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RESPONSE ON THE GROUND OF BARK BEETLE AND WEEVIL SPECIES COLONIZING CONIFER STUMPS AND ROOTS TO TERPENES AND ETHANOL

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Abstract—Responses of three *Hylastes* species, *Dryocoetes autographus*, and two *Hylobius* species to terpenes and ethanol were studied in field experiments on clear-cut forest sites in Sweden using baited ground traps. α-Pinene alone did not attract any of the six species. A terpene blend (spruce turpentine consisting mainly of α-pinene, β-pinene, and 3-carene) attracted *Hylastes cunicularius*, *H. brunneus*, and *Hylobius abietis* in some experiments, but not in others. The attractiveness of ethanol also varied; the only species consistently attracted was *H. abietis*. Baits containing both terpenes and ethanol, particularly the combination of spruce turpentine and ethanol, were attractive to all species except *Hylobius pinastri*. In *H. abietis*, the terpene plus ethanol/ethanol catch ratios increased during early summer. Seasonal differences in catch levels were observed in *H. cunicularius* and *H. abietis*. The addition of α-pinene reduced the attractiveness of the combination of spruce turpentine and ethanol to *H. cunicularius*, *H. opacus*, and *D. autographus*. The differences in response to the volatiles between species are probably related to differences in reproductive behavior and host preferences.

Key Words—*Hylastes cunicularius*, *Hylastes brunneus*, *Hylastes opacus*, *Dryocoetes autographus*, *Hylobius abietis*, *Hylobius pinastri*, α-pinene, terpenes, turpentine, ethanol, ground traps, Coleoptera, Scolytidae, Curculionidae.

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INTRODUCTION

Many coleopterans living in the inner bark and wood of conifers recognize suitable host material by its odor. Important volatiles mediating host recognition are monoterpenes, e.g., α-pinene, and degradation products such as ethanol (e.g., Borden, 1982; Fatzinger, 1985; Ikeda et al., 1980; Schroeder, 1988). Differences among insect species in their response to host volatiles may be related to differences among the volatiles released by the specific host substrates. For example, the ambrosia beetle *Trypodendron lineatum* Oliv., which breeds in conifer wood in a somewhat advanced stage of deterioration, is attracted during flight by ethanol, but not by α-pinene. In contrast, *Tomicus piniperda* L., which breeds in pine trees that have recently died, is strongly attracted by α-pinene, while high release rates of ethanol may decrease attraction to the host terpene (Schroeder and Lindelöw, 1989).

During the flight period, root-colonizing Coleoptera in the scolytid genus *Hylastes* and the weevil genus *Hylobius* search for suitable breeding substrate, which for most of the species is conifer stumps and roots that are dying or have recently died. The adults immigrate in large numbers into clear-felled areas. They are often abundant at rich sources of conifer volatiles such as fresh sawdust and chips, cut timber, and stacks of pulpwood. Flying *Hylastes cunicularius* Er. are attracted by stored spruce wood (Lindelöw et al., 1992) and by combinations of α-pinene and ethanol (Schroeder and Lindelöw, 1989). A combination of turpentine and ethanol strongly attracted flying *Hylastes salebrosus* Eichh., whereas the single compounds showed no or only a low attractiveness (Phillips, 1990).

After their flight period, the adult insects walk on the ground in search of reproductive substrate, which is usually hidden under the soil surface. Little is known about the orientation behavior of *Hylastes* species during this phase. They burrow deeply into the soil and can find breeding material at a depth of more than 50 cm (Stark, 1952; Hedqvist, 1961; Eidmann et al., 1977). *H. cunicularius* breeds mainly in the roots of spruce stumps and is rarely found in pine (Lekander et al., 1977). In laboratory experiments, this scolytid readily accepted both spruce and pine and had only a slight preference for spruce (Eidmann et al., 1991). The scolytid *Dryocoetes autographus* Ratz. is often found together with *H. cunicularius* and seems to have similar preferences regarding host material, but in contrast to the latter, *D. autographus* may also breed in spruce stems above ground level.

Host odors attract walking *Hylobius abietis* (L.) and induce the weevils to enter the soil in search of reproductive substrate (Nordlander et al., 1986); the combination of host odors and ethanol has a synergistic effect on attraction (Tilles et al., 1986; Nordlander, 1990). *Hylobius pinastri* (Gyllenhal) is also attracted to combinations of terpenes and ethanol. The response of *H. abietis* to these substances appears to vary with the phases of the adult life cycle (Nordenhem and Eidmann, 1991). In the field, ground traps baited with α-pinene and ethanol attract not only *Hylobius* but also *Hylastes* and other beetles (Zumr and Stary, 1991).

The host orientation behavior of *Hylastes* species and pine weevils, particularly *H. abietis*, is of practical interest because of the damage these insects cause in reforestation areas. The adults feed on conifer seedlings and often cause high seedling mortality.

Trapping methods have been used widely for estimating the relative size of insect populations and damage risks. For instance, a method employing baited ground traps has been suggested for use in monitoring *H. abietis* (Nordlander, 1987). *H. cunicularius* can cause heavy losses of seedlings (Lindelöw, 1992), but no evaluations of damage risks have been published. The first estimates of *H. cunicularius* populations have been documented recently (Lindelöw, unpublished).

Earlier field experiments have shown that low numbers of *H. cunicularius* can be captured in ground traps baited with α-pinene and ethanol. If a more attractive blend could be found, it might be possible to develop an efficient method for estimating *H. cunicularius* populations. Spruce turpentine, a terpene blend consisting mainly of α-pinene, β-pinene, and 3-carene, was considered to be a promising candidate.

The present study had several aims: to evaluate the response of *Hylastes* and *Hylobius* species on the ground to α-pinene, spruce turpentine, and ethanol, used singly and in various combinations, and to establish if there are differences in response among species or seasonal differences within species.

METHODS AND MATERIALS

The responses of walking adults to volatiles were studied using ground traps on several sites during various periods in 1989 and 1990. Experimental sites were located in clear-felled areas in central Sweden. The ages (years elapsed) of the clear-fellings at the time of the experiment are denoted according to the definition used by Bejer-Petersen et al. (1962). Thus, the growing season immediately following cutting is referred to as year A; A + 1 refers to the following year, and so on. Experiments 1 and 6 were conducted on the same site; likewise, experiments 3 and 7 were on the same site. Site ages and trapping periods are given in Table 3 below.

The experiments were conducted using a randomized block design with 10 replicates. One treatment in each experiment consisted of unbaited traps. The minimum distance between traps was 4 m. The ground traps used were slightly conical with holes along the upper margin at ground-surface level. They were similar to the traps used by Nordlander (1987), but smaller (8 cm diameter, 12 cm height). Water containing a detergent in the bottom of the traps prevented escape.

The substances used were ethanol (96% technical), (−)-α-pinene (Fluka 97%, $[\alpha]_D^{20}$ − 42 ± 3°), and spruce turpentine (SCA) containing 440 g/liter α-pinene, 210 g/liter β-pinene, 76 g/liter 3-carene, and 13 g/liter unspecified minor components. The substances were released separately from open glass tubes containing a strip of filter paper. The approximate release rates were 300–400 μl/day for ethanol, 100 μl/day for α-pinene, and 100 μl/day for spruce turpentine.

The traps were inspected at intervals varying in length from a few days to two weeks. At each inspection, the insects were collected and the baits (chemicals and dispensers) were renewed. All bark beetles and pine weevils from experiments 1 and 2 were determined to species and counted in the laboratory. In the other experiments, only *H. cunicularius* and *H. abietis*, the species of prime interest, were counted. In the results, only data for species trapped in numbers larger than 10 in at least one of the treatments are presented.

Catches of *H. abietis* in unbaited traps generally are low, variable, and not very reliable as a basis for comparison among treatments. For that reason the relative catches of *H. abietis* over time in traps baited with ethanol and with combinations of terpene and ethanol in experiments 1 and 2 are expressed as catch ratios, i.e., number caught by combined treatment/number caught by ethanol-baited traps (Figure 2 below).

The statistical treatment comprised a nonparametric analysis of variance, according to Friedman, followed by Dunnet's test for differences between controls and treatments (Zar, 1984). The Wilcoxon signed-rank test was used to test for synergism between terpenes and ethanol, namely, whether the combined treatments caught significantly more insects than the sum of the two separate treatments with single substances. The level of significance was set at $P < 0.05$.

RESULTS

The experiments summarized in Tables 1 and 3 revealed significant differences in the catches of each species (except *H. pinastri* in experiment 2 and *H. cunicularius* in experiment 7) between one or several of the treatments and the untreated controls. All scolytid and weevil species were caught in significantly larger numbers in ethanol-baited traps than in unbaited control traps on the 1-year-old area in experiment 1 (Table 1). On the freshly clear-felled area in experiment 2, only *H. abietis* was caught in higher numbers in traps baited with ethanol than in unbaited traps. In experiments 1 and 2, traps baited with α-pinene or spruce turpentine alone did not catch significantly more insects than the

TABLE 1. NUMBER OF BEETLES CAUGHT IN GROUND TRAPS IN EXPERIMENT 1 (SITE AGE A + 1; 30 APRIL TO 29 JUNE 1990) AND EXPERIMENT 2 (SITE AGE A; 11 MAY TO 21 JUNE 1990)[a]

	Treatment							
Species	U	αP	St	Et	αP & Et	St & Et	St & Et & αP	St & Et & αP/St & Et[b]
Experiment 1								
Hylastes cunicularius	77	63	76	151[c]	207[c]	458[c]	211[c]	0.46**
H. opacus	31	8	14	76[c]	76[c]	236[c]	49	0.21**
H. brunneus	5	23	24[c]	32[c]	81[c]	144[c]	123[c]	0.85
Dryocoetes autographus	10	19	25	90[c]	304[c]	820[c]	352[c]	0.43**
Hylobius abietis	12	43	29	305[c]	507[c]	600[c]	476[c]	0.79
H. pinastri	8	10	8	69[c]	67[c]	44[c]	40[c]	0.91
Experiment 2								
H. cunicularius	27	28	24	20	71[c]	107[c]	57[c]	0.53*
H. opacus	3	5	8	14	33[c]	70[c]	18	0.26*
H. brunneus	1	3	12	7	31[c]	45[c]	35[c]	0.78
D. autographus	2	9	4	27	46[c]	100[c]	35[c]	0.35**
H. abietis	26	63	33	239[c]	426[c]	509[c]	439[c]	0.86*
H. pinastri	3	5	4	12	15	10	15	1.50

[a] Traps were baited with α-pinene (αP), spruce turpentine (St), or ethanol (Et) alone or in various combinations. Unbaited (U) traps were used as controls.
[b] Significant difference between St & Et & αP and St & Et; $*P < 0.05$, $**P < 0.01$, Wilcoxon signed-rank test.
[c] Significant difference between treatment and unbaited control traps; $P < 0.05$, Dunnet's test.

unbaited controls, with the exception of catches of *Hylastes brunneus* Er. with spruce turpentine in experiment 1.

In these two experiments, all species except *H. pinastri* in experiment 2 were caught in higher numbers by the combinations of α-pinene and ethanol or spruce turpentine and ethanol than in unbaited traps. The difference between the catch with the combination of α-pinene and ethanol and the sums of catches in α-pinene baited traps and ethanol baited traps was significant for *D. autographus* and *H. abietis* in experiment 1 (Table 2). In experiment 2 this was true only for *H. brunneus*. Traps baited with a combination of spruce turpentine and ethanol caught significantly higher numbers of *H. cunicularius*, *Hylastes opacus* Er., *H. brunneus*, and *D. autographus* than the sum of the two separate treatments. In experiment 2 this was also true for *H. abietis*.

The addition of α-pinene to the combination of spruce turpentine and ethanol significantly decreased the catches of *H. cunicularius*, *H. opacus*, and *D. autographus* as compared with the combination of spruce turpentine and ethanol in

TABLE 2. SUMS OF BEETLES CAUGHT IN GROUND TRAPS IN EXPERIMENTS 1 AND 2[a]

Species	Sum αP & Et	Com αP & Et	Sum St & Et	Com St & Et
Experiment 1				
Hylastes cunicularius	214	207	227	458[b]
H. opacus	84	76	90	230[b]
H. brunneus	55	81	56	144[b]
Dryocoetes autographus	109	304	115	820[b]
Hylobius abietis	348	507[b]	334	600
H. pinastri	79	67	77	44
Experiment 2				
H. cunicularius	48	71	44	107[b]
H. opacus	19	33	22	70[b]
H. brunneus	10	31[b]	19	45[b]
D. autographus	36	46	31	100[b]
H. abietis	302	426	272	509[b]
H. pinastri	17	15	16	10

[a]Sum = sum of separate catches with α-pinene (αP) or spruce turpentine (St) alone and ethanol (Et) alone. Com = catch with α-pinene or spruce turpentine and ethanol together in same trap.
[b]Significant difference between Sum and Com; $P < 0.05$; Wilcoxon signed-rank test.

experiments 1 and 2. Its addition did not affect *H. brunneus* or *H. pinastri* catches, and for *H. abietis* the reduction was significant only in experiment 2.

In experiment 1 on an A + 1 site, the numbers of *H. abietis* caught in traps baited with ethanol, with α-pinene and ethanol, and with spruce turpentine and ethanol decreased significantly as the trapping period progressed (Figure 1). No such decrease was observed on the fresh clear-cutting in experiment 2, nor did the numbers of *H. cunicularius* caught by these treatments show a tendency to decrease significantly during the trapping period in either of the experiments. For *H. abietis*, the catch ratios (αP & Et/Et and St & Et/Et) exceeded unity at all inspections in both experiments and increased towards the end of the trapping periods (Figure 2). The corresponding catch ratios for *H. cunicularius* varied in both experiments 1 and 2 but did not increase significantly towards the end of the trapping periods. Catches of *H. cunicularius* at the consecutive inspections in these experiments were generally higher with spruce turpentine and ethanol than with α-pinene and ethanol.

In the seven experiments covering various time periods (Table 3), catches of *H. cunicularius* and *H. abietis* were lower in the latter part of the season. No major temporal differences in the response of *H. cunicularius* to any of the treatments were observed, with the exception of a decrease over time in attraction to the combination of α-pinene and ethanol. The combination of spruce turpentine and ethanol was consistently most attractive. Terpenes (spruce tur-

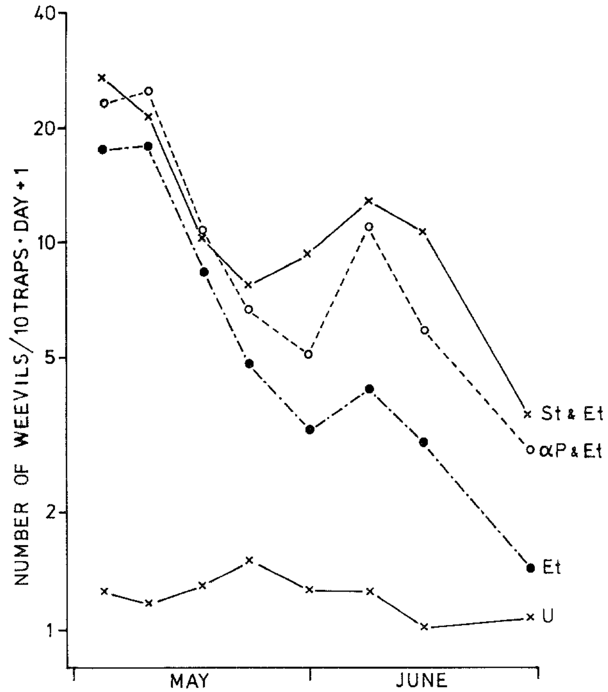

FIG. 1. Mean daily catches (1990) of *Hylobius abietis* in ground traps in four treatments of experiment 1. Treatments: U = unbaited control, Et = ethanol, αP & Et = α-pinene and ethanol, St & Et = spruce turpentine and ethanol. Ordinate: number of beetles/10 traps · day + 1, logarithmic scale.

pentine) caught significantly more *H. cunicularius* than unbaited traps in experiment 4, but not in any of the other experiments. *H. abietis* was also strongly attracted to the combinations of terpene and ethanol, and there was no major difference in this respect between spruce turpentine and α-pinene. In experiment 3, traps baited with spruce turpentine alone caught significantly more *H. abietis* than the unbaited traps.

DISCUSSION

In the present study, spruce turpentine occasionally attracted *H. cunicularius* (experiment 4), *H. brunneus* (experiment 1), and *H. abietis* (experiment 3), but none of the other three coleopteran species. α-Pinene did not significantly attract any of the species, but the catches of *H. abietis* with α-pinene in experiments 1 and 2 were two to three times higher than in unbaited controls. Attrac-

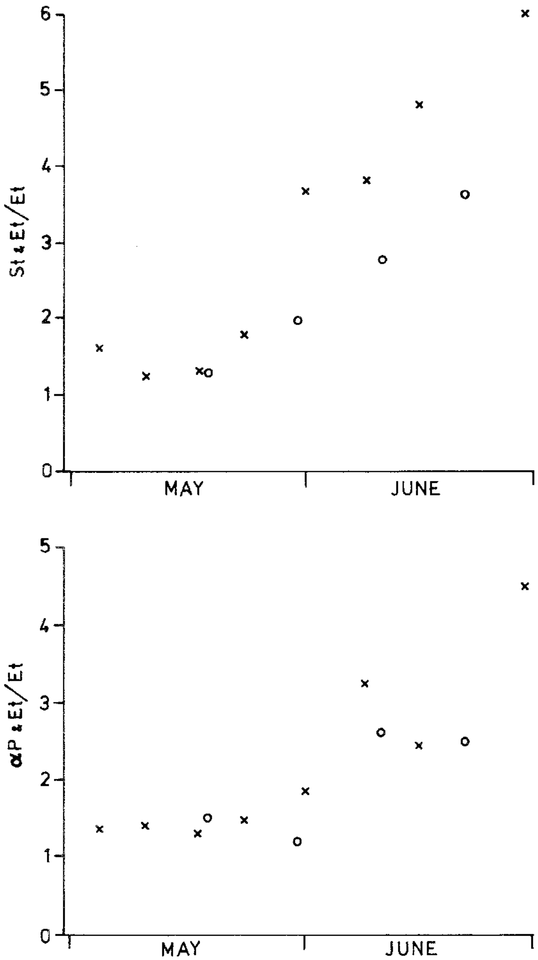

FIG. 2. Catches (1990) of *Hylobius abietis* in experiments 1 (×) and 2 (○) expressed as terpene and ethanol/ethanol catch ratios. Above: spruce turpentine and ethanol/ethanol catch ratio (St & Et/Et). Below: α-pinene and ethanol/ethanol catch ratio (αP & Et/Et).

tion of *H. abietis* and *H. pinastri* on the ground to α-pinene has been observed earlier (Nordlander, 1990). The attractiveness of ethanol varied among experiments, and only *H. abietis* was consistently attracted by ethanol. In earlier studies, traps baited with ethanol caught low numbers of *H. abietis* (Tilles et al., 1986), *H. radicis*, and *Pachylobius picivorus* (Hunt and Raffa, 1989). In contrast, the combinations of terpenes and ethanol were highly attractive, and the substances tended to act synergistically. Synergism between turpentine or stem section and ethanol was also found in the responses of *H. pales*, *H. radicis*,

TABLE 3. NUMBERS OF *Hylastes cunicularius* AND *Hylobius abietis* CAUGHT IN GROUND TRAPS BAITED WITH ETHANOL (Et), α-PINENE (αP), SPRUCE TURPENTINE (St), AND COMBINATIONS αP & Et AND St & Et[a]

			Treatment					
Exp.	Site age	Trapping period	U	αP	St	Et	αP & Et	St & Et
Hylastes cunicularius								
1	A + 1	Apr. 30–June 29, 1990	77	63	76	151[b]	207[b]	458[b]
2	A	May 11–June 21, 1990	27	28	24	20	71[b]	107[b]
3	A + 1	May 13–26, 1989	25	7	37		61[b]	
4	A + 2	May 31–June 13, 1989	15		58[b]		36	291[b]
5	A, A + 1	July 20–25, 1989	7		19	18		50[b]
6	A + 1	Sept. 1–19, 1990	20	3	21	15	6	68[b]
7	A + 1	Sept. 1–14, 1989	1	3	21	5	3	31
Hylobius abietis								
1	A + 1	Apr. 30–June 29, 1990	12	43	29	305[b]	507[b]	600[b]
2	A	May 11–June 21, 1990	26	63	33	239[b]	426[b]	509[b]
3	A + 1	May 13–26, 1989	9	8	36[b]		342[b]	
4	A + 2	May 31–June 13, 1989	0		3		31[b]	35[b]
5	A, A + 1	July 20–25, 1989	0		7	30[b]		92[b]

[a] Unbaited (U) traps used as controls. Age A refers to the first season after clear-felling according to Bejer-Petersen et al. (1962).
[b] Significant difference in catch between treatment and unbaited traps; $P < 0.05$, Dunnet's test.

and *P. picivorus*, while the single substances were not attractive (Raffa and Hunt, 1988; Hunt and Raffa, 1989).

The attraction of *H. cunicularius* and *D. autographus* to spruce turpentine and ethanol is in accordance with their preferred host substrate, which is spruce roots. On the other hand, both *H. brunneus* and *H. opacus*, living in pine roots (cf. Lekander et al., 1977) are also attracted to spruce turpentine and ethanol. The composition of the turpentine may reflect a common conifer odor, not specific enough to be recognized and accepted as spruce or pine by the beetles.

The addition of α-pinene to the combination of spruce turpentine and ethanol reduced attractiveness to *H. cunicularius*, *H. opacus*, and *D. autographus*, but not to *H. brunneus*. Apparently the species differ in their response to α-pinene. The reduced attractiveness suggests that the host orientation of these species is influenced by the amounts or proportions of the released volatiles. This is probably related to the host specificity of the species.

The lower attractiveness of the combination of α-pinene and ethanol to *H. cunicularius* in autumn suggests seasonal differences in response. Another seasonal difference is the generally low catch of *H. cunicularius* and *H. abietis* at the end of July. Low catches of *H. abietis* with terpene and ethanol baits late in the season are in agreement with earlier studies (Nordenhem and Eidmann, 1991), which indicated that the prereproductive new generation in autumn is attracted by natural host material, but not by the combination of α-pinene and ethanol.

The gradual increase observed in the relative attractiveness to *H. abietis* of the combinations of terpenes and ethanol as compared with ethanol alone or, inversely, the decrease in relative attractiveness of ethanol, has not been demonstrated before. This change was observed on a freshly clear-cut area, comprising mostly young, newly immigrated, and reproductive weevils (cf. Nordenhem, 1989), as well as on a 1-year-old area comprising predominantly old, reproductive weevils that had immigrated the previous year. We doubt that this change in relative attractiveness has been caused by temperature-related differences in release rates during the trapping period, since the release rates of ethanol and α-pinene in ground traps were previously found to be similarly influenced by temperature (Nordlander, 1990). Instead, the change may reflect a short-term change of behavior in individual weevils. Alternatively, it could have been an indirect result of a numerical change in the proportions of weevil groups with different orientation behavior.

The combination of spruce turpentine and ethanol trapped about 3–20 times more *H. cunicularius* and about 20–50 times more *H. abietis* than unbaited traps. Thus, spruce turpentine and ethanol may prove useful in practical applications such as population estimates.

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