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The oriental beetle has become the most important white grub species in turfgrass in New Jersey, southeastern New York, Connecticut, and Rhode Island. Research at Rutgers University demonstrates the potential of using sex pheromones to disrupt mating to control this serious turfgrass pest.

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# Mating Disruption of Oriental Beetle with Sprayable Sex Pheromone Formulations

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## SUMMARY

The feasibility of mating disruption in the oriental beetle, *Anomala orientalis*, with microencapsulated sprayable formulations of its sex pheromone was evaluated in turfgrass. The effect of the applications was measured by monitoring male oriental beetle captures in pheromone-baited traps throughout the flight period and estimating oriental beetle larval densities in September in soil/sod samples. The results showed:

- Trap captures were 90 - 100% lower in the treated areas during the first 7 - 10 days after treatment, but started to increase thereafter. Therefore applications were repeated after 14 days in most treatments.
- The most effective formulation reduced trap captures by 87 - 88% with two applications of each 5 or 20 g pheromone per acre, but only by 74% by a single application of 30 g pheromone per acre.
- Significant amounts of the pheromone remained on grass foliage after application, but 51 and 73% of this residue was washed off the foliage with 1/8" and 1/4" post-treatment irrigation, respectively.
- Shoes walked at one day after treatment through pheromone-treated areas were sufficiently contaminated with pheromone to attract high numbers of oriental beetle males in non-treated areas.
- Mating disruption is a promising strategy for oriental beetle management in turfgrass. However, more persistent formulations need to be developed that have a lower potential to contaminate shoes and other clothing articles with pheromone.

The oriental beetle, *Anomala orientalis*, is part of a complex of white grub species (Coleoptera: Scarabaeidae) that damages turfgrass throughout the Northeastern U.S. It has been

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erroneously considered a relatively minor pest until recently because adults are cryptic and largely go unnoticed and the larvae of the Japanese beetle, *Popillia japonica*, and oriental beetle are indistinguishable without magnification.

The oriental beetle has become the most important white grub species in turfgrass in New Jersey, southeastern New York, Connecticut, and Rhode Island (1). In addition it is also the major white grub species in ornamental nurseries and blueberries, and causes losses in cranberries, strawberries, raspberries, peaches, and sweet potatoes. An increase in oriental beetle significance may occur in other areas where it is already established, i.e., all of coastal New England and Middle Atlantic states as well as Ohio, Virginia, North Carolina, South Carolina, West Virginia, and Tennessee (1, 8, 9).



Male (bottom) oriental beetle in pursuit of female (top). The adult beetles only live for about two weeks and do not cause significant damage. After mating, the females lay eggs among the roots of host plants, and the eggs hatch in two to three weeks.

## Oriental Beetle Biology

The oriental beetle has a one-year life cycle similar to that of other important white grub species. At the latitude of New Jersey, oriental beetle flight occurs from early June through early August with peak flight activity typically in late June/early July. The adult beetles only live for about two weeks and do not cause significant damage. After mating, the females lay eggs among the roots of host plants, and the eggs hatch in two to three weeks. The first and second instar each last around three weeks so that by mid-September the majority of the larvae are in the third instar (8, 9).

After overwintering below the frost line, the third instars resume feeding until pupation in late spring. The extensive feeding activity of the larger larvae can kill large areas of grass from mid-August into mid-October, especially under warm dry conditions. In addition, vertebrate predators (i.e. raccoons, opossums, skunks, crows) often damage the turf to feed on the grubs.



Male oriental beetle screening air for sex pheromone

## Oriental Beetle Mating Behavior

Sex pheromone-mediated mate finding and copulation of oriental beetles occur at or near the soil surface, immediately after female emergence from the soil, close to the emergence site (4, 5, 6). Males respond to female-released pheromone by a combination of flying upwind and walking short distances. Both sexes are most active between 6 and 10 pm. The oriental beetle sex pheromone consists of a 9:1 blend of (Z)-7-tetradecen-2-one and (E)-7-tetradecen-2-one (3, 10). Field trapping studies have indicated that (Z)-7-tetradecen-2-one alone is as effective as a 9:1 blend containing both compounds in attracting males (3).

## Need For Alternatives to Chemical Control

Despite the proximity of turf to people, chemical insecticides are still the primary tools for white grub management. However, the implementation of the Food Quality Protection Act of 1996 (FQPA) has resulted in the loss of many insecticides for white grub control. Mating disruption with sex pheromones is widely used as an environmentally safe, non-toxic alternative to broad-spectrum insecticides for several moth species (2).

The sex pheromones of scarab beetles have been studied intensively and are used for monitoring purposes. But only recently has mating disruption technology been considered as a possibility for management of white grubs. Polavarapu et al. (7) have already shown the feasibility of mating disruption in oriental beetle in large-scale field experiments in blueberries and ornamental nurseries with a microencapsulated sprayable formulation of its sex pheromone.

## Mating Disruption Field Trials

To determine the feasibility of mating disruption technology in turfgrass, we conducted field trials with sprayable microencapsulated formulation of the oriental beetle sex pheromone. Two methods were used to determine the effect of



Trap used for trapping oriental beetle males (left). Red rubber septum is lured with oriental beetle pheromone. In addition to evaluating the ability of oriental beetle males (right) to locate a pheromone source, trapping was also used to monitor oriental beetle male flight and optimize the application timing of sex pheromones.

treatments on the mating success of oriental beetles. The first method measured the ability of oriental beetle males to locate a pheromone source similar to a female by determining the number of oriental beetle males captured in traps. The traps consisted of standard Japanese beetle traps (Trécé, Salinas, CA) baited with red rubber septa lures containing (Z)-7-tetradecen-2-one and were fitted into a hole in the ground so that only the funnel portion remained aboveground.

Trapping was also used to monitor oriental beetle male flight and optimize the application timing. The traps were placed in each plot at least 66 feet from the plot's border and any other trap. In 2002, four traps with 300  $\mu\text{g}$  (Z)-7-tetradecen-2-one per septum were placed per plot, and septa were replaced once after four weeks. In 2003 and 2004, three traps with 30  $\mu\text{g}$  (Z)-7-tetradecen-2-one per septum were placed per plot, and septa were replaced twice after three weeks use. Traps were first placed in early June of each year and emptied every three to four days and directly

before treatment applications until beetle flight was very low. Captured males were counted and killed.

The second method estimated oriental beetle larval densities during September following the applications by taking 30 soil/sod cores (4.25" diam  $\times$  4" depth) with a standard golf hole cup cutter in a grid pattern at least 50 feet within the plot's border. Scarab larvae found in the cores were identified to species using the raster pattern.

Field plots were situated in turfgrass areas at the Rutgers Research Station in large lawn areas and in golf course rough areas (typically between tee and fairway) in Monmouth County, NJ. The plots measured between 0.8 and 1.4 acres in size and were separated from other plots by a minimum of 300 feet. All plots were watered as needed to prevent excessive drought stress.

The treatment plots were broadcast sprayed once or twice with microencapsulated oriental beetle sex pheromone using locally available spray equipment such as tractor-drawn boom

sprayers and self-propelled boom sprayer units. The first spray was applied about 10 days after the first oriental beetle males were captured in traps. Where applicable, a second spray was applied about 14 days after the first spray. The control plots were not sprayed. All plots received 1/4" overhead irrigation after treatment.

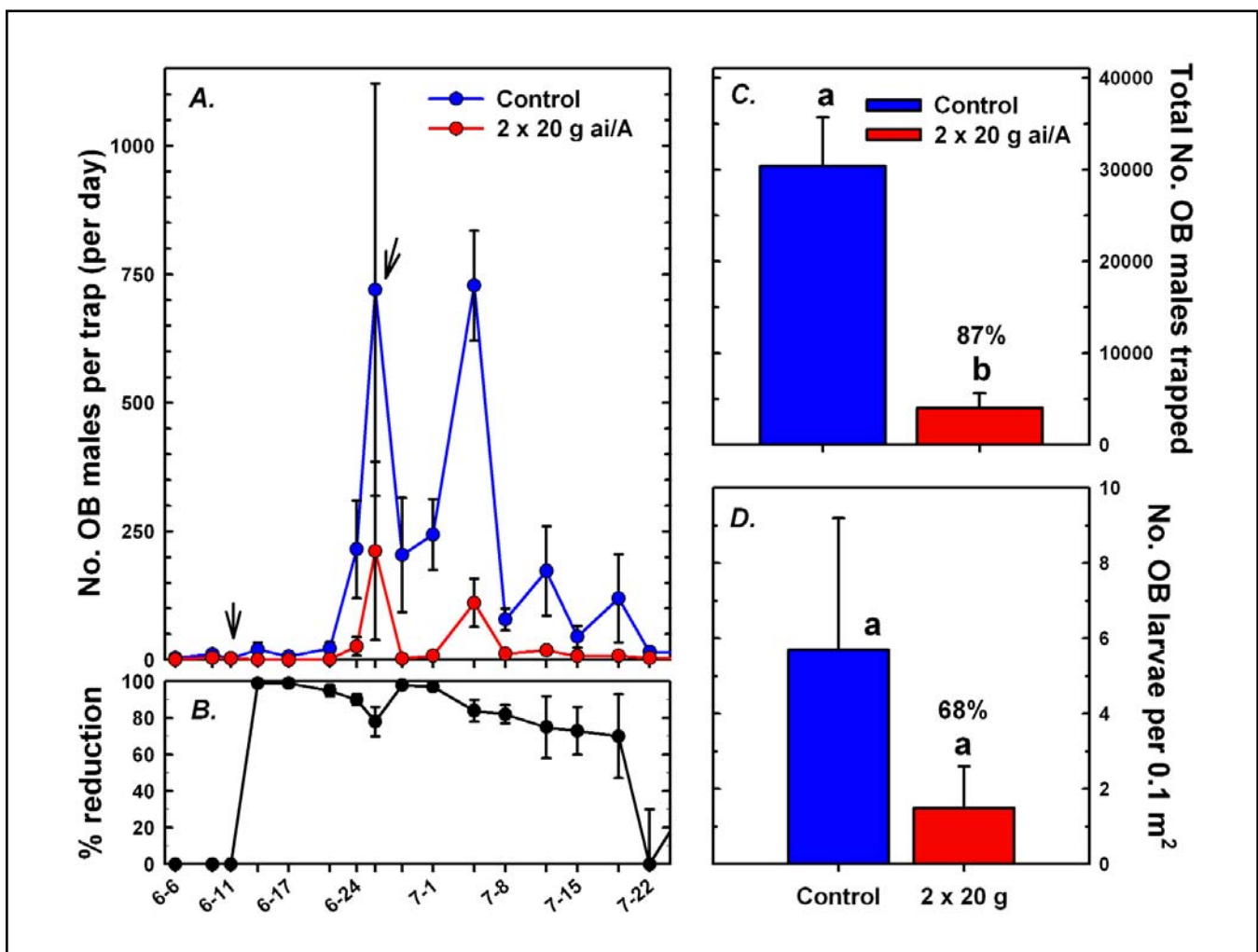
In 2002, one treatment was applied consisting of two sprays of 20 g ai/acre of a formulation developed by 3M Canada Company (London, Ontario) containing 20% (Z)- and (E)-7-tetradecen-2-one at a 93:7 ratio. In 2003, two treatments were applied consisting of one spray of 30 g ai/acre or two sprays of 5 g/acre of the 3M formulation. Because 3M discontinued the production

of its formulation, two formulations developed by Suterra LLC (Bend, OR) were used in 2004 containing 5.35% (Suterra 03) and 24.11% (Suterra 04), respectively, (Z)-7-tetradecen-2-one, both applied twice at 10 g ai/acre.

## Results

### Mating Disruption Field Trials

Oriental beetle larval densities in spring and oriental beetle male trap captures before treatment did not differ significantly between plots designated for the controls and treatments in any



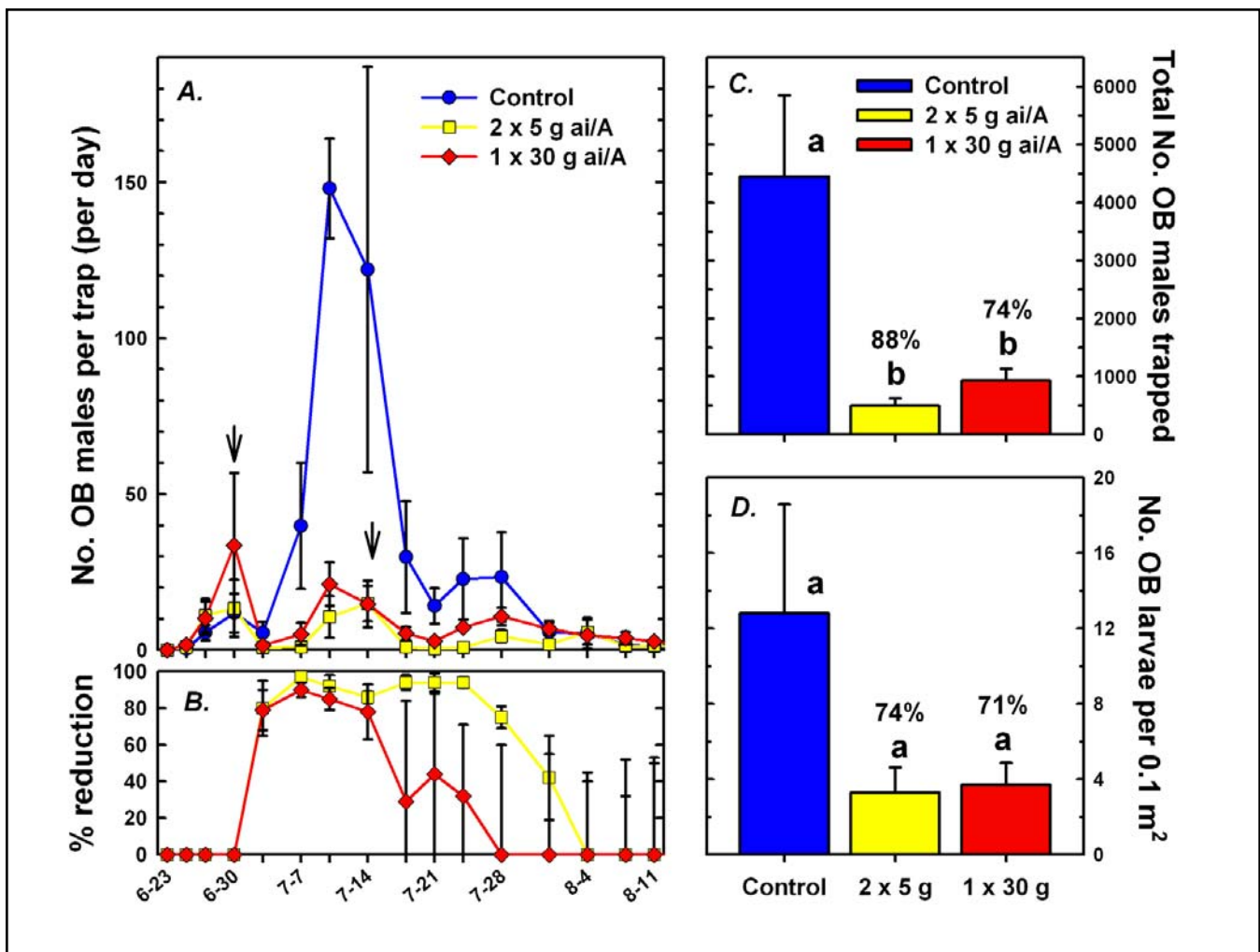
**Figure 1.** Field season 2002 oriental beetle mating disruption trial. A. Twice-weekly male trap captures (arrows indicate application dates), B. Percentage reduction in twice-weekly trap captures, C. Total seasonal trap captures, D. *A. orientalis* larval densities in September following application. A; asterisk above data points indicates significant difference among means on sampling date. Arrow indicates pheromone application dates. C, D; means with same letter above bars are not significantly different, and figures above bars indicate percent reduction compared to control.

experiment. Larval populations at the time of evaluation in September consisted of 65 - 95% oriental beetles, the remainder typically being larvae of Japanese beetle, Asiatic garden beetle, and/or northern masked chafer.

In 2002, oriental beetle male flight started in the first week of June and trap captures had two distinct peaks on June 25 and around July 5 (Figure 1A). Percent reduction in trap captures (Figure 1B) in the treated plots was 96 - 100% for the first week after each application, but started to drop during the second week. Total trap captures were 87% lower in the treated plots than in the control plots (Figure 1C). Oriental beetle larval densities in September were 68% lower in the treated plots than in the controls but due to high

variation in the control plots, the reduction was not statistically significant.

In 2003, oriental beetle male flight started in the last week of June (after an unusually cool spring), and trap captures had a distinct peak around July 10-14, and continued elevated activity until July 28 (Figure 2A). Percent reduction in trap captures (Figure 2B) in the treated plots was 96 - 100% for the first week after each application but started to drop during the second week. Total trap captures were significantly lower in the 1 × 30 g ai/acre treatment (74% reduction) than in the control, and significantly lower in the 2 × 5 g ai/acre treatment (88% reduction) than in the 1 × 30 g ai/acre treatment (Figure 2C). Oriental beetle larval densities in September were 71 - 74%



**Figure 2.** Field season 2003 *A. orientalis* mating disruption trial. A. Twice-weekly male trap captures (arrows indicate application dates), B. Percentage reduction in twice-weekly trap captures, C. Total seasonal trap captures, D. *A. orientalis* larval densities in September following application. A; asterisk above data points indicates significant difference among means on sampling date. Arrow indicates pheromone application dates. C, D; means with same letter above bars are not significantly different, and figures above bars indicate percent reduction compared to control.

lower in the treated plots than in the controls but due to high variation in the control plots, the reduction was not statistically significant (Figure 2D).

In 2004, oriental beetle male flight started in the first week of June, had an extended peak between June 17 and July 5 and continued elevated activity until about July 20. Total trap captures were significantly lower for the Suterra 03 (68% reduction) and Suterra 04 formulations (70% reduction) (both applied at  $2 \times 25$  g ai/ha) compared to the untreated control. The effect of the pheromone started to decline in the second week after each application. Oriental beetle larval densities in September compared to the control were 22% higher and 56% lower for Suterra 03 and Suterra 04 formulations, respectively. Due to the high variation in larval densities, there were no significant differences among treatments.

#### **Effect of Post-application Irrigation on Pheromone Adherence to Grass Blades**

After spray application, a significant amount oriental beetle pheromone may remain on the grass foliage rather than drip off into the thatch and soil. Removal of grass clippings from mow-

ing could then reduce the efficacy of the pheromone application.

To determine whether post-application irrigation is necessary to wash the pheromone off the foliage and into the thatch and upper soil layers, areas were sprayed with 30 g ai/acre of the 3M formulation and overhead-irrigated with 0", 1/8", or 1/4" after treatment. Then the grass was cut just above the thatch surface, collected, and the pheromone extracted. The amount of pheromone in the clippings extract was determined with gas chromatography-mass spectrometry. In samples taken directly after application, significantly less pheromone was detected in clippings taken from plots watered with 1/8" ( $3.6 \pm 0.7$   $\mu$ g) and 1/4" ( $2.7 \pm 0.3$   $\mu$ g) than in the non-watered plots ( $7.3 \pm 1.3$   $\mu$ g). No pheromone could be detected in samples taken after seven days.

#### **Adsorption of Sex Pheromone to Shoes**

Oriental beetle pheromone can adsorb to surfaces it comes into contact with such as shoes on treated turf areas. These can then attract male oriental beetles over an extended period of time. To test whether shoes can be contaminated with enough oriental beetle pheromone to cause poten-



Shoes walked at one day after treatment through pheromone-treated areas were sufficiently contaminated with pheromone to attract high numbers of oriental beetle males in non-treated areas.



tial nuisance to golfers, one pair of athletic shoes was walked for 30 minutes through each of the areas treated with oriental beetle pheromone in the large field trials at one or eight days after treatment. From each pair, one shoe was used for pheromone extraction, the other in a bioassay.

In the bioassay, the shoes were lined up on the surface of a non-pheromone treated turfgrass area perpendicular to the prevailing wind direction in a continuous line of three groups with each group containing one shoe from each treatment and a non-pheromone exposed shoe with 15-ft distance between shoes. Oriental beetle males were collected from the shoes for 45 minutes. No males were attracted to the control shoes. Significantly fewer males were attracted to shoes walked at eight days after treatment (average 1.8; range 0 - 10) compared to shoes walked at one day after treatment (average 42.3; range 6 - 81).

The shoe not used in the bioassay was rinsed with acetone for 10 minutes and the amount of pheromone in the extract analyzed by gas chromatography. From shoes walked one day after treatment  $62.1 \pm 15.3 \mu\text{g}$  per shoe were detected. No pheromone was detected on the shoes walked eight days after treatment and on the control shoes.

## Conclusions

This study demonstrates the feasibility of mating disruption in the turfgrass system. However, the effect of the pheromone spray started to wane after about 10 days, making a second application after 14 days necessary. The two Suterra formulations used in the 2004 turfgrass experiment persisted even shorter than the 3M formulation. Due to the inherently high variability of white grub populations within and among turfgrass sites, the larval counts in our experiments, particularly in the non-treated areas, were too variable to allow for the detection of statistically significant difference. Nevertheless, the trend in the 2002 and 2003 field seasons using the 3M formulation was very consistent with 68 - 74% lower oriental beetle larval populations in the treated areas.

The efficacy of mating disruption using sprayable formulations could be improved with more frequent applications, probably even with lower pheromone application rates than used in this study. However, the availability of insecticides that are highly effective and only require one seasonal application (i.e., imidacloprid, clothianidin) will limit the acceptance of mating disruption unless a formulation can be developed that is more effective and/or requires only one seasonal application. We don't believe that this goal can be achieved using microencapsulated sprayable formulations. In addition, the potential contamination of shoes and other clothing articles by the sprayable formulation and the ensuing attraction of male beetles to these articles outside of treated areas present a drawback of these formulations.

Dispersible pheromone formulations consisting of numerous broadcast small pheromone sources or fewer larger sources may solve the problems of limited persistence as well as contamination of clothing articles in the turfgrass system. Our ongoing studies with dispersible formulations suggest that mating disruption can be an effective, safe, environmentally and economically sound, easily implementable, durable, and highly IPM-compatible option for oriental beetle management in turfgrass.

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