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THE SCARAB BEETLE *Anomala cuprea* UTILIZES THE SEX PHEROMONE OF *Popillia japonica* AS A MINOR COMPONENT

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Abstract—GC-EAD analyses revealed that the scarab beetle *Anomala cuprea*, the cupreous chafer, utilizes, in addition to the previously identified major sex pheromone (*R*,*Z*)-5-(−)-(oct-1-enyl)oxacyclopentan-2-one, a minor component, (*R*,*Z*)-5-(−)-(dec-1-enyl)oxacyclopentan-2-one, which has been previously identified as the sex pheromone of the Japanese beetle. Release of the sex pheromone blend did not significantly differ when collected from feeding or starving female beetles, nor did it differ from volatiles collected in the scoto- and photophase. However, after mating, the amount and the ratio of the two components changed. Field tests revealed that traps baited with the synthetic sex pheromone captured more beetles than traps containing only virgin females. Based on field experiments, 10 mg of a 90:10 blend of the pheromone was suggested as appropriate for monitoring of the cupreous chafer, although the optimal ratio for attractiveness is yet to be established. The occurrence of minor components in the pheromone system of other scarab beetles is also discussed.

Key Words—Japanese beetle, cupreous chafer, GC-EAD, (*R*,*Z*)-5-(−)-(oct-1-enyl)oxacyclopentan-2-one, (*R*,*Z*)-5-(−)-(dec-1-enyl)oxacyclopentan-2-one, sex pheromone, *Anomala cuprea*, *Popillia japonica*, Coleoptera, Scarabaeidae.

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INTRODUCTION

Herbivorous scarab beetles (Coleoptera: Scarabaeidae) include various economically important pests in agriculture, horticulture, and forestry. Both larvae and adults damage crops by feeding on underground and aerial parts, respectively. In Japan, the most important scarab species is *Anomala cuprea* Hope, the cupreous chafer, which is a severe pest of a wide variety of crops, mainly soybean, grape, and strawberry. Due chiefly to the difficulty of controlling this pest with conventional insecticides, there has been an increasing interest in environmentally sound alternative methods of control. Sex pheromones of only a few species of scarab beetles have been identified so far, but they are so successfully applied in the field that they are seriously considered as alternatives in combination with other IPM techniques in order to minimize or even replace hard chemicals. Therefore, identification of these semiochemicals is of utmost importance to investigate their relevance for field application.

Nevertheless, identification of scarab sex pheromones is a hard task, with the lack of uniform and consistent laboratory bioassays being the main obstacle. This difficulty can be exemplified by the fact that sex pheromone evidence has been proven for beetles in the genera *Phyllophaga*, *Lachnosterna*, *Popillia*, *Pachypus*, *Polyphylla*, *Plectis*, *Melolontha*, *Costelytra*, *Rhizotrogus*, *Rhaepaea* (Bestmann and Vostrowsky, 1988), *Cyclocephala* (Potter, 1980), and *Cotinis* (Domek and Johnson, 1987), but until recently sex pheromones of scarabs were identified only in *Costelytra zealandica* (Henzell and Lowe, 1970), *Popillia japonica* (Tumlinson et al., 1977), and *Anomala rufocuprea* (Tamaki et al., 1985).

In order to alleviate the difficulties incurred in isolating sex pheromones by monitoring only with bioassays (either in wind tunnels or in field tests), a GC-EAD technique has been applied (Leal et al., 1992a). That work led to the identification of (*R*,*Z*)-5-(−)-(oct-1-enyl)oxacyclopentan-2-one as a sex pheromone of *Anomala cuprea* (Leal, 1991). Although all the sex pheromones identified from scarab beetles hitherto were single components, we kept trying to identify any possible minor component. Recent findings showed that *Anomala daimiana* utilizes a binary mixture, whose individual components are sex pheromones of other *Anomala* spp. (Leal et al., 1993). This result further stimulated our investigation on the minor components of other scarab species. We describe here the utilization by *Anomala cuprea* of (*R*,*Z*)-5-(−)-(dec-1-enyl)oxacyclopentan-2-one, the sex pheromone of the Japanese beetle, as a minor component. Field evaluations of the binary pheromone blend will also be described.

METHODS AND MATERIALS

Chromatographic and MS Analyses. GC analyses were performed on a Hewlett-Packard 5890 equipped with either an HP-1 column (12 m × 0.2 mm; 0.33 μm) or a DB-wax column (30 m × 0.25 mm; 0.25 μm). The oven was

operated at 50°C for 1 min, programmed at 4°C/min to 180°C, held at this temperature for 1 min, programmed again at 10°C/min to 210°C and held at this temperature for 30 min. Both internal and external standards were used for quantitative analyses. Mass spectra were recorded on a Hewlett-Packard 5891 mass selective detector using either HP-1 or DB-wax columns.

GC-EAD. The responses of *A. cuprea* antennae were recorded with a previously described GC-EAD system (Leal et al., 1992a) utilizing two left antennae of males. The system was tested before and after analysis by the introduction of vapor of the major sex pheromone or air (Burger et al., 1991). This was done by puffing volatiles in a Pasteur pipet into the interface of the GC exit and the EAD glass transfer line.

Rearing of Insects. A. cuprea was raised according to a previously reported method (Hatsukade et al., 1984).

Aeration. The airborne volatiles of either male or female beetles were collected as previously reported (Leal et al., 1992a). In order to investigate the effect of starvation on the release of the sex pheromones, the volatiles of groups of 10–20 virgin female beetles were collected for 24 hr either in the presence of foodstuff (grape leaves) or without it. Aeration was also carried out in both scotophase (1800–0600 hr) and photophase (0900–1700 hr) with virgin females.

Effect of Mating on Pheromone Release. A group of 20 virgin female beetles of nearly the same age (12 days old on average) was placed together with 20 unmated males for three days. Then the mated females were isolated and their pheromones were collected after one, two, and three days.

Syntheses. (*R*,*Z*)-5-(−)-(oct-1-enyl)oxacyclopentan-2-one was synthesized as previously reported (Leal, 1991) and (*R*,*Z*)-5-(−)-(dec-1-enyl)oxacyclopentan-2-one was prepared according to a reported method (Senda and Mori, 1983). Their optical purities were >99%, i.e., >97% ee, as revealed by GC using a Chiraldex GTA column (20 m × 0.25 mm; 0.125 μm) (Leal, 1991).

Field Experiments. Evaluation of the pheromone system in the field was conducted at the National Institute of Sericultural and Entomological Science field in Tsukuba and at Chiba Prefectural Agricultural Experiment Station field in Chiba, Japan in the summer of 1992. The traps used were either funnel traps (Japan Tobacco Inc.), hereafter called JT traps, or water pan traps (Sankei Chemical), hereafter called Sankei traps. The chemicals (10 mg, unless otherwise mentioned), dissolved in hexane, were applied to rubber septa (Daburu Kyappu No. 2, Araki Rubber Co. Ltd., Osaka), and the solvent was allowed to evaporate in a fume hood in an airstream for one day. The baits were then used in the field or stored at −30°C. The traps were set at 1.5 m above the ground at 10-m intervals near Japanese chestnut orchards or sweet potato experimental fields. The experiments were done with at least three replicates, and the positions of the traps were randomized from time to time to avoid any effect of trap location. Virgin females were placed in plastic bottles provided along with

JT traps. Small holes were opened and the individuals were renewed daily. Capture data were transformed to log $(x + 1)$, and differences between means were tested for significance by ANOVA. Throughout this paper, treatments followed by the same letters are not significantly different at a 5% level in the Scheffe F test.

RESULTS AND DISCUSSIONS

Identification of Minor Component. GC-EAD analyses of the airborne volatiles of virgin female beetles fed on grape leaves revealed the occurrence of two EAD-active peaks (Figure 1), which appeared at 40.32 and 46.23 min on a DB-wax capillary column. The peak with the shorter retention time has been previously detected (Leal et al., 1992a) from volatiles of field-captured female beetles. However, the EAD response to the peak at 46.23 min was often missed, most probably due to differences in response of individual antennae rather than due to the origin of samples. In order to improve the system, we used two male antennae in the biodetector to minimize the individual effect. This method gave an improvement in the signal-to-noise ratio, but although the signal of the major component was reproducible, response of the minor component was detected only in less than 8% of the trials with males of different ages and origins. Nevertheless, the signals were generated from volatiles of virgin as well as field-captured female beetles. Two EAD peaks appeared also on a HP-1 capillary column at 24.74 and 30.47 min.

The major EAD-active peak was identified as (*R*,*Z*)-5-(−)-(oct-1-enyl)oxacyclopentan-2-one (Leal, 1991), based mainly on its MS (Figure 2A). The minor peak, on the other hand, gave a similar MS (Figure 2B) displaying the base peak at $m/z = 111$ and the molecular ion peak at $m/z = 224$. This was reasoned to be due to a lactone analog of the major component, having a chain longer by two methylenes. Such a compound, (*R*,*Z*)-5-(−)-(dec-1-enyl)oxacyclopentan-2-one, has been previously identified as the sex pheromone of the Japanese beetle (Tumlinson et al., 1977). In fact, synthetic japonilure gave the same MS and retention times on both columns and was EAD active. Due to the amount of the natural product available, its absolute configuration was not confirmed, but the fact that the Japanese beetle and the cupreous chafer both utilize *R* enantiomers strongly suggested the *R* configuration (later corroborated by bioassay). Regarding the double bond, it was possible to confirm its configuration as *Z* by comparison with synthetic isomers; the *E* isomer appeared at 46.62 min on the DB-wax column.

Sex Pheromone Release. Feeding of the green June beetle, *Cotinis nitida*, has been demonstrated to stimulate aggregation of conspecific males (Domek and Johnson, 1988). Therefore, we examined whether this would also happen

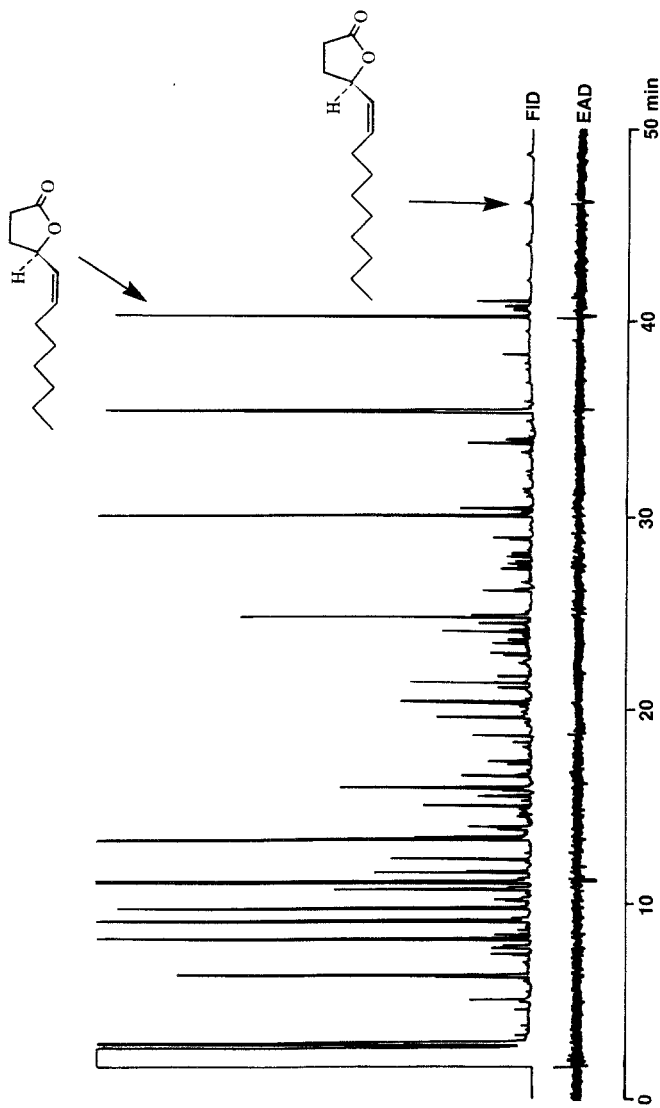

FIG. 1. Coupled GC-EAD response of *A. cuprea* male antennae to the airborne volatiles of virgin female beetles fed on grape leaves.

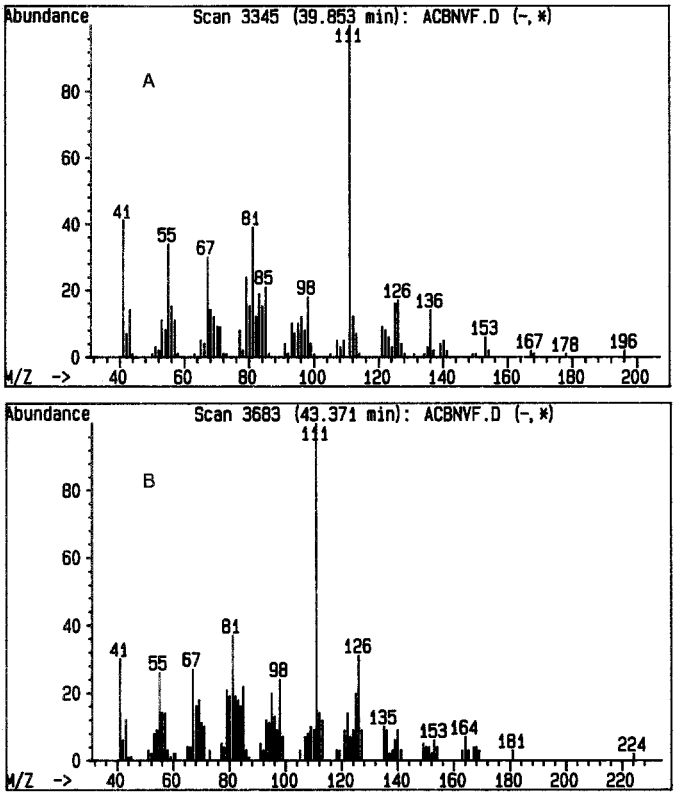

FIG. 2. EI-MS of the two EAD-active peaks. (A) Major peak identified as (*R*,*Z*)-5-(−)-(oct-1-enyl)oxacyclopentan-2-one. (B) Minor component identical to the Japanese beetle sex pheromone.

with sex pheromone release by *A. cuprea*. However, the cupreous chafer fed on grape leaves did not significantly release more pheromone than starving beetles (Figure 3). Furthermore, nearly equal amounts of the semiochemicals were collected when aeration was done in scoto- or photophase (Figure 4). The fact that the total amount of pheromone collected in the latter experiments differs from the one-day long aeration (Figure 3) might be due to the difference in collection times.

One day after mating, the amount of the major pheromone (but not of the minor) significantly decreased. Interestingly, however, as the isolation period increased, the amount of pheromone released increased for the major component and there was a trend to decrease for the minor component (Figure 5). That

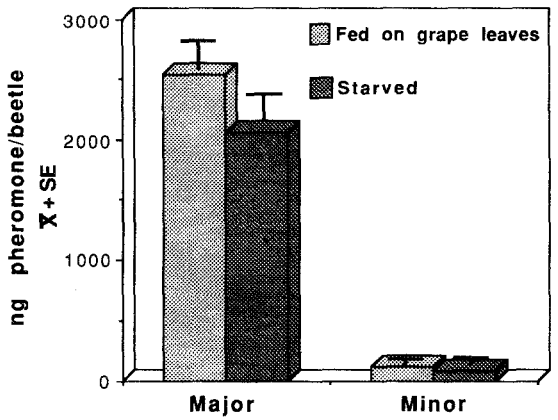

FIG. 3. Amount of (*R*,*Z*)-5-(−)-(oct-1-enyl)oxacyclopentan-2-one (major) and (*R*,*Z*)-5-(−)-(dec-1-enyl)oxacyclopentan-2-one (minor) collected from the headspace of *A. cuprea* virgin females fed on grape leaves and starved females.

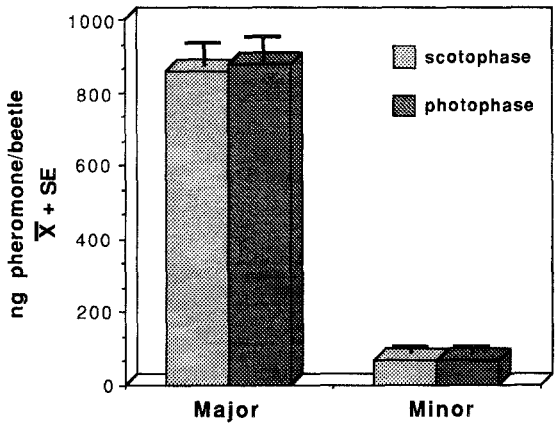

FIG. 4. Amount of sex pheromone released by *A. cuprea* virgin female during scoto- and photophase.

mated females can again produce the major component seems reasonable, since mating occurs several times. However, the reason for a major/minor ratio change remains unclear. One possible explanation would be a selective advantage of virgin females against mated females, giving the former a higher mating probability.

Catches of Cupreous Chafer. Preliminary experiments were carried out with a binary mixture of (*R*,*Z*)-5-(−)-(oct-1-enyl)oxacyclopentan-2-one (92.5%) and (*R*,*Z*)-5-(−)-(dec-1-enyl)oxacyclopentan-2-one (7.5%), which was on aver-

age the ratio detected from virgin females (see Figure 5). A large number of beetles were captured in Tsukuba at the beginning of the flight season (July 2–9). Baited traps caught an average of 52 beetles per trap per day, whereas no beetles were captured in the control traps. Catches in July 8 were limited by the capacity of the trap, i.e., 105 beetles/trap. In Chiba, the synthetic pheromone blend was compared to the catches by two virgin female beetles in tests conducted July 1–6. Pheromone-baited traps caught significantly more beetles than virgin female traps (Figure 6).

A study was carried out to determine the response of the cupreous chafer to different dosages of the 92.5:7.5 pheromone blend. In the experiments of July 18–24, the 100-mg treatment dosage captured significantly more beetles

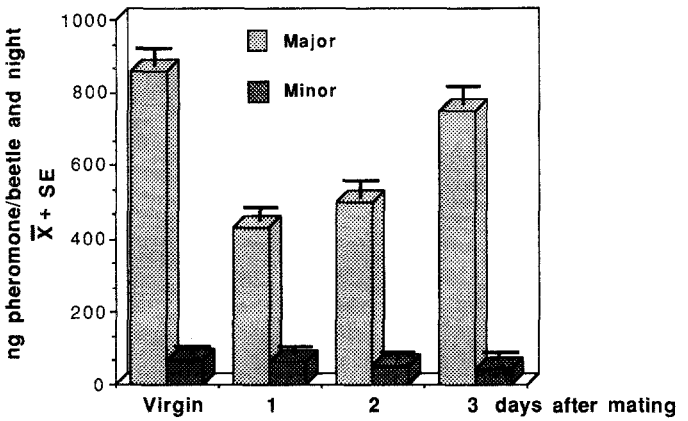

FIG. 5. Effect of mating and isolation on the amount of pheromone released by *A. cuprea*. Major, (*R*,*Z*)-5-(−)-(oct-1-enyl)oxacyclopentan-2-one; minor, (*R*,*Z*)-5-(−)-(dec-1-enyl)oxacyclopentan-2-one.

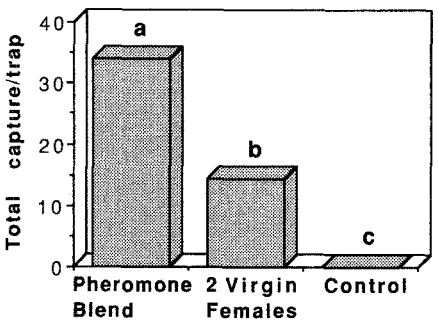

FIG. 6. Response of *A. cuprea* males to traps baited with rubber septa containing the sex pheromone blend, two virgin female beetles, and control.

than the 10-mg dosage (Figure 7). Although later experiments confirmed this trend, the captures at that time were not significantly different, probably due to the decrease of the insect population in the field. Based on these results, as well as economic reasons, the 10-mg dosage was considered to be appropriate for monitoring applications.

In another series of experiments (July 7–12), traps were baited with the pheromone blend in various ratios, in order to establish the optimum for maximum attractiveness to the cupreous chafer. Although traps baited with the pheromone blend containing 10, 15, and 20% of the minor component captured significantly more beetles than those baited with 1 and 5%, there was no significant difference in captures among the 10, 15, or 20% baits (Figure 8). A new series of tests was carried out at the end of the flight season (August 24–September 9), using traps baited with the three ratios of the minor component, namely, 10, 15, and 20%. Again there was no significant difference in captures among the three treatments. This was carried out at two dosages, 10 and 20

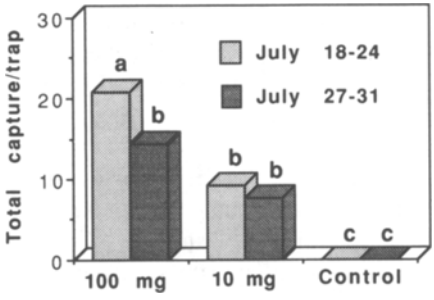

FIG. 7. Effect of the pheromone amount on the capture of *A. cuprea* males.

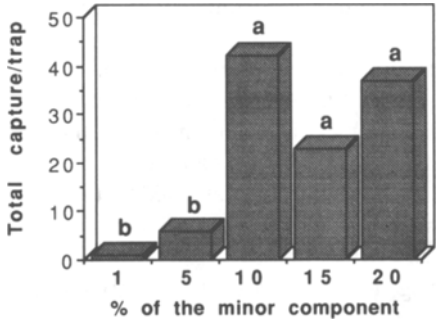

FIG. 8. Response of *A. cuprea* males to traps baited with different ratios of (*R*,*Z*)-5-(−)-(oct-1-enyl)oxacyclopentan-2-one (major)/(*R*,*Z*)-5-(−)-(dec-1-enyl)oxacyclopentan-2-one (minor).

mg, and there was no significant difference in the catches with these ratios. As demonstrated by the pheromone release experiments, the ratio of the two components undergoes great changes, according to the sexual stage of females. Therefore, it is hard to establish one optimal major/minor ratio. Nevertheless, we propose as a rule of thumb that a mixture of 90% (*R*,*Z*)-5-(−)-(oct-1-enyl)oxacyclopentan-2-one and 10% (*R*,*Z*)-5-(−)-(dec-1-enyl)oxacyclopentan-2-one can be considered a workable ratio for monitoring applications. Further experiments, however, will be carried out during the next season(s) to establish the optimal ratio.

Although it was suggested as early as 1964 (Wright, 1964) that multicomponent pheromones would be widely used because they carry a greater amount of information, only now did we obtain concrete evidence that some scarab beetles utilize binary mixtures. This was confirmed to be the case in two species of the genus *Anomala*, namely, *A. cuprea* and *A. daimiana* (Leal et al., 1993). In both cases, these species utilize a pheromone produced by other scarab beetles as the minor component of their own blend. *Holotrichia parallela* also utilizes a minor component, which was identified as *R*-(−)-linalool, as will be described elsewhere (Leal et al., in preparation). On the other hand, there was no evidence of such minor components in *A. schonfeldti* (Leal et al., 1992c) and *Blitopertha orientalis* (Leal, 1993). The latter utilizes a *Z*/*E* mixture, whose significance has yet to be investigated. Based only on the attractiveness of their single-component pheromone systems in the field, *Costelytra zealandica*, *Popillia japonica*, and *A. rufocuprea* do not seem to possess minor components. However, as already pointed out by other authors (Francke, 1992), satisfactory attractiveness in field tests may mask the occurrence of minor components. Results of field experiments with *H. parallela* stress this point. Traps baited only with the major pheromone, L-isoleucine methyl ester (Leal et al., 1992b), captured 26 male beetles per trap per night, which is a satisfactory figure for most applications. The captures, however, tremendously increased to 86 male beetles per trap per night when the minor component, (*R*)-(−)-linalool, was added (Leal, unpublished data).

Interestingly, results of field applications of the sex pheromones of the Japanese beetle, *P. japonica*, and the soybean beetle, *A. rufocuprea*, simultaneously applied in the same traps demonstrated that the Japanese beetle sex pheromone tremendously inhibits the captures of *A. rufocuprea*, but that the catches of the Japanese beetle were not affected by the soybean beetle pheromone (Ono, personal communication).

In conclusion, *A. cuprea* utilizes a sex pheromone blend that can be utilized in field for monitoring the occurrence of this agricultural pest and, thus, minimize pesticide applications.

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