

**CLASSICAL BIOLOGICAL CONTROL OF MEDITERRANEAN FRUIT
FLY, *Ceratitis capitata* (WIEDEMANN), (DIPTERA: TEPHRITIDAE):
NATURAL ENEMY EXPLORATION AND NONTARGET TESTING**

A Dissertation

by

MARCIA KATHERINE TROSTLE DUKE

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

May 2005

Major Subject: Entomology

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May 2005

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ABSTRACT

Classical Biological Control of Mediterranean Fruit Fly, *Ceratitis capitata*, (Wiedemann),
(Diptera: Tephritidae): Natural Enemy Exploration and Nontarget Testing. (May 2005)

Marcia Katherine Trostle Duke, B.S., Texas A&M University

Chair of Advisory Committee: Dr. Robert A. Wharton

This work covers stages one through seven (of nine stages) of a classical biological control program for Mediterranean fruit fly (=medfly), *Ceratitis capitata* (Wiedemann). Major research objectives concentrate on stage five (exploration and collection of natural enemies), and stage seven (testing and selecting natural enemies for additional work).

Coffee was collected monthly from three locations in Kenya from November 1997 through July 1999. Four species of tephritid flies and ten parasitoid species were recovered. Four guilds of parasitoids were recorded, and two egg-prepupal endoparasitoids, *Fopius caudatus* (Szépligeti) and *F. ceratitivorus* (Wharton), were discovered. The oviposition behavior of these two species is contrasted. Domination of this tropical parasitoid assemblage by koinobionts is discussed relative to the dominance of temperate fruit-infesting tephritid systems by idiobionts.

Fruit handling procedures were examined for impact on overall percent emergence and specifically percent emergence of flies versus parasitoids. It was determined that stirring samples had a significant positive effect on overall emergence, however daily misting of fruit did not. The only treatment without a significant bias in fly emergence over parasitoids was the stirred/dry treatment. Effects of these results on rearing procedures are discussed.

Host specificity and host suitability of parasitoids reared from coffee were examined via:
(1) association of parasitoids with host flies based on characteristics of the fly puparia from

which parasitoids emerged, (2) rearing of cucurbit infesting tephritids and their parasitoids in Kenya, (3) rearing of flowerhead infesting tephritids and their parasitoids in Kenya and Hawaii, and (4) host range testing of *Psytalia* species in Kenya and Hawaii. These results are discussed in terms of their utility for predicting nontarget effects.

Psytalia concolor (Szépligeti) was shipped to Hawaii and tested against the nontarget gall forming tephritid *Procecidochares utilis* Stone introduced to control the weed *Ageretina adenophora* (Maui pamakani). *Psytalia concolor* failed to attack the gall-forming *P. utilis* both in choice and no-choice tests, but readily attacked tephritid larvae offered in fruit in choice tests.

Recommendations for further testing and release of the parasitoids from Kenya are discussed for Hawaii and Latin America.

To my parents,
Mark and Susan,
who have supported me unconditionally and endured 30 years
of questions, answering when necessary and sending me on my
way when it was mine to figure out alone. Amazingly never
getting angry; though once in a while asking for a short five
minutes of quiet.

To my new husband,
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may he have the patience to support me and endure my
questions for the rest of his lifetime.

To my best friend,
Sunny Ruth, DVM,
this would have not been possible without your friendship, love
and sacrifice.

And

to the Lord,
Jesus looked at them and said, "With man this is impossible, but
with God all things are possible."
Matthew 19:26

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I also appreciate the rest of my committee members, Drs. Jim Woolley, Tom Linton, Marvin Harris and George Teetes. I especially thank Dr. Harris for replacing Dr. Teetes when the latter retired. Their service to my education has been invaluable. I am appreciative to many faculty members in the Department of Entomology for their assistance particularly Drs. Kevin Heinz, Larry Keeley and Ted Wilson. I am also indebted to the professors who have taught my courses and shared their knowledge.

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CHAPTER I

INTRODUCTION

Medfly: An Economic Threat to the United States

The Mediterranean fruit fly (=medfly), *Ceratitis capitata* (Wiedemann) is endemic to tropical, sub-Saharan Africa (Gasparich *et al.*, 1997), and can complete development in the fruiting bodies of over 400 plant species in tropical, subtropical and temperate regions (Copeland *et al.*, 2002; Liquido *et al.*, 1998). Thus, medfly is one of the most widespread and serious pests of edible fruit (Weems, 1981; Liquido *et al.*, 1990, 1991; White and Elson-Harris, 1992). Damage to fruit initiates when a female oviposits under the fruit's outer skin. Eggs hatch one to two days after oviposition and larvae immediately begin feeding on the fruit's pulp. Rapid deterioration results from this feeding and the fruit is quickly rendered unmarketable and inedible.

Through movement of infested fruit during the nineteenth and twentieth centuries, medfly became established in the Mediterranean Region, Hawaii, numerous Atlantic and Indian Ocean Islands, and most major continental regions except Asia and North America (White and Elson-Harris, 1992). It has been estimated that if medfly populations were to establish on the U.S. mainland, it would cost the U.S. economy 1.5 billion dollars per year, not only in reduced crop yields, but also through export sanctions, loss of markets, and treatment costs (USDA/APHIS, 1993).

In an effort to reduce the potential for such enormous economic losses, one current focus

of the Fruit Fly Exclusion and Detection Program within USDA/APHIS/PPQ is to implement programs minimizing the risk of medfly introductions to the U.S. Mainland. Two central goals are suppression of foreign adventive populations and inspection of non-Mainland originating passengers and cargo (USDA, 1997; USDA/APHIS/PPQ, 2002).

The public, especially in urban areas, is vehemently opposed to the current control measures that use insecticide bait sprays as a supplement to sterile insect technique (SIT). In addition to poor public support, concerns about the impact of insecticides on biodiversity and the environment in general have increased the urgency of implementing non-insecticide control programs. Thus, a third goal of the USDA/APHIS policy regarding medfly is to search for control tactics that will decrease the amount of harsh, broad-spectrum insecticides released into the environment during suppression activities in adventive areas and eradication of incipient introductions on the U.S. Mainland (USDA, 1997; USDA/APHIS/PPQ, 2002). With attention focused on alternatives, there has been a renewed interest in biological control (Wharton, 1989; Knipling, 1992; Headrick and Goeden, 1996; Sivinski, 1996; Purcell, 1998), and in particular the use of parasitoids as an integral part of an IPM approach to the effective management of medfly (USDA, 1997).

Current Perspectives in Medfly Adventive Areas That Directly Affect the United States

Central America

U.S. government involvement in foreign eradication programs began in the late 1970's when medfly's rapid range expansion from Central America toward the southern U.S. began to threaten U.S. agriculture. In response to medfly's movements, the U.S. joined the Mexican and

Guatemalan governments in a cooperative effort known as MOSCAMED. MOSCAMED's objective was to halt the northward spread of medfly by eradicating the insect from México and Guatemala through a combination of malathion bait sprays, SIT releases and regulatory procedures. Largely due to Mexican efforts, MOSCAMED declared in 1982 that medfly had been eradicated from México and control efforts were pushed into Guatemala (CICP, 1988).

Currently, chemical eradication programs and SIT releases are used in an attempt to maintain México as a fly free zone. Due to the increasingly "leaky" barrier zone along the Mexican/Guatemala border a biocontrol project directed against medfly and other tephritid pests was initiated, joining collaborators in the U.S. with those from several foreign countries. One goal of this program was to lay the scientific groundwork for introducing host-specific biocontrol agents that could be used in conjunction with SIT in Guatemala. Introduction of biocontrol agents should lead to less insecticide use and possibly reduce the threat of medfly's northward movement by establishing a natural barrier in the mountainous region between México and Guatemala.

Hawaii

The area that is now the state of Hawaii has been a focal point for introductions of biocontrol agents since the early 1900s. At least a dozen parasitoids have been successfully established (Clausen, 1978), including several that have contributed substantially to the reduction of fruit fly pest populations. Parasitoids currently used for augmentative biocontrol of medfly are capable of attacking a wide of range of tephritid hosts including Oriental, melon, and solanum fruit flies, as well as medfly. These species of tephritid parasitoids, routinely cultured

in Hawaii and elsewhere, are potentially available for augmentative programs in other areas of the world.

Knipling (1992) developed the theoretical framework for expanding the role of classical biocontrol to an inundative, area wide, augmentative approach for eradicating fruit fly pests. However, none of the parasitoids currently in culture are native to sub-Saharan Africa, medfly's native home, nor were any of these initially obtained from medfly in areas where medfly is adventive. It therefore seems advisable, in developing this type of an augmentative program for medfly, to search for host specific parasitoids (those that have the least probable propensity for nontarget effects) by exploring areas where medfly is endemic.

Since medfly populations are significantly higher in some parts of Hawaii than others, there continues to be interest in exploring the native home of medfly for co-evolved parasitoids that could be introduced to Hawaii. Newly discovered or previously untested parasitoids from Africa may prove beneficial for reducing populations in those parts of Hawaii where existing parasitoids (primarily of Asian origin and originally introduced to control Oriental fruit fly) are less effective.

Essential Elements of a Classical Biological Control Program

Based on the focus of USDA as mentioned above and the constant threat of medfly introduction to the United States, this thesis represents a resurrection of the search for medfly biological control agents. Van Driesche and Bellows (1996) list the essential elements of a successful classical biological control program. The nine steps feed directly into one another, with careful completion of each step providing needed information for successive steps. This project will follow these steps. The nine steps include: (I) target selection and assessment, (II)

preliminary taxonomic and survey work, (III) selecting areas for exploration, (IV) selecting natural enemies for collection, (V) exploration, collection and shipment of candidate natural enemies, (VI) quarantine and exclusion, (VII) testing and selecting natural enemies for additional work, (VIII) field colonization and evaluation of effectiveness, and (IX) agent efficacy and program evaluation.

This research program takes the reader through stages one to seven of a classical biological control program for medfly. Although all seven steps are addressed in this program, the major research objectives concentrate on steps five and seven. Suggestions and recommendations are also made for addressing stages eight and nine.

Social Responsibility: Assessing Nontarget Effects

A new element, non-target testing, has been added to the protocol of classical biological control since the last time a concerted effort was made to look for medfly natural enemies in sub-Saharan Africa (Steck *et al.*, 1986, Gilstrap and Hart, 1987). As concerns about nontarget effects have dominated the biological control literature in recent years (Howarth, 1983, 1985, 1991, 2000; Lockwood, 1993, 1997; Simberloff and Stiling, 1996; Purcell *et al.*, 1997; Louda *et al.*, 1997, 2003; Follett and Duan, 2000; Louda and O'Brien, 2002; Stiling, 2002; Hoddle, 2004; Louda and Stiling, 2004) it is essential to consider potential nontarget impacts before any parasitoids can be released in a new area. The work of J. Duan, R. Messing and their colleagues (Messing and Jang, 1992; Duan *et al.*, 1996, 1997a, b; 1998, 2000; Duan and Messing, 1996, 1997, 1998, 1999, 2000a, b, c), in particular, has highlighted the need for more thorough assessment of parasitoids prior to their release against tephritid pests.

Hoddle (2004) states, it is apparent that selection of natural enemies with narrow host ranges and high host fidelity protects nontarget species because physiological, behavioral, ecological or geographical attributes make native organisms unsuitable for exploitation by introduced natural enemies (Strand and Obrycki, 1996, Frank, 1998). And, although it can be argued that host range expansion through evolutionary adaptation by specialized natural enemies is theoretically possible, it is a rare phenomenon (Nechols *et al.*, 1996; Onstad and McManus, 1996) that no one may ever be able to predict using conventional tests. Thus, in today's ever changing social climate, high levels of host specificity do the most to ensure that the perceived benefit of controlling the host will not change unless the perceived value of the pest changes (Hoddle, 2004). Assessing nontarget effects thus must be done prior to all releases to address the social responsibility of practicing biological control.

Foreign Exploration: A Return to the Aboriginal Home of Medfly

Few attempts have been made to study the parasitoid community associated with medfly in its aboriginal home. In fact, the only parasitoids established from medfly's aboriginal home are from the 1913 and 1914 foreign explorations (Clausen, 1978). This has resulted in a paucity of knowledge about the basic biology of the parasitoids in this community. Modern advances in transportation and a renewed interest in parasitoid biological control demand a return to medfly's aboriginal home to examine the biology of all parasitoids associated with medfly (Gilstrap and Hart, 1987; Wharton, 1989). Since medfly originated in tropical sub-Saharan Africa (Steck *et al.*, 1996; Gasparich *et al.*, 1997), this is a logical place to begin further investigations for medfly parasitoids.

Kenya was selected as the primary location for foreign exploration in this project because of the availability of excellent laboratory facilities at the International Centre for Insect Physiology and Ecology (ICIPE), access to coffee plantations nearby, and as part of medfly's aboriginal home, the area should yield parasitoids with a propensity to be host specific. Kenyan coffee should yield biological control agents that have a greater propensity for being host specific for several reasons. One is the possibility of the coevolution of medfly and parasitoids in coffee. Coffee is native to sub-Saharan Africa (Smith, 1985) and one of the preferred hosts of medfly (Wharton *et al.*, 2000; Copeland *et al.*, 2002). In addition, Abasa (1973) confirmed that medfly and other tephritids are readily collected from coffee in Kenya. The second reason, few insecticides are used on coffee plantations, as fungicides are more important to the health of the crop (Bardner, 1985). Thus, we can sample coffee from large commercial plots in which the natural enemy component has not been devastated and has rarely been influenced by pesticide applications.

Third, the project will concentrate on the collection of koinobiont endoparasitoids. Koinobiont endoparasitoids tend to exhibit greater host specificity than ectoparasitoids, as they must overcome the immune responses of an active host to complete their own development (Gauld and Bolton, 1988). Although, Hawkins *et al.* (1990) stated that the majority of parasitoid species of endophytic hosts support idiobionts (potential generalists), and exophytic hosts support mainly koinobionts (potential specialists), Hoffmeister and Vidal (1994) refute this trend for fruit fly parasitoids. Hoffmeister and Vidal (1994) contend that fruit-infesting species are attacked mainly by koinobiont larval parasitoids, to a lesser extent by pupal parasitoids, and only by a few idiobiont larval parasitoids.

Objectives

I set the objectives of this research within the context of a classical biological control program for medfly. Coupled with other non-invasive methods of pest control such as SIT based IPM programs, host specific parasitoids could provide ecologically sound possibilities for medfly control in several foreign countries as well as in Hawaii. The first objective addresses facets associated with the exploration for natural enemies in the aboriginal home of the pest, including characterization of the biology of previously unknown species. The second and third objectives examine two separate but closely related issues of primary importance for every biological control program undertaken today: the host specificity and nontarget impact of a candidate natural enemy. The specific objectives of this research are:

1. To characterize the parasitoid guilds associated with fruit-infesting tephritids in coffee in Kenya and to test the hypothesis that koinobiont parasitoids dominate this assemblage (Chapter II).
2. To test the hypothesis that parasitoids reared from coffee in central Kenya are host specific to medfly on coffee in Kenya (Chapter III).
3. To test the hypothesis that *Psytalia concolor* (Szépligeti), a parasitoid of medfly collected from coffee in Kenya, will attack the nontarget, gall-forming tephritid, *Proceidochares utilis* Stone in Hawaii (Chapter IV).

CHAPTER II

GUILD STRUCTURE OF THE PARASITOIDS OF MEDFLY AND RELATED TEPHRITIDS IN KENYAN COFFEE: A PREDOMINANTLY KOINOBIONT ASSEMBLAGE* †

Introduction

Medfly and Related Tephritids

The Mediterranean fruit fly (=medfly), *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae), is one of the most polyphagous and important pests of edible fruits worldwide (Weems, 1981; Liquido *et al.*, 1991, 1998; Copeland *et al.*, 2002). The Natal fly is an equally serious pest of many edible fruits, but is limited in distribution to Africa, Mauritius, and La Réunion (Commonwealth Institute of Entomology, 1985; White and Elson-Harris, 1992). The common name, Natal fly, has been applied historically to *Ceratitis rosa* Karsch, but recent work by De Meyer (2001) revealed the existence of two species residing under this name: *C. rosa* and *C. fasciventris* (Bezzi).

Medfly is indigenous to Africa (Silvestri, 1913). Steck *et al.*, (1996) and Gasparich *et al.* (1997) suggested that the high genetic diversity of populations from tropical, sub-Saharan Africa (Fig. 1) provides strong evidence for the origin of medfly from this region. Medfly is the most

*Part of this chapter is reprinted with permission from “Biosystematics of the *Psytalia concolor* Species Complex (Hymenoptera: Braconidae: Opiinae): The Identity of Populations Attacking *Ceratitis capitata* (Diptera: Tephritidae) in Coffee In Kenya” by S. W. Kimani-Njogu, M. K. Trostle, R. A. Wharton, J. B. Woolley, and A. Raspi, 2001. *Biological Control* **20**, 167-174. ©2001 by Academic Press.

†Part of this chapter is reprinted with permission from “Parasitoids of Medfly, *Ceratitis capitata*, and Related Tephritids in Kenyan Coffee: a Predominantly Koinobiont Assemblage” by R. A. Wharton, M. K. Trostle, R. H. Messing, R. S. Copeland, S. W. Kimani-Njogu, S. Lux, W. A. Overholt, S. Mohamed, and J. Sivinski, 2000. *Bulletin of Entomological Research* **90**, 517-526. ©2000 by CAB International.

widespread of the fruit-infesting tephritid pests, having been introduced to Australia, Hawaii, the Mediterranean Region, most of tropical America and numerous islands (Fig. 2) (White and Elson-Harris, 1992). An enormous amount of information has been published on medfly, but much of our knowledge comes from efforts to control this pest in areas where it has been introduced (see Quaintance (1912) and Back and Pemberton (1918) for earlier studies and Fletcher (1989) for a more recent review). Relatively few studies have been conducted on medfly in regions of its presumed origin (e.g. Abasa, 1973). As both medfly and Natal fly are native to sub-Saharan Africa, data on factors that may limit population growth in the aboriginal home should be of some value to pest management programs.

In East Africa, coffee cherries (especially *Coffea arabica* L.: Rubiaceae) are an important reservoir for both *C. capitata* and *C. fasciventris*. It is important to note that although coffee grows in a tropical climate, the occurrence and relative abundance of medfly and other tephritids found in coffee varies regionally as well as seasonally (Greathead, 1972; Abasa, 1973; Waikwa, 1978; Steck *et al.*, 1986; Mukiyama and Muraya, 1994). In addition differences in tephritid species composition among coffee species have also been noted (Greathead, 1972; Mukiyama and Muraya, 1994).

Tephritids usually cause little or no economic damage in coffee (Hamilton, 1967; Le Pelley, 1968; Abasa, 1973), facilitating the acquisition of samples under relatively insecticide-free conditions. Coffee is thus a potentially useful host for examining the effects of natural enemies and other factors on populations of medfly and the related pest *C. fasciventris*. Nevertheless, some economic damage can occur when fly densities are so excessively high that a significant amount of oviposition occurs in unripe cherries (a non-preferred stage) (Back and Pemberton, 1918).

Native Parasitoids of Medfly and Other Fruit-Infesting Tephritidae in Africa

Tephritid Fruit Flies and Their Braconid Parasitoids: A General Overview

Medfly and other tephritid fruit flies are attacked by several species of opiine Braconidae (Hymenoptera) as well as other parasitoids. The braconid parasitoids that attack medfly larvae have two strategies for development: koinobiont endoparasitoid and idiobiont ectoparasitoid. In those species utilizing the koinobiont endoparasitoid strategy, females oviposit into the fruit fly larvae without killing the host. The host continues normal development, including exiting the fruit and forming a puparium in the soil. After formation of the host's puparium, but prior to pupation, the parasitoid larva consumes the insect host and completes development, eventually emerging from the host puparium. Other parasitoid species utilize the idiobiont ectoparasitoid strategy. In this strategy, adult females prevent further development of the host larvae by paralyzing it immediately prior to oviposition. The female then lays an egg on or in the general vicinity of the host. The larva consumes the host, spins a cocoon, and pupates inside the fruit.

Data on the parasitoids and other natural enemies of East African, coffee-infesting tephritids are largely lacking. Greathead (1972) recorded several parasitoids of *Trirhithrum coffeae* Bezzi (a ceratitidine tephritid) in robusta coffee (*Coffea canephora* Pierre ex Froehner) from Uganda, and this is undoubtedly the best quantitative data available for East Africa. Unfortunately, there were very few specimens of medfly and Natal fly in his samples. Other reports of parasitoids from coffee samples collected in East Africa are largely anecdotal (Bianchi and Krauss, 1937; Clausen *et al.*, 1965; Waikwa, 1978). Steck *et al.* (1986) provided a quantitative assessment of parasitism of tephritids in coffee from West Africa, but medfly was

also rare in their samples, and Natal fly was absent. Thus, most of our knowledge about parasitoids of sub-Saharan fruit-infesting tephritids comes from fruits other than coffee, and where medfly was rare or absent (Silvestri, 1913, 1914, 1915; Fullaway, 1914; Bridwell, 1914; Bianchi and Krauss, 1936; Van Zwaluwenburg, 1936; Clausen *et al.* 1965; Neuenschwander, 1982). Table 1 lists the species most commonly reared from wild fruits in sub-Saharan Africa prior to the beginning of this project. In addition to those species listed in Table 1, many other parasitoids were reared, but never adequately identified and still others, though obtained from fruit collections, were never confirmed as being reared from tephritid fruit flies (Bianchi and Krauss, 1936; Van Zwaluwenburg, 1936).

Almost all of our information regarding the parasitoids listed in Table 1 comes from sampling programs conducted during the foreign exploration phases of classical biological control programs. The few notable exceptions (e.g. *Psytalia concolor* (Szépligeti)) are treated in the sections below. Greathead (1976) and Gilstrap and Hart (1987) provide more exhaustive tabular summaries; Clausen (1978) and Gilstrap and Hart (1987) provide additional details on the sampling programs.

Taxonomy of *Psytalia concolor* Species Complex

One of the dominant parasitoids attacking tephritids in coffee in central Kenya is a species of *Psytalia* that appears morphologically identical to the common Mediterranean species *P. concolor* and the South African species *P. humilis* (Silvestri). To use this native Kenyan species more effectively in biological control, some long-standing problems in the systematics of the *Psytalia concolor* species complex need to be solved. This species complex includes a series of nominal species that have been separated from one another primarily by subtle differences in

the length of the ovipositor and the size of the eye (Silvestri, 1914; Wharton and Gilstrap, 1983). This group includes (among others) *P. concolor*, *P. humilis*, and *P. perproxima* (Silvestri). These three parasitoids have frequently been treated as synonyms of one another, but the matter is far from being resolved (Fischer, 1987; Wharton, 1987, 1988, 1997). Resolution of these particular taxonomic problems is of interest because members of the *P. concolor* species complex have been used extensively in both classical and augmentative biological control programs directed against tephritid pests. *Psytalia humilis* was at one time the most effective parasitoid introduced to Hawaii against medfly (Pemberton and Willard, 1918a; Willard and Mason, 1937), and *P. concolor* is still routinely used in the Mediterranean Region for augmentative releases against olive fly (Raspi, 1995). For a detailed review of taxonomic problems associated with the *P. concolor* species complex, see Kimani-Njogu *et al.* (2001). This paper contains the work that I did in collaboration with Susan Kimani-Njogu on the differentiation of various *Psytalia* populations using morphometrics and hybridization experiments.

Parasitoid Assemblages

Fruit-infesting tephritids are exploited in a variety of ways by numerous parasitic Hymenoptera, most notably those in the families Braconidae, Chalcididae, Diapriidae, Eulophidae, Eupelmidae, Eurytomidae, Figitidae (Eucoilinae), Ichneumonidae, and Pteromalidae (Clausen *et al.*, 1965; Wharton *et al.*, 1981; Hoffmeister, 1992). Nearly all of these parasitoids attack the host when it is concealed inside the fruit (as an egg or larva) or in the substrate (as a puparium). Mills (1994) proposed a classification of parasitoid guilds that provides a convenient method for organizing data on parasitoid biology and placing this information in the context of

community structure. Mills' (1994) classification focuses on the host stage attacked, host stage from which the parasitoid emerges, and koinobiont versus idiobiont strategies. While guild placement can be extracted from publications on *P. concolor* and a few other Afrotropical species successfully introduced for biological control (Silvestri, 1913; Pemberton and Willard, 1918a; Willard and Mason, 1937; Feron, 1954; Biliotti and Delanoue, 1959; Stavraki-Paulopoulou, 1966; Neuenschwander, 1982; Ramadan and Wong, 1990; Raspi and Loni, 1994; Purcell *et al.*, 1996; Loni, 1997; Canale, 1998; Canale and Raspi, 2000; Raspi and Canale, 2000), several east African tephritid parasitoids still have not been adequately characterized.

An issue associated with structure of parasitoid assemblages (numbers of idiobionts versus koinobionts) on concealed hosts has been broadly addressed by Hawkins *et al.* (1990) and with respect to fruit-infesting tephritids by Hoffmeister (1992) as well as Hoffmeister and Vidal (1994). Their carefully documented findings, that idiobionts outnumber koinobiont parasitoids, are based largely on temperate communities and may not be applicable to tropical systems, since many of the tropical tephritids are multivoltine.

The overall goal of this chapter is to characterize the assemblage of parasitoids attacking tropical, fruit-infesting tephritids, specifically those from coffee in Kenya. This goal was accomplished within the context of a multi-institutional, collaborative exploration for medfly natural enemies in Kenya. This is the most detailed survey to date of this pest from a preferred host in a region of endemism. The results of the survey are presented and then used to develop a complete picture of the guild structure of the parasitoids of tephritids infesting coffee in Kenya. Finally, tropical and temperate tephritid parasitoid assemblages are compared by testing the hypothesis that koinobionts dominate the parasitoid assemblage of medfly and related tephritid in coffee.

Materials and Methods

The acquisition of parasitoids through international collaboration for use in a medfly classical biological control program is beset with numerous challenges. In this regard, the determination of guild structure proved to be a convoluted process, requiring the resolution of several peripheral issues. As a result, I address this chapter's main objective within the context of three separate, but interrelated goals. These goals are: (1) collaborating with ICIPE staff to collect and process samples of coffee (a preferred host of medfly) in Kenya (an endemic area for medfly) in order to obtain candidate wasps for classical biological control of medfly; (2) determining biological attributes of collected wasps to complete the guild structure, test the idiobiont/koinobiont hypothesis, and facilitate rearing of parasitoids for biological control programs in areas where medfly is adventive; and (3) determining how coffee sampling practices impact estimation of infestation levels by flies and rates of parasitization by wasps.

Collection of Seasonal Data on Tephritid Fruit Flies and Their Parasitoids

Many people were involved in collecting and processing the coffee samples in Kenya as well as processing the shipments of puparia sent to Hawaii. As I was the only person who worked for an extended period of time in both places, I have compiled the general coffee data in Appendix C and have included the materials and methods for all collections here. Results of special collections I undertook to specifically answer questions concerning guild structure or sample handling practices are presented in the results section of this chapter.

Coffee Sampling Sites

Principal Collection Sites

Samples of coffee were obtained from three principal sites in Kenya: Rurima, Ruiru and Koru (Fig. 3). Coffee was chosen for study as it provides a readily available, approximately year-round source of medfly and its natural enemies. The sites were selected by project leaders Wharton, Overholt, and Sivinski on the basis of results from preliminary samples, which yielded a somewhat different parasitoid complex at each of the three sites. Rurima farm is a commercial coffee plantation located in the southern part of central Kenya, near Embu, at $0^{\circ}38.39'S$, $37^{\circ}29.69'E$, and an elevation of *c.* 1228m. The other two sites are experimental field stations of the government-run, Coffee Research Foundation (CRF). Most of the coffee at Rurima is unshaded and none of the coffee from these two other sites is shaded. CRF-Ruiru (hereafter referred to as Ruiru) is also located in south central Kenya, at $1^{\circ}5.72'S$, $36^{\circ}54.22'E$, and an elevation of 1609 m. It is approximately 15 km north of the International Centre of Insect Physiology and Ecology (ICIPE) laboratories where all coffee samples were processed. CRF-Koru (hereafter referred to as Koru) is located in the western Kenyan highlands at $0^{\circ}8.16'S$, $35^{\circ}16.87'E$, and an elevation of 1513 m. Rurima and Ruiru are located in the central highlands, on the eastern side of the Rift Valley and form part of a more or less continuous band of commercial coffee farms. The major coffee season in the Ruiru area is from October to December. A smaller coffee-harvesting period occurs from April to July. Koru is located on the western side of the Rift Valley where coffee farming is far less prevalent and there is more tea and sugarcane. Koru has one long coffee season, with most of the coffee produced from July to November. For all samples, coffee cherries were handpicked and returned to an ICIPE laboratory in Nairobi on the same day (Ruiru and Rurima) or the following day (Koru).

Alternate Sample Localities

Although mature coffee plants are capable of bearing fruit all year long, coffee cherries are routinely stripped from all plants as a means of reducing coffee-pest populations during non-peak seasons. Stripping occurred in mid-December at all three principal sites, resulting in little or no coffee available for sampling during the first few months of the year. In an attempt to fill this gap, small samples of arabica coffee were sometimes obtained from adjacent farms where stripping was more sporadic. Farms adjacent to the Coffee Research Foundation provided nearly all of the coffee at Ruiru from November 1997 through July 1998.

Coffee Varieties Collected at Sample Sites

Robusta coffee was available at both Ruiru and Koru, but sampling was restricted to arabica coffee to facilitate among-site comparisons. Earlier reports (e.g. Greathead, 1972) suggest that there are distinct differences in tephritid species composition in robusta and arabica coffee. Most coffee cherries collected from Koru were collected from the variety K7 or Ruiru 11. K7 is a coffee berry disease (CBD) resistant cultivar. Ruiru 11 is a CBD and rust resistant variety of arabica coffee developed at the Ruiru CRF. The coffee collected at Ruiru was collected from a “museum” plot that had arabica varieties from all over the world. The variety of coffee collected at Rurima is unknown.

Meteorological Data

Precipitation and mean maximum and minimum temperatures are given in Wharton *et al.* (2000) for the principal sites. Ruiru and Rurima have similar rainfall patterns with both a long and a short rainy season each year. Koru receives more rainfall than Ruiru or Rurima, with a distinct peak during the long rainy season from March to May. Rurima is both drier and warmer than the other two sites (Wharton *et al.*, 2000).

Coffee Sampling Periods

Samples were taken from the three localities, beginning with the first collection made by Wharton, Overholt and Sivinski in November 1997. Subsequent collections were made by me when I was in Kenya (Appendix D) and otherwise by the fruit fly project staff at ICIPE. These routine samples were completed in November 1999, however data are not available for all months due to a variety of sampling and processing difficulties that occurred mostly during my absence.

Although each site could not be sampled every month during this period, arrangements were made to sample the sites as often as possible. These routine samples provided phenological data, information on levels of infestation and an overall picture of parasitoid and fly composition at each site. Material reared from these samples was also used for the initiation of cultures for the biological control efforts in Hawaii and Guatemala and for laboratory experiments. (See sections below and Materials and Methods sections of Chapters III and IV.)

In addition to the routine samples, I collected and processed several additional samples from August to November 1999. Some of these samples were used to calculate infestation rates,

while others were used to survey for pupal and egg parasitoids. In November of 1999 an additional, 40kg sample was collected from Koru to examine the effects of different processing or sample handling procedures on the emergence of flies and parasitoids.

Sample Processing

Sample Size

Sample sizes varied in weight from 1 to 10 kg, depending on two unrelated factors: 1) coffee availability and 2) size of coffee cherries collected. When scales were available at the CRF stations, samples were weighed immediately after picking. Otherwise, weights were estimated as follows: a 5 kg sample was weighed and placed into a 20L bucket at the start of the sampling program. A fill line was then drawn on the bucket at the level reached by the 5 kg sample. This line, in subsequent samples, was used as the measure for 5 kg samples of coffee. Later measurements using a precision balance showed this estimation procedure had an error rate of up to 10%, depending largely on the size of the coffee cherries.

Sample Processing Procedures

Technical staff of the fruit fly program at ICIPE processed approximately 85% of the coffee samples. Trostle processed the remaining coffee samples.

Conditions in the laboratory at ICIPE reflected ambient outdoor temperature and relative humidity in the shade. Samples were held in either 60 X 48 X 60 or 60 X 88 X 60 cm, wooden-bottomed and framed rearing cages covered on three sides and the top with fine white mesh and in the front by a sheet of removable Plexiglas. Fruits were distributed between two stacked, plastic rearing trays, each with slits in the bottom through which larvae could drop to moistened

sand at the bottom of the cage. When the fruit started to desiccate, it was sprayed with water, then rolled and mixed by hand to ensure all fruits were moistened. The sand at the bottom of the cage was sieved at 12–14 days and again five days later. Coffee was thrown away after 19 days. Half of the puparia obtained from these two sievings were placed in Petri dishes and held in smaller rearing cages for emergence of flies and parasitoids. The other half of the puparia was shipped to Hawaii.

Shipments to Hawaii

A sub-sample of the puparia obtained from the first sieving (approximately half of the puparia collected) was shipped to the Hawaii Department of Agriculture (HDOA) quarantine facility. Splitting the sample served two purposes: facilitating culture of parasitoids using the medfly mass-rearing facilities in Hawaii and providing a safeguard (at ICIPE) against losses incurred through shipping to Hawaii.

The puparia were shipped under permit to the Honolulu International Airport. There, the specially packaged puparia were picked up and immediately transported to the HDOA Quarantine facility. Inside the Quarantine facility, the puparia were further segregated to protect other projects in the facility as well as to prevent escape of new tephritid fruit flies into the Hawaiian environment. To accomplish this, all puparia were kept in Petri dishes inside a Plexiglas opening cage that was inside a special screened-in-room.

Until December of 1998, when he left the project, Nathan Peabody processed the puparia sent to the Hawaiian Quarantine Facility. From January to July 1999, Trostle processed these Hawaiian shipments. In August of 1999, Trostle traveled to Kenya and shipments to Hawaii ceased for the time being as there was no one in Hawaii to process the Hawaiian portion

of the sample. However, the samples were still split in half and processed separately in Kenya for the remainder of 1999.

Handling of Puparia from Coffee Collections at ICIPE and Hawaii

At ICIPE, puparia obtained from fruit samples were transferred to smaller cages with fine mesh on at least two sides. Emerging insects were provided with water-soaked cotton wool, honey droplets, and a yeast/sugar diet. Adult tephritids emerging at ICIPE were held for about five days then killed and pinned, or identified while alive and used to establish and maintain colonies (medfly and *C. fasciventris*). Initially, at ICIPE, hymenopterous parasitoids were killed immediately in 95% ethanol and kept for subsequent identification. Beginning in August of 1998, with the arrival of Trostle in Kenya, the focus shifted from preserving parasitoids for identification to elucidating the biology of the parasitoids and establishing parasitoid colonies. (See sections on Characterization of Guild Structure, Oviposition Cues, and Parasitoid Culture Initiation in this chapter.)

In Hawaii, all emerging flies were killed immediately inside the opening cage in quarantine to prevent any chance of escape. When parasitoids emerged they were moved to screen cages inside the screen room. Here, under sub-optimal light conditions, further attempts were made to elucidate the biology of the parasitoids in order to establish lab cultures. The USDA Fruit Fly Rearing facility at Manoa provided tephritid flies and their eggs for these rearing attempts.

Shipments to Guatemala

Beginning in late 2000, shipments of puparia collected from coffee were made to the USDA International Services quarantine facility in Petapa, Guatemala by the ICIPE technical

staff. Miguel Lopez oversaw efforts to initiate cultures of the parasitoids in the quarantine facility in Guatemala based on information provided by Trostle on the host stage attacked by each Kenyan parasitoid species. In January 2001, Trostle traveled to Guatemala to resolve problems associated with identification of live individuals from Kenya so that pure cultures of each could be established. While in Guatemala, she conducted experiments on the oviposition cues and behavior of *Fopius caudatus* (Szépligeti) and *F. ceratitivorus* Wharton. (For Materials and Methods, see sub-sections on Oviposition Cues under Characterization of Guild Structure in this chapter.)

Estimates of Infestation Rates

Despite repeated efforts, it was impossible to obtain reliable estimates of infestation rates for coffee samples collected and processed during my absence from Kenya. Thus, infestation rates per fruit were obtained only once in August 1998 from Ruiru (my first field season) and again from August–September 1999 (my second field season). Infestation rates were estimated in each case by dissecting several hundred field-collected cherries and recording the numbers of tephritid eggs and larvae in each fruit. Infestation rates were measured twice at Ruiru and Rurima and one time at the Koru location. For use in scaling the coffee samples so comparisons could be made amongst them, coffee cherries were weighted on a precision balance so infestation rates could be calculated on per fruit and per kilogram basis. Average number of coffee cherries per kg was measured seven times: two times each for the Koru and Rurima sites, and three times for the Ruiru site.

Assessment of Phenology from Coffee Samples

The equations below approximate the number of fly and parasitoid individuals present if emergence from all coffee collections samples is 100%. This approximation was made so that the two halves of each sample (Kenya and Hawaii) could be recombined to represent the initial sample taken in Kenya. After combining sample portions, the data were further normalized by dividing the adjusted total number of flies or parasitoids by the number of kilograms collected for each sample. These adjustments remove shipping bias as well as the difficulty of comparing or combining samples with differing percent emergence. It also provides a more accurate picture of the number of flies and parasitoid present per kilogram at each site.

The total puparia emerged, T_{PE} , is calculated by

$$T_{PE} = F_E + F_{DOE} + P_E, \quad [1]$$

where F_E is the total number of flies eclosed, F_{DOE} is the number of flies dead on emergence (These flies partially emerged from, but did not exit the puparium. Often their species identity could not be determined, but it was obvious they were flies and were thus counted as dead on emergence) and P_E is the total number of parasitoids eclosed. The percentage of flies in the eclosed portion of the sample, F_{PE} is calculated by Eq. [2]

$$F_{PE} = (F_E + F_{DOE}) / T_{PE}. \quad [2]$$

While the total number of unemerged puparia is known from physical sample counts, it is not known what part of this value can be attributed to flies. Equation [3],

$$F_U = F_{PE} * T_{PU}, \quad [3]$$

is used to estimate the total number of flies unclosed, where F_U is the total number of flies unclosed and T_{PU} is the total number of puparia unclosed in the sample. To adjust for percent emergence, an eclosion index, the *Ratio for % Eclosion Adjustment (Fly)*, is calculated as

$$\text{Ratio for \% Eclosion Adjustment (Fly)} = (F_U + F_{DOE}) / F_E. \quad [4]$$

where the species identities of both F_U and F_{DOE} are unknown.

Multiplying the *Ratio for % Eclosion Adjustment (Fly)* by the number of flies emerged for a given species, FEx_i , produces $FUNKx_i$, an estimate of the unknown flies (flies unclosed + flies dead on emergence) for the given species, x_i . In Eq. [5],

$$FUNKx_i = FEx_i * \text{Ratio for \% Eclosion Adjustment}, \quad [5]$$

x_i is the value represented by each fly species. Knowing $FUNKx_i$, the estimated number of unknown flies for a given species, fly species can be adjusted to represent 100% emergence by

$$FAPEx_i = FUNKx_i + FEx_i \quad [6]$$

where x_i is the value represented by each fly species and $FAPEx_i$ is the estimated number of the i th fly species at 100% eclosion. For each Hawaiian or Kenyan processed sample, Eq. [7],

$$\sum_i FAPEx_i = \sum_i FUNKx_i + \sum_i FEx_i \quad [7]$$

where x_i is the value for each species, gives the value of all fly species at 100% emergence.

After each sample part was adjusted to represent 100% emergence, samples with both Hawaiian and Kenyan components were summed. (If only one portion of the sample was available, it was included in further calculations as a stand-alone entity.) Once samples were combined, the data were normalized for sample size by Eq. [8],

$$\text{Flies per Kg (Single Species)} = FAPEx_i / \text{Kgs Collected in sample.} \quad [8]$$

To normalize the number of flies in the entire sample, Eq. [9],

$$\text{Flies per Kg (All Species)} = \sum_i FAPEx_i / \text{Kgs Collected in Sample} \quad [9]$$

normalizes the data to facilitate overall comparisons among samples and Eq. [8] allowed comparison within samples as well as among samples for each species of fly.

An identical set of equations was used to facilitate comparisons of parasitoids within and among samples. Substitution of P for F and y_i for x_i in the above equations gives the following:

$$P_{PE} = P_E / T_{PE}. \quad [10]$$

$$P_U = P_{PE} * T_{PU}, \quad [11]$$

$$\text{Ratio for \% Eclosion Adjustment (Parasitoid)} = P_U / P_E. \quad [12]$$

$$PUNKy_i = PEy_i * \text{Ratio for \% Eclosion Adjustment (Parasitoid)}, \quad [13]$$

$$PAPEy_i = PUNKy_i + PEy_i \quad [14]$$

$$\sum_i P A P E y_i = \sum_i P U N K y_i + \sum_i P E y_i \quad [15]$$

$$\text{Parasitoids per Kg (Single Species)} = P A P E y_i / \text{Kgs Collected in sample.} \quad [16]$$

$$\text{Parasitoids per Kg (All Species)} = \sum_i P A P E y_i / \text{Kgs Collected in Sample.} \quad [17]$$

The results of the coffee sampling data are presented in Appendix C.

Assessment of Sample Processing Procedures

Dissections of Coffee Cherries in Insectary

Late in the program, I discovered that after normal processing of fruit to obtain puparia, some fruit still contained puparia despite the fact that these tephritids normally exit the fruit to pupate in the soil. As the fruit was normally discarded after sand beneath the fruit was sieved two times to extract the puparia, the levels of infestation of the coffee cherries were apparently being underestimated.

To determine the relative percentage of puparia remaining in the fruit, 100 fruits from the 15.ix.99 Rurima sample were dissected after the second sieving and all puparia remaining in the fruit were counted. Using the weight of the sample and the number of cherries per kilogram in this sample, the number of larvae pupating inside the fruit was estimated for the entire sample. Since many of these puparia were inadvertently damaged during extraction of puparia from the dried, shriveled fruit, no attempt was made to determine the relative percentage of flies and parasitoids in these puparia.

Examination of Sample Handling Practices

To examine the influence of sample handling practices on percent emergence of flies and parasitoids from puparia a four-part experiment was conducted. It was hypothesized that the normal sample handling practices were adversely affecting percent emergence of the samples; and might also be causing “hidden” effects such as skewing the ratio of flies to parasitoids by preferentially causing a lower emergence rate of parasitoids.

To test these hypotheses, forty, 0.5 kg samples were isolated in small fruit holding cages. These cages consisted of plastic containers, 14 X 14 X 20.3 cm, with holes cut in the lids. The holes cut in the lids were covered with organza. The inside bottom of the cage was covered with sand to serve as a larval pupation site. To hold the coffee cherries above the sand, pieces of welded wire mesh (1.5 X 1.5cm mesh) were cut to fit inside the cage. These pieces of wire were suspended at approximately the midpoint on the wall of the cage. For each cage, 0.5 kg of coffee cherries was placed on the mesh. One of four treatments was assigned to each cage. These four treatments were: 1) misted with water and stirred each day, 2) not misted with water, but stirred each day; 3) misted with water each day but not stirred; and 4) not misted with water and not stirred. The first treatment (misted with water and stirred each day) mimicked normal sample handling practices and served as the control. At the end of the experiment all puparia were counted and all unemerged puparia were dissected to determine whether they contained a fly or a parasitoid. Data were analyzed with a 2X2 Factorial ANOVA.

To examine for possible “hidden” effects, a Chi-squared test was conducted on the expected versus observed emergence of flies and parasitoids for each of the four treatments. Expected values were calculated using the equations described above (previous section). Expected values for each individual fly or parasitoid species were not calculated since only the

overall fly and parasitoid values were compared. After dissection, some specimens could still not be identified. These unidentified individuals were subtracted from the totals to give observed values for use in the Chi-squared tests.

Fly Culture Initiation

None of the research objectives could have been accomplished without a culture of medfly to provide host material for parasitoids. Cultures of hosts are integral to basic biological research such as characterization of guild structure and host specificity studies. Since existing fly cultures at ICIPE (previously used for behavioral work on tephritids fruit flies) were inadequate for rearing parasitoids when the parasitoid work began in 1999, a separate fly culture was established by Trostle specifically for rearing hosts for parasitoid development. Flies used to initiate this culture were obtained from coffee collected at Ruiru.

In addition to establishing a separate host culture for the parasitoid work, two major changes were made to the general ICIPE fruit fly rearing practices to improve both the quantity and the quality of flies used in the overall program. First, fruit fly rearing protocols were adopted from the USDA/ARS fruit fly laboratory in Honolulu, Hawaii (USDA/ARS, 1997), but were modified to Kenyan conditions and availability of materials. The adopted protocol included using yeast hydrolysate (not previously used in Kenya to rear fruit flies) as a protein source for the adult flies. (Yeast hydrolysate was kindly provided by USDA/ARS lab in Honolulu.) A mixture of yeast hydrolysate powder and sugar was free fed to adult flies at a 4:1 ratio. The second change was the adoption of a new female medfly oviposition unit. Bananas are readily available in Kenya and Trostle discovered that they were more attractive to female adult medfly for oviposition than the parafilm covered balls of larval diet then in use at ICIPE.

Hundreds of small holes drilled in the peel (pericarp) of each banana with an insect pin provided a much higher production of fly larvae.

The “banana unit” was exposed in a Plexiglas rearing cage to a cohort of adult medfly. After a banana was exposed for 2 days, the banana was removed from the adult fly cage and any larvae inside the banana were placed in the carrot-based fruit fly larval diet (Hooper, 1987). (See Mohamed *et al.* (2003) for specific larval diet ingredients and their amounts as used at ICIPE). The subsequent transfer from bananas to diet was essential because bananas do not provide adequate nutritional support to complete larval development of medfly, even though they are very attractive for oviposition. The diet/banana medium was placed in a solo cup elevated above a layer of sand sprinkled on the bottom of a 2.5-gallon plastic bucket (Fig. 4). This arrangement allowed larvae space to “pop” and exit the cup after they completed feeding, thus allowing the larvae to follow their natural behavior of exiting fruit and pupating in the soil. The middle of the lid on the bucket was cut out and replaced with mesh to allow air circulation. Upon emergence, adult flies were placed in the fly colony for rearing purposes. The temperature in the adult medfly cages was maintained at 27-28°C. The photoperiod was 12L:12D.

Due to the success of this method with *C. capitata*, these protocols were adopted for the cultures of *C. fasciventris* and *Ceratitis cosyra* (Walker). (Cultures of the latter two species had also been established at ICIPE previously for use in studies of fruit fly behavior.) However, mangos, instead of bananas, were used as an oviposition unit for *C. cosyra*. At the completion of the experiments reported here and in Chapter III, the medfly cultures became the property of ICIPE.

Species Identification

Routine Identifications

Several research groups provided assistance in the identification of the host flies and their parasitoids. Taxonomic research on tephritids was conducted concurrently by Drs. Ian White (International Institute of Entomology, London, U. K.) and Marc De Meyer (Royal Museum for Central Africa, Tervuren, Belgium). They provided keys and diagnostic features that facilitated routine identification of all flies reared from coffee and other fruits (mango, squash, etc.) used in this study. Preliminary identification of parasitoids was provided by Drs. John LaSalle (International Institute of Entomology, London, U. K.) for tetrastichine Eulophidae and Robert Wharton (Texas A and M University) for all other taxa. As with the host flies, several parasitoid taxonomic problems were resolved during the course of this investigation (Wharton, 1999; Kimani-Njogu *et al.*, 2001; LaSalle and Wharton, 2002; Yoder and Wharton, 2002). Voucher specimens for all species used in this study are deposited in the Texas A and M University Insect Collection.

Although experts provided initial identifications, Trostle identified all of the flies and parasitoids in the samples she processed. In addition, she provided assistance in identification to collaborators in Hawaii and Guatemala and trained other graduate students, researchers and technicians at ICIPE.

The *Psytalia concolor* (Szépligeti) Species Group

One of the parasitoid species was selected for more detailed investigation (in collaboration with Dr. Susan Kimani-Njogu at ICIPE) because of the inability to distinguish it from *P. concolor*, a North African species widely used in augmentative biological control of olive fly, *Bactrocera oleae* (Gmelin), in the Mediterranean Region. For this purpose, a permit was obtained to receive an Italian strain of *P. concolor* and rear it in quarantine at ICIPE. Once the cultures of the Kenyan *Psytalia* species from coffee were established at ICIPE, the population from Italy was compared to populations from Kenya using morphometric analysis and hybridization experiments. To ensure adequate materials for these and other experiments, cultures of both Italian and Kenyan *Psytalia* were subsequently shipped to the HDOA quarantine facility in Honolulu, Hawaii.

The results of these experiments were published in Kimani-Njogu *et al.* (2001). The first part of the paper details a preliminary morphometric analysis conducted jointly by Trostle and Kimani-Njogu to test the overall similarity of appearance between the material reared from coffee in Kenya and from the Italian *P. concolor* cultures. The second part, a hybridization experiment, also conducted jointly assessed the mating compatibility and determined the ability to produce viable female offspring through two generations, using reciprocal crosses. For more detailed information on the materials and methods used, see Kimani-Njogu *et al.* (2001).

Characterization of Guild Structure

Guild classification was based on a paper by Mills (1994) in which he defined the structure of parasitoid communities using 12 guilds. To characterize the guild structure of

parasitoids attacking tephritids in coffee, the following data were obtained for each species of parasitoid, with the exception of *Fopius silvestrii* (Wharton): (1) host stage attacked by the parasitoid; (2) the host stage killed by the parasitoid and (3) mode of parasitism (koinobiont or idiobiont; ectoparasitic or endoparasitic) (Mills, 1994). For *F. silvestrii*, the host stage attacked was hypothesized based on ovipositor morphology and the host stage attacked by other *Fopius* species with a similar morphology. The relative number of species utilizing the following host categories was also tabulated: (1) egg, (2) larva, (3) prepupa, (4) pupa and (5) adult parasitoids. Since arabica coffee is attacked by at least three species of Tephritidae at each sample site an assessment of host suitability was also needed to identify parasitoids of medfly (see Chapter III). The three sampling sites were compared for qualitative differences in parasitoid species composition.

Larval and Prepupal Guilds: Rearing Koinobiont and Idiobiont Parasitoids from Coffee Samples

Egg-Prepupal and larval-prepupal early and late emerging koinobiont endoparasitoids were reared from monthly coffee samples processed as described above (see Sample Processing section). These endoparasitoids all emerge from the host puparium and are thus easily acquired through normal rearing protocols. Idiobiont larval ectoparasitoids were collected from 40 separate 0.5-kg samples kept in carefully sealed, escape-proof cages. It was necessary to alter routine collection processing to collect ectoparasitoids as ectoparasitoids kill their larval hosts and pupate inside the coffee cherry making it impossible to collect them without holding the coffee in an escape-proof container (suitable for retaining, for example, small chalcidoid wasps). When ectoparasitoid taxa were initially recovered, fruits were dissected to verify hosts (since

coffee cherries also harbor larval beetles and moths that could serve as hosts of ectoparasitoids). Sampling for ectoparasitoids was much more labor-intensive than the normal protocols, thus comparisons of the number of idiobiont and koinobiont species are limited to those reared from these 0.5-kg samples.

Rearing Egg and Pupal Parasitoids: Modified Sampling of Coffee

Additional samples were taken to obtain data on egg and pupal parasitoids to complete the assessment of parasitoid guild structure. To determine the presence of egg parasitoids, coffee cherries were sampled on four different occasions from Ruiru, three times from Koru, and once from Rurima. Six hundred seventy-four cherries were dissected to recover tephritid eggs. Two hundred twenty eggs were recovered. All 220 eggs were placed in Petri dishes on the associated hull of the coffee cherry from which they were extracted. The Petri dishes were then taped shut to prevent escape of any egg parasitoids, and held until eggs hatched.

Preliminary attempts to recover pupal parasitoids by extracting puparia from soil beneath coffee bushes were unsuccessful, yielding only flies and koinobiont larval–prepupal parasitoids. Thus, during September 1999, fully fed third instar larvae were removed from the laboratory culture, immediately taken to Ruiru, and allowed to enter the soil to pupate. Four hundred fifty larvae were dispersed in the field at a rate of 50 per coffee bush. Samples beneath five of the bushes were recovered after a 3-day period and the remaining four samples were recovered after a one-week period. Of 450 third instar larvae released under coffee plants, only 249 puparia were recovered, and these were held individually in the laboratory for emergence of flies and parasitoids. This experiment was severely limited by the availability of transportation into the field and the numbers of larvae available for exposure.

Host Stage Killed by Koinobiont Endoparasitoids: Prepupa or Pupa

Towards the end of the fly's third larval instar, pupariation begins. The outer cuticle of the third instar larva becomes hardened into the shell known as the puparium, inside which a pupa will eventually be formed. It is important to differentiate between pupariation (the formation of the puparium) and the distinctly later process of pupation and development of a true pupa (Ferrar, 1987). This stage before true pupation is referred to as the prepupal stage.

Thirty-four dissections were done of puparia containing both developing flies and parasitoids to determine the stage killed (pupa or prepupa) by the parasitoid. Additionally, 30 puparia from which both flies and parasitoids emerged were examined for the presence of a fly pupal exuvium and/or a parasitoid cocoon.

Host Stage Attacked

Published information on host stage attacked was available for four of the seven species that we routinely reared from arabica coffee in Kenya. There is, for example, considerable information on the biology and use of *P. concolor* in biological control (Féron, 1952; Biliotti and Delanoue, 1959; Delanoue, 1960; Arambourg, 1962; Monastero and Delanoue, 1966; Genduso, 1967; Étienne, 1973; Raspi and Loni, 1994; Loni, 1997). In addition, Pemberton and Willard (1918b) described in great detail the biology of both *Diachasmimorpha fullawayi* (Silvestri) and *Tetrastichus giffardianus* Silvestri, while Neuenschwander (1982) noted that *Bracon celer* Szépligeti attacked third instar larvae.

For *P. concolor* and for *D. fullawayi*, previously shown to have a propensity for attacking hosts in their later instars, cherries artificially infested with 3rd instar larvae were exposed to the

parasitoids for culture initiation and verification of host stage attacked (the two objectives being essentially inseparable). These exposures were accomplished by collecting late instar larvae from the fly colony, opening non-infested coffee cherries, removing the two beans inside and then using soft forceps to place the larvae inside the cherries in the space previously occupied by the beans. Three to five larvae were placed in each cherry depending on availability of larvae. Carrot based larval diet for tephritids (Hooper, 1987) was placed inside the cherry as well as over the open end of the cherry to prevent escape of the larvae. These modified cherries were exposed for one hour to females in 12-dram glass shell vials. Four cherries were exposed at one time to one female in the vials. Later, as we became more experienced in rearing *P. concolor*, the exposure medium changed from 1) one female in a glass vial with four cherries, to 2) several stuffed coffee cherries in a Petri dish with three to four females to 3) oviposition units consisting of larvae inside balls of larval diet wrapped in parafilm and exposed to a cage of approximately 50 females parasitoids.

To verify the host stage attacked by the ectoparasitoid *B. celer*, it was necessary to dissect coffee cherries from samples producing this species. *Bracon celer* produces a distinctive cocoon to which the host remains are frequently attached. Since it is an idiobiont ectoparasitoid, the host stage attacked is the same as the host stage killed. In order to focus more efforts on the braconids, no additional work was undertaken on the biology of either of the species of *Tetrastichus* reared from the coffee samples.

Wharton (1987, 1997, 1999) contains drawings and electron microscopy figures of the ovipositors of *Fopius* species, including those reared in Kenya, and provides the only published clues to the host stage attacked by these species. He noted that the ovipositors of *Fopius caudatus* (Szépligeti) and *Fopius ceratitivorus* Wharton exhibit morphologies similar to that of

Fopius arisanus (Sonan), a parasitoid species with a well-known biology of attacking the egg stage of tephritid hosts.

To test the hypothesis that these two Kenyan parasitoids share ovipositor morphology as well as host stage attacked with the Indo-Pacific species *F. arisanus*, five freshly picked and uninfested cherries were initially placed on two leaves from a coffee bush covering the bottom of a three and one-half inch Petri dish. Gravid female medfly were exposed to coffee cherries for “natural” oviposition for 24 hours. After 24 hours, the cherries were exposed to the female parasitoids for one hour to observe their response to the newly infested cherries. Upon observing ovipositional probing by *F. caudatus* and *F. ceratitivorius* in this set-up, further experiments to confirm host stage attacked as well as addressing oviposition cues were undertaken for these two species.

No work was done with the third species of *Fopius*, *F. silvestrii*, as it was rarely recovered at only one collection site.

The experiments to confirm the host stage attacked by *F. caudatus* and *F. ceratitivorius* were undertaken inside the Petapa, Guatemala quarantine facility. Petapa is approximately 50 km south of Guatemala City. Female wasps for all Guatemalan experiments were shipped as pupae to Guatemala from Kenya, representing samples collected at Koru either in late November or early December of 2000. Both parasitoids were exposed to medfly eggs as well as 1st, 2nd, and 3rd instar larvae. All exposures took place at 25-27°C and 55-61% RH. All cages used in the experiments were 22 cm³ and were positioned to receive natural sunlight through a window.

Exposing these parasitoids to the egg and first instar stages of medfly was accomplished using a method that attempts to closely mimic natural conditions. Entire branches of coffee with berries and leaves were obtained from the field in Guatemala. As soon as the branches arrived at the quarantine facility in Petapa, they were placed in water to prevent desiccation of the coffee

cherries and leaves. Any cherries with previous oviposition scars were removed from the branches to prevent interference with the experiment.

The branches of coffee were placed in a cage of male and gravid female medfly for the natural infestation of the cherries. To ensure exposure to the egg stage of the fly, after 24 hours the branches were removed from the medfly cage and placed in the parasitoid cage. The parasitoids were allowed to oviposit for 24 hours, after which the cherries were removed from the parasitoid cage and dissected to remove the medfly eggs. The medfly eggs were then dissected on a slide in a drop of water to observe if they contained parasitoid eggs. Fifty-six medfly eggs exposed to *F. caudatus* were dissected. Due to time constraints, only 10 of the eggs exposed to *F. ceratitivorus* were dissected.

For exposure to first instars of medfly, the same “natural” method of infestation of coffee cherries was used. However, instead of exposing the cherries to the parasitoids immediately after medfly oviposition, the cherries were held for three days to ensure all exposures consisted of first instar larvae. During the interim, the cherries were misted with water to keep them viable. On the fourth day after exposure to medfly females, the cherries were exposed to the parasitoids for 24 hours. After this 24-hour period the cherries were removed from the cage and dissected to remove the first instar medfly. The medfly larvae were then dissected on a slide in a drop of water to observe if any parasitoid eggs were present. From the *F. caudatus* exposures, 41 first instar larvae were dissected and from the *F. ceratitivorus* exposures, 58 larvae were dissected.

First, second and third instar medflies from the SIT mass rearing facility in Guatemala were also exposed to the same cages *F. caudatus* and *F. ceratitivorus* as used above. Three to five larvae were placed in a coffee cherry following the same method described above for the rearing of *P. concolor*. These coffee cherries with larvae inside were exposed to the parasitoids

in Petri dishes 6cm in diameter. Exposures lasted one hour in the same conditions as the Petapa, Guatemala quarantine facility.

Oviposition Cues and Behavior of Egg-Prepupal Parasitoids

Oviposition Cues

Developing a parasitoid rearing program requires not only the knowledge of the host stage attacked, but also information on the cue(s) required to initiate oviposition. Thus, I also conducted experiments on the cue or set of cues used by females of *F. caudatus* and *F. ceratitivorus* to initiate probing (the first step in the oviposition sequence) into coffee cherries. Experiments were designed to test the hypothesis that both species require a host fly oviposition mark to initiate probing. Knowledge of such cues is extremely important for initiating a colony as well as for assessing possible nontarget effects. (See chapter IV for discussion of how probing cues can translate into assessing possible nontarget effects.) These experiments were done at the USDA quarantine facility in Petapa, Guatemala in January, 2001, with comparable experiments done on *F. arisanus* in Hawaii in May, 2001.

Based on the results from the previous section, it was known that both *F. caudatus* and *F. ceratitivorus* would probe and oviposit into cherries naturally infested by medfly. Thus, the goal of this experiment was to determine whether these parasitoids would initiate probing either without the fly pheromone or without the presence of a host egg. For the experiment, coffee cherries still attached to their branches were obtained from the field. Only completely clean (without fly oviposition marks) coffee cherries were chosen from the branches and used in the experiment. The experiment consisted of two treatments and a control. Two replicates of the

experiment were undertaken. Five cherries were used in each treatment and five cherries were used in the control, for a total of 15 cherries per replicate.

Treatment one consisted of artificially infesting five cherries with medfly eggs. Five holes were made in each cherry with a pair of fine forceps. Using a fine-tipped paintbrush, three medfly eggs obtained from the USDA fruit fly rearing facility in Guatemala were placed inside each artificially made hole. This treatment combined the egg cue and the damage cue but excluded the female fly oviposition cue. Treatment two consisted of artificially damaging the five cherries. Five holes, once again using a pair of fine forceps, were made in each cherry. In this treatment, though, no eggs were placed inside the holes. This treatment concentrated on damage as a cue for probing. The two treatments, by separating the different parts of a natural infestation, thus enabled assessment of whether a combination of all the cues or if only one cue was of greater importance in host location by these species.

The control for this experiment consisted of five non-modified cherries placed among the cherries of treatment one and two. All the treatment one cherries and three control cherries (8 cherries total) were placed in a single Petri dish, 6 cm in diameter. All the treatment two cherries and two control cherries (7 cherries total) were placed in a separate Petri dish of the same size. To stabilize the cherries, two green and rinsed coffee plant leaves were placed in the bottom of each Petri dish prior to the placement of the treatment and control cherries.

Both Petri dishes were exposed simultaneously to a cage of approximately 25 female *F. caudatus* or *F. ceratitivorus* for one hour. The Petri dishes were placed approximately 10 cm from the floor on the sunny side of a small, 22 cm³ Plexiglas cage with one ventilation hole covered in organdy. The organdy was streaked with honey as a food source and water was provided through a wick attached to a lidded cup full of water. Temperature was maintained at 27-28°C and 56-60 %RH.

In a separate experiment, both *F. caudatus* and *F. ceratitivorus* were exposed to medfly eggs placed in a Petri dish and their responses were recorded.

Oviposition Behavior: *F. caudatus* and *F. ceratitivorus* Compared to *F. arisanus*

The behavior of *F. caudatus*, *F. ceratitivorus*, and *F. arisanus* during searching and ovipositing sequences was recorded. Specifically, antennal position during searching and during oviposition as well as whether the antennae were used to guide the ovipositor during probing and initiation of oviposition were recorded.

Initiating Cultures of Kenyan Parasitoids in Hawaii

Kenyan parasitoids destined for shipment to Hawaii were obtained from the routine samples of coffee cherries from the sites at Koru, Ruiru, and Rurima. As previously mentioned, one-half of the puparia harvested from these coffee samples were sent primarily by express mail delivery service, i.e. DHL, UPS and others, directly to the Hawaii Department of Agriculture (HDOA) Quarantine Facility in Honolulu, Hawaii.

Taxonomic expertise acquired during the work in Kenya was essential for screening species in quarantine since several species from Kenya are very similar in appearance. The first successful shipments were hand-carried by Trostle to Hawaii and personally screened in quarantine. Quarantine personnel were then trained to recognize the different species for subsequent shipment by express mail.

Although several species of tephritid parasitoids emerged from coffee collections that were shipped to Hawaii during 1998 and 1999, only one of these, the *P. concolor* population

from Kenya, was successfully established as a permanent culture. This work was done in conjunction with N. Peabody who was responsible for receiving, culturing and examining the host suitability of the Kenyan parasitoids until December of 1998. Additionally, a culture of *P. concolor* originating from Italy was established in quarantine in Hawaii (See Chapter III, Laboratory Host Range Testing in Hawaii). To establish and maintain the two *P. concolor* colonies, host larvae were exposed to parasitoids using the protocols of Wong and Ramadan (1992). Second and third instar larvae of *C. capitata* and *Bactrocera latifrons* (Hendel) were acquired from the USDA Tropical Fruit and Vegetable Laboratory in Manoa, and were used as hosts. These two colonies were maintained separately in quarantine in Honolulu.

In late 1999, the Plant and Animal Advisory Committee of Hawaii approved the release of *P. concolor* for additional laboratory testing including host suitability and nontargets studies. These were conducted at the University of Hawaii, Manoa Campus in Gilmore Hall. These studies are contained in Chapter III.

All attempts to culture parasitoids in Hawaii were carried out in the screen room of the HDOA quarantine facility. Since it was suspected (based on ovipositor morphology) that Kenyan species of *Fopius* attacked eggs or first instar larvae, various methods were developed for exposing eggs and first instar larvae to the parasitoids in quarantine. These methods were all dependent on the availability of coffee cherries, which were difficult to obtain in Honolulu. When coffee cherries could be obtained, eggs and first instars of medfly, acquired from the USDA lab in nearby Manoa, were artificially placed in the uninfested cherries. Additionally, female medflies were obtained from the Manoa facility and allowed to oviposit in the fruit, providing a more natural infestation. Although these attempts at culturing were unsuccessful in Hawaii, the methodology developed there proved useful in successful attempts to culture *Fopius* in Guatemala where more natural lighting conditions prevailed in quarantine.

Results

The Species of Tephritidae Infesting Coffee in Kenya

Identity

From November 1997 through to November 1999, 31,944 puparia were obtained from arabica coffee samples collected at Koru (2661 of these were from the November, 1999 20-kg sample that was divided into forty 0.5 kg sub-samples), 19,486 puparia from Ruiru, and 13,511 puparia from Rurima. Four species of ceratitidine Tephritidae were reared from these samples: *Ceratitis anonae* Graham, *C. capitata*, *C. fasciventris* and *T. coffeae*. Females of *C. anonae* are virtually indistinguishable from *C. fasciventris*, and it thus was possible to overlook the presence of *C. anonae* in those Koru samples where *C. fasciventris* was abundant.

During the course of these investigations, two important studies affecting the names of the species of Tephritidae recorded here were completed. First, De Meyer (2001) discovered that the Natal fly consisted of two distinct species (*C. rosa* and *C. fasciventris*), and that both are found in Kenya. *Ceratitis rosa* occurs along the Kenyan coast and *C. fasciventris* is an inland species. Though our earlier reports (e.g. Wharton *et al.*, 2000) list the species from Ruiru, Rurima, and Koru as *C. rosa*, the species at all these sites should now be referred to as *C. fasciventris* based on the findings of De Meyer (2001). For a more in-depth discussion of the distribution of *C. rosa* and *C. fasciventris*, see De Meyer (2001). Second, Ian White's revision of the genus *Trirhithrum* (White *et al.*, 2003) confirmed the identification of the *Trirhithrum* emerging from our Kenyan coffee samples as *T. coffeae*. At least two other names had previously been applied to species of *Trirhithrum* attacking coffee in East Africa: *Trirhithrum*

nigerrimum (Bezzi) and *Trirhithrum nigrum* Graham. The latter is a misidentification and the former is a polyphagous species that is widespread in Kenya and commonly reported from robusta coffee. These species of *Trirhithrum* are relatively dark, with a uniformly black scutellum, and are thus readily separated from the species of *Ceratitis* reported from coffee, which are more extensively pale. Otherwise, the fruit fly species recorded here can be readily identified using a combination of the keys in White and Elson-Harris (1992) and Hancock and White (1997).

Relative Abundance

The relative abundance of the four fly species at each of the three primary sampling sites is shown in Figs. 5, 6, and 7. Medfly dominated the samples from Ruiru and Rurima, whereas *C. fasciventris* was the dominant species at Koru. *Ceratitis anonae* was recovered only from Koru, but the other three species occurred at all three primary sample sites. *Trirhithrum coffeae* was occasionally abundant at Koru, sporadic at Ruiru, but recovered only very rarely from Rurima.

Table 2 shows the arithmetic mean number of flies per kilogram for each fly species at the three sites in Kenya. Although the fly populations may vary seasonally, this table gives a broad overview of the dominant species at each site. At Koru the dominant fly species is *C. fasciventris*, followed by *C. capitata*, *T. coffeae* and finally *C. anonae*. At both Ruiru and Rurima, *C. capitata* was obviously the dominant species followed by *C. fasciventris* and relatively low populations of *T. coffeae*. Sample sizes were unequal, with 96.5 kg collected at Koru, 189 kg at Ruiru, and 75.5 kg at Rurima.

Infestation Rates

Infestation rates (shown in Table 3 as sample means of number of eggs and larvae per coffee cherry) ranged from 0.87–1.4 at Ruiru, 1.2–1.4 at Rurima, and 0.4–1.5 at Koru over the latter half of 1999. Table 3 below shows infestation rates for the samples I dissected immediately upon arrival at ICIPE in 1999. The lower values from Koru may have been affected by a larger percentage of unripe cherries in the samples, but unfortunately this was not quantified. A smaller sample collected in Ruiru on 4 August 1998 was unusually heavily infested, with a mean of 2.0 individuals per coffee cherry.

Phenology

Sampling problems prevented critical assessment of within-site and seasonal variations. The gross coffee data showing phenology of the flies collected from coffee is included in Appendix C, Tables 1, 2, and 3. Fruit stripping by coffee growers was particularly problematic at Ruiru, for example, and several other samples were lost to poor handling practices. Nevertheless, some patterns are evident (Figs. 8, 9 and 10). Medfly showed a peak of abundance in mid-year at all three sites, even though the degree of infestation and extent of the peak period varied among sites. Maximum rate of infestation was recorded in February at Rurima, giving a bimodal pattern at this site (Fig 9). Fruit stripping at the other two sites made it impossible to determine patterns of abundance at the beginning of the year, and to verify bimodal peaks in abundance as a general phenomenon.

No seasonal patterns were evident for *C. anonae*, which always exhibited very low levels of infestation (Fig. 10). Similarly, seasonal patterns were not apparent for *T. coffeae* at

either Rurima or Ruiru (Figs. 8 and 9). At Koru, where *T. coffeae* was much more abundant, peak activity extended from June - August, thus extending two months beyond the peak of medfly abundance at this site. For *C. fasciventris*, seasonal fluctuations were most evident at Koru, where this species was very abundant, with the primary peak (August) occurring after peak abundance of medfly (April - June).

Parasitoids of Tephritidae in Coffee in Kenya

Identity and Distribution

Ten species of parasitoids were reared from the tephritids infesting coffee cherries in Kenya (Table 4). These include two species of opiine Braconidae, *Fopius caudatus* and *F. silvestrii*, found only at Koru and a third opiine, *Psytalia cosyrae* (Wilkinson), found only at Rurima. *Fopius ceratitivorus* Wharton, present at Rurima and Ruiru, was never found at the wetter site in Koru. Two other opiines, *Diachasmimorpha fullawayi* and *Psytalia* cf. *concolor*, were found at all three sites, as were the braconine braconid *Bracon celer* and the eulophid *Tetrastichus giffardianus*. Puparia were field collected only at Ruiru and thus the pupal parasitoid *Coptera robustior* (Silvestri) was recovered only from this site. *Tetrastichus giffardii* Silvestri was collected at Koru, and may also have occurred at the other two localities but was probably overlooked because of general problems associated with identification and collection of the two *Tetrastichus* species.

Taxonomic works, conducted by others during this program, lead to the description of *F. ceratitivorus* as a new species (Wharton, 1999) and facilitated the identity of three of the other parasitoids listed above (LaSalle and Wharton, 2002; Yoder and Wharton, 2002). *Psytalia* cf.

concolor, whose identity was investigated as part of the dissertation, is covered in the next section.

Identity of *Psytalia* cf. *concolor*

Results of the morphometric analyses have now been published (Kimani-Njogu *et al.*, 2001). A species of *Psytalia* reared from cucurbits, tentatively identified as *P. phaeostigma* (Wilkinson), was used for comparison because of its distinctly longer ovipositor. Although females from the Ruiru population had slightly shorter ovipositors than those from Rurima and Italy, there was no evidence of postcopulatory incompatibility between individuals from the Italian population and those from either of the two populations from coffee in Kenya. Relatively few individuals were available for backcrosses due to the difficulty of establishing and maintaining isolated cultures of Kenyan parasitoids and the relatively long developmental time that prevented rapid buildup of parasitoid cultures. Nevertheless, all backcrosses resulted in viable female offspring.

Behaviorally, males and females from Italy were much more eager to mate under caged conditions and artificial lighting than were males and females from either Ruiru or Rurima. This was true for controls and for the experimental crosses between the Italian and Kenyan populations. Males from the Italian cultures were aggressive in both intra-strain and hybrid crosses. Males from Rurima and Ruiru, however, showed only mild interest in mating, especially when isolated as pairs in larger cages. When grouped together in small containers, there was more evidence of wing fanning (an important prelude to copulation) on the part of Kenyan males in both intra-strain and hybrid crosses.

Individuals from the two populations in Kenya were similar in their developmental biology to those from Italy. Generation time for individuals from Kenya fell consistently within the range of Italian *P. concolor* reported by Loni (1997). Although females from Kenyan populations failed to recognize the Petri dish oviposition units during the first few generations after laboratory colonization, hybrid females readily accepted both Petri dish oviposition units and coffee berries for oviposition.

Relative Abundance

The relative abundance of each of the sampled parasitoid species is listed by sample locality in Figs. 11, 12 and 13. *Psytalia cosyrae*, *F. silvestrii* and *C. robustior* were rarely collected, with fewer than ten individuals of each species. *Coptera robustior*, for example, was represented by six individuals reared from the 249-medfly puparia recovered from soil samples at Ruiru.

The relative abundance of the two *Tetrastichus* species was difficult to estimate in the larger samples due to their gregarious nature (6-10 individuals developing in each medfly puparium) in combination with their small size. The latter factor made it easy for them to be overlooked by technicians, and they often escaped from the rearing cages. Thus, data for the two species are combined in Table 5. Nevertheless, *T. giffardianus* was much more commonly encountered than *T. giffardii* in those samples producing sufficient numbers of *Tetrastichus* for examination.

Table 5 shows the arithmetic mean number of parasitoids per kilogram for the dominant parasitoid species at each of the three sites in Kenya. Although the parasitoid population numbers often fluctuated dramatically from one sampling period to the next, Table 5 gives a

broad overview of the dominant species at each site. As noted above for the tephritids, sample sizes were unequal, with 172 kg collected at Koru, 96.5 kg collected at Ruiru, and 75.5 kg at Rurima.

At Koru the dominant parasitoid was *F. caudatus* followed by *P. concolor* and *Tetrastichus* (Fig. 13). Ruiru's dominant parasitoid was *D. fullawayi* (Fig. 11). Despite the fact that the species was unknown prior to the survey, *F. ceratitivorus* was the most frequently reared species at Rurima (Fig. 12). *Psytalia concolor* was nearly equal in abundance in most samples taken at this site, though this is not apparent from Fig. 12, which reflects the influence of a single sample from December, 1997 that was overwhelmingly dominated by *F. ceratitivorus* (Appendix C, Table 5).

Data on the larval ectoparasitoid, *Bracon celer*, were obtained from separate 0.5 kg samples taken at Koru in November of 1999. The sample yielded 2.7 *B. celer* per kg, roughly equivalent to the yield of *F. caudatus* from routine monthly samples (Table 5). However, the numbers of *F. caudatus* in these 0.5 kg samples were much higher than recorded for most monthly samples.

Phenology

Seasonal patterns in abundance are shown in Figs. 14, 15, and 16 for dominant parasitoids at each site. Parasitoid data from coffee collections for each site are shown in Appendix C, Tables 4, 5 and 6. In general, at Koru, *F. caudatus* (Fig. 13) was the dominant parasitoid. However, in July and August of 1998, *P. concolor* (Fig. 16) and *Tetrastichus* spp. dominated the parasitoid assemblage. This pattern did not repeat in 1999.

At Ruiru, although *D. fullawayi* was the most abundant parasitoid overall, with *F. ceratitivorus* following as a distant second (Fig. 11), this is the result of very high numbers of flies and parasitoids for the four months data were collected in 1999. In 1998, *P. concolor* was the dominant parasitoid from June to November. December was the only month in 1998 with *D. fullawayi* showing a slight dominance over the other parasitoids. In 1999 the number of flies per kilogram increased dramatically, and although there were still high numbers of *P. concolor*, *D. fullawayi* and *F. ceratitivorus* exhibited much greater numbers than in the pervious year (Appendix C, Tables 1 and 4).

At Rurima, early in the year, the population of *F. ceratitivorus* was either too low to be recorded or the infestation levels were less than one individual per kilogram. There were obvious peaks in activity in July (1998) and November/December (1997 and 1998) for both *F. ceratitivorus* and *P. concolor* (Fig. 15 and Appendix C, Table 5).

Assessment of Sample Processing Procedures

Estimating Rates of Infestation

Examination of fruit in the holding cages revealed the presence of numerous puparia and some pupating larvae following the second (and last) sieving of sand from the bottom of the collection cages. As the fruit decays and begins to dry, the mass of fruit becomes a suitable pupation site with some larvae pupating in the fruit and others exiting the fruit but pupating against the pericarps of adjacent fruits. Fifty-three percent of all larvae recovered from the September 1999 Rurima sample pupated in or on the fruit rather than in the sand beneath the

fruit trays. Thus, under normal fruit processing procedures, infestation rates would be grossly underestimated.

Impact of Moisture and Stirring of Fruit Samples on Emergence Rates

The number of adults emerging from 0.5 kg sub-samples of the November 1999 Koru collection was not significantly affected by different moisture treatments ($F = 2.08$; $df = 1$; $P = 0.1584$) in the 2X2 factorial ANOVA. There was, however, a significantly higher emergence of flies and parasitoids from samples that were stirred ($F = 7.65$; $df = 1$; $P = 0.0091$).

Although from a purely numerical perspective, there were seven to ten more unclosed flies than parasitoids per sample, all four sample-handling procedures resulted in a bias in the percentage of parasitoids unclosed. The four procedures resulted in a mean of 21.91% unclosed flies in comparison to 35.30% unclosed parasitoids. Overall, after dissection of unclosed portions of the sample to identify fly and parasitoid remains, flies accounted for 59.64% of the sample while parasitoids accounted for 25.96%, with the remaining 14.90% representing unclosed puparia whose occupants were still unidentifiable after dissection. These totals represent our most accurate assessment of the actual rate of infestation of coffee in Kenya as well as the overall level of parasitism.

Looking at each sample independently, in the control sample of wet and stirred coffee the observed versus expected numbers for flies and parasitoids were significantly different ($\chi^2 = 7.226$; $df = 1$; $P = 0.0072$). For the treatment dry and not stirred, differences between the observed versus expected numbers were highly significant ($\chi^2 = 18.109$; $df = 1$; $P = 0.0001$). The same result was also seen in the wet and not stirred treatment ($\chi^2 = 21.574$; $df = 1$; $P = 0.0001$). The difference in the expected versus observed flies and parasitoids for the dry and stirred sample,

although different, was not quite statistically significant ($\chi^2=3.250$; $df=1$; $P=0.0714$). Thus, the expected numbers overestimate the actual number of flies in the sample and underestimate the actual number of parasitoids because of the greater percentage of unclosed parasitoids relative to unclosed flies.

Fly Culture Initiation

The increase in the numbers of fruit fly larvae reared by ICIPE staff for maintenance of host fly colonies after the mid-August addition of yeast hydrolysate to the adult fruit fly diet and bananas as oviposition units is shown in Tables 6 and 7. By the end of October 1999 production of *C. capitata* larvae had increased 2000% from a mean of 98 larvae produced each week in late July and early August to mean of 1975 larvae produced each week in October. Similar increases can be seen with *C. fassiventris*. Production of larvae was increased 1900%, from a mean of 123 larvae per week in late July and early August to a mean of 2374 larvae per week in early October.

The Guild Structure of Parasitoids of Tephritid Fruit Flies in Coffee in Kenya

The Parasitoid Assemblage

The parasitoid assemblage of tephritid fruit flies in coffee in Kenya, as modeled after Mills (1994), includes: 1) egg-prepupal endoparasitoids, 2) early and late attacking larval-prepupal endoparasitoids, 3) larval ectoparasitoids, and 4) pupal endoparasitoids. Other guilds delineated by Mills (1994) were not found even though sampling protocols were modified to

detect them (Table 8). Table 8 records the confirmed number of parasitoid species in each guild. In addition, it also addresses the possibility of parasitoids residing in each guild that were not recovered in this study.

Dissection of developing tephritids containing parasitoids clearly demonstrated that the host is killed as a prepupa, after the puparium is formed but prior to pupation. Thus the term larval parasitoid, as used in some publications, is more accurate than the occasionally used larval-pupal parasitoid. Confirmation of utilization of prepupa as a resource was accomplished by observing that third instar parasitoid larvae were never found inside a host pupa.

In addition, the use of the prepupal stage was confirmed by the presence of a non-silken pupal exuvium inside the puparium containing a pair of pupal spiracles on the posterior end when a fly emerged versus the absence of a fly pupal case or its pupal spiracles when a parasitoid emerged.

The following categories are based on: (1) host stage attacked, (2) host stage used as the major resource for parasitoid development and (3) mode of development (endo or ectoparasitic; koinobiont or idiobiont).

Egg Parasitoids (Designation - E1)

No true egg parasitoids were found. All of the 211-tephritid eggs isolated from 540 field-collected coffee cherries produced tephritid larvae. If egg parasitoids are present they are rare and/or seasonal.

Egg-prepupal Endoparasitoid (Designation - Pre1)

The biology of *F. caudatus* and *F. ceratitivorius* was previously unknown, though Wharton (1987) suggested that *F. caudatus* might oviposit in the host egg based on ovipositor

morphology. Trostle observed both *F. caudatus* and *F. ceratitivorius* ovipositing into host eggs in the laboratory in Guatemala and at ICIPE and the experimental results confirmed these observations. From the 56-medfly eggs exposed to *F. caudatus*, four parasitoid eggs were recovered during dissection. Of the ten *C. capitata* eggs dissected after exposure to *F. ceratitivorius*, five parasitoid eggs were recovered. For both of these *Fopius* species, only one parasitoid egg was recovered per host egg. In the experimental exposures, no attacks on second or third instar medfly larvae were observed, but *F. ceratitivorius* did attack 1st instar larvae (see next section). In addition, both species were successfully reared on host eggs through at least one generation for establishment of lab cultures in Guatemala by the staff in the Guatemala quarantine. Thus, based on these experiments and supplemental observations and information, *F. caudatus* is exclusively an egg-prepupal endoparasitoid but *F. ceratitivorius* is both an egg-prepupal and an early attacking larval-prepupal endoparasitoid.

All three of the *Fopius* species that were reared from coffee in Kenya were confirmed to kill the host in the prepupal stage (Table 9). In addition they were all confirmed to be koinobiont endoparasitoids (Table 9) as they all emerged from tephritids that were field-collected as larvae. Thus, *F. caudatus* and *F. ceratitivorius* are egg-prepupal parasitoids and *F. silvestrii* is probably an early attacking larval-prepupal parasitoid, although confirmation of host stage attacked is needed.

Larval-prepupal Endoparasitoids (Designation – Pre2)

Early attacking larval-prepupal endoparasitoids (designation – Pre2A). Only *F. ceratitivorius* was confirmed as attacking first instar tephritid larvae (Table 9). Of the 58 first instar *C. capitata* exposed to *F. ceratitivorius*, one parasitoid egg was recovered. Lopez et al. (2003) further confirm *F. ceratitivorius* oviposits into first instars. Of the 41 first instar medfly

exposed to *F. caudatus*, no parasitoid eggs were recovered, and it is therefore excluded from the larval-prepupal guild.

Biology of *F. silvestrii* was not confirmed, but based on ovipositor morphology, which resembles that of *Fopius vandenboschi* (Fullaway), it is hypothesized to be an early attacking larval-prepupal parasitoid (Table 9).

Late attacking larval-prepupal endoparasitoids (designation – Pre2B).

Diachasmimorpha fullawayi, *P. cosyrae*, *P. concolor* and *T. giffardianus* were all confirmed as late attacking larval-prepupal endoparasitoids based on rearing the parasitoids from isolated puparia and laboratory exposure to and successful attack of third instar medfly larvae (Table 9). All larval-prepupal parasitoids were confirmed to kill the host prepupal stage (Table 9).

All larval-prepupal parasitoids emerged from puparia of tephritids that were field-collected as larvae, and thus are koinobiont endoparasitoids (Table 9). *Tetrastichus giffardianus* and *T. giffardii* are morphologically similar and gregarious, with 6-10 individuals emerging from each host puparium. This biology, as well as overall morphological similarity between the two suggests that *T. giffardii* may also be a larval-prepupal endoparasitoid, but this needs confirmation (Table 9).

Larval Ectoparasitoids (Designation – L3)

Standard rearing protocols facilitated confirmation of the above koinobiont species as parasitoids of Tephritidae since tephritid larvae normally exit from the fruit to pupate in the soil and thus their puparia can easily be gathered and isolated in the laboratory for the emergence of parasitoids. Modification of standard rearing protocols to holding fruit in a Plexiglas cage yielded several parasitoids that emerge from the fruit. Dissections of fruits to locate host remains lead to discovery of only one of these species as a parasitoid of Tephritidae: the larval

ectoparasitoid *Bracon celer*. Based on host remains, *B. celer* attacks third instar larvae. However, since only a limited number of host remains were discovered, a confident statement cannot be made about whether this is the only host stage that can be successfully attacked.

As an idiobiont, *B. celer* paralyzes its host and then feeds ectoparasitically. Since the parasitoid paralyzes its host, the host stage killed is also the larval stage that is attacked.

Pupal Endoparasitoids (Designation – P2)

Coptera robustior was the only pupal parasitoid recovered from medfly puparia recovered from the soil beneath coffee plants at Ruiru. The widespread, polyphagous pupal ectoparasitoid *Pachycrepoideus vindemmiae* (Rondani) (Hymenoptera: Pteromalidae), commonly used in augmentative programs against tephritid pests, was recovered from *Dacus* puparia infesting squash at ICIPE, but not from coffee. On occasion, adult wasps of this species were also found crawling on the inside of the rearing room windows (undoubtedly originating from the drosophilids associated with the older coffee samples).

Silvestri (1913) describes the biology of *C. robustior* as endoparasitic by recording the insertion of the egg into the body of the pupa. The samples from Ruiru confirm that *C. robustior* kills the pupal stage as the parasitoid emerges from the host puparium. As a pupal parasitoid, by definition it is an idiobiont (Table 9).

Searching and Oviposition Behavior of F. caudatus, F. ceratitivorus, and F. arisanus

General Behavior

Fopius caudatus females search for prospective oviposition sites in short bursts. The females roll the ends of their antennae into a circle and use the outer surface of the circle to search the surface of the fruit for cues (Fig. 17). Between bursts of searching with antennae rolled, the female will stop and straighten her antennae. After a short pause she again rolls her antennae and returns to searching the surface of the coffee cherry. During searching the antennae are held in line with the body. When the female finds a suitable oviposition site, the antenna remain rolled and are brought in closer together so each antenna is in contact with possible oviposition site. As probing of the site begins, the rolled antennae stay in contact with the site and guide the ovipositor into the oviposition site. Thus, as the female is guiding the ovipositor into the site, she is in a characteristic C-shape.

Although *F. ceratitivorus* also searches for oviposition sites in short bursts, this species uses a very different search tactic. *Fopius ceratitivorus* searches for oviposition sites using flattened antennae held out in front of the body, sweeping back and forth. When the female finds a suitable oviposition site she brings the antennae together except for the last few segments, which are held apart, creating a Y-shaped figure. Then she lifts the antennae, holding them out straight. She does not use them to guide the ovipositor into the hole but rather lifts the ovipositor and probes in circles on the cherry surface until the ovipositor disappears into the hole.

Fopius arisanus searches in the same manner as *F. ceratitivorus*.

Ovipositional Cues

Both *F. caudatus* and *F. ceratitivorus* were observed inserting their ovipositors into coffee cherries naturally infested with eggs of *C. capitata*. *Fopius caudatus* also probed cherries artificially damaged and artificially infested with medfly eggs (treatment 1) and artificially damaged cherries without eggs (treatment 2). *Fopius caudatus* did not respond to the control cherries (no damage and no eggs) or to eggs presented alone. *Fopius ceratitivorus*, however, did not probe either treatment 1 or treatment 2 cherries. They also did not respond to the control cherries or to eggs presented alone. *Fopius arisanus* probed both treatment 1 and 2 cherries as well as eggs presented alone. They did not respond to the control cherries, but they did probe the plastic of the Petri dish.

Koinobiont Versus Idiobiont Parasitoids

Only two idiobionts, the larval ectoparasitoid, *B. celer*, and the pupal endoparasitoid, *C. robustior*, were collected. All other species (Table 9) are koinobionts that oviposit in the egg or larval stage of the host and emerge from the puparium. Host specificity of these parasitoids, relative to the various species of tephritids infesting coffee in Kenya is an important part of any discussion of community-level interactions, and is covered in Chapter III.

Discussion

As seen above acquisition of parasitoids for use in classical biological control programs is beset with challenges running the gamut from locating the pest and collecting its natural

enemies to quarantine and regulatory issues. Specific difficulties associated with acquisition of parasitoids for use in biological control of tephritid pests fall under the following categories: (1) field sampling techniques, (2) processing of samples in the laboratory, (3) estimates of rates of infestation and parasitization, and culturing hosts and parasitoids. The relevance of these problems to successful biological control programs is discussed in the first part of this section while the second part focuses on the guild structure of parasitoids in arabica coffee in Kenya, including the relative contributions of koinobiont and idiobiont parasitoids.

Sampling

General Considerations

The issue of when to sample fruits is a problem for all tephritid programs. A related problem is how to recover pupal parasitoids from hosts that pupate in the soil. As noted by Sivinski *et al.* (1993) the best estimates of parasitism by koinobionts infesting fruit are obtained by waiting until the larvae begin to emerge from the fruit to pupate in the soil to collect the fruit. (This assumes, of course, that larvae normally exit the fruit to pupate, which is true of coffee-infesting tephritids and generally true of tropical fruit-infesting pests but there are exceptions.) If fruit is sampled too early (when coffee is still green or just turning from green to reddish), parasitism by species that preferentially oviposit in third instar larvae, such as *P. concolor* and *D. fullawayi*, will be underestimated relative to koinobionts such as *F. caudatus* that oviposit in the host egg. If fruit is sampled too late, many larvae will have already exited and thus infestation rates will be underestimated. There is thus a trade-off between ease of sampling (which often translates to larger or more samples being processed) and logistically cumbersome modifications

to the sampling program (Sivinski *et al.*, 1993) that facilitate collection of hosts that have received the maximum amount of exposure to parasitoids.

Samples collected at some distance from the laboratory (e.g. Koru relative to Ruiru and Rurima) have added problems of increased mortality during transport. Constant jarring of fruit on rough roads, for example, can lead to premature emergence of larvae from fruit and lower rates of successful pupation. Samples generate a considerable amount of heat when many fruits are packed together in closed containers during transport, resulting in fairly high rates of mortality of larval instars for large samples. These problems can be solved at least in part by open, well-aerated containers and care in packaging and transportation.

Fruit-infesting tephritids are traditionally sampled by collecting fruit, holding the fruit until emergence of larvae to form puparia in soil, vermiculite, or other substrates beneath the mass of fruit, then placing the recovered puparia in a separate emergence cage. This eliminates potential for sampling egg and pupal parasitoids, as well as larval ectoparasitoids that kill the host prior to formation of the puparium. Thus, these other guilds must be sampled in non-traditional ways to complete the picture of the community structure.

Weather Effects on Coffee Sampling

Starting in the middle of 1997 and extending through early 1998 a very strong warming episode in the tropical Pacific (commonly known as “el niño”) was in effect. Warm episodes of this nature cause dry weather from December to February in East African areas such as Kenya (NOAA, 2004b). In response to the warming episode, a strong cold episode effect (commonly known as “la niña”) began in mid 1998 lasting through mid 2000. Cold episodes cause wetter weather in East African areas including Kenya (NOAA, 2004a). It is highly probable that the

dryer and then much wetter than normal weather that affected Kenya during the time of the project had an impact on our results. For instance, the cold episode effect and the resulting wetter weather in Kenya during early 1999 may have been the cause for the dramatic increase in fly numbers per kilogram at both Ruiru and Rurima in the early months of 1999.

Infestation Rates and Impact of Sample Handling Procedures

Sample results presented in Appendix C do not accurately represent infestation rates because of two problems associated with sample processing that were only discovered towards the end of the program: (1) pupation of larvae in the coffee mass that were overlooked and thus not included in the sample results and (2) larval mortality from time of field collection until recovery of pupae in the lab, exclusive of mortality caused by ectoparasitoids. For example, in the sample from Rurima collected in September, 1999, 53% of the larvae was estimated to have pupated in the mass of coffee fruit (based on counts of individuals pupating in the fruit mass and on comparison of infestation rates calculated from the number of larvae and eggs in a subsample of dissected fruit with those based on number of puparia recovered from the two sievings of sand beneath the fruit sample). Accurate infestation rates (such as those presented in Table 3) can only be obtained by dissecting cherries and counting the larvae and eggs contained initially upon collection of the sample. Thus, the number of puparia recorded from samples in Appendix C reflects only a portion of the true level of infestation, whereas Table 3, which gives infestation rates from numbers of larvae and eggs dissected from fruit immediately after collection, gives a much more accurate picture of infestation rates.

Though laborious, it is essential to dissect fruit samples immediately after collection for accurate assessment of infestation rates rather than rely solely on numbers of collected puparia.

Dissection of fruits immediately upon collection also leads to the generation of valuable information on the age structure of the infestation. Once a series of such dissections has been made, however, a mortality factor for laboratory handling can be established by comparing the infestation rate based on dissections with the number of puparia recovered, as was done for the September Rurima sample noted above. This will facilitate acquisition of a more reliable estimate of the rate of infestation without the need to dissect a subsample each time. From the results of the sample handling experiment, it was determined that stirring but keeping samples dry provided the best combination for higher sample emergence and less bias against parasitoid emergence. The number of adults emerging from 0.5 kg sub-samples of the November 1999 Koru collection was not significantly affected by moisture treatments but there was a significantly higher number of adults emerging from samples that were stirred. All sample combinations except stirred and dry showed significant bias in emergence of flies over parasitoids. Thus, the stirred and dry combination provides the best emergence data.

This “dry but stirred” recommendation is a departure from the rearing protocol of stirring and misting samples daily as used in this project and reflected in the data shown in Appendix C. Based on the results of these experiments, we can expect the data in Appendix C to overestimate the number of flies and underestimate the number of parasitoids. For the November, 1999 sample, parasitoids would need to be adjusted upwards by 11.7% and flies adjusted downward by 4.2% for the expected numbers to equal the actual observed numbers after the sample was dissected. (Expected numbers for this sample were calculated following the same method as in Appendix C where the percentage of emerged flies and parasitoids was assumed to be same in the unemerged puparia as it was in the emerged.)

While it may not seem obvious, determining the effects of coffee sampling practices on the resulting parasitoid guild structure is important in order to better understand possible

mechanisms and constraints for parasitoid community development. In this particular case, the degree of stacking of the larval-prepupal guild and the absence of stacking in the egg-prepupal guild would not be readily apparent.

Phenology

In general the data are not complete enough to determine if the cyclical patterns for parasitoids shown in Figs. 14-16 are based on abundance of a fly species, abnormal changes in weather patterns, or normal seasonal variations. An obvious exception is the peak in parasitoid activity at Koru that appears during the middle part of the year from April to August (Fig. 15) in both 1998 and 1999. This trend follows the peak months for *C. capitata* and *T. coffeae*. None of the parasitoid numbers appear to follow the abundance of *C. fasciventris* at Koru.

When mean abundance values for the two years are combined and presented as monthly averages, it is more obvious that *D. fullawayi* tracks the abundance of *C. capitata* (Figs. 8 and 14) than when each year is examined alone. The same conclusion can be drawn for *F. ceratitivorus*.

Phenology of parasitoids seems to be affected by the stripping of all coffee cherries in December to reduce coffee-pest populations during non-peak seasons. This effect seems to be especially evident during the early part of the year for *F. ceratitivorus*, when populations of this parasitoid are extremely low. Other factors cannot be ruled out, but the observed trend was consistent over a two year period.

Flies

Host Flies

Distribution of the tephritid hosts in arabica coffee, as determined from our survey, has already been published in Wharton *et al.* (2000). This paper contains the work that I did in collaboration with others during this project. Wharton *et al.* (2000) used the name *C. rosa* for Natal fly from the Koru, Ruiru and Rurima sites, as did Abasa (1973) in his study of coffee in the central highlands. The common name, Natal fly, has been applied historically to populations throughout Africa, but recent work by De Meyer (2001) revealed the existence of a second species residing under this name: *C. fasciventris*. We confirmed that all of our material should be called *C. fasciventris* rather than *C. rosa*.

Arabica coffee supports a complement of both *Trirhithrum* and *Ceratitis* species in Kenya (Abasa 1973; Wharton *et al.*, 2000) with most of our samples producing 2-3 species of flies. Robusta coffee, however, seems to be a poor host for medfly and other species of *Ceratitis* (Greathead, 1972; Steck *et al.*, 1986). Thus robusta and arabica coffee host different complements of tephritid flies. Although we sampled arabica coffee almost exclusively because of our interest in medfly, we did obtain a collection of robusta coffee at Ruiru in February 1999. This sample yielded only flies of the genus *Trirhithrum*, thus further supporting the idea that robusta coffee is mainly attacked by flies in this genus.

Importance of Host Cultures

Many previous classical biological control explorations have failed because of the inability to establish cultures of host flies and/or parasitoids. For example, *F. caudatus* has been recovered numerous times beginning with its discovery in 1912 (Silvestri, 1913; Clausen *et al.*, 1965; Steck *et al.*, 1986). Yet, due to a complicated biology coupled with paucity of cultures in areas like Kenya, this parasitoid has never been reared. .

Host cultures are just as important as parasitoid cultures in a biological control program. They are essential for augmentative release programs and are critical for developing the understanding of biological characteristics that is needed for the successful rearing of these parasitoids. USDA protocols for rearing medfly are very thorough and work very well for most tephritid fly species. In a majority of the cases these protocols should be adopted as closely as possible with modifications as required by limitations of local supplies. For example, in this project we modified the USDA fly oviposition unit as wild flies, unaccustomed to oviposition in plastic units, were being used to establish colonies. While we could not get the flies to oviposit into plastic containers normally used by the USDA in rearing tephritid fruit flies, they readily oviposited in fruit, even unperferred hosts such as banana. Since bananas were readily available in Kenya, they were used as an oviposition unit for medfly as well as other flies reared from coffee. The novelty and ease of using bananas as a cheap and readily available oviposition unit can be adapted for tephritids worldwide, but especially under third world conditions. It is always important to meet the nutritional needs of both the immature and adult stages of the host. For fruit fly adults, protein, usually provided in the form of yeast, is necessary. Larvae cannot generally develop in unperferred hosts such as banana and must be transferred to a more accommodating diet for survival.

*Parasitoids****Psytalia concolor* Species Complex: The Identity of the Kenyan *Psytalia***

The production of viable female offspring from all the experimental crosses strongly suggests that the populations of *Psytalia* from coffee in central Kenya are *P. concolor*. Our work thus supports the findings of Fischer (1958) for northern African populations. A certain amount of behavioral incompatibility was noted, however, and this suggests the need for some caution in interpretation of results and the need for further experimentation. Mating often seemed forced by aggressive males, especially in the male Italian X female Kenyan experiments. A major problem was the general reluctance of Kenyan individuals to mate under any of the experimental conditions, even for the within-population crosses. Thus, it is uncertain whether the behavioral differences that we observed (particularly the reluctance of females from Kenya to remain quiescent during mating with males from Italy) are an artifact of caged conditions or reflect a potentially important behavioral barrier to mating in the wild.

Kenya has a diverse array of habitats, including arid thorn scrub, montane forests, and lush, coastal lowland tropical forests. Members of the genus *Psytalia* have been reared from a variety of tephritids in fruits representing numerous plant families from these various habitats. As their host flies exhibit two to threefold difference in body size, the *Psytalia* also vary tremendously in size. The more obvious differences in size and color appear to be governed largely by the host on which they develop, though experimental evidence is lacking for Kenyan populations. Continued comparisons of members of the *P. concolor* species complex from these different regions and different hosts should further elucidate this speciation problem and enable

us to determine whether there is just a single, widespread, polyphagous species or a complex of species sorting by host and/or region.

***Psytalia concolor* Species Complex: Relevance to Biological Control**

Correct identification of a natural enemy is essential for its effective use in biological control, and establishing the identity of *P. concolor* on *Ceratitis capitata* in coffee in Kenya should facilitate its use in biological control of medfly. With renewed interest in obtaining parasitoids more effective than those currently available (Messing, 1996; Sivinski, 1996), a logical focus of attention is the indigenous parasitoids of medfly in sub-Saharan Africa (Headrick and Goeden, 1996). Most of the parasitoids currently in culture (primarily opiine Braconidae) develop successfully on medfly, and laboratory cultures are often maintained on this host. In nearly all cases, however, these opiine species were originally collected and reared from tephritids of the genus *Bactrocera*, outside of the native range of *Ceratitis*, and then were introduced to Hawaii and elsewhere, where they attack medfly and other introduced tephritid pests (Silvestri, 1914; Clausen *et al.*, 1965). Thus, populations of *Diachasmimorpha longicaudata* (Ashmead), *D. tryoni* (Cameron), *Fopius arisanus*, *F. vandenboschi*, and *Psytalia incisi* (Silvestri) that attack medfly all represent new host associations. Similarly, the cultures of *P. concolor* reared on medfly in Italy and elsewhere represent a new association because this parasitoid was originally obtained from olive fly in Tunisia. Thus, the discovery of populations of *P. concolor* that occur naturally in coffee in Kenya should be of substantial interest for those concerned with lowering populations of medfly in areas where coffee is a primary host.

Psytalia concolor has been credited with some level of success in augmentative programs against tephritid pests, though documentation is frequently inadequate. Our results

suggest that significant behavioral changes may occur when this species is kept in culture for multiple generations. These changes affect both mating and oviposition. Our results further suggest that there may be a trade-off between ease of mass-rearing and field efficacy. Behavioral traits that make this species ideal for culturing (adoption of Petri dish oviposition units and loss of responses to cues that inhibit mating under artificial conditions) may limit their ability to use the chemical cues that enable them to mate successfully and find hosts in the field.

Discovery of Two New Egg/Prepupal Parasitoids of Medfly

Two egg-prepupal parasitoids, *F. caudatus* and *F. ceratitivorus*, were recovered during the course of this research. This biological trait was previously unknown for tephritid parasitoids native to Africa, and one of these (*F. ceratitivorus*) represents a new species first discovered during this program. There is great excitement surrounding the possibilities for these two parasitoids, as their congener *F. arisanus*, which is also an egg-prepupal parasitoid, is widely regarded as the most successful tephritid fruit fly parasitoid used in biological control.

Comparisons of *Fopius caudatus* and *F. ceratitivorus* with *F. arisanus*: Oviposition

Behavior and Associated Cues

The following description of the oviposition behavior of *F. arisanus* is from Haramoto's (1953) thesis (the species was identified as *Opius oophilis* at that time, and the target pest was placed in the genus *Dacus* rather than *Bactrocera*).

Opius oophilis females search for prospective ovipositional sites with the antennae spread wide apart and directed into onto the fruit surface. The female moves in short dashes, lifting the antennae back to their normal position after each brief stop. As she approaches the fruit fly egg cavity,

the antennae are drawn closed together except for the last few segments which are spread apart to give a Y-shaped appearance. After a brief examination of the potential site, the female raises her antennae to their normal position and moves forward until the hind legs straddle the egg cavity. (Indications are that the ovipositional site is first located by the sense of smell, and then the host eggs are located by the sense of touch). She then raises her abdomen, brings her ovipositor to a vertical position and probes delicately up and down until the opening to the cavity of the *D. dorsalis* eggs is found.

Fopius ceratitivorius follows very similar oviposition behavior patterns to *F. arisanus*. However, *F. caudatus* exhibits an extremely different oviposition behavior. In the latter, the antennae are rolled while searching and thus the dorsal portions of the antennae touch and are used to search the fruit surface. The antennal rolling is unique to *F. caudatus* among the *Fopius* I have observed, as is the use of the dorsal surface of the antenna. In addition, once *F. caudatus* has found a suitable oviposition site, her antennae remain rolled and in contact with the site to guide the ovipositor into position. This behavior is unlike that exhibited by *F. ceratitivorius* or *F. arisanus* or any other *Fopius* species.

Haramoto (1953) conducted an experiment to determine whether the egg or the fruit attracted *F. arisanus*. Sections of guava were exposed in the following manner: (1) sections punctured by a needle, (2) sections punctured by *Bactrocera dorsalis* females with the fruit fly egg removed, (3) sections punctured by a needle and fruit fly egg placed the bottom of the punctures. Searching and probing were observed in treatments two and three but not in treatment one. In addition, fruit fly eggs were placed on a cellulose sponge. The parasitoids did not show any interest in these eggs. Thus the conclusion was made that both the fruit and the egg are necessary for effective parasitism.

Similar sets of experiments were conducted during the course of this investigation, with *F. arisanus* used for comparison. Initially this experiment was designed with the naturally infested coffee cherries as a choice. This choice had to be removed as the parasitoids, when

naturally infested coffee cherries were present, did not respond to any of the other choices. Based on these preliminary observations, it can be concluded that both *F. caudatus* and *F. ceratitivorus* use the fly pheromone mark to some degree in finding a host.

Following removal of the naturally infested cherries, *F. caudatus* responded to artificial damage of coffee cherries both with and without eggs present. Thus, it is possible that although they use the fly's marking pheromone, they are also using damage as one of their cues for probing and oviposition. *F. ceratitivorus* did not respond to any of the treatments. They must rely on the fly pheromone cue to initiate the probing sequence. Thus, *F. caudatus* uses plant damage (possibly including associated volatiles) to initiate oviposition behavior whereas *F. ceratitivorus* does not. Contrary to the findings of Haramoto (1953), *F. arisanus* responded to all the treatments including probing the plastic. Generations of rearing in the laboratory has obviously changed cues or selected for parasitoids that accept different cues than those initially acceptable to *F. arisanus*.

Based on differences in cues, oviposition behavior, and geographical distribution (separated by the Rift Valley), *F. ceratitivorus* and *F. arisanus* may be more closely related to each other than either is to *F. caudatus*.

Culturing Parasitoids

Part of the failure to culture African parasitoids in Hawaii (other than *P. concolor*) may have been due to the facilities available in quarantine. All of the material sent to Hawaii was assigned to a small, screened room for assessment. The screened room did not have adequate airflow and the only available sunlight came indirectly through a tempered window. Based on observations made in Kenya and Guatemala, the lack of airflow and sunlight probably had the

greatest impact on the difficulty of rearing the two species of *Fopius* that were frequently shipped to Hawaii, *F. caudatus* and *F. ceratitivorius*. Similar problems were encountered when *F. arisanus* and *F. vandenboschi* were first cultured during the oriental fruit fly program (Hagen, 1953). Even today it is much harder to rear *F. arisanus* than any of the other parasitoids established in Hawaii. Many of the same problems with rearing and mating of the Kenyan species of *Fopius* also occurred in Guatemala, but with brand new facilities designed specifically for rearing tephritid parasitoids, these problems were easier to solve and a culture of *F. ceratitivorius* was successfully established. Nevertheless, the culture of *F. caudatus* has not yet been sustained beyond the seventh generation (P. Rendon, J. Sivinski, pers. comm.).

Based on examination of oviposition behavior (previous section), *F. ceratitivorius* may be very difficult to rear using artificial methods. However, using the initial work done in this thesis, Lopez *et al.* (2003) were able to establish lab cultures of *F. ceratitivorius* using infested coffee cherries. *Fopius arisanus*, even in the 1950's, fresh from the field, would probe an artificial hole containing fresh fruit fly eggs (Haramoto, 1953). This early acceptability to eggs in fruit, without the fly mark, probably explains why today *F. arisanus* is able to be reared using eggs that are only hours old, placed in an artificial substrate. *Fopius caudatus* should also be easier to rear as it cues in on damage as well as the fly mark and egg. The problem to date with *F. caudatus* seems to be the tendency of the culture to turn to a male bias. It is highly probable that this male bias is due to a lack of mating. *Fopius caudatus* may need sunlight (like *F. arisanus*) for mating.

Guild Structure

Defining and Utilizing the Concept of a Guild

In our biological control program we classified the potential release candidates into guilds, groups that exploit the same class of environmental resources in a similar way. Classifying natural enemies in this manner facilitates nontarget testing both in terms of selecting which natural enemies to test as well as streamlining experimental design. Mills (1994) used three criteria to delimit parasitoid guilds in his attempt to define the structure of endopterygote insect host communities. These three criteria are: 1) host stage attacked by the parasitoid, 2) the host stage killed by the parasitoid, and 3) the mode of parasitism (ectoparasitic or endoparasitic). Based on these factors he delimited a series of 12 guilds. Of the 12 guilds he defines, we recovered four of these attacking tephritids in Kenyan coffee. They are 1) egg-prepupal endoparasitoid, 2) larval-prepupal endoparasitoid, 3) larval ectoparasitoid, and 4) pupal endoparasitoid. The publication by Wharton *et al.* (2000) incorporated preliminary characterization of these guilds based on these studies.

The Guilds of Tephritid Parasitoids Associated with Arabica Coffee in Kenya

Most surveys of tephritid parasitoids do not sample for egg parasitoids. This lack of sampling has resulted in few if any legitimate records of tephritid egg parasitoids (the idiobiont egg parasitoid guild). It is therefore difficult to determine, based on previous studies of tephritid parasitoids, whether the egg parasitoid niche is truly empty or simply inadequately sampled. The results reported above, however, confirm the paucity of idiobiont egg parasitoids, at least in

Kenya. No egg parasitoids were recovered from the 211 eggs segregated from field-collected fruit. If egg parasitoids are present, they either occur seasonally or in extremely low frequency. Since the tephritids we examined insert their eggs deep inside fleshy fruits, the moist, sticky environment may preclude access by egg parasitoids. Thus, the paucity of recorded egg parasitoids may not be an artifact of inadequate sampling.

Wharton *et al.* (2000) treated the egg-prepupal and the larval-prepupal endoparasitoids as a single guild of koinobiont endoparasitoids that develop at least in part on the larval stage of the host and emerge from the host puparium. I, however, consider the egg-prepupal parasitoids and the larval-prepupal parasitoid guilds to comprise two separate guilds with a distinct potential for interference competition.

While we found several parasitoids coexisting in the larval-prepupal endoparasitoid guild, whereas the egg-prepupal endoparasitoid guild only contained one parasitoid at each collection site. At Rurima and Ruiru, on the east side of the Rift Valley, *F. ceratitivorus* was recovered as the only egg-prepupal parasitoid. *Fopius caudatus* was collected on the west side of the Rift Valley at Koru. (Based on ovipositor morphology, *Fopius silvestrii* is most likely an early instar parasitoid like *Fopius vandenboschi*, thus no overlap in the egg-prepupal guild.) Since there is only one egg-prepupal parasitoid at each site, there is no competition within this guild. Parasitoids in the larval-prepupal guild exhibited a much different strategy. There tended to be four to five parasitoids in this guild at each site (Table 4).

In multiparasitism studies using parasitoids previously introduced into Hawaii to control tephritids, it has been shown that the first parasitoid present in a host generally wins the competition for survival (Pemberton and Willard, 1918a; Wang and Messing, 2003; Wang *et al.*, 2003). It is possible, because of the intense competition between parasitoids in the highly

occupied larval-prepupal guild, that there has been strong selection for oviposition into younger hosts.

If the *Fopius* species from Africa behave like *F. arisanus* (and we predict they will), then they will consistently win the interference competition with the larval-prepupal endoparasitoids. This puts the egg-prepupal endoparasitoids at a distinct advantage. Certain species in the genus *Fopius* may have developed this capability by learning new oviposition cues and ovipositing in successively earlier instars and finally the host egg.

The discovery of two species of egg-prepupal parasitoids capable of attacking medfly is of particular interest to biological control. This interest stems in part from the fact that the most successful parasitoid to date in the biological control of tephritid pests, *Fopius arisanus* (Sonan), is an egg-prepupal parasitoid (Clausen et al. 1965; Clausen, 1978; Haramoto and Bess, 1970). Further, there have been repeatedly expressed concerns that fruit fly pests of larger commercial fruits, such as mango, common guava, and citrus, are largely free from attack by most parasitoids because the latter cannot reach the larvae feeding deep within these larger fruits with their ovipositors (Sivinski, 1991). Unfortunately, egg-prepupal parasitoids have been notoriously difficult to culture (Harris and Okamoto, 1983). With the successful colonization of *F. ceratitivorus* (Lopez et al. 2003) and a greater appreciation of rearing constraints for *F. caudatus*, these two species should be excellent candidates for use in biological control. In addition to associations established via host remains, *F. ceratitivorus* was recovered from at least two large samples of coffee from which only Medfly was reared. In this study, *F. ceratitivorus* was not reared from any other host, though it is premature to conclude that this parasitoid is host specific to medfly.

As the name of the guild implies, the egg-prepupal and the larval-prepupal guilds do not kill their host until the prepupal stage, a fact that has been overlooked in several previously

published studies of tephritid parasitoids. Though the parasitoids may not attack the larvae when it is fully developed (just prior to pupariation), they are nonetheless utilizing the maximum host capacity by waiting to kill the host until the prepupal stage. This indicates size, or the amount of resources available for development, may be an important consideration for successful parasitoid development (Askew and Shaw, 1979; Mills, 1994).

The fourth guild considered here consists of idiobiont ectoparasitoids of the larval stages. *Bracon celer* was the only member of this guild reared from tephritids in Kenyan coffee. We found this larval ectoparasitoid at all three sites, but only after modifying the standard rearing techniques. Wharton *et al.* (2000) concluded that larval ectoparasitoids of frugivorous tephritids appear to be rare. If intensive sampling were undertaken, specifically for larval ectoparasitoids, it is plausible that other species might be recovered but based on the size of the samples that I collected for the special processing needed to recover ectoparasitoids, additional species either do not exist, or they are rare and/or seasonal in arabica coffee.

In our study, only a single pupal parasitoid (*Coptera* sp.), representing the pupal endoparasitoid guild, was recovered from coffee plantations. During the same period, however, two other pupal parasitoids (in the genera *Pachycrepoideus* and *Dirhinus*) were reared from cucurbit-infesting *Dacus* hosts in Kenya. It is extremely labor intensive and difficult to recover pupal parasitoids of tephritid fruit flies. Either the ground and litter beneath a fruit fly infested area must be collected and sieved for the collection of puparia or traps must be placed over the same area for collection of live adults as they emerge from the ground. If live adults are collected, then the parasitoid/host association must be verified by culturing them in the laboratory on tephritid hosts.

As I had limited access to the field and was unable to set up traps to capture live adults, I was unable to fully explore this guild. Since we only recovered one pupal endoparasitoid at one

location (Ruiru) in coffee, it is highly probable that if a more extensive collection were made, other species would be found. Nevertheless, my samples indicate that the attack rate by pupal parasitoids is low (2.4% parasitism), and that pupal parasitoids are relatively rare.

Relevance of Guild Structure to Biological Control

It is apparent that the larval-prepupal guild contains several species of parasitoids. Our data from Kenyan coffee are comparable in this regard to the findings of Steck *et al.* (1986) for coffee in West Africa. Similarly, frugivorous tephritids in tropical America host several species of koinobiont larval-prepupal parasitoids in both cultivated and wild settings (Gilstrap and Hart, 1987; Ovruski *et al.*, 2000). Thus, the multiple introductions and establishment in Hawaii of several species from this guild mirrors conditions found in regions where pests are endemic. This provides a powerful argument in favor of multiple species introductions in the larval-prepupal guild. Our data were not sufficient to elucidate seasonal patterns in parasitoid presence. Thus, although we know that parasitoids such as *P. concolor*, *D. fullawayi* and *T. giffardianus* coexist at periods of peak abundance, we do not know if they are present together throughout the fruiting season.

There may be several reasons for only one egg-prepupal endoparasitoid at each site, and these could have important implications for future biological control introductions of these parasitoids. First, it is much wetter at the Koru location where *F. caudatus* is present, than at the drier locations where *F. ceratitivorus* is present. Thus, climate may be an important factor limiting the distribution of these two species and should be carefully considered when introducing either of these egg-prepupal parasitoids to Latin America and/or Hawaii. Second, these parasitoids may have different host preferences. From my dissections the host fly puparia

from which parasitoids have emerged, *F. ceratitivorus* attacks medfly while *F. caudatus* prefers *C. fasciventris*, although *F. caudatus* will attack and successfully develop on other species such as medfly. Third, it is also possible the egg-prepupal endoparasitoid guild is not capable of sustaining two parasitoids in the same immediate area. Thus, in Hawaii, where *F. arisanus* is already very successful at controlling populations of fruit flies, broad scale, augmentative releases may not be the best course of action for liberation of the new African egg-prepupal parasitoids. Instead, it will be important to concentrate releases of *F. ceratitivorus* in areas with high medfly densities and *F. caudatus* in wet areas where these African parasitoids may be better able to control specific fruit fly populations than *F. arisanus*.

Fopius arisanus, an egg-prepupal endoparasitoid, is the most successful parasitoid introduced against tephritids. Because of its success, biological control practitioners have been continually searching for other egg-prepupal parasitoids for use against tephritid pests. Since *F. arisanus* is such a successful parasitoid, it is logical to devote a significant amount of effort to develop tactics to rear these two new biological control candidates that show so much promise.

Thus, one of the most significant and exciting findings of this program was the discovery of a previously undescribed species of egg-prepupal parasitoid attacking medfly in coffee (*F. ceratitivorus*). We also reared and elucidated the life history of a second egg-prepupal parasitoid (*F. caudatus*) that no one has been able to culture successfully since its original description 90 years ago.

Parasitoid Assemblage: Koinobiont Versus Idiobiont

Hawkins *et al.* (1990) state that the majority of parasitoid species of exophytic hosts are koinobionts, whereas the endophytic hosts support mainly idiobionts. From the data presented

(Hawkins *et al.*, 1990), this appears to be a general trend across hosts in the combined orders Coleoptera, Diptera, Lepidoptera, and Hymenoptera. Hoffmeister (1992) asserts the same idea, that the number of idiobionts attacking tephritid hosts outnumbers koinobionts. He concludes that idiobiont parasitoids outnumber koinobiont species in all seven parasitoid complexes examined (which included both pulp and seed feeding tephritids), thus confirming the statements by Hawkins *et al.* (1990).

The above may be true for endopterygote hosts in general and temperate tephritid systems in particular, but the same conclusion cannot be drawn for tropical frugivorous tephritids. In our system, koinobionts grossly outnumbered idiobionts, eight to two, however the pupal idiobiont parasitoid guild was not completely explored. This is an important point since, as Hoffmeister (1992) points out, a critical assessment of the temperate tephritid systems reveals that while there are a large number of idiobiont pupal parasitoids attacking pulp feeders, the parasitoids attacking the larval stages are exclusively koinobionts. However, we believe that even if a concentrated sampling effort were to be undertaken to complete the guild of idiobiont pupal parasitoids in coffee in Kenya, it is still unlikely that idiobionts will out-number koinobionts, and in any case they would certainly never dominate the parasitoid assemblage.

Hoffmeister (1992) also states that competition amongst idiobiont parasitoids is limited to within their own guild. For example, ectoparasitoids kill the host larvae while it is still in the fruit and thus cannot be hyperparasitized by pupal parasitoids. In addition, they can develop as parasitoids of any koinobiont species already present in the host. Thus, Hoffmeister (1992) states that more idiobiont than koinobiont species should be able to coexist in the parasitoid community.

We did not find this conclusion to hold true for our tropical tephritid system. Of the ten parasitoids recovered during our sampling program, we found eight species to be koinobiont

endoparasitoids and only one larval idiobiont ectoparasitoid. In addition, in Hoffmeister's own (1992) paper, if the pulp feeding tephritids are separated from the seed feeding tephritids, then all parasitoids attacking larval tephritids in the pulp are koinobiont endoparasitoids. Parasitoids attacking the seed feeders are dominated by larval ectoparasitoids while still sharing the parasitoids attacking the pulp feeders. In a re-examination of the data, Hoffmeister and Vidal (1994) found that tephritids feeding on flowerheads and stems are mainly attacked by idiobiont larval parasitoids while those feeding on leaves and fruit are mainly attacked by koinobiont larval parasitoids and a few idiobiont larval parasitoids.

While conclusions can be drawn for a specific ecological habitat (i.e. pulp feeders), comparisons should not be made across different types of concealed habitats in an effort to extrapolate broad-based conclusions for all tephritids (such as the relative dominance of idiobiont versus koinobiont). Each type of ecological host habitat should be examined separately, conclusions should be drawn concerning each habitat, and only then may comparisons be made between habitat types.

At the guild level, tephritid fruit flies in Kenya are attacked by the same guilds of parasitoids (egg-prepupal, larval-prepupal, larval ectoparasitoid, and pupal) on both the East and West side of the Rift Valley in Kenya. The same guilds attacking tropical frugivorous tephritids also attack temperate tephritids (Prokopy and Webster, 1978, Wharton, 1997, Hoffmeister, 1992). However as already discussed, the distribution of parasitoids within these guilds is different between the temperate and tropical areas. We hypothesize this tropical/temperate difference is also based on ecological factors.

In our tropical system frugivorous tephritids are attacked by eight koinobiont endoparasitoids, and we hypothesize a small number of pupal parasitoids. In the temperate system studied by Hoffmeister (1992), there are only three koinobiont endoparasitoids and nine

idiobiont pupal parasitoids. Temperate species are generally univoltine with host flies overwintering as pupae in the soil. This fact easily explains the abundance of pupal parasitoids, as hosts are available for a long period of time in the soil. Tropical species are multivoltine with flies almost always available in the larval stage. (Man induced stripping at the end of the coffee season is the main reason flies may become unavailable, otherwise coffee fruits throughout the year.) The pupal stage of tropical tephritids is not prolonged as in the temperate system. With a short period for pupae to be in the soil and several species of flies to parasitize in the larval stage, it is easy to explain the greater number of koinobiont egg-prepupal and larval-prepupal parasitoids in the tropical system. Thus, although the guilds attacking frugivorous tephritids are the same in temperate and tropical systems the distribution of parasitoids within the guilds may be based on relative availability of pupae.

Although the underlying basis for the differences between tropical and temperate tephritid communities still needs to be explored, our data provide a sharp contrast to the findings of Hawkins *et al.* (1990) that endophytic hosts are attacked predominately by idiobionts. Either fruit-infesting tephritids in the tropical setting are just a system that breaks the parasitoid community rules that most other systems follow (and thus it should be studied for why it is different) or there are not enough well studied systems to actually draw broad based conclusions regarding whether koinobionts preferentially attack exophytic hosts or whether there are more idiobionts dominating parasitoid systems in endophytic hosts.

CHAPTER III

HOST SPECIFICITY AND HOST SUITABILITY OF MEDITERRANEAN FRUIT FLY PARASITIDS RECOVERED FROM COFFEE IN KENYA

Introduction

In the age of environmental awareness, parasitoids showing a potential for biological suppression of pests must be tested for possible nontarget effects before they are introduced into a new region. It is logical, then, due to both financial and time constraints involved in testing a potential biological control agent, to first examine the host specificity and host suitability in the native range of the candidate organism. If the organism(s) in question exhibits a propensity for attacking several families of hosts or finds suitable hosts among several orders in its native area, then there is no reason to invest time or money exploring, rearing or testing the organism for release in a classical biological control program. Once it has been determined that the organism in question shows potential for acceptable host specificity in its native area, it is then necessary to examine the candidate biological control organism for nontarget effects in the area where it is to be introduced.

Host Specificity of Ectoparasitoids versus Endoparasitoids

Insect parasitoids vary widely in their host specificity. Some parasitoid species attack a wide range of hosts, while others are relegated to developing in one or a few species (Godfray, 1994). Often, egg, pupal, and ectoparasitoids attack several species. Generally, the offspring of

these parasitoids are not required to overcome the host immune system to complete development. As a result, some parasitoids in these groups, (e. g. *Bracon mellitor* (Say) and some species of *Trichogramma*), may even be found attacking insects across several orders. Larval endoparasitoids, however, tend to be more host-specific as their intimate association with their host requires physiological compatibility (Strand and Pech, 1995; Rutledge and Wiedenmann, 1999).

Eight of the 10 species of parasitoids recovered from tephritids in coffee in Kenya were koinobiont endoparasitoids. Six of these belong to the braconid subfamily Opiinae while the other two are in the family Eulophidae. To complete development, these endoparasitoids must overcome the host's immune system, thus creating the intimate association mentioned earlier. This chapter will focus on the koinobiont endoparasitoids in the subfamily Opiinae.

Opiines are a large (about 1500 described species), relatively host specific group, with all host records in the cyclorrhaphous Diptera. Within the cyclorrhaphous Diptera, the families Agromyzidae and Tephritidae contain the vast majority of opiine hosts. Thus, opiines make excellent initial candidates for biological control screening.

Classification of Tephritidae into Developmental Strategies

White and Elson-Harris (1992) categorize pests in the family Tephritidae by their developmental strategies: 1) those that attack fruit; 2) pests of leaves, stems and roots; and 3) flower head pests. About 1800 (46%) of the described species of Tephritidae are flower head associated. An estimated 600 (16%) of the described tephritids have larvae that mine the leaves, stems or roots of their host plants. Finally, approximately 1500 (38%) of the described tephritids are fruit associated. Most tephritids are non-pests or are beneficial species that fit in these three

categories, however the literature is mainly devoted to the small percentage of tephritids considered pest species.

There is no clear taxonomic division between flower, fruit and bud associated species within the two subfamilies containing the most economically important tephritid pests, the Dacinae (e.g. *Bactrocera* Macquart, *Ceratitis* MacLeay, and *Dacus* Fabricius) and the Trypetinae (e.g. *Anastrepha* Schiner and *Rhagoletis* Loew) to guide selection of nontargets for testing (White and Elson-Harris, 1992; Messing, 2001). For example, taxonomic records indicate that members of the genus *Psytalia* Walker are restricted to tephritid hosts. However, approximately 80% of the described species of *Psytalia* attack fruit-infesting tephritids while 20 % attack flower head or seed infesting species.

Host Specificity/Host Range Testing and Host Suitability

Host Specificity/Host Range Testing

In order to understand the range of hosts that a parasitoid will attack in the field, an in-depth examination of parasitoid host specificity, also sometimes referred to as host range testing, is essential. For parasitoids of fruit-infesting Tephritidae, a logical approach for initiating a study of host specificity is to test hosts within the known habitat of the target pest, in this case coffee cherries, then move to hosts developing in other types of fruits (e.g. squash), and finally tephritids that live in non-fruit plant parts (e.g. flower heads).

Host range testing can be done in the laboratory or in the field. There are three possible steps and two possible types of host range testing. The three steps include testing host range in quarantine, testing in a laboratory outside of quarantine and field-testing in cages. Although

field-testing is preferred, the permits needed for field tests require that the biological control agents have already shown a high degree of host specificity in the laboratory. The two types of host range testing are ecological (=host habitat location and host location) and physiological (=host suitability).

Host Suitability

Host suitability testing is done in the laboratory. A host is offered to a parasitoid; often in a manner ensuring the parasitoid will attack the host. Based on dissection of the host and/or development or non-development of progeny the host is determined suitable or not suitable for development of the parasitoid (Murdoch *et al.*, 1985). Physiological host range estimates the greatest possible host range for a parasitoid, but generally overestimates the actual number of hosts that would be utilized under natural or host range testing conditions (Rutledge and Wiedenmann, 1999).

Ecological Host Range Testing

Ecological host range testing encompasses a complex of long and short-range visual, olfactory, gustatory, mechanoreceptor, and auditory signals (Salt, 1937; Lewis *et al.*, 1976; Vinson 1981, 1984a, b; van Alphen and Vet, 1986; Lewis and Martin, 1990, Vet and Dicke, 1992). Ecological host range testing addresses which host a parasitoid will attack at an ecological level, i.e., the host that the parasitoid actually encounters and uses in nature. It is limited by factors such as phenology, the host life cycle, and climate (Cate and Maddox, 1994; Rutledge and Wiedenmann, 1999). It is also limited by the behavior of the parasitoid itself. The

parasitoid must first choose a habitat and then search within that habitat for the host (Vinson, 1976). It is important to note, the ability of a parasitoid to locate a host in an experimental arena can be affected by cage size and how choices, if there are choices, are offered. Thus, ecological host range tests should attempt to minimize these effects and results should be carefully interpreted.

Materials and Methods

Overview of Host Specificity Testing for Kenyan Parasitoids

The host specificity of Kenyan parasitoids reared from coffee was examined in three ways: (1) association of Kenyan coffee parasitoids with their host flies based on species-level characteristics of the field-collected puparia from which they emerged, (2) rearing of cucurbit infesting tephritids and their parasitoids in Kenya, and (3) rearing of flower head infesting tephritids and their parasitoids in Kenya and Hawaii. For two species of *Psytalia*, *P. concolor* (Szépligeti) and *P. phaeostigma* (Wilkinson), host specificity was examined in two additional ways: (1) by exposing tephritids reared from non-coffee host plants to the two *Psytalia* species and (2) by exposing the tephritids present in Kenyan coffee as well as the invasive frugivorous tephritids in Hawaii to *P. concolor*.

Cucurbits were chosen for examination in this study for two reasons. First, they represent a fruit that is generally infested by a different group of tephritid fruit flies than those normally infesting coffee and other fruits growing on trees and shrubs. Second, and not related to host suitability, there is interest in parasitoids from cucurbits for control of melon fly,

Bactrocera cucurbitae (Coquillett) in Hawaii. Melon fly is an introduced pest in both Kenya and Hawaii.

Flower heads were selected for this study as they are a separate ecological habitat attacked by tephritids and there are parasitoids in the genus *Psytalia* that are known to attack flower heads. In addition, in Hawaii, collections of nontarget flower heads were made to conduct retrospective analysis of the effect that previously released tephritid parasitoids have had on tephritids infesting flower heads.

Members of the genus *Psytalia* were selected for the host suitability portions because of their predominance at localities where medfly was the dominant host in coffee (Chapter II), but also because of rearing success. *Psytalia concolor* was the first parasitoid from this project that was cultured in Kenya and in Hawaii as well as the first to be released from quarantine into the laboratory in Hawaii.

Association of Kenyan Coffee Parasitoids with Their Host Flies

As a cyclorrhaphous fly (such as a tephritid) begins to transform into its pupal stage, the cuticle of the third larval instar hardens forming a puparium (Ferrar, 1987). During pupariation the mouthhooks and the rest of the cephalopharyngeal skeleton remain attached to the hard outer skin of the puparium. Sclerotization of the anterior and posterior spiracles also takes place during pupariation.

The heavily sclerotized mouthhooks as well as the rest of the cephalopharyngeal skeleton remain inside the puparium after both flies and parasitoids have emerged. Thus, they can be used along with anterior and posterior spiracles to identify the host fly after parasitoids have emerged from the puparium. Since nine of the 10 species of parasitoids reared from coffee-

infesting Tephritidae emerged from the host puparium, puparia from field-collected samples were the primary source of data on host use by parasitoids in coffee.

To accomplish the task of associating these parasitoids with their hosts, I individually isolated over a thousand puparia from the monthly coffee samples (see Chapter II for sampling methods) in 1 or 2-dram glass shell vials stoppered with a cotton plug. These were then held for emergence of either a fly or parasitoid. To ensure correct association of a parasitoid and its host fly, relationships first had to be established between each fly species and the diagnostic characteristics of its puparium. The three most distinguishing characteristics of tephritid puparia are: 1) Cephalopharyngeal skeleton (cps) of the third instar larvae that remains inside the cap of the puparium; 2) the anterior spiracles, projecting laterally on each side of what was T1 (the first thoracic segment of the larvae); and 3) the posterior spiracles on the caudal segment (also known as A8, the last abdominal segment of the larva) (Phillips, 1946; Ferrar, 1987).

In this study, the presence or absence of a preapical tooth on the mouthhooks proved to be the most useful characteristic. Thus, on the cps, only the mouthhooks were examined. Anterior spiracles were not used to identify the puparia, as they were not mounted on the slides well enough to distinguish the individual lobes. Posterior spiracles were scored for two distance factors and the relative amount of branching on the interspiracular processes. Ferrar (1987) defined a Spiracle Distance Factor (S. D. F.) as:

$$S. D. F. = \text{Distance between plates} / \text{Diameter of one plate.} \quad [18]$$

Measurements of the distance between postspiracular plates and the diameter of one post spiracular plate are illustrated in Figs. 18 and 19, respectively. The second distance factor, the button to button distance factor (B. B. D. F.), is defined as:

$$B. B. D. F = \text{Diameter of one plate} / \text{Distance from button to button.} \quad [19]$$

The measurement from button to button between the two posterior spiracular plates is illustrated in Fig. 20. For both S. D. F. and B. B. D. F. the reticle was always placed so it covered the center of the two buttons to ensure consistency in measurements.

To establish the correct relationship between the emerged fly species and the morphological features described above, the cap of the puparium (containing both mouthhooks and anterior spiracles) as well as the bottom portion of the puparium (containing the posterior spiracles) were dissected and mounted on slides. Prior to mounting, the extra tissue surrounding the three crucial parts was first removed by soaking the puparium in a 10% potassium hydroxide solution for 4 hours. Then the contents were transferred to glacial acetic acid for 4 hours. Afterwards the structures were rinsed with 95% ethanol (Ferrar, 1987). The final step, separation of the structures from their surrounding tissue, was a delicate and tedious process accomplished using insect pens with their tips bent into hooks. After separation from the surrounding tissues the mouthhooks and anterior and posterior spiracles were mounted on a slide using euparal, and examined under a compound microscope at 400x.

Distinguishing morphological features were scored for ten *Ceratitis capitata* (Wiedemann) (medfly), ten *C. fasciventris* (Bezzi), and fifteen *Trirhithrum coffeae* Bezzi, the three fly species most commonly found in coffee. *Ceratitis anonae* Graham was present only in very low numbers in the Koru samples and thus variation in a character previously used to distinguish this species (large number of anterior spiracular lobes) (White and Elson-Harris, 1992) could not be evaluated due to low availability of individuals as well as problems with the mounting of anterior spiracles.

Following the discovery of diagnostic features on the puparium for the three most abundant tephritids reared from coffee, the mouthhooks and the anterior and posterior spiracles of puparia from which parasitoids had emerged were mounted on slides. The species of fly(s) attacked in the field by the parasitoid was then determined using the characteristics noted above.

Rearing Tephritids and Associated Parasitoids from Non-coffee Host Plants

Cucurbit Collections in Kenya

In both 1998 and 1999, cucurbits (yellow squash and zucchini) were planted at our request in two gardens that contained tephritid-infested coffee and tephritid-infested flower heads. Both gardens were either at or adjacent to ICIPE in Nairobi. As cucurbit fruits matured, they were brought into the lab at ICIPE for processing. Processing began with placing the mature squash fruit in Perspex cages (of various sizes). Paper towels were placed on the bottom of the cage to absorb the liquid of the decaying squash as well as to provide a pupation medium for the tephritid larvae. Puparia were either collected from the cage and isolated in 1-2 dram vials or were allowed to remain in the cage until emergence (for establishment of fly and parasitoid colonies). Cultures of *Dacus ciliatus* Loew, *D. bivittatus* (Bigot), and *B. cucurbitae* were established (the first two by M. Trostle and *B. cucurbitae* by S. Mohamed) by first placing male and female flies in a cage for mating and then placing an uninfested mature squash in the cage for oviposition. The remaining rearing protocol is the same as discussed in Chapter II for rearing flies and parasitoids from field collections. R. Wharton initially identified flies and parasitoids from the squash.

In 1998, puparia from the laboratory were put in the soil beneath rotting, tephritid infested squash in an effort to increase the possibility of recovering a pupal parasitoid. This experiment was only done one time due to the time constraints imposed by attempts to rear pupal parasitoids from coffee (see chapter II). One hundred puparia were placed under 7 rotting squash. After one week, soil beneath the squash was collected and brought back to the lab. There the soil was sieved to collect puparia. The puparia were held for emergence of flies and/or pupal parasitoids.

Characteristics of the puparium were scored for identification of the hosts attacked by the parasitoids reared from squash, using the methods described above for the puparia from coffee samples. Puparia of 20 *D. bivittatus* were examined, but due to the limited number of good quality specimens and the availability of previous descriptions of their puparia (Ferrar, 1987; White and Elson-Harris, 1992), only three *D. ciliatus* and three *B. cucurbitae* were scored.

Flower Head Collections in Kenya and Hawaii

Asteraceae (Zinnia) Collections in Kenya

In Kenya, middle and older stages of *Zinnia* (Asteraceae) flowers heavily infested with tephritids were collected. The flowers were collected in two gardens where infested coffee and cucurbits were yielding species of *Psytalia* and *Tetrastichus* Haliday, thus assuring that fruit-infesting parasitoids were in the immediate area. One collection of 54 flower heads was made at the KISI garden August 5, 1998. The other collection, August 18, 1998, was made at the Duduville guesthouse at ICIPE. Seventy-nine flower heads were collected in the latter sample. Puparia were dissected from each flower head and placed in Ziploc® bags to ensure any parasitoids emerging from flower heads were associated with a tephritid host. To determine the infestation rate, the puparia were counted and a mean infestation rate was determined for each of

the samples. Dr. Ian White (Natural History Museum, London) identified the fly emerging from the puparia and Trostle and Wharton identified the parasitoids.

Asteraceae Collections in Hawaii

In 2001, 882 flower heads were collected at two sites on the island of Oahu, Hawaii where at least seven species of tephritid parasitoids are known to be established from releases made against medfly and Oriental fruit fly. One site was along the intersection of Route 61 (Pali Highway) and Highway 83 (the Kamehameha Highway). Two collections were made at this site: July 11 and 18, 2001. The second site was along the Old Pali Road hiking trail. Two collections were made at this site also: June 18 and 25, 2001. The flowers collected were *Bidens fondosa* L., devils beggarticks, and a species of *Sonchus* (most probably *S. oleraceus* L.). Both of these flowers are in the family Asteraceae. The flowers were individually isolated in Ziploc® bags and held for the emergence of flies and parasitoids.

*Laboratory Host Range Testing of *Psytalia* Species in Kenya*

To test the ability of parasitoids to attack and successfully develop in different tephritid hosts, two species of *Psytalia* were reared in the laboratory and exposed to various species of hosts. *Psytalia concolor* was cultured on *C. capitata* and *P. phaeostigma* on *D. ciliatus*. See chapters II for details of culture methods for flies and parasitoids.

The *C. capitata* and *P. concolor* colonies were initiated using individuals originally obtained from *Coffea arabica* L. at Ruiru, near Nairobi. The *P. concolor* used for this study came from two sources, Italy and Kenya. A mass culture of *P. concolor* is maintained in Pisa, Italy for basic research and augmentative releases against olive fly (Raspi and Loni, 1994).

Raspi shipped several hundred *P. concolor* pupae (inside host puparia) to Nairobi, Kenya where cultures of the Italian strain were established in the ICIPE quarantine facility by Trostle. Two East African populations of *P. concolor* were initially reared at ICIPE in Kenya. One of these populations was sampled from coffee cherries at the Coffee Research Foundation, Ruiru (about 15 km N of Nairobi) and the other population was from a coffee plantation at Rurima, near Embu, about 110 km NE of Nairobi (Kimani-Njogu *et al.*, 2001). The colonies of the two Kenyan populations were mixed and reared as one for these studies, but the colony of Italian *P. concolor* was maintained separately.

Flies reared from field-infested squash from the KISI garden adjacent to ICIPE were used to establish laboratory colonies of *D. bivittatus* (1998 and 1999), *B. cucurbitae* (1998 and 1999) and *D. ciliatus* (1999 only). *Psytalia phaeostigma* was colonized on *D. ciliatus* and *D. bivittatus* cultures using the methods of Wong and Ramadan (1992). A small culture of *C. fasciventris* (originally obtained from *C. arabica* at Ruiru and cultured on artificial diet) was also established for these tests. In addition, small numbers of *Ceratitis cosyra* (Walker) and *C. anonae* larvae were obtained from ICIPE cultures previously used for studies on fly behavior. Tephritid larvae belonging to the genus *Craspedoxantha* Bezzi were obtained directly from zinnias growing at ICIPE and in the KISI garden.

Exposures of *Psytalia* spp. to Tephritid Larvae from Coffee, Squash and Flower Heads

Mated females of *P. concolor* with prior oviposition experience on *C. capitata* were exposed to third instar larvae of *C. fasciventris*, *C. cosyra*, *C. anonae*, *D. ciliatus*, *D. bivittatus*, and an undetermined species of *Craspedoxantha* from flower heads. Ten larvae of both *C. capitata* and *C. fasciventris* were exposed to *P. concolor* for a period of six hours. After six

hours the larvae were removed and held for 24 hours. After 24 hours the larvae were dissected to look for evidence of oviposition scars, encapsulation and/or melanization of parasitoid eggs in each of these species. The larvae were placed on a clean slide in a drop of water, examined for oviposition scars and then dissected under a dissecting scope. After receiving instructions from Trostle on how to dissect larvae to determine whether or not parasitoid eggs were encapsulated, S. Mohamed expanded this work as part of her dissertation (done concurrently and initially using the same cultures) by including exposures of *C. cosyra* and *C. anonae* to both *P. concolor* and *P. cosyrae* (Wilkinson) and increasing the number of exposures to *C. capitata*, *C. fasciventris*, and *D. ciliatus* (Mohamed et al., 2003).

A different procedure was used for the three host species that were not cultured on an artificial diet. Ten larvae each of *D. ciliatus* and *D. bivittatus* were removed from squash and placed into coffee cherries, which were then exposed to females of *P. concolor* by placing the infested coffee cherries in the *P. concolor* rearing cage. Similarly, 20 larvae of *Craspedoxantha* were extracted from flower heads and placed in coffee cherries (5 larvae per cherry, stacked in a chimney fashion) and exposed to *P. concolor* females from the laboratory culture. Mated females of *P. phaeostigma* reared from tephritids in squash were then exposed to third instar larvae of *C. capitata* in coffee cherries in a fashion similar to that used for *P. concolor*. Females of *P. phaeostigma* were exposed to squash infested by either *D. ciliatus* or *D. bivittatus*.

The above experiments were used to acquire preliminary data for the design of more detailed experiments on host specificity (this chapter as well as Mohamed *et al.* (2003)). Although I did the preliminary host suitability work with these fly species and *P. concolor*, the majority of the work on six of the host species was done at ICIPE by S. Mohamed, as noted above.

Host Suitability of Psyttalia species in Hawaii

***Psyttalia concolor* Exposed to the Invasive Frugivorous Tephritids in Hawaii**

Host Suitability

In Hawaii, the populations of Italian and Kenyan parasitoids were mixed and the combined culture was reared on *Bactrocera latifrons* and *C. capitata* after methods in Wong and Ramadan (1992). Preliminary trials with naïve females proved problematic as few oviposition events were observed. To ensure experience in oviposition and willingness to oviposit, adult females observed attacking larvae during colony maintenance activities were selected for use in this experiment. Exact age of the females was thus unknown, but varied from 7-40 days.

For these experiments a clear, inverted pint size plastic cup was used as the cage. The cup had a corked hole in the side for transfer of wasps and organdy covering the mouth of the cup. This unit was placed over an inverted glass petri dish on which third instar larvae of the species of fruit fly to be tested were placed. To begin the experiment, five parasitoids were placed in the cup. Each oviposition attempt on the larvae was scored for duration. Any oviposition attempt not lasting for at least 30 seconds (indicating probing as opposed to actual oviposition) was not counted. Individual larvae were removed after successful oviposition by a parasitoid. To avoid superparasitism, one of the parasitoids was removed with a stick if two females were observed attacking the same larvae. The species of fruit flies exposed to *P. concolor* during these trials were the exotic (to Hawaii) pests *C. capitata*, *B. latifrons* (Hendel) (the Malaysian fruit fly), *B. dorsalis* (Hendel) (the Oriental fruit fly), and *B. cucurbitae* (the melon fly). The USDA Manoa laboratory provided all tephritid larvae. After exposure to parasitoids, fruit fly larvae were placed back on artificial diet and allowed to pupate over sand.

Adults and pupae produced by these larvae were counted after one month. As a control for fly colony quality, an equal number of unexposed third instar larvae were allowed to pupate over sand. Adult and pupae of the flies for the control were counted in the same manner as parasitized larvae. Nathan Peabody did the majority of this work prior to his leaving the project, however I provided limited assistance. The results are presented here as they were done as a companion to my work on encapsulation (see next paragraph) and have not been presented elsewhere, but are an important part of the discussion on host suitability of *P. concolor*. Two hundred and seventy-seven *B. latifrons*, 165 *C. capitata*, 148 *B. cucurbitae*, and 135 *B. dorsalis* were exposed to *P. concolor* in this experiment.

Encapsulation

Larvae for the encapsulation tests were provided in the same method as described above in the Hawaiian host suitability experiments, except only third instar larvae were used. Parasitoids were the same except experienced *P. concolor* females were from 5-25 days old. In Hawaii, larvae were exposed to parasitoids for 12 hours. Twenty-four hours after larvae were removed from the experimental arena, they were dissected in a drop of water on a slide under a dissecting scope. Thirty-one *B. dorsalis*, 61 *B. cucurbitae*, 50 *B. latifrons*, and 32 *C. capitata* were exposed for this experiment.

Psytalia phaeostigma* Exposed to *B. cucurbitae

Psytalia phaeostigma was brought to Hawaii (through the Texas A & M quarantine facility) as adults. In the Hawaii Department of Agriculture quarantine, female *P. phaeostigma*

were exposed to larvae of *B. cucurbitae* in the same method as described above for *P. concolor*. The USDA Manoa laboratory provided larvae of *B. cucurbitae*.

Results

Identification of Flies Based on Characteristics of the Puparium

A preapical tooth was present on the mouthhook of both *C. fasciventris* and *C. anonae* (Fig. 22). The interspiracular processes were branched and longer in *C. fasciventris* (Fig. 23). These two features easily differentiated *C. fasciventris* from the other two coffee infesting tephritids *C. capitata* and *T. coffeae*. No attempt was made to differentiate *C. fasciventris* from *C. anonae* based on pupal characteristics other than those already published (White and Elson-Harris, 1992) because, as noted above, *C. anonae* was rarely collected and was present only at one of the three sampling sites.

Trirhithrum coffeae lacks a preapical tooth on the mouthhook (Fig. 24). *Ceratitis capitata*, in most cases, also lacked a preapical tooth (Fig. 25), but rarely had a very small tooth present (Fig. 26). *Ceratitis capitata* had a greater SFD ratio and button-to-button diameter than *T. coffeae*. *Ceratitis capitata* displayed branching in the dorsal and ventral interspiracular processes on the posterior spiracles (Fig. 27), while *T. coffeae* had a moderate to large amount of branching (Fig. 28).

A key is presented (Table 10) to facilitate identification of puparia from coffee in Kenya. The three species of tephritids collected from squash can be readily distinguished from one another by the size of the preapical tooth. *Dacus ciliatus*, widespread in Africa and throughout southern Asia, has a very large preapical tooth (much larger than that of *C. fasciventris*: Fig. 22).

Dacus bivittatus also has a very conspicuous preapical tooth, comparable in size to the preapical tooth on the mouthhook of *C. fasciventris*. Additionally, this sub-Saharan species is almost twice the size of *D. ciliatus*, and thus has conspicuously larger puparia and mouthhooks. The preapical tooth on the mouthhook of the adventive (to Africa) *B. cucurbitae* is distinct, but much smaller than in the two species of *Dacus*, and similar in degree of development to those specimens of *C. capitata* with a small preapical tooth (as in Fig. 26). The puparia features of Kenyan *D. ciliatus* and Kenyan *B. cucurbitae* matched the previous descriptions for these species found in Ferrar (1987) and White and Elson-Harris (1992) respectively.

Identification of Parasitoids Based on the Characteristics of the Host Puparium

Based on examination of mouthhooks and posterior spiracles of the individually isolated puparia from which parasitoids emerged, the following host/parasitoid associations were made (Table 11). *Ceratitis capitata* is attacked by *Fopius caudatus* (Szépligeti), *F. ceratitivorus* Wharton, *P. concolor*, *Diachasmimorpha fullawayi* (Silvestri), *Coptera robustior* (Silvestri) and the *Tetrastichus spp.* reared from coffee. *Ceratitis fasciventris* was only attacked by *F. caudatus*. *Fopius caudatus*, *P. concolor*, *D. fullawayi* and *Tetrastichus spp.* attack *T. coffeae*. Thus, in these samples, *F. ceratitivorus* was only recovered from medfly; *D. fullawayi*, *P. concolor* and *Tetrastichus spp.* successfully developed on medfly and *T. coffeae*; and *F. caudatus* developed on all three of these potential hosts.

Rearing Tephritids and Associated Parasitoids from Non-coffee Host Plants

Cucurbitaceae

Three species of Tephritidae were reared from the yellow squash and zucchini: *D. bivittatus*, *D. ciliatus*, and *B. cucurbitae*. Two species of parasitoids were reared from the tephritids in squash: the opiine braconid *P. phaeostigma* and the eulophid *Tetrastichus giffardii* Silvestri. One pupal parasitoid, a chalcidid from the genus *Dirhinus*, was reared from the exposures of puparia beneath the soil of rotting squash. The eulophid *T. giffardii* was the only parasitoid found in coffee that was also reared from field-collected squash.

Flower Heads

Asteraceae (Zinnia) Collections in Kenya

The tephritid flies reared from zinnias in Kenya were all members of the genus *Craspedoxantha*, and probably *Craspedoxantha marginalis* (Wiedemann). This species has previously been reported as a pest of zinnias in South Africa (Munro, 1964 as noted in White and Elson-Harris, 1992). Two species of parasitoids, one a eulophid and the other a eurytomid, were reared from this tephritid. No parasitoids infesting coffee were reared from flower heads. Eurytomids were never reared from any of our coffee samples, and the eulophid reared from flower heads was not a member of the genus *Tetrastichus*, the only genus of eulophid recovered from coffee-infesting Tephritidae.

Infestation rates in zinnias were estimated from two separate collections in August 1998. On August 5, 54 *Zinnia* flower heads were collected. Upon dissection, 218 larvae, pupae or

emerged puparia were found in these flower heads. On August 18, 79 Zinnia flower heads were collected. Forty-six larvae or pupae were recovered from dissection of these flower heads. The mean number of tephritids per flower head for August 5 and 18 was 4.03 and 0.58 respectively.

Asteraceae Collections in Hawaii

From the 882 flower heads, 439 specimens of two non-frugivorous tephritid species were reared. One hundred ninety-four specimens of a species in the agromyzid genus *Melanagromyza* Hendel was also reared from these flower heads. Although 70 chalcidoids and 82 specimens of a eucoiline figitid, *Nordlandiella semirufa* (Kieffer), were reared from the flower heads, neither the species of *Tetrastichus* nor the species of *Dirhinus* Dalman that were established in Hawaii for frugivorous tephritids were recovered from the sample. Most importantly, no braconids were reared from the tephritid-infested flower heads, thus clearly demonstrating that the opiines released against frugivorous tephritid pests in Hawaii do not attack flower-infesting flies.

Laboratory Host Range Testing of Psyttalia Species in Kenya

Psyttalia concolor

Psyttalia concolor can successfully develop in *C. capitata* and *C. cosyra*. *Psyttalia concolor* cannot develop in *C. fasciventris* or *C. anonae* and the parasitoid eggs are invariably encapsulated and melanized in these two hosts (Tables 12 and 13). However, as observed by both Trostle and Mohamed, females from the Kenyan populations of *P. concolor* readily probed *C. fasciventris* larvae with their ovipositors. Dissection of *C. fasciventris* host larvae (exposed

for six and dissected after 24 hours) revealed that eggs were laid, but that they were encapsulated and usually melanized within 24 hours. The number of oviposition scars present on *C. fasciventris* larvae was approximately five times higher than the number of parasitoid eggs recovered from the larvae. This is in extreme contrast to the superparasitism that occurs when larvae are exposed to *C. capitata* for a similar period of time, suggesting *P. concolor* is able to discriminate, at least partially, between suitable and non-suitable hosts.

Problems were experienced with exposures of *P. concolor* to *D. ciliatus* and *D. bivittatus*. Larvae of each *Dacus* species were placed in coffee cherries for exposure to *P. concolor*, as the parasitoids did not respond well to infested squash. However, the *Dacus* larvae could not complete development in the coffee cherries. Thus, the larvae from these exposures died. However, the fly larvae were dissected upon death and viable parasitoid larvae were recovered, with no signs of encapsulation or melanization. Thus, the preliminary results suggest both *D. ciliatus* and *D. bivittatus* are suitable hosts for *P. concolor*.

Females of *P. concolor* exposed to five *Craspedoxantha* larvae placed in coffee cherries neither probed the host fruit with their ovipositors nor oviposited in the host larvae. These flower head infesting larvae remained immobile when placed in coffee cherries, unlike larvae of fruit-infesting species, which actively moved inside the fruit even when they were not feeding.

Psytalia phaeostigma

Psytalia phaeostigma, obtained from tephritids infesting squash, successfully oviposited into and completed development on medfly larvae. *Psytalia phaeostigma* was also reared from both *D. bivittatus* and *D. ciliatus* in the laboratory and these two were confirmed as field hosts based on examination of puparia recovered from squash (n = 3 and 14 respectively).

Host Suitability Tests of Psytalia Species in Hawaii

Psytalia concolor

Psytalia concolor can successfully develop in *B. latifrons* and *C. capitata*. *Psytalia concolor* cannot develop in either in *B. dorsalis* or *B. cucurbitae* (Tables 12, 13, 14, 15, 16). Table 15 shows the combined data of Trostle and Peabody.

The eggs of *P. concolor* are never encapsulated or melanized in *B. latifrons* or *C. capitata*. *Psytalia concolor* eggs are always encapsulated, and melanization occurs within 24 hours 82% and 83% of the time in *B. cucurbitae* and *B. dorsalis* respectively (Tables 14, 16).

Females of *P. concolor* oviposited much less frequently in those larvae that would not support development of parasitoid young. For example *P. concolor* females were observed probing the larvae of *B. dorsalis* and *B. cucurbitae* and many scars were clearly evident on the host larvae during dissection, but very few eggs were deposited in these hosts. Those hosts that received eggs were never superparasitized, and eventually all parasitoid eggs became encapsulated. However, when *P. concolor* was given medfly larvae, superparasitization was commonplace and encapsulation was never observed. The results thus suggest that *P. concolor* is able to differentiate to a considerable extent between hosts that can support development of larvae and those that cannot.

Development of Tephritid Hosts: Attack by *P. concolor*

For *B. cucurbitae* and *B. dorsalis*, there was a noticeable difference between mortality of host flies in the control group and host flies exposed to *P. concolor*. Only 6.67% of the *B. dorsalis* and 24.90% of the *B. cucurbitae* emerged after exposure to *P. concolor* versus a mean percent emergence in the control of 86.75% and 88.20% for *B. dorsalis* and *B. cucurbitae* respectively. Unfortunately, there are not enough replications for a two-tailed Wilcoxon Signed-Rank Test to produce statistically significant results.

Psytalia phaeostigma* Exposed to *B. cucurbitae

Bactrocera cucurbitae larvae invariably encapsulated and melanized the larvae of *P. phaeostigma*.

Discussion

Host Fidelity of Afrotropical Frugivorous Tephritidae

Unlike in the temperate parts of the world where fruits generally support a single species of tephritid fruit fly, Afrotropical fruits often host two or more species of frugivorous tephritids (Steck et al, 1986; Copeland et al, 2002). As a further contrast to the work on host specificity of species within the genus *Rhagoletis* (Bush, 1966, 1969a, b), tropical species in genera such as *Ceratitis*, *Trirhithrum* Bezzi, and *Bactrocera* contain a mixture of stenophagous and polyphagous species. From a parasitic wasp's perspective, any given fruit may conceal both

suitable and unsuitable hosts that cannot be distinguished by the parasitoid until the host larva is probed with the ovipositor through the fleshy part of the fruit. This provides interesting challenges for both the parasitoid and the investigator.

Although at least five species of Tephritidae have been reared from coffee cherries in East Africa, we only recovered four species from the samples of *C. arabica* taken during this study (Chapter II). *Trirhithrum nigerrimum* (Bezzi) is common throughout Kenya, where it attacks fruits of various Rubiaceae and other plant families (White *et al.*, 2003), but seems to attack coffee only rarely in East Africa (Greathead, 1972). This species was originally reared from coffee in West Africa (Silvestri, 1913) where it has subsequently been reared in substantial numbers from this host (Steck *et al.*, 1986). Its congener, *T. coffeae*, may be a coffee specialist in East Africa. Of the four species we reared from coffee, at least three (medfly, *C. fasciventris*, and *C. anonae*) are polyphagous (Copeland *et al.*, 2002). Though medfly is polyphagous, attacking over 400 hosts, arabica coffee is a preferred host (Wharton *et al.*, 2000; Copeland *et al.*, 2002). None of the three polyphagous species found in coffee were reared from our squash or zucchini samples.

Numerous species of dacine Tephritidae have been reared from cucurbits in Africa and some of these are serious pests (White and Elson-Harris, 1992). Yellow squash and zucchini growing near ICIPE produced three species of tephritids, two native (*D. ciliatus* and *D. bivittatus*) and one introduced (*B. cucurbitae*). A fourth cucurbit pest, *Dacus punctatifrons* Karsch, was also present, but was only collected in sweep net samples at ICIPE. Cucurbit-infesting tephritid pests such as the ones we reared tend to attack many hosts within the Cucurbitaceae, but are rarely recorded on fruits outside this plant family (White and Elson-Harris, 1992), and are never found in coffee.

Diagnostic Features of Tephritid Puparia

The presence or absence of a preapical tooth was the most useful diagnostic character for separating fruit-infesting tephritids into two major groups, both in coffee and cucurbits. These groups largely corresponded to current classification (based on adult morphology) of the tribe Dacini (Dacina and Ceratitidinia) (Norrbom et al. 1998). Thus, a preapical tooth is present in all three species of the subgenus *Pterandrus* for which mouthhooks have been described: *C. (P.) fasciventris*, *C. (P.) anonae* and *C. (P.) rosa* Karsch. There is also increasing evidence that the genus *Trirhithrum* is only marginally distinct from *Ceratitis*, though the two have been kept separate for decades largely because the best-studied species of *Trirhithrum* are so much darker than the species in the genus *Ceratitis*. Similarly, the relative development of the preapical tooth enabled me to separate the one introduced species of *Bactrocera* from the two species of *Dacus* in my samples of squash.

The combination of characters on the cephalopharyngeal skeleton and posterior spiracles was sufficient for separation of all species that we reared from coffee. However, the number of available characters and character states is small, and would probably be inadequate for separation of large numbers of species reared on a regional basis from more than one host plant.

Host Suitability

Eight of the 10 parasitoids reared from coffee in Kenya are capable of successfully developing on medfly, and none of these eight species was found attacking tephritids in either cucurbits or flower heads. We were unable to determine specific hosts in coffee for either *Tetrastichus giffardii* or *Fopius silvestrii* (Wharton), but *T. giffardii* was also reared from

tephritids in squash. The work presented here provides essential baseline data for further selection of parasitoids that could be used in the management of tephritid pests.

Tables 12 and 13 show that four of the seven species of tephritids tested here, representing two genera in two different subtribes, encapsulate the eggs of *P. concolor* and as a result this parasitoid cannot complete development on these species. In all cases this was an all or none phenomenon, thus differing from the well studied system of the *Drosophila melanogaster* Meigen (Diptera: Drosophilidae) subgroup attacked by the parasitoid *Asobara tabida* (Nees) (Hymenoptera: Braconidae), where a gradient of lamellocytes, plasmatocytes and crystal cells explain differing, or “relative”, levels of encapsulation of the parasitoid (Eslin and Prevost, 1998).

If parasitoid host suitability patterns could be delimited for tephritid fruit flies based on phylogeny of the host fly, then this information could be used to predict nontarget effects. However, my results, as well as those of Mohamed *et al.* (2003) show that host phylogeny is not an adequate predictor of host suitability. As shown in Table 16, both *B. latifrons* and *B. dorsalis* are in the same subgenus of *Bactrocera*, yet *P. concolor* can develop in *B. latifrons* and not *B. dorsalis*. On the other hand, *P. concolor* develops in *Ceratitis (Ceratitis) capitata* and *C. (Ceratalapis) cosyra*, but not in any members of the subgenus *Pterandrus* tested. *Trirhithrum coffeae* is also a suitable host for *P. concolor*.

Parasitoids from Coffee

Medfly is the host used for mass rearing *P. concolor* in the Mediterranean region for augmentative releases against of olive fly, *Bactrocera oleae* (Gmelin). Thus, the ability of *P. concolor* to develop on medfly has been known for decades. However, we do not advise using

this culture of parasitoids as a source for releasing *P. concolor* in areas where coffee is a preferred host of medfly. It would be more suitable to release *P. concolor* originating from Kenyan coffee for augmentative releases against medfly in the New World than to rely on *P. concolor* from the Mediterranean, which originated on olive fly.

Ceratitis fasciventris and its sibling species, *C. rosa*, are the most important pests of non-cucurbit fruits in Africa. Unlike medfly, however, they have not become established outside sub-Saharan Africa except on some islands in the Indian Ocean. Populations of *P. concolor* from coffee in Kenya are not capable of successfully attacking *C. fasciventris*. However, the results presented in Table 11 show that the egg-prepupal parasitoid *F. caudatus* is capable of successfully developing on both medfly and *C. fasciventris*, and would therefore likely be a suitable candidate for introduction against the latter. *Fopius caudatus* should also be tested for host suitability against *C. rosa* in Reunion, where there has been a long-standing interest in biological control of this pest (S. Quilici, pers. comm.).

It is highly probable that *F. ceratitivorus* is host specific to medfly. If confirmed, *F. ceratitivorus* holds great promise for the biological control of medfly. It is an egg-prepupal parasitoid, like the successful *F. arisanus* (Sonan), and if host specific, would cause little concern to nontargets species. *Fopius ceratitivorus* should be a major focus of the medfly biological control program. Areas of future emphasis should include confirming host specificity to medfly and then designing a mass-rearing program for field trials and augmentative releases.

Parasitoids from Cucurbits

Cucurbits were chosen for examination in this study for two reasons. First, they represent a fruit that is generally infested by a different group of tephritid fruit flies than those normally infesting coffee and other fruits growing on trees and shrubs. Medfly and Oriental fruit fly, for example, attack fruits from hundreds of plant species, but very rarely attack cucurbits. However, since there are a few records suggesting a limited amount of exchange of flies and parasitoids between these two hosts types, cucurbits were a logical choice for exploring the effect of host habitat on host range.

There is considerable interest in discovery of natural enemies of *B. cucurbitae* for release against this pest in Hawaii. Though *B. cucurbitae* is not native to Africa, it was introduced to this area several decades ago, and some of the native parasitoids attacking cucurbit-infesting flies may have moved onto this species. Unfortunately, based on preliminary studies done here, *B. cucurbitae* successfully encapsulates eggs of both *P. concolor* and *P. phaeostigma* when and if eggs are laid in larvae of this fly. Following these preliminary results, no further study was undertaken.

Although *B. dorsalis* and *B. cucurbitae* are not suitable hosts for *P. concolor*, it is very interesting that attack by *P. concolor* on these species greatly reduces the number of flies emerging compared to the control group (Table 15). Although this is a wasted progeny resource by the female parasitoid, it is beneficial to biological control if the same effects are seen in the wild. Given the results of the encapsulation studies, however, I do not recommend further studies on either of these species in the context of biological control of melon fly.

Predicting Non-target Effects: Host Habitats

Data obtained here from rearing tephritids and their parasitoids from three different host habitats suggest that habitat plays a significant role in the ultimate choice of hosts by the parasitoid. While this phenomenon has been known for some time (Salt, 1937) and has been explored extensively (Lewis *et al.*, 1976, Vinson, 1976, 1984, Glas and Vet, 1983, Vet, 1983, 1985, 1988, 1999, Vet and van Opzeeland, 1984, Vet and van Alphen, 1985, Vet *et al.*, 1990, 1991, 1993), there seems to be little acknowledgement of this body of work by those most concerned about non-target effects in biological control.

The larval host plant may be relatively unimportant for pupal parasitoids that search for their hosts in the soil, such as the widespread, polyphagous pteromalid *Pachycrepoideus vindemmiae* (Rondani). The distinction between cucurbits growing on the soil surface and coffee growing up in bushes may also be relatively unimportant to species such as the eulophid *T. giffardii*, that may preferentially attack hosts in fruit that have aborted. In our studies, however, none of the opiine parasitoids collected from coffee were reared from field-collected cucurbits and no opiines were reared from the several hundred flower heads that were sampled. Although *P. phaeostigma* successfully attacked medfly in the laboratory, it doesn't encounter medfly in the field and similarly *P. concolor* was not attracted to the large, heavily infested cucurbits in our plots and thus had no opportunity to attack cucurbit-infesting tephritids. Along similar lines, cucurbit-infesting flies are never found in coffee and widespread, polyphagous species such as medfly and *C. fasciventris* are almost never found in cucurbits. In our studies, medfly was unable to develop successfully in the squash from which *Dacus* and *Bactrocera* were reared, but there are a few records (Weems, 1981; Copeland *et al.*, 2002) that demonstrate medfly's capacity for developing in some of the cucurbit species with smaller fruits.

Flower heads clearly did not fall within the realm of habitats explored by the parasitoids of frugivorous tephritids. *Psytalia concolor* from our cultures was not attracted to flower heads, but more importantly, the flower head tephritids do not have the same mobility as fruit-infesting tephritids and thus do not send the same vibration cues that are essential to host location by this or any other parasitoid ovipositing in the larval stages of the host. Nevertheless, there are records of opiines attacking flower-infesting tephritids in both the New and Old World tropics. For example, there is a species of *Psytalia* in South Africa, *Psytalia* cf. *vittator* (Brues), attacking flower head seed feeding tephritids in the genus *Mesoclanis* Munro (Edwards, 1998). Wharton (1997), however, has already shown that the large genus *Psytalia*, with over 50 described species, consists of two morphologically distinct groups, one of which feeds on tephritids in flower heads and one on tephritids infesting fruits. *Psytalia vittator* and related species belong to the group of species attacking flower head tephritids. Based on our investigations, the likelihood that a species in the *P. concolor* species group would attack and be able to develop in a flower head tephritid is so low as to be inconsequential.

Gall-forming tephritids are as much of a nontarget concern as flower head tephritids. In Duan *et al.* (1997), less than 3% of female *Psytalia fletcheri* (Silvestri) probed *Eutreta xanthochaeta* Aldrich galls and none of the larvae in the galls received parasitoid eggs. Duan and Messing (1996) exposed *P. fletcheri* to *E. xanthochaeta* in modified substrate (screen dish), but the eggs that were oviposited never hatched and no *P. fletcheri* were reared from *E. xanthochaeta*. We predict that *P. concolor* will respond in the same manner and thus is no threat to gall-forming tephritids in Hawaii.

As discussed in Chapter II, parasitoid complexes attacking tephritid fruit flies differ in their distribution of idiobiont versus koinobiont parasitoids species. Flower head infesting tephritids are mainly attacked by idiobiont larval parasitoids while pupal parasitoids are almost

absent. The same pattern can be seen in stem-inhabiting hosts. Fruit-infesting tephritids are attacked by mainly koinobiont larval parasitoids, to a lesser degree by pupal parasitoids and by a few idiobiont larval parasitoids. Leaf-mining tephritid parasitoid complexes resemble the patterns in fruit-infesting species (Hoffmeister and Vidal, 1994).

These conclusions allow a succinct look at the effects host feeding site have on koinobiont and idiobiont parasitoid assemblage composition. Because the host feeding site is so different frugivorous endoparasitoids present little risk to flower head and gall-forming tephritids in the field.

Predicting Nontarget Effects Based on Classification of the Parasitoids

The generic level is the first major division where a pattern for nontarget effects can be distinguished in the three opiine braconids (*Diachasmimorpha* Viereck, *Fopius* Wharton and *Psytalia*) reared from medfly in coffee in Kenya. The taxonomy of opiine parasitoids of fruit-infesting tephritids has been well studied and the placement of the parasitoids in their respective genera is relatively stable (Wharton, 1997). Thus, we feel confident discussing, at the generic level, previous nontarget experiments and in the case of *Fopius*, ecological host finding characteristics that will predict nontarget effects as well as which genera to select parasitoids from for further study and possible release in a biological control program.

Tephritid Parasitoids in the Genus *Diachasmimorpha*

In *Diachasmimorpha*, the monophyletic *longicaudata* species group includes several parasitoids previously released in Hawaii for the biological control of tephritid pests. The three currently established in Hawaii are *D. kraussii* (Fullaway), *D. longicaudata* (Ashmead) and *D. tryoni* (Cameron). Native hosts of the parasitoids in this group are generally *Bactrocera* species. However, one species in this group, *D. paeoniae* (Tobias), has been reared from *Macrotrypeta ortalidina* Portschinsky, a pest of flower buds (Wharton, 1997). In addition, in nontarget tests and field rearing of nontarget hosts, *D. tryoni* has been shown to attack gall-forming tephritids (see below).

The only species of *Diachasmimorpha* that we reared in Kenya, *D. fullawayi*, is morphologically distinct and is placed in a species group that differs in several respects from the other species groups within *Diachasmimorpha* (Wharton, 1997). Thus far, *D. fullawayi* has only been reared from ceratitine tephritids (Wharton, 1997). This species was released in Hawaii, but has not been recovered in Hawaii since 1949, even though it was one of the dominant parasitoids of medfly from 1913-1933 (Willard and Mason, 1937).

Diachasmimorpha tryoni, originally from eastern Australia, attacks the late instars of several species of tephritid fruit flies in areas where it has been introduced, including species in the genera *Anastrepha* and *Ceratitis*, as well as several species of *Bactrocera* and *Rhagoletis* (Wharton and Gilstrap, 1983). *Diachasmimorpha tryoni* was introduced to Hawaii in 1913 (Silvestri, 1914), and prior to the introduction of *Fopius arisanus* to Hawaii, it was one of the most important parasitoids of *C. capitata* (Pemberton and Willard 1918a,b; Willard and Mason, 1937). *Diachasmimorpha longicaudata* is native to the Indo-Australian region. It was introduced to Hawaii during the Oriental fruit fly program of the 1950's. It attacks hosts in the

genera *Anastrepha*, *Ceratitis*, *Dacus*, and *Bactrocera*. *Diachasmimorpha kraussii* is an Australian species that has been reared on several *Bactrocera* species, but not *B. dorsalis* nor *B. cucurbitae*. *Diachasmimorpha kraussii* has recently been re-released in Hawaii for the control of the Malaysian fruit fly, *B. latifrons*, even though it was shown to have a weak response to some nontarget tephritids.

The biology of *D. tryoni* has been well-studied (Fischer, 1971), although its host finding behavior is of greater interest now as it continues to be cited for its ability to attack some nontarget tephritids in laboratory experiments and field collections (Purcell *et al.*, 1997; Duan *et al.*, 1997, 1998, 2000; Duan and Messing 1999, 2000). To date, *D. tryoni* has been shown to attack two nontarget gall-forming tephritids in Hawaii, *E. xanthochaeta* (Clancy, 1950, Duan and Messing, 2000) and *Procecidochares utilis* Stone (Bess and Haramoto, 1959, 1972), but it has never been reared from flower head tephritids (Duan *et al.*, 1996; Duan and Messing 1998a, b). Like *D. tryoni*, *D. longicaudata* has been exposed to gall-forming and flower head infesting tephritids under a variety of experimental conditions (Duan and Messing, 1996, 1997, 2000b; Duan *et al.*, 1997a,b; Purcell *et al.*, 1998), but does not seem to show the propensity in the field for attacking gall-forming tephritids that *D. tryoni* does (Duan *et al.*, 1998). In laboratory experiments, *D. longicaudata* rarely landed on *Procecidochares alani* Steyskal galls (Purcell *et al.*, 1998) and lacked a positive response to the flower head infesting *T. dubautiae* (Duan and Messing, 1997). In an experiment where *D. longicaudatus* was argumentatively released over a 2-month period in a patch of *E. xanthochaeta*, only 0.8% was parasitized by *D. longicaudata* (Duan *et al.* 1997a). Duan and Messing (2000) exposed *D. kraussii* to four nontarget tephritids including 1) *Trupanea dubautiae* (Bryan) (in flower heads), 2) *Ensina sonchi* (L.) (in flower heads), 3) *E. xanthochaeta* (in galls) and 4) *P. utilis* (in galls). Regardless of the presence of a preferred host (*B. latifrons*), *D. kraussii* probed nontarget host plant substrates.

When offered a choice between host in fruit/diet versus hosts in galls or flower heads all these species showed a decided preference for the fruit-infesting hosts over the nontarget hosts in galls and flower heads. Thus, differential behavioral response to different types of microhabitats may play a strong role in limiting potential interactions between these species and nontarget, non-frugivorous tephritids.

Based on the above studies, and the relative success of *D. fullawayi* against medfly in Hawaii between 1914 and 1933, we predict that this species would be a useful addition to the medfly biological control arsenal in Guatemala. Despite the extensive testing showing relatively low risks, the species of *Diachasmimorpha* that have been studied in Hawaii do show a slight propensity for nontarget effects. As a consequence, *D. fullawayi* would have to undergo considerable scrutiny before it could ever be released in Hawaii. Because of the lengthy nontarget tests that *D. fullawayi* would have to undergo in Hawaii and the difficulty of showing ecological impact in caged trials, effort may be better spent on more promising species such as those in the genus *Fopius*.

Tephritid Parasitoids in the Genus *Fopius*

Following Wharton (1997, 1999), *Fopius* is presently known from Africa/Madagascar, the Eastern and Southern Pacific as well as the Indo-Pacific region stretching into Southeastern Russia. The *Fopius persulcatus* species group, found from the Indo-Pacific region to southeastern Russia, contains two species introduced to Hawaii: *F. arisanus* and *F. vandenboschi* (Fullaway). The members of this species group primarily attack *Bactrocera*, but have also been reared from *Carpomya* Costa, *Myoleja* Rondani, *Rhagoletis* and *Ceratitis*. *Fopius arisanus* has been shown to be the best biological control agent of frugivorous tephritids

in Hawaii (Purcell, 1998). The *F. silvestrii* species group closely resembles species in the *persulcatus* group but is found in Africa where they have been reared from species of *Ceratitis*, *Trirhithrum* and *Dacus* (Wharton, 1987; Steck *et al.*, 1986; Wharton *et al.*, 2000). *Fopius caudatus*, also from Africa, is the sole member of its own species group. *Fopius silvestrii* and *F. caudatus* have previously been sent to Hawaii for introduction, but never successfully established (Wharton, 1987).

These few species of *Fopius* that have been studied in detail attack the egg or early instar larvae of tephritid fruit flies. As cues from flies may be very important, especially for finding eggs for oviposition, these parasitoids potentially all exhibit very high levels of host habitat specificity.

Fopius arisanus is an egg/prepupal parasitoid. It attacks hosts in the following genera: *Anastrepha*, *Ceratitis*, *Carpomyia*, and *Bactrocera* (Wharton and Gilstrap, 1983). It has been credited with the successful reduction of the Oriental fruit fly infestation in Hawaii, and probably a reduction of medfly populations as well (van den Bosch *et al.*, 1951; Bess and Haramoto, 1961; Bess *et al.*, 1963; Newell and Haramoto, 1968; Haramoto and Bess, 1970). *Fopius arisanus* has never been cited for nontarget effects on other Diptera (Bess and Haramoto, 1959, 1972). This is most likely due to the fact that it uses chemical cues to locate eggs or early instars of the host rather than the vibrational cues used by other opiines to attack later larval instars. *Fopius arisanus* has been the center of many competition studies with the other fruit fly parasitoids also introduced into Hawaii to discover which parasitoid is intrinsically the most competitive when two species are both present in the same host (Wang and Messing, 2002).

Fopius vandenboschi attacks species in the genera *Ceratitis*, *Bactrocera*, and possibly *Carpomyia*. Its native range includes northern India, Java, Malaysia, Philippines, Taiwan, and Thailand. *Fopius vandenboschi* attacks the earliest larval instars of its tephritid fruit fly hosts

and has never been tested for nontarget effects in Hawaii. It has, however, been included in competition studies (Wang and Messing, 2002).

Based on their ability to attack the host egg stage or early instars of hosts in fruit, parasitoids in the genus *Fopius* are excellent candidates for tephritid biological control per their propensity toward high host habitat specificity. A specific set of chemical cues for oviposition (see chapter II) ensures that parasitoids in the genus *Fopius* are attracted to tephritid-infested fruit, not flower heads or galls. Thus, based on the biological control success and lack of nontarget effects of *F. arisanus*, additional egg-preupal koinobiont parasitoids such as *F. caudatus* or *F. ceratitivorus* should be given high priority by those interested in new or additional tephritid parasitoids for biological control. Specifically, *F. caudatus* is an excellent candidate for possible control of *C. rosa* in adventive areas such as Reunion or for release in wetter climates in Latin America. *Fopius ceratitivorus*, as a specialist on medfly in coffee, represents a very low nontarget risk for release in Latin America and Hawaii for control of this pest in drier climates. However, there is one caveat: possible nontarget effects on the successful *F. arisanus* must be addressed prior to release of additional *Fopius* species in Hawaii.

Tephritid Parasitoids in the Genus *Psytalia*

Psytalia, an Old World genus, contains more species than either *Diachasmimorpha* or *Fopius*. Host records are available for approximately 40% of described species in the genus *Psytalia* (Wharton, 1997). These host records represent only one host family: Tephritidae, with approximately 80% of these species attack fruit-infesting tephritids. The remaining 20% attack flower-infesting tephritids. The *P. vittator* species group forms a morphologically distinct grouping of those *Psytalia* attacking flower-infesting tephritids (Wharton, 1997), with no known

records of *Psytalia* that attack both flower- and fruit-infesting tephritids. (See Chapter IV for further discussion of the relationship between the species of *Psytalia* that attack tephritids in flower heads and those attacking fruit-infesting tephritids.)

Two morphologically similar species of *Psytalia* are established in Hawaii for biological control of tephritid fruit flies: *P. fletcheri* and *P. incisi* (Silvestri). *Psytalia fletcheri* is endemic to an area that includes India, Malaysia, Sri Lanka, and Taiwan. Hosts include species in the genus *Bactrocera* that infest cucurbits. *Psytalia incisi* is endemic to a region including Malaysia, India and Thailand. It also attacks hosts in the genus *Bactrocera*, but not those that attack cucurbits. Though these species of *Psytalia* are from the Indo-Pacific Region (both originally described from India), they cannot develop in the same host species. *Psytalia fletcheri* attacks and develops in *B. cucurbitae*, but not *B. dorsalis* (Clausen *et al.*, 1965). *Psytalia incisi* attacks *B. dorsalis*, but not *B. cucurbitae* (Clausen *et al.*, 1965). A few nontarget studies have been undertaken with *P. fletcheri* in Hawaii, but none with *P. incisi*. In an ecological host range setting, *P. fletcheri* has shown little positive response to gall-forming and no response to flower head infesting tephritids (Messing *et al.*, 1996; Duan and Messing, 1996, 1997). In addition, *P. fletcheri* is unable to develop physiologically in *E. xanthochaeta* (Duan and Messing, 1996).

In Kenya, the two species that I examined, *P. concolor* and *P. phaeostigma*, are members of a large species complex occurring throughout Africa. My laboratory studies demonstrated that these two species are capable of developing in some of the same hosts, but my field studies indicate that they do not encounter the same hosts in the field because of host habitat specificity. The available information thus suggests that there are several *Psytalia* species, many of them morphologically similar, each attacking a different Old World tropical tephritid (or small group of tephritids), with both habitat specificity and host suitability playing a role in the overall degree

of host specificity. Following this reasoning, I predict that there is a species of *Psytalia* that attacks *C. fasciventris* (and/or its sibling species, *C. rosa*). The populations of *P. concolor* reared from coffee in Kenya clearly cannot develop on *C. fasciventris*, but given the economic importance of this and the related species of *Ceratitis* (*Pterandrus*), more effort should be made to locate populations of *Psytalia* that do attack these species.

Psytalia concolor, reared from coffee in Kenya, has recently been shipped to Hawaii, where laboratory colonies have been established. Although *P. concolor* was the first species in this program to be introduced to Hawaii, primarily because it was the first species to be successfully cultured in Kenya, the work done here supports this choice from a biological perspective. From a taxonomic standpoint, the *Psytalia* reared from coffee does not belong to the *P. vittator* species group and therefore should not pose a risk to flower head tephritids (results of nontarget experiments on gall forming tephritids are presented in Chapter IV). *Psytalia concolor* from the Mediterranean region, including Italy, has been widely introduced against medfly and olive fly with little success. However, the behavioral differences between the Italian *P. concolor* and the Kenyan *P. concolor* noted in Chapter II (due, no doubt, to inadvertent selection for more aggressive males and females attracted to volatiles in fly diet after generations of laboratory rearing) lead me to predict that the Kenyan *P. concolor* will have better success against medfly in the field due to its preference for medfly in coffee. There are two caveats to this prediction. One, *P. concolor* from Kenya may be a seasonal parasitoid. Two, long-term rearing in cages will also decrease the effectiveness of the Kenyan parasitoids in the field.

CHAPTER IV

HOST RANGE TESTING OF *PSYTTALIA CONCOLOR* IN HAWAII: ADDRESSING THE ISSUE OF POSSIBLE NONTARGET EFFECTS

Introduction

The introduction of natural enemies for use in biological control of pests is not a panacea without risks (Howarth, 1983). In the past, untested biocontrol agents have attacked unintended, nontarget hosts (Louda *et al.*, 2003; Lockwood, 1993, Boettner *et al.*, 2000; Henneman and Memmott, 2001). The lack of a scientifically based protocol for testing proposed introductions of biocontrol agents for nontarget effects has resulted in increased complaints about biological control programs (Howarth, 1983,1985,1991; Lockwood, 1993,1997; Simberloff and Stiling 1996) and consequent restrictions against importation of natural enemies. These restrictions have nearly halted the introduction of biocontrol agents in Hawaii due to the inability to address, in a scientific manner, potential nontarget effects and possible degradation of the ecosystem by introduced exotic species.

Host Specificity a Key Element to Successful Classical Biocontrol

Although introduction of host specific parasitoids could provide increased control of frugivorous flies in Hawaii, the level of controversy surrounding any biological control release is such that studies addressing nontarget effects, though not legally required, are virtually

mandatory prior to release. Thus, potential nontarget effects must be addressed prior to release of any parasitoids from Kenya.

It is impossible to test parasitoids against every nontarget (which could be construed to include every species in an ecosystem) prior to release. For insect biological control there are no protocols listing specific steps to disclose potential nontarget, as there are for weed biological control, and thus, any nontarget concern can impede the release of new biocontrol agents. To facilitate development and implementation of new classical and augmentative biological control programs against frugivorous pests, future investigations must concentrate on carefully designed experiments that can assess broad categories of potential negative impacts.

For future introductions of frugivorous fruit fly parasitoids in Hawaii there are the two nontarget groups of concern. These are: (1) tephritids that are endemic to Hawaii and associated with native plant species, and (2) tephritids that were deliberately introduced to Hawaii for the biological control of economically important weeds. Jian Duan and R. Messing, along with several colleagues (Messing and Jang, 1992; Purcell *et al.*, 1997; Duan *et al.*, 1996, 1997a,b, 1998, 2000; Duan and Messing, 1996, 1997, 1998, 1999, 2000a,b,c) are now examining the nontarget effects of the frugivorous parasitoids released in Hawaii during the first half of the 20th century. These retrospective studies can serve as a basis for the development of a rational approach and possible model for testing the nontarget effects that future introductions of biological control agents might pose to the environment.

Tephritids in Hawaii: Targets and Nontargets

Fruit-infesting Tephritids

There are four fruit-infesting tephritid flies adventive to Hawaii. They are: Mediterranean fruit fly (=medfly), *Ceratitis capitata* (Wiedemann); Oriental fruit fly, *Bactrocera dorsalis* (Hendel); melon fly, *B. cucurbitae* (Coquillett); and Malaysian fruit fly, *B. latifrons* (Hendel). There are no native or beneficial fruit-infesting Tephritidae in Hawaii. There are also no indigenous parasitoids of fruit-infesting tephritids in Hawaii. This is important to note for three reasons. One, there is no concern about the affect of parasitoids on flies in fruit as all flies are pests. (This situation is different in Latin America as there are native *Anastrepha* Schiner species.) Second, if a parasitoid can be shown to search for hosts solely in a fruit microhabitat, then it can be hypothesized that the parasitoid should be safe for release. Third, since there are no native parasitoids that attack fruit-infesting tephritids, there is no indirect, nontarget concern to address in the area of parasitoid competition affecting native species. However, arguments have been made that competition studies should be undertaken between previously released and potential candidates prior to the release of additional tephritid parasitoids. I will not address this point as other have already begun this testing (Wang and Messing, 2002, 2003).

Gall-forming Tephritids

Gall-forming tephritids can be beneficial, when used in weed biological control, or detrimental, when they kill or stunt ornamentals. In this chapter we are concerned with nontarget effects on the biological control of weeds. There are several well-documented

examples of parasitoids of fruit-infesting tephritids attacking gall-forming tephritids (Bess and Haramoto 1959, 1972; Wong *et al.*, 1991; Duan *et al.*, 1996; see discussion of *Diachasmimorpha* Viereck in Chapter III). Although, in general these parasitoids do not have an immense impact on the nontarget tephritids, it is still crucial to test parasitoids for this propensity prior to release.

Flower Head-infesting Tephritids

Throughout the islands of Hawaii there are many species of flowers, both native and introduced weed species, which are infested by tephritids. Of particular interest to those concerned with nontarget effects are flowers in the “Silver Sword Alliance”; a group of species that contains endangered and non-endangered native Hawaiian plants. For example, the flower heads of the endangered Silver Sword are infested with a tephritid, *Trupanea cratericola* (Grimshaw) (U.S. Fish and Wildlife Service, 1997). As many of the flowers in the “Alliance” are rare they cannot be used in testing. However, they belong to the family Asteraceae that contains many weed species that are infested by tephritids. These weed species in the family Asteraceae can serve as representatives for the rare species in nontarget test. It is important to ensure that any release of frugivorous tephritid parasitoids would not adversely impact native plants or the tephritids that inhabit them and/or tephritids released for weed biological control. Nontarget effects on flower heads in Hawaii by the previously introduced frugivorous tephritids are discussed in Chapter III.

Nontarget Testing Involving Tephritid Fruit Flies and Their Parasitoids in Hawaii

Messing and his colleagues in Hawaii started studying the nontarget effects of tephritid fruit fly parasitoids in the mid-1990's. Initially, emphasis was placed on host suitability and ecological host range was rarely addressed. In their experiments, larvae of nontarget tephritid species were exposed to frugivorous parasitoids in three ways. One, the nontarget host was removed from its natural (i.e. ecological) habitat and exposed in a screened dish. Two, the natural habitat of the nontarget species was modified (e.g. by shaving off one side of a gall) and exposed to frugivorous fruit fly parasitoids. Or, three, the host finding component was removed from the experiment by placing the parasitoids immediately in the vicinity of the nontarget host (i.e. on a gall containing a nontarget host). Thus, many of the first experiments only looked at the suitability of nontarget hosts for a specific fruit fly parasitoid that had previously been released in Hawaii. While this is invaluable information in the current search for elucidating what entices a parasitoid to probe and attack a nontarget host, the results were not presented this way. These early studies gave the impression that since the parasitoids could develop in these hosts, there was a nontarget impact (Duan and Messing, 1996, 1997, 1998; Duan et al., 1997b; Purcell et al., 1997). In the late 1990's and early 2000's, as ecological host range testing became more popular, Duan, Messing and colleagues switched to a more ecologically based form of testing. This form of ecological testing has provided valuable information on nontarget impacts by correlating laboratory results with field experiments.

Nontarget Gall-forming Tephritids

Gall-forming tephritids studied by Messing and colleagues include the following 3 species: 1) *Eutreta xanthochaeta* Aldrich, lantana gall fly, a weed biocontrol agent introduced to Hawaii to control the highly invasive *Lantana camera* L.; 2) *Procecidochares utilis* (Stone), the Maui pamakani gall fly, a deliberately introduced weed biological control agent of *Ageretina adenophora* (Sprengel) R. King and H. Robinson; 3) *Procecidochares alani* Stekyskal, Hamakua pamakani gall fly, a deliberately introduced weed biological control agent of *Ageretina riparia* (Regel) R. King and H. Robinson.

Objectives

The objective of this chapter is to test the hypothesis that *Psytalia concolor* (Szépligeti), a parasitoid of medfly collected from coffee in Kenya, will attack the nontarget, gall-forming tephritid, *Procecidochares utilis* in Hawaii. I choose to test and study the nontarget effects of *P. concolor*, as it is a species already destined for potential release against frugivorous tephritids in the Hawaiian setting. Releases of the Kenyan parasitoids have also been proposed in Latin America. Although the testing in this chapter is focused on Hawaii, there is hope the results can be used in assessing nontarget risk for parasitoid release in Guatemala and other Latin America countries infested with medfly.

This objective was accomplished through ecological host-range testing of *P. concolor* in the laboratory. Using the data acquired under this objective (Chapter IV) and the other two objectives (Chapters II and III), I discuss the propensity of parasitoids of medfly in coffee in Kenya to attack nontarget hosts in Hawaii.

Materials and Methods

Nontarget Experiments –The Gall-forming Tephritid Procecidochares utilis and Maui Pamakani

During both 2000 and 2001, *P. concolor* was tested against *P. utilis*, a gall-forming tephritid used in the biological control of Maui pamakani, *A. adenophora*. *Psytalia concolor* was the first parasitoid from this study chosen for nontarget studies in Hawaii, as it was the first Kenyan parasitoid approved for laboratory studies outside of quarantine by the HDOA.

During 2000, Maui pamakani galls used in the experiments were collected along the Old Pali Road on Oahu. The road is now a hiking trail that starts at the Nu'uaniu Pali lookout and runs parallel to the current Pali Highway, Route 61. During 2001, galls for the experiment were collected in an area heavily infested with Maui pamakani along the Maunawili trail. This trail is a few miles east of the Nu'uaniu Pali lookout and is also accessible from Route 61. In 2001, prior to the laboratory portion of the experiment, PVC flagging tape was tied around newly formed *P. utilis* galls on Maui pamakani in the wild to prevent parasitization and the consequent appearance of parasitoid ovipositional scars on the larvae developing in the galls. It was important to prevent field parasitization as the galls were dissected after a 48-hour experimental exposure to parasitoids in the laboratory, and ovipositional scars and/or parasitoid eggs or first instar larvae were recorded as evidence of probing or oviposition, respectively. One week after marking with PVC tape the galls were collected and returned to the lab along with an equal number of non-galled stems. In 2000, the galls from the field were not marked with tape prior to collection as these galls were used in one-hour experiments where the wasps were observed the entire experiment.

Galls were offered to parasitoids under a variety of choice and no choice conditions, described below. Kumquats (*Fortunella japonica* (Thunb.) Swingle) were used for comparison in several of the choice tests. The kumquats were obtained at a local grocery store. Since kumquats were obtained from a retail outlet, the outer skin (pericarp) was removed to prevent pesticide or other interference with the experiment.

As a control for the experiments, after each female was exposed to all three experiments (galled versus non-galled pamakani plants, galled plants versus infested kumquats and kumquats alone), she was exposed to an oviposition dish in a separate cage to ensure she was willing and able to oviposit normally. If the female did not show a positive oviposition response to the dish within one hour, the trial was discarded.

In 2000, female wasps used in the experiment were selected as follows: 1) a normal oviposition unit was placed in the maintenance culture, 2) female wasps showing an interest in oviposition (i.e. attraction to oviposition dish), but prior to oviposition, were removed from the cage and placed in a 1 dram glass vial. These wasps were then used in experiments on the following day. In 2001, naïve female *P. concolor* were used for all experiments. All experiments were conducted in 0.5m³ cages. The cages were always placed in the same area by a window, but no direct sunlight shone onto the cage. Temperature in the laboratory varied between 73°F and 78°F. Relative humidity varied from 45 to 50%.

Galled Versus Non-galled Plants; No Preferred Host

For this experiment, two galled and two non-galled stems were randomly placed in the corners of the cage (Fig. 29). The plants were placed in vials filled with water to ensure the freshness of the pamakani throughout the experiment. In 2000, a female *P. concolor* was released in the middle of the cage and then removed after one hour. There were 21 replicates since the cultures were small and relatively few parasitoids were available for experiments. In 2001 there were 50 replicates.

Data analysis in 2000 and 2001 consisted of comparisons between the two possible outcomes: (1) wasps that responded to non-galled plants only, (2) wasps that respond to galled plants only. In 2000, a G test statistic was used with a p set at 0.05. We used the non-parametric G test since the number of replicates was low (21) and the G test, unlike the Chi-square, is powerful enough to handle smaller replicate numbers. In 2001, there were 50 replicates so the data were analyzed using Chi-square with p set at 0.05. In 2001, data analysis also consisted of comparison among three possible outcomes: (1) wasps landing on non-galled plants only, (2) wasps landing on galled plants only, and (3) wasps landing on neither type of plant.

Galled Stems (Non-preferred Host) Versus Kumquats (Preferred Host)

This experiment was conducted using the same methods as the galled/non-galled experiment, except the non-galled plants were exchanged for a preferred host; kumquats infested with five late instar medfly larvae. The kumquats were placed on diet cup lids (Fig. 30) to ensure the bottom of the cages would not become permanently contaminated with the scent of kumquats. Even though precautions were taken to prevent kumquats from contaminating the

cage, after each experiment the bottom of the cage was wiped with a wet cloth as a precaution against contamination. For this experiment, the wasps were considered host specific if the females showed a preference for infested fruit over galls, with p set at 0.05.

Data analysis in 2000 and 2001 consisted of comparisons between the two possible outcomes: (1) wasps that responded to kumquats only, (2) wasps that respond to galled plants only. Since the number of replicates (20) was low in 2000, a G test statistic was used. In 2001, there were 45 replicates and therefore a Chi-square statistic was used. In 2001 data analysis also consisted of comparison between three possible outcomes: (1) wasps landing on kumquat only, (2) wasps landing on galled plants only, and (3) wasps landing on neither plant nor kumquat.

Kumquats: No Choice Test

This experiment was performed in 2001 primarily to verify that infested kumquats are attractive to wasps and would be suitable for choice tests with galled stems. Coffee cherries were very difficult to obtain and since kumquats were much more readily available we used them as the preferred host. The same methods as in the galled/non-galled experiment were used except all the pamakani plants were replaced with infested kumquats. Eighteen replicates were conducted. The experiment allowed the female access to four kumquats, each containing five *C. capitata* larvae. Data recorded included relative numbers landing on and inserting ovipositors in kumquats. These data were compared to the routine responses to the oviposition dish in the laboratory culture.

Galled Versus Non-galled Plants; No Preferred Host; Comparison of *P. concolor* to *D. tryoni*

During 2001 both *P. concolor* and *D. tryoni* (Cameron) (known to attack *P. utilis* in the field) were exposed to a galled and non-galled stems for 48 hours. Aside from the increased exposure time and the use of organdy for all sides of the exposure cage the methods were the same as for the galled/non-galled experiment described above. After a 48-hour exposure, the wasps were removed from the cage. Twenty-four hours after the end of the experiment, the larvae in the galls were dissected in water under a dissecting microscope. Prior to dissection, the presence of oviposition scars on the larvae was recorded. Since the melanization of oviposition scars on the host does not occur immediately after the initial parasitoid sting, the objective for waiting 24 hours after the end of the experiment for dissection was to ensure any ovipositional scars would be visible and recorded. As the larvae were dissected, the numbers of eggs or early instars of parasitoids (if present) were recorded. This experiment had 22 replicates for both *P. concolor* and *D. tryoni*. No statistical analysis was needed as neither species attacked the galls in this experiment.

Results

Galled Versus Non-galled Test with No Preferred Host Present

Psytalia concolor did not attack the galls of the pamakani plant. Regardless of the experimental design, *P. concolor* was neither attracted to nor probed galls of pamakani plants. There was no significant difference in the number of female wasps landing on the gall and non-

galled plants in 2000 (overall $G=0.5053$; $df=1$; $P=0.25$) and 2001 ($\chi^2 = 0.50$; $df=1$; $P= 0.4795$) (Table 17). In a separate analysis (2001) including wasps that never landed on any plants, there was no significant difference between number of wasps landing on galled plants, nongalled plants and the number of wasps flying to and remaining on the side of the cage ($\chi^2 = 0.656$; $df=2$; $P= 0.7204$) (Table 18). Thus, it can be inferred that galled plants are not more attractive to these wasps than the side of the cage or nongalled plants.

Gall (Non-preferred)/ Kumquat (Preferred) Tests

Although *P. concolor* did land on the leaves of the galled plants, the wasps did not search for the galls, and thus did not probe or attack the tephritids within the galls on the pamakani. In 2001, significantly more wasps landed on the kumquats than on galled plant leaves ($\chi^2 = 12.565$; $df=1$; $P= 0.0004$). In 2000, the same result was realized (overall $G=5.0620$; $df=1$; $P=0.025$) (Table 19). The wasps also probed and oviposited in the kumquats infested with *C. capitata*. In a separate analysis (2001) including wasps that never landed on plants or kumquats, there was a significant difference between number of wasps landing on galled plants, kumquats and remaining on the side of the cage ($\chi^2 = 14.53$; $df=2$; $P= 0.0007$) (Table 20). The results clearly demonstrate that *P. concolor* prefers fruit and does not cue to or express interest in galled plants except as a resting place.

No Choice Experiment with Kumquats

There was no significant difference in the numbers of *P. concolor* responding to infested kumquats in a no choice situation (four kumquats) versus infested kumquats presented in a

choice situation (gall/kumquats) ($\chi^2 = 0.8348$; $df=1$; $P= 0.8348$) (Table 21). However, there was a significant difference in the response of female wasps to the infested kumquats when compared to their response to the oviposition dish used as the control ($\chi^2 = 9.615$; $df=1$; $P= 0.0019$) (Table 22). An even greater difference was seen when comparing oviposition into infested kumquats with oviposition into larvae in the dishes ($\chi^2 = 19.105$; $df=1$; $P= 0.0001$) (Table 23). These data thus infer that the parasitoids have learned the “bad habit” (from a biological control perspective) of responding to a dish and the smells of larval diet relative to odors from infested fruits.

Galled Versus Non-galled Plants with No Preferred Host: Comparison of P. concolor and D. tryoni

Upon dissection of the larvae from the galls of the Maui pamakani plants, none of the tephritid larvae were found to have oviposition scars or contain parasitoid eggs. Thus, no comparisons could be made between *D. tryoni* and *P. concolor*.

Discussion

The Case for Introduction of Additional Frugivorous Tephritid Parasitoids in Hawaii

The case for introduction of additional parasitoids to Hawaii is supported on two fronts. First, few of the parasitoid species discovered during the early explorations for natural enemies of tephritid fruit flies were successfully introduced or established in Hawaii. Of the almost 50 species reared during government sponsored explorations, only 10 were ever recorded as established in Hawaii (Gilstrap and Hart, 1987). Most of the species established were the

easiest to rear and the least host specific parasitoids collected. These traits, especially the lack of host specificity, played an important role in the establishment of these parasitoids. However, biocontrol opponents now cite these same traits as nontarget problems.

Previously unreleased or non-established parasitoids should be reexamined for potential benefit to current biological control programs. Technological advances in transportation and a renewed interest in biological control make it imperative to return to medfly's aboriginal home to reexamine the host associations of medfly and its natural enemies (Wharton, 1989; Gilstrap and Hart, 1987). Only by reexamining these relationships, through host range studies in the parasitoids' native home (Chapter III), and nontarget studies in the potential release area (this chapter) can we ascertain which parasitoids exhibit the key element of host specificity.

The First Kenyan Parasitoid Cultured

The first parasitoid from our program to be cultured in Hawaii was *P. concolor*. *Psytalia concolor* was introduced to Italy in an effort to control olive fly shortly after its discovery in Tunisia. Its early use in Italy was well documented (Silvestri, 1939; Delucchi, 1957), as was its subsequent use in augmentation programs following development of mass rearing techniques using medfly as host (Raspi, 1995). As a result of these efforts, there is a considerable amount of information on the developmental biology of *P. concolor*, as well as other facets related to its utility for biological control of fruit pests (Féron, 1952; Biliotti and Delanoue, 1959; Delanoue, 1960; Arambourg, 1962; Monastero and Delanoue, 1966; Genduso, 1967; Étienne, 1973; Raspi and Loni, 1994; Loni, 1997).

Relationship Between *Psytalia concolor* and *Psytalia humilis*

There are several geographically distinct populations of *Psytalia* Walker, extending from northern to southern Africa, that are morphologically indistinguishable. In some cases, because the relationships among these geographically distinct populations have not been explored, these populations have been described as separate species. *Psytalia concolor*, *P. humilis* (Silvestri), and *P. perproxima* (Silvestri) are three examples relevant to our work. In my research we were able to study the relationship between *P. concolor* originally from Tunisia and the Kenyan population of *Psytalia* cf. *concolor* (Chapter I). However, we were unable to study the relationship between *P. concolor* and *P. humilis*. *Psytalia humilis* was one of the parasitoids collected in South Africa in 1912 and released in Hawaii by Filippo Silvestri in 1913 during his exploration for parasitoids of medfly in Africa (Silvestri, 1913,1914).

***Psytalia humilis* in Hawaii**

The braconids *P. humilis*, *Diachasmimorpha fullawayi* (Silvestri), and *D. tryoni* have been credited with initial suppression of medfly throughout the Hawaiian Islands (Back and Pemberton, 1916). *Psytalia humilis* was initially the most effective parasitoid. Then, for a short period, *D. fullawayi* exceeded parasitization of both the other opiines. Eventually, however, *D. tryoni* provided the greatest control of medfly until other parasitoids were later released during the Oriental fruit fly program (Willard and Mason, 1937; Willard and Bissell, 1930).

Pemberton and Willard (1918a,b) became concerned about the effect of the cannibalistic nature of *D. tryoni* on *P. humilis*. They believed *P. humilis* to possess the most desirable traits for effective medfly control and concluded that mortality of *P. humilis*, as caused by other

parasitoids, reduced total parasitism and overall medfly control. As noted by Gilstrap and Hart (1987), the work of Pemberton and Willard (1918a,b) has been used by proponents of the concept that future biological control programs should ascertain in advance the best parasitoid for release to avoid decreasing the percentage of control through interference. Smith (1929) makes an excellent rebuttal to this argument and asserts that medfly control was improved by the collective action of all the parasitoids even when *P. humilis* was not dominant. These arguments are important for continued exploration efforts against medfly, other fruit flies, and biological control as a scientific discipline (Gilstrap and Hart, 1987).

Prior to the Oriental fruit fly program, *P. humilis* disappeared from the Hawaiian landscape. There are two main hypotheses to explain the disappearance. One, a genetic bottleneck may have led to the demise of the species. There were only three female individuals released in 1913 from Silvestri's initial collections. The resulting population may not have been able to overcome inbreeding depression. An alternative hypothesis is that *Fopius arisanus* (Sonan), when it was released in the late 1940's and early 1950's, out-competed and eliminated *P. humilis*. This alternative hypothesis has some support from studies showing that *F. arisanus* which oviposits in the host egg, out competes species attacking late larval instars in laboratory studies (e.g. van den Bosch and Haramoto, 1953; Bess *et al.*, 1961; Wang and Messing, 2002, 2003). However, this theory does not seem plausible as not a single individual of *P. humilis* was recovered, even at the beginning of the Oriental fruit fly sampling program.

Based on the initial success of *P. humilis* after its release and the high probability of extirpation by inbreeding depression, it would seem reasonable to introduce the very similar *P. concolor* to Hawaii. *Psytalia concolor* exhibits qualities that show it to be a parasitoid capable of adapting to different climatic situations. It is the only braconid parasitoid reared in this project that was collected at all three Kenyan collection sites. In particular, Kenyan parasitoids,

as they are collected at higher elevations, might be more successful in controlling medfly at the higher elevations in Hawaii. Medfly competes more successfully with the fruit fly *B. dorsalis* at higher elevations and is thus more abundant and needs greater control in these areas.

Psytalia concolor in the Americas

California

Psytalia concolor was released in California against olive fly in 2000. *Dacus oleae* (Gmelin) is a stenophagous species with only one commercial host (olives). Its quick establishment and rapid dispersal within California precluded an eradication program especially since it does not pose the threat to the entire fruit industry that medfly does. Thus, *P. concolor*, with a wealth of published biological information, was a good choice for the release against olive fly.

Latin America

No work has been done on the ability of *P. concolor* to attack pest and non-pest species of *Anastrepha* indigenous to Guatemala and the rest of Latin America. Before *P. concolor* or other fruit fly parasitoids from Africa are released in Guatemala, nontarget work ensuring these rediscovered parasitoids would not adversely affect the environment will be necessary. If *P. concolor* shows little or no propensity for nontarget effects in Guatemala, it would be a cheap parasitoid to begin mass rearing as so much work has already been done on this species in Europe. Although previous releases of the Italian *P. concolor* in Costa Rica were ineffective, as

demonstrated by the failure to recover *P. concolor* during an on-going augmentative program (Wharton *et al.*, 1981), there is anecdotal evidence, supported by our work, that long-term acclimatization to laboratory conditions precludes effectiveness in the field. This needs to be explored for *P. concolor*.

Host Location: The Importance of the Host Habitat

Although a female fruit fly parasitoid can be enticed to oviposit into a nontarget host by placing the larvae in an attractive substrate (Duan and Messing, 1996,1997; Purcell *et al.*, 1997), or by placing the parasitoids in very close proximity of the nontarget host i.e. on a galled stem (Duan *et al.*, 1997), it is the ecological process of host finding that is most important in assessing nontarget risks (Lockwood, 2000).

Despite their earlier studies under artificial conditions in small arenas, Duan and Messing (1998) state that for accurate assessment of susceptibility to parasitism, nontarget species should be exposed to a parasitoid in the natural microhabitat instead of being removed from their host plants. If a nontarget species in its natural substrate is not acceptable for parasitoid oviposition (e.g., because the parasitoids do not accept (probe) the substrate, as in Duan and Messings' study), the nontarget species should be considered safe from attack, regardless of its physiological suitability for parasitoid progeny development.

Cues Used in Host Location

In order to examine nontarget risks in a natural setting, one must understand how a parasitoid locates its host. Feeding by fruit fly larvae inside the fruit causes bacteria-related decay of the host fruit. This decay releases chemical stimuli that are known to be important for the location and recognition of fruit fly larvae by tephritid parasitoids that attack later larval instars (Nishida, 1956; Greany *et al.*, 1977, Messing, 1999). In contrast, feeding by gall fly larvae does not cause host plant tissue decay, but rather stimulates the growth of gall tissue (Hapai and Chang, 1986). Thus, decaying fruit and a developing or mature gall represent two ecologically distinct host habitats (Messing, 1999) from the standpoint of chemical cues useful in location of a concealed host.

Parasitoids of fruit-infesting tephritids are known to use other cues to locate hosts, and vibrational cues are perhaps the best known of these for wasps that oviposit into the active larval stage of the host. The combination of chemical cues and vibrational cues enable the parasitoid to locate the host microhabitat chemically and detect larvae buried within the fruit by means of sound waves passing through the fruit due to movement and feeding by the host. Both *D. tryoni* and *D. longicaudata*, two of the opiine parasitoids of tephritids established in Hawaii, are known to use a combination of chemical and vibrational cues to locate hosts (Greany *et al.*, 1977; Lawrence, 1981; Duan and Messing, 1999). Egg parasitoids, however, do not use vibrational cues, and therefore must rely entirely on chemical or other cues such as color, size, and shape. Chemical cues for egg parasitoids may include not only host-plant derivatives, but also ovipositional deterrents deposited on the fruit surface by the host fly (Prokopy and Webster, 1978).

While there has been some work on the importance of size and shape in host location (Leyva *et al.*, 1991), color is a relatively unexplored cue for parasitoids of fruit-infesting tephritids. Color is, however, known to be important in host selection by flies. If color and shape or size plays some role in host location, this may explain some of the nontarget findings for parasitoids of fruit-infesting tephritids, and would facilitate assessment to risk of nontargets. For example, the use of color, size, and shape as subsidiary cues may explain why some species of *Diachasmimorpha*, which rely heavily on odor cues to locate infested fruit, occasionally attack tephritids in certain galls despite the difference in chemical cues emanating from the galls versus infested fruits. The consistently low attack rate on gall-forming tephritids, despite decades of association in Hawaii also provides a strong argument against the unrealistic demand of zero risk to nontargets in pre-release experiments.

Evolutionary Implications

It is known that at least some species of *Psytalia* attack tephritids in developing flower heads. This has been used as an argument against the use of *Psytalia* for biological control of frugivorous tephritids in areas where flower head-infesting tephritids are used for weed biocontrol. *Psytalia vittator* (Wilkinson), for example, attacks tephritids developing in flowers of *Chrysanthemoides* Fabricius. *Chrysanthemoides* consists of two species, both endemic to southern and eastern Africa. These two species have fleshy fruit, which immediately separates *Chrysanthemoides* from all other plants in the Asteraceae and makes it more fruit like than flower like. It is plausible that because *Chrysanthemoides* has fleshy fruit that turns a brown, blackish or purple color when ripe and contains tephritids in the seeds, that *P. vittator* is the evolutionary link between those *Psytalia* that attack fruit-infesting tephritids and those that

attack tephritids in flower heads; and that color, size, and shape cues used in host habitat location facilitated this evolutionary transition. Nevertheless, the repeated failure to find parasitoids of frugivorous Tephritidae in flower heads and parasitoids of flower head infesting tephritids in fruit suggests that this transition occurred once, a long time ago.

Duan and Messing (2000) state that from an evolutionary point of view, it would be parsimonious for a specialist parasitoid to evolve only one type of sensory modality, using either vibration or chemical cues (instead of both) for host searching in a patch. For a generalist parasitoid, on the other hand, evolution of different types of sensory modalities may be necessary for exploitation of different types of hosts. Thus, species of *Diachasmimorpha* that have multiple sensory modalities to locate hosts can be predicted to be generalists, according to Duan and Messing (2000). This argument is at its best simplistic. It overlooks the complexity of the host habitat as well as the need for detecting concealed hosts, in addition to host microhabitats that exist only as ephemeral resources. For parasitoids that attack hosts concealed within plant tissue, ovipositor probing often is an essential element of host-searching behavior (Vet and van Alphen, 1985; van Dijken and van Alphen, 1998; Messing, 2001). The role of learning (Vet 1983,1988, Vet and van Opzeeland, 1984, Vet and van Alphen, 1985, Lewis and Tumlinson, 1988, Lewis and Takasu, 1990, Lewis *et al.*, 1990, Vet *et al.*, 1990) also needs to be considered. Thus, regardless of whether the parasitoid is a specialist or generalist, multiple sensory modalities are essential for tracking hosts, and parasitoids, like most animals, are well equipped with a variety of sensory modalities. The use of specific chemical cues for host recognition once the host is located may be an easier argument to use when comparing specialists and generalists.

Messing, in his 2001 paper on centrifugal phylogeny as a basis for non-target host testing in biological control makes the assessment that nontarget testing in arthropod biocontrol is different than for weed biocontrol and thus cannot follow the same guiding principles of

centrifugal phylogeny testing as is called for in protocols for weed biological control. (Centrifugal phylogeny testing is the idea that a weed biocontrol agent is exposed to a sequence of plants from those most closely related to the target weed to progressively more distantly related plants (Wapshere, 1974).) Messing asserts that for many parasitoids, host range is dictated in large part by microhabitat rather than by the hosts themselves. He gives the example of species in the genera *Pachycrepoideus* Ashmead, *Spalangia* Latreille and *Dirhinus* Dalman attacking tephritid pupae in the soil and ignoring related tephritids in nearby galls and flower heads. However, parasitoids in the first two genera will attack housefly and dung fly pupae in addition to tephritids and can hardly be considered host specific. Although this example highlights the role of host habitat, it provides almost no information of relevance to nontarget testing. Pupal parasitoids, for example, are notorious for being generalists, in part, perhaps, because they do not have to overcome the same difficulties with the immune system that larval endoparasitoids must. Some pupal parasitoids, however, do seem to be relatively host specific and species in the genus *Dirhinus* may fall into this category.

Expanding the microhabitat argument beyond pupal parasitoids, Messing hypothesizes that the fruit fly parasitoids attacking larval tephritids in fruit (i.e. coffee) will attack any small reddish-tinged sphere that vibrates from internal feeding. If this were true, all the larval opiine parasitoids previously released in Hawaii would be found in lantana in the field. Instead, only one of the five species attacks lantana gall flies, and only in small numbers. In reality, the parasitoids are not highly attracted to galls, however they will attempt to probe and may oviposit into the gall-forming tephritids if the wasp does land on an infested gall or a stem near a gall. As further evidence that it is a sequence of cues that elicits a probing response from frugivorous parasitoids, and not simply an attraction to round reddish objects, fruit fly parasitoids do not attempt to oviposit in old galls that the tephritids have already emerged from. In essence,

Messing advocates adopting Lockwood's (2000) argument that we should define nontargets as ecological processes, rather than species or entities. This may be appropriate, but if it leads to the conclusion that galls are ecological equivalents to fruit; then essential details of the ecological process have been missed and inappropriate conclusions about the risk to nontargets will be (and have been) made.

Parasitoid Habitat Types

I suggest an alternative definition of parasitoid habitat types in relation to frugivorous tephritid fruit flies for use in biological control. Habitat type I is represented by solitary, larval-prepupal endoparasitoids. These parasitoids use a combination of chemical, visual and vibratory cues to find their host. Examples of Habitat Type I parasitoids from our collections of coffee in Kenya include *D. fullawayi* and *P. concolor*.

Habitat type I parasitoids may have minor nontarget effects on gall-forming tephritids, not necessarily based on an attraction to the habitat or the host itself, but based on using vibratory cues after landing on a gall. These nontarget effects manifest themselves in the field in the genus *Diachasmimorpha*, but not *Psytalia*, leading to interesting questions regarding the difference between these two either in long-range, host habitat finding (patch location) or short range response to vibrational cues. This brings us back to one of the conclusions of Chapter III: for those parasitoids that attack frugivorous tephritids, nontarget effects can be predicted on the basis of the genus to which the parasitoid belongs. Answers to the question of why *Diachasmimorpha* and *Psytalia* exhibit different nontarget effects may allow biological control workers to predict how parasitoids find hosts and thus better predict and test for nontarget effects.

Habitat Type II consists of the egg-prepupal and early larval prepupal endoparasitoids. Examples from our collections in coffee include *F. ceratitivorus* Wharton, *F. caudatus* Szépligeti, and *F. silvestrii* (Wharton). Habitat Type II parasitoids use different cues to find their hosts than the late larval–prepupal endoparasitoids. They use a cue on the surface of the fruit e.g. a chemical cue, a damage cue, or a female fly's oviposition mark or hole to find their host and not vibratory cues like parasitoids in Habitat Type I.

Fopius arisanus, a congener of the Habitat II Kenyan parasitoids listed above, has been established in Hawaii since the 1950's, however none have been reared from galls or flower heads. These parasitoids, based on their host finding mechanism do not pose any threat to galls or flower heads. Nontarget concerns for Habitat Type II parasitoids in Hawaii are largely limited to possible interference effects on other parasitoids that have already been released for fruit fly biological control. We know from Kenya that there is only one egg-prepupal parasitoid in each biogeographic region of the country (on either side of the Rift Valley). Is this a result of competition? Or is it just an artifact of the development of parasitoids throughout evolutionary history? If species of *Fopius* Wharton are released in Hawaii they should be released in areas of high concentrations of medfly, thus limiting the potential interaction with the highly successful *F. arisanus*. Nontarget concerns for potential Habitat type II parasitoids to be released against medfly in Latin America are different than those in Hawaii as *F. arisanus* is not a successful parasitoid in Latin America. However, there is another difficulty. Unlike in Hawaii, there are native tephritids in the genus *Anastrepha* with a compliment of native parasitoids. *Fopius ceratitivorus*, a parasitoid that potentially attacks only medfly, could present a potential solution to this problem.

Where Does This Lead Us?

Opiine braconids parasitoids of fruit-infesting Tephritidae have never been reared from hosts outside the family Tephritidae. All known species of opiines are koinobiont endoparasitoids. For these parasitoids, the combination of ecological and physiological factors result in a relatively restricted host range that eliminates most nontarget concerns. Nontargets of interest, then, are limited to members of the family Tephritidae, and in particular those used in the biological control of weeds (flower-infesting and gall-forming species) or native fly species.

I conducted nontarget experiments with the larval parasitoid, *P. concolor*, as it was the first species I was able to culture. I recommend releasing *P. concolor* in Hawaii based on the results of the nontarget experiments and the successful nature of *P. humilis* in the early 1900's. There is already a large guild of larval-prepupal parasitoids, but as we saw in Kenya stacking of parasitoids is the natural composition of parasitoid complexes in a tropical setting. Additionally, in Hawaii, any new parasitoids will increase numbers of flies attacked by filling a slightly different niche (Smith, 1929). The two *Fopius* species need more nontarget work on their possible effect on *F. arisanus* before they are released in Hawaii, since the latter is already considered a success. Again, citing Smith (1929), a release of host specific parasitoids like *F. ceratitivorus* should increase parasitism of *C. capitata*, thus reducing host reservoirs that serve as potential sources of flies introduced to the mainland (a major goal of the program).

However, even though I was unable to conduct nontarget testing with the two Kenyan *Fopius* species, I would recommend future programs concentrate on these two species for rearing and release. Tephritid parasitoids that oviposit in the host egg, despite the fact that they tend to be difficult to culture, exhibit a propensity to be highly specialized in host location behavior.

Since timing is critical for location of host eggs, this again suggests a specific set of host and host habitat cues.

Specific recommendations for Latin America include: (1) before *Psytalia* is released tests must be performed to insure *P. concolor* does not attack and produce a negative impact on native *Anastrepha* species and (2) the release of *F. ceratitorus* in Latin America as well as the further study of *F. caudatus* for potential release.

Fopius arisanus has been released in Latin America, but is not a major control factor of fruit flies as it is in Hawaii. Hence, there is no worry about potential interactions with a highly successful parasitoid. Special consideration should be given to *F. ceratitorus*, as it seems to be host specific to medfly in coffee. As it is host specific to medfly, no negative impact on native *Anastrepha* parasitoids species should be realized. Also, as a Host Habitat Type II parasitoid, essentially its potential for other nontarget effects are negligible. Preventing medfly from moving north is one of the major tactics to prevent medfly expansion into mainland America (USDA, 1997; USDA/APHIS/PPQ, 2002). Releasing *Fopius ceratitorus* in Latin America shows the greatest potential to augment control of medfly in Guatemala with the least propensity for nontarget effects, thus keeping medfly from moving north through Mexico to the United States. *Fopius caudatus*, the other Host Habitat Type II parasitoid that we reared in numbers from coffee in Kenya, must be tested for nontarget effect on native *Anastrepha* parasitoids as our results have shown that it is not a medfly specific parasitoid.

The frugivorous tephritid/parasitoid system in Hawaii is atypical (in part because of the lack of native fruit-infesting tephritids) and thus cannot be used as a model for introduction of tephritid parasitoids throughout the world. However, results of the nontarget studies that have been accomplished in Hawaii can be used to predict possible nontarget effects on non-frugivorous tephritids in other areas of the world. The frugivorous tephritid/parasitoid system in

Guatemala is more typical and thus could be used as a model for introducing tephritid parasitoids of medfly throughout the world. This model consists of considering each potential habitat for release of tephritid parasitoids for its own potential nontarget effects, then categorizing the potential release candidates into Host Habitat Types. Potential release candidates can be prioritized for evaluation based in part on what is known about the nontarget potential of other members of the respective genera. If it is not known what nontarget effects the parasitoids in a particular genus have previously shown, then cues for host habitat finding must be elucidated. Then based on the Host Habitat Type the parasitoids must be tested for nontarget effects prior to release.

CHAPTER V

CONCLUSIONS

A Pertinent Question: Can Introducing More Parasitoids Increase Control of Fruit Flies?

If utilized properly, biocontrol is the most powerful ecological technology and one of the least disruptive, environmentally safe methods available for pest management (Pimentel *et al.*, 1984; Lockwood, 1996). The introduction of parasitoids for biological control of fruit flies in Hawaii has resulted in significant control of medfly even though very few of the parasitoids collected in early expeditions were actually reared from medfly. Control of medfly in Hawaii may not be complete, but the reduction in pest reservoirs has decreased the potential for accidental introductions to the mainland and is indicative of the possibility for similar levels of suppression in Central America and elsewhere. Thus parasitoids, either through classical or augmentative releases in conjunction with SIT, could be used to establish a natural border to northward movement of medfly by suppressing wild populations in the mountainous areas of the México/Guatemala border (Gilstrap and Hart, 1987).

Early explorations suggest a rich natural enemy component attacking fruit-infesting Tephritidae in Africa. Our results confirm this for medfly, with ten species recovered from infested coffee in Kenya, at least seven of these confirmed during this study as capable of successfully attacking medfly. One of the parasitoids, *F. ceratitivorus*, is a newly discovered parasitoid species that is thus far only known from medfly. Two of the species, *F. ceratitivorus* and *F. caudatus* oviposit in host eggs, a highly desirable trait.

Knipling (1992) advocated large augmentative releases of tephritid parasitoids endemic to Asia for complete control of Asian flies in all adventive areas. Theoretically then, when medfly is the target, parasitoids from Africa, where medfly is endemic, could be similarly effective. Yet, few of the African parasitoids of Tephritidae have been released against adventive populations of medfly, and only two are currently established in the Western Hemisphere (both in Hawaii).

Based on limited potential for nontarget impacts, as demonstrated in this study, it would be beneficial to introduce both *Psytalia concolor* and the two species of *Fopius* that are egg-prepupal parasitoids of medfly against medfly in the Western Hemisphere.

Some in the biocontrol arena are concerned though; that niches of new parasitoid species may overlap too much with one another or with previously introduced species, resulting in competitive exclusion. This is an unsubstantiated concern for primary parasitoids of fruit flies. Smith (1929) in his rebuttal to Pemberton and Willard (1918a) demonstrated that two species working together, despite overlap, would destroy a greater percentage of the hosts than one species working alone. The Oriental fruit fly program, where several species were introduced and established, supports Smith's argument. Even though one species eventually became the dominant parasitoid in the Oriental fruit fly program, every recovery of a different species adds to the overall level of parasitization.

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APPENDIX A

FIGURES

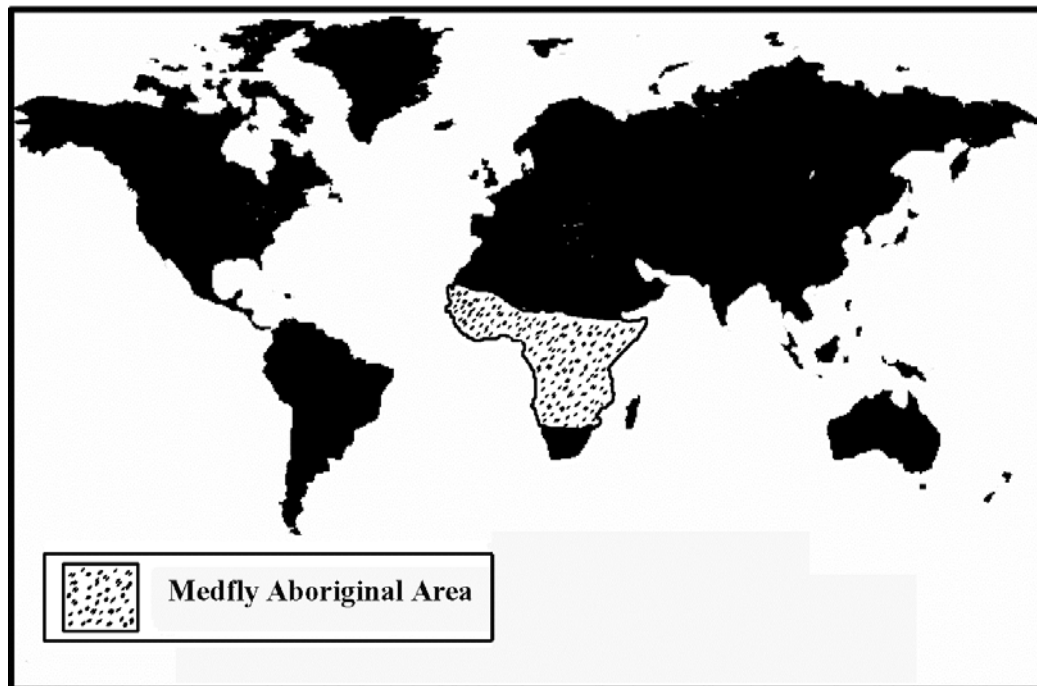


FIG. 1. Area representing medfly's most probable aboriginal home.

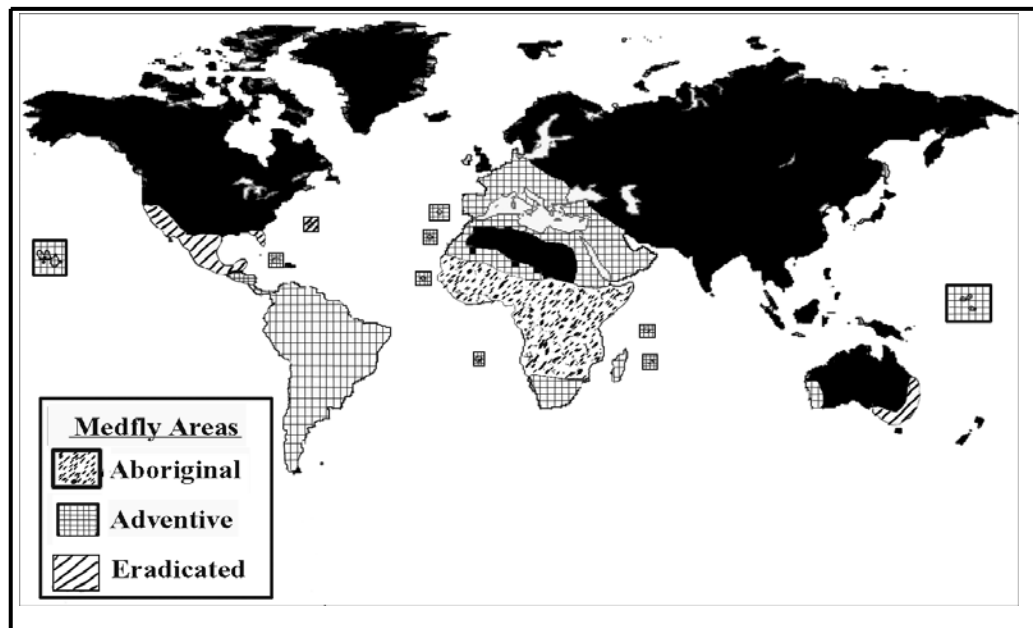


FIG. 2. Aboriginal, adventive and eradicated medfly areas throughout the world.

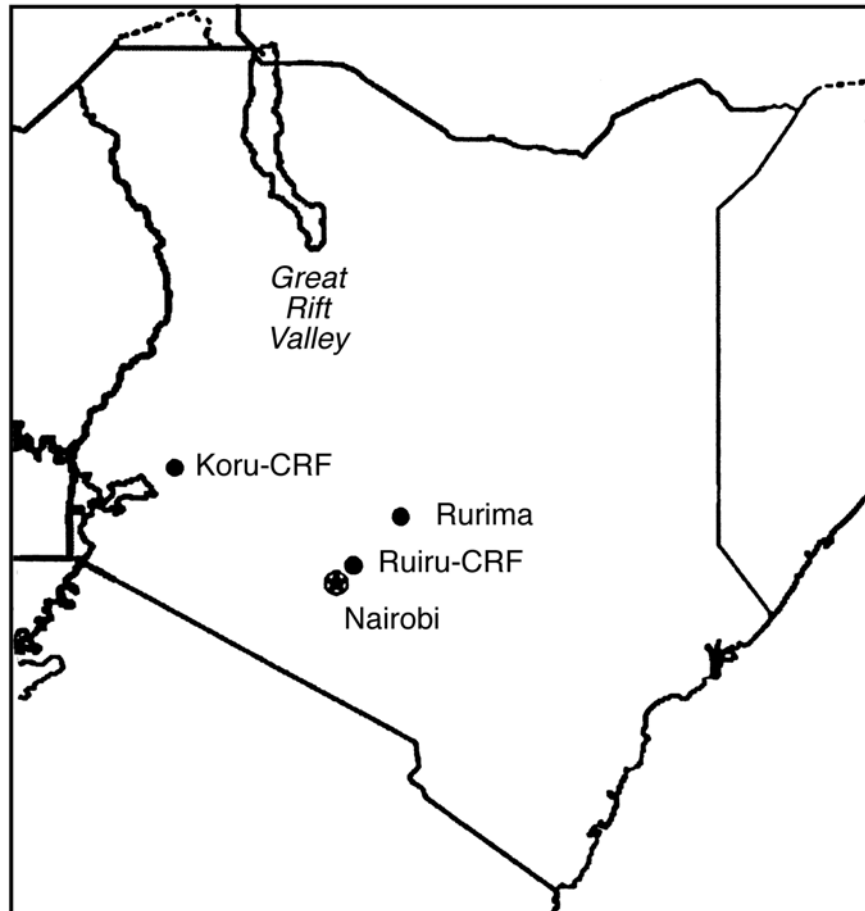


FIG. 3. Map of Kenya showing locations of primary collecting sites.

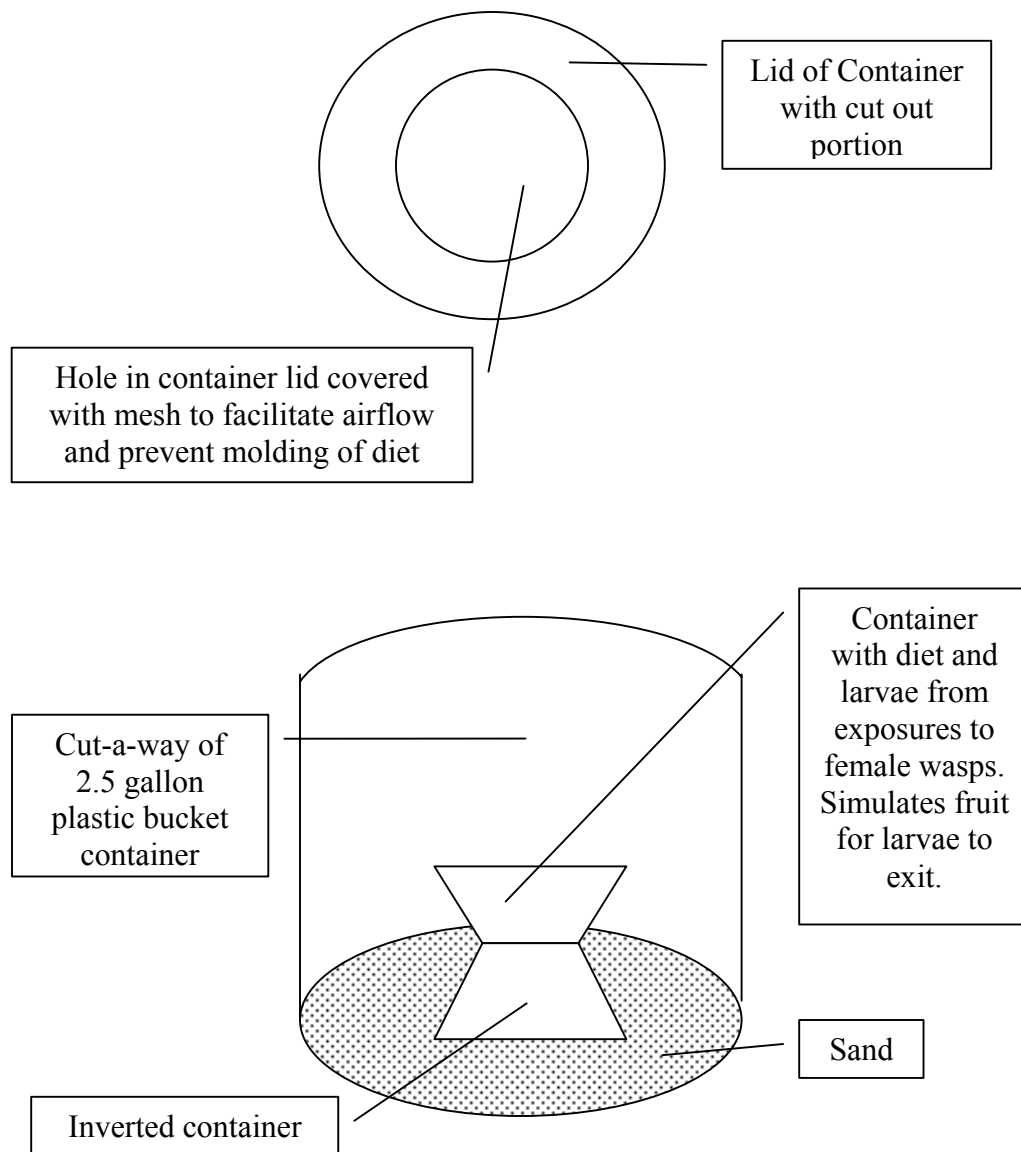


FIG. 4. Modified 2.5 gallon plastic bucket for stimulation of the natural larval behavior of exiting the fruit and pupating in soil

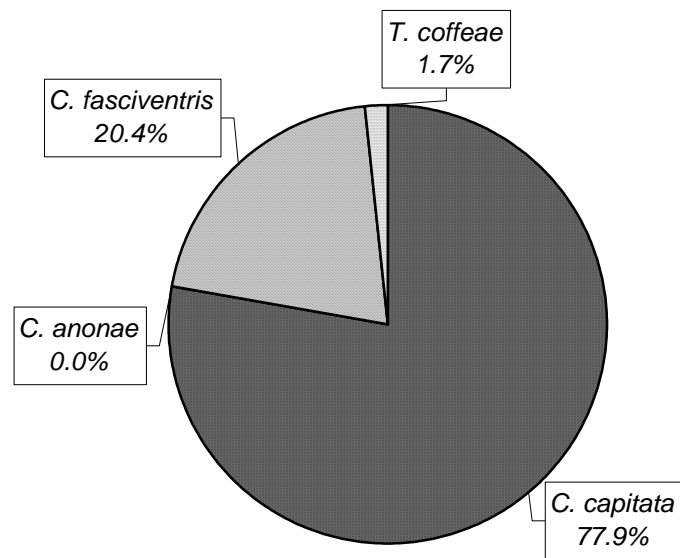


FIG. 5. Ruiru - The relative abundance of each fly species. Adjusted for percent emergence and kilograms collected (Eq. [8], see p. 25).

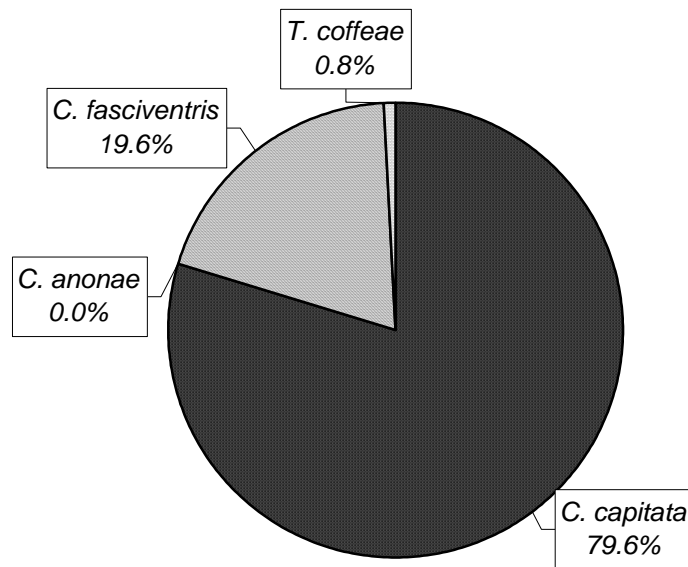


FIG. 6. Rurima – The relative abundance of each fly species. Adjusted for percent emergence and kilograms collected (Eq. [8], see p. 25).

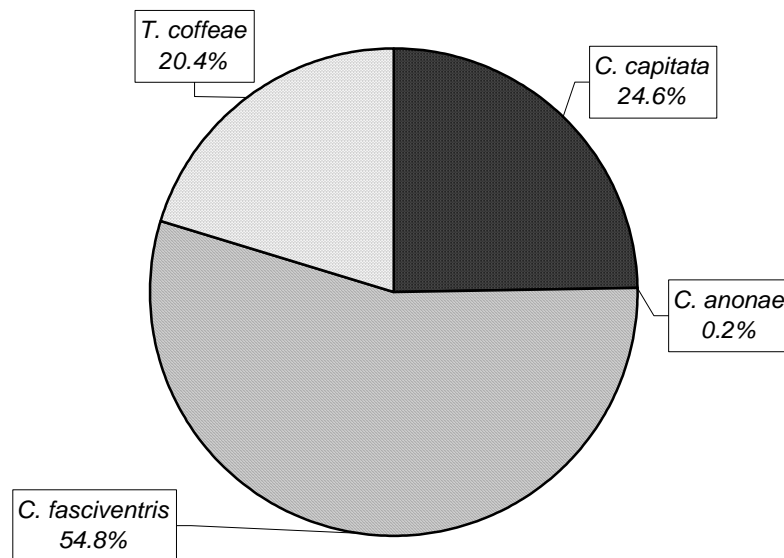


FIG. 7. Koru – The relative abundance of each fly species. Adjusted for percent emergence and kilograms collected (Eq. [8], see p. 25).

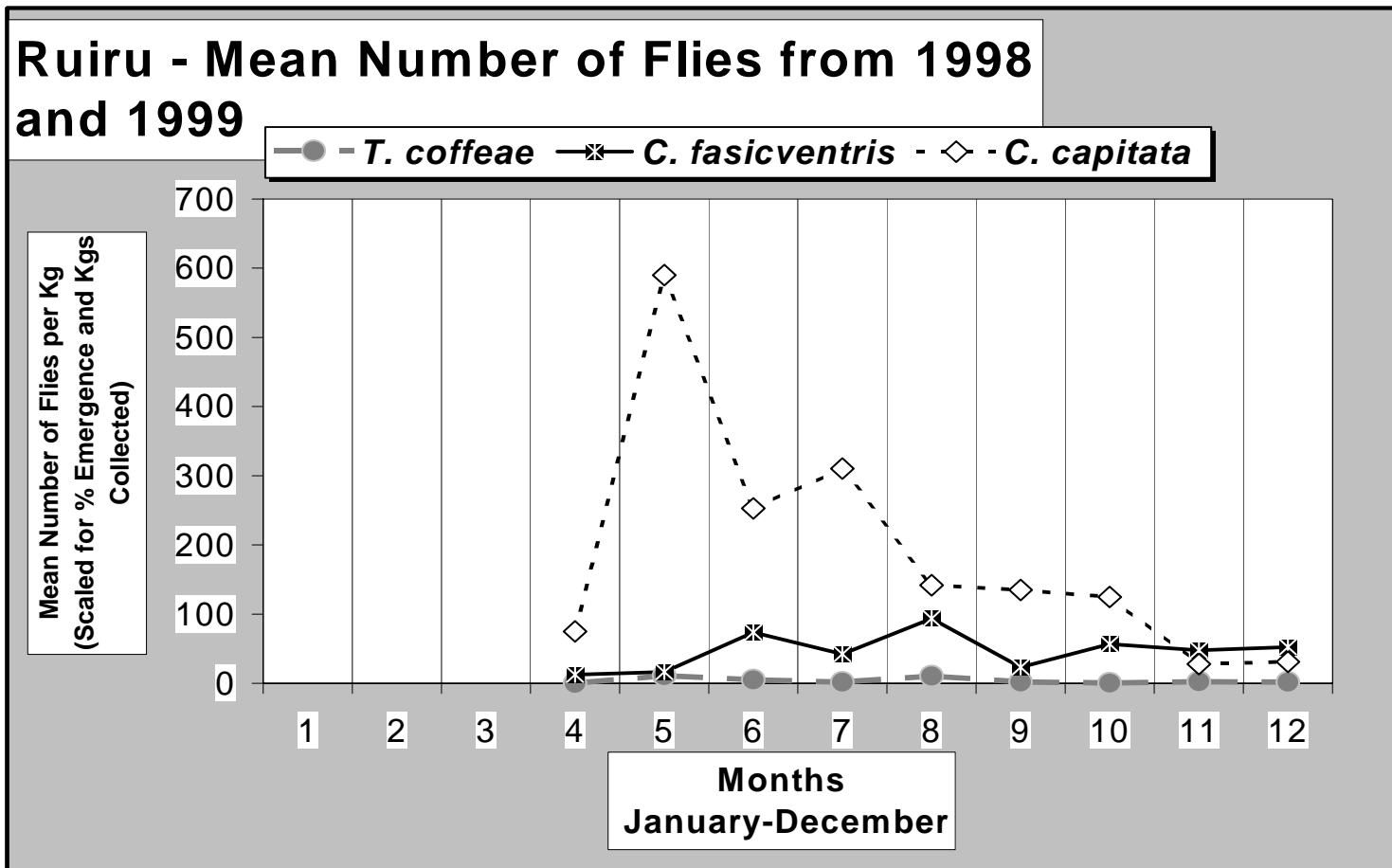


FIG. 8. Seasonal abundance of tephritid fruit flies at Ruiru. Figure represents the monthly mean for all data collected and is scaled for percent emergence and kilograms collected (Eq. [8], see p. 25).

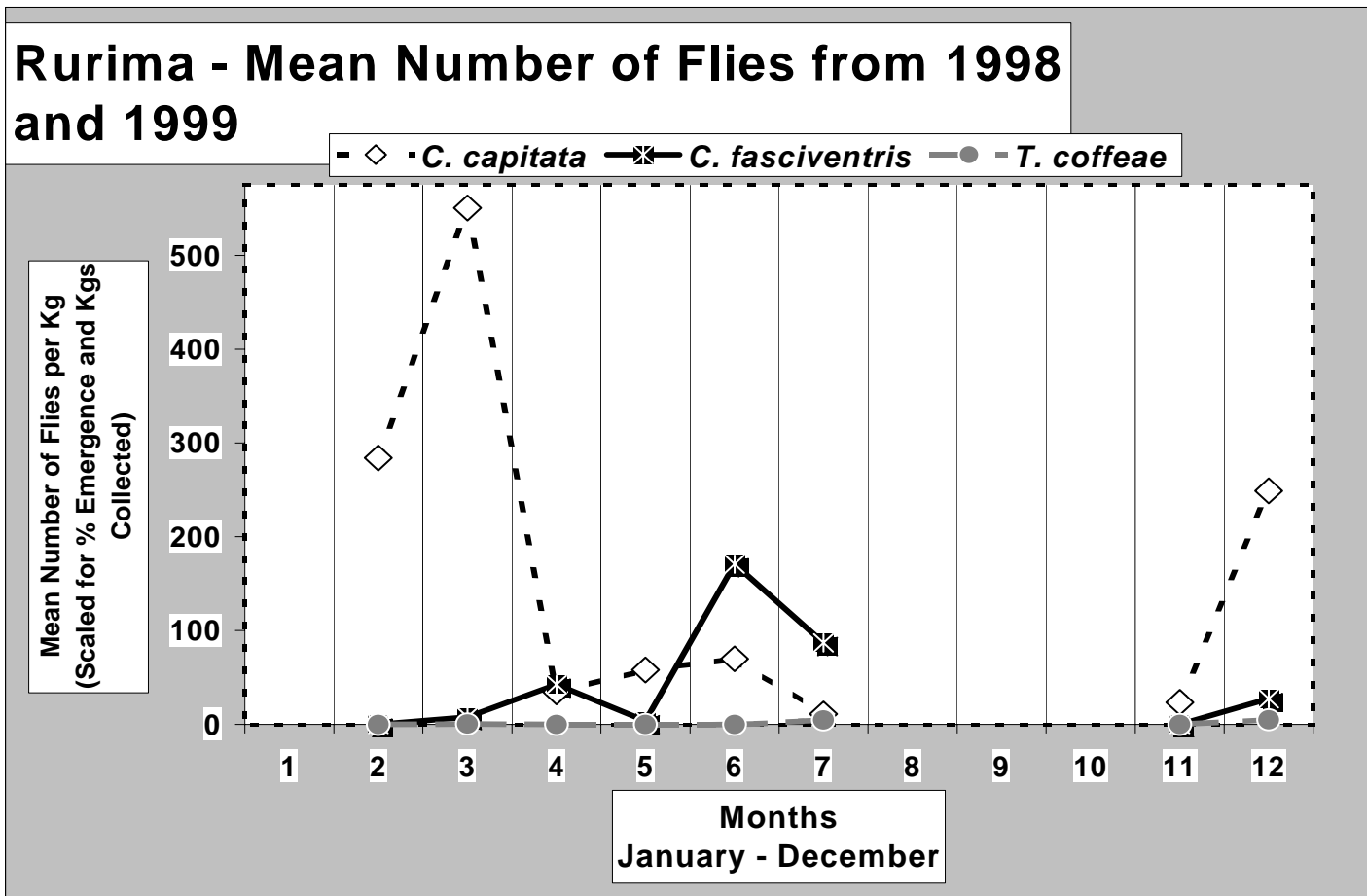


FIG. 9. Seasonal abundance of tephritid fruit flies at Rurima. Figure represents the monthly mean for all data collected and is scaled for percent emergence and kilograms collected (Eq. [8], see p. 25).

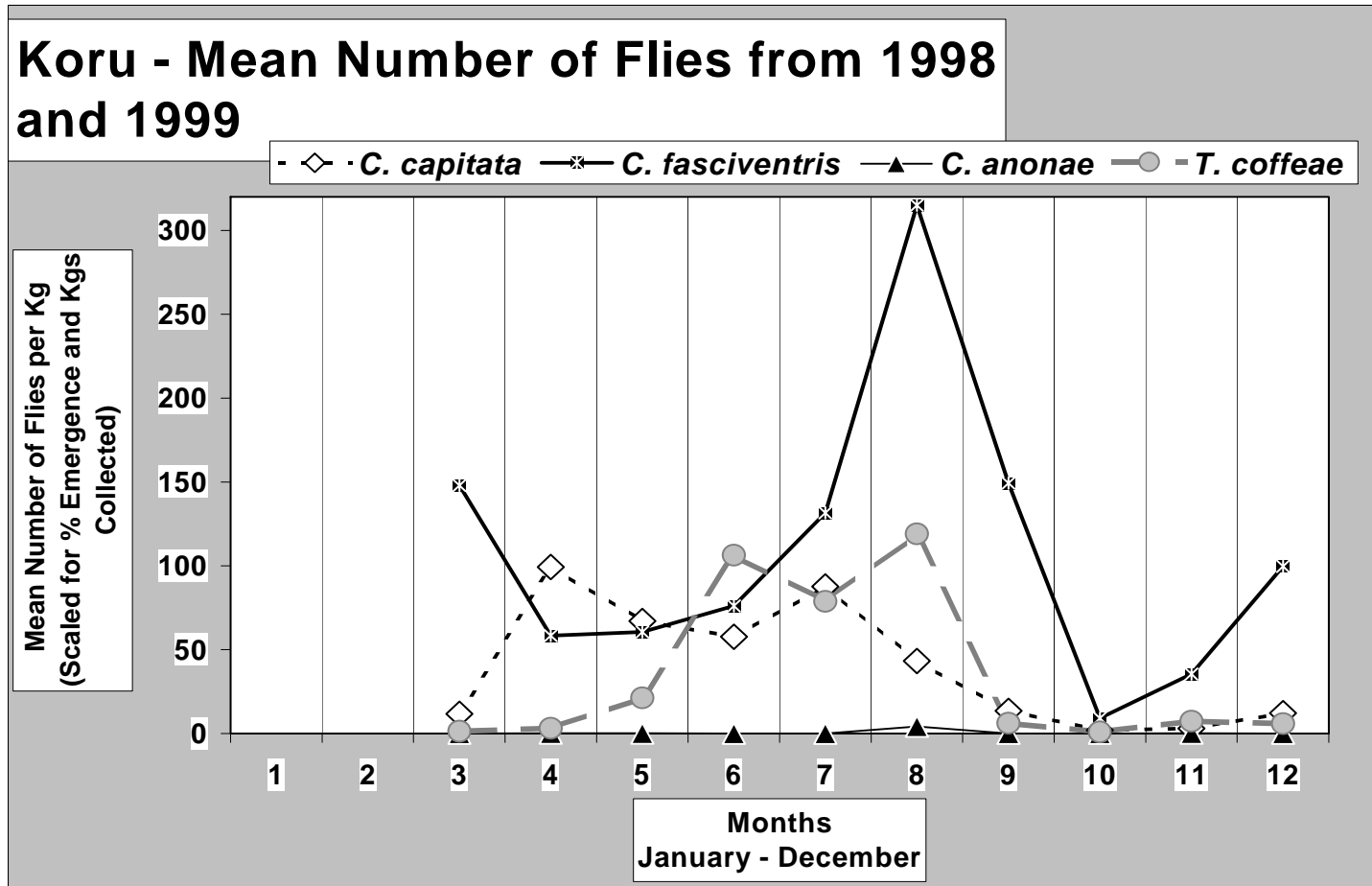


FIG. 10. Seasonal abundance of tephritid fruit flies at Koru. Figure represents the monthly mean for all data collected and is scaled for percent emergence and kilograms collected (Eq. [8], see p. 25).

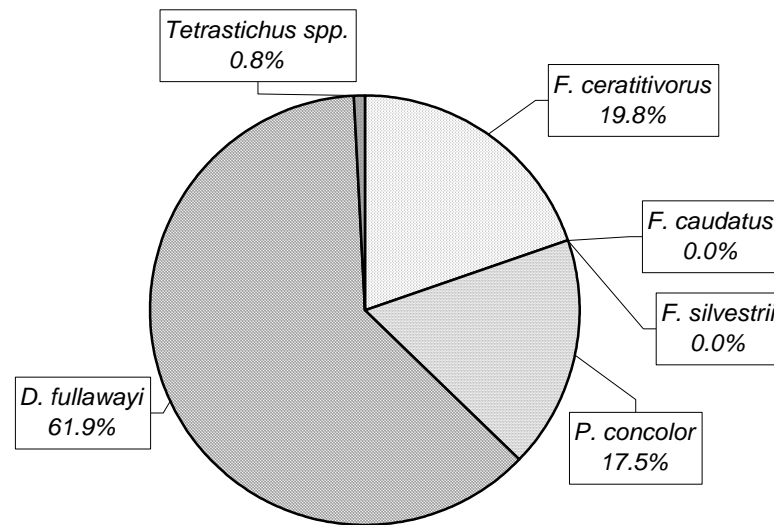


FIG. 11. Ruiru – The relative abundance of each parasitoid species. Adjusted for percent emergence and kilograms collected (Eq. [16], p. 26).

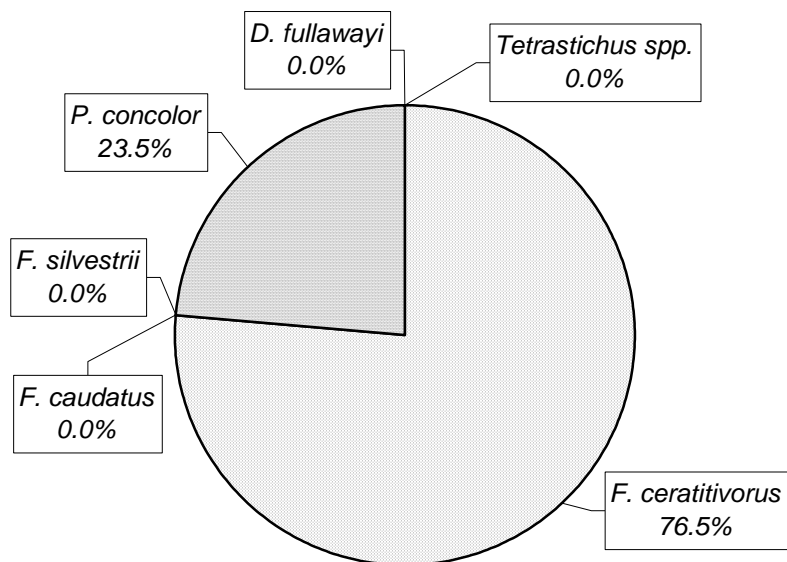


FIG. 12. Rurima – The relative abundance of each parasitoids species. Adjusted for percent emergence and kilograms collected (Eq. [16], p. 26).

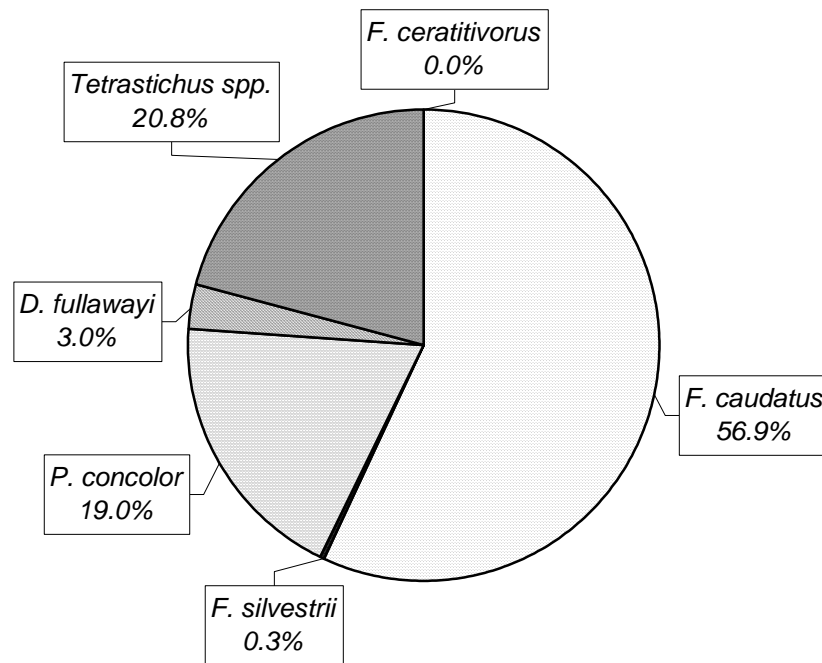


FIG. 13. Koru - The relative abundance of each parasitoid species. Adjusted for percent emergence and kilograms collected (Eq. [16], p. 26).

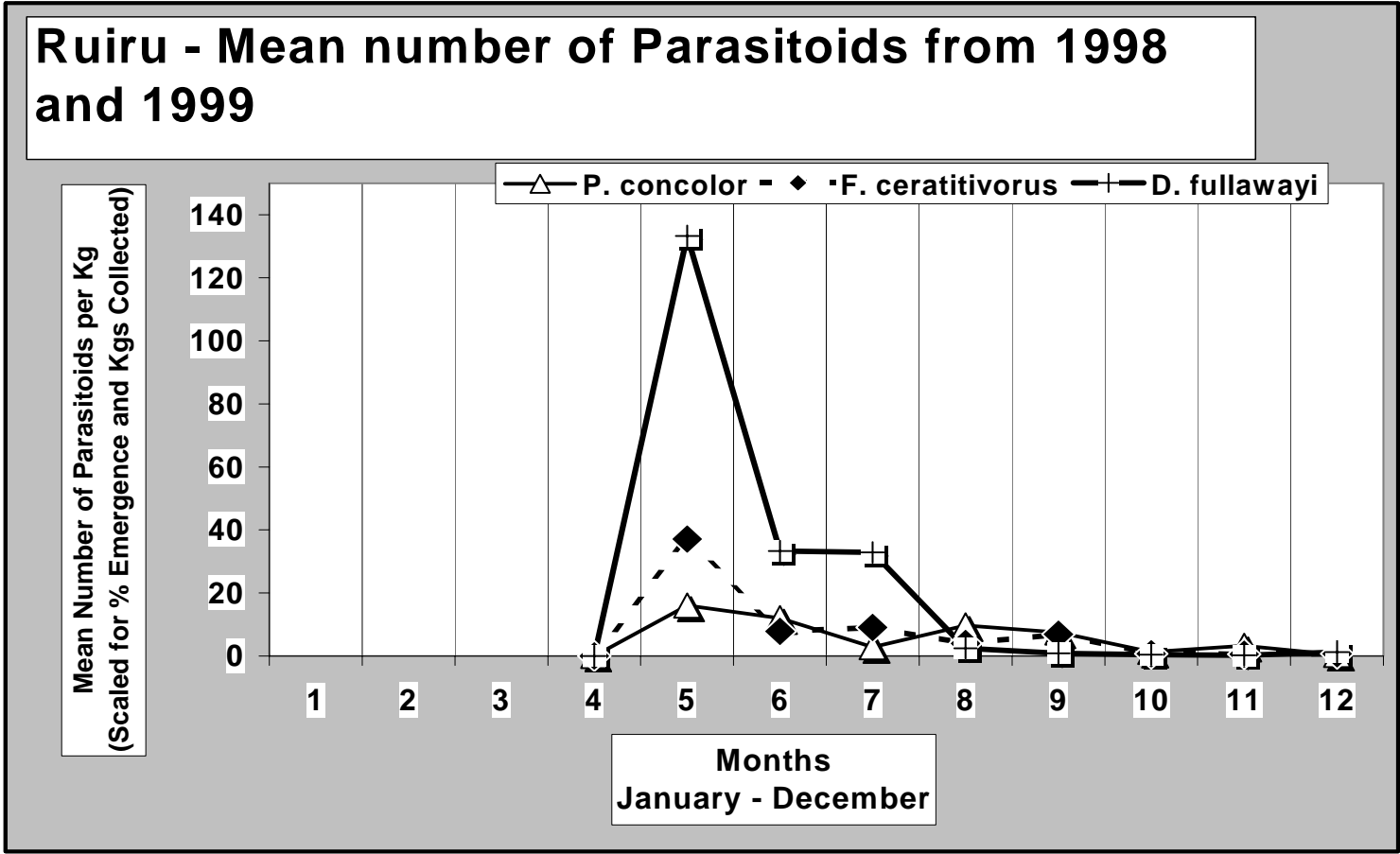


FIG. 14. Seasonal abundance of tephritid fruit fly parasitoids at Ruiru. Figure represents the monthly mean for all data collected and is scaled for percent emergence and kilograms collected (Eq. [16], p. 26).

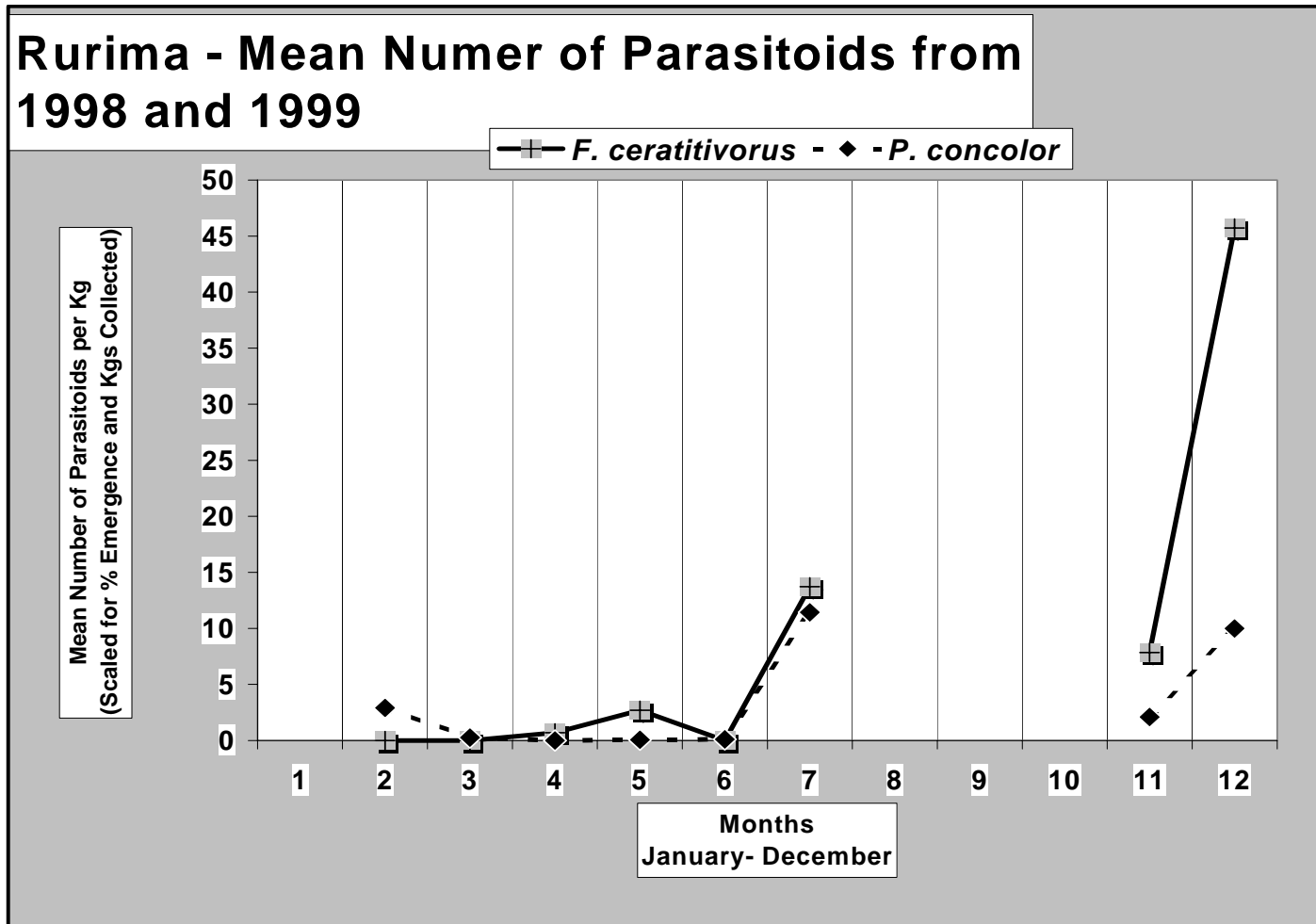


FIG. 15. Seasonal abundance of tephritid fruit fly parasitoids at Rurima. Figure represents the monthly mean for all data collected and is scaled for percent emergence and kilograms collected (Eq. [16], p. 26).

Koru - Mean Number of Parasitoids from 1998 and 1999

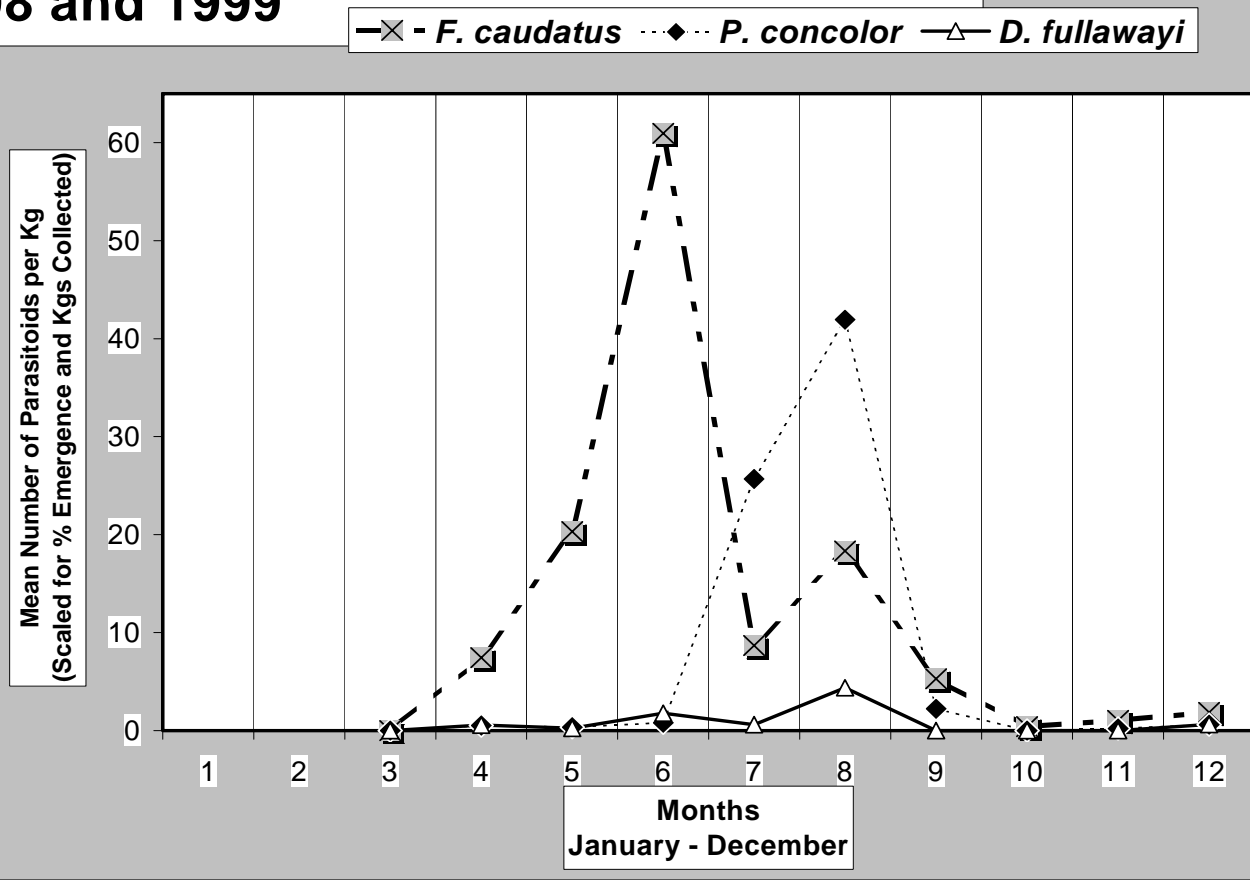


FIG. 16. Seasonal abundance of tephritid fruit fly parasitoids at Koru. Figure represents the monthly mean for all data collected and is scaled for percent emergence and kilograms collected (Eq. [16], p. 26).

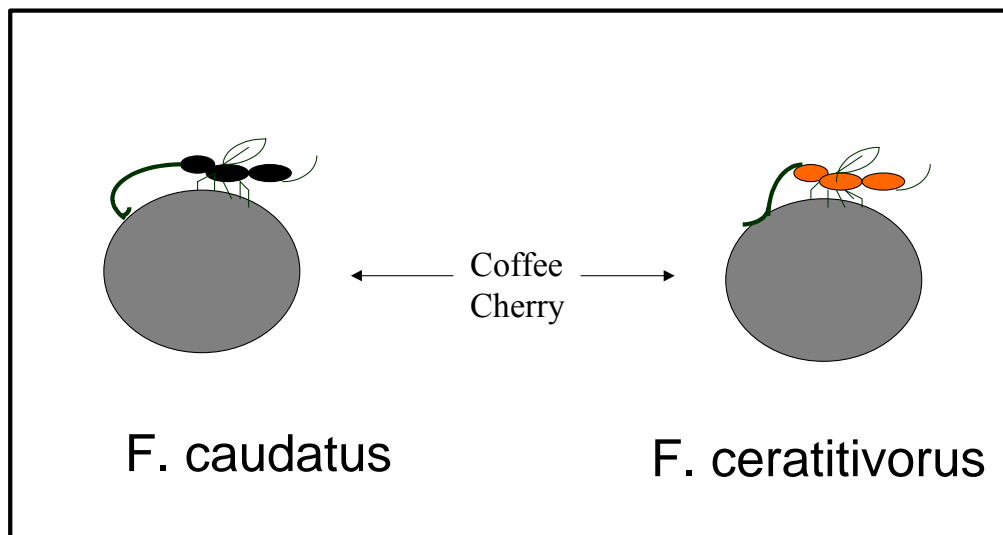


FIG. 17. Antennal position of *F. caudatus* and *F. ceratitorus* when foraging on coffee cherries

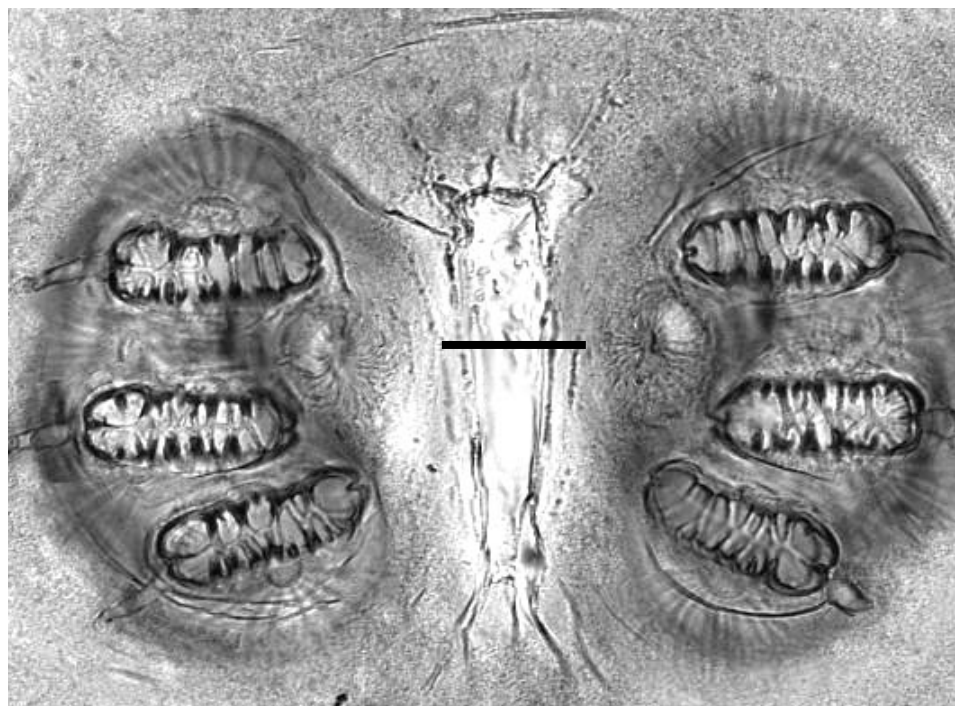


FIG. 18. Bar represents distance between the two plates used for figuring SFD. Measured with the reticle passing through center of both buttons.

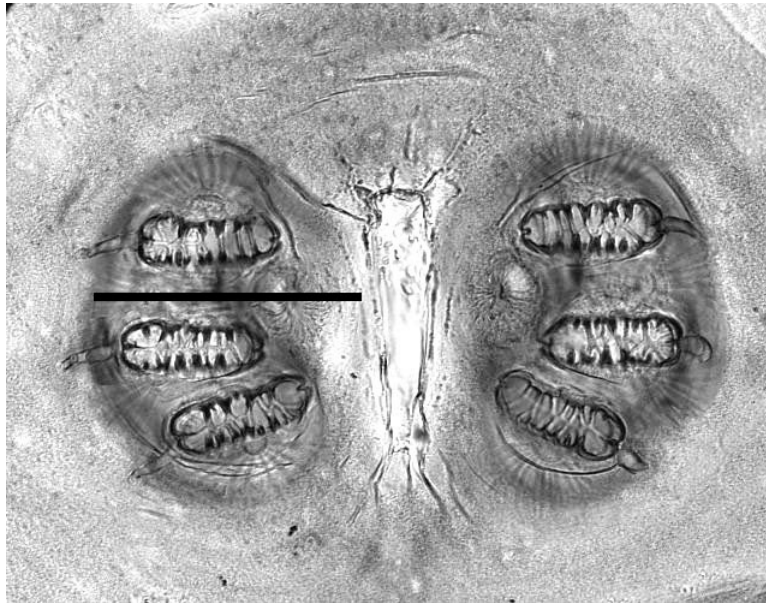


FIG. 19. Bar represents the diameter of one spiracular plate used in figuring SFD. Measured with the reticle passing through center of both buttons.

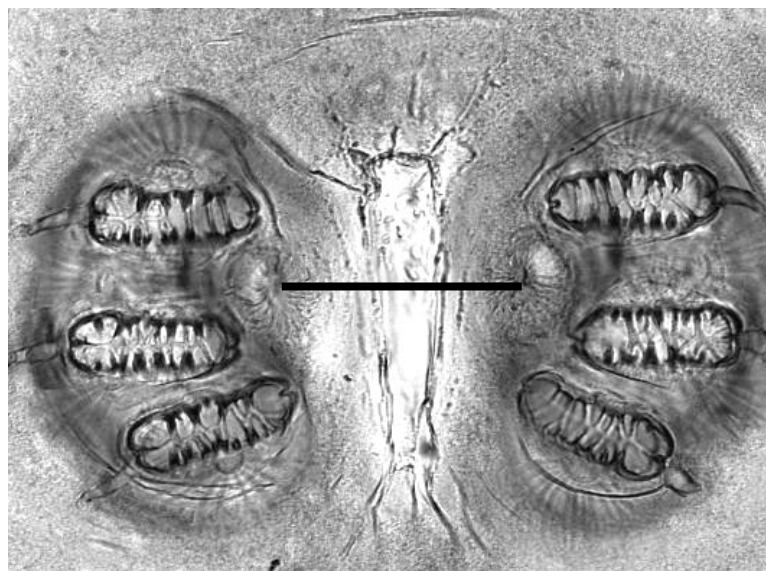


FIG. 20. Bar represents the button to button distance. Measured with the reticle passing through center of both buttons.

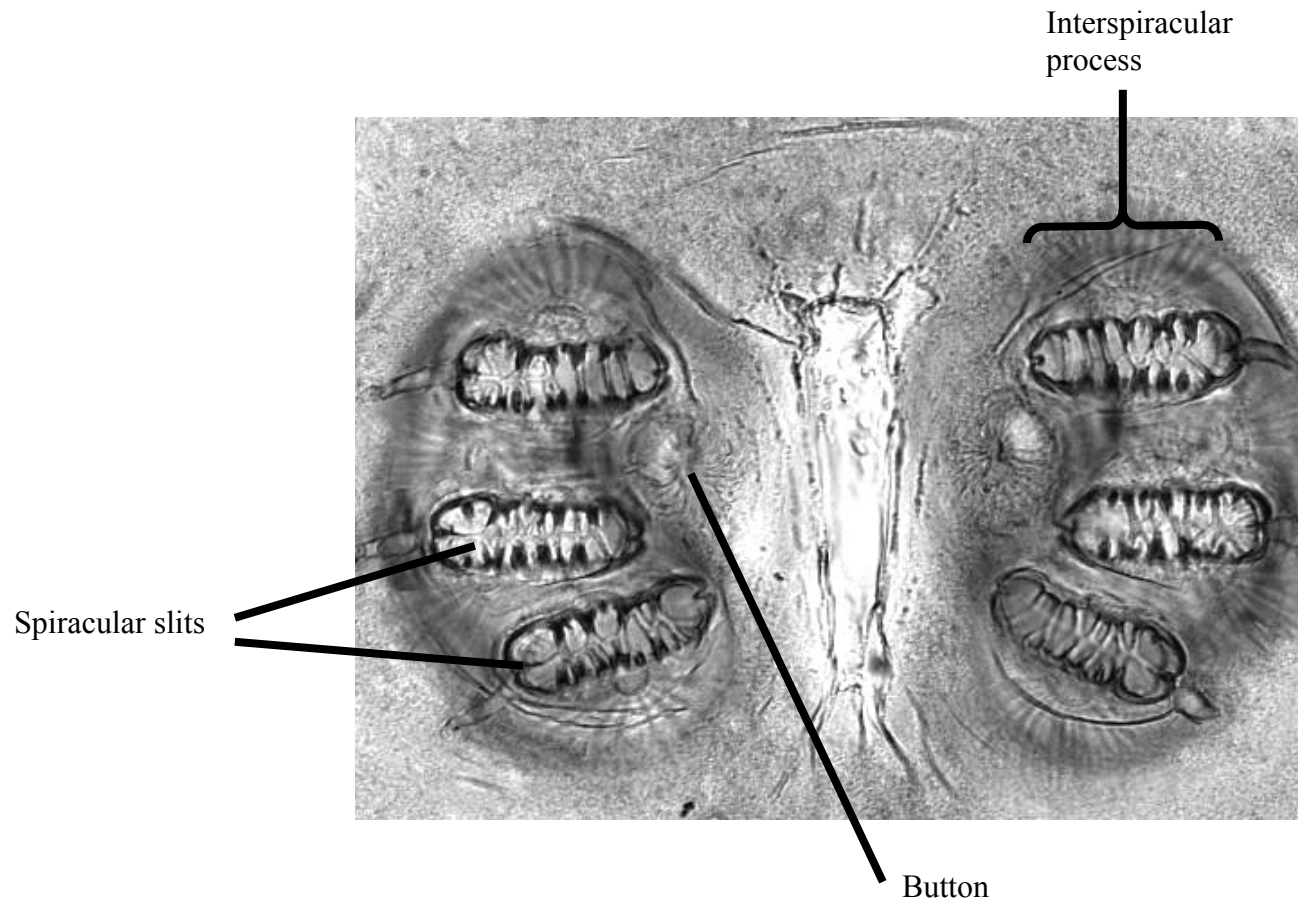


FIG. 21. General structure of the posterior spiracles of a tephritid fruit fly.



FIG. 22. *Ceratitis fasciventris* mouthhook with large preapical tooth.

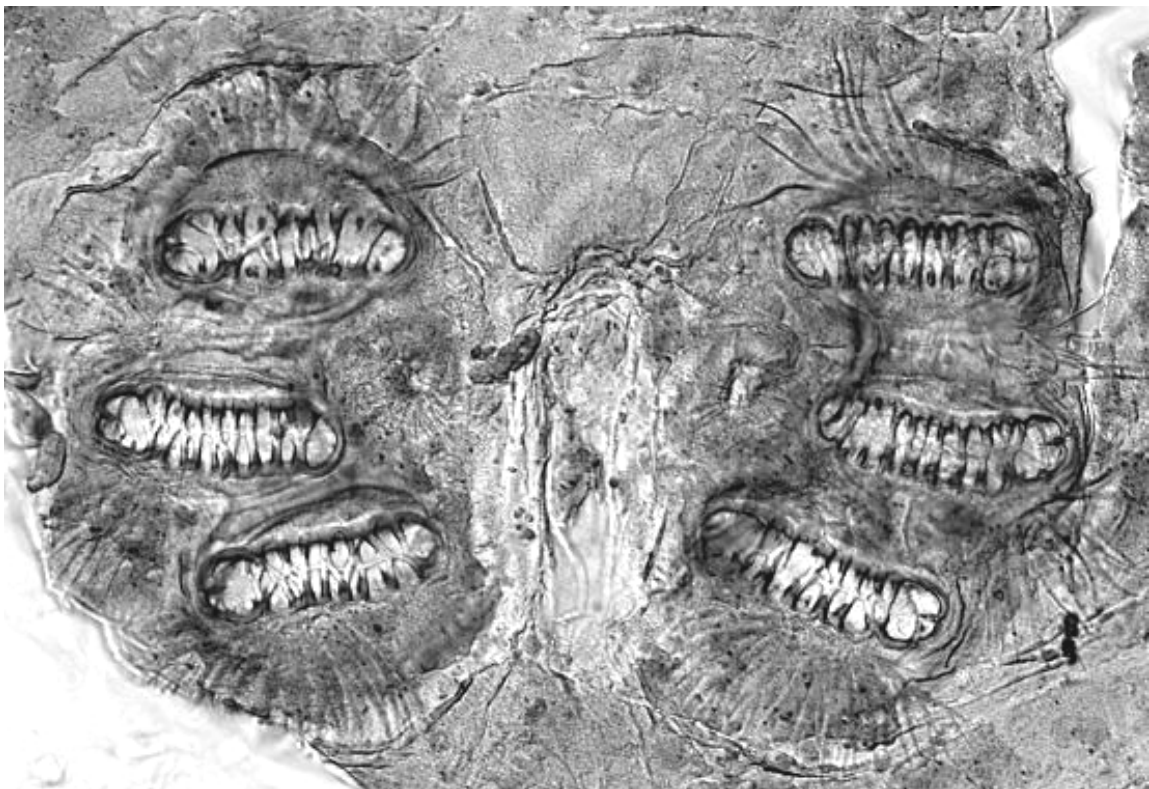


FIG. 23. Posterior spiracles of *Ceratitis fasciventris*.



FIG. 24. *Trirhithrum coffeae* mouthhook.

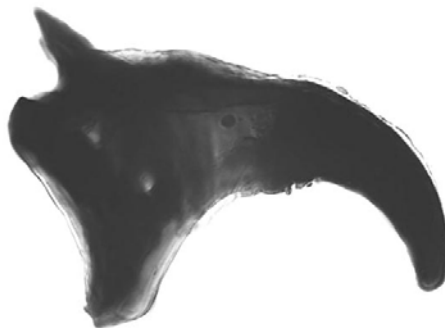


FIG. 25. *Ceratitis capitata* mouthhook without small preapical tooth present.



FIG. 26. *Ceratitis capitata* mouthhook with small preapical tooth present.

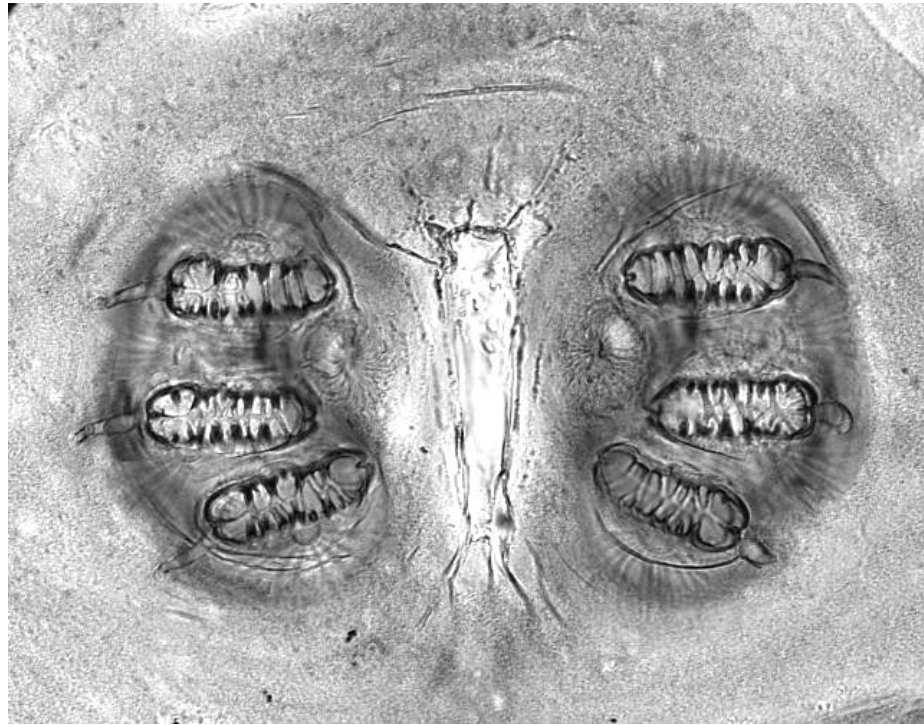


FIG. 27. Posterior spiracles of *Ceratitidis capitata*.



FIG. 28. Posterior spiracles of *Trirhithrum coffeae*

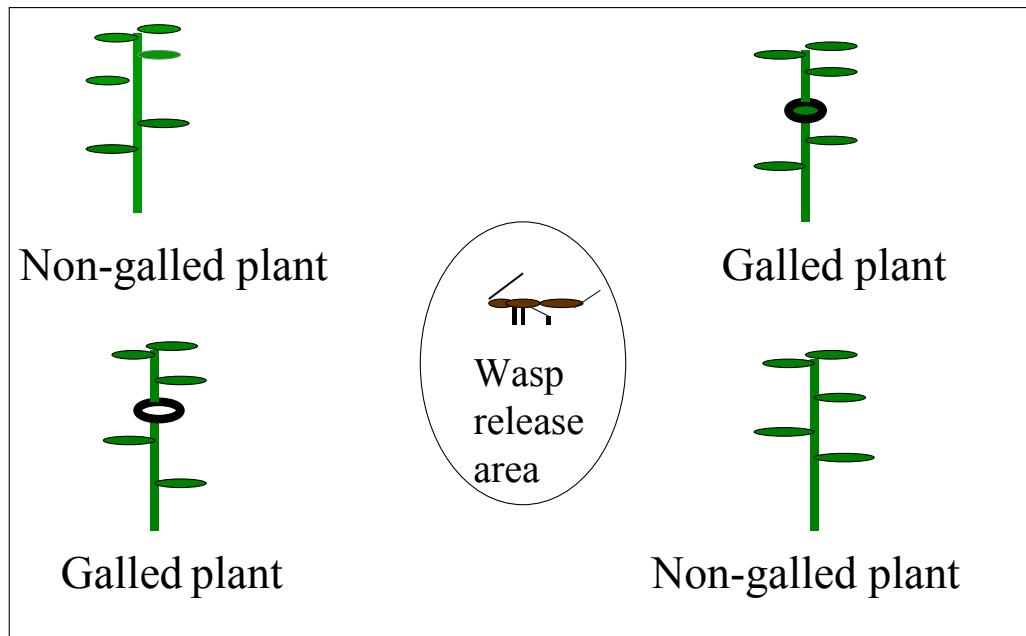


FIG. 29. Arrangement of galled and non-galled pamakani plants for exposure to *P. concolor* in Hawaii.

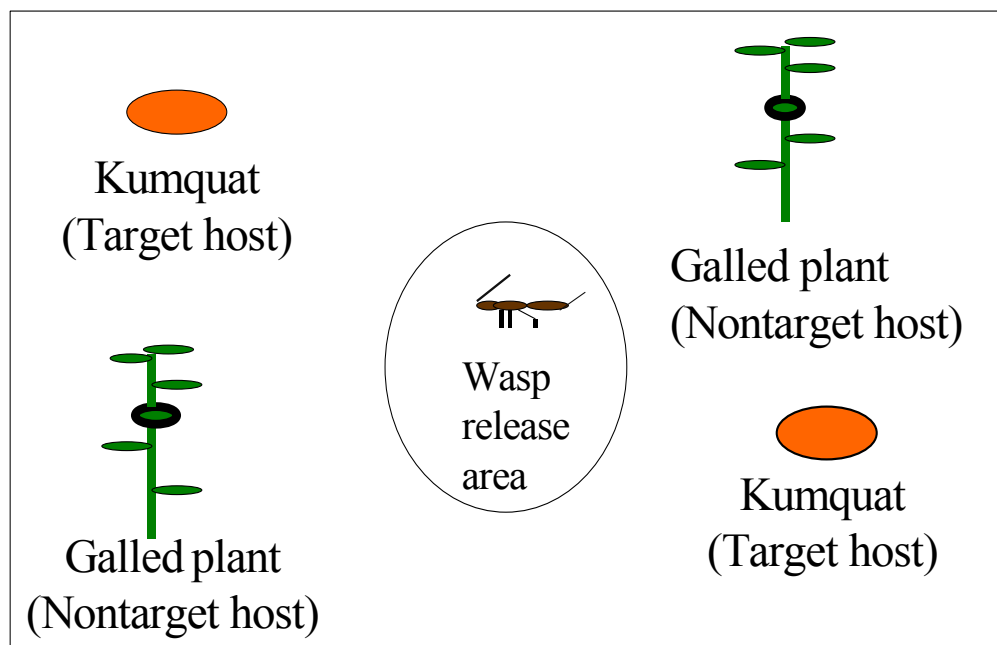


FIG. 30. Arrangement of artificially infested kumquats and field collected galled pamakani plants for exposure to *P. concolor* in Hawaii.

APPENDIX B**TABLES**

TABLE 1Tephritid Parasitoids Collected from Sub-Saharan Africa 1913-1982.^a**Superfamily Ichneumonoidea**

Family Braconidae

Subfamily Opiinae

Diachasmimorpha carinata (Szépligeti, 1910)*Diachasmimorpha fullawayi* (Silvestri, 1913)*Fopius bevisi* (Brues, 1926)*Fopius caudatus* (Szépligeti, 1913)*Fopius desideratus* (Bridwell, 1919)*Fopius silvestrii* (Wharton, 1987)*Psytalia concolor* (Szépligeti, 1910)*Psytalia inconsueta* (Silvestri, 1913)*Psytalia humilis* (Silvestri, 1913)*Psytalia lounsburyi* (Silvestri, 1913)*Psytalia perproxima* (Silvestri, 1913)*Psytalia phaeostigma* (Wilkinson, 1927)*Utetes africanus* (Szépligeti, 1910)

Subfamily Braconinae

Bracon celer Szépligeti, 1913**Superfamily Chalcidoidea**

Family Chalcididae

Dirhinus ehrhorni Silvestri, 1913*Dirhinus giffardii* Silvestri, 1913

Family Eulophidae

Tetrastichus giffardianus Silvestri, 1915*Tetrastichus giffardii* Silvestri, 1913*Tetrastichus oxyurus* Silvestri, 1913

Family Eupelmidae

Eupelmus afer Silvestri, 1914*Eupelmus urozonus* Dalman, 1820

Family Pteromalidae

Halticoptera daci Silvestri, 1914*Halticoptera* sp.*Pachycrepoideus vindemmiae* (Rondani, 1875)*Spalangia* sp.**Superfamily Cynipoidea**

Family Figitidae

Subfamily Eucoilinae

Ganaspis sp.**Superfamily Proctotrupeoidea**

Family Diapriidae

Coptera magnificus (Nixon, 1930)*Coptera robustior* (Silvestri, 1913)*Coptera silvestrii* (Kieffer, 1913)*Trichopria capensis* Kieffer, 1913^a Species are listed by their current valid name as used by Wharton, 1997; Yoder and Wharton, 2002; LaSalle and Wharton, 2002

TABLE 2

Mean Number of Tephritid Fruit Flies per Kilogram (\pm SD) for Each Fly Species at Each Site in Kenya.

	Ruiru	Rurima	Koru
<i>C. capitata</i>	199.46 \pm 187.77	137.08 \pm 160.82	39.81 \pm 47.37
<i>C. fasciventris</i>	52.31 \pm 31.01	33.66 \pm 54.01	88.78 \pm 75.63
<i>C. anonae</i>	0	0	0.32 \pm 1.04
<i>T. coffeae</i>	4.30 \pm 4.68	1.39 \pm 3.10	33.16 \pm 56.83
<i>Total</i>	256.07 \pm 67.27	172.13 \pm 163.49	162.07 \pm 122.84

TABLE 3

Infestation Rates of Coffee Cherries Determined by Dissection. Infestation per Kilogram Based on Counts of Total Number of Cherries per Sample.

	Sample Date	Larvae per cherry	# Cherries Dissected	Cherries per kg	Larvae per kg
RUIRU	4.viii.1998	2.03 ± 1.42	34	na ^a	1320.5 - 1612.2 ^b
	19.viii.1999	0.87 ± 1.14	100	794.2 ± 14.35	691.0
	9.ix.1999	0.92	na ^a	650.5 ± 0.71	598.5
	21.x.1999	1.4	na ^a	793.8 ± 13.2	1111.3
RURIMA	18.viii.1999	1.39 ± 1.36	36	792.3	1101.3
	15.ix.1999	1.42 ± 1.27	100	650.5	923.7
	28.x.1999	1.2	na ^a	na ^a	780.6 - 950.8 ^b
KORU	1.ix.1999	0.6	na ^a	na ^a	375.2 - 376.5 ^b
	30.ix.1999	0.42 ± 0.65	100	627.5 ± 20.5	263.6
	15.x.1999	na	na ^a	625.3	na ^a
	11.xi.1999	1.0	na ^a	na ^a	625.3 - 627.5 ^b
	30.xi.1999	1.54	na ^a	na ^a	963.0 - 966.4 ^b

^aData not available for this date.

^bLarvae per cherry multiplied by the highest and lowest numbers of cherries per kg for each coffee collection site to estimate infestation levels for dates when data not available for number of cherries per kilogram.

TABLE 4
Known Occurrence of Parasitoids by Collection Site.

Parasitoid species	Collection Site		
	Ruiru	Rurima	Koru
<i>Bracon celer</i>	X	X	X
<i>Coptera robustior</i>	X		
<i>Diachasmimorpha fullawayi</i>	X	X	X
<i>Fopius caudatus</i>			X
<i>Fopius ceratitivorius</i>	X	X	
<i>Fopius silvestrii</i>			X
<i>Psytalia concolor</i>	X	X	X
<i>Psytalia cosyrae</i>		X	
<i>Tetrastichus giffardianus</i>	X	X	X
<i>Tetrastichus giffardii</i>			X

TABLE 5

Mean Number of Koinobiont Parasitoids per Kilogram (\pm SD) for Each Parasitoid Species at Each Site in Kenya.

	Ruiru	Rurima	Koru
<i>Fopius caudatus</i>	0	0	13.77 \pm 23.24
<i>Fopius ceratitivorus</i>	7.30 \pm 11.13	11.54 \pm 23.88	0
<i>Fopius silvestrii</i>	0	0	0.08 \pm 0.3
<i>Psytalia concolor</i>	6.43 \pm 7.35	3.54 \pm 4.99	4.60 \pm 11.80
<i>Diachasmimorpha fullawayi</i>	22.77 \pm 42.75	0 ^a	0.74 \pm 1.18
<i>Tetrastichus spp.</i> ^b	0.30 \pm 0.61	0	5.04 \pm 15.59
<i>Total</i>	36.80 \pm 58.44	15.08 \pm 26.00	24.21 \pm 35

^a *Diachasmimorpha fullawayi* occurs at Rurima, but data on this species were not kept for this site.

^b It is likely there is more *Tetrastichus spp.* than those recorded as emergence cages were not built to hold very small chalcidoids.

TABLE 6

Number of *C. capitata* and *C. fasciventris* Larvae Produced per Week at ICIPE, July 27 – October 15, 1999.

Fly Species	Week of	Production of Larvae per Week	Week of	Production of Larvae per Week
<i>C. capitata</i>	July 26	100	July 26	140
	August 2	137	August 2	200
	August 9	57	August 9	30
	August 16 ^a	329	August 16 ^a	188
	August 23	421	August 23	373
	August 30	1374	August 30	1594
	September 6	1800	September 6	997
	September 13	1571	September 13	971
	September 20	2666	September 20	1026
	September 27	2964	September 27	1521
	October 4	1672	October 4	2155
October 11	2279	October 11	2593	

^a Yeast Hydrolysate and Banana units added to production system of host flies at ICIPE.

TABLE 7

Increase in Numbers of Fruit Fly Larvae Reared by ICIPE Staff for Maintenance of Fly Colonies After Addition of Yeast Hydrolysate to the Adult Fruit Fly Diet and Bananas as Oviposition Units.

Time Period	Mean Production of Larvae Each Week ^a	
	<i>Ceratitis capitata</i>	<i>Ceratitis fasciventris</i>
Before I arrived in Nairobi - 1999	98	123
One week after I arrived (August 16 th) through end of first week in September 1999	708	718
September 1999	2250	1129
October 1999 (through 15 th)	1975	2374
Production increase	2000% greater production than when I arrived	1900% greater production than when I arrived

^a Number of larvae produced were recorded every Monday, Wednesday and Friday.

TABLE 8

Confirmed and Hypothesized Guild Structure of Parasitoids in Coffee in Kenya.^a

Host Stage Used for Majority of Parasitoid Development	Guild	Designation	Number of Species in Coffee in Kenya	
			Confirmed	Hypothesized
EGG	Egg	E1	0	If present, very rare or seasonal
LARVA	Early-Larval Endo	L1	0	0
	Late-Larval Endo	L2	0	0
	Larval Ecto	L3	1	More may be present, but probably rare
PREPUPA	Egg-Prepupal Endo	Pre1	2	0
	Larval-Prepupal Endo Early Attackers	Pre2A	1	(1) <i>F. silvestrii</i>
	Larval-Prepupal Endo Late Attackers	Pre2B	5	(1) <i>Tetrastichus giffardii</i> . More may be present, but are rare
	Larval-Prepupal Ecto	Pre3	0	0
	Prepupal-Pupal Ecto	Pre4	0	0
PUPA	Larval-Pupal Endo	P1	0	0
	Pupal Endo	P2	1	More must exist, but will take extensive sampling to recover
ADULT	Larval-Adult Endo	A1	0	0
	Adult Endo	A2	0	0

^a Guilds defined following Mills (1994) paper on resource utilization.

TABLE 9

Guild Structure Characterization of Parasitoids from Coffee in Kenya.

Parasitoid Species	Guild(s) ^a	Host Stage Attacked				Host Stage Killed				Mode of Parasitization			
		Egg	Early Larval	Late-Larval	Pupal	Egg	Larval	Prepupal	Pupal	Endo	Ecto	Koinobiont	Idiobiont
<i>F. caudatus</i>	Pre1, Pre2A	Confirmed	Probable	no	no	no	no	Confirmed	no	X	-	X	-
<i>F. ceratitivorus</i>	Pre1, Pre2A	Confirmed	Confirmed	no	no	no	no	Confirmed	no	X	-	X	-
<i>F. silvestrii</i>	Pre1, Pre2A	no	Probable	no	no	no	no	Confirmed	no	X	-	X	-
<i>P. concolor</i>	Pre2B	no	no	Confirmed	no	no	no	Confirmed	no	X	-	X	-
<i>P. cosyrae</i>	Pre2B	no	no	Confirmed	no	no	no	Confirmed	no	X	-	X	-
<i>D. fullawayi</i>	Pre2B	no	no	Confirmed	no	no	no	Confirmed	no	X	-	X	-
<i>T. giffardianus</i>	Pre2B	no	no	Confirmed	no	no	no	Confirmed	no	X	-	X	-
<i>T. giffardii</i>	Pre2B	no	no	Probable	no	no	no	Confirmed	no	X	-	X	-
<i>B. celer</i>	L3	no	no	Confirmed	no	no	Confirmed	no	no	-	X	-	X
<i>C. robustior</i>	P2	no	no	no	Confirmed	no	no	no	Confirmed	X	-	-	X

^a See Table 8 for Designation of Guilds.

TABLE 10

Key to the Puparia Collected from Coffee in Kenya.

1. Mouthhook of cephalopharyngeal skeleton with preapical tooth present (Figs. 22 and 26).
2
- Mouthhook of cephalopharyngeal skeleton with preapical tooth absent (Figs. 24 and 25).
3
2. Preapical tooth conspicuous and large at 400x (Fig. 22).
 *C. fasciventris* (*C. anonae* rarely)
- Preapical tooth (if present) very small at 400x (Fig. 26).
 some *C. capitata*
3. S. D. F. nearly always (90%) less than 0.4 and B. B. D. F. at least 1.3 (Fig. 27).
 most *C. capitata*
- S. D. F. nearly always (93%) greater than 0.6 and B. B. D. F. less than 1.1 (Fig. 28).
 *T. coffeae*

TABLE 11Host Fly/Parasitoid Associations from Coffee in Kenya.^a

Parasitoid Species	Number of Puparia Examined	Host Flies		
		<i>C. capitata</i>	<i>C. fasciventris</i>	<i>T. coffeae</i>
<i>F. caudatus</i>	46	Yes	Yes	Yes
<i>F. ceratitivorus</i>	14	Yes	No	Unknown
<i>P. concolor</i>	22	Yes	No	Yes
<i>D. fullawayi</i>	3	Yes	Unknown	Yes
<i>Tetrastichus spp.</i>	17	Yes	Unknown	Yes
<i>Coptera robustior</i>	6	Yes	Unknown	Unknown

^aBased on examination of field-collected puparia from which parasitoids emerged.**TABLE 12***Psytalia concolor* Egg Viability by Fruit Fly Species.

Viable eggs	Fruit Fly Species	Eggs encapsulated or melanized
Eggs viable	<i>Ceratitis capitata</i>	
Eggs viable	<i>Ceratitis cosyra</i>	
	<i>Ceratitis rosa</i>	All eggs encapsulated
	<i>Ceratitis fasciventris</i>	All eggs encapsulated
	<i>Ceratitis anonae</i>	All eggs encapsulated
	<i>Bactrocera cucurbitae</i>	All eggs encapsulated
	<i>Bactrocera dorsalis</i>	All eggs encapsulated
Eggs viable	<i>Bactrocera latifrons</i>	

TABLE 13*Psytalia concolor* Host Suitability by Tephritid Species.

Suitable for development (Progeny produced)	Fruit Fly Species	Unsuitable for development (no progeny produced)
X	<i>Ceratitis capitata</i>	
X	<i>Ceratitis cosyra</i>	
	<i>Ceratitis rosa</i>	X
	<i>Ceratitis fasciventris</i>	X
	<i>Ceratitis anonae</i>	X
	<i>Bactrocera cucurbitae</i>	X
	<i>Bactrocera dorsalis</i>	X
X	<i>Bactrocera latifrons</i>	

TABLE 14Dissections of Larvae Exposed to Female *Psytalia concolor* in Hawaii.

	Number of Larvae Dissected	Number of Larvae with Parasitoid Eggs	Number of Parasitoid Eggs Encapsulated	Number of Parasitoid Eggs Melanized	Number of Parasitoid Eggs Neither Encapsulated nor Melanized
<i>Bactrocera dorsalis</i>	31	12	12	10	0
<i>Bactrocera cucurbitae</i>	61	17	17	14	0
<i>Bactrocera latifrons</i>	50	41	0	0	41
<i>Ceratitis capitata</i>	32	20	0	0	20

TABLE 15*Psytalia concolor*: Host Suitability of the Four Invasive Frugivorous Tephritids in Hawaii.

	<i>C. capitata</i>	<i>B. latifrons</i>	<i>B. cucurbitae</i>	<i>B. dorsalis</i>
Number of Trials	7	12	5	4
Mean Number of Larvae Exposed per Trial	23.57 ± 10.36 ^a	23.08 ± 7.79	29.60 ± 0.89	30.0 ± 0
Mean Number of <i>P. concolor</i> Emerged	15.86 ± 9.39	13.17 ± 5.2	0	0
Mean Percent Host Flies Emerged After Exposure to <i>P. concolor</i>	0.95 ± 1.63	0	24.9 ± 25.47	6.67 ± 7.20
Mean Percent Emergence of <i>P. concolor</i>	63.29 ± 20.20	60.08 ± 19.30	0	0
Mean Percent Emergence of Fly Control Group	92.8 ± 8.23	89.55 ± 10.53	88.20 ± 11.52	86.75 ± 4.72

^aM ± SD**TABLE 16**

Tephritid Fruit Fly Phylogeny:
 Not an Adequate Predictor of *P. concolor*'s Ability to Overcome the Host's Immune System and Produce Offspring.

Genus	Subgenus	Fly Species	Encapsulation present
<i>Ceratitis</i>	<i>Ceratitis</i>	<i>C. capitata</i>	Never
	<i>Ceratalaspis</i>	<i>C. cosyra</i>	Never
	<i>Pterandrus</i>	<i>C. rosa</i>	Yes, always, in all three species
		<i>C. anonae</i>	
<i>Bactrocera</i>	<i>Bactrocera</i>	<i>B. dorsalis</i>	Always in <i>B. dorsalis</i> ,
		<i>B. latifrons</i>	Never in <i>B. latifrons</i>
	<i>Zeugodacus</i>	<i>B. cucurbitae</i>	Yes, always

TABLE 17

Nontarget Exposures of *P. concolor* to Galled and Nongalled Plants of Maui Pamakani, *Ageretina adenophora*.

Experiment	Year	Landings on Galled Plants	Landings on Nongalled Plants	<i>df</i>	G or χ^2	P	
Galled vs. Nongalled	2000	3	5	1	G = 0.5053	0.25	n.s. ^a
	2001	14	18	1	$\chi^2 = 0.50$	0.4795	n.s.

^a Not significant at $p \leq 0.05$.

TABLE 18

Nontarget Exposures of *P. concolor* to Galled and Nongalled Plants of Maui Pamakani, *Ageretina adenophora* Including Parasitoids Not Landing on Either Type of Plant.

Landings on Galled Plants	Landings on Nongalled Plants	Landings on Neither Galled or Nongalled Plants	<i>df</i>	G or χ^2	P	
14	18	18	2	$\chi^2 = 0.656$	0.7204	n.s. ^a

^a Not significant at $p \leq 0.05$.

TABLE 19

Nontarget Exposures of *P. concolor* to Galled Plants of Maui Pamakani, *Ageretina adenophora* and Kumquats Infested with Medfly.

Experiment	Year	Landings on Galled Plants	Landings on Kumquats	<i>df</i>	G or χ^2	P
Galled vs. Kumquat	2000	1	7	1	G = 5.062	0.025
	2001	3	20	1	$\chi^2 = 12.565$	0.0004

TABLE 20

Nontarget Exposures of *P. concolor* to Galled Plants of Maui Pamakani, *Ageretina adenophora* and Kumquats Infested with Medfly Including Parasitoids Not Landing on Either Choice.

Landings on Galled Plants	Landings on Kumquats	Landings on Neither Galled Plants or Kumquats	<i>df</i>	G or χ^2	P
3	20	22	2	$\chi^2 = 14.53$	0.0007

TABLE 21

Comparison of Numbers of *P. concolor* Oviposit in Kumquats in a No Choice Setting Versus Kumquats in a Choice Setting with Pamakani Galls.

Oviposition in Kumquats in No Choice Setting	Oviposition in Kumquats in a Choice Setting	<i>df</i>	G or χ^2	P
12	11	1	$\chi^2 = 0.8348$	0.8348 n.s. ^a

^a Not significant at $p \leq 0.05$.

TABLE 22

Control Exposure of *P. concolor* to an Oviposition Dish Compared to Oviposition in Kumquats in a No Choice Setting.

Experiment	Response to Kumquats	Response to Dish	<i>df</i>	G or χ^2	P
Kumquats vs. Oviposition Dish	20	45	1	$\chi^2 = 9.615$	0.0019

TABLE 23

Control Exposure of *P. concolor* to an Oviposition Dish Compared to Oviposition in Kumquats in a No Choice Setting.

Experiment	Oviposition in Kumquats	Oviposition in Dish	<i>df</i>	G or χ^2	P
Kumquats vs. Oviposition Dish	12	45	1	$\chi^2 = 19.105$	<0.0001

APPENDIX C

COFFEE COLLECTION DATA

Ruiru Coffee Collections – Overall Sample and Fly Collection Data

Collection Month		6	7	8	8	9	10	11	12	4	5	6	7	SUM	MEAN	STANDARD DEVIATION
Sampling Date		8.vi.98	6.vii.98	24.viii.98	28.viii.98	10.ix.98	7.x.98	4.xi.98	1.xii.98	13.iv.99	10.v.99	16.vi.99	14.vii.99			
Part of Sample for Which Data is Available		Kenya, Hawaii	Kenya	Kenya, Hawaii	Kenya, Hawaii	Kenya, Hawaii	Kenya, Hawaii	Kenya, Hawaii	Kenya, Hawaii	Hawaii	Hawaii	Hawaii	Kenya			
Kgs Collected	Sample Total	12	6.5	8	8	10	12	10	20	2.5	2.5	2.5	2.5	96.5	8.04	± 5.28
Puparia Collected	Sample Total	2630	833	1856	2331	1752	2207	827	1766	219	2010	1380	1675	19486	1623.83	± 699.88
Puparia Uncollected	Sample Total	318	298	613	1710	1095	1014	490	756	135	1057	537	1186	9209	767.42	± 456.46
Puparia Eclosed	Sample Total	2312	535	1243	621	657	1193	337	1010	84	953	843	489	10277	856.42	± 573.23
Adjusted by																
- % Emergence	All Flies	212.54	126.48	226.75	264.46	160.01	181.50	77.76	85.30	87.60	617.55	450.51	582.31	3072.76	256.06	± 67.27
- Kgs Collected																
Adjusted by																
- % Emergence	All Flies	2550.42	822.10	1813.96	2115.66	1600.08	2177.97	777.65	1706.08	219	1543.88	1126.26	1455.78	17908.84	1492.40	± 664.58
Uncollected	All Flies	308.42	270.15	598.96	1530.66	1003.08	1000.97	460.65	720.08	135	798.57	405.78	1030.78	8263.09	688.59	± 404.72
Dead on Eclosion	All Flies	102	43	87	92	59	20	11	0	0	12	51	0	477	39.75	± 38.22
Eclosed	All Flies	2140	485	1128	493	538	1157	306	986	84	720	637	425	9099	758.25	± 542.45
Each Fly Species	<i>C. capitata</i>	163.13	89.45	115.15	168.06	135.03	124.61	27.98	31.02	75.09	590.11	342.30	531.62	2393.53	199.46	± 187.77
Adjusted by																
- % Emergence	<i>C. fasciventris</i>	40.69	36.51	97.77	89.32	23.14	56.53	47.64	52.51	12.51	16.30	106.79	47.96	627.68	52.31	± 31.01
- Kgs Collected	<i>C. anonae</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	± 0.00
	<i>T. coffeae</i>	8.71	0.52	13.83	7.07	1.84	0.36	2.15	1.77	0	11.15	1.41	2.74	51.55	4.30	± 4.68
Each Fly Species	<i>C. capitata</i>	1957.55	581.40	921.21	1344.50	1350.26	1495.30	279.80	620.42	187.71	1475.27	855.75	1329.04	12398.21	1033.18	± 543.50
Adjusted by																
- % Emergence	<i>C. fasciventris</i>	488.32	237.31	782.12	714.57	231.44	678.40	476.39	1050.26	31.29	40.74	266.98	119.89	5117.71	426.48	± 325.66
	<i>C. anonae</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	± 0.00
	<i>T. coffeae</i>	104.56	3.39	110.62	56.59	18.37	4.27	21.45	35.39	0	27.88	3.54	6.85	392.92	32.74	± 38.64
Number of Each Fly Species	<i>C. capitata</i>	313.55	238.40	345.21	1023.50	899.26	708.30	169.80	217.42	115.71	787.27	371.75	941.04	6131.21	510.93	± 334.75
Uncollected + Dead on Eclosion	<i>C. fasciventris</i>	80.32	97.31	296.12	549.57	151.44	310.40	287.39	481.26	19.29	21.74	115.98	84.89	2495.71	207.98	± 176.22
(Calculated #)	<i>C. anonae</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	± 0.00
	<i>T. coffeae</i>	16.56	1.39	44.62	49.59	11.37	2.27	14.45	21.39	0	14.88	1.54	4.85	182.92	15.24	± 16.48
Number of Each Fly Species Eclosed from Sample	<i>C. capitata</i>	1644	343	576	321	451	787	110	403	72	688	484	388	6267	522.25	± 409.31
	<i>C. fasciventris</i>	408	140	486	165	80	368	189	569	12	19	151	35	2622	218.50	± 191.27
	<i>C. anonae</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	± 0.00
	<i>T. coffeae</i>	88	2	66	7	7	2	7	14	0	13	2	2	210	17.50	± 28.53

Rurima Coffee Collections – Overall Sample and Fly Collection Data

Collection Month		11	12	4	5	6	7	11	12	2	3	5				
Sample Date		19.xi.97	17.xii.97	17.iv.98	6.v.98	11.vi.98	13.vii.98	18.xi.98	8.xii.98	26.ii.99	26.iii.99	11.v.99				
Parts of Sample from Which Data is Available		Kenya, Hawaii	Kenya, Hawaii	Kenya	Kenya, Hawaii	Kenya, Hawaii	Kenya	Kenya	Kenya	Kenya, Hawaii	Kenya, Hawaii	Kenya		SUM	MEAN	STANDARD DEVIATION
Kgs Collected	Sample Total	10	10	5	10	12	3	5	4	6	5	0.5		75.5	6.41	± 3.58
Puparia Collected	Sample Total	561	3652	388	292	2891	684	54	422	1721	2796	50		13511.00	1228.27	± 1306.95
Puparia Eclosed	Sample Total	435	1784	333	224	2434	399	44	363	814	2172	40		9042.00	822.00	± 877.47
Puparia Unclosed	Sample Total	126	1868	55	68	457	285	10	59	907	624	10		4469.00	406.27	± 565.96
Adjusted by																
- % Emergence	Fly Total	36.24	277.91	76.90	28.66	240.82	202.86	10.80	81.38	283.93	558.94	95.00		1893.43	172.13	± 163.49
- Kgs Collected																
Adjusted by																
- % Emergence	Fly Total	362.43	2779.11	384.50	286.57	2889.86	608.57	54.00	325.51	1703.56	2794.68	47.50		12236.30	1112.39	± 1183.99
Unclosed	Fly Total	77.43	1425.11	54.50	66.57	456.86	253.57	10	45.51	894.56	623.68	9.50		3917.30	356.12	± 459.76
Dead on Eclosion	Fly Total	30	64	27	5	162	59	3	55	45	100	8		558	50.73	± 47.21
Eclosed	Fly Total	255	1290	303	215	2271	296	41	225	764	2071	30		7761	705.55	± 810.09
Each Fly Species	<i>C. capitata</i>	36.24	213.87	34.52	21.05	69.86	111.02	10.80	81.02	283.93	550.57	95.00		1507.87	137.08	± 160.82
Adjusted by	<i>C. fasciventris</i>	0	54.36	42.38	7.47	170.85	87.04	0	0.36	0	7.83	0		370.30	33.66	± 54.01
- % Emergence	<i>C. anonae</i>	0	0	0	0	0	0	0	0	0	0	0		0	0	± 0.00
- Kgs Collected	<i>T. coffeae</i>	0	9.68	0	0.14	0.11	4.80	0	0	0	0.54	0		15.26	1.39	± 3.10
	<i>C. capitata</i>	362.43	2138.67	172.58	210.55	838.30	333.07	54.00	324.06	1703.56	2752.83	47.50		8937.54	812.50	± 944.54
Each Fly Species	<i>C. fasciventris</i>	0	543.62	211.92	74.66	2050.26	261.11	0	1.45	0	39.17	0		3182.19	289.29	± 608.17
Adjusted by	<i>C. anonae</i>	0	0	0	0	0	0	0	0	0	0	0		0	0	± 0.00
- % Emergence	<i>T. coffeae</i>	0	96.82	0	1.36	1.30	14.39	0	0	0	2.68	0		116.56	10.60	± 28.91
Number of Each Fly	<i>C. capitata</i>	107.43	1153.67	36.58	52.55	179.30	171.07	13.00	100.06	939.56	712.83	17.50		3483.54	316.69	± 413.06
Species Unclosed +	<i>C. fasciventris</i>	0	283.62	44.92	18.66	439.26	134.11	0	0.45	0	10.17	0		931.19	84.65	± 146.63
Dead on Eclosion	<i>C. anonae</i>	0	0	0	0	0	0	0	0	0	0	0		0	0	± 0.00
(From Calculations)	<i>T. coffeae</i>	0	51.82	0	0.36	0.30	7.39	0	0	0	0.68	0		60.56	5.51	± 15.52
Number of Each Fly	<i>C. capitata</i>	255.00	985.00	136.00	158.00	659.00	162.00	41.00	224.00	764.00	2040	30		5454.00	495.82	± 602.75
Species Eclosed from	<i>C. fasciventris</i>	0	260	167.00	56.00	1611.00	127.00	0	1.00	0	29.00	0		2251.00	204.64	± 474.37
Sample	<i>C. anonae</i>	0	0	0	0	0	0	0	0	0	0	0		0	0	± 0.00
	<i>T. coffeae</i>	0	45.00	0	1.00	1.00	7.00	0	0	0	2.00	0		56.00	5.09	± 13.40

Koru Coffee Collections – Overall Sample and Fly Collection Data

Collection Month		11	12	1	4	5	6	7	8	9	10	11	12	3	4	5	6	SUM	AVERAGE	STANDARD DEVIATION
Sample Date		21.xi.97	9.xii.97	23.i.98	9.iv.98	15.v.98	25.vi.98	24.vii.98	26.viii.98	23.ix.98	28.x.98	27.xi.98	2.xii.98	5.iii.99	12.iv.99	21.v.99	21.vi.99			
Part of Sample for Which Data is Available		Kenya, Hawaii	Kenya	Hawaii	Kenya, Hawaii	Kenya, Hawaii	Kenya, Hawaii	Kenya, Hawaii	Kenya	Kenya, Hawaii	Kenya	Kenya, Hawaii	Kenya, Hawaii	Kenya, Hawaii	Kenya, Hawaii	Hawaii	Hawaii			
Kgs Collected	Sample Total	20	10	10	20	16	12	12	5	10	5	10	10	12	10	5	5	172	10.75	± 4.75
Puparia Collected	Sample Total	358	1441	500	1675	3143	4874	4738	2805	1765	62	771	964	1933	2535	714	1005	29283	1830.19	± 1459.30
Puparia Unclosed	Sample Total	50	817	419	649	1846	3585	3646	2163	1191	32	280	624	1064	839	572	714	18491	1155.69	± 1116.21
Puparia Eclosed	Sample Total	307	624	83	996	1297	1287	1092	642	574	30	491	340	869	1728	152	301	10813	675.81	± 493.97
Adjusted by - % Emergence - Kgs Collected	Fly Total	16.85	142.95	44.15	79.90	169.57	307.69	298	481.48	168.98	11.99	75.26	92.37	161.08	242.02	128.61	172.65	2593.53	162.10	± 122.87
Adjusted by - % Emergence Unclosed	Fly Total	336.93	1429.45	441.52	1597.98	2713.14	3692.26	3576.01	2407.41	1689.77	59.93	752.59	923.66	1933	2420.18	643.03	863.26	25480.13	1592.51	± 1123.58
Dead on Eclosion	Fly Total	0	9	0	37	90	131	37	94	75	4	9	33	56	47	0	0	622	38.88	± 40.74
Eclosed	Fly Total	291	610	73	930	1033	849	842	457	475	25	473	292	813	1592	135	256	9146	572	± 417.86
Each Fly Species Adjusted by - % Emergence - Kgs Collected	<i>C. capitata</i>	2.10	17.34	0.60	33.11	80.89	11.54	87.64	43.20	13.46	1.92	3.89	6.89	11.72	165.44	53.35	103.86	636.93	39.81	± 47.37
	<i>C. fusciventris</i>	9.79	114.82	7.26	40.58	51.63	89.51	131.57	315.02	149.36	9.11	61.10	84.39	147.99	76.21	69.54	62.72	1420.60	88.79	± 75.63
	<i>C. anonae</i>	0	0	0	0.08	0.25	0	0	4.21	0	0	0.38	0	0	0.24	0	0	5.17	0.32	± 1.04
	<i>T. coffeae</i>	4.96	10.78	36.29	6.13	36.80	206.64	78.79	119.05	6.15	0.96	9.89	1.08	1.38	0.12	5.72	6.07	530.82	33.18	± 56.88
Each Fly Species Adjusted by - % Emergence	<i>C. capitata</i>	41.93	173.41	6.05	662.21	1294.17	138.43	1051.68	215.98	134.63	9.59	38.86	68.89	140.63	1654.41	266.74	519.30	6416.91	401.06	± 507.65
	<i>C. fusciventris</i>	195.70	1148.25	72.58	811.50	826.07	1074.14	1578.83	1575.09	1493.60	45.55	611.04	843.94	1775.85	762.13	347.71	313.60	13475.59	842.22	± 562.92
	<i>C. anonae</i>	0	0	0	1.60	4.07	0	0	21.07	0	0	3.80	0	0	2.43	0	0	32.97	2.06	± 5.26
	<i>T. coffeae</i>	99.30	107.79	362.89	122.67	588.83	2479.69	945.50	595.27	61.53	4.79	98.88	10.83	16.52	1.22	28.58	30.35	5554.65	347.17	± 632.43
Number of Each Fly Species Unclosed + Dead on Eclosion (From Calculations)	<i>C. capitata</i>	6.93	99.41	5.05	287.21	809.17	107.43	750.68	174.98	94.63	5.59	13.86	48.89	80.63	600.41	210.74	365.30	3660.91	228.81	± 267.29
	<i>C. fusciventris</i>	34.70	658.25	60.58	328.50	503.07	829.14	1250.83	1276.09	1078.60	26.55	195.04	574.94	1030.85	227.13	274.71	220.60	8569.59	535.60	± 436.72
	<i>C. anonae</i>	0	0	0	0.60	2.07	0	0	17.07	0	0	0.80	0	0	0.43	0	0	20.97	1.31	± 4.24
	<i>T. coffeae</i>	4.30	61.79	302.89	51.67	365.83	1906.69	732.50	482.27	41.53	2.79	69.88	7.83	8.52	0.22	22.58	21.35	4082.65	255.17	± 490.08
Number of Each Fly Species Eclosed from Sample	<i>C. capitata</i>	35	74	1	375	485	31	301	41	40	4	25	20	60	1054	56	154	2756	172.25	± 276.29
	<i>C. fusciventris</i>	161	490	12	483	323	245	328	299	415	19	416	269	745	535	73	93	4906	306.63	± 204.89
	<i>C. anonae</i>	0	0	0	1	2	0	0	4	0	0	3	0	0	2	0	0	12	0.75	± 1.29
	<i>T. coffeae</i>	95	46	60	71	223	573	213	113	20	2	29	3	8	1	6	9	1472	92	± 146.51

Ruiru Coffee Collections – Parasitoid Collection Data

Collection Month		6	7	8	8	9	10	11	12	4	5	6	7	SUM	MEAN	STANDARD DEVIATION
Sample Date		8.vi.98	6.vii.98	24.viii.98	28.viii.98	10.ix.98	7.x.98	4.xi.98	1.xii.98	13.iv.99	10.v.99	16.vi.99	14.vii.99			
% Parasitism	Sample Total	3.0%	1.3%	2.3%	9.2%	8.7%	1.3%	6.0%	3.4%	0%	23.2%	18.4%	13.1%	-	7.5%	± 0.07
Adjusted by																
- % Emergence	Parasitoid Total	6.63	1.68	5.25	26.92	15.19	2.42	4.94	3	0	186.45	101.49	87.69	441.65	36.80	± 58.44
- Kgs Collected																
Adjusted by	Parasitoid Total	79.58	10.90	42.04	215.34	151.92	29.03	49.35	59.92	0	466.12	253.74	219.22	1577.16	131.43	± 137.17
- % Emergence																
Uneclosed	Parasitoid Total	9.58	3.90	14.04	179.34	91.92	13.03	29.35	35.92	0	245.12	98.74	155.22	876.16	73.01	± 81.57
Eclosed	Parasitoid Total	70	7	28	36	60	16	20	24	0	221	155	64	701	58.42	± 65.97
Each Parasitoid	<i>F. caudatus</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	± 0.00
Species Adjusted	<i>F. ceratitivorus</i>	1.23	0.24	0.95	6.95	6.87	0.71	0.54	0.75	0	37.12	14.41	17.81	87.58	7.30	± 11.13
by	<i>F. silvestrii</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	± 0.00
- % Emergence	<i>P. concolor</i>	1.71	1.44	4.12	15.24	7.44	1.28	3.24	0.34	0	16.03	22.26	4.11	77.21	6.43	± 7.35
- Kgs Collected	<i>D. fullawayi</i>	1.70	0	0.18	4.73	0.89	0.43	0.19	1.23	0	133.30	64.83	65.77	273.24	22.77	± 42.75
	<i>Tetrastichus spp.</i>	1.99	0	0	0	0	0	0.96	0.68	0	0	0	0	3.63	0.30	± 0.62
Each Parasitoid	<i>F. caudatus</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	± 0.00
Species Adjusted	<i>F. ceratitivorus</i>	14.82	1.56	7.63	55.56	68.67	8.53	5.40	14.98	0	92.80	36.01	44.53	350.50	29.21	± 30.25
by	<i>F. silvestrii</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	± 0.00
- % Emergence	<i>P. concolor</i>	20.48	9.34	32.94	121.96	74.39	15.37	32.41	6.83	0	40.07	55.66	10.28	419.74	34.98	± 34.98
	<i>D. fullawayi</i>	20.45	0	1.46	37.82	8.86	5.12	1.92	24.53	0	333.24	162.06	164.42	759.89	63.32	± 103.92
	<i>Tetrastichus spp.</i>	23.84	0	0	0	0	0	9.62	13.58	0	0	0	0	47.04	3.92	± 7.75
Number of Each	<i>F. caudatus</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	± 0.00
Parasitoid	<i>F. ceratitivorus</i>	1.82	0.56	2.63	48.56	37.67	3.53	3.40	8.98	0	48.80	14.01	31.53	201.50	16.79	± 19.26
Species	<i>F. silvestrii</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	± 0.00
Uneclosed	<i>P. concolor</i>	2.48	3.34	10.94	102.96	49.39	7.37	20.41	3.83	0	21.07	21.66	7.28	250.74	20.89	± 29.22
(From Calculations)	<i>D. fullawayi</i>	2.45	0	0.46	27.82	4.86	2.12	0.92	14.53	0	175.24	63.06	116.42	407.89	33.99	± 56.73
	<i>Tetrastichus spp.</i>	2.84	0	0	0	0	0	4.62	8.58	0	0	0	0	16.04	1.34	± 2.72
Number of Each	<i>F. caudatus</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	± 0.00
Parasitoid	<i>F. ceratitivorus</i>	13	1	5	7	31	5	2	6	0	44	22	13	149	12.42	± 13.51
Species Eclosed	<i>F. silvestrii</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	± 0.00
from Sample	<i>P. concolor</i>	18	6	22	19	25	8	12	3	0	19	34	3	169	14.08	± 10.41
	<i>D. fullawayi</i>	18	0	1	10	4	3	1	10	0	158	99	48	352	29.33	± 49.81
	<i>Tetrastichus spp.</i>	21	0	0	0	0	0	5	5	0	0	0	0	31	2.58	± 6.11

Rurima Coffee Collections – Parasitoid Collection Data

Collection Month		11	12	4	5	6	7	11	12	2	3	5	SUM	MEAN	STANDARD DEVIATION
Sample Date		19.xi.97	17.xii.97	17.iv.98	6.v.98	11.vi.98	13.vii.98	18.xi.98	8.xii.98	26.ii.99	26.iii.99	11.v.99			
% Parasitism	Sample Total	35.4 %	23.9%	0.9%	1.9%	0.04%	11.0%	0%	22.9%	1.0%	0.1%	5.0%	-	9.28%	± 0.12
Adjusted by															
- % Emergence	Parasitoid Total	19.86	87.29	0.70	0.54	0.10	25.14	0	24.12	2.91	0.26	5.00	165.92	15.08	± 26.00
- Kg Collected															
Adjusted by															
- % Emergence	Parasitoid Total	198.57	872.89	3.50	5.43	1.14	75.43	0	96.49	17.44	1.32	2.50	1274.70	115.88	± 258.65
Unclosed	Parasitoid Total	48.57	442.89	0.50	1.43	0.14	31.43	0	13.49	12.44	0.32	0.50	551.70	50.15	± 131.21
Eclosed	Parasitoid Total	150	430	3	4	1	44	0	83	5	1	2	723	65.73	± 129.91
Each Parasitoid	<i>F. caudatus</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	± 0
Species Adjusted	<i>F. ceratitivorus</i>	15.67	81.27	0.70	0.41	0	13.71	0	10.17	0	0	5.00	126.93	11.54	± 23.88
by	<i>F. silvestrii</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	± 0
- % Emergence	<i>P. concolor</i>	4.19	6.02	0	0.14	0.10	11.43	0	13.95	2.91	0.26	0	38.99	3.54	± 4.99
- Kgs Collected	<i>D. fullawayi</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	± 0.00
	<i>Tetrastichus spp.</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	± 0.00
Each Parasitoid	<i>F. caudatus</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	± 0.00
Species Adjusted	<i>F. ceratitivorus</i>	156.68	812.69	3.50	4.07	0	41.14	0	40.69	0	0	2.50	1061.27	96.48	± 242.08
by	<i>F. silvestrii</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	± 0.00
- % Emergence	<i>P. concolor</i>	41.89	60.19	0	1.36	1.14	34.29	0	55.80	17.44	1.32	0	213.43	19.40	± 24.16
	<i>D. fullawayi</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	± 0.00
	<i>Tetrastichus spp.</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	± 0.00
Numbers of Each	<i>F. caudatus</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	± 0.00
Parasitoid Species	<i>F. ceratitivorus</i>	38.68	409.69	0.50	1.07	0	17.14	0	5.69	0	0	0.50	473.27	43.02	± 122.19
Unclosed	<i>F. silvestrii</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	± 0.00
(from Calculations)	<i>P. concolor</i>	9.89	33.19	0	0.36	0.14	14.29	0	7.80	12.44	0.32	0	78.43	7.13	± 10.30
	<i>D. fullawayi</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	± 0.00
	<i>Tetrastichus spp.</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	± 0.00
Number of Each	<i>F. caudatus</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	± 0.00
Parasitoid Species	<i>F. ceratitivorus</i>	118	403	3	3	0	24	0	35	0	0	2	588	53.45	± 121.13
Eclosed from	<i>F. silvestrii</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	± 0.00
Sample	<i>P. concolor</i>	32	27	0	1	1	20	0	48	5	1	0	135	12.27	± 16.82
	<i>D. fullawayi</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	± 0.00
	<i>Tetrastichus spp.</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	± 0.00

Koru Coffee Collections – Parasitoid Collection Data

Collection Month		11	12	1	4	5	6	7	8	9	10	11	12	3	4	5	6	SUM	MEAN	STANDARD DEVIATION
Sample Date		21.xi.97	9.xii.97	23.i.98	9.iv.98	15.v.98	25.vi.98	24.vii.98	26.viii.98	23.ix.98	28.x.98	27.xi.98	2.xii.98	5.iii.99	12.iv.99	21.v.99	21.vi.99			
% Parasitism	Sample Total	5.6%	0.8%	12.1%	2.8%	13.7%	24.2%	24.5%	14.7%	4.3%	3.3%	2.4%	4.8%	0	5.8%	11.3%	15.1%	-	9.0%	± 0.08
Adjusted by - % Emergence - Kg Collected	Parasitoid Total	1	1.15	6.05	2.35	26.87	98.31	96.83	79.52	7.52	0.41	1.84	4.03	0	14.68	16.19	30.35	387.12	24.20	± 34.87
Adjusted by - % Emergence	Parasitoid Total	20.07	11.55	60.48	47.02	429.86	1179.74	1161.99	397.59	75.23	2.07	18.41	40.34	0	146.82	80.97	151.74	3823.87	238.99	± 386.10
Unclosed	Parasitoid Total	4.07	6.55	50.48	18.02	255.86	872.74	948.99	306.59	51.23	1.07	9.41	25.34	0	57.82	63.97	106.74	2778.87	173.68	± 301.57
Eclosed	Parasitoid Total	16	5	10	29	174	307	213	91	24	1	9	15	0	89	17	45	1045	65.31	± 90.28
Each Parasitoid Species	<i>F. caudatus</i>	0.62	0	4.39	2.35	24.40	94.26	8.69	18.35	5.29	0.41	1.48	3.72	0	12.52	16.19	27.65	220.33	13.77	± 23.25
	<i>F. ceratitivorus</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	± 0.00
Adjusted by - % Emergence - Kgs Collected	<i>F. silvestrii</i>	0	1.20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.20	0.08	± 0.30
	<i>P. concolor</i>	0.07	0	0	0	0.67	1.62	25.69	41.94	2.23	0	0.37	0	0	1.02	0	0	73.62	4.60	± 11.80
	<i>D. fullawayi</i>	0	0	1.46	0	0.45	1.52	0.60	4.37	0	0	0	0.31	0	1.14	0	2.02	11.87	0.74	± 1.18
	<i>Tetrastichus spp.</i>	0.31	0	0	0	1.35	0.91	61.84	14.86	0	0	0	0	0	0	0	1.42	80.69	5.04	± 15.58
Each Parasitoid Species	<i>F. caudatus</i>	12.42	0	43.86	47.02	390.39	1131.11	104.34	91.75	52.90	2.07	14.76	37.24	0	125.18	80.97	138.26	2272.25	142.02	± 280.29
	<i>F. ceratitivorus</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	± 0.00
Adjusted by - % Emergence	<i>F. silvestrii</i>	0	12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	12	0.75	± 3
	<i>P. concolor</i>	1.38	0	0	0	10.76	19.48	308.30	209.72	22.34	0	3.66	0	0	10.24	0	0	585.87	36.62	± 88.95
	<i>D. fullawayi</i>	0	0	14.62	0	7.18	18.22	7.25	21.85	0	0	0	3.09	0	11.40	0	10.12	93.72	5.86	± 7.39
	<i>Tetrastichus spp.</i>	6.27	0	0	0	21.53	10.93	742.10	74.28	0	0	0	0	0	0	0	7.12	862.22	53.89	± 184.47
Number of Each Parasitoid Species	<i>F. caudatus</i>	3.42	0	37.86	18.02	238.39	837.11	84.34	70.75	35.90	1.07	6.76	23.24	0	47.18	63.97	97.26	1565.25	97.83	± 205.91
	<i>F. ceratitivorus</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	± 0.00
Unclosed (From Calculations)	<i>F. silvestrii</i>	0	7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7	0.44	± 1.75
	<i>P. concolor</i>	0.38	0	0	0	4.76	14.48	238.30	161.72	15.34	0	2.66	0	0	5.24	0	0	442.87	27.68	± 68.89
	<i>D. fullawayi</i>	0	0	12.62	0	3.18	13.22	6.25	16.85	0	0	0	2.09	0	5.40	0	7.12	66.72	4.17	± 5.61
	<i>Tetrastichus spp.</i>	0.27	0	0	0	9.53	7.93	620.10	57.28	0	0	0	0	0	0	0	7.12	702.22	43.89	± 154.31
Number of Each Parasitoid Species	<i>F. caudatus</i>	9	0	6	29	152	294	20	21	17	1	8	14	0	78	17	41	707	44.19	± 76.90
	<i>F. ceratitivorus</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	± 0.00
	<i>F. silvestrii</i>	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0.31	± 1.25
Eclosed from Sample	<i>P. concolor</i>	1	0	0	0	6	5	70	48	7	0	1	0	0	5	0	0	143	8.94	± 20.10
	<i>D. fullawayi</i>	0	0	2	0	4	5	1	5	0	0	0	1	0	6	0	3	27	1.69	± 2.18
	<i>Tetrastichus spp.</i>	6	0	0	0	12	3	122	17	0	0	0	0	0	0	0	3	163	19.18	± 30.23

APPENDIX D

TIMELINE OF PROJECT AND TRAVELS OF M. TROSTLE

Timeline of Project and Travels of M. Trostle

1998

January 1998-

Two weeks in Honolulu, HI

January 1998 – June 1998

Washington D.C.

June 1998 - August 1998

College Station, TX

August 1998 – September 1998

Nairobi, Kenya (ICIPE)

September 1998-December 1998

College Station, TX

1999

January 1999-August 1999

Honolulu, HI

August 1999-December 1999

Nairobi, Kenya (ICIPE)

2000

January 2000-March 2000

Honolulu, HI

March 2000-December 2000

College Station, TX

2001

January 2001

Two weeks in Guatemala

January 2001-May 2001

College Station, TX

May 2001-August 2001

Honolulu, HI

2001-2004

August 2001 – Present

College Station, TX

VITA

Marcia Katherine Trostle Duke

PERSONAL DATA:

Born in Arlington, Texas on Tuesday, 21 May 1974. Married to Thadeus Allen Duke. Personal name, Marcia Katherine Trostle Duke. Mother, Susan Katherine Ferguson Trostle; father, Mark Robert Trostle.

EDUCATION:

Texas A&M University, College Station, TX. B.S. Entomology, Magna Cum Laude, 1997.
Texas A&M University, College Station, TX. Ph.D. Entomology, 2005. Major Professor - R. A. Wharton.

PUBLICATIONS:

- Lopez, M., Sivinski, J., Rendon, P., Holler, T., Bloem, K., Copeland, R., Trostle, M., Aluja, M. 2003. Colonization of *Fopius ceratitivorus*, a newly discovered African egg-pupal parasitoid (Hymenoptera: Braconidae) of *Ceratitis capitata* (Diptera: Tephritidae). *Florida Entomologist* **86**, 53-60.
- Wharton, R. A., Trostle, M. K., Messing, R. H., Copeland, R. S., Kimani-Njogu, S. W., Lux, S., Overholt, W. A., Mohamed, S., Sivinski, J. 2000. Parasitoids of medfly, *Ceratitis capitata*, and related tephritids in Kenyan coffee: a predominantly koinobiont assemblage. *Bulletin of Entomological Research* **90**, 517-526.
- Kimani-Njogu, S. W., M.K. Trostle, R.A. Wharton, J.B. Woolley and A. Raspi. 2001. Biosystematics of the *Psytalia concolor* species complex (Hymenoptera: Braconidae: Opiinae): The identity of populations attacking *Ceratitis capitata* (Diptera: Tephritidae) in coffee in Kenya. *Biological Control*, **20**, 167-174.
- Trostle M., Carrejo N. S., Mercado I., Wharton R. A. 1999. Two new species of Phaenocarpa Foerster (Hymenoptera : Braconidae : Alysiinae) from South America. *Proceedings of the Entomological Society of Washington* **101**, 197- 207.

HONORS AND AWARDS

- Department of Entomology Biological Control Assistantship
- Who's Who of American Colleges and Universities
- Texas A&M College of Agriculture and Life Sciences Outstanding Senior Merit Award
- Texas A&M Department of Entomology Outstanding Undergraduate Award

INTERNSHIPS

National Cotton Council Policy Internship
Texas A&M University Agriculture and Natural Resources Policy Internship

PERMANENT ADDRESS:

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