EVIDENCE OF A SEX PHEROMONE IN THE GREEN JUNE BEETLE, *COTINIS NITIDA* (COLEOPTERA: SCARABAEIDAE)[1]

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ABSTRACT

A trapping study was conducted in an area of heavy green June beetle (GJB), *Cotinis nitida* (L.) (Coleoptera: Scarabaeidae), emergence to collect evidence in support of the hypothesis that unmated females attract males with a sex pheromone. Yellow-painted baffle and funnel traps, baited with unmated females, mated females, unmated males or no beetles (control), were arranged in a Latin-cube design and randomized daily for four consecutive days. Trap catch was not significantly affected by trap placement (row or column) or time. Significantly more male beetles were caught in traps baited with unmated females than in any other treatments (P = 0.05, Duncan's multiple range test).

Key Words: Green June Beetle, *Cotinis nitida,* sex pheromone.

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INTRODUCTION

Larvae of the green June beetle (GJB), *Cotinis nitida* (L.)., endemic to the southeastern United States, have long been noted as a pest of lawns, pastures, and plant beds and as an adult pest of ripening tree and vineyard fruit (Howard 1898; Davis and Luginbill 1921; Dominick 1950; Wylie 1969). Larvae feed nocturnally on detritus and may damage plant roots as they tunnel through soil (Davis and Luginbill 1921; Miner 1951). In collecting the egg and larval stages of the GJB in Arkansas we have noted that they can often be found in soil enriched with plant residue or animal manure, such as that near barns and in grazed pastures. We have observed many hundreds of GJBs fly from such areas to vineyards and apple and peach orchards.

The economic importance of this beetle has been attributed to the opportunistic feeding by large aggregations of adults on ripe fruit, e.g. apple, blackberry, fig, grape, nectarines, peach, pear, plum, raspberry, young corn, and new growth of various hardwood trees (Davis and Luginbill 1921). More recently, there have been reports of GJB adults causing economic losses to the new releases of early maturing table grapes, apples, and peaches (Wylie 1969; Johnson and Mayes 1982). The high cost of insecticide treatment of pastures precludes chemical control of GJB larvae. In addition, Wylie (1969) and Johnson and Mayes (1982) reported that GJB adults cause considerable economic damage to fruit despite its frequent treatment with carbaryl. Insecticide applications for GJB control also may interfere with other fruit pest management techniques and reduce overall control.

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The first reference to a possible olfactory attraction of male to female GJBs was based on the observation that males could locate females hidden in grass (Davis and Luginbill 1921). Remnants of mower-killed females were noted to attract males, which indicated that sight, sound or motion of females were not key elements in this process (Patton 1956). In 1985, a preliminary test was conducted of GJB field response to severed abdomens of male and unmated female GJBs. Results indicated that only unmated female abdomens were attractive to males and that male abdomens were not attractive to either sex. Other trials showed that a paper strip or other object could be made attractive to male GJBs for about 40 seconds if it was touched to the ovipositor of an unmated female (kept in a sealed vial). Males flew upwind, landed on or near the apparent source, and in a few cases, everted their genitalia. Observations of male GJB flight during the mating period showed that they generally flew upwind but alternated from left to right in a series of 90° turns. This pattern may provide them with an energy conservative method of sampling air to locate a source of volatile attractant(s). This observation parallels those of early workers with the Japanese beetle, *Popillia japonica* Newman, which indicated a similar upwind attraction of many males to virgin females (Smith and Hadley 1926). The presence of a volatile sex pheromone in the Japanese beetle was later field demonstrated (Ladd 1970; Goonewardene et al. 1970), structurally identified and synthesized (Tumlinson et al. 1977; Doolittle et al. 1980). Japanese beetle pheromone has been used in various combinations with several food-type lures to locally reduce populations and to survey for new infestations (Klein et al. 1981; Ladd et al. 1981).

The purpose of this study was to collect evidence in support of the hypothesis that unmated females attract males with a sex pheromone. This is a prerequisite to isolation, identification, and synthesis of the presumed sex pheromone and its potential employment possibly in conjunction with other synergistic attractants in control of the GJB.

MATERIALS AND METHODS

On 23 June 1985, emergence of a large number of GJBs was observed in a 8.1 ha (20 acre) area of pasture near Goshen, AR. This site was selected for the trapping study reported herein. The study area was bordered to the north by a steep slope that dropped 60 m to a lake, to the south by an incline interspersed with trees and rock outcroppings, to the east by heavy growth of annual weeds and to the west by mixed hardwoods and conifers. A Latin-cube design was used to account for possible treatment location effect. The number of treatments was equal to the number of replicates (4), and beetles were trapped on 4 consecutive days, from 30 June to 3 July 1986. After each day of trapping, the 16 traps were relocated so that every treatment occurred once at each of the 16 trap locations during the 4-day course of the experiment.

Traps were made from 0.45 mm galvanized steel and consisted of a baffle, painted bright yellow (Lemon yellow, Sherwin-Williams, Chicago, IL), attached to a funnel. The 4-winged baffle, 40 cm wide and 30 cm high, had a 110 mm × 70 mm wide opening in its center for placement of a screen cage containing the treatment. The baffle was bolted to the large end of a 45 cm high × 40 cm diameter funnel that tapered to a 30 mm diameter bottom opening. A 3 liter plastic jug, attached to the funnel bottom, held beetles caught by the trap. The trap was suspended by a hook welded to the top center of the baffle. The trap was supported by a 1.25

cm diameter × 2 m long electrical conduit bent in a J-shape, inverted and slipped over a 1.2 cm diameter × 1 m long steel rod driven into the ground. Traps were spaced 23 m apart in a 4 row × 4 column arrangement. The screen cage part of the trap that held the treatment was about 90 cm above the ground.

The 4 treatment baits in the screen cages were 1) six unmated females, 2) six mated females, 3) six unmated males and 4) no beetles. Unmated female and unmated male beetles were individually reared from soil cells dug from the same pasture several weeks prior to emergence. Mated females were obtained by placing 3 males with one unmated female until copulation was completed. A 40 ml plastic cup packed with polyester fiber and saturated with distilled water was placed in all cages as a water source for beetles.

Trapping began at 0730 hrs CDT and ran daily until 1830 hrs CDT. After each daily trapping period, trap containers holding beetles were emptied into Ziploc® polyethylene bags, placed in a cooler with ice and brought to the lab. Beetles were sexed and counted.

An analysis of variance of the square root transformed data was conducted using the Statistical Analysis System (SAS Institute 1982). Duncan's multiple range test (Duncan 1955) was used to separate significantly different treatment means of square root transformed data.

RESULTS AND DISCUSSION

An analysis of variance of the trap catch data showed no significant trap placement (row, column) or time effects. Treatment effects were significant (Treatment $F_{3,51}$ = 56.8, P = 0.0001) (Table 1).

Table 1. Mean number of male GJB caught per trap per day by each treatment.

Treatment	Mean number of males/ trap/day*	S.E. of mean
Unmated females	112.25 a	25.31
Mated females	4.06 b	2.58
Unmated males	0.63 b	0.22
No beetles-control	0.06 b	0.06

* Means followed by different letters are significantly different (P = 0.05, N = 16, Duncan's multiple range test). Mean separations are based on square root transformed data.

No females and very few males were caught in traps baited with unmated males, indicating that males do not attract either sex from a distance. Mated females attracted a much lower number of males than did unmated females. Ladd (1970) found that traps baited with mated females or males caught only a few Japanese beetles, whereas unmated females were highly attractive to males. The trapping data herein suggested that a similar reduction or cessation of pheromone release occurred after GJB females were mated. Field observations suggested that females were somewhat attractive to males immediately after mating, but only to those within 15 - 20 cm. Often, those were males that were apparently drawn to the vicinity minutes before by a calling female. However, recently mated females tried to escape from or used their hind legs to push at males attempting copulation. Traps baited with unmated females caught an average of 112.3 males per trap per day. This was significantly different (P = 0.05, Duncan's multiple range test) from the other 3 treatment means, none of which differed significantly (Table 1). Unmated female GJBs are clearly the most attractive to males which indicates a sexual basis for this behavior. This inference is supported by our field observations of female GJB mate calling and mating behavior on about 40 occasions. Females, upon emergence from the soil, separated the terminal abdominal tergite and sternite, exposing the ovipositor. Almost immediately, many males were observed flying upwind toward the female. No courtship behavior was observed. The first male to reach a receptive female usually mated successfully, although physical aggression was common between competing males in the vicinity of the calling female.

Results of this study, considered along with supporting observations of a calling behavior by unmated females followed by a males-only response, plus the attractiveness of a substance from intact or severed, unmated female abdomens, provide strong evidence that unmated female GJBs produce and release a sex pheromone to recruit males. Studies to develop a laboratory bioassay for male response to unmated females and to collect, identify and synthesize the probable pheromone involved are underway.

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