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Capture of *Zeugodacus cucurbitae* (Diptera: Tephritidae) in traps baited with torula yeast solution versus cucumber volatile plugs

Todd Shelly[1,], Rick Kurashima[1], Jon Nishimoto[1], and Earl Andress[2]*

Abstract

Several species of invasive fruit flies (Diptera: Tephritidae) are among the most damaging pests of agricultural crops in the world. Detection of these pests relies primarily on traps baited with male-specific lures. Traps with protein food baits are also employed to attract females, but these baits are fairly weak, and there is considerable interest in developing more powerful lures for female tephritids. Recently, a plug that emits cucumber volatiles was developed as a lure for cucurbit-infesting species, and a recent field study showed that traps baited with these plugs attracted more females of the Australian cucurbit pest *Bactrocera cucumis* (French) than traps containing a protein bait. The objective of the present study was to gather additional data regarding captures of a closely related species, the melon fly, *Zeugodacus cucurbitae* (Coquillett), in traps baited with a torula yeast and borax (TYB) solution (a standard food lure) compared with traps baited with the cucumber volatile (CV) plug. Data collected from 5 locations on Oahu, Hawaii, showed that traps baited with the TYB solution captured significantly more melon flies than traps baited with the CV plugs. At 4 sites, where hosts were scattered, melon fly captures were relatively low, and males were trapped in significantly greater numbers than females in both TYB- and CV-baited traps. At the remaining site, hosts were very abundant, melon fly captures were high, and females were trapped in significantly greater numbers than males in both TYB- and CV-baited traps. Possible explanations for the superior performance of TYB-baited traps are discussed.

Key Words: melon fly; fruit fly; trapping; lure; *Bactrocera cucurbitae*

Resumen

Varias especies de moscas invasoras de la fruta (Diptera: Tephritidae) están entre las plagas más dañinas de los cultivos agrícolas en el mundo. La detección de estas plagas se basa principalmente en trampas cebadas con señuelos específicos para los machos. Se emplean con cebos de alimentos de proteínas también para atraer a las hembras, pero estos cebos son bastante débiles, y hay un interés considerable en el desarrollo de señuelos más efectivos para las hembras tefrítidas. Recientemente, un tapón que emite volátiles de pepino fue desarrollado como un señuelo para las especies que infestan las cucurbitáceas, y un reciente estudio de campo mostró que las trampas cebadas con estos tapones atrajeron más hembras de la plaga australiana de cucurbitáceas *Bactrocera cucumis* (French). El objetivo del presente estudio fue recopilar datos adicionales sobre la captura de una especie estrechamente relacionada, la mosca del melón, *Zeugodacus cucurbitae* (Coquillett), en trampas cebadas con una solución de levadura de torula y borax (LTB) (un señuelo de alimento estandard), en trampas cebadas con el tapón del volátil del pepino (VP). Los datos recopilados en 5 localidades de Oahu, Hawai, mostraron que las trampas cebadas con la solución de TYB capturaron significativamente más moscas de melón que las trampas cebadas con los tapones de VP. En 4 sitios, donde los hospederos fueron dispersos, las capturas de la mosca del melón fueron relativamente bajas, y los machos fueron atrapados en un número significativamente mayor que las hembras tanto en las trampas cebadas con TYB y CV. En el sitio restante, los hospederos fueron muy abundantes, las capturas de moscas de melón fueron altas y las hembras fueron atrapadas en números significativamente mayores que los machos en trampas con cebos con TYB y VP. Se discuten las posibles explicaciones para el desempeño superior de las trampas con cebo con TYB.

Palabras Clave: mosca de melón; mosca de la fruta; captura; señuelo; *Bactrocera cucurbitae*

Several species of invasive fruit flies (Diptera: Tephritidae) are among the most damaging pests of agricultural crops in the world (Enkerlin 2005). Collectively, females of these pestiferous taxa deposit eggs, and larvae subsequently develop, in dozens of important fruits and vegetables (White & Elson-Harris 1992). These fruit flies cause direct damage to the crops and disrupt international trade via quarantines or other trade restrictions (Follett & Neven 2006; Jang et al. 2014). Because of this large agricultural threat, many countries operate surveillance and detection programs for invasive tephritids (IPRFFSP 2006; Gonzalez & Troncoso 2007; Jessup et al. 2007). Detection of invasive fruit fly pests relies primarily on the area-wide deployment of traps baited with male-specific lures such as cue-lure, which is attractive to males of the melon fly, *Zeugodacus cucurbitae* (Coquillett) (Jang & Light 1996). The new generic classification of the melon fly is here adopted (De Meyer et al. 2015; Virgilio et al. 2015).

In addition to male lures, food baits are used in trapping programs, because they have general attractiveness and are neither sex- nor species-specific (Heath et al. 1995). In addition, males of many tephritid species do not respond to known male lures (IAEA/FAO 2013), and consequently food lure trapping is the only feasible monitoring method. Food

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lures are typically delivered as an aqueous slurry of torula yeast and borax (Lopez et al. 1971; Burditt 1982) or as dry synthetic bait composed of several components (ammonium acetate and putrescine with or without trimethylamine; Epsky et al. 1999; Thomas et al. 2001). Compared with male lures, however, available food baits are relatively weak (Calkins et al. 1984), and there is considerable interest in developing more powerful lures specifically targeting female tephritids. Improved female lures would not only increase trapping sensitivity but would serve as an effective tool in programs to suppress fruit fly populations via direct removal of females and their future progeny (Jang & Light 1996).

Host fruit odors represent an alternative type of female-biased lures, though the presence of competing natural fruit volatiles may limit their effectiveness (Reissig et al. 1982). The use of fruit odors in traps has been developed most extensively for the apple maggot fly, *Rhagoletis pomonella* (Walsh), where apple volatiles have been used in control programs based on attract-and-kill trapping (Bostanian & Racette 2001). Siderhurst & Jang (2006, 2010) identified compounds in host fruit odors that elicited electroantennogram and behavioral responses in females of *Bactrocera dorsalis* (Hendel) and *Z. cucurbitae*. Regarding the latter species, Siderhurst & Jang (2010) identified 31 compounds in cucumber, *Cucumis sativus* L. (Cucurbitaceae), a preferred host of *Z. cucurbitae* (Miller et al. 2004), that elicited electroantennogram responses from melon fly females, and the attractiveness of individual compounds was screened in behavioral assays. Various synthetic blends were subsequently tested in a large outdoor cage, and a 9-component blend (Lure #7) was found to be most attractive to females. Importantly, in field trials, this blend was more attractive to *Z. cucurbitae* females than a protein bait (Solulys AST). Based on these results, a cucumber volatile plug was developed and is now commercially available. In the only published study on this new plug, Royer et al. (2014), working on a species (*Bactrocera cucumis* [French]) whose males are unresponsive to any male lure, found that traps baited with this cucumber plug captured more flies than traps baited with food lures (either orange ammonia or Cera Trap®).

The objective of the present study was to gather additional data comparing captures of *Z. cucurbitae* in traps baited with the standard torula yeast and borax solution versus traps baited with the cucumber volatile plug. Results are presented for 6 or 8 wk trapping intervals conducted at 5 locations on Oahu, Hawaii.

Materials and Methods

STUDY SITES

Trapping was conducted in 2011 at 5 locations on Oahu, Hawaii, namely A) Kapolei (Aloun Farm) during Jan–Mar, B) Waialua during Apr–May, C) Waimanalo during May–Jun, D) Waipio (Aloun Farm) during Jun–Jul, and E) Haleiwa during Oct–Nov. Aside from the Waipio location, host plants (Cucurbitaceae) were scattered and not particularly abundant at the trapping sites and consisted primarily of zucchini (*Cucurbita pepo* L.), bitter melon (*Momordica charantia* L.), and ivy gourd (*Coccinia grandis* [L.] Voigt). The Kapolei site is agricultural but was fallow at the time of trapping, the Waialua and Waimanalo sites are largely residential areas with backyard gardens, and the Haleiwa site is a coffee planting where host plants, notably bitter melon, grow in gullies along the field's perimeter. In contrast, the agricultural Waipio site, when sampled, had an abundance of ripe zucchini, as reflected in the high melon fly captures at this location (see results). All sites were at low elevation (<100 m) with comparable temperatures (with daily minimum and maximum temperatures generally between 20 and 24 °C and between 27 and 31 °C, respectively; AccuWeather.com).

BAITS, TRAPS, AND SAMPLING PROTOCOL

Capture of melon flies was compared between 2 baits—torula yeast and borax (TYB) solution and cucumber volatile (CV) plugs—, both of which were presented in Multilure traps (Better World Manufacturing, Fresno, California). Traps baited with TYB are hereafter referred to as TYB traps, and those baited with CV plugs are termed CV traps. The TYB solution was prepared by placing 1 TYB pellet (5 g total = 2.25 g of torula yeast + 2.75 g of borax; Scentry Biologicals Inc., Billings, Montana) per 100 mL of a 90% aqueous solution of commercial antifreeze (a 14% solution of propylene glycol, Splash RV & Marine Antifreeze, Fox Packaging Co., St. Paul, Minnesota). After a 2 d ageing period, an aliquot of 300 mL of this solution was placed in individual Multilure traps, which are plastic McPhail-type traps with a transparent top and yellow base (which holds the liquid) containing a central, open invagination to allow fly entry (IAEA/FAO 2013). Attracted flies drown in the food bait slurry.

The CV plugs (0.9 g total weight, 0.3 g a.i.; Scentry Biologicals Inc., Billings, Montana) contain 7 major volatiles of cucumber fruits, which were among the most attractive compounds in previous assays (Siderhurst & Jang 2010). These 7 compounds are 1-octen-3-ol, (*Z*)-6-nonenal, *E*-2-nonenal, (*E*,*Z*)-2,6-nonadienal, hexanal, 1-hexanol, and (*Z*)-6-nonen-1-ol (A. Ramsay, personal communication). The CV plugs were also deployed in Multilure traps, with 1 plug placed in a perforated well in the upper part of the trap and with 300 mL of the same propylene glycol solution described above (without TYB pellets) placed in the yellow base.

With a few exceptions, the same trapping protocol was used at the 5 sites. At each site (except Waipio), 15 Multilure traps containing TYB solution and 15 Multilure traps containing CV plugs were deployed in alternating fashion along service roads at farms (Kapolei, Waipio), rural streets in residential areas (Waialua, Waimanalo), or the perimeter of a commercial coffee planting (Haleiwa). At Waipio, 10 Multilure traps were deployed for each attractant. Traps were placed on non-host trees (most commonly haole koa, *Leucaena leucocephala* (Lamarck) de Wit' Fabaceae) at 2 to 3 m above ground in shaded locations, and adjacent traps were separated by a minimum of 30 m. All traps were serviced on a weekly basis over a 6 wk period (except for Kapolei, where trapping was conducted for 8 wk). The liquid was replaced weekly for all traps, with the used liquid poured through a sieve in the field to collect captured insects. In contrast, the CV plugs were used continually (i.e., were not replaced) during a sampling period. The TYB and CV treatments were alternated at trap positions between successive sampling intervals. Melon flies were identified by sex and counted using a magnifying lamp in the laboratory.

DATA ANALYSES

For all traps, catch data were tabulated on a weekly basis and expressed as flies per trap per day (FTD). Preliminary inspection of these values suggested that the 5 sites could be assigned to 3 categories: sites B, C, and E had low captures (average weekly FTD were less than 6 for both sexes for both attractants), site A had intermediate captures (average weekly FTD were 2–6 for females and 6–12 for males for both attractants), and site D had high captures (average weekly FTD were generally greater than 10 for both sexes for both attractants). Although all were based on ANOVA, the analyses differed somewhat among these 3 categories as described below. In all cases, raw FTD values were $\log_{10}$ transformed to normalize the data, and upon detection of a significant effect, the Holm–Šídák method for multiple comparisons was employed to identify pairwise differences. Note that, although normality was not achieved in all cases, the deviations did not appear to influence the analyses markedly, as performing the tests using ranked data in 2-way (Conover & Iman 1981) and 1-way (Kruskal–Wallis test) non-parametric ANOVA yielded similar results.

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Although derived from different locations and at different times, data for the low capture sites were pooled for analysis, not only because trap catch was similar among them, but also because captures varied independently of time at all sites. In preliminary analyses, 2-way ANOVAs, with week and bait type as the main effects, were conducted separately for females and males at each site, and in no instance was week found to have a significant effect on captures. Moreover, in no test was the interaction (week × bait type) significant, indicating that the differences observed between bait types were consistent across time. Based on these results, pooled data from the 3 low-capture sites were analyzed—independently of sampling week—using a 2-way ANOVA with sex and bait type as the main effects. Inspection of the plotted data indicated large temporal variation in captures at the intermediate (A) and high (D) sites; consequently, analyses for these sites involved 3-way ANOVA with the main factors of sex, bait type, and week. Note that inter-site comparisons were not drawn because our focus was on potential variation in trap catch between 2 bait types, not spatiotemporal variation in melon fly populations (see Leblanc et al. 2012, who supply this information for Oahu).

Results

For the sites with low capture levels, sex ($F_{1, 1076}$ = 10.0; P = 0.002) and bait type ($F_{1, 1076}$ = 197.1; P < 0.001) had significant effects on captures of *Z. cucurbitae*. However, the interaction was significant as well ($F_{1, 1076}$ = 6.2; P = 0.01). This latter result reflected the fact that the sexual difference in trap captures resulted largely from a difference between females and males in their response to TYB traps relative to CV traps. Specifically, CV traps captured similar numbers of males and females, whereas TYB traps captured greater numbers of males than females (Fig. 1).

For site A with intermediate captures, sex ($F_{1, 448}$ = 100.2; P < 0.001), bait type ($F_{1, 448}$ = 181.8; P < 0.001), and week ($F_{7, 448}$ = 3.4; P = 0.001) all had significant effects on captures. Interpretation is confounded, however, by significant interactions for sex × week ($F_{7, 448}$ = 3.1; P = 0.003) and bait type × week ($F_{7, 448}$ = 3.8; P < 0.001). Neither the sex × bait type nor the 3-way interactions were significant (P > 0.05 in both cases). Examination of the plotted data (Fig. 2) allows clarification of these results. First, TYB traps had higher trap catch than CV traps for both females (t = 9.1; P < 0.001) and males (t = 9.5; P < 0.001. Second, more males were captured than females in both TYB (t = 6.2; P < 0.001) and CV (t = 5.8; P < 0.001) traps. The significance of interaction terms involving week reflected the finding that captures of the 2 sexes and the 2 trap types were not consistent over time. For example, female captures were relatively high in wk 5 and low in wk 6, whereas male captures showed the opposite trend. Similarly, aside from wk 8, female captures in CV traps were uniform over time, whereas male catch in CV traps showed much greater temporal variability.

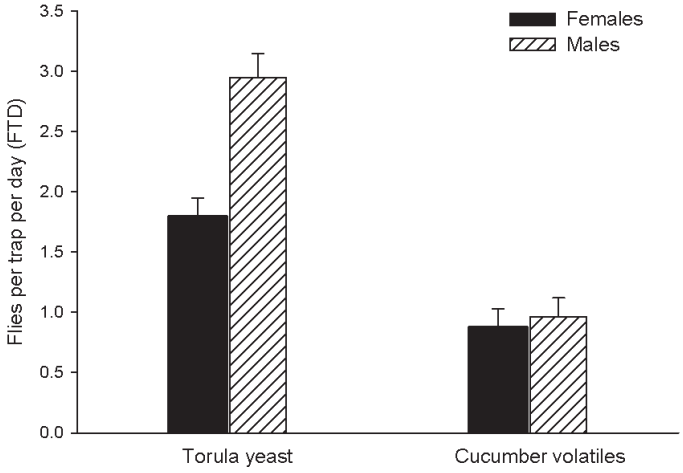


Fig. 1. Captures of melon flies, *Zeugodacus cucurbitae*, in Multilure traps baited with torula yeast borax solution or cucumber volatile plugs at low-capture sites (B, C, and E). Data were pooled among sites and over sampling weeks as described in the text. Bar heights represent means (± 1 SE) of 270 values (3 sites × 15 traps per trap type × 6 wk).

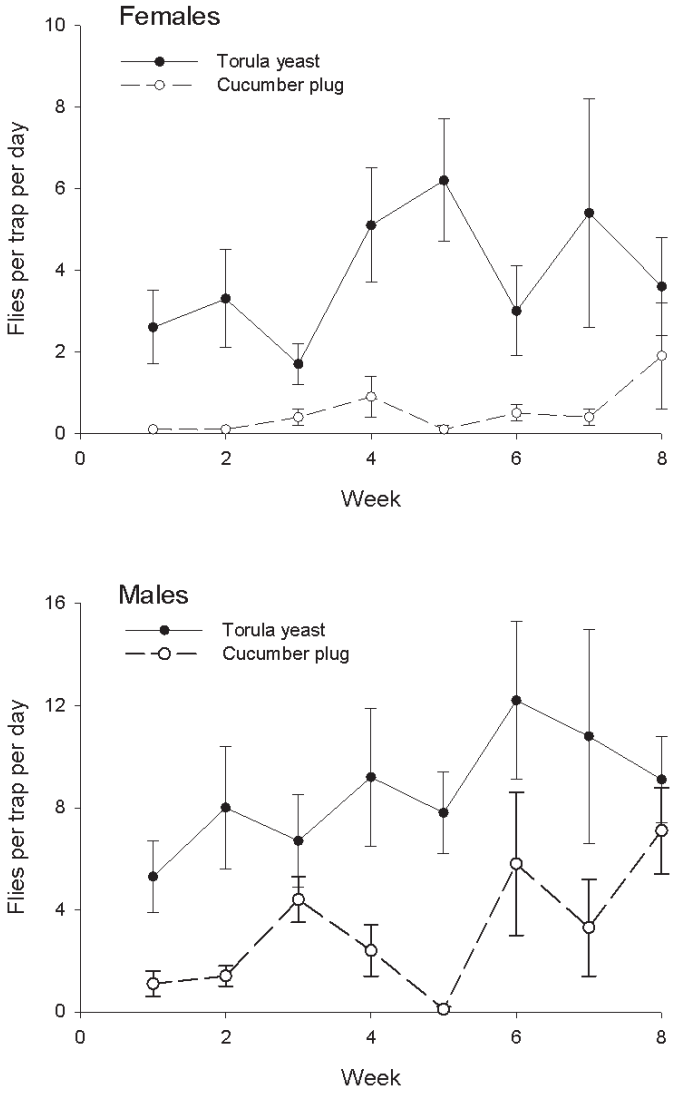


Fig. 2. Captures of melon flies, *Zeugodacus cucurbitae*, in Multilure traps baited with torula yeast borax solution or cucumber volatile plugs over an 8 wk period at the intermediate-capture site (A). Points represent means (± 1 SE) of 15 traps per lure type.

For the high-capture site (D), sex ($F_{1, 216}$ = 10.8; P = 0.001), bait type ($F_{1, 216}$ = 37.5; P < 0.001), and week ($F_{5, 336}$ = 18.9; P = 0.02) all had significant effects on captures. None of the interactions were significant (P > 0.05 in all cases). Unlike the previous sites, greater numbers of females than males were captured at site D (t = 3.3; P = 0.001), but as noted for the other sites, the TYB traps captured more flies than the CV traps (t = 6.1; P < 0.001; Fig. 3).

Discussion

Similar to Siderhurst & Jang (2010) and Royer et al. (2014), we compared fly catch in traps baited with a host (cucumber) odor versus a food

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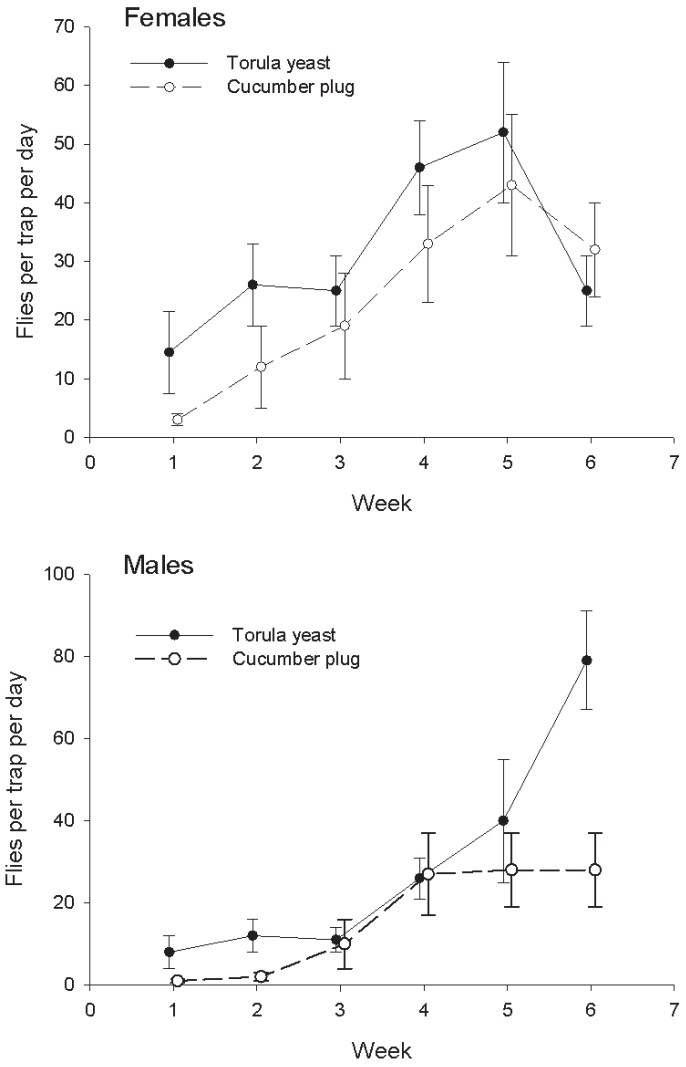


Fig. 3. Captures of melon flies, *Zeugodacus cucurbitae*, in Multilure traps baited with torula yeast borax solution or cucumber volatile plugs over a 6 wk period at the high-capture site (D). Points represent means (± 1 SE) of 10 traps per lure type.

(protein) odor. In contrast to those earlier studies, however, the present findings show that the food-based lure was more attractive than synthetic cucumber volatiles. Moreover, this pattern was observed over 5 study sites and at different times of the year. At present, any explanation of the difference between our study and previous ones is conjecture, but broadly interpreted, it likely reflects variation in the relative availability of proteinaceous food and host plants in the different habitats where CV traps were tested and/or differences in the age structure of the populations sampled.

Protein consumption is critical for the reproductive success of female tephritid fruit flies. As in other tephritid species, females of *Z. cucurbitae* are anautogenous (Drew & Yuval 2000) and must consume protein to initiate egg development when immature and, after mating, to continue egg development to maximize egg output (Meats & Leighton 2004). Although males likely have lower protein requirements than females, data from *B. dorsalis* (Shelly et al. 2005), *Z. cucurbitae* (McInnis et al. 2004), and *Bactrocera tryoni* (Froggatt) (Perez-Staples et al. 2007) reveal that protein intake, at least during sexual maturation, is necessary to obtain copulations.

Focusing on females, several studies have documented the importance of physiological state upon responsiveness to food versus host fruit odors. Experimental manipulation of the nutritional history has demonstrated that, in laboratory or field-cage trials, protein-deprived and/or immature females with small egg loads oriented preferentially to protein odors over host fruit odors, whereas the opposite was true for protein-fed, mature females with large egg loads (Prokopy et al. 1991; Cornelius et al. 2000a; Miller et al. 2004; Piñero et al. 2011; Balagawi et al. 2014). Field observations are consistent with these results (Cornelius et al. 1999, 2000b). Data on *Bactrocera* males are more variable: fruit-baited traps caught significantly more *B. dorsalis* males than protein-baited traps in one field study (Cornelius et al. 2000b), whereas captures were similar between the 2 trap types in another (Cornelius et al. 2000a).

In light of these data, the present finding that *Z. cucurbitae* females were captured in greater numbers in TYB traps than CV traps likely reflects lower availability of proteinaceous food relative to the habitats sampled by Siderhurst & Jang (2010) and Royer et al. (2014). The alternative explanation of differing age structures among populations seems less likely, because it implies a consistently higher proportion of immature, protein-hungry females in the populations sampled in the present study. However, as noted, trapping was conducted throughout the year and hence was unlikely to have sampled populations of similar age structure. It also seems unlikely that the lower female catch of CV traps observed in the present study reflected greater competition from natural host fruit odors. Such competition may have occurred at site D (Waipio), where hosts and *Z. cucurbitae* were abundant, but host plants were not common at the remaining 4 sites.

For site D, a commercial planting of zucchini, the higher catch of females in TYB traps than CV traps is contrary to prior speculation and data. In their study of the melon fly in Hawaii, Nishida & Bess (1957) made sweep samples of flies both in and around a tomato (*Solanum lycopersicum* L. [Solanaceae], a host plant in Hawaii) field and reported a strong female bias in captures made inside the crop. They further speculated that females entered the tomato plot to oviposit rather than to feed; indeed, most females captured there were found to be gravid upon dissection. Feeding was thought to occur in non-host vegetation surrounding the crop area. Siderhurst & Jang (2010) and Royer et al. (2014) conducted their studies in commercial plots containing host plants, and their common finding—CV traps captured more females than TYB traps—is consistent with Nishida & Bess's (1957) suggestion. However, our data suggest that although the female bias in captures in a crop area likely reflects intended oviposition, females in crop areas also feed opportunistically on available protein. Based on the nutritional status (total body nitrogen and carbon) of wild *B. tryoni*, Balagawi et al. (2014) proposed that females are not protein limited in the field, which may explain the low attractancy of protein bait sprays to this species (Balagawi et al. 2012). Again, however, the present results suggest that in our study areas, *Z. cucurbitae* females were protein limited, and the demonstrated effectiveness of protein bait sprays in suppressing melon fly populations in Hawaii supports this interpretation (Prokopy et al. 2003; Jang et al. 2008; Piñero et al. 2009).

The present data on male captures also differed from those reported by Siderhurst & Jang (2010) and Royer et al. (2014). Here, as with females, *Z. cucurbitae* males were captured in greater numbers in TYB traps than CV traps at all 5 study sites. In contrast, and consistent with Cornelius et al. (2000a), Siderhurst & Jang (2010) found similar numbers and Royer et al. (2014) found similar proportions of males in food-baited and CV-baited traps. Reasons for the discrepancy between the present and previous studies are unknown but again may involve the scarcity of protein sources in the 5 habitats sampled herein. Additionally, males of *Z. cucurbitae* appear to establish mating aggregations (leks) primarily on non-host plants (Iwahashi & Majima 1986). As males do not defend host fruits as mating territories, they would presumably show relatively weak attraction to host plant odors.

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Siderhurst & Jang (2010) and Royer et al. (2014) conducted trapping in crop areas, but the present study also compared captures in CV traps and TYB traps in non-crop areas (sites A, B, C, and E). Surprisingly, in areas with scattered hosts and relatively low densities of *Z. cucurbitae* populations, males were caught in significantly greater numbers than females in both trap types. Given the lower protein requirements of males, it is not known why a male-bias characterized the trap catch in low-density populations.

In conclusion, the relative attractiveness of food- and host-baited traps to tephritid fruit flies is not static but varies temporally with changes in the availability of different food and oviposition sources in the environment and the physiological state of the majority of the adults. Understanding the dynamic nature of trap attractiveness is difficult given the large number of relevant parameters and the associated interactions among them. Much progress has been made on understanding the importance of fly physiological state (Díaz-Fleischer et al. 2009 and references therein), while far less is known about the impact of available food and oviposition resources on trap effectiveness. It is generally assumed that, with respect to its availability to fruit flies, nitrogen is scarce in the environment and thus is a central target of tephritid foraging behavior (Drew & Yuval 2000 but see Balagawi et al. 2014). Although the present study did not measure the physiological and nutritional condition of trapped flies, the consistently greater captures of both males and females of *Z. cucurbitae* in TYB over CV traps strongly suggest protein sources were scarce in the 5 sites sampled on Oahu.

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