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TERPENES AS POTENTIAL SEMIOCHEMICALS FOR THE COCONUT PEST, *RHYNCHOPHORUS FERRUGINEUS* (COLEOPTERA: CURCULINOIDAE): AN ELECTROANTENNOGRAM ASSAY.

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Abstract : Electroantennogram (EAG) responses of male and female *Rhynchophorus ferrugineus* F. (Coleoptera: Curculionidae) to sixteen terpenes were determined. The chemoreceptive sensitivity of *R. ferrugineus* was sensitive to the size and the position of the oxygen function, degree of unsaturation and the arrangement of olefinic bonds in the molecules.

A significant ($P < 0.001$, t-test) reduction of the EAGs of terpene alcohols was observed when hydroxyl groups were acetylated. Thus linalool and terpineol (EAGs 17.50 and 17.52 respectively) elicited EAGs of 11.24 and 12.36 respectively with the corresponding acetates. The antennal responsiveness of *R. ferrugineus* to terpene alcohols did not differ significantly from their corresponding carbonyl analogues. Terpenes bearing non-terminal OH/C=O had higher EAG potency significantly different ($P < 0.001$) from those with terminal -OH/ C=O.

Combining the above findings with the olefinic moieties of the molecule, the terpenes could be divided into two classes *viz.* a) Terpenes with 3 unsaturations, 1-3 unsaturation with non terminal -OH /C=O and cyclic structure with non-terminal -OH/C=O b) Terpenes having isolated diene systems, terpenes with terminal OH/C=O and terpenes having both of the above characters. Comparison of EAG activities of the two classes indicated that the former category had greater EAG potency (class mean 18.6) significantly different ($P < 0.001$, t-test) from the latter (class mean 14.9). However even the most favoured structure elicited lesser EAG response than the natural host attractant, isolated from the steam volatiles of coconut bark, (EAG 31.49).

Key words : *Rhynchophorus ferrugineus*, red weevil, coconut pest, EAG potency, terpenes, high unsaturation, non terminal -OH or C = O.

INTRODUCTION

Host finding behaviour of Coleopterans is an outcome of an intriguing system of chemical communication which in many respects resembles that of the social insects.[1] The most thoroughly studied are the family scolytidae whose attraction to aggregation on a suitable host was known as early as 1787.[2] It is known now that monoterpene alcohols such as ipsdienol, ipsenol, cis & trans - verbenol, exo-brevicomin, frontalin and other aliphatic alcohols with a maximum of 10 carbon atoms are used by scolytids as their attractants. Coleopterans also use the same type of compounds with the exception that the carbonyl function is also common in their attractants.[3]

Rhynchophorus ferrugineus F. (Coleoptera: Curculionidae) commonly known as the red weevil, is one of the five major pests on coconut palm in Sri Lanka.[4] The damage caused by this pest to coconut palms in Sri Lanka and the inadequacy of protective measures has been described previously.[5] As an alternative approach semiochemical baited traps could be used in the field to lure the adult pest in the field. Such an early detection of adult weevils in the field could be an early warning to the cultivators in order to take suitable precautionary measures.

In a previous behavioural assay, it was shown that *R. ferrugineus* is strongly attracted to the steam volatiles of the young coconut bark, and four simple aliphatic alcohols.[5] The olfactory basis for the antennal responsiveness of the red weevil to compounds belonging to four different classes *viz* . lower aliphatic alcohols, simple aromatics, long chain aliphatics and terpenes has been elucidated previously and the terpenes as a class had high electroantennogram (EAG) potency.[6] The present study was undertaken to investigate the preferred structural requirements for the antennal receptors of *R. ferrugineus*. It was expected that the information obtained might lead to the formulation of an artificial attractant.

METHODS AND MATERIALS

Chemicals: The source and gas liquid chromatographic (GC) purity of chemicals subjected to EAG assay are listed in Table 1. Ethanol and hexane were purchased from Merc. Chemical Co. Germany.

Table 1: Source and purity of test compounds used in EAG assay

Compound	Source of supply	Chemical purity (%)*
1) Terpinyl acetate	Holzminden, Germany	99
2) Terpinene	Holzminden, Germany	99
3) α - Terpineol	Holzminden, Germany	96
4) Linalool	Holzminden, Germany	96
5) Linalool acetate	Haarmann Reimer GmbH	98
6) Caryophyllene	Haarmann Reimer GmbH	99
7) Pulegone	Haarmann Reimer GmbH	98
8) β-ionol	Haarmann Reimer GmbH	97
9) Limonene	Haarmann Reimer GmbH	96
10) Ips dienol	Haarmann Reimer GmbH	94
11) Myrcene	Haarmann Reimer GmbH	96
12) 3-pinanol	Sigma Chem. Co, Germany	96
13) Neral	Sigma Chem. Co, Germany	94
14) Pulegol	Sigma Chem. Co, Germany	95
15) Nereol	Haarmann Reimer GmbH	98
16) β-Ionone	Haarmann Reimer GmbH	98

*Purity was estimated by GLC

EAG assay: Newly emerged *R. ferrugineus* between age 1-5 days were air lifted from Sri Lanka to Germany. They were maintained in a photoperiod regime of 10 hr light

14 hr dark at 29 ± 2^0C and were fed apples and water. One weevil (age 3-8 days) was anesthetized with a stream of CO_2 and the antenna was cut immediately as close to the base as possible and isolated. Following the methodology originally described by Schneider[7] (1957) the antenna was fixed on two Ag-AgCl capillary electrodes filled with insect ringer solution.[8] The recording electrode was inserted into the antennal club and the indifferent electrode was inserted into the base of the antenna. Stimuli were delivered at 3 min intervals as 10 μl aliquots on filter papers (20 x 7 mm) placed inside glass cartridges (75 mm long, 5 mm int.diam.) and oriented towards the antennal preparation from ca. 1 cm. A stream of filtered air was purged through the cartridge for 1 sec. A vacuum outlet was positioned approximately 10 cm behind the setting of the antenna in order to remove attractant- containing air from the area surrounding the antenna. A stream of filtered air (0.5 L/min) continuously bathed the antenna except during the time that the stimuli were sent. The responses of the antenna to test compounds were recorded as peaks and the distance between the base of the peak and the highest point was measured. Test compounds were presented to the antenna on a random basis and each compound was assayed on both male and female antennae. The life-time of an antenna of *R. ferrugineus* was approximately 30 min and therefore to test 16 compounds a total of 6 (3 male and 3 female) antennae were used. Each test compound was presented to the antenna a minimum of 6 and maximum of 13 times. Interspersed between every fifth test odor stimulation were control stimulations throughout the course of the experiment and were used to "correct" for individual and time dependent alterations in responses.[9] Mean EAG responses versus structural features of the individual terpenes were examined and the two classes recognised consequently, were compared using Student's t-test.[10]

RESULTS

EAG responses of *R.ferrugineus* males and females to the control, ethanol (EAG = 5.48, SD = 1.79 and EAG = 4.83, SD = 2.3 respectively) did not differ significantly ($P > 0.05$, t-test) and therefore the data were pooled. The mean responses of male and female red weevils to the control, was -0.50 mV (SD = 0.10, n = 6). The results of the EAG activities to test compounds are presented in Figure 1.

The antennal responses of *R. ferrugineus* to terpene alcohols were significantly different ($P < 0.001$, t-test) from the corresponding acetates. For example, terpineol and terpineol acetate elicited EAGs 17.52 (SD = 3.87, n = 7) and 12.36, (SD = 1.82, n = 5) respectively and linalool and linalool acetate had EAGs 17.50 (SD = 4.53, n = 13) and 11.2 (SD = 1.66, n = 6) respectively. The mean responses of *R. ferrugineus* to terpene alcohols however did not differ significantly ($P > 0.05$, t-test) from those of the corresponding carbonyl analogues. The examples are pulegol (EAG 18.55, SD = 4.4, n = 8) and pulegone (EAG 19.97, SD = 5.72, n = 6); β-ionone (EAG 17.36, SD = 3.65, n = 7) and β-ionol (EAG 20.92, SD = 3.94, n = 9); nereol (EAG 14.73, SD = 4.33, n = 6) and neral (15.91, SD = 3.55, n = 7).

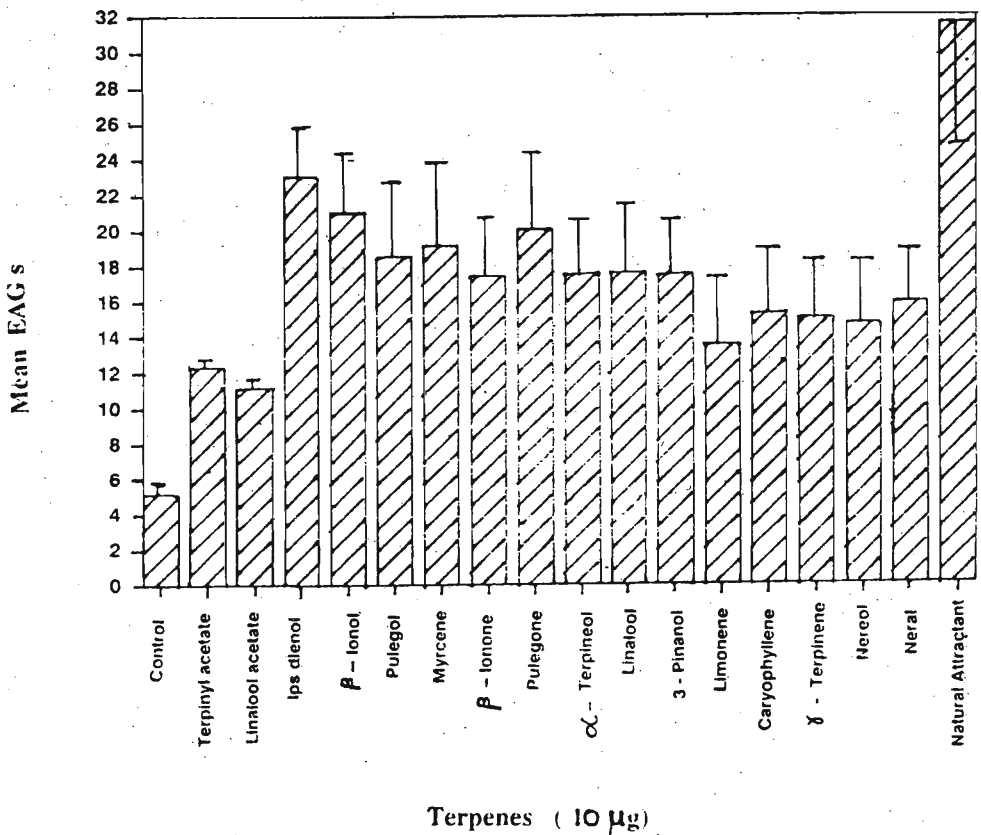

Figure 1: Mean Electroantennogram Responses (± SD) of R. ferrugineus to 10 mg doses of different terpenes.
*** Ethanol was used as the control and the control response was approximately -0.50 mv.**

Based on the EAGs elicited in the bioassay two categories of terpenes were recognized a) Terpenes with 3-unsaturations, 1-3 unsaturations with non-terminal OH or C=O, and cyclic structure with non terminal -OH or C=O b) Terpenes with isolated diene systems, terpenes with terminal -OH or C=O and terpenes with both of the above. The decreasing order of EAG responses of *R. ferrugineus* to the terpenes belonging to above two categories are as follows; ipsdienol (EAG 23.0, SD=3.0, n=10), β-ionol >pulegone >myrcene (EAG 19.1, SD=5.7, n=6) > pulegol >linalool >α -terpineol > 3-pinanol (EAG 17.45, SD=4.11, n=6) β-ionone α-terpinene (EAG 15.52, SD=3.80, n=7) caryophyllene (EAG 15.27, SD=4.38, n=9) limonene (EAG 13.53, SD=5.39, n=6). The natural attractant of the red weevil resulted from the steam distillation of the coconut bark[5] however had the highest EAG potency of EAG 31.5 (SD =6.28) compared to all other single terpenes tested.

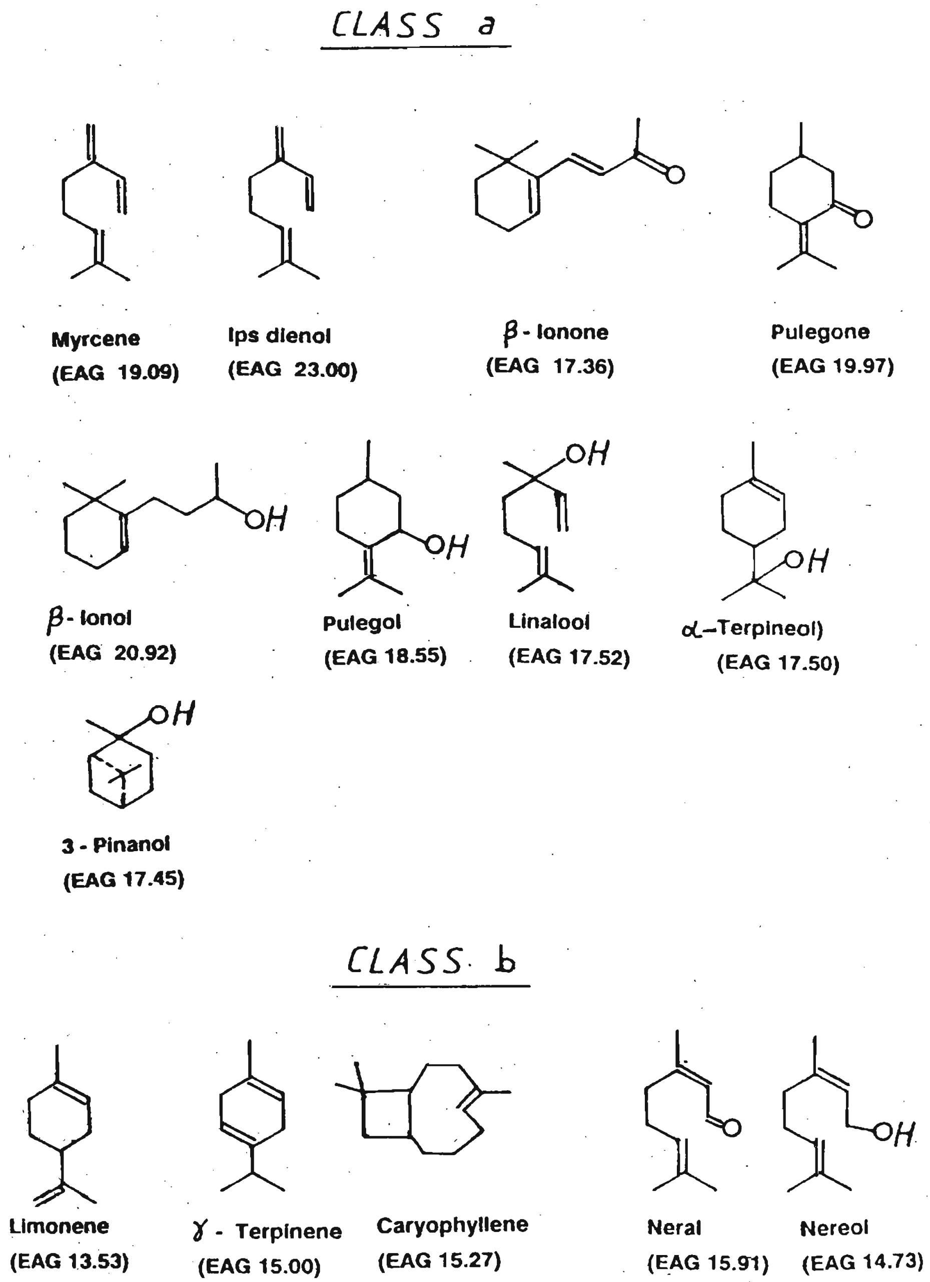

Figure 2: Two classes of candidate terpenes based on EAG values and structural features.

DISCUSSION

High responsiveness of the antennal receptors of *R. ferrugineus'* receptors to some structural features of terpenes has been demonstrated in the present study. The size of the oxygen functionality was identified as one of the important factors influencing the EAG activity of *R. ferrugineus*. Significant differences in the EAGs between terpene alcohols and those of the corresponding acetates were exemplified by terpineol and linalool and their respective acetates. From three dimensional considerations, the size of an -OH is expected to be two/three times smaller than that of an acetate group. Further the EAG responses of *R. ferrugineus* to alcohols did not differ significantly from those of the carbonyl analogues as exemplified by pulegol /pulegone (18.55/19.78); ionol/ionone (20.92/17.36) and neral /nereol (15.91/14.73). Preference of the red weevil antennal receptors to alcohol/ ketone functions is in accordance with the reported data because unlike lepidopteran attractants where the dominating candidates are acetates, those of coleopterans are alcohols and ketones.[3]

Of the terpene candidates, differing in their olefinic moieties and the position of -OH/C=O , two classes were recognized based on the EAG results. Terpenes having greater EAG potency had one of the following characteristic structures, a) 3 unsaturations of which two are conjugated, 1-3 unsaturation with non terminal OH/C=O, cyclic structure with non terminal OH/C=O while those with lower EAG potency had either isolated diene system or terminal -OH/C=O or both (Fig 2). The type a) represented by ipsdienol (EAG 23.0) and β-ionol (20.92) were the most potent EAG stimulants. The second highest EAG stimulants were pulegone (19.97) and myrcene (19.0) while other terpenes belonging to this category were terpineol(17.5), linalool(17.5), 3-pinanol (17.45), β-ionone (17.36).

The fact that myrcene, a terpene hydrocarbon (EAG 19.0) had high EAG comparable to most of the other oxygenated terpene candidates in the same category was an important observation because it contradicted the general understanding that hydrocarbon terpenes are less active than the oxygenated terpenes.[11,12] Perhaps the reason for high EAG potency of this terpene hydrocarbon is the unique carbon skeleton (three olefinic bonds of which two are conjugated) found also in ipsdienol. It is possible that high unsaturation is vital for high EAG activity. Further, the increment from EAG value of 19.0 (myrcene) to a value of 23.0 (ipsdienol) perhaps shows the importance of the -OH function in the molecule. The class of terpenes with lower EAG potency are exemplified by caryophyllene, limonene and α-terpinene which only had isolated diene moieties. Nereol and neral which had terminal -OH/CHO represent the second type of structures which also gave low EAGs (range of 14-15) in the antenna of red weevil.

From the above considerations it is possible to conclude that the antennal receptors of *R. ferrugineus* favour high unsaturation or cyclic low unsaturated structures with nonterminal -OH/C=O functionality in the molecule. These findings provide a basis for further investigations into the receptor sensitivity of *R. ferrugineus* and the composition of multicomponent formulations for use as attractants.

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