Synergism Between Ethanol and Conifer Host Volatiles as Attractants for the Pine Weevil, *Hylobius abietis* (L.) (Coleoptera: Curculionidae)

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ABSTRACT Field tests using pitfall traps were conducted to test the relative attractiveness of conifer volatiles and ethanol to *Hylobius abietis* (L.). Traps baited with stems of Scots pine, *Pinus sylvestris* L., plus ethanol caught about twice as many weevils as those with stems alone. When tested separately, α-pinene, a terpene blend, and ethanol were much less attractive than ethanol combined with any of the terpene treatments.

KEY WORDS *Hylobius abietis, Pinus sylvestris,* host attraction, host volatiles, ethanol, α-pinene

THE PINE WEEVIL, *Hylobius abietis* (L.), is a widespread Palearctic forest insect that can cause extensive damage to conifer seedlings throughout its range. The weevil breeds in the dead and dying roots of its conifer hosts and is strongly attracted to host volatiles (e.g., Ohnesorge 1953, Hesse et al. 1955, Kauth & Madel 1955, Dässler 1958, Selander et al. 1973, 1974, 1976, Mustaparta 1975a,b, Löyttyniemi & Hiltunen 1976).

The pine weevil is about equally attracted to stem sections cut from its two major hosts in North Europe, Scots pine, *Pinus sylvestris* L., and Norway spruce, *Picea abies* (L.) Karst (D.A.T., unpublished data). Weevils are not attracted to uninjured, living conifers for breeding purposes, although such material releases volatile compounds. Ethanol is present in dead or weakened pines but absent or present in lesser amounts in healthy trees (Cade et al. 1970; Crawford & Baines 1977; K.S., unpublished data). Ethanol has been reported to attract numerous scolytid species attacking dead and dying trees (Moeck 1970, Montgomery & Wargo 1983). Nordlander et al. (1986) found that pine weevils were attracted to underground sources of ethanol in the laboratory. We describe field studies to determine the attractant properties of ethanol and terpene volatiles from Scots pine.

Materials and Methods

All tests were conducted ca. 20 km east of Uppsala, Sweden, on a 6-month-old, clear-cut area with scattered Scots pine and Norway spruce stumps. Pitfall traps used to catch weevils were constructed from lengths (20 cm) of PVC plastic drainpipes (2 mm thick and 11 cm diam). Both ends of the tubes were covered with plastic caps. Eight holes (1 cm diam) were drilled at 4 cm intervals around the circumference of each trap, 3 cm below its top. Traps were buried at a depth of 17 cm so the holes rested at ground level and allowed responding weevils access to them. Sources of volatiles were placed in the bottom of the traps. A thin layer of Fluon (ICI, Herts, England) was applied to the inside walls of the trap to prevent weevil escape. Traps were set out in groups of three to form the apexes of an equilateral triangle with sides 2 m long. A row consisted of five trap groups placed at 10-m intervals. Rows were spaced apart by at least 25 m. We noted that the ethanol and the terpenes continued to be released throughout the test periods (i.e., material was still left in the release devices at the end of each test).

Test 1: Pine/Pine Plus Ethanol. On 4 July 1983, two traps in each of 15 groups (three rows) were baited with pieces of fresh Scots pine stem (4 cm long and 2–3 cm diam) wrapped in insect net to eliminate any contact between the pine and responding weevils. The third trap in each group was not used in this test. In addition, a micro test tube (6 by 35 mm) filled with 70% ethanol (0.5 ml) was placed in one of the two traps in each group. Traps were emptied 72 h later. The captured weevils were counted and their sex was determined.

Test 2: Terpene Blend/Ethanol/Terpene Blend Plus Ethanol. Monoterpene compounds were mixed together in amounts to roughly estimate their relative proportions in Scots pine. The major components of this mixture were (by GC analysis): α-pinene (47%), 3-carene (29%), β-pinene (7%),

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Table 1. Catch of *H. abietis* in pitfall traps baited with pine, or a combination of pine and ethanol, 4–7 July 1983[a]

Treatment	Total weevils captured		
	♂♂	♀♀	Total
Pine	44	51	95
Pine + 0.5 ml 70% ethanol	116	70	186

Number of weevils caught by the combined treatment is significantly different from the number caught by pine alone ($P < 0.01$; two-sided Wilcoxon [1945] signed rank test.

[a] n = 15 sets of 2 traps.

limonene (6%), terpinolene (5%), myrcene (5%), and camphene (0.5%).

On 17 July, we set out 20 groups of three traps in four rows. One trap in each group was baited with a capillary tube (20 μl) containing the terpene blend and another with a micro test tube (6 by 35 mm) filled with 70% ethanol (0.5 ml). The third trap in each group was baited with both treatments contained in separate tubes. Traps were emptied 48 h later. The number of captured weevils was counted and their sex was determined. The release devices were then refilled, the traps were rotated, and a second collection and count was made after another 48 h.

Test 3: α-pinene/Ethanol/α-pinene Plus Ethanol. On 20 and 29 July, the procedures described in test 2 were repeated on another location on the same clear-cut, but 20 μl crude α-pinene—a mixture of (+)- and (−)-α-pinene—was substituted for the terpene blend and only ten sets of traps were used.

Statistical Treatment. In test 1, a two-sided Wilcoxon signed rank test was used for pairwise comparisons between the pine and pine plus ethanol treatments. In tests 2 and 3, data from the first and second counts were analyzed with Friedman's test, a nonparametric analysis of variance by ranks, followed by a nonparametric multiple comparison procedure (Zar 1984). A one-sided Wilcoxon signed rank test (Wilcoxon 1945) was used to test for synergism between ethanol and α-pinene or the terpene blend (i.e., whether the combined treatment caught significantly more weevils than the sum of the two separate treatments). Differences between or among treatments in the sex ratio of responding weevils were tested for by using a χ^2 test.

Results

Test 1. The numbers of weevils caught in traps baited with pine plus ethanol were about twice as high as the catch in traps baited with pine alone (Table 1). The differences among treatments in terms of numbers of weevils caught were significant for males as well as females ($P < 0.01$). The sex ratio of weevils caught differed significantly between the treatments ($P < 0.05$; χ^2 test); the ratio of males/females was higher in the pine plus ethanol treatment.

Table 2. Catch of *H. abietis* in pitfall traps baited with a terpene blend, ethanol, or both, 1983[a]

	Treatment	Total weevils captured		
		♂♂	♀♀	Total
17–19 July	Terpene blend	9	8	17
	Ethanol	5	8	13
	Terpene blend + ethanol	94	75	169
19–21 July	Terpene blend	8	2	10
	Ethanol	3	8	11
	Terpene blend + ethanol	40	46	86

Number of weevils caught by the combined treatment is significantly different from the number caught by either of the separate treatments ($P < 0.05$; Friedman's test followed by a nonparametric multiple comparison [Zar 1984]).

[a] n = 20 sets of 3 traps.

Test 2. Results of the experiment with a terpene blend and ethanol are shown in Table 2. Friedman's test revealed significant differences between the treatments ($P < 0.01$). The terpene blend plus ethanol treatment caught ca. 10 fold as many pine weevils as either the terpene blend alone or ethanol alone; this difference was significant ($P < 0.05$). A Wilcoxon sign rank test showed the combined treatment (terpene blend plus ethanol) trap catch was significantly higher than the catches of the terpene blend treatment and the ethanol treatment added together ($P < 0.01$). There were no significant differences in sex ratios among the treatments: males and females responded similarly.

Test 3. The results of the test with α-pinene, ethanol, and a combination of them (Table 3) were similar to those described in Test 2. There were significant differences between the treatments ($P < 0.005$), and the combined treatment (α-pinene plus ethanol) caught significantly more weevils than either of the separate treatments ($P < 0.01$). The sum of the weevils caught in the separate treatments was still significantly less than the trap catch in the combined treatment (α-pinene plus ethanol)

Table 3. Catch of *H. abietis* in pitfall traps baited with α-pinene, ethanol, or both, 1983[a]

	Treatment	Total weevils captured		
		♂♂	♀♀	Total
20–22 July	α-pinene	11	8	19
	Ethanol	4	1	5
	α-pinene + ethanol	35	32	67
29–31 July	α-pinene	2	3	5
	Ethanol	3	3	6
	α-pinene + ethanol	19	21	40

Number of weevils caught by the combined treatment is significantly different from the number caught by either of the separate treatments ($P < 0.01$; Friedman's test followed by a nonparametric multiple comparison [Zar 1984]).

[a] n = 10 sets of 3 traps.

($P < 0.05$). There were no significant differences in sex ratios among treatments.

Discussion

The combination of ethanol and terpenes was highly attractive to *H. abietis* in contrast to either the terpene blend, α-pinene, or ethanol presented separately (Tables 2 and 3). Ethanol combined with conifer volatiles showed a synergistic, not an additive effect (Table 2) (i.e., the trap catch with the terpene blend plus ethanol treatment was significantly higher than the sum of the catches for the two individual treatments). A similar synergistic effect also occurred when ethanol was combined with α-pinene. *H. abietis* may use α-pinene and possibly other conifer terpenes to recognize a host species, while it uses ethanol as a signal indicating host deterioration.

Minor constituents of the terpene blend and impurities in the α-pinene could also have affected weevil behavior. Clearly, however, the terpene blend, α-pinene, and ethanol alone were poor attractants, while the ethanol/terpene combinations were highly attractive.

In laboratory tests, Selander et al. (1974) reported attraction of *H. abietis* to α-pinene, 3-carene, and to Scots pine phloem fractions containing monoterpene alcohols. However, they found certain monoterpene hydrocarbon fractions (including α-pinene and 3-carene) to be either attractive or repellent, depending on concentration. Thomas & Hertel (1969) reported that *H. pales* (Herbst) was attracted to $(+)$-α-pinene but not $(-)$-α-pinene in the laboratory. In both studies, many of the bioassays were conducted using ethanol as a solvent for the test substances. Since ethanol synergism could have increased the response of weevils to the terpenes, results of these previous investigations should be interpreted with care.

Our experiments show that an artificial blend of compounds can be used for trapping pine weevils. A standardized attractant will be useful in our efforts to design a system for surveying pine weevil populations in reforestation areas. Goyer & Smith (1984) stressed the need for a method of monitoring *H. pales* populations. Since the biologies of these two closely related species are similar, our results may apply to the North American species as well.

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