RESPONSES OF THE BLACK VINE WEEVIL (*OTIORHYNCHUS SULCATUS*) TO WEEVIL AND HOST-PLANT ODOURS

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Summary
Four host plants, while damaged by black vine weevil (*Otiorhynchus sulcatus*) were tested in a dual-choice olfactometer. Only *Taxus baccata* and *Euonymus fortunei* attracted large number of weevils. *Fragaria x ananassa* and *Rhododendron* were not attractive. Undamaged *Euonymus* in the presence of weevils was not attractive whereas *Euonymus* freshly damaged by weevils attracted weevils. A positive correlation between weevil/frass odour and attraction was found in the control treatment and a treatment with pre-damaged *Euonymus*. It is concluded that feeding-induced volatiles are involved in host finding of this parthenogenetic weevil species.

INTRODUCTION

The black vine weevil (*Otiorhynchus sulcatus* F., Coleoptera: Curculionidae) is a serious pest in many ornamental and small fruit crops. At present no trap or attractant is available for monitoring of this weevil species. Food preference, electroantennogram (EAG) recordings and weevil refuge attraction (van Tol & Visser, 1998; Pickett *et al*, 1996) indicate that specific plant volatiles and possibly aggregation pheromones are involved in the attraction of the weevil towards feeding sites. Of the EAG tested volatiles only a number of general green leaf volatiles and some more specific plant compounds gave large responses (van Tol & Visser, 1998).

Attraction to plant volatiles is known for a number of weevil species. Also the importance of host plants for pheromone release is evident in several phytophagous insects. In some weevil species, pheromones are passed and released with faeces, providing a strong relationship between host-plant chemistry and pheromones (Landolt & Phillips, 1997). Several studies also reveal the importance of feeding damage for attraction. Broken beans attracted more females of *D. abbreviatus* (Harari & Landolt, 1997), and a strong indication for attraction of male plum curculio *Conotrachelus nenuphar* to damaged plums has been reported (Leskey *et al*, 1998). The Japanese beetle *Popillia japonica* is attracted towards leaves of crabapple which were damaged overnight by the beetle, but not to artificially damaged or freshly beetle damaged leaves (Loughrin *et al*, 1995).

In this study we developed an olfactometer for the black vine weevil and tested the attraction towards several host plants. In addition we investigated the effects of feeding damage and the presence of weevils on the attraction.

MATERIAL AND METHODS

Weevil collection

Weevils were collected from *Astilbe chinensis* plants cultivated in 3-litre pots. The plants were inoculated with eggs of *O. sulcatus* in the summer and placed into a cold room at 4°C in the autumn. Three months before the start of the trials, plants were placed in a greenhouse at 20°C. Emerging weevils were fed with leaves from *Taxus baccata* and *Euonymus fortunei cv.* 'Dart's Blanket' under 16-hour light conditions at 22°C. In the summer weevils were also collected from an infested field in Boskoop. The collected weevils were treated in the same way as the weevils collected from the infested pots.

Bioassay performance

A dual-choice olfactometer was constructed in which laboratory bioassays were conducted to test the response of adult weevils. Before entering the olfactometer, the airflow is purified by passage through a charcoal filter (Merck) and then passed through washbottles. The airflow is set to 5.2-cm/s (13.2 ml/s). Silicone tubing and glass tubes connect bottles and both arms of the olfactometer. The olfactometer is placed in a black box with one halogen lamp (12 V DC, 10 VA) in the center of the ceiling of the box illuminating the Y-junction. Light intensity was set to 0.2 lux.

Weevils were starved 24 hours prior to testing. The olfactometer is a closed system. Responding weevils can not be removed during the test. Compensation for weevil presence is done by placing 10 starved weevils in washbottles on each side in the olfactometer. A fluon barrier prevents weevils from leaving the washbottles.

For each test 40 weevils are released in a box connected to the entering point of the olfactometer. Each test lasted for 2 hours and started when the weevils were 3 to 4 hours in the dark period. To compensate for humidity differences between left and right side, each washbottle contained 80 grams of sterilized silver-sand wetted with 20 ml demineralized water. Each test was repeated four times (4-block trial). Treatment side (left or right) was alternated for the repetitions.

Trial 1: Host-plant odour attraction

Four plant species known to be seriously affected by weevil damage, were chosen for this trial. The plant species tested were *Taxus baccata*, *Euonymus fortunei cv.* 'Dart's Blanket', *Rhododendron cv.* 'Catawbiense Boursault' and *Fragaria x ananassa cv.* 'Elsanta'. The odour was applied at one side of the olfactometer by filling one washbottle with undamaged plant parts. For *Euonymus*, *Taxus* and *Rhododendron* we used the top parts of stems with leaves or needles still attached in order to minimize damage. For strawberry we used excised leaves. Stems were placed in the silver-sand before wetting the sand with demineralized water. The weight of the leaves used in the test was tuned to equivalent leaf areas for all plant species: on average, *Euonymus* 6 g, *Rhododendron* 7 g, *Taxus* 8 g and *Fragaria* 3 g. On both sides of the olfactometer 10 weevils were placed in the washbottles with the plant material. After 2 hours the number of weevils in the washbottles on both sides were counted. The control treatment consisted of two washbottles on both sides, with 10 weevils placed in each bottle.

Trial 2: Feeding damage and weevil attraction

In the first trial we tested the olfactory response of weevils to plants in contact with starved weevils. During the tests weevils were eating from the plants in the washbottles. To determine the effects of feeding damage we separated weevils and plants by serially connecting washbottles in the olfactometer setup. For this trial we used *Euonymus* as test plant. In each washbottle either plant parts (Eu) or starved weevils (w) were present. Weevils were unable to leave the washbottles to come in contact with the plant. By changing the sequence of the washbottles containing weevils or plants, the weevils in the washbottles were offered either clean air or air that had passed over *Euonymus* foliage. In order to further specify the effects of feeding damage, twenty starved weevils were allowed to feed on foliage for 2 hours in the dark period. Subsequently the damaged foliage (denoted by Eu*) was removed and placed in a clean washbottle and used in the bioassay. Weevils released in the olfactometer could only enter the first empty washbottle on either side of the Y-junction. Fluon prevented weevils from moving to the other washbottles containing the odour stimuli under study. The dual-choice tests always consisted of a treatment on one side versus 10 weevils in a washbottle on the other side. The following treatments were performed:

1. *Euonymus* (freshly damaged) with 10 weevils in one washbottle (Eu+w)
2. *Euonymus* (undamaged) in a washbottle connected to a washbottle with 10 weevils (Eu ➡ w) , the air of *Euonymus* passing over the weevils
3. *Euonymus* (pre-damaged) in a washbottle connected to a washbottle with 10 weevils (Eu* ➡ w), the air of pre-damaged *Euonymus* passing over the weevils
4. 10 weevils in a washbottle connected to a washbottle with *Euonymus* (pre-damaged) (w ➡ Eu*), the air of weevils passing over the pre-damaged *Euonymus*

RESULTS AND DISCUSSION

Trial 1: Host-plant odour attraction

The results of trials have been analyzed for participation and for choice. Participation is the number of weevils that respond in the olfactometer (trapped into left or right washbottle) compared to weevils not responding (not entering one of both washbottles in the olfactometer). The choice-analysis is looking at the left-right distribution of the weevils.

Of the host plants tested, *Taxus* and *Euonymus* gave significantly larger participation (83 and 81%, respectively) than the control treatment (58%). The participation in tests with *Fragaria*, *Rhododendron* and in the control treatment are not significantly different. These results show that the odour of *Taxus* and *Euonymus* while damaged by the weevils, activate the weevils so that they move upwind into the olfactometer.

Of the participating weevils only *Euonymus* and *Taxus* attract significantly more than 50% of the weevils (Figure 1A, 90 and 88% respectively) while *Fragaria* and *Rhododendron* are neutral in this respect. The control treatment indicated no preference for the left or right side.

Trial 2: Feeding damage and weevil attraction

The participation of the weevils in the different treatments, including the control, does not differ significantly and varies between 46 and 65%. The results (Figure 2) show that *Euonymus* damaged by weevils during the trial and *Euonymus* pre-damaged by weevils, attracts significantly more weevils. Undamaged *Euonymus*, i.e., undamaged *Euonymus* and weevils separated (Eu ➡ w), is not attractive for the weevils. The treatments where odour of pre-damaged *Euonymus* subsequently passes over weevils in a washbottle before entering the choice area (Eu* ➡ w) is as attractive as the treatment where air passes the weevils first and subsequently the pre-damaged *Euonymus* (w ➡ Eu*). There is, however, an indication that a pheromone or weevil kairomone is involved in weevil attraction. A typical weevil/frass odour was positively correlated to the weevil preference in the control treatment and the treatment with pre-damaged *Euonymus* (Eu* ➡ w).

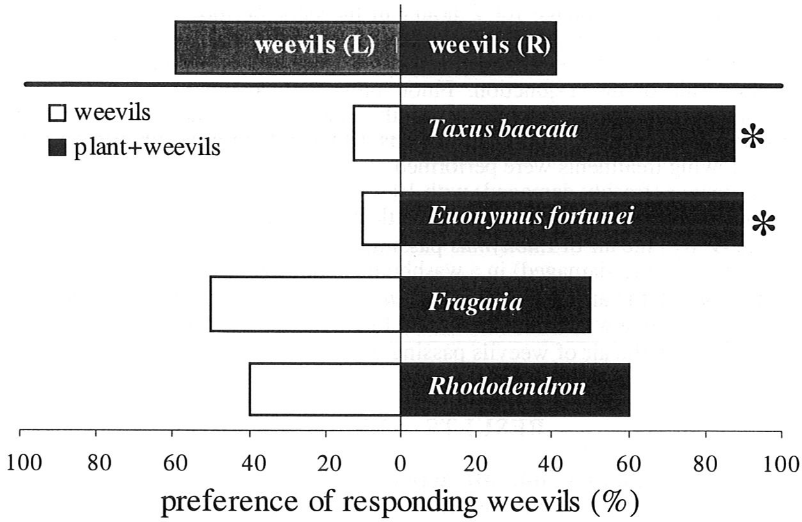

*Figure 1. Preference of O.sulcatus to odour of host plants while damaged by weevils. * = significantly different from even distribution at p=0.05.*

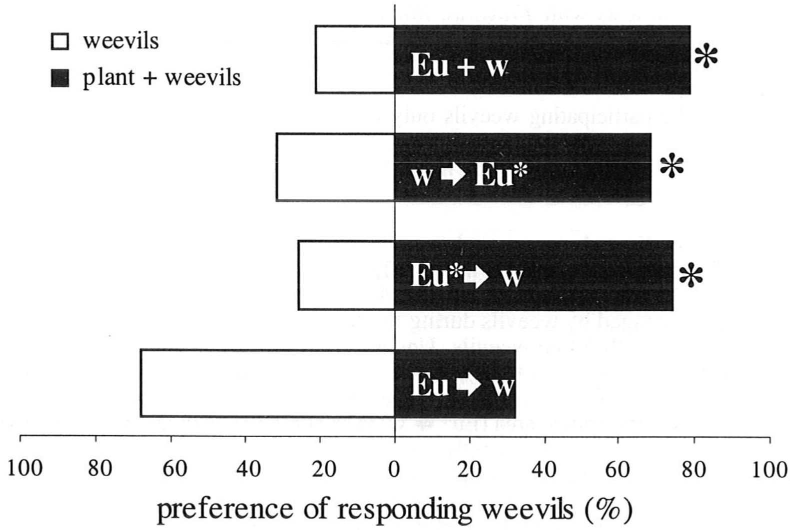

Figure 2. Preference of O.sulcatus to odour of undamaged (Eu➡w), pre-damaged (Eu➡w and w➡Eu*) or freshly damaged (Eu+w) Euonymus fortunei in the presence of weevils. * = significantly different from even distribution ($p \leq 0.05$).*

The volatile attraction to *Taxus* and *Euonymus* is well related to the feeding preference and oviposition of the black vine weevil on these crops (Hanula, 1988; Nielsen & Dunlap, 1981; Smith, 1932; van Tol & Visser, 1998). *Rhododendron* odour is not attractive. On *Rhododendron* the oviposition rate of weevils is low and the mortality is high as compared to the performance on other plant species (Hanula, 1988; Nielsen & Dunlap, 1981; van Tol & Visser, 1998; van Tol, unpublished). Only when there is no choice the weevils will feed on *Rhododendron*. Several cultivars of *Rhododendron* are resistant to black vine weevil (Doss, 1983) but the cultivar we used is well accepted by the weevils. The nearby absence of suitable crops and the clearly visible feeding damage to this evergreen shrub on nurseries explains why it is considered as one of the main host plants for the black vine weevil. The tested cultivar of strawberry is not attractive for the black vine weevil in our olfactory trials. Though reports of damage, oviposition and feeding on strawberry are variable. Smith (1932) reports no preference for strawberry and lower oviposition rates. Both Smith (1932) and Evenhuis (1978) mention a relative high mortality of larvae feeding on the roots of strawberry. Hanula (1988) found a relative high oviposition rate on strawberry, but clearly lower than on *Taxus*. Most reports on strawberry fecundity are, however, unrelated to other plant species. Recently we found that the weevils feed well on strawberry in both choice and no-choice situation, but that the oviposition rate is generally lower and the mortality higher compared to feeding on several other crops like *Taxus* and *Aronia* (van Tol, unpublished).

A strong attraction to insect damaged plant foliage is evident from our results. The low number of weevils attracted to undamaged *Euonymus* states the importance of volatiles released by freshly damaged *Euonymus* for weevil attraction. The high EAG response for several green leaf volatiles (van Tol & Visser, 1998) and the bioassay result suggest an important rôle of these volatiles in attraction to host plants. Some other attractive or repelling substances in plants probably also affects attraction since weevil-damaged *Rhododendron* and *Fragaria* (Trial 1) are not attractive. Further more, odour released by the weevils in their faeces, is possibly playing a rôle in attraction.

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