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Attraction and mortality of oriental fruit flies to SPLAT-MAT-methyl eugenol with spinosad†

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Abstract

Studies were conducted in 2007 and 2008 in Hawaii, USA to quantify attraction and feeding responses resulting in mortality of the male oriental fruit fly, *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae), to a novel male annihilation treatment (MAT) formulation consisting of specialized pheromone and lure application technology (SPLAT) in combination with methyl eugenol (ME) and spinosad (=SPLAT-MAT-ME with spinosad) in comparison with Min-U-Gel-ME with naled (Dibrom). Our approach involved a novel behavioral methodology for evaluation of slow-acting reduced-risk insecticides. Methyl eugenol treatments were weathered for 1, 2, 4, and 8 weeks in California, USA, and shipped to Hawaii for bioassays. In field tests involving bucket traps to attract and capture wild males, and in toxicity studies conducted in 1 m^3 cages using released males of controlled ages, SPLAT-MAT-ME with spinosad performed similar to or outperformed the standard formulation of Min-U-Gel-ME with naled for material aged for up to 8 weeks in the 2008 tests. In laboratory feeding tests in which individual males were exposed for 5 min to the different ME treatments, mortality induced by SPLAT-MAT-ME with spinosad recorded at 24 h did not differ from that caused by Min-U-Gel ME with naled at 1, 2, and 4 weeks. Spinosad has low contact toxicity, and when mixed with SPLAT offers a reduced-risk alternative for control of *B. dorsalis*, without many of the negative effects to humans and non-targets of broad-spectrum contact poisons such as naled. Our results indicate that SPLAT-MAT-ME with spinosad offers potential for control of males in an area-wide integrated pest management (IPM) system without the need for conventional organophosphates.

Introduction

Traditionally, fruit flies (Diptera: Tephritidae) of the genus *Bactrocera* have been controlled throughout the Pacific using protein bait (against females) and male annihilation treatments (MAT) using the highly attractive kairomone lures, methyl eugenol (ME) and cue-lure (C-L) (Metcalf & Metcalf, 1992), depending on the target fly species, against males (Mau et al., 2007). The bait spray and MAT strategy dramatically reduces the amount of pesticides used and it has been used successfully in eradication campaigns (Steiner et al., 1965, 1970) and more recently in area-wide control of fruit flies (Vargas et al., 2008a), including the oriental fruit fly, *Bactrocera dorsalis* (Hendel). This highly polyphagous species is native to tropical Asia and is considered to be among the five most damaging and aggressive pest fruit flies in the world (Allwood et al., 1999; Leblanc & Putoa, 2000).

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Since the late 1950s the most common toxicants used in fruit fly bait spray and MAT formulations have been organophosphate insecticides such as malathion and naled (Steiner et al., 1965; Roessler, 1989). However, organophosphate insecticides have been largely implicated in negative effects on natural enemies and human health (Carson, 1962). Recently, a new protein bait (Peck & McQuate, 2000; Vargas et al., 2001) and MAT sprays (Vargas et al., 2008a) containing the reduced-risk insecticide spinosad have been researched and developed in Hawaii for use against pestiferous fruit flies in several regions of the USA. Spinosad is a mixture of spinosyns A and D, the soil fermentation products of the soil bacterium *Saccharopolyspora spinosa* Mertz and Yao, and is active at low application rates, has low mammalian toxicity, and reduced impact on natural enemies (DowElanco, 1994; Stark et al., 2004).

As part of a 10-year program to promote area-wide integrated pest management (IPM) methods in Hawaii for fruit fly suppression (Mau et al., 2007; Vargas et al., 2008a), GF-120 NF Naturalyte Fruit Fly Bait with spinosad has replaced protein bait with malathion, thereby greatly reducing the use of organophosphate insecticides. However, 'attract and kill' male-lure devices without organophosphate insecticides are still being evaluated and registered with the U.S. Environmental Protection Agency. Of particular interest are sprayable formulations with reduced-risk insecticides such as spinosad (Vargas et al., 2008a). Recently, Vargas et al. (2008b) formulated and tested sprayable 'attract and kill' dispensers containing the specialized pheromone and lure application technology (SPLAT^{TM}), spinosad, and ME or C-L against *B. dorsalis* and *Bactrocera cucurbitae* (Coquillett), respectively. The SPLAT-MAT-ME and SPLAT-MAT-C-L spinosad formulations show promise as substitutes for current liquid organophosphate insecticide formulations used for area-wide suppression of *B. dorsalis* and *B. cucurbitae* in Hawaii.

SPLAT is a proprietary base matrix formulation of biologically inert materials used to control the release of semiochemicals with or without pesticides. The recently developed SPLAT matrix emits semiochemicals at effective pest suppression levels for a time interval ranging from 2 to 16 weeks and, by having a wide range of viscosities and application methods (e.g., applicator sprays, aerial applicator sprays, and caulking gun type tubes), increases productivity by mechanizing the application of pheromone-dispensing points (Stelinski et al., 2007). The amorphous and flowable quality of this highly adaptable product allows for an easy transition from small-scale manual applications to large-scale manual or mechanical applications.

The objective of the present study was to investigate the performance of SPLAT-MAT-ME with spinosad weathered under Californian climatic conditions to attract and kill male *B. dorsalis* in Hawaii. In local attraction experiments conducted using bucket traps, and in toxicity tests involving field cages and feeding tests in the laboratory, we compared the novel SPLAT-MAT-ME formulation with spinosad to the standard Min-U-Gel MAT ME formulation with naled. Min-U-Gel is a fine grade of attapulgite clay (anhydrous magnesium aluminum silicate) that was developed for spot applications in male annihilation programs in California for eradication of *B. dorsalis* (Chambers et al., 1974; Cunningham & Suda, 1985). One disadvantage of Min-U-Gel and similar thickened formulations is that they are short-lived when used in areas subject to high temperatures and high rainfall (Cunningham et al., 1975a,b; Cunningham & Suda, 1985; Vargas et al., 2000). Furthermore, use of naled in residential areas has met increasing opposition from home-owners and environmental groups.

Materials and methods

Insects

Bactrocera dorsalis pupae were obtained from a wild colony established from infested papaya and reared for no more than two generations at the USDA-ARS, United States Pacific Basin Agricultural Research Center (USPBARC) in Hilo, HI, USA, following the methods described in Vargas (1989). Adult flies were allowed to emerge inside cubical screen cages (30 cm^3) with a 3:1 mixture of sucrose and USB enzymatic yeast hydrolysate (United States Biochemical, Cleveland, OH, USA) as a food source and water ad libitum. All experimental flies were held in a laboratory maintained at 22 ± 3 °C and 60–80% r.h., under a L12:D12 photoperiod. All male flies were tested after they reached an age of 25–28 days.

Methyl eugenol treatments

Three ME treatments were evaluated: (1) SPLAT-MAT-ME + spinosad [2% active ingredient (a.i.)]; (2) SPLAT-MAT-ME without spinosad (control); and (3) Min-U-Gel ME with naled (5% a.i.) (Dibrom® Concentrate; Valent USA, Walnut Creek, CA, USA), currently in use in California. A metering gun (ISCA Technologies, Riverside, CA, USA) was used to apply an average amount of 1.89 g of each treatment to the surface of wooden tongue depressors (2 × 5 cm) (Puritan Medical Products, Guilford, ME, USA). A small hole was drilled in the non-treated end of each tongue depressor. Depressors were hung on a weathering line in direct

sunlight in Riverside, CA, USA. Mean (±SEM) daily air temperature and cumulative rainfall values for the weathering site were: 13.6 ± 0.4 °C (range: 2.2–30.5 °C) and 115.6 mm for the 2007 (trial 1) study, and 20.4 ± 0.54 °C (7.8–37.2 °C) and 21.3 mm for the 2008 (trial 2) study, respectively. Each of three different treatments was tested for each of three aging periods (1, 4, and 8 weeks) in 2007 and four aging periods (1, 2, 4, and 8 weeks) in 2008. At the prescribed intervals, each depressor was placed inside an individual 50-ml polyethylene conical tube and shipped to Hawaii.

Field attraction experiment

The 2007 tests (trial 1) were conducted from November 20, 2007 to January 11, 2008, whereas the 2008 evaluations (trial 2) took place from May 6 to June 27, 2008. For this experiment, we used plastic bucket traps. Each trap was constructed from a plastic container (Highland Plastics, Mira Loma, CA, USA) (20 cm height × 21.5 cm diameter) which was drilled on the sides to create eight 1-cm holes. A plastic 1.5-ml microcentrifuge tube (Fisher Scientific, Hampton, NH, USA) that had the tip cut off (diameter of the resulting hole: 6 mm) was inserted in each hole to permit responding males to enter. Each trap had a plastic lid through which a 30-cm steel wire was placed and secured with a drop of glue. On a given test day, four sets (i.e., four replicates) of three bucket traps containing each of the three treatments were deployed simultaneously in vegetation surrounding the USPBARC facility at Hilo, Hawaii (trial 1) and the University of Hawaii Experiment Station at Waiakea, Hawaii (trial 2) to quantify the relative attractiveness of each ME formulation. Starting at 08:30 hours traps were hung from branches of fruiting strawberry guava trees (in 2007) and from non-host plants that faced a papaya orchard (in 2008) for a 30-min period, allowing wild *B. dorsalis* males to enter traps. Every 10 min, traps were rotated clockwise to avoid positional bias. After the 30-min period, the micro-centrifuge tube lids were closed on each trap and all traps were brought back to the laboratory where numbers of males captured were recorded. Four persons were needed to service the trap sets. Four replicates of each ME treatment were conducted simultaneously on each test day (eight replicates over a 2-day period).

Cage attraction/mortality experiment

The relative toxicity of the two ME treatments associated with a toxicant (spinosad or naled) was quantified in 1-m^3 organdy cages deployed at the University of Hawaii Waiakea Experiment Station using the laboratory-reared (F_1 or F_2) male *B. dorsalis*. The ME treatment without spinosad was evaluated to determine whether males exposed continuously to SPLAT-MAT-ME for a maximum period of 24 h would die from feeding upon ME even in the absence of a toxicant. For each of the four aging periods on each test day, one treated wooden tongue depressor was hung inside each of three organdy cages containing an aluminum tray with food (6–8 sugar cubes) and water. Twenty-five laboratory-reared males were released per cage between 08:30 and 10:00 hours. After flies were released, an observer recorded the number of dead males at 4 and 24 h after release. Four replicates (one per day) were carried out. A total of 300 males was tested for each aging period (2 100 males in all).

Feeding tests

The relative toxicity of the three ME formulations described above was assessed inside a laboratory maintained at 22 ± 2 °C, 50–60% r.h., and a L12:D12 photoperiod using the same type of laboratory-reared males (F_1 or F_2) used for the cage tests. Individual males were introduced into an experimental cage (30 cm^3) containing a particular treatment and gently placed onto the test material. Each male was allowed to feed for at least 1 min and up to 5 min (maximum time); actual feeding time was recorded. Given the compulsive feeding behavior that *B. dorsalis* males exhibit when in contact with ME (Steiner, 1952), males not feeding for 5 min were rather uncommon. After the 5-min feeding period, each male was introduced into an inverted small plastic cup (labeled) containing a small cotton wick saturated with a sugar/water solution. Mortality was recorded after 4 and 24 h. Because 10 males were exposed in sequence to the same material, mortality was calculated as a proportion (no. males dead/10 males that fed on a given treatment). For each of the three (in 2007) and four (in 2008) aging periods, there were 5–6 replicates of each of the three ME treatments. In all, 1 210 males were individually tested.

Statistical analysis

For the field attraction study involving bucket traps, captures of male *B. dorsalis* in the three ME treatments over a 30-min period were compared using one-way ANOVA, on $\sqrt{(x + 0.5)}$-transformed data whenever needed to stabilize variances. Means were compared using the Fisher-protected least significant differences test with $\alpha = 0.05$. For the cage and the feeding studies, rates of mortality induced by SPLAT-MAT-ME with spinosad were compared, for every week period, against that of Min-U-Gel-ME with naled using a t-test. Data on proportions were arcsin-transformed before analysis.

Table 1 Captures (mean number ± SEM; n = 8) of wild male *Bactrocera dorsalis* in bucket traps baited with various methyl eugenol (ME) treatments that were weathered for either 1, 4, or 8 weeks (trial 1) or 1, 2, 4, or 8 weeks (trial 2) in California and shipped to Hawaii for bioassays. Traps were hung from branches of fruiting strawberry guava trees (trial 1) and from non-host plants that faced a papaya orchard (trial 2), for 30 min. Study conducted from 20 November 2007 to 11 January 2008 (trial 1) and from 6 May to 27 June, 2008 (trial 2)

		Time period (weeks)			
Year	ME treatment	1	2	4	8
2007 (trial 1)	SPLAT-MAT-ME	25.75 ± 6.89a	–	35.25 ± 7.72a	10.62 ± 1.83b
	SPLAT-MAT-ME spinosad	22.00 ± 3.88a	–	38.62 ± 8.57a	9.75 ± 1.34b
	Min-U-Gel ME with naled	20.37 ± 3.23a	–	35.12 ± 6.02a	15.37 ± 1.16a
2008 (trial 2)	SPLAT-MAT-ME	7.50 ± 2.31a	24.62 ± 6.25a	23.12 ± 2.88a	13.75 ± 2.53a
	SPLAT-MAT-ME spinosad	6.50 ± 1.08a	16.75 ± 6.17a	22.12 ± 4.68a	7.75 ± 1.28b
	Min-U-Gel ME with naled	3.25 ± 0.81a	17.62 ± 4.43a	12.37 ± 2.60b	1.75 ± 0.70c

For each year and within each week category, values in a column followed by different letters are significantly different according to ANOVA at the 0.05 level LSD (for trial 1: week 1: $F_{2,21} = 0.31$, $P = 0.735$; week 4: $F_{2,21} = 0.07$, $P = 0.933$; week 8: $F_{2,21} = 4.21$, $P = 0.029$; for trial 2: week 1: $F_{2,21} = 1.23$, $P = 0.313$; week 2: $F_{2,21} = 0.58$, $P = 0.567$; week 4: $F_{2,21} = 3.73$, $P = 0.041$; week 8: $F_{2,21} = 19.54$, $P<0.001$).

Tables show untransformed data. All statistical analyses were conducted using STATISTICA (StatSoft, 2001).

Results

Field attraction experiment

In the 2007 studies, *B. dorsalis* captures were not significantly different among the three ME treatments for weeks 1 and 4 ($P>0.05$); however, by week 8, *B. dorsalis* captures were significantly greater for Min-U-Gel ME with naled than for the two SPLAT-MAT-ME treatments (Table 1). In the 2008 studies, *B. dorsalis* captures were not significantly different among the three ME treatments for weeks 1 and 2. At weeks 4 and 8, the two SPLAT-MAT-ME treatments attracted significantly more males than Min-U-Gel-ME with naled (Table 1).

Cage attraction/mortality experiment

For the 2007 cage tests, mortality recorded 4 h after release did not differ significantly between SPLAT-MAT-ME with spinosad and Min-U-Gel-ME with naled for material weathered for 1 week in 2007; however, at weeks 4 and 8 mortality recorded 4 h after release was significantly greater for Min-U-Gel ME with naled than for SPLAT-MAT-ME with spinosad. Cumulative mortality recorded at 24 h was not significantly different for the two insecticide-containing ME treatments even after 8 weeks in the 2007 tests (Table 2). For the 2008 tests, no significant differences in the 4 and 24 h cumulative mortality were recorded between SPLAT-MAT-ME with spinosad and Min-U-Gel-ME with naled for any of the four aging periods (Table 2).

Feeding tests

Results obtained in both trials revealed the same pattern of response. In both trials the 4-h mortality did not vary significantly between SPLAT-MAT-ME with spinosad and Min-U-Gel ME with naled for material weathered for 1 week, but it differed significantly between these two ME treatments at weeks 2 (for trial 2, conducted only in 2008), 4, and 8 (Table 3). Cumulative mortality recorded at 24 h reached 100% for the two insecticide-containing ME treatments weathered for 1 or 2 weeks, and remained statistically similar at week 4. By week 8, significantly higher mortality (both 4 and 24 h after exposure) was recorded for Min-U-Gel-ME with naled than for SPLAT-MAT-ME with spinosad (Table 3).

Discussion

In the present study the relative attractiveness and toxicity of SPLAT-MAT-ME containing the reduced-risk insecticide spinosad to male *B. dorsalis* was compared with that of the standard Min-U-Gel-ME with naled, a very toxic organophosphate insecticide. Our behavior-based experimental approach allowed for the quantification of the relative levels of attractiveness of the ME treatments to wild males in the field (using bucket traps) as well as their residual toxicity in two additional bioassays (in 1-m^3 cages and in feeding tests conducted in the laboratory) using males of controlled ages. In terms of attraction, SPLAT-MAT-ME with and without spinosad outperformed the current standard of Min-U-Gel-ME with naled after 8 weeks in our 2008 trial. In terms of toxicity, in both trials we demonstrated that

Table 2 Mortality (mean % ± SEM; n = 4) of laboratory-reared male *Bactrocera dorsalis* induced by two methyl eugenol (ME) treatments associated with a toxicant (either spinosad or naled) or without a toxicant (SPLAT-MAT-ME). Materials were weathered in California and shipped to Hawaii for bioassays. For each test day, groups of 25 males were released inside 1-m^3 cages with one ME treatment and food (sugar) and water

			Time period (weeks)			
Year	Mortality	ME treatment	1	2	4	8
2007 (trial 1)	4 h	SPLAT-MAT-ME	0	–	1.00 ± 1.00	0
		SPLAT-MAT-ME spinosad	78.92 ± 7.85a	–	60.60 ± 4.90b	64.00 ± 10.71b
		Min-U-Gel ME with naled	89.33 ± 4.81a	–	85.80 ± 4.20a	90.00 ± 3.83a
	24 h[1]	SPLAT-MAT-ME	3.00 ± 1.00	–	2.00 ± 1.10	5.00 ± 3.00
		SPLAT-MAT-ME spinosad	95.00 ± 3.00a	–	93.00 ± 2.52a	73.00 ± 11.36a
		Min-U-Gel ME with naled	89.00 ± 5.25a	–	97.00 ± 1.91a	95.00 ± 1.91a
2008 (trial 2)	4 h	SPLAT-MAT-ME	0	0	0	0
		SPLAT-MAT-ME spinosad	87.00 ± 6.81a	97.00 ± 1.91a	77.36 ± 1.92a	81.44 ± 2.25a
		Min-U-Gel ME with naled	96.00 ± 2.83a	100.00 ± 0.00a	96.03 ± 2.44a	80.37 ± 8.49a
	24 h[1]	SPLAT-MAT-ME	1.00 ± 1.00	3.00 ± 1.91	1.00 ± 1.00	0
		SPLAT-MAT-ME spinosad	100a	99.00 ± 1.00a	89.00 ± 11.00a	97.00 ± 1.91a
		Min-U-Gel ME with naled	99.00 ± 1.00a	100a	100a	88.22 ± 4.33a

For each year, and within each mortality period, values in a column followed by different letters are significantly different according to t-test at the 0.05 level. Comparison made only for ME treatments containing toxicant (spinosad or naled) (for trial 1, mortality 4 h: week 1: t = 1.13, d.f. = 5, P = 0.311; week 4: t = 3.64, d.f. = 6, P = 0.010; week 8: t = 2.50, d.f. = 6, P = 0.046; mortality 24 h: week 1: t = 0.83, d.f. = 6, P = 0.44; week 4: t = 1.02, d.f. = 6, P = 0.347; week 8: t = 2.41, d.f. = 6, P = 0.052; for trial 2, mortality 4 h: week 1: t = 1.13, d.f. = 6, P = 0.300; week 2: t = 1.68, d.f. = 6, P = 0.143; week 4: t = 0.96, d.f. = 6, P = 0.375; week 8: t = 0.11, d.f. = 6, P = 0.917; mortality 24 h: week 1: t = 1.00, d.f. = 6, P = 0.356; week 2: t = 1.00, d.f. = 6, P = 0.356; week 4: t = 1.00, d.f. = 6, P = 0.356; week 8: t = 2.18, d.f. = 6, P = 0.072).
[1]Cumulative mortality.

the cumulative 24-h mortality did not differ between the two insecticide-containing ME treatments after 8 weeks (in the cage study) or after 4 weeks (in the feeding study in the laboratory).

In this study we evaluated the performance of SPLAT, a new matrix formulation of biologically inert materials used to control the release of semiochemicals, in this case ME, in combination with the reduced-risk insecticide spinosad. Previously, carriers used throughout the Pacific in MAT included canec fiberboard blocks, cotton wicks, Min-U-Gel, and molded paper fiber (Vargas et al., 2000, 2005). For example, fiberboard blocks impregnated with ME and various organophosphate insecticides (e.g., naled and malathion) were previously used to eradicate *B. dorsalis* from Rota (Steiner et al., 1965), Saipan (Steiner et al., 1970), Okinawa (Koyama et al., 1984), and papaya fruit fly, *Bactrocera papayae* Drew & Hancock, from Australia (Cantrell et al., 2002). In earlier comparative trials in Hawaii using traps, the SPLAT-ME formulation outperformed the Min-U-Gel-ME formulation after 7–12 weeks (Vargas et al., 2008b). The most popular organophosphate insecticides used for male annihilation have included naled, malathion, and 2,2-dichlorovinyl dimethyl phosphate (Vargas et al., 2003). These insecticides, in particular naled and malathion, are highly toxic and pose serious concerns in terms of potential negative effects on human and environmental health. Furthermore, organophosphate insecticides (i.e., malathion and naled) are very unpopular with residential home-owners, where accidental fruit fly introductions often occur. As stated before, Min-U-Gel with naled is currently used in California and Florida for eradication of *B. dorsalis* (Chambers et al., 1974; Cunningham & Suda, 1985). Of particular concern in California has been the use of naled in residential areas on telephone poles and tree trunks during eradication programs. The SPLAT-spinosad products are unique in that they offer a novel and convenient ready-to-use MAT formulation that contains both a powerful lure and a reduced-risk insecticide for fruit fly control by farmers and home gardeners and thus SPLAT-MAT-ME could be deployed in these environmentally sensitive areas.

Our field study involving bucket traps showed that attraction of male *B. dorsalis* to the SPLAT-MAT-ME treatments equaled or outperformed Min-U-Gel-ME with naled after 4 weeks (in both trials) and after 8 weeks (only in the 2008 trial). Similarly, our field cage results indicated excellent killing power of SPLAT-

Table 3 Mortality (mean % ± SEM; n = 5–6) of laboratory-reared male *Bactrocera dorsalis* in the feeding study in the laboratory. Individual males (10 per replicate) were fed for 5 min (except for the ME treatment involving naled which killed males within seconds) on one of the three ME treatments that were weathered in California and shipped to Hawaii for bioassays

			Time period (weeks)			
Year	Mortality	ME treatment	1	2	4	8
2007 (trial 1)	4 h	SPLAT-MAT-ME	0	–	0.38 ± 0.38	2.85 ± 1.84
		SPLAT-MAT-ME spinosad	98.00 ± 2.00a	–	81.27 ± 6.67b	35.00 ± 23.60b
		Min-U-Gel ME with naled	100a	–	100a	100a
	24 h[1]	SPLAT-MAT-ME	0	–	0	5.71 ± 2.97
		SPLAT-MAT-ME spinosad	100a	–	91.43 ± 5.95a	42.50 ± 25.29b
		Min-U-Gel ME with naled	100a	–	100a	100a
2008 (trial 2)	4 h	SPLAT-MAT-ME	0	3.33 ± 3.33	0	0
		SPLAT-MAT-ME spinosad	90.00 ± 7.75a	78.33 ± 9.10b	42.00 ± 18.28b	10.00 ± 6.83b
		Min-U-Gel ME with naled	100a	100a	100a	100a
	24 h[1]	SPLAT-MAT-ME	0	3.33 ± 3.33	0	0
		SPLAT-MAT-ME spinosad	100a	100a	98.00 ± 2.00a	30.00 ± 16.33b
		Min-U-Gel ME with naled	100a	100a	100a	100a

For each year, and within each mortality period, values in a column followed by different letters are significantly different according to t-test at the 0.05 level. Comparison made only for ME treatments containing toxicant (spinosad or naled) (for trial 1, mortality 4 h: week 1: $t = 1.10$, d.f. = 9, $P = 0.296$; week 4: $t = 2.50$, d.f. = 9, $P = 0.034$; week 8: $t = 2.89$, d.f. = 5, $P = 0.027$; mortality 24 h: week 4: $t = 1.12$, d.f. = 9, $P = 0.292$; week 8: $t = 2.62$, d.f. = 6, $P = 0.039$; for trial 2, mortality 4 h: week 1: $t = 0.02$, d.f. = 7, $P = 0.981$; week 2: $t = 3.47$, d.f. = 10, $P = 0.006$; week 4: $t = 5.31$, d.f. = 9, $P<0.001$; week 8: $t = 20.96$, d.f. = 10, $P<0.001$; mortality 24 h: week 4: $t = 1.11$, d.f. = 9, $P = 0.296$; week 8: $t = 6.20$, d.f. = 10, $P<0.001$).
[1]Cumulative mortality.

MAT-ME with spinosad weathered for 8 weeks when compared with Min-U-Gel-ME with naled after a 4-h period of male exposure to these materials in 2008 but not in 2007. During the overall aging period of the ME treatments in Riverside, California, rainfall was about five times greater in 2007 (cumulative rainfall: 115.6 mm) compared with 2008 (21.3 mm) and thus the negative impact of rainfall on the ME treatments including SPLAT may have been greater in 2007 than in 2008. Regardless of the amount of rainfall, however, we observed that most Min-U-Gel had fallen from the depressors after only 2 weeks of exposure to outdoor California conditions. This result was expected as Min-U-Gel is known to often last less than 2 weeks (Vargas et al., 2000) in particular when weathered in areas with high temperatures and high rainfall (Cunningham *et al*., 1975a, b; Cunningham & Suda, 1985; Vargas et al., 2000). Conversely, SPLAT has a waxy outer coating that acts as a reservoir with time release properties which allows the lure to last longer than Min-U-Gel when applied to surfaces. The present study thus represents the second report (after Vargas et al., 2008b) documenting that SPLAT performs at least similarly or better than other commercial carriers such as Min-U-Gel in delivering ME as an attractant against *B. dorsalis*. In addition, SPLAT, like Min-U-Gel, can be sprayed from small sprayers, trucks, and aircraft making the technology convenient and flexible.

Our feeding tests also indicate that when males were allowed to feed for only 5 min on SPLAT-ME with spinosad weathered for 8 weeks, less than 50% (30–42.5%) of the males died in a 24-h period. In contrast, when males were exposed to the same material for 24 h inside a field cage the resulting mortality (73–97%) was comparable with that induced by Min-U-Gel with naled. It is conceivable that this contrasting result is due to presumably greater amounts of spinosad ingested by the males in the cage tests, given that males had continuous access to the SPLAT-MAT-ME formulation with spinosad. Given the compulsive feeding behavior that male *B. dorsalis* exhibits towards ME (Steiner, 1952), it would be reasonable to assume that under field conditions SPLAT-MAT-ME with spinosad would be accessible to males for much longer than for the 24 h period of maximum exposure in the cages.

Our combined results, when coupled with findings from previous tests conducted in Hawaii (Vargas et al., 2008b), indicate that spinosad can be considered an excellent replacement for organophosphate insecticides in MAT sprays. MAT approaches should be combined with environmentally friendly bait spray treatments such as the spinosad-based GF-120 NF Naturalyte Fruit Fly

Bait for successful area-wide suppression of ME and C-L-responding fly species in regions of the world where they are serious economic pests as well as for eradication of accidental introductions of into the USA mainland and other Pacific countries.

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