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Host-plant acceptance on mineral soil and humus by the pine weevil *Hylobius abietis* (L.)

Running title: **Host-plant acceptance on mineral soil and humus**

Niklas Björklund, Göran Nordlander and Helena Bylund
Department of Entomology, Swedish University of Agricultural Sciences, P.O. Box 7044, SE-750 07 Uppsala, Sweden

Correspondence: N. Björklund. Tel.: + 46 (0)18 672327; fax: + 46 (0)18 672890; e-mail: Niklas.Bjorklund@entom.slu.se

Abstract

1 The pine weevil *Hylobius abietis* (L.) (Coleoptera, Curculionidae) is an economically important pest of conifer forest regenerations in Europe and Asia.

2 Soil scarification, which usually exposes mineral soil, is widely used to protect seedlings from weevil attack. However, the mechanism behind this protective effect is not yet fully understood.

3 Field experiments were conducted to determine the pine weevil's responses to visual and odour stimuli from seedlings when moving on mineral soil and on the undisturbed humus surface.

4 One experiment measured the number of pine weevils approaching seedlings, with and without added host odour, on mineral soil and undisturbed humus. Seedlings with added host odour attracted more weevils on both soil types. Unexpectedly, somewhat more weevils approached seedlings surrounded by mineral soil.

5 In a similar experiment, feeding attacks on seedlings planted directly in the soil were recorded. Only half as many seedlings were attacked on mineral soil as on undisturbed humus.

6 In the first experiment, the weevils were trapped 2.5 cm from the bases of the seedlings' stems, whereas they could reach the seedlings in the experiment where seedlings were planted directly in the soil. We conclude that the pine weevils' decision on whether or not to feed on a seedling is strongly influenced by the surrounding soil type and that this decision is taken in the close vicinity of the seedling. The presence of pure mineral soil around the seedling strongly reduces the likelihood that an approaching pine weevil will feed on it.

Keywords Curculionidae, host plant acceptance, host volatiles, *Hylobius abietis*, olfactory orientation, large pine weevil, *Picea abies*, pitfall trap, reforestation, scarification, seedling damage.

Introduction

The pine weevil *Hylobius abietis* (L.) (Coleoptera, Curculionidae) is an economically important forest pest as the adults feed on newly planted conifer seedlings, among other food sources (Eidmann 1974; Heritage *et al.* 1989; Örlander & Nilsson 1999). Large numbers of pine weevils are attracted to fresh clear-cuttings during their migration flight in spring. After arrival, their flight muscles regress, and they remain on the ground at the clear-cutting for the rest of the season (Långström 1982; Nordenhem 1989; Örlander *et al.* 1997, 2000). Treatment of seedlings with the insecticide permethrin has long been the most common way to limit damage caused by the pine weevil. However, this insecticide will be prohibited in the EU as from 2004 (Anonymous 2000). Thus, there is an urgent need to find and improve alternative control methods.

Soil scarification, which usually exposes mineral soil, is a widely used method in forest regeneration to create a good environment for the seedlings (Örlander *et al.* 1990) and to reduce damage caused by pine weevils (Lindström *et al.* 1986; Sydow 1997; Örlander & Nilsson 1999; Thorsén *et al.* 2001). Seedlings are an attractive food source and if planted in undisturbed humus (untreated ground surface) high mortality rates (80-90%) are commonly reported (e.g. Petersson & Örlander, manuscript). If the soil is scarified and seedlings are planted in the mineral soil, the mortality rates are usually less than half as high (Örlander & Nilsson 1999; Thorsén *et al.* 2001; Petersson & Örlander, manuscript). However, it is not fully understood why mineral soil has such a strong impact on damage caused by pine weevils, even though several studies have examined this issue. In a laboratory study where movement in relation to soil type was analysed, Kindvall *et al.* (2000) found that the pine weevils did not stop or turn back when they reached the border of a mineral soil area, but they moved faster on mineral soil than on humus, reducing the time spent on mineral soil. This difference in movement patterns should only slightly reduce the probability of weevils encountering a seedling planted in a patch of mineral soil. Nordlander (1998) suggests that pine weevils avoid staying on mineral soil because of the risk of overheating due to sudden exposure to solar radiation. In support of this hypothesis, adults of the related species *Hylobius radicis* and *H. pales* go into heat stupor within less than two minutes when the ground temperature exceeds 50°C (Wilson 1968; Corneil & Wilson 1984). Thus, avoiding the risk of lethally high temperatures may be the underlying reason why pine weevils cause less seedling damage on mineral soil than on undisturbed humus (Nordlander 1998). However, shading and irrigation experiments indicate that temperature, humidity and shading *per se* do not cause differences in weevil responses that could explain the difference in attack rates between mineral soil and humus (Nordlander *et al.* 2000). Thus, greater knowledge of factors that influence weevil responses is needed to improve soil scarification methods.

Pine weevils use host odours when locating food, and it has been shown that a small wound made on the stem of a seedling increases its probability of being attacked about five-fold on undisturbed humus (Nordlander 1991). In the present study we investigated how soil type around seedlings affects pine weevil responses to different levels of host odours. For this, we used traps measuring the numbers of weevils passing within 2.5 cm of the base of the seedling, and also recorded pine weevil attacks on planted seedlings. By comparing rates of approaching weevils and feeding attacks on mineral soil and undisturbed humus, it was possible to determine if responses leading to attacks were predominantly initiated in the close vicinity (<2.5 cm) of the seedling or further away. Two levels of release of host volatiles, and thus of host attraction (Nordlander 1991), were set by using intact and wounded seedlings. A hypothesis tested was that pine weevils often do not respond to host-odour stimuli when moving rapidly, as they do on mineral soil (Kindvall *et al.* 2000). According to this hypothesis the number of weevils approaching a seedling should be lower on mineral soil than on undisturbed humus, and the number of approaching weevils should increase less with increased host-odour levels on mineral soil than on undisturbed humus.

Materials and Methods

The number of adult pine weevils approaching wounded and intact seedlings placed in traps on mineral soil and undisturbed humus was measured in a field experiment. In a second field experiment the presence or absence of feeding scars caused by pine weevils was recorded amongst seedlings planted in mineral soil or undisturbed humus. The experiments were conducted on a fresh clear-cutting 32 km NNW of Uppsala in central Sweden. Before felling, the old forest consisted of a mixed Scots pine (*Pinus sylvestris*) and Norway spruce (*Picea abies*) stand. It was harvested during the winter of 1999-2000, and the experiments were carried out during June and July 2000. Containerised two-year-old Norway spruce seedlings (of the provenance Rezekne) were used for the stimulus treatments, when applicable.

Trapping around seedlings

The trapping experiment was set-up as a randomised block-design with two levels. Treatments included two types of ground cover and three types of stimulus presented in the centre of the traps. The 12 blocks included one representative of each treatment combination (each stimulus treatment on each ground cover), giving a total of 72 traps. The traps were emptied, baits were renewed and the stimuli were randomly replaced within blocks on six occasions at one-week intervals.

In each block, six circular areas (0.2 m radius) were chosen that were as similar as possible with regard to vegetation and other factors. There was a minimum distance of 3 m between the periphery of the circles. Slash was removed from the area within a 1 m radius of each circular area. Three of the six areas in each block were randomly chosen to be covered with mineral soil. The mineral soil, taken from a sandpit dug close by (with 74 % by weight of the grains between 0.1-0.5 mm), was added in a layer as thin as possible while completely covering the ground. To avoid colonisation by vegetation, supplementary mineral soil was added each week. The remaining three areas in each block were left undisturbed, i.e. a plain surface of humus with some litter and sparsely growing grasses. The spatial location of the areas with different types of soil cover was not changed between the weeks but the stimuli treatments in the traps were randomised after each recording in order to minimise the risk of site effects.

A pitfall trap was placed in the middle of each circular area. In the centre of the traps was a wounded seedling, an intact seedling or no stimulus. The wounded seedling treatment involved cutting a 3 cm long knife cut through the bark of the seedlings. From the fourth week of the experiment, the wounded seedling treatment was replaced by adding three "host-odour pegs" beside an intact seedling to increase odour emission. These consisted of 5 cm long stem sections taken from the same lot of seedlings as those used in the experiment. The height of the seedling gradually doubled during the six weeks the experiment lasted.

The pitfall traps surrounding the seedlings were constructed with the aim of catching all pine weevils approaching the seedling (Fig. 1). There was a distance of only 2.5 cm from the stem base of the seedling to the sloping rim from which the weevils fell. Therefore, it was assumed that the trapped weevils either had detected or would have detected the seedling if the trap had not been there. A thin border (≈2 cm) of mineral soil was added around the traps on undisturbed soil in order to standardise the trap efficiency on different types of ground cover.

The traps were constructed from Polyethylene terephthalate (PET) bottles and filled with water, plus one drop of detergent, to 6 cm from the top. No glue was used to avoid odour influence. A slippery surface was created on the fall rim, and on the inner side of the trap, by applying a layer of Fluon® (ICI, Herts, England). Laboratory studies before the field experiment confirmed that pine weevils passing the rim were effectively trapped (unpublished data).

An analogous field experiment comparing traps with and without water was conducted in order to investigate whether the presence of water influenced the catches. The experiment included twelve blocks with four types of treatments: empty traps, water, host-odour pegs and host-odour pegs plus water. The traps were emptied on four occasions at 2-day intervals.

Feeding on planted seedlings

Another experiment with a similar design but without traps was carried out on the same clear-cutting. Treatments included seedlings with and without wounds planted in areas (0.2 m radius) with and without added mineral soil. Each treatment combination was represented once in each of the 51 blocks, 37 of the blocks were recorded twice with new randomised seedling treatments, i.e. 352 seedlings were used. One week after planting it was recorded whether or not the seedlings had feeding scars caused by pine weevils.

Statistics

The pine weevil catch around seedlings was calculated as the total catch, summarised for six sampling occasions, per treatment combination and block. The mean catch per treatment (n=12 per treatment) was then calculated. To account for between block variation the average catch for each block was subtracted from the total block catch of each treatment. Hence, the block factor could be omitted in the statistical analyses. An analysis of variance of average weevil catch per treatment was performed (procedure GLM, SAS Inst.), using host odour (two levels) and ground cover as main factors (both treatments fixed factors). The same procedure was used to analyse the effect of water in the traps. Data were found to fulfil the assumption of homogenous variances and were thus not transformed.

A logit model for qualitative predictors (2*2*2 contingency table) (Agresti 1996) of feeding attacks was used to test possible effects of ground cover and host-odour level and to determine if there were any interactions between ground cover and host-odour level (procedure GENMOD, SAS Inst.).

Results

The main factors, host odour and ground cover, showed significant effects in the analysis of variance testing trap catches around seedlings. The trap catches were larger around seedlings with added host odour ($P<0.05$, d.f. =1, F=5.28) and also larger in traps placed on mineral soil compared to undisturbed humus ($P<0.001$, d.f. =1, F=17.03). There was no interaction between ground cover and host-odour level (P=0.193, d.f. =1, F=1.75) (Fig. 2). In the experiment evaluating the presence of water no difference in trap catches was found between traps with and without water (P=0.056, d.f. =1, F=3.85) (Fig. 3). There was, however, an interaction between water and host odour, showing that the presence of water amplified the attraction of the host odour ($P<0.05$, d.f. =1, F=5.76).

Among the planted seedlings, about half as many were attacked on mineral soil compared to those on undisturbed humus (d.f. =1, χ^2=12.495, $P<0.001$). Extra host odour from wounds more than doubled the rate of feeding attacks (d.f. =1, χ^2=39.134, $P<0.001$) compared with intact seedlings. A deviance value of 0.741 (d.f. =1, P=0.389) suggests there was a reasonable fit to the model and thus no interaction between ground cover and host-odour level (Fig. 4).

Discussion

Slightly higher numbers of weevils were caught in pitfall traps with seedlings when the seedlings were surrounded by mineral soil rather than by undisturbed humus. This may at first seem as a surprising result since only half as many seedlings were attacked on mineral soil compared to humus in our experiment with seedlings planted directly in the soil. Several earlier studies have also shown that seedlings planted in mineral soil suffer considerably less from pine weevil damage than seedlings planted in humus (Lindström *et al.* 1986; Sydow 1997; Örlander & Nilsson 1999; Thorsén *et al.* 2001). However, seedlings planted directly in the soil could be reached by the pine weevils, whereas in the trapping experiment there was a 2.5 cm gap between the stem base of the seedling and the rim from which the weevil fell. We conclude that the weevils' decision on whether or not to feed on the seedling is strongly influenced by the surrounding soil type and that this decision is taken in close vicinity (less than 2.5 cm) of the seedling. The presence of a top layer with pure mineral soil a few centimetres deep around the seedling strongly reduced the likelihood that an approaching pine weevil would feed on the seedling. It is possible that the presence of good hiding or burrowing places, as provided by undisturbed humus, close to seedlings is the essential factor. Pine weevils may stay for days, hiding during the hot hours of the day and repeatedly return to the same seedling.

Our hypothesis that pine weevils do not respond to host odour when moving rapidly, as they do on mineral soil (Kindvall 1999), was not supported by the results of the trapping experiment. Trap catches were not lower on mineral soil than on undisturbed humus, and no interaction effect was found between odour treatment and soil type. Thus, the response to raised odour level was similar regardless of the soil type the weevils were moving on. On both mineral soil and humus the addition of host odour resulted in a relatively low increase in trap catch 2.5 cm from the seedling, but it increased the frequency of attacks on planted seedlings more than threefold. This implies that the increased probability of attack for wounded seedlings (Nordlander 1991) was mainly due to stimuli acting very near or at the wounded seedlings.

The considerable numbers of weevils caught in the empty traps both on mineral soil and undisturbed humus suggest that pine weevils walk around to such an extent that almost every spot on a clear-cutting is passed during one season. Consequently, almost all seedlings should be “visited” by pine weevils, although large proportions of those planted in mineral soil are never attacked. The substantial catch in control traps was not due to attraction to water, as shown by our control experiment. There was, however, an amplification of the attraction to the added host odour (“host-odour pegs”) in the presence of water, but this did not bias the main experiment since all traps were filled with water.

Pine weevils walk faster on mineral soil than on humus, but they do not turn back when they come to a border of an area with mineral soil (Kindvall *et al.* 2000). Because of their higher speed on mineral soil, slightly smaller catches of weevils would be expected on mineral soil than on humus, at least in empty control traps not affected by visual or olfactory orientation. However, for all three categories of traps, more weevils were caught in traps on mineral soil than on undisturbed humus in our experiment. This might have been because the higher speed on the mineral soil increased the probability of the weevils falling into the traps, although our tests prior to the main experiment proved that the efficiency of the traps was very high.

The areas with mineral soil in this study resembled areas affected by soil scarification. Based on the results of this study we suggest that pine weevils often do not feed on seedlings planted in mineral soil even if they pass by very closely. Further research on the protective effects of soil scarification should concentrate on identifying factors that strongly influence feeding decisions made in the close vicinity of the seedlings.

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Figure legends

Figure 1 Pitfall trap used in experiments. The distance between the stem of the seedling (placed in the central container) and the sloping rim from which the weevils fell was 2.5 cm.

Figure 2 Average total numbers of pine weevil caught, summed over six occasions, in pitfall traps with three levels of host odour and on two types of soil surface (n=12 for each treatment). Fresh clear-cutting, 1 June - 13 July.

Figure 3 Pine weevil catches in pitfall traps with empty traps, traps with water, with host odour and with them in combination (n=12 for each treatment). Fresh clear-cutting, 14 July - 1 August.

Figure 4 Proportions of seedlings attacked by pine-weevils with and without added host odour and planted on two types of ground surface. Numbers above the bars give the total number of seedlings in the respective classes (n=51 for each treatment). Fresh clear-cutting, 23 June -18 July.

Fig. 1

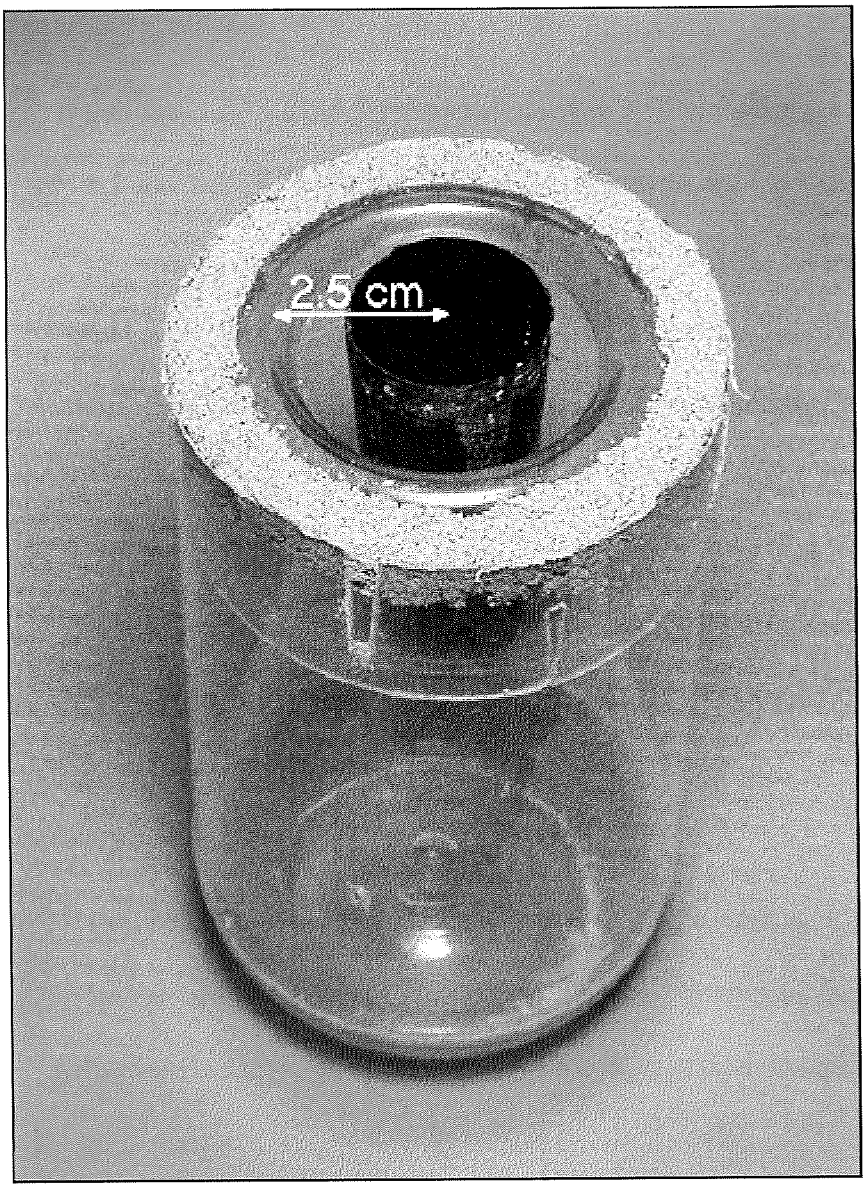


Fig. 2

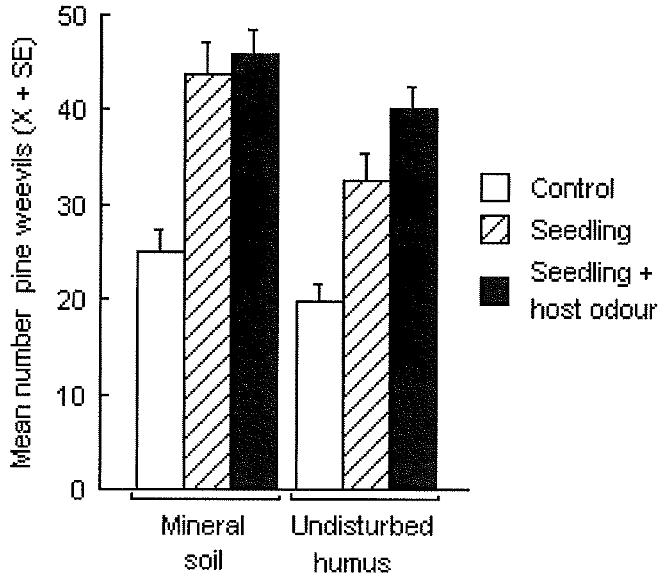

Mean number pine weevils (X + SE)
50
40
30
20
10
0
Control
Seedling
Seedling +
host odour
Mineral
soil
Undisturbed
humus

Fig. 3

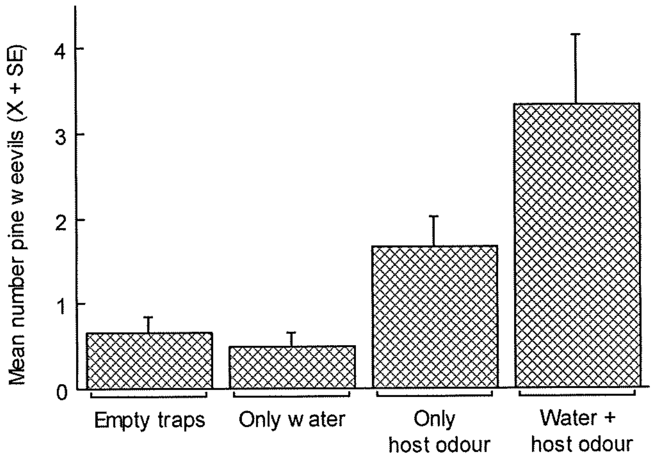
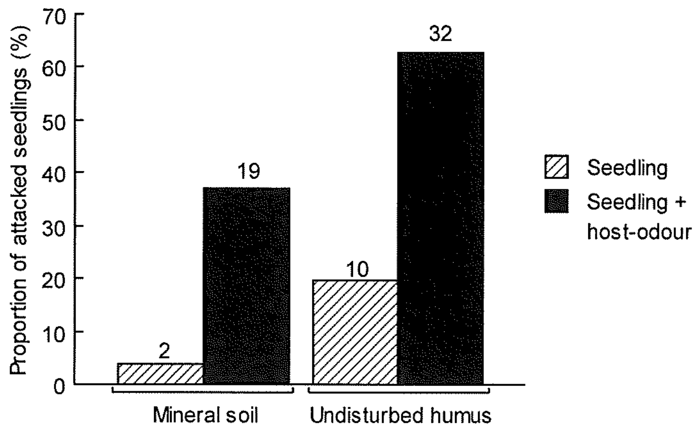

Mean number pine w eevils (X + SE)
0
1
2
3
4
Empty traps
Only w ater
Only
host odour
Water +
host odour

Figure 4


Proportion of attacked seedlings (%)
70
60
50
40
30
20
10
0
2
19
10
32
Mineral soil
Undisturbed humus
Seedling
Seedling +
host-odour