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ORIGINAL PAPER

Season-long mating disruption of *Grapholita molesta* (Lepidoptera: Tortricidae) by one machine application of pheromone in wax drops (SPLAT-OFM)

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Abstract A novel emulsified wax dispenser (SPLAT-OFM) of pheromone was evaluated in concert with a custom-built, tractor-mounted applicator, designed for fast application of dispensers for mating disruption of Oriental fruit moth, *Grapholita molesta* (Busck), in apple. The formulation consisted of microcrystalline wax emulsified in water. It was loaded with *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) at 10% by weight. The hydraulically driven applicator dispensed the wax formulation as discrete particles from a rotating double-orifice distributor positioned directly above the tree canopy. Wax-drop size averaged (±SEM) 0.38 ± 0.16 g and 4.3 ± 0.5 drops adhered per tree. Following a single mechanized application of SPLAT-OFM on 24 April at 8 ml per tree (1.6 kg/ha) to 0.8 ha blocks of apple, male *G. molesta* orientation to optimally attractive pheromone traps was disrupted by 98% relative to untreated control plots for the whole season. Furthermore, on 17 weekly deployments of tethered virgin females (1,016 females deployed and 732 recovered for dissection) throughout the season, no mating was detected in SPLAT-OFM-treated blocks, while mating in control blocks averaged 27%. During the first 17 days following deployment in sticky traps, SPLAT-OFM drops attracted ca. 1/46th of the number of male *G. molesta* attracted to optimized synthetic lures. However, following 17 days of field aging, SPLAT-OFM drops became equally attractive to optimized synthetic lures for the remainder of the season. The release rate of pheromone from wax drops 0–14 and 15–76 days following deployment averaged 21.4 and 5.3 μg/h, respectively. The trapping and release rate data were consistent with competitive attraction as the mechanism mediating disruption. Shoot injury following the first moth generation was sevenfold less in SPLAT-OFM treated blocks compared with controls and fruit injury at the end of the season in treated blocks was approximately half of that recorded in controls.

Keywords Applied chemical ecology · *Grapholita molesta* · Mating disruption · SPLAT-OFM · Competitive attraction

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Introduction

The Oriental fruit moth, *Grapholita molesta* (Busck), is a key worldwide pest of stone fruit (Rothschild and Vickers 1991), pears (Il'ichev et al. 2004) and apples (Kovanci et al. 2005; Myers et al. 2006). After mating, female *G. molesta* lay eggs on host foliage or fruit. Feeding by first-generation larvae causes damage to sprouting shoot growth in the spring and early summer, while internal feeding by larvae from later summer generations destroys fruit (Rothschild and Vickers

1991). This pest can cause up to 50% crop loss and prolonged use of broad-spectrum insecticides, as a main control tactic, has resulted in pronounced resistance to organophosphorous, carbamate, and pyrethroid insecticides (Kanga et al. 2003).

The possibility for mating disruption for *G. molesta* was successfully demonstrated three decades ago using hand-applied hollow fiber dispensers of pheromone deployed at 1,700 points sources per ha (Cardé et al. 1977). This behavioral-modification tactic represents a promising alternative to broad-spectrum neurotoxins (Gut et al. 2004). Currently, the predominant commercially available formulations for disruption of *G. molesta* are hand-applied polyethylene tube dispensers such as Isomate®-M, M 100 and M (OFM) Rosso (Pacific Biocontrol Co., Litchfield Park, AZ, USA) (Pfeiffer and Killian 1988; Audemard et al. 1989; Rice and Kirsch 1990; Pree et al. 1994; Trimble et al. 2001; Atanassov et al. 2002; Trimble et al. 2004). These dispensers are loaded with 75–250 mg of *G. molesta* pheromone and applied by hand at 500–1,000 units/ha (typically results in 1–4 dispensers/tree). Pheromone release per hr from one of these dispensers ranges between ca. 600–1,000-fold more than the amount released by a calling female. Although this technology has proven effective in and among wide-ranging moth population densities, crops, and environmental conditions (references above), a commonly cited drawback is the labor cost associated with hand application of many hundreds of units per ha of crop. Furthermore, Stelinski et al. (2005) documented that *G. molesta* males can de disrupted more effectively by deploying approximately eight thousand 0.1-ml drops of paraffin wax per ha (each containing ca. 1% of the total pheromone active ingredient loaded into a standard Isomate dispenser) than with the label rate of 500 Isomate M Rosso dispensers per ha, despite using less total pheromone/ha with the former treatment. Also, there is mounting corroborating evidence that disruption of various moths is superior via higher rather than lower densities of pheromone release sites at common overall release rates of pheromone per ha (Charlton and Cardé 1981; Palaniswamy et al. 1982; Suckling et al.1994; de Lame 2003; Stelinski et al. 2005; Epstein et al. 2006; Miller et al. 2006a,b).

Sprayable formulations of microscopic capsules that release pheromone over time have proven useful in *G. molesta* control (Trimble et al. 2004; Kovanci et al. 2005; Il'ichev et al. 2006). These formulations are applied with standard air-blast sprayers; they can be tank-mixed and co-applied with other orchard-management chemicals, and are considered a cost-saving alternative to hand-applied dispensers (Kovanci et al. 2005; Il'ichev et al. 2006). Although effective against *G. molesta* when properly applied, sprayable pheromone formulations require more frequent applications (every 2–4 weeks) compared with hand-applied reservoir dispensers, which maintain effectiveness for 24–28 weeks (Trimble et al. 2004; Kovanci et al. 2005; Il'ichev et al. 2006). Other drawbacks of sprayable microencapsulated formulations include wash-off of microcapsules by heavy rains and degradation of active ingredients due to UV radiation, both reducing longevity of effective disruption (Knight et al. 2004; Waldstein and Gut 2004).

Recently, paraffin-wax dispensers were developed for releasing *G. molesta* pheromone (Atterholt 1996; Rice et al. 1997; Atterholt et al. 1998; Meissner et al. 2000; de Lame 2003). These dispensers are biodegradable, inexpensive, and easy to produce (Stelinski et al. 2005). When applied by hand from syringes as 0.1 ml drops containing 5% pheromone by weight, wax drops effectively disrupted *G. molesta* for 4–6 weeks (one moth generation) (Stelinski et al. 2005). Such longevity would require three applications for effective season-long control of *G. molesta* in Michigan, USA.

Following the successful development of a paraffin-wax formulation for *G. molesta* disruption, a mechanical applicator was built that delivered ca. 160 wax drops (0.04 ml of wax/drop) per tree at an approximate rate of 20 min/ha of crop (Stelinski et al. 2006). This formulation disrupted *G. molesta* at >98%; however, efficacy of this formulation was maintained for only 7–10 days in mid- to late-summer under elevated temperatures (Stelinski et al. 2006). Although this initial applicator prototype successfully deployed high densities of wax drops per ha of crop, the limited length of disruption efficacy during summer months was economically prohibitive.

The current research tested the hypothesis that increasing the size of dispensed drops above the 0.04 ml average volume achieved by the initial applicator prototype, as well as, re-formulating the wax to allow for a higher initial pheromone loading concentration for longer release over time in hot temperatures would maintain efficacy and improve longevity (Stelinski et al. 2006). The specific objectives were to: (1) modify the mechanical applicator to release larger wax drops, and (2) evaluate an experimental microcrystalline wax formulation of *G. molesta* pheromone (SPLAT-OFM). We show here that one high-speed, mechanized application of SPLAT-OFM in early spring (April 24th) effectively disrupts *G. molesta* in apples for the duration of an entire growing season and reduces crop damage.

Materials and methods

Wax formulation and application protocol

We evaluated a newly developed formulation of emulsified wax for releasing insect pheromones (SPLAT-OFM, ISCA, Riverside, CA, USA). It is a proprietary microcrystalline wax formulation composed of biologically inert materials similar to those reported by Rice et al. (1997) and Atterholt et al. (1998). The active ingredient [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)] was incorporated at 10% by weight.

This formulation was deployed with an improved version of a tractor-mounted, mechanized applicator detailed by Stelinski et al. (2006). The wax was pressurized by a piston to 100 psi in a 20 l reservoir. A gear pump metered the wax through hoses feeding a spinning distributor at the end of a boom arm (Fig. 1a) used to position the distributor above the tree canopy (Fig. 1b). This spinning distributor rotated two steel nozzles (10-mm internal diameter) spaced 180° apart at 100–150 rpm via a hydraulic motor (Fig. 1a). A remotely controlled hydraulic valve adjusted the rpm of the metering gear pump to dispense the desired flow rate of wax. A digital readout monitored the rpm of the metering gear-pump, allowing verification or modification of the flow rate. Eight ml of wax was dispensed per tree when traveling at 7.4 km/h. Wax drops were applied to treatment plots (described below) on 24 April.

Plots and experimental design

This experiment was conducted in commercially farmed and unmanaged portions of an apple orchard in South Lyon, MI, USA described by Stelinski et al. (2006). Two treatments were assigned to eight 0.8 ha replicate blocks in a randomized complete block design. The treatments were: (1) mechanically applied SPLAT-OFM at 8 ml/tree (1.6 kg/ha) and (2)

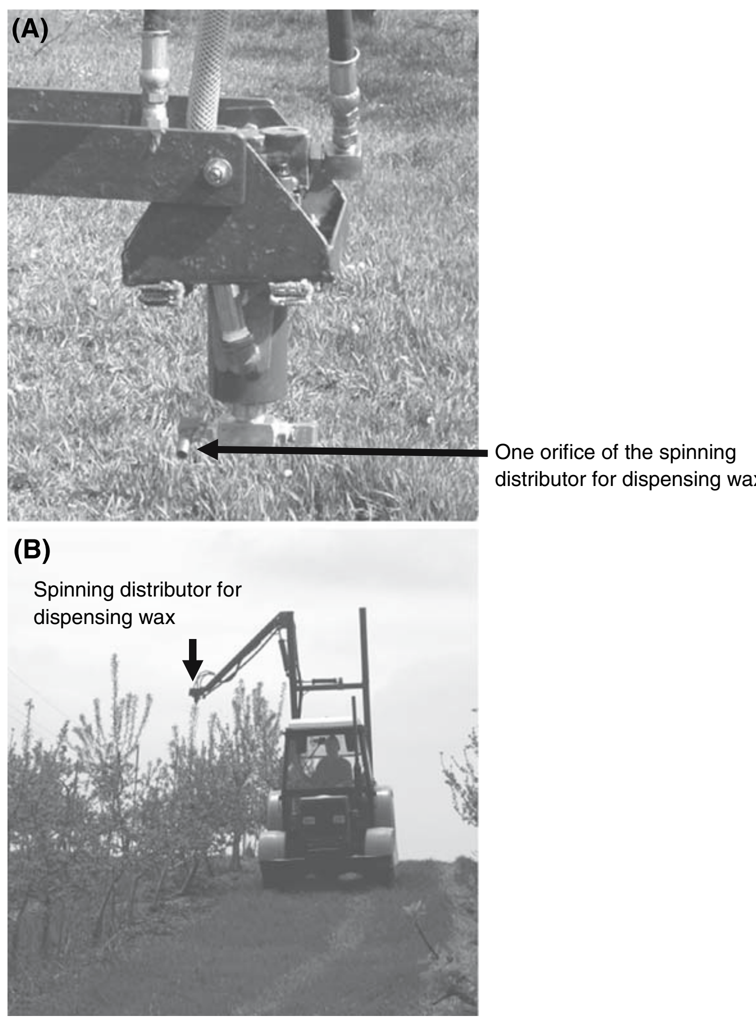

Fig. 1 Spinning distributor for dispensing discrete particles of wax-based dispensers of pheromone (**a**). Mechanized application of SPLAT-OFM dispensing wax particles from above the tree canopy (**b**)

untreated check. Replicate orchards (blocks) were separated by 15–40 m and treatment plots by 10–20 m. Two replicates of each treatment were established in a recently abandoned portion of the apple orchard which had not been sprayed with pesticides or harvested since 2001. These plots were managed by mowing the orchard floor throughout the experiment and by pruning trees. The other two replicates were established on commercially farmed portions of the orchard. This area received one spray of thiacloprid (0.58 l/ha) on 5 May; however, no broad-spectrum organophosphates were applied. The two replicates in the commercial portion of the orchard were also treated with Isomate-C Plus dispensers for mating disruption of *Cydia pomonella* (L.) at a rate of 1,000/ha, each containing 182.3 mg of a 60:33:7 blend of (*E*,*E*)-8,10-dodecadien-1-ol, 12OH, and 14OH.

Evaluation of wax-drop size, deployment density, and longevity of adherence

Average mass of SPLAT-OFM drops deployed from the spinning distributor was quantified by dispensing drops onto sheets of heavy construction paper in the same way as they were deployed onto trees. Twenty individual drops were removed from the construction paper and weighed. Wax-drop deposition per tree was quantified by examining 20 randomly chosen trees immediately following application in the commercially managed plots. Wax drops found on tree wood and foliage were counted. Forty of these (all on wood) were randomly chosen and marked with flagging tape. These flagged drops were checked weekly 29 April through 5 June and on 17 July, 17 August, and 13 September to assess longevity of drop adherence following application.

Disruption of male orientation

Disruption of male *G. molesta* orientation to synthetic sex pheromone was assessed using four pheromone traps (LPD Scenturian Guardpost, Suterra, Bend, OR, USA) deployed per replicate plot. Two traps were deployed 15 m apart in the central row of plots and two traps were placed two rows from plot borders. Traps were baited with red septa (The West Company, Linville, PA, USA) 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. Traps were deployed approximately 3–4 m above-ground in the upper third of the tree canopy following application of wax. New pheromone lures were deployed at the onset of each moth generation resulting in 2 replacements over the course of the season. Moths captured in traps were counted and removed twice weekly.

Disruption of female mating

Disruption of moth mating was also directly assessed by deploying tethered virgin female moths in plots weekly for the duration of the experiment. Female *G. molesta* were drawn from a 5-year-old laboratory colony at Michigan State University (East Lansing, MI, USA) originally collected as larvae from apple orchards in Southwest Michigan. Rearing and handling protocols of moths have been described by Stelinski et al. (2005).

Eight to 15 female moths (2–4-day old) were tethered per plot during each of 17 deployment dates (10, 18, 24, 30 May; 7, 14, 24, 29 June; 4, 13, 19, 25 July; 03, 10, 17, 24, and 31 August) throughout the season. Female moths were attached to branches of trees with polyester thread tied to the base of one wing. They were tethered on 50 cm of thread, which allowed moths to relocate themselves on branches following placement. Of the 1,016 moths deployed, 72% were recovered. Females were collected from the field approximately 18–20 h following deployment. Dissections were conducted to determine mating status by removal of the bursa copulatrix and inspection for the presence of a spermatophore.

Shoot and fruit injury evaluation

Damage to shoots by *G. molesta* larvae was assessed after the first and second moth generations on 7–9 June and 7–8 August, respectively. During both evaluations, 20 shoots were removed at random from 20 trees per replicate block. Ten of the shoots were removed at eye-level and 10 from the upper part of the canopy, 1–1.5 m from tree tops using pole pruners. Collected shoots were dissected to confirm that damage was caused by *G. molesta*.

Fruit damage was assessed on 22–29 August using random samples of 30 apples (15 from upper canopy and 15 from lower canopy) from 20 trees per replicate block. All damaged fruit were cut open for identification of *G. molesta* larvae. Larvae were preserved in 70% ethanol and later examined under a microscope to differentiate *G. molesta* from *C. pomonella* by presence of an anal comb.

Trapping study

An experiment was conducted to assess ability of 0.38 g drops of SPLAT-OFM (average size of drops dispensed by the mechanized applicator; see Results) to elicit moth capture in sticky delta traps. Captures of *G. molesta* males in traps baited with SPLAT-OFM drops were compared with captures in traps baited with highly attractive rubber septum lures impregnated with pheromone as described above. This test was conducted at the Trevor Nichols Research Complex (TNRC) of Michigan State University in Fennville, MI, USA in plots described by Stelinski et al. (2004). SPLAT-OFM drops, weighing 0.38 g, were pipetted onto 2 × 5 cm strips of aluminum foil and air-dried for 24 h. Treatments were inserted into plastic delta traps (described above) deployed in unsprayed 0.4 ha plots of apples. Unbaited traps were used as a negative control. The experiment was arranged in a randomized complete block design with six replicates. Treatments were spaced by 8–12 m apart. Traps were hung ca. 1.5–2 m above ground level in the upper third of the tree canopy. Rubber septum lures were replaced every 4–5 weeks to maintain maximum attractiveness while wax drops were not replaced because the goal was to determine whether their attractiveness changed over time as pheromone release rate diminished. Moths captured in traps were counted and removed twice weekly. The experiment was conducted 5 June–27 September.

Release rate analysis

SPLAT-OFM drops, approximating the 0.38 g drops applied in the field, were applied to 1.9 × 15.3 cm pieces of flat, wooden Jumbo Craft Sticks (Forster Crafts, Cloquet, MN, USA). These sticks were screwed to the top of a 38 cm long section of 2 × 5 cm wood with 26 sticks per board. Each stick was labeled as to location, and each assembly (array) was labeled. Two arrays were attached with zip-ties to the branches of five 5–7 m tall apple trees at a height of 1.5–2 m within the tree canopy. These were attached so that one array faced upwards, and received direct sunlight, and one array faced downwards and was shaded. Samples held within each tree comprised one block.

Samples were maintained in the field from 17 May to 1 August. A randomized collection scheme was developed prior to application. A set of 20 samples was collected immediately upon application to determine the amount of pheromone in the wax drops at test onset. Thereafter, samples were collected approximately every 2 days for the first 16 days and every 3–4 days for the remainder of the trial. On each sampling date, one sample was collected from each of the ten arrays. Upon collection, samples were placed into 20 ml scintillation vials and 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/l methyl tridecanoate (99%+, MP Biomedicals, Inc., Solon, OH, USA) in acetonitrile was added to each scintillation vial. Samples were then placed into a water bath shaker (Shaking Water Bath Model 406015, AO Scientific Instruments, Keene, NH, USA) at 80–85°C for 10 min; removed and agitated vigorously by hand and then vortexed and replaced in the water bath. The bath was then set to shake at a moderate level for 30 min. During this 30 min period, each sample was removed, agitated and vortexed two more times. Samples were then stored at −20°C for 16 h, during which time the wax precipitated out of solution.

Prior to analysis, vials were thawed and agitated by hand. At least one ml 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, USA) through another Pasteur pipette fitted with a small piece of Kimwipe (Kimberly-Clark Corp., Roswell, GA, USA) 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 Co.). The GC was fitted with a DB-23 polar column (model # 122-2332, J&W Scientific, Folsom, CA, USA) of length 30 m and internal diam. 250 μm. The initial GC temperature was held at 50°C for 5 min and then ramped at a rate of 25°C/min to 133°C, where it was held for 5 min. Afterward, the temperature was increased by 0.5°C/min to 137°C, and then ramped by 25°C/min to a final temperature of 200°C which was held for 3 min. The carrier gas, He, entered the column at 13 psi. The pheromone content of the samples was calculated using the internal standard method (McNair and Miller 1998).

Data analysis

Trapping data were transformed to $\ln(x + 1)$ (to normalize the distributions and homogenize variance) and then subjected to analysis of variance (ANOVA). Shoot and fruit injury data as well as tethered female mating data were arcsine transformed prior to ANOVA. Differences in pairs of means were separated using least significant difference tests (SAS Institute 2000). In all cases, the significance level was $\alpha < 0.05$. Percent orientational disruption was calculated as 1 − (mean moth catch per trap in the pheromone-treated block/mean moth catch per trap in the control block) × 100.

Results

Wax-drop size, deployment density, and longevity of adherence

The average (±SE) weight of a SPLAT-OFM drop dispensed from the mechanized applicator ($N = 20$) was 0.38 ± 0.16 g. An average of 4.3 ± 0.5 drops was found adhering per treated tree ($N = 20$). Of the 40 drops flagged on 28 April directly following the application, all 40 were found remaining on wood on 23 May. On 29 May, 38 of the drops were found and on 5 June 35 were located. The remaining 35 drops were successfully located on 17 July, 17 August, and near the end of the study on 13 September.

Disruption of male orientation

Following a single mechanized application of SPLAT-OFM on April 28th, 98% inhibition of male catch (Fig. 2a) was recorded season long. The mean (±SE) number of males caught per block per generation was significantly (F's = 72.3, 27.4, 41.7; $df = 1, 3$; $P < 0.05$) greater in control plots (9.1 ± 2.8, 10.9 ± 1.9, 21.3 ± 4.1) than in pheromone-treated plots (0.02 ± 0.02, 0.02 ± 0.02, 0.9 ± 0.3) for moth generations 1–3, respectively. Inhibition of moth catch in traps was >99% for generation 1 and 2 and 96% for the third generation.

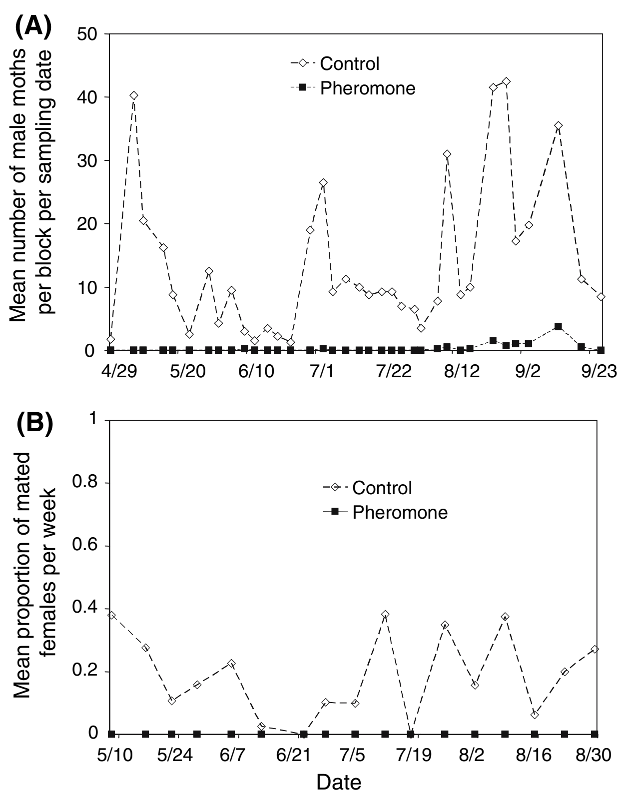

Fig. 2 Mean captures of *Grapholita molesta* males per week throughout the 2006 season in replicated 0.8 ha pheromone-treated and control plots (**a**). Mean proportion of virgin female *Grapholita molesta* mating during 18–20 hr of field deployment in plots receiving a mechanized application of SPLAT dispensers of pheromone versus untreated control blocks (**b**). Application of SPLAT was made on 24 April

Disruption of female mating

Excluding days on which no mated females were recovered from both pheromone-treated and control plots (21 June and 19 July), the proportion of female moth mating in control plots averaged 27% over the course of the season ($N = 32$–60 per treatment per sampling date) (Fig. 2bB). Mating of tethered virgin female *G. molesta* was completely disrupted in pheromone-treated blocks season long.

Shoot and fruit injury evaluation

Following the first generation of moth flight, the mean (±SE) percent of shoot injury ($N = 1{,}600$ per treatment) in pheromone-treated plots (0.38 ± 0.07) was reduced sevenfold compared with that in control plots (3.1 ± 0.98) ($F = 7.9$; $df = 1, 3$; $P = 0.06$). Following the second generation, the difference between mean shoot injury ($N = 1{,}600$ per treatment) in pheromone-treated (0.38 ± 0.2) and control (0.8 ± 0.1) plots was not significant ($F = 4.9$; $df = 1, 3$; $P = 0.1$). At season's end, the mean proportion of fruit injury ($N = 2{,}400$ per treatment) due to *G. molesta* infestation found in pheromone-treated plots (0.25 ± 0.2) was less than half of that in control plots (0.67 ± 0.4); however, the difference was not statistically significant ($F = 2.2$; $df = 1, 3$; $P = 0.2$).

Trapping study

During the first 17 days of deployment (10–27 July), the mean (±SE) number of male moths captured in traps baited with optimized 0.1 mg rubber septum lures (60.4 ± 11.4) was significantly ($F = 45.7$; $df = 1,10$; $P = 0.05$) greater than the mean number of males captured with SPLAT drops (1.3 ± 0.03). Only one male *G. molesta* was captured in a blank negative control trap during this 17-day interval. However, following 17 days of aging in the field, the mean number of moths captured with wax drop-baited traps (4.7 ± 0.02) was nearly identical ($F = 1.6$; $df = 1,10$; $P = 0.2$) to that captured in traps baited with optimized 0.1 mg lures (4.7 ± 0.4) for the remainder of the test (31 July–27 September). Three male *G. molesta* in total were captured in blank traps during this remaining interval.

Release rate analysis

The release rate profile of pheromone is shown in Fig. 3a fitted with an exponential decay curve; the curve fit yielded an R^2 of 0.95 and a decay constant of –0.024. The average release rate of pheromone from a wax drop was 21.4 µg/h over the first 14 days of release in the field (Fig. 3b). Between day 14 and 63, release rate decreased to ca. 9.7 µg/h (Fig. 3b). After day 63, the release rate leveled off at ca. 2.8 µg/h (Fig. 3b).

Discussion

Only one early season application of SPLAT-OFM from a re-designed mechanical applicator was required for nearly complete disruption of *G. molesta* male orientation to traps and mating of tethered virgin females for an entire growing season. Orientational disruption of males exceeded 99% for the majority of the season; however, there was a slight decrease in efficacy near mid-September during the third moth generation. The mechanical applicator was calibrated to dispense ca. 8 ml of wax per tree, which could have at most resulted in approximately 20 0.4-g drops per tree. Our quantification of drops actually deposited on trees directly following application revealed that only ca. 1/5 of dispensed drops adhered to trees. Thus, of the 160 g of active ingredient applied/ha, only about 30 g of pheromone/ha may have accounted for the season-long disruption efficacy. About 88% of those drops successfully deposited onto trees remained for the duration of the experiment. Future investigations are needed to determine whether wax having landed on the ground contributed to disruption efficacy.

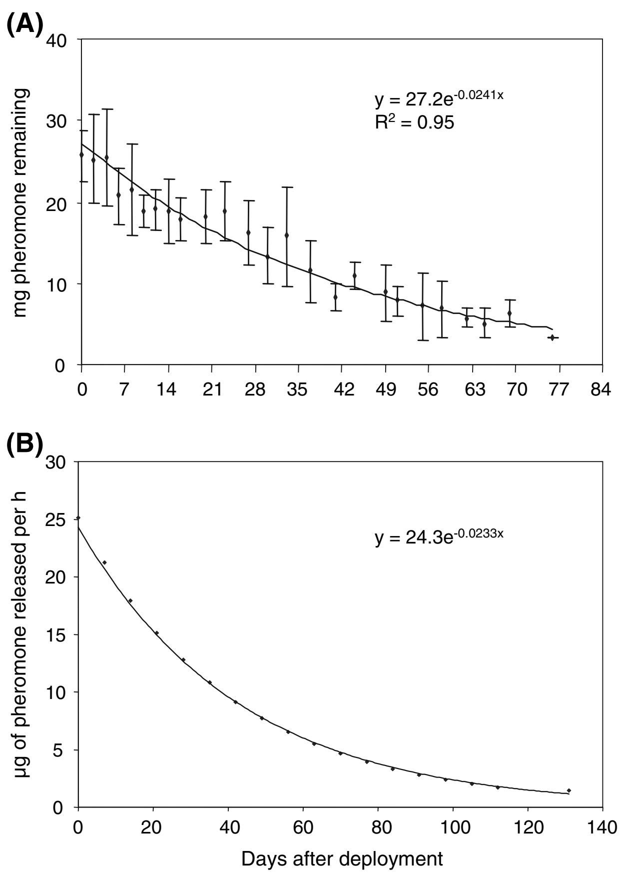

Fig. 3 Release profile of 0.38 g wax drops (SPLAT-OFM) containing 10% pheromone fitted with an exponential decay curve (**a**) µg's of pheromone released per h. Data after day 77 are an extrapolation (**b**)

The 160 g of pheromone applied per ha with mechanically applied SPLAT-OFM in the current study exceeded the 125 g a.i./ha label-rate of the Isomate M-Rosso reservoir dispenser widely used for disruption of *G. molesta* throughout the world. Further improvement of the applicator to dispense drops such that a greater proportion of the total material deployed adheres onto trees could lower the amount of a.i. required per ha. However, even with the current prototype, there is a cost-saving advantage of mechanical application. A single operator can treat a hectare of crop with the current mechanized applicator in ca. 20 min (Stelinski et al. 2006). This is approximately 3.4 times faster than three people hand applying Isomate M Rosso dispensers (de Lame 2003). Considering the labor-saving advantage of mechanical application, the currently evaluated SPLAT-OFM formulation represents an economical alternative to hand-applied reservoir dispensers for high-performance mating disruption of *G. molesta*. However, until machine applicators are affordable to individual growers (Stelinski et al. 2006), this would require application of SPLAT provided as a service by the manufacturer and/or distributor given the need for initial investment in the applicator. Other potential advantages of deploying SPLAT over current mechanically applied formulations include: longer lifespan of efficacy than that achieved with current sprayable microencapsulated formulations (Knight et al. 2004; Waldstein and Gut 2004; Stelinski et al. 2005), and lesser susceptibility to rainfall and sunlight degradation than with pheromone microcapsules (Knight et al. 2004; Waldstein and Gut 2004; Stelinski et al. 2005).

Deploying a high density of pheromone sources per area of crop has been shown to contribute to achieving effective disruption of moth communication for several species including *G. molesta* (Charlton and Cardé 1981; Palaniswamy et al. 1982; Suckling et al. 1994; de Lame 2003; Stelinski et al. 2005; Epstein et al. 2006; Miller et al. 2006b). One major advantage of mechanically applied wax dispensers is that more point sources of pheromone can be delivered per tree on an as-needed basis than can be achieved with reservoir dispensers. It has been recently suggested that competitive attraction may be a leading mechanism mediating disruption of moth pests by synthetic pheromones (Miller et al. 2006a,b). Wax formulations such as SPLAT, which can be easily applied as numerous discrete point sources per area of crop, have the potential of maximally exploiting this mechanism.

Our combined trapping and release rate studies revealed that the initial rate of pheromone release from drops of SPLAT-OFM (21.4 μg/h) was too high to elicit a high visitation rate of males to within centimeters of the dispensers for at least the first 17 days following deployment. This result does not falsify the hypothesis that male *G. molesta* engaged in false-plume following to drops of SPLAT during this initial interval. *G. molesta* males initiate upwind flight, in equal frequencies, to rubber septa loaded with 1,000 and 100 μg of pheromone (Cardé et al. 1975; Baker and Roelofs 1981), which release ca. 12 and 219 ng of pheromone/hr, respectively (Baker et al. 1980). The drawing range of the 1,000 μg septum deployed in traps is ca. twofold and fourfold greater than that of septa loaded with 100 and 10 μg of pheromone, respectively (Baker and Roelofs 1981). However, average flight termination distances away from 1,000 and 100 μg septa are 155 and 20 cm, respectively (Baker and Roelofs 1981). Septa loaded with 10 μg of pheromone, which release ca. 1.2 ng of pheromone/hr (Baker et al. 1980), are optimal for catching males in traps; however, their drawing range is considerably smaller than that of 1,000 μg septa. Thus, it is likely that during the first 2–3 weeks of deployment, male *G. molesta* oriented toward SPLAT drops but terminated their upwind progress at a certain distance at which the pheromone concentration was above the upper threshold for response. Similar results have been observed directly in the field for orientations to Isomate M Rosso dispensers (Stelinski et al. 2004). However, at 17 days of pheromone release in the field, drops of SPLAT reached a highly attractive release rate (ca. 15 μg/h) that resulted in moth captures in traps equaling those elicited by highly attractive rubber septum lures. This attractiveness was maintained for the remainder of the summer (62 days total) at an average release rate of 5.3 μg/h. Collectively, these data support the hypothesis that competitive attraction between dispensers of SPLAT-OFM and feral *G. molesta* females was an operating mechanism mediating disruption throughout the majority of this study.

To our knowledge this is the first peer-reviewed report evaluating the commercially manufactured formulation SPLAT (ISCA Technologies, Riverside, CA, USA) that is patterned after the wax formulation originally developed by Rice et al. (1997) and Atterholt et al. (1998). The product was highly effective and represents a potential alternative to reservoir-style dispensers that require hand application. This formulation could have broad application for many pest species provided that the pheromonal active ingredients can be adequately stabilized and protected from degradation (Millar 1995) and that they are not phytotoxic so as to damage foliage and/or mark fruit (Giroux and Miller 2001).

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