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RESIDUAL ATTRACTIVENESS OF A SPINOSAD-CONTAINING PROTEIN-BASED BAIT AGED UNDER VARIABLE CONDITIONS TO *BACTROCERA DORSALIS* AND *B. CUCURBITAE* (DIPTERA: TEPHRITIDAE) WILD FEMALES IN HAWAII

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ABSTRACT

Key to the effectiveness of fruit fly (Diptera: Tephritidae) suppression efforts using insecticidal bait sprays is the determination of how long the bait remains attractive to adult flies after application. Using a comparative approach, field studies were conducted in commercial papaya (*Carica papaya* L.; Brassicales: Caricaceae) orchards in Hawaii with the goal of quantifying the response of *Bactrocera dorsalis* (Hendel) and *Bactrocera cucurbitae* (Coquillett) wild females to yellow bait stations treated with 2 dilutions (40% and 20%) of GF-120 NF Naturalyte™ Fruit Fly Bait that had been aged either outdoors (for 3 and 7 days) or indoors (for 1, 2, 3, and 7 days). Important variations in the level of female response to the baits were documented between these 2 fruit fly species, and the response levels were modulated by bait dilution, duration of aging and aging conditions. For *B. dorsalis* wild females, the attractiveness of 40% GF-120 and aged outdoors for either, 3 or 7 days did not differ significantly from the fresh bait, whereas for *B. cucurbitae* wild females a significant reduction (48%) in bait attractiveness was recorded within 1 day of aging indoors independent of percent dilution of the bait. Environmental conditions, in particular mean temperature and relative humidity, prevailing during bait aging outdoors differed significantly from those recorded indoors, and these differences appeared to have influenced the attractiveness of GF-120. The types of variations in the level of responses to the aged baits documented in this study between fruit fly species within a genus, and potentially across genera need to be considered when developing suppression programs for fruit flies that involve the use of bait sprays.

Key Words: GF-120 NF Naturalyte Fruit Fly Bait, residual attractiveness, integrated pest management, bait station

RESÚMEN

Un aspecto clave que debe ser considerado para la supresión efectiva de moscas de la fruta (Diptera: Tephritidae) usando cebos insecticidas asperjados es la determinación de por cuánto tiempo el cebo se mantiene atractivo para las moscas después de una aplicación foliar. Usando un método comparativo, se realizaron estudios en huertos comerciales de papaya (*Carica papaya* L.; Brassicales: Caricaceae) en Hawai con la finalidad de cuantificar la respuesta de dos diluciones (40% y 20%) del cebo GF-120 NF Naturalyte™ Fruit Fly Bait que fueron añejados usando estaciones cebo tanto al exterior (por 3 y 7 días) como en interiores (por 1, 2, 3, y 7 días) para hembras de la mosca Oriental, *Bactrocera dorsalis*, y la mosca del melón, *B. cucurbitae*. Variaciones importantes en el nivel de respuesta de las hembras a los cebos entre especies fueron documentadas, y dicha respuesta fue modulada por el nivel de dilución y condiciones de añejamiento del cebo. Para hembras de *B. dorsalis*, la atracción del GF-120 diluido al 40% y añejado por 3 ó 7 días no fue estadísticamente diferente de la atracción al cebo fresco mientras que la respuesta de hembras de *B. cucurbitae* al GF-120 se redujo significativamente (48%) un día después de preparar el cebo, independientemente de la dilución. Las condiciones ambientales, en particular temperatura y humedad relativa registradas durante los periodos de añejamiento al exterior fueron significativamente diferentes a aquellas registradas en interiores, y dichas diferencias al parecer afectaron el nivel de atracción del GF-120. Los tipos de variaciones en el nivel de respuesta a los cebos añejados documentados entre especies de moscas dentro de un mismo género deben ser considerados para el desarrollo de un programa de supresión de moscas de la fruta que usan aplicaciones de cebos proteicos asperjados como un componente de un programa de Manejo Integrado de Plagas.

Palabras Clave: GF-120 NF Naturalyte Fruit Fly Bait, atracción residual, Manejo Integrado de Plagas, estaciones cebo

Translation by the authors.

Application of protein baits mixed with a killing agent is a common and effective attract-and-kill approach to fruit fly (Diptera: Tephritidae) management (Roessler 1989). This behavioral tactic targets female fruit flies primarily based on the female's need to ingest and search for protein for adequate egg production (Hagen & Finney 1950). For many years, protein baits that included organophosphate insecticides such as malathion as killing agents were applied primarily against invasive fruit fly species (Steiner 1952; Roessler 1989; Vargas et al. 2001).

To be effective, insecticidal bait formulations ought to induce high levels of attraction and stimulate flies to ingest a lethal dose of the toxicant upon contact (Mangan 2009). GF-120 NF Naturalyte™ Fruit Fly Bait (Dow AgroSciences, 2009), is a mixture of the toxicant spinosad, phagostimulants, and protein-based feeding attractants, among other components (Mangan & Moreno 2004). This bait has proven effective at suppressing invasive fruit flies such as Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (McQuate et al. 2005; Vargas et al. 2010), melon fly, *Bactrocera cucurbitae* (Coquillett) (Jang et al. 2008), and oriental fruit fly, *B. dorsalis* (Hendel) (Piñero et al. 2009a, 2010) in the Hawaiian Islands. However, when applied to tree foliage, various factors influence the effectiveness of GF-120 including (1) rainfall events – because the bait is highly water soluble (Piñero et al. 2009a; Teixeira et al. 2009), and (2) a rapid loss of attractive volatile compounds even in the absence of rainfall (Revis et al. 2004; Piñero et al. 2011a,b). Determining how long GF-120 remains attractive to adult fruit flies after a bait spray application is, therefore, an important question that needs to be addressed for effective fruit fly control. Previous studies aimed at assessing the residual attractiveness of baits have commonly relied on small scale greenhouse-type tests. For example, the effects of aging and dilution have been addressed for *B. cucurbitae* by Revis et al. (2004) using field cages. These authors documented an 11-fold decrease in bait attractiveness for baits that were aged for only 2 h when compared to fresh bait. Common conclusions and recommendations from these studies are that baits need to be frequently applied to obtain maximum benefits (Revis et al. 2004; Prokopy et al. 2003, 2004).

The objective of this study was to quantify the response of *B. dorsalis* and *B. cucurbitae* wild females to 2 dilutions of GF-120 as a function of

bait age and aging conditions (i.e., outdoors vs. indoors), using a standardized comparative approach that involved use of visually-attractive bait stations.

MATERIALS AND METHODS

This study was conducted from 8 Jan 2010 to 15 Mar 2011 in a ca. 25 ha commercial papaya (*Carica papaya* L.; Brassicales: Caricaceae) orchard located in Keaau (N 19° 37' 15" W 155°04' 22", 208 m asl and 254 cm of annual average precipitation), Hawaii Island. Papaya trees were subjected to ca. weekly fungicide applications but no insecticides were applied. One orchard block of approximately 3 ha was selected for the observations.

Visually Attractive Bait Stations

All olfactory treatments were evaluated using yellow attract-and-kill bait stations that were developed in Hawaii as a way of protecting GF-120 against rainfall (Piñero et al. 2009b). This bait station serves as an open system onto which insecticidal baits can be applied. Bait stations have proven valuable in comparative studies (e.g., Piñero et al. 2011), because they provide a standardized way of evaluating, inter alia, bait dilutions and aging periods; and thus allowing for proper comparisons across fruit fly species and bait formulations. We constructed the bait stations from inverted plant pot saucers (36 cm outer diam; 5 cm deep) and painted them with a yellow paint as described in Piñero et al. (2009b).

Bait Preparation and Aging

Two dilutions of GF-120 NF Naturalyte Fruit Fly Bait were evaluated: (1) a 40% (vol/vol) solution = the recommended application rate (Dow AgroSciences 2006)], and (2) a 20% dilution (vol/vol). Olfactory treatments were prepared at the aging site (described below) and applied onto bait stations (10 mL) using a hand-held sprayer (500 mL in capacity) (ACE Hardware). For each bait dilution, 2-3 sets of bait stations were sprayed every other day to ensure that baits of a particular age would be available for testing in the papaya orchard on given observation day. This spray application method closely simulated an actual bait spray application to foliage of trees as done in previous studies, e.g., Piñero et al. (2009a).

For GF-120 bait aged outdoors, 4 olfactory treatments were evaluated simultaneously in the papaya orchard: (1) fresh GF-120, (2) 3-day old bait, (3) 7-day old bait, and (4) a 20% sugar/water (wt/vol) solution as a control to assess the relative contribution of the visual stimulus. Bait aging took place at the University of Hawaii Agricultural Research Station, Panaewa, Hawaii, located about 5 km from the commercial papaya orchard. All sprayed bait stations were attached to metallic posts that supported a fence around a grassy area and they were fully exposed to daily fluctuations of temperature, wind, and rain.

For baits aged indoors, 6 olfactory treatments were evaluated simultaneously: (1) fresh GF-120, (2) 1-day old bait, (3) 2-day old bait, (4) 3-day-old bait, (5) 7-day old bait, and (6) a 20% sugar/water solution. Two sets of bait stations were sprayed with 1 of the 2 bait solutions following a spraying schedule that was prepared so that, for each bait dilution, all 6 treatments could be tested simultaneously in the papaya orchard. Baits were aged inside a large room at the USDA-ARS, United States Pacific Basin Agricultural Research Center (USPBARC) in Hilo, Hawaii, kept under relatively constant conditions. For each type of bait aging, efforts were made to evaluate, on a given observation day, each of the 2 bait dilutions using 2 different sections of the orchard that were at least 300 m apart.

Observation Protocol

For each observation day, bait stations with aged baits were taken from the aging station and brought to the papaya orchard. After in situ application of the fresh bait and the sugar/water solution, bait stations were attached to the tree trunk of perimeter-row trees at eye height (150-170 cm), using zip ties. For each bait dilution, olfactory treatments were 15 m apart and the initial position of baits of each aging period was assigned randomly. Subsequently, the number of male and female *B. dorsalis* and *B. cucurbitae* that responded (i.e., that alighted in the interior of the bait station) was recorded every 15 min for a 2-h period. At each fly census, bait stations were rotated clockwise. After carefully recording the number of flies (by species and sex) that responded, flies were captured by rapidly coupling one Tangletrap-coated bait station with the test bait station. This ensured that fly responders were counted only once. Observations were conducted on both on sunny and overcast days (temperature range: 24-29 °C) during the period of highest fly activity in the field and typically started by 09:00-09:30 a.m. and ended by 11:00-11:30 a.m. Depending on the availability of aged material and on environmental conditions, observations were made 1-3 times a week until 8-15 replicates of each dilution were completed.

Statistical Analysis

For each of the 2 bait dilutions tested, the attractiveness of the freshly prepared bait was expected to outperform that of the aged material. Aged baits were considered to still be attractive to fruit flies if the average fly response exceeded that recorded for the control bait stations. The effects of aging on the numbers of females that responded in a 2-h period were compared separately for each fly species, for each type of bait aging (e.g., outdoors and indoors) and for each bait dilution with a one-way ANOVA. Data were transformed to $\sqrt{(x + 0.5)}$ prior to analysis to stabilize variances. Whenever appropriate, means were separated by a Fisher-protected Least Significant Differences (LSD) test at the $P = 0.05$ level. A comparison of temperature and relative humidity indoors versus outdoors was done using a t-test. To test whether aging conditions had an effect on the number of females that responded to baits aged either 3 or 7 days, we (1) calculated the percent reduction of females of each species that responded relative to the response to the fresh bait and (2) compared the effects of aging conditions by computing the significance level for the difference between the two proportions, where the P level was computed based on the t value for the respective comparison (Statsoft 2001).

RESULTS

For baits aged outdoors, the attractiveness of the 3- and 7-day old 40% dilution of the GF-120 bait to *B. dorsalis* wild females did not differ significantly from the corresponding freshly prepared bait, and all bait treatments were significantly more attractive to females than the control ($F = 3.44$; $df = 3, 27$; $P = 0.030$) (Fig. 1A). The 3-day old 20% dilution of the GF-120 bait was as attractive to female *B. dorsalis* as the fresh bait, whereas the corresponding 7-d old bait was no longer attractive to females when compared to the control treatment ($F = 6.24$; $df = 3, 30$; $P = 0.002$) (Fig. 1B). In contrast for *B. cucurbitae*, the attractiveness of the 3- and 7-d old baits was significantly reduced, regardless of bait dilution, when compared to the fresh bait, but the 2 aged bait dilutions were significantly more attractive than the control treatment ($F = 18.51$; $df = 3, 70$; $P < 0.001$ and $F = 33.27$; $df = 3, 72$; $P < 0.001$, for the 40% and 20% dilutions, respectively) (Fig. 1A,B).

For baits aged indoors, and for both bait dilutions, similar numbers of female *B. dorsalis* responded to the fresh and 1-day old baits while 2, 3, and 7-old baits were no longer attractive to the females when compared with the control treatment ($F = 3.01$; $df = 5, 18$; $P = 0.037$ and $F = 5.74$; $df = 5, 24$; $P = 0.001$, for the 40% and

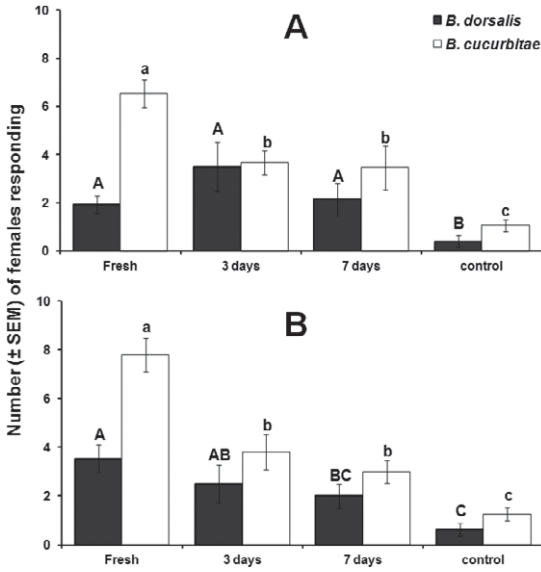


Fig. 1. Effect of aging the fruit fly bait outdoors. Numbers (mean \pm SEM) of *Bactrocera dorsalis* and *B. cucurbitae* wild females that responded in 2 h (i.e., landed on a visually-attractive bait station) to 2 dilutions (A = 40%; B = 20%) of GF-120 NF Naturalyte Fruit Fly Bait presented either, fresh or aged outdoors for 3 or 7 days. For each fly species, bars with same letters are not significantly different (ANOVA followed by Fisher-protected LSD tests, $P > 0.05$).

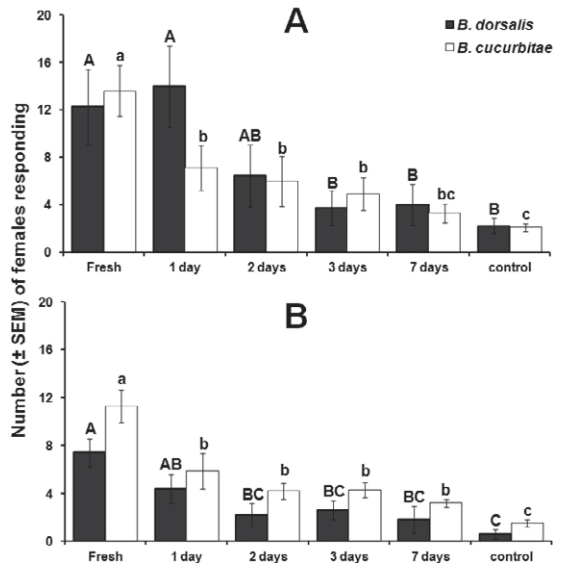


Fig. 2. Effect of aging the fruit fly bait indoors. Numbers (mean \pm SEM) of *Bactrocera dorsalis* and *B. cucurbitae* wild females that responded in 2 h (i.e., landed on a visually-attractive bait station) to 2 dilutions (A = 40%; B = 20%) of GF-120 NF Naturalyte Fruit Fly Bait presented either, fresh or aged indoors for 1, 2, 3, and 7 days. For each fly species, bars with same letters are not significantly different (ANOVA followed by Fisher-protected LSD tests, $P > 0.05$).

20% dilutions, respectively) (Fig. 2A,B). For female *B. cucurbitae*, a significant reduction in bait attractiveness was recorded within 1 day of aging, but no further decreases in bait attractiveness were noted after that aging period. For both bait dilutions, all aged materials were significantly more attractive to females than the control treatment ($F = 4.53$; $df = 5, 54$; $P = 0.002$ and $F = 12.62$; $df = 5, 54$; $P < 0.001$, for the 40% and 20% dilutions, respectively) (Fig. 2A,B).

Environmental conditions during bait aging are presented in Table 1. Daily average temperature was significantly greater at the indoor aging site than in the outdoor site and, conversely,

relative humidity was significantly greater at the outdoor aging site than in the indoor aging site. Important differences were noted between fly species when the mean response of the female flies to the 3 and 7 day old baits was converted to percent reduction/increase relative to that recorded with the fresh bait. Table 2 shows that for *B. dorsalis* the levels of response (expressed in percentage) to GF-120 diluted to 40% and aged indoors either for 3 or 7 days were 82% and 11.4% greater, respectively, than those recorded for fresh bait. In contrast, for the same aged baits and same dilutions, the aging of bait indoors resulted in a 69.4% and 67.3% reduction in bait at-

TABLE 1. ENVIRONMENTAL CONDITIONS AT THE TWO SITES USED FOR AGING OF GF-120 NATURALYTE™ FRUIT FLY BAIT IN HILO, HAWAII ISLAND. BAITS WERE AGED OUTDOORS AT THE UNIVERSITY OF HAWAII AGRICULTURAL RESEARCH STATION, PANAWEA, HAWAII, AND INDOORS AT THE UNITED STATES PACIFIC BASIN AGRICULTURAL RESEARCH CENTER (USPBARC), HILO, HAWAII.

	Outdoors	Indoors	t-value (df)	P-value
Temperature ¹ (°C) (mean \pm SEM)	22.2 \pm 0.1 a	26.8 \pm 0.1 b	33.3 (299)	< 0.001
Temperature range (°C)	18.2 - 31.5	24.7 - 29.5	—	—
Relative humidity ¹ (%) (mean \pm SEM)	84.4 \pm 0.6 b	60.5 \pm 0.5 a	31.1 (299)	< 0.001
Relative humidity range (%)	53.8 - 99.1	48.7 - 67.7	—	—
Total rainfall (mm)	23.1	—	—	—

¹Values originated from daily determinations during the aging periods of the baits.

TABLE 2. EFFECT OF DURATION OF AGING, CONDITION UNDER WHICH AGING OCCURRED, AND DILUTION OF GF-120 NATURALYTE™ FRUIT FLY BAIT ON ITS ATTRACTANCY TO WILD FEMALES OF *BACTROCERA DORSALIS* AND *B. CUCURBITAE*. THE TREATMENTS CAUSED CHANGES IN RESPONSES OF THE FEMALES, I.E., PERCENT CHANGE IN TRAP CAPTURE WITH A REDUCTION INDICATED BY A NEGATIVE NUMBER, AND AN INCREASE INDICATED BY A POSITIVE NUMBER.

Fly species	Aging period	Bait Dilution	Mean percent change relative to fresh bait		P-value
			Bait aged indoors	Bait aged outdoors	
<i>B. dorsalis</i>	3 days	40%	- 69.4	82.0	—
<i>B. cucurbitae</i>	3 days	40%	- 64.0	- 43.9	0.335
<i>B. dorsalis</i>	7 days	40%	- 67.3	11.4	—
<i>B. cucurbitae</i>	7 days	40%	- 75.7	- 46.9	0.166
<i>B. dorsalis</i>	3 days	20%	- 64.9	- 29.3	0.148
<i>B. cucurbitae</i>	3 days	20%	- 61.9	- 51.3	0.563
<i>B. dorsalis</i>	7 days	20%	- 75.7	- 43.1	0.192
<i>B. cucurbitae</i>	7 days	20%	- 71.7	- 61.5	0.558

For each row, statistical comparisons of reduction in response (proportions) between bait aged indoors and outdoors were conducted by computing the significance level for the difference between the 2 proportions, where the *P* level is computed based on the *t* value for the respective comparison (Statsoft 2001). Data are presented in percentages. In those cases where both a reduction and an increase in response were recorded indoors and/or outdoors for the same treatment, proportions were not compared statistically.

tractiveness, respectively. The level of reduction in bait attractiveness to *B. dorsalis* females was similar for baits diluted at 20% regardless of bait age and aging conditions.

For *B. cucurbitae*, the level of reduction in bait attractiveness was 61.9% for GF-120 20% dilution aged indoors for 3 days and 75.7% for GF-120 20% dilution aged indoors for 7 days (Table 2). In contrast the outdoor aging of the bait diluted to 40% reduced its attractiveness to *B. cucurbitae* wild females by 43.9% at 3 days and by 46.9% at 7 days.

DISCUSSION

Results of our study suggest that the response of female *B. dorsalis* and *B. cucurbitae* to GF-120 can vary according to both the bait aging period and aging conditions. For instance, for *B. dorsalis* wild females 40% GF-120 aged outdoors for either 3- or 7-days was as attractive as the freshly prepared bait. In contrast, for *B. cucurbitae* wild females the attractiveness of the bait was consistently and significantly reduced (~48%) within a 24 h period when compared to the fresh bait. Nevertheless, aged baits attracted significantly more *B. cucurbitae* wild females than the control (20% sugar/water solution), which suggested that aged baits remain effective against this pest. However the responses of *B. dorsalis* wild females increased by 82% and 11.4% to 40% GF-120 aged outdoors for 3 and 7 days, respectively, compared to fresh bait.

Our experimental approach utilized visually-attractive yellow bait stations, and they provide a reliable way of experimentally evaluating bait formulations under field conditions as demon-

strated in previous studies (e.g., Piñero et al. 2009b, 2010, 2011a, b). As indicated before, previous research aimed at assessing the residual attractiveness of baits such as GF-120 had been conducted largely in field cages (e.g. Miller et al. 2004; Barry et al. 2006; Vargas & Prokopy 2006). In greenhouse-type tests in Hawaii, Revis et al. (2004) documented that the toxicity of GF-120 over time was greatly affected by rainfall, temperature, and relative humidity and that rainfall exerted the greatest impact. In the present study, application of GF-120 onto bait stations simulated a bait spray application, and bait aging took place under two differing environmental conditions. Conditions outdoors (in particular mean temperature and relative humidity) differed significantly from those recorded indoors, and these differences influenced the attractiveness of GF-120. GF-120 that was aged outdoors was exposed to lower temperatures and greater relative humidity values and the aged bait had a wetter appearance. In contrast, GF-120 that was aged indoors were subjected to comparatively lower relative humidity values and significantly greater temperatures than baits aged outdoors, which resulted in a 'drier' bait spray. All baits were transported to the papaya orchard and tested within 30 minutes after removing them from the aging stations, and therefore difference in fruit fly response can be attributed to differences in the bait aging conditions.

The persistence of an insecticidal bait can be affected by various factors including bait composition, dilution, aging conditions, and application methods, amongst other factors. GF-120 includes in its formulation attractants such as Solulys® (the proteinaceous component from corn steep li-

quor) and ammonium acetate, feeding stimulants such as Invertose® (high fructose corn syrup that also has the greatest humectant properties of the sugars), dispersant-conditioners such as polyethylene glycol (also a humectant), water, and spinosad as the toxicant (Moreno & Mangan 2002), as well as proprietary refinements that improve the overall effectiveness of the bait. The manufacturer of GF-120 suggests that bait attractiveness is regained at 48-96 h after bait spray application. We found that not to be the case for *B. cucurbitae* because bait attractiveness was not regained at all, regardless of bait dilution and of the conditions under which the bait was aged. For *B. cucurbitae* the documented extended period of bait attractiveness might be explained by the yellow color of the bait stations, as it is known that this species shows a particularly strong response to objects that reflect the most light (Piñero et al. 2006).

Approaches taken to increase and extend the attractiveness of proteinaceous baits include the addition of vegetable gums and starches as supplementary thickeners/conditioners to the GF-120 proteinaceous base to produce a gelled mixture termed named Solgel bait (Mangan & Moreno 2007). In that study, solgel bait remained attractive to the Mexican fruit fly, *Anastrepha ludens* (Loew), at ages > 150 days; however, such an extended period of bait attractiveness seem to have resulted in loss of spinosad toxicity. In later tests they mixed the bait at 10-fold higher spinosad concentration (800 ppm) to the same bait mixture. Mangan & Moreno (2007) also addressed the importance of bait stations by indicating that the solgel bait “must be situated in a station structure that protects the material from weather and intruders, but it will allow attractant volatiles to escape and flies to enter and feed with minimal interference”.

In summary, under the conditions of this study GF-120 lost ~50% of its attractiveness to female *B. cucurbitae* within 24 h, regardless of bait dilution and aging conditions, a finding that is in agreement with previous reports for this fly species (e.g., Revis et al. 2004). In contrast, GF-120 can remain as attractive to female *B. dorsalis* as the fresh bait even if it is aged for up to 7 days, depending on aging conditions and dilution. Our findings suggest that a 40% dilution of GF-120 is likely to be effective at attracting *B. dorsalis* over a longer period of time (compared to a 20% dilution) under comparatively dry climates whereas a 20% dilution may be enough to elicit good responses for up to a week in more humid environments. Reduced bait attractiveness caused by “drying” needs to be addressed in local areas where temperatures can be extremely high and humidity low. Probably this can be done by adding humectants to

gain maximum recovery of water as suggested by Mangan & Moreno (2007). The variations in the levels of response to the aged baits documented between fruit fly species within a genus (the focus of this study) and potentially across genera need to be considered when developing a suppression program for fruit flies that uses bait sprays as an IPM component.

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