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Effect of Physiological State on Female Melon Fly (Diptera: Tephritidae) Attraction to Host and Food Odor in the Field

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Abstract

Foraging behavior of wild female melon fly, *Bactrocera (Zeugodacus) cucurbitae* Coquillett, a worldwide pest of economically important cucurbit crops, was examined through mark and recapture studies in both wild (*Kona*: dominated by the invasive weed ivy gourd, *Coccinea grandis* [L.] Voigt [Cucurbitaceae]), and cultivated (*Kapoho*: dominated by papaya, *Carica papaya* L. [Caricaceae] orchards) habitats on Hawaii Island. In particular, the extent to which wild melon flies and color-marked F_2 females responded to cucumber odor and Solulys yeast hydrolysate laced with ammonium acetate (1%, wt/vol) according to sexual maturity stage and degree of protein hunger was documented. Kona results indicated that more wild and color-marked F_2 females responded to cucumber (*Cucumis sativus* L. [Cucurbitaceae]) odor than to protein odor with the exception of captured wild flies without eggs, which responded similarly to protein bait and cucumber odor. Results with captured wild females and color-marked F_2 females in Kapoho suggested a significant preference for cucumber odor over protein odor regardless of whether or not they had eggs in their ovaries with the exception of protein-deprived color-marked F_2 females, which responded to both odors in equal numbers. Implications of these new findings based on wild melon flies in natural habitats are discussed with respect to integrated pest management control strategies with protein bait sprays used in Hawaii. The possibility of adding cucurbit volatiles to protein-based baits is discussed.

Key words: foraging behavior, management, protein bait spray, cucumber volatile

How insects locate and exploit essential resources depends on the interplay of several factors such as the genetic structure, physiological state, and sensory capabilities of the insect as well as environmental conditions, including host availability and suitability, all of which influence foraging behavior (Prokopy et al. 1991). For instance, dietary history and age (an indicator of reproductive state) are physiological factors influencing the response of female fruit flies (Diptera: Tephritidae) to food and host odors (Tschinkel 1985, Barton-Browne 1993, Rull and Prokopy 2000, Piñero et al. 2002). In terms of age effects on fruit fly foraging behavior, after emergence, the adults of all species of frugivorous fruit flies have a preoviposition period during which their activities are more devoted to dispersal and location of food sources rather than oviposition sources (Fletcher 1973, Fletcher and Prokopy 1991). Consequently, female fruit flies often exhibit a behavioral switch from searching primarily for protein sources after eclosion to searching for host fruit after ovarian development and mating (Jang 1995, Prokopy et al. 1996, Miller 2004).

Although most investigations have studied the need for protein for ovarian development and production of eggs, there are a few investigations with *Bactrocera* species documenting that sexually mature females are more attracted to host fruit odor than to proteinaceous odors. For example, Cornelius et al. (2000a) documented that twice as many sexually mature female oriental fruit flies, *Bactrocera dorsalis* (Hendel) were captured in McPhail traps baited with host fruit odors than in protein-baited traps. Such preference of sexually mature females for host-based odor over protein baits is known to influence the efficacy of protein bait sprays (Prokopy et al. 2003, Miller et al. 2004).

Native to Southeast Asia (Virgilio et al. 2010), the melon fly, *Bactrocera (Zeugodacus) cucurbitae* Coquillett, has been the most important pest of economically important cucurbit crops in the Hawaiian Islands since its introduction about 1895 (Back and Pemberton 1917, Nishida 1953). 'Attract-and-kill' strategies that utilize attractive proteinaceous baits mixed with insecticides are commonly implemented to reduce the application of pesticides (Steiner 1955, Nishida and Bess 1957, Roessler 1989, Vargas et al. 2001, Prokopy 2003). Previously, the most commonly used bait formulation was Nu-Lure Insect Bait (Miller Chemical and Fertilizer Corporation, Hanover, PA), a yeast-based protein attractant mixed with the contact poison malathion (Mangan and Moreno 1995). More recently, use of this product has mostly been replaced in Hawaii by GF-120 NF Naturalyte Fruit Fly Bait (Vargas et al. 2009, 2015), which utilizes the toxicant spinosad in conjunction with phagostimulants and protein-based attractants (Dow Agrosciences 2009).

As stated above, protein baits are expected to be most effective when applied against sexually immature, protein-seeking female fruit flies. However, it has been documented (J. C. Piñero et al., unpublished data) that *B. cucurbitae* females as young as 1-wk old are attracted to and feed upon the main host, cucumber fruit, and that the response to cucumber odor increases steadily according to age, with about 70% of the females responding to cucumber odor when they reach sexual maturity. Such a comparatively high level of response of young females to cucumber odor could present an additional challenge to the effectiveness of bait sprays that are commonly applied to the borders of cucurbit fields.

Evaluations of protein baits have usually been done in large field cages with laboratory-reared flies of known physiological states (Miller et al. 2004, Revis et al. 2004, Vargas and Prokopy 2006). Results from these studies could vary from outcomes observed in the field with wild flies where physiological states are not known. Assessing the effectiveness of particular baits to attract females of different physiological states is particularly complicated in wild populations of fruit flies due to the difficulty in determining age and degree of hunger. Rull and Prokopy (2000) developed a technique to overcome these problems in studying apple maggot fly, *Rhagoletis pomonella* Walsh, in apple orchards by releasing color-marked lab-reared apple maggot flies of known ages and physiological states and by comparing their response to environmental cues to that of wild flies.

The objective of this study was to assess the extent to which wild melon flies responded to host fruit odor and protein baits according to sexual maturity stage and degree of protein hunger in two habitats. The first site was on the dry leeward side of Hawaii Island in vegetation dominated by the invasive weed *Coccinea grandis* (L.) Voigt (Cucurbitaceae), and the second site was on the windward side in papaya (*Carica papaya* L. [Caricaceae]) orchards. Papaya is a less preferred host plant of *B. cucurbitae* (Piñero et al. 2017a).

Materials and Methods

Released Flies

All flies used for marked release in this study were F_2 generation, and all adults were reared in papayas in the laboratory. Parental flies were collected as larvae from fallen fruit of papaya, *C. papaya* L., in the Kapoho area on the east side of Hawaii Island. After eclosion, adult flies were held in cages (30 × 30 × 30 cm). Flies were kept in caged cohorts with equal numbers of males and females (~150 each sex). All flies were given sugar and water *ad libitum*. Flies were divided into three dietary groups for Kona and two groups for Kapoho. Females in the first group (designated as protein-fed flies) were fed a 3:1 mixture of USB enzymatic yeast hydrolysate (United States Biochemical, Cleveland, OH) and sugar from eclosion, and they were released and tested when they were 4-wk old. Females in the second group (designated as 48-h protein-starved) were fed with the yeast hydrolysate–sugar mixture until 48 h before they were released and tested. This group represented females with an assumed moderate level of protein hunger; females were also 4-wk old when tested. The third group (designated as protein-starved flies) included females that were provided sugar but no protein throughout their adult lives, and they were tested when they were 2- to 3-wk old. Female fruit flies require protein sources for adequate egg production (Epsky et al. 2014 and references therein). For the study at Kapoho, the treatment involving 48-h protein-starved females was dropped because of insufficient fly supply and similarity of fly response recorded from the Kona study, compared with protein-fed females.

Test Plots

Experiments were conducted on both the windward and leeward sides of Hawaii Island, HI, to examine the behavior of wild melon flies from two distinct habitats. On the windward side, experiments were conducted in papaya orchards, which were devoid of ground cover, and located in the Kapoho area, in the district of Puna. On the leeward side, experiments were conducted approximately 1 km from Kealakekua Bay in the district of South Kona. Habitat in this area consisted of patches of the invasive weed ivy gourd (*C. grandis* Voigt), an important melon fly host (Uchida et al. 1990), growing as vines in the branches of kiawe (*Prosopis pallida* [Humb. & Bonpl. ex Willd.] Kunth) trees. Tall grass (~50 cm) covered the area. Experiments were conducted each week for a period of 4 wk in south Kona and over a period of 7 wk in Kapoho. Mean temperatures for Kona and Kapoho were 26 ± 1.5 and 28 ± 2°C (mean ± SD), respectively, during testing. Mean rainfall during the days of testing were 2.05 ± 2.39 cm for Kona and 0.18 ± 0.19 cm (mean ± SD) for Kapoho. Each location contained two experimental plots (5 × 5 m each) located 20 m apart. Each plot had four baited stations with odor arranged to form four corners of a square. Each station consisted of a black plant propagation flat (40 × 40 × 6 cm) turned upside down and placed on top of a piece of blue fiberboard that extended out 2 cm on each side of the flat. Two yellow-painted (Cadmium Yellow Medium; Windsor and Newton Finity Series, Ltd., London, United Kingdom) plastic hemispheres (8 cm diameter) covered in Tangletrap insect coating (Tanglefoot Company, Grand Rapids, MI) were wired to the top of each black flat to capture arriving flies. A 10- × 15-cm white tray was placed beneath each flat (i.e., on top of each blue fiberboard). In each square, there were two bait stations with protein odor and two with host fruit odor. Bait stations with like odors were positioned across from each other diagonally in the square. The stations with protein odor contained ~200 ml of a solution of 4- to 5-d-old Solulys yeast hydrolysate (Corn Products, Argo, IL; 88% water, 8% Solulys, and 4% Borax) containing ammonium acetate (1%, wt/vol; Sigma Aldrich, Milwaukee, WI). The protein bait was poured into the bottom of the white tray to cover eight cotton dental wicks (15 cm long) placed in the bottom of the tray. Preliminary tests indicated that the addition of 1% ammonium acetate (wt/vol) increased attraction by threefold. A similar tray containing eight fresh slices (2–3 cm thick) of cucumber (*Cucumis sativus* L. [Cucurbitaceae]) was present at each of the two bait stations containing host fruit odor. Cucumber pieces in the trays were sliced fresh every 15 min.

Release and Testing

Release and testing was conducted three times a day during 1-h intervals. Testing intervals were at 8:30, 12:00, and 15:30. The previous day female melon flies were marked on the pronotum with a dot of paint (Gloss Enamel; Testors, Rockford, IL). A different color was used to mark each dietary treatment. Twenty-five females of each treatment were put in plastic boxes (11 × 18 × 6 cm [width by length by depth]) with screened lids secured with Velcro (Velcro Industries B.V., Manchester, NH) closures. A box containing each of the female groups was placed in the center of 5 × 5 m square in the shade beneath foliage. The boxes were opened at the beginning of each test period, and marked flies were allowed to leave the containers. A combined total of 61 releases were made in this manner, and a total of 3,525 marked flies were set free (Table 1). The odor stations were rotated one position clockwise every 15 min during each 1-h trial to minimize positional effects. Each station was surveyed (every 2 min), and all wild and released flies were removed with forceps from the yellow hemispheres. Removed flies were preserved in ethanol and brought back to the laboratory where they were dissected to evaluate egg load.

Statistical Analysis

Initially, a three-way Proc Mixed (SAS Institute 2013) analysis was done to compare the responses of flies to the two olfactory stimuli (protein and cucumber), conducted at three times of the day (AM, noon, and PM), at the two locations (Kapoho and Kona). The mixed model had date, time of day, olfactory stimuli, protein state, and their interactions as fixed effects. The random effects were field, time of day by olfactory stimuli by field, and protein state by time of day by olfactory stimuli by field (the variance component estimates for the two interaction random effects turned out to be zero for both the wild and lab analyses). The log(count + 1) transformation was used given that data followed a negative binomial distribution. Our focus was on the olfactory stimuli by protein state interaction for the two sites. The interaction was highly significant for the Kapoho site (wild flies: $F_{6,83} = 5.53$, $P < 0.0001$; F_2 flies: $F_{1,83} = 3.23$, $P < 0.001$). Due to an imbalance in the data for the Kona site, the Proc Mixed results are presented for only three dates (wild flies: $F_{1,23} = 3.85$, $P = 0.0619$; F_2 flies: $F_{1,23} = 14.76$, $P = 0.0008$). Thereafter, for clarity and based on our main research question, separate *t*-tests with a significance level of $P = 0.05$ were conducted to compare the response of nongravid and gravid wild females and of F_2 females of each dietary group to the olfactory stimuli (STATISTICA 13, TIBCO 2017). These analyses are presented in the results.

Results

Recapture Rates and Female Egg-Loads

Of the 1,425 color-marked females that were released in Kona and 2,100 females that were released in Kapoho, 20.3 and 13.7%, respectively, responded on average to either olfactory stimulus. Percentage recovery rates of color-marked F_2 females ranged from 13.1 to 22.7% (Table 1). In Kona, 68 wild females responded to yellow hemispheres harboring either odor, whereas in Kapoho, 1,036 wild females arrived to yellow hemispheres. In Kona, of the 68 wild females that responded, 31 females had no mature eggs in their ovaries and 37 had an average of 22 eggs. In Kapoho, of the 1,036 females that responded 639 had no mature eggs in their ovaries and 392 females were gravid and they had 28.5 eggs, on average. F_2 females released in Kapoho had an average of 50.9 eggs (Table 1).

Female Response to Olfactory Stimuli

In Kona, for wild *B. cucurbitae* females that upon dissection showed not to have any eggs (i.e., they were nongravid), a similar level of response was recorded for the Solulys protein bait and for cucumber odor ($t_{36} = 1.47$; $P = 0.15$). Wild females that had fully developed eggs responded in significantly greater numbers to cucumber than to protein ($t_{36} = 3.00$; $P < 0.01$; Fig. 1A). For color-marked F_2 females, there was a significant preference for cucumber odor over protein odor regardless of the dietary history of the females (protein-fed: $t_{35} = 2.86$; $P < 0.001$; 48-h protein-starved: $t_{36} = 5.85$; $P < 0.001$; protein-starved: $t_{36} = 2.05$; $P < 0.001$; Fig. 1A). The difference in the magnitude of the response to the two olfactory treatments was 22-fold for protein-fed, 9-fold for 48-h protein-starved, and 2-fold for protein-starved females.

In Kapoho, a significant preference for cucumber odor over Solulys protein odor was documented for both types of wild females (nongravid: $t_{77} = 2.17$; $P = 0.03$; gravid: $t_{77} = 4.20$; $P < 0.001$; Fig. 1B). Color-marked F_2 females showed a significant preference for cucumber odor over protein odor when they were protein-fed ($t_{82} = 7.12$; $P < 0.001$). The difference in the magnitude of the response to the two olfactory treatments was 8-fold for protein-fed females. In contrast, when females were protein-starved, they responded in similar numbers to the olfactory treatments ($t_{82} = 0.90$; $P = 0.37$; Fig. 1B).

Discussion

We examined the behavior of female *B. cucurbitae* foraging for food and oviposition sources as a function of dietary history, under natural conditions. We found that, under a variety of conditions, females significantly preferred cucumber odor over Solulys (protein) odor. Only for wild, nongravid females in Kona and for F_2, protein-starved females in Kapoho, the response to either olfactory stimulus did not differ statistically. In no instance was protein more attractive

Table 1. For each of the two locations and type of *Bactrocera cucurbitae* female (wild vs color-marked F_2 females of various physiological states), mean number of eggs ± SEM per female and recovery rate (of color-marked F_2 females only)

Location and type of female	Mean (SEM) eggs (*n*)	Number released	Number recovered	Percentage recovery
Kona				
F_2—protein-fed	—	475	91	19.1
F_2—48-h protein-starved	—	475	90	18.9
F_2—protein-starved	0.0 (108)	475	108	22.7
Wild—gravid	22.0 ± 7.3 (37)	—	68*	—
Wild—non gravid	0.0 (31)	—		—
Kapoho				
F_2—protein-fed	50.9 ± 1.3 (133)	1,050	137	13.1
F_2—protein-starved	0.0 (150)	1,050	150	14.3
Wild—gravid	28.5 ± 0.7 (392)	—	1,036*	—
Wild—nongravid	0.0 (639)	—		—

Numbers in parentheses show the number of females that were recovered; all captured females were dissected except for F_2 females in Kona. Numbers with asterisk are total wild female flies trapped in each location.

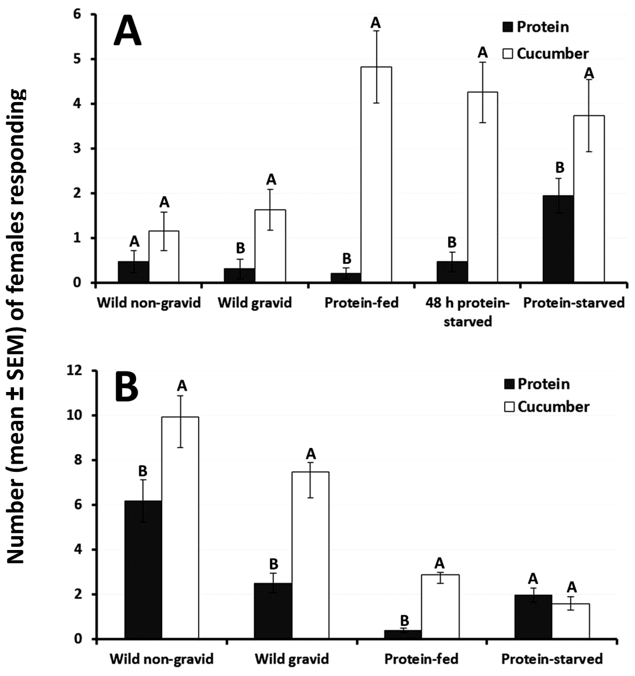

Fig. 1. Number (means ± SEM) of wild and released, color-marked F2 female *Bactrocera cucurbitae* captured at yellow-painted hemispheres baited with Solulys yeast hydrolysate with 1% ammonium acetate (wt/vol) or cucumber odor (eight fresh slices [2–3 cm thick] of cucumber fruit) at (A) Kona, HI and (B) Kapoho, HI. For each fly type, bars with different letters are significantly different according to *t*-tests at $P = 0.05$.

to females than cucumber odor. The lack of significance (at $P = 0.05$) for wild, nongravid females in Kona may have been due to lower number of wild females responding. Overall, comparatively greater rates of recovery of color-marked F_2 females were recorded at Kona than at Kapoho.

Several studies have been conducted on other tephritid species involving presentation of competing host fruit and proteinaceous food. For example, Prokopy and Vargas (1996) documented that the odor of Nu-Lure was significantly more attractive than coffee fruit odor to mature-age, protein-deprived *Ceratitis capitata* (Wiedemann) females, whereas the reverse result was observed for mature-age, protein-fed females. Vargas et al. (2009) documented that sexually mature *B. cucurbitae* females responded more strongly to cucumber odor than 1-wk old, sexually immature females. Our results indicating that wild gravid *B. cucurbitae* females and F_2 protein-fed females (all of which were gravid) responded more to host odor than to protein odor was expected given their reduced need for protein and greater drive to oviposit.

Our findings with protein-starved F_2 females in Kapoho indicating a similar response to both sources of olfactory stimuli suggest that variations possibly due to differences in environmental conditions at the two testing locations. The Kapoho location was mostly devoid of vegetation, other than a papaya canopy, due to herbicide application. However, the Kona test site was highly diverse in ground-covering grasses and weeds under trees containing *Coccinia grandis* vines. This habitat may have contained more alternative sources of protein from animal feces, which have been shown to be more attractive than protein baits (Prokopy et al. 1992). Alternatively, it is conceivable that the greater visual exposure of the yellow hemispheres at Kapoho compared with Kona may have influenced the types of responses shown by females. Female *B. cucurbitae* are known to possess a remarkable ability to use visual cues for host location (Piñero et al. 2006, 2017a), and the extent to which the visual response to the yellow color overrides the olfactory response depends to a large extent on the level of attractiveness of the olfactory stimuli. For example, Piñero et al. (2017a) documented that when a low attractiveness olfactory stimulus (represented by papaya [*C. papaya* L.] odor) was presented alone and in association with a yellow stimulus, the behavioral response could be explained by the visual stimulus alone. In contrast, when a highly attractive olfactory stimulus (represented by cucumber odor) was used, the strongest response occurred when both types of stimuli were presented simultaneously. From the present study, we can also infer that physiological state may influence the type of response exhibited to visual and olfactory stimuli.

Cornelius et al. (2000b) found that protein-deprived oriental fruit fly, *B. dorsalis*, arrived to both host and protein odors in equal numbers when attraction was tested in field cages, while protein-fed flies preferred host fruit odors over protein. Similarly, Miller et al. (2004) found comparable results with melon flies when tested in cages. In these studies, protein-deprived females came to cucumber and protein in equal numbers, while protein-fed flies showed a 4-fold greater level of attraction to cucumber. Female *B. cucurbitae* have consistently shown to be more attracted to commercially available proteins such as Nu-Lure and GF-120 NF Fruit Fly Bait than female *B. dorsalis* (Piñero et al. 2011, 2017b). In spite of this comparatively high response to proteins, in this study cucumber odor was more attractive to *B. cucurbitae* females under a variety of conditions than was Solulys laced with ammonium acetate, which is a very attractive material to females. Cucumber fruits contain 0.65 g of protein per 100 g of fruit weight (USDA 2017). Whether volatiles emitted by cucumber fruit that attract female melon flies signal for the presence of protein is unknown. However, if this is true, then in the melon fly, sites of proteinaceous food acquisition and sites of oviposition may not be as spatially distinct as reported for *R. pomonella* Walsh, an oligophagous species that reproduces on plants belonging to the family Rosaceae (Prokopy et al. 1995). In other words, it is possible that young, nongravid *B. cucurbitae* females foraging for food may benefit from feeding on cucumber fruits that are damaged by birds and other animals.

The relevance of our findings also lies in the context of protein bait sprays used to protect highly attractive, high-value crops such as cucumber. Previous research conducted by Prokopy et al. (2003) under field conditions in the context of protein bait sprays using GF-120 NF Fruit Fly Bait revealed that a 50-cm-wide swath of GF-120 NF Fruit Fly Bait containing spinosad sprayed to a sorghum border surrounding cucumbers was more effective against released protein-deprived melon fly females but was less effective against released protein-fed females. Location, plant species, and density of these border plants were found to alter the effectiveness of protein bait sprays (Vargas et al. 2009). As documented here, in the absence of a barrier, most females responded strongly to volatiles emitted by freshly sliced cucumber fruits. A nine-component lure that is attractive to female *B. cucurbitae* was identified by Siderhurst and Jang (2010). The Hawaii Area-Wide Fruit Fly Pest Management Program was based on ecological approaches that included an integrated pest management program for *B. cucurbitae*, which comprised sanitation, protein baits, and male-lure stations incorporating reduced-risk insecticides (Nishida 1953; Vargas et al. 2016). Addition of cucurbit volatiles to the protein-based baits could increase the number of female *B. cucurbitae* responding to protein baits, depending on the age structure and the alternative sources of protein available in nature. This possibility will be investigated further for improved management of melon fly in Hawaii.

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