

HOW EFFECTIVE IS GF-120 FRUIT FLY BAIT SPRAY APPLIED TO BORDER AREA SORGHUM PLANTS FOR CONTROL OF MELON FLIES (DIPTERA: TEPHRITIDAE)?

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ABSTRACT

Application of bait spray to non-host sorghum plants bordering host plants of melon flies, *Bactrocera cucurbitae* Coquillett, is a common practice for melon fly control in Hawaii. In a field study conducted in 2003 in Hawaii, we first asked whether GF-120 Fruit Fly bait spray applied to sorghum plants that bordered only two (opposite) sides of a patch of cucumbers was as effective in protecting cucumbers against melon flies as similar spray applied to sorghum plants that bordered all four sides of a cucumber patch. Second, we asked whether mature melon fly females carrying a high egg load but deprived of protein during the previous 24 h were more responsive to bait spray than mature females having continuous access to protein. Color-marked melon fly females were released outside of patches of sorghum-bordered cucumbers. We found no significant differences between two-sided and four-sided patches of sorghum or between protein-deprived (for 24 h) and protein-fed (continuously) mature females in percentages of released females that found cucumbers in bait-sprayed plots. Moreover, none of these percentages was significantly less than percentages of released females that found cucumbers in unsprayed plots, indicating an overall ineffectiveness of bait spray application. During the 24 h after alighting on cucumbers, released females that were captured alive on cucumbers and placed in cups with cucumbers laid on average almost as many eggs (insignificantly fewer) when taken from bait-sprayed plots as when taken from unsprayed plots. An overriding factor may have been the presence of just a narrow swath of sorghum (arising from a single row of plants), which may have permitted females easy access to cucumbers and masked potential differences among treatments. Bait spray applied to broader swaths of sorghum may be more effective.

Key Words: *Bactrocera cucurbitae*, GF-120 Fruit Fly bait, bait spray, spinosad, sorghum, cucumbers.

RESUMEN

La aplicación de cebos rociados sobre plantas de sorgo (no hospederas) que rodean plantas hospederas de la mosca del melón, *Bactrocera cucurbitae* Coquillett, es una práctica común para el control de esta especie de mosca en Hawaii. En un estudio de campo realizado en 2003 en Hawaii, nos preguntamos primero si el cebo para moscas de la fruta GF-120 aplicado a plantas de sorgo ubicadas en sólo dos lados (opuestos) de un parche de pepinos era tan efectivo protegiendo pepinos contra el ataque de moscas del melón como una aplicación de cebo en plantas ubicadas en los cuatro lados del parche. En segundo término nos preguntamos si hembras maduras conteniendo una alta carga de huevos pero privadas de proteína por 24 horas respondían más al cebo aplicado que hembras maduras mantenidas con acceso continuo a proteína. Estas preguntas fueron contestadas liberando hembras marcadas en la orilla de cada uno de estos tipos de parche que rodeaban el área conteniendo pepinos. No encontramos diferencias significativas entre parches de sorgo teniendo dos o cuatro lados, o entre hembras privadas de proteína por 24 horas y hembras que tuvieron acceso continuo a proteína en cuanto al porcentaje de hembras liberadas que encontraron pepinos en los parches que tuvieron aplicación de cebo. Además, ninguno de estos porcentajes fue significativamente menor que los porcentajes de hembras liberadas que encontraron pepinos en parches no aplicados, lo que indica una general inefectividad del cebo aplicado. Durante las 24 horas posteriores al contacto con los pepinos, hembras liberadas que fueron capturadas vivas y colocadas posteriormente en recipientes de plástico conteniendo pepinos ovipositaron en promedio casi el mismo número de huevos (insignificamente menos) cuando fueron tomadas de parches aplicados con cebo en comparación con parches no aplicados. Un factor determinante posiblemente fue la presencia de solamente una franja angosta de sorgo (originada por una sola hilera de plantas) lo cual pudo haber permitido a las hembras acceder fácilmente a

los pepinos, enmascarando posibles diferencias entre tratamientos. Una aplicación de cebo en franjas de sorgo más anchas pudiera ser más efectiva.

Translation provided by the authors.

The melon fly, *Bactrocera cucurbitae* Coquillet, is an important pest of cucurbits in Asia, several islands in the Pacific ocean, and Africa (White & Elson-Harris 1992). Beginning in the 1950s, bait sprays containing protein (as an attractant and feeding stimulant) plus an insecticide (as a toxicant) have been used widely for control of melon flies (e.g., Steiner 1955; Nishida et al. 1957; Gupta & Verma 1982; Stonehouse et al. 2002). Recently, GF-120 Fruit Fly bait (Dow Agrosciences, Indianapolis, IN) containing spinosad as toxicant has emerged as an effective and environmentally safe alternative to traditional bait sprays (containing organophosphorus insecticide) for control of several different pest tephritid flies (e.g., King & Hennessey 1996; Peck & McQuate 2000; Burns et al. 2001; Vargas et al. 2001).

In 2002 in Hawaii, we investigated the effectiveness of GF-120 Fruit Fly bait spray applied to border area plants (*Sorghum* sp.) that completely surrounded (on all four sides) patches of cucumbers (*Cucumis sativus* L.), a favored host of melon flies. Application of bait spray to sorghum or other non-host vegetation surrounding host plants of melon flies is a common practice for melon fly control in Hawaii. The intent (after Nishida et al. 1957; Nishida 1958) is to attract (with bait spray droplets) immigrating melon fly females to sprayed sites, where they ingest feeding stimulant and insecticide before entering cultivated fields of hosts. As shown by Nishida (1953), most gravid melon fly females overnight on favored non-host plants in border areas adjacent to cultivated hosts before entering cultivated fields during the day to oviposit. In our 2002 study, we found that GF-120 Fruit Fly bait spray applied to a broad and dense swath of sorghum (50 cm deep) was very effective in preventing 4-week-old released protein-deprived (since eclosion) melon fly females from entering cucumber patches. It was significantly less effective, however, against 4-week-old released protein-fed (since eclosion) melon fly females (Prokopy et al. 2003).

On some Hawaiian islands, fields of cucurbit crops are more frequently bordered by sorghum or other vegetation on two (opposite) sides rather than on all four sides. As our first question here, we asked are bait sprays applied to sorghum plants that border only two (opposite) sides of a patch of cucumbers as effective as bait spray applied to sorghum plants that border four sides of a cucumber patch?

Under certain field conditions, newly-eclosed melon fly females could encounter absence of sufficient protein (to support egg development) for

extended periods, possibly even several weeks. Under other field conditions, feral females might encounter sufficient protein to develop a full complement of mature eggs during the 3-4 weeks that precede maturity but after reaching maturity fail to find protein during the course of a given day. As our second question, we asked are melon fly females carrying a high egg load but deprived of protein during the previous 24 h more responsive to bait spray than mature females having continuous access to protein?

In tests conducted in Hawaii in 2003, our objective was to answer these two questions. Our experimental approach was similar to that used in our 2002 investigation (Prokopy et al. 2003).

MATERIALS AND METHODS

Fly Origin and Maintenance

All melon fly females evaluated here were of the F_2 generation. Grand-parental flies oviposited in field-collected fruit of papaya, *Carica papaya* L. Parental flies and flies used here originated from papaya held in laboratory containers together with flies of the preceding generation. Following eclosion, F_2 adults were held in groups of ~150 females and ~150 males for 28-32 days at ~25°C, ~60% RH and ~13 h of natural light in 30 × 30 × 30 cm laboratory cages to permit mating. During this time, all flies were provided continuously with sucrose, USB enzymatic yeast hydrolysate (United States Biochemical, Cleveland, OH) and water (but no fruit).

Test Plots

A large open area of mowed grass (~70 × ~170 m), bordered by woods and located on the grounds of the Hawaii Agricultural Experiment Station at Kainaliu on Hawaii Island, was selected as the site for establishment of rotatable test plots (Fig. 1). For two of the test plots, on each test day we arranged potted sorghum plants in a square measuring 6 × 6 m (Fig. 1). Three sides of the square consisted of a single row of abutting pots of sorghum (24 plants per pot, 25 pots per row) that gave rise to a swath of sorghum ~25 cm wide × ~150 cm tall. The fourth side consisted of two fewer pots, thus allowing a 50-cm gap at the end of the row through which an observer could enter the plot. Sorghum plants were held upright by sandwiching them between strands of rope (50 cm apart; 75 and 125 cm above ground) attached to

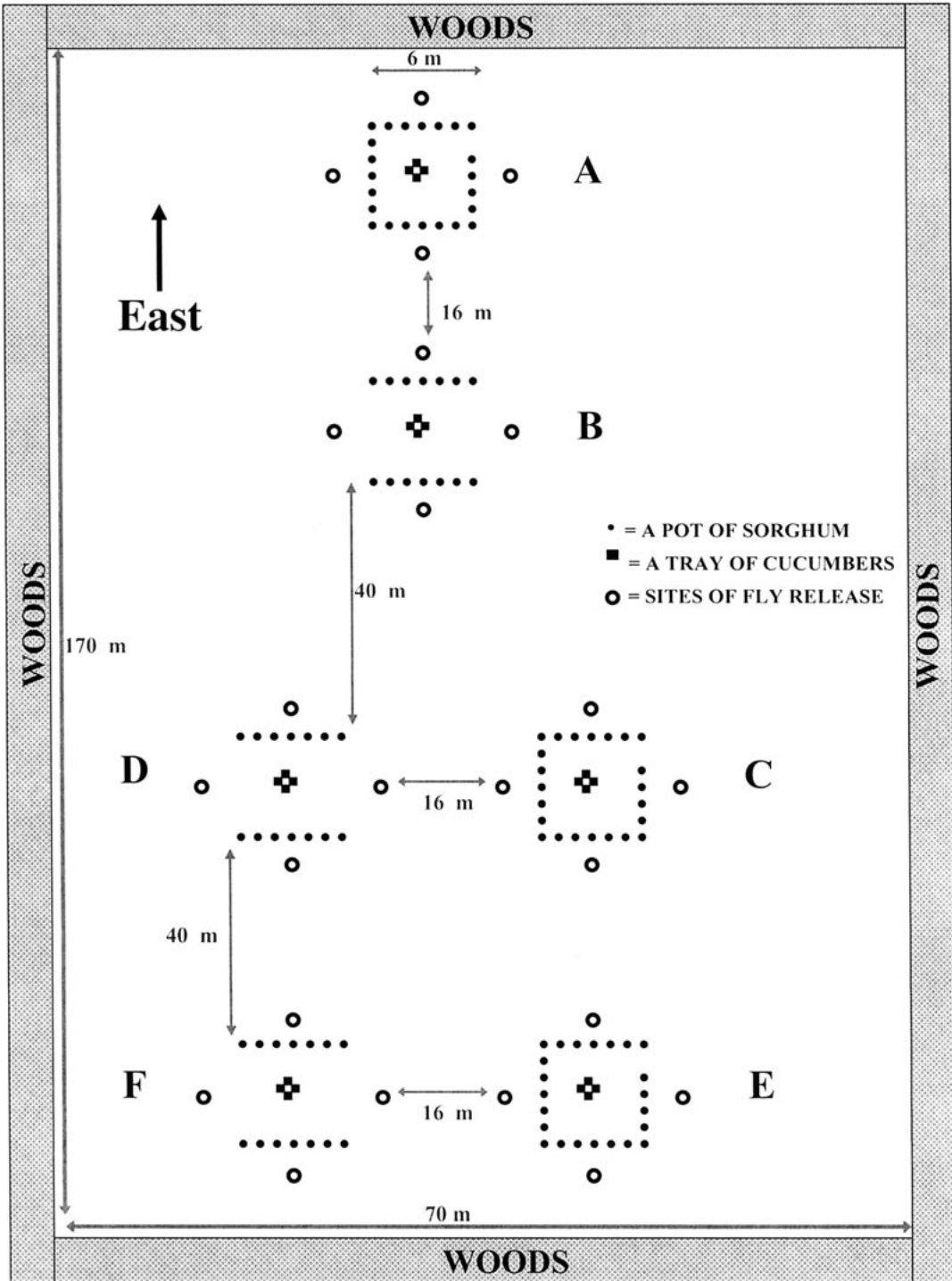


Fig. 1. Schematic arrangement of field test plots. Plots A, C, and E were bordered by a row of potted sorghum plants on four sides. Plots B, D, and F were bordered by a row of potted sorghum plants on two sides. Rows of sorghum plants in Plots A and B (in replicates 1 and 2) and Plots E and F (in replicates 3 and 4) received bait spray. Rows of potted sorghum plants in Plots C and D (in replicates 1 and 2) and Plots A and B (in replicates 3 and 4) did not receive bait spray. Rows of sorghum plants in Plots A and B were switched with those in Plots E and F after the second replicate. The entire area circumscribed by woods was mowed. Distances between plots and between plots and woods are not drawn to scale.

metal stakes. For two other test plots, on each test day we arranged sorghum plants (as above) in two parallel rows, 6 m long, that bordered the east and west sides of a plot, leaving the north and south sides open (Fig. 1).

For each test plot, we established four positions at which flies were released (Fig. 1). Each position was 5 m from the center of the plot (2 m outside of a row of sorghum) and received six pots of sorghum, arranged in a tight circle around a central stake. The plants were enveloped with rope so as to form a dense canopy of foliage that offered flies resting places after departure from release containers attached 80-120 cm above ground to the central stake.

For each test plot, we placed four black plastic trays (50 × 50 cm) on the ground 1 m from the center of the plot (Fig. 1). Each tray received eight cucumbers (purchased at a local supermarket and washed thoroughly before use) that served as potential ovipositional sites for released melon flies. A narrow slice (~5 mm thick) was cut from one end of each cucumber at 0830 h (the time of fly release) and every 30 min thereafter until 1630 h to enhance the emission of fresh odor.

Spray of GF-120

Each test day, two of the four test plots (one bordered by four rows of sorghum, the other by two rows of sorghum) received bait spray at label-recommended amount per hectare applied in the same manner as described in Prokopy et al. (2003). Briefly, using a hand-pumped back-pack sprayer, we applied 60 ml of freshly-made aqueous solution of GF-120 Fruit Fly bait (containing 80 ppm of spinosad) in a continuous swath 50 cm wide (75-125 cm above ground) to the outer perimeter (6 m long) of sorghum plants comprising each row of a plot. The batch of GF-120 Fruit Fly Bait used here was manufactured 21 months before our tests and was considered by the manufacturer to be fully potent at the time of use.

All bait spraying was done at 0815 h, 15 min before fly release. Because our supply of sorghum was limited, we could not apply spray to a new set of sorghum plants for each replicate. Hence, the day after completing a replicate, we thoroughly hosed all sprayed sorghum plants with an amount of water equivalent to ~20 mm of rainfall that, according to Prokopy et al. (2003), effectively removed any residual bait spray. We then waited 4 d before commencing the next replicate. To guard against any possible lingering effects of bait spray on released females, we chose the same set of sprayed sorghum plants for all replicates requiring sprayed plants and a second set of washed but unsprayed sorghum plants for all replicates requiring unsprayed plants. In all, there were four replicates. No rain fell during the conduct of any of the replicates.

Marking and Release of Flies

Two days before release, 640 females were marked on the pronotum with a dot of paint (Gloss Enamel, Tester Corp., Rockford, IL). Different two-color combinations were used to mark each of 16 sets of 20 females designated as protein-fed and each of 16 sets of 20 females designated as protein-deprived. To ensure flight capability of released females, only females that were observed to fly just after marking were used. After marking, 20 same-colored females were placed in a polyethylene box (12 cm wide × 18 cm tall × 5 cm deep) provided with sucrose, enzymatic yeast hydrolysate, and water. An opening (8 × 8 cm) was cut into the lid of the box and covered with removable netting to permit introduction of flies and their departure after release. At 0800 h on the day before release, yeast hydrolysate was removed from boxes containing flies designated to be protein-deprived for 24 h. It remained in boxes containing flies designated to be protein-fed. Dissections revealed that average loads of fully developed eggs for protein-deprived and protein-fed females at time of release were 38.8 ± 2.0 (SEM) and 36.5 ± 2.1 (SEM) eggs per female, respectively ($n = 30$ females per type).

At 0820 h each test day, one box of protein-deprived and one box of protein-fed females was attached, in vertical orientation, to each of the four stakes positioned 5 m from the center of each of four test plots. The opening of each box faced the center of the plot. In all, each test plot received 80 distinctively colored protein-deprived females and 80 distinctively colored protein-fed females. At 0830 h, netting was removed from each box to permit fly exit. At 1700 h, we censused each box and subtracted the number of flies therein from the number (20) originally intended for release. Across all treatments and replicates, only 3.5% of marked flies failed to exit from the boxes (Table 1). The majority exited by 1000 h.

Censusing Fly Presence in Test Plots

Beginning at 0900 h and every 30 min thereafter until 1700 h (when very few flies were observed), one observer stationed at each of the four simultaneously operative test plots carefully censused the number of color-coded melon flies observed on the foliage of sorghum and on cucumbers. For each census, each of the 6 m rows of sorghum was examined for 3 min and the cucumbers in each plot were examined for 15 min. Dead flies on sorghum and alive flies on cucumbers (none were found dead on cucumbers) were removed. Each of the first 16 females to arrive on cucumbers in a plot was aspirated into a separate net-covered plastic cup supplied with sucrose, water, and a piece of cucumber (1 × 2 × 2 cm) wrapped in parafilm except for a parafilm-free area (1 × 1 cm) punc-

TABLE 1. PERCENTAGES OF RELEASED PROTEIN-DEPRIVED FOR 24 H (P-DEP) AND PROTEIN-FED (P-FED, CONTINUOUSLY) MELON FLY FEMALES OBSERVED DEAD ON SORGHUM PLANTS AND ALIVE ON CUCUMBERS IN PLOTS BORDERED ON TWO OR FOUR SIDES BY BAIT-SPRAYED OR UNSPRAYED SORGHUM PLANTS (ACROSS ALL CENSUS PERIODS).

Flies	Sorghum sprayed	No. plot sides with sorghum	Total no. flies released	Mean percentages (\pm SEM) ¹	
				Dead on sorghum ²	Alive on cucumbers ²
P-DEP	Yes	4	313	5.4 \pm 2.9 a	17.5 \pm 7.9 a
		2	309	0.3 \pm 0.3 b	16.5 \pm 1.3 a
P-FED	Yes	4	306	1.0 \pm 0.7 b	17.0 \pm 8.6 a
		2	303	0.3 \pm 0.3 b	12.9 \pm 4.2 a
P-DEP	No	4	312	0.0 \pm 0.0 b	19.7 \pm 5.1 a
		2	302	0.0 \pm 0.0 b	21.7 \pm 6.5 a
P-FED	No	4	311	0.0 \pm 0.0 b	26.2 \pm 7.4 a
		2	313	0.0 \pm 0.0 b	21.2 \pm 8.4 a

¹Means in each column not followed by the same letter are significantly different according to ANOVA and LSD tests at $P = 0.05$.

²During each census, all females observed dead on sorghum or alive on cucumbers were removed.

tured with a needle to create a hole suitable for egg deposition. Cups with flies were returned to the laboratory. At 24 h after fly capture, pieces of cucumber were removed and eggs were counted. At 24 and 72 h after fly capture, females were assessed for mortality. Ambient temperature in field plots was recorded every 30 min from 0830-1700 h each test day and averaged 26°C (range 22-30°C).

Data Analysis

Proportions of each type of female released in each of the four replicates of each of the four test plot treatments (eight treatments in all) that were found dead on sorghum or alive on cucumbers were submitted to arcsin transformation before analysis of variance (ANOVA), which was followed by least significant difference (LSD) tests ($P = 0.05$) for comparison of treatment means where appropriate (significant P value from ANOVA).

Numbers of eggs laid by females of each type in cucumber over a 24-h period in cups subsequent to their capture in each test plot treatment were submitted to square root transformation ($\chi + 0.5$) before ANOVA. Proportions of such females that died during 24 h after capture were submitted to arcsin transformation before ANOVA. For all ANOVAS performed, "replicate" was used as random factor. For a given treatment in each analysis, we included only those females released within 5 m of plot center and subsequently observed in that plot. No females originating from another plot were included.

RESULTS

For females found dead on sorghum, ANOVA revealed a significant effect of treatment on mortality ($F = 3.32$; $df = 7, 21$; $P = 0.015$). Mortality was significantly greater among protein-deprived

females in four-sided bait-sprayed plots than for any other treatment (Table 1). Even though numerically more protein-deprived and protein-fed females were found dead on sorghum in four-sided and two-sided plots that were bait-sprayed compared with unsprayed plots, percent mortality per treatment in sprayed plots was low (range = 0.3-5.4% of released flies) (Table 1).

For females found alive on cucumbers, ANOVA revealed no significant effect of treatment on numbers observed ($F = 1.08$; $df = 7, 21$; $P = 0.41$). There was a consistent numerical trend toward fewer numbers of both protein-deprived and protein-fed females observed on cucumbers in each type of bait-sprayed plot (range = 12.9-17.5% of released flies) than in comparable unsprayed plots (range = 19.7-26.2% of released flies) (Table 1).

For females captured alive on cucumbers and transferred to cups with food, water, and a piece of cucumber for oviposition for 24 h after capture, ANOVA revealed a significant effect of treatment on mortality ($F = 5.76$; $df = 7, 21$; $P < 0.002$). For protein-deprived as well as protein-fed females taken from sprayed four-sided as well as sprayed two-sided plots, mortality after 24 h in cups was significantly greater (range = 25.0-38.1%) than for females of either type taken from either type of unsprayed plot (range = 0.0- 6.5%) (Table 2). For the last two of our four replicates, we observed mortality after 72 h but found no additional death among any flies.

For captured females transferred to cups, ANOVA revealed no significant effect of treatment on numbers of eggs laid per female during the 24 h after capture ($F = 1.92$; $df = 7, 21$; $P = 0.07$). For all four treatments involving flies taken from cucumbers in bait-sprayed plots, oviposition averaged numerically less (range = 20.7-27.1 eggs laid per female) than for flies taken from comparable unsprayed plots (range = 28.6-34.2 eggs laid per female) (Table 2).

TABLE 2. DURING THE 24 H AFTER CAPTURE OF RELEASED MELON FLY FEMALES ALIVE ON CUCUMBERS IN FIELD PLOTS BORDERED BY BAIT-SPRAYED OR UNSPRAYED SORGHUM PLANTS, PERCENTAGES OF CAPTURED FEMALES THAT DIED AND AMOUNT OF OVIPOSITION BY CAPTURED FEMALES INTO CUCUMBER.

Flies	Sorghum sprayed	No. plot sides with sorghum	Total no. flies captured ¹	Mean percent dead (\pm SEM) ²	Mean no. eggs laid (\pm SEM) ^{2,3}
P-DEP	Yes	4	26	34.6 \pm 9.7 a	27.1 \pm 3.5 a
		2	30	33.3 \pm 11.1 a	20.7 \pm 4.4 a
P-FED	Yes	4	21	38.1 \pm 14.0a	23.3 \pm 5.1a
		2	24	25.0 \pm 8.4 a	24.2 \pm 4.9 a
P-DEP	No	4	22	4.5 \pm 4.0 b	28.6 \pm 4.0 a
		2	31	6.5 \pm 6.3 b	32.0 \pm 4.0 a
P-FED	No	4	30	0.0 \pm 0.0 b	28.8 \pm 3.6 a
		2	26	3.8 \pm 3.8 b	34.2 \pm 3.7 a

¹Across all four replicates, 32 females per treatment were captured, placed in netted cups, and returned to the laboratory. While there, some inadvertently escaped from cups, accounting for the reduction from 32 per treatment.

²Means not followed by the same letter are not significantly different according to ANOVA and LSD tests at $P = 0.05$.

³Means are based on total numbers of females placed in cups, regardless of whether females were alive or dead after 24 h in cups.

Of the 2,560 color-marked females originally placed in release boxes, 2,469 (96.4%) left the boxes during test periods. Of these 2,469, 477 (19.3%) were observed on cucumbers in plots immediately adjacent to sites of release, 50 (2.0%) were observed on cucumbers in the nearest of the other three plots, and 11 (0.4%) were observed on cucumbers in the two most distant plots.

DISCUSSION

Our findings indicate that GF-120 Fruit Fly Bait containing spinosad at 80 ppm in aqueous solution applied as a spray to a single row of potted sorghum plants that surrounded a patch of cucumbers on all four sides (north, east, south, west) was not effective against released melon fly females deprived of protein for 24 h. Only 5.4% were observed dead on sprayed sorghum, whereas 17.5% were found alive on cucumbers. It was equally ineffective against released protein-fed females. Only 1.0% were observed dead on sprayed sorghum, whereas 17.0% were found alive on cucumbers. Furthermore, the same type of spray applied to single rows of potted sorghum plants that bordered a patch of cucumbers on two sides (east, west) was even less effective against released melon fly females: only 0.3% of released protein-deprived and 0.3% of released protein-fed females were observed dead on sprayed sorghum. For neither type of female (protein-deprived or protein-fed) and neither structure of plot (a row of sorghum on four or two sides) was the percentage of released females found on cucumbers significantly less for bait-sprayed than unsprayed plots.

For two of the treatments (protein-fed females released adjacent to plots surrounded on all four sides by bait-sprayed or unsprayed sorghum), the experimental protocol here was identical to that used for these two treatments in our 2002 test

(Prokopy et al. 2003), except in one respect. Here, only a single row of potted sorghum plants (foliage 25 cm wide) surrounded each plot, whereas in 2002 two abutting rows of sorghum plants (foliage 50 cm wide) surrounded each plot. For plots with unsprayed sorghum, results for each year were similar: 0% of released females observed dead on sorghum each year and 31.2% (2002) vs. 26.2% (2003) observed alive on cucumbers. For plots with bait-sprayed sorghum, however, results were quite different between years: 14.0% (2002) vs. 1.0% (2003) of released females observed dead on sorghum and 10.9% (2002) vs. 17.0% (2003) observed alive on cucumbers. If we presume (as affirmed by the manufacturer) that the GF-120 Fruit Fly Bait used in 2003 was as potent as that used in 2002, we are left to conclude that the much-reduced mortality of flies released adjacent to baited-sprayed plots in 2003 and the greater percentage of released flies observed on cucumbers in 2003 was due principally (or exclusively) to the presence of only a single row rather than a double row of potted sorghum plants. Compared with a double row of potted sorghum plants, a single row could have permitted a greater amount of attractive odor from cucumbers to flow through the sorghum to fly-release sites or afforded less shelter to foraging females (thereby reducing the amount of time females would spend in the presence of bait spray). Evidence from a study in 2004 by Revis et al. (unpublished data) supports the latter explanation.

We anticipated substantially greater mortality than observed of protein-deprived (for 24 h) compared with protein-fed (continuously) females on bait-sprayed sorghum based on the expectation that 24 h of protein deprivation would enhance hunger for protein. We also anticipated observation of substantially more (not fewer) females of each type on cucumbers in bait-sprayed plots

with sorghum on two sides compared with four sides based on presence of half as many bait-sprayed rows of sorghum. These expectations were not met, perhaps because the strong influence of cucumber odor relative to the bait spray odor masked or overrode anticipated effects of fly hunger and test plot structure.

One could argue that the effect of spinosad on target insects may not be immediate mortality but delayed mortality (24, 48, or 72 h later) and that following ingestion of spinosad, target insects may be subjected to sub-lethal effects. Among released females captured on cucumbers at interiors of bait-sprayed plots and held for 24 h in cups, there was indeed substantial mortality (33.3-34.6% for protein-deprived females and 25.0-38.1% for protein-fed females) above that observed on sorghum during the 8 h after fly release (there was no additional mortality from 24-72 h). Even so, on average only 23% fewer eggs were laid by such females during 24 h after capture compared with eggs laid by females captured on cucumbers at interiors of unsprayed plots (Table 2).

In conclusion, our findings here suggest that GF-120 Fruit Fly Bait spray may not be an effective control measure against melon flies if applied only to a narrow or thin swath of sorghum bordering a cultivated field of attractive melon fly hosts, such as cucumbers. This could be true regardless of whether immigrating females have fed on protein within the previous 24 h or not, and regardless of whether cultivated host fields are bordered by sorghum on two or four sides. If sorghum is to be used effectively as a site for bait spray application, we suggest that it be planted in a broad or dense swath that could provide effective shelter for foraging flies, thereby enhancing the probability of local encounter with bait spray droplets.

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