

# Evaluation of semiochemicals potentially synergistic to $\alpha$ -pinene for trapping the larger European pine shoot beetle, *Tomicus piniperda* (Col., Scolytidae)

T. M. Poland<sup>1</sup>, P. de Groot<sup>2</sup>, R. A. Haack<sup>1</sup> and D. Czokajlo<sup>3</sup>

<sup>1</sup>USDA Forest Service, North Central Research Station, East Lansing, MI, USA; <sup>2</sup>Natural Resources Canada, Canadian Forest Service, Great Lakes Forestry Centre, Sault Ste. Marie, ON, Canada; <sup>3</sup>IPM Tech., Inc., Portland, OR, USA

Ms. received: March 2, 2004; accepted: July 1, 2004

**Abstract:** The pine shoot beetle, *Tomicus piniperda* (L.) (Col., Scolytidae) is an exotic pest of pine, *Pinus* spp., in North America. It is attracted strongly to host volatiles ( $\pm$ )- $\alpha$ -pinene, (+)-3-carene, and  $\alpha$ -terpinolene. Attraction to insect-produced compounds is less clear. Other potential attractants include *trans*-verbenol, myrtenol, myrtenal, nonanal and  $\alpha$ -pinene oxide. We conducted a series of field experiments to determine if any of these compounds would increase attraction of *T. piniperda* to  $\alpha$ -pinene, either individually or in various combinations. None of the individual compounds increased attraction. Although several combinations that included *trans*-verbenol, nonanal, myrtenol, or myrtenal increased attraction, results were variable between experiments.

**Key words:** *Tomicus piniperda*, attraction,  $\alpha$ -pinene, myrtenol, myrtenal, nonanal, *trans*-verbenol

## 1 Introduction

The pine shoot beetle, *Tomicus piniperda* (L.) (Col., Scolytidae), is an exotic pest of pine, *Pinus* spp., discovered in the Great Lakes region of North America in 1992 (HAACK and POLAND, 2001). *Tomicus piniperda* is native to Europe, Asia, and parts of northern Africa (LÅNGSTRÖM, 1983; SCHROEDER and EIDMANN, 1987; YE, 1991).

Overwintering adult beetles become active in early spring (BAKKE, 1968). They use host volatiles to locate suitable pine brood material such as severely stressed or weakened trees, freshly killed trees, or recently cut stumps and slash (BYERS et al., 1985). Progeny adults emerge in early summer and feed in the shoots of healthy pine trees throughout the summer while they complete sexual maturation. When temperatures cool in autumn, beetles move down the trunk to overwinter in the bark at the base of trees where they feed on shoots (LÅNGSTRÖM, 1983; PETRICE et al., 2002).

*Tomicus piniperda* is strongly attracted to host volatiles including ( $\pm$ )- $\alpha$ -pinene, (+)-3-carene, and  $\alpha$ -terpinolene (BYERS et al., 1985; SCHROEDER and EIDMANN, 1987). The addition of ethanol, a degradation product from the phloem of weakened and dying trees, to host monoterpenes can either increase (VITÉ et al., 1986; ZUMR, 1989) or decrease (SCHROEDER, 1988; BYERS, 1992) attraction by *T. piniperda*. The response by *T. piniperda* appears to be dependent on the ratio and release rates of  $\alpha$ -pinene and ethanol (SCHROEDER and

LINDELÖW, 1989; BYERS, 1992) and on ambient temperatures (CZOKAJLO and TEALE, 1999).

Lures consisting of host volatiles are available commercially for *T. piniperda* in Europe and in North America. In Europe, the commercial bait contains ( $\pm$ )- $\alpha$ -pinene and terpinolene released at 40 mg/day (Witasek, Kärnten, Austria). In North America, the commercial bait consists of  $\alpha$ -pinene [95% (-)-enantiomer] released at approximately 300 mg/day (Phero Tech., Inc., Delta, BC, Canada; IPM Tech., Inc., Portland, OR, USA).

Although attraction of *T. piniperda* to host volatiles has been demonstrated clearly, attraction to insect-produced compounds has been equivocal. Several studies found no evidence for pheromone attraction because beetles were attracted equally to uninfested pine bolts and bolts infested with *T. piniperda* (BYERS et al., 1985; LÖYTTYNIEMI et al., 1988). Similarly, in laboratory bioassays, LÄNNE et al. (1987) found that *T. piniperda* was attracted strongly to pine logs with no increase in attraction when females were added. However, SCHÖNHERR (1972) found *T. piniperda* was attracted to Scots pine bolts colonized by females but not to bolts colonized by males.

Several compounds have been identified from the hindguts of *T. piniperda*, including myrtenol, *trans*-verbenol and verbenone in females (FRANCKE and HEEMANN, 1976); 3-carene-10-ol, myrtenol, *trans*-verbenol and verbenone in both sexes but at higher levels in

males (LANNE et al., 1987); and 3-carene, verbenone, *trans*-verbenol and myrtenol in both sexes (ZHOU et al., 1997). Physiological and behavioural responses have been reported for a number of these compounds. LANNE et al. (1987) found antennal responses to 3-carene-10-ol, myrtenol, *trans*-verbenol and verbenone. They also found that logs baited with *trans*-verbenol and 3-carene-10-ol captured significantly higher numbers of the closely related lesser pine shoot beetle, *Tomicus minor* (Hart.), and appeared to capture more *T. piniperda* than unbaited logs. Sticky traps baited with *trans*-verbenol and 3-carene-10-ol in addition to the monoterpenes  $\alpha$ -pinene, terpinolene, and (+)-3-carene, captured significantly more *T. piniperda* than traps baited with the monoterpenes alone. KANGAS et al. (1970) found *T. piniperda* was attracted to *trans*-verbenol alone or combined with phloem extracts in laboratory tests. The addition of *cis*-verbenol or verbenone inhibited responses. NIEMEYER et al. (1996) found ethanol,  $\alpha$ -pinene,  $\beta$ -pinene, terpinolene, *trans*-verbenol and myrtenol were inactive for *T. piniperda* when tested alone but demonstrated weak attraction when tested in various combinations. ZHOU et al. (1997) found 3-carene, verbenone, *trans*-verbenol and myrtenol were highly attractive in laboratory bioassays, but had low activity in the field except when combined in mixtures. Using coupled gas chromatographic electro-antennographic detection analysis of volatiles collected from beetles and host material, CZOKAJLO (1998) identified several antennally active compounds for *T. piniperda* including  $\alpha$ -pinene oxide, nonanal, (-)-myrtenal, *trans*-verbenol and (-)-myrtenol.

We conducted field trapping experiments to investigate the attraction of *T. piniperda* to the antennally active compounds  $\alpha$ -pinene oxide, nonanal, (-)-myrtenal, *trans*-verbenol, and (-)-myrtenol. Our objective was to determine whether any of these candidates increased attraction of *T. piniperda* to the standard North American attractive lure,  $\alpha$ -pinene, when added singly or in various mixtures.

## 2 Materials and Methods

A series of field experiments were conducted in Michigan and Indiana in the United States and in Ontario, Canada. Field sites consisted of Scots pine, *Pinus sylvestris* L., Christmas

tree plantations infested with *T. piniperda*. Scots pine trees were 1.5–2.5 m tall and 6–12 years old. The US sites were located near DeWitt, Ingham County, Michigan (42°44'N, 84°35'W) and Rolling Prairie, LaPorte County, Indiana (41°37'N, 86°43'W). The Canadian sites were located near Barrie, Essa Township, Ontario (44°14'N, 79°48'W) and Angus, Adjala Township, Ontario (44°19'N, 79°58'W).

At each site, 12-unit multiple funnel traps (Phero Tech., Inc.) were laid out in randomized complete blocks with at least 15 m between traps. Traps were baited with  $\alpha$ -pinene alone or in combination with one or more of the five candidate attractants. Unbaited control traps were not included because it is well documented that they are not attractive to *T. piniperda* and capture very few beetles (POLAND and HAACK, 2000; SCHLYTER et al., 2000). Furthermore, our objective was to determine if any of the treatment combinations increased attraction compared with  $\alpha$ -pinene alone. All semiochemical release devices were supplied by IPM Tech., Inc. Release rates and release devices for semiochemicals used in all experiments are presented in table 1.

Experiments 1 and 2 tested *T. piniperda* responses to  $\alpha$ -pinene alone or combined singly with each of the test compounds released at either a high or low release rate (table 1). The high release rate of each compound was used in experiments 3–6. Experiment 1 was conducted in Rolling Prairie, Indiana from 6 March to 18 April 2000. It consisted of 20 replicates of five treatments: (i)  $\alpha$ -pinene; (ii)  $\alpha$ -pinene plus myrtenol high release rate; (iii)  $\alpha$ -pinene plus myrtenol low release rate; (iv)  $\alpha$ -pinene plus *trans*-verbenol high release rate; and (v)  $\alpha$ -pinene plus *trans*-verbenol low release rate. Experiment 2 was conducted in Angus, Ontario from 8 March to 19 April 2000. It comprised 10 replicates of seven treatments: (i)  $\alpha$ -pinene; (ii)  $\alpha$ -pinene plus  $\alpha$ -pinene oxide high release rate; (iii)  $\alpha$ -pinene plus  $\alpha$ -pinene oxide low release rate; (iv)  $\alpha$ -pinene plus nonanal high release rate; (v)  $\alpha$ -pinene plus nonanal low release rate; (vi)  $\alpha$ -pinene plus myrtenal high release rate; and (vii)  $\alpha$ -pinene plus myrtenal low release rate.

Experiments 3 and 4 tested *T. piniperda*'s responses to  $\alpha$ -pinene alone or with binary combinations of the attractant candidates. Experiment 3 was conducted in Rolling Prairie, Indiana from 6 March to 18 April 2000. It included 10 replicates of five treatments that tested all pairwise combinations of *trans*-verbenol plus one of the other compounds,  $\alpha$ -pinene oxide, myrtenol, myrtenal or nonanal. Experiment 4 was comprised of 10 replicates of eight treatments that tested all possible pairwise combinations of *trans*-verbenol, myrtenol, myrtenal and nonanal. It was conducted in DeWitt, Michigan from 20 March to 2 May 2000.

Experiments 5 and 6 tested *T. piniperda* responses to  $\alpha$ -pinene plus ternary combinations of the potential attrac-

Semiochemical name	Code	Experiment	Purity (%)	Release device	Release rate (mg/day)
$\alpha$ -pinene [76%(-)]	ap	1, 2, 3, 5, 6	98	PE bulb	310
(-)-myrtenol high	mol H	1, 3, 5, 6	97	PA	0.8
(-)-myrtenol low	mol L	1	97	PA	0.2
(-)-myrtenal high	mal H	2, 3, 4, 6	98	PA	4.8
(-)-myrtenal low	mal L	2	98	PA	0.7
(-)- <i>trans</i> -verbenol high	tv H	1, 4, 5, 6	99	PA	0.5
(-)- <i>trans</i> -verbenol low	tv L	1	99	PA	0.3
( $\pm$ )- $\alpha$ -pinene oxide high	apox H	2, 3, 4, 5	97	PE vial	1.7
( $\pm$ )- $\alpha$ -pinene oxide low	apox L	2	97	PE vial	0.4
Nonanal high	non H	2, 3, 4, 5, 6	95	PE vial	2.8
Nonanal low	non L	2	95	PE vial	0.2

Release rates were determined gravimetrically at 20°C.  
PE bulb, airtight polyethylene bulb; PA, paper absorbent saturated with semiochemical and enclosed in ultraviolet protective pouch; PE vial, polyethylene vial enclosed in ultraviolet protective pouch.

**Table 1.** Description of semiochemical release devices used during 2000 in field trapping experiments for *Tomicus piniperda*

tants. Experiment 5 was conducted in Barrie, Ontario from 8 March to 18 April 2000. It comprised 10 replicates of five treatments that tested *T. piniperda* responses to  $\alpha$ -pinene and all possible ternary combinations of  $\alpha$ -pinene oxide, *trans*-verbenol, myrtenol and nonanal. Experiment 6 was conducted in Angus, Ontario from 21 March to 3 May 2001 and comprised 10 replicates of six treatments that compared all possible ternary combinations of *trans*-verbenol, nonanal, myrtenol and myrtenal.

For all experiments, beetles were collected from the traps every 2 weeks and then frozen until counted. Up to 30 beetles from each trap were sexed to determine the sex ratio. Beetles from each collection period were pooled to obtain the total number of beetles captured per trap over the entire experimental period. The total number of beetles captured in each trap during the entire trapping period was transformed by  $\log(x + 1)$  to satisfy assumptions of normality and homoscedasticity and then analysed using two-way ANOVA with model factors for treatment and replicate in each experiment. Differences between treatments were compared using the Ryan–Einot–Gabriel–Welch multiple *Q*-test (REGW; SAS INSTITUTE INC., 1996). An  $\alpha$ -level of 0.05 was used in all tests.

### 3. Results

In experiment 1 there were no significant differences ( $F = 0.45$ ,  $P = 0.77$ , d.f. = 4) in the number of *T. piniperda* captured in traps baited with  $\alpha$ -pinene alone or combined with high or low release rates of myrtenol or *trans*-verbenol added individually (table 2).

Similarly, in experiment 2, there was no significant difference between the number of *T. piniperda* captured

in traps baited with  $\alpha$ -pinene alone or with high or low release rates of  $\alpha$ -pinene oxide, nonanal or myrtenol added individually (table 3). Significantly more *T. piniperda* were captured in traps baited with  $\alpha$ -pinene plus the high release rate of nonanal than in traps baited with  $\alpha$ -pinene and the low release rate of myrtenol ( $F = 2.93$ ,  $P = 0.01$ , d.f. = 6) with all other treatments being intermediate (table 3).

In experiment 3, significantly more *T. piniperda* were captured in traps baited with  $\alpha$ -pinene plus the binary combinations of *trans*-verbenol and myrtenal; *trans*-verbenol and myrtenol; and *trans*-verbenol and nonanal compared with  $\alpha$ -pinene alone ( $F = 6.28$ ,  $P = 0.0006$ , d.f. = 4) (table 4). The addition of *trans*-verbenol and  $\alpha$ -pinene oxide did not increase attraction of *T. piniperda* compared with  $\alpha$ -pinene alone (table 4).

However, in experiment 4, addition of  $\alpha$ -pinene to the binary combinations of *trans*-verbenol and myrtenal; *trans*-verbenol and myrtenol; and *trans*-verbenol and nonanal did not increase attraction significantly compared with  $\alpha$ -pinene alone ( $F = 1.42$ ,  $P = 0.21$ , d.f. = 7), nor did any of the other binary combinations tested (i.e., myrtenal and nonanal; myrtenal and myrtenol; and myrtenol and nonanal) (table 5).

In experiment 5, the combination of  $\alpha$ -pinene, *trans*-verbenol,  $\alpha$ -pinene oxide, nonanal and myrtenol was significantly more attractive than the combination without nonanal ( $F = 2.93$ ,  $P = 0.03$ , d.f. = 4) (table 6). In experiment 6, significantly more *T. piniperda* were captured in traps baited with  $\alpha$ -pinene plus the ternary

**Table 2.** Mean  $\pm$  SEM number and sex ratio of *Tomicus piniperda* captured in experiment 1 in multiple funnel traps in Rolling Prairie, Indiana (6 March to 18 April 2000). Baits consisted of  $\alpha$ -pinene (AP) released at 310 mg/day, myrtenol released at 0.8 mg/day (high) or 0.2 mg/day (low), and *trans*-verbenol released at 0.5 mg/day (high) or 0.3 mg/day (low) ( $n = 20$ )

Treatment	Mean number of <i>T. piniperda</i> captured	Sex ratio (♂:♀)	Normalized response relative to $\alpha$ -pinene
$\alpha$ -pinene (AP)	13.1 $\pm$ 2.8 a	0.98	1.00
AP + myrtenol high	9.1 $\pm$ 2.1 a	1.02	0.69
AP + myrtenol low	10.2 $\pm$ 2.4 a	1.15	0.78
AP + <i>trans</i> -verbenol high	13.9 $\pm$ 3.9 a	1.07	1.06
AP + <i>trans</i> -verbenol low	13.6 $\pm$ 3.9 a	1.03	1.04

Mean values followed by the same letter are not significantly different, Ryan–Einot–Gabriel–Welsch test on data transformed by  $\log(x + 1)$ ,  $P \leq 0.05$ .

**Table 3.** Mean  $\pm$  SEM number and sex ratio of *Tomicus piniperda* captured in experiment 2 in multiple funnel traps in Angus, Ontario (8 March to 19 April 2000). Baits consisted of  $\alpha$ -pinene (AP) released at 310 mg/day,  $\alpha$ -pinene oxide released at 1.7 mg/day (high) or 0.4 mg/day (low), nonanal released at 2.8 mg/day (high) or 0.2 mg/day (low), and myrtenal released at 4.8 mg/day (high) or 0.7 mg/day (low) ( $n = 10$ )

Treatment	Mean number of <i>T. piniperda</i> captured	Sex ratio (♂:♀)	Normalized response relative to $\alpha$ -pinene
$\alpha$ -pinene (AP)	18.3 $\pm$ 2.6ab	0.79	1.00
AP + $\alpha$ -pinene oxide high	12.9 $\pm$ 2.1ab	1.05	0.70
AP + $\alpha$ -pinene oxide low	16.4 $\pm$ 2.4 ab	1.00	0.90
AP + nonanal high	26.4 $\pm$ 4.4 a	1.00	0.90
AP + nonanal low	14.8 $\pm$ 2.5 ab	1.08	0.80
AP + myrtenal high	19.1 $\pm$ 3.2 ab	0.80	1.04
AP + myrtenal low	11.6 $\pm$ 2.6 b	1.00	0.63

Mean values followed by the same letter are not significantly different, Ryan–Einot–Gabriel–Welsch test on data transformed by  $\log(x + 1)$ ,  $P \leq 0.05$ .

**Table 4.** Mean  $\pm$  SEM number and sex ratio of *Tomicus piniperda* captured in experiment 3 in multiple funnel traps in Rolling Prairie, Indiana (6 March to 18 April 2000). Baits consisted of  $\alpha$ -pinene (AP) released at 310 mg/day, trans-verbenol (tv),  $\alpha$ -pinene oxide (apox), myrtenal, myrtenol and nonanal released at 0.5, 1.7, 4.8, 0.8 and 2.8 mg/day ( $n = 10$ )

Treatment	Mean number of <i>T. piniperda</i> captured	Sex ratio ( $\delta$ : $\varphi$ )	Normalized response relative to $\alpha$ -pinene
$\alpha$ -pinene (AP)	15.6 $\pm$ 3.1 b	1.26	1.00
AP + tv + apox	28.0 $\pm$ 4.6 ab	0.53	1.79
AP + tv + myrtenal	33.5 $\pm$ 4.7 a	0.90	2.14
AP + tv + myrtenol	41.7 $\pm$ 4.3 a	0.67	2.67
AP + tv + nonanal	38.0 $\pm$ 6.3 a	0.96	2.43
Mean values followed by the same letter are not significantly different, Ryan–Einot–Gabriel–Welsch test on data transformed by $\log(x + 1)$ , $P \leq 0.05$ .			

**Table 5.** Mean  $\pm$  SEM number and sex ratio of *Tomicus piniperda* captured in experiment 4 in multiple funnel traps in DeWitt, Michigan (20 March to 2 May 2001). Baits consisted of  $\alpha$ -pinene (AP) released at 310 mg/day, myrtenal, myrtenol, nonanal (non) and trans-verbenol (tv) released at 5, 5, 6 and 5.5 mg/day ( $n = 10$ )

Treatment	Mean number of <i>T. piniperda</i> captured	Sex ratio ( $\delta$ : $\varphi$ )	Normalized response relative to $\alpha$ -pinene
$\alpha$ -pinene (AP)	14.3 $\pm$ 5.2 a	0.91	1.00
AP + myrtenal + nonanal	16.5 $\pm$ 3.8 a	1.29	1.15
AP + myrtenal + myrtenol	11.2 $\pm$ 3.5 a	1.15	1.10
AP + myrtenol + nonanal	15.8 $\pm$ 3.5 a	1.15	1.10
AP + tv + myrtenal	24.4 $\pm$ 5.3 a	1.05	1.71
AP + tv + myrtenol	19.1 $\pm$ 4.8 a	2.18	1.71
AP + tv + nonanal	11.5 $\pm$ 1.9 a	1.25	0.80
All	19.5 $\pm$ 5.1 a	0.96	1.36
Mean values followed by the same letter are not significantly different, Ryan–Einot–Gabriel–Welsch test on data transformed by $\log(x + 1)$ , $P \leq 0.05$ .			

**Table 6.** Mean  $\pm$  SEM number and sex ratio of *Tomicus piniperda* captured in experiment 5 in multiple funnel traps in Barrie, Ontario (8 March to 18 April 2000). Baits consisted of  $\alpha$ -pinene (AP) released at 310 mg/day,  $\alpha$ -pinene oxide (apox), myrtenol, nonanal (non) and trans-verbenol (tv) released at 1.7, 0.8, 2.8 and 0.5 mg/day ( $n = 10$ )

Treatment	Mean number of <i>T. piniperda</i> captured	Sex ratio ( $\delta$ : $\varphi$ )
AP + tv + apox + non	196.4 $\pm$ 26.9 ab	1.00
AP + tv + non + myrtenol	190.6 $\pm$ 21.6 ab	0.74
AP + tv + apox + myrtenol	144.8 $\pm$ 21.8 b	0.94
AP + apox + non + myrtenol	175.8 $\pm$ 19.7 ab	0.73
All	205.1 $\pm$ 23.0 a	0.74
Mean values followed by the same letter are not significantly different, Ryan–Einot–Gabriel–Welsch test on data transformed by $\log(x + 1)$ , $P \leq 0.05$ .		

combinations of trans-verbenol, myrtenol and nonanal; or trans-verbenol, myrtenol and myrtenal compared with traps baited with  $\alpha$ -pinene alone ( $F = 2.55$ ,  $P = 0.04$ ,  $df = 5$ ). When added to  $\alpha$ -pinene, the other ternary combinations (i.e. trans-verbenol, nonanal and myrtenal; and myrtenol, nonanal and myrtenal) and the combination of all four compounds, trans-verbenol, nonanal, myrtenol and myrtenal, resulted in trap catches that were intermediate (table 7).

#### 4. Discussion

Some combinations of the tested compounds resulted in increased numbers of *T. piniperda* captured compared with  $\alpha$ -pinene alone. However, the individual

compounds tested including trans-verbenol, nonanal,  $\alpha$ -pinene oxide, myrtenol and myrtenal, had rather low levels of activity when added to  $\alpha$ -pinene alone (tables 2 and 3). Results for different combinations were variable between experiments. In experiment 3, the binary combinations of trans-verbenol plus myrtenol, trans-verbenol plus myrtenal and trans-verbenol plus nonanal were most attractive (table 4); however, in experiment 4, none of the binary combinations significantly increased attraction of *T. piniperda* compared with  $\alpha$ -pinene alone (table 5). In experiment 5, the combination of  $\alpha$ -pinene plus trans-verbenol,  $\alpha$ -pinene oxide, nonanal and myrtenol was significantly more attractive than the same combination without nonanal, suggesting that inclusion of nonanal in the blend increased attraction (table 6). In experiment 6,

**Table 7.** Mean  $\pm$  SEM number and sex ratio of *Tomicus piniperda* captured in experiment 6 in multiple funnel traps in Angus, Ontario (21 March to 3 May 2001). Baits consisted of  $\alpha$ -pinene (AP) released at 310 mg/day, myrtenal, myrtenol, nonanal (non) and trans-verbenol (tv) released at 5, 5, 6, and 5.5 mg/day ( $n = 10$ )

Treatment	Mean number of <i>T. piniperda</i> captured	Sex ratio ( $\sigma$ : $\rho$ )	Normalized response relative to $\alpha$ -pinene
$\alpha$ -pinene (AP)	6.3 $\pm$ 1.0 b	0.85	1.00
AP + myrtenol + tv + non	11.0 $\pm$ 1.0 a	0.72	1.75
AP + myrtenol + tv + myrtenol	11.6 $\pm$ 1.7 a	0.97	1.84
AP + tv + non + myrtenal	9.9 $\pm$ 2.4 ab	0.94	1.57
AP + myrtenol + non + myrtenal	10.0 $\pm$ 2.4 ab	0.89	1.59
All	11.9 $\pm$ 3.1 ab	0.98	1.88

Mean values followed by the same letter are not significantly different, Ryan–Einot–Gabriel–Welsch test on data transformed by  $\log(x + 1)$ ,  $P \leq 0.05$ .

the combinations of trans-verbenol, myrtenol and myrtenal, and trans-verbenol, myrtenol and nonanal significantly increased attraction of *T. piniperda* when added to  $\alpha$ -pinene; however the combination of all four compounds did not (table 7). Combinations that included trans-verbenol, myrtenol and myrtenal increased attraction in experiments 3 and 6 (tables 4 and 7) and combinations that included trans-verbenol and nonanal increased attraction in experiments 3, 5 and 6 (tables 4, 6 and 7). While variable, the results suggest that some combination of trans-verbenol, nonanal, myrtenol and myrtenal may enhance attraction of *T. piniperda* to  $\alpha$ -pinene.

The results of this study generally support those of POLAND et al. (2003) in which trans-verbenol, on its own or combined with myrtenol and/or nonanal, was found to consistently and significantly increase attraction of *T. piniperda* to  $\alpha$ -pinene. Our results also support previous laboratory and field studies demonstrating compounds were inactive individually but were attractive when tested in various combinations (NIEMEYER et al., 1996; ZHOU et al., 1997).

Although combinations that included trans-verbenol and myrtenol significantly increased attraction of *T. piniperda* to  $\alpha$ -pinene in two experiments (tables 4 and 7), the increase was not significant in a third experiment (table 5) and trans-verbenol did not significantly increase attraction on its own (table 2). In contrast, POLAND et al. (2003) found that trans-verbenol increased attraction significantly on its own and in various combinations including myrtenol. However, higher release rates of trans-verbenol were used (1.5 mg/day) by POLAND et al. (2003) than in this study (high = 0.5 mg/day, low = 0.3 mg/day). Lure release rate has been found to be one of the most important factors affecting the capture of insects in pheromone-baited traps MINKS (1977). For instance, capture of *Ips typographus* increased with increasing release rates of its pheromones, cis-verbenol and methyl butenol (SCHLYTER et al., 1987; FRANKLIN and GREGOIRE, 2001). Douglas-fir beetle, *Dendroctonus pseudotsugae* Hopkins, trap catches increased significantly to a plateau with increasing release rates of frontalin and seudenol (ROSS and DATERMAN, 1998).

The activity of trans-verbenol in attraction of beetles to host compounds is more pronounced and consistent in the closely related species, *T. minor* (LANNE et al., 1987). However, *T. piniperda* produces trans-verbenol

in greater quantities than does *T. minor* and thus may require higher release rates to elicit strong responses.

Significant quantities of trans-verbenol are formed by autoxidation of  $\alpha$ -pinene (Hunt et al. 1989); therefore,  $\alpha$ -pinene baits may release an unknown quantity of trans-verbenol. This phenomenon could partially explain the variable responses of *T. piniperda* to trans-verbenol because the rate at which autoxidation occurs varies with environmental conditions.

Responses by males and females to the different treatments were similar, as indicated by sex ratios generally close to 1.0. In a few cases, the sex ratio differed from 1.0; however, these were likely random deviations rather than true sex-specific differences in response to particular treatments. For example, the response to  $\alpha$ -pinene + trans-verbenol + myrtenol was female-biased in experiment 3 (sex ratio = 0.67; table 4) while the response to the same treatment was male-biased in experiment 4 (sex ratio = 2.18; table 5).

$\alpha$ -Pinene released at 310 mg/day was selected as the standard attractive lure for comparison in this study. Although the monoterpenes (+)-3-carene and terpinolene enhance attraction to  $\alpha$ -pinene at low release rates of 30 mg/day (BYERS et al., 1985), we have found the high release rate of  $\alpha$ -pinene is significantly more attractive than the low release monoterpene blend and addition of (+)-3-carene and terpinolene to the high release rate of  $\alpha$ -pinene did not increase attraction (POLAND et al., 2003). The high release rate of  $\alpha$ -pinene is the recommended lure for use in North America (LINDGREN, 1997).

The results of this study, combined with those of POLAND et al. (2003), suggest that addition of semiochemical combinations that include trans-verbenol, nonanal, myrtenol and myrtenal can significantly increase the capture of *T. piniperda*.

#### Acknowledgements

We thank C. Bowman, S. Dunn, R. Disipio, S. Geib, A. George, A. Holmes, M. Montgomery, R. Nott, T. Petrice, R. Scarbach, and C. St. John for assisting us with field experiments; D. Huber and N. Siegert for reviewing an earlier version of the manuscript; W. Dudeck for allowing us to use his Christmas tree plantation; IPM Technologies, Inc., for donating release devices; the Ontario Ministry of Natural Resources, and the Forestry Ecosystem Processes, and

Enhanced Timber Production and Protection Networks, Canadian Forest Service, Natural Resources Canada, for providing funding and logistical support for the studies conducted in Ontario.

## References

- BAKKE, A., 1968: Ecological studies on bark beetles (Coleoptera: Scolytidae) associated with Scots pine (*Pinus sylvestris* L.) in Norway with particular reference to the influence of temperature. *Medd. Nor. Skogforsk.* **21**, 443–602.
- BYERS, J. A., 1992: Attraction of bark beetles, *Tomicus piniperda*, *Hylurgops palliates*, and *Trypodendron domesticum* and other insects to short-chain alcohols and monoterpenes. *J. Chem. Ecol.* **18**, 2385–2402.
- BYERS, J. A.; LÄNNE, B. S.; LÖFQVIST, J.; SCHLYTER, F.; BERGSTRÖM, G., 1985: Olfactory recognition of host-tree susceptibility by pine shoot beetles. *Naturwiss.* **72**, 324–326.
- CZOKAJLO, D., 1998: Semiochemicals for the larger pine shoot beetle (*Tomicus piniperda* L.) and its clerid predators. PhD Dissertation, Syracuse, NY: State University of New York, 164 pp.
- CZOKAJLO, D.; TEALE, S. A., 1999: Synergistic effect of ethanol to  $\alpha$ -pinene in primary attraction of the larger pine shoot beetle, *Tomicus piniperda*. *J. Chem. Ecol.* **25**, 1121–1130.
- FRANCKE, W.; HEEMANN, V., 1976: The odour-bouquet of *Blastophagus piniperda* L. (Col., Scolytidae). *J. Appl. Entomol.* **82**, 117–119.
- FRANKLIN, A. J.; GREGOIRE, J. C., 2001: Dose-dependent responses and preliminary observations on attraction range of *Ips typographus* to pheromones at low release rates. *J. Chem. Ecol.* **27**, 2425–2435.
- HAACK, R. A.; POLAND, T. M., 2001: Evolving management strategies for a recently discovered exotic forest pest: the pine shoot beetle, *Tomicus piniperda* (Coleoptera). *Biol. Invasions* **3**, 307–322.
- HUNT, D. W. A.; BORDEN, J. H.; LINDGREN, B. S.; GRIES, G., 1989: The role of autoxidation of  $\alpha$ -pinene in the production of pheromones of *Dendroctonus ponderosae* (Coleoptera: Scolytidae). *Can. J. For. Res.* **19**, 1275–1282.
- KANGAS, E.; OKSANEN, H.; PERTTUNEN, V., 1970: Responses of *Blastophagus piniperda* L. (Coleoptera, Scolytidae) to *trans*-verbenol, *cis*-verbenol, and verbenone, known to be population pheromones of some American bark beetles. *Acta Entomol. Fenn.* **36**, 75–83.
- LÄNGSTRÖM, B., 1983: Life cycles and shoot-feeding of the pine shoot beetles. *Stud. For. Suec.* **163**, 1–29.
- LÄNNE, B. S.; SCHLYTER, F.; BYERS, J. A.; LÖFQVIST, J.; LEUFVEN, A.; BERGSTRÖM, G.; VAN DER PERS, J. N. C.; UNELIUS, R.; BÄCKSTRÖM, P.; NORIN, T., 1987: Differences in attraction to semiochemicals present in sympatric pine shoot beetles, *Tomicus minor* and *T. piniperda*. *J. Chem. Ecol.* **13**, 1045–1067.
- LINDGREN, B. S., 1997: Optimal release rate of the host monoterpene  $\alpha$ -pinene for trapping the European pine shoot beetle, *Tomicus piniperda* (Coleoptera: Scolytidae). *Proc. Entomol. Soc. Ontario* **128**, 109–111.
- LÖYTTYNIEMI, K.; HELIÖVAARA, K.; REPO, S., 1988: No evidence of population pheromone in *Tomicus piniperda* (Coleoptera: Scolytidae): a field experiment. *Ann. Entomol. Fenn.* **54**, 93–95.
- MINKS, A. K., 1977: Trapping with behavior-modifying chemicals: feasibility and limitations. In: *Chemical Control of Insect Behavior: Theory and Applications*. Ed. by SHOREY, H. H.; MCKELVEY, J. J. New York, NY: John Wiley and Sons, **414**, 385–394.
- NIEMEYER, H.; WATZEK, G.; LU, N.; NIU, J., 1996: Field investigation into the attraction of *Tomicus piniperda* L. (Col., Scolytidae) to monoterpenes and ethanol. *J. Appl. Entomol.* **120**, 265–267.
- PETRICE, T. R.; HAACK, R. A.; POLAND, T. M., 2002: Selection of overwintering sites by *Tomicus piniperda* (Coleoptera: Scolytidae) during fall shoot departure. *J. Entomol. Sci.* **37**, 48–59.
- POLAND, T. M.; HAACK, R. A., 2000: Pine shoot beetle, *Tomicus piniperda* (Col., Scolytidae), responses to common green leaf volatiles. *J. Appl. Entomol.* **124**, 63–69.
- POLAND, T. M.; de GROOT, P.; BURKE, S.; WAKARCHUK, D.; HAACK, R. A.; NOTT, R.; SCARR, T., 2003: Development of an improved attractive lure for the pine shoot beetle, *Tomicus piniperda* (Coleoptera: Scolytidae). *Agr. For. Entomol.* **5**, 293–300.
- ROSS, D. W.; DATERMAN, G. E., 1998: Pheromone-baited traps for *Dendroctonus pseudotsugae* (Coleoptera: Scolytidae): influence of selected release rates and trap designs. *J. Econ. Entomol.* **91**, 500–506.
- SAS INSTITUTE, 1996: SAS System for Personal Computers, release 6.04. Cary, NC: SAS Institute.
- SCHLYTER, F.; BYERS, J. A.; LÖFQVIST, J., 1987: Attraction to pheromone sources of different quantity, quality, and spacing: Density-regulation mechanisms in bark beetle, *Ips typographus*. *J. Chem. Ecol.* **13**, 1503–1523.
- SCHLYTER, F.; ZHANG, Q-H.; ANDERSON, P.; BYERS, J. A.; WADHAMS, L. J.; LÖFQVIST, J.; BIRGERSSON, G., 2000: Electrophysiological and behavioural responses of *Tomicus piniperda* and *Tomicus minor* (Coleoptera: Scolytidae) to non-host leaf and bark volatiles. *Can. Entomol.* **32**, 965–981.
- SCHÖNHERR, J., 1972: A new pheromone excreted by the pine-bark beetle, *Myelophilus piniperda* L. (Coleoptera Scolytidae). *J. Appl. Entomol.* **71**, 410–413.
- SCHROEDER, L. M., 1988: Attraction of the bark beetle *Tomicus piniperda* and some other bark- and wood-living beetles to the host volatiles  $\alpha$ -pinene and ethanol. *Entomol. Exp. Appl.* **46**, 203–210.
- SCHROEDER, L. M.; EIDMANN, H. H., 1987: Gallery initiation by *Tomicus piniperda* (Coleoptera: Scolytidae) on Scots pine trees baited with host volatiles. *J. Chem. Ecol.* **13**, 1591–1599.
- SCHROEDER, L. M.; LINDELÖW, Å., 1989: Attraction of scolytids and associated beetles by different absolute amounts and proportions of  $\alpha$ -pinene and ethanol. *J. Chem. Ecol.* **15**, 807–817.
- VITÉ, J. P.; VOLZ, H. A.; PAIVA, M. R.; BAKKE, A., 1986: Semiochemicals in host selection and colonization of pine trees by the pine shoot beetle, *Tomicus piniperda*. *Naturwiss.* **73**, 39–40.
- YE, H., 1991: On the bionomy of *Tomicus piniperda* (L.) (Col., Scolytidae) in the Kunming region of China. *J. Appl. Entomol.* **112**, 366–369.
- ZHOU, N.; LI, L.; JIANG, Z.; LIO, H., 1997: Study on effect of chemical attractants to *Tomicus piniperda*. *Yunnan Acad. For. Sci.* **2**, 20–38.
- ZUMR, V., 1989: Attractiveness of the terpene  $\alpha$ -pinene to the larger pine shoot beetle, *Blastophagus piniperda* (L.) (Col., Scolytidae). *J. Appl. Entomol.* **107**, 141–144.

**Author's address:** Therese M. Poland (corresponding author), USDA Forest Service, North Central Research Station, 1407 S. Harrison Road, Rm. 220, East Lansing, MI 48823, USA, e-mail: tpoland@fs.fed.us