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Environmental Entomology
Section: Population Ecology

Disruption of Sexual Communication of Oriental Beetles (Coleoptera: Scarabaeidae) in Highbush Blueberries with Retrievable Pheromone Sources

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ABSTRACT

The feasibility of disrupting sexual communication in oriental beetle, *Exomala orientalis* (Waterhouse), was evaluated by placing 50-75 ChemTica dispensers per ha (50-75 g active ingredient per ha) releasing (*Z*)-7-tetradecen-2-one, the major sex pheromone component of oriental beetle, or red rubber septa deployed at the same density, but loaded with only 5-7.5 g active ingredient per ha in 2002 and 2003. In both years, pheromone trap captures in plots treated with dispensers and rubber septa were significantly lower compared with pheromone trap captures in untreated control plots. Significantly fewer grubs were found in 2002 in blueberry plants placed in the two disrupter treatments compared to untreated control plots. In 2003, fewer females recovered from disrupter plots were found mated compared to females placed in untreated control plot. These studies indicate that sexual communication in oriental beetle can be disrupted by deploying retrievable dispensers releasing high rates of pheromone at lower densities than previously thought.

Keywords: mating disruption, *Exomala orientalis*, (Z)-7-tetradecen-2-one

The oriental beetle, *Exomala orientalis* (Waterhouse) is a major pest of blueberries, ornamental nurseries, and turfgrass in the northeastern United States (Polavarapu 1996, Polavarapu et al. 2002). In New Jersey, the oriental beetle is the most important white grub species infesting blueberries and nursery stock, followed by the Asiatic garden beetle, and the Japanese beetle (Polavarapu 1996). The range of this non-native beetle has expanded from shipment of infested nursery stock (Alm et al. 1999).

The 1-yr life cycle of the oriental beetle is similar to Japanese beetle. In New Jersey, adults start to emerge in the middle of June, reaching a peak in early July (Polavarapu 1996). After mating, females lay eggs singly in the soil, which hatch after several weeks (Vittum et al. 1999). Most grubs reach first and second instars by the end of August, and third instars by the middle of September (Polavarapu 1996). Grubs move downwards in the soil as winter temperatures drop and remain here throughout the winter. In early spring, as soil temperatures increase, grubs start to move upwards, resume feeding, enter the prepupal stage beginning the third week of May, and emerge in June (Polavarapu 1996, Polavarapu et al. 2002).

The sex pheromone was isolated independently from Japanese (Leal 1993, Leal et al. 1994) and North American populations of oriental beetle and has been identified as a 9:1 blend of (*Z*)- and (*E*)-7-tetradecen-2-one (Zhang et al. 1994). Sex pheromone-mediated mate acquisition and copulation was found to occur at or near soil surface, shortly after emergence, close to the emergence site (Facundo et al. 1999). This pheromone has been used to determine the distribution of oriental beetle in the United States (Alm et al. 1999).

The development of sprayable microencapsulated formulations (3M Canada, London, Canada) has facilitated distribution of oriental beetle pheromone near the soil surface and further improved prospects for commercialization of this technology (Polavarapu et al. 2001). Previous studies have indicated that percentage of reduction in trap captures (Disruption Index) in plots treated with the 9:1 blend of (*Z*)- and (*E*)-7-tetradecen-2-one was comparable to reductions in trap captures among plots treated with only (*Z*)-7-tetradecen-2-one, the major component, at the same rates (Polavarapu et al. 2002). Using sustained-flight tunnel assays Zhang et al. (1994) found no differences between the blend and major component in eliciting male activation, upwind flight, and source contact. Facundo et al. (1994) found similar numbers of beetles captured in traps baited with the blend and major pheromone component. Polavarapu et al. (2002) stated that the cost of synthesis of 93:7 blend is significantly lower compared with pure (Z)-7-tetradecen-2-one (>99% purity), as such it is this blend that is used in our study. The 93:7 blend was found to be within the range of blend variability in natural populations of oriental beetle (Zhang et al. 1994).

The sex pheromone of the oriental beetle, being a ketone, does not qualify for EPA tolerance exemptions on food crops unlike the other arthropod pheromones containing acetate, alcohol, and aldehyde moieties. However, arthropod pheromones formulated in retrievable polymeric dispensers have been granted tolerance exemption for use on food and non-food crops (see Weatherston and Minks 1995).

The objective of this study was to compare the potential of retrievable reservoir-type plastic dispensers and red rubber septa in disrupting pheromone-mediated communication of oriental beetle in highbush blueberries.

Materials and Methods

Mating Disruption, 2002. Experimental trials were conducted in 0.8 ha plots of highbush blueberry in Hammonton, NJ. Two replicates were located on Variety Farms Inc. and one on Atlantic Blueberry Company. Untreated control and pheromone-treated plots were separated by a minimum of 100 m, and were downwind from the untreated control plots. All experimental plots received the usual pesticide and fertilizer applications throughout the season, as part of a routine crop management program.

Plots contained either plastic dispensers (ChemTica Internacional TA, San Jose, Costa Rica) or red rubber septa (Thomas Scientific, Swedesboro, NJ) loaded with 1 and 0.1 g, respectively of the oriental beetle sex pheromone (93:7 blend of (Z)- and (E)-7-tetradecen-2-one; Bedoukian Research Inc., Danbury, CT), respectively. Plastic dispensers and red rubber septa were placed in plots on 10 June and deployed at a rate of 75/ha (Fig. 1a). Both types of disrupters were hung from poles 20 cm above the soil surface. Populations of oriental beetles were assessed using four Japanese beetle traps (Great Lakes IPM, Vestaburg, MI) per plot, each baited with 300 µg of (*Z*)-7-tetradecen-2-one. Traps were on hung from poles so the trap bottom was touching the ground. Traps were checked at weekly intervals until 5 August. Pheromone lures in traps were replaced after 3-4 weeks. Three replicates were completed.

Disruption Assessment with Virgin Females, 2002. A procedure similar to that developed by Polavarapu et al. (2002) was used to estimate the effectiveness of mating disruption treatments. This assay was necessary because many of the fields had been treated with insecticides and it avoided destructive sampling of bushes on commercial farms.

Oriental beetle larvae were collected from Rutgers University Research Farm, Adelphia, NJ and maintained individually in plastic cylinders (3 cm in height, 5 cm in diameter), which contained Pro-mix BX (Premier Horticulture Inc., Quakertown, PA) and perennial rye grass. After eclosion adult beetles were sexed. Trials were conducted at two of the three sites used in 2002 (one at Atlantic Blueberry Company and one at Variety Farms Inc.). For each treatment, ten virgin females were placed in each of five pots (33 cm in diameter; 25 cm in height), containing a 2-3 yr-old blueberry plant. Pots were located near the center of the plot (Fig. 1a). Females were tethered to the plant using a loop of 5 pound test fishing line (Stren, Madison, NC) carefully tied between the thorax and the abdomen of the beetle. There was 25 cm of line between the plant and beetle. Pots with tethered beetles were placed in the field at 1500 h on 27 June. Pots were retrieved on 5 September and numbers of oriental beetle grubs in each pot were determined by destructive sampling. (No behavioral observations were made of tethered females.)

Mating Disruption, 2003. Experimental trials were similar to those in 2002. Trials were conducted in 0.8 ha plots of highbush blueberry. Two replicates were located on Variety Farms Inc., Hammonton, NJ; one on Emery's Blueberry Acres, Tinton Falls, NJ; and one on Emery's Blueberries, New Egypt, NJ. Untreated control and pheromone-treated plots were separated by a minimum of 100 m, and were downwind from the untreated control plots. All experimental plots received the usual pesticide and fertilizer applications throughout the season, as part of a routine crop management program.

Treatment plots contained either plastic dispensers or red rubber septa loaded with 1 and 0.1 g, respectively of the oriental beetle sex pheromone (93:7 blend of (Z)- and (E)-7-tetradecen-2-one; Bedoukian Research Inc.). Plastic dispensers and red rubber septa were placed in plots on

2 July and deployed at a rate of 50/ha (Fig. 1b). Both types of disrupters were hung from poles 20 cm above the soil surface. Populations of oriental beetles were assessed using four Japanese beetle traps per plot, each baited with 300 μg of (Z)-7-tetradecen-2-one. Traps were hung on 29 June, and after an initial 4 d pre-treatment check, were inspected at weekly intervals until 14 August. Pheromone lures in traps were replaced after 3-4 weeks.

Disruption Assessment with Virgin Females, 2003. A new method was used to assess mating disruption, because the tethered female procedure was labor intensive and did not give information specific to each female. Oriental beetle larvae were collected from Medford, NJ and maintained individually in plastic cylinders (30 ml), which contained Pro-mix BX and perennial rye grass. After eclosion, 120 female beetles were selected for trials that were performed at one site (Atlantic Blueberry Company). In each treatment, 8 virgin females were placed in each of five plastic pots (18 cm in diameter, 10 cm in height), containing a peat/vermiculite soil mixture (Fig. 1b). A wire mesh (0.7 cm grid; 20 cm x 20 cm) was placed on top of the pot, which limited females from escaping by flight and allowed males to enter and mate with females. Beetles were placed in pots at 1400 h. After 48 hr, adult females remaining in the pot were collected and maintained individually in 30 ml cups with moist sand. Oviposited eggs were monitored for one month to determine egg hatch.

Statistics. Analysis of variance (ANOVA) tests were used to compare square root-transformed ($x + 0.5$) data for numbers of beetles in traps for both years, and numbers of grubs in 2002. Means were separated using Fisher's least significant difference (LSD) tests (SAS Institute 1999). Chi-square tests were used to analyze the number of grubs in 2003 trial. To assess reduction in trap captures, a disruption index (DI) was calculated using DI = (C-

T)/C)*100, where C = average beetle captures per trap in control plot, T = average beetle captures per trap in treatment plot.

Results

Mating Disruption, 2002. The number of beetles captured before disrupters were placed was not significantly different among treatments ($F = 1.10$; df = 2, 6; $P = 0.392$; Table 1). The presence of disrupters resulted in traps placed in treatment plots capturing significantly lower numbers of males than the control ($F = 24.93$; df = 2, 6; $P = 0.001$). There were no significant differences between the types of disrupters. In less than one week after disrupter deployment there was a substantial decrease in the numbers of male beetles captured (Fig. 2a). The disruption index was greater than 95% for both types of disrupters.

Disruption Assessment with Virgin Females, 2002. Treatments significantly affected the numbers of grubs that were found in potted plants, with the two types of disrupters having lower numbers of grubs than the control. ($F = 6.36$; df = 2, 7; $P = 0.0423$; Fig. 3). No pots had grubs in the septa treatment and only two of fifteen pots had grubs in the dispenser treatment. This assay does not allow determination of how many females laid eggs resulting in the grubs that were found or survivorship of virgin females.

Mating Disruption, 2003. The number of beetles captured before disrupters were placed was not significant different between treatments ($F = 0.53$; df = 2, 9; $P = 0.606$; Table 2). The presence of disrupters resulted in treatment plots capturing significantly lower numbers of males than the control ($F = 75.96$; df = 2, 9; $P < 0.0001$). There were no significant differences between treatments. Both disrupters resulted in decreased captures of male beetles in less than one week after deployment (Fig. 2b). The disruption index was greater than 95% for both types of disrupters.

Disruption Assessment with Virgin Females, 2003. After two nights in the field 13, 15, and 17 females were recovered in untreated, rubber septa, and plastic dispenser plots, respectively. Treatment had a significant effect on the number of females that were fertile (χ^2 = 31.682, df = 2, $P < 0.0001$). In the plastic dispenser plot, one of the 17 females that remained laid fertile eggs, whereas none of the females laid fertile eggs in the rubber septa treatment. Fewer than 50% of the virgin females were recovered after two nights in the field. Ant predation and beetles escaping from the cages were significant factors affecting recovery.

Discussion

Evidence from virgin female trials and males caught in traps indicates that the use of red rubber septa and plastic dispensers did result in mating disruption of oriental beetle. The performance of these two disrupters was comparable, even though the plastic dispensers had 10x more active ingredient (AI) than the red rubber septa (50-75 and 5-7.5 g [AI]/ha, respectively). Polavarapu et al. (2002) achieved comparable disruption indices using sprayable pheromones at 40 – 111 g [AI]/ ha. Our trials demonstrate that this rate is effective at a rate almost 10x lower, when using red rubber septa.

In 2002 and 2003 the disruption indices were similar, even though 33% fewer dispensers were employed in 2003 relative to 2002 (Tables 1 and 2). This reduction in the number of disrupters is important from an efficacy, economical, and environmental standpoint. Future work is needed to determine the minimum deployment of traps and the amount of pheromone [AI]/ha necessary to achieve an effective level of mating disruption (i.e., DI > 90%).

At Tinton Falls preliminary trap results from 2000 and 2001 field seasons revealed an extremely large and well-established population of oriental beetles within an 18-acre field of blueberries. Japanese beetle traps, baited with 300 μg of (*Z*)-7-tetradecen-2-one, that were placed during the peak flight in July were entirely filled or overflowing within 4 d, containing over 3000 beetles per trapping station. Semio-chemical strategies for mass population reduction are inversely density-dependent, because they have more effective control of the pest as the pest-to-trap ratio decreases. This relationship is also found with the sterile insect technique, where pest control is weakened as the ratio of laboratory-reared sterile flies to wild flies decreases (Knipling 1955, Krafsur 1998). At one site with maximum pest pressure from oriental beetle, several significant measures of control were determined that point to a much higher-level potency of this trapping system.

In the two pheromone treatments, trap catches were greatly reduced, virgin females went unmated, few eggs were laid, and next-generation grubs were eliminated or reduced well below economic thresholds. This result occurred in two different seasons at two separate sites. This consistency appears to equal or exceed efficacy found in other innovative insect management strategies such as cucurbitacin for corn rootworm, mating disruption for codling moth, and sterile male releases for screwworm (Brown et al. 1992, Krafsur 1998, Pingel et al. 2001)

Oriental beetle threatens several economically valuable crops; annual sales in NJ alone exceed $45 million for highbush blueberry and over $300 million for landscape-nursery, sod and greenhouse production (New Jersey Agricultural Statistics Service 2004). The wide-ranging behavior of this invasive pest has led to increasing populations throughout the northeastern United States. Its ease of immigration through infested nursery stock and diverse host plant diet may threaten the ecological stability of native and agricultural plant systems in the near future.

The convenient installation and removal of the retrievable disrupters offers an innovative and environmentally sensitive solution.

Retrievable dispensers releasing high rates of pheromones deployed at low density appear to hold promise in disrupting sexual communication in oriental beetle especially in food crops where residue data may be required for registration of sprayable pheromone formulations. We believe the prudent next step would be to initiate widespread, commercial sized testing in IPM systems for blueberry, nursery, and turf throughout the region.

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Table 1. **Oriental beetle sexual communication disruption in highbush blueberries, 2002**

Treatment[1]	Disrupters/ha	Loading (g)/ Disrupter	Beetles/trap (mean ± SE) Pre-treatment (6 - 10 Jun)	Beetles/trap (mean ± SE) Post-treatment (11 Jun - 5 Aug)	DI[2]
Control	0	0	67.9 ± 13.8	1055.2 ± 152.3a	---
Dispenser	75	1.0	55.2 ± 19.3	50.3 ± 8.3b	95.2
Rubber septa	75	0.1	56.7 ±13.6	34.0 ± 2.9b	96.8

Means within a column with the same letter are not significantly different (Fisher's LSD > 0.05)
[1]n=5
[2]Disruptive index (DI) = [Control - (Treatment/Control)]*100

Table 2. **Oriental beetle sexual communication disruption in highbush blueberries, 2003**

			Beetles/trap (mean ± SE)		
Treatment[1]	Disrupters/ha	Loading (g)/ Disrupter	Pre-treatment (29 Jun – 2 Jul)	Post-treatment (3 Jul – 14 Aug)	DI[2]
Control	0	0	46.9 ± 8.1	340.8 ± 41.7a	---
Dispenser	50	1.0	22.7 ± 5.4	6.6 ± 1.8b	98.1
Rubber septa	50	0.1	37.8 ±12.7	2.3 ± 0.6b	99.3

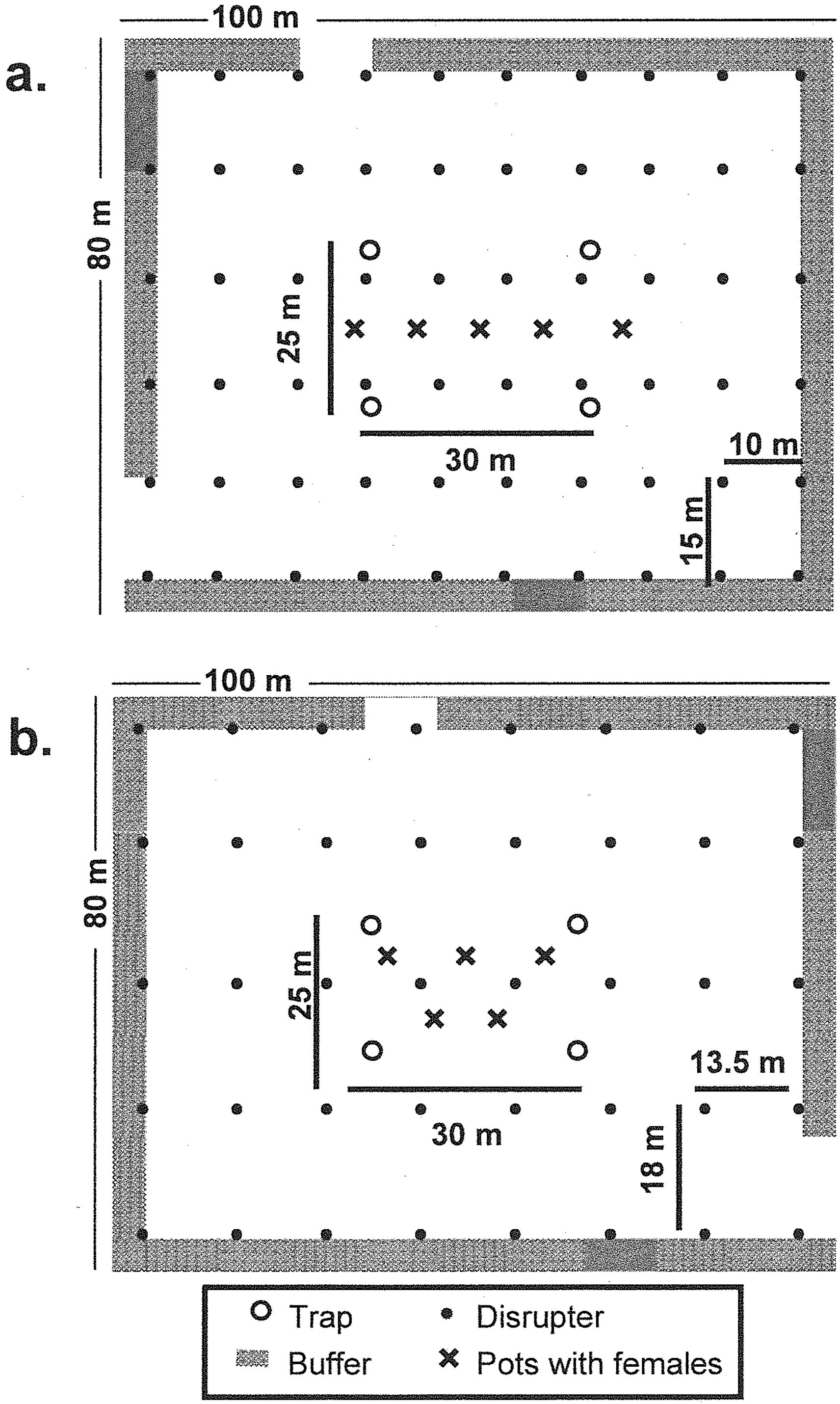
Means within a column with the same letter are not significantly different (Fisher's LSD > 0.05)
[1]n=5
[2]Disruptive index (DI) = [Control - (Treatment/Control)]*100

Figure Legends

Fig. 1. Diagram of mating disruption trials occurring in blueberry fields in 2002 (a) and 2003 (b). Plots were 0.8 ha.

Fig. 2. Captures of male oriental beetles in pheromone-baited traps placed in blueberry fields in 2002 (a) and 2003 (b). Treatments included red rubber septa, plastic dispensers, and an untreated control.

Fig. 3. Number of grubs from virgin female trials in 2002.



a.
100 m
80 m
25 m
30 m
10 m
15 m
b.
100 m
80 m
25 m
30 m
13.5 m
18 m
Trap
Disrupter
Buffer
Pots with females

Fig. 1

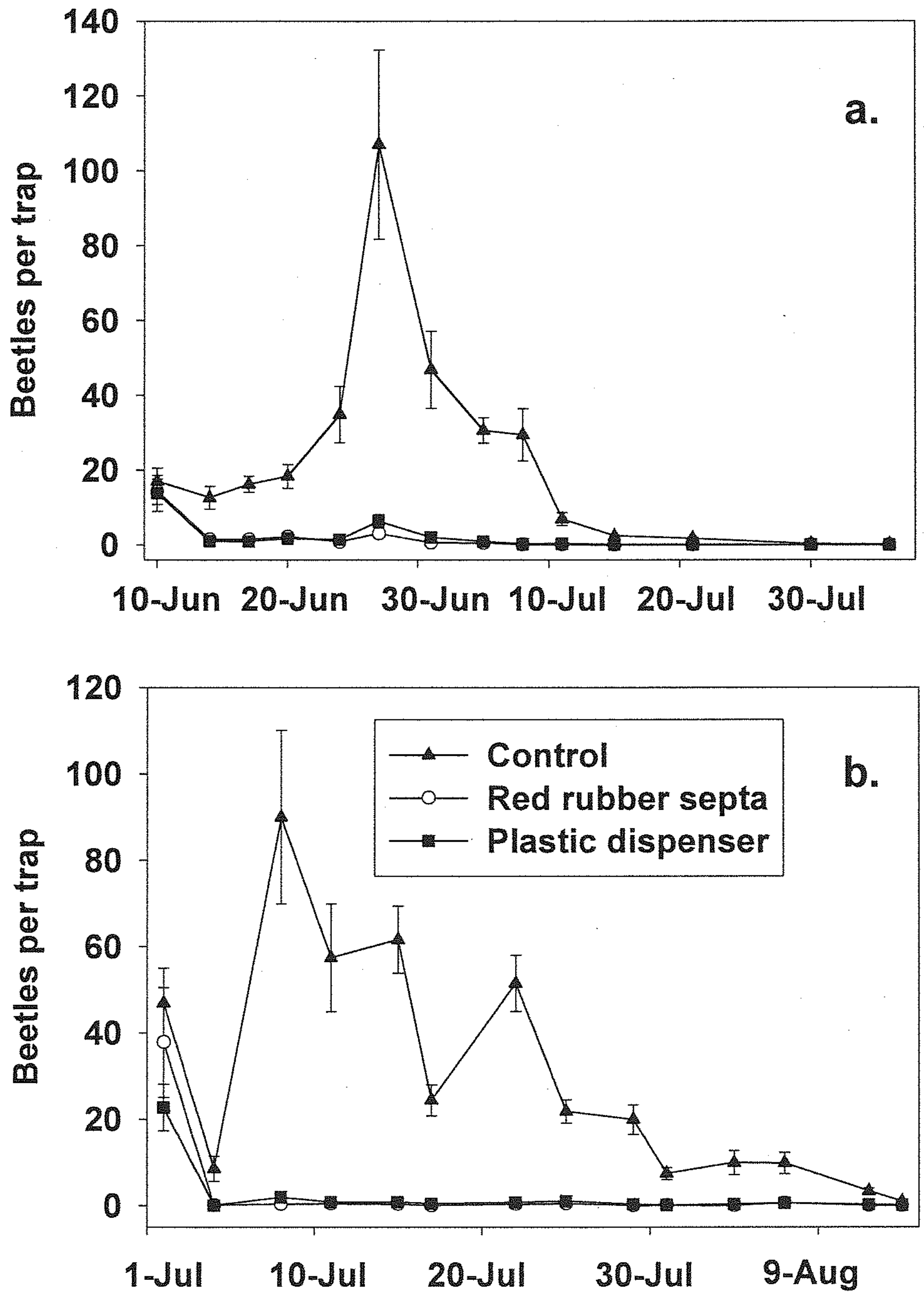

a.
Beetles per trap
140
120
100
80
60
40
20
0
10-Jun
20-Jun
30-Jun
10-Jul
20-Jul
30-Jul
b.
Control
Red rubber septa
Plastic dispenser
Beetles per trap
120
100
80
60
40
20
0
1-Jul
10-Jul
20-Jul
30-Jul
9-Aug

Fig. 2.

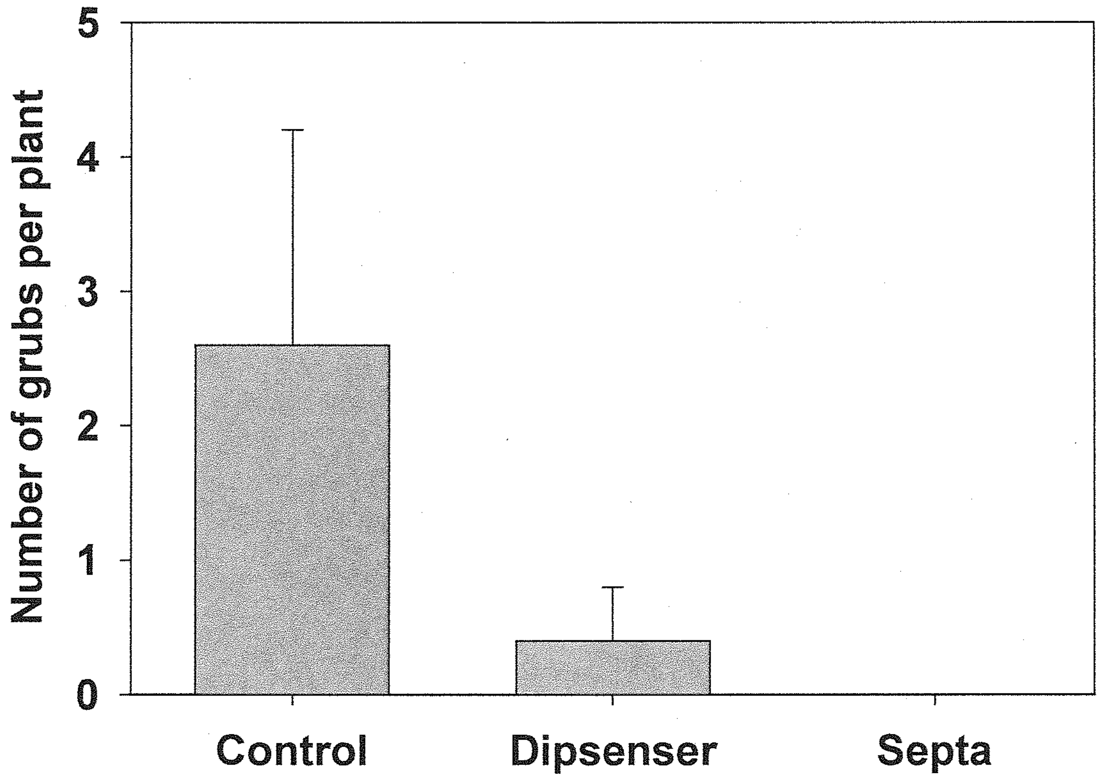


Fig. 3.