Identification of the Aggregation Pheromone of the Invasive Guam Strain of Coconut Rhinoceros Beetle, *Oryctes rhinoceros*, and Determination of Stereochemistry

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Introduction

The coconut rhinoceros beetle, *Oryctes rhinoceros* (Linnaeus 1758) (Coleoptera: Scarabaeidae: Dynastinae) (CRB), is endemic to tropical Asia where it damages both coconut and oil palm (Fig. 1). A new invasion by CRB occurred on Guam in 2007 and eradication attempts failed using commonly applied *O. rhinoceros* nudivirus (OrNV) isolates. This and subsequent invasive outbreaks were found to have been caused by a previously unrecognized haplotype, CRB-G, which appeared to be tolerant to OrNV.

Fig. 1. The coconut rhinoceros beetle, *Oryctes rhinoceros* (Coleoptera: Scarabaeidae: Dynastinae) (CRB) and the damage caused to a coconut plantation.

The male-produced aggregation pheromone of the susceptible strain of *O. rhinoceros* (CRB-S) was previously identified as ethyl *(S)*-4-methyloctanoate. Following reports from growers that commercial lures containing the racemic compound were not attractive to CRB-G, the aim of this work was to identify the pheromone of CRB-G.

Material and Methods

Insects

Insects of the Guam strain of *O. rhinoceros* (CRB-G) were collected in plantations of the Guadalcanal Plains Palm Oil Limited (GPPOL) in the Solomon Islands.

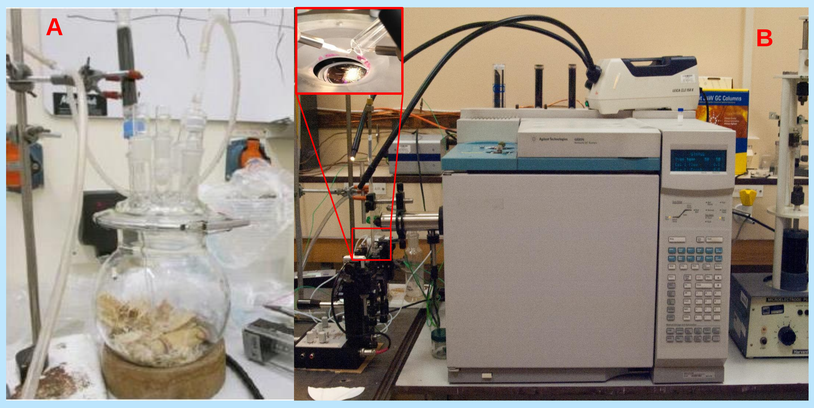

Fig. 2. A: Entrainment apparatus for collecting volatiles from beetles, B: Gas Chromatography coupled Electroantennography (GC-EAG) apparatus.

Collection and analysis of volatiles

Volatiles were collected from individual beetles on Porapak resin (Fig. 2A) and eluted with dichloromethane. Collections were analysed by gas chromatography (GC) coupled to mass spectrometry (MS) on polar (DBWax), non-polar (VF5) and CP-Chirasil-Dex CB columns. Collections were also analysed by GC coupled to electroantennographic (EAG) recording from a beetle antenna (Fig. 2B).

Field trapping experiments

Field trials were carried out in plantations of GPPOL in the Solomon Islands. Traps were constructed from PVC pipe (3 m long x 15 cm diameter) with open ends positioned vertically with the lower end in a bucket on the ground to capture beetles (Fig. 5). Catches were compared in traps baited with racemic, (*S*)- and (*R*)-enantiomers of ethyl 4-methyloctanoate, combinations of racemic, (*S*)- and (*R*)-enantiomers of ethyl 4-methyloctanoate with the corresponding acid and a standard ChemTica lure (containing a racemic mixture).

Results and Discussion

Male CRB-G beetles were shown to produce ethyl (*R*)-4-methyl octanoate and the corresponding acid by GC analysis of the acid on an enantioselective column (Fig. 3). Male CRB-S beetles were also shown to produce the (*R*)-enantiomers.

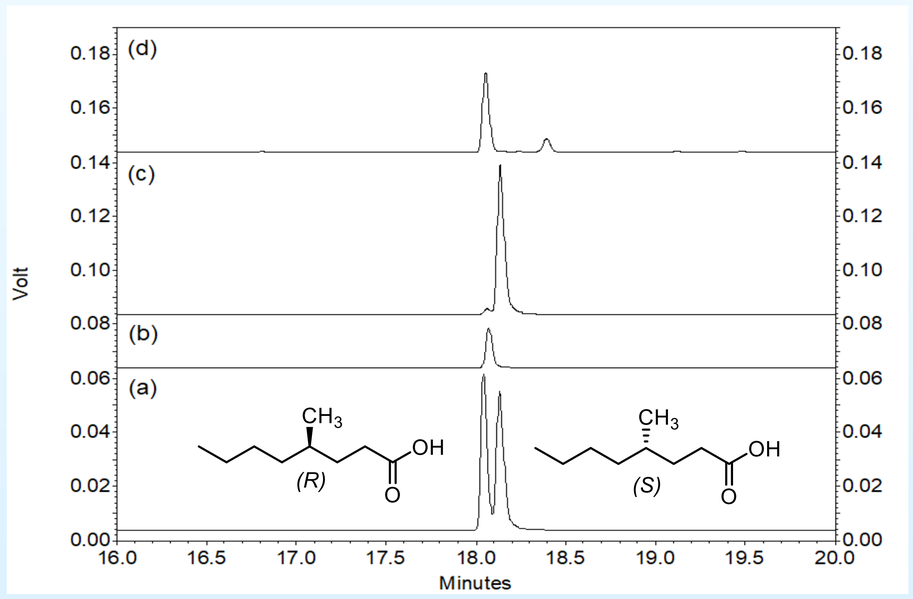

Fig. 3. Analyses on enantioselective cyclodextrin GC column of (from bottom) (a) racemic 4-methyloctanoic acid, (b) (*R*)-4-methyloctanoic acid, (c) (*S*)-4-methyloctanoic acid, and (d) a volatile collection from male Guam strain Oryctes rhinoceros beetles (CRB-G) after hydrolysis of ethyl 4-methyloctanoate to 4-methyloctanoic acid, showing this is exclusively the (*R*)-enantiomer

In GC-EAG analyses of synthetic standards a consistent response was observed from antennae of both males and females to ethyl 4-methyloctanoate (Compound (II), Fig. 4) and not to other potential pheromone components.

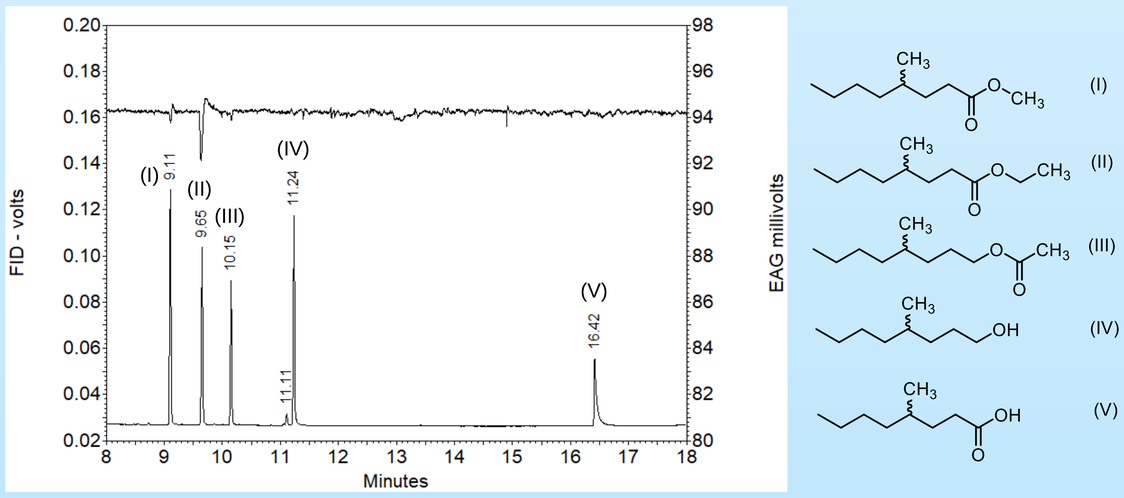

Fig. 4. GC-EAG Analysis of synthetic standards with antenna of male Guam strain Oryctes rhinoceros (CRB-G). Upper trace EAG, lower trace FID

Catches of CRB-G beetles in traps baited with racemic or the (*R*)-enantiomer of ethyl 4-methyloctanoate were significantly higher than those in traps baited with the (*S*)-enantiomer (Fig. 5). Addition of the corresponding acid increased catches when added to the racemate.

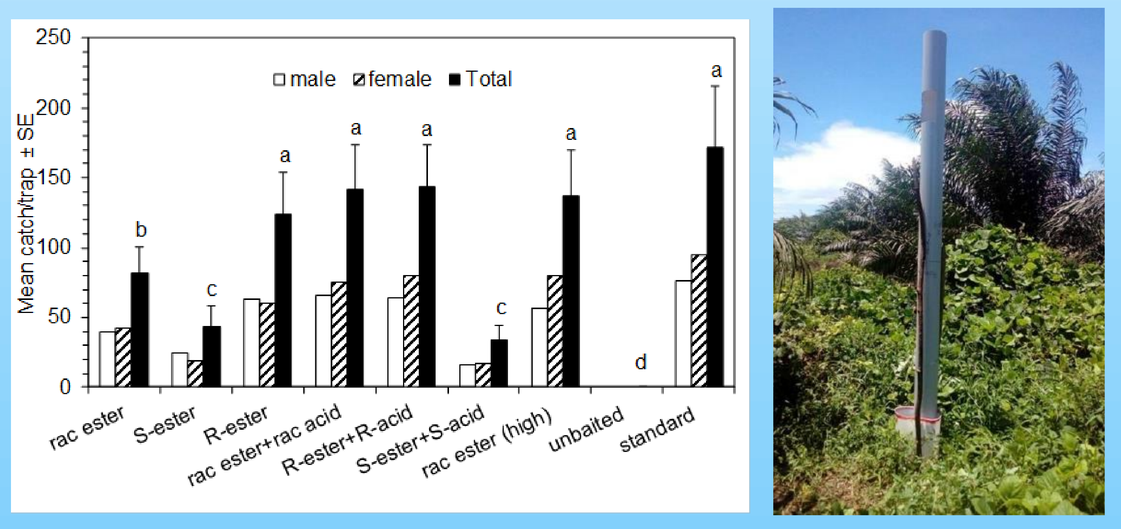

Fig. 5. Mean catches of Guam strain Oryctes rhinoceros (CRB-G) beetles in traps baited with individual chemicals (20 June – 11 July 2017; N = 6). Means with different letters are significantly different ($P < 0.05$). Ester is ethyl 4-methyloctanoate; acid is 4-methyloctanoic acid; standard is Sime Darby/ChemTica lure.

Reasons for the difference in configuration of the pheromone components found here and that reported previously are being investigated..

ACKNOWLEDGEMENTS

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