Small Plot Trials Documenting Effective Mating Disruption of Oriental Fruit Moth by Using High Densities of Wax-Drop Pheromone Dispensers

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ABSTRACT In 2004 field experiments, we compared the effectiveness of various deployment densities of 0.1-ml paraffin wax drops containing 5% pheromone versus Isomate M-Rosso "rope" dispensers for disruption of *Grapholita molesta* (Busck). Treatments were evaluated in 0.05-ha (12-tree) plots of 'Delicious' apples receiving regular maintenance according to growers' standards, but not sprayed with insecticides. The application densities of 0.1-ml wax drops were 3 per tree (820/ha), 10 per tree (2,700/ha), 30 per tree (8,200/ha), and 100 per tree (27,300/ha). Wax drops were compared with 3-ml dispensers of pheromone-containing paraffin wax or Isomate M-Rosso ropes at 1.8 per tree (500/ha) and untreated control plots. Treatments were applied before the start of each of three moth generations. Orientational disruption, as measured by inhibition of moth captures in pheromone-baited delta traps, was greatest in plots that received 100 drops per tree (99.2%) and 30 drops per tree (99.4%). More than 55% of tethered, virgin females were mated in control plots after one night of deployment. However, no mating was recorded at the two highest application densities of wax drops where orientational disruption of traps exceeded 99%. Mating ranged from 7 to 20% among the other treatments, including Isomate rope dispensers. *G. molesta* males were observed closely approaching pheromone dispensers in plots containing ropes and wax drops, documenting competitive attraction between synthetic pheromone sources and feral females. The majority of observed *G. molesta* males approached within 60 cm of wax drops or pheromone ropes and departed within 20 s by flying upwind. Thirty wax drops per tree yielded higher mating disruption of *G. molesta* than did Isomate M-Rosso dispensers deployed at the recommended rate of 500/ha (1.8 per tree). Measurement of release rates confirmed behavioral data indicating that paraffin wax dispensers would need to be applied once per *G. molesta* generation in Michigan. Paraffin wax drops are a promising technology for moth mating disruption. They are cheaper and easier to produce, require less total pheromone per annual application, and produce better mating disruption at appropriate deployment densities compared with Isomate M-Rosso dispensers under high *G. molesta* population densities. The cost-effectiveness of this approach will require an appropriate mechanized applicator for wax drops.

KEY WORDS mating disruption, emulsified wax, competitive attraction, false-plume following

ORIENTAL FRUIT MOTH, *Grapholita molesta* (Busck), is a long-standing target of efforts to develop mating disruption programs (Gentry et al. 1974, 1975; Cardé et al. 1977, 1979) because of its status as a major pest of stone fruit trees (Rosaceae) worldwide (Rothschild and Vickers 1991). Polyethylene tube dispensers (Isomate-M, Shin-Etsu Chemical Co., Tokyo, Japan) that release *G. molesta* pheromone into orchards have become an industry standard (Vickers 1990). Isomate-M 100 and M-Rosso dispensers have proven effective in numerous field trials (Pfeiffer and Killian 1988; Audemard et al. 1989; Rice and Kirsch 1990; Pree et al. 1994; Trimble et al. 2001, 2004; Atanassov et al. 2002). However, desired improvements include: decreased cost of the product, mechanized deployment to reduce cost of hand-labor, and increased efficacy at high pest densities without the use of companion insecticides.

An ideal pheromone-release device should remain effective for a prolonged period, not waste active ingredients, be inexpensive to produce and apply in the field, and be nontoxic (Plimmer 1981). Furthermore, it is highly desirable that pheromone dispensers be amenable to application at varying densities and deployment dates, depending on pest pressure. If false-plume following (competitive attraction) is a prominent mechanism mediating mating disruption, as suggested by recent studies (Maini and Accinelli 2000; Stelinski et al. 2004a, b), many rather than few pheromone dispensers would be desirable. In the United States, machine application of pheromone dispensers would be favored over manual labor. Advantageous attributes of machine applied formulations include capability to be pumped from a storage reservoir and sprayed upon the crop, as well as longlasting adhesion of dispensed particles to plant surfaces.

Recently, biodegradable, paraffin wax dispensers have been developed for releasing *G. molesta* pheromone (Atterholt 1996, Atterholt et al. 1998, de Lame 2003). These paraffin wax dispensers release pheromone at effective rates for extended periods and inhibited male moth captures in pheromone traps for at least 200 d in the field under moderate population densities (Atterholt 1996, de Lame 2003). They contain 5% *G. molesta* pheromone by weight and have been effectively deployed as ≈3-g deposits at ≈580/ha (de Lame 2003). One of the initial formulations (30% paraffin wax emulsified in water, vitamin E, soy oil, and antioxidant) proved as effective as Shin-Etsu, Isomate-M 100 polyethylene-tube dispensers for 75 d in the field, as measured by orientational disruption to pheromone traps (Delwiche et al. 1998). Subsequently, a single application of a more viscous version of the above-described paraffin wax dispenser provided the same level of season-long disruption of *G. molesta* as Isomate-M 100 dispensers, yet the paraffin wax dispensers were hand-applied in the field in half the time required to apply Isomate-M 100 dispensers (de Lame 2003).

The current investigation was part of a collaboration to develop cost-effective and robust mating disruption of *G. molesta* through improvements in paraffin wax dispensers. We sought a formulation that could eventually be applied as a sprayable and one that used pheromone judiciously. Experiments were conducted in managed orchards not receiving insecticide sprays and containing high population densities of *G. molesta.* The major objective was to compare four application densities of 0.1-ml wax drops containing 5% *G. molesta* pheromone with standard application densities of Isomate M-Rosso dispensers in replicated, 0.05-ha (12-tree) apple plots. Comparison of treatments versus controls was carried out by measuring orientational disruption of males to optimally baited pheromone traps and disruption of mating using tethered, virgin females. Additional objectives were to directly observe pheromone dispensers in the field determining whether feral male moths visit these dispensers and to quantify the release profile of pheromone from paraffin wax dispensers.

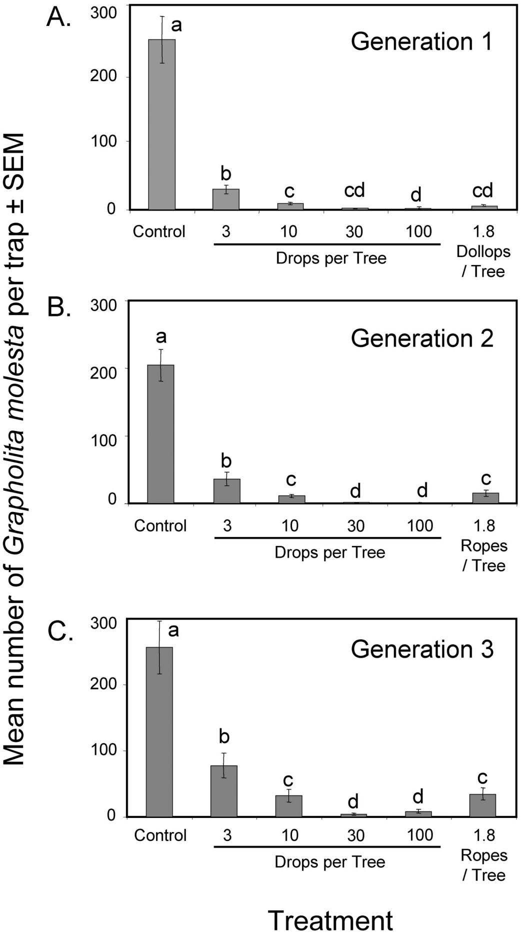

Fig. 1. Mean captures of *G. molesta* males in lure-baited delta traps in plots containing various pheromone treatments per generation. Means followed by the same letter are not significantly different at $\alpha < 0.05$. Dollops (3-ml wax dispensers) were deployed during the first generation 26 April through 29 June. Isomate M-Rosso dispensers (ropes) were deployed during the second and third generations 30 June through 15 September.

Materials and Methods

General Methods for Field Study. This study was conducted in spring and summer 2004 at the Trevor Nichols Research Complex (TNRC) of Michigan State University in Fennville, MI. Experiments were conducted within plots of 19-yr-old 'Delicious' apple trees with ≈2.5–4.5-m canopy heights. Trees were planted on a 3-m within- and 6-m between-row spacing. Plots were pruned and received fungicide and herbicide applications according to growers' standards in Michigan, but they did not receive insecticide applications. As measured by weekly captures of male *G. molesta* in the orchards used in this study (Fig. 1) and the surrounding TNRC orchards (data available online at http://www.maes.msu.edu/tnrc/), population densities of this species were substantial and occurred as three discernible generations.

Pheromone Release Devices. Paraffin wax dispensers were formulated using the protocol of de Lame (2003) adapted from Atterholt (1996). Briefly, the paraffin wax formulation consisted of 30% paraffin wax (Gulf wax, Royal Oak Sales, Inc., Roswell, GA), 4% soy oil (Spectrum Naturals, Inc., Petaluma, CA), 2% Span 60 (Sorbitan monostearate, Sigma, St. Louis, MO), 1% vitamin E [(±)-α-tocopherol, Sigma], 5% *G. molesta* pheromone [93:6:1 blend of (*Z*)-8-dodecen-1-yl-acetate:(*E*)-8-dodecen-1-yl-acetate: Z-8-dodecen-1-ol (Shin-Etsu Chemical Co., Ltd., Tokyo, Japan, confirmed by gas chromatography), and 58% deionized water. The dispensing medium was formulated by separately heating the paraffin wax and water to 60–65°C and 65–70°C, respectively. The remaining ingredients were then incorporated into the wax, followed by the addition of the hot water. These components were mixed for 5 min in a 4-liter industrial laboratory blender (Waring Commercial, Torrington, CO). The resulting emulsion was then cooled gradually to room temperature in a cold water bath. Immediately before field application, carpet adhesive (3% by weight of emulsion) (Roberts Premium Indoor/Outdoor Carpet Adhesive 6700, Roberts Consolidated Industries, Inc., Boca Raton, FL) was thoroughly mixed into the emulsion using a power drill-driven paint mixer (de Lame 2003). This glue was used to ensure adherence of the wax drops to tree wood.

Isomate M-Rosso "rope" dispensers were used as a standard positive control treatment given their broad commercial acceptance. These dispensers contained 250 mg of 88.5% (Z)-8-dodecen-1-yl-acetate, 5.7% (*E*)-8-dodecen-1-yl-acetate, 1.0% Z-8-dodecen-1-ol, and 4.8% inert ingredients.

Treatments Evaluated. Mating disruption trials were conducted comparing four application densities of 0.1-ml drops of paraffin wax containing 5% pheromone as described above. Wax drops were hand-applied to branches of trees using 5-ml plastic syringes. Densities of wax drops compared were three per tree (820/ha, 3.3 g [AI]/ha), 10 per tree (2,700/ha, 11 g [AI]/ha), 30 per tree (8,200/ha, 33 g [AI]/ha), and 100 per tree (27,300/ha, 109 g [AI]/ha). During the first moth generation (Fig. 1), the two other treatments evaluated were large 3-ml wax dollops with 5% pheromone deployed at 1.8 per tree (500/ha, 33 g [AI]/ha) and a no pheromone control. During the second and third generations (Fig. 1), the two additional treatments were Isomate M-Rosso dispensers described above at 1.8 per tree (500/ha, 199 g [AI]/ha) and a no pheromone control. Wax-based pheromone dispensers were always prepared using the formulation described above. "Drops" are defined as 0.1-ml aliquots of pheromone-containing wax, whereas "dollops" are 3-ml aliquots of wax. The treatments were applied before the start of each moth generation. The experimental design was a randomized complete block with treatments applied to 0.05-ha (12-tree) plots and replicated five times. Replicate orchards (blocks) were separated by at least 35 m and treatment plots by at least 15 m.

Measuring Orientational Disruption. Disruption of male moth orientation to sex pheromone was assessed using two pheromone traps (LPD Scenturian Guardpost, Suterra, Bend, OR) placed in the center two trees of each plot. Care was taken that wax or Isomate dispensers were never placed closer than 50 cm from monitoring traps. Traps were baited with red septum (The West Company, Linville, PA) lures loaded with 0.1 mg of (Z)-8-dodecenyl acetate:(*E*)-8-dodecenyl acetate:(Z)-8-dodecen-1-ol in a 100:6:10 blend. Pheromone solutions used to load rubber septa were prepared in HPLC grade hexane and stored at $-18°C$. Traps were hung ≈2–3 m above ground level in the upper third of the tree canopy. New pheromone lures were deployed at the onset of each moth generation for a total of three times throughout the season. Moths captured in traps were counted and removed twice weekly.

Deployment of Tethered Virgin Females: Measurement of Mating Disruption. In addition to measuring orientational disruption to traps, mating disruption was measured using tethered, virgin females. Female *G. molesta* used for tethering were drawn from a 3-yr-old laboratory colony at Michigan State University (East Lansing, MI) originally collected as larvae from apple orchards in southwestern Michigan. They were reared at 24°C and 60% RH on pinto bean-based diet (Shorey and Hale 1965) under a photoperiod of 16:8 (L:D) h. Females were sorted in the pupal stage and placed into 50-ml plastic cages containing 5% sucrose in plastic cups with cotton dental wick protruding from their lids.

Female *G. molesta* were deployed for 24 h within plots on five dates coinciding with peak moth presence during the second and third generations (9, 22, and 26 July; 17 and 22 August). Three or four female moths were tethered per treatment replicate during each deployment period. Female moths were secured to branches of trees with polyester thread (Jo-Ann Stores, Inc., Hudson, OH) tied to the base of the left wing. They were given at least 60 cm of thread and were observed for at least 30 s after deployment to ensure they remained tethered and mobile upon the branch. Approximately 70 g of Insect Tangle-foot (Great Lakes IPM, Vestaburg MI) was spread on the base of branches bearing moths in an effort to deter arrival of predators. Approximately 73% of tethered females were recovered over the five deployments. Female moths were dissected after collections from plots. Mating status was determined by the presence or absence of a spermataphore in the bursa copulatrix.

Moth Observations. Observations of male moth visits to pheromone wax drops or ropes were conducted directly within tree canopies of each treatment plot on 26 nights throughout the three generations of *G. molesta* flight. The control plot had a single pheromone-containing wax drop randomly placed in one tree for observation; these plots were otherwise untreated. Two or three observers rotated among plots conducting 20-min observational bouts per treatment such that multiple treatments were under observation concurrently. The order of observations across treatments was randomized nightly. Observed events were spoken into a hand-held microcassette audio recorder by an investigator standing 0.75 m from the pheromone dispenser under observation. Data recorded included: anemotactic orientations to the dispenser, time during the diel period, closest approach to the dispenser, and duration of visits. Observations after dusk were assisted by night-vision goggles (Rigel 3100, Rigel, DeWitt, IA) as described by Stelinski et al. (2004 b).

Trapping Studies. Two experiments were conducted comparing captures of male *G. molesta* in traps baited with various sizes of paraffin wax drops formulated as described above with that of optimized rubber septum lures described above and known to be highly attractive. These tests were conducted in plots not being used for mating disruption studies. In the first experiment (conducted 3 May–21 June), 0.1-, 1.0-, and 3.0-ml dispenser sizes of paraffin wax were compared with rubber septum lures. Paraffin wax dispensers containing pheromone were pipetted onto 2 by 5-cm strips of aluminum foil and allowed 24 h to dry. Treatments were inserted into plastic delta traps deployed in unsprayed 0.4-ha plots of apples, as described above. Unbaited traps were used as a negative control. The experiment was arranged in a randomized complete block with six replicates. Treatments were spaced by at least 26 m and rotated weekly. Traps were hung ≈1.5–2 m above ground level in the upper third of the tree canopy. Moths captured in traps were counted and removed twice weekly.

In the first trapping experiment, captures of male *G. molesta* in traps baited with paraffin wax drops were much lower than captures for traps baited with rubber septa (see *Results*). We postulated that the release rate of pheromone from wax drops was too high preventing moths from entering traps. Thus in experiment 2 (conducted 23 June–30 August), we reduced drop size and compared 25-, 50-, and 100-μl drops with rubber septum lures. Again, unbaited traps were the negative control. The experimental design and trap maintenance protocol was identical to that described for experiment 1.

Release Rate Measurements from 0.1-ml Wax Drops. Paraffin wax drops (0.1 ml), formulated as described above, were applied to one by 5.5-cm pieces of flat, wooden Craft sticks (Diamond Brands, Cloquet, MN) by using an Eppendorf repeat pipetter. Resulting drops were ≈7 mm in diameter and 2 mm thick. Before application of wax, the Craft sticks were notched with a rotary tool (Dremel Professional High Speed Rotary Tool, S-B Power Tool Co., Racine, WI) equipped with a high speed cutter to roughen the ends receiving wax so as to promote adhesion. The sticks were then screwed to the bottom of 38-cm-long sections of 2 by 5-cm wooden boards fitting 25 sticks per board. Half of the resulting racks holding wax drop samples were covered by a 25 by 53-cm sheet of wood, yielding a 7.5-cm overhang over all edges of the rack to provide shading. The other half of racks containing samples were left without supplemental shading. The roof overhangs were designed to minimize pheromone degradation due to UV radiation. The purpose of these two treatments was to determine the release rate of the samples as well as the extent of pheromone degradation in wax drops not receiving supplemental shade. One of each rack type (shaded and nonshaded) was attached with zip-ties to the branches of five 5–7-m-tall apple trees at a height of 2–3 m within the tree canopy. Samples held within each tree comprised one block.

Samples were maintained in the field from 3 August to 8 October. Five samples were collected immediately at application to determine the amount of pheromone in the wax drops at test onset. Thereafter, samples were collected approximately every 24 h for 4 d and then every other day for 10 d. Collection intervals were increased as time went on to increase the longevity of the experiment as necessary to see the full release rate of the samples. During each collection, one sample was randomly collected from each rack of each tree and placed in a 20-ml scintillation vial. Vials were stored at –20°C until extraction.

Samples were extracted using a procedure modified from Meissner et al. (2000). Ten milliliters of an internal standard solution, 230 μl/liter methyl tridecanoate (98%, Aldrich, Milwaukee, WI) in acetonitrile, was added to each scintillation vial. Samples were then placed into a water bath shaker (Shaking Water Bath model YB-531, American Scientific Products, McGraw Park, IL) at 80–85°C for 3 min and then shaken for 7 min. The samples were momentarily removed from the water bath and agitated by hand for 30 s and then returned for an additional 3 min of shaking. Finally, samples were frozen at −20°C for at least 8 h, during which time the wax precipitated.

Before analysis, vials were thawed and vortexed. One milliliter of the solution was removed from each sample with a disposable glass Pasteur pipette and filtered into a 2.0-ml GC vial (Supelco, Bellefonte, PA) through another Pasteur pipette fitted with an 4.5 by 6.5 by 8-cm triangular piece of Kimwipe (Kimberly-Clark Corp., Roswell, GA) folded to form a plug at the tapered end of the pipette. Pheromone within samples was quantified using a gas chromatograph (GC) (HP-6890, Hewlett–Packard, Palo Alto, CA). The GC was fitted with a DBWAXETR polar column (model 122-7332, J&W Scientific, Folsom, CA) of length 30 m and internal diameter 250 μm. The initial GC temperature was held at 130°C for 2 min and then ramped at a rate of 2.5°C/min to 160°C, where it was held for 2 min. The program then ran at 40°C/min to a final temperature of 230°C. The carrier gas, He, entered the column at 20 psi. The pheromone content of the samples was calculated using the internal standard method (McNair and Miller 1998).

Statistical Analysis. For orientation disruption and trapping studies, data were transformed to ln $(x + 1)$ (which normalized the distributions) and then subjected to analysis of variance. Differences in pairs of means were separated using the least significant difference test (SAS Institute 2000). In all cases, the significance level was $\alpha < 0.05$.

Results

Orientational Disruption Trials. During the first generation, significantly more ($F = 17.5$; df = 5, 20; $P < 0.05$) male *G. molesta* were captured in control plots compared with any of the pheromone treatments (Fig. 1A). Furthermore, significantly more ($P < 0.05$) males were captured in plots treated with three drops per tree (820 drops/ha) compared with the higher application densities of wax drops tested and large 3-ml dollops deployed at 1.8 per tree (500/ha). Likewise, during the second and third moth generations, traps in control plots captured significantly ($F = 80.4$ and 35.9, respectively; df = 5, 20; $P < 0.05$) more *G. molesta* than any of the pheromone treatments (Fig.

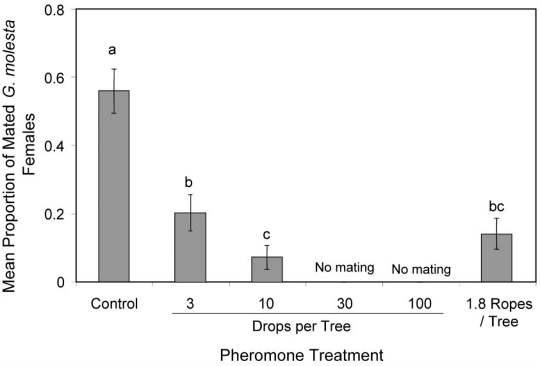

Fig. 2. Mean proportion of virgin female *G. molesta* mating during 24 h of field deployment in plots receiving various pheromone treatments. $n = 55–59$ female *G. molesta* dissected per treatment. Means followed by the same letter are not significantly different at $\alpha < 0.05$.

1B and C). Also, during both of these generations the highest moth captures recorded in a pheromone treatment were in plots treated with three drops per tree (820 drops/ha) (Fig. 1B and C). The second highest captures were recorded in plots with 1.8 ropes per tree (500/ha) and 10 drops per tree (2,700/ha); moth captures were statistically equal ($P > 0.05$) for these two treatments. Finally, the fewest moths captured were in plots treated with 30 and 100 drops per tree (8,200 and 27,300/ha, respectively); moth captures were statistically equal ($P > 0.05$) for these two treatments.

Mating Disruption Trials: Tethered Virgin Females. Female mating was inversely related to the number of wax pheromone point sources; no mating occurred only at the two highest densities where orientational disruption as measured by traps exceeded 99%: 30 drops per tree (8,200/ha, 99.4% orientational disruption) and 100 drops per tree (27,300 drops/ha, 99.2% orientational disruption) (Fig. 2). Significantly ($F = 33.8$; df = 5, 20; $P < 0.05$) more tethered females were mated after 24 h in the control plots compared with any of the pheromone treatments evaluated (Fig. 2). The highest proportion of mating in pheromone-treated plots (20%) was documented in those receiving 3 drops per tree (820/ha), but this proportion was not statistically different ($P > 0.05$) from the proportion of mating in plots treated with Isomate ropes at 1.8 per tree (500/ha) (Fig. 2). The proportion of mating recorded in plots treated with Isomate ropes at 1.8 per tree (500/ha) and those treated with wax drops at 10 per tree (2,700/ha) was not statistically different ($P > 0.05$) (Fig. 2).

Moth Observations. Males were observed visiting all pheromone treatments except those within the 30 per tree (8,200/ha) treatment (Fig. 3). As expected under competitive attraction, relatively few moths were also observed approaching the one wax drop under observation in plots treated with 27,000 drops/ha. All male *G. molesta* observed approached within 130 cm of all pheromone dispenser types and >60% approached within 60 cm. Observed *G. molesta* moths landing near wax dispensers or ropes wing-fanned vigorously and walked rapidly; they remained in motion for the duration of their stay. More than 70% of the *G. molesta* observed departed wax drops or ropes within 20 s, and none remained near pheromone dispensers for >2 min. All departing moths flew in an upwind direction.

Trapping Studies. In both trapping experiments, red septa captured significantly ($F = 25.2$ and 78.8, respectively; df = 4, 29; $P < 0.05$) more *G. molesta* than did the other treatments (Fig. 4A and B). In the first experiment, 0.1-ml wax drops captured significantly ($P < 0.05$) more *G. molesta* compared with larger drop sizes (Fig. 4A). In the second experiment, traps baited with wax drops ranging from 25 to 100 μl captured statistically equivalent ($P > 0.05$) numbers of *G. molesta* (Fig. 4B).

Pheromone Release Rates from Wax Drops. The release profiles are shown in Fig. 5, fitted with exponential decay curves. The two curves are nearly indistinguishable, indicating no detectable difference in pheromone release with level of shading from direct sunlight. Samples without supplemental shading yielded an R^2 of 0.97 and a decay constant value of −0.054 and shade samples an R^2 of 0.97 and a decay constant value of −0.057.

Discussion

Paraffin wax drops containing 5% pheromone and deployed at 30 and 100 per tree (8,200 and 27,300/ha) completely disrupted mating of *G. molesta* under heavy population densities as measured by tethered female moths and disrupted orientation of feral males to optimally baited traps above 99% relative to control plots. In addition, the level of mating disruption using high-application densities of wax drops was better than with label-recommended applications of Isomate M-Rosso dispensers, which resulted in ≈17% mating of tethered virgin females (Fig. 2). Further-

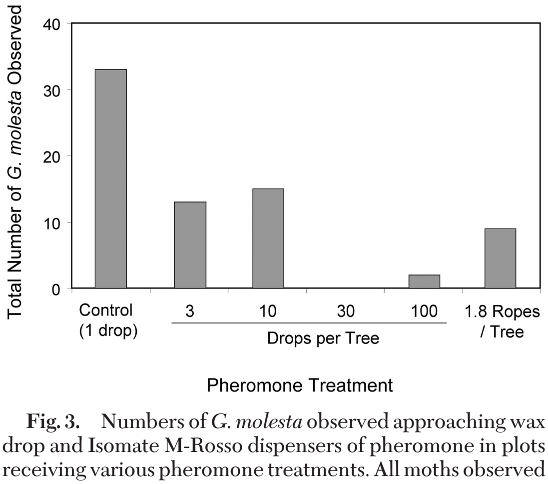

Fig. 3. Numbers of *G. molesta* observed approaching wax drop and Isomate M-Rosso dispensers of pheromone in plots receiving various pheromone treatments. All moths observed approached within 130 cm of dispensers. Observations were conducted over 26 evenings during generations 1–3.

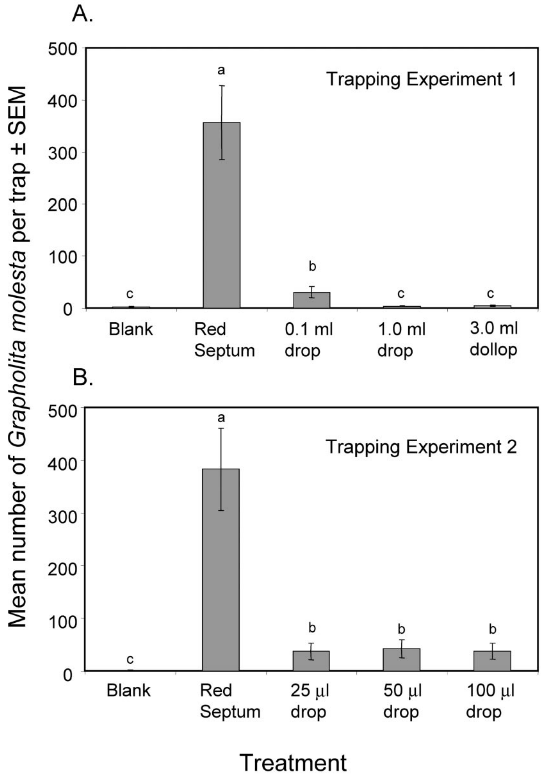

Fig. 4. Mean numbers of *G. molesta* males captured in delta traps containing various sizes of wax drops with 5% pheromone versus rubber septum lures. Means followed by the same letter are not significantly different at $\alpha < 0.05$.

more, the application of 8,200 wax drops/ha required less total pheromone per season (99 g pheromone/ha) compared with Isomate M-Rosso ropes at 500/ha (199 g pheromone/ha). Given that 30 drops per tree (8,200/ha) resulted in >99% disruption of traps and 100% disruption of mating, higher application rates of drops would not be necessary at the moth densities recorded in these trials. Three such applications would be required in Michigan, by using approximately one-half the total active ingredient that would be deployed with one seasonal application of Isomate M-Rosso dispensers.

Previous trials evaluating the effectiveness of paraffin wax dispensers for mating disruption of *G. molesta* also have reported success as measured by disruption of male orientation to lure- and female-baited traps (Atterholt 1996, Atterholt et al. 1998, Delwiche et al. 1998, de Lame 2003). However, this is the first report to show complete mating disruption of tethered, virgin female moths in heavily infested plots. Importantly, disruption of pheromone-baited traps seemed to be a more conservative estimate of pheromone treatment efficacy than mating of tethered virgin females; percentage of mating was typically higher than percentage of orientational disruption in treatment plots relative to controls (Figs. 1 and 2).

In addition, previous experiments have reported technical difficulties associated with the fluidity of commercial versions of paraffin wax dispensers (Gowan Co., Yuma, AZ) and the need for use of more pheromone per hectare compared with label-recommended applications of Isomate polyethelene-tube dispensers, given the required multiple applications of wax per moth generation (de Lame 2003). The formulation described here is viscous, easy to apply, and releases pheromone slowly so as to provide generation-long disruption of *G. molesta.* In our release-rate studies, wax drops receiving direct sunlight dispensed pheromone indistinguishably from those provided with supplemental shade. This suggests that degrada-

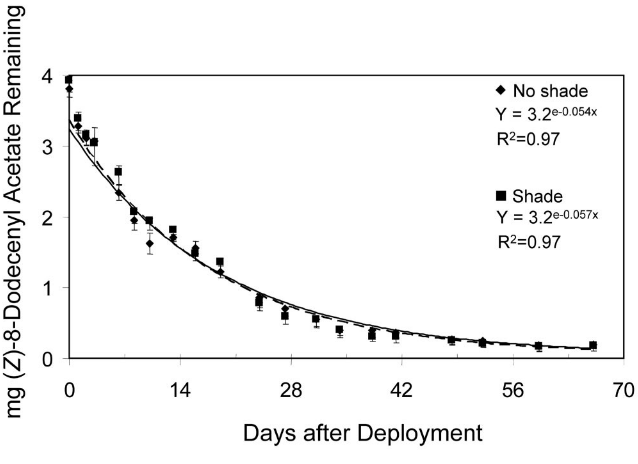

Fig. 5. Release profiles of 0.1-ml wax drops containing 5% pheromone fitted with exponential decay curves. Samples without supplemental shade received only the natural shading of the tree canopy, whereas shade samples were supplemented with a wooden roof to block direct sunlight and rain.

tion of pheromone due to UV exposure was negligible. Our studies proved that one application of 0.1-ml drops disrupted male orientation for 5–6 wk, which is the duration that wax drops release between 0.15 and 0.05 mg of pheromone per day (Fig. 5). This release rate from wax drops is higher than that from rubber septa loaded with 0.1 mg of *G. molesta,* which is likely in the range of 290 ng per day (Baker et al. 1980).

Pheromone point source density and distribution is known to affect the level of mating disruption (Charlton and Cardé 1981, Palaniswamy et al. 1982, Alford and Silk 1983, Suckling et al. 1994, Suckling and Angerilli 1996, de Lame 2003). Typically, higher numbers of point sources emitting pheromone yield higher levels of mating disruption. Our data corroborate this pattern in that our two highest deployment densities of wax drops performed better than the lower densities. In addition, plots receiving 2,700 drops/ha (11 g pheromone/ha) performed equally to or better than 500 Isomate M-Rosso dispensers/ha (199 g pheromone/ha). Thus, our data prove that point-source density was a more important factor in disrupting *G. molesta* than the total amount of pheromone released into the apple orchard atmosphere.

Our extensive field observations revealed that male *G. molesta* briefly (<30 s) approached within 130 cm of wax drops. Although some individuals approached within 10 cm, the majority (75%) remained 20–60 cm away from drops. This finding was corroborated by our trapping study that revealed that fewer male *G. molesta* were captured in traps baited with 0.1-ml wax drops compared with optimally tuned rubber septum lures. This suggests that the pheromone blend and/or release rate dispensed from wax drops was suboptimally attractive compared with that released from rubber septum lures. A more attractive wax formulation might improve disruption when population densities are extremely high. Although male visits to wax drops were brief and the majority of observed moths did not make direct contact with these dispensers, competitive attraction between attractive sources seems to be an important mechanism of communicational disruption in our study. In addition to directly documenting male *G. molesta* visits to wax drops, we found that higher point source densities rather than more total pheromone per hectare deployed into the crop atmosphere resulted in greater mating disruption. These results support false-plume following rather than camouflage as the leading mechanism of disruption for this pest. Notably, plots receiving only 820 drops/ha (3.3 g pheromone/ha) realized 86% disruption of male orientation to traps and 65% disruption of female mating. It is difficult to imagine that camouflage was an important mechanism mediating disruption in plots receiving only 3.3 g pheromone/ha, given that concentrations of pheromone quantified in plots receiving recommended densities (500–1000 dispensers/ha) of polyethylene tube dispensers are between 1 and 2 ng/m^3 (Koch et al. 2002). Additionally, false-plume following to high-release Isomate M-Rosso dispensers has been recently documented as a contributing factor to mating disruption of *G. molesta* (Stelinski et al. 2004 b). However, camouflage has not been excluded as a possible contributing mechanism in plots that received the two highest densities of pheromone wax drops evaluated.

Our results show that paraffin wax can be used as an effective dispenser for mating disruption of high population densities of *G. molesta.* Such dispensers are inexpensive and easy to produce, consisting mostly of paraffin wax and water. They were more effective than Isomate M-Rosso polyethylene tubes at application rates that used less total pheromone per season. The higher application densities of wax drops tested produced the best results, probably because these treatments created the highest level of competitive attraction between dispensers and feral female *G. molesta.*

Currently, Michigan State University is developing a mechanized applicator for quick and inexpensive deployment of paraffin wax drops in commercial orchards. A prototype exists that dispenses drops of appropriate size at correct application densities (J.R.M. et al. unpublished). Although paraffin wax drops will not likely be appropriate dispensers for all moth pests given potential phytotoxicity of certain pheromone components (Giroux and Miller 2001) and degradation of certain pheromones, broader testing of this formulation at various densities is warranted for a wide range of insect pests. The tactic of deploying high densities of female-equivalent release dispensers for mating disruption shows considerable promise and is not limited to wax drops.

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