Mark-Release-Recapture Experiments on the Effectiveness of Methyl Eugenol–Spinosad Male Annihilation Technique Against an Invading Population of *Bactrocera dorsalis*

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Abstract. *Bactrocera dorsalis* is a pest of major concern in fruit-growing areas where it is not established. Control and eradication often employs male annihilation technique, using methyl eugenol as an attractant (MAT-ME). We conduced a small-scale mark-release-recapture study comparing two densities of MAT-ME ("high" = 225 spots per km^2; "low" = 100 spots per km^2) with a control by counting males recaptured in sentinel traps baited with ME 40 m from the release point. We hypothesized that recaptures would be reduced under the two MAT treatments by equivalent amounts compared with the control, reflecting male mortality from the treatments. We found a large degree of variation in trap recaptures between replicates and treatments, and no significant difference between recaptures under the high treatment and control. Recaptures were significantly lower under the low treatment, indicating greater mortality compared with control and high. We propose the "MAT-ME saturation hypothesis" to explain this result: increasing the number of stations per square mile increases mortality of receptive males until too many stations create a high enough background of ME that the males don't effectively follow a gradient to MAT sources. Our findings highlight that further research into the effect of increasing MAT-ME spot density on male mortality is needed.

Key words: mark-release-recapture, SPLAT-MAT-ME, male annihilation, eradication

The oriental fruit fly, *Bactrocera dorsalis* (Hendel) is a serious quarantine pest associated with about 480 host plant species and 100 commercial crops (CABI 2015, Liquido et al. 2017, McQuate and Liquido 2017). These include such high-value crops as apple, (*Malus sylvestris*), apricot (*Prunus armeniaca*), avocado (*Persea americana*), sweet cherry *(Prunus avium*), cucumber (*Cucumis sativas*), fig (*Ficus carica*), grapefruit (*Citrus paradisi*), lemon (*Citrus limon*), pear (*Pyrus communis*), nectarine (*Prunus persica* var. *nectarina*), sweet orange (*Citrus sinensis*), pepper (bell or chilli) (*Capsicum annuum*), persimmon (*Diospyros spp.*), plum (*Prunus americana*), and tomato (*Lycopersicon esculentum*) (White and Elson-Harris 1992). Native to Southeast Asia, the oriental fruit fly and close relatives the melon fly (*Bactrocera cucurbitae*), the Queensland fruit fly, (*Bactrocera tryoni*) as well as the Mediterranean fruit fly (*Ceratitis capitata*) constitute some of the greatest threats to agriculture in the U.S. Each year exotic fruit fly invasions into the continental U.S. result in significant state and federal costs associated with eradication. Eradication of Tephritid fruit flies to undetectable levels represents the basis of declaration of fly-free status among production areas and a key discussion point for bilateral trade negotiations between countries involved in agricultural products. In states such as Florida, California, and Texas, international trade in fruit fly host commodities is worth millions to billions of dollars, so state and federal efforts are focused on detection and eradication (Gilbert et al. 2013, USDA/APHIS 2013).

For the past fifty years, semiochemical-based male attractants, termed parapheromones by Cunningham (1989), have been used for detection and, in some cases, control of oriental fruit fly and other invasive pests (Chambers et al. 1974). Methyl eugenol (ME) (1-2-dimethoxy-4-allylbenzene) is a phenylpropanoid known to occur naturally in some plants, and is highly attractive to male oriental fruit flies (Metcalf et al. 1975). When flies are detected in surveillance traps, a control method termed the male annihilation technique (MAT) (Steiner and Lee 1955, Steiner et al. 1965) is routinely applied in California and other oriental fruit fly-free areas (discrete spots of attractant + insecticide), with the aim of luring and killing nearly all males in the population over time. This approach has been successful in eradication and area wide campaigns over the years (Steiner et al. 1965, 1970; Koyama et al. 1984; Vargas et al. 2008).

Ideas on the number of spots or stations per area required for effective MAT against *B. dorsalis* vary significantly. One of the earliest recommendations was for 30 stations per mi^2 (about 12 per km^2) with a large amount of attractant in each station (30 ml of methyl eugenol plus naled) (Steiner and Lee 1955). Subsequent research implemented 85–230 impregnated cane fiberboard squares per km^2 (Steiner et al. 1970), 12–40 squares per km^2 (Chambers et al. 1974), and 50 spots per km^2 (Cunningham 1981). In an extreme case, Vargas et al. (2014) employed a density equivalent to about 5000 spots per km^2 in an area on Hawaii island with a large standing population of *B. dorsalis* in order to measure a reduction in the numbers of males captured in traps. The general perception is that, all else being equal, more spots per unit area leads to increasing effectiveness of MAT, though it has been argued that there are limiting returns due to economic costs (Cunningham 1981).

The large variation in number of spots used for MAT in different contexts is reflected in the various values used by the California Department of Food and Agriculture (CDFA) for eradication of invading *B. dorsalis* (between 256 and over 1000 per square mile). Currently, CDFA employs a protocol calling for a minimum of ~230 methyl eugenol–naled treatment sites per km^2 (600 spots/mi^2) in its oriental fruit fly eradication programs. The treatments are applied to street trees, utility poles, etc. within a few feet of the edge of paved roads. It is becoming increasingly difficult to reach this density of treatment sites as power and phone lines are put underground and street trees are planted further away from the edge of paved roads.

In order to compare higher and lower MAT application rates, we undertook a small-scale mark-release-recapture study in Hawaii (where *B. dorsalis* is established), comparing control conditions (no MAT) with 100 and 225 spots/km^2 of ME and insecticide, tested in a ca. 1 km^2 area of a macadamia nut orchard. Our initial hypothesis was that recaptures of released *B. dorsalis* at sentinel traps baited with ME would be reduced under the two MAT treatments by equivalent amounts compared with the control, reflecting male mortality from the treatments.

For this study we used a formulation of methyl eugenol containing the insecticide spinosad in a wax matrix (SPLAT), registered for use as SPLAT-ME which replaces the organophosphate chemical naled in older formulations (Vargas et al. 2009).

Materials and Methods

We conducted mark-release-recapture experiments in the Island Princess macadamia nut orchard in Keaau, Hawaii (N19 36.725, W155 05.084) between August 2014 and April 2015. The vegetation within the orchard consists primarily of macadamia nut (*Macadamia integrifolia* Maiden & Betche) trees with Norfolk Island pine (*Araucaria heterophylla* (Salisb.) Franco) as windbreaks inside and around the orchard. Commercial host fruit orchards of guava (*Psidium guajava* L.) and papaya (*Carica papaya* L.) border the south and east edges of the macadamia nut plantation, respectively. Eucalyptus (*Eucalyptus* spp.) and strawberry guava (*Psidium cattleyanum* Sabine) forests surround the rest of the site. Keaau has a continuously wet tropical climate with year-round rain, high humidity, and relatively constant temperatures (NOAA 2011).

A square area in the center of the orchard, 1 km per side, was established for the experiments on the effect of varying the number of SPLAT-MAT-ME stations per unit area. Within this area we set up a release site, with Jackson traps set at chest height about 40 m from a central release point. The Jackson traps were baited with 6 ml methyl eugenol with 1% Dimethyl 1,2-dibromo-2,2-dichloroethylphosphate (“dibrom”) soaked into a wick (following procedures used in California; see Gilbert et al. 2013), which was placed in the trap using a basket-type holder and a coarse screen to keep the flies from contacting the wick. One trap was placed upwind from the release point (in a roughly northeast direction) and another downwind (southwest). Fresh baited traps were hung when a new release was conducted more than 2 weeks after the last one.

MAT was implemented via application of “SPLAT-MAT-ME” (Vargas et al. 2009). The MAT formulation consisted of a specialized pheromone and lure application technology (SPLAT) in combination with methyl eugenol (ME) and spinosad (ISCA technologies, Riverside CA). SPLAT is a proprietary matrix formulation of inert materials used to control the release of semiochemicals with or without pesticides. Spinosad is a reduced-risk biopesticide composed of spinosyns A and D, the soil fermentation products of the bacterium *Saccharopolyspora spinosa* Mertz and Yao, which has low mammalian toxicity, and low impact on natural enemies (Stark et al. 2004).

Three different treatments were evaluated: (1) “Control” (no SPLAT-MAT-ME application), (2) “low rate” (a 10 x 10 grid of SPLAT-MAT stations for a density of 100 per km^2, approximately 256 stations per mi^2), and (3) “high rate” (a 15 x 15 grid of SPLAT-MAT-ME stations for a density of 225 per km^2, approximately 600 stations per mi^2). In all cases, we applied 5 g SPLAT-MAT-ME to 10 cm x 7 cm pieces of 7 mm-thick plywood and attached these to the trees with wire in order to allow rapid treatment removal. SPLAT-MAT-ME blocks were distributed in a regular grid within the experimental area of the orchard. Fresh applications were used whenever a new release was conducted more than 2 weeks after the last one.

B. dorsalis were obtained from the research colony at the USDA-ARS Daniel K. Inouye United States Pacific Basin Agricultural Research Center (DKI-PBARC) in Hilo, Hawaii. This colony was established from wild flies collected in Puna, Hawaii island, in 1984. It has since been maintained in the laboratory on artificial diet (Tanaka et al. 1969) using a standard rearing protocol (Vargas 1989) in large (0.6 m w x 1.18 m h x 1.32 m d) mixed cages at a density of about 50,000 per cage with a 12:12 photoperiod, and were periodically refreshed with wild flies from Hawaii island to maintain genetic diversity. Adult male flies were 12–14 days old at the time of release.

Adult males were marked 2–4 days prior to release using fluorescent paint, applied using a small piece of sponge affixed to the end of a chopstick, to the dorsal thorax of chilled males (Rust-Oleum Corp., Vernon Hills, IL). When releases overlapped, different colors were used to indicate different replicates. We used as many as three different colors at a time.

Recapture consisted of removing the sticky card insert from each Jackson trap in the grid during the morning and replacing it with a fresh insert. Inserts were transported to DKI-PBARC with any captured flies to check them for marking under UV light.

Results

Results per release date are given in Table 1. Significant variation in the proportion recaptured over the first few days is evident for two of the treatments: For the control releases, recaptures varied from about 10% to almost 65%, and for the high rate treatment recaptures varied between 4% and 67%. However, recapture proportions for the low rate treatment were consistently low, between 0% and 8%.

One-tailed two-sample z-tests were used to compare proportion recaptures in order to accommodate unequal variances. These tests indicated a statistically significant difference between the control and the low rate ($z = 1.86, p = 0.032$) and between the high rate and the low rate ($z = 2.44, p = 0.007$). There was no statistically significant difference between the control and the high rate treatments ($z = 0.04, p = 0.515$). The means, standard errors and results of statistical comparison are shown in Figure 1.

Discussion

There was a large amount of variation in the proportion of released males recaptured between replicates, within and between treatments. Part of this variation is attributable to variation in environmental conditions (Hooper and Drew 1979, Liu and Ye 2006, Deepa et al. 2009). There is also likely significant variation in the cohorts of males used for each release, particularly in the proportion responsive to methyl eugenol (Shelly and Edu 2010, Manoukis et al. 2015). Any remaining variation might result from the small number of males released for each replicate in order to simulate a detection scenario, which by sampling could introduce significant additional variation.

Under control conditions we measured an average recapture proportion around 0.25 from a distance of 40 m. This is significantly lower than previous studies in Hawaii and with sterile males in California, which indicate around 50% recapture at a distance of 50 m (Shelly and Edu 2010, Shelly et al. 2010, Shelly and Nishimoto 2011) and also lower than studies with grids of traps on Hawaii island, which suggest a 65% recapture rate at a distance of 40 m (Manoukis et al. 2015). Examination of Table 1 shows that similar recapture rates were attained in this study, but not consistently. In addition, the experimental design used here is less robust to variation than the studies mentioned above, which employ multiple release points or a network of traps (Manoukis et al. 2014). For these reasons, we feel that the absolute value of the recapture proportion reported here should be considered unusually low.

Table 1. Mark-release-recapture results by date and treatment.

Release date	**Check (days)**	**Treatment**	***N***[*]	***R***[+]	**Proportion recaptured**
11 Aug 2014	4	Control	25	16	0.64
10 Dec 2014	2	Control	34	3	0.09
12 Dec 2014	3	Control	25	4	0.16
15 Dec 2014	2	Control	33	6	0.18
03 Sep 2014	6	Low	25	2	0.08
10 Sep 2014	2	Low	25	2	0.08
21 Oct 2014	3	Low	25	0	0.00
24 Oct 2014	4	Low	25	0	0.00
29 Oct 2014	2	Low	25	0	0.00
27 Feb 2015	3	High	25	6	0.24
02 Mar 2015	2	High	25	6	0.24
04 Mar 2015	5	High	24	16	0.67
04 Apr 2015	3	High	26	1	0.04
06 Apr 2015	2	High	25	8	0.32
08 Apr 2015	2	High	23	3	0.13

[*] Number of marked males released; [+] Number of marked males recaptured.

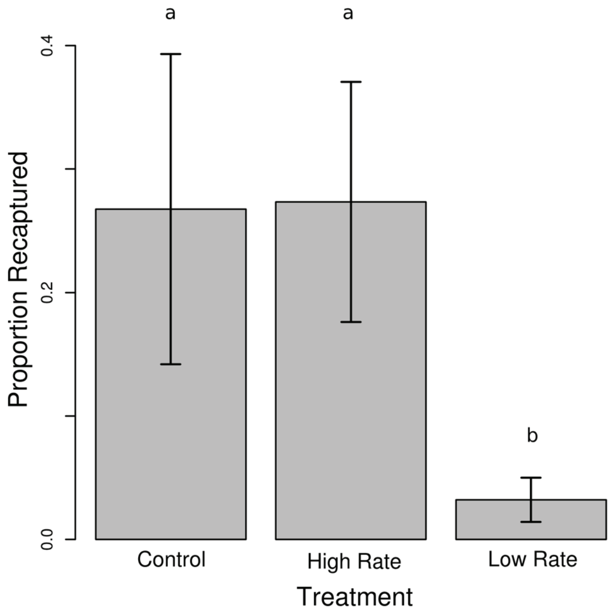

Figure 1. Mean proportion recaptured by treatment. Whiskers indicate standard errors. Letters indicate statistically significant differences at $\alpha = 0.05$ via two-sample *z*-test (see text for details)

Despite the variation and generally low recapture rates mentioned above, we found a statistically significant and large reduction in the proportion of males recaptured under the low rate of SPLAT-MAT-ME application compared with the control and high rate. The control and high rate treatments were remarkably similar, an unexpected result. Two elements must be discussed to interpret this finding: (1) the potential causes of a low recapture rate, and (2) factors that might impact the efficacy of SPLAT-MAT-ME in unexpected ways.

In terms of the meaning of recapture rates in this study, we interpret lower rates as resulting from either increased mortality or emigration from the trapping area. Mortality in the SPLAT-MAT-ME treatments would result from flies following odor plumes from bait spots and then eating the spinosad. Emigration could be driven by many factors, including environmental conditions, but we don't expect these to vary significantly in correlation with the treatments, though there may have been seasonal effects (Table 1, see replicate dates).

Our results indicate, therefore, that mortality was similar between the control without SPLAT-MAT-ME and the high treatment, but much higher under the low rate of SPLAT-MAT-ME application. This was unexpected, and runs contrary to the usual idea that increasing numbers of spots leads to increasing effectiveness (Cunningham 1981). However, the result can be explained based on what we know about point source finding in other species. We propose the "MAT-ME saturation hypothesis" to explain this result (Figure 2).

Under the MAT-ME saturation hypothesis, increasing the number of stations per square mile increases mortality of receptive males up to a point, when too many stations create a high enough background of methyl eugenol that the males have trouble following odor gradients to small sources (SPLAT-MAT-ME spots) but can still find large sources with a very high concentration of ME (the traps). The inability of individuals to locate small sources under a situation of high background is probably exacerbated by the high sensitivity of *B. dorsalis* to methyl eugenol, similar to that demonstrated for the sex pheromone of the silkworm moth (Metcalf et al. 1975). Both plume finding and navigation along the plume could therefore be impacted by a background level of attractant (Bau and Cardé 2015). In the context of trapping networks, this sort of saturation is sometimes termed trap interference or competition (Wall and Perry 1978, Elkington and Carde 1984, Yamanaka 2006), and has been measured in the field for various pest insects (Suckling et al. 2015).

A separate study conducted at the same time and in the same experimental grids by our group similarly suggested reduced effectiveness of MAT at high density compared with lower density (Manoukis et al. 2017). In that study, the actual daily survivorship of a separate group of *B. dorsalis* was estimated via MRR yielding values for males of 0.751 under control conditions, 0.704 at 225 spots/km^2 and 0.211 at 100 spots/km^2. This is in agreement with the results presented here obtained via a different method, but it is important to note that the two studies were not completely independent. We are conducting further experiments to confirm these findings.

Unexpected results from MAT against *B. dorsalis* are not new, despite the high attractiveness of ME to males of this species. Steiner and Lee (1955) found an increase in fruit infestation rate in a small guava orchard treated with MAT, presumably due to migration of *B. dorsalis* from neighboring areas. Ineffective MAT under situations of low isolation and large standing *B. dorsalis* populations are not unusual

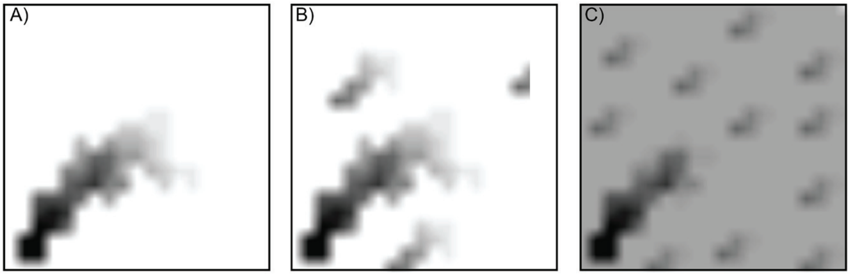

Figure 2. Visual representation of the MAT-ME saturation hypothesis. Darker areas represent higher concentrations of attractant odor in the air column, represented from above. (A) A single sentinel trap baited with 6 ml of methyl eugenol under control conditions. A release of males around the center of the area would lead some of the males to find the plume and successfully follow it to the trap for capture. (B) A single sentinel trap with some MAT-ME spots (low rate). More attractant is available, but gradients to point sources are still clear; note that the MAT-ME spots have a lower overall concentration at the source. (C) A single sentinel trap with a high density of MAT-ME spots. A haze of attractant exists, making gradients shorter. The sentinel trap is still about as effective as before due to higher final concentration, but the less concentrated MAT-ME spots are harder to find.

(Cunningham and Suda 1986, Vargas et al. 2014). These studies indicate that the strong attractiveness of ME to *B. dorsalis* does not exclude the possibility of MAT being ineffective. This study suggests a novel way in which ME-based MAT might fail to reach expected effectiveness against *B. dorsalis*.

The results presented here suggest that there may be an optimal number of MAT stations per unit area, and that mortality might actually be reduced above this level. We expect that the number of stations needed for maximum attraction and mortality is contingent on many factors, including the lure being used, search and flight characteristics of the males, weather conditions (especially wind), and environmental variables such as natural sources of the attractant.

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