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Attractant Semiochemicals of the Engraver Beetle, Ips perturbatus, in South-Central and Interior Alaska

Edward H. Holsten, Roger E. Burnside, and Steven J. Seybold





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Abstract

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From 1996 through 1999, field tests of various engraver beetle (*Ips perturbatus* (Eichhoff)) semiochemicals in funnel traps were conducted in south-central and interior Alaska in stands of Lutz (*Picea* × *lutzii* Little) and white spruce (*P. glauca* (Moench) Voss). The European spruce beetle (*I. typographus* (L.)) is believed to be taxonomically similar to *I. perturbatus*. Commercially available European spruce beetle lures, which include 2-methyl-3-buten-2-ol, however, were no more attractive to *I. perturbatus* than the combination of racemic ipsdienol and 83%-(+)-*cis*-verbenol. The addition of >97%-(—)-ipsenol to ipsdienol and *cis*-verbenol, however, was more attractive than the binary combination alone. Racemic ipsenol dispersed from bubble caps did not prevent *I. perturbatus* from colonizing fresh logging debris. Thus ipsenol was found to function as an attractant rather than as an antiaggregant as previously shown.

Keywords: Bark beetles, *Ips perturbatus*, semiochemicals, pheromones, aggregation pheromones, antiaggregation pheromones, white spruce, *Picea glauca*, Lutz spruce, *Picea ×Iutzii*, Alaska (interior, south-central).

Summary

Data from 3 years of field and laboratory studies on the efficacy of potential engraver beetle (*Ips perturbatus* (Eichhoff)) semiochemicals are summarized. The addition of *cis*-verbenol to racemic ipsdienol caught significantly higher numbers of beetles than ipsdienol alone. The addition of 2-methyl-3-buten-2-ol did not affect trap catches. Ipsenol [racemic or >97%-(—)] did not reduce trap catches as anticipated. Rather, when combined with ipsdienol and *cis*-verbenol, ipsenol more than doubled trap catches. Future research will concentrate on the role of verbenone as an antiaggregant of *I. perturbatus* to interrupt its response to ipsdienol, *cis*-verbenol, and ipsenol or to naturally derived aggregation pheromone.

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Introduction

The engraver beetle (*Ips perturbatus* (Eichhoff)) (Coleoptera: Scolytidae), is distributed transcontinentally in the boreal region of North America, generally after the distribution of white spruce (*Picea glauca* (Moench)) Voss (Bright 1976, Wood 1982). It colonizes standing white spruce and Lutz spruce (*P. ×lutzii* Little) stressed by natural disturbances such as drought; flooding; wind, ice, and snow damage; and human disruptions such as logging activity and rights-of-way clearance (Holsten and Werner 1987). It also readily colonizes fallen trees and tree debris from these disruptions. Normally, endemic populations may infest individual standing spruce trees, but during warm, dry summers after mild winters, engraver beetle populations can increase significantly and kill groups of standing spruce trees.

After a 1983 wildfire and excessive ice and snow damage in the Fairbanks, Alaska, area, high engraver beetle populations in 1986 and 1987 killed trees on more than 6475 ha (Holsten 1986). In Alaska, most damage by *I. perturbatus* has occurred in interior white spruce forests. Historically, only limited tree mortality has been caused by this beetle in Lutz spruce forests in south-central Alaska. In 1996, however, more than 47 percent 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 apparently led to this rapid increase in *Ips* activity in 1996 (Holsten 1996, 1997, 1998). Increases in tree mortality in Alaska caused by *Ips* species has stimulated research efforts in developing new management strategies using semiochemical approaches.

Aggregation pheromone production has been described and reviewed for over 30 bark beetle species (Borden 1982, 1985). Research on pheromone isolation and identification has prompted much interest during the last decade in the application of pheromones and other semiochemicals to manipulate bark beetle populations (Salom and Hobson 1995, Shea 1994).

From 1977 through 1992, field tests of the efficacy of various bark beetle semiochemicals showed that ipsdienol (ID = 2-methyl-6-methylene-2,7-octadien-4-ol) and 2-methyl-3-buten-2-ol (MB) were generally attractive to *I. perturbatus*, whereas ipsenol (IE = 2-methyl-6-methylene-7-octen-4-ol), 3-methylcyclohex-2-enone (MCH), and verbenone were generally inhibitory (Werner 1993). Racemic ID alone caught 84 percent more beetles than racemic IE alone and 62 percent more beetles than the combination of racemic ID and racemic IE. The addition of racemic IE to MB reduced beetle trap catches by 38 and 92 percent, respectively. Also, the addition of MCH and verbenone to ID-baited traps reduced trap catches by 31 and 19 percent, respectively. No beetles were caught in traps baited with either *cis*- or *trans*-verbenol, but these two compounds were never tested in combination with ID.

Surprisingly, Werner (1993) also showed racemic *exo*-brevicomin (a *Dryocoetes* spp. and *Dendroctonus* spp. attractant) was slightly attractive alone to *I. perturbatus* and when combined with racemic ID, elicited the highest field response in one experiment. Field studies utilizing bubble cap (bc) formulations of racemic IE to reduce the number of attacks on standing trees yielded mixed results. Werner (1993) also reported that newly emerged adult *I. perturbatus* moving to hibernation sites were not responsive to racemic ID.

Building on research results of Werner (1988, 1993) and stimulated in 1996 by increased *lps* activity in south-central Alaska, efforts to apply *lps* attractants and antiaggregants for population manipulation were renewed in south-central and interior Alaska.

From 1996 through 1998, the following objectives were developed and tested through laboratory and field studies:

- 1. Determine which attractant and antiaggregant pheromones are produced by *I. perturbatus* through chemical (gas chromatography [GC] and mass spectrometry [MS]), and neuronal analyses (electroantennogram detection [EAD])
- 2. Determine the efficacy of various compounds and their enantiomers of *I. perturbatus* attractants and antiaggregants through field studies with baited funnel traps
- 3. Determine the efficacy of candidate antiaggregants as deterrents to colonization by *I. perturbatus* of white and Lutz spruce logging slash in south-central Alaska

Immature and mature *I. perturbatus* were collected in infested Lutz spruce logging debris from the Granite Creek campground area, 150 km south of Anchorage, Alaska, in June 1998 and shipped to the University of Nevada, Reno for rearing (Browne 1972) and analyses of semiochemicals. To date, no method has been developed to distinguish the sexes of this species without dissecting genitalia. Sex can be determined when insects are dissected while making semiochemical extracts, but sex cannot be determined in living insects. Porapak or solid phase microextrac-tion (SPME) collections cannot therefore be made on pure-sex groups of beetles.

Gas chromatography and MS analyses were conducted on Porapak extracts of volatiles produced by newly emerged adults feeding in green logs of Lutz spruce and on abdominal extracts of males and females that had been previously fed on Lutz spruce phloem or treated topically with juvenile hormone III (JH III). Specific methods for preparing extracts and for chemical analysis will be published separately, but techniques generally followed those described in Seybold and others (1995a, 1995b) and Tillman and others (1998).

In addition to these analyses, Porapak extract of both sexes of *I. perturbatus* feeding in green logs of Lutz spruce was analyzed by using GC-MS.¹ This extract also was used as the stimulus in a lab study using coupled GC-EAD² with unknown sexes of *I. perturbatus* at Simon Fraser University.

Field studies in Alaska were conducted on three individual stands having different characteristics:

Mature white spruce stands, 300 m in elevation in the Bonanza Creek Experimental Forest, 40 km west of Fairbanks, were 165 years old with an average diameter of 32 cm, an average height of 30 m, and a stand density of 875 trees per ha.

Young white spruce stands 500 m in elevation near Tok, Alaska, originated from a fire about 80 years ago. Recently, these stands have been thinned to about 1,400 stems per ha with an average diameter of 9.5 cm and an average height of 10 m, including 20 stems per ha of quaking aspen (*Populus tremuloides* Michx.) in the residual stands.

Materials and Methods

Laboratory Studies of Production and Response to Semiochemicals by *lps perturbatus*

Field Studies of Response to Semiochemicals by Ips perturbatus

¹ Professor Wittko Franke. 1999. Institute of Organic Chemistry, University of Hamburg, Edmund-Siemers-Allee ID-20146. Hamburg, Germany.

² Regine Gries. 1999. Simon Fraser University, 8888 University Drive, Burnaby, BC V5A 156.

Table 1—Release rates of synthetic semiochemicals used in *lps perturbatus* trapping studies, Alaska, 1997-99^a

| Treatment | Stereochemistry | Bubble cap amount | Release rate |
|---|-----------------|-------------------|--------------|
| | | Mg | Mg per day |
| Ipsdienol | Racemic | 40 | 0.2 |
| Ipsenol | Racemic | 20 | .2 |
| Ipsenol | 97%-(—) | 20 | .2 |
| cis-verbenol | 83%-(+) | 75 | .6 |
| <i>lps</i> Lure ^R and Ecolure ^R : | | | |
| 2-methyl-3-buten-ol | | 1400 | 40.0 |
| and (-)-cis-verbenol | () | 70 | 2.0 |
| and ipsdienol | Racemi | 15 | .6 |

^a All semiochemicals have chemical purity greater than 98 percent.

Trap Placement

Shrub cover in both the Fairbanks and Tok sites 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 high bush-cranberry (*Viburnam edule* (Michx.) Raf.) occupying the sites.

Young, residual Lutz spruce stands 250 m in elevation in the Granite Creek campground area, 150 km south of Anchorage, Alaska, were about 90 years old with an average diameter of 7.5 cm, an average height of 10 m, and a stand density of about 600 trees per ha. Shrub cover in the Granite Creek area was sparse, being occupied mostly by blue-joint reedgrass (*Calamagrostis canadensis* (Michx.) Beauv.) and *Salix* spp.

Semiochemicals (table 1) were dispersed from slow-release polyethylene bc (PheroTech, Inc, Delta, BC, Canada³) placed in 12-unit Lindgren multiple funnel traps (Lindgren 1983). Funnel traps were either hung from branches of nonhost trees or dead spruce or suspended from a nylon rope tied between two trees in such a way that the collection container of the trap was 0.3 m aboveground. All traps were placed at least 30 m apart. Beetles were collected from the traps weekly from late May through the end of July. Trapped insects were placed in labeled plastic bags and frozen for later identification and counting.

³ 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.

Table 2—Effect of *cis*-verbenol on the response of *lps perturbatus* to racemic ipsdienol released from Lindgren funnel traps, Fairbanks and Granite Creek, Alaska, 1997

| | Number of beetles caught (mean ± SE) | |
|------------------------------------|--------------------------------------|----------------------|
| Treatment | Fairbanks | Granite Creek |
| Racemic ipsdienol | 8.1 <u>+</u> 2.3 | 44.5 <u>+</u> 30.9 |
| Racemic ipsdienol and cis-verbenol | 54.7 <u>+</u> 19.4 | 448.0 <u>+</u> 165.6 |
| Unbaited control | 3.6 <u>+</u> 2.4 | 14.2 <u>+</u> 9.6 |

Experimental Design

Results and Discussion

Laboratory Studies of Production and Response to Semiochemicals by *Ips perturbatus*

Field Studies of Response to Semiochemicals by Ips perturbatus Treatments for all field tests were replicated at least five times in either randomized complete block designs or line transects. Treatments consisted of various semiochemicals with unbaited traps as controls.

Gas chromatography and GC-MS analyses of a Porapak extract from living insects of both sexes feeding in green Lutz spruce logs for 1 week revealed IE [>99%-(—)], ID [~90%-(+)], *cis*-verbenol, *trans*-verbenol, and verbenone in the volatile headspace. This analysis was confirmed in part at the University of Hamburg (see footnote 1). Also, using the same Porapak extract in collaborative studies as those used at Simon Fraser University (see footnote 2), IE, ID, and an unknown compound each elicited neuronal responses from the antenna of an unsexed live *I. perturbatus* in a GC-EAD assay. Finally, extracts taken from dissected abdominal tissue of beetles of known sex showed that JH treatment induced IE, ID, and *cis*-verbenol synthesis in male *I. perturbatus*.⁴

In 1996, *I. perturbatus* populations increased to damaging levels in south-central Alaska (Holsten 1996, 1997, 1998). The *Ips* species was believed to be *I. tridens* (Mannerheim). Funnel trap studies were initiated in 1997 near Fairbanks (interior Alaska) and in the Granite Creek area (south-central Alaska). Traps were baited with racemic ID + *cis*-verbenol, an attractant pheromone previously identified for *I. tridens* in Canada (Moeck and others 1985). In south-central Alaska, addition of *cis*-verbenol caught at least 10 times more beetles than ID alone (table 2). Later during the 1997 field season, the most abundant *Ips* species in south-central Alaska was determined to be *I. perturbatus* not *I. tridens*. It seems that adding *cis*-verbenol greatly enhances the response of *I. perturbatus* to ID.

⁴ Seybold, S.J. Identification of *Ips perturbatus* attractant pheromones through gas chromatography and mass spectrometry. Manuscript in preparation. On file with: S.J. Seybold, Departments of Entomology and Forest Resources, University of Minnesota, St. Paul, MN 55108.

Table 3—Effect of 2-methyl-3-buten-2-ol on the response of *lps perturbatus* to racemic ipsdienol and *cis*-verbenol released from Lindgren funnel traps, Granite Creek, Alaska, 1998

| | No. of beetles caught (mean <u>+</u> SE) | |
|--------------------------------------|---|--|
| Treatment | | |
| Ipsdienol and <i>cis</i> -verbenol | 87.1 <u>+</u> 15.8 | |
| Ipsdienol and cis-verbenol | | |
| and 2-methyl-3-buten-2-ol (Ips Lure) | 94.3 <u>+</u> 29.5 | |
| Ipsdienol and cis-verbenol | | |
| and 2-methyl-3-buten-2-ol (Ecolure) | 67.0 <u>+</u> 16.8 | |
| Unbaited control | 19.1 <u>+</u> 10.9 | |

Ips perturbatus is believed by some to be taxonomically similar to *I. typographus*, a serious killer of conifers in Europe (Wood 1982). Bakke (1982) identified the attractant pheromone complex of *I. typographus*. The blend is composed of ID + *cis*-verbenol+ 2-methyl-3-buten-2-ol (MB). We tested two commercially available *I. typographus* pheromone baits (Ips Lure^R and Ecolure^R, both with high dosages of MB) and compared them to our two-component attractant (ID + *cis*-verbenol). We found no difference in response of *I. perturbatus* to the commercial *I. typographus* baits or to the two-component bait we developed (table 3). Earlier, Werner (1993) showed an antiattractant effect when MB was added to ID. Werner, however, did not test ID + *cis*-verbenol, which may explain the mixed results that occurred in his earlier studies and why we were not able to inhibit the response of *I. perturbatus* to *cis*-verbenol and ID with MB.

In 1999, we undertook two *lps* pheromone field studies—one in Tok (interior Alaska) and the other at the Granite Creek area. The objective of the Granite Creek study was to treat fresh logging slash with bc filled with racemic IE to keep engraver beetle populations from breeding in this highly susceptible host material. Untreated slash served as a control. The choice of racemic IE was based on results reported by Werner (1993). The experiment had three treatments, replicated five times, and plot size was 10 m²: (1) 9 racemic IE bc/plot, (2) 25 racemic IE bc/plot, and (3) an untreated check. Bubble caps were stapled on the south side of a 1-m stake and uniformly spaced throughout the plot. Contrary to our expectations, the plots treated with IE apparently had higher *l. perturbatus* attack rates, as determined by linear 0.3-m samples, than the untreated slash (table 4).

Table 4—Effect of racemic ipsenol on the response of *lps perturbatus* to fresh logging debris, Granite Creek, Alaska, 1999

| | Number of attacks per linear 0.3 m |
|-----------------------------|------------------------------------|
| Treatment | (mean <u>+</u> SE) |
| 9 bubble caps ^a | 8.2 <u>±</u> 1.3 |
| 25 bubble caps ^a | 10.7 <u>+</u> 4.9 |
| Unbaited control | 5.8 <u>+</u> 0.7 |

^aRacemic ipsenol.

Table 5—Effect of 97%-(—)-ipsenol on the response of *Ips perturbatus* to racemic ipsdienol and *cis*-verbenol in baited funnel traps, Granite Creek and Tok, Alaska, 1999^a

| | No. of beetles caught (mean <u>+</u> SE) | |
|------------------------------------|--|-------------------|
| Treatment | Granite Creek | Tok |
| Racemic ipsdienol and cis-verbenol | 191.1 <u>+</u> 51.2 | 330 <u>+</u> 13.3 |
| Racemic ipsdienol and cis-verbenol | | |
| and >97%-(—)-ipsenol | 962.3 <u>+</u> 190.7 | 555 <u>+</u> 33.1 |
| >97%-(—)-ipsenol | | 110 <u>+</u> 30.0 |
| Unbaited trap | 4.0 <u>+</u> 0.6 | 5.5 <u>+</u> 1.1 |

^a Tok trapping study had 1 additional treatment: >97%-(—)-ipsenol alone.

Based on these findings, it seemed racemic IE acted as an attractant to *I. perturbatus*. Having determined *I. perturbatus* produces >99%-(—)-IE, we obtained >97%-(—) IE bcs from PheroTech and completed a replicated funnel trap study in the Granite Creek area comparing control (empty traps), ID and *cis*-verbenol, and >97%-(—)-IE and ID and *cis*-verbenol. Each treatment was replicated seven times. Based on the work of Werner (1993), we expected to see a significant reduction in trap catches with the addition of >97%-(—)-IE. Results (table 5) of this study, however, indicated that the addition of >97%-(—)-IE significantly enhanced the response to the two-component attractant (ID and *cis*-verbenol).

The 1999 studies near Tok involved replicated funnel traps comparing the following treatments: (1) *cis*-verbenol + racemic ID; (2) *cis*-verbenol + racemic ID + >97%-(—)-IE; (3) >97%-(—)-IE alone; and (4) control (empty traps). Results demonstrated that adding >97%-(—) IE increased, from two to seven times, the number of engraver beetles trapped compared to the two-component attractant (table 5).

Conclusions

We now have a strong attractant for *I. perturbatus* (ID + *cis*-verbenol + IE). Because IE is produced by male beetles after JH treatment, appears in the headspace above feeding males and females, elicits an antennal response in EAD assays, and enhances response to ID and *cis*-verbenol in field studies, it should be regarded as an attractive pheromone for *I. perturbatus* and an important component in its synthetic attractant blend. This result is in contrast to the earlier results found by Werner (1993); perhaps because of variation in population or to differences in trapping technique. Ipsdienol also seems to be an attractive pheromone for *I. perturbatus* given its production and antennal activity combined with field tests. The *cis*-verbenol is produced by *I. perturbatus* and enhances field response, but we had no evidence for its activity in the EAD assays; perhaps because it was present in low quantities in the Porapak extract. Additional EAD assays with *cis*- and *trans*-verbenol as well as verbenone may be needed.

Future laboratory and field studies will concentrate on the role of verbenone in the semiochemical complex of *I. perturbatus*. Verbenone is produced and released when both sexes feed in Lutz spruce logs for 1 week, and perhaps is released late in the colonization process as an interruptant. As previously noted, Werner (1993) showed a 19-percent reduction in trap catch when verbenone was added to ID. Laboratory bioassays with *Ips paraconfusus* (McPheron and others 1997) showed that increasing concentrations of verbenone resulted in slower responses by beetles reaching an attractant source of naturally produced male pheromone volatiles. Similar laboratory assays could be conducted with verbenone as an interruptant for a Porapak extract of male and female *I. perturbatus* volatiles.

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English Equivalents

| When you know: | Multiply by: | To find: |
|------------------|----------------|------------|
| Celsius (°C) | 1.8 and add 32 | Fahrenheit |
| Centimeters (cm) | 2.54 | Inches |
| Hectares (ha) | 2.47 | Acres |
| Kilometers (km) | 0.621 | Miles |
| Meters (m) | 3.281 | Feet |
| Millimeters (mm) | 0.254 | Inches |

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