EVALUATION OF ZINGERONE AS A MALE LURE OF MELON FLY,

BACTROCERA CUCURBITAE COQUILLET (DIPTERA: TEPHRITIDAE): BEHAVIORAL MODIFICATIONS
UPON CONSUMPTION AND FIELD ATTRACTION

A THESIS SUBMITTED TO THE GRADUATE DIVISION OF THE
UNIVERSITY OF HAWAII AT MANOA IN PARTIAL FULFILLMENT OF THE
REQUIREMENTS FOR THE DEGREE OF

MASTERS OF SCIENCE
IN
ENTOMOLOGY
MAY 2016

By
Jess R. Inskeep

Thesis Committee:
Helen Spafford, Chairperson
Todd Shelly
Roger Vargas

Keywords: melon fly, Bactrocera cucurbitae, male lures, zingerone
ABSTRACT

The males of many Bactrocera species (Diptera: Tephritidae) are strongly attracted to, and feed upon, a few natural compounds (and their synthetic analogs), commonly termed ‘male lures’ or ‘lures’. Research has shown that lure feeding enables males to mate more successfully with conspecific females, and females are more attracted to the pheromones of lure-fed males. However, it has long been unclear exactly why lure feeding enhances male mating success. Furthermore, little is known about a recently discovered natural lure, zingerone which is found in the flowers of some orchids (Bulbophyllum spp.). Zingerone has unique properties relative to other male lures, and has garnered recent attention due to its potential as an attractant for ecological surveys and fruit fly pest management. This thesis investigates zingerone and its role as a modifier of melon fly (Bactrocera cucurbitae) behavior and biology when consumed by males. Furthermore, this thesis assesses zingerone as an attractant of the melon fly in the field. These two areas of study are detailed in chapters 2 and 3 of this thesis.

As outlined in chapter two, zingerone was fed to male B. cucurbitae to test if they mated more successfully. Furthermore, select life parameters of zingerone-fed males and females mated to zingerone-fed males were tested, including virgin male longevity, female fecundity, egg viability, and mated female longevity. Feeding on zingerone was found to enhance male mating success, but only for one day after zingerone feeding. Virgin males that fed on zingerone did not die at different rates than un-fed males, and females mated to zingerone-fed males had the same levels of fecundity and egg hatch as compared to females mated to un-fed males. These findings differ from those obtained in a previous study that observed higher fecundity associated with females mating with zingerone-fed males. However, different species were tested and therefore the effects of lure feeding on female fecundity may be species-specific. Interestingly females mated to zingerone-fed males had higher mortality when compared to females mated to un-fed males, suggesting females that mate with zingerone-fed males may incur a negative effect on fitness.
As outlined in chapter three, the attraction of wild melon flies to zingerone is assessed through field trapping using zingerone, cue-lure, and mixtures of the two lures at a local farm on the island of Oahu, Hawaii. This experiment is the first to mix two different male lures that are both attractive to the same fruit fly species, and the potential for synergistic or inhibitory effects of mixing were examined. It was observed that traps containing zingerone caught fewer flies than cue-lure, and there was only an additive effect when the two were mixed. These results suggest zingerone is unlikely to be effective at detecting or managing pestiferous populations of melon fly.

The studies conducted in this thesis expand upon previous research on the phenomenon of lure attraction by male Bactrocera fruit flies. Results suggest that the fitness benefit of lure feeding may not be obvious, and future research should focus on other fitness parameters across more species. Nevertheless, lures have tremendous value to fruit fly management, and elucidating questions surrounding the evolved trait of lure attraction may be uncover new ways to use lures for management purposes. Zingerone itself does not appear to be highly attractive to B. cucurbitae, and does not appear to interfere with cue-lure when mixed together as a single attractant of the melon fly. Thus, this observation suggests that zingerone, when added to cue-lure in the same wick, does not reduce the trap capture of B. cucurbitae assuming the quantity of cue-lure is sufficient for management purposes.
ACKNOWLEDGEMENTS

This thesis was completed with generous support from my advisor, Dr. Helen Spafford, and committee members Drs. Todd Shelly and Roger Vargas. I am grateful for their insightful comments and critical thinking. To Dr. Todd Shelly, I am further grateful for many productive discussions during the beginning of the research, and for his willingness to lend male lures and assistance. I am thankful to Dr. Luc Leblanc for sharing his broad knowledge of fruit flies, and his enthusiasm to engage in long conversations which he often filled with practical advice. I am also thankful to Dr. Nicanor Liquido for his insightful comments and generous support.

I extend my gratitude to Mike McKenney for providing lab flies from the USDA facility in Hilo, Hawaii. My sincere thanks to Susan Migita and Neil Ho for allowing me to run studies at their farms. I also thank Gee Sarat for his assistance in the field. To Dr. Will Haines I am thankful for his willingness to share limited space in the UH rearing room, which was often filled with fruit flies.

For encouraging me to pursue entomology, I must also thank Drs. Mark Schwarzlaender and Edward Bechinski at the University of Idaho for their imparting enthusiasm. Most importantly I am grateful for the endless support of my parents, Russell and Kristina Inskeep, as well the support of my many family members.

Finally, this thesis would not have been possible without funding through a cooperative agreement between USDA-APHIS and the University of Hawaii at Manoa, and I am grateful for that.
# TABLE OF CONTENTS

ABSTRACT ............................................................................................................................................ 1

ACKNOWLEDGEMENTS .................................................................................................................. 3

TABLE OF CONTENTS ..................................................................................................................... 4

LIST OF FIGURES ............................................................................................................................ 6

CHAPTER 1.0: REVIEW OF LITERATURE ......................................................................................... 7

1.1 General introduction .................................................................................................................. 8

1.1.1 Methyl Eugenol .................................................................................................................. 11

1.1.2 Raspberry ketone/cue-lure ............................................................................................... 14

1.1.3 Zingerone .......................................................................................................................... 15

1.2 Male lures as modifiers of mating behavior ............................................................................. 17

1.3 Research objectives .................................................................................................................. 19

1.3 References .................................................................................................................................. 22

CHAPTER 2.0: EFFECTS OF ZINGERONE FEEDING ON THE MATING BEHAVIOR AND FITNESS OF BACTROCERA CUCURBITAE (DIPTERA: TEPHRITIDAE) ........................................................................................................... 27

2.1 Introduction .................................................................................................................................. 28

2.2. Materials and Methods .......................................................................................................... 31

2.2.1 Mating trials ....................................................................................................................... 31

2.2.2 Female fecundity, egg hatch, female mortality, and male mortality ................................. 33

2.3. Results ..................................................................................................................................... 37

2.3.1 Mating trials ....................................................................................................................... 37

2.3.2 Female fecundity, egg viability, female mortality, male mortality ..................................... 37

2.4 Discussion .................................................................................................................................. 38

2.5 Conclusion ............................................................................................................................... 42

2.6 Figures ...................................................................................................................................... 44

2.7 References .................................................................................................................................. 49

CHAPTER 3.0: ATTRACTION OF MALE MELON FLIES, BACTROCERA CUCURBITAE (DIPTERA: TEPHRITIDAE), TO MIXTURES OF CUE-LURE AND ZINGERONE IN THE FIELD ......................................................................................................................... 52

3.1 Introduction .................................................................................................................................. 53

3.2 Methods ...................................................................................................................................... 56
LIST OF FIGURES

**Figure 1.1.** Molecular structure of zingerone, methyl eugenol, raspberry ketone, and cue-lure. .......... 10

**Figure 1.2.** Fate of ingested lures (methyl eugenol and zingerone) by *Bactrocera dorsalis*. ................. 13

**Figure 1.3.** Fate of ingested lures (raspberry ketone, cue-lure, and zingerone) by *Bactrocera cucurbitae* and *B. tryoni*. ................................................................................................................. 15

**Figure 2.1.** Number of copulations by zingerone-fed males (treated), and unfed males (control). .......... 44

**Figure 2.2.** Cumulative number of eggs laid/female/week (mean ±SE) of females mated to zingerone-fed males (treated) or unfed males (control). ........................................................ ........................................... 45

**Figure 2.3.** The percent hatch rate (mean ±SE) of eggs laid by females mated to zingerone-fed males (treated) or unfed males (control). ................................................................................................. 46

**Figure 2.4.** The percent of females surviving (mean ±SE) after mating with zingerone-fed males (treated) or unfed males (control). .................................................................................................. 47

**Figure 2.5.** Cumulative percent (mean ±SE) survival of zingerone-fed males (treated) or unfed males (control) over 10 weeks. ......................................................................................................... 48

**Figure 3.1.** Experimental layout of Ho farms in Kahuku, Oahu, Hawaii. ...................................................... 63

**Figure 3.2.** Cumulative capture of *B. cucurbitae* in traps baited with lures (mean ±SE) over six weeks. .. 64

**Figure 3.3.** Mean (±SE) capture of *B. cucurbitae* by host and week. ............................................................ 65
CHAPTER 1.0: REVIEW OF LITERATURE
1.1 General introduction

*Bactrocera* (Diptera: Tephritidae) is a large genus containing over 500 described species arranged in roughly 28 subgenera (Drew and Hancock, 2000; Smith *et al*., 2003). The majority of these species are monophagous or oligophagous, preferring non-economic hosts in the plant families Asclepiadaceae, Passifloraceae and Cucurbitaceae, however, approximately 70 species are polyphagous and feed on a wide array of commercial fruits and vegetables spanning many plant families (White and Elson-Harris, 1992; Metcalf and Metcalf, 1992). These fruit flies frequently invade warm Mediterranean and tropical habitats and are considered among the most destructive agricultural pests globally. While few studies have attempted to quantify the economic damages associated with fruit flies, losses in the state of California could approach $1 billion dollars annually from the establishment of invasive fruit flies (White and Ellis-Harris, 1992), and California currently spends over $15 million annually in fruit fly prevention measures (Vargas *et al*., 2010). In Australia, where some pest species exist and others could become established, costs associated with fruit flies have been estimated as high as $100 million annually (White and Ellis-Harris, 1992). However, fruit fly damages are arguably most severe in developing nations due to international trade restrictions on fresh produce exportation and food security threats from a general lack of management resources (Vayssieres *et al*., 2014; Vayssieres *et al*., 2009; McGregor, 2000; Clarke *et al*., 2005).

Fruit flies are damaging agricultural pests, although certain management tools can be highly effective. Past research efforts focused on behavior have led to the discovery of certain phenylpropanoids and phenylbutanoids that are attractive to male *Bactrocera*. These compounds, tremendously valuable to fruit fly management, are commonly referred to as ‘male lures’ or simply ‘lures’. *Bactrocera* populations are monitored and/or suppressed by combining these lures with insecticides to create attract-and-kill baits.
Two common and effective methods of employing male lures are male annihilation technique (MAT) and surveillance monitoring. In MAT, male lure traps are deployed to attract-and-kill males in such high numbers that virgin females do not encounter males to mate with, or must fly away from the trapping area to mate. MAT can be highly effective at reducing fruit fly populations, and is an important component of many area-wide fruit fly management programs (Vargas et al., 2014; Vargas et al., 2007; Vargas et al., 2010; Ali et al., 2010; Leblanc et al., 2013). MAT has even been used to successfully eradicate fruit fly populations from various regions around the world (Steiner et al., 1965; Steiner et al., 1970; Koyama et al., 1984; Cantrell et al., 2002). The other application for male lures, surveillance trapping, is useful in regions where fruit flies populations do not exist but are nevertheless at risk of invasion (i.e. when conditions favor a reproducing population) (Jang et al., 2014; Meats, 2014). Surveillance programs utilize many traps spread out over a wide area to give early-warning of incipient fruit fly populations, which allows for the possibility of successful eradication.

Many male lures have been synthesized (Metcalf et al., 1983; Metcalf et al., 1986), but some occur naturally (Tan, 2009; Tan and Nishida, 2012). Of the naturally occurring lures, methyl eugenol (ME), raspberry ketone (RK), and zingerone (Fig. 1.1) are believed to be most common, and these three lures are produced in the flowers of some orchid species (Orchidaceae) (Tan, 2009). Male flies have been observed consuming lures on the flowers of these orchids and performing the role of pollinator while feeding (Tan, 2009).

In fruit fly management only two males lures are widely used, ME and cue-lure (CL, the more volatile synthetic analog of RK [Fig. 1.1]). It is mentionable that species which respond to ME are not responsive to RK/CL, and likewise species that respond to RK/CL are not responsive to ME. The majority of Bactrocera species show attraction to either ME or RK/CL, although roughly 33% of the described species are non-responsive to either of these two lures (Raghu, 2004). Zingerone is not widely used in fruit fly management, because it is generally considered to be a much weaker attractant compared to
ME and CL. But zingerone nevertheless contains properties that suggest it may be useful in some situations. Zingerone is useful in ecological surveys, as it has been used to attract previously undocumented species in the *Dacus* and *Bactrocera* genera (Fay, 2012; Dominiak *et al*., 2015; Royer, 2015). Furthermore, zingerone feeding appears to alter the survival and metabolism of *B. tryoni* males (Kumaran *et al*., 2013; Kumaran *et al*., 2014b) and the survival and fecundity of mated *B. tryoni* females (Kumaran *et al*., 2013), suggesting this lure may, at least for this particular species, provide a fitness benefit and may be useful in explaining the evolved phenomenon of lure attraction.

**Figure 1.1.** Molecular structure of common male lures of *Bactrocera* fruit flies, zingerone (A), methyl eugenol (B), raspberry ketone (C), and cue-lure (D).
1.1.1 Methyl Eugenol

The discovery of male lures began with Frank Milburn Howlett, a British chemist and entomologist working at the Pusa Research Institute in New Delhi, India (Verghese et al., 2013). While searching for female fruit fly attractants, Howlett (1912) discovered that male *Dacus zonatus* (=*Bactrocera zonatus*) and *D. ferrugineus* (=*Bactrocera dorsalis*) were strongly attracted to oil of citronella, a mosquito repellant. Howlett (1915) later identified the attractive component of citronella as a single compound, methyl eugenol (ME). Howlett’s discovery is widely regarded as a hallmark in the field of chemical ecology and predated the discovery of the first insect sex pheromone, bombykol, by over 40 years. However, it wasn’t until *B. dorsalis* invaded Hawaii in 1946 that ME was first employed for control of this pest, in the form of ME combined with insecticide to create attract-and-kill baits (Steiner, 1952).

Today we know that ME-responding fruit flies are attracted to citronella grass, *Cymbopogon nardus* L. (of which oil of citronella is derived) and ME is produced naturally in >450 known plants, spanning 80 plant families and 38 plant orders (Tan and Nishida, 2012). It is unknown why so many plants produce ME, although it is believed to deter herbivores and pathogens and/or prevent cellular damage from foreign compounds (Tan and Nishida, 2012). However, in the case of some orchids (*Bulbophyllum* spp.), ME is produced as a floral attractant of fruit flies, which are the sole pollinators of these plants (Tan, 2009; Tan et al., 2006).

Upon consumption of ME by a male *B. dorsalis*, the lure is converted into E-coniferyl alcohol (CF) and 2-allyl-4,5-dimethoxy cinnamyl alcohol (DCA) in the crop before the two metabolites are transported, via the hemolymph, to the rectal gland (the storage site of the male sex pheromone) (Fig. 1.2) (Nishida et al., 1988). Other ME-responding species also metabolize ME into CF and DCA (in varying ratios of the two), and incorporate these metabolites into their pheromone blends (Tan and Nishida,
It is widely assumed that these metabolites enhance the attractiveness of the male pheromone blend, which is released to attract females for mating.

Attraction of *B. dorsalis* males to ME is strongest following sexual maturation, although immature males will occasionally feed on ME sources. But immature males which consume ME will accumulate very few ME metabolites in their rectal glands, and subsequently their pheromone blend is not believed to be as attractive as mature adults who have fed on ME (Wong *et al*., 1989; Shelly *et al*., 2008). Contrary to *B. dorsalis*, another ME responding species, *Bactrocera carambolae*, exhibits attraction to ME only after sexual maturity has been reached (Wee and Tan, 2000).

Male *B. dorsalis* mate more frequently with conspecific females after feeding on ME, and a mating advantage has been observed for over 35 days following a one-time consumption of ME (Shelly and Dewire, 1994). However, after feeding on a large amount of ME males often appeared sluggish and mate significantly less often on the same day of ME consumption (Shelly and Dewire, 1994), even though ME metabolites are transported to the rectal gland as soon as 15 minutes after lure feeding (Wee *et al*., 2007). Interestingly, Shelly (2000b) did not observe a reduced mating effect on the same day after ME consumption when flies were allowed to feed upon a natural ME-producing flower, and males gained a mating advantage for 21 days after a one-time feeding from this floral ME source. Thus it is possible that natural or artificial sources of ME have varying effects on male fruit flies or that the quantity of ME consumed is an important factor in predicting mating success.

The enhanced mating benefit afforded to ME-fed males is well documented (Shelly, 2010). However, it is unknown if the female fly receives a benefit from mating with a lure-fed male. *B. dorsalis* females do not lay more eggs over an 8-week period, and egg survival rates and development times do not differ with respect to whether or not the male mating partner had fed on ME or not (Shelly 2000a). However, feeding on ME appears to make male *B. dorsalis* wing-fan (a courtship behavior before
mating) more often (Shelly and Dewire, 1994), a behavior also observed in cue-lure fed *B. cucurbitae* (Shelly and Villalobos, 1995).

Finally, while many lures have been discovered to attract *B. dorsalis* males (Metcalf *et al.*, 1983; Metcalf *et al.*, 1986; Tan and Nishida, 2000), only ME has been explored as a mating attractiveness enhancer for this species. Exploration into other male lures may uncover differing effects on mating behavior.

**Figure 1.2.** Fate of ingested lures (methyl eugenol and zingerone) by the ME-responding species, *Bactrocera dorsalis*. 
1.1.2 Raspberry ketone/cue-lure

In contrast to the discovery of ME, an effective male lure of RK/CL responding flies was discovered by systematically screening thousands of chemicals when searching for an effective lure of the melon fly, *Bactrocera cucurbitae*, in Hawaii. From these tests, anisyl acetone was discovered to be effective (Barthel *et al*., 1957), which was later supplanted by the discovery of a more attractive compound, cue-lure (Beroza *et al*., 1960). Cue-lure (CL) is not found in nature, but quickly hydrolyzes into raspberry ketone immediately after consumption by the male (Nishida *et al*., 1990; Tan and Nishida, 1995). Raspberry ketone (RK) is found in 17 different plant genera (Zorn *et al*., 2003), including raspberries (*Rubus idaeus* L.) (Honkanen *et al*., 1980), strawberries (*Fragaria* spp.) (Vaughn *et al*., 1993), and some orchid species in the genera *Bulbophyllum* and *Dendrobium* (Tan, 2009). Male flies which are attracted to these wild RK-producing orchids are believed to be the sole pollinators of these plants (Tan, 2009). RK/CL responding species are sometimes simply called CL-responders, in reference to the widespread use of cue-lure in fruit fly management. RK itself is an effective lure, however, it volatilizes at a slower rate than cue-lure, making it less attractive (Metcalf and Metcalf, 1992).

*B. cucurbitae* males feed upon RK and CL, and males gain a mating advantage from feeding on RK (Shelly, 2000c) or CL (Shelly and Villalobos, 1995). However, this mating advantage lasts for only 1 day after feeding on either lure (see aforementioned studies). Another RK/CL-responding species, *B. tryoni*, also has a short mating advantage of 3 days after feeding on CL. These examples contrast the >35 day mating advantage that is conferred by ME-feeding in *B. dorsalis* (Shelly and Dewire, 1994). Also, while ME is converted and stored in the rectal gland as two metabolites (CF and DCA), CL or RK are sequestered as RK in the rectal gland of *B. cucurbitae* (Nishida *et al*., 1990) and *B. tryoni* (Tan and Nishida, 1995; Kumaran *et al*., 2014a). In these cases, RK is released as a component of the male pheromone blend (Fig. 1.3).
Figure 1.3. Fate of ingested lures (raspberry ketone, cue-lure, and zingerone) by the RK/CL-responding species, *Bactrocera cucurbitae* and *B. tryoni*.

1.1.3 Zingerone

Zingerone is another naturally occurring male lure that, like ME and RK, is produced in some *Bulbophylum* orchid flowers. Zingerone is the only known naturally occurring compound that attracts both ME and RK/CL responding *Bactrocera* fruit flies (Tan and Nishida, 2000). Furthermore, zingerone is incorporated into the pheromones of both ME and RK/CL responding male flies and improves the attractiveness of the male pheromone blend to conspecific females (Tan and Nishida, 2000; Khoo and
Tan, 2000; Tan and Nishida, 2007). Male *B. dorsalis* which feed on pure zingerone accumulate zingerone and zingerol in the rectal gland at a ratio of 5:95 (Fig. 1.2) (Tan and Nishida, 2000). Likewise, Tan and Nishida (2000) observed female *B. dorsalis* approaching a pure zingerol source in a wind tunnel, and it is therefore assumed that zingerol is attractive to females when incorporated into the pheromone. In the RK/CL responding *B. cucurbitae* and *B. tryoni*, ZN is sequestered unchanged into the rectal gland following consumption by the males (Fig. 1.3) (Tan and Nishida, 2000; Kumaran et al., 2014a). In the only study testing the effects of zingerone-feeding on the mating rate of fruit flies, Kumaran et al. (2013) observed *B. tryoni* males mated more often for 1 day after feeding on zingerone.

Zingerone shares a similar molecular structure to capsaicin (chili pepper), piperine (black pepper) and vanillin (vanilla bean), and expresses a natural pungent spicy taste with a sweet vanilla aroma (Liu and Simon, 1996). Zingerone is not widely distributed in nature and has been recorded from only six *Bulbophyllum* orchid species endemic to Malaysia, Indonesia, Papua New Guinea and Australia (Tan, 2009). Zingerone is commonly referred to as an ‘essence of ginger’ and while it is derived from ginger root, it does not occur in fresh ginger. Instead, fresh ginger rhizomes must be heated or cooked, at which point gingerol (present in fresh ginger) transforms into zingerone. Interestingly, galangal rhizomes (*Zingiberaceae*), a relative of ginger, contain insecticidal properties when exposed to *B. dorsalis* (Sukhirun et al., 2011). The rhizomes can be soaked in ethyl alcohol to extract two compounds (hexane and acetate dichloromethane), and the resulting liquid is sprayed on adult flies to inhibit enzyme production and cause death (Sukhirun et al., 2011).

Zingerone attracts a wide range of *Bactrocera* fruit flies including ME-responders (*Bactrocera cacuminata*, *B. carambolae*, *B. dorsalis*, *B. endiandrae*, *B. indonesiae*, and *B. umbrosa*), RK/CL-responders (*Bactrocera aeruginosa*, *B. alyxiae*, *B. albistragata*, *B. breviaculeus*, *B. bryiniae*, *B. caudata*, *B. cucurbitae*, *B. frauenfeldi*, *B. jarvisi*, *B. neohumeralis*, *B. rufofuscula*, *B. silvicola*, *B. secamoneae*, *B. tau*, and *B. tryoni*), and species non-responsive to either ME or RK/CL (*Bactrocera aurea*, *B. aglaiae*, *B.
Notable among these species is *Bactrocera indonesiae*, a member of the *B. dorsalis* complex which responds to both ME and zingerone yet is more responsive to zingerone relative to other species in the *B. dorsalis* complex (Tan and Nishida, 2007). Tan and Nishida (2007) postulate the enhanced attraction to zingerone could be due to a mutualistic relationship with the zingerone-producing orchid, *Bulbophyllum baileyi* F. Muell., which shares an endemic habitat with *B. indonesiae*. Another notable species, *Bactrocera jarvisi*, is an endemic pest to Queensland, Australia which displays attraction to both CL and zingerone. However, it significantly prefers the later and is the first example of a *Bactrocera* which prefers zingerone over either ME or RK/CL (Fay, 2012). In Australia zingerone could be used as a highly effective lure for the monitoring and control of this species.

*Dacus*, a genus of fruit flies closely related to *Bactrocera*, contains many species that respond to zingerone (Fay, 2012; Dominiak et al., 2015; Royer, 2015; Michael San Jose, personal communication). Zingerone attracts RK/CL responding species (*D. absonifacies*, *D. aequalis*, *D. anaxus*, *D. bellulus*, and *D. secamoneae*), as well as species that do not respond to any other known lure (*D. newmani* and two undescribed species in Royer, 2015) (Fay, 2012; Dominiak et al., 2015; Royer, 2015). When compared to CL, zingerone appears to be highly attractive to many of these *Dacus* species. However there have been few surveys with zingerone, and trapping has been limited to Queensland, Australia (Fay, 2012), Sydney, Australia (Dominiak et al., 2015; Royer, 2015), and Vietnam (Michael San Jose, personal communication).

### 1.2 Male lures as modifiers of mating behavior

Male lures offer no caloric or nutritional value to male fruit flies and are not classified as a ‘food source’ in the strict sense (Tan, 2009; Teck et al., 2011; Tan and Nishida, 2007). Instead, male flies mate
more often with conspecific females after consuming them (Shelly and Dewire, 1994; Shelly and Villalobos, 1995; Kumaran et al., 2013; Orankanok et al., 2011; Wee et al., 2007). This enhanced mating effect is believed to motivate the males to seek out and consume lures in nature.

It remains unclear why lure-fed males mate more often, although many theories have been developed (Metcalf and Metcalf, 1992; Raghu, 2004; Shelly, 2000a; Kumaran et al., 2013). The addition of male lures (either as metabolites or unchanged) increases the attractiveness of the male pheromone to conspecific females (Hee and Tan, 1998; Wee et al., 2007; Khoo and Tan, 2000), and the mating behavior of fruit flies involves female-choice (Sivinski et al., 2000). For these reasons it is widely assumed that lure-fed males mate more often because females preferentially choose these males as mating partners. Nevertheless, it remains unresolved which (if any) fitness benefits a female would receive by mating with a lure-fed male over an un-fed male.

Previous studies on *B. dorsalis* observed no demonstrable reproductive benefit of females mating with lure-fed males (Shelly, 2000a). On the basis of available evidence, Shelly (2000a) proposed that the heightened mating propensity of lure-fed males may be explained by runaway selection (Fisher, 1930), and that females receive no direct reproductive benefit by mating with a lure-fed male over an un-fed male. Instead, by mating with a lure-fed male, a female gains the increased likelihood that her sons will in turn have a greater ability of seek out and consume lures in nature and therefore have an enhanced mating attractiveness to other females, which prefer lure-fed males for the same reason (=‘sexy-sons’ bias in a positive feedback system).

This theory is bolstered by Kumaran and Clarke (2014) who demonstrated that *B. tryoni* males sired by lure-fed males were significantly more attracted to lures than adult males sired by unfed males. These findings suggest multigenerational changes following lure feeding and support a positive feedback theory, which could result in runaway selection. Additionally, Kumaran et al. (2014b) discovered that lure-feeding by male *B. tryoni* induced changes in gene expressions associated with male aggression,
pheromone release, and courtship and mating. Likewise, weight loss and increased mobility was observed in lure-fed males (Kumaran et al., 2014b). Lure-fed males also signal more frequently to nearby females (Shelly and Dewire, 1994; Shelly and Villalobos 1995), again suggesting that lures may make males more active. These finding suggest that females may receive indirect (i.e. ‘sexy-sons’) benefits by mating with lure-fed males.

However, the presence of indirect benefits does not mutually exclude the possibility for direct benefits as well. Recently, Kumaran et al. (2013) discovered that B. tryoni females laid more eggs over their lifetime when mating with lure-fed males. These are the first observations of a direct reproductive benefit associated with lure-feeding in any Bactrocera species. Suggesting that, for this species at least, females and males benefit from male lure-feeding because they generate more offspring. This observation differs from those obtained for B. dorsalis, which has no increased lifetime fecundity associated with ME feeding (Shelly, 2000a). Thus, fecundity associated with lure feeding may differ among Bactrocera species. However, few studies have investigated the evolutionary driver of lure response in Bactrocera males, and further investigations may create a more robust understanding of this phenomenon.

1.3 Research objectives

The research presented in this thesis focuses on the melon fly, Bactrocera cucurbitae. Zingerone attracts male B. cucurbitae (Tan and Nishida, 2000) but the utility of zingerone as a lure for this species needs further investigation. Furthermore, trapping for B. cucurbitae relies on its responsiveness to CL but there is a demonstrated need to evaluate other lures and ways of increasing the probability of capture. This research expands on recent studies investigating the usefulness of zingerone in
understanding the biology of fruit flies, as well as investigations into more effective management via trapping with male lures.

1. To examine whether zingerone consumption by male *B. cucurbitae* conveys an enhanced mating effect with conspecific females in the laboratory. A further aim investigates whether zingerone consumption imparts an effect on the longevity of male *B. cucurbitae* and select life history parameters (=fecundity, egg viability, and longevity) to female *B. cucurbitae* mated with zingerone-fed males.

2. To examine the attraction of wild *B. cucurbitae* males to zingerone, cue-lure, and various mixtures of the two male lures.

Objective 1 is detailed in Chapter 2. A study observed the potential for enhanced mating, and the duration of enhanced mating (in days), conferred to zingerone-fed male *B. cucurbitae*. For this study wild-type male *B. cucurbitae* were mated with wild-type conspecific females. Previous studies have observed an enhanced mating effect on only 1-day after lure feeding when male *B. cucurbitae* fed upon raspberry ketone (Shelly, 2000c) and cue-lure (Shelly and Villalobos, 1995), and a non-effect to mating on the same day of lure feeding (see previous sources). However, the present study is the first to measure the mating benefit conveyed by zingerone in this species, and it is hypothesized that zingerone feeding confers an enhanced mating effect similar to raspberry ketone and cue-lure feeding. A further series of experiments investigates the effects of zingerone feeding on males (=virgin male longevity) and various parameters on females mated to zingerone-fed males (=fecundity, egg viability, and longevity). These flies were derived from a lab colony that is >300 generations removed from the wild. It is hypothesized that higher mortality will be observed in zingerone-fed males and females mated to zingerone-fed males, because these effects have been observed in a separate species, *Bactrocera tryoni*,

20
when zingerone was used in a similar study (see Kumaran et al., 2013). Likewise, it is hypothesized that females mated to zingerone-fed males will lay more eggs, because this effect was observed in the B. tryoni species when females mated with zingerone-fed males (see aforementioned study). Furthermore, the hatch rate of eggs from females mated to lure-fed males has not been observed in B. cucurbitae, and these observations are included. Lure feeding has not been recoded to effect egg hatch rates in other Bactrocera species (Kumaran et al., 2013; Shelly, 2000a), and it is hypothesized that a non-effect is likewise present in B. cucurbitae.

The second objective is evaluated in Chapter 3. A field study investigates zingerone as a lure attractant of wild male B. cucurbitae, including relative attraction to cue-lure and various mixtures of zingerone and cue-lure. Based on previous studies showing that zingerone is attractive to male B. cucurbitae (Tan and Nishida, 2000), it was expected that wild B. cucurbitae male would respond to traps baited with zingerone lures. However zingerone attraction relative to cue-lure is not known for this species. Furthermore, mixing two lures to target one fly species has yet to be studied.
1.3 References


CHAPTER 2.0: EFFECTS OF ZINGERONE FEEDING ON THE MATING BEHAVIOR AND FITNESS OF BACTROCERA CUCURBITAE (DIPTERA: TEPHRITIDAE)
2.1 Introduction

Sexual selection provides a strong evolutionary pressure in a wide variety of animal species. Female-choice and competition among sexually mature males is often involved, whereby the males which display superior qualities are preferred as mating partners by females (Darwin, 1871). Such a sex bias is favored due to a couple limiting factors, 1) the female investment in reproduction (i.e. eggs) requires more resources than the male investment (i.e. sperm), and 2) females are limited in the number of eggs they can produce, whereas males are far less limited in their ability to produce sperm, leading to choosy females. Thus, in female-choice mating systems males may evolve attributes to attract females to mate, and females may perform a kind of ‘cost-benefit analysis’ of mating with a courting male or moving on in search of a more favorable male (while risking death or scarcity of acceptable males in the process).

Examples of sexual selection exist across Tephritidae (Diptera). Male flies of various species form leks and engage in complex courtship behaviors consisting of acoustic, visual and chemical cues, each of which plays a role in attracting conspecific females (Sivinski et al., 2000). In the genus Bactrocera, courtship behaviors are performed by males to attract females (although the courtship behavior in Bactrocera is generally not as elaborate as those evident in many other tephritid genera). However, another aspect of the mating behavior that is prevalent in many, but not all, Bactrocera species is the attraction to, and consumption of, naturally occurring phenylpropanoids and phenylbutanoids by males (Shelly, 2010). These compounds are commonly termed ‘male lures’ or simply ‘lures’. Upon ingestion, the male lure is transported from the crop to the rectal gland (the storage site of the male pheromone) either unchanged or as metabolites. The male lure (or metabolites of the compound) are incorporated into the pheromone and released by the male both to attract females and during the courtship behavior when conspecific females are nearby (Tan et al., 2014). Consuming male lures (or exposure to the volatiles) has been shown to improve the mating success of males in a variety
of *Bactrocera* species (Shelly and Dewire, 1994; Shelly and Villalobos, 1995; Haq *et al*., 2014; Haq *et al*., 2016; Kumaran *et al*., 2013; Orankanok *et al*., 2011; Wee *et al*., 2007) and improve the attractiveness of the male pheromone blend to conspecific females (Hee and Tan, 1998; Wee *et al*., 2007; Khoo and Tan, 2000). It’s likely that the attraction of males to male lures is a result of the garnered benefit of enhanced mating success, however, it remains unclear if females receive direct fitness benefits from mating with lure-fed males.

Past research on lures ingested by male *Bactrocera* flies has focused on methyl eugenol (ME), raspberry ketone (RK), and cue-lure (CL, the synthetic analog of RK) primarily because these lures have tremendous value to the management and monitoring of *Bactrocera* pests across the globe (Tan *et al*., 2014). *Bactrocera* species differ in their response to these lures and are commonly placed into one of three categories: 1) species that respond to ME, 2) species that respond to RK and CL, and 3) species that do not respond to either of these male lures. These categories are widely referenced, but they may not include all known associations between fruit flies and male lures. Zingerone (ZN), another naturally occurring lure of fruit flies, has been found to attract both ME and RK/CL responding species (Tan and Nishida, 2007). Furthermore, ZN is highly attractive to some *Bactrocera* species and this lure has been the subject of many recent research efforts (Fay, 2012; Dominiak *et al*., 2015).

Studies investigating the effects of lure-feeding on the behavior and biology of *Bactrocera* flies have largely focused on *B. tryoni* (RK/CL-responder) and *B. dorsalis* (ME-responder). Kumaran *et al*. (2013) observed that *B. tryoni* females laid more eggs after mating with lure-fed (both CL- and ZN-fed) males. Furthermore, Kumaran *et al*. (2014) discovered that ZN feeding by males induced weight loss, and resulted in the upregulation of genes which are believed to influence aggression, pheromone synthesis, mating, and accessory gland protein synthesis in various *Drosophila* species. These findings support a conventional sexual selection explanation for this species, whereby females mate with lure-fed males because they gain the fitness benefit of increased fecundity. Males in this case benefit from
enhanced mating success in addition to fathering more offspring due to the increased fecundity of their mates. It was further observed by Kumaran et al. (2013) that lure feeding reduced the lifespan of lure-fed males and females mated to these males, and lure-fed males were more likely to mate with multiple females.

In the case of *B. dorsalis*, no direct fitness benefit associated with ME-feeding is evident, as Shelly (2000a) found no difference in the number of eggs laid by wild-type females mated with ME-fed males compared to females mated with control males. For *B. dorsalis*, Shelly (2000a, b) proposed two scenarios that may explain the enhanced mating success of lure-fed males. First, a ‘sensory bias’ may exist in which females preferentially mate with lure-fed males, because male lures trigger a strong pre-existing sensory response that evolved in a different context (e.g. host searching). Second, females may prefer lure-fed males due to a Fisherian runaway selection scenario (Fisher, 1930). In this scenario females receive no direct fitness benefits from mating with lure-fed males. Instead, by mating with a lure-fed male, the female increases the likelihood that her sons will in turn have a greater ability to seek out and consume lures in nature and therefore have enhanced mating success. Presumably foraging for lures increases the possibility of mortality for males, nevertheless the benefit garnered from enhanced mating success likely outweighs the possibility of death. Assuming the fitness cost doesn’t outweigh the mating benefit a runaway selected trait will continue unchecked and lead to an exaggerated trait by the males (Fisher, 1930; Dawkins, 1986). In the case of *B. dorsalis*, females preferentially mating with lure-fed males may reinforce a runaway selection by giving birth to 1) sons that are more likely to respond to and feed on lures due to paternal habits, and 2) daughters that are more likely to mate with lure-fed males due to maternal habits.

The present study expands upon existing work by examining lure feeding and female benefits in *Bactrocera cucurbitae* (melon fly). This test species was chosen due to its abundance in Hawaii, and a general paucity of research relative to other *Bactrocera* pests. The male lure ZN was chosen given the
aforementioned findings of Kumaran et al. (2013, 2014) for B. tryoni. The present study tested fitness parameters associated with ZN feeding by male B. cucurbitae, including female mate choice, direct female benefits (=longevity, fecundity, and egg viability), and direct male benefits (=virgin longevity). It was predicted that male B. cucurbitae mating success will be enhanced by ZN feeding based on parallel earlier studies (Shelly and Villalobos 1995; Shelly 2000c) showing mating enhancement following male exposure to RK or CL. It was also predicted that female fecundity will be enhanced after mating with ZN-fed males, akin to observations made with female B. tryoni mated to ZN-fed males (Kumaran et al., 2013).

2.2. Materials and Methods

2.2.1 Mating trials

Study insects

Adult Bactrocera cucurbitae (n=300-350 adults) were reared from infested cherry tomatoes (Solanum lycopersicum L.) and Japanese cucumber (Cucumis sativus L.) collected at Ho Farms in Kahuku, Oahu, Hawaii. Infested fruits were collected in May-June 2014 (tomatoes) and December 2014 (cucumbers). Adults reared from these fruits were given unrestricted access to a 3:1 (vol:vol) mixture of white granulated sugar and hydrolyzed yeast protein as a food source and cotton dental wicks placed upright in small cups filled with water. Both food and water sources were replaced once per week. When adult flies had reached sexual maturity, zucchinis (Cucurbita pepo L.) were punctured and placed into rearing cages for 20 minutes for oviposition.

The infested fruits were placed on newspaper resting on a metal grate suspended over a layer of fine corncob granules (Grit-o’cobs® item #2040) in enclosed plastic bins. Larvae fed on the fruits and
pupated in the corncob granules below, which were sifted twice weekly to collect pupae. Flies were 3-9 generations removed from the wild when used in the mating trials.

Colonies were maintained in a mesh cage (0.6m x 0.6m x 1.0m) (n=1,500-2,000 adults) at the University of Hawaii at Manoa in a large room illuminated with artificial (fluorescent) and natural (windows) light throughout the daytime. At 1700 hours, the artificial lights were extinguished, and only natural lighting was available until sundown. The room was maintained at 23.4°C (±0.3°C) with a relative humidity of 69.0% (±3.0%).

Mating trials

Male flies were marked with different colors of enamel paint to distinguish between control and treated individuals. Males were captured in vials and placed on ice for several minutes, and after they were immobilized, a small dot of enamel paint was placed on the thorax. The marked males resumed normal activity within minutes of exposure to ambient temperature. Females were not cooled and marked. Previous studies have not observed any noticeable effects of cooling and marking the males (Shelly and Villalobos, 1995; Shelly et al., 2005; Wee et al., 2007; Kumaran et al., 2013), and preliminary mating trials of marked males in the present study confirmed a non-effect.

At least two days following marking, males (18-20 days old) of one color group were exposed to zingerone (ZN) for two hours. A filter paper (7cm diameter) soaked in 5ml of a ZN solution (5g of lure mixed with 50ml of water) was placed into cages containing males, and a high feeding response from the males was observed within 15 seconds. Males were exposed to the lure from 0800-1000, in groups of 50-100 in wooden cages (27cm x 27cm x 27cm) with mesh walls. These lure-fed males were referred to as ‘treated males’. The males of the other color group were not exposed to ZN, instead receiving water, and these males were referred to as ‘control males’.
Mating trials were performed shortly after sexual maturity was reached, when flies were 18-24 days old. Males are more likely to display courtship behavior in the presence of other males. Therefore, 2 treated males, 2 control males, and 1 female were placed in clear plastic cages (30cm x 30cm x 30cm). An artificial plant with large green leaves was placed in each cage to mimic a natural mating environment. *B. cucurbitae* only mate at dusk, therefore eight hours before sunset the cages were placed in front of a west-facing window and were not disturbed until after sunset. Artificial lights were extinguished at 1600 hours, and the cages received direct sunlight at dusk via the window.

Mated males/females remain paired throughout the night. However, flies do not mate after the sun has set (i.e. complete darkness). Therefore, cages were checked for mating pairs at 2200 hours, after complete darkness, and mated pairs were captured in vials. The males which paired with a female were identified to treatment group by observing the color of enamel paint on their thorax.

Observations of mating between females, treated males, and control males were carried out 0 (same day), 1, 2, and 3 days after treated males were exposed to ZN. Data from 70 successful mating replicates were collected for each of the four exposure periods.

**Statistics**

A binomial test was used to assess the null hypothesis of random mating between females and treated males or control males.

2.2.2 Female fecundity, egg hatch, female mortality, and male mortality

**Study insects**

*Bactrocera cucurbitae* pupae were obtained from a colony maintained at the USDA-ARS facility in Hilo, Hawaii. These flies were >300 generations removed from the wild and reared on an artificial diet
using standard mass-rearing procedure for this species (Vargas, 1989). These flies were believed to have low variation in the length of the pre-oviposition period and enhanced ability to mate in lab conditions due to adaptation to lab conditions over many generations, and because of these traits they were preferred over wild-type flies in the current lab study. Adults were sexed and separated within three days of eclosion, well before sexual maturity at 12-14 days. Preliminary tests revealed that males of this rearing colony were most responsive to ZN at 14 days after eclosion (DAE). Therefore, at this age males were provided access to a filter paper (7cm diameter) soaked in 5ml of a ZN solution (5g mixed with 50ml of water). Flies were allowed to feed on the ZN for three minutes, and only the ZN-fed males were collected and moved to a separate cage. Males not exposed to ZN were used as controls.

Mating was performed with groups of 150 males (14 DAE) and 150 females (14 DAE) together in large cages. Females were placed in cages containing either ZN-fed males or control males. These cages were placed in front of a window receiving natural light. Artificial lights were extinguished 4 hours before sunset. Mating began at sunset, and mating pairs were collected 4 hours later, so as not to disrupt the mating process and allow ample time for sperm and seminal fluid transfer. Females mated to ZN-fed males were referred to as ‘treated females’, and females mated to non-lure fed males were referred to as ‘control females’.

**Female fecundity**

Fifteen mated females were placed into clear plastic cages (30cm x 30cm x 30cm) with mesh ventilation holes. There were 6 cages for both treatment groups, for a total of 12 cages arranged in a randomized complete block design. All females were given unrestricted access to food (protein hydrolysate and white sugar in a 3:1 vol:vol mixture) and water (cotton wicks placed in cups of water). Food and water sources were replaced weekly.
Three times per week (Monday, Wednesday, and Friday) females in each cage were provided access to green-yellow colored egg collection cups (9 oz. paper cups [Dixie®] coated in a 1:4 [vol:vol] mixture of green and yellow gloss enamel paints [DecoArt®]) that were punctured with holes and lined with a paper towel soaked in exactly 1.5ml of pure cucumber juice. Females were allowed to oviposit into the cups for 4 hours and eggs were counted within 6 hours of removal from the cages. The number of eggs laid by females in each cage was recorded for 6 weeks, as this length of time was sufficient to produce significant results in *B. tryoni* (Kumaran *et al.*, 2013). Dead females were removed and recorded on egg collection days.

**Egg viability**

Egg viability was assessed for each of the oviposition days over the 6 week period. On oviposition days, a subsample of 50 eggs was taken from each egg collection cup and eggs were carefully placed onto a moist black filter paper. The filter paper was placed in an enclosed petri dish and sealed with parafilm to avoid egg desiccation. Eggs were maintained at 23.4°C (±0.3°C) with a relative humidity of 69.0% (±3.0%), and hatch rate was observed under a microscope 48-52 hours after eggs were laid.

**Female mortality**

Each cage was checked for dead females on egg collection days. Dead females were removed from the cages and recorded.

**Male mortality**

Lab-type virgin males were used to assess male mortality, because they were believed to have low variation in the length of time needed before sexual maturity was reached, as well as to keep consistency with the fecundity experiments were lab-type males were mated to lab-type females. Males
were sexed 3 DAE and moved to a large cage provisioned with food and water (see previous food and water sources). At 13 DAE the males were randomly separated into one of two large cages and again provided food and water. The next day the males in one cage were provided access to a filter paper (7cm diameter) soaked in 5ml of a ZN solution (5g mixed into 50ml of DI water) for three minutes. Males that fed on the ZN were collected and moved to a separate cage. The ZN-fed males were held for one day to allow them time to preen and remove excess lure from their bodies. ZN-exposed males were referred to as ‘treated males’ and males from the second cage that were never exposed to ZN were referred to as ‘control males’. Males were then placed in clear plastic experimental cages (30cm x 30cm x30cm) with ventilation holes covered in mesh. Each experimental cage received 30 males of the same treatment (ZN-fed or control), and 6 cages were allotted to each treatment. Cages were supplied with food and water, which were replaced weekly. Each cage was checked daily (Monday-Friday) for dead males, which were recorded and removed from the cage. All flies that died over the weekend (Saturday and Sunday) were counted on Monday and removed. Male deaths were recorded for 10 continuous weeks.

Statistics

To analyze Female fecundity data the number of eggs were counted in each cage and divided by the number of living females in the cage. Means were produced by summing the number of eggs/female for all three collection days (Monday, Wednesday, and Friday) in a given week. Means were analyzed using a one-way ANOVA. Egg viability data was analyzed by averaging the egg-hatch rate of the 50-egg subsamples for a given week in each cage. Means were analyzed using a one-way ANOVA. Female and male mortality data was assessed using a chi-squared log-rank test to test changes in mortality patterns over time. Differences in the number of flies living at the conclusion of the observations were analyzed using a one-way ANOVA. Analyses were performed with SAS JMP Pro Statistical Software (Version 12.0).
2.3. Results

2.3.1 Mating trials

ZN feeding enhanced the mating success of treated males, but only on the first day after lure feeding (Fig. 2.1). On this day treated males mated 62.9% (44/70) of the time compared to control males which mated 37.1% (26/70) of the time (p<0.05; binomial test). On all other test days there were no detectable differences in the number of matings obtained by treated and control males.

2.3.2 Female fecundity, egg viability, female mortality, male mortality

Female fecundity

The mean number of eggs produced by females mated to treated or control males was not different after 6 weeks (F₁,70=0.46, p>0.05, one-way ANOVA) (Fig. 2.2). At the end of 6 weeks the number of eggs laid by females mated to treated or control males was 430.53 ±37.46 and 404.23 ±61.56, respectively. The average number of eggs laid per week by females mated to treated and control males was 71.75 ±26.47 and 67.37 ±28.32, respectively. The number of eggs laid was highest in the first week after mating, and gradually declined as the experiment progressed.

Egg viability

There was no difference between the mean hatch rate of eggs laid by females mated to treated males or control males over 6 weeks (F₁,70=0.023, p>0.05, one-way ANOVA) (Fig. 2.3). Over the first week, the hatch rate of eggs laid by females mated to treated males was 91.22% ±9.9%, and the hatch rate of eggs laid by control males was 93.22% ±4.56%. On the 6th week the hatch rate of eggs laid by females mated to treated males was 22.89% ±16.90%, and the hatch rate of eggs laid by control males was 25.43% ±12.64%. Egg hatch rates declined by an average of 13.67% per week for females mated to treated males, and 13.56% for females mated to control males.
Female mortality

The pattern of survival differed significantly between females mated to treated males and control males ($\chi^2=4.08$, $p<0.05$, Log-rank test) (Fig. 2.4). This difference largely reflected the high mortality of females mated to treated males between weeks 1 and 2. During this period 10.0% of females mated to treated males died compared to only 1.1% of females mated to control males. Likewise, after 6 weeks there was a difference ($F_{1,70}=5.46$, $p<0.05$, one-way ANOVA) in the number of surviving females mated to treated males (69.42% ± 6.53%) and females mated to control males (77.09% ± 9.87%).

Male mortality

There was no difference in the survival of treated and control males ($\chi^2=0.0032$, $p>0.05$, log-rank test) (Fig. 2.5). Between weeks 1 and 2 there was a greater rate of mortality in treated males (4 deaths, 2.25% of the total population) relative to control male (0 deaths), however, this figure was not statistically significant ($p>0.05$, Tukey HSD test). After 10 weeks the survival of treated males (89.25% ± 6.53%) and control males (90.00% ± 4.60%) was not different ($F_{1,116}=0.078$, $p>0.05$, one-way ANOVA).

2.4 Discussion

Mating success

Male lures are known to enhance the mating success of males that feed on them (Shelly, 2010). Zingerone (ZN) has a similar molecular structure to other male lures and elicits a feeding response by the males of many Bactrocera species (Tan and Nishida, 2000). Thus it has been assumed that a mating advantage is conferred to males that consume this lure (Tan and Nishida, 2000; Khoo and Tan, 2000). The present study is the first to demonstrate this effect for B. cucurbitae. ZN consumption was found to
enhance the mating success of males but only on the first day after lure feeding. This one-day mating advantage has likewise been demonstrated when *B. cucurbitae* males feed on raspberry ketone (Shelly, 2000c) and cue-lure (CL) (Shelly and Villalobos, 1995).

Interestingly, in the present study ZN-fed males mated less often than un-fed males (although the difference was non-significant) on the same day of ZN feeding, a result also observed for raspberry ketone fed *B. cucurbitae* (Shelly, 2000c) and methyl eugenol (ME) fed *B. dorsalis* (Shelly and Dewire, 1994). There remains no explanation for this effect. However, *B. cucurbitae* and *B. dorsalis* are described as appearing “sluggish” or lethargic shortly after consuming a male lure (see aforementioned studies), and this may explain fewer matings observed on the same day of lure-feeding. However, the current study did not include behavioral observations of males after feeding on ZN, thus it is unknown whether consumption of ZN similarly induces male lethargy.

It has been suggested that feeding male lures to sterile flies could improve their mating success with wild fertile females in the field. Indeed the benefit may be substantial for some ME-responding species, such as *Bactrocera dorsalis* which mate more often for >35 days after feeding on ME (Shelly and Dewire, 1994). However, our results suggest feeding zingerone to sterile male *B. cucurbitae* would likely have a minimal effect as mating success is enhanced for only one day. Nevertheless, the current study explored wild-type males in lab settings, and lure feeding may have a different effect with lab-type males in wild settings.

**Female fecundity**

The present study provides no evidence that female fecundity was affected when *B. cucurbitae* females mated with ZN-fed males. These results differ from those obtained for *B. tryoni*, where females mated to CL-fed or ZN-fed males lay more eggs over their lifetime (Kumaran *et al.*, 2013). However, the
results from the present study are similar to those observed in *B. dorsalis*, where fecundity is not affected when females mate to ME-fed males (Shelly, 2000a).

Two non-mutually exclusive interpretations may be offered to account for the observed variation in these studies. First, the effects of lure feeding may differ between species, and some experience enhanced fecundity (e.g. *B. tryoni*), while others do not (e.g. *B. dorsalis* and *B. cucurbitae*). A second possibility is that the observed differences can be explained by the experimental designs of the various studies. Two potentially significant differences in methodology merit consideration.

First, in the study with *B. tryoni* (Kumaran et al., 2013), male lures remained in the experimental cages for the entire duration of the 8 week experiment. The *B. tryoni* males were presumably able to consume a large amount of lure, because they were given unrestricted access to it. However, the present study and Shelly (2000a) allowed males to feed on lures only once. In these two studies *B. cucurbitae* and *B. dorsalis* fed on ZN (5g diluted in 50ml water) for 3 minutes and pure ME for 30 seconds, respectively. These experiments were careful to ensure that all ‘lure fed males’ had, in fact, fed on the respective lure sources. It is possible, however, that these males did not consume enough lure in the allotted time to affect female fecundity. It is also possible that, in the study with *B. tryoni*, the volatiles emitted from the lure source influenced the females to lay more eggs. Indeed male lures have a similar molecular structure to many compounds which are known to stimulate oviposition in female fruit flies (Metcalf et al., 1983).

Second, in the study with *B. tryoni*, females and males were placed in the same cages for the duration of the 8 week observations (Kumaran et al., 2013). Presumably, male and female *B. tryoni* mated multiply during the 8 weeks, as multiple mating is common in this species (Radhakrishnan et al., 2009). Frequent mating may shorten female lifespan and increase fecundity in Dacine fruit flies (Whittier and Shelly, 1993; Shelly, 2000a; Chinajariyawong et al., 2010). If multiple mating occurred more often in cages where females cohabited with lure-fed males then an increase in fecundity could have resulted. In
contrast, the females in the current study and Shelly (2000a) were allowed to mate only once with either a lure-fed male or control male.

**Egg hatch**

No difference was found in the hatch rate of eggs laid by females mated to ZN fed males versus control males. Similar observations have been made for *B. dorsalis* (Shelly, 2000a) and *B. tryoni* (Kumaran et al., 2013). Throughout the study egg hatch rate gradually declined, and this effect is likely due to depletion of sperm reserved by the singly-mated females (as is the case with *Ceratitis capitata*, Blay and Yuval, 1999).

**Female and male survival**

The number of surviving females mated to ZN-fed males declined sharply between weeks 1 and 2. During this period there was also a decline in the number of surviving treated males, but the male decline was not statistically significant.

It is possibility that ZN contains toxic properties, and some of the lure may have remained on the male after lure feeding and could have been transferred to the female while mating. If true, small amounts of the lure could have harmed the flies during the experiment, leading to higher mortality. Another explanation is that lure feeding enhances the male seminal fluids, either causing the male to produce more proteins or a higher quality of proteins, which could have an effect on his own fitness as well as that of his mate. For example, in *Drosophila melanogaster* (Diptera: Drosophilidae) at least 85 different proteins are passed from male to female during copulation, and these have a wide range of effects on female behavior (Chen, 1996). Many of these proteins are known to reduce the lifespan of females (Chapman, 2001). In male *B. tryoni*, Kumaran et al. (2014) demonstrated that ZN-feeding altered the expression of genes with homologues linked to various seminal proteins in *Drosophila* species, and
some of these proteins are believed to impact female longevity when transferred from the male during copulation. It is currently unknown if ZN feeding alters male seminal proteins in *B. cucurbitae*, or if female longevity would be impacted, but investigations into such an effect may be insightful. The current results suggest that females would benefit from not mating with zingerone-fed males, because there appears to be no positive benefit associated with fecundity and egg hatch rate but there is a clear negative benefit of increased chance of mortality. Nevertheless this negative effect does not appear to reduce fitness enough to deter females from avoiding lure-fed males, as there is a mating bias. Instead, high mortality may be indicative of physiological changes in the females that mate to zingerone-fed males and future studies should investigate this possibility.

2.5 Conclusion

The mating trials with ZN-fed males produced the same results as other studies performed with *B. cucurbitae* males feeding on the male lures cue-lure, and raspberry ketone (Shelly, 2010). Although different methods were followed in the present study, our results suggest that male consumption of ZN may confer a similar effect on *B. cucurbitae* mating behavior as these other male lures.

Male ZN feeding had no effect on mated female fecundity or egg hatch rate. Survival patterns were significantly different in females mated to ZN-fed males, however it is unknown what attributed to this effect. Thus the present study lacks evidence suggesting that lure attraction confers direct fitness benefits to *B. cucurbitae*, and we find no reason to reject the ‘sensory-bias’ and/or ‘runaway selection’ sexual selection hypotheses, neither of which assume direct fitness benefits (Shelly 2000a,b). Nevertheless it is possible that other effects exist that were not directly tested. For example, the investigations into metabolism increases and male seminal fluid proteins alterations following lure feeding in male *B. tryoni* are compelling (Kumaran *et al.*, 2014). Investigating seminal fluid alterations in
other *Bactrocera* species may provide evidence as to why reproduction is enhanced after lure feeding in *B. tryoni*, but not in *B. cucurbitae* or *B. dorsalis*. 
2.6 Figures

**Figure 2.1.** Number of copulations by zingerone-fed males (treated), and unfed males (control). Asterisk denotes statistical significance at p<0.05.
Figure 2.2. Cumulative number of eggs laid/female/week (mean ±SE) of females mated to zingerone-fed males (treated) or unfed males (control).
Figure 2.3. Percent hatch rate (mean ±SE) of eggs laid by females mated to zingerone-fed males (treated) or unfed males (control).
Figure 2.4. Percent females surviving (mean ±SE) after mating with zingerone-fed males (treated) or un-fed males (control). Females represented here are those used in the fecundity experiment (see methods).
Figure 2.5. Cumulative percent (mean ±SE) survival of zingerone-fed males (treated) or unfed males (control) over 10 weeks.
2.7 References


CHAPTER 3.0: ATTRACTION OF MALE MELON FLIES, BACTROCERA CUCURBITAE (DIPTERA: TEPHritidae), TO MIXTURES OF CUE-LURE AND ZINGERONE IN THE FIELD
3.1 Introduction

*Bactrocera* (Diptera: Tephritidae) is a large genus containing over 500 described species of fruit flies arranged in approximately 28 subgenera (Drew and Hancock, 2000; Smith et al., 2003). The majority of these species prefer non-economic hosts in the plant families Asclepiadaceae, Passifloraceae, and Cucurbitaceae, however, ~70 species are polyphagous pests of many commercial fruits and vegetables (White and Elson-Harris, 1992; Metcalf and Metcalf, 1992). Many of these pestiferous fruit flies frequently invade warm Mediterranean and tropical habitats and require extensive management.

Efforts to manage and monitor fruit fly pests rely largely upon certain male-attractive compounds. Most, but not all, *Bactrocera* species are attracted to (and subsequently consume) a few naturally occurring phenylpropanoids and phenylbutanoids (Shelly, 2010). These compounds (commonly termed ‘male lures’ or simply ‘lures’) are combined with insecticides to create baits capable of attracting and killing mature male fruit flies.

Male lure traps are widely used in male annihilation technique (MAT), a method by which large numbers of male lures are deployed to attract-and-kill males in such high numbers that females cannot find males to mate or must fly away from the trapping area to mate. MAT can be highly effective at reducing fruit fly populations, and is an important component of many area-wide fruit fly management programs (Vargas et al., 2014; Vargas et al., 2007; Vargas et al., 2010; Ali et al., 2010; Leblanc et al., 2013). MAT has even been used to eradicate fruit fly populations in various regions around the world (Steiner et al., 1965; Steiner et al., 1970; Koyama et al., 1984; Cantrell et al., 2002).

Male lures are also used in the surveillance and monitoring of fruit flies (Jang et al., 2014; Meats, 2014). Traps are employed in agriculture fields to assess the population size and seasonal fluctuation of fruit fly populations. In many regions where fruit flies populations do not exist, but are nevertheless at risk of invasion (i.e. when conditions favoring sustained fruit fly reproduction are present), large scale surveillance programs are employed using an array of male lure traps capable of detecting select fruit fly...
species over a wide area. These surveillance programs give early-warning of incipient fruit fly populations and allow for the possibility of successful eradication.

Only two male lures have been widely adopted for managing and monitoring *Bactrocera* fruit flies, methyl eugenol and cue-lure. Methyl eugenol (ME) is a naturally occurring compound that was first observed to attract male flies over 100 years ago (Howlett, 1912), however, it wasn’t until *Bactrocera dorsalis* invaded Hawaii in 1946 that ME was first used for control and monitoring (Steiner, 1952). Cue-lure (CL), on the other hand, is a synthetic compound discovered by systematically screening thousands of chemicals when searching for an effective lure of *Bactrocera cucurbitae* in Hawaii (Beroza et al., 1960) and has been used widely since its discovery. It is an interesting and relevant point that many species of *Bactrocera* are attracted to ME (but not CL), while many other species are attracted to CL (but not ME). Thus, both lures are used in surveillance trapping programs so that ME- and CL-responding *Bactrocera* species can be targeted. Finally, an estimated 1/3 of described *Bactrocera* species are not responsive to either ME or CL (Raghu, 2004), and detection and management of these flies is limited without the use of male lures.

Both ME and CL have been used successfully for over 50 years. However, much attention has been given to developing new, more effective and/or safer male lures, such as raspberry ketone formate (Metcalf and Metcalf, 1992; Jang et al., 2007) and fluorinated ME (Khrimian et al., 2009; Jang et al., 2011). Another male lure, zingerone, has shown recent promise, and unlike the aforementioned synthetic lures in development, zingerone is a floral compound known to attract male *Bactrocera* flies in nature (Tan and Nishida, 2000). Zingerone uniquely attracts both *Bactrocera* species that respond to ME and other species that respond to CL, as well as a wide range of species in the *Dacus* genus which are generally (often weakly) responsive to CL (Tan and Nishida, 2000; Tan and Nishida, 2007; Fay, 2012; Dominiak et al., 2015; Royer, 2015; Michael San Jose, personal communication). However, investigations into zingerone trapping have been limited to studies in Queensland, Australia (Fay, 2012), Sydney,
Australia (Dominiak et al., 2015; Royer, 2015) and Vietnam (Michael San Jose, personal communication). Dominiak et al. (2015) stated that zingerone can be an effective lure of the highly destructive Queensland fruit fly, B. tryoni (a CL-responding species), and Fay (2012) discovered that zingerone is highly attractive to Bactrocera jarvisi, a moderate pest of mangos in northern Australia. These findings suggest that zingerone could be a useful lure of these Australian-endemic fruit fly pests.

Zingerone may in time be proven to be a practical and useful lure for fruit fly management outside of Australia, but additional research is needed. The present study investigates the field attraction of melon fly (Bactrocera cucurbitae), a CL-responding species, to zingerone and various mixtures of zingerone and CL at a Hawaiian farm. Melon fly was first introduced to Hawaii in 1895 (Back and Pemberton, 1917) and has since become a serious pest of commercially grown cucurbits. This fruit fly has spread globally, invading Africa, Southeast Asia, China and various Pacific islands from its endemic range of the Indian subcontinent (CABI, 2015; White and Elson-Harris, 1992). The melon fly is among the most well-known and destructive fruit fly pests and is a primary target of fruit fly surveillance programs. At least 137 different species of fruiting plants are hosts of the melon fly under natural field conditions, and species in the Cucurbitaceae family are generally preferred (Liquido et al., 2015).

The melon fly was chosen as a test species primarily because of the promising results observed in other CL-responding pest fruit flies from zingerone trapping studies in Australia (e.g. B. tryoni and B. jarvisi). However, the melon fly was also the only fruit fly species in Hawaii that showed high attraction to the lure in preliminary lab trials. Males from lab colonies of Ceratitis capitata (Mediterranean fruit fly) and Bactrocera latifrons (Malaysian fruit fly) showed no attraction to zingerone (Inskeep, unpublished data). Bactrocera dorsalis (oriental fruit fly) males landed on a zingerone source when the lure was presented at close proximity, but relative attraction was considerably low when compared to ME (Inskeep, unpublished data). Research into a more effective lure of the melon fly may be valuable, because CL is generally considered to be a weak attractant when compared to ME, and the ability to
detect melon flies from a distance with CL may be limited (Shelly et al., 2010; Shelly and Nishimoto, 2011). Furthermore, in a caged lab study, Shelly and Villalobos (1995) observed only a 33% response rate of sexually mature melon fly males to CL when the lure was presented to them. Thus, if these results are indicative of attraction rates of wild flies (i.e. only ~33% of flies coming to CL-baited traps) then the ability to control and detect *B. cucurbitae* with CL may be greatly limited.

CL is widely used in managing *B. cucurbitae*, but this lure may not be completely effective in all cases. The relative attraction of wild *B. cucurbitae* to zingerone is unknown, and furthermore, mixtures of two male lures attractive to the same fruit fly species, has yet to be examined. For these reasons, we investigated the field attraction of *B. cucurbitae* to zingerone and mixtures of zingerone and CL in effort to enhance understanding of lure attraction and seek out more effective lures of this pest.

### 3.2 Methods

*Preparation of lures*

The field experiment assessed five lure treatments containing cue-lure (CL), zingerone (ZN), or a unique ratio of the two lures mixed together. The treatments were: 1) 100% CL, 2) 75% CL and 25% ZN, 3) 50% CL and 50% ZN, 4) 25% CL and 75% ZN, and 5) 100% ZN.

In preparation of the lures, treatments 2 (75% CL and 25% ZN) and 3 (50% CL and 50% ZN) were created by dissolving the solid ZN in the proportional amounts (by weight) of CL, which is a strong solvent. In treatment 4 (25% CL and 75% ZN), the amount of CL was insufficient to fully dissolve the ZN. Therefore the solid ZN was melted using a heated bath method, and the melted ZN was combined with CL and mixed vigorously. In treatment 5 (100% ZN), ZN was melted using a heated bath method. Five grams of each lure treatment was applied to an individual 1cm x 7.5cm cotton dental wick.

Clear plastic containers (1L) were used to create standard bucket traps. Each trap was fitted with two entrance holes (2.5cm diameter), and a metal wire was used to attach the traps to the branches of
trees. Cotton dental wicks soaked in the various lure treatments were placed individually in perforated plastic baskets (Scentry Biological Inc., Billings, MT) and suspended in the top center of traps with a metal wire. A single 25 x 50mm strip containing 10% dichlorvos insecticide (Vaportape II, Hercon Environmental, Emigsville, PA) was placed next to the lure, which was replaced with a fresh strip three weeks later.

**Trap locations**

*Bactrocera cucurbitae* were trapped at Ho Farms in Kahuku, on the island of Oahu, Hawaii, from July 24 until September 4, 2015. Traps were placed 10m from the edge of an agriculture field in a split plot design. Three monocropped fields (0.81 hectares each) consisting of preferred hosts of *B. cucurbitae* were treated as main plots, tomato (*Solanum lycopersicum* L.), cucumber (*Cucumis sativus* L.), and eggplant (*Solanum melongena* L.). For each host, a set of traps was placed along the North West field edge and another set along the South East field edge, and these constituted sub-plots. Each sub-plot contained five traps (one trap for each lure treatment), and the location of traps within each sub-plot was randomized. Sub-plots were spaced at least 100m apart, and traps within sub-plots were spaced 35m apart. Fig. 3.1 presents a schematic diagram of the trapping array.

Traps were placed 1.2-1.5 meters above the ground in non-host (or rarely infested) trees (i.e. milo tree, *Thespesia populnea* L. Sol. Ex Correa, strawberry guava, *Psidium cattleyanum* Sabine, Haole koa, *Leucaena leucocephala* Lam. de Wit., and turkey berry, *Solanum torvum* Sw.). Sub-plots along the North West field edge were dominated by a dense overstory of milo trees, and sub-plots along the South East field edge were dominated by an understory of grass (*Poa* spp.) and an overstory of haole koa with interspersed strawberry guava, and turkey berry.
**Trap monitoring**

Traps were monitored every seventh day for six weeks. The contents of each trap were emptied into bags, and specimens frozen at the University of Hawaii at Manoa. In traps containing fewer than 600 flies, all flies were counted and the exact number was recorded. In traps containing more than 600 flies, four groups of 100 flies were randomly chosen and the exact weights were acquired using an electronic scale. The entire batch of flies was weighed, and divided by the average weight/fly from the weighed sub-samples, to obtain an estimated total number of flies in the trap.

**Statistics**

Using a split plot design, plant hosts (i.e. tomato, cucumber and eggplant) were treated as main plots and field edge (i.e. North West or South East) were treated as sub-plots (Fig. 3.1). Data on weekly trap captures were transformed to the natural log of X + 1 to obtain adequate fit to a normal distribution, and means were analyzed using a standard least-squares fit model Tukey HSD test with level of significance at α=0.05. To compare capture between hosts, a one-way ANOVA compared total trap capture over the six weeks of trapping. All analyses were performed with SAS JMP Pro Statistical Software (Version 12.0).

An adjusted r-squared value was obtained using a standard least-squares fit model (SAS JMP Pro v12.0) to test for synergistic or inhibitory effects of adding zingerone to cue-lure in the various lure treatments. The least squares fit model compared measured and estimated trap capture for the lure treatments (with values transformed to the natural log of X + 1) with week, host, and field edge as independent variables.
3.3 Results

The number of flies captures in traps was affected by the lure treatment (least-squares fit, df=5, p<0.0001) (Fig. 3.2). Traps baited with 100% cue-lure (CL) (1,724 ±1,452 flies per week) captured the most flies (significantly more than all other lure treatments, p<0.05), while traps baited with 100% zingerone (ZN) (39 ±54 flies per week) captured the fewest flies (significantly less than all other lure treatments, p<0.0001). The number of flies captured in traps baited with the lure treatments ZN(50%)+CL(50%) and ZN(25%)+CL(75%) was statistically the same (least-squares means Dunnett test, p=0.99).

The ratio of CL in the lure treatments was a strong predictor of the amount of flies trapped in our model (adj-$r^2$=0.65). Generally traps with more CL caught a higher quantity of flies, and the high adj-$r^2$ value suggests a linear relationship. Thus the amount of zingerone in the trap appeared to have no synergistic or antagonistic effect when combined with CL.

The number of flies captured was different between host plants (least squares fit, df=2, p>0.01) (Fig. 3.3). More flies were trapped near the plots where tomatoes were being grown than those where eggplant was grown (p<0.01), but trap capture near cucumbers was not significantly different from tomato or eggplant (p>0.05).

The number of captured flies also varied by week (least-squares fit, df=5, p>0.0001). Trap capture dipped in weeks 2 and 4, uniformly across treatments and trap location, coinciding with large storm events in the area.

3.4 Discussion

Previous investigations into lure mixing have focused on combinations of methyl eugenol (ME) and cue-lure (CL), so that a greater range of fruit fly pest species can be targeted (i.e. both ME- and CL-
responding species) (Shelly et al., 2004; Liu, 1989; Hooper, 1978; Vargas et al., 2000a). Interestingly, Shelly et al. (2004), Liu (1989) and Hooper (1978) observed a greater attraction of *B. cucurbitae* to CL and ME mixtures when compared to CL alone, and an evolutionary basis for this response has been suggested (see Shelly et al., 2004). Vargas et al. (2000a) on the other hand, using a similar experimental design as the present study, obtained different results. They found little effect of using different mixture on *B. cucurbitae* captures (no difference at 100, 75, and 50% but differences at 25% CL). However, over time captures of *B. dorsalis* were reduced with the amount of ME initially incorporated on the wick (100> 75> 50> 25% ME). As chemical analysis did not accompany the earlier Vargas et al. (2000a) studies, the following two factors were not recognized and have recently been verified through chemical analysis (Vargas et al., 2015): 1) high volatility of ME and low volatility of CL/RK, with exponential loss of ME over time, but a low constant loss of CL/RK over time; and 2) when using mixtures of CL/RL and ME, this volatility difference could be compensated for by using small amounts of CL/RK and large amounts of ME. However, results in areas with high *B. dorsalis* populations and high amounts of ME and low amounts of CL in traps may cause competitive interactions; whereby, high activity of *B. dorsalis* around traps reduces numbers of *B. cucurbitae* entering the trap (Vargas et al., 2000a, 2015). Therefore, habitat, type of trap and ratio of mixtures (i.e. high amounts of ME and very low amounts of CL) may influence trap captures in traps containing multiple dispensers. While these findings provide valuable practical information on lure mixtures, these research efforts were primarily conducted to investigate a lure combination that can attract two *Bactrocera* pest species. In contrast the present study is the first to investigate lure combinations that target the same fruit fly species. Also, this is the first study comparing attraction of *B. cucurbitae* to zingerone relative to CL.

In the current study, the addition of zingerone to CL appeared to have no synergistic or inhibitory effect on the attraction to *B. cucurbitae*. Furthermore, the attraction of zingerone alone was low, as traps baited with 100% zingerone attracted only 2.5% as many flies as traps baited with 100% CL.
Interestingly, trap capture between the lure treatments ‘ZN(50%)+CL(50%)’ and ‘ZN(25%)+CL(75%)’ was the same. It’s possible that the distance between traps (35m) and the dosage of the lures (5g per trap) influenced these treatments. Traps may have interfered with each other due to high lure dosage and/or close proximity, although such an interaction was not apparent with the other lure treatments. A further possibility is that the ZN(25%)+CL(75%) lures may have been randomly placed in locations where fewer flies were present. For all other lure treatments there was an overall trend that trap capture was linearly dependent on the amount of CL in the lure, with higher amounts of CL resulting in more flies captured. Thus the effects of adding zingerone to CL in a bait trap appears to have no interactive effect on the capture of *B. cucurbitae* as has been observed when ME is added to CL.

Capture of *Bactrocera cucurbitae* was highest near the tomato fields, likely because large amounts of fallen mature tomato fruits were available for oviposition throughout the entirety of the experiment. In contrast, fallen mature eggplants and cucumbers were first observed beginning on the 1st and 2nd week, respectively. The presence of mature eggplants did not appear to increase trap capture near these fields, but a spike in flies captured near the cucumber fields beginning in the 5th week could have coincided with the onset of mature fruits three weeks prior, if flies were breeding on these fruits. *B. cucurbitae* males will only exhibit attraction to lures when sexual maturity is reached (Wong *et al.*, 1991), and this can take multiple weeks (Vargas *et al.*, 2000b).

It is possible that the Hawaiian population of *B. cucurbitae* is less sensitive to zingerone. The population present in Hawaii arrived over 100 years ago (Back and Pemberton, 1917), and it is generally unknown if/how lure attraction changes in geographically separated populations of *Bactrocera*. Nevertheless, our results suggest that employing zingerone traps (either alone or mixed with CL) for the detection and control of Hawaiian *B. cucurbitae* would likely be ineffective.

However, previous investigations clearly suggest zingerone may be useful in the broader context of fruit fly management. Fay (2012) observed zingerone traps attracting nearly 700 times as many
*Bactrocera jarvisi* (a pest in Australia) males as compared to CL traps. The attraction of zingerone to this species is so strong that the lure is considered useful for control and detection. Further trapping observations in Australia by Dominiak *et al.*, 2015 suggest that zingerone can be an effective lure of *Bactrocera tryoni*, considered to be one of the most destructive CL-responding fruit fly species globally. However, in contrast, Fay (2012), captured significantly fewer *B. tryoni* in zingerone traps, and trap placement may explain the differences observed by the two experiments (see Dominiak *et al.*, 2015; Fay, 2012).

Zingerone appears to be highly attractive to many Australian *Dacus* species, including cryptic species previously believed to not respond to male lures (Fay, 2012; Royer, 2015; Dominiak *et al.*, 2015). The majority of *Dacus* species yielded from zingerone-trapping surveys appear to either be more attracted to zingerone than CL or attracted to zingerone and not CL. Additional zingerone-trapping in Vietnam yielded similar results with *Dacus* species and suggests that heightened attraction of *Dacus* to zingerone may not be confined to only taxa endemic to Australia (Michael San Jose, Personal communication). There are many *Dacus* species considered to be significant agricultural pests. It is unknown whether pestiferous *Dacus* respond well to zingerone, although investigating this topic may be valuable.

Finally, zingerone has significant ecological value to fruit fly research. A number of undescribed species, never before captured in CL or ME baited traps, have been captured in zingerone traps in Australia (Fay, 2012; Dominiak *et al.*, 2015; Royer, 2015). Similar results in a Vietnam survey also yielded undescribed species. These results indicate that the categories commonly used to describe *Bactrocera* species (i.e. 1. ME-responders, 2. CL-responders, and 3. non-responders) may be misleading because they do not include species that respond to zingerone but not ME or CL. Further zingerone-trapping surveys in areas with endemic fruit fly populations may yield more undescribed species, as well as elucidate general questions surrounding the phenomenon of evolved lure attraction by fruit flies.
3.5 Figures

Figure 3.1. Experimental layout of Ho farms in Kahuku, Oahu, Hawaii. Dark circles denote individual traps, and brackets denote subplots. Trap location was randomized within subplots. Traps were spaced 35m apart and subplots spaced at least 100m apart (between and across from hosts). Tomato and cucumber fields measured roughly 2 acres in size, while the eggplant field measured 2.5 acres.
Figure 3.2. Cumulative capture of *B. cucurbitae* in traps baited with lures (mean ±SE) over six weeks. ‘Z’ and ‘CL’ denote zingerone and cue-lure, respectively. Each lure treatment contained five grams of lure, and percentage of Z and/or CL for the treatments is given.
Figure 3.3. Mean (±SE) capture of *B. cucurbitae* by host and week.
3.6 References


CHAPTER 4.0: CONCLUSION
4.0 Concluding remarks

Many fruit fly species exhibit profound attraction to male lures, yet there has been a surprising paucity of research on the evolutionary context of male lure feeding. Based on previous studies with an Australian tephritid species, *Bactrocera tryoni*, zingerone has been demonstrated to improve the fitness of males that feed on zingerone and females that mate with zingerone-fed males. Furthermore, zingerone has demonstrated potential as a lure of many wild tephritid flies for ecological surveys and management trapping. The aim of this thesis was to evaluate the value of zingerone for *B. cucurbitae* life history and its potential as a management tool.

The first objective of this research, outlined in chapter two, was to investigate zingerone feeding as a modifier of male mating success, and to make observations on select life parameters of zingerone-fed males and females mated to zingerone-fed males. The life parameters tested were virgin male longevity, female fecundity, egg viability, and mated female longevity. It was observed that zingerone feeding enhanced the mating success of males, but only on the first day after feeding. The enhanced mating success of zingerone-fed males, albeit not long lasting, is comparable to the mating enhancement afforded by cue-lure feeding and raspberry ketone feeding in this species. This outcome was predicted. However, when female fecundity and egg viability were tested, there were no discernable differences between females mated to either zingerone-fed males or un-fed (control) males. Interestingly, females mated to zingerone-fed males had higher mortality over the first few weeks after mating, suggesting that females mated to zingerone-fed males have reduced fitness. These findings support a runaway selection hypothesis as I observed no demonstrable direct fitness benefits afforded to females that mate with zingerone-fed males over un-fed males. This hypothesis was first postulated to explain lure attraction in *Bactrocera dorsalis*. Nevertheless, another study did observed direct benefits (=higher fecundity) afforded to females mated with lure-fed males of a different species, *Bactrocera tryoni*. These differing results call in to question why lure feeding by males would enhanced
mated female fecundity in one species (=B. tryoni) but not others (=B. dorsalis and B. cucurbitae). The results obtained from B. cucurbitae in the present study were unexpected as they contrast the results obtained for B. tryoni, another cue-lure/raspberry ketone responding species. The present research also does little to clarify the evolved association with lures in Bactrocera, but rather provides opposing results suggesting more research is needed. Investigations into the effects of lure feeding on male seminal proteins and male metabolism may be insightful, per compelling data gathered on the aforementioned study with B. tryoni. Furthermore, specific investigations into the methodology of the aforementioned studies may uncover hidden interactions influencing the results obtained for the different species. Nevertheless, further work should focus on uncovering the evolved phenomenon of lure attraction in male flies as male lures have tremendous value to fruit fly management.

The second objective of this study, outlined in chapter three, was to examine the attraction of wild B. cucurbitae males to zingerone, cue-lure, and various mixtures of the two male lures. Zingerone has garnered much recent attention due to its potential as an attractant for ecological surveys and fruit fly pest management. Many Bactrocera species are attracted to zingerone, and many species in the Dacus genus appear exceptionally attracted to the lure. However, few studies have explored the relative attraction of fruit flies to zingerone when compared to the more conventional lures, methyl eugenol and cue-lure. I found zingerone to be only a weak attractant of B. cucurbitae when compared to cue-lure, the standard lure of this species. Furthermore, the addition of zingerone to cue-lure (in the same wick) appeared to have no synergistic or inhibitory effect on the trap capture. Previous research has shown mixtures of methyl eugenol and cue-lure does enhance the attractiveness to B. cucurbitae. Thus there appears to be different interactions affecting B. cucurbitae attraction to cue-lure when it is mixed with methyl eugenol or zingerone.
This research within this thesis has demonstrated for the first time that zingerone consumption by male *B. cucurbitae* has benefits for mating success but not similar benefits for females that mate with zingerone-fed males. Zingerone alone is unlikely to be effective at managing or monitoring wild *B. cucurbitae* populations, and mixing zingerone with cue-lure has only an additive effect on attraction. Nevertheless, past research clearly suggests that zingerone is useful in the broader context of fruit fly management, as per surveys in Australia and Vietnam. Future research with zingerone should focus on additional ecological surveys, and investigate its attraction to select non-responding *Bactrocera* species as well as pestiferous *Dacus* species. Such trapping studies may even elucidate general questions surrounding the phenomenon of evolved lure attraction by fruit flies.