Aggregation Pheromone of the African Rhinoceros Beetle, *Oryctes monoceros* (Olivier) (Coleoptera: Scarabaeidae)

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Ethyl 4-methyloctanoate is a male-produced aggregation pheromone of the African rhinoceros beetle, *Oryctes monoceros* (Olivier). It was identified by coupled gas chromatographic-electroantennographic detection (GC-EAD) and coupled GC-mass spectrometry (MS) of Porapak Q-trapped male-produced volatiles. Natural and synthetic ethyl 4-methyloctanoate elicited comparable antennal responses. In a field experiment (La Me Research Station, Côte d'Ivoire), synthetic ethyl 4-methyloctanoate released at 30 mg/day attracted both male and female *Oryctes*, whereas the known, non-beetle-produced attractant, ethyl chrysanthemate, did not attract any *Oryctes*. Upon improvement of trap design and placement, and the identification of synergistic (plant) volatiles, pheromone-based mass trapping may become an alternative and/or additional strategy to manage *O. monoceros*, one of the most destructive pests of commercial coconut, oil, and date palms in Africa.

Introduction

The African rhinoceros beetle, *Oryctes monoceros* (Olivier) (Coleoptera: Scarabaeidae), is one of the most destructive pests of commercial coconut, oil and date palm in Africa (Hill, 1983). While the larvae develop in decomposing organic matter, adults feed inside of unopened fronds and meristem of palms. Beetle attacks kill young palms, provide entry holes for lethal diseases and other destructive insects, damage inflorescences and reduce photosynthetically active foliage, thereby diminishing revenue of oil and coconut production (Mariau *et al.*, 1981). Introduction of pathogenic baculovirus, *Rhabdionvirus oryctes*, suppressed populations of the rhinoceros beetle, *O. rhinoceros*, in parts of Asia (Bedford, 1986; Zelazny and Alfiler, 1987, 1991; Young, 1986) but did not effect *O. monoceros* in Africa (Julia and Mariau, 1976a).

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O. monoceros is currently controlled by silvicultural methods (Hinckley, 1973; Ouvrier, 1980) and removal of adults from young palms and larvae from decomposing logs. Pheromone-based trapping would be an ideal alternative and/or additional strategy to manage rhinoceros beetles in Africa. We report the identification of a male-produced aggregation pheromone in *O. monoceros*.

Materials and Methods

Male (15) and female (18) *O. monoceros* were collected in oil palm plantations 40–50 km northeast of Abidjan, Côte d'Ivoire, and placed together in a modified Nalgene desiccator (Oehlschlager *et al.*, 1992). An aspirator-driven charcoal-filtered airstream was maintained through the desiccator for 5 days, collecting male- and female-released volatiles on Porapak Q. In a second experiment, 11 females and 13 males were aerated separately for 7 days. Volatiles were eluted from Porapak Q with pentane, concentrated by distillation and subjected to gas chromatographic (GC) analysis with flame ionization (FID) and electroantennographic detection (EAD) (Arn *et al.*,

1975), employing a Hewlett-Packard (HP) 5890 A gas chromatograph equipped with a 30 m×0.25 mm ID SP-1000-coated, fused silica column (Supelco Inc., Bellefonte, PA 16823). Coupled GC-mass spectrometry (MS) was conducted using a HP 5985 B GC-MS fitted with the same column.

The candidate pheromone ethyl 4-methyloctanoate was synthesized by conjugate addition of an organocuprate to ethyl acrylate (Corey and Boaz, 1985; Matsuzawa *et al.*, 1989). This was prepared by addition of CuCN (10 mol%) to 2-hexylmagnesium bromide in THF at −40 °C. After stirring 30 min, the solution was cooled to −78 °C, and trimethylchlorosilane and HMPA (2.4 equiv. each) and ethyl acrylate (2 equiv.) in THF were added dropwise *via* cannula. After 2 h, work up of the reaction followed by column chromatography gave ethyl 4-methyloctanoate (95% pure) in 56% yield.

This compound was tested 8–17 May 1993 in 3- to 4-year-old oil palm plantations at the La Me Research Station, Côte d'Ivoire. 15 l Plastic buckets (Oehlschlager *et al.*, 1993) 1–2 m apart from palms were employed as pitfall traps (side entrance holes at ground level) in randomized complete blocks with traps at 27 m intervals and blocks 27–500 m apart. A 4-treatment, 8-replicate experiment tested attraction of the known, non-beetle-produced attractant ethyl chrysanthemate (EC) (Maddison *et al.*, 1973; Julia and Mariau, 1976 b) (30 mg/day, @25 °C) and the candidate pheromone ethyl 4-methyloctanoate released at 3 doses (0.3, 3, or 30 mg/day, @25 °C). Petrolatum (Anachemia, Rouses Point, N.Y. 12979) on the inner bucket surface below side entrances and a wet sponge treated with insecticidal Evisect "S" (3% thiocylam-hydrogenoxalate in water) at the bottom of the bucket, assured retainment of captured beetles. Weakly effective insecticide and petrolatum were probably unnecessary because captured beetles could not climb the smooth plastic bucket.

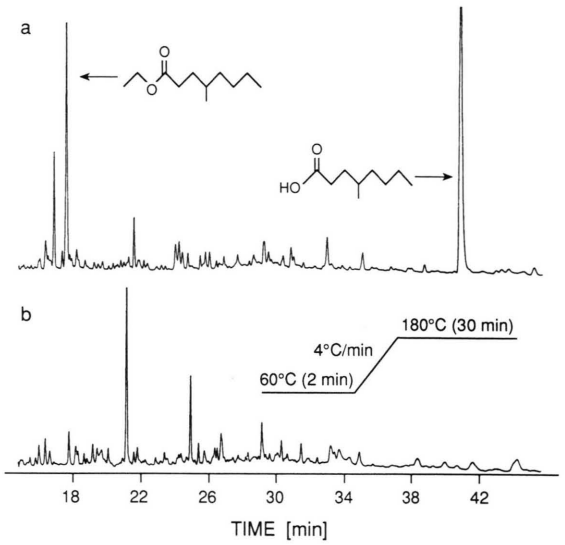

Fig. 1. Gas chromatograms of volatiles from male (n = 13) (a) and female (n = 11) (b) *O. monoceros* maintained in aeration chambers for 168 h without provision of food. Chromatography: Hewlett-Packard 5830 A equipped with a glass capillary column (30 m×0.5 mm ID) coated with SP-1000.

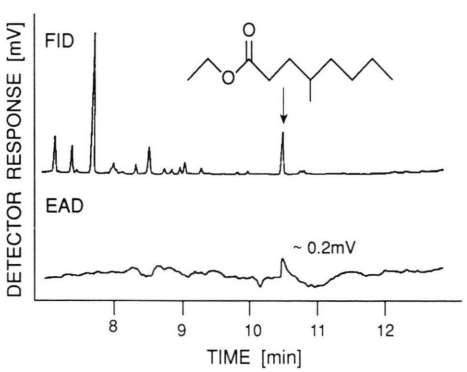

Fig. 2. Representative recording (n = 9) of FID and EAD responses to volatiles obtained from male and female *O. monoceros*. The antennal recording was carried out with an antenna of a female beetle. Chromatography: Hewlett-Packard (HP) 5890 A gas chromatograph (GC) equipped with a fused silica column (30 m×0.25 mm ID) coated with SP-1000; 1 min at 70 °C, 10 °C/min to 180 °C.

Results and Discussion

GC and GC-EAD analyses of Porapak Q-trapped volatiles obtained from aerations of either *Oryctes* males, females or both sexes combined revealed two male specific compounds (Fig. 1), of which the early eluting volatile elicited responses from male and female antennae (Fig. 2). This male-specific compound was not detected by FID, GC-MS or EAD in female-produced volatiles (Fig. 1). GC-MS of the antennally active compound (Fig. 3) indicated an ethyl ester (MW =

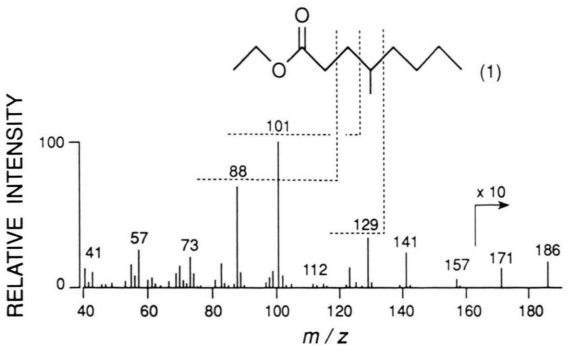

Fig. 3. Electron impact (70 eV) mass spectrum of ethyl 4-methyloctanoate. Chromatography: Hewlett-Packard 5985 B GC-mass spectrometer; column and temperature program as in Fig. 2.

186) (Jennings and Shibamoto, 1980) with a retention index lower than that of isomeric, straight chain ethyl nonanoate. Based on the increased intensity of the *m/z* 101 and *m/z* 129 fragmentation ions (Fig. 3), we hypothesized that the compound was ethyl 4-methyloctanoate. Identical retention and mass spectrometric characteristics as well as comparable antennal activity of synthetic and male-produced ethyl 4-methyloctanoate confirmed this structural assignment.

In a field experiment, ethyl 4-methyloctanoate released at 30 mg/day attracted 6 males and 5 females in 9 days, whereas the known attractant ethyl chrysanthemate at 30 mg/day did not attract any *Oryctes*. Lower release rates of the pheromone were not attractive.

In assessing absolute trap captures, low relative abundance of these very large insects must be taken into account. In 1992 *e.g.*, weekly removal of *Oryctes* from palms had revealed ~9 adults per hectare per month in these plantations (M. Zebeyou, unpublished). Had the pheromone experiment been conducted in beetle-preferred coconut rather than oil palm stands, and not prior to but in the middle of the raining season during which *Oryctes* is more abundant (Mariau *et al.*, 1981), trap catches probably would have been higher. However, capture of 11 *Oryctes* to ethyl 4-methyloctanoate *versus* none to the known attractant EC clearly indicates superior attraction of the aggregation pheromone. Addition of as yet unknown plant volatiles will likely enhance attraction of the aggregation pheromone. Palm weevil aggregation pheromones in the *Rhynchophorinae, e.g.*, are hardly attractive by themselves and require the presence of synergistic plant volatiles (Gries *et al.*, 1993; Hallett *et al.*, 1993; Weissling *et al.*, 1994). Capture rates of *Oryctes* might also be improved by refining and optimizing pheromone formulation, and trap design and placement.

Molecular structures of scarab beetle pheromones are diverse. (*R,Z*)-5-(1-Decenyl)dihydro-2(3H)-furanone (Tumlinson *et al.*, 1977), methyl (*Z*)-5-tetradecenoate (Tamaki *et al.*, 1985) and (*R,Z*)-5-(−)-(oct-1-enyl)oxacyclopentan-2-one (Leal, 1991) are female-produced sex pheromones of Japanese beetle, *Popillia japonica,* soybean beetle, *Anomala rufocuprea,* and cupreous chafer beetle, *A. cuprea,* respectively. Females of the grass grab beetle, *Costelytra zealandica,* utilize phenol to attract males (Henzell and Lowe, 1970), whereas males of the dung beetle, *Kheper lamarcki,* release hexadecanoic acid, 2,6-dimethyl-5-heptenoic acid and (*E*)-nerolidol along with a polypeptide pheromone carrier (Burger *et al.*, 1983, 1990). Although only 5 insects were caught in a field experiment, Leal *et al.* (1992) provide evidence that female large black chafer, *Holotrichia paralellela,* attract males with L-isoleucine methyl ester, a unique amino acid derived sex pheromone.

Unlike these previously identified sex pheromones in the Scarabaeidae, ethyl 4-methyloctanoate is a novel aggregation pheromone for which we propose the trivial name "oryctelure". Future research targets the development and implementation of oryctelure for control of *O. monoceros* in commercial oil, coconut, and date palms in Africa.

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