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Sex Pheromones of Plant-Feeding Scarab Beetles

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ABSTRACT From a chemist's perspective, Scarabaeidae is one of the most exciting group of insects to work on the isolation, identification, and synthesis of sex pheromones because-as opposed to Lepidoptera, which by and large utilizes straight chain alcohols, aldehydes, and acetates -the pheromonal chemistry of scarab beetles is remarkably diverse. While species in the subfamily Rutelinae utilize pheromone constituents, which are presumably fatty acid derivatives, the more primitive species in the subfamily Melolonthinae use phenolic, amino acid derivative, and terpenoid compounds. Here, I discuss the recent advances we have accomplished in the identification of scarab sex pheromones with especial emphasis on their chemical diversity. Also, I discuss the potential role of these sex pheromones in insect pest management. Field tests revealed that, in contrast to what has been frequently observed in the Lepidoptera, the higher the dosage of sex pheromone loaded in the traps the greater the capture of scarab beetles. These data suggest that mass trapping is more likely to be useful for scarab pest management than mating distruption

KEY WORDS Sex pheromones, scarabaeidae, rutelinae, melolonthinae, chemical diversity, GC-EAD, GC-BB

Herbivorous scarb beetles (Coleoptera scarabaeidae) include various economically important pests in agriculture, horticulture, and forestry. Both larvae (grubs) and adult damage crops by feeding on underground and aerial parts, respectively. In spite of the fact that many species are economically important pests throughout the world and that alternative methods of control are highly sought after, few studies have been designed to identify sex pheromones of scarab beetles and evaluate their potential in pest management. I have initiated a comprehensive cooperative project to investigate the feasibility of applying semiochemicals in the integrated pest management (IPM) of various scarabs in Japan. As a result, the sex pheromones of some economically important pests have been identified and this brought to light the fact that the chemical diversity of these sex pheromones is remarkably different from that of Lepidoptera species

EFFORT TOWARD SHORT-CUT BIOASSAYS

One of the main barriers in the task of identifying sex pheromones from scarab beetles is the lack of uniform and consistent bioassays due to the complicated behavior of these insects. In order to identify the sex pheromone of the Japanese beetle, for example, attempts to develop a laboratory bioassay in a number of olfactometers and wind tunnels did not consistently yield any quantifiable orientation behavior and ultimately field tests (which are more time- and sample-consuming) were conducted (M. G. Klein, personnal communication)

Coupling gas chromatography (GC) with a biological detector having the insect antenna as the sensing element (EAD) has been demonstrated to be a powerful technique to overcome the difficulty incurred for the lack of appropriate bioassay (Leal *et al* 1992a). The insect antenna is placed in an acrylic stage in such a way to allow the club to be open during the measurements.

Identification of active compounds can also be achieved by behavioral observation of insects placed in an arena (Fig. 1) directly linked to a GC flow (GC-BB) (Leal *et al*. 1992b). Males responded to female sex pheromone by walking or flying towards

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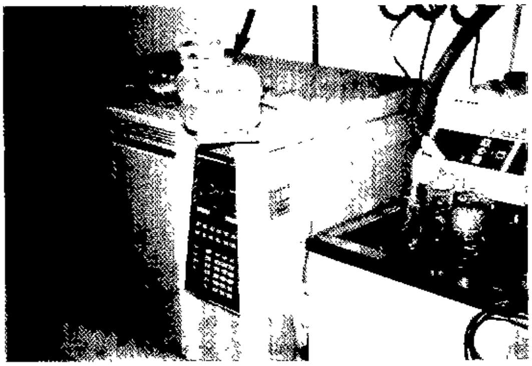


Fig. 1. Gas chromatography-behavior bioassay (GC-BB) setup Males are placed in a plastic box arena (arrow) connected to the GC effluent.

the GC outlet, and gathering on a plastic mesh, allowing one to locate the active peak(s) (Fig. 2).

Wind tunnels (Fig. 3a) are widely applied in pheromone research. Although it could not be used for the Japanese beetle, some species like *Anomala diamiana* fly nicely in a wind tunnel in response to sex pheromone (Fig. 3b) and land on the pheromone source where they search for females (Fig. 3c). This behavior can be quantified and used for the identification of active compounds. For some species like *Anomala cuprea* (Fig. 4), the attractancy to sex pheromone can be also tested in simple walking behavior bioassay in a small plastic box (6×22 ×30 cm)

Use of these different approaches combined with field tests led to the identification of the sex pheromone of various species (Leal 1991; Leal *et al.* 1992a,b,c; 1993a,b,c; Leal 1993, Leal *et al.* 1994a, b,c)

CLASSFICATION AND PHYLOGENY OF SCARABS

Scarabaeidae is one of the 8 families in the superfamily Scarabaeoidea (=Lamellicornia) (Parker 1982) (Fig. 5a). Out of 20 subfamilies of the Scarabaeidae, four are the most notorious because their members are agricultural pests (Fig 5b). Sex pheromones have been identified only from Rutelinae and Melolonthinae species According to Howden (1982), Melolonthinae is the most primitive of these two subfamilies

SEX PHEROMONES OF MELOLONTHINES

The sex pheromone of four Melolonthines have been identified thus far (Table 1) and this indicate that these more primitive species utilize phenolic, amino acids, and terpenoid compounds, which by and large are likely to have amino acids as precursors. Even an amino acid derivative has been identi-

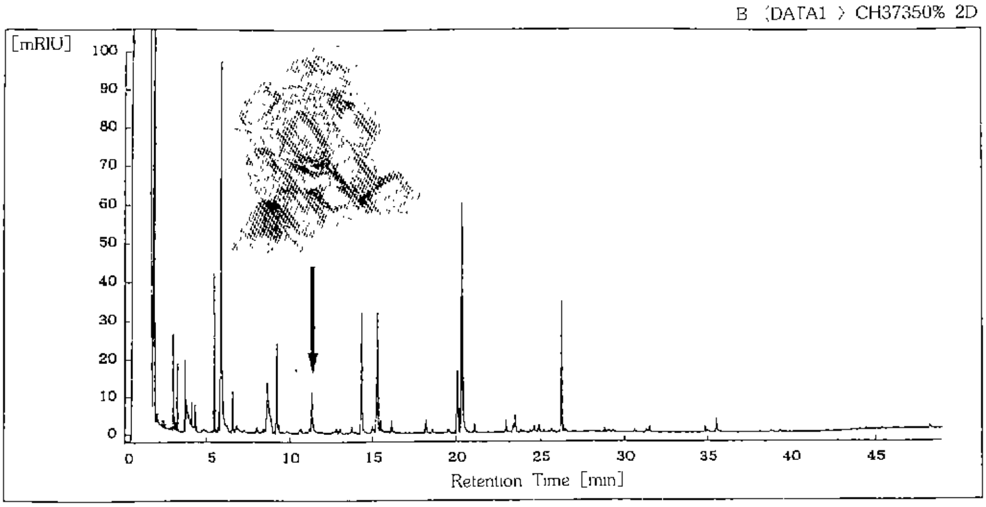


Fig. 2. GC-BB result. *Anomala schonfeldti* males gather on the GC outlet in response to the active peak in the female airborne volatiles at ca. 11.4 min

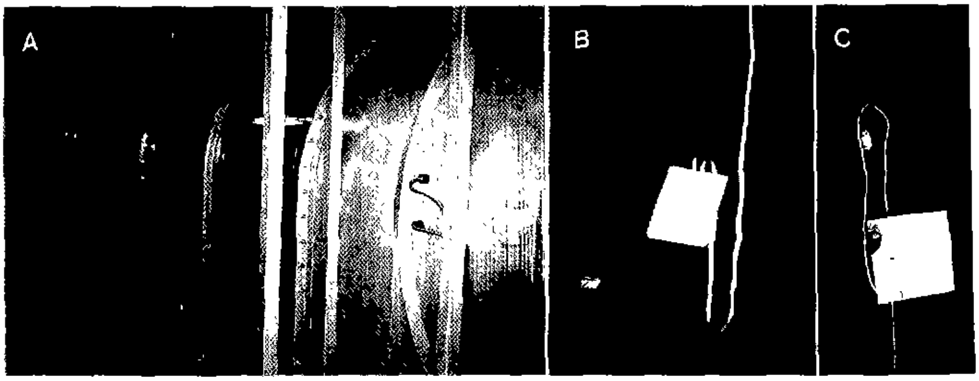


Fig. 3. Behavior of *Anomala daimiana* in a wind tunnel (A). Male flying toward a pheromone source (B) and searching for female after landing on the filter paper (C)

Fig. 4. *Anomala cuprea* males, attracted to a filter paper impregnated with female sex pheromones, try to copulate with each other

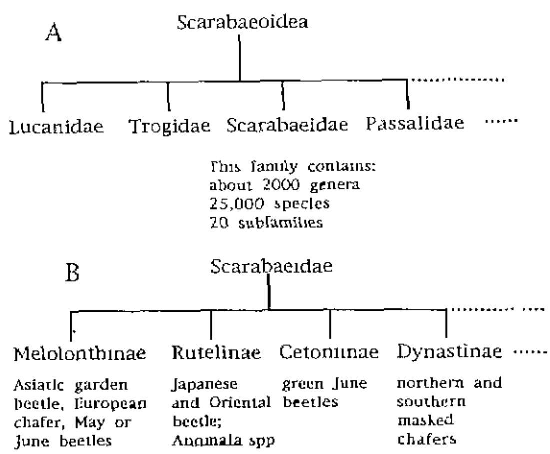


Fig. 5. Classification of scarab beetles.

fied as the major constituent of a *Holotrichia* species. L-Valine methyl ester, found in trace amounts in the female glands of the same species, was inactive to males, but it is the major constituent of a pheromone blend produced by an American *Phyllophaga* species (Leal *et al.* unpublished data). Some of these compounds showed antimicrobial activity (Leal and Shirata 1992a, b). These findings along with the evidence that attractancy of *H. parallela* sex pheromone is not affected by non-natural enantiomers and diastereomers (Matsuyama *et al.* 1994) suggest that these semiochemicals evolved from a more primitive role as defensive substances (Leal 1995).

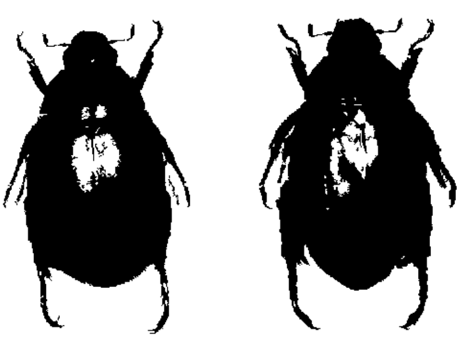
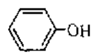
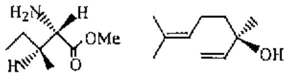
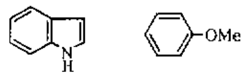
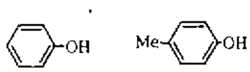


Fig. 6. *Anomala albopilosa sakishimana* (left) and *A cuprea* utilize the same sex pheromone blend, but are geographically isolated.

Table 1. Sex pheromones of mololonthines

Costelytra zeolandica		(Henzell and Lowe, 1970)
Holotrichia parallela		(Leal *et al.* 1992c, 1993b)
Holotrichia consanguinea		(Leal *et al* unpublished data)
Phyllophaga cuyabana		(Leal *et al* unpublished data)

SEX PHEROMONES OF RUTELINES AND ITS POSSIBLE ORIGIN

Although the biosynthesis of scarab sex pheromones have not been studied thus far, it is likely that most of them are derived from fatty acids. Japonilure and methyl 5-(Z)-tetradecenoate could be produced from oleic acid by two cycles of β-oxidation, which by esterification would produce the ester utilized by *Anomala rufocuprea*. while allylic oxidation, followed by cyclization would give rise to japonilure (Leal 1995).

Despite to the fact that Rutelines utilize closely related compounds, species isolation is acheived because species using even the same pheromone blend are geographically and/or seasonally isolated, *A. albopilosa sakishimana* and *A. cuprea* (Fig. 6) use a mixture of two lactone compounds (Table 2), but cross-attraction does not take place because the former is restricted to Miyakojima Island in the South of Japan whereas the latter occurs in the mainland of the Japanese archipelago. On the other hand, *A. octiescostata* and *A. cuprea* use also the same blend of lactones (Table 2), but the former occurs in early spring whereas the flight season of the latter is in summer

APPLICATION OF SCARAB SEX PHEROMONES

Because of the difficulty of control most scarab pests with insecticides and the demand for safer agrochemicals, the identification of these sex pheromones paved the way for their use in integrated pest management Seven formulations for monitoring of scarab species are now produced by Fuji Flavor and commercialized by Japan Tobbaco. Although these products are registered only for monitoring of some species, preliminary field experiments suggest that they may be useful for pest control.

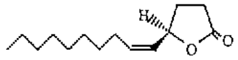
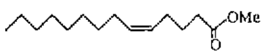
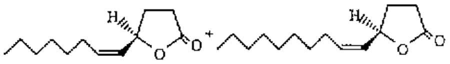
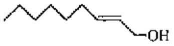
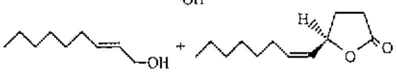
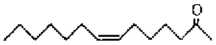
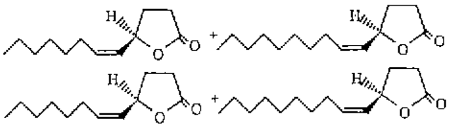
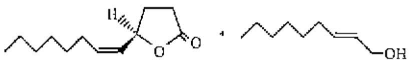
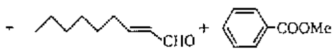
For species that utilize a binary mixture as a sex pheromone blend, it has been observed that the ratio of the two compounds is not a critical factor as for the case of moths. For example, deviation from the natural ratio (8 : 2) of the sex pheromone emission by female *A. octiescosta* did not significantly diminish the attraction of males to traps in the field (Leal *et al.* 1994b). In Lepidoptera the response of males diminishes markedly even with slight deviations from the natural proportion. In addition, saturation due to an increased dosage of sex pheromone, which is commonly found in Lepidoptera, has not been observed for the species studies These findings suggest that these sex pheromones may be more feasible for mass trapping than to mating disruption, although none of these techniques has been evaluated in the field. Preliminary experiments on the application of these sex pheromones for pest management are underway.

CONCLUSIONS

The lack of uniform and consistent bioassays for the identification of scarab beetle sex pheromones can be overcome by the use of "short-cut" techniques, such as GC-EAD and GC-BB.

The identification of sex pheromones of other scarab species will allows us to test our hypothesis

Table 2. Sex pheromones of rutelines

Popillia japonica		(Tumlinson et al 1977)
Anomala rufocuprea		(Tamaki et al. 1985)
Anomala cuprea		(Leal 1991 and Leal et al. 1993a)
Anomala schonfeldti		(Leal et al. 1992b)
Anomala daimiana		(Leal et al. 1993c)
Exomala orientalis		(Leal 1993 and Leal et al 1994a)
Anomala octiescostata		(Leal et al 1994b)
Anomala albopilosa sakishimana		(Leal et al. 1994c)
Anomala japonica		(Leal et al. unpublished data)

that sex pheromones of the most primitive group, Melolonthinae, have evolved from primary defensive role and that sex pheromone of the most evolved one, Rutelinae, are derived from fatty acids.

These sex pheromones can be used for monitoring of scarab populations and are likely to be useful in mass trapping of some species.

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