Mechanized Applicator for Large-Scale Field Deployment of Paraffin-Wax Dispensers of Pheromone for Mating Disruption in Tree Fruit

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ABSTRACT A tractor-mounted mechanized applicator was developed for large-scale deployment of paraffin-wax dispensers of pheromone for mating disruption of oriental fruit moth, *Grapholita molesta* (Busck). The wax formulation was mostly water and emulsified paraffin wax containing 5% (by weight) pheromone [93:6:1 blend of (*Z*)-8-dodecen-1-yl-acetate:(*E*)-8-dodecen-1-yl-acetate:(*Z*)-8-dodecen-1-ol]. Ten milliliters of wax was applied per tree as ≈160 deposits (0.04 ml of wax per drop). An average of 23 min was required to treat 1 ha of crop. Disruption efficacy of mechanically applied wax was measured relative to an untreated control in replicated 0.4-ha blocks within a recently abandoned apple orchard. From 6 May to 27 June, 100% disruption of tethered virgin females and 97% inhibition of pheromone traps was achieved for 52 d with two applications of wax. However, during mid- to late summer (July–August), this level of efficacy was maintained for only ≈1 wk after each of two applications. Higher temperatures later in the season may have accounted for abbreviated efficacy of the applied small drops. Mechanically applied paraffin-wax technology may increase adoption of mating disruption given that a higher level of efficacy was achieved despite deploying less active ingredient per hectare relative to that used with reservoir dispensers. The savings in labor by not requiring hand application of reservoir dispensers could be directed toward cost of machinery. However, the short duration of efficacy obtained with the current wax formulation and mechanical applicator is judged uneconomical given the eight or more applications that would have been required for high-performance disruption over the full season. Larger drops with lower surface area-to-volume ratios are expected to prolong pheromone release for extended efficacy and desirable overall economics.

KEY WORDS oriental fruit moth, *Grapholita molesta*, emulsifiable wax, competitive attraction

The oriental fruit moth, *Grapholita motesta* (Busck), is a worldwide pest of stone fruit and apples (Rothschild and Vickers 1991). Pheromone-based mating disruption, executed by broadcasting synthetic pheromones into crops or stored-product warehouses to disrupt mate finding (Cardé and Minks 1995), has been successfully implemented for oriental fruit moth (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). Reservoir-style dispensers made of polyethylene tubes (Isomate-M, Shin-Etsu Chemical Co., Tokyo, Japan), releasing oriental fruit moth pheromone across a full growing season, are the industry standard (Vickers 1990). Although reasonably effective (see citations above), such dispensers require labor-intensive hand application.

Recently, we (Stelinski et al. 2005a) evaluated an improved paraffin-wax formulation (Atterholt 1996, Atterholt et al. 1998, de Lame 2003) for oriental fruit moth that maximizes competition between dispensers and feral females for males. Paraffin-wax drops at 8,200 and 27,300 per ha (30 and 100 per tree, respectively) provided higher levels of mating disruption (100%) of tethered virgin females than standard applications of Isomate-M Rosso dispensers (83%) for an entire oriental fruit moth generation (4–5 wk) (Stelinski et al. 2005a). This high-performance disruption was achieved despite deployment of less total pheromone per hectare per season (99 g) compared with Isomate-M Rosso dispensers (200 g) (Stelinski et al. 2005a). In addition to being highly effective, paraffin-wax dispensers are inexpensive and easy to produce (Stelinski et al. 2005a).

The next step toward cost-effective use of paraffin-wax dispensers for oriental fruit moth disruption was development of a mechanized applicator for rapid deployment of high densities of wax drops. The current report describes such an applicator and quantifies the resultant efficacy against oriental fruit moth across a full growing season.

Materials and Methods

Pheromone Dispenser Formulation and Mechanized Applicator. The paraffin-wax emulsion of pheromone was formulated as per de Lame (2003) and Stelinski et al. (2005a). The formulation comprises 40% paraffin wax (Gulf wax, Royal Oak Sales, Inc.,

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Roswell, GA), 4% soy oil (Spectrum Naturals, Inc., Petaluma, CA), 2% Span 60 (Sorbitan monostearate, Sigma-Aldrich, St. Louis, MO), 1% vitamin E [(±)-α-tocopherol, Sigma-Aldrich], 5% oriental fruit moth 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 48% (by total weight) deionized water. Immediately before field deployment, carpet adhesive (3% by weight of emulsion) (Roberts Premium Indoor/Outdoor Carpet Adhesive 6700, Roberts Consolidated Industries, Inc., Boca Raton, FL) was admixed into the emulsion using a power-drill-driven paint mixer (de Lame 2003) to guarantee adherence of the wax drops to tree wood and foliage.

The wax formulation was deployed in the field with a newly designed mechanized applicator (Fig. 1). The applicator was mounted to the tractor via a standard three-point hitch (Fig. 1A). The emulsified wax was loaded into a plastic cylinder reservoir (10.2 liter) (Fig. 1B) from which it was precisely metered by a hydraulically driven gear-pump serving as a rotary metering valve. Material in the reservoir was pressurized (100 psi) by a piston and metered via the gear pump through hoses feeding a spinning-hub distributor at the end of a boom arm (Fig. 1C). The articulated boom arm was hydraulically controlled by the operator to position the spinning hub just above tree canopies (Fig. 1C). The spinning hub was a slight modification of that used by the Proptec sprayer (Proptec, Bath, MI) (Fig. 1D). It was rotated by an internal gear hydraulic motor. Droplet size and number delivered per min were influenced by the rate of wax flow into the hub, the number and size of orifices in the hub walls, hub rotational speed (rpm), and wax viscosity. This dispenser had two 7-mm orifices spaced 180° apart and rotated at 100–450 rpm. The wax formulation was extruded through the orifices of the spinning hub by centrifugal force so as to form filaments that broke into discrete drops (Fig. 1D). Calibration of the applicator was accomplished by determining a desired flow rate based on the formula trees/min = (trees/ha × km/h × width [m]/tree row)/600; multiplying trees/min × ml/tree resulted in a flow rate in ml/min. A remotely controlled hydraulic valve adjusted the rpm of the metering gear pump to dispense the desired flow rate of wax to the atomizer. A digital readout monitored the rpm of the metering gear-pump, allowing the operator to verify or change the rate of flow if desired. Field calibrations were based on dispensing 10 ml of wax formulation per tree when traveling at 7.4 km/h. Wax drops were applied on 6 and 27 May, 11 July, and 3 August 2005.

Field Plots. The experiment was conducted in a recently abandoned apple (*Malus* spp.) orchard in South Lion, MI, which had not been sprayed with pesticides or harvested since 2001. The orchard floor was mowed throughout the experiment, and large overgrown branches and shoots were pruned before the start of the experiment. Our intent was to evaluate the effectiveness of mechanically applied wax under high oriental fruit moth population densities with no insecticidal input. Two treatments were assigned to eight 0.4 ha replicate blocks in a randomized complete block design. Tree composition and canopy size are given in Table 1. Treatments were 1) mechanically applied paraffin wax at 10 ml per tree (42.5–46.5 g/ha) and 2) untreated check. Replicate orchards (blocks) were separated by at least 15 m and treatment plots by at least 10 m.

Evaluation of Wax-Drop Density, Size, and Deployment Speed. Wax-drop deposition on foliage and on the ground beneath trees was quantified. Cardboard was placed underneath trees ($n = 15$) such that the entire surface directly beneath tree canopies was covered. In addition, sheets of heavy construction paper (0.9 by 6.7 m) were draped horizontally over trees ($n = 10$) of replicates 1–3 (Table 1). Immediately after mechanical application of wax, the number of drops on cardboard beneath trees and on paper draped over trees was counted. The 6-m^2 surface area of construction paper draped over a tree made up approximately one-eighth of the total surface area of a tree canopy, which was estimated by measuring tree canopy height and width. To quantify average size of individual wax drops deployed from the spinning hub, drops were dispensed onto sheets of heavy construction paper, described above, in a manner identical to deploying material onto trees. Forty drops were removed from the construction paper after application and weighed. Application speed was timed by stop watch ($n = 4$ blocks). Daily temperature data were recorded by the Michigan Automated Weather Network (http://www.agweather.geo.msu.edu/mawn).

Evaluation of Moth Disruption. Disruption of male oriental fruit moth orientation to synthetic sex pheromone was assessed using two pheromone traps (LPD Scenturian Guardpost, Suterra, Bend, OR) in the centers of plots and four traps placed one row from plot borders. Traps were baited with red septa (The West Company, Linville, PA) 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 hung ≈3–4 m above ground in the upper one-third of the tree canopy. Traps were either replaced after application of wax drops or briefly removed before application and subsequently replaced. 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.

Table 1. Description of tree composition and age used in evaluation of novel mechanized applicator for deployment of paraffin-wax pheromone dispensers

Replicate	Cultivar	Tree age (yr)	Canopy ht (m)	Tree spacing (m)
1	'Macintosh'	8	3.5–4.5	3 by 6
2 and 3	'Macintosh'	10	3.5–4.5	3 by 6
	'Empire'	10	3.5–4.5	3 by 6
4	'Paula Red'	20	4.5–5.5	4 by 7

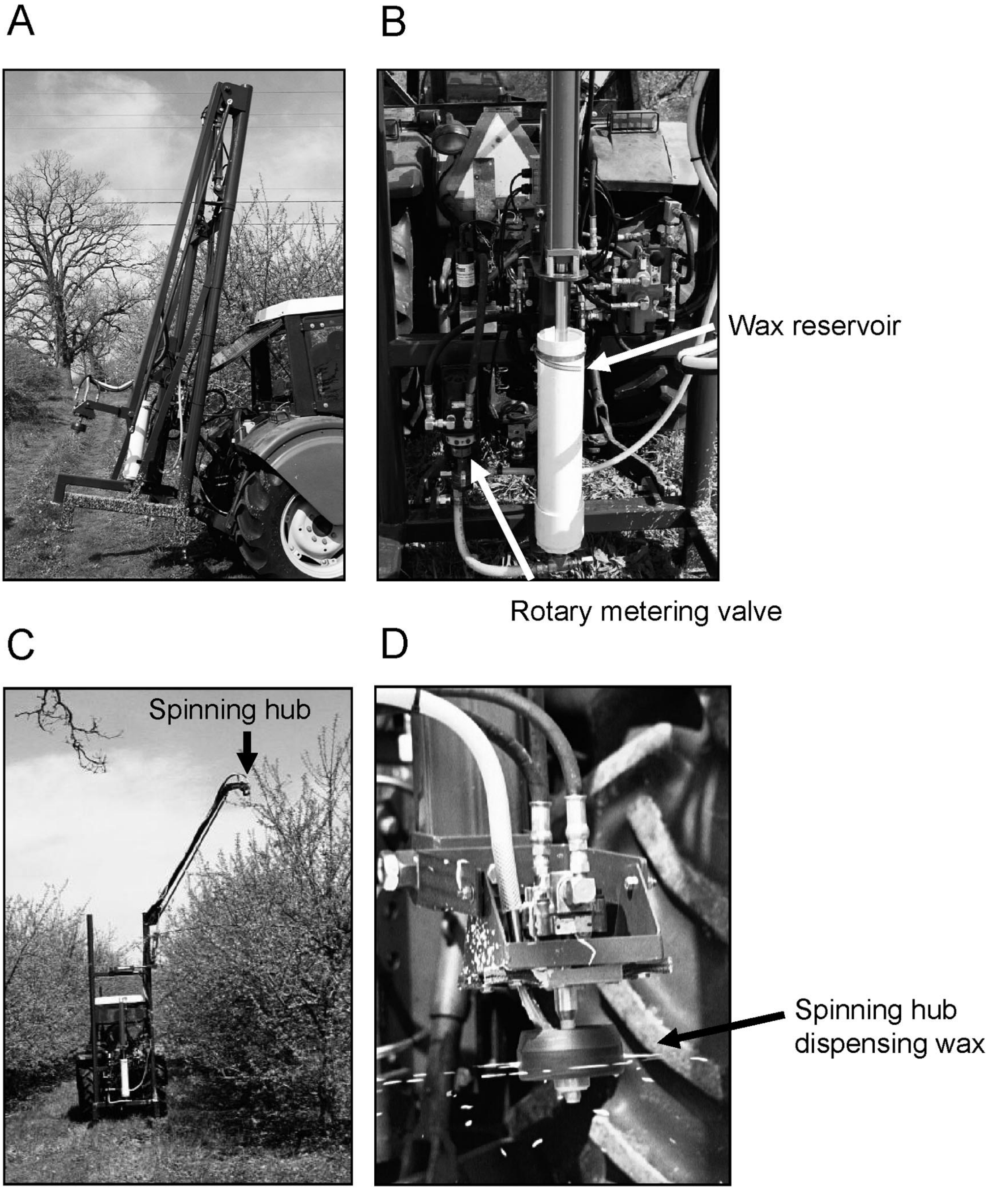

Fig. 1. (A) Tractor-mounted mechanized applicator for deploying wax dispensers of pheromone. (B) Polyvinyl chloride wax reservoir with internal piston. (C) Wax applicator with extended maneuverable, hydraulically operated boom placed above tree canopy. (D) Hydraulically driven spinning hub dispensing discrete drops of emulsified wax.

Mating disruption also was directly assessed by deploying tethered, virgin females. These female oriental fruit moth were drawn from a 4-yr-old laboratory colony at Michigan State University (East Lansing, MI) originally collected as larvae from apple orchards in southwestern Michigan. Rearing and handling protocol of moths are described by Stelinski et al. (2005b).

Eight to 12 female moths were tethered per plot during each of 16 deployment dates (11 and 20 May; 2, 8, 16, 23, and 29 June; 8, 19, and 28 July; 3, 10, 18, and 26 August; and 1 and 8 September) throughout the season. 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 tethered on at least 60 cm of thread and observed for 30 s after deployment to ensure they remained attached and mobile upon the branch. Approximately 82% of tethered females were recovered. Females were recovered 18–20 h after deployment, and mating status was determined by the presence or absence of a spermatophore in the bursa copulatrix.

Shoot Injury Evaluation. Damage to shoots by oriental fruit moth larvae was assessed once on 20–21 June, after the end of the first generation. Twenty shoots were removed at random from 20 trees per replicate block. Ten of the shoots were removed at eye level and 10 shoots from the upper part of the canopy, 1–1.5 m from tree tops, via pole pruners. Collected shoots were dissected to confirm that damage was caused by oriental fruit moth. A fruit injury evaluation at the end of the season was not possible given negligible fruit set in the unmanaged orchard.

Statistical Analyses. Trapping data were transformed to ln $(x + 1)$ (to normalize the distributions and homogenize variance) and then subjected to analysis of variance (ANOVA). Shoot injury and tethered female mating data were arcsine transformed before ANOVA. Differences in pairs of means were separated using least significant difference (LSD) tests (SAS Institute 2000). Percentage of 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

Characterization of Applicator and Wax-Drop Density and Size. The mean number of drops counted on cardboard beneath tree canopies was 33.7 ± 7.5. The mean number of drops counted on paper sheets (0.9 by 6.7 m) draped over one-eighth of each tree canopy was 20.4 ± 3.3; thus, an estimated 160 drops were delivered per tree, of which 130 drops were deposited on the target. The mean volume of a mechanically applied wax drop was 0.042 ± 0.022 ml. The wax treatment was applied at an average rate of 22.6 ± 3.0 min/ha.

Disruption of Male Orientation to Pheromone Traps. The mean number of oriental fruit moth captured in edge versus central traps was not different; therefore, these data were combined for comparison between catches in wax treatment and control. Ninety-seven percent disruption of moth catch was achieved 6 May–27 June with two applications of wax (Fig. 2A). During this interval, significantly ($F = 35.1$; df = 1, 3; $P < 0.01$) more oriental fruit moth males were captured in control plots (24.8 ± 5.1) than in pheromone-treated plots (0.7 ± 0.3). After 52 d, efficacy was lost between 27 June and 11 July, there was no significant ($F = 1.4$; df = 1, 3; $P = 1.1$) difference between the mean number of moths captured in control (10.1 ± 3.8) versus pheromone-treated (8.1 ± 2.6) blocks. After the third application on 11 July, a high level of disruption of moth catch (88%) was maintained for only 11 d (Fig. 2A). During this period, the mean number of moths captured in control plots (5.7 ± 0.5)

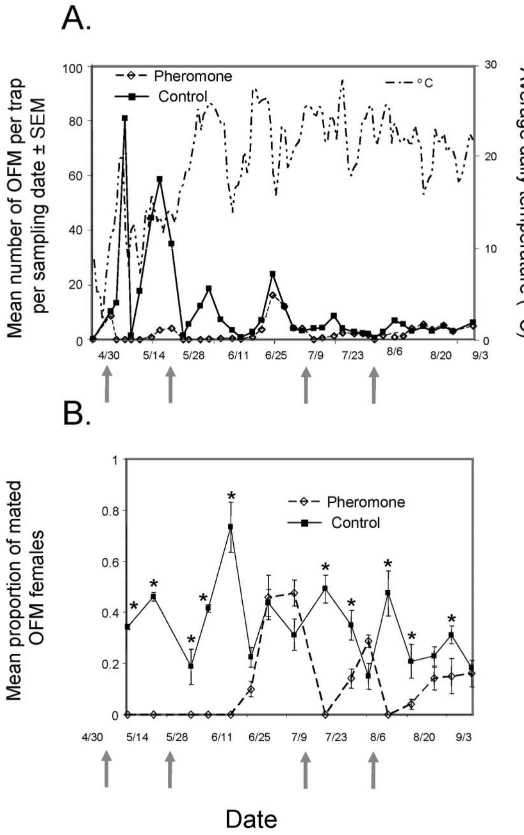

Fig. 2. (A) Mean captures of oriental fruit moth males per week throughout the 2005 season in replicated 0.4-ha pheromone-treated and control plots. Arrows indicate date of mechanized application of wax drops. Superimposed are average daily temperature values (°C) throughout the season. (B) Mean proportion of virgin female oriental fruit moth mating during 24 h of field deployment in plots receiving a mechanized application of wax dispensers of pheromone versus untreated control blocks. $n = 62–79$ females dissected per treatment over the season. Means accompanied by asterisk (*) are significantly different at $\alpha < 0.05$. Arrows indicate date of wax application.

was significantly ($F = 23.4$; df = 1, 3; $P < 0.01$) higher than in pheromone-treated plots (0.7 ± 0.1). Disruption was not significant ($F = 1.9$; df = 1, 3; $P = 0.9$) after 22 July (Fig. 2A). Eighty-eight percent disruption of moth catch was achieved for 13 d after a fourth application on 3 August (Fig. 2A); significantly ($F = 18.6$; df = 1, 3; $P < 0.01$) more moths were captured in control blocks (4.0 ± 0.5) than in pheromone-treated blocks (0.5 ± 0.1) during this interval.

Efficacy of mechanically applied wax dispensers was high and relatively long-lasting in the spring and early summer (11 May–16 June). High and low daily air temperatures during this interval averaged 23.8 ± 0.8 and 11.2 ± 0.8°C, respectively (Fig. 2A). However, during mid- to late summer when high and low daily temperatures averaged 27.4 ± 0.4 and 15.2 ± 0.6°C (Fig. 2A), respectively, disruption of traps lasted for ≈1 wk.

Disruption of Tethered Female Mating. Mating of tethered, virgin females was completely disrupted in pheromone-treated plots on five deployment dates between 11 May and 16 June (Fig. 2B). The mean proportion of mated females in control plots averaged 39.4 ± 8.8 (range 19–73%) during this period (Fig. 2B); this is considered reasonable given that, on average, 60% of females from our laboratory culture call during peak sexual activity (Stelinski et al. 2006). After the third application of wax drops in July, 100% disruption of female mating was recorded only on the first sampling date within 8 d of machine application of wax (until 20 July), and the proportion of mated females was significantly ($F = 16.7$; df = 1, 3; $P < 0.01$) reduced in pheromone-treated (0.14 ± 0.06) versus control (0.35 ± 0.1) plots until 28 July, 17 d after application of wax (Fig. 2B). In August, 100% disruption was recorded 7 d after the fourth application and the proportion of mated moths was significantly ($F = 28.2$; df = 1, 3; $P < 0.01$) reduced 15 d after application (until 18 August) in pheromone-treated plots (0.04 ± 0.01) compared with control plots (0.21 ± 0.08) (Fig. 2B).

Shoot Injury. Percentage of shoot injury in plots treated with mechanically applied wax (1.2 ± 0.4) was less than one-half of that quantified in control plots (2.5 ± 0.3) on 20–21 June; however, this difference was not significant ($P = 0.1$).

Discussion

Pheromone-based mating disruption is one of the most effective and broadly practiced pest control tactics serving as an alternative to broad-spectrum insecticides (Cardé and Minks 1995, Gut et al. 2004). However, pheromonal active ingredients are more costly per hectare than insecticides, efficacy of the technique is variable depending on factors such as pest density, and control is generally achieved through a combination of disruptant and supplemental insecticide sprays (Gut et al. 2004). Further adoption of mating disruption, especially as a stand-alone control tactic, will likely require nearly 100% efficacy, especially at high pest densities. In addition, mating disruption will need to be economically competitive.

Mating disruption has been more widely adopted in California, Oregon, and Washington (≈35,500 ha in 2004) than in eastern states, such as Michigan (≈2000 ha in 2005). The higher cost of the active ingredient and labor to apply the most commonly used dispenser technology, hand-applied reservoir devices, have been advanced as major obstacles to greater adoption of mating disruption (Thomson et al. 1998, Gut et al. 2004). Growers are reluctant to make the up-front investment in an expensive and risky technology, as is required if they are to successfully use hand-applied formulations.

Mechanically applied paraffin-wax technology could potentially increase adoption of mating disruption, given that less active ingredient may be required per hectare relative to that used with reservoir dispensers (Stelinski et al. 2005a), labor costs for application would be lower, and wax could be applied on an as-needed basis targeting key periods of adult activity (Gut et al. 2004). One operator using the current prototype applicator deployed paraffin-wax drops 3.4 times faster than three people hand-applying polyethylene-tube dispensers for oriental fruit moth (de Lame 2003). A dual-boom applicator for commercial use could realize a field deployment rate of six ha/h. The potential long-term savings in labor costs might require an estimated initial up-front investment of US$20,000 for an applicator of this type. This estimate is based on the costs for design, construction labor, and field evaluation. However, it also is envisioned that a distributor of a commercial wax-based formulation would invest in such an applicator providing a service to growers who purchase this type of disruption product. This is currently true of mechanically applied hollow fibers (Scentry Biologicals, Inc., Billings, MT) for mating disruption of codling moth, *Cydia pomonella* L., in Michigan (L.G., unpublished). A wax formulation patterned after the one described herein is currently under development for commercial application (ISCA Technologies, Riverside, CA). Development of mechanical application technologies for such a product could promote its adoption.

Paraffin-wax drops are considered "matrix-type" dispensers where the active ingredient is dispersed throughout a polymer matrix, and its release is governed by Fick's law (Fan and Singh 1989). Accordingly, the rate of release of an active ingredient is directly proportional to the area of the polymer matrix as well as the concentration gradient of active ingredient, and it is inversely proportional to the distance the active ingredient must travel through the matrix as well as the viscosity of the medium. Thus, multiple factors influence the release rate of oriental fruit moth pheromone from paraffin-wax drops. Additionally, increases in temperature can decrease matrix viscosity. If the free volume of amorphous space within the paraffin matrix increases, diffusion of active ingredient increases (Fan and Singh 1989). The ≈7°C higher daily temperatures in the summer than spring may have contributed to an elevated release rate of pheromone, reducing longevity of dispensers.

A higher surface area-to-volume ratio results in a higher release rate of pheromone per milligram of wax formulation (Atterholt 1996). The 0.04-ml drops dispensed by the mechanized applicator in the current study were smaller than the 0.1-ml drops for which we were aiming (Stelinski et al. 2005a). They were also flatter than the hemispherical 0.1-ml drops that we hand-applied in a previous study (Stelinski et al. 2005a). That study demonstrated that 0.1-ml wax drops were attractive to oriental fruit moth males and that increasing the density of drops per hectare increased the efficacy of mating disruption (Stelinski et al. 2005a). It is likely that the 0.04 ml, flat drops dispensed by the current version of the mechanized applicator lost efficacy after 1–2 wk in the summer heat, because the release rate of pheromone after this interval fell below that attractive to males. Photodegradation of pheromone also may have occurred at a faster rate in the flatter and smaller mechanically applied drops than in the larger drops dispensed by syringe in our previous study.

The current report demonstrates that speedy, mechanical application of paraffin-wax drop dispensers of pheromone is possible in tree fruit. The applicator was easily attached in minutes to a typical orchard tractor and used standard hydraulic remotes for operation. Cleanup of wax was easily accomplished by power-washing with water. Cleanout of the metering pump system and dispenser hub was simple, requiring only water. Cost of a retail commercial applicator is yet to be determined and will depend on how much remote control is built into the applicator; however, cost of materials for the current machine was ≈US$9,000. This type of applicator could be readily adopted for deployment of wax dispersers in other crops including from aircraft. Although the mechanically applied drops in the current study were highly effective upon deployment, the relatively short duration of efficacy in the second half of the growing season made these given wax deposits uneconomical, given that as many as eight applications would have been required per season to maintain >95% efficacy throughout. Efforts to improve the formulation and applicator for sustained efficacy are underway. These efforts include increasing wax viscosity, modifying the applicator to release larger drops at reduced impact velocities, and increasing the load of active ingredient per deposit.

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