Effects of Application Rate and Interval on the Efficacy of Sprayable Pheromone for Mating Disruption of the Oriental Fruit Moth *Grapholita molesta*

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The efficacy of microencapsulated sprayable pheromone was evaluated at different application rates and intervals for mating disruption of the oriental fruit moth, *Grapholita molesta* (Busck), in apple orchards during 2002. The following treatments were arranged in a randomized complete block design with three replications: (i) a low rate of pheromone (6.2 g a.i. ha^{-1}) applied at 14-day intervals, (ii) a medium rate of pheromone (12.4 g a.i. ha^{-1}) applied at 28-day intervals, (iii) a high rate of pheromone (24.7 g a.i. ha^{-1}) applied at 28- day intervals, and (iv) a non-pheromone control (insecticides only). The combination of a single insecticide application against first generation *G. molesta* at petal fall with one pheromone application each for the second, third and fourth generations at 12.4–24.7 g a.i. ha^{-1} successfully controlled low populations. Pheromone-treated blocks had significantly lower trap catches than those in the insecticide-treated control blocks. Among pheromone treatments, significantly more moths were caught in the 6.2 g compared with the 12.4 and 24.7 g rates. Fruit damage was $< 1\%$ at harvest and there were no significant differences among treatments. Low rate frequent applications of sprayable formulation appeared to be effective under low pest pressure but efficacy declined with increasing populations. Further studies are needed to demonstrate the effectiveness of this approach under higher pest pressure.
KEY WORDS: *Grapholita molesta* (Busck); oriental fruit moth; mating disruption; sprayable pheromone; integrated pest management; apples.

INTRODUCTION

The oriental fruit moth (OFM), *Grapholita molesta* (Busck), has long been a serious pest of peaches, with only sporadic infestations observed in apples throughout the world (19). However, it has risen to a key pest status on apples in the eastern USA in recent years and 464 loads of apples were rejected because of the presence of live OFM larvae within fruit in Pennsylvania in 2000 (11). The reason for the sudden rise of the OFM on apples is unknown, but the increased use of more narrow-spectrum insecticides due to Food Quality Protection Act restrictions by the United States Department of Agriculture and resistance to organophosphate insecticides may have contributed to the problem (17,24).

Four generations of OFM occur per year on apples grown in monoculture in western North Carolina (NC). OFM populations can sustain themselves in apples alone in NC,

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where there are no nearby stone fruit orchards or other fruit crops as alternative hosts (14). Chemical control of the OFM has been insufficiently effective in many locations because of the long and continuous presence of ovipositing OFM adults during August–September (2). Most insecticides do not provide the necessary residual activity to allow growers to spray later-maturing varieties in mid-August, and this is presumably the reason for the higher incidence of live larvae in apples later in the season. The increasing importance of late-season OFM damage on apple has increased interest in the use of alternative management tactics that have proved successful in controlling this pest in stone fruit.

Mating disruption, the release of large amounts of synthetic insect pheromone into the cropping environment to prevent or reduce sexual communication, has proven to be a viable alternative for control of many tortricid pests in a number of crops including codling moth, *Cydia pomonella* (L.) in pears (15) and apples (1); *Eupoecilia ambiguella* Hübner (25) and *Lobesia botrana* Den. & Schiff. in grapes (21); and OFM in peaches (19) and apples (14). Several types of pheromone-dispensing systems have been developed for mating disruption including hand-applied dispensers, paraffin emulsions, and microencapsulated sprayable formulations (12). Sprayable formulations consist of a blend of pheromone encapsulated in 15–150 μm polymer microcapsules that are suspended within a liquid carrier. Following the application of sprayable pheromone, these capsules adhere to the leaves and foliage. Once dried, the capsules begin emitting small amounts of pheromone throughout the tree canopy (26).

The efficacy of microencapsulated sprayable pheromone for mating disruption of OFM was first evaluated by Gentry *et al*. (9). Small-plot studies with microencapsulation formulation of 5 g OFM pheromone + 15 g dodecyl alcohol/ha contained within gelatin-based microcapsules (50–250 μm diameter) resulted in suppression of adult trap captures for 2 weeks. Later, water-based microcapsules (15–60 μm diameter) containing 20% of OFM pheromone and 80% inert ingredients such as stickers, spreaders and UV-protectants, were tested against the OFM to increase the longevity of sprayable pheromone in the field (10). Gut and Wise reported that improved sprayable formulations applied at a rate of 19–37 a.i. g ha^{-1} greatly inhibited moth capture in pheromone traps up to 3 weeks (10). Recently, sprayable pheromone products have been successfully used for the OFM and many other tortricid pests, including *Rhopobota naevana* (Hübner), *Sparganothis sulfureana* (Clemens), *Cydia pomonella* (L.) and *Endopiza viteana* (Clemens) (5,8,16,22,23).

The high cost of mating disruption relative to conventional insecticide management is a major impediment to its more widespread adoption (18). One way to reduce the cost of sprayable pheromone application is by using lower dosage rates. In addition, frequent applications of low rates of sprayable pheromone could improve the efficacy of mating disruption by maintaining high levels of pheromone titer throughout the adult flight period. However, little is known about the effects of application rates and intervals on the field performance of sprayable pheromone formulations. The objectives of this study were to (i) compare the efficacy of different rates of sprayable pheromone formulations, and (ii) evaluate the use of low rate frequent applications of sprayable pheromone for management of the OFM in apples.

MATERIALS AND METHODS

Study sites Large block trials were carried out in three commercial apple orchards in Henderson County, North Carolina (USA) in 2002. The experiment was conducted using a randomized complete block design with three replications (orchards). The Coston orchard was a 10.5-ha block of 'Golden Delicious' and 'Rome Beauty' apples, and was divided into a 4 ha conventional insecticide block, and three sprayable pheromone blocks each approximately 2–2.5 ha in size. The Dalton orchard was a 7.5-ha block of Golden Delicious apples that consisted of three adjacent blocks. Located approximately 500 m from these plots was an insecticide-treated block. Each treatment block was approximately 2.5 ha in size. The Marlowe orchard was a 10-ha block of Rome Beauty apples, and was divided into four plots ranging in size from 2 to 3.2 ha. One treatment (12.4 g rate) was separated from other treatments by a shrubbery area, composed mainly of kudzu *Pueraria montana* (Lour.) Merr. var. *lobata* (Willd.) Maesen and Almeida, eastern poison ivy *Toxicodendron radicans* (L.) Kuntze and false poison sumac *Rhus michauxii* Sarg.

Description of treatments The efficacy of OFM sprayable pheromone (Phase V, 3M Canada Company, London, ON, Canada) containing 18.6% Z-8-dodecen-1-yl acetate (Z8-12:Ac), 1.2% E-8-dodecen-1-yl acetate (E8-12:Ac), 0.2% Z-8-dodecen-1-ol (Z8-12:OH) and 80% inert ingredients was evaluated at different application rates and intervals. In each orchard, the following treatments were compared: *(a)* a low rate of pheromone (6.2 g a.i. ha^{-1}) applied at 14-day intervals; *(b)* a medium rate of pheromone (12.4 g a.i. ha^{-1}) applied at 28-day intervals; *(c)* a high rate of pheromone (24.7 g a.i. ha^{-1}) applied at 28-day intervals; and *(d)* a non-pheromone control (insecticides). All blocks were sprayed with carbaryl (Sevin 50WP, 80WPS and/or XLR, Aventis, Research Triangle Park, NC, USA) to control the first generation OFM larvae at petal fall (14). Sprayable pheromone was applied by grower cooperators with an airblast sprayer delivering 1000–1400 l ha^{-1} of water. For each pheromone treatment, the initial application of sprayable pheromone was made on 9 June in all orchards.

In sprayable pheromone-treated orchards, codling moth was managed with three applications of tebufenozide (Confirm 2F, Dow Agrosciences, Indianapolis, IN, USA), one application each in May, July and August. One tebufenozide application was made against each of the two tufted apple bud moth *Platynota idaeusalis* (Walker) generations, in June and mid August. Tebufenozide was used in sprayable pheromone treatments because it has relatively low toxicity to the OFM (2). Due to increasing populations of apple maggot *Rhagoletis pomonella* (Walsh) in sprayable pheromone blocks, two organophosphate insecticide applications using azinphos-methyl (Guthion 50WP, Bayer Crop Science, Research Triangle Park, NC, USA) and/or phosmet (Imidan 70WP, Gowan, Yuma, AZ, USA) were unavoidable: one in late June and one in early July.

A non-pheromone-treated conventional block was included at each site, and sprayed with five to seven applications of organophosphate insecticides using azinphos-methyl and/or phosmet for OFM, codling moth and apple maggot control, and tebufenozide for tufted apple bud moth control. Organophosphate insecticides were timed to coincide with egg laying periods of each of the three generations of codling moth (two applications for the first generation in May and early June, one or two applications for the second generation in July, and one application against the third generation in late August to early September). One tebufenozide application was made against each of the two tufted apple bud moth generations, in June and mid August.

Assessment of treatment efficacy The efficacy of treatments was evaluated by comparing captures of adult male OFM in pheromone-baited traps and by inspection of fruit for

OFM larval feeding injury at harvest.

The capture of moths in sex pheromone traps in pheromone-treated blocks relative to those captured in traps placed in insecticide-treated blocks was used as an indirect measure of the efficacy of the sprayable pheromone treatment. Wing-style pheromone traps (Pherocon 1C Trap, Trécé, Salinas, CA, USA) were used to monitor OFM populations in each treatment. For each treatment, traps were hung at a density of one trap per 0.4 ha, and each trap was placed in the upper third of the canopy based on the results of Kovanci (Ph.D. dissertation, 2003), who reported catching significantly more moths in traps placed within 0.5 m of the top of the canopy compared with those placed at eye level ($\sim$1.6 m). Rubber septa (Thomas Scientific, Swedesboro, NJ, USA) were loaded with OFM pheromone (100 μg, Bedoukian Research Inc., Danbury, CT, USA). A blend (95.5% purity) of pheromone consisting of 90.4% Z8-12:Ac, 6.1% E8-12:Ac, 1.1% Z8-12:OH, and 2.4% inert materials in 25 μl hexane was loaded on a septum (3). Pheromone lures were changed every 4 weeks. Traps were checked weekly from 4 June to 31 September. Trap bottoms were replaced as needed.

Fruit damage was assessed at harvest on 23 September. Three types of OFM damage were recognized: ‘sting’ represented surface blemishes caused by a complex of lepidopterous larvae, ‘entry’ represented larval tunneling into the fruit flesh, and ‘live larvae’ was used for fruit infested with a live larva. Within each treatment, fruit damage was evaluated by picking 100 fruit arbitrarily from each of ten trees per treatment. The trees were randomly selected and all together 80 trees were sampled, representing 1% of the total trees. All fruits were cut to check for the presence of internal lepidopterous damage. Larvae were collected and identified to species using morphological criteria (4). The larva of the *Grapholita* can be distinguished from the *Cydia* larva by the presence of an anal comb, which the *Cydia* larva is lacking. Of the two *Grapholita* species, lesser appleworm *Grapholita prunivora* (Walsh.) larva is known to retain pinkish pigment in the integument, whereas the body color of *G. molesta* larva is altered to whitish or creamy after the larvae are killed in boiling water and preserved in 70% ethyl alcohol.

Data analysis Pooled data were subjected to analysis of variance (ANOVA) (20). Data are presented as mean cumulative moth catches per trap, but counts were transformed to log (x+0.5) before ANOVA. If there were significant interaction effects, LSMEANS comparisons were used to identify these effects. Mean percentage fruit damage was transformed using arcsine square root and data were analyzed using ANOVA.

RESULTS

Pheromone trap catches Mean weekly catches per pheromone trap for each conventional insecticide block in the Coston, Dalton and Marlowe orchards are shown in Figure 1. Capture data from conventional insecticide blocks indicated that the second, third and fourth generation flight peaks of OFM adults occurred in late June, late July and late August, respectively. Based on pooled data from the three locations where studies were conducted, season total pheromone trap captures varied significantly with the rate of pheromone applied (F=8.35; df=3,24; P=0.02). At all rates of sprayable pheromone, significantly fewer moths were captured compared with the conventional treatment, and significantly fewer moths were caught in the 12.4 and 24.7 g rates compared with the 6.2 g rate (Table 1).

TABLE 1. Mean cumulative oriental fruit moth pheromone trap captures in apple blocks treated with 6.2, 12.4 and 24.7 g a.i. ha^{-1} of sprayable pheromone, and conventional organophosphate insecticides during the study period (Henderson County, NC, 2002)

Location	Pheromone rate	n	Moths per trap (±SEM)	% Reduction^x
Coston	6.2	4	2.0 (0.4)b	87
	12.4	4	4.3 (1.9)b	71
	24.7	4	1.3 (0.3)b	92
	Control^z	4	15.3 (0.8)a	-
Dalton	6.2	4	7.8 (2.6)b	87
	12.4	4	3.5 (1.4)c	94
	24.7	4	0.8 (0.5)c	99
	Control	4	61.0 (10.5)a	-
Marlowe	6.2	8	27.4 (7.1)a	0
	12.4	4	1.3 (0.8)b	90
	24.7	4	1.3 (0.8)b	90
	Control	4	12.8 (1.9)a	-
Pooled	6.2	16	16.1 (4.5)b	46
	12.4	12	3.0 (0.9)c	90
	24.7	12	1.1 (0.3)c	96
	Control	12	29.7 (7.4)a	-

^{z}Controls consisted of insecticide treatment.
^{y}Within the same location, means followed by the same letter do not differ significantly by Fisher's protected LSD test ($P<0.05$). Data were analyzed using log (x + 0.5), but data shown are back transformations.
^{x}Percentage reduction calculated using (C-T/C)×100, where C = mean cumulative trap catches in insecticide-treated control, and T = mean cumulative trap catches in treatment.

TABLE 2. Mean percentage (±SEM) fruit damage at harvest averaged across three different locations treated with 6.2, 12.4 and 24.7 g a.i. ha^{-1} of sprayable pheromone and conventional organophosphate insecticides (Henderson County, NC, 2002)^z

Pheromone rate	n	Sting	Entry	Live larvae
6.2	3	0.6 (0.3)	0.6 (0.3)	0.1 (0.1)
12.4	3	0.2 (0.1)	0.1 (0.0)	0.0 (0.0)
24.7	3	0.1 (0.1)	0.0 (0.0)	0.0 (0.0)
Control (insecticide)	3	0.8 (0.3)	3.0 (1.7)	0.6 (0.4)

^{z}Within columns, there were no significant differences according to Fisher's protected LSD test ($P<0.05$). Data were analyzed using arcsine square root, but data shown are back transformations.

Trap captures did not vary among locations (F=1.03; df=2,24; P=0.37) but there was a significant treatment x location interaction (F=4.14; df=6,24, $P<0.01$). At Coston, there were no differences among pheromone treatments. At the Marlowe and Dalton sites, trap captures differed significantly among pheromone treatments. At both locations, late season trap captures increased to significantly higher numbers in the 6.2 g rate compared with the 12.4 and 24.7 g rates.

Sprayable pheromone treatments resulted in reduced trap shutdown at all locations except for the 6.2 g treatment in Marlowe (Table 1). Treatment with the 6.2 g rate in Marlowe inhibited OFM captures through June but the pheromone treatments at this rate had no effect on OFM orientation to traps, with consistent moth captures during both the third and fourth generation flights. Overall, average inhibition of moth captures in pheromone-baited traps ranged from 46% to 96% in all sprayable pheromone-treated

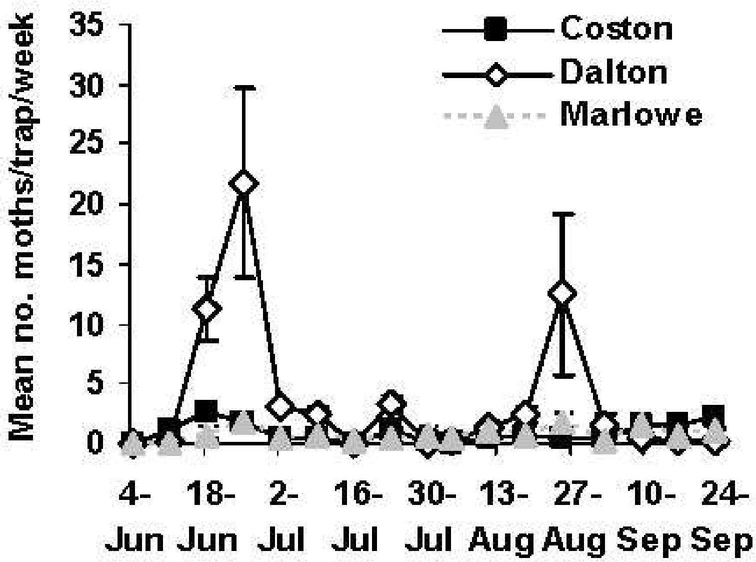

Fig. 1. Mean weekly (±SEM) oriental fruit moth pheromone trap captures averaged over four traps in each conventional insecticide block in the Coston, Dalton and Marlowe orchards (Henderson County, NC, USA) during the study period 2002.

blocks, indicating a low to high level of trap catch shutdown.

Fruit damage Damage in all categories was low in all treatments except for the number of entries in the conventional insecticide blocks (Table 2). Sprayable pheromone treatments were highly successful in minimizing insect damage and less than 0.7% of fruit exhibited larval entries and stings. The highest incidence of mean larval stings was found in the insecticide-treated blocks (0.8%), but no significant differences were detected among treatments (F=2.06, df=3,6, P=0.21). Similarly, the mean percentage of entries in the sprayable pheromone- and insecticide-treated blocks did not differ significantly (F=2.97, df=3,6, P=0.12). No larval entry damage or live larvae were found in the 24.7 g rate of sprayable pheromone, whereas the conventional treatment averaged 3.0% and 0.6% larval entries and fruit with live larvae, respectively. However, the mean percentage of infested fruits in sprayable pheromone blocks was not significantly different compared with that in conventional insecticide blocks (F=1.92, df=3,6, P=0.23). A total of four live OFM larvae were recovered in the 6.2 g rate of sprayable pheromone at Marlowe.

DISCUSSION

Our results showed clearly that the combination of a single insecticide application against first generation *G. molesta* at petal fall with one pheromone application each for the second, third and fourth generations at 12.4–24.7 g a.i. ha^{-1} successfully controlled low populations. Insecticide application is needed to reduce initial OFM populations in NC because first generation OFM has the largest moth population, reaching up to 250 moths/trap/week in unmanaged orchards (27).

Following the reduction of initial populations by an insecticide, medium and high rate pheromone applications at monthly intervals provided an average of, respectively, 90% and 96% inhibition of OFM moth catch in pheromone traps during subsequent pest generations. In contrast, low rate (6.2 g a.i. ha^{-1}), frequent applications had substantially weaker effects in disrupting communication (46%). However, low level of mating disruption was likely due to consistently increasing edge and interior trap captures during August–September at one location (Marlowe), suggesting an incomplete disruption with sprayable formulations, as reported previously by Trimble *et al.* (22). Incomplete disruption may be caused by the uneven release rates from microcapsules over time (13). Based on the electroantennogram measurements of sprayable pheromone of *Sesamia nonagrioides* Lefebvre, Koch *et al.* showed a very high initial pheromone release rate followed by an exponential decay in release to the antennal threshold levels after 8–10 days (13).

It is also important to note that moth captures were very low in insecticide-treated control blocks, which may have masked the level of trap shutdown in the field. This was particularly evident in the 6.2 g treatment at Marlowe, where no effects of mating disruption could be detected from pheromone trap catches. Unfortunately, it was not possible to compare mating disruption with an untreated control in this study because of economic constraints.

Grower adoption of mating disruption as a management strategy can be adversely affected by the relatively high cost of mating disruption compared with chemical control (14). Following the insecticide application at petal fall for first generation OFM, only one application of Isomate-M 100 dispensers in late May proved effective (14), while at least three applications were necessary with sprayable formulations to provide season-long control in North Carolina. Currently, Isomate M-100 costs approximately US$100 ha^{-1}, and an additional $15 ha^{-1} for application, whereas the cost of three applications of sprayable pheromone at 24.7g a.i. ha^{-1} totals $135 ha^{-1}, costing $45 ha^{-1} each (14). Because the 12.4 g rate was found to be as effective as 24.7 g a.i. ha^{-1}, the cost of a single application could potentially be decreased by 50%. This could not only significantly reduce the cost of mating disruption but also encourage the greater adoption of sprayable formulations.

In North Carolina, OFM sprayable pheromone is typically applied three times per season at rates ranging from 24.7 to 37.1 g a.i. ha^{-1}. Trap catches and fruit inspections in blocks treated with 24.7 g a.i. ha^{-1} indicate that the Phase V formulation of sprayable pheromone remained effective for 21 to 28 days. However, field trials conducted in Michigan and in other states suggest that high rates are not the most effective or economical way to use sprayable pheromones (6,7). The effectiveness of the pheromone treatment was shown to be correlated with rain wash-off of the microcapsules that contain the pheromone (26).

A more economical alternative to high rate applications of sprayable formulations might be the frequent application of sprayable pheromone at a low rate. Under a low-rate, frequent-application program, OFM sprayable pheromone is currently labeled at 5–10 g a.i. ha^{-1} on a spray schedule of every 7–10 days during the flight period. Based on our results, low-rate, frequent applications (6.2 g a.i. ha^{-1}) of sprayable pheromone were effective in suppressing trap catches under low OFM pressure at Coston for up to 14 days, but efficacy declined with increasing population densities encountered at the Dalton and Marlowe orchards.

Although cumulative moth catches in insecticide-treated blocks at the Dalton orchard (61.0 moths/trap) were higher than those at Marlowe (12.8 moths/trap), the 6.2 g rate of sprayable pheromone appeared to perform better at the Dalton than at the Marlowe site. Environmental variables such as precipitation, temperature and wind velocity can all affect the pheromone release rates and aerial concentrations, and differences in these variations among study sites may account for the efficacy differences. In addition, temperature, rainfall and sunlight can impact the adherence of microencapsulated pheromone to trees (26). The late season increase in trap captures observed in the sprayable pheromone treatments may also be related to the adherence of microcapsules to older leaves. Waldstein and Gut showed that there was a greater propensity of microcapsules to adhere to branches and immature apple foliage compared with mature foliage (26).

The results of large plot trials demonstrated that OFM mating disruption with sprayable formulations was successful in managing low populations of this insect when combined with chemical control of the first generation. The use of mating disruption reduced the use of conventional insecticides considerably by eliminating the four or five organophosphate applications per season. This study showed clearly that sprayable pheromones offer growers considerable flexibility in the rate and timing of application when tailoring mating disruption programs. However, further studies are needed to demonstrate the effectiveness of sprayable formulations under higher pest pressure.

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