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The influence of ethylene on primary attraction of the olive beetle, *Phloeotribus scarabaeoides* (Bern.) (Col., Scolytidae)

R. González[a,]* and M. Campos[b]

[a]*Departamento de Biología Animal, Vegetal y Ecología, Universidad de Jaén, Paraje "Las Lagunillas" S/N, E-23071 Jaén (Spain), Fax +34 53 212141*
[b]*Estación Experimental del Zaidín (CSIC), Departamento de Agrobiología y Protección de Cultivos, Profesor Albareda 1, E-18008 Granada (Spain)*
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Abstract. Two experiments have been carried out to verify the effect of ethylene on the primary attraction of *Phloeotribus scarabaeoides* under both laboratory and field conditions. The experiments were based on the enhancement of ethylene production by olive branches and trunks after the application of (2-chloroethyl)phosphonic acid. Under laboratory conditions, the beetles' response was strongly stimulated on the treated branches. Under field conditions, the treatment aided the location of the olive wood by the flying beetles, and the attack density was significantly greater in the treated wood at the end of the experiment.
Key words. Primary attraction; Scolytidae; olive beetle; *Phloeotribus scarabaeoides*; ethylene.

The olive beetle, *Phloeotribus scarabaeoides* (Bern.), is associated with species from the Oleaceae family[1] and particularly with the olive tree (*Olea europaea* L.), and is found throughout the Mediterranean area[2]. This scolytid species is intermediate between species that attack healthy trees and those that only reproduce in dead trees. They reproduce in weak or unhealthy trees, such as pruning wastes, which are present in abundance in olive groves as a result of the annual pruning (late winter-early spring). Their offspring emerge in early summer and feed throughout the season on the young buds of healthy trees. This is the stage when the main crop damage occurs, as the presence of feeding galleries causes the small branches to break, leading to an important loss of yield[1,3]. From August onwards the adults abandon the feeding galleries and shelter in the cortex of the trunk and branches, where they build small cells in which they remain until the following spring.

During the feeding stage, reproductive cycles can be accidentally initiated locally in the same branches in which the bettles are feeding if these are torn off by the wind[3]. During the winter this can be artificially provoked if the branches are submitted to temperatures adequate for scolytid reproduction[4,5]. Pioneer females are attracted to the host material by the emission of non-specific odours from the olive wood[1,2,6]. According to Neuenschwander and Alexandrakis[7], these volatile substances are also concentrated in the smoke produced on combustion of the olive wood. Ethylene beetle response has been recorded in laboratory bioassays using the electroantennogram technique (EAG)[8]. Moreover, the attraction induced by ethylene itself has recently been demonstrated by a set of experiments carried out with a glass olfactometer in which pure ethylene from a gas cylinder was bioassayed directly. Maximal responses were observed up to 0.7 bar ethylene in both males and females[9]. These observations have enabled us to verify and evaluate the insect response to ethylene, and allowed us to design an integrated management system by using a barrier of ethylene-treated trap-trees[10].

Ethylene is a modulatory phytohormone and secondary effector of other growth regulatory phytohormones, and is liberated when the plant undergoes developmental changes such as flowering and fruiting[11], as well as in response to environmental stress[11]. In the olive tree the greatest release occurs in spring and summer, usually in the zones of most active growth such as the buds, and young shoots bearing flowers or fruit[12,13]. These are the zones where the *P. scarabaeoides* feeding galleries are found during the two annual feeding periods (beginning of spring and summer[5]). Likewise, the level of ethylene released from the olive logs significantly increases after pruning (up to 1400 pl/g · h[14]), providing an ideal material for *P. scarabaeoides reproduction*[2,5]. Moreover, the studies recently conducted show that the level of ethylene released from olive logs is significantly greater in those damaged after *P. scarabaeoides* attack[14]. The increase in ethylene released as a result of insect damage is a phenomenon previously reported in *Allium cepa* L. attacked by *Thrips tabaci* Lindeman[15], in *Medicago sativa* L. by *Therioaphis maculata* (Buckton)[16], and it has also been recorded after the inoculation of fungi vectored by bark beetles in *Pinus* spp.[17].

A role of ethylene in insect-plant interactions has been reported in lepidopteran[18] and thysanopteran species[15], but has not yet been shown to be involved in primary attraction in Scolytidae. Our own observations suggested

* Corresponding author.

a possible function of ethylene in the primary attraction of *P. scarabaeoides* to wood. This study therefore aims to prove the function of ethylene in the stimulation of insects during their reproduction and to identify its role in host location during spring flight dispersion.

Materials and methods

Description of the study area. The study was carried out on an olive farm 20 km north of Granada (between longitudes 4403 and 4444 UTM). An area of 371 ha is dedicated to olive trees, and the average age of the trees is between 50 and 80 years.

Experimental set-up in experiment 1: induction of the beetle attack under controlled conditions. This experiment was carried out in 1989 with the aim of testing insect response to increasing levels of ethylene released from tree branches containing overwintering beetles.

A total of 15 branches strongly infested with overwintering adults were cut from trees during the winter of 1989 (January and February). All the branches were taken from trees in the same part of the olive grove, from the northeast region (height > 2 m) of trees, because of the higher scolytid population density in these areas[5]. Maximum diameter of the main axis of the branches was between 4 and 6 cm, and the surface area susceptible to phytophage attack ranged from 3 to 7 dm^2; the density of beetle infestation ranged from 105 to 125 overwintering galleries per branch. The day following pruning, a third of the branches were sprayed with a 0.5% solution of Ethrel48 (Etisa, Barcelona, Spain). Ethrel48 is a formulation which contains an aqueous solution of 48% (2-chloroethyl)phosphonic acid, also known as Ethephon. This compound hydrolyses easily to phosphoric acid, liberating chloride and ethylene. Treated branches were placed in a room at temperature between 20 °C and 25 °C; RH = 40–45% and photoperiod = 16 L:8 D). The remaining branches were treated with distilled water (10 units) and 5 of these, comprising control group B, were placed in the same room as the treated branches approximately 2 m away. The other 5 branches, control A, were placed in another room under similar conditions.

Branches were observed daily and the number of attacks in each was recorded. Due to the differences in the density of infestation between branches, data of attack density in each observation and branch unit were normalized as the percentage of the final density reached the end of the experiment.

Experiment 2: Attack induction under field conditions. This series of experiments was carried out in 1988, 1992 and 1993, with the aim of determining the effect of an increase in levels of ethylene released by the wood on the rhythm of insect attack under natural conditions.

Treatment consisted of spraying cut trunks with an aqueous solution of 0.5% Ethrel. From early March each year, two groups of trunks (treated and control) of 10–20 units each were placed in the olive grove. Trunk length ranged from 30 to 60 cm and the diameter from 3.5 cm to 11 cm. Each group was placed at the base of a tree 50–200 m apart. In each group the trunks were spread out on the northern side of the tree perpendicular to the trunk.

The humidity of the wood is an important factor in triggering beetle attraction, and to be suitable for scolytid attack, it must have lost a minimum of 10% of its initial weight by evaporation[7]. In natural conditions this state is reached 7–11 days after pruning, and therefore in our study all logs were suitable for *P. scarabaeoides* reproduction from the moment they were introduced into the olive grove.

Weekly observations were carried out on every log from both groups, and the total number of *P. scarabaeoides* entrance holes produced during that week was recorded.

Statistical analysis. Data were compared with a normal distribution using the chi-square test. Data distributions corresponding to each of the periodical observations were adjusted using the Kolmogorov-Smirnov (K-S) test.

To determine differences in the number and density of attacks in treated and control trunks/branches, one-way analysis of variance (ANOVA) and the Kruskal-Wallis (K-W) analysis of variance by rank were used. These tests were also used to determine differences in total production of offspring in both types of logs/branches. The Mann-Whitney (M-W) test was used for the comparison of attack densities of control and treated trunks/logs at each observation. This was also used to compare total attack density and emergence between them.

Results and discussion

Experiment 1. In the treated branches the attack began the day after the experiment had begun (the second day after pruning), giving the maximum value (fig. 1). The density was greater than in control group A on the first five days (one-way ANOVA, $F > 3.9$, $p < 0.05$). The latter group presented a symmetrical curve in which the attack began on the third day, reaching a maximum on the sixth day. Figure 2 shows that 70% of total accumulated attack was reached in the treated group, when the control group A only showed 40% of the total accumulated attack. The difference in the treated branches compared with control group A is attributed to the additional ethylene production in the former[19], attracting the individuals in the overwintering galleries. Control group B, which had not been treated but had been left in the same room as the treated logs, presented an attack curve intermediate between the treated group and control group A (figs 1 and 2). As in the treated group the attack began on the second day, but the

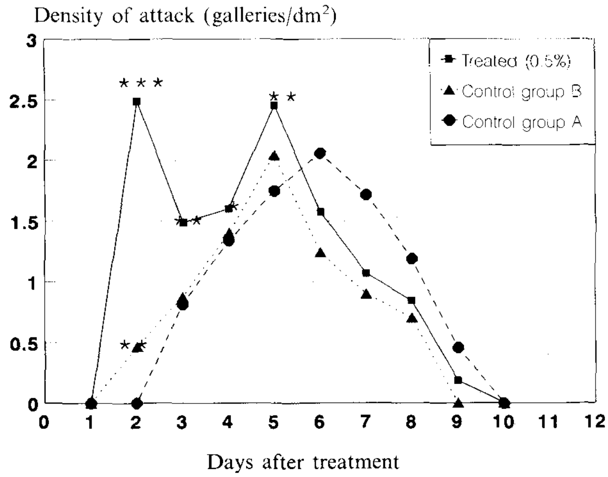

Figure 1. *P. scarabaeoides* attack density curves in branches after experiment 1. Asterisks note statistically significant differences (Mann-Whitney test): (*) = $p < 0.1$; (**) = $p < 0.05$; (***) = $p < 0.001$.

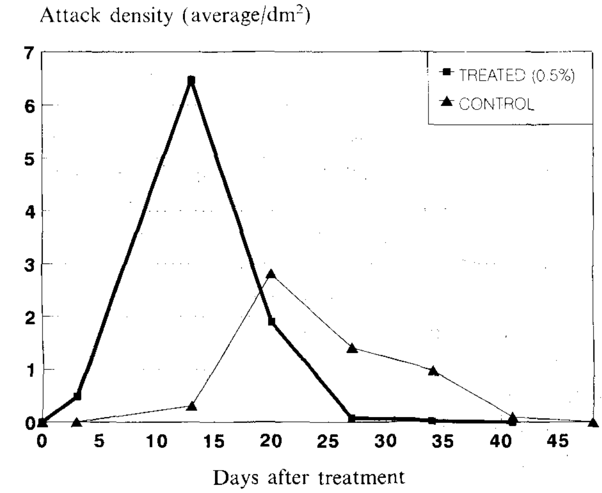

Figure 3. Attack density curves of *P. scarabaeoides* on control and treated trunks in 1992 [day of treatment and positioning of the olive trunks on the olive grove (day 0) corresponds to 10th of April].

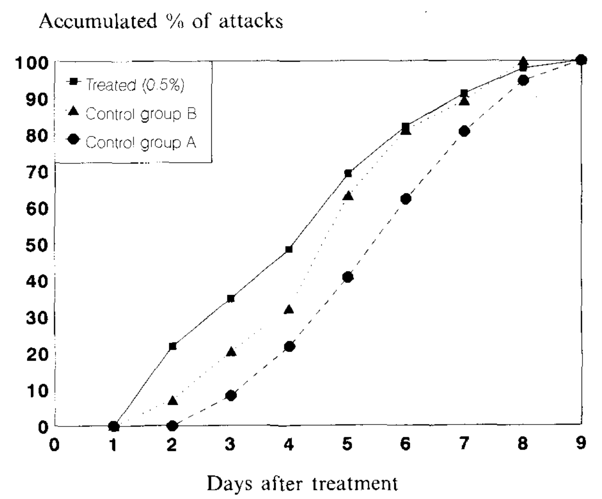

Figure 2. Accumulated percentage of attack for the three groups of branches in experiment 1.

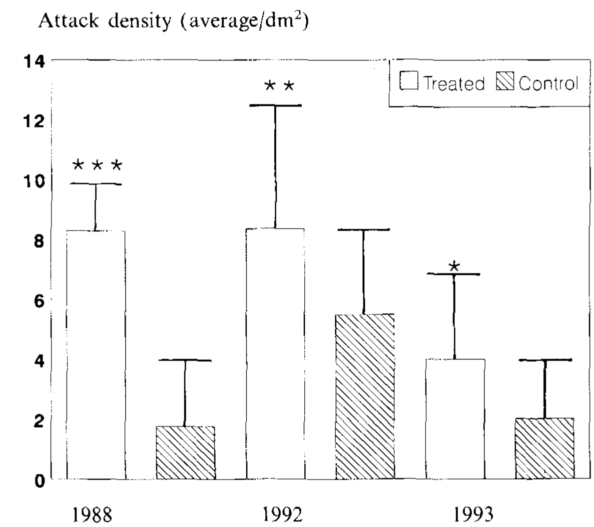

Figure 4. Mean density of attacks of *P. scarabaeoides* in the control and treated trunks for the three years of experiment 2. Asterisks indicate statistically significant differences (Kruskal-Wallis and one-way ANOVA): (*) = $p < 0.1$; (**) = $p < 0.05$; (***) = $p < 0.001$.

overall intensity of attack was less. Insect behaviour of this group can be attributed to an indirect effect of ethylene released from the treated group accumulated in the room.

Experiment 2. Scolytid attack started 5 and 15 days earlier in the treated group than in the controls in 1992 and 1993 respectively, except for 1988, when the attack began at the same time. In the control piles of wood, the attack curve for *P. scarabaeoides* of wood usually spanned 6–8 weeks and consisted of three clearly defined stages representing the change in the rate of arrival of the scolytids[5] (fig. 3). In the treated piles the period of attack was shorter, ranging from 4 to 6 weeks. The earlier attack curve in the treated group is closely related to the results observed in experiment 1, and is in accordance with the attractivity induced by a source of pure ethylene, as observed in the bioassays using the olfactometer[5,9] and the electroantennogram[8]. On the other hand, the earlier attack by *P. scarabaeoides* on the treated wood is in agreement with the studies recently conducted, which shows that a significant increase in the level of ethylene released by treated olive is produced during the four weeks after treatment[20]. An increase in the level of ethylene released from the wood after treatment aids its location by the insects, thus reinforcing the primary attraction mechanism.

Attack density in the control piles of wood varied in different years (fig. 4), and the lowest density was recorded in 1988, when only 80% of trunks were attacked by *P. scarabaeoides*. In 1992 and 1993, 100% of trunks were attacked.

The modification of *P. scarabaeoides* attack density in the logs by the treatment is shown by the significant differences in each of the three years between the densities in the treated and control groups (K-W test; Wilcoxon $T = 13$, $p < 0.001$ for 1988; one-way ANOVA, $F > 2.8$, $p < 0.05$ and $p < 0.1$ for 1992 and 1993 respectively, fig. 4). Total density in the treated group at the end of the scolytid attack curve was significantly greater than corresponding values in the control (M-W test, $p < 0.001$ for 1988; Student's t test, $p < 0.05$ and 0.1 for 1992 and 1993 respectively). Density values in the treated group were significantly greater in 1988 throughout the entire period of attack (M-W test, $p < 0.001$) and in 1992 and 1993 only at times of maximum attack (Student's t test, $p < 0.005$ and $p < 0.001$, respectively).

During the dispersion flight of *P. scarabaeoides*, pruning wastes are present in abundance in the olive grove as a result of the annual pruning, and constitute an important focus of attraction for beetle attack. The location in the olive grove of the resulting olive wood strongly varies from year to year, because pruning the whole olive grove takes a period of 3–4 years. This fact implies differences in the relative placement and distance of the woodpiles used in our study respect to the main focus of attraction on the olive grove, which changed every year. This could explain the differences observed in the level of attack on the woodpiles used in our study, and the oscillation in the level of significance of attack density between treated and control groups. Mechanical damage caused by the feeding of insects has been reported to stimulate a greater production of ethylene in *Allium cepa* L. after the attack of *Thrips tabaci* Lindeman[15], in *Medicago sativa* L. infested by the spotted alfalfa aphid, *Therioaphis maculata* (Buckton)[16], in *Gossypium hirsutum* L. attacked by the cotton fleahopper, *Pseudatomoscelis seriatus* (Reuter)[21], and in *Tilia americana* L. after the attack of *Thrips calcaratus* Uzel[22]. This increase in the level of ethylene has also been observed in olive wood infested by *P. scarabaeoides*[20], and it has been reported as a phytochemical defensive host response[15,17]. Moreover, in our study the increase in the level of scolytid attack on the olive-treated wood is observed when the higher level of ethylene is being released[20], suggesting a density-dependent mechanism. It is not surprising that beetle-induced ethylene emission was synergized by the exogenous ethylene produced by the treatment. The simultaneous arrival of beetles attracted by the presence of ethylene on treated logs could favour a sharp increase in the concentration of ethylene released by the mechanical damage provoked, thus explaining the steeper slope of the attack curve in the treated wood (fig. 3), and the greater level of attack.

Ethylene has not yet been shown to be involved in primary attraction in Scolytidae. In 'secondary' bark beetles, the ethanol generated in sapwood and phloem tissue has frequently been reported to play an important role in the primary attractant mechanism[23]. Its effect synergizes with other host odours in *Hylurgops palliatus* (Gyll.) and *Hylastes* spp.[24] and/or with attractant pheromones in the case of *Dryocoetes autographus* (Ratz.)[24], *Trypodendron* spp.[25] and *Gnathotrichus* spp.[26]. These observations point out that future efforts should try to elucidate its possible role in the primary attraction mechanism of *P. scarabaeoides* to olive wood.

Conclusions

The results indicate that ethylene release favours the triggering of adult insect reproductive responses. Likewise, changes in ethylene levels have been shown to affect wood attractiveness strongly, increasing the ease of its location by pioneer individuals during spring dispersion flight. These results allow us to assume that ethylene is one of the main components involved in host location during beetle primary attraction.

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