Effects of Pheromone Loading, Dispenser Age, and Trap Height on Pheromone Trap Catches of the Oriental Fruit Moth in Apple Orchards

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The effects of field aging (0–28 days) and pheromone loading rate on the longevity of red rubber septa loaded with the sex pheromone blend of the oriental fruit moth *Grapholita molesta (*Busck), were evaluated in North Carolina apple orchards in 2002. Separate field tests examined the influence of trap height and pheromone loading rate of rubber septa on trap catches of adult *G. molesta* males in an abandoned orchard. The loss of the major pheromone component, (Z)-8-dodecenyl acetate (Z8-12:OAc), from red rubber septa over a 4-week period exhibited a relatively constant release rate with 30, 100 and 300 μg pheromone. Trap catch was significantly higher in pheromone traps placed in the upper canopy than in those in the lower canopy. Pheromone traps baited with 100-μg lures caught more moths compared with those loaded with 300 μg. There was no apparent relationship between pheromone trap catch and septa age, with trap catch appearing to be primarily a function of *G. molesta* population density.

KEY WORDS: *Grapholita molesta;* (Z)-8-dodecenyl acetate; apple; red rubber septa; release rate; septa dose; trap placement.

INTRODUCTION

The Oriental fruit moth (OFM) *Grapholita molesta* (Busck) is a key pest of stone fruit throughout the world (32). It has recently become problematic in apple (*Malus domestica* Borkh.) orchards in the eastern USA, especially in areas where peach and apple orchards are located in close proximity or in areas free of stone fruit orchards (13,19). In North Carolina (NC), OFM completes four generations on apples grown in monoculture (18) and it has become even more damaging than the codling moth *Cydia pomonella* (L.) (20). The presence and emergence of adult OFM males in apple orchards can be detected by using pheromone traps baited with a four-component OFM sex pheromone, consisting of (Z)-8-dodecenyl acetate (Z8-12:OAc), (E)-8-dodecenyl acetate (E8-12:OAc), (Z)-8-dodecen-1-ol (Z8-12:OH), and dodecan-1-ol (12:0H) (6). Trap catches have also been used for timing insecticide applications, and assessing the efficacy of OFM mating disruption (25,26,30).

Pheromone lures used in monitoring traps ideally should release an effective dose of pheromone at a constant rate throughout the trapping period (38,39). Controlled-release

Received July 2, 2005; accepted Dec. 7, 2005; http://www.phytoparasitica.org posting May 14, 2006.
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pheromone lures have included cotton dental wicks, rubber septa, polyethylene vials, laminates, hollow fibers, membranes, and polymeric systems (13). Rubber septa have been the most widely used substrate for the controlled release of many insect sex pheromones (5,40). Flint *et al.* (10) showed that rubber septa were an excellent substrate for the controlled release of gossyplure for pink bollworm *Pectinophora gossypiella* (Saunders), and rubber septa baited with codlemone have been the most common substrate for the codling moth (27).

The selection of an appropriate dose of pheromone in lures is also critical for successful monitoring. Unlike many moth species, OFM is not attracted to high concentrations of pheromone (7), and Roelofs *et al.* (29) reported that pheromone traps using rubber septa baited with 200 μg of OFM pheromone caught significantly more males than traps baited with 1, 10 or 1000 μg in peach orchards. Complete arrest of male flight at doses of 1000 μg has been observed (3,33). Therefore, high loading of OFM lures to extend longevity is not an option. However, although studies were conducted to evaluate the dose response of OFM males to lures baited with various amounts of sex pheromone in peaches, no such information is available for populations of OFM in apple.

The longevity of field-aged red rubber septa depends on their pheromone release rate and the initial loading (9,21). Maitlen *et al.* (21) showed that codlemone exhibited a first-order release rate from rubber septa, where initially a high amount of codlemone was released followed by a gradual decline in the release rate over time. Based on their calculations, they reported that a 1-mg lure should provide optimal attraction for codling moth for up to 4 weeks, whereas a 5-mg lure would remain effective for more than 4 months. Yet, Kehat *et al.* (14) showed that both the release rate and the attractiveness of 1-mg lures declined rapidly after 2 weeks in the field. This apparent inconsistency was attributed to the effects of high summer temperatures.

For reliable OFM monitoring, pheromone lures should be replaced regularly, usually every 4 weeks (23,28,35,37). However, little information is available on factors influencing the longevity of field-aged rubber septa. Using pheromone lures without a full understanding of how the initial pheromone dose and dispenser age may influence the attractiveness of lures could lead to unreliable trap catches (17,36).

The objective of this study was to determine the effects of field aging and initial loading on the release rate and longevity of OFM pheromone from red rubber septa. In addition, field tests were conducted to determine the attractiveness of traps that were baited with different amounts of OFM pheromone and placed at different heights within apple orchards.

MATERIALS AND METHODS

Preparation of lures Red rubber septa (no. 1780J07, Thomas Scientific, Swedesboro, NJ, USA) were used in all experiments. Septa were first ultrasonicated for 6 h in hexane, and then extracted three times with hexane for a total of 24 h, and air-dried in a fume hood for 48 h before loading. This procedure resulted in clean septa that later could be extracted for analysis of pheromone. Septa were impregnated with 30, 100 or 300 μg of OFM pheromone (95.5% pure; Bedoukian Research Inc., Danbury, CT, USA), which consisted of 90.4% Z8-12:OAc, 6.1% E8-12:OAc, 1.1% Z8-12:OH, and 2.4% inert materials, in 25 μl hexane. Hexane (50 μl) was added to aid in the penetration of pheromone into the rubber. Septa were stored at –20°C until used in experiments.

Evaluation of field-aged septa To determine the release rate of pheromone from rubber septa aged in the field, lures loaded with different amounts of pheromone were placed in the field. Pre-extracted pheromone-loaded septa were pinned with brass safety pins (size 00) and attached 1 cm apart to wire hangers. Hangers were deployed at a height of 1.7 m in an apple orchard at the Mountain Horticultural Crops Research Station (Fletcher, NC, USA) on 11 June 2002 for an early season trial, and on 23 August for a late-season trial. Septa were exposed to natural weathering. In the early season trial, a total of 42 septa of each dose were placed in the orchard; seven septa per dose (30, 100 and 300 μg) were removed 0, 1, 7, 14, 21 and 28 days after placement in the orchard. For the late-season trial, which included 36 septa, six septa per dose were removed 0, 1, 7, 14, 21 and 28 days after placement in the orchard. After removal from the field, septa were stored at –20°C until pheromone was extracted.

To extract pheromone, each septum was individually subjected to intermittent vortexing for 90 min in a glass vial containing 3 ml of hexane and 30 μg of hexadecyl acetate (16:OAc), the latter used as an internal standard. The septum was extracted again with 3 ml hexane with shaking for 90 min. Both extracts were combined and capped with a Teflon-lined cap, and stored at –20°C. This combined extract recovered 97.95$\pm$0.54% of the loaded Z8-12:OAc. For chemical analyses, the volume of each sample was reduced under a gentle N_2 stream to $\sim$1 or 2 ml (depending on initial dose, resulting in approximately the same amount of pheromone in each sample) and $\sim$1 μl was injected into a gas chromatograph (GC). Analyses were conducted on a HP5890 Series II GC (Agilent, Palo Alto, CA, USA) equipped with a splitless injector (250°C) and a flame-ionization detector (250°C) and interfaced with a ChemStation. The column (HP-5, 30 m $\times$ 0.32 mm $\times$ 0.25 μm) was operated at 50°C for 2 min, and the oven temperature was increased 20°C per min to 245°C and held for 5 min. Helium was used as the carrier at a flow rate of 30 cm sec^{-1}.

Field trapping study To determine the response of OFM male moths to red rubber septa loaded with 100 and 300 μg of pheromone, a trapping study was conducted in a 2-ha abandoned orchard in Henderson County, NC, USA, in 2002. A total of 16 Scentry wing traps (Scentry Biologicals, Billings, MT, USA) were placed in the orchard in a randomized complete block design with four replications: eight traps were placed in the lower and eight in the upper third of the canopy. Four traps at each height were baited with septa loaded with 100 μg and four with 300 μg pheromone. The low traps were placed on the periphery of trees at eye level ($\sim$1.6 m), whereas high traps were hung within 0.5 m of the top of the canopy, often near the trunk of a tree. Traps were placed 50 m apart. They were monitored weekly from 11 June to 7 July in the early season trial, and from 16 August to 13 September in the late season trial. Traps were rotated among trees each week so that each trap appeared at each tree for a one-week period.

Data analysis Pheromone extraction data were analyzed using a 2 (season) x 3 (dose) x 6 (field age) factorial experiment. The effects of pheromone dose and field age during the two trial periods were tested with analysis of variance (ANOVA; 34). Fisher's protected LSD test was used for mean separation (P=0.05). Exponential and linear regression analysis was used to fit the release of pheromone from septa over time. The average daily release rate of Z8-12:OAc from lures during each time interval was estimated by calculating the difference in mean sex pheromone content at the beginning and end of successive sample periods, and dividing by the number of days between sample periods.

TABLE 1. Oriental fruit moth captures (mean ± SEM) in pheromone traps baited with 100 or 300 μg pheromone, and traps placed in the upper and lower canopy in the early and late season trials (Henderson County, NC, USA; 2002)

Trial	Septa load (μg)	Trap height	Moths per trap[z]			
			Week 1	Week 2	Week 3	Week 4
Early season	100	Low	0.8 (0.3)a	2.3 (0.6)a	1.0 (0.4)a	0.3 (0.3)a
		High	3.0 (0.9)a	2.8 (1.3)a	1.5 (0.9)a	1.5 (1.0)a
	300	Low	1.5 (0.5)a	1.0 (0.4)a	0.0 (0.0)a	1.0 (0.6)a
		High	0.8 (0.3)a	1.5 (0.6)a	2.0 (0.4)a	0.8 (0.5)a
Late season	100	Low	2.3 (0.5)a	4.0 (0.8)a	2.5 (1.3)a	4.3 (2.0)a
		High	3.5 (1.2)b	7.0 (0.9)b	4.3 (1.6)ab	9.3 (3.1)a
	300	Low	1.3 (0.3)b	2.5 (0.5)ab	2.0 (1.4)b	5.3 (2.4)a
		High	2.5 (0.6)b	2.8 (0.8)b	3.3 (0.5)ab	7.3 (2.1)a

[z] Within rows, means followed by a common letter do not differ significantly according to Fisher's protected LSD test (P<0.05). Data were analyzed using log (x + 0.5), but data shown are back transformations.

Captures in pheromone traps over the 4-week trapping period for the early- and late-season trials were analyzed using ANOVA. Based on inspection of plots of residuals, data were transformed using log (x + 0.5) before ANOVA, but data are presented as back transformations. Fisher's Protected LSD test was used to compare treatment means (P=0.05). If there were significant interaction effects, LSMEANS comparisons were used to identify these effects (34). Because there was a significant interaction effect between season and field aging on trap catch, the results for field aging effects were presented and analyzed separately for each season.

RESULTS

Pheromone release from septa The release of pheromone from septa loaded with 30, 100 and 300 μg of the three-component OFM pheromone (27.1, 90.4 and 271.2 μg Z8-12:OAc) was best described by linear regressions in both the early- (Fig. 1A) and late-season trials (Fig. 1B). The equations were similar for the two trials (for 30 μg lures: y=38.7–0.62x [R^2=0.73] in the early season and y=37.2–0.66x [R^2=0.82] in the late season; for 100 μg lures: y=121.8–2.36x [R^2=0.87] in the early season and y=114.6–1.99x [R^2=0.82] in the late season; for 300 μg lures: y=310.3–4.32x [R^2=0.62] in the early season and y=295.1–4.93x [R^2=0.74] in the late season). Applying an exponential decay function to these data failed to improve the fit for 30 μg and 100 μg, and only improved the R^2 for late-season septa loaded with 300 μg to 0.76. Therefore, these data indicate relatively constant release rates from all lures in both early- and late-season trials.

As for total pheromone released over each season, the release rates from the 30, 100 and 300 μg lures also did not differ significantly between seasons (F=0.11; df=1, 110; P=0.74). The differences in the release rate per day (slope of the linear regression, Fig. 1) between the early and late season were 6.5%, 18.5% and 12.5% for the 30, 100 and 300 μg lures, respectively. We detected no significant interactions between lure dose and field aging on release rate (F=0.79; df=8, 110; P=0.61).

Field trapping study Total OFM captures were significantly higher in the late-season compared with the early-season trial (F=61.30; df=1, 97; P<0.01). The effect of field aging (1–4 weeks) was not significant averaged over seasons (F=2.25; df=1, 97; P=0.09). However, there was a significant interaction between season and field aging (F=5.36; df=3,

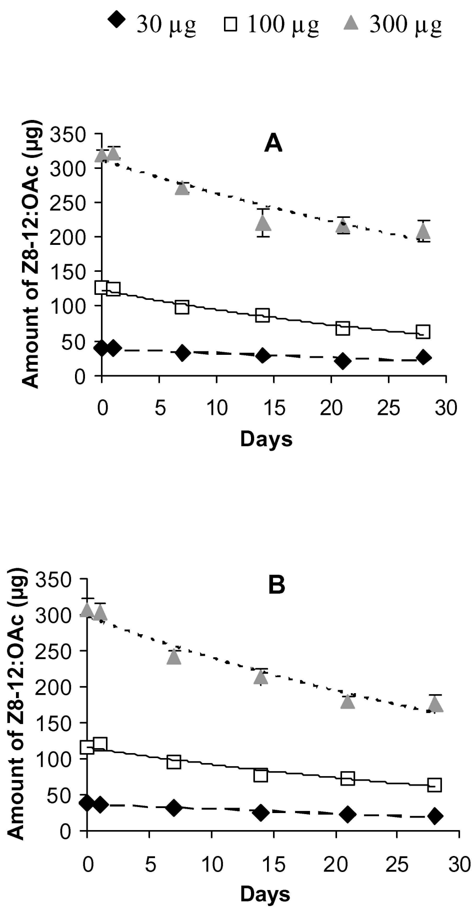

Fig. 1. Loss of Z8-12:OAc in the field from red rubber septa loaded with 30, 100 and 300 μg OFM pheromone (A) in the early season and (B) in the late season in Henderson County, in 2002. Means and SEM are offset from their respective data points for clarity. Linear regressions are drawn through the raw data, not through the means.

97; $P<0.01$). When analyzed separately for each season (Table 1), there was no significant effect of lure age on trap catch in the early season (F=2.21; df=3, 36; P=0.10). In contrast, lure age had a significant effect in the late season (F=6.13; df=3, 36; $P<0.01$). Significantly more moths were captured after 4 weeks of lure placement compared with those after one and 3 weeks when averaged over trap height and pheromone load.

Trap height (F=14.35; df=1, 97; $P<0.01$) and pheromone dose in septa (F=4.73; df=1, 97; P=0.03) were both significant factors affecting moth catch in pheromone traps. Total moth capture was significantly higher in pheromone traps placed in the upper *vs* lower canopy when averaged across doses and seasons (Fig. 2A). The height effect was consistent over seasons, whereas the height x season interaction was not significant (F=0.43; df=1,

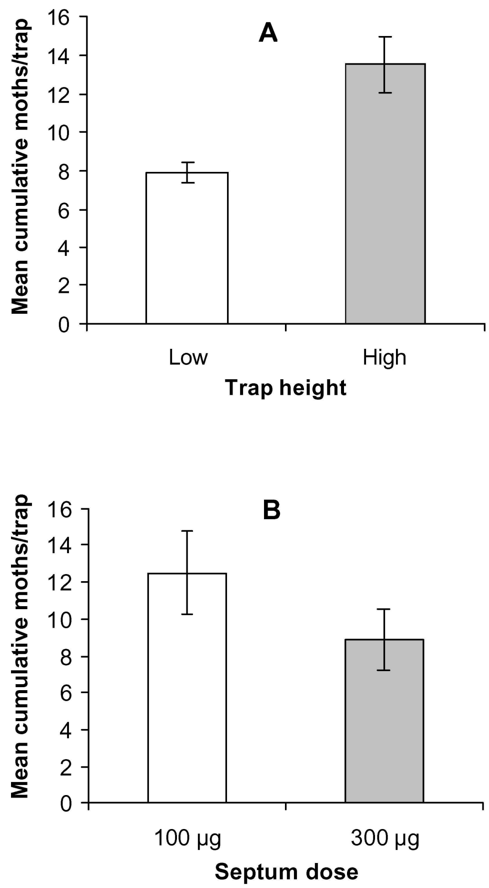

Fig. 2. Mean ($\pm$ SEM) cumulative oriental fruit moth catches in (A) pheromone traps placed in the lower and upper canopy averaged across doses and seasons and (B) pheromone traps baited with 100 or 300 μg pheromone per septum averaged across trap heights and seasons in Henderson County abandoned orchard in 2002.

97; *P*=0.51). Pheromone traps baited with 100 μg septa lures caught more total moths compared with 300 μg averaged over both heights and seasons (Fig. 2B), and this difference between doses was significant (F=14.26; df=1, 97; P<0.01). The septa dose x season interaction was not significant (F=0.04; df=1, 97; P=0.85).

DISCUSSION

Our results showed that rubber septa loaded with a three-component OFM pheromone blend released pheromone in an approximately linear fashion over a 4-week period (Fig. 1). Nevertheless, slightly higher rates of pheromone release from septa lures generally occurred during the first 2 weeks. This was most evident among septa loaded with different amounts of pheromone. For example, between days 1 and 7, the average release rates from 30, 100 and 300 μg lures (averaged across trial dates), respectively, were 1.0, 3.2 and 8.0 μg/day (3.7%, 3.5% and 2.9% of loaded dose), whereas they were similar between days 21 and 28, being 0.2, 0.9 and 0.8 μg/day (0.7%, 0.9% and 0.3% of loaded dose). Similarly, the pheromone release from rubber septa loaded with 0.2–20 mg of the honeydew moth *Cryptoblabes gnidiella* (Millière) pheromone ((Z)-11-hexadecenal) was shown to be initially constant for 18 days, and then gradually declined (1). Conversely, Gut and Brunner (11) and Kehat *et al.* (14) demonstrated a rapid decline in the release rates of pheromone lures for codling moth within 2 weeks, but this rapid decline was attributed to the chemical instability of codlemone, a conjugated diene alcohol (4).

Pheromone release from septa lures is generally expected to be higher with increasing temperature (24). However, in our trials ambient temperature was probably not a factor in OFM pheromone release from septa, because temperatures were similar during the two study periods: average daily temperatures were 21.2°C and 20.3°C during the early and late season trials, respectively.

During the early season trial, the highest moth counts averaged over the two trap heights for each pheromone load occurred when lures were 2 weeks old, a period of time when pheromone release from lures was highest, based on extractions and gas chromatography. Whether this response was due to septum performance, changing ambient temperature or declining OFM populations is unknown. Some studies have reported a decreasing trend in trap catches of tortricid pests including codling moth and European vine moth *Lobesia botrana* Den. & Schiff. as the septum age increased (2,14,17), whereas others found no relationship between septa age and moth capture (15).

Results of the late season trial, when total OFM captures were significantly higher compared with the early season trial, suggest that moth response did not decline in response to lure age. During the late season trial there was a trend of increased moth capture with lure age. In fact, the highest moth captures were recorded in traps when lures were 4 weeks old for each pheromone load. This was likely due to increasing OFM populations later in the season rather than a positive response of moths to aging lures, although these effects could not be separated in this study. Based on pheromone extraction studies, septa were releasing $\sim$1 μg/day during this time.

The relative efficiency of pheromone traps depends on factors such as proper placement of traps (22). According to our results, OFM trap catches were consistently higher in pheromone traps placed in the upper canopy. This finding supports the earlier reports by Rothschild and Minks (31) in peach orchards that the variation in OFM adult catches with canopy height stemmed from the mating activity of moths in the upper canopy. An increasing catch trend in the upper canopy has also been observed for codling moth males in both pheromone-treated and non-treated orchards (8,16). However, although high load codlemone lures (10 mg) were shown to outperform low load lures (1 mg) (8), high load OFM lures (300 μg) did not improve the catching efficiency of traps. On the contrary, traps loaded with 100 μg of OFM pheromone captured a greater number of moths than 300 μg lures in this study, as reported previously by Baker *et al.* (3).

In conclusion, red rubber septa loaded with 100 μg and 300 μg OFM pheromone effectively monitored OFM over a 4-week period in apple orchards. Current recommendations are to replace OFM lures every 4 weeks (23,28,37). Based on release rates, which were determined to be $<$1.2 μg/day between days 21 and 28 in this study, pheromone lures are likely to remain effective for at least 6 weeks. However, further field studies will be required to confirm these estimates.

ACKNOWLEDGMENTS

We thank Charles Thayer, Dorit Eliyahu and Steve Schoof for technical assistance, and Cavell Brownie (NC State University) for statistical advice. This research was supported in part by USDA-CSREEC Grant no. 00-34381-9567, the Gerber Products Company, and the North Carolina Agricultural Research Service.

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