

Olfactory responses of the vine weevil, *Otiorhynchus sulcatus*, to tree odours

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Abstract. A Y-tube olfactometer and a still-air olfactometer were developed to determine the attractiveness of several host plants for the vine weevil (*Otiorhynchus sulcatus* (F.); Coleoptera: Curculionidae). Odours of weevil-damaged yew (*Taxus baccata*) and spindle trees (*Euonymus fortunei*) are attractive to the vine weevil, but *Rhododendron* and strawberry (*Fragaria × ananassa*) are not. Undamaged *Euonymus* is attractive to the weevils in springtime but not in late summer. When clean air or undamaged *Euonymus* is the alternative, weevils strongly prefer weevil-damaged *Euonymus* foliage, and this preference is retained throughout the year. Hence, plant damage plays a role in attraction of the vine weevil. In contrast to the permanent attractiveness of weevil-damaged *Euonymus*, mechanically damaged plants gradually lose the attractiveness that they have early in the growing season. This suggests that emission of volatiles, produced by the plants in response to weevil damage, is important for attraction of the weevils because the weevils may use these plant odours to find suitable food plants throughout the season. Apart from weevil-damage-related plant volatiles, green leaf volatiles must also play a significant role, as indicated by the fact that weevils prefer: early season, undamaged *Euonymus* over clean air; early season, mechanically damaged *Euonymus* over undamaged *Euonymus*; and, throughout the season, had no preference when mechanically damaged *Euonymus* is tested against weevil-damaged *Euonymus*. Thus, monitoring traps may be developed by the use of green leaf volatiles and/or herbivore-induced volatiles, as attractants.

Key words. Bioassays, *Euonymus*, *Fragaria*, herbivory, kairomones, olfactometer, plant odours, *Rhododendron*, semiochemicals, *Taxus*.

Introduction

The vine weevil (*Otiorhynchus sulcatus* (F.); Coleoptera: Curculionidae) is one of the most serious insect pests in nursery stock and small fruit production in North America and Northern Europe (Moorhouse *et al.*, 1992). Currently, effective pesticides against weevil larvae are not available and large-scale biocontrol of weevil larvae with parasitic nematodes is expensive. In the Netherlands the only

economically feasible method to control adult weevils is by spraying acephate. This pesticide is not compatible with integrated control as it kills natural enemies of other pests. Hence, there is a need to apply it at the right site and the right time. This necessitates the development of monitoring tools, such as odour-baited traps. As the vine weevil is polyploid and reproduces by thelytokous parthenogenesis, sex pheromones do not exist. However, it is possible to focus on food-related attractants. In this paper, the results of olfactometer tests are described in an attempt to find attractive blends of host-plant-related volatiles.

Numerous species of weevils have been shown to be attracted to (single or blends of) volatiles of their host plants. Examples are the cabbage seed weevil, *Ceutorhynchus assimilis* (Evans & Allen-Williams, 1993; Bartlett *et al.*,

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1997), the pea weevil, *Sitona lineatus* (Landon *et al.*, 1997) the plum curculio, *Conotrachelus nemophar* (Butkewich & Prokopy, 1993; Leskey *et al.*, 2001) and the sugarcane rootstalk borer weevil, *Diaprepes abbreviatus* (Harari & Landolt, 1997). Some studies also reveal the importance of feeding damage for attraction of various weevil species (Harari & Landolt, 1997; Leskey *et al.*, 1998). All these studies involve attraction of monophagous or oligophagous weevil species to their host plant and host-plant odours. There are, however, no studies on host-plant attraction of polyphagous weevil species, such as *O. sulcatus*.

Although the vine weevil is polyphagous, it prefers plant species in the families *Rosaceae*, *Ericaceae* and *Taxaceae* (Smith, 1932; Evenhuis, 1978; Masaki *et al.*, 1984). In choice situations, vine weevils prefer certain plant species for feeding (Van Tol & Visser, 1998). *Taxus baccata* (L.) and *Euonymus fortunei* ((Turcz.) Hand.-Mazz.) are preferred over *Rhododendron* (Catawbiense Group) 'Boursault', whereas all three plant species are reported to be heavily damaged by weevils in the field. In no-choice situations the weevils feed well on this rhododendron cultivar, but fecundity is low and mortality high compared to that on *Taxus* or *Euonymus* (van Tol, unpublished data). The latter two host plants stand out as suitable and one of these, *Euonymus fortunei*, is already used by some growers as a monitoring tool in the field (Van der Horst & van Tol, 1995; Van Tol, 1996). All these findings suggest that the vine weevil discriminates between potential host-plant species, but we do not know whether this results from contact chemoreception or from olfaction. Food preference, electroantennogram (EAG) recordings and aggregation behaviour (Pickett *et al.*, 1996; van Tol & Visser, 1998; Van Tol & Visser, 2002) suggest that some plant volatiles and aggregation pheromones are involved in the attraction of the weevils towards feeding sites. Hence, there is every reason to hypothesize that olfactory senses play a role in host-plant detection and discrimination.

In this paper, we present the results of olfactometer studies to assess the attractiveness of several host plants for the vine weevil. For one of the tested host plants, *Euonymus fortunei*, we also determine how the olfactory response is influenced by mechanical leaf damage, feeding damage by adult weevils and the time of the year.

Materials and methods

Insects

A population of *Otiorynchus sulcatus* was kept at 22 °C in a climate room under long-day conditions (LD 16:8 h). Weevils were maintained on *Astilbe chinensis* (Franch. & Sav.) plants cultivated in 3-L pots. The plants were inoculated with eggs of *O. sulcatus* in the summer and placed in a room at 4 °C. Three months before the start of the trials, plants were placed in a greenhouse at 20 °C. Emerging weevils were fed a mixture of cuttings from *Taxus baccata*

and *Euonymus fortunei* 'Dart's Blanket'. In summer, weevils were additionally collected from an infested field with *Taxus* and *Euonymus* at the Research Station in Boskoop. Weevils collected from pots and field were kept separately in the climate room. Trials were repeated with both populations to control for an influence of their origin. In all trials weevils were used that had emerged from the soil not more than 6 months previously. Weevils were returned to the stock population after each test, so that they could be re-used in tests that took place at least a week later.

Plants

The main host plant under test was *Euonymus fortunei* 'Dart's Blanket'. For reasons of comparison, three other plant species were tested, *Taxus baccata*, *Rhododendron* (Catawbiense Group) 'Boursault' and *Fragaria × ananassa* 'Elsanta'. All these plant species were grown in a field at the Research Station in Boskoop. Stems were cut from these stock plants and these cuttings were used for the trials within 24 h.

Bioassay set-up

Two olfactometers were constructed for laboratory bioassays to test the response of adult vine weevils.

1. A Y-tube olfactometer (Fig. 1) consisted of a glass Y-tube (base tube 12 cm long; Y-arms 9 cm long; internal tube diameter 18 mm) connected with screw caps to glass arms (26 cm long; internal diameter 18 mm). The glass arms were connected to washbottles (500 mL) with screw caps. A weevil release box (14 cm diameter) was connected to the base of the Y-tube. Airflow was purified by passage through a charcoal filter and then passed through washbottles and connecting arms before entering the Y-tube. For this trial, three serially connected washbottles were connected to each glass arm. The airflow was set to 5.2 cm/s (13.2 mL/s). The olfactometer was placed in a black box with a halogen lamp (12 DC, 10 V) in the top centre to illuminate the Y-junction. Light intensity was set to $0.3 \times 10^{-3} \text{ W/m}^2$. Weevils were deprived of food for 24 h prior to testing. For each test, 40 weevils were released in the weevil release box. Single weevil release was abandoned because pre-testing revealed a limited response in contrast to multiple weevil release. As responding weevils could not be removed during the test, 10 weevils were placed in one washbottle on each side of the Y-tube before the start of the trials to compensate partly for possible effects of changing weevil accumulation on one of the two sides of the Y-tube during the test. The results of the tests therefore only reveal the differences between plants in the presence of weevils vs. weevils alone. Each test lasted 2 h, starting when the weevils were in the active period for food searching (3–4 h after sunset). Pre-testing indicated that after 2 h no more significant changes occurred in the weevils' choice. Weevils could choose to remain in the

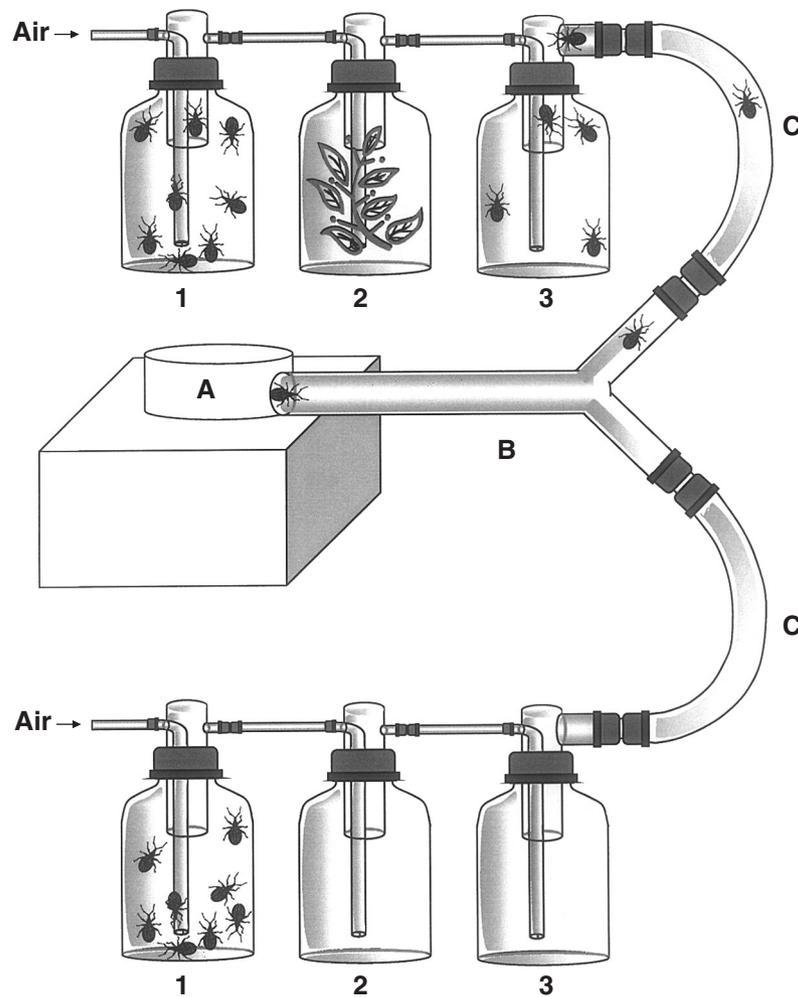


Fig. 1. A Y-tube olfactometer. Weevils released in box A can enter the washbottles 3 through a glass Y-tube B connected with screw caps to glass arms C. The glass arms are connected to the washbottles 3 with screw caps. Airflow was purified by passing through a charcoal filter and then passed through washbottles 1–3 and connecting arms C before entering the Y-tube B. The olfactometer is placed in a black box with a halogen lamp in the top centre to illuminate the Y-junction of B.

weevil release box, Y-tube, connecting arm parts or enter the washbottles directly connected to the arm parts. Weevils were prevented from returning to the arms and Y-tube after entering the washbottle by a fluon barrier. A fluon barrier also prevented weevils from entering the serially connected washbottles. To compensate for humidity differences between left and right side, each washbottle contained 80 g of sterilized silver-sand wetted with 20 mL of demineralized water. Each test was repeated four times with independent samples of weevils. For each replicate treatment and control side were interchanged between the left and the right odour-inlet of the olfactometer.

2. A still-air olfactometer, similar to that used by Prokopy *et al.* (1995) for behavioural tests with the plum curculio (*Conotrachelus nenuphar*), was modified to prevent contact of the weevils with plant material (Fig. 2). The olfactometer consisted of a glass Petri dish (140 mm diameter ×

20 mm high) with two holes, 12 mm diameter and 95 mm apart, in the Petri dish lid. Small glass tubes (length 25 mm; diameter 12 mm) were positioned on top of the holes in the lid. A glass cylinder (length 65 mm; diameter 60 mm) was placed on the lid such that the small glass tube was in its centre. A glass cup (length 60 mm; diameter 60 mm) with a rim (length 15 mm; diameter 67 mm), was placed on top of each glass cylinder interlocking the cylinder and cup. Rigid nylon gauze (mesh 1 mm) was clamped between cylinder and cup. Weevils were deprived of food for 24 h prior to testing. Several olfactometers were placed in a non-airtight black box. For each test, 10 weevils were released in the Petri dish. Each test lasted 2 h, starting when the weevils were in the active period for food searching (3–4 h after sunset). Weevils could choose to remain in the dish or enter the cylinder parts through the inner surface of the glass tubes on the Petri dish lids. The nylon gauze prevented

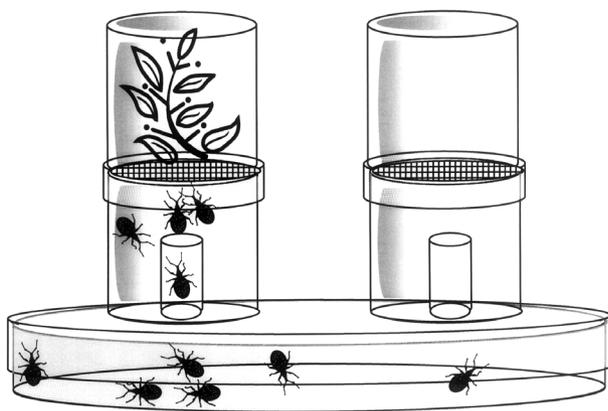


Fig. 2. A two-choice still-air olfactometer adapted from Prokopy *et al.* (1995). Weevils released in the Petri dish can choose to enter one of both cylinders through the tubes on top of the Petri dish lid. Nylon gauze allows diffusion of odours but prevents the weevils from entering the cups on top of the cylinders.

the weevils from entering the glass cups on top of the cylinders. No attempt to correct for weevil accumulation effects was made during the still-air olfactometer trials. Plant parts, with the base of the stems wrapped in wet cotton wool balls, were placed in the cups several minutes prior to the start of the test. To compensate for humidity effects small, wet cotton wool balls were also placed in the control treatment cups. Each test was repeated at least eight times. For each replicate the Petri dishes were turned through 90° (four different orientations of the treatment and control side).

Attraction to different species of weevil-damaged host plants

First, *Euonymus fortunei* 'Dart's Blanket' was tested, against clean air, for its attractiveness to weevils in the Y-tube olfactometer (Fig. 1, with only one washbottle on each side). In addition, three other host-plant species of the vine weevil were tested for comparison: *Taxus baccata*, *Rhododendron* (Catawbiense Group) 'Boursault' and *Fragaria* × *ananassa* 'Elsanta'. The odour was applied at one side of the olfactometer by filling one washbottle with cuttings from the stock plants. For *Euonymus*, *Taxus* and *Rhododendron*, the top parts of stems were used with leaves or needles still attached in order to minimize damage. For *Fragaria*, excised leaves were used. Stems or petioles were placed in the silver-sand before wetting the sand with demineralized water. The ratios of weight and leaf area for the four plant species were very different. Therefore, it was decided to tune the weight of the cuttings used in the test to present equivalent leaf areas for all four plant species: on average, *Euonymus* 6 g, *Rhododendron* 7 g, *Taxus* 8 g and *Fragaria* 3 g. Ten weevils were placed in the plant-containing washbottles on both sides of the Y-tube olfactometer,

directly connected to the Y-tube. Two hours after release of 40 weevils at the base of the Y-tube the number of weevils in the washbottles on both sides were counted. The control treatment consisted of washbottles on both sides, with 10 weevils placed in each bottle. Each experiment was repeated four times.

Herbivory-induced odour attraction

To determine the effect of feeding damage on host-plant attraction, weevils and plants were separated by serially connected washbottles in the Y-tube olfactometer (Fig. 1). *Euonymus fortunei* was used as the only test plant. The washbottles were either empty or contained *Euonymus* cuttings or 10 starved weevils. Weevils were unable to reach washbottles with plants or starved weevils. By changing the sequence of the washbottles containing weevils or plants (washbottles 1 and 2 in Fig. 1), the weevils in the washbottles were offered either clean air or air that had passed over *Euonymus* foliage. Weevil-damaged *Euonymus* was prepared by offering 20 starved weevils fresh plant cuttings for 2 h prior to starting a test. Subsequently, the damaged foliage, after removal of the 20 weevils, was placed in a clean washbottle for use in the bioassay. The two-choice tests consisted of a treatment with *Euonymus* (undamaged or feeding damaged) in a washbottle serially connected to a washbottle with 10 weevils and an empty washbottle on one side vs. one washbottle with 10 weevils connected to two empty washbottles on the other side. Three serially connected washbottles on each side with 10 weevils in the middle washbottles on both sides served as one control treatment and the same set-up with 10 weevils in the middle washbottle on one side vs. *Euonymus* cuttings + 10 weevils in the middle washbottle on the other side served as the other control treatment.

Herbivore- vs. mechanically induced odour preference

The preference of vine weevils for odour from feeding-damaged, mechanically damaged and undamaged cuttings of *Euonymus* was compared relative to each other and to clean air in the still-air olfactometer (Fig. 2). Weevil-damaged or mechanically damaged cuttings from *Euonymus* were placed in the cups of the olfactometer prior to the release of 10 weevils in the Petri dishes. After 2 h the number of weevils in both glass cylinders and in the Petri dish present were counted. In this olfactometer, the preference of the weevils was tested to odour from fresh mechanically damaged and fresh or one-day-old weevil-damaged *Euonymus* when compared with a control ($n = 24$, 16 and 9, respectively) or to undamaged *Euonymus* ($n = 34$, 22 and 22, respectively). Furthermore, it was tested whether the weevils preferred the odour of fresh or one-day-old mechanically damaged *Euonymus* when odour of fresh ($n = 22$) or one-day-old weevil-damaged *Euonymus* ($n = 8$)

was the alternative. Fresh weevil-damaged *Euonymus* cuttings were fed on by 20 starved weevils 2 h before a test. The weevils were removed from the plants prior to the start of the test. Fresh mechanically damaged leaves were cut (six cuts per leaf) with a pair of scissors 15 min before the start of a test. Attractiveness of one-day-old damaged *Euonymus* cuttings (mechanically and weevil-damaged) was tested on separate plants 24 h later. The control treatment consisted of two empty cups with a wet cotton wool ball on each side. To determine the influence of season on plant attractiveness for the vine weevil, several treatments were repeated for two years at different times of the year.

Statistics

Data of all trials were analysed by Generalized Linear Model (GLM) with binomial distribution and logit link and with the different test combinations as independent variables ($P=0.05$; one-tailed analysis for experiments where an odour source was compared to a control and two-tailed analysis for all other experiments). The response was measured as the fraction of active weevils (activity) and the fraction of weevils choosing one treatment side (choice) using the Genstat 5 computer program. Activity was measured as the number of weevils that showed behavioural activity in the olfactometer (trapped into left or right washbottle or glass cylinder) compared to weevils not responding (not entering washbottles or glass cylinders in the olfactometer). Choice was measured as the number of active weevils choosing one or the other side in the olfactometer. After the GLM analysis the estimated fractions of activity and choice (logit transformed data) were analysed with *t*-tests. For activity these functions were compared with each other and for choice the fractions different from zero (indicating the fractions different from 50% were determined). Extra GLM analysis, with Year and Day of the year as independent variables, was performed within each test combination to see whether the choice fraction (on the logit scale) decreased in a linear fashion with time (Day of the year).

Results

Attraction to weevil-damaged host-plant species (Y-tube olfactometer)

Of the weevil-damaged host plants tested, odour from *Taxus* and *Euonymus* stimulated significantly more weevils to become active (83%, $P=0.01$, and 81%, $P=0.02$, respectively) than the control treatment (58%). The activity in tests with *Fragaria* (68%, $P=0.29$) and *Rhododendron* (63%, $P=0.56$) did not differ from the control treatment.

The active weevils were strongly attracted to *Euonymus* (90% preference; $n=4$, $P=0.005$) and *Taxus* (88% preference; $n=4$, $P=0.005$), whereas the response to odour from *Fragaria* (52% preference; $n=4$, $P=0.86$) and *Rhododendron*

(61% preference; $n=4$, $P=0.19$) was neutral. The control treatment indicated no preference for the left or right side of the Y-tube (left 47% and right 53%; $n=4$, $P=0.43$). Analysis on influence of the weevil populations used in this study revealed no differences ($F=0.59$, d.f. = 1, 3, $P=0.63$).

Herbivore-induced odour attraction (Y-tube olfactometer)

The percentage of responding weevils under the different treatments and the controls did not differ significantly ($F=0.57$, d.f. = 7, 12, $P=0.77$) and varied between 43 and 69%.

The odours of *Euonymus*, fed on by weevils ($P=0.05$), and weevil-damaged *Euonymus*, after removal of weevils prior to testing ($P=0.02$ for test where odour of weevil-damaged *Euonymus*, subsequently passes over weevils before entering the choice area) were attractive for the weevils (Fig. 3), but did not differ in attractivity (Student's *t*-test: $P(t)=0.90$). Undamaged *Euonymus*, i.e. undamaged *Euonymus* and weevils separated by connecting washbottles in the olfactometer, was not attractive to the weevils. In contrast, although not significant ($P=0.08$), more weevils were found at the side with clean air. Although the treatment where air passed over the weevils first before passing over weevil-damaged *Euonymus* was not significantly attractive for the vine weevils ($P=0.08$), it was still as attractive as the treatment where air passed weevil-damaged *Euonymus* first and subsequently weevils in a washbottle before entering the choice area (Student's *t*-test: $P(t)=0.44$). The control treatment revealed no significant differences between the left and right side ($P=0.48$ for the right side).

Herbivore- vs. mechanically-induced odour preference (still-air olfactometer)

The percentage of weevils responding was generally high for all trials (83–95%). Significant differences in activity were mainly found between the control treatment and treatments with *Euonymus* ($F=0.015$, d.f. = 9, 236, $P<0.001$), with the control treatment having the lowest number of weevils responding (Fig. 4).

The results of preference experiments (Fig. 4) with *Euonymus* were performed with plant material picked from stock plants in the field in the period March–September over two consecutive years. Data in Fig. 4 are overall results and hence not corrected for effects of time of the year. Odours from undamaged ($n=71$, $P<0.001$), mechanically damaged ($n=24$, $P<0.001$) and herbivore-damaged ($n=16$, $P<0.001$) plants were strongly preferred by the vine weevil when clean air was the alternative. Weevil-damaged *Euonymus* was still attractive 24 h after weevil damage ($n=9$, $P<0.001$). One-day-old weevil-damaged *Euonymus*, however, was not preferred when undamaged ($n=22$, $P=0.61$) or one-day-old mechanically damaged *Euonymus* ($n=8$, $P=0.27$) was the alternative choice (Fig. 4b). When weevils could choose between fresh weevil-damaged and

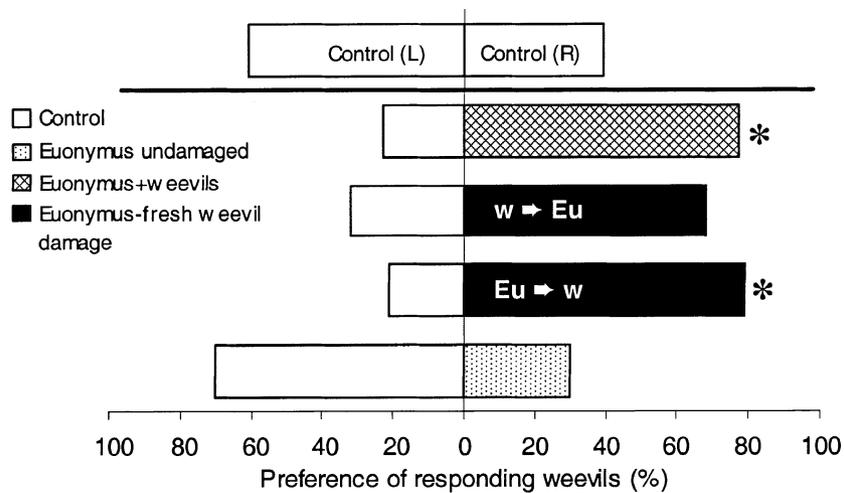


Fig. 3. Preference of *O. sulcatus* in an Y-tube olfactometer to odour of undamaged or weevil-damaged *Euonymus fortunei* ($n = 4$). *Euonymus* + weevils = 10 weevils feeding on the cuttings while tested; *Euonymus*-fresh weevil damage = cuttings fed on by 20 weevils 2 h prior to testing; $w \rightarrow Eu$ = clean air passing subsequently over 10 weevils and *Euonymus* foliage before entering the olfactometer; $Eu \rightarrow w$ = clean air passing subsequently over *Euonymus* foliage and 10 weevils before entering the olfactometer. Asterisks indicate significant differences from even distribution at $P = 0.05$ (*). The mean values shown are predicted values from the regression analysis.

mechanically damaged *Euonymus*, there was no preference for either odour source ($n = 22$, $P = 0.25$; Figs 4 and 5f). There was, however, a preference for mechanically damaged ($n = 34$, $P = 0.001$) and herbivore-damaged ($n = 22$, $P = 0.001$) plants when undamaged *Euonymus* was the alternative option (Figs 4a, 4c, 5d,e). Analysis on 'time-of-the-year' effects revealed that this positive preference was correlated to season-dependent growth of the plants (Fig. 5). The tested cuttings in spring (freshly budded sprouts) were preferred by the vine weevil, whereas olfactory preference for undamaged and mechanically damaged *Euonymus* decreased during the season (Figs 5a and b). For mechanically damaged cuttings this decrease in preference was lower than for undamaged cuttings, resulting in loss of preference for undamaged *Euonymus* in September and still significant preference for mechanically and herbivore-damaged *Euonymus* in September and October (Figs 5a–c). The absence of preference for undamaged *Euonymus* in Fig. 3 is in agreement with these results. These trials were performed with plant material picked in September, whereas the preference for *Euonymus* as shown in Fig. 4 presents overall results of tests performed in the period from March to September. Mechanically damaged as well as herbivore-damaged plants were also more preferred than undamaged *Euonymus* from March to July (Figs 5d and e). In late summer, this preference remained high for herbivore-damaged *Euonymus*, but was lost for mechanically damaged plants. Generally, herbivore-damaged *Euonymus* remained attractive up to late summer, whereas mechanically damaged *Euonymus* decreased in attractiveness with the time of the year. There was, however, no preference for weevil-damaged or mechanically damaged *Euonymus* when given a choice. This absence of

preference was not influenced by time of the year (Fig. 5f). The influence of time of the year on preference for one-day-old damaged *Euonymus* (Fig. 4b) was not tested. Results presented in Fig. 4b were tests performed with plant material exclusively picked in September. The control treatment revealed no bias in the olfactometer ($n = 33$, $P = 0.48$).

Discussion

Host-plant odour attraction

The vine weevil is attracted to the odour of some, but not all, host plants tested. *Taxus* and *Euonymus* are attractive when damaged by weevils but weevil-damaged *Rhododendron* and *Fragaria* are not. The discrimination for host-plant odours is promising for developing a monitoring tool or a pest control method based on 'attract and kill'. The attraction to volatiles from weevil-damaged *Taxus* and *Euonymus* is positively related to the feeding preference and high oviposition rate of the vine weevil on these crops (Smith, 1932; Nielsen & Dunlap, 1981; Hanula, 1988; Van Tol & Visser, 1998). The absence of attraction to odour from *Rhododendron* is in accordance with the low fecundity and high mortality of the weevils as compared with the performance on other plant species (Nielsen & Dunlap, 1981; Hanula, 1988; Van Tol & Visser, 1998; Van Tol, unpublished data). Only when there is no alternative plant will the vine weevil feed on *Rhododendron*. Several cultivars of *Rhododendron* are resistant to vine weevil (Doss, 1983), but the cultivar we used is accepted. The absence of suitable crops nearby and the clearly visible

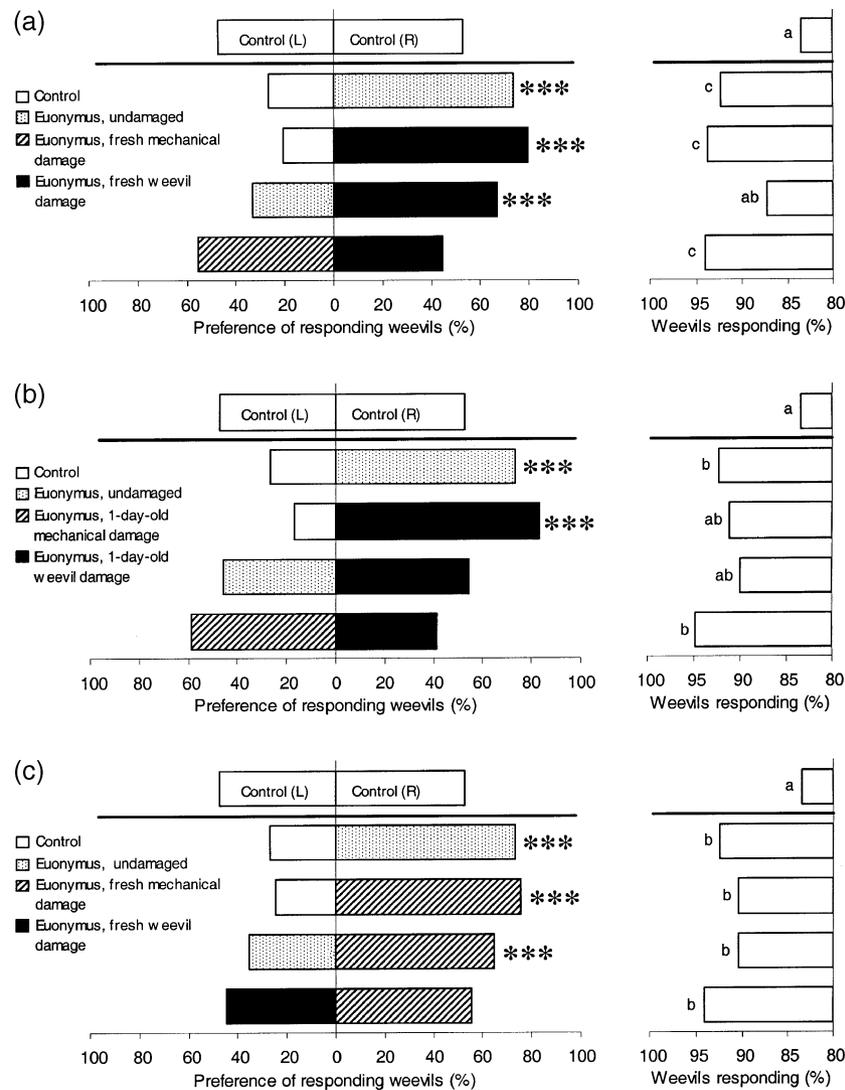


Fig. 4. Preference and activity of *O. sulcatus* in a still-air olfactometer to odour of undamaged *Euonymus fortunei* ($n = 71$) when a control was the alternative and to odour of *E. fortunei* that was (a) fed upon by 20 weevils 2 h prior to testing when a control ($n = 16$), undamaged ($n = 18$) or fresh mechanically damaged *Euonymus* ($n = 22$) was the alternative, (b) fed upon by 10 weevils 24 h prior to testing when a control ($n = 9$), undamaged ($n = 22$) or one-day-old mechanically damaged *Euonymus* ($n = 8$) was the alternative, and (c) freshly mechanically damaged when a control ($n = 24$), undamaged ($n = 34$) or fresh weevil-damaged *Euonymus* ($n = 22$) was the alternative. Asterisks indicate significant differences from even distribution at $P = 0.05$ (*), $P = 0.01$ (**) or $P = 0.001$ (***). Activity is expressed as percentage weevils responding, and bars followed by a different letter (a, b and c) indicate significant different activity of the weevils between the treatments. The mean values shown are predicted values from the regression analysis.

feeding damage to this evergreen shrub in nurseries explains why it is considered as one of the main host plants for the vine weevil. The strawberry cultivar tested is not attractive for the vine weevil according to our olfactory trials. 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 relatively high mortality of larvae feeding on the roots of

strawberry. Hanula (1988) found a relatively 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 situations, but that the oviposition rate is generally lower and the mortality higher compared to feeding on several other crops such as *Taxus* and *Aronia* (Van Tol, unpublished data).

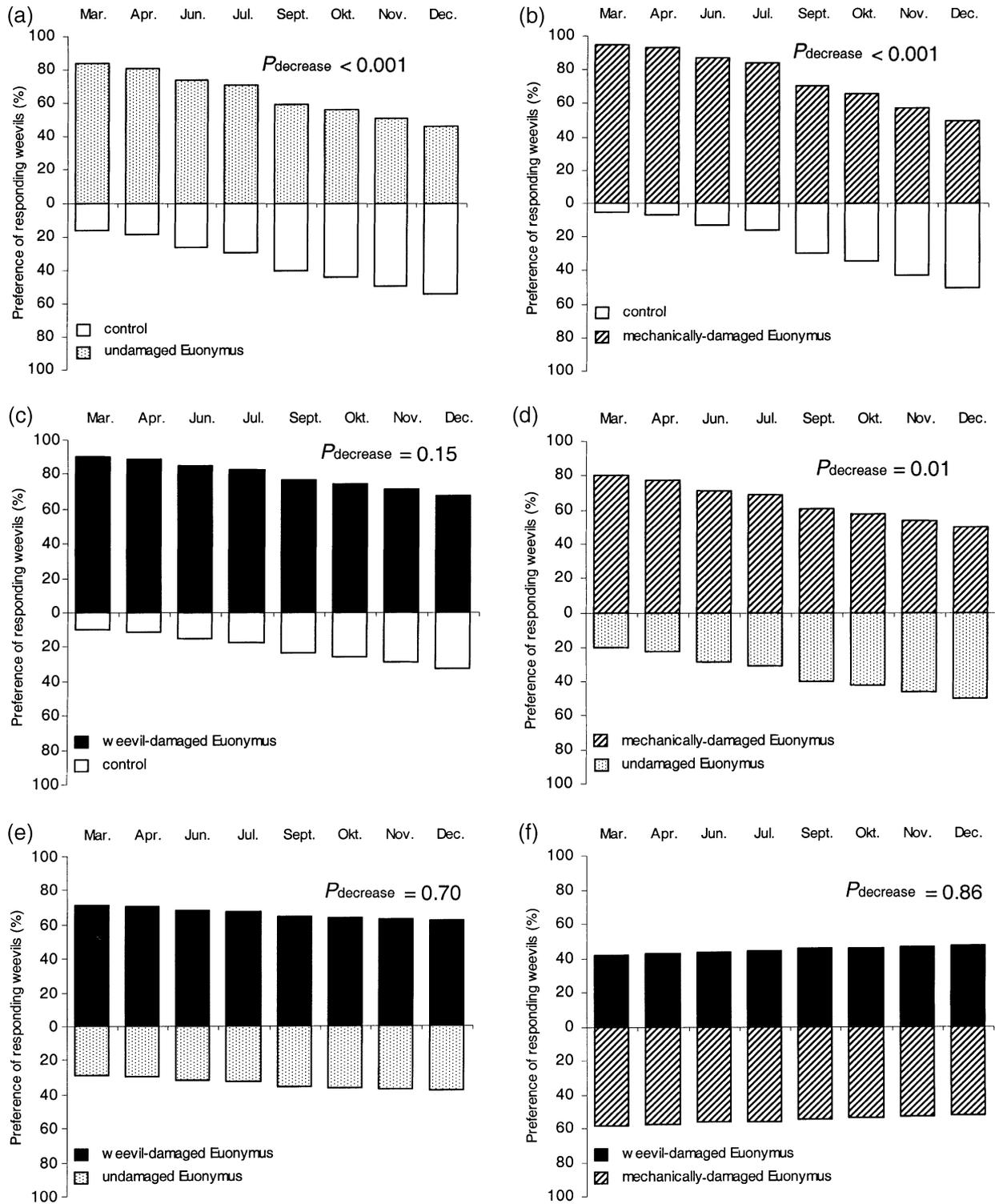


Fig. 5. Influence of season-dependent growth of *Euonymus fortunei* on preference of *O. sulcatus* in a still-air olfactometer for (a) undamaged ($n=71$), (b) mechanically damaged ($n=24$) and (c) weevil-damaged ($n=16$) *E. fortunei* when a control was the alternative, for (d) mechanically damaged ($n=34$) and (e) weevil-damaged ($n=22$) *E. fortunei* when undamaged *Euonymus* was the alternative, and for (f) weevil-damaged ($n=22$) *E. fortunei* when mechanically damaged *Euonymus* was the alternative choice. Decrease in time for preference is significant at $P_{\text{decrease}} = 0.001$ (***) , $P_{\text{decrease}} = 0.01$ (**) or $P_{\text{decrease}} = 0.05$ (*).

Herbivore- vs. mechanically induced attraction to Euonymus

The present results show that herbivore-damaged foliage of *Euonymus*, separated from the weevils prior to the start of the test is as attractive as *Euonymus*, while being fed upon by the weevils during the test. In this trial odour of weevils was present on both sides of the Y-tube olfactometer. Although it is clear that the plant odour is attracting the weevils, the influence of weevil odour combined with plant odour cannot be excluded as being responsible for the attraction in this experiment. Clearly some influence of weevil odour, perhaps in relation to faeces production, is possible.

A bioassay based on still-air was developed to determine the influence of plant damage caused by weevil feeding or mechanical damage on weevil attraction. Diffusion of odour in this bioassay enables the weevils to discriminate for odours as effectively as with the airflow in the Y-tube olfactometer. Because this two-choice olfactometer is easy to use and cheap to make, more parallel trials could be performed in a short period by using more olfactometers at one time. In this bioassay weevils strongly prefer mechanically as well as weevil-damaged foliage of *Euonymus* when clean air is the alternative. Weevil-damaged *Euonymus* is still attractive 24 h after the insects are removed. Because vine weevils strongly prefer odours released by mechanically as well as herbivore-damaged foliage of *Euonymus*, it may be that volatile components from wounded leaves play an important role in attraction of the vine weevil. The role of induced plant response to plant damage seems to be of minor importance in the first instance. It is, however, still possible that the preference for damaged foliage is caused by other volatiles produced in response to damage of *Euonymus*. Preference for one-day-old damaged leaves of *Euonymus* may still be caused by increased release of several green leaf volatiles. Loughrin *et al.* (1997) showed that grape plants damaged by Japanese beetles (*Popillia japonica*) emitted higher levels of several volatiles on the second day after damage when compared with freshly damaged plants. We suspect that these volatiles play an important role in attraction of the vine weevil to their preferred food plants because several volatiles also dominate strongly the EAG response profile of this weevil species (Van Tol & Visser, 2002).

Effect of season on plant attractiveness

Attractiveness of *Euonymus* for the vine weevil differs with season. In early spring (March–April) the young undamaged leaves are attractive to the vine weevil, whereas undamaged leaves in late summer (September) lose their attractiveness. Mechanically and herbivore-damaged foliage, however, remain attractive for the weevil from spring to late summer. Obviously, young undamaged leaves emit substantial amounts of attractive volatiles that are not released or released in lower amounts in late summer. The odour of damaged *Euonymus* signals to the weevils that it is a suitable food plant. Consumption of foliage from plant species such

as *Taxus* and *Euonymus* is limited to very few insects and no vertebrates because of the toxic properties of these plant species (Van Genderen *et al.*, 1996). The vine weevil is the sole insect that feeds on the needles of *Taxus* and only a few lepidopteran species within the family of *Yponomeutidae* feed on the leaves of *Euonymus* (Menken *et al.*, 1992). The caterpillars of these insect species, however, have left the plant by June and no overlap with weevil damage will occur. Therefore, feeding vine weevils most likely cause the increased emission of volatiles from *Euonymus*, and orientation to this plant odour is a guarantee for the weevils to find a suitable host plant. The preference of vine weevils for herbivore-damaged *Euonymus* does not decline during the season, unlike that for mechanically damaged *Euonymus*. This suggests that emission of volatiles, produced by the plants in response to weevil damage, may be more essential for attraction of the weevils because the weevils use these plant odours to find suitable food plants throughout the season.

The vine weevil is a polyphagous insect that can live and reproduce on many different plant species. Nevertheless, it clearly discriminates between plant species by olfaction. This olfactory response seems to be closely correlated to the fecundity of the weevil for the plant species tested. As the olfactory tests with *Euonymus* show, volatiles from damaged plants play a key role in attraction. The strong preference of the vine weevil for mechanically and weevil-damaged *Euonymus*, combined with the results of electroantennogram study (Van Tol & Visser, 2002), indicate an important role for the green leaf volatiles in attraction. In future studies, gas chromatography combined with electroantennogram detection and bioassays with attractive plant extracts will be used to determine the compounds and their composition that are attractive to the vine weevil.

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