

Trapping Male Melon Flies, *Zeugodacus cucurbitae* (Coquillett) (Diptera: Tephritidae), Using Mixtures of Zingerone and Cue-Lure in the Field

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Abstract. The males of many fruit flies (Diptera: Tephritidae) are strongly attracted to, and feed upon, a few natural compounds (and their synthetic analogs; commonly referred to as “lures”). Zingerone is a lure that has garnered recent attention for its use as an attractant for ecological surveys and pest management of select fruit flies. We investigated the attraction of male melon flies, *Zeugodacus cucurbitae* (Coquillett), to zingerone and mixtures of zingerone and cue-lure (the typical lure for this species) at a commercial farm on Oahu, Hawaii. Our findings indicate that zingerone and mixtures of zingerone and cue-lure are less attractive than cue-lure alone to male melon flies in the field, and the number of captured flies is positively and linearly correlated to the quantity of cue-lure in traps.

Key words: *Zeugodacus cucurbitae*, zingerone, cue-lure, trapping

Fruit flies (Diptera: Tephritidae) are among the most destructive agricultural pests globally and frequently invade warm Mediterranean and tropical habitats (Drew and Hancock 2000, White and Elson-Harris 1992, Metcalf and Metcalf 1992). In Hawaii, four non-native fruit flies are important pests to most horticultural fruits grown across the state, they are *Ceratitis capitata* (Wiedemann), *Bactrocera dorsalis* (Hendel), *Bactrocera latifrons* (Hendel), and *Zeugodacus cucurbitae* (Coquillett) (Vargas et al. 2016). Of these, *Z. cucurbitae* has been a serious agricultural pest in Hawaii since its introduction to the islands in 1895 (Back and Pember-ton 1917). The fly has also invaded Africa, Southeast Asia, China and various Pacific islands from its endemic range of the Indian subcontinent (CABI 2017, White and Elson-Harris 1992). It infests at least

136 species of fruiting plants under natural field conditions, with a preference for fruits in the Cucurbitaceae and Solanaceae families (McQuate et al. 2017). In Hawaii, growers of *Z. cucurbitae* host fruits often sustain considerable economic loss from direct infestation of marketable fruits and restrictions on fruit transportation.

Effectively managing *Z. cucurbitae*, as well as other pest fruit flies, often relies upon certain integrated pest management (IPM) techniques (Vargas et al. 2016). Central to these IPM techniques are male-attractive phenylpropanoids and phenylbutanoids, commonly termed “male lures” or simply “lures.” Male flies gain enhanced mating success after consuming these lures, which presumably explains their attraction to them (Segura et al. 2018, Inskeep et al. 2018, Haq et al. 2018). For management, lures are typically combined

with insecticides to attract and kill male flies for area-wide management programs (Vargas et al. 2014, Vargas et al. 2007, Vargas et al. 2010, Ali et al. 2010, Leblanc et al. 2013), for small-scale local management (Cunningham 1989, Alyokhin et al. 2001, Stonehouse et al. 2007, Ndlela et al. 2016), for eradication of invasive populations (Steiner et al. 1965, Steiner et al. 1970, Bateman et al. 1973, Ushio et al. 1982, Koyama et al. 1984, Cantrell et al. 2002), and in surveillance programs to give early-warning of incipient populations (Jang et al. 2014, Meats 2014).

The most effective lure of *Z. cucurbitae* is cue-lure (CL), having been utilized since its discovery over 50 years ago (Beroza et al. 1960). However, CL is often considered a weak attractant for sampling and detecting *Z. cucurbitae* and inadequate at long distances (Shelly et al. 2010, Shelly and Nishimoto 2011). Furthermore, only about one-third of sexually mature male *Z. cucurbitae* respond to CL at even short distances (Shelly and Villalobos 1995). Reducing or eradicating fly populations using CL can be inefficient, which has prompted efforts to develop new lures such as melolure (Metcalf and Metcalf 1992) and raspberry ketone formate (Metcalf and Metcalf 1992, Jang et al. 2007). These lures are more attractive than CL to *Z. cucurbitae* but are prohibitively expensive under normal IPM schemes.

Another lure that has shown promise for managing and detecting some fruit fly species is zingerone (Tan and Nishida 2000). This lure is naturally produced in the flowers of some orchids in the genus *Bulbophyllum* (Thouars), for which zingerone attracts male fruit flies for pollination (Tan 2009). The earliest research on zingerone as a tool for IPM and ecological studies found the lure to be highly attractive to many *Dacus* species in Australia, including some previously undescribed species (Fay 2012, Royer 2015, Dominiak

et al. 2015). Many of these species were even more attracted to zingerone than CL. As it pertains to notable pest species, Dominiak et al. (2015) found zingerone to be an effective lure of *Bactrocera tryoni* (Froggatt), a highly destructive fruit fly pest in eastern Australia. Fay (2012) found zingerone to be the most attractive known lure to *Bactrocera jarvisi* (Tryon), a pest of mangos in northern Australia. In the latter study zingerone-baited traps captured 700 times as many males as traps baited with equal amounts of CL. Furthermore, in South Africa, Manrakhan et al. (2017) reported a strong response to zingerone from *Dacus frontalis* (Becker), a pest of cucurbits.

Following recent interest in zingerone as a male lure for fruit fly IPM, we investigated the field attraction of *Z. cucurbitae* to zingerone on the island of Oahu, Hawaii. Furthermore, we combined zingerone and CL to reveal any synergistic or inhibitory effects when these lures are used together.

Materials and Methods

Preparation of lures. The field experiment assessed five lure treatments containing zingerone (ZN), cue-lure (CL), or a unique ratio of the two lures mixed together. The treatments were: (1) 100% ZN, (2) 75% ZN + 25% CL, (3) 50% ZN + 50% CL, (4) 25% ZN + 75% CL, and (5) 100% CL. In preparation of the lures, treatments 4 (25% ZN + 75% CL) and 3 (50% ZN + 50% CL) were created by dissolving the solid ZN in the proportional amounts (by weight) of CL, which is a strong solvent. In treatment 2 (75% ZN + 25% CL), the amount of CL was insufficient to fully dissolve the ZN, therefore, the solid ZN was placed in a glass beaker and heated over boiling water for 5 minutes or until completely melted, and the melted ZN was combined with CL and mixed vigorously. In treatment 1 (100% ZN), the solid ZN

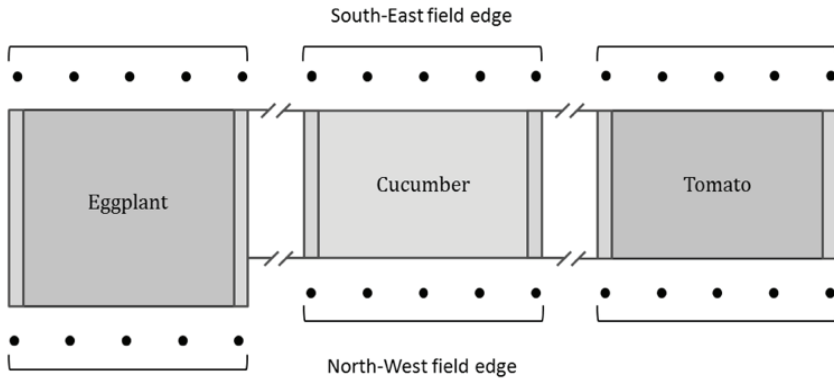


Figure 1. Experimental layout of Ho Farms in Kahuku, Oahu, Hawaii. Dark circles and brackets denote individual traps and blocks, respectively. Tomato and cucumber fields measured 0.8 ha in size, while the eggplant field measured 1.0 ha.

was melted using the heated bath method above. The zingerone is unlikely to have been altered by this melting method as it has high thermostability (Gopi et al. 2016), and similar studies have heated zingerone to its melting point using conventional microwave ovens (Shelly 2017, Royer 2015, Royer et al. 2017). Five grams of each lure treatment was applied to individual 1 x 7.5 cm cotton dental wicks.

Bucket traps were made from clear plastic containers (1 L vol.). Each trap was fitted with two entrance holes (2.5 cm diameter), and a metal wire was used to attach the traps to tree branches. Cotton dental wicks soaked with 5 g of 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 50 mm strip containing 10% dichlorvos insecticide (Vaportape II, Herculon Environmental, Emigsville, PA) was placed next to the lure, which was replaced with a fresh strip three weeks later.

Trap locations. *Zeugodacus cucurbitae* were trapped at Ho Farms in Kahuku, on the island of Oahu, Hawaii, from July 24 until September 4, 2015. Traps were placed 10 m from the outer edge of three

monocropped fields of *Z. cucurbitae* hosts: tomato (*Solanum lycopersicum* L.), cucumber (*Cucumis sativus* L.), and eggplant (*Solanum melongena* L.). Tomato and cucumber fields were 0.8 ha in size and the eggplant field was 1.0 ha.

For each host, five traps (one trap of each lure treatment) were placed randomly along the north-west field edge of each host and another five traps were placed randomly along each south-east field edge. Blocks of five traps were spaced at least 100 m apart, and traps within blocks were spaced 35 m apart. Separating traps far enough apart to eliminate interference between them requires knowledge that is currently incomplete of (1) CL and ZN volatility, and (2) relative *Z. cucurbitae* attraction to these lure volatiles. Therefore, traps were spaced far apart relative to similarly designed studies (see Royer 2015, Royer et al. 2017, Vargas et al. 2000, Vargas et al. 2010). Traps were placed at the edge of the field primarily to avoid disturbance from farm workers, but also because *Z. cucurbitae* are typically abundant on non-host plants bordering fields containing hosts (Prokopy et al. 2003, Nishida and Bess 1957, McQuate and Vargas 2007). Figure 1 presents a

schematic diagram of the trapping array.

Traps were placed 1.2–1.5 m above the ground in non-host (or rarely infested, see Vargas et al. 1990) trees; these were milo (*Thespesia populnea* L. Sol. Ex Correa), strawberry guava (*Psidium cattleianum* Sabine), haole koa (*Leucaena leucocephala* Lam. de Wit.), and turkey berry (*Solanum torvum* Sw.). Blocks along the north-west field edge were dominated by a dense overstory of milo, and blocks along the south-east field edge were dominated by an overstory of haole koa trees and an understory of grass with interspersed strawberry guava and turkey berry.

Trap monitoring. Traps were monitored weekly 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. A small number of *B. dorsalis* were captured in 100% ZN traps (which trapped fewer than 600 flies in all cases, and the total number of flies were counted). Trapped *B. dorsalis* were discarded and accounted for only <2% of flies in 100% ZN traps, therefore for other traps containing less ZN (and more CL) the number of *B. dorsalis* captured was likely negligible compared to relatively high numbers of *Z. cucurbitae*. 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. The relationship between lure treatment and trap capture was tested using a general linear model (GLM, RStudio 2016), with lure treatment, host, and week as fixed variables. Pairwise comparisons of lure treatment, host, and week were made using a Least-Squares

Means Tukey test. To test for synergistic or inhibitory effects of adding zingerone to CL an adjusted R-squared value was obtained using a standard least-squares fit model (LS, SAS Institute 2016) with quantity of CL as a continuous variable and the data from the 3 hosts pooled for a single analysis. For all tests the weekly trap capture figures were transformed using $\ln(X + 1)$ to obtain an adequate fit to a normal distribution.

Results

There was a significant difference between lure treatments (Fig. 2) (GLM, $F_{4,168} = 163.60$, $p < 0.0001$), hosts (GLM, $F_{2,168} = 5.21$, $p < 0.05$), and weeks (GLM, $F_{5,168} = 22.23$, $p < 0.0001$), with the model accounting for most of the variability in the data ($R^2 = 0.93$). The numbers of *Z. cucurbitae* captured generally declined with less CL, these treatments were “100% CL” (1,724 \pm 242 flies/week), “75% CL + 25% ZN” (943 \pm 141 flies/week), “50% CL + 50% ZN” (953 \pm 121 flies/week), “25% CL + 75% ZN” (551 \pm 110 flies/week), and “100% ZN” (39 \pm 9 flies/week). However, there was no difference in capture between the treatments “75% CL + 25% ZN” and “50% CL + 50% ZN” (Tukey, $p = 0.98$). The quantity of CL in the lure treatments was a strong predictor of the number of flies trapped (LS, $\text{adj-}R^2 = 0.65$), suggesting a linear relationship and a lack of synergistic or inhibitory effects from adding zingerone. Hosts differed in trap capture because fly capture was significantly greater in tomatoes (1,105 \pm 151 flies/trap) than in cucumbers (877 \pm 150 flies/trap) or eggplants (544 \pm 72 flies/trap) (tomatoes decomposing on the ground were in abundance throughout the study and they harbored many larvae, but few decomposing cucumbers and eggplants were observed). Differences between weeks is due to lower capture in weeks 2 (573 \pm 121 flies/trap) and 4 (283 \pm 61 flies/

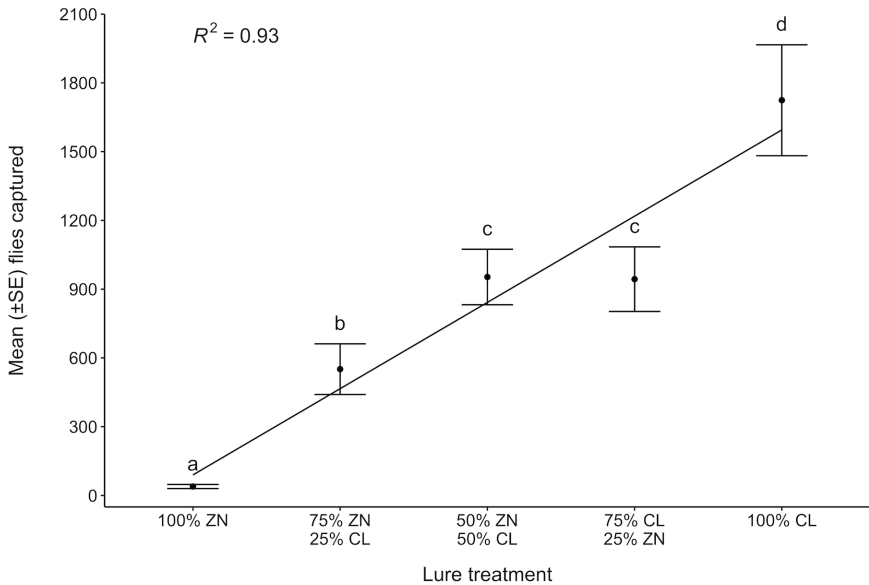


Figure 2. Mean (\pm SE) melon flies (*Z. cucurbitae*) captured weekly in individual traps. “ZN” and “CL” denote zingerone and cue-lure, respectively. Each trap contains 5 grams of total lure with percentages of ZN and CL given.

trap) compared to all other weeks (1,049 \pm 104 flies/trap) (weeks with lower capture coincided with major storm events).

Discussion

Previous work with mixing male lures has focused on combining ME and CL into one lure matrix to attract both ME- and CL-responding fruit fly species. Such mixtures could reduce the number of traps and labor hours needed to build and maintain large trapping arrays (Shelly et al. 2004, Liu 1989, Hooper 1978, Vargas et al. 2000). Interestingly, multiple studies have observed greater attraction of *Z. cucurbitae* to CL- and ME mixtures when compared to CL alone (Shelly et al. 2004, Liu 1989, Hooper 1978). However, Vargas et al. (2000), using a similar experimental design to the present study, found little effect of CL and ME mixing on *Z. cucurbitae* capture rates, except that reduced *Z.*

cucurbitae capture was observed when ME in a high dosage and CL in a low dosage (vol:vol) were combined. This effect was likely attributed to the different volatilities of the two lures (for chemical analysis see Vargas et al. 2015) as well as possible interference from high numbers of oriental fruit fly males, which may have reduced the number of *Z. cucurbitae* males entering the traps.

The present study observed no synergistic or inhibitory effects of mixing zingerone and CL as observed for ME and CL mixtures. Instead, trap captures were linearly and positively dependent on the amount of CL in the lure, with higher amounts of CL resulting in more flies captured. One treatment (“75% CL + 25% ZN”) deviated from this trend, which may have been due to trap interference from close proximity between traps (35 m) and high lure dosage (5 g per trap).

Furthermore, traps containing this lure treatment may have been randomly placed in locations where fewer flies were present. However, any interaction was not apparent with the other lure treatments. The ineffectiveness of zingerone may be due to its low volatility compared to CL (Hanssen 2015). Lures must volatilize into the atmosphere, where males fruit flies can detect the compound and orient themselves towards it. Other male lure formulations, such as raspberry ketone formate and melolure (Metcalf and Metcalf 1992) have managed to attract higher numbers of *Z. cucurbitae* by increasing volatility. The low volatility of zingerone is also evident in other field trapping studies, where zingerone showed no marked reduction in attraction over 23 months (Dominiak et al. 2015), 3 months (Fay 2012), and 8 weeks (Royer 2015, Royer et al. 2017).

Further trapping research with zingerone may have significant ecological value to fruit fly research. Several undescribed species, never before captured in CL or ME baited traps, have been captured in zingerone traps (Fay 2012, Dominiak et al. 2015, Royer 2015, Royer et al. 2017). These results indicate that zingerone may be an important lure for some fruit fly species in nature, whereas previously fruit flies have only been observed feeding on raspberry ketone and ME in nature. Further zingerone-trapping surveys in areas with established fruit fly populations may yield more undescribed species, as well as elucidate general questions surrounding the phenomenon of lure attraction by male fruit flies. Further investigation into zingerone may also produce applications for managing and detecting select fruit flies, as has been the clear case for *B. jarvisi* in Australia and *D. frontalis* in Africa. Nevertheless, our observations suggest that employing zingerone traps (either alone or mixed with CL) for the detection and control of Hawaiian *Z. cucurbitae* would

likely be ineffective.

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