Mauritius

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CONTENTS

Abstract Yeast and yeast products are widely considered alternative sources of protein for baits used in fruit fly suppression. The objective of this study was to develop new protein bait formulations from locally available materials in Mauritius, thereby making baits more affordable and reducing the cost of the fruit fly monitoring and control programs. Locally available waste brewery yeast (WBY) was modified in a digester. The WBY was exposed to different boiling and proteolysis conditions. A two-choice bioassay was conducted and each of the 64

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resulting baits were tested against water in a noncompetitive situation with two fruit fly species of economic importance: the peach fruit fly, *Bactrocera zonata* (Saunders), and the melon fly, *Zeugodacus cucurbitae* (Coquillett). Three baits, F1, F2, and F3, showed significantly more fly attraction ranging from 0.7 to 1.1 mean fly catches. These baits were used for further testing for optimal concentrations (7.5, 10, 12.5, and 15% v/v) in field cages. With *B. zonata*, bait attractiveness increased significantly with increasing bait concentrations for both male and female flies. With *Z. cucurbitae*, an increase in attraction was observed but attractiveness was not significantly different. Bait concentration (10% v/v) was selected for open field trials using the three preselected baits (F1, F2, and F3). Two cucurbit plantations were chosen to test the baits against commercial protein hydrolysate in Tephri Traps®, targeting *Z. cucurbitae*. A similar trial was conducted in a fruit orchard to test selected baits in attracting *B. zonata*. The results of traps baited with modified WBY at 10% v/v were comparable to commercial protein hydrolysate in attracting flies. A 5-year cost-benefit analysis indicated that a net benefit of US$283,558.60 is possible if modified WBY is used instead of imported commercial protein hydrolysate. Thus, modified WBY is a promising cost-effective alternative to the imported costly protein hydrolysate in fruit fly suppression programs for Mauritius.

9.1 INTRODUCTION

Fruit flies (Diptera: Tephritidae) are some of the most destructive and important pests of fruits and vegetables worldwide. Fruit fly problems in Mauritius date back to the beginning of this century (Sookar et al. 2006). Fruit flies can be controlled by regular insecticide applications of cover sprays; however, there are known negative side effects, including residues of insecticides in crops, health problems for farmers, contamination of water and soil, development of insecticide resistance, and a decrease in natural enemy populations (Guaman Sarango 2009).

The sterile insect technique (SIT) is one of the major corner stones for fruit fly control programs (Barnes 2008). However, SIT is not a stand-alone technique and should always be integrated with other control methods including baiting (Anon. 2018b).

Flies require sugars and proteinaceous food to survive and mature and are attracted to high-quality protein and sugar baits (Vargas et al. 2002; Bharati et al. 2004). Studies conducted by Prokopy and Roitberg (1984) on searching behavior for food, water, mating, and egg-laying have led to new methodologies by using food baits for monitoring and control of several important fruit fly species. Ammonia-releasing compounds in lures such as protein hydrolysate play an important role in fruit fly attraction (Thomas et al. 2008). The success of any suppression program relies on the ability of protein-based bait formulations to induce good levels of attraction (Mazor et al. 1987).

Protein hydrolysate is the highest-cost component of bait in Mauritius, comprising 15% of the total cost. The import cost of commercial protein amounts to US$15/L. The annual requirement is estimated to be 9000 L, to be used mainly for spraying with respect to the local fruit fly suppression program, for monitoring purposes, and for free distribution to planters and backyard owners. Moreover, because this protein bait needs to be imported, there is always a risk of shortage or delay in procurement as a result of shipping problems (Gopaul et al. 2001). Hence, there is an imperative need to develop new bait formulations, preferably from locally available materials, to bring down the cost of protein used in SIT programs and make bait more affordable. The formulation must be effective, economically feasible, and lack environmental and health hazards.

Lloyd and Drew (1997) reported that waste brewery yeast (WBY) could be developed into suitable locally produced baits because WBY is a rich source of B-complex vitamins, protein, and minerals. Extensive field trials have been conducted in Tonga using Royal Tongalure, which is a protein bait derived from brewer's yeast. Royal Tongalure bait is as effective as the expensive imported

protein bait. Preliminary studies have been carried out on the modification of WBY as a protein source for the control of *Zeugodacus cucurbitae* (Coquillett) in Mauritius (Sookar et al. 2002). There is presently no protocol for developing fruit fly bait from free local WBY, which is available from the Phoenix beer factory.

This study aims to (i) develop and test various formulations using local brewery yeast, (ii) develop a protocol for producing an optimum formulation, and (iii) assess its attractancy and efficacy against *Bactrocera zonata* (Saunders) and *Zeugodacus cucurbitae*.

9.2 MATERIALS AND METHODS

The study was divided into three main parts. The first section included laboratory studies to narrow down the number of prospective baits. The second part involved using field cages to determine the most effective bait's concentration for fly capture. Field studies were then conducted using identified hosts for *Z. cucurbitae* in cucurbit fields and for *B. zonata* in an orchard setting.

9.2.1 Laboratory Bioassay

The WBY was boiled and cooled before addition of papain enzyme. Different conditions were tested by altering:

1. Boiling time (24, 48, 72, and 96 hrs) at 95°C;
2. Proteolysis time (24, 48, 72, and 96 hrs) at 60°C;
3. Papain concentration (0.1%, 0.2%, 0.3%, and 0.4% w/v).

B. zonata flies were reared under standard rearing conditions for 210 generations and *Z. cucurbitae* flies were reared for 30 generations. A two-choice bioassay was used with each of the resulting 64 baits tested independently against water in a non-competitive situation using flies of 10–16 days of age.

From eclosion, adult *B. zonata* and *Z. cucurbitae* flies were fed sugar, water, and protein. Two days prior to testing, they were deprived of the protein source. On the testing day, 20 female flies were released in a small fiberglass cage (30 cm × 30 cm × 30 cm) without sugar or water. Four replicates for each tested bait were set under control conditions of 28 ± 1°C and relative humidity varying from 70% to 75%.

Two dry square sponges (4 cm^2) were placed on top of the gauze cages in diagonally opposite corners. This step enabled flies to get acquainted with the dry sponge before introducing the test bait. At the start of each test in each test cage, 1 mL of water was applied in one sponge (control) and 1 mL of diluted bait in the second sponge.

At the start of the experiment, the impregnated sponge was inverted on the top of the cage to enable flies to have direct access to both water and the test bait. Five measurements were taken; that is, every 2 minutes the number of flies on each sponge were counted across a 10-minute duration. After 5 minutes, the test cages were rotated 180° to ensure optimal light conditions and reduce potential position and light biases.

The total number of flies attracted to each sponge during the observation time was recorded as the attraction of the bait versus the water control. Flies were used only once and then were discarded. A clean cage and new sponges were used for each individual test to reduce any potential interference from possible bait residue.

Tests were run four times daily: from 08:30 to 09:30 am, from 09:30 to 10:30 am, from 10:30 to 11:30 am, and from 11:30 am to 12:30 pm. Each bait was tested 16 times. The attraction of each bait relative to the standard was expressed as the ratio of the mean maximum number of flies attracted to the standard control.

Data were analyzed using an analysis of variance (ANOVA) in Minitab, and means were separated by Tukey's test ($P < 0.05$). Interactions among the three factors (boiling time, proteolysis time, and papain concentration) were tested using the factorial plot of the general linear models.

9.2.2 Comparison of Attraction of Different Concentrations of Modified WBY in Field Cages for *B. zonata* and *Z. cucurbitae*

Three baits, namely F1 (boiled for 72 hrs, hydrolyzed for 72 hrs with 0.2% w/v papain), F2 (boiled for 72 hrs, hydrolyzed for 72 hrs with 0.3% w/v papain), and F3 (boiled for 72 hrs, hydrolyzed for 72 hrs with 0.4% w/v papain), showing significant relative attraction ranging from 0.7 to 1.1 mean fly catches, were selected for further trials.

The experiment was conducted in field cages (2.9 m × 2.9 m × 2 m) resembling semi-natural conditions using six potted fruit trees consisting of guava, mango, and Annona species for *B. zonata* flies. For *Z. cucurbitae*, cucurbit plants (*Cucurbita pepo*) were placed in the field cages. Tephri Traps® with 300 mL of bait solution were used for trappings. There were five treatments with five replicates and each experiment was repeated independently four times with male and female flies using different concentrations of modified WBY (Table 9.1).

Traps were hung 50 cm above the plant canopy on an H-shaped base and arranged along a 1-m-radius imaginary circle around the center of the field cage. Four traps with treatments F1, F2, F3, and protein hydrolysate were placed randomly at the beginning of each experiment and then moved 90° clockwise along the circle every 2 hours to minimize position bias. The fifth trap was placed at the center as a control, where it received exposure comparable to the other traps. Once the traps were in place, 100 flies (males or females) were released in each cage. The traps were serviced after 24 hours. Flies were collected in vials at the end of the experiment and counted. The attraction of the different baits, at varying concentrations for FI, F2, and F3, was analyzed with an analysis of variance (ANOVA) in Minitab for the determination of the differences between the means, and when significant differences were observed, a Tukey's test was used for mean separation. A P value of 0.05 or less was considered for statistical significance level. Analysis of treatment values across rows and down columns was done using a one-way ANOVA.

9.2.3 Comparison of Fly Attraction of Selected Baits in Tephri Traps® in Cucurbit Fields Targeting *Z. cucurbitae*

The experiment was carried out in two local regions, namely Saint Pierre and Albion. Saint Pierre is situated at 20.22° S latitude and 57.52° E longitude, with minimum and maximum temperatures of 10°C–31°C, respectively. Albion is situated at 20.21° S latitude and 57.4° E longitude, with minimum and maximum temperatures of 12°C–35°C, respectively (Anon. 2017a).

TABLE 9.1
Comparison of Different Concentrations of Selected Baits in Field Cage Trials

SN	Treatments	Amount of Protein Bait Used (v/v)			
1	Control	Water only			
2	F1	7.5%	10%	12.5%	15%
3	F2	7.5%	10%	12.5%	15%
4	F3	7.5%	10%	12.5%	15%
5	Protein Hydrolysate	2%	2%	2%	2%

TABLE 9.2
Concentration of Baits Used as Treatments in St Pierre, Albion, and Labourdonnais

SN	Treatments	Amount of Bait Used (v/v)	Borax (w/v)
1	Control	Water only	2%
2	Protein Hydrolysate	2%	
3	F1	10.0%	
4	F2	10.0%	
5	F3	10.0%	

The trial in St. Pierre started on February 16, 2017, and were conducted in a pumpkin plantation when crops were in the fruiting stage. The second trial started on May 17, 2017, and was conducted in Albion in a bitter gourd plantation in the fruiting stage. The five treatments are shown in Table 9.2.

Borax (Sodium Tetraborate) purchased from Loba Chemie PVT. Ltd (India) was added to all the treatments (food baits) at 2% w/v to help prevent disintegration of the fruit flies (Sookar et al. 2002). Tephri Traps® with 300 mL of bait solution were placed at 10-m intervals and hung just above the crop canopy using bamboo stands in a randomized complete block design. Traps were serviced weekly with trap washing and addition of fresh solution, and collected insects were placed in vials with ethanol (70%) for subsequent identification in the laboratory. Trials were conducted across a 5-week period with traps rotated sequentially within a block at each service.

Trapping data recorded from the five treatments (Table 9.2) were converted to flies per trap per day and then log transformed to meet the assumption of homogeneity of variance. The transformed data from the Randomized block design (RBD) experiment were subjected to an analysis of variance (ANOVA) using Minitab and a Tukey's means separation test was used if F values were significant ($P < 0.05$).

9.2.4 Comparison of Fly Attraction of Selected Baits in Tephri Traps® in a Fruit Orchard Targeting *B. zonata*

The experiment was conducted in a 45-hectare area located at Mapou in the Labourdonnais Orchard, having an annual fruit production of around 500 tons/year (Anon. 2018a). Mapou is situated at 20.07° S latitude and 57.62° E longitude, with Minimum and maximum temperatures of 24°C–30°C, respectively (Anon. 2017b).

Trials were conducted in a guava plantation in the fruiting stage. The trial started in August 2017 and lasted 5 weeks. The same treatments shown in Table 9.2 were used. The same methods and procedures for locating traps, setting traps, servicing traps, identifying trap catches, and analyzing data used in the previous test with *Z. cucurbitae* were used in this test.

9.2.5 Cost–Benefit Analysis

A cost–benefit analysis template provides a simple tool for calculating financial futures. A free Excel online version was used for the purpose of this study. This program facilitated financial forecasting over a 5-year period using an estimated yearly increase rate of 2%. All the costs associated with the production of the novel bait were inserted as costs versus the benefit of not importing commercial protein hydrolysate.

9.3 RESULTS

9.3.1 Laboratory Bioassay

A total of 64 baits were obtained following the treatments applied to the crude WBY (i.e., after boiling and proteolysis). The laboratory bioassay on *Z. cucurbitae* indicated that increasing proteolysis time increased fly attraction to the modified baits (F statistic (F) = 1.60, degrees of freedom (df) = 15, p-value (P) = 0.242). Additionally, increasing the papain concentration significantly increased fly attraction (F = 3.62, df = 15, P = 0.046). Increasing boiling time from 24 to 96 hrs significantly increased fly attraction (F = 6.01, df = 15, P = 0.01) (Figures 9.1 through 9.4).

Time of test influenced the results, with fly attraction being significantly higher when the baits were tested between 08:30 and 09:30 am (F = 6.82, df = 15, P = 0.006), compared to other time periods (Figure 9.5).

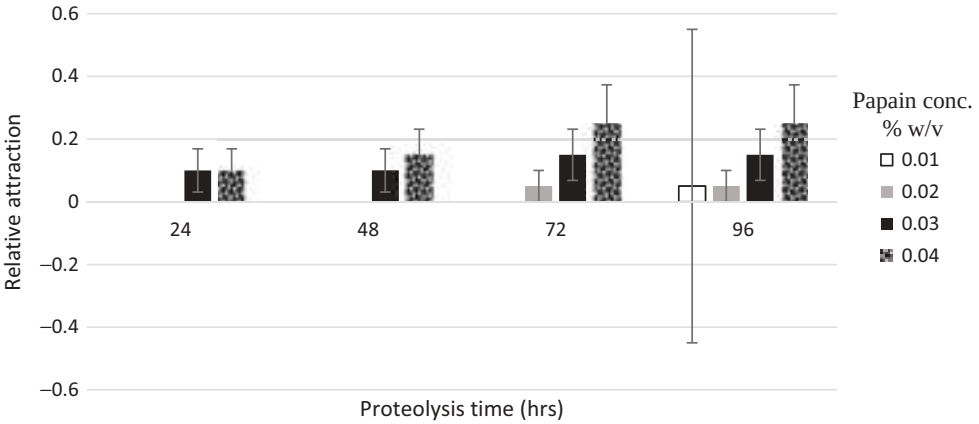

FIGURE 9.1 *Zeugodacus cucurbitae* relative attraction to baits (mean ± SE) boiled for 24 hrs, modified with papain enzyme powder at different proteolysis times.

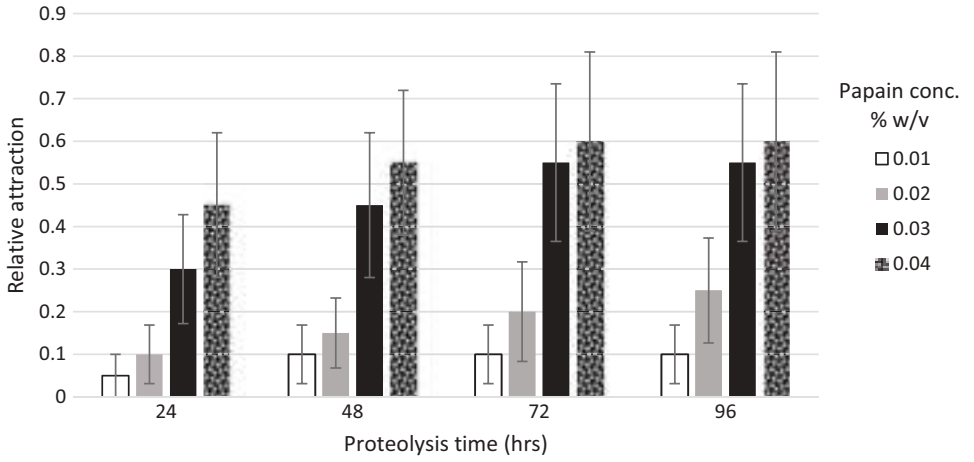

FIGURE 9.2 *Zeugodacus cucurbitae* relative attraction to baits (mean ± SE) boiled for 48 hrs, modified with papain enzyme powder at different proteolysis times.

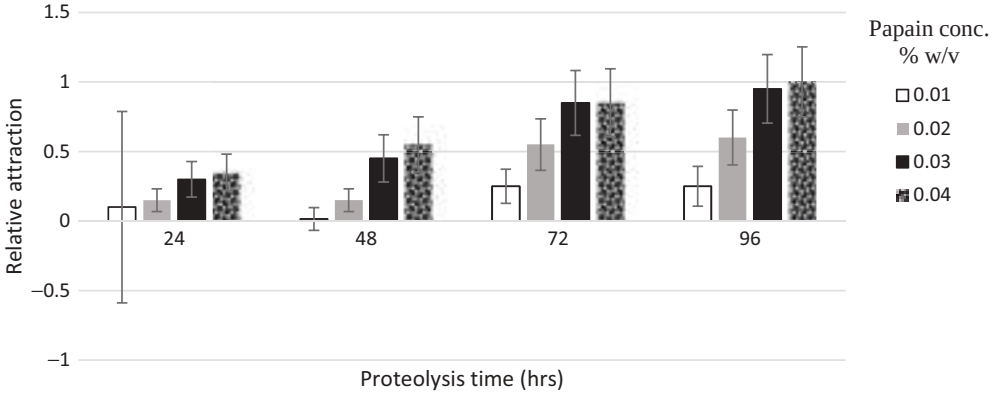

FIGURE 9.3 *Zeugodacus cucurbitae* relative attraction to baits (mean ± SE) boiled at 72 hrs, modified with papain enzyme powder at different proteolysis times.

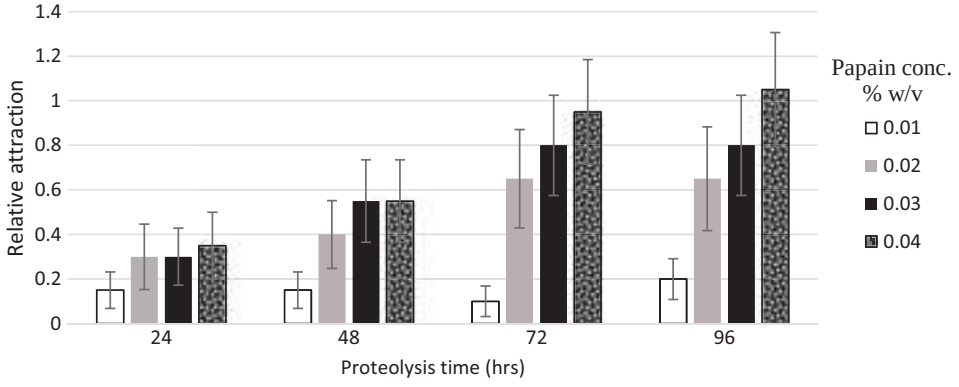

FIGURE 9.4 *Zeugodacus cucurbitae* relative attraction to baits (mean ± SE) boiled at 96 hrs, modified with papain enzyme powder at different proteolysis times.

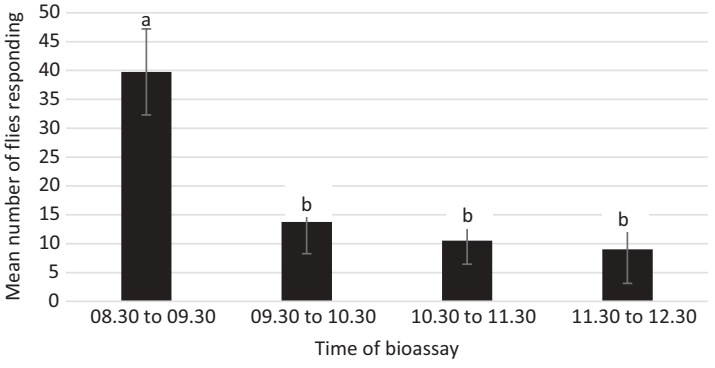

FIGURE 9.5 Number of *Zeugodacus cucurbitae* flies responding (Mean ± SE) by collection time. Means followed by the same letter were not significantly different at $P > 0.05$ level.

Further analyses to determine the effect of the interactions revealed the following: (i) boiling time and proteolysis time was significant (F = 2.46, df = 9, P = 0.023), (ii) boiling time and papain concentration was significant (F = 2.61, df = 9, P = 0.016), and (iii) proteolysis time and papain concentration was not significant (F = 1.00, df = 9, P = 0.445).

In the case of *B. zonata*, increased proteolysis time had a positive effect on fly attraction (F = 0.98, df = 15, P = 0.288). Increasing the concentration of papain during proteolysis increased fly attraction (F = 1.4, df = 15, P = 0.433). Fly attraction increased significantly with increasing boiling time of waste brewery yeast from 24 to 96 hrs (F = 7.93, df = 15, P = 0.004) (Figures 9.6 through 9.9).

Fly attraction was significantly higher when tested between 08:30 and 09:30 am and between 09:30 and 10:30 am, compared to other time periods (F = 6.67, df = 15, P = 0.007) (Figure 9.10).

An interaction effect occurs when the effect of one variable depends on the value of another variable, and the data analysis revealed the following interaction effects: (i) boiling time and proteolysis time was significant (F = 2.60, df = 9, P = 0.017), (ii) boiling time and papain concentration was significant (F = 2.31, df = 9, P = 0.031), and (iii) proteolysis time and papain concentration was not significant (F = 1.34, df = 9, P = 0.245).

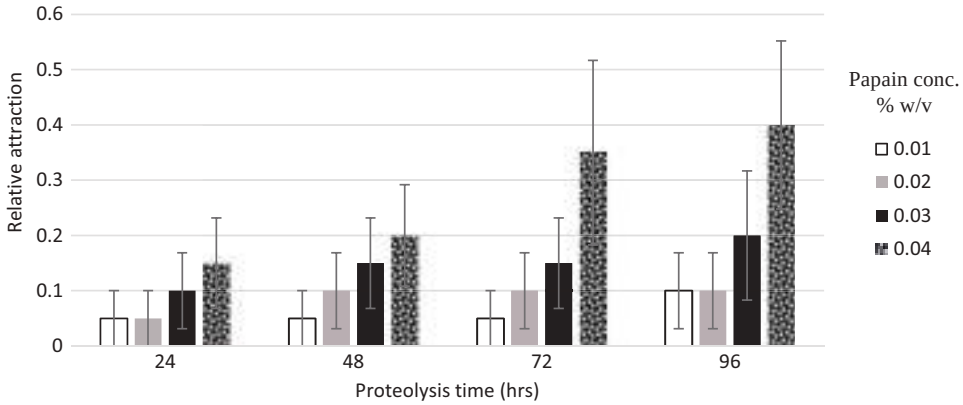

FIGURE 9.6 *Bactrocera zonata* relative attraction to baits (mean ± SE) boiled at 24 hrs, modified with papain enzyme powder at different proteolysis times.

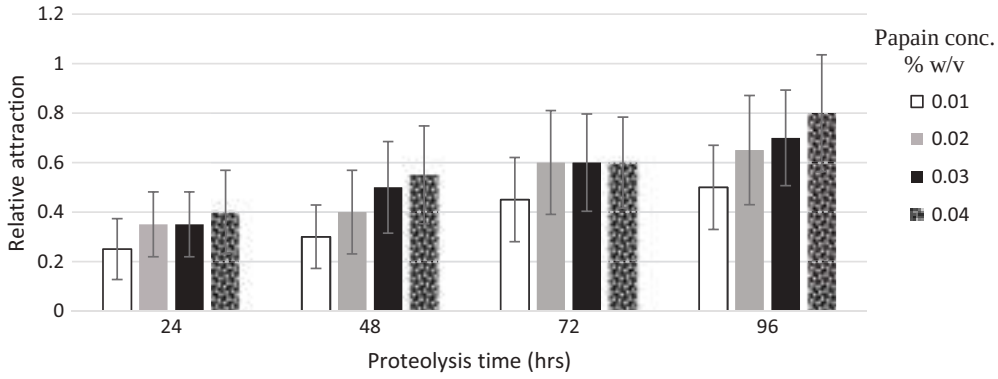

FIGURE 9.7 *Bactrocera zonata* relative attraction to baits (mean ± SE) boiled at 48 hrs, modified with papain enzyme powder at different proteolysis times.

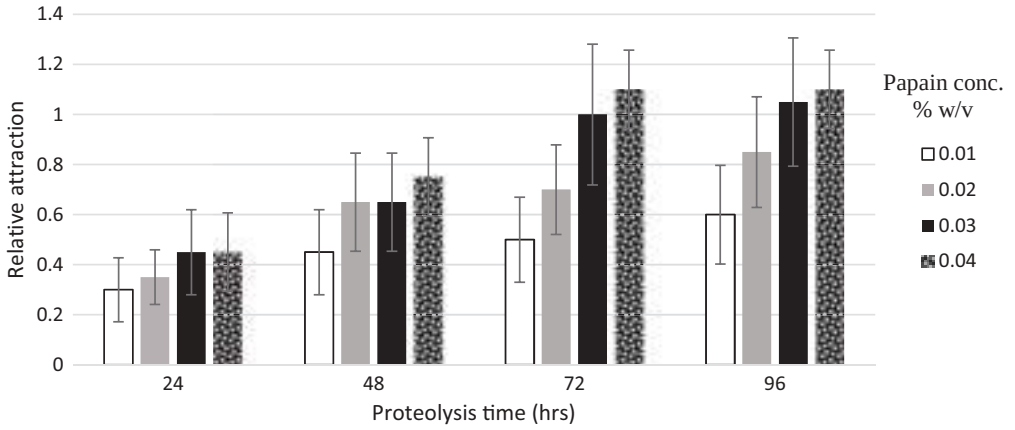

FIGURE 9.8 *Bactrocera zonata* relative attraction to baits (mean ± SE) boiled at 72 hrs, modified with papain enzyme powder at different proteolysis times.

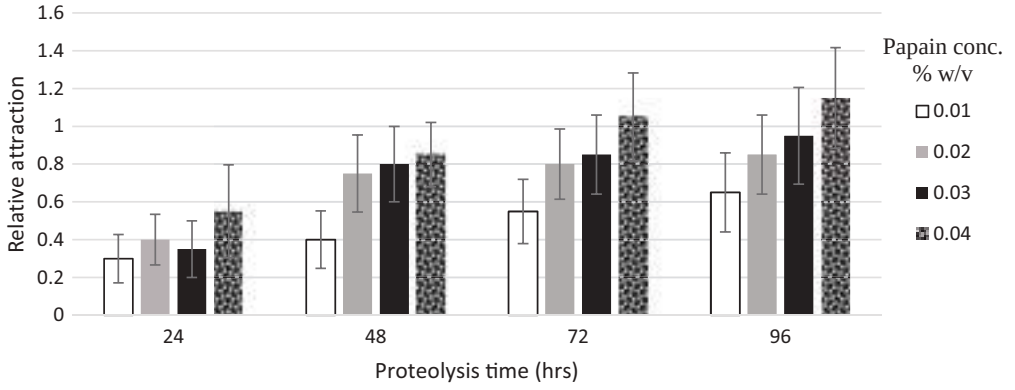

FIGURE 9.9 *Bactrocera zonata* relative attraction to baits (mean ± SE) boiled at 96 hrs, modified with papain enzyme powder at different proteolysis times.

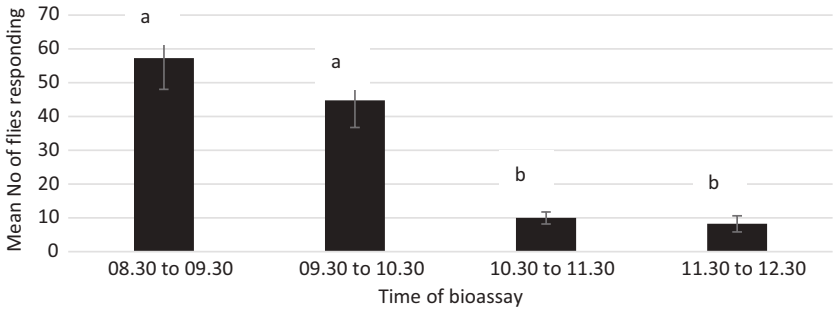

FIGURE 9.10 Number of *B. zonata* flies responding (Mean ± SE) by collection time. Means followed by the same letter were not significantly different at $P > 0.05$ level.

9.3.2 Comparison of Attraction of Different Concentrations of Modified WBY in Field Cages for *B. zonata* and *Z. cucurbitae*

The mean number of *B. zonata* (male and female) collected in Tephri Traps® increased with increasing concentration of WBY from 7.5% v/v to 15% v/v. Trap catches from the different bait concentrations were significantly different for both female flies ($F = 7.08$, df = 11, $P = 0.012$) (Table 9.3) and male flies ($F = 6.65$, df = 11, $P = 0.014$) (Table 9.4). There was no significant difference in attractiveness among the three baits F1, F2, and F3 when they were compared to each other for both female flies ($F = 1.52$, df = 11, $P = 0.269$) and male flies ($F = 1.60$, df = 11, $P = 0.254$). There was also no significant difference when protein hydrolysate was compared to the modified WBY for both male flies ($F = 1.82$, df = 15, $P = 0.197$) and female fruit flies ($F = 1.52$, df = 15, $P = 0.259$).

In the case of *Z. cucurbitae*, the mean number of male and female flies collected in Tephri Traps® increased with increasing concentration of WBY from 7.5% v/v to 15% v/v, as shown in Tables 9.5 and 9.6. Trap catches for different bait concentrations were not significantly different for either female ($F = 2.24$, df = 11, $P = 0.161$) or male flies ($F = 2.59$, df = 11, $P = 0.126$).

TABLE 9.3
Fly Attraction of Bait Treatments at Different Concentrations for Adult *Bactrocera zonata*

	No. of Female Flies Captured/Trap/Day				
WBY	**7.5% v/v**	**10.0% v/v**	**12.5% v/v**	**15.0% v/v**	**Pooled Mean**
Water	0.1 + 0.1 cA	0.2 + 0.1 cA	0.4 + 0.1 bA	0.4 + 0.13c A	0.275 b
Protein (20% v/v)	13.4 + 1.1 aA	12.7 + 0.9 aA	13.9 + 1.2 aA	11.8 + 1.1b A	13.0 a
WBY F1	5.7 + 0.8 bA	7.9 + 0.5 bA	11.4 + 1.4 aA	12.0 + 1.3 bA	9.3 a
WBY F2	7.4 + 0.6 bB	10.6 + 0.7 abAB	13.1 + 1.6 aAB	15.5 + 1.1 abA	11.7 a
WBY F3	8.8 + 1.0 bB	11.7 + 0.6 aAB	15.9 + 1.8 aAB	18.5 + 1.0 aA	13.7 a
Mean (WBY)	7.3 b	10.1 a, b	13.5 a	15.3 a	

In a column or row, means followed by the same letter (capital letters within a row and small letters within a column) are not significantly different at $P > 0.05$ level.
WBY, waste brewery yeast.

TABLE 9.4
Fly Attraction of Bait Treatments at Different Concentrations for Adult *Bacterocera zonata*

	No. of Male Flies Captured/Trap/Day				
WBY	**7.5 % v/v**	**10.0 % v/v**	**12.5 % v/v**	**15.0 % v/v**	**Pooled Mean**
Water	0.1 + 0.7 d A	0.1 + 0.1 cA	0.2 + 0.1 bA	0.2 + 0.09 cA	0.15 b
Protein (20% v/v)	8.9 + 0.6aA	9.5 + 0.9 aA	9.1 + 0.9 aA	9.9 + 0.8 abA	9.35 a
WBY F1	3.9 + 0.4 c B	4.9 + 0.4bB	7.8 + 0.8 aA	8.7 + 0.8bA	6.3 a
WBY F2	4.9 + 0.4 bcC	5.9 + 0.5bBC	8.5 + 0.8 aAB	10.4 + 1.1abA	7.4 a
WBY F3	5.8 + 0.6bC	8.2 + 0.6 aBC	11.9 + 1.1 aAB	13.0 + 1.2 aA	9.73 a
Mean (WBY)	4.9 b	6.3 a, b	9.4 a, b	10.7 a	

In a column or row, means followed by the same letter (capital letters within a row and small letter within a column) are not significantly different at $P > 0.05$ level.
WBY, waste brewery yeast.

TABLE 9.5
Fly Attraction of Bait Treatments at Different Concentrations for Adult *Zeugodacus cucurbitae*

	No. of Male Flies Captured/Trap/Day				
WBY	**7.5% v/v**	**10.0% v/v**	**12.5% v/v**	**15.0% v/v**	**Pooled Mean**
Water	0.35 + 0.2 dA	0.35 + 0.2 eA	0.3 + 0.1 cA	0.35 + 0.2 cA	0.338 c
Protein (20% v/v)	15.2 + 0.6 cA	15.65 + 0.7 aA	14.85 + 1.0 aA	16.0 + 1.3 aA	15.43 a
WBY F1	4.8 + 0.4cB	5.3 + 0.4 dB	6.4 + 0.5 bB	9.3 + 0.9 bA	6.45 b
WBY F2	6.75 + 0.7b cC	7.9 + 0.5 cC	11.2 + 0.7 aB	14.15 + 0.4 aA	10.0 ab
WBY F3	8.5 + 0.6 bC	10.5 + 0.6 bBC	14.85 + 0.8 aAB	17.7 + 1.2 aA	12.89 a
Mean (WBY)	6.683 a	7.9 a	10.817 a	13.72 a	

In a column or row, means followed by the same letter (capital letters within a row and small letter within a column) are not significantly different at $P > 0.05$ level.
WBY, waste brewery yeast.

TABLE 9.6
Fly Attraction of Bait Treatments at Different Concentrations for Adult *Zeugodacus cucurbitae*

	No. of Female Flies Captured/Trap/Day				
WBY	**7.5% v/v**	**10.0% v/v**	**12.5% v/v**	**15.0% v/v**	**Pooled Mean**
Water	0.4 + 0.1 dA	1.25 + 0.3 dA	0.55 + 0.2 cA	0.5 + 0.2 c A	0.68 d
Protein (20% v/v)	21.8 + 1.3aA	19.05 + 1.3 aA	19.85 + 0.9 a A	22.6 + 0.7 a A	16.40 a
WBY F1	5.95 + 0.7 cA	6.75 + 0.8 cA	9.1 + 1.1bA	10.35 + 1.0 bA	8.04 c
WBY F2	8.3 + 0.9bcB	10.9 + 1.0 bc AB	17.1 + 1.3 aA	18.25 + 1.5 aA	13.64 bc
WBY F3	10.6 + 1.0 bC	14.45 + 1.0 abBC	18.95 + 1.3 aAB	21.6 + 1.6 aA	16.40 ab
Mean (WBY)	8.28 a	10.7 a	15.05 a	16.73 a	

In a column or row, means followed by the same letter (capital letters within row and small letter within a column) are not significantly different at $P > 0.05$ level.
WBY, waste brewery yeast.

When protein baits were compared to each other, there was a significant difference in attractiveness for both female flies ($F = 8.54$, df = 15, $P = 0.003$) and male flies ($F = 7.26$, df = 15, $P = 0.005$).

9.3.3 Comparison of Attraction of Selected Baits in Tephri Traps® in Cucurbit Fields (St. Pierre) Targeting *Z. cucurbitae*

The mean number ± standard error (SE) of melon flies (male and female) trapped per day by each bait treatment is shown in Table 9.7. An analysis of variance was done on transformed data using log (x + 1). A significantly higher number of melon flies were caught in the protein baits compared to the water control ($F = 7.50$, df = 29, $P = 0.0001$). There was no significant difference ($F = 0.39$, df = 23, $P = 0.765$) between the tested protein hydrolysate and modified protein baits F1, F2, and F3. Similar results were obtained when the analysis was done for captured females only ($F = 8.29$, df = 29, $P = 0.000$). Results were not significant when the analysis was done for males only ($F = 2.43$, df = 29, $P = 0.074$).

The sex ratio was significantly different ($F = 8.99$, df = 9, $P = 0.017$), with a higher number of female melon fly catches compared to male melon fly catches.

TABLE 9.7
Mean Number ± SE of Melon Flies Collected per Day in St. Pierre Using Different Attractants

Catch	Protein	Water	F1	F2	F3	Mean
Females	0.41 + 0.1 aA	0.04 + 0.03 aA	0.51 + 0.2 aA	0.60 + 0.2 aA	0.68 + 0.3 aA	0.45 a
Males	0.05 + 0.01 bA	0.01 + 0.01 aA	0.11 + 0.04 aA	0.13 + 0.05 bA	0.19 + 0.1 aA	0.10 b
Mean	0.23 a	0.025 b	0.31 a	0.37 a	0.44 a	

In a column or row, means followed by the same letter (capital letters within a row and small letter within a column) are not significantly different at $P > 0.05$ level.
SE, standard error.

9.3.4 Comparison of Fly Attraction of Selected Baits in Tephri Traps® in Cucurbit Fields (Albion) Targeting *Z. cucurbitae*

The mean number of melon flies (male and female) trapped per day is shown in Table 9.8. Significantly higher catches of melon flies were noted for the protein baits when compared to water (F = 23.79, df = 24, $P = 0.001$). Similar results were observed when captured males (F = 15.32, df = 24, $P = 0.001$) and captured females were analyzed separately (F = 21.93, df = 24, $P = 0.0001$). However, the ratio of male-to-female catches was not significantly different (F = 1.43, df = 9, $P = 0.266$).

TABLE 9.8
Mean Number ± SE of Melon Flies Collected per Day in Albion Using Different Attractants

Catch	Protein	Water	F1	F2	F3	Mean
Females	1.15 + 0.2 aA	0.06 + 0.04 aB	0.70 + 0.2 aAB	0.90 + 0.2 aA	0.97 + 0.2 a A	1.16 a
Males	1.55 + 0.4 aA	0.08 + 0.04 aB	1.18 + 0.3 aAB	1.44 + 0.3aA	1.54 + 0.3aA	0.76 a
Mean	1.35 a	0.07 b	0.94 ab	1.17 ab	1.26 ab	

In a column or row, means followed by the same letter (capital letters within a row and small letter within a column) are not significantly different at $P > 0.05$ level.
SE, standard error.

9.3.5 Comparison of Fly Attraction of Selected Baits in Tephri Traps® in a Fruit Orchard Targeting *B. zonata*

Total trap catches for the three selected baits (F1, F2, and F3) were not significantly different (F = 0.35, df = 14, $P = 0.709$). Trap catches in protein hydrolysate were significantly lower (F = 29.97, df = 24, $P = 0.0001$) compared to modified WBY (Table 9.9).

TABLE 9.9
Mean Capture ± SE of Peach Fruit Flies per Day in Labourdonnais Using Different Attractants

Catch	Protein	Water	F1	F2	F3	Total
Females	0.19 + 0.02 aA	0 aB	0.39 + 0.1 aA	0.26 + 0.00 aA	0.25 + 0.02 a A	1.09 a
Males	0.17 + 0.03 aB	0 aC	0.19 + 0.02 aB	0.34 + 0.04 aAB	0.39 + 0.02 a A	1.09 a
Total	0.36 a	0	0.58 a	0.60 a	0.64 a	

In a column or row, means followed by the same letter (capital letters within a row and small letter within a column) are not significantly different at $P > 0.05$ level.
SE, standard error.

The ratio of male-to-female trap catches was not significantly different between protein hydrolysate (F = 0.29, df = 9, $P = 0.608$) and bait F2 (F = 3.09, df = 9, $P = 0.117$), whereas trap catches were significantly different between F1 (F = 5.62, df = 9, $P = 0.045$) and F3 (F = 22.53, df = 9, $P = 0.01$).

9.3.6 Cost–Benefit Analysis

Total benefits over 5 years were US$ 811,010.62, and the total costs amounted to US$ 527,452.02. The resulting net benefits were US$ 283,558.60 (Table 9.10) when using modified WBY instead of imported protein hydrolysate.

9.4 DISCUSSION

Ekesi et al. (2016) stated that WBY has promising chemical properties. However, to be effective, there is a need to release the ammonium compound and its derivatives efficiently, which serve as volatile cues to locate protein rich food (Piñero et al. 2017). In the laboratory bioassay, boiling of the WBY served to remove as much alcohol as possible (Ekesi et al. 2016), increasing solid content (Lloyd and Drew 1997) and causing lysis of the yeast cell wall to release protein compounds that are very attractive to tephritids (Vargas and Prokopy 2006). Papain, known to digest most protein substrates (Anon. 2015), was added to accelerate this process and an increase in bait attraction was observed by increasing papain concentration from 0.1% to 0.4% w/v. The higher the concentration of the enzyme, the higher the proteolysis of the yeast cells; thus, higher amounts of free amino acids were available in the protein bait. A direct correlation between dry matter content of a protein bait, which is the result of proteolysis of the yeast cell content, and relative attractiveness has been reported by Sookar et al. in 2003 (Aggrey-Korsay 2014). Results of the laboratory bioassay demonstrated that combining boiling for more than 72 hrs with proteolysis for more than 72 hrs did not have a significant effect. Therefore, it is not required to boil for 96 hrs followed by proteolysis for 96 hrs.

The response of the flies to the time at which the tests were carried out was significantly different for both species. This agrees with a study carried out by Prokopy and Roitberg (1984) on the foraging behavior and daily activity of different fruit fly species which varied based on time of the day.

TABLE 9.10
5-Year Cost–Benefit Analysis Using an Estimated Increase Rate of 2%

	Current Year (CY) ($)	CY + 1 ($)	CY + 2 ($)	CY + 3 ($)	CY + 4 ($)	CY + 5 ($)	
Costs							
Cost of Digestor	73, 529.00						
Electrical Cost	1, 233.00	1, 257.66	1, 282.81	1, 308.47	1, 334.64	1, 361.33	
Water Cost	2, 136.00	2, 178.72	2, 222.29	2, 226.74	2, 312.08	2, 358.32	
Cost of Papain	4, 764.00	4, 859.28	4, 956.47	5, 055.59	5, 156.71	5, 259.84	
Cost of Potassium Sorbate	36, 529.00	37,259.58	38, 004.77	38, 764.87	39, 540.16	40, 330.97	
Cost of Diesel	6, 195.00	6, 318.90	6, 445.28	6, 574.18	6, 705.67	6,839.78	
Labor	11, 847.00	12, 083.94	12,325.62	12, 572.13	12, 823.57	13, 080.05	
Transport Cost	466.00	475.32	484.83	494.52	504.41	514.50	
Training Cost	150.00	153.00	156.06	159.18	162.36	165.61	
Infrastructure	73, 529.00						
Total Costs (Future Value)	210, 378.00	64, 586.40	65,878.13	67, 195.69	68, 539.60	69, 910.40	
Total Costs (Present Value)	210, 378.00	63, 320.00	63, 344.35	63, 392.16	63, 462.60	63, 554.91	527, 452.02
Benefits	135, 000.00	137,700.00	140, 454.00	143, 263.08	146, 128.34	149, 050.91	
No Importation of Protein Hydrolysate							
Total Benefits (Future Value)	135, 000.00	137,700.00	140, 454.00	143, 263.08	146, 128.34	149, 050.91	
Total Benefits (Present Value)	135, 000.00	135,000.00	135, 051.92	135, 153.85	135, 304.02	135, 500.83	811,010.62
	Cost–Benefit Analysis (US dollars)						
	Total PV Benefits		$811,010.62				
	Total PV Costs		$527,452.02				
	NET BENEFIT		$ 283,558.60				

Sookar et al. (2002) reported that all their tested baits caught all flies before 11:30 am. The results obtained in this study are consistent with previous findings, as fly attraction for *Z. cucurbitae* was most significant between 08:30 and 09:30 am, compared to *B. zonata* flies, which were most responsive between 08:30 and 10:30 am. Thus, food baited catches reflect the feeding activity of fruit flies (Bharati et al. 2004). This information is important for a more effective planning of fruit fly spraying programs using attract and kill methods targeted toward specific fly species.

To be effective at suppressing fruit fly populations, protein-based bait formulations must induce good levels of attraction. Ammonia is the principal component of the protein bait that attracts fruit flies, mainly females (Mazor et al. 1987; Piñero et al. 2015). Attraction of baits increases with increased concentration or amount of ammonia emitted (Bateman and Morton 1981). The field cage trial with *B. zonata* showed a significantly increased fly attraction with increased bait concentration of the three selected baits (F1, F2, and F3) from 7.5% to 15% v/v. Field cage trials with *Z. cucurbitae* showed that increasing bait concentration did not significantly increase fly attraction. This may be due to the fact that different fly species vary in their response to a particular stimulus (Kotikal and Math 2017). For both species, the number of female fruit flies caught in the protein baits was much higher than the number of male fruit flies. The main purpose of liquid protein baits is to capture female fruit flies. The bait targets the female fruit fly's need for protein for the development and maturation of eggs. With protein attractants, recently emerged female fruit flies enter the trap, get caught, and eventually die by drowning in the capture fluid (Anon. 2016).

Protein sources are an important component of food baits and commercial lures for *Z. cucurbitae* (Steiner 1952; Narayanan and Batra 1960; Vijaysegaran 1985; Satpathy and Rai 2002; Fabre et al. 2003 cited in Nagaraj et al. 2014). In trials conducted in open vegetable fields, protein baits were highly attractive for capturing both male and female *Z. cucurbitae* flies. Results indicated that, for melon flies, there was no significant difference in attractiveness between the selected baits and the commercial protein hydrolysate, which indicates an acceptable quality of the prepared baits. Comparable results were obtained with *Bactrocera invadens* (now *B. dorsalis*) in Nigeria using modified brewery waste and commercial torula (Umeh and Onukwu 2010).

Female fruit flies require a protein source to mature sexually and to develop eggs (Bateman 1972). This is supported by Kotikal and Math (2017), who concluded that baits offer one of the most effective methods of control, especially in the pre-oviposition stage when fruit flies require plenty of water to drink and are easily attracted to protein sources. This helps to explain the significantly higher mean trap catches of female melon flies compared to male melon flies in St. Pierre. In Albion, the ratio of male-to-female captures was not significantly different. One possible reason could be the higher prevailing temperatures (Anon. 2017a), which would increase the attraction of males to a wet trap (Barry et al. 2006).

Climate plays a critical role either directly or indirectly as a control for Tephritids. The results of the open field trials conducted in the north of Mauritius (Labourdonnais orchard) showed promising results on the attraction of *B. zonata* to the low-cost prepared bait. Trap catches from the modified WBY were significantly higher compared to the trap catches from the commercial protein hydrolysate, indicating the quality of the prepared baits. Trap catches of male and female *B. zonata* were not significantly different. The climatic conditions prevailing in the region, which has a hot climate (Anon. 2017b), may explain similar male and female captures. A significant positive correlation has been reported between trap catches and maximum temperature in studies carried out by Gajalakshmi et al. (2011) and Boopathi et al. (2013). Flies were attracted to a wet trap irrespective of whether they were male or female.

The results of the field trial targeting the two fruit fly species shows a marked attraction of *Z. cucurbitae* compared to *B. zonata*. Piñero et al. (2017) documented an inherent stronger response to protein baits by *Z. cucurbitae* compared to *B. dorsalis*. These findings are supported by studies done by Vargas and Prokopy (2006) on attraction and feeding propensity of different fly species relative to different protein baits.

9.5 CONCLUSION

An effective protocol has been developed and tested for the modification of WBY. The study determined the most effective boiling time (72 hrs at 95°C), proteolysis time (72 hrs at 60°C), and concentration of papain (0.4% w/v) required to prepare an effective protein bait. The protocol has been evaluated in both field cage and open field trials against both *B. zonata* and *Z. cucurbitae*.

An important finding from the study was determining the time (early morning) at which the flies are most active. The overall results are encouraging and indicate that the prepared WBY bait may prove to be a useful monitoring tool for both male and female flies of the two species. One drawback of the modified WBY is its non-specificity, which causes non-target flies to be attracted to the bait, resulting in large fly captures. However, this non-specific attractiveness can aid in general surveillance programs to detect other pest species of economic importance.

The cost–benefit analysis supports the feasibility of the project by supporting the objective of this study in developing a cost-effective replacement for the expensive protein hydrolysate imported from Europe or North America. The developed WBY bait is financially promising for smaller growers. The WBY bait will also enhance the sustainability of fruit fly monitoring and fruit fly management programs by making them more economically feasible. The horticulture industry as a whole is assumed to receive the benefits from avoided production loss and gains in export value from improved international market access (Abdalla et al. 2012).

Further research is required on the amount of bait solution needed per trap and the effective servicing intervals when using WBY as a monitoring tool. During hot summer days, the baits dry up faster and may have a negative effect on fruit fly monitoring results. Assessment of the shelf life of the prepared bait requires investigation for production, especially if switching to a large-scale basis.

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Section IV

Risk Assessment, Quarantine, and Post-Harvest

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10 International Database on Commodity Tolerance (IDCT)

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CONTENTS

Abstract An important factor for increasing the commercialization of phytosanitary irradiation (PI) is the adoption of generic doses in international and national regulatory frameworks. A limiting factor to accelerating the use of PI is the availability of information on commodity tolerance for the wide range of horticultural products that might be eligible for treatment with PI. The International Database on Insect Disinfestation and Sterilization (IDIDAS) created by the Joint Food and Agriculture Organization/International Atomic Energy Agency (FAO/IAEA) Program for Nuclear Techniques in Food and Agriculture contains an extensive collection of international research on PI. The International Database on Commodity Tolerance (IDCT; https://nucleus.iaea.org/sites/naipc/IDCT/Pages/default.aspx), also created under the auspices of the Joint Program, contains information on the reaction of fresh horticultural commodities including fruit, vegetables, flowers, roots, and tubers, to radiation. Data were extracted from scientific publications from 1950 to the present. The procedure was to collect defined data elements for reporting in a consistent manner. The information used for the database focused on the parameters of specific treatments and conditions. The concepts of "market acceptance" and "market rejection" were determined based on factors associated with how the radiation dose (or range of doses) affected acceptance or rejection of the commodity by taking into account the damage from the treatment and handling conditions in each research scenario. Approximately 415 articles were reviewed: 336 articles corresponded to 48 different fruit species; 47 articles corresponded to tubers and vegetables; and 35 articles covered 21 species of flowers. The database can be searched by commodity using the common name, cultivar, or Latin name as well as by genus and family. Each study lists the respective reference and listings are illustrated with the Google photo gallery. The availability of this information in the IDCT database greatly facilitates the process of identifying potential trade opportunities using PI and helps highlight where commodity tolerance research is sufficient or is still needed.

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10.1 BACKGROUND

One of the primary uses of food irradiation is as a phytosanitary treatment for fresh commodities (IPPC 2003). Another benefit of this treatment can be to increase the shelf life of commodities in some instances (Arvanitoyannis et al. 2009). Doses in the range of 0.05–2.5 kGy are useful to achieve both purposes in some fresh horticultural products (Bustos-Griffin et al. 2012).

Unlike other phytosanitary treatments (e.g., fumigation and heat or cold treatment) the efficacy of phytosanitary irradiation (PI) is not measured on the basis of acute mortality of the target pests but on preventing the development of the life stages (e.g., non-emergence of adults when larvae are irradiated) or in affecting the ability of the pest to reproduce (e.g., reproductive sterility of irradiated adults) as when irradiated females lay eggs that hatch, but the F_1 neonates die (Hallman et al. 2016). Radiation at the doses applied for PI alters certain physiological processes in the commodities while controlling the pests that may be associated with the fresh products. PI offers an alternative to many traditional quarantine treatments such as fumigation and creates new treatment opportunities where commodity treatments were not previously available.

PI was first used in 1986 when a commercial shipment of irradiated mango from Puerto Rico was shipped to markets in Florida, USA (Phillips 1986). Commercialization followed slowly because of challenges associated with the lack of regulations, concerns about consumer acceptance, and the absence of adequate facilities, among others (Bustos-Griffin et al. 2015). As many other quarantine treatments were banned, such as ethylene Dibromide (EDB) (Ruckelshaus 1984), PI has steadily grown as a mainstream quarantine treatment.

Over time, a number of countries including the United States, Australia, New Zealand, India, Thailand, Vietnam, Mexico, South Africa, and Malaysia have become involved in the import and export of PI-treated fresh horticultural products (Bustos-Griffin et al. 2015). This growth can be attributed in large part to changing perceptions, market forces, and the adoption of international phytosanitary standards that provide the basis for national programs that incorporate PI as a viable treatment option. These conditions have helped to better position PI for large-scale commercialization.

One important characteristic of PI that distinguishes it from other quarantine treatments is the potential to adopt generic treatments for similar pest species across a broad range of commodities (APHIS 2006). For example, studies done on different species of the Tephritidae family demonstrate that a 150 Gy treatment dose can control their normal development (Hallman 2012). Once the dose for a pest is established by research, it is the same no matter what commodity is being treated. Thus, the dose is "generic" for the pest in all commodities that can tolerate the dose. When this dose is then expanded to whole groups of pest organisms, it opens the door for many new commodity treatments with generic doses. The key to taking advantage of this opportunity is having available PI host tolerance information. This information explains which commodities are potential candidates for PI using generic doses.

The quality of most fresh horticultural commodities is not affected by the radiation doses used for PI. However, a few commodities (such as avocado) cannot tolerate the doses required to control most common pest species (Balock et al. 1966). Before investing in PI programs and treatment facilities, marketers need to understand which aspects of quality are most important for the commodities in question and the effects of the radiation dose under specific commercial conditions.

The scientific literature on commodity tolerance to PI is highly variable and can be confusing with vague or incomplete conclusions and conflicting reports. For instance, the quality of several avocado cultivars is negatively affected at doses irradiated below or equal to 250 Gy (Balock et al. 1966; Arevalo et al. 2002), but Simon and Vietes (2014) report the quality of avocado is not affected at 1000 Gy. Interpretation and synthesis of the information in a consistent and easily searchable format is important to make it useful for the business decisions needed to commercialize PI.

The International Database on Insect Disinfestation and Sterilization (IDIDAS) was created by the Joint Food Agriculture Organization/International Atomic Energy Agency (FAO/IAEA) Program for Nuclear Techniques in Food and Agriculture as a searchable international repository for

irradiation treatment research relevant to agricultural applications, including PI. The International Database on Commodity Tolerance (IDCT) has been created to complement IDIDAS with consolidated commodity tolerance information based on critical review and analysis of the available literature. The objective was to create a database that substantially supports the decision-making processes associated with the expansion and commercialization of PI.

10.1.1 Phytosanitary Irradiation

Extensive studies have demonstrated that irradiation used for phytosanitary purposes offers significant benefits and has many characteristics that make it unique as a phytosanitary treatment (Hallman 2011). Irradiation offers a range of possible responses other than only mortality. The treatment response can also be sterility, limited fertility, limited development, non-emergence of adult from pupae (Bustos et al. 2004), devitalization in the case of seed (Wage and Kwon 2007), inactivation in the case of microorganisms (Dickson 2001), and sprout inhibition (ICGFI 1991). The integrity of the treatment is assured by research that identifies the appropriate dose, and dosimetry to assure that treatment achieves the dose under specific conditions.

A key difference between PI and all other phytosanitary treatments is that the presence of live pests post-treatment is acceptable by plant protection organizations. Quarantine security is not compromised by the presence of live target pests if research has shown that they are unable to grow or produce viable offspring after treatment (Bakri et al. 2005; Hallman 2012).

The source of radiation for a PI treatment can be a radioactive gamma source such as Co-60 or Cs-137, or machine-generated radiation in the form of X-rays or electron beams (FAO 1984). Dosimetry assures that the minimum dose is absorbed at every point in the treatment load. The dose is the same for every commodity treated for the same pest. There should be no need for additional commodity dose research or regulatory approvals for each commodity (IPPC 2003).

In the case of generic doses, research has demonstrated the effectiveness of one dose for groups of pests. For example, 150 Gy is currently recognized by the International Plant Protection Convention as the generic dose for all tephritid fruit flies, (IPPC 2008), and 400 Gy is recognized by the Animal and Plant Health Inspection Service (APHIS) and New Zealand for all insects except lepidopteran pupae and adults. These doses create an enormous opportunity to treat a wide range of commodities affected by the pests of concern if the commodity can tolerate the dose, hence the importance of commodity tolerance for accelerating the commercialization of PI.

10.1.2 Tolerance of Irradiated Fresh Commodities

The concept of tolerance is related to the desired characteristics of the final product following treatment. Tolerance is not a regulatory parameter, but it is crucial to determining if a treatment is practical and the treated product is acceptable for marketing purposes. PI that effectively treats a pest but renders the product unusable for the desired market is not commercially viable.

Consumers expect safety and quality of their food. The safety of irradiated food has been demonstrated with extensive research carried out in many countries over many decades. One of the principal studies was The International Project on Food Irradiation that was active from 1970 to 1982. Its work included feeding studies contracted to cover a range of commodities irradiated at very high doses. None of the studies gave any indication of the presence of radiation induced carcinogens or other toxic substances (Elias and Cohen 1983). The data generated by this project and other related investigations were reviewed by the Expert Committee on the Wholesomeness of Irradiated Food at World Health Organization (WHO) Headquarters. After several meetings and extensive review, this committee, which was formed by members of the WHO, IAEA, and FAO concluded that the irradiation of any commodity up to an overall average dose of 10 kGy (10,000 Gy) presented no toxicological hazard and no special nutritional or microbiological problems. Hence, toxicological testing of foods so treated was no longer required (FAO 1984).

The quality characteristics in fresh horticultural products for consumers are shelf life, appearance, odor, flavor, firmness, and texture. Some of these characteristics can be subjectively judged by simple observation (e.g., shape, appearance, defects, color, odor) and others can be precisely measured (texture, color, size) or another characteristic may be more important depending on the commodity and end use. This means that acceptable quality is variable. For example, appearance will be more important for apples to be sold as fresh fruit than apples that will be used for applesauce.

The ability to extend commodity shelf life is generally a desirable characteristic, and some fresh horticultural products benefit from this characteristic of PI. Treatment of radio-phylic commodities such as mango, papaya, and rambutan either delay the ripening or the senescence process or tolerate high doses (1 kGy) with little or no negative effects (Kader 1986). At the opposite end of the scale are radio-phobic commodities such as avocado, soursop, and some leafy vegetables that are very susceptible to damage by radiation even at low doses (Kader 1986). Because of their sensitivity to irradiation treatment, they cannot be considered viable commodities for PI. Between these extremes is a range of commodities that tolerate some level of radiation under specific conditions. PI may be a viable treatment for this large category of commodities, but the successful use of PI requires a clear understanding of the tolerance of the commodity and the optimal conditions for treatment at the dose required for the pest(s) of concern.

Fresh horticultural commodities contain a high percentage of water, and this compound is an excellent medium for chemical and biochemical reactions in living tissues. Exposure to irradiation excites molecules in the product, creating free radicals and ions, which almost instantaneously initiate reactions that affect the metabolism of the commodity. The resulting physiological changes will be reflected in characteristics that may affect the quality of an irradiated fresh product, depending on the dose (Arvanitoyannis et al. 2009).

10.2 METHODS

10.2.1 The IDCT Project

As interest in the commercialization of irradiation for phytosanitary purposes has grown, IAEA through its Joint FAO/IAEA Program recognized the need for information that supports business decisions as well as research. Commodity tolerance is a central point to trust in the viability of PI on a commercial scale.

Some commodity tolerance information is available from specific studies in peer-reviewed literature. Other information is found in related research, proceedings, and industry studies. The IDCT aims at identifying, collecting, reviewing, analyzing, and summarizing the information in a form that makes it easily searchable and useful. Researchers, regulators, marketers, and investors benefit from being able to quickly determine the viability of a particular commodity treatment and whether additional research is needed or not for a commodity tolerance for a particular dose and under specific storage or shipment conditions.

10.2.2 Collecting and Selecting Information

The review includes available research from 1950 to 2018. Information regarding commodity tolerance was extracted, interpreted, compiled, and submitted in a defined format for inclusion in an IAEA database created for the purpose. Information on the quality of different irradiated fresh commodities was provided. The information was adjusted to a format that met the objectives of the database (i.e., to be both useful and user friendly). The scope of the database was limited to specific information on commodity tolerance for irradiated fresh plant products. Live plants, seeds, wood, and other nonhorticultural products were not included. The procedure was to collect defined data elements for reporting in a consistent manner (see Table 10.1). The information used for the database focused on the conditions of pre-treatment, treatment, and post-treatment for each commodity. The concepts of “Market acceptance” and “Market rejection” listed in the summaries refer to the parameters used or insinuated by the authors of each study to determine if an irradiated commodity was marketable.

TABLE 10.1
IDCT Data Collection Framework

Conditions						
		Post-Treatment				
Pre-Treatment	**Treatment**	**Bioassay**	**Market Acceptance**	**Market Rejection**	**Dose (Gy)**	**References**
Commodity origin (country, city), maturity etc.	Radiation source, dose rate, DUR.	Tests done on tolerance and quality of commodities irradiated (T, RH, air, packaging, time tested parameters, quality assessment), etc.	Basis for acceptance	Basis for rejection	Minimum to maximum range yielding acceptance; marketability of the doses tested	Published results of each study.

Abbreviations: DUR, dose uniformity ratio; IDCT, International Database on Commodity Tolerance; RH, relative humidity; T, temperature.

10.3 RESULTS

Tolerance information was obtained from scientific articles published in peer-reviewed journals, proceedings of symposia, reports, and any sources that could be cited. Approximately 415 sources were initially reviewed: 336 corresponded to fruit and covered 48 different fruit; 47 articles corresponded to tubers and vegetables, and 35 articles were reviewed for flowers, covering 21 different flowers for each commodity including cultivars or varieties. Table 10.2 identifies the commodities reviewed. This list is continually being expanded as new information comes to our attention.

The analysis of the results shows that the tolerance of a product is not only a function of the radiation dose, but also other parameters such as the species and cultivar or variety, the stage of maturity at the time of treatment, and the physical conditions before, during, and after the treatment (e.g., temperature, relative humidity, type of package, and type/time of storage and transportation).

TABLE 10.2
Fresh Horticultural Products in the IDCT as Well as Those Planned to Be Added in the Future

Fruit	Apple, apricot, avocado, banana, blackberry, blueberry, cantaloupe, cherry, clementine, curuba, custard apple, Dragon fruit, durian, feijoa, fig, granadilla, grapes, grapefruit, guava, gulupa, hazelnut, kiwifruit, lemon, lime, longan, lychee, lulo, mandarin, mango, mangosteen, nectarine, orange, papaya, passion fruit, peach, persimmon, pear, pineapple, plantain, plum, pomelo, rambutan, starfruit or carambola, soursop, strawberry, uchuva
Vegetables, bulbs, and tubers	Asparagus, capsicum, cucumber, eggplant, mushroom, onion, potato, tomato, spinach, sweet potato, zucchini
Flowers	Bellflower, bird of paradise, Bouvardia, carnation, chrysanthemum, foliage, freesia, gentian, gerbera, ginger, gladiolus, gloriosa, hoary tock, iris, lily, orchids, palm, Rosa, statice, summer tulip, sweet pea

Abbreviation: IDCT, International Database on Commodity Tolerance.

TABLE 10.3
Dose Tolerated in Different Cherry Cultivars

Cultivar	Dose Tolerated (Gy)	References[a]
Bing	≤600 or 2000	Salunkhe (1961), Drake (1997), Drake (1998), Neven and Drake (2000)
Lambert	1000 or <2000	Salunkhe (1961), Eaton (1970)
Napoleon	<2000	Salunkhe (1961)
Rainer	300 or ≤600 or 1000	Drake (1994), Drake (1997), Drake (1998), Neven and Drake (2000)
Van	1000	Eaton (1970)
Windsor	<2000	Salunkhe (1961)
0900Zirat	300	Akbudak (2008)

[a] See the complete citation on the International Database on Commodity Tolerance (IDCT) database.

The range of variables affecting tolerance may be manifest in differences in research results. Studies done at different times in different countries with different conditions can have different results for the same variety. As can be seen in Table 10.3, the doses tolerated by different cultivars of cherry are highly variable. Additional cases can be found by exploring IDCT. For this reason, it is important to consider the range of results and conditions affecting commodity tolerance.

It is important to note that the search was principally for fresh commodities. The idea is to later complement the IDCT with other plant products that need PI, such as nuts. Some of these, like grapes, apples, and strawberries, combine irradiation with low temperature or modified atmosphere, but the commodities are always fresh. It is also important to mention that there are some other very particular commodities that belong to specific regions or countries. All dosage information was normalized to gray units (Gy) and temperatures to degrees Celsius (°C).

10.3.1 Database Structure

The IDCT (2018) was launched by the IAEA Insect Pest Control Section of the Joint FAO/IAEA Division on March 9, 2017. The database can be searched by commodity using the common name, the cultivar, or Latin name as well as by genus and family. Each study lists the respective reference, and all commodity listings are illustrated with links to a Google photo gallery. The database tracks user traffic metrics because the number of hits and visits are generated in a monthly report by Google analytics.

10.4 CONCLUSIONS

The phytosanitary applications for irradiation have become mainstream tools for safe trade in horticultural commodities. To realize the full potential of PI, efforts that have previously emphasized research and regulatory frameworks must also support commercialization. This means that the experience and information developed over the past decades needs to be made available in a way that facilitates decision making for regulators, marketers, and investors to clearly understand the opportunities and limitations of the technology. A central strategy in this transformation is providing easily accessed and digestible information on commodity tolerance.

IDCT is an important source of collective scientific knowledge to learn and know about the quality of irradiated commodities. It clearly demonstrates that the response of a commodity to irradiation is not only a function of the radiation dose but also conditions of handling and

storage. The data contained in this system also indicates that the commodity cultivar type and the country or region of origin are also important parameters to consider in the application of the treatment.

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11 Gamma-H2AX
A Promising Biomarker for Fruit Fly Phytosanitary Irradiation Exposure

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CONTENTS

Abstract DNA double-strand breaks (DSBs) are one of the most biologically significant DNA damage lesions. Exposure to ionizing radiation (IR) causes DSBs in living organisms, which trigger intrinsic DNA repair mechanisms. Phosphorylation of the C-terminal of the core histone protein H2AX (termed γH2AX when phosphorylated) is an early known response to DNA DSBs. Quantification of the γH2AX response offers a highly sensitive and specific assay for detecting DSB formation and repair. Postharvest exposure to IR of 150–400 Gy is an increasingly prominent phytosanitary measure in a variety of Australian (and imported) fruit. The radiation-induced γH2AX response has been shown to be highly persistent in the Queensland fruit fly ("Q-fly"; *Bactrocera tryoni*), Australia's most economically damaging insect pest of horticultural crops, lasting at least 17 days after exposure to IR. The presence of persistent γH2AX, indicating ongoing repair of impaired DNA, can be used to assess irradiation exposure in fruit flies. A direct and reliable assay using γH2AX as a marker of prior IR exposure in fruit flies has the potential to facilitate domestic and international trade in commodities that have been irradiated for disinfestation.

11.1 INTRODUCTION

Fruit flies are the most economically damaging insect pest of Australian horticulture. Between 2006 and 2009, the average value of fruit fly susceptible production in Australia was approximately AU$5.3 billion and exports of fruit fly susceptible horticulture products were around AU$406.9 million (Abdalla et al. 2012; Hyam 2007; Plant Health Australia 2018). The risk of exotic fruit flies—in the form of eggs, larvae, pupae, or adult—entering and establishing in Australia is increasing (Abdalla et al. 2012; Hallman 2011; Hallman et al. 2011). Phytosanitary treatments, such as fumigation and other chemical and physical (e.g., heat, cold) treatments, are commonly used to disinfest imported and exported commodities of quarantine pests (Hallman 2011; Hallman et al. 2011, 2018). Over the past 40 years, the standard postharvest insect disinfestation chemicals dimethoate and fenthion have provided phytosanitary assurance, but the use of these insecticides has been

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greatly restricted (Richard et al. 2003). Finding alternatives to chemical treatments is necessary to prevent introduction and establishment of exotic pests in new areas (Hallman 2011).

Ionizing radiation (IR) is a safer alternative than fumigation and other chemical and physical (heat/cold) disinfestation methods (Follett 2009, Follett et al. 2011; Hallman 2011; IAEA-TECDOC-1427 2004). Numerous countries use IR to disinfest fruit and vegetables from a multitude of quarantine pests (Richard et al. 2003; Hallman 2011; Hallman et al. 2011), including approximately 30,000 metric tons (and increasing by ~10% each year) of sweet potatoes. Increasing quantities of irradiated tropical fruit, such as mangoes, papayas, litchis, capsicums, and tomatoes, are now successfully being exported from Australia to New Zealand consumer markets (Lynch 2010; Lynch and Nalder 2015).

For biosecurity treatments, fresh produce in finished pallet loads is exposed to a minimum generic dose of 150–400 Gy of IR (e.g., electron beam, X-ray, or gamma ray from cobalt-60) (Follett 2009; Hallman et al. 2011). When IR comes into contact with a cell of a pest insect, it breaks chemical bonds in DNA and other molecules, rendering the insect unable to complete development and to reproduce, and thus preventing the establishment of viable pest populations. Verifying irradiation treatment is difficult because quarantine pests are often found alive during inspection in exported and imported commodities. Currently, the only means of assessing quality of imported and exported fruit is through quarantine audits and treatment facility certification. For commercial disinfestation, a regulatory framework exists with the use of generic irradiation doses for a wide range of pest groups. However, the lack of a reliable test to retrospectively confirm radiation exposure can reduce market confidence in a situation where live pests are detected in exported and imported fruit and costs must be incurred to destroy or export the infested consignment.

11.1.1 Can Gamma-H2AX Be Used as a Biomarker of Phytosanitary Radiation?

On exposure to IR, DNA double-strand breaks (DSBs) are induced in the nuclei of all living cells, inducing a DNA repair mechanism characterized by the phosphorylation of the histone protein H2AX (producing the active form gamma-H2AX [γH2AX]) (Rogakou et al. 1998, 1999). Gamma-H2AX is highly conserved across a wide taxonomic range of organisms and is a well-characterized histone protein known to be responsive to IR-induced DNA DSBs (Downs et al. 2000; Foster and Downs 2005; Redon et al. 2002). Gamma-H2AX assay is a standard and well-established method for biological dosimetry of IR exposure. Quantification of the γH2AX response has been used widely as a highly sensitive and specific assay in radiation biodosimetry and cellular radiosensitivity responses during chemotherapy and radiotherapy and to identify regions of the genome where DSBs fail to repair (Bhogal et al. 2010; Ivashkevich et al. 2012; Redon et al. 2012). However, the γH2AX test has not yet been exploited as a retrospective test for identifying the irradiation status of live insects found in exported or imported consignments of fruit and vegetables.

In the γH2AX assay, the DSB level and corresponding IR dose exposure in the nuclei of cells are measured either by measuring the overall γH2AX protein level or by counting discrete "foci" in individual nuclei, which can be visualized and quantified using numerous methods, including fluorescence microscopy and flow cytometry (Figure 11.1) (Hamasaki et al. 2007; Nakamura et al. 2006; Pilch et al. 2004). Two types of γH2AX foci have been found in cells: the first is transient γH2AX foci that are associated with rapid DSB repair and dephosphorylation of γH2AX to H2AX, usually in minutes to hours (Markova et al. 2007, 2011). The second type of γH2AX foci is residual and tends to persist for days to months (Figure 11.1). The measurement of persistent γH2AX signals has been widely used in many applications in recent years, such as for monitoring cancer patients' response to chemotherapy and radiotherapy, radiation biodosimetry, drug biodosimeters, environmental genotoxicity, and in disease (Siddiqui et al. 2015). A study on mini-pig skin cells showed that γH2AX was significantly elevated in irradiated cells after 70 days post-IR exposure (Ahmed et al. 2012). Another study on mouse skin found γH2AX signals up to 7 days post exposure