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Response of *Phloeotribus scarabaeoides* (Coleoptera, Scolytidae) to ethylene in an olfactometer

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Abstract. The role of ethylene, pure or in formulation, in the colonization behaviour of the olive bark beetle, *Phloeotribus scarabaeoides* (Coleoptera, Scolytidae) has been investigated in the laboratory. Ethylene has been found to be attractive in both sexes; the formulation ethrel 48 was active in an olfactometer up to several months. Ethylene, whose concentration varies with the developmental stage or the condition of the tree or its wood, may play an important role in the primary attraction of these scolytids to their host.
Key words. Ethylene; kairomone; *Phloeotribus scarabaeoides*; Coleoptera; Scolytidae.

Ethylene, one of the most important natural plant hormones, is released in very large amounts by olive trees when they undergo developmental changes such as flowering or fruit formation[1–3]. In addition, when a plant or a plant organ suffers from any particular stress, for example as a result of pruning, a sharp and rapid increase in ethylene release is observed[4].

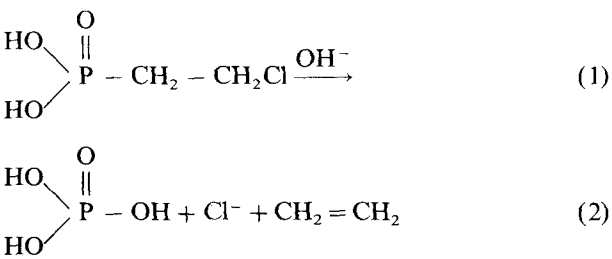
The olive bark beetle, *Phloeotribus scarabaeoides* is a pest of olive trees. It reproduces in waste wood resulting from pruning, and produces serious losses when it flies back to feed on the young olive branches, thus affecting olive production[5,6]. Bark beetles colonize a host tree as a result of a complex mechanism[7–11] which includes host selection, and population aggregation brought about by beetle-emitted pheromones. In the selection of a suitable host, the most aggressive species are believed to respond to the odour of terpenoids, while less aggressive species—those which colonize dying or decaying trees—are generally attracted to a combination of terpenoids and ethanol[12–13] which results from the decomposition of plant tissues.

Although ethylene was not included among the host volatiles mentioned above, it has been reported as being active in connection with several insect behaviour patterns. Ramos and Ramos[14] indicated that this compound, when present at high levels might inhibit the attraction of *Prays oleae* females to the fruit. Recently, ethylene has been described as a chemical signal involved in the sexual behaviour of a moth, *Helicoverpa zea*[15] and as a defense mechanism of conifers against the attack of two bark beetle species, *Dendroctonus frontalis* and *D. ponderosae*[16].

A casual observation in the laboratory (R. González, pers. commun.) indicated an attraction of the olive bark beetle, *Phloeotribus scarabaeoides*, to ethrel 48, a formulation employed to release ethylene. It was then shown[17,18] that when logs were treated with a 0.5% aqueous solution of ethrel 48 one day after pruning, the attack of *P. scarabaeoides* was enhanced and advanced with respect to a control. As a part of a wider project aiming at the identification of semiochemicals that might be involved in the attack on the olive tree by the olive bark beetle, *P. scarabaeoides*, the effect of ethylene on the behaviour of this scolytid has been studied by means of a laboratory bioassay.

Materials and methods

Ethrel 48 (Etisa, Barcelona, Spain) is a formulation which contains an aqueous solution of 48% 2-chloroethyl phosphonic acid, also known as Ethephon (1). This compound hydrolyses easily to phosphoric acid, liberating chloride and ethylene (2). Hyrolysis is enhanced in basic media.



(1)

(2)

Insects. Beetles reared in the laboratory[19] (24 ± 2 °C, 60 ± 5% relative humidity, 18:6 h photoperiod) were sexed on emergence and maintained for at least 30 min under the conditions used for the bioassay (1192 lux and 22 ± 2 °C).

Walking bioassay. A glass olfactometer (50 cm × 3 cm i.d.) was employed under conditions previously optimized[20]. Ten individuals of each sex were observed at a time, and the position of the beetles noted after 10 min. Ten µl of each sample was added to a glass ball, the solvent was allowed to evaporate, and the bioassay was run under a constant flow of synthetic air at ca 0.2 $m.s^{-1}$. The beetles were used only once. Blanks

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were run at the beginning of each set of bioassays, and glass olfactometers were discarded if contamination was suspected. Each sample was tested on two different days with a total of six replications. Results are expressed as the attractivity index[20], which is a measure of the attraction power of a tested sample.

Concentration of ethrel. Ethrel 48 was tested undiluted and in 1, 2 and 3% aqueous solutions, as well as in a 1:1 mixture with 0.1 M sodium hydroxide (Panreac, Barcelona, Spain).

Duration of the effect of ethrel 48. Ten µl of undiluted ethrel 48 was added to the glass ball. A blank test was carried out before the first bioassay and, as the test went on, a second blank test was carried out in another olfactometer to verify that the beetles were behaving normally. In the first week, bioassays were repeated at least 5 times a day to detect possible differences in the response according to time of emergence. Between the 10th and 38th days a minimum of 4 replicates a day were carried out. From the 7th week on, a minimum of two replicates a day were carried out. Results for a day are given as the average value.

Influence of time of day. Data from the experiment described above, in which bioassays with ethrel 48 were carried out at least 5 times a day for 5 days, made it possible to investigate possible differences in the response according to time of day.

Ethylene. Pure ethylene from a gas cylinder was directly bioassayed in the glass olfactometer. Ethylene was premixed with air and the mixture passed through the olfactometer. Ethylene back pressure varied from 0.2 to 4 bar.

Statistical analyses for non-parametric tests were used (Mann-Whitney and Kruskal-Wallis tests[21]).

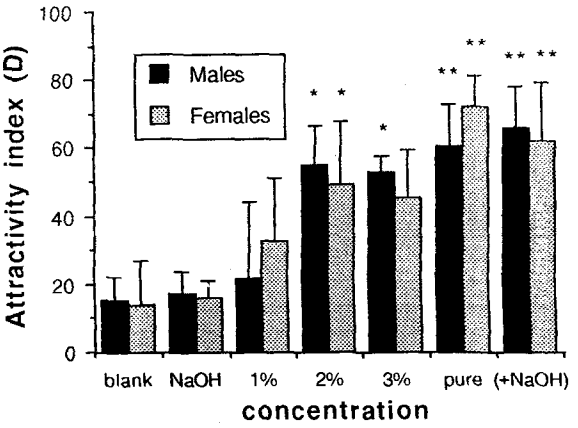

Figure 1. Behavioural responses in a glass olfactometer of *P. scarabaeoides* to aqueous solutions of ethrel 48 of different concentrations, or a mixture with 0.1 M NaOH.
Asterisks indicate significant differences from the blank (Mann-Whitney U test, $*p \leq 0.05$, $**p \leq 0.01$). Vertical bars represent standard deviation.

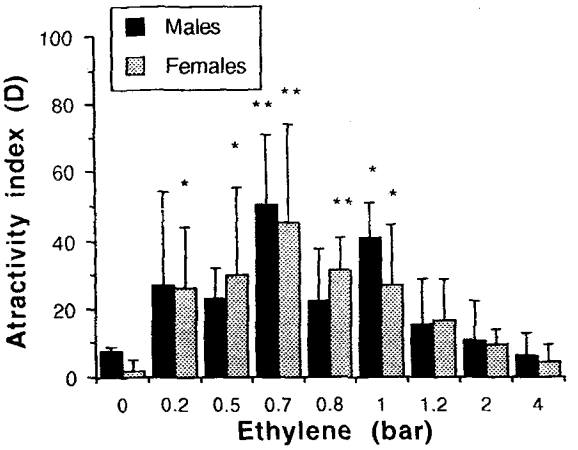

Figure 2. Behavioural responses of *P. scarabaeoides* in a glass olfactometer to different ethylene back pressures. See legend to figure 1.

Results and discussion

Ethrel 48 was biologically active at different dilutions in water (fig. 1). Two percent and 3% solutions were already significantly different ($p < 0.05$) from the blank except in one case for the females. Differences between the blank and pure ethrel, and between the blank and the mixture of ethrel:0.1 M sodium hydroxide (1:1), added to enhance release of ethylene, were highly significant for both sexes ($p < 0.01$). Sodium hydroxide was bioassayed on its own as a control at the same concentration, and elicited no behavioural response.

To make sure that the response to ethrel 48 was due to ethylene and not to any other compound released by the formulation, some bioassays were performed with pure ethylene at different flow-rates (fig. 2). Maximal responses were observed up to 1 bar ethylene for both sexes, but no statistically significant differences were obtained above the latter value. Therefore, from these results a response of olive bark beetles to low concentrations of ethylene was evident, although as concentration increased attractivity diminished, owing probably to a saturation of the receptors or to a repellent effect. As part of the bioassay to study the duration of the effect of ethrel 48, the response of the bark beetles to this compound was tested at different times of day to determine whether the time that had elapsed from the beginning of the photoperiod had an influence on the behaviour of this scolytid. As can be seen in figure 3, although the results after 2 hours were slightly lower than at any other time of day, no statistically significant differences were observed. Averages of the results from the same day were used in the study of the duration of the effect of the ethrel 48 formulation.

It was important to find out how long the formulation would be effective in releasing ethylene. After a single application of ethrel 48, bioassays were run for several weeks. All data were globally compared using the Kruskal-Wallis test, which showed a statistically signifi-

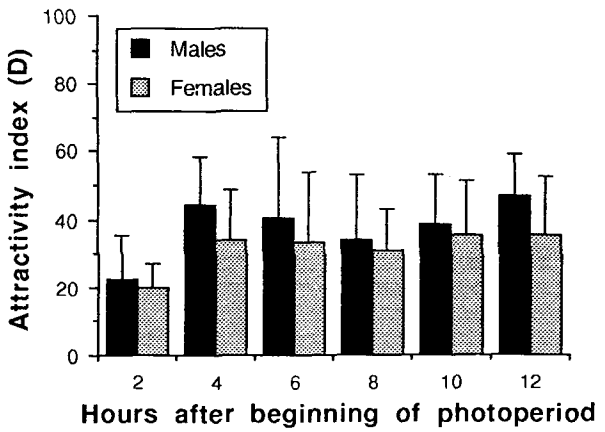

Figure 3. Behavioural responses of *P. scarabaeoides* in a glass olfactometer, using undiluted ethrel 48 at different times of day. No differences were found (Kruskal-Wallis test, $p = 0.192$). Vertical bars represent standard deviation.

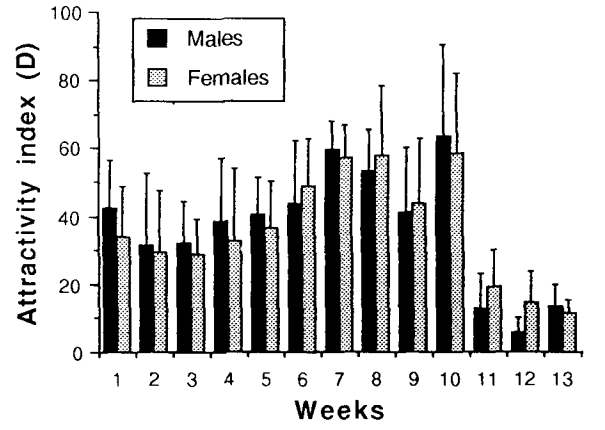

Figure 4. Behavioural responses in a glass olfactometer of *P. scarabaeoides* after a single application of 100% ethrel 48. Vertical bars represent standard deviation. For statistical significance see text.

cant difference among them at $p < 0.01$ (fig. 4). However, as the number of repetitions in the last three weeks was small, these data were grouped and compared with those of the three previous weeks with the Mann-Whitney test. The statistical difference, as expected, was significant ($p < 0.01$), indicating that the formulation used released ethylene at a concentration above the bark beetles' perception threshold for ten weeks.

In the case of *D. frontalis* and *D. ponderosae*, two bark beetles which attack living trees, the production of ethylene has been associated with a defence mechanism of the host[16]. For *P. scarabaeoides*, ethylene may act more as an indicator of host suitability (as reported, though with a different mechanism, for the corn earworm, *Helicoverpa zea*[15]). Olive bark beetles dig their reproduction galleries in offcuts from pruning, whose attractivity is determined by different factors. Ethylene concentration, which is higher for the logs more recently cut[4], may play an important role in the primary attraction of *P. scarabaeoides* to the logs, and may indicate to the pioneer females those logs which are more suitable for starting reproduction galleries, and in which their progeny will have a better chance to survive.

Preliminary field tests indicated an increase in attack density for logs treated with ethrel 48. Olive logs or trees with ethrel 48, with or without an insecticide[18,22], could be used to concentrate the olive bark beetles' attack or divert them from on living trees.

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