# Verbenone interrupts the response to aggregation pheromone in the northern spruce engraver, *Ips perturbatus* (Coleoptera: Scolytidae), in south-central and interior Alaska

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## ABSTRACT

Field tests of verbenone, a potential antiaggregation pheromone of the northern spruce engraver, *Ips perturbatus* (Eichhoff), were conducted in south-central and interior Alaska in stands of Lutz spruce, *Picea* x*lutzii* (Little), and white spruce, *P. glauca* (Moench) Voss, respectively. Addition of 84%-(–)-verbenone at a high release rate to the three-component aggregation pheromone of *I. perturbatus* (racemic ipsenol, racemic ipsdienol, and 83%-(-)-*cis*-verbenol), significantly reduced trap catches. The results of this study, combined with previous results on the presence of verbenone in extracts of volatiles collected from feeding *I. perturbatus* and GC-EAD data, are consistent with antiaggregant behavioral activity of verbenone for *I. perturbatus*.

**Key words:** Bark beetles, *Ips perturbatus*, semiochemicals, pheromones, antiaggregation pheromones, verbenone, white spruce, *Picea glauca*, Lutz spruce, *Picea* x*lutzii*, Alaska

## INTRODUCTION

The northern spruce engraver, *Ips perturbatus* (Eichhoff) (Coleoptera: Scolytidae), is distributed transcontinentally in the boreal region of North America, generally following the distribution of white spruce, *Picea glauca* (Moench) Voss (Bright 1976; Wood 1982; Robertson 2000). In Alaska it colonizes standing white spruce and Lutz spruce, *Picea* x*lutzii* Little, that are stressed by natural disturbances such as drought, flooding, wind, ice, and snow damage. Human activities such as logging and right-of-way clearance also provide significant amounts of potential host material (Holsten and Werner 1987; Holsten 1996, 1997, 1998). Normally, endemic populations may infest individual standing spruce trees, but during warm, dry summers following mild winters, engraver beetle populations can increase significantly and kill groups of standing spruce trees. Historically, only limited tree mortality has been caused by this beetle in Lutz spruce forests in south-central Alaska. However, damage caused by *I. perturbatus* and other *Ips* spp. may assume greater economic importance as more habitat is provided through climate change and human activities (Robertson 2000). For example, in 1996 more than 47% of the residual spruce in a thinned area near Granite Creek in south-central Alaska became infested with *I. perturbatus* and *I. tridens* (Mannerheim). Spring drought conditions as well as the recent overall "warming" of the Kenai Peninsula apparently led to this rapid increase in *Ips* activity in 1996 (Anon. 1999; Holsten 1996, 1997, 1998).

Increased tree mortality in Alaska caused by *Ips* spp. has stimulated research on new management tactics utilizing semiochemicals. From 1977 through 1992, field tests of the efficacy of various bark beetle semiochemicals showed that ipsdienol (2-methyl-6-methylene-2,7-octadien-4-ol) and 2-methyl-3-buten-2-ol were generally attractive to *I. perturbatus*. Ipsenol (2-methyl-6-methylene-7-octen-4-ol), 3-methylcyclohex-2-enone, and verbenone (4,6,6-trimethylbicyclo[3.1.1]hept-3-en-2-one) were found to be generally inhibitory (Werner 1993). Seybold and co-workers found that *I. perturbatus* produce >99%-(-)-ipsenol, ~90%-(+)-ipsdienol, and *cis*-verbenol (unpublished data), while Holsten *et al.* (2000) found that a combination of racemic ipsenol, racemic ipsdienol, and 83%-(–)-*cis*-verbenol[1] was attractive to *I. perturbatus* in field assays.

The presence of verbenone in an extract of volatiles trapped from the headspace above male and female *I. perturbatus* feeding on *P. xlutzii*, and verbenone's activity in coupled gas chromatographic-electroantennographic detection (GC-EAD) assays suggest that verbenone may be behaviorally active for *I. perturbatus*. Verbenone collected from the volatile headspace above feeding male and female *I. perturbatus* may be synthesized by the insects, by microbes, or through autooxidation of host $\alpha$-pinene (Seybold *et al.* 2000). Verbenone has been shown to interrupt aggregation in other *Ips* spp. and in a variety of other scolytids (Borden 1997). For example, laboratory bioassays with *I. paraconfusus* Lanier (McPheron *et al.* 1997) showed that increasing concentrations of verbenone resulted in slower responses by beetles reaching an attractant source of naturally produced male pheromone volatiles. In limited field studies of verbenone's effect on aggregation of *I. perturbatus*, Werner (1993) showed a 19% reduction in trap catch when verbenone was added to ipsdienol.

Building on semiochemical studies by Holsten *et al.* (2000) and Werner (1988, 1993) and in response to increased *Ips* activity in south-central Alaska in 1996, efforts to apply *Ips* attractants and antiaggregants for population manipulation (Shea 1994; Salom and Hobson 1995) have been renewed in south-central and interior Alaska.We tested the efficacy of one enantiomeric blend of verbenone as an antiaggregant for *I. perturbatus* at two locations in Alaska.

## MATERIALS AND METHODS

**Study area characteristics.** In the south-central Alaska site (Kenai Peninsula, 150 km south of Anchorage), characterized by a transitional climate, traps were placed among Lutz spruce trees located at 250 m elevation in the Granite Creek campground area. This stand contained trees that were about 90 years old with a mean diameter at 1.3 m height of 7.5 cm, a mean height of 10 m, and a stand density of about 600 per ha. Shrub cover was sparse, consisting mostly of blue-joint reedgrass, *Calamagrostis canadensis* (Michx.) Beauv., and *Salix* spp.

In the interior site, characterized by a continental climate, traps were placed among white spruce trees located at 500 m elevation near Tok. This stand contained trees that

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[1] The enantiomeric composition of *cis*-verbenol in Holsten et al. (2000) was incorrectly reported as 83%-(+). It was 83%-(–).

originated from a fire about 80 years ago and had recently been thinned to about 1400 stems per ha, including 20 stems per ha of quaking aspen, *Populus tremuloides* Michx.. White spruce trees had a mean diameter at 1.3 m height of 9.5 cm and a mean height of 10 m. Shrub cover was sparse with only green alder, *Alnus crispa* (Ait.) Pursh, crowberry, *Empetrum nigrum* L., Labrador tea, *Ledum groenlandicum* L., mountain cranberry, *Vaccinium vitis-idaea* L., and highbush cranberry, *Viburnam edule* (Michx.) Raf., occupying the site.

**Trap Placement and Semiochemicals.** Twelve-unit, multiple funnel traps (Lindgren 1983) were hung from branches of non-host or dead spruce trees, or on nylon rope suspended between host trees. Traps at both sites were hung at least 10 m apart (Bakke *et al.* 1983) with collection containers 0.3 m aboveground. Traps were baited with semiochemicals (Table 1) dispensed from polyethylene bubble cap release devices (PheroTech, Inc., Delta, BC, Canada[2]). To reduce cost, racemic semiochemicals were used where available. The three-component attractant of ipsenol, ipsdienol, and *cis*-verbenol was used because it is more attractive than ipsdienol alone (Holsten *et al.* 2000). Each component of the attractant and verbenone were released from separate bubble caps. Beetles were collected from traps weekly from late May through July. Trapped insects were placed in labeled plastic bags and frozen for later identification and counting.

**Table 1**

Release rates of synthetic semiochemicals used in *Ips perturbatus* trapping studies, Alaska, 2000[1].

| Semiochemical | Enantiomeric composition | Bubble cap load (mg) | Bubble cap release rate (mg/day)[2] |
|---|---|---|---|
| Ipsdienol | Racemic | 40 | 0.2 |
| Ipsenol | Racemic | 20 | 0.2 |
| *cis*-verbenol | 83%-(−) | 75 | 0.6 |
| Verbenone | 84%-(−) | 790 | 3.5 |

[1]All semiochemicals have chemical purity >98 percent.
[2] Release rates determined at 22$^0$C.

**Experimental Design and Statistical Analyses.** Treatments were completely randomized in each field test and were initially replicated at least ten times at each location. However, in some instances trap catches were discarded from the experiment because neighboring trees became infested with *I. perturbatus* and these natural aggregations influenced trap catches. Thus, the number of replicates varied from 9 to 10 (Granite Creek) or 7 to 10 (Tok) (Table 2). Treatments were: 1) Attractant (ipsenol + ipsdienol + *cis*-verbenol), 2) Attractant + high dosage (two bubble caps) of verbenone[3], 3) Attractant + low dosage (one bubble cap) of verbenone, 4) Verbenone alone (low and high dosages used at Granite Creek; low dosage used at Tok), and 5) Unbaited traps as controls. Statistical analyses

[2] The use of trade or firm names in this publication is for reader information and does not imply endorsement by the U.S. Department of Agriculture of any product or service.

[3] As the enantiomeric composition of verbenone associated with feeding *I. perturbatus* has not yet been determined, we used "standard" verbenone bubble caps (PheroTech Inc.) typically applied for mountain pine beetle, *Dendroctonus ponderosae*.

were completed using "Statistix 7" software[4]. Numbers of *I. perturbatus* caught by each treatment were first examined by the Shapiro-Wilk Test to determine whether data conformed to a normal distribution. Since they did not, data were transformed using the natural log + 1 before being subjected to ANOVA followed by Tukey's (1953) comparison of means test ($\alpha$= 0.05). Untransformed means are reported in the results.

**Table 2**

Effect of 84%-(–)-verbenone on the response of *Ips perturbatus* to an attractant composed of racemic ipsdienol, racemic ipsenol , and 83%-(–)-*cis*-verbenol in multiple funnel traps, Granite Creek and Tok, Alaska, 2000[1].

| | No. of beetles caught (mean $\pm$ SE)[2] | |
|---|---|---|
| Treatment | Granite Creek (n[3]) | Tok (n) |
| Attractant | 288.8 $\pm$ 66.7 [a] (10) | 731.5 $\pm$ 101.8 [a] (7) |
| Attractant + Low Verbenone | 112.0 $\pm$ 16.9 [a] (10) | 194.6 $\pm$ 47.0 [b] (7) |
| Attractant + High Verbenone | 46.5 $\pm$ 8.7 [b] (9) | 136.2 $\pm$ 23.5 [b] (8) |
| High Verbenone | 1.2 $\pm$ 0.4 [c] (9) | |
| Low Verbenone | 0.4 $\pm$ 0.1 [c] (10) | 3.0 $\pm$ 0.9 [c] (9) |
| Unbaited trap | 1.6 $\pm$ 0.8 [c] (10) | 5.9 $\pm$ 3.3 [c] (10) |

[1] Granite Creek trapping study had one additional treatment; high release rate of verbenone alone (two release devices).

[2] Mean and standard error values followed by same letter within each column are not significantly different, P< 0.05, Tukey's comparison of means test.

[3] Number of replications

## RESULTS

The overall effect of treatment was significant at both locations (Granite Creek, $F$ = 98.2, df = 5,52, $P$ < 0.001; Tok, $F$ = 64.6, df = 4,36, $P$ < 0.001). The ternary blend of racemic ipsdienol, racemic ipsenol, and 83%-(-)-*cis*-verbenol was significantly more attractive to *I. perturbatus* than the unbaited trap (Table 2). Because sexes of *I. perturbatus* cannot be differentiated by external morphology, the sex ratio of the captured beetles was not determined. Verbenone released at a high rate reduced mean trap catches at both Tok and Granite Creek by a factor of five relative to the attractant (Table 2). Addition of verbenone at a low release rate also significantly reduced trap catches of *I. perturbatus* at Tok (Table 2). There was no significant difference at either location between the responses to verbenone alone and to the unbaited control (Table 2).

## DISCUSSION

The dose-dependent effect of verbenone on the response of *I. perturbatus* to its attractant (noted at Granite Creek) has also been demonstrated for other species of *Ips* (Miller *et al.*1995, McPheron *et al.*1997). With *I. perturbatus*, Werner (1993) showed a 19% reduction in trap catch when verbenone [87%-(–) and 5 mg/day] was added to racemic ipsdienol (0.2 mg/day). However, we have demonstrated higher reductions (62% to 84%) in trap catches when verbenone [84%-(–) and 3.5 or 7 mg/day] was added to the three-component attractant. The enantiomeric blends and release rates of verbenone used by us and by Werner (1993) were similar, while the ipsdienol used in each study was

[4] "Statistix 7", 2000, Analytical Software, PO Box 12185, Tallahassee FL 32317-2185.

identical in chemical composition and release. Differences in the effect of verbenone on trap catches between our study and that of Werner (1993) could have been due to variation in *I. perturbatus* populations, to differences in trapping technique, or particularly to differences in attractant used as the positive control for interruption. In as much as Werner (1993) only used one of the three components we used in our attractant, it is possible that his 19% reduction might have been higher if all three pheromone components had been used.

We now have identified an antiaggregánt for *I. perturbatus* (verbenone) that is associated with feeding *I. perturbatus* adults (unpublished data). Although we do not know the exact enantiomeric composition of verbenone associated with *I. perturbatus* or the timing of its release during host colonization, we have achieved a significant reduction in trap catches using the commercially available, and relatively inexpensive verbenone bubble cap containing 84%-(−)-verbenone. If verbenone is released late in the colonization process, it may function naturally as an antiaggregation pheromone to minimize further attacks on host material. As has been demonstrated for *Ips pini* (Say) in lodgepole pine, *Pinus contorta latifolia* (Engelmann) Critchfield (Borden *et al.* 1992; Devlin *et al.* 1994), treatment of white and Lutz spruce logging debris with commercially available verbenone may reduce the level of colonization of host material and population increase by this forest pest.

## ACKNOWLEDGMENTS

We thank John Hard, USDA Forest Service PNW Research Station (ret.), Kathy Matthews and Ken Zogas, Forest Health Management, State and Private Forestry, Alaska Region, Anchorage, Alaska, for help with the installation of the field studies and trap collections, personnel from Tanana Chiefs, Fairbanks, Alaska, for assistance in the field studies conducted in Tok, and K.E. Gibson, A.D. Graves, P.J. Shea, B.L. Strom, and two anonymous reviewers for review of an earlier draft of this paper. Manuscript preparation and laboratory research on *I. perturbatus* by S.J. Seybold were supported by Cooperative Agreements #PSW-0028-CA and #USDA/FS-00-CA-11272138 between the USDA Forest Service Pacific Southwest Research Station and S.J. Seybold.

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