Journal of Chemical Ecology, Vol. 25, No. 5, 1999

SYNERGISTIC EFFECT OF ETHANOL TO α-PINENE IN PRIMARY ATTRACTION OF THE LARGER PINE SHOOT BEETLE, *Tomicus piniperda*

DARIUSZ CZOKAJLO[1,*] and STEPHEN A. TEALE

State University of New York
College of Environmental Science and Forestry
1 Forestry Dr., 133 Illick Hall
Syracuse, New York 13210

(Received December 19, 1997; accepted January 9, 1999)

Abstract—α-Pinene and ethanol were released in the approximate proportions 1:0.1, 1:0.9 and 1:9 (at 21°C). Ethanol, released in the range of 3–279 mg/day, generally synergized the attraction of *T. piniperda* to α-pinene (30 mg/day at 21°C), although attraction to the mixtures varied within and between years. The low release rate of ethanol together with α-pinene attracted a significantly higher number of beetles than α-pinene alone in 1995, April of 1996, and in 1997. Lures with the medium release rate of ethanol were the most attractive only in March of 1996. The high dose of ethanol significantly synergized attraction to α-pinene in 1995 and 1997. The variable attraction of *T. pinipeda* to ethanol and α-pinene at various release rates and proportions may be due to the temperature dependent nature of beetle antennal sensitivity. At ambient temperatures of 10–13°C. *T. piniperda* was most attracted to the lures with α-pinene and high release rates of ethanol, at 14–17°C it was most attracted to those with medium release rates of ethanol, and at 18°C and higher it was most attraacted to those with low release rates of ethanol.

Key Words—*Tomicus piniperda*, synergism, α-pinene, ethanol, ambient air temperature, release rates.

INTRODUCTION

The pine shoot beetle, *Tomicus piniperda* (L.) (Coleoptera, Scolytidae) is one of the most destructive insect pests affecting pines in its native range of Europe and Asia (Långström, 1980; Ye, 1991; Långström and Hellqvist, 1993). Accidentally

*To whom correspondence should be addressed.
[1]Current address: IPM Technologies, Inc., 4134 N. Vancouver Ave. Suite 105, Portland, OR 97217.

0098-0331/99/0500-1121/$16.00/0

introduced to North America, it was first discovered near Cleveland, Ohio, in July 1992 (Haack et al., 1997). Surveys in 1997 showed that eight states in the Great Lakes region and southern Ontario were infested (Haack et al., 1997; NAPIS, 1998).

The strong attraction of *T. piniperda* to Scots pine odors is well documented (Perttunen et al., 1970; Oksanen et al., 1971; Byers et al., 1985; Klimetzek et al., 1986; Vité et al., 1986; Schroeder and Eidmann, 1987; Zumr, 1989; Byers, 1992). Although emission of secondary attractants by females has been reported by some authors (Schönherr, 1972; Carlé, 1974; Francke and Heemann, 1976), others report that secondary attraction is unlikely (Byers et al.,1 985; Löyttyniemi et al., 1988).

Tomicus piniperda reproduces in recently cut, windbroken, windthrown, or otherwise severely weakened pines. These damaged or weakened trees emit monoterpenes (Ikeda et al., 1980; Strömvall and Petersson, 1991), as well as ethanol, which forms as a result of degradation processes in the tissue of hosts suitable for colonization by *T. piniperda* (Moeck, 1970; Ikeda et al., 1980; Sjödin et al., 1989). Ethanol has been reported to both synergize (Vité et al., 1986; Klimetzek et al., 1986; Schroeder and Eidmann, 1987; Schroeder and Lindelöw, 1989; Zumr, 1989; Byers, 1992) and inhibit (Klimetzek et al., 1986; Schroeder, 1988) attraction of *T. piniperda* to monoterpenes. Vité et al. (1986) reported approximately an eightfold increase in trap catches when baited with ethanol and the Scots pine monoterpenes α-pinene and terpinolene compared to that of monoterpenes alone. Schroeder and Eidmann (1987) reported that *T. piniperda* was attracted to and attacked healthy Scots pines baited with ethanol. Schroeder and Lindelöw (1989) found that attraction of *T. piniperda* to α-pinene is synergized by ethanol at low release rates of α-pinene, but very high release rates of α-pinene caught more beetles than those combined with ethanol. Zumr (1989) reported increasing attraction with increasing release rates of ethanol together with α-pinene (tested in the proportions of 1 : 1, 1 : 3, 1 : 5, and 1 : 7). Byers (1992) reported that ethanol released at increasing rates (0.08–800 mg/day) does not affect *T. piniperda* attraction to the Scots pine monoterpenes α-pinene, Δ-3-carene, and terpinolene released at rates of 28, 6, and 2.5 mg/day, respectively, but ethanol increased attraction when tested together with lower release rates of monoterpenes (3.3% of the higher rates). However, Klimetzek et al. (1973) reported that ethanol released at 24 mg/day synergizes the attractiveness of *T. piniperda* to α-pinene and terpinolene, and attraction decreases witih increasing release rates of ethanol. Schroeder (1988) reported that ethanol released at rates from 36 mg/day to 50 g/day inhibit the attractiveness of *T. piniperda* to α-pinene released at 240 mg/day.

Such contradictory results are difficult to interpret. The response of *T. piniperda* to different amounts of ethanol and monoterpenes may be affected by ambient temperatures during different field experiments. Temperature has

been reported to affect chemically mediated communication in insects (Cardé and Roelofs, 1973; Comeau et al., 1976; Castrovillo and Cardé, 1979; Linn et al., 1988, 1991; Bento et al., 1993; Charlton et al., 1993; Facundo et al., 1994). Moreover, Bestmann and Dippold (1983) reported that moth antennal response varied at different temperatures with a constant pheromone stimulus.

In this study the synergistic effect of ethanol on *T. piniperda* attraction to α-pinene was examined. The trap catch data were also correlated with the ambient temperatures during *T. piniperda* reproductive flight.

METHODS AND MATERIALS

Experiments were conducted in a 35-year-old Scots pine stand 5 km east of Lockport, Niagara County, New York in 1995, 1996, and 1997. This 4.8-ha unmanaged stand has been damaged by *T. piniperda* for more than a decade (Czokajlo et al., 1997). No trees were harvested in the stand for several years prior to this study, and all freshly snow-damaged trees were removed prior to *T. piniperda* reproductive flight to avoid competition from natural sources.

Beetles were caught in 36, 8-unit multiple-funnel traps (Lindgren, 1983) spaced 15 m or more, and hung with the collection cup ca. 30 cm above the ground. The chemicals used were α-pinene (Aldrich, 98% $[\alpha]^{22}$-0°) and 95% ethanol. The compounds were released separately from 2-ml glass septum vials through glass capillaries inserted into the vial septum or from uncapped vials. There were six treatments: (1) blank trap, (2) α-pinene (30 mg/day at 21°C), (3) ethanol (28 mg/day at 21°C), and (4–6) α-pinene (30 mg/day at 21°C) + ethanol in the proportions of 1 : 0.1, 1 : 0.9, and 1 : 9 (small, medium, and high release rates of ethanol), respectively. Each treatment was replicated six times. Traps were emptied and lures refilled weekly. The release rates of α-pinene and ethanol were estimated gravimetrically in the laboratory at the constant temperatures of 10, 15.5, and 21°C.

Data from all field tests were separately subjected to single factor ANOVA. This analysis was performed on two data sets: (1) pooled among and within each year all α-pinene + ethanol treatments to test for overall synergism of ethanol to α-pinene, and (2) seasonal data sets to test for synergism of different release rates of ethanol in *T. piniperda* attraction to α-pinene. Data from laboratory estimated release rates and proportions of α-pinene and ethanol were subjected to two-way ANOVA with replication. All data were transformed to satisfy ANOVA assumptions as follows: pooled over year trap catches were log-transformed, trap catches from 1995 were rank-transformed, trap catches from March of 1996 were log-transformed, and trap catches from April of 1996 and 1997 were converted to the proportions of the catch of each replicate and then log-transformed.

The laboratory estimated release rates of α-pinene and ethanol were log-transformed and their proportion square root-transformed. The LSD test was used to compare means (Stat Soft, Inc., 1995).

RESULTS

The reproductive flight activity of *T. piniperda* during experimental years and corresponding maximum daily temperatures are given in Table 1.

Synergism of Ethanol to α-Pinene. Ethanol, released in the range of 3–279 mg/day (21°C) (Table 2), had a synergistic effect on the attraction of *T. piniperda* to α-pinene (30 mg/day at 21°C) (LSD, $P = 0.011$). In 1995 and 1997, pooled captures in traps baited with α-pinene + all release rates of ethanol were significantly higher than those of α-pinene alone (LSD, $P = 0.009$ and $P = 0.017$, respectively). Captures in traps baited with α-pinene + the three release rates of ethanol from both 1996 data sets were not significantly different from those of α-pinene alone.

Dose Effect of Ethanol. In 1995, significantly more beetles were attracted to the traps baited with α-pinene and low (3 mg/day at 21°C) and high (142 mg/day at 10°C) (Table 2) release rates of ethanol than to the traps baited with α-pinene alone (LSD, $P = 0.003$ and $P = 0.043$; Figure 1). Captures in traps baited with α-pinene and ethanol at a medium release rate were slightly higher but not significantly different from those of α-pinene alone. In March of 1996, captures in traps baited with α-pinene and the medium release rate of ethanol (19 mg/day at 15.5°C) were significantly higher than those baited with α-pinene alone (LSD, $P = 0.049$). Significantly fewer beetles were captured in traps baited

TABLE 1. MAXIMUM DAILY TEMPERATURES DURING REPRODUCTIVE FLIGHT OF *T. piniperda* NEAR LOCKPORT, NIAGARA COUNTY, NEW YORK IN 1995–1997

Flight season	Dates of flight	Maximum daily temperatures (°C)[a]
1995	Mar 15–20	21, 18, 12, 10, 10, 21
March 1996[b]	Mar 31–Apr 1	17, 15
April 1996[b]	Apr 11, 15–17	18, 19, 18, 17
1997	Mar 28–Apr 3	24, 16, 7, 1, 12, 18, 18

[a]Temperature data for 1995 and 1996 were obtained from the Climatological Station, Lockport 2NE located 5 km west of the experimental site (NOAA, 1995, 1996), and for 1997 recorded in a nearby stand on Omnidata Pod Model DP220 (Omnidata International, Inc., Logan, UT) (Knodel and Barak, 1997).

[b]In 1996 *T. piniperda* had two reproductive flight peaks that were separated by nine days of cold weather.

TABLE 2. ATTRACTION OF *Tomicus piniperda* TO TRAPS BAITED WITH α-PINENE AND ETHANOL AND RELATIONSHIP TO AMBIENT TEMPERATURE NEAR LOCKPORT, NIAGARA COUNTY, NEW YORK IN 1995–1997

Year	Temp. range (No. of days)[a]	Percent of beetles captured[b] with α-pinene (30 mg/day–ethanol in lure		
		1 : 0.1	1 : 0.9	1 : 9
1995	A(3); C(3)	54[c]	11	35[c]
Mar. 1996	B(3)	9	65[c]	26
Apr. 1996	C(4)	61[c]	16	23
1997	A(1); B(1); C(3)	36[c]	21	43[c]

[a]Temperature ranges are as follows: (A) 10–13°C, (B) 14–17°C, (C) 18°C and higher.
[b]Captures are expressed as percent of total captures in traps baited with ethanol and α-pinene for a given year.
[c]Captures differ significantly from those of α-pinene alone.

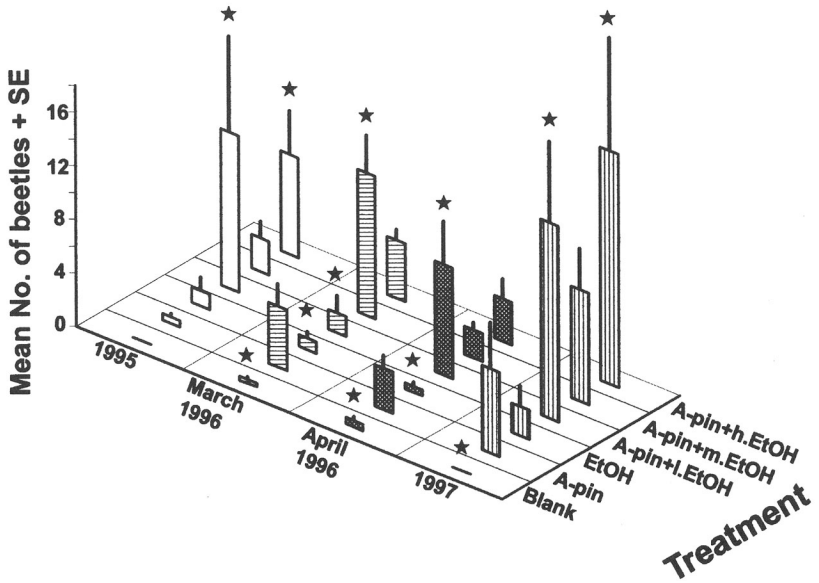


FIG. 1. Mean number of *Tomicus piniperda* caught in Lindgren funnel traps baited with α-pinene (A-pin, 30 mg/day at 21°C), ethanol (EtOH, 28 mg/day at 21°C), A-pin + l.EtOH, A-pin + m.EtOH, and A-pin + h. EtOH α-pinene (30 mg/day at 21°C) with ethanol in the release rate proportions 1 : 0.1, 1 : 0.9 and 1 : 9, respectively, near Lockport, New York. Stars indicate catches significantly different from that of α-pinene alone (A-pin) (LSD, $N = 6$, $P < 0.05$).

TABLE 3. RELEASE RATES OF α-PINENE AND RELEASE PROPORTIONS OF α-PINENE AND ETHANOL DETERMINED IN LABORATORY AT DIFFERENT TEMPERATURES ($N = 36$)[a]

Temp. (°C)	Release of α-pinene (mg/day)[b]	Proportion of ethanol in lure (±SD) at release rate		
		Low	Medium	High
10	15.25	0.11 (0.81)	0.88 (2.98)	9.25 (34.54)
15.5	20.17	0.12 (1.45)	0.95 (3.69)	9.28 (29.14)
21	30.79	0.09 (0.57)	0.89 (4.93)	9.02 (32.21)

[a]Proportions among release rates of α-pinene and three release rates of ethanol are not modified by changes in ambient temperature ($P = 0.99$).
[b]For each temperature the quantity of α-pinene corresponds to the proportion 1 in the lure composition.

with α-pinene and the low release rate of ethanol than to α-pinene alone (LSD, $P = 0.036$). Captures in traps baited with α-pinene and the high release rate of ethanol were not different from those of α-pinene alone. In April of 1996, only captures in traps baited with α-pinene and low release rates of ethanol (3 mg/day at 21°C) were significantly higher than those of α-pinene alone (LSD, $P = 0.046$). Attraction to the traps baited with α-pinene and medium and high release rates of ethanol was not different from that to α-pinene alone. In 1997, significantly more beetles were captured in traps baited with α-pinene and low (3 mg/day 21°C) and high (142 mg/day 10°C) release rates of ethanol than to the traps baited with α-pinene alone (LSD, $P = 0.016$ and $P = 0.023$, respectively). Captures in traps baited with α-pinene and the medium release rates of ethanol were not different from those baited with α-pinene alone.

Release Rates and Proportions of α-Pinene and Ethanol. Release rates of α-pinene and ethanol varied among tested temperatures but their proportions remained unchanged (1 : 0.1, 1 : 0.9, and 1 : 9) (LSD, $P > 0.3$) (Tables 3 and 4). Proportions between release rates of α-pinene and three release rates of ethanol

TABLE 4. ANOVA TABLE FOR ANALYSIS OF RELEASE PROPORTIONS OF α-PINENE AND ETHANOL ESTIMATED IN LABORATORY UNDER CONSTANT TEMPERATURES OF 10, 15.5, AND 21°C

	df effect	*MS* effect	*df* error	*MS* error	*F*	*P* level
Release proportion	2	216.3	315	0.04	6064.8	0
Temperature	2	0.04	315	0.04	1.15	0.31
Rel. prop. × temp.	4	0.003	315	0.04	0.09	0.99

TABLE 5. ANOVA TABLE FOR ANALYSIS OF RELEASE RATES OF ETHANOL ESTIMATED IN LABORATORY UNDER CONSTANT TEMPERATURES OF 10, 15.5, AND 21°C

	df effect	*MS* effect	*df* error	*MS* error	*F*	*P* level
Release rate	2	645.6	369	0.12	5384.1	0
Temperature	2	14.2	369	0.12	118.1	0
Rel. rate × temp.	4	0.1	369	0.12	0.88	0.47

are not modified by changes in ambient temperature (Table 4). Release rates of ethanol at 10, 15.5, and 21°C were different (LSD, $P < 0.001$ for all) among the three tested levels regardless of temperature (Table 5). Release rates of α-pinene and medium release rates of ethanol were not significantly different within each tested temperature (LSD, $P > 0.05$).

DISCUSSION

Ethanol plays an important role in the host searching behavior of *T. piniperda.* Various release rates of ethanol synergized attractiveness of α-pinene to the beetle. Attraction to the mixture varied among and within years: the low release rate of ethanol toghether with α-pinene attracted a higher number of beetles than α-pinene alone in 1995, April of 1996 and 1997. The medium release rate of ethanol was the most attractive only in March of 1996, and the high release rate of ethanol synergized attraction to α-pinene in 1995 and 1997.

The role of ethanol in the chemical communication of *T. piniperda* has been the focus of numerous studies (Klimetzek et al., 1986; Vité et al., 1986; Schroeder and Eidmann, 1987; Schroeder, 1988; Schroeder and Lindelöw, 1989; Zumr, 1989; Byers, 1992). High release rates of ethanol have been reported to be both increasingly attractive (Zumr, 1989; Byers, 1992), and inhibitory (Klimetzek et al., 1986; Schroeder, 1988). Inhibition obtained by Schroeder (1988) resulted from release rates of ethanol and α-pinene higher than those observed in nature (more than 3 g/day and 240 mg/day, respectively) (Moeck, 1970; Ikeda et al., 1980; Byers et al., 1985). In our study, synergism of ethanol to α-pinene was similar to that of Vité et al. (1986) and Schroeder and Lindelöw (1989).

Changes in ambient temperature may affect *T. piniperda* antennal activity sensitivity to various release rates and proportions of ethanol and α-pinene. Bestmann and Dippold (1983) reported changes in antennal responses of three moth species when stimulated with an identical amount of sex pheromone at various temperatures. In all studied species, the smallest antennal responses were recorded at minimum and maximum temperatures. The strongest antennal responses were recorded at all medium range temperatures for one moth species,

at the lower range of mean temperatures for a second moth species, and at the temperature close to the maximum tested for the third species. Temperature has also been reported to affect the time of day and intensity of insect reproductive flight (Cardé and Roelofs, 1973; Comeau et al., 1976; Linn et al., 1988; Bento et al., 1993), performance during upwind flight (Castrovillo and Cardé, 1979; Charlton et al., 1993; Facundo et al., 1994), or perception of odor quality in insects (Linn et al., 1991).

Ambient air temperature has not previously been considered as a factor affecting the olfactory sensitity of *T. piniperda.* Our data suggest that beetle responsiveness to varying proportions to ethanol and α-pinene may be temperature dependent. In 1995, beetles were most attracted to the lures with low and high release rates of ethanol and α-pinene at maximum daily temperatures of 10–12°C for three days and 18–21°C for another three days of reproductive flight. On March 31 and April 1, 1996, the maximum daily temperatures were 15–17°C, and beetles were most attracted to the lures with medium release rates of ethanol and α-pinene. On April 11 and 15–17, 1996, the maximum daily temperatures were 17–19°C, and beetles were most attracted to the lures with low release rates of ethanol and α-piene. In 1997, the maximum daily temperatures were 12, 16, and 18–24°C, and beetles were most attracted to the lures with low and high release rates of ethanol and α-pinene. Our data suggest that at a low range of maximum daily temperatures (10–13°C), *T. piniperda* is most attracted to the lures with α-pinene and high release rates of ethanol, at 14–17°C it is most attracted to those with medium release rates of ethanol, and at 18°C and higher it is most attracted to those with low release rates of ethanol. Our data suggest that the amount of released ethanol has a direct effect on its synergism in *T. piniperda* attraction to α-pinene since the proportions between their release rates across measured temperatures remained unchanged.

Acknowledgments—We thank J. C. Warren and A. I. Cognato for help with the field experiments and Mr. Warren for helpful comments on this manuscript. This research was supported by grant 210-L109 from the USDA McIntire-Stennis Cooperative Forestry Research Program, Cooperative Agreement No. 23-95-41 from the USDA Forest Service, and Cooperative Agreement No. 95-8100-0304 (CA) from USDA APHIS to S. A. Teale.

REFERENCES

BENTO, J. M. S., DELLA LUCIA, T. M. C., and FRIGHETTO, R. T. S. 1993. Male response to natural sex pheromone of *Migdolus fryanus* Westwood (Coleoptera: Cerambycidae) females as affected by daily climatic factors. *J. Chem. Ecol.* 19:2347–2351.

BESTMANN, H. J., and DIPPOLD, K. 1983. Temperaturabhängigkeit von Elektroantennogrammen bei Lepidopteren. *Naturwissenschaften* 70:47–48.

BYERS, J. A. 1992. Attraction of bark beetles, *Tomicus piniperda*, *Hylurgops palliatus*, and *Trypodendron domesticum* and other insects to short-chain alcohols and monoterpenes. *J. Chem. Ecol.* 18:2385–2402.

BYERS, J. A., LANNE, B. S., LÖFQVIST, J., SCHLYTER, F., and BERGSTRÖM, G. 1985. Olfactory recognition of host-tree susceptibility by pine shoot beetles. *Naturwissenschaften* 72:324–326.

CARDÉ, R. T., and ROELOFS, W. L. 1973. Temperature modification of male sex pheromone response and factors affecting calling in *Homomelina immaculata* (Lepidoptera: Arctiidae). *Can. Entomol.* 105:1505–1512.

CARLÉ, P. 1974. Mise en evidence d'une attraction secondaire d'origine sexuelle chez *Blastophagus destruens* Woll. (Col., Scolytidae). *Ann. Zool.* 6:539–550.

CASTROVILLO, P. J., and CARDÉ, R. T. 1979. Environmental regulation of female calling and male response periodicities in codling moth (*Laspeyresia pomonella*). *J. Insect Physiol.* 25:659–667.

CHARLTON, R. E., KANNO, H., COLLINS, R. D., and CARDÉ, T. 1993. Influence of pheromone concentration and ambient temperature on flight of the gypsy moth, *Lymantria dispar* (L.), in a sustained-flight wind tunnel. *Physiol. Entomol.* 18:349–362.

COMEAU, A., CARDÉ, R. T., and ROELOFS, W. L. 1976. Relationship of ambient temperatures to diel periodicities of sex attraction in six species of Lepidoptera. *Can. Entomol.* 108:415–418.

CZOKAJLO, D., WINK, R. A., WARREN, J. C., and TEALE, S. A. 1997. Growth reduction of Scots pine, *Pinus sylvestris* L. caused by the larger pine shoot beetle, *Tomicus piniperda* (L.) (Col., Scolytidae) in New York State. *Can. J. For. Res.* 27:1394–1397.

FACUNDO, H. T., ZHANG, A., ROBBINS, P. S., ALM, S. R., LINN, C. E., JR., VILLANI, M. G., and ROELOFS, W. L. 1994. Sex pheromone responses of the oriental beetle (Coleoptera: Scarabaeidae). *Environ. Entomol.* 23:1508–1515.

FRANCKE, VON W., and HEEMANN, V. 1976. Das Duftstoff-Bouquet des Grossen Waldgärtners *Blastophagus piniperda* L. (Coleoptera, Scolytidae). *Z. Angew. Entomol.* 82:117–119.

HAACK, R. A., LAWRENCE, R. K., MCCULLOUGH, D. C., and SADOF, C. S. 1997. *Tomicus piniperda* in North America: An integrated response to a new exotic scolytid, pp. 62–72, *in* J. C. Gregoire, A. M. Liebold, F. M. Stephen, K. R. Day, and S. M. Salom (eds.). Proceedings: Integrating Cultural Tactics into the Management of Bark Beetle and Reforestation Pests. USDA Forest Service General Technical Report NE 236.

IKEDA, T., ENDA, N., YAMANE, A., ODA, K., and TOYODA, T. 1980. Attractants for the Japanese pine sawyer, *Monochamus alternatus* Hope (Coleoptera: Cerambycidae). *Appl. Entomol. Zool.* 15:358–361.

KLIMETZEK, D., KÖHLER, J., and VITÉ, J. P. 1986. Dosage response to ethanol mediates host selection by "secondary" bark beetles. *Naturwissenschaften* 73:170–272.

KNODEL, J. J., and BARAK, A. 1997. Development of a pest management program for minimizing the economic impact of pine shoot beetle in pine nurseries and plantations of New York. *In* Proceedings of 1997 Regulatory Review of Japanese Beetle and Pine Shoot Beetle, Louisville, Kentucky, Feb. 24–26, 1997, USDA APHIS, Riverdale, Maryland.

LÅNGSTRÖM, B. 1980. Distribution of pine shoot beetle attacks within the crown of Scots pine. Studia For. Suecica No. 154.

LÅNGSTRÖM, B., and HELLQVIST, C. 1993. Induced and spontaneous attacks by pine shoot beetles on young Scots pine trees: Tree mortality and beetle performance. *J. Appl. Entomol.* 115:25–36.

LINDGREN, B. S. 1983. A multiple funnel trap for scolytid beetles (Coleoptera). *Can. Entomol.* 115:299–302.

LINN, C. E., CAMPBELL, M. G., and ROELOFS, W. L. 1988. Temperature modulation of behavioural thresholds controlling male moth sex pheromone response specificity. *Physiol. Entomol.* 13:59–67.

LINN, C., JR., CAMPBELL, M., and ROELOFS, W. 1991. The effects of different blend ratios and temperature on the active space of the Oriental fruit moth sex pheromone. *Physiol. Entomol.* 16:211–222.

LÖYTTYNIEMI, K., HELIÖVAARA, K., and REPO, S. 1988. No evidence of population pheromone in *Tomicus piniperda* (Coleoptera, Scolytidae): A field experiment. *Ann. Entomol. Fenn.* 54:93–95.

MOECK, H. A. 1970. Ethanol as the primary attractant for the ambrosia beetle, *Trypodendron lineatum* (Coleoptera: Scolytidae). *Can. Entomol.* 102:985–995.

NAPIS. 1998. Website: www.ceris.purdue.edu:80/napis/pests/psb.

NOAA (National Oceanic and Atmospheric Administration). 1995, 1996. Climatological data. New York. National Climatic Center, Asheville, North Carolina.

OKSANEN, H., PERTTUNEN, V., and KANGAS, E. 1971. Studies on the chemical factors involved in the olfactory orientation of *Blastophagus piniperda* (Coleoptera, Scolytidae). *Contrib. Boyce Thompson Inst.* 24:299–304.

PERTTUNEN, V., OKSANEN, H., and KANGAS, E. 1970. Aspects of the external and internal factors affecting the olfactory orientation of *Blastophagus piniperda* (Coleoptera, Scolytidae). *Contrib. Boyce Thompson Inst.* 24:293–297.

SCHÖNHERR, VON J. 1972. Pheromon beim Kiefern-Borkenkäfer "Waldgärtner," *Myelophilus piniperda* L. (Coleoptera, Scolytidae). *Z. Angew. 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 α-pinene and ethanol. *Entomol. Exp. Appl.* 46:203–210.

SCHROEDER, L. M., and 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., and LINDELÖW, Å. 1989. Attraction of scolytids and associated beetles by different absolute amounts and proportions of α-pinene and ethanol. *J. Chem. Ecol.* 15:807–817.

SJÖDIN, K., SCHROEDER, L. M., EIDMANN, H. H., NORIN, T., and WOLD, S. 1969. Attack rates of scolytids and composition of volatile wood constituents in healthy and mechanically weakened pine trees. *Scand. J. For. Res.* 4:379–391.

STAT SOFT, INC. 1995. Statistica for Windows (Computer program manual). Tulsa, Oklahoma.

STRÖMVALL, A. M., and PETERSSON, G. 1991. Conifer monoterpenes emitted to air by logging operations. *Scand. J. For. Res.* 6:253–358.

VITÉ, J. P., VOLZ, H. A., PAIVA, M. R., and BAKKE, A. 1986. Semiochemicals in host selection and colonization of pine trees by the pine shoot beetle *Tomicus piniperda. Naturwissenschaften* 73:39–40.

YE, H. 1991. On the bionomy of *Tomicus piniperda* (L.) (Coleoptera: Scolytidae) in the Kunming region of China. *J. Appl. Entomol.* 112:366–369.

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.

New lure for the larger pine shoot beetle, *Tomicus piniperda* - attractant/trap design combinations tested in North America and Europe.

D. Czokajlo[1], B. Hrasovec[2], M. Pernek[2], J. Hilszczanski[3], A. Kolk[3], S. Teale[4], J. Wickham[4], P. Kirsch[1].

[1] IPM Tech, Inc. 4134 N. Vancouver Ave, Suite 105, Portland, OR 97217, USA
[2] University of Zagreb, Zagreb, Croatia
[3] Forest Research Institute, Warsaw, Poland
[4] State University of New York, College of Environmental Science and Forestry, Syracuse, NY 13210, USA

Abstract

An optimized, patented lure for the larger pine shoot beetle, *Tomicus piniperda* has been developed and tested in the United States, Poland, and Croatia. Seven different beetle attractants were tested: α-pinene, α-pinene oxide, ethanol, nonanal, myrtenal, myrtenol, and *trans*-verbenol. α-pinene was tested alone or in combination with two or more of the remaining compounds. Attraction of all candidate lures was compared to attraction of Tomodor, a Polish commercial lure for *T. piniperda*, using the Intercept™ Panel Trap (PT). A lure containing α-pinene, α-pinene oxide, nonanal, myrtenal, myrtenol, and *trans*-verbenol was used to compare trap captures in Intercept PT with 12-unit multi-funnel traps in USA, Theyson trap in Croatia, and IBL-3 trap in Poland. This study demonstrated that at least a quaternary semiochemical combination, including α-pinene, nonanal, *trans*-verbenol, and myrtenol is required to assure maximum trap captures. The best IPM Tech lure was significantly more attractive than Tomodor when tested in Poland and Croatia. Catches of *T. piniperda* in the Intercept PT were significantly higher than in the IBL-3 trap or Theyson trap.

Keywords

larger pine shoot beetle, *Tomicus piniperda*, attractant, Intercept Panel Trap (PT), insect monitoring

Introduction

The larger pine shoot beetle, *Tomicus piniperda,* is native to Europe and Asia. In 1992, *T. piniperda* was discovered in the United States (Haack et al. 1997). Czokajlo et al. (1996) demonstrated that the beetle had been introduced to North America as early as the late 1970's or early 1980's. Since then, *T. piniperda* has spread through 12 USA States and two Canadian Provinces (NAPIS 2002). The chemical ecology of *T. piniperda* is not well understood, and consequently, this pest is difficult to monitor and manage. Presently in North America, only α-pinene is used as a commercial lure (Phero Tech, Inc., Delta, BC, Canada). In Europe, Tomodor (Z.D. Chemipan, Poland) and Tomowit (Bio/Technik/Chemie WITASEK, Austria) are the only known commercially available lures, however none of these lures attract a satisfactory number of beetles. Several trap designs are used for monitoring beetle populations. The multi-funnel trap (Phero Tech, Inc., Delta, BC, Canada) has been the most common trap design used in North America. Several other trap designs have been used in Europe and Asia, e.g. Theyson trap, drain-pipe trap, barrier traps, and multi-funnel trap. Forest managers and land owners need an

effective *T. piniperda* monitoring system.

IPM Tech has developed an improved lure for the larger pine shoot beetle based on previous research (Czokajlo 1998; Czokajlo and Teale 1999; Teale et al. 2001) and unpublished field results. The main objective of this study was to validate an optimal blend and optimal release rates of semiochemicals, along with field test trap designs in order to determine the most effective system for trapping *T. piniperda* during its reproductive flight in spring.

Materials and Methods

Experiments were conducted in the United States, Poland, and Croatia in the spring of 2002. In the United States, the experiment was conducted in an isolated (5 ha), unmanaged, 50-year-old Scots pine stand near Syracuse, NY. In Croatia, the experiment was conducted in a mixed 90% *Pinus sylvestris*, 10% *P. nigra* uneven age (40 to 80-year-old) forest. In Poland, the experiment was conducted in a 65-year-old, even-aged Scots pine forest near Suprasl.

Beetles were caught in Intercept™ Panel Trap (Int PT, IPM Tech, Inc., Portland, OR, USA) and in: 12 unit multi-funnel trap in the United States, IBL-3 funnel trap in Poland, and Theyson trap in Croatia (Fig. 1). Traps were spaced at 15 m or more, with the collection cup or container about 30 cm above the ground. The chemicals used were: α-pinene (Berje, Inc., 98%), α-pinene oxide (Elf-Atochem, 96%), ethanol (Aaper Alcohol and Chemicals, Co., 100%), nonanal (Polarome, 98%), myrtenal (Aldrich, 98%), myrtenol (Aldrich 98%), and *trans*-verbenol (IPM Tech, Inc., 99%). The compounds were released separately and combined into mesh bags for different treatments (Fig 2). Lure combinations, release dispensers and rates of release are provided in Table 1. There were eight treatments in the US experiment and seven treatments in Poland and Croatia. Each treatment was replicated ten times. Beetles were collected weekly.

Figure 1. Intercept PT (left), Multi-funnel trap (center), IBL-3 trap (top right), Theyson trap (bottom right).

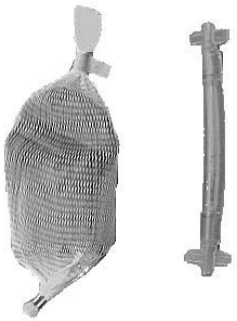


Figure 2. *Tomicus piniperda* IPM Tech's lure (left) and Tomodor (right).

The field data was subjected to a single factor ANOVA. Trap catches from the United States were log transformed and trap catches from Croatia were square root transformed to satisfy ANOVA assumptions. The HSD test was used to compare means in Poland and LSD test was used to compare means in Croatia and in the United States (Stat Soft, Inc., 1995).

Results

The best IPM Tech lure was significantly more attractive than Tomodor and α-pinene lures when tested in Poland (Fig. 3) and Croatia (Fig. 4). Data from the United States was inconclusive (Fig. 5). In Poland, IPM Tech's best lure attracted 638% more beetles than

Table 1. Candidate semiochemicals for *Tomicus piniperda.*

Common Name	Abbreviation	Release device	Release rate (mg/24h)	Treatment*
α-Pinene	α-P	2 LDPE bulbs	300	A,B,C,D,E,F
α-Pinene Oxide	α-P-ox	2 LDPE vials	4	D,E
Nonanal	N	2 LDPE vials	16	B,C,D,E,F
(-) Myrtenal	M-al	LDPE pouch	12	C,D,E
trans-Verbenol	t-V	LDPE pouch LDPE	4	B,C,D,E,F
(-) Myrtenol	M-ol	pouch LDPE pouch	4	B,C,D,E
Ethanol	E		70	E
Tomodor				Tomodor

* Treatment D was used to compare captures in Intercept PT, Theyson trap, and Multi-funnel trap.

Tomodor and 176% more beetles than α-pinene. In Croatia, the best IPM Tech lure attracted 650% more beetles than Tomodor and 233% more beetles than α-pinene. In the United States, captures in traps baited with the best IPM Tech lure, Tomodor, or α-pinene were not different. However, the data collected in the United States was probably inconclusive because in 2002, the reproductive flight of *T. piniperda* occurred unusually late in the spring and was extended over a period of several weeks; in addition, population levels were unusually low. Also, in Poland and Croatia, the Tomodor lure attracted significantly less beetles than α-pinene (by 363% and 278%, respectively); however this was not the case in the United States. Captures of *T. piniperda* in Intercept PT in Croatia were significantly higher than in the Theyson trap (by 216%).

Fig. 3. Captures of *Tomicus piniperda* in Intercept PT and IBL-3 traps baited w/ various IPM Tech lure combinations and Tomodor (Polish lure), Poland, 2002.

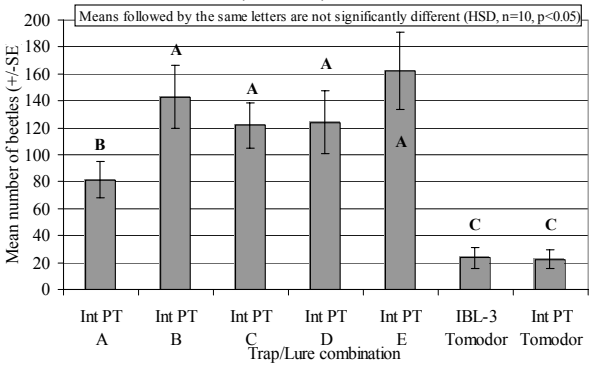
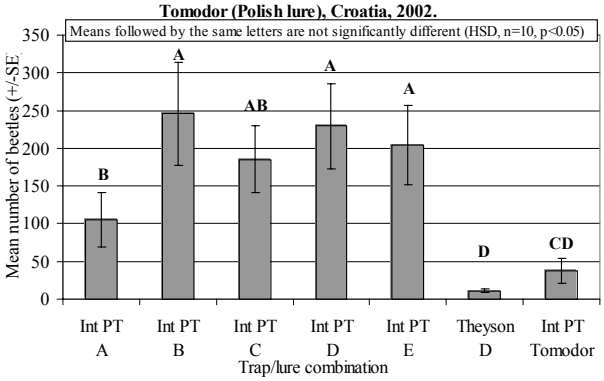


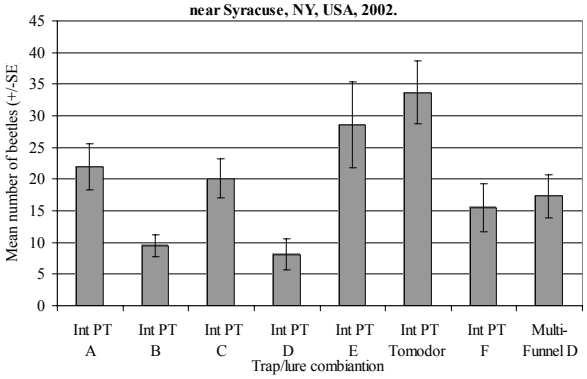
Fig. 4. Trap captures of *Tomicus piniperda* in Int PT & Theyson traps baited with various IPM Tech lures and Tomodor (Polish lure), Croatia, 2002.



Conclusions

The new IPM Tech trap (Intercept PT) and lure for *T. piniperda* proved to be superior to those used commercially in Europe. IPM Tech's lure for *T. piniperda* performs better than commercially available European lures and better than the α-pinene lures used in the United States. Our research indicates that at least a quaternary semiochemical combination, including α-pinene, nonanal, *trans*-verbenol, and myrtenol is required to assure maximum trap captures. Further, IPM Tech's Intercept PT proved to be the best trap compared to any of the European trap designs tested in this study.

Fig. 5. Trap captures of *Tomicus piniperda* in Int PT & Multi-funnel traps baited with various IPM Tech lures and Tomodor (Polish lure), near Syracuse, NY, USA, 2002.



Czokajlo, D. and S.A. Teale. 1999. Synergistic effect of ethanol to α-pinene in primary attraction of the larger pine shoot beetle, *Tomicus piniperda*. J. Chem. Ecol. 25(5): 1121-1130.

Czokajlo, D. 1998. Semiochemicals for the larger pine shoot beetle, *Tomicus piniperda* L. And its clerid predators. Ph.D. Dissertation. State University of New York, College of Environmental Science and Forestry. 164 pp.

Czokajlo, D., R.A. Wink, J.C. Warren, and S.A. Teale. 1997. Growth reduction of Scots pine, *Pinus sylvestris* L. Caused by the larger pine shoot beetle, *Tomicus piniperda* (L.)(Col., Scolytidae) in New York State. Can. J. For. Res. 27, 1394.

Haack, R.A., R.K. Lawrence, D.G. McCullough, and C.S. Sadof. 1997. *Tomicus piniperda* in North America: An integrated response to a new exotic scolytid. In Proceedings: Integrating cultural tactics into the management of bark beetle and reforestation pests, J.C. Grégoire, A.M. Liebhold, F.M. Stephen, K.R. Day, and S.M. Salom, Eds. (USDA For. Ser. Gen. Tech. Rep. NE-236), pp. 62-72.

NAPIS. 2002. Website: www.ceris.purdue.edu/napis/pests/psb

Stat Soft, Inc. 1995. Statistica for windows (Computer program manual). Tulsa, Oklahoma.

Teale, S.A., D. Czokajlo, and F.X. Webster. 2001. Pine shoot beetle attractant composition. US Patent # US 6,203,786 B1.

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

T. M. Poland[1], P. de Groot[2], R. A. Haack[1] and D. Czokajlo[3]
[1]USDA Forest Service, North Central Research Station, East Lansing, MI, USA; [2]Natural Resources Canada, Canadian Forest Service, Great Lakes Forestry Centre, Sault Ste. Marie, ON, Canada; [3]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 (±)-α-pinene, (+)-3-carene, and α-terpinolene. Attraction to insect-produced compounds is less clear. Other potential attractants include *trans*-verbenol, myrtenol, myrtenal, nonanal and α-pinene oxide. We conducted a series of field experiments to determine if any of these compounds would increase attraction of *T. piniperda* to α-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 pinperda*, attraction, α-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 (±)-α-pinene, (+)-3-carene, and α-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 α-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 (±)-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 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, Lanne 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-caren-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-caren-10-ol, myrtenol, *trans*-verbenol and verbenone. They also found that logs baited with *trans*-verbenol and 3-caren-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-caren-10-ol in addition to the monoterpenes α-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, 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. 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 α-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 α-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, α-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 α-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 α-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 α-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) α-pinene; (ii) α-pinene plus myrtenol high release rate; (iii) α-pinene plus myrtenol low release rate; (iv) α-pinene plus *trans*-verbenol high release rate; and (v) α-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) α-pinene; (ii) α-pinene plus α-pinene oxide high release rate; (iii) α-pinene plus α-pinene oxide low release rate; (iv) α-pinene plus nonanal high release rate; (v) α-pinene plus nonanal low release rate; (vi) α-pinene plus myrtenal high release rate; and (vii) α-pinene plus myrtenal low release rate.

Experiments 3 and 4 tested *T. piniperda*'s responses to α-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, α-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, myrtena and nonanal. It was conducted in DeWitt, Michigan from 20 March to 2 May 2000.

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

Semiochemical name	Code	Experiment	Purity (%)	Release device	Release rate (mg/day)
α-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
(−) -*trans*-verbenol high	tv H	1, 4, 5, 6	99	PA	0.5
(−) -*trans*-verbenol low	tv L	1	99	PA	0.3
(±)-α-pinene oxide high	apox H	2, 3, 4, 5	97	PE vial	1.7
(±)-α-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 α-pinene and all possible ternary combinations of α-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 α-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 α-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 α-pinene alone or with high or low release rates of α-pinene oxide, nonanal or myrtenol added individually (table 3). Significantly more *T. piniperda* were captured in traps baited with α-pinene plus the high release rate of nonanal than in traps baited with α-pinene and the low release rate of myrtenal ($F = 2.93$, $P = 0.01$, d.f. $= 6$) with all other treatments being intermediate (table 3).

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

However, in experiment 4, addition of α-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 α-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 α-pinene, *trans*-verbenol, α-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 α-pinene plus the ternary

Table 2. *Mean ± 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 α-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 *T. piniperda* captured	Sex ratio (♂:♀)	Normalized response relative to α-pinene
α-pinene (AP)	13.1 ± 2.8 a	0.98	1.00
AP + myrtenol high	9.1 ± 2.1 a	1.02	0.69
AP + myrtenol low	10.2 ± 2.4 a	1.15	0.78
AP + *trans*-verbenol high	13.9 ± 3.9 a	1.07	1.06
AP + *trans*-verbenol low	13.6 ± 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 ± 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 α-pinene (AP) released at 310 mg/day, α-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 *T. piniperda* captured	Sex ratio (♂:♀)	Normalized response relative to α-pinene
α-pinene (AP)	18.3 ± 2.6ab	0.79	1.00
AP + α-pinene oxide high	12.9 ± 2.1ab	1.05	0.70
AP + α-pinene oxide low	16.4 ± 2.4 ab	1.00	0.90
AP + nonanal high	26.4 + 4.4 a	1.00	0.90
AP + nonanal low	14.8 ± 2.5 ab	1.08	0.80
AP + myrtenal high	19.1 ± 3.2 ab	0.80	1.04
AP + myrtenal low	11.6 ± 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 ± 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 α-pinene (AP) released at 310 mg/day,* trans*-verbenol (tv), α-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 *T. piniperda* captured	Sex ratio (♂:♀)	Normalized response relative to α-pinene
α-pinene (AP)	15.6 ± 3.1 b	1.26	1.00
AP + tv + apox	28.0 ± 4.6 ab	0.53	1.79
AP + tv + myrtenal	33.5 ± 4.7 a	0.90	2.14
AP + tv + myrtenol	41.7 ± 4.3 a	0.67	2.67
AP + tv + nonanal	38.0 ± 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 ± 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 α-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 *T. piniperda* captured	Sex ratio (♂:♀)	Normalized response relative to α-pinene
α-pinene (AP)	14.3 ± 5.2 a	0.91	1.00
AP + myrtenal + nonanal	16.5 ± 3.8 a	1.29	1.15
AP + myrtenal + myrtenol	11.2 ± 3.5 a	1.15	1.10
AP + myrtenol + nonanal	15.8 ± 3.5 a	1.15	1.10
AP + tv + myrtenal	24.4 ± 5.3 a	1.05	1.71
AP + tv + myrtenol	19.1 ± 4.8 a	2.18	1.71
AP + tv + nonanal	11.5 ± 1.9 a	1.25	0.80
All	19.5 ± 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 ± 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 α-pinene (AP) released at 310 mg/day, α-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 *T. piniperda* captured	Sex ratio (♂:♀)
AP + tv + apox + non	196.4 ± 26.9 ab	1.00
AP + tv + non + myrtenol	190.6 ± 21.6 ab	0.74
AP + tv + apox + myrtenol	144.8 ± 21.8 b	0.94
AP + apox + non + myrtenol	175.8 ± 19.7 ab	0.73
All	205.1 ± 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 α-pinene alone ($F = 2.55$, $P = 0.04$, $df = 5$). When added to α-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 α-pinene alone. However, the individual compounds tested including *trans*-verbenol, nonanal, α-pinene oxide, myrtenol and myrtenal, had rather low levels of activity when added to α-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 α-pinene alone (table 5). In experiment 5, the combination of α-pinene plus *trans*-verbenol, α-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 ± 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 α-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 *T. piniperda* captured	Sex ratio (♂:♀)	Normalized response relative to α-pinene
α-pinene (AP)	6.3 ± 1.0 b	0.85	1.00
AP + myrtenol + tv + non	11.0 ± 1.0 a	0.72	1.75
AP + myrtenol + tv + myrtenol	11.6 ± 1.7 a	0.97	1.84
AP + tv + non + myrtenal	9.9 ± 2.4 ab	0.94	1.57
AP + myrtenol + non + myrtenal	10.0 ± 2.4 ab	0.89	1.59
All	11.9 ± 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 α-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 α-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 α-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 α-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 α-pinene (Hunt *et al.* 1989); therefore, α-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 α-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).

α-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 α-pinene at low release rates of 30 mg/day (Byers et al., 1985), we have found the high release rate of α-pinene is significantly more attractive than the low release monoterpene blend and addition of (+)-3-carene and terpinolene to the high release rate of α-pinene did not increase attraction (Poland et al., 2003). The high release rate of α-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. Skogforsok. **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.; Lanne, 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 α-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 α-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.

Långström, B., 1983: Life cycles and shoot-feeding of the pine shoot beetles. Stud. For. Suec. **163,** 1–29.

Lanne, B. S.; Schlyter, F.; Byers, J. A.; Löfqvist, J.; Leufven, A.; Bergström, G.; van der Pers, J. N. C.; Unelius, R.; Baeckström, 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 à-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 α-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 α-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