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Development of an improved attractive lure for the pine shoot beetle, *Tomicus piniperda* (Coleoptera: Scolytidae)

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Abstract

1 The pine shoot beetle, *Tomicus piniperda* (L.) (Coleoptera: Scolytidae), is an exotic pest of pine, *Pinus* spp., and was first discovered in North America in 1992.

2 Although primary attraction to host volatiles has been clearly demonstrated for *T. piniperda*, the existence and role of secondary attraction to insect-produced pheromones have been widely debated.

3 Currently, commercial lures for *T. piniperda* include only the host volatiles α-pinene in North America and α-pinene, terpinolene and (+)-3-carene in Europe. Several potential pheromone candidates have been identified for *T. piniperda*.

4 We tested various combinations of host volatiles and pheromone candidates in Michigan, U.S.A., and Ontario, Canada, to determine an optimal blend.

5 Attraction of *T. piniperda* was significantly increased when *trans*-verbenol (95% pure, 3.2% *cis*-verbenol content) was added with or without myrtenol to α-pinene or to blends of α-pinene and other kairomones and pheromone candidates.

6 Our results, together with other research demonstrating that *trans*-verbenol is produced by *T. piniperda*, support the designation of *trans*-verbenol as a pheromone for *T. piniperda*. A simple operational lure consisting of α-pinene and *trans*-verbenol is recommended for optimal attraction of *T. piniperda*.

Keywords Myrtenol, pine shoot beetle, α-pinene, *Tomicus piniperda*, *trans*-verbenol.

Introduction

The pine shoot beetle, *Tomicus piniperda* (L.) (Coleoptera: Scolytidae), is a native pest of pine, *Pinus* spp., in Europe, Asia, and parts of northern Africa (Schroeder and Eidmann, 1987; Långström and Hellqvist, 1991; Ye, 1991, 1997; Hui and Lieutier, 1997). Established populations of *T. piniperda* were first discovered in the Great Lakes region of North America in 1992 (Haack *et al.*, 1997). As of January 2003, it had been found in 12 states in the north central and north-eastern regions of the U.S.A. (NAPIS, 2002) and in the adjacent Canadian provinces of Ontario and Quebec (CFIA, 2002).

Tomicus piniperda is univoltine throughout its native and North American range. Overwintering adult beetles become active in early spring when they fly in search of suitable brood material such as stumps and slash or trees that have been severely stressed, weakened, freshly killed, or recently cut. Brood adults emerge in late spring or early summer and feed in the shoots of healthy pine trees throughout the summer to complete sexual maturation. When temperatures cool in autumn, beetles move down the trunk to overwinter in the bark at the base of trees in which they have shoot-fed (Bakke, 1968; Långström, 1983). In milder climates, such as

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in the Mediterranean area and southern China, *T. piniperda* may remain in the shoots throughout the winter.

Shoot-feeding by *T. piniperda* may cause serious economic damage to pine forests throughout the beetle's native range. In Europe, losses of 20–45% in annual growth increment and volume have been reported (Långström, 1980; Långström and Hellqvist, 1991). In North America, severe shoot damage including tree mortality has been found in some unmanaged plantations of Scots pine, *Pinus sylvestris* L., in New York State (Czokajlo *et al.*, 1997). In southern Ontario, *T. piniperda* has damaged native species of pines, *P. resinosa* Ait, *P. banksiana* Lamb and, to a lesser extent, *P. strobus* L. (Scarr *et al.*, 1999). Because several species of North American pines are suitable hosts for *T. piniperda* (Långström and Hellqvist, 1985; Sadof *et al.*, 1994; Långström *et al.*, 1995; Lawrence and Haack, 1995; Haack and Lawrence, 1997), it may pose a significant threat as it spreads throughout North America.

Considerable effort has been expended in delineating and limiting the spread of *T. piniperda* in North America (Haack and Poland, 2001). The beetle is monitored and detected primarily through the use of semiochemical-baited multiple funnel traps. Investigations on the role of semiochemicals in host and mate finding by *T. piniperda* have produced conflicting results. Kangas *et al.* (1970) found that *T. piniperda* was attracted to natural phloem extracts from Scots pine and to *trans*-verbenol either alone or combined. Oksanen *et al.* (1970) and Perttunen *et al.*, 1970) reported that the host volatiles α-pinene and 3-carene were inhibitory to *T. piniperda* in laboratory tests and that α-terpineol alone or combined with *cis*- or *trans*-carveol was attractive. Byers *et al.* (1985) demonstrated strong attraction of *T. piniperda* in the field to uninfested pine bolts (+/–)-α-pinene (+)-3-carene and α-terpinolene. Schroeder and Eidmann (1987) found that *T. piniperda* was attracted to sticky traps baited with fresh pine chips and that attacks could be induced on healthy Scots pine trees by baiting them with ethanol or with (–)-α-pinene, (+)-3-carene and terpinolene, either separately or mixed together. The addition of ethanol, a degradation product from the phloem of weakened and dying trees, to α-pinene can either increase (Zumr, 1989) or decrease (Schroeder, 1988) attraction by *T. piniperda*. The response by *T. piniperda* appears to be dependent on the ratio and release rates of α-pinene and ethanol (Schroeder, 1988) and on ambient temperatures (Czokajlo and Teale, 1999).

Currently, attractive lures consisting of host volatiles are available commercially for *T. piniperda* in Europe and in North America. In Europe, the attractive bait contains (+/–)-α-pinene and terpinolene released at 40 mg/day (Witasek, Kärnten, Austria). In North America, the commercial bait consists of α-pinene [95% (–)- enantiomer] released at 300 mg/day (Phero Tech, Inc., Canada).

Although primary attraction to host volatiles has been clearly demonstrated, the existence and role of secondary attraction to insect-produced compounds have been widely debated. Byers *et al.* (1985) found no evidence for pheromone attraction because beetles were equally attracted to uninfested pine bolts and to bolts that had been infested with *T. piniperda* females, males or pairs. Similarly, Löyttyniemi *et al.* (1988) found that equal numbers of *T. piniperda* were attracted to uninfested and infested Scots pine bolts. In laboratory bioassays, Lanne *et al.* (1987) found that *T. piniperda* adults were strongly attracted to pine logs with no apparent increase in attraction when conspecific females were present in the log. On the other hand, in an unreplicated study, Schönherr (1972) found that *T. piniperda* was attracted to Scots pine bolts colonized by females but not to bolts colonized by males. He also found an unidentified compound in female hindgut extracts that was not present in male extracts. Francke and Heemann (1976) identified several compounds from the hindguts of boring *T. piniperda,* including myrtenol, *trans*-verbenol, verbenone and one unidentified compound in virgin females. Kangas *et al.* (1970) found that *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. Lanne *et al.* (1987) identified 3-caren-10-ol, myrtenol, *trans*-verbenol, and verbenone from the hindguts of *T. piniperda*, and they found *trans*-verbenol to be the most active in electroantennograms. In laboratory bioassays and field tests using sticky traps surrounding logs or synthetic baits, *T. piniperda* was attracted to uninfested pine logs and host monoterpene hydrocarbons whereas the sympatric species, *Tomicus minor* Htg., was more strongly attracted to logs infested with conspecific females or baited with *trans*-verbenol and 3-caren-10-ol (Lanne *et al.*, 1987). Zhou *et al.* (1997) identified 3-carene, verbenone, *trans*-verbenol, and myrtenol from the hindguts and frass of female *T. piniperda.* The individual components were highly attractive in laboratory bioassays but had low activity in the field except when combined in mixtures. Niemeyer *et al.* (1996) found that ethanol, α-pinene, β-pinene, terpinolene, *trans*-verbenol and myrtenol were inactive for *T. piniperda* when tested alone but demonstrated weak attraction when tested in various combinations.

Czokajlo (1998), using coupled gas-chromatographic electroantennographic detection, determined that the following compounds had some activity either individually or in mixtures: α-pinene oxide, nonanal (–)-myrtenal, *trans*-verbenol and (–)-myrtenol. McLaughlin *et al.* (2001) found that combinations that included *trans*-verbenol combined with myrtenol or nonanal were the most attractive and resulted in trap catches that were significantly higher than α-pinene alone.

Our objective was to determine whether an improved operational lure could be developed for *T. piniperda,* incorporating pheromone candidates and other host volatiles in addition to α-pinene. For commercial application, the lure would have to be significantly more attractive than α-pinene alone and be able to perform with reliable and consistent results.

Materials and methods

Field experiments were conducted in Michigan and southern Ontario to test *T. piniperda* responses to different lure combinations. All experiments were conducted with 12-unit multiple funnel traps (PheroTech Inc.) in infested Scots pine

Christmas tree plantations. Experiments were replicated in Michigan and Ontario to verify that responses were consistent in different geographical locations.

In 2000, the Michigan field site was located at an abandoned Christmas tree plantation in DeWitt, Ingham County (42°73′N, 84°58′W). The plantation was composed primarily of Scots pine planted in uniform blocks. Trees were approximately 8–10-years old and 2.5–3 m tall. The site was no longer managed for Christmas tree production, and no trees had been cut there for 2 years. Thus, there was very little breeding material available, and the population of *T. piniperda* at the site was fairly low, as indicated by low levels of shoot-feeding damage. In 2001, the Michigan field site was located at a Christmas tree plantation in Mason, Ingham County (42°58′N, 84°44′W). The plantation was composed primarily of Scots pine and trees were 7–8-years old and 2–2.5 m tall. The plantation was being managed for cut-your-own Christmas trees and abundant breeding mater ial was available. Therefore, trees were heavily infested with *T. piniperda*. In 2002, the Michigan field site was located in Shepherd, Isabella County (43°52′N, 84°69′W). The plantation was composed of 10–12-year-old Scots pines that were no longer managed for Christmas tree production and were heavily infested with *T. piniperda*.

A single site was used in Ontario from 2000 to 2002. The site was an operational Scots pine Christmas tree plantation located near Barrie, Essa Township (44°14′N, 79°48′W) in an area where high populations of *T. piniperda* were established. Trees at the site were 6–8-years old and 1.5–2.5 m tall.

Experiment 1 was conducted from 28 February to 21 April 2000 in Michigan and from 1 March to 18 April 2000 in Ontario. It compared α-pinene alone with a blend of host volatiles used in Europe, and with a blend that included antennally active pheromone candidates (Czokajlo, 1998). It comprised four treatments: (i) α-pinene; (ii) α-pinene, (+)-3-carene and terpinolene; (iii) α-pinene, nonanal, α-pinene oxide and myrtenol; and (iv) α-pinene, (+)-3-carene, terpinolene, nonanal, α-pinene oxide and myrtenol. The α-pinene lures were the standard North American commercial baits (Phero Tech, Inc.) consisting of 95% (–)-α-pinene (96.1% chemical purity) released from two 15-mL plastic vials at a combined release rate of 300 mg/day. The blend of 95% (–)-α-pinene (99% chemical purity), (+)-3-carene (91.2% chemical purity) and terpinolene (96.5% chemical purity) (2 : 1 : 1) was released from a plastic vial at 300 mg/day. The blend of 76% (–)-α-pinene (96.1% chemical purity), nonanal (93.9% chemical purity) and α-pinene oxide (2 : 1 : 1) was released from two 15-mL plastic vials at a combined release rate of 300 mg/day and myrtenol released from bubble caps at 1.5 mg/day.

Experiment 2 was conducted in Michigan and Ontario from 22 March to 2 May 2001. It compared α-pinene alone with blends of host volatiles and antennally active compounds. In Michigan, Experiment 2 included four treatments: (i) unbaited control traps; (ii) α-pinene; (iii) α-pinene, (+)-3-carene, terpinolene and nonanal; and (iv) α-pinene, (+)-3-carene, terpinolene, nonanal, *trans*-verbenol and myrtenol. The α-pinene lures were the standard North American commercial baits as used in Experiment 1. The blend of 76% (–)-α-pinene (96% chemical purity), terpinolene (96–97% chemical purity), (+)-3-carene (91% chemical purity) and nonanal (94% chemical purity) (2 : 1 : 1 : 1) was released from two plastic vials at 300 mg/day. The mixture of *trans*-verbenol (95% chemical purity, 3.2% *cis* content) and myrtenol (94% chemical purity) (1 : 1) was released from bubble caps at 1.5 mg/day.

In Ontario, Experiment 2 included two additional treatments using different enantiomers of α-pinene: 75%-(+)-α-pinene (96% chemical purity) and (+/–)-α-pinene (97% chemical purity). It included six treatments: (i) unbaited control traps; (ii) (95%-) α-pinene; (iii) (95%-) α-pinene, (+)-3-carene, terpinolene and nonanal; (iv) (75% +) α-pinene, (+)-3-carene, terpinolene and nonanal; (iv) (+/–) α-pinene, (+)-3-carene, terpinolene and nonanal; and (vi) (95%-) α-pinene, (+)-3-carene, terpinolene, nonanal, *trans*-verbenol and myrtenol.

Experiment 3 was conducted from 28 February to 16 May 2002 in both Michigan and Ontario. It compared *T. piniperda* attraction with nonanal, myrtenol, and *trans*-verbenol added to α-pinene individually and in all possible combinations. Experiment 4 had eight treatments: (i) α-pinene; (ii) α-pinene and nonanal; (iii) α-pinene and *trans*-verbenol; (iv) α-pinene and myrtenol; (v) α-pinene, nonanal and *trans*-verbenol; (vi) α-pinene, nonanal and myrtenol; (vii) α-pinene, *trans*-verbenol and myrtenol; and (viii) α-pinene and all of the volatiles above. The α-pinene lures were the standard North American baits. Nonanal (94% chemical purity), *trans*- verbenol (95% chemical purity, 3.2% *cis*), and myrtenol (94% chemical purity) were released individually from bubble caps at 13, 1.5, and 0.75 mg/day, respectively (Phero Tech, Inc.).

All experiments were laid out in randomized complete blocks with at least 10 replicates per treatment. Traps were set out between rows of trees in the plantations with at least 15 m between traps. All semiochemical products were provided by Phero Tech, Inc. Insects were collected at approximately 2-week intervals throughout each experiment and were stored in sealed plastic bags in a freezer until they were counted and sexed. All *T. piniperda* in each trap were counted, and 20–30 insects were sexed to determine the proportion of females and males captured. Treatment positions were not re-randomized between collection periods, and the numbers of beetles captured during the different collection periods were pooled for each trap. The data were transformed by $\log(x+1)$ to satisfy assumptions of normality and homoscedasticity and then were analysed by two-way analysis of varaince with factors for replicate and treatment (SAS Institute, 1990). The Ryan–Einot–Gabriel–Welsch multiple comparison procedure was used to determine differences among treatments. For Experiment 4, the effects of the individual compounds were compared using orthogonal contrasts.

Results

Responses by *T. piniperda* in Experiment 1 differed between Ontario and Michigan. In Ontario, both male and female

T. piniperda were attracted in significantly higher numbers to the complete blend of α-pinene (+)-3-carene, terpinolene, nonanal, α-pinene oxide and myrtenol compared to α-pinene alone (Table 1). In Michigan, significantly more male and female *T. piniperda* were captured in traps baited with α-pinene alone than in any other treatment.

The results for Experiment 2 were very similar in Michigan and Ontario. In both locations, significantly more male and female *T. piniperda* were captured in traps baited with the blend of α-pinene, (+)-3-carene, terpinolene, nonanal, *trans*-verbenol and myrtenol than in traps baited with α-pinene alone (Table 2). The blend that included α-pinene, (+)-3-carene, terpinolene and nonanal resulted in numbers of *T. piniperda* captured that were intermediate regardless of the enantiomeric composition of α-pinene used in Ontario.

In Experiment 3, in Michigan, significantly more *T. piniperda* (both sexes combined) were captured in traps baited with the combinations of α-pinene and *trans*-verbenol; α-pinene, *trans*-verbenol and myrtenol; and α-pinene, *trans*-verbenol, myrtenol and nonanal compared to α-pinene alone. Addition of nonanal to α-pinene did not enhance attraction of *T. piniperda*, and responses to the other combinations were intermediate between those to α-pinene alone and those to α-pinene plus *trans*-verbenol (Table 3). In Ontario, all of the treatment combinations resulted in significantly higher numbers of *T. piniperda* captured compared to α-pinene alone (Table 3). Considering the effects of the three compounds, *trans*-verbenol, nonanal and myrtenol, in both Michigan and Ontario, *trans*-verbenol had the greatest effect in increasing attraction. The results for orthogonal contrasts of treatments that contained *trans*-verbenol vs. treatments that did not, were significant in both Michigan and Ontario. Contrasts of treatments that contained nonanal vs. treatments that did not were significant only in Ontario. There was no significant difference between treatments that contained myrtenol vs. treatments that did not contain myrtenol in either Michigan or Ontario (Table 4).

Discussion

The results of these experiments, together with other research demonstrating that *trans*-verbenol is produced by *T. piniperda*, support the designation of *trans*-verbenol as a pheromone for *T. piniperda*. In Experiment 2, only the blend that included *trans*-verbenol and myrtenol resulted in significantly higher trap catches (214–255% higher) than α-pinene alone (Table 2). Similarly, in Experiment 3 in Michigan, addition to α-pinene of *trans*-verbenol alone or in combination with myrtenol or myrtenol and nonanal significantly enhanced attraction of *T. piniperda* by 175%, 144% and 155%, respectively (Table 3). In Ontario, addition of *trans*-verbenol, myrtenol and nonanal individually, or in any combination, significantly increased attraction of *T. piniperda* by up to 200% compared to α-pinene alone (Table 3). In both Michigan and Ontario, treatments that contained *trans*-verbenol captured significantly more *T. piniperda* when contrasted to traps that did not contain *trans*-verbenol (Table 4). Results for Experiment 1, that did not include *trans*-verbenol, were inconsistent between Michigan and Ontario. However, the overall number of beetles captured in Experiment 1 in Michigan was very low, making it difficult to draw conclusions.

Myrtenol and *trans*-verbenol have been isolated and identified from hindgut extracts of boring females (Francke and Heemann, 1976; Lanne *et al.*, 1987; Zhou *et al.*, 1997). However, strong attraction by myrtenol and *trans*-verbenol in the field has not been consistently demonstrated. Kangas *et al.* (1970) found that *trans*-verbenol increased attraction in laboratory bioassays. Zhou *et al.* (1997) found that myrtenol and *trans*-verbenol were

Table 1 Mean (±SEM) number of *Tomicus piniperda* captured in Experiment 1 in multiple funnel traps in DeWitt, Michigan (28 February to 21 April 2000) and in Barrie, Ontario (1 March to 18 April 2000)

		Mean number of *T. piniperda* captured/trap		
Treatment	Number of traps	Males	Females	Total
Michigan				
α-pinene	23	15.1 ± 2.0^{a}	10.4 ± 1.8^{a}	25.5 ± 3.5^{a}
α-p + terp + 3-c	21	1.6 ± 0.5^{c}	1.3 ± 0.5^{c}	2.9 ± 0.8^{c}
α-*P* + α-pox + non + myr	25	3.6 ± 0.6^{b}	3.0 ± 0.4^{b}	6.6 ± 0.9^{b}
α-p + terp + 3-c + α-*P* + α-pox + non + myr	24	3.5 ± 0.8^{b}	$2.3 \pm 0.5^{b,c}$	5.8 ± 1.3^{b}
Ontario				
α-pinene	10	$55.2 \pm 8.0^{b,c}$	$62.1 \pm 7.3^{b,c}$	$117.3 \pm 14.2^{b,c}$
α-p + terp + 3-c	10	42.4 ± 9.5^{c}	44.4 ± 7.0^{c}	86.8 ± 14.4^{c}
α-*P* + α-pox + non + myr	10	$76.4 \pm 10.7^{a,b}$	66.7 ± 6.6^{b}	$143.1 \pm 16.6^{a,b}$
α-p + terp + 3-c + α-*P* + α-pox + non + myr	10	97.8 ± 12.1^{a}	109.5 ± 25.8^{a}	207.3 ± 36.9^{a}

Means within a column, for each site separately, followed by the same superscript letter are not significantly different, Ryan–Einot–Gabriel–Welsch test on data transformed by $\log(x+1)$, $P \leq 0.05$. The total number of *T. piniperda* captured in each trap was counted, and up to 30 were sexed to determine the sex ratio and estimate the number of males and females. Baits consisted of α-pinene released at 300 mg/day; α-pinene: terpinolene: 3-carene (2 : 1 : 1) released as a mixture at 300 mg/day (α-p + terp + 3-c); and a two-part lure consisting of α-pinene, α-pinene oxide and nonanal (2 : 1 : 1) released as a mixture at 300 mg/day and myrtenol released at 0.75 mg/day from a separate device (α-*P* + α-pox + non + myr).

Table 2 Mean (± SEM) number of *Tomicus piniperda* captured in Experiment 2 in multiple funnel traps in Mason, Michigan and Barrie, Ontario (22 March to 2 May 2001) ($n = 10$ traps per treatment)

	Mean number of *T. piniperda* captured/trap		
Treatment	Males	Females	Total
Michigan			
Unbaited control	3.8 ± 2.3^{c}	0.6 ± 0.2^{c}	4.4 ± 2.2^{c}
α-pinene	97.5 ± 15.6^{b}	102.7 ± 18.8^{b}	200.2 ± 32.6^{b}
(76%-)α-p + terp + 3c + non	105.2 ± 12.2^{b}	$138.5 \pm 27.3^{a,b}$	243.7 ± 36.3^{b}
α-p + terp + 3c + non + tv + myr	246.1 ± 40.5^{a}	263.8 ± 50.6^{a}	509.9 ± 88.6^{a}
Ontario			
Unbaited control	3.0 ± 2.4^{c}	1.7 ± 0.8^{c}	4.7 ± 3.3^{c}
α-pinene	16.4 ± 2.2^{b}	13.6 ± 1.8^{b}	30.0 ± 3.7^{b}
(76%-)α-p + terp + 3c + non	$20.9 \pm 2.5^{a,b}$	$19.0 \pm 2.4^{a,b}$	$39.9 \pm 4.6^{a,b}$
(+/–)α-p + terp + 3c + non	$19.8 \pm 2.6^{a,b}$	17.2 ± 2.1^{b}	$37.0 \pm 4.4^{a,b}$
(75% +)α-p + terp + 3c + non	$18.8 \pm 2.1^{a,b}$	$18.3 \pm 2.5^{a,b}$	$37.1 \pm 4.4^{a,b}$
α-p + terp + 3c + non + tv + myr	33.5 ± 4.6^{a}	30.7 ± 4.0^{a}	64.2 ± 8.1^{a}

Means within a column followed by the same superscript letter are not significantly different, Ryan–Einot–Gabriel–Welsch test on data transformed by $\log(x+1)$, $P \leq 0.05$. The total number of *T. piniperda* captured in each trap was counted, and up to 30 were sexed to determine the sex ratio and estimate the number of males and females. Baits consisted of α-pinene (95%-) released at 300 mg/day; α-pinene (76% –, 75% +, or +/–): terpinoline: (+) 3-carene: nonanal (2 : 1 : 1 : 1) released as a mixture at 300 mg/day (α-p + terp + 3-c + non); and a two-part lure consisting of α-pinene (76% –): terpinoline: (+) 3-carene: nonanal (2 : 1 : 1 : 1) released as a mixture at 300 mg/day and (–) *trans*-verbenol (84.4% -): (–) myrtenol (1 : 1) released from a separate device at 1.5 mg/day (α-p + terp + 3-c + non + tv + myr).

attractive in laboratory bioassays as individual components, but that combinations of various pheromone components were required for attraction in the field. Lanne *et al.* (1987) found that *trans*-verbenol plus 3-caren-10-ol was attractive to *T. minor* in the field but not to *T. piniperda*; however, in combination with host terpenes, it was highly attractive. The inconsistent activity of *trans*-verbenol in the field may be partially explained by its purity. Kangas *et al.* (1970) found that *cis*-verbenol was inhibitory to *T. piniperda*. In their field tests, Lanne *et al.* (1987) used *trans*-verbenol that contained 12% *cis*-verbenol. In the present study, we used 95% pure *trans*-verbenol that contained only 3.2% *cis*-verbenol.

The (+)- and (–)-enantiomers of α-pinene appear to be equally attractive to *T. piniperda*. There were no significant

Table 3 Mean (±SEM) number of *Tomicus piniperda* captured in Experiment 3 in multiple funnel traps in Shepherd, Michigan and in Barrie, Ontario (28 February to 3 May 2002) ($n = 10$ traps per treatment)

	Mean number of *T. piniperda* captured/trap		
Treatment	Males	Females	Total
Michigan			
α-p	$108.4 \pm 11.7^{b,c}$	92.2 ± 11.6^{b}	200.6 ± 18.1^{c}
α-*P* + non	84.9 ± 12.7^{c}	112.2 ± 14.0^{b}	197.1 ± 22.9^{c}
α-*P* + tv	179.4 ± 40.8^{a}	171.0 ± 28.5^{a}	350.4 ± 58.5^{a}
α-*P* + myr	$109.9 \pm 14.9^{b,c}$	108.2 ± 16.5^{b}	$218.0 \pm 30.3^{b,c}$
α-*P* + non + tv	$120.2 \pm 11.2^{a,b,c}$	$119.9 \pm 14.7^{a,b}$	$240.1 \pm 24.4^{a,b,c}$
α-*P* + non + myr	$143.8 \pm 19.3^{a,b}$	$142.1 \pm 23.9^{a,b}$	$285.9 \pm 40.2^{a,b,c}$
α-*P* + tv + myr	$142.9 \pm 14.8^{a,b}$	$145.4 \pm 22.2^{a,b}$	$288.3 \pm 34.9^{a,b}$
α-*P* + non + tv + myr	$167.8 \pm 23.2^{a,b}$	$142.1 \pm 19.5^{a,b}$	309.9 ± 38.9^{a}
Ontario			
α-p	16.2 ± 2.5^{b}	15.3 ± 1.7^{b}	31.5 ± 2.7^{b}
α-*P* + non	$23.1 \pm 2.5^{a,b}$	25.0 ± 2.9^{a}	48.1 ± 7.2^{a}
α-*P* + tv	$24.9 \pm 3.0^{a,b}$	29.1 ± 3.9^{a}	54.0 ± 6.6^{a}
α-*P* + myr	$22.2 \pm 3.0^{a,b}$	23.6 ± 1.5^{a}	45.8 ± 4.6^{a}
α-*P* + non + tv	$23.1 \pm 2.3^{a,b}$	30.8 ± 3.4^{a}	53.9 ± 8.0^{a}
α-*P* + non + myr	$23.4 \pm 2.8^{a,b}$	26.7 ± 4.0^{a}	50.1 ± 8.4^{a}
α-*P* + tv + myr	24.3 ± 1.7^{a}	26.3 ± 2.8^{a}	50.6 ± 5.4^{a}
α-*P* + non + tv + myr	26.7 ± 3.4^{a}	34.2 ± 5.7^{a}	60.9 ± 11.9^{a}

Means within a column followed by the same superscript letter are not significantly different, Ryan–Einot–Gabriel–Welsch test on data transformed by $\log(x+1)$, $P \leq 0.05$. The total number of *T. piniperda* captured in each trap was counted, and up to 30 were sexed to determine the sex ratio and estimate the number of males and females. Baits consisted of α-pinene (α-p) released at 300 mg/day, nonanal (non) released at 13 mg/day, *trans*-verbenol (tv) released at 1.5 mg/day, and myrtenol (myr) released at 0.75 mg/day.

Table 4 Results of orthogonal contrasts examining the effects of trans-verbenol, nonanal and myrtenol on the number of *Tomicus piniperda* captured in Experiment 3 in multiple funnel traps in Shepherd, Michigan and in Barrie, Ontario (28 February to 3 May 2002)

	Statistics		
Contrast	d.f.	*F*	*P*
Michigan			
With *trans*-verbenol vs. without *trans*-verbenol	1	21.38	< 0.0001
With nonanal vs. without nonanal	1	0.05	0.8157
With myrtenol vs. without myrtenol	1	2.76	0.1017
Ontario			
With *trans*-verbenol vs. without *trans*-verbenol	1	9.71	0.0028
With nonanal vs. without nonanal	1	4.47	0.0384
With myrtenol vs. without myrtenol	1	2.35	0.1307

Orthogonal contrasts performed on data transformed by $\log(x+1)$, $P \leq 0.05$. Treatments are listed in Table 3.

differences in the numbers of *T. piniperda* captured in traps baited with the blend containing 76% (–)-α-pinene and the two special blends containing 75% (+)-α-pinene or racemic α-pinene (Table 2). Similarly, in Europe, attraction of *T. piniperda* has been found to (+/–)-α-pinene (Byers, 1992), (+)-α-pinene (Zumr, 1989) and (–)-α-pinene (Schroeder and Eidmann, 1987). In Europe, the standard commercial lure for *T. piniperda* contains (+/–)-α-pinene whereas, in North America, the standard commercial lure contains 95% (–)-α-pinene (Phero Tech, Inc.). The natural enantiomeric composition of α-pinene in Scots pine is somewhat variable, ranging from 3–30% (–) (Borg-Karlson *et al.*, 1997) to 40% (–) (Hiltunen and Laakso, 1995) or even 50% (–) (Klimetzek and Francke, 1980). Genetic, environmental, and seasonal factors may influence the concentrations of monoterpenes present in Scots pine resin (Sadlowska, 1987; Baradat and Yazdani, 1988). Therefore, it would be adaptive for *T. piniperda* to be attracted to both enantiomers of α-pinene in various ratios.

In North America, a high release rate (300 mg/day) of the single host volatile α-pinene is currently used as the standard commercial lure (Phero Tech, Inc.) whereas, in Europe, a low release rate (40 mg/day) host blend is used (Witasek, Austria). We have found that α-pinene released at 300 mg/day is more attractive than the blend of of α-pinene, (+)-3-carene and terpinolene released at 40 mg/day. In a field experiment in Michigan, in which 10 12-unit multiple funnel traps were baited with each treatment, we found that 233.2 ± 33.7 (mean ± SE) *T. piniperda* were captured in traps baited with the standard North American high release α-pinene lure and only 9.5 ± 3.3 were captured with the low release European blend of α-pinene, terpinolene and (+)-3-carene (Poland *et al.*, unpublished data). Increasing release rates of α-pinene have been found to increase attraction of *T. piniperda*. Schroeder and Lindelöw (1989) demonstrated a clear dose–response by *T. piniperda* to α-pinene in the range of 0–250 mg/day. Lindgren (1997) found that the release rate of α-pinene at 300 mg/day was optimal and that, at 450 mg/day, there was no significant difference in the number of *T. piniperda* captured. In Experiment 1, the blend of α-pinene, terpinolene and (+)-3-carene released at 300 mg/day did not significantly increase attraction of *T. piniperda* compared to α-pinene alone released at the same rate (Table 1).

Nonanal is a volatile found in several herbaceous and deciduous plant species (Buchbauer *et al.*, 1994; Borden *et al.*, 1998; Quiroz and Niemeyer, 1998; Soriano-Cano *et al.*, 1998; Zhang *et al.*, 1999). In general, volatiles such as nonanal from nonhost deciduous species have been found to disrupt attraction of conifer-infesting bark beetles (Borden *et al.*, 1998; Deglow and Borden, 1998). Non-host volatiles have also been found to disrupt attraction of *T. piniperda* (Schroeder, 1992; Poland and Haack, 2000; Schlyter *et al.*, 2000). In the case of *T. piniperda,* nonhost alcohols were found to disrupt attraction whereas aldehydes did not (Poland *et al.*, 2000). Nonanal was found to be an antennally active component of the volatiles collected from Scots pine infested with *T. piniperda* and was found to be attractive to *T. piniperda* in laboratory bioassays (Czokajlo, 1998) and in the field (Czokajlo, 1998; McLaughlin *et al.*, 2001).

Overall, our results indicate that addition of *trans*-verbenol to either α-pinene alone (Table 3), or to blends of α-pinene and other kairomones and pheromone candidates (Table 2), significantly enhanced attraction of *T. piniperda.* On the other hand, addition of host volatiles or pheromone candidates [terpinolene, (+)-3-carene, α-pinene oxide, or nonanal] in the absence of *trans*-verbenol (Table 1) or *trans*-verbenol and myrtenol (Table 2) did not significantly enhance attraction of *T. piniperda* to α-pinene. Traps that were baited with combinations that included *trans*-verbenol caught significantly more *T. piniperda* than traps that did not (Table 4). Therefore, a simple lure consisting of α-pinene and *trans*-verbenol would be an optimal operational lure for *T. piniperda.* An economically viable commercial lure must be simple to use, relatively inexpensive to manufacture, and produce reliable, consistent results. The combination of α-pinene and low *cis* content *trans*-verbenol meets these criteria. It is a simple, two-component lure that can be readily manufactured. In both Michigan and Ontario, traps that contained *trans*-verbenol consistently captured more *T. piniperda* than traps that did not. Overall, α-pinene plus *trans*-verbenol was nearly twice as attractive as the standard North American lure of α-pinene alone. An improved lure for *T. piniperda* should greatly increase the efficacy of monitoring, detection and management programs.

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