

# Novel bait stations for attract-and-kill of pestiferous fruit flies

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## Abstract

A novel, visually-attractive bait station was developed in Hawaii for application of insecticidal baits against oriental fruit fly, *Bactrocera dorsalis* (Hendel), melon fly, *Bactrocera cucurbitae* (Coquillett), and Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (all Diptera: Tephritidae). The bait station developed represents a supernormal visual stimulus of papaya foliage and takes advantage of the flies' strong response to the high light-reflecting properties of yellow color and of their need for shelter, while fully protecting the bait against rainfall. Field studies revealed that the behavioral response of female fruit flies, in particular *C. capitata* and *B. cucurbitae*, to yellow-painted bait stations sprayed with GF-120 NF Naturalyte Fruit Fly Bait was significantly enhanced compared with similarly sprayed bait stations that mimicked the green color of fully grown papaya leaves. Field studies conducted with *B. cucurbitae* indicated that the period of bait attractiveness can be extended for at least 1 week after bait application due to the rain-fastness properties of the bait stations and the use of a visually-attractive color. Our studies provide the behavioral basis for the development of improved attract-and-kill bait stations for fruit flies in Hawaii. These devices also provide a standardized way of evaluating bait spray formulations, thus allowing for proper comparisons over time, across species, and among geographical areas.

## Introduction

Current approaches for more environmentally-friendly management of herbivorous insect pests call for use of both less toxic insecticides as well as more efficient ways of applying lures and other semiochemicals in crop as well as border areas. Behavioral approaches to pest management represent an excellent alternative to the conventional application of broad spectrum insecticides (Shelton & Badenes-Perez, 2006; Cook et al., 2007). One such approach is the attract-and-kill method, which involves deployment of positive visual/olfactory stimuli in association with a killing agent to allure pestiferous insects to selected areas (Foster & Harris, 1997; Vincent et al., 2003).

For management of pestiferous fruit flies (Diptera: Tephritidae), one of the best examples of an attract-and-kill tactic is the use of apple fruit mimics to control

the apple maggot fly, *Rhagoletis pomonella* (Walsh), in eastern North America. This effective control tactic was developed by the late RJ Prokopy and collaborators (for a recent review, see Leskey et al., 2009) and relies on a comprehensive understanding of the orientation behavior and movement of adult flies to food and oviposition resources. By surrounding blocks of orchard trees with attractive odor-baited sticky red spheres to intercept adults immigrating into orchards from nearby feral hosts, the level of control of apple maggot in orchard blocks in Massachusetts subjected to this behavioral control was comparable to that achieved in orchard blocks sprayed with conventional organophosphate insecticides (Prokopy et al., 2005).

Bait stations represent another type of attract-and-kill approach to fruit fly management (Cook et al., 2007). Various types of bait stations have been developed for use against *Bactrocera* spp. fruit flies in Hawaii using male-specific lures. For example, Vargas et al. (2008a, 2009a) tested sprayable attract-and-kill dispensers with spinosad and the highly attractive male-specific lures methyl euge-

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nol and cue-lure for area-wide suppression of oriental fruit fly, *Bactrocera dorsalis* (Hendel), and melon fly, *B. cucurbitae* (Coquillett), respectively. Mangan & Moreno (2007) developed tent- and cylindrical-shaped bait stations with a protein and phloxine B (a toxicant) for use against *Anastrepha ludens* (Loew) and Heath et al. (2009) designed a wax-based lure and bait station for use against *Anastrepha suspensa* (Loew).

The protein bait GF-120 NF Naturalyte Fruit Fly Bait has been used in Hawaii against introduced fruit fly species as part of a 10-year program implemented to promote area-wide Integrated Pest Management (IPM) methods for fruit fly suppression (Mau et al., 2007; Vargas et al., 2008b). For example, this protein bait has been used with success for area-wide suppression of *B. cucurbitae* (Jang et al., 2008), against *B. dorsalis* in papaya (*Carica papaya* L.) orchards (Piñero et al., 2009) and against Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (McQuate et al., 2005; Vargas et al., 2009b), in selected areas of Hawaii. However, the efficacy of foliar applications of insecticidal baits can be negatively affected by the high levels of rainfall that occur during or shortly after the bait sprays (Piñero et al., 2009). Consequently, developing a device that will protect insecticidal baits such as GF-120 NF Naturalyte Fruit Fly Bait from being washed away by rainfall is highly desirable in rainy areas where foliar bait applications may not represent the most efficient application method unless frequent applications are achieved, resulting in more costly applications.

In this study, we report on the development and performance of a novel attract-and-kill bait station for use against tephritid flies in Hawaii. For its development, several aspects such as simplicity in design and low cost were considered, in an attempt to increase the likelihood of adoption by fruit and vegetable growers in Hawaii. In addition to describing the physical characteristics (spectral reflectance and surface area) of the bait stations [hereafter referred to as papaya leaf mimics (PLMs)] compared with fully grown papaya leaves, we conducted field experiments aimed at (1) quantifying the response of wild female *B. dorsalis*, *B. cucurbitae*, and *C. capitata* to high (yellow) and low (sap green) light-reflecting PLMs baited with GF-120 NF Naturalyte Fruit Fly Bait under field conditions, and (2) comparing the residual attractiveness to wild female *B. cucurbitae* of two dilutions of bait weathered for 3 and 7 days using PLMs. Information gained from this study was expected to provide the basis for development of improved management methods against the three highly polyphagous (Christenson & Foote, 1960; Weems et al., 2001) fruit fly species investigated here, all of which are established in the Hawaiian Islands.

## Materials and methods

### Characteristics of bait stations

Papaya leaf mimics were constructed using plant pot saucers (36 cm outer diameter, 5 cm height of the lip) (Anderson Die & MFG, Portland, OR, USA). A metallic shelf bracket (20.3 × 25.4 cm) (Home Depot, Atlanta, GA, USA) was attached to the interior of the saucer using screws and glue (Gorilla Glue, Cincinnati, OH, USA). This simple design allowed for easy deployment to vertical structures such as the trunks of papaya trees (Figure 1). To increase adherence of the protein bait, the interior area of each saucer was scraped in a circular fashion using a wire-wheel brush (7.6 mm in diameter) (ACE hardware, Oak Brook, IL, USA) attached to an electric drill. With the grooves created by this brushing and using the hand-held sprayer to apply GF-120 NF Naturalyte Fruit Fly Bait (Dow AgroSciences, Indianapolis, IN, USA), virtually no bait dripping was observed. Subsequently, a primer (Rust-Oleum, Vernon Hills, IL, USA) was applied. Each bait station was then painted either cadmium yellow medium or sap green using artist's pigments (Windsor & Newton Finity Series, London, UK) for use in the various experiments. Cost of all materials was ca. US\$ 6.50 per bait station.



**Figure 1** A yellow papaya leaf mimic (PLM) attached to a papaya tree trunk with adult *Bactrocera dorsalis* and *Bactrocera cucurbitae* feeding on GF-120 NF Naturalyte Fruit Fly Bait.

### Reflectance spectra quantification

The reflectance spectra of 10 fully grown green and 10 fully grown yellowing (i.e., senescing) papaya leaves were measured using a portable spectroradiometer (FieldSpec-HandHeld; Analytical Spectral Devices, Boulder, CO, USA) focusing on a spectral range of 400–680 nm and using a spectral resolution of 3.5 nm. This spectral range includes the dominant foliar reflectance-transmittance hues of 500–580 nm (Prokopy & Owens, 1983). Leaves were sampled from 3-year-old (about 2.5 m tall) papaya trees located in commercial papaya orchards in Keaau, Hawaii Island. Leaves were selected so that their visual appearance was not affected by insect injury or fungal disease. On each of 2 days (19 and 20 August, 2008) five leaves of each type were collected, placed inside plastic RubberMaid containers with pieces of paper towel that was slightly moistened to prevent desiccation, and transported to USDA-ARS, United States Pacific Basin Agricultural Research Center (USPBARC) in Hilo, Hawaii, for the spectral measurements. The reflectance spectra of two yellow-painted PLMs (one for each day) were also recorded. Measurements took place outdoors, at noon on sunny days.

### Surface area estimation

The surface area of the same 10 green and 10 yellow papaya leaves that were used for spectral reflectance estimation was measured in the laboratory. Outlines of the leaves were first drawn on brown paper and then cut out. Their area was estimated by passing the leaf outlines through a CI-203 laser area meter (CID, Camas, WA, USA), equipped with a CI-203CA stand (CID). The area of two PLMs was estimated by pressing the brown paper inside the saucer and cutting the paper that covered the surface and the curved lip. The area of this paper was then measured with the laser area meter as described above for the papaya leaves.

### Experiment 1: response of three fruit fly species to yellow and green papaya leaf mimics

The first behavioral experiment was aimed at testing the hypothesis that yellow PLMs enhance the behavioral response of wild female *B. dorsalis*, *B. cucurbitae*, and *C. capitata* to GF-120 NF Naturalyte Fruit Fly Bait when compared with similarly-baited green surrogates of papaya leaves. Yellow is a high light-reflecting color that is visually very attractive to *B. dorsalis* (Vargas et al., 2001a; Stark & Vargas, 1992), *B. cucurbitae* (Piñero et al., 2006), and *C. capitata* (Epsky et al., 1996; Uchida et al., 1996). In turn, sap green is a low light-reflecting color previously shown by Piñero et al. (2006) to be considerably less visually attractive to female *B. cucurbitae* than the cadmium yellow

medium pigment used here. Spectral reflectance of the sap green color is provided in Drew et al. (2003). Green-painted PLMs were used as a surrogate of a fully developed papaya leaf, given their similarity in both size and reflectance pattern compared with real papaya leaves. In addition, use of sap green-painted PLMs permitted the accurate application of 10 ml of the olfactory stimuli used for the experiments, an amount that is difficult to apply onto a single papaya leaf.

Observations with *B. dorsalis* and *B. cucurbitae* took place from 8 September to 27 October 2008 in a large (ca. 25 ha) unsprayed commercial papaya orchard located in Keaau (19°37'N, 155°04'W, average elevation: 208 m), Hawaii Island. One orchard block of about 1 ha was selected for the observations. Treatments evaluated were (1) a yellow PLM sprayed with GF-120 NF Naturalyte Fruit Fly Bait [10 ml of a 40% (vol/vol) solution = the recommended application rate (Dow AgroSciences, 2006)], (2) a yellow PLM sprayed with 10 ml of a 20% (wt/vol) sugar/water solution to assess the relative contribution of the visual stimulus, (3) as in (1) but using a green-painted PLM, and (4) a green PLM with sugar/water solution. Olfactory treatments were prepared in the field and applied onto each PLM using a hand-held sprayer (500 ml in capacity) (ACE hardware) that was calibrated to spray 10 ml of each material.

For each observation day, each of the four PLMs was attached to the tree trunk of perimeter-row trees, at eye height, using zip ties. PLMs were 15 m apart and the initial position of each color/odor treatment was assigned randomly. Observations typically started by 09:00 and ended by 11:00 hours. After spraying the bait and the sugar/water solution, the number of male and female *B. dorsalis* and *B. cucurbitae* that responded (i.e., that alighted in the PLM interior) was recorded every 15 min for a 2-h period. At each fly census, PLMs were rotated clockwise. A yellow PLM whose interior side was coated with Tangletrap glue (Great Lakes IPM, Vestaburg, MI, USA) was used to capture all responding flies. This procedure ensured that all responders were counted only once. Observations were made once a week, on sunny days, for a total of eight replicate weeks. Mean ( $\pm$ SEM) daily air temperature and r.h. values for the observations in the papaya orchard were  $28.8 \pm 0.2$  °C (range: 24.1–31.7 °C) and  $54.7 \pm 0.6\%$  (range: 47–67%).

Observations with *C. capitata* took place on 23–27 February 2009 in a very large unsprayed coffee [*Coffea arabica* L. cv. Arabica (Rubiaceae)] plantation in Kalaheo (21°54'36"N, 159°32'54"W, average elevation: 122 m), on Kauai Island. One ca. 1-ha block that had a perimeter row of at least 300 m was selected for the observations. On the

opposite side of the perimeter row there was a single row of 30 m tall ironwood trees [*Casuarina cunninghamiana* Miq. (Casuarinaceae)], which served as windbreak. Coffee plants were about 2 m tall and some branches from the top portion of the canopy had to be clipped to permit PLM attachment using zip ties. Experimental protocol (e.g., fly censusing) was the same as described for *B. dorsalis* and *B. cucurbitae*. Two replicates were done simultaneously per day (between 09:00 and 11:00 hours) for 5 days using two areas that were about 100 m apart. Mean ( $\pm$ SEM) daily air temperature and r.h. values for the observations in the coffee plantation were  $23.1 \pm 0.3$  °C (range: 20.7–26.9 °C) and  $55.5 \pm 1.2\%$  (range: 42–72%).

**Experiment 2: residual attractiveness of two dilutions of weathered GF-120 NF Naturalyte Fruit Fly Bait to female *Bactrocera cucurbitae***

The overall objective of this field experiment, conducted from 24 November 2008 to 19 March 2009, was to determine the effect of dilution and bait weathering on the response of wild adult *B. cucurbitae*. Data for *B. dorsalis* were also recorded but the population level was very low, thus sufficient data could not be obtained to be reported here. The following five olfactory treatments were evaluated using yellow-painted PLMs in two parallel experiments (2a and 2b): (1) a 40% solution of GF-120 NF Naturalyte Fruit Fly Bait applied fresh, (2) a 40% solution of this bait weathered either for 3 days (in Experiment 2a) or weathered for 7 days (in Experiment 2b) (see weathering procedure below), (3) a 20% solution of fresh bait, (4) a 20% solution of bait weathered either for 3 days (in Experiment 2a) or for 7 days (in Experiment 2b), and (5) a 20% sugar/water solution.

GF-120 NF Naturalyte Fruit Fly Bait was prepared fresh and applied (10 ml per PLM) as indicated in the first experiment at the University of Hawaii Waiiaka Agricultural Experiment Station, located about 5 km from the commercial papaya orchard used in Experiment 1. Two sets of PLMs were sprayed with one of the two bait solutions almost on a daily basis so that they could be tested in the papaya orchard whenever the bait reached a particular weathering period. After spraying, PLMs were attached using zip ties to metallic posts that supported a fence around a grassy area where they were fully exposed to daily temperature fluctuation, wind, and rain. Mean ( $\pm$ SEM) daily air temperature values during the weathering periods were  $17.4 \pm 0.5$  °C (range: 13.3–28.9 °C) and  $19.2 \pm 0.4$  °C (range: 13.2–29.8 °C), for Experiment 2a (involving 3-day-old bait) and Experiment 2b (involving 7-day-old bait), respectively. Mean ( $\pm$ SEM) rainfall values were  $23.1 \pm 6.0$  mm (range 0–61.7 mm) and  $37.0 \pm 6.6$  mm (range 3.3–73.7 mm) for the 3- and for the 7-day weathered periods, respectively.

The field evaluations took place on sunny or overcast days only. For most days, only one of the two weathering periods was evaluated and thus only one person was needed to conduct the observations. For the few days in which two sets of weathering periods were available, two persons conducted the observations using two different sections of the orchard that were at least 600 m apart. Mean ( $\pm$ SEM) daily air temperature and r.h. values during the observations in the papaya orchard were  $25.3 \pm 0.3$  °C (range: 20.2–28.6 °C) and  $59.9 \pm 0.8\%$  (range: 42–76%) for Experiment 2a, and  $25.8 \pm 0.3$  °C (range: 20.3–31.6 °C) and  $63.6 \pm 0.8\%$  (range: 48–84%) for Experiment 2b.

For each of the two bait dilutions tested, the attractiveness of the freshly prepared baits was expected to outperform that of the weathered material. Weathered material was considered to be still attractive to fruit flies if the average fly response exceeded that recorded for yellow PLMs that were sprayed with a 20% sugar/water solution. During deployment, a PLM that was sprayed with freshly made bait of a particular dilution rate was always deployed next (15 m apart) to a PLM with weathered bait of the same dilution. As in the first experiment, each observer quantified every 15 min and for a 2-h period the number of male and female *B. cucurbitae* that responded (responders were removed) and each PLM was rotated one position clockwise at each fly census. Each of these two tests was replicated 15 times.

**Statistical analysis**

For each of the three species tested, there are very powerful male-specific lures (methyl eugenol for *B. dorsalis*, cue-lure for *B. cucurbitae*, and trimedlure for *C. capitata*) (Metcalf & Metcalf, 1992). Therefore, for the statistical analyses we focused on female responses. For Experiment 1, data on the number of females responding were analyzed, for each fly species, using a two-way analysis of variance (ANOVA) using a factorial model with an interaction term with color and olfactory treatments as main effects. For Experiment 2, numbers of female *B. cucurbitae* responding to the treatments were analyzed separately for each weathering period (3 and 7 days) using one-way ANOVA. Data from both experiments were transformed to  $\sqrt{(x + 0.5)}$  prior to analysis to stabilize variances. Means were separated by a Fisher-protected Least Significant Differences (LSD) test at the  $P = 0.05$  level. In addition, Pearson's correlation analysis (Pearson, 1896) was used to determine the extent to which the residual attractiveness of each of the two bait dilutions was correlated with the amounts of rainfall that fell during the weathering periods. Residual attractiveness values were calculated for each bait dilution and for each replicate by dividing the numbers of responders to the

weathered material by the numbers of responders to the fresh material  $\times 100$ . All figures show untransformed data. Statistical analyses were conducted using STATISTICA (StatSoft, 2001).

## Results

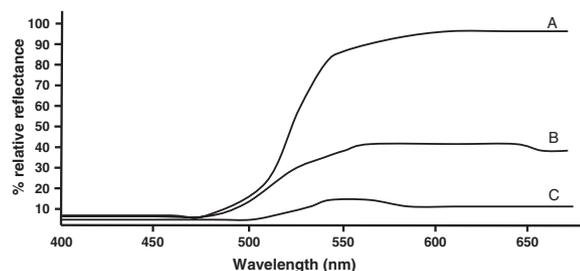
### Physical characteristics of the novel bait stations

Owing to their concave shape and coarsely-sanded interior surface, the novel bait stations provided excellent adherence and protection of GF-120 NF Naturalyte Fruit Fly Bait against rainfall. The surface area of a papaya leaf mimic (1 312.6 cm<sup>2</sup>) fell within the 95% CI of the area offered by fully grown green (range: 981.3–1 518.9 cm<sup>2</sup>; mean  $\pm$  SEM: 1 250.1  $\pm$  118.8 cm<sup>2</sup>; n = 10) or yellow (1 230.8–1 711.3 cm<sup>2</sup>; 1 471.0  $\pm$  106.2 cm<sup>2</sup>; n = 10) papaya leaves. In terms of spectral reflectance, the peak of total energy reflected by a yellow PLM occurred at 460 nm. At this particular wavelength, a PLM reflected about eight times more light than that reflected by a green papaya leaf and about 2.3 times more light than that reflected by a yellowing (senescing) papaya leaf (Figure 2).

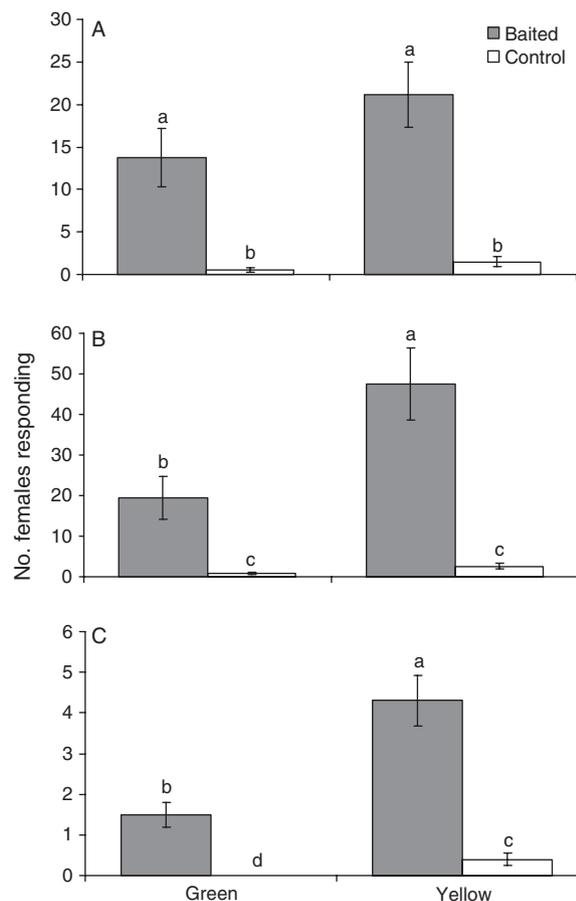
### Experiment 1: response of three fruit fly species to yellow and green papaya leaf mimics

The field response of female *B. dorsalis* was significantly affected by the olfactory stimuli (ANOVA:  $F_{1,28} = 26.20$ ,  $P < 0.001$ ) with a non-significant effect of color ( $F_{1,28} = 1.01$ ,  $P = 0.32$ ) and a non-significant interaction effect ( $F_{1,28} = 0.02$ ,  $P = 0.88$ ). Regardless of color, PLMs baited with GF-120 NF Naturalyte Fruit Fly Bait attracted more females than PLMs with sugar water (Figure 3A). Furthermore, even though the differences were not significant, yellow PLMs baited with GF-120 NF Naturalyte Fruit Fly Bait attracted 28% more *B. dorsalis* females than similarly-baited green PLMs.

For female *B. cucurbitae*, there was a significant effect of both olfactory stimuli ( $F_{1,27} = 82.94$ ;  $P < 0.001$ ) and color



**Figure 2** Spectral reflectance curves of (A) a papaya leaf mimic painted cadmium yellow medium using Windsor & Newton artist's pigments, (B) a fully grown, yellowing papaya leaf, and (C) a fully grown, green papaya leaf.



**Figure 3** Response of wild female (A) *Bactrocera dorsalis*, (B) *Bactrocera cucurbitae*, and (C) *Ceratitis capitata* to yellow- and sap green-painted papaya leaf mimics according to olfactory treatment: baited = 10 ml of a 40% solution (= recommended rate) of GF-120 NF Naturalyte Fruit Fly Bait; control = 10 ml of a 20% sugar/water solution. For each fly species, bars with same letters are not significantly different (two-way ANOVA followed by Fisher-protected LSD tests:  $P > 0.05$ ).

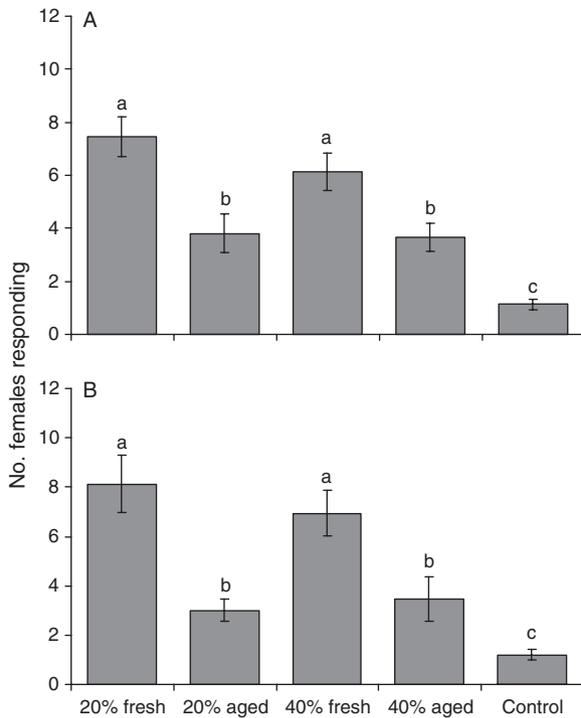
( $F_{1,27} = 4.13$ ,  $P = 0.048$ ), with a non-significant interaction effect ( $F_{1,27} = 0.42$ ,  $P = 0.52$ ). Papaya leaf mimics baited with GF-120 NF Naturalyte Fruit Fly Bait attracted significantly more females than PLMs baited with sugar and water, and yellow PLMs baited with GF-120 NF Naturalyte Fruit Fly Bait attracted significantly more (77% more) females than similarly-baited green PLMs (Figure 3B).

For female *C. capitata* there was a significant effect of both color ( $F_{1,36} = 22.33$ ,  $P < 0.001$ ) and olfactory stimuli ( $F_{1,36} = 84.10$ ,  $P < 0.001$ ) with a significant interaction effect ( $F_{1,36} = 7.41$ ,  $P = 0.01$ ). Irrespective of color, PLMs baited with GF-120 NF Naturalyte Fruit Fly Bait attracted significantly more female *C. capitata* than PLMs baited with sugar and water. As for the interaction, yellow PLMs

baited with GF-120 NF Naturalyte Fruit Fly Bait attracted nearly three times more females than similarly-baited green PLMs. In turn, yellow PLMs baited with sugar/water solution outperformed similarly-baited green PLMs, which did not attract any fly (Figure 3C).

**Experiment 2: residual attractiveness of two dilutions of weathered GF-120 NF Naturalyte Fruit Fly Bait to female *Bactrocera cucurbitae* in the field**

In Experiment 2a, significantly more females responded to PLMs with freshly made baits than to PLMs with 3-day-old baits (ANOVA:  $F_{4,70} = 20.95$ ,  $P < 0.001$ ). Baits aged for 3 days were still attractive to females as determined by the significantly greater response to 3-day-old baits compared with control PLMs (Figure 4A). Overall, 3-day-old baits retained 52.2% (for the 20% solution) and 71.3% (for the 40% solution) of their attractiveness, and the residual attractiveness of the two 3-day-old bait dilutions was found to be independent from rainfall during the weathering periods ( $r^2 = 0.10$ ,  $P = 0.25$  and  $r^2 = 0.004$ ,  $P = 0.83$  for the 20% and 40% solutions, respectively).



**Figure 4** Response of wild female *Bactrocera cucurbitae* to two dilutions of GF-120 NF Naturalyte Fruit Fly Bait weathered outdoors for either (A) 3 days, or (B) 7 days, using papaya leaf mimics. For each bait aging period, bars with same letters are not significantly different (ANOVA followed by Fisher-protected LSD tests:  $P > 0.05$ ).

In Experiment 2b, which involved bait weathered for 7 days, the pattern of female response to the fresh and weathered baits was very similar to the one described above with fresh bait outperforming weathered baits ( $F_{4,70} = 17.18$ ,  $P < 0.001$ ). Remarkably, PLMs with bait that was weathered for 7 days were significantly more attractive to female *B. cucurbitae* than were control PLMs (Figure 4B). The 7-day-old baits retained 41.8% (for the 20% solution) and 50.8% (for the 40% solution) of their attractiveness, and the residual attractiveness of the two 7-day-old baits was not significantly correlated with rainfall during the weathering periods ( $r^2 = 0.09$ ,  $P = 0.30$  and  $r^2 = 0.03$ ,  $P = 0.55$  for the 20% and 40% solutions, respectively).

**Discussion**

Initial field research and development of the current GF-120 NF Naturalyte Fruit Fly Bait as a foliar spray replacement for malathion bait sprays against fruit flies were done in Hawaii against *C. capitata* in coffee (Peck & McQuate, 2000; Vargas et al., 2001b; McQuate et al., 2005). Subsequently, this bait was evaluated for control of *B. cucurbitae* in vegetable crops (Prokopy et al., 2003, 2004; Jang et al., 2008). More recently, use patterns of GF-120 NF Naturalyte Fruit Fly Bait as a reduced-risk foliar treatment against *B. dorsalis* were evaluated in commercial papaya orchards by Piñero et al. (2009). In the latter study, the need to develop a bait station to apply this bait given the high cost of this product and the high amounts of rainfall that fell during or shortly after foliar applications was recognized. The novel bait station developed here is termed a papaya leaf mimic (PLM) because it represents a supernormal visual stimulus (Prokopy, 1972; Prokopy & Owens, 1983) of papaya foliage. Our combined behavioral data suggest that PLMs have the potential to be used as an open system to which insecticidal baits can be applied. PLMs not only protect baits such as GF-120 NF Naturalyte Fruit Fly Bait against rainfall but, as conclusively shown here, when painted yellow PLMs not only enhanced the behavioral response of wild females (in particular *B. cucurbitae* and *C. capitata*) to this bait in comparison to sap green-painted PLMs but they also extended the period of attractiveness of both bait dilutions for at least 1 week.

To provide maximum effectiveness against target insects, insecticidal baits such as GF-120 NF Naturalyte Fruit Fly Bait must attract and stimulate flies to feed on the bait (Prokopy et al., 2003; Dow AgroSciences, 2006). Previous reports indicate that the residual activity of the bait sprays containing spinosad is dependent on the protein components which can quickly lose attractiveness over time due to rapid volatilization of compounds such as

ammonium acetate (Prokopy et al., 2003; Yee, 2007). In Hawaii, Prokopy et al. (2003) and Revis et al. (2004) documented that the attractiveness of GF-120 NF Naturalyte Fruit Fly Bait to *B. cucurbitae* declined considerably within 24 h after application and that nearly all was lost after direct exposure of the bait to ca. 8 mm of rainfall. Similar results were found by Vargas & Prokopy (2006) and by Barry et al. (2006). In our second experiment, we conclusively showed that when exposed to at least three times the rainfall reported in the above studies, nearly half (or more) of the attractiveness of either dilution of 3- and 7-day-old GF-120 NF Naturalyte Fruit Fly Bait was retained when compared with fresh bait. Such a residual attractant effect of bait weathered using PLMs is likely to be due to the preservation of some of the attractive bait components and the important contribution of the yellow color in fruit fly attraction to olfactory stimuli (e.g., Vargas et al., 2001a; Stark & Vargas, 1992; Epsky et al., 1996; Piñero et al., 2006).

From a methodological viewpoint, previous evaluations of the efficacy of GF-120 NF Naturalyte Fruit Fly Bait against various fruit fly species have relied on various levels of evaluation such as direct observations of fly behavior in the laboratory (Yee 2008), in field cages (Miller et al., 2004; Barry et al., 2006; Vargas & Prokopy, 2006), in small plot trials (Prokopy et al. 2003, 2004), in the field either by directly applying droplets of this bait onto host tree leaves (Pelz-Stelinski et al., 2006), by using traps such as sticky yellow panel traps (Yee, 2007), or through foliar bait sprays (Piñero et al., 2009). Some of the variability in bait attractiveness and toxicity reported from these studies, even for the same fly species, may be partially due to the different experimental methods. Based on our results, we hypothesize that use of yellow PLMs might improve the response of fruit fly species for which GF-120 NF Naturalyte Fruit Fly Bait has shown not to be very attractive, such as *Rhagoletis mendax* Curran (Pelz et al., 2005), *Rhagoletis cingulata* (Loew), (Pelz-Stelinski et al., 2006), and *Rhagoletis indifferens* Curran (Yee & Chapman, 2005). We believe that PLMs should be tested in various settings and against several fruit fly species as they provide an opportunity to be used for standardized evaluations of bait spray formulations thus allowing for proper comparisons.

Two of the long-term goals of the highly successful Hawaii Area-Wide Fruit Fly Pest Management Program (Mau et al., 2007, Vargas et al., 2008b) have been to develop simple, effective, safe, and sustainable methods to suppress different species of fruit flies and also to increase the likelihood of grower adoption of the technology developed. The novel bait station developed is simple, durable (PLMs can endure at least 2 years of continuous weathering; JC Piñero, RFL Mau & RI Vargas, unpubl.) and it can also be

deployed easily in the field. In terms of costs, the cost of materials used to make one PLM was ca. US\$ 6.50 but this amount can be reduced nearly by half if cheaper materials (e.g., a zip tie or Velcro) instead of shelf brackets are used for attachment onto tree trunks of papaya trees or onto branches of host trees in other agroecosystems. In addition, waste of bait moving onto the ground or onto undesirable areas of the target tree or plant (e.g., trunk, fruit) can also be avoided. Additional advantages of PLMs are that they circumvent leaf phytotoxicity observed in the field, which are likely caused by one or more ingredients in the bait matrix (DeLury et al., 2009), and also minimize degradation of spinosad by photolysis (Mangan et al., 2006).

Optimization of existing traps, lures, and mass trapping techniques and development of 'attract-and-kill' systems including bait stations for fruit fly control are currently priority research areas in several regions of the world (IAEA, 2007). Our studies provide the behavioral basis for development of an efficient visually-attractive bait station, and future applications are expected in the context of improved environmentally friendly attract-and-kill systems for fruit fly control in Hawaii. Results of a large-scale study aimed at comparing the efficacy of GF-120 NF Naturalyte Fruit Fly Bait applied either onto PLMs or as foliar sprays in reducing female *B. dorsalis* population density and fruit infestation in papaya orchards in Hawaii indicate that PLMs have the potential of being used for fruit and vegetable production in Hawaii (JC Piñero, RFL Mau & RI Vargas, unpubl.). Further research should be conducted to determine, in papaya orchards, the optimal bait density and within-orchard distribution and impact of factors such as field sanitation. The performance of PLMs in other agroecosystems should also be investigated.

In closing, multiple insect sensory modalities such as olfaction and vision should be exploited for pest monitoring control as IPM tools that do not rely on a single cue are likely to work more reliably under various environmental conditions (Dorn & Piñero, 2009). Some trapping systems have been optimized by taking advantage of the known interaction between visual and olfactory stimuli in tephritid flies (Heath et al., 1996; Epsky & Heath, 1998; Piñero et al., 2006) and the bait station developed takes advantage of such interaction. PLMs were evaluated in this study in association with GF-120 NF Naturalyte Fruit Fly Bait; however, their use could be extended to application of other recently developed insecticidal baits such as Solgel (Mangan et al., 2006) as well as male annihilation treatments such as specialized pheromone and lure application technology (SPLAT) in combination with methyl eugenol and spinosad (Vargas et al., 2009a).

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