Orientation of the pine weevil *Hylobius abietis* to underground sources of host volatiles

Göran Nordlander[1], Hubertus H. Eidmann[1], Ulla Jacobsson[2], Henrik Nordenhem[1] & Kristina Sjödin[2]
[1]*Division of Forest Entomology, Swedish University of Agricultural Sciences, P.O. Box 7044, S-750 07 Uppsala, Sweden;* [2]*Department of Organic Chemistry, Royal Institute of Technology, S-100 44 Stockholm, Sweden*

Keywords: *Hylobius abietis,* pine weevils, Curculionidae, olfactory orientation, primary attraction, bioassay, terpenes, *Pinus sylvestris*

Abstract

Adults of *Hylobius abietis* (L.) (Coleoptera: Curculionidae) were found to locate conifer roots suitable for oviposition by utilizing host volatiles diffusing through the soil. Underground sources of host volatiles were presented to weevils in a laboratory bioassay. A cold-trapping condensate of Scots pine, *Pinus sylvestris* L., and fractions of it were tested. Various fractions containing host terpenes attracted weevils in the bioassay, but the complete pine condensate caused the highest response. Ethanol was also found to be attractive. Weevils caged underground in the absence of host material did not attract weevils on the surface.

Introduction

The pine weevil *Hylobius abietis* is of considerable economic importance in Palaearctic coniferous forests. The adults cause damage by feeding on phloem tissue on the stems of young conifer plants. Heavy seedling mortality is common, particularly where seedlings have been planted on recently clear-cut areas (Eidmann, 1974).

Oviposition and larval development occur in the phloem of freshly killed or dying conifer roots and stems in close contact with the soil. The roots of a felled tree are usually suitable for oviposition from the time of felling until the following summer and sometimes still longer. The oviposition period of *H. abietis* in Northern Europe lasts from late May or early June until August.

It has long been known that adults of *H. abietis* are able to penetrate into the soil and oviposit on suitable breeding substrate which does not reach the surface of the ground (Butovitsch, 1931; Hedqvist, 1961; Eidmann, 1974). Based on the data of Butovitsch (1931), Elton *et al.* (1964) concluded that weevils are attracted by odours from the breeding material diffusing through the soil.

Field observations made prior to this study confirmed that weevils walking on the ground surface can locate underground roots suitable for oviposition (Nordlander, unpublished). These observations were made during an experiment in which the soil was first removed within a circular area surrounding freshly cut trees of Scots pine, *Pinus sylvestris* L., and then the soil was sifted and replaced around the stumps. Vertical holes made by weevils in the moist, sandy soil were detected during the following weeks. Numerous holes were located directly above the paths of the major roots while none were observed in areas between roots (Fig. 1); neither could any holes be found above the roots of uncut trees surrounded by sifted soil.

Several recent laboratory studies have dealt with the perception of and reaction to host volatiles in *H. abietis* (Selander *et al.*, 1973, 1974; Mustaparta, 1975; Selander & Havukkala, 1976). However, none of these studies was specifically intended to investigate the orientation to breeding material.

The aim of this study was to confirm that weevils walking on the ground use host volatiles diffusing through the soil to locate suitable breeding material before they start excavating a hole. We also at-

Entomol. exp. appl. 41: 91–100, 1986

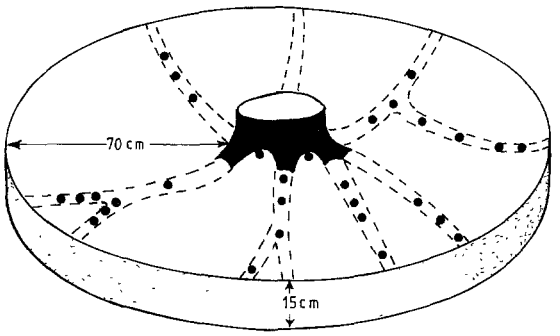

Fig. 1. A fresh pine stump surrounded by sifted soil. See holes made by adults of *H. abietis* above the roots.

tempted to determine the substances that are involved in this orientation by presenting underground sources of various host volatiles to weevils in a newly developed bioassay.

To better interpret the results of the bioassay we needed more information regarding the digging behaviour of the weevils. Is there, for instance, an increased probability for a second weevil to locate an odour source after a weevil has excavated a hole? This would be the case if weevils orient towards other digging weevils, to holes in general, or if the host odour emanating from holes is of considerably higher concentration than odour diffusing up through the soil. Aggregation pheromones (Selander, 1978) and stridulation (Selander & Jansson, 1977) may also affect orientation to holes already containing weevils.

Materials and methods

The following sections describe three behavioural experiments performed during the development of a digging bioassay as well as the bioassay itself. In all of these experiments weevils were placed on the smooth surface of a sand layer covering either one or several odour sources. The weevils responded by burrowing in the sand straight above the odour source which they had located.

Sources of host volatiles presented to weevils included: (1) freshly cut stem sections of Scots pine (diam. about 4 cm), (2) volatiles collected from chopped stems of Scots pine, (3) fractions of the collected pine volatiles, and (4) individual substances. Collection and fractionation techniques are described below.

Weevils used in the experiments were collected during their flight period in June at sawmills in the province of Uppland in central Sweden. The weevils were stored in darkness at 10 °C and were provided with pine bolts as a source of food. Mortality during storage was very low and the weevils were used until early spring the following year without any observable behavioural changes.

One week before use the weevils were transferred to a room kept at +20 ± 0.5 °C with an L20:D4 regime. All tests were carried out under the same environmental conditions during the light period. Artificial fluorescent light provided about 100 lux at the sand surface.

The bioassay and the behavioural tests were conducted in two different kinds of boxes, each supplied with a layer of moist, clean sand on the bottom. The sand consisted of a mixture of grain sizes less than 1.2 mm. We had found that when a grain size less than 0.5 mm was used, the sand became hard and probably less permeable to volatiles. When using a grain size between 0.8 and 1.2 mm, the surface layer soon dried out and the bottom layer became too wet. In both cases the weevils dug considerably less than in the blend of grain sizes used in the experiments.

Aggregation test. The objective of this experiment was to determine whether weevils aggregate when they are allowed to choose between several similar odour sources. Tests were conducted in an 80 × 80 × 50-cm plexiglass cage with a nylon mesh top. The bottom of the cage was covered with a 10 cm thick layer of sand. Four equally sized sections of Scots pine stem were buried at four sites situated 30 cm from each corner of the cage. Each piece of host material was buried at a depth of 5 cm in the centre of an open glass jar (diam. 135 mm, 75 mm deep) with its rim situated a few mm beneath the sand surface. Ten male and ten female weevils were used in each test. These weevils were individually marked on their elytra with 'Tipp-Ex Correction Fluid' prior to each test. During the first 3 h of the experiment all digging events were recorded by continuous observation. The four glass jars were removed after 48 h and the number of weevils in each jar was recorded.

Attraction to other weevils. This test was conducted to determine whether odours or sounds of weevils already present underground can attract weevils on the surface. Weevils used as lures were caged in plastic petri dishes with perforated lids. The batches of caged weevils consisted of six males, six females, or three males and three females. Each batch was used only once. Similar petri dishes with freshly chopped pine were used as controls to gauge the responsiveness of the test insects. Each petri dish was then buried under 5 cm of sand in the centre of a 55 × 36 × 28 cm white plastic box with a metal mesh lid.

Batches of 20 males, 20 females, or 10 males and 10 females were then released on the surface of the sand and their responses were recorded. These batches of weevils were kept in separate containers and provided with pine bolts as food between tests. All possible combinations of lures and batches of test animals were tried.

These tests were conducted on three different occasions which differed in experimental design and treatment of the weevils as follows: (1) Test periods lasting for either 7 or 24 h. (2) Test weevils which had either been stored at +10 °C (see above) or had been stored outdoors for a few weeks between collection and testing. (3) Weevils used as lures which either had been provided with food until the start of the experiment or had been starved during the preceding 18 h.

Response to holes. Aggregation of weevils to premade holes in the sand was studied in this experiment. Twenty-one glass jars (diam. 27 mm) were buried in the sand so that their rims were on a level with the sand surface in an 80 × 80 × 50 cm plexiglass cage. The glass jars were equally spaced forming a circle with a diam. of 70 cm. The jars contained three randomly assigned treatments as follows: (1) A piece of freshly cut pine placed in the jar and covered with sand. (2) As in (1) but with a hole (diam. 7 mm) made in the sand extending down to the pine in the centre of the jar. (3) A hole in the sand as in (2) but omitting the pine in the jar.

Batches of ten male and ten female weevils were released at the centre of the circle of the buried jars. Each time a weevil attempted to enter a hole or to initiate digging, the event was recorded and the responding weevil was removed. Weevils leaving the area circumscribed by the circle of jars were also removed. Each batch of weevils was used for 15 min and then replaced by a new batch; 22 batches were used in the first test and 18 batches in the second test. Each individual was used only once.

Bioassay of host volatiles. In this bioassay we used white plastic boxes (55 × 36 × 28 cm) covered with a metal mesh lid. Each box contained an 80 mm thick layer of sand. A circular glass jar (diam. 135 mm, 75 mm deep), containing the odour source and filled with sand, was placed with its rim just beneath the level of the sand surface in the centre of each box. Weevils were recorded as responding to the odour if they were found inside this jar at the end of the 7-h test period. The sand in the jars was discarded after each test, and equal amounts of clean sand were added to the boxes.

Host volatiles were presented in a 1-μl glass capillary tube (inner diam. 0.20 mm) suspended inside a glass tube which was buried horizontally in the sand 5 cm beneath the surface. The glass tube was placed with its open end at the centre of the glass jar (Fig. 2). The volatiles to be compared were presented twice and in reverse order to each of ten batches of weevils consisting of ten males and ten females. The responses of each batch were summed for the two 7-h test periods. Each batch of weevils was stored separately and provided with fresh pine as food during the 17-h period between the daily test periods.

A condensate of pine volatiles and fractions of it

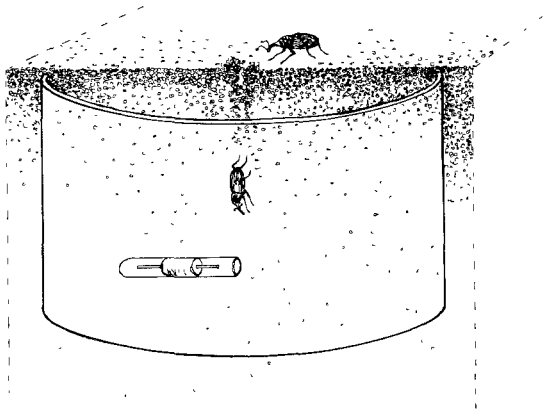

Fig. 2. Centre of bioassay box. A glass capillary tube is filled with test substances and suspended inside a glass tube. Weevils located odour source and are moving towards it.

were presented neat as well as diluted 10 × in silicon oil (DC 200 Fluid, 1 cSt, hexamethyldisiloxane). This silicon oil had no visible effects on the behaviour of the weevils in the digging tests in contrast to some organic solvents tested (see results). The silicon oil has a viscosity suitable for use in the same kind of 1-μl capillary tubes used for the undiluted host volatiles.

Some tests were also made with individual monoterpenes which are common conifer wood constituents. Crude samples from our stock of monoterpenes were used in these tests. The purities according to GC-analysis were as follows (the major impurities in brackets): (−)-α-pinene 89% (β-pinene 5%, unknown 4%), (+)-3-carene 95% (unknown 3%), (+)-limonene 96% (3-carene + myrcene 2%), terpinolene 92% (unknown 2%). Both enantiomers of α-pinene are known to be present to various extents in Norway spruce (M. Lindström, pers. comm.) while (+)-α-pinene probably dominates in Scots pine (Bukala & Kuczynski, 1952; Piriatinski *et al.*, 1947). The enantiomeric composition of limonene in Scots pine is uncertain but probably (−)-limonene is the main component (Bukala & Kuczynski, 1952; Piriatinski *et al.*, 1947). Methanol, ethanol, and pentane, commonly used solvents, were also tested. They were presented in 1- and 10-μl capillary tubes (inner diam. 0.20 and 0.55 mm).

Collection and fractionation of host volatiles. Volatiles can be collected using solvent extraction, adsorption-desorption, and cold-trapping methods. Since some solvents affect the behaviour of the weevils (see Results) we needed fairly large amounts of solvent-free material and, therefore, the cold-trapping method was chosen.

Condensates of volatiles from both stems and roots have been tested and found to be equally attractive (author's observ.). Thus stems were used since they are more readily available than roots. The pine wood was stored in a freezer prior to use.

A 50-l, stainless steel container was filled with chopped pine stems (14 kg). Nitrogen was chosen as the carrier gas to maintain an inert atmosphere. The gas was led through the container from bottom to top (ca. 100 ml/min) and then allowed to pass through two cold-traps. Most of the water condensed in the first cold-trap which was cooled with ice. The second trap, which was cooled to −80 °C with methylene chloride and dry ice, collected volatile pine constituents as an oil together with some water (see Table 1). All connecting tubes were made of teflon. The system was run continuously for five days and the condensed volatiles were collected twice a day after separation from the water. The yields varied between 1 and 3 ml of oil per 5-day period.

Table 1. Composition of the major constituents (≥0.02%) of the collected condensate of pine volatiles (CPV) and of the fractions F1, F2 and F3 (%).

Identified substances[1]	CPV	F1	F2	F3
tricyclene	0.2	0.3		0.03
α-pinene	*56*	*98*	0.07	6.2
unknown	0.06	0.09		
unknown	0.03	0.2		0.7
camphene	0.5	1.3		6.6
β-pinene	3.0	0.1	0.1	*17*
sabinene	1.2			0.02
unknown				0.03
unknown			0.1	0.3
3-carene	*34*	0.02	*89*	*48*
myrcene	1.7		0.6	*14*
α-phellandrene[2]	0.02		0.8	0.9
unknown	0.07			0.6
α-terpinene	0.06		0.8	1.5
unknown	0.02			
limonene	0.5	0.07	2.3	3.2
β-phellandrene	0.9		2.0	0.3
unknown				0.02
γ-terpinene[3]	0.09	0.02	1.5	0.4
unknown			0.03	0.03
unknown			0.02	
p-cymene	0.2		0.4	
unknown	0.06		0.1	
terpinolene	0.8		2.3	0.2
unknown			0.02	

[1] Except for constituents included above, volatile components exist; amounts not determined: hidden under solvent peak in GC-analysis.

[2] Determination of amounts of α-phellandrene uncertain due to bad separation from myrcene.

[3] γ-Terpinene may largely be a product of decomposition in GC system (Hiltunen & Räisanen, 1981).

The condensate of pine volatiles (CPV) was fractionated by using preparative gas chromatography (10% OV-101 on Chromosorb W, 100 °C). Three fractions, F1, F2, and F3, were collected according to the chromatogram shown in Fig. 3. The separate fractions were collected, one at a time, in a straight glass tube (length 12 cm, inner diam. 2 mm). The

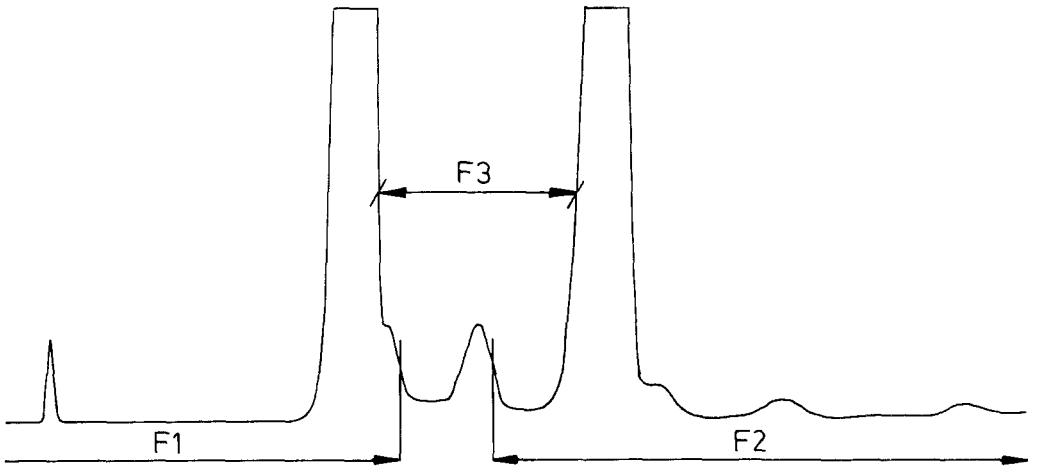

Fig. 3. Preparative gas chromatogram showing fractionation of CPV to F1-F3 Fractions collected one at a time (10% OV-101 on Chromosorb W, 100 °C).

tube was indirectly cooled *via* a metal rod immersed into liquid nitrogen. The rod was attached to a metal cylinder which surrounded the glass tube. A condensate that had been allowed to pass through the column without fractionation (C1) did not show any loss of activity in the bioassay (see Results).

The compositions of CPV, F1, F2, and F3 are listed in Table 1. The analysis was made by GC using a WG-11 coated glass capillary column (50 m, inner diam. 0.2–0.3 mm), and the compositions were calculated as integrated area divided by total integrated area (%). The substances were identified by comparing the retention times with those of known constituents in reference mixtures. The identity of these reference constituents was determined using GC (reference samples) and/or GC/MS taking into account previous investigations on *Pinus sylvestris* (Hiltunen *et al.*, 1975; Vité *et al.*, 1980).

Results

Aggregation test. Responding weevils were frequently found to aggregate when given a choice between several similar sources of pine volatiles (Table 2). In four out of six tests the distribution of weevils differed significantly from a random distribution.

There was also a strong correlation between observed digging at a particular site during the initial

Table 2. Underground aggregation of *H. abietis* at four equally attractive pieces of pine buried in sand at four sites in a cage. Sex of first responding weevil at each site recorded by continuous observation during first 3 h of the 48 h tests. Ten ♂♂ and ten ♀♀ were used in each test.

Test	Site	Sex first responder	No. responding weevils				Chi-square ♂ + ♀
			♂	♀	♂ + ♀	Total (n = 20)	
1	a	♂	2	0	2		
	b	–	0	0	0		
	c	♂	6	6	12		
	d	–	0	0	0	14	28.3***
2	a	♂	5	3	8		
	b	–	0	0	0		
	c	♂	4	5	9		
	d	♀	1	1	2	19	12.4**
3	a	–	0	0	0		
	b	♂	2	0	2		
	c	♀	8	6	14		
	d	–	0	1	1	17	30.3***
4	a	♂	8	7	15		
	b	–	0	0	0		
	c	–	1	2	3		
	d	–	0	1	1	19	30.5***
5	a	–	0	0	0		
	b	♀	3	1	4		
	c	–	5	3	8		
	d	♂	2	4	6	18	7.78
6	a	♀	2	1	3		
	b	♀	2	1	3		
	c	♀	5	5	10		
	d	♀	1	3	4	20	6.80

3 h of the test and the presence of weevils at that site after 48 h (Table 2). No digging was observed at any of the sites lacking weevils at the end of a test. Equal numbers of males and females (7♂, 7♀) were recorded as first responders. The sex of the first responder at a particular site had no observable effect on the probability that an aggregation would occur at that site.

Attraction to other weevils. Neither male nor female weevils responded positively to other weevils (♂, ♀, or ♂ + ♀) present in underground cages. No positive responses were recorded among a total of 1340 observations in three different test series. For comparison, more than 50% of the weevils responded to underground sources of pine.

Response to holes. The number of weevils responding to a piece of buried pine with and without a pre-made hole in the sand above it was significantly different in both tests (Table 3). In these two tests six and eight times as many weevils responded to pine plus hole than to pine alone. There was also a significant difference between the response to holes associated with pine and to holes alone. More weevils responded to holes with pine odour. This preference was more pronounced in test 1 than in test 2 (heterogeneity χ^2, $p<0.05$).

Table 3. Numbers of *H. abietis* attempting to dig into the sand above a piece of buried pine, or attempting to enter into a hole with pine present beneath or into a hole not associated with pine.

Test	Sex	No. responding weevils			Chi-square	
		Pine	Pine + hole	Hole	Pine/ pine + hole	Hole/ pine + hole
1	♂	4	24	7		
	♀	6	34	5		
	♂ + ♀	10	58	12	32.5***	28.9***
2	♂	4	22	14		
	♀	3	32	16		
	♂ + ♀	7	54	30	34.7***	6.30*

In these experiments, weevils were immediately removed as they began entering a hole. Otherwise, as we observed, they would soon have emerged from the holes without host volatiles. No digging attempts were recorded in areas lacking holes or odour sources.

Bioassay of host volatiles. The condensate of pine volatiles (CPV; see Table 1) was found to be highly attractive to *H. abietis*. The response was similar to that achieved with a piece of pine as odour source. On the average, half of the test weevils responded

Table 4. Bioassay of CPV, C1, and the GC fractions F1 – F3 (see Table 3). Dilution 10× in silicon oil is indicated with a 'd'. An additional mean calculated only for the responding weevil groups is given within parentheses.

Test	Treatment	Mean no. responding weevils (n = 40)	Statistics[1]	No. nonresponding groups (n = 10)
1	CPV	21.2	a	0
	C1	20.3 (22.5)	a	1
	F1	13.9	b	0
	F2	12.1 (13.4)	b	1
2	CPV	16.7 (18.6)	a	1
	F1 + F2	20.4	a	0
3	CPV	19.2	a	0
	F3	7.6 (10.9)	b	3
4	F1d	8.1 (11.6)	-	3
	F2d	5.2 (7.4)	-	3
5	CPVd	6.3 (10.5)	-	4
	F3d	3.1 (4.4)	-	3

[1] Means followed by same letter not significantly different. Test 1: randomized block ANOVA followed by Duncan's multiple range test ($p<0.05$). Test 2 – 3: paired sample t-test ($p<0.05$). Test 4 – 5: not statistically tested.

to 1 μl of CPV during the 7-h test period. There was no significant difference in responses to CPV and C1 (CPV which had passed the GC column). The responses to CPV and C1 differed significantly from the responses to fractions F1 and F2; responses to F1 and F2 were about 30–40% lower than the responses to CPV and C1 (Table 4, test 1). A 1:1 mixture of F1 and F2 elicited a response similar to that elicited by CPV (Table 4, test 2).

The responses to F3 and CPV were significantly different; less weevils responded to F3 than to CPV (Table 4, test 3). The number of weevils responding to F3 was also lower than the numbers responding to F1 and F2 in the previous comparisons (F3 was prepared later than F1 and F2 and therefore no statistically testable comparison could be made).

The use of CPV, F1, F2, and F3 diluted 10× in silicon oil reduced the number of responding weevils by 40–70% as compared with the response to the undiluted preparations (Table 4, tests 4 and 5). However, several weevil groups did not respond to the diluted samples of CPV or its fractions, and therefore the total numbers of responding weevils should not be directly compared (see Discussion).

1-μl capillary tubes containing samples of either α-pinene, 3-carene, limonene, terpinolene or various combinations of these monoterpenes all elicited responses in about half the number of weevil groups compared to all 12 groups for CPV; the total number of weevils responding was less than half the number responding to CPV. There were no significant differences in the responses to these monoterpenes or to their various combinations.

Ethanol released from a 10-μl capillary tube elicited a digging response in several batches of weevils while 1-μl capillary tubes with ethanol elicited no response. No weevils responded to methanol or to pentane in the amounts of 1 or 10 μl. Methanol sometimes seemed to increase the activity of the weevils around the odour source. Some tests indicated that the presence of pentane together with host terpenes diminished the response to the terpenes.

Discussion

This study shows that *H. abietis* uses olfaction to locate suitable underground breeding material. Both sexes respond similarly to host volatiles and males are as likely as females to be the first individual locating an underground odour source (Table 2).

Both sexes mate repeatedly during the oviposition period and copulation may take place anywhere that weevils meet. The strong response of males to host material therefore suggests that it is important for them to be able to locate oviposition sites in order to mate with females shortly before they lay an egg (females lay only one or two eggs/day). Such male behaviour would be particularly advantageous to the male if the last male to copulate with a female before she oviposits also fertilizes her eggs. Sperm displacement is common in insects (see Parker, 1970; Walker, 1980; Thornhill & Alcock, 1983) and has been noted, for example, in the North American white pine weevil, *Pissodes strobi*, by Jaynes & Godwin (1957). In species where both sexes mate repeatedly, as in *H. abietis,* partial or complete sperm precedence is prevalent (McCauley & Reilly, 1984). Complete sperm displacement would maximize genetic diversity within a female's offspring if egg batches are deposited after each mating (Walker, 1980). It is notable that females may have direct control over the amount of sperm displacement as shown for the boll weevil, *Anthonomus grandis* (Villavaso, 1975).

Weevils frequently aggregated at certain sites containing pine although several equally attractive pine sources were also available (Table 2). Our results indicate that these aggregations were partly due to the holes made by the first responding weevil which aided other weevils in locating the odour source. Holes appeared to be visually attractive and were frequently investigated by weevils (Table 3). Furthermore, the concentration of host volatiles ought to be higher above a hole leading to an odour source than on the undisturbed surface above an identical odour source. Increased amounts of host volatiles emanating from tissues damaged by feeding weevils may be another factor causing weevils to aggregate at host material.

Contrary to what was reported by Selander (1978) we have not found any evidence indicating the existence of an aggregation pheromone in *H. abietis,* and concurrent field studies (Tilles *et al.*, 1986a) also support our observations. Thus, we have no reason to assume that first-responding weevils in our bioassay were affecting the behaviour of other test animals except through the indirect ef-

fects caused by the presence of holes in the sand.

The bioassay which we have developed is based on a behavioural response observed in nature, i.e., weevils localize and burrow in the soil to a source of host odours. The weevils were found to excavate holes only above the odour source and not in surrounding odourless areas. Therefore we consider that the use of this unequivocal response makes our bioassay advantageous to many other bioassays in which the test animals are restricted to choose between being outside or inside one or more odour-permeated areas (e.g. Thomas & Hertel, 1969; Selander *et al.*, 1974). Our bioassay resembles digging tests with *Hylastes nigrinus* (Mannerheim) conducted by Rudinsky & Zethner-Møller (1967), but these tests were not aimed at comparing different odours. Merker (1953) also described a similar method in which two different odour sources were presented simultaneously to *Hylastes cunicularius* Erichson and to some other bark beetle species. We deemed it necessary to present only one odour source at a time to *H. abietis,* since weevils usually aggregate at the odour source where the first hole is made.

Both sexes of *H. abietis,* which were in their reproductive phase when tested, varied in responsiveness to host odour over time. During a 7-h test period, up to 50% of our test weevils did not respond to pine odour. The use of a single individual in each replicate of the test would therefore require a large number of replicates and consequently large quantities of samples of volatiles. By using groups of test animals, 'false' negative recordings due to non-responsive individuals are avoided and the number of replicates can be reduced.

When the first responding weevil in a group has made a hole above an odour source, the other weevils in this group do not have to locate the odour source through the undisturbed sand surface. This situation is, however, similar for all responding groups. The number of responding weevils may therefore be used as a measure of attractiveness when there is at least one response in all groups used in a test. When attraction is low and not all groups respond, only the number of responding groups should be compared between treatments. If this is the case, the number of replicates usually will have to be increased or it may be preferable to compare the response thresholds of the compounds in question.

Although the tested condensate fractions (F1, F2, and F3) and monoterpene hydrocarbons were all active, the responses to them were weaker than were the responses to the complete condensate. F1 and F2 contained almost entirely different subsets of the CPV constituents (Table 1) while F3 contained most of the constituents of CPV, although in different proportions. Though F3 was the most complete of the three fractions with regard to its qualitative content, it was not more attractive than either F1 or F2. These results indicate that several host substances are used by weevils to locate breeding material. Optimal attraction appears to be achieved only for a more complete set of substances in 'naturally occurring proportions'. This is a rather broad concept since the conifer hosts show considerable intra- and interspecific variation (Thorin & Nommik, 1974; Harborne & Turner, 1984). Therefore it can be expected that weevils will respond to a wide range of compounds in rather variable proportions.

An electrophysiological study by Mustaparta (1975) showed that certain olfactory cells of *H. abietis* antennae respond strongly to common host terpenes such as α-pinene, β-pinene, camphene, 3-carene, limonene, and terpineol. Selander *et al.* (1974, 1976) reported that these terpenes and some others were attractive in olfactometer tests. Negative responses were also reported in some cases; weevils were attracted by low concentrations but were repelled by higher concentrations (Selander *et al.*, 1976). It may be pointed out that it is difficult to compare the concentrations in a closed olfactometer of the kind used by Selander and coworkers with those affecting the weevils in our open system.

We conclude that several host substances can be used by *H. abietis* to locate its breeding material and that the more complete bouquets elicit the strongest attraction. Concurrent field studies have shown that the attraction by host terpenes can be strongly enhanced by adding ethanol, although ethanol alone is no strong attractant (Tilles *et al.*, 1986b). Ethanol can always be detected in the trunks and roots of forest trees (Crawford & Baines, 1977) and the amount in wood has been found to increase under anaerobic conditions (Moeck, 1970). However, our method for collecting the CPV gives a low yield of ethanol ($\leq 0.02\%$, see Table 1), since most of it should remain in the discarded water phase. It

is not known whether such small amounts of ethanol can affect the attractiveness of CPV and fractions thereof. The same applies to other volatile constituents which were hidden under the solvent peak in the GC analysis (see Table 1).

For monitoring purposes it may be possible to combine a few major terpene components and ethanol to achieve a standardized and sufficiently effective trap bait. Work is in progress in this area.

Acknowledgements

We thank Peter Baeckström, Mikael Lindström, Torbjörn Norin, Sture Strömberg, and David Tilles for discussions and Helena Bylund for technical assistance. Carried out within the Swedish project 'Odour signals for control of pest insects' funded by the Swedish Research Councils FRN, NFR, and SJFR. A fellowship from 'Ingenjör Ernst Johnsons fond' is acknowledged (K.S.).

Zusammenfassung

Die Orientierung des Rüsselkäfers Hylobius abietis *zu unterirdischen Quellen flüchtiger Inhaltsstoffe des Wirtsmaterials*

Rüsselkäfer (*Hylobius abietis*) können auf der Erdoberfläche die Lage im Boden verborgenen Brutmaterials feststellen und Wurzeln senkrecht grabend auffinden. Mit einer neu entwickelten Methode für Laborversuche, die sich auf die spezifische Reaktion des Eingrabens zum Brutmaterial gründet, wurden das Orientierungsverhalten der Käfer und die zum Brutmaterial führenden Geruchsstoffe des Wirtes studiert.

Beide Geschlechter von *H. abietis* reagierten gleichartig mit Eingraben auf die Geruchsstoffe des Wirtes. In Wahlversuchen zwischen gleichwertigen Anlockungspunkten kam häufig Aggregation der Käfer an einem Punkt vor. Die stärkere Ansammlung an einzelnen Stellen stand im Zusammenhang mit dem Vorhandensein einer Erdröhre zur Quelle der Geruchsstoffe. In den folgenden Versuchen wurde deshalb den Tieren jeweils nur eine Geruchsstoffquelle angeboten. Weder Weibchen noch Männchen im Boden übten eine Anziehung auf Käfer an der Oberfläche aus.

Kiefernstücke und Kondensate flüchtiger Kieferinhaltsstoffe sowie Kondensat nach Passage durch die Kolonne des Gaschromatographen waren stark attraktiv. Alle durch präparative Gaschromatographie hergestellten Fraktionen des Kondensats waren ebenfalls, aber schwächer attraktiv. Zehnfache Verdünnung des Kondensats und der Fraktionen verminderte ihre Attraktivität mit etwa 40–70%. Auch Äthanol, Methanol und Pentan wurden geprüft; nur Äthanol hatte eine mässig anlockende Wirkung.

References

Bukala, M. & H. K. Kuczynski, 1952. Studies on the composition of the Polish turpentines obtained from *Pinus silvestris*. Roczn. Chem. 26: 58–84. [In Polish].

Butovitsch, V., 1931. Beitrage zur Bekampfung und Biologie des grossen braunen Russelkafers, *Hylobius abietis* L. Mitt. Forstwirtsch. Forstwiss. 1931(2): 434–480.

Crawford, R. M. M. & M. A. Baines, 1977. Tolerance of anoxia and the metabolism of ethanol in tree roots. New Phytol. 79: 519–526.

Eidmann, H. H., 1974. *Hylobius* Schonh. In: W. Schwenke (ed.), Die Forstschädlinge Europas II. Paul Parey, Hamburg, Berlin: 275–293.

Elton, E. T. G., H. F. H. Blankwaardt, H. C. Burger, W. F. Steemers & L. G. Tichelman, 1964. Insect communities in barked and unbarked pine stumps, with special reference to the large pine weevil (*Hylobius abietis* L., Coleoptera: Curculionidae). Z. angew. Ent. 55: 1–54.

Harborne, J. B. & B. L. Turner, 1984. Plant Chemosystematics. Academic Press, London: 562 pp.

Hedqvist, K.-J., 1961. Uber das Vermogen von *Hylastes brunneus* Er., Brutplatze im Boden aufzusuchen. Ent. Tidskr. 82: 211–212.

Hiltunen, R. & S. Raisanen, 1981. Selection of liquid phases for glass capillary gas chromatography of monoterpene hydrocarbons. Planta med. 41: 174–185.

Hiltunen, R., M. v. Schantz & K. Loyttyniemi, 1975 The effect of nitrogen fertilization on the composition and the quantity of volatile oil in Scots pine (*Pinus silvestris* L.). Communs. Inst. For. Fenn. 85: 1–14.

Jaynes, H. A. & P. A. Godwin, 1957. Sterilization of the white-pine weevil with gamma radiation J. econ. Ent. 50: 393–395.

McCauley, D. E. & L. M. Reilly, 1984. Sperm storage and sperm precedence in the milkweed beetle *Tetraopes tetraophthalmus* (Forster) (Coleoptera: Cerambycidae). Ann. ent. Soc. Am. 77: 526–530.

Merker, E., 1953. Lockstoffe und Nahrstoffe in Wirtspflanzen einiger Waldschadlinge. Allg. Forst- u. Jagdztg 124: 138–145.

Moeck, H. A., 1970. Ethanol as the primary attractant for the ambrosia beetle, *Trypodendron lineatum* (Coleoptera; Scolytidae) Can. Ent. 102: 985–995.

Mustaparta, H., 1975. Responses of single olfactory cells in the pine weevil *Hylobius abietis* L. (Col.: Curculionidae). J. comp. Physiol. 97: 271–290.

Parker, G. A., 1970. Sperm competition and its evolutionary consequences in the insects. Biol. Rev. 45: 525–567.

Piriatinski, A. L., K. B. Bardysheva & O. I. Cherniaeva, 1947. On occurrence of β-pinene in gum turpentine of common pine. Zh. prikl. Khim. 20: 1308–1319.

Rudinsky, J. A. & O. Zethner-Møller, 1967. Olfactory responses of *Hylastes nigrinus* (Coleoptera: Scolytidae) to various host materials. Can. Ent. 99: 911–916.

Selander, J., 1978. Evidence of phermone-mediated behaviour in the large pine weevil, *Hylobius abietis* (Coleoptera, Curculionidae). Annls ent. fenn. 44: 105–112.

Selander, J., I. Havukkala & P. Kalo, 1976. Olfactory behaviour of *Hylobius abietis* L. (Col., Curculionidae). II. Response to 3-carene and α-terpineol during three stages of its life cycle. Annls ent. fenn. 42: 63–66.

Selander, J. & A. Jansson, 1977. Sound production associated with mating behaviour of the large pine weevil, *Hylobius abietis* (Coleoptera, Curculionidae). Annls ent. fenn. 43: 66–75.

Selander, J., P. Kalo, E. Kangas & V. Perttunen, 1974. Olfactory behaviour of *Hylobius abietis* L. (Col., Curculionidae). I. Response to several terpenoid fractions isolated from Scots pine phloem. Annls ent. fenn. 40: 108–115.

Selander, J., E. Kangas, V. Perttunen & H. Oksanen, 1973. Olfactory responses of *Hylobius abietis* L. (Col., Curculionidae) to substances naturally present in pine phloem or their synthetic counterparts. Annls ent. fenn. 39: 40–45.

Thomas, H. A. & G. D. Hertel, 1969. Responses of the pales weevil to natural and synthetic host attractants. J. econ. Ent. 62: 383–386.

Thorin, J. & H. Nommik, 1974. Monoterpene composition of cortical oleoresin from different clones of *Pinus sylvestris*. Phytochemistry 13: 1879–1881.

Thornhill, R. & J. Alcock, 1983. The evolution of insect mating systems. Harvard University Press, Cambridge, Mass., London: 547 pp.

Tilles, D. A., G. Nordlander, H. Nordenhem, H. H. Eidmann, A.-B. Wassgren & G. Bergström, 1986. Why does the pine weevil *Hylobius abietis* (L.) (Coleoptera: Curculionidae) aggregate in the field. Environ. Ent. 15 (in press).

Tilles, D. A., K. Sjödin, G. Nordlander & H. H. Eidmann, 1986. Synergism between ethanol and conifer host volatiles as attractants for the pine weevil *Hylobius abietis* (L.) (Coleoptera: Curculionidae). J. econ. Ent. 79 (in press).

Villavaso, E. J., 1975. Functions of the spermathecal muscle of the boll weevil, *Anthonomus grandis*. J. Insect Physiol. 21: 1275–1278.

Vité, J. P., U. Brümmer & W. Francke, 1980. Investigation and discussion of the systematic listing of the volatile constituents of pine resin. Dragoco Report: 243–259.

Walker, W. F., 1980. Sperm utilization strategies in nonsocial insects. Am. Nat. 115: 780–799.

Accepted: December 11, 1985.