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Olfactory and visual stimuli used in orientation to conifer seedlings by the pine weevil *Hylobius abietis*

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Abstract

A field experiment was conducted to determine the influence of non-contact plant cues on the likelihood that individual conifer seedlings will be found by walking adults of the pine weevil *Hylobius abietis* (L.) (Coleoptera, Curculionidae). Pine weevils were caught in traps when approaching different combinations of olfactory and visual stimuli and their numbers recorded. Treatments with solely odour or solely visual stimuli caught significantly more weevils than a non-stimuli treatment, and the combination of odour and visual stimuli caught more weevils than odour and visual stimuli alone. There was a slight interaction between odour and visual stimuli, in the direction of competition rather than synergism. However, the effect was small compared to the response to the separate stimuli and principally the response can be regarded as additive. The reaction to odour and visual stimuli was similar for three phases of the pine weevil’s life cycle associated with three ages of clear-cuttings. Visual stimulus was at least as important as odour for the pine weevil in finding an undamaged conifer seedling. We suggest that the significance of visual stimuli in resource finding may be generally underestimated because of the use of unnaturally strong odour stimuli in many studies.
Introduction

The behavioural events leading a phytophagous insect to feed on a host plant can be divided into three sequential steps: finding food, examining food, and consuming food (Miller & Strickler, 1984). Finding food can be further subdivided into levels, e.g. finding the habitat and finding the resource item itself, where the influence of olfactory and visual stimuli may differ (Prokopy, 1986). This study concerns the influence of non-contact plant cues on the likelihood that individual conifer seedlings will be found by walking adults of the pine weevil *Hylobius abietis* (L.) (Coleoptera, Curculionidae).

Many phytophagous insects orient towards odours from host plants (Bell, 1984; Bernays & Chapman, 1994; Visser, 1986), but most of the evidence for this is based on laboratory experiments and may not be relevant under natural conditions with many competing stimuli (Jermy *et al.*, 1988; Morris & Kareiva, 1991; Schoonhaven *et al.*, 1998). Baited traps emitting very high concentrations of host odour often catch large numbers of walking adult insects but this does not prove the significance of host odour in plant finding. The evidence is scarce for the orientation solely towards odour released from a plant in the field by walking adult insects. It has been proposed that distant olfactory attraction to host plants in nature may be much rarer than has been thought (Jermy *et al.*, 1988). The use of visual stimuli in detecting plants, although much less investigated, has also been shown for several species (Prokopy & Owens, 1983). As is the case with odours, many studies have been carried out in the laboratory, e.g. walking *Pissodes strobi* Peck (Curculionidae) adults orient preferentially towards silhouettes that are: vertical rather than oblique, 3 cm in width rather than other diameters, taller rather than shorter (VanderSar & Borden, 1977). These results are in good agreement with the characteristics of spruce twigs attacked by *P. strobi* in the field. There are also examples from the laboratory where a visual stimulus alone does not elicit a response,
e.g. the adult plum curculio *Conotrachelus nenuphar* (Herbst) (Curculionidae) walking on
tree twigs does not respond to visual fruit characteristics (Butkewich & Prokopy, 1993). In the
field, attraction towards solely visual stimuli has been shown for the desert locust, which
walks towards conspicuous vegetation from at least 1.5 m (Kennedy, 1939).

Multiple sensory modalities are often used before an insect arrives to a resource (reviewed in
(Miller & Strickler, 1984; Prokopy, 1986)). Authors often claim that they have found an
“interaction” between odour and visual stimuli, but the interpretation of this term differs. If,
for instance, the response to a combination of the stimuli is as large as the sum of the
individual responses some would claim that there is an interaction. Others, using statistical
terms, would claim that there is no interaction in this case but refer to it as an additive
response. In this study the statistical definition of interaction will be used (Slinker, 1998).

Several studies have shown that insects respond stronger when, for example, a visual stimulus
is added to an odour stimulus. It is tempting to conclude that the odour and visual stimuli
together are stronger than the odour by itself, but the visual stimulus may alone be as strong
as the combination. It is therefore important that the stimuli are tested both individually and
together. This has been done in several studies. For example, it has been shown that the
cabbage moth landed more often when an artificial leaf with added plant odour were
presented than when an artificial leaf or plant odour were presented separately (Rojas &
Wyatt, 1999). Similarly, the mean number of captured adult cabbage root flies tended to be
higher when odour and visual stimuli were combined than when presented separately (Tuttle
*et al.*, 1988).
If a treatment with none of the stimuli is included it is possible to determine the “true response” of the other treatments, that is the fraction above the control level. A control is also necessary to test if the stimuli acts synergistically, i.e. if the total effect is greater than the sum of effects taken independently (Slinker, 1998). Odour and colour have been reported to act synergistically on alighting for a species of leafhopper in an observation chamber (Todd et al., 1990) and for the onion fly on three-dimensional traps in onion fields (Judd & Borden, 1991). Odour and visual stimuli, i.e. yellow vs. transparent, appears to act synergistically on the number of aphids landing on traps in the field (Chapman et al., 1981). An example of where the effect of odour and visual stimuli appears to be additive rather than synergistic is the response to tree models by adult plum curculio (Butkewich & Prokopy, 1996). For walking adult insects much less has been done, but in a laboratory experiment the grasshopper Melanoplus sanguinipes (F.) approached odour and visual stimuli from plant sources more frequently than the control, and the response to odour and visual stimuli combined was even larger. The present study is, to our knowledge, the first that in the field has investigated if odour and visual non-contact stimuli act synergistically during resource finding for a walking insect.

The physiological state of an insect could influence resource-oriented behaviours (reviewed in Barton Browne, 1993). Responses to non-contact odour and visual stimuli from a resource have been shown to be influenced by age and reproductive status of the insect and the season (Barata & Araújo, 2001; Borden et al., 1986; Brevault & Quilici, 1999; Hoffman et al., 1997; Landon et al., 1997; Mathieu et al., 2001; Prokopy, 1977). Also in H. abietis there is some evidence for that the reaction to odour may be dependent on in which phase in the adult life cycle the pine weevils are, which in turn is related to the age of the clear-cutting (Nordenhem & Eidmann, 1991; Nordlander, 1990). The present experiment was therefore performed on
clear-cuttings of different ages, with weevils in different phases of their life cycle, to evaluate
if the response to odour and visual stimuli differ.

Large numbers of pine weevils *H. abietis* fly during late spring to fresh clear-cuttings, which
provides plenty of breeding material. After arrival their flight muscles regress and they remain
on the ground for the rest of the season (Långström, 1982; Nordenhem, 1989; Örlander *et al.*, 1997; Örlander *et al.*, 2000). The pine weevil is most active at twilight (Sibal *et al.*, 1999) and
feeds on several tree species (Leather *et al.*, 1994; Manlove *et al.*, 1997). It is a serious pest in
areas where clear-cutting with subsequent replanting is practised, since the adults feed on the
stem bark of newly planted conifer seedlings (Day *et al.*, 2004; Day & Leather, 1997;
Långström & Day, 2004) but feeding also occurs in the crowns and roots of mature conifer
trees (Örlander *et al.*, 2000).

It has been shown in other pest systems that an increased knowledge of the basic processes of
resource finding could bring about more effective protection methods (Duan & Prokopy, 1995; Foster & Harris, 1997), which should be valid also for the pine weevil system. The aim
of this study was to determine: 1) if the number of pine weevils arriving to an individual
conifer seedling is influenced by odour and visual stimuli presented separately 2) if there is a
synergistic effect between odour and visual stimuli (which requires the following treatments:
*only* odour stimuli, *only* visual stimuli, odour and visual stimuli, and control (Slinker, 1998)),
and 3) if these reactions differ among the three phases of the pine weevil's life cycle
associated with three ages of clear-cuttings. To answer these questions it was necessary that
the stimuli used were of the same magnitude as those found in nature.
Materials and methods

In a field experiment, adult pine weevils were caught in traps when approaching different combinations of olfactory and visual stimuli and their numbers recorded. The experiment was conducted near Uppsala in central Sweden during three periods of time: 1) during six weeks in August and September 2000 on a 1-year-old clear-cutting (newly emerged pre-reproductive weevils, prior to hibernation) 2) during six weeks in May and June 2001 on a 2-year-old clear-cutting (pre-reproductive, pre-migratory, maturation feeding weevils), and 3) during four weeks in June and July 2000 on a fresh clear-cutting (reproductive, immigrant weevils) (Nordenhem & Eidmann, 1991). Before clear felling the stands consisted of mixed Scots pine *Pinus sylvestris* (L.) and Norway spruce *Picea abies* (L.) Karst.. A randomised block-design was used with 12 blocks on each clear-cutting and six traps with different treatments within each block. Each week the traps were emptied, the numbers of trapped pine weevils recorded, baits renewed, and the treatments randomly replaced within blocks. Within each block, trap positions were chosen so that the surrounding vegetation and other factors were as similar as possible. There was at least 3 m between traps and at least 60 m between a trap and the edge of the clear-cutting. Slash was removed within a 1-m radius of the traps.

Pitfall traps, described by Björklund et al. (2003), caught approaching pine weevils at a 2.5-cm radius from the treatment (Fig. 1). A slippery surface was created on the sloping fall rim and on the inner side of the trap by applying Fluon® (ICI, Herts, U.K.). The traps were made of polyethylene terephthalate (PETP) bottles, constructed without glue to avoid odour influence, and filled with water with a few drops of detergent added to drown responding weevils and to supply the conifer seedlings with water.
The following treatments were included: 1) control 2) pine odour 3) dummy-seedling 4) dummy-seedling + pine odour 5) seedling 6) seedling + pine odour. Dummy-seedlings were used to obtain an odourless visual stimulus, treatment 5 and 6 were used as positive controls. The seedlings used were two-year-old containerized Norway spruce seedlings (provenance Rezekne). Pine odour was achieved by burying three 5-cm long freshly cut stem pieces, from the same lot of seedlings, in soil in the centre of the trap with their upper end at level with the soil surface. The dummies was composed of a green metal wire (3 mm in diameter) as stem and 6-cm long thin metal wires covered with green plastic (CLIP’N’TWIST (Buryrite)) twisted around the stem as twigs (Fig. 1). The dummies reached 25 cm above ground, which was approximately the same height as the seedlings.

Statistics

The positive controls, treatment 5 and 6, were not included in the following analyses. The pine weevil catch was calculated as the total catch, summarised for each period, per treatment and block. The homogeneity among blocks of the responses to the different treatments were tested with Fisher’s exact test (period 1 and 2) or a $\chi^2$-test (period 3) for separate two-way contingency tables (12 blocks × 4 treatments) (procedure FREQ, SAS Institute 1999). The homogeneity among periods of the responses to the treatments among the periods were tested by a $\chi^2$-test for a two-way contingency table (3 periods × 4 treatments) (proc. FREQ, SAS Inst.), which requires homogeneity among blocks of each period. A confidence interval for the response of treatment k, k=1,2,3,4, was calculated as $\hat{p}_k \pm 1.96 \times SE(\hat{p}_k)$, when $\hat{p}_k = x_k / x$. with $x_k$ equal to the number of weevils caught at treatment k and x. equal to the total number of weevils. By using facts about the binomial distribution the standard error was calculated as $SE(\hat{p}_k) = [\hat{p}_k (1 - \hat{p}_k) / x_k]^{1/2}$. To determine if there was any interaction between
odour and visual stimuli a $\chi^2$-test was used for a two-way contingency table (combinations of absence and presence of odour and visual stimuli) (proc. FREQ, SAS Inst.).

Results

In total, 118 pine weevils were caught on the 2-year-old clear-cutting, 750 on the fresh clear-cutting, and 96 on the 1-year-old clear-cutting. The distributions of catches on the different treatments were similar during the three time periods (Fig. 2). The response to the positive controls (conifer seedling and conifer seedling + pine odour) were similar although stronger than the response to their experimental counterparts (dummy-seedling and dummy-seedling + pine odour). The positive controls were not included in the following analysis. There was no difference in responses to the different treatments among blocks for period 1 (Fisher’s exact test $P=0.18$), period 2 (Fisher’s exact test $P=0.65$) or period 3 ($\chi^2=30.92$, $P=0.57$). It was therefore appropriate to test for differences in responses to the different treatments among periods, however no differences were found ($\chi^2=6.8$, $P=0.34$). In the following analysis the data from the three periods were pooled. Treatments with solely odour and solely visual stimuli caught significantly more weevils than the non-stimuli treatment, and the combination of odour and visual stimuli caught more weevils than odour and visual stimuli alone (Fig. 3). There was a weak interaction between odour and visual stimuli ($\chi^2=3.95$, $P=0.049$) in the direction of competition rather than synergism (Fig. 3).
Discussion

The positive controls, i.e. conifer seedling and conifer seedling + pine odour, confirmed that the stimuli used were of the same magnitude as those emitted by seedlings. Further, the effect of adding pine odour to the artificial visual stimuli (dummy-seedling) was similar to the effect of adding pine odour to a spruce seedling. The non-stimuli treatment is considered to measure the probability that a spot is passed by chance. In support of this it has earlier been shown that the water present in this type of traps does not increase trap catches (Björklund et al., 2003).

The rather high catches in traps without any stimuli suggest that pine weevils should frequently encounter conifer seedlings even in the absence of visual and olfactory stimuli. The high catches in traps without stimuli emphasises the importance of having a non-stimuli treatment since even a slightly deterrent stimuli could have caught a significant amount of insects but still less than a non-stimuli treatment. Without a non-stimuli treatment results may therefore be misinterpreted. Both odour and visual stimuli may act as deterrents (Strom et al., 1999). The relationship between non-stimuli, seedling and seedling + pine odour was very similar to those found in a previous study (Björklund et al., 2003).

Pine odour alone increased the number of approaching walking pine weevils significantly. Several species have been shown to walk towards odours in the laboratory (Bell, 1984; Bernays & Chapman, 1994; Szentesi et al., 1996). However, field studies with odours emitted in similar amounts as emitted from natural plants are necessary to evaluate the significance of odours for finding food. For flying insects, some field experiments have shown that solely odour increase the number of approaching insects (e.g. (Butkewich & Prokopy, 1996; Chapman et al., 1981). For many insects odour and visual stimuli from the resource are always provided together but for others it is sometimes necessary to use solely odour stimuli. *Hyllobius abietis* is able to locate roots when walking on the ground and burrow down to them.
for ovipositing or feeding (Nordlander et al., 1986; Nordlander et al., 2000; Örlander et al., 2000).

Solely the visual stimuli of a dummy-seedling increased the number of approaching pine weevils significantly. This is noteworthy since “... visual responses often occur only in the presence of an appropriate olfactory signal.” (p.531 Bernays, 2002). However, orientation towards solely visual stimuli has been shown for several species, both in the field and in the laboratory (Prokopy & Owens, 1983; Prokopy & Roitberg, 1984). In some cases rather unspecific visual stimuli have elicited a positive response in the laboratory (Meyer, 1975; Meyer & Raffensperger, 1974; Szentesi et al., 1996). The sometimes observed lack of response in the field may be due to that the resource provides a too unspecific stimulus to be useful for the insect or that the tested visual stimuli do not sufficiently resemble the natural resource. It has, however, been shown in the field that the desert locust *S. gregaria* walks towards solely the visual stimuli of vegetation (Kennedy, 1939). Considering that it has been suggested that monophagous insects should be greater visual specialists than polyphagous insects (Prokopy & Owens, 1978), it is interesting that the polyphagous pine weevil shows such a strong reaction to visual stimuli.

When both odour and visual stimuli were provided, more pine weevils approached than when odour and visual stimuli were provided separately. There was a slight interaction between odour and visual stimuli, in the direction of competition rather than synergism. However, the effect was small compared to the response to the separate stimuli and principally the response can be regarded as additive. An additive response to non-contact odour and visual stimuli has been reported in several studies. The response of walking and flying adult plum curculio to odour and visual stimuli from tree models in the field appears to be additive (Butkewich &
Prokopy, 1996). Similarly, the effect of odour and colour of paper strips on the number of
approaching flying flies were additive in a laboratory study (Harris et al., 1993). Several
examples of synergism also exist. Odour and visual stimuli from traps in the field act
synergistically on the number of aphids landing on traps (Chapman et al., 1981). A
synergistic reaction to odour and colour on the number of alighting has been shown in the
laboratory for a leafhopper (Todd et al., 1990) and in the field for the onion fly (Judd &
Borden, 1991). Whether responses are additive or synergistic may depend on the strength of
the involved stimulus. This is supported by studies of the relative influence of different
stimuli. When the visual stimulus became weaker the relative influence of odour increased for
the apple maggot fly (Aluja & Prokopy, 1993), and for a grasshopper it has been shown that
when the olfactory stimulus increased the relative influence of visual stimuli decreased
(Szentesi et al., 1996). These relationships between stimuli in many studies have probably
lead to an underestimation of the significance of visual stimuli in resource finding.

It is not possible to determine the exact relative influence of odour and visual stimuli with the
experimental setup used in this study, and it has been questioned if it is possible at all (Harris
& Miller, 1988). However, the odour-less dummy-seedling increased the number of
approaching weevils with approximately half as much as an undamaged conifer seedling,
which suggest that the visual stimulus is at least as important as odour for finding an
undamaged conifer seedling. This reasoning is possible since the response to odour and visual
stimuli was principally additive. The relative influence of odour for a damaged seedling is of
course larger since it emits a higher concentration of odour, and an increased odour emission
also increase the number of approaching weevils (this study; Björklund et al., 2003) and the
number of attacks (Björklund et al., 2003; Nordlander, 1991).
For *H. abietis* and closely related species, several studies have been conducted using traps resembling small stumps. However, the trap type used precluded the possibility to have a treatment with no visual stimulus at all, thus a true response to the visual stimulus could not be determined. Further the results differed, i.e. *H. abietis* was caught in unbaited traps made of tubes, with 4 cm of the tube above ground (Nordlander, 1987) whereas the closely related *Pachylobius picivorus* (Germar) was not caught in similar unbaited traps (Hunt & Raffa, 1989). With another type of trap *P. picivorus* and *Hylolobius pales* (Herbst) were caught in unbaited traps, again no control without visual stimuli was used (Mizell & Tedders, 1999). In studies where it has been tested if additional visual stimuli can increase catches in baited traps the results are again hard to interpret. When an additional visual cue resembling a 20 cm high stump was provided to baited traps, the catches of *H. abietis* did not increase (Nordlander, 1987). Similar baited traps painted white caught larger numbers of *P. picivorus* and *H. pales* than those painted black (Hunt & Raffa, 1991), whereas the opposite results were shown with two other types of traps (Fatzinger, 1985; Mizell & Tedders, 1999). In conclusion, the discrepancy among the results of all the studies presented above suggests that responses to visual stimuli need to be investigated in further detail. The results of the present study suggest that it should be possible to increase trap catches with a combination of plant odour and an appropriate visual stimulus.

The reaction to odour and visual stimuli was similar for the different time periods which indicates that neither reproductive status, age of the weevils, age of the clear-cutting or season influenced the response to non-contact odour and visual stimuli from conifer seedlings. The result that there was no interaction between odour orientation and period is in agreement with a study in which the proportion of attacks on wounded seedlings compared to intact seedlings was similar during the same periods as investigated in the present study (Nordlander, 1991).
However, different responses to odours (limonene, α-pinene and α-pinene + ethanol) have been observed in studies where baited pitfall traps (Nordlander, 1987) have been used (Nordenhem & Eidmann, 1991; Nordlander, 1990). This may be due to that these traps mainly catch weevils searching for breeding material which is not relevant for pre-reproductive weevils. A lower proportion of pre-reproductive individuals in similar types of traps has been shown also for some related *Hylobius* species (Hoffman *et al.*, 1997).

An increased number of insects arriving to a resource is not necessarily related to the probability that the resource will be utilised. For example more pine weevils approached conifer seedlings planted in mineral soil than in humus but fewer seedlings were attacked on mineral soil in a study by Björklund *et al.* (2003). It was proposed that this was due to that the humus provided hiding and burrowing places. It has also been shown that more onion flies alighted on cylinders larger than onion stems than on cylinders of the same size as onion stems, but the larger cylinders received a lower percentage of the eggs (Harris & Miller, 1984). However, an increased number of insects arriving to a suitable resource in a suitable environment should increase the probability that the resource is utilised. In support of this it has been shown that an increased pine odour emission increase both the number of pine weevils approaching a conifer seedling and the number of attacks (Björklund *et al.*, 2003).

Similarly, it has been shown that yellow stems, compared to blue or grey, increased the number of alighting and also the number of eggs laid by the onion fly (Harris & Miller, 1983). In the present study it was shown that both odour and visual stimuli are used by pine weevils for locating conifer seedlings.
References


(Accepted).


Fatzinger, C. W. (1985) Attraction of the black turpentine beetle (Coleoptera: Scolytidae) and other forest Coleoptera to turpentine-baited traps, Environmental Entomology, 14, 768-775.


Rojas, J. C. & Wyatt, T. D. (1999) Role of visual cues and interaction with host odour during the host-finding


Curculionidae), *XXIV Nordic Congress of Entomology*, 163-166.


finding in the southern pine beetle, *Ecological Applications*, 9, 1028-1038.

sanguinipes*, to visual, olfactory and wind stimuli and their combinations, *Entomologia Experimentalis et Applicata*, 80, 539-549.

Todd, J. L., Phelan, P. L. & Nault, L. R. (1990) Interaction between visual and olfactory stimuli during host-

Tuttle, A. F., Ferro, D. N. & Idoine, K. (1988) Role of visual and olfactory stimuli in host finding of adult
cabbage root flies, *Delia radicum*, *Entomologia Experimentalis et Applicata*, 47, 37-44.

VanderSar, T. J. D. & Borden, J. H. (1977) Visual orientation of Pissodes strobi Peck (Coleoptera:
Curculionidae) in relation to host selection behavior, *Canadian Journal of Zoology*, 55, 2042-
2049.

Legends to figures

Fig. 1

Dummy-seedling in the pitfall trap used for the experiments.

Fig. 2


Fig. 3

Mean proportion (±95% confidence interval) of *H. abietis* caught in traps with the following treatments: control, pine odour, dummy-seedling, and dummy-seedling + pine odour. The shaded area represents the size of the interaction effect.
Fig. 2

Period 1

Total number of H. abietis

Period 2

Total number of H. abietis

Period 3

Total number of H. abietis

C  P  D  DP  S  SP

C  P  D  DP  S  SP

C  P  D  DP  S  SP
Fig. 3

Visual stimuli

<table>
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<th>Yes</th>
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<td>Control</td>
<td>Dummy-seedling</td>
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<tr>
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<tr>
<td>(0.12, 0.16)</td>
<td>(0.20, 0.25)</td>
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<tr>
<td>Spruce odour</td>
<td>Dummy-seedling + Spruce odour</td>
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<tr>
<td>0.29</td>
<td>0.35</td>
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<td>(0.26, 0.32)</td>
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