

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

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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. piniperda* 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 attracted 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

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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., 1985; 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 with 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% $[\alpha]^{22-0^\circ}$) 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

^aTemperature 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).

^bIn 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

^aTemperature ranges are as follows: (A) 10-13°C, (B) 14-17°C, (C) 18°C and higher.
^bCaptures are expressed as percent of total captures in traps baited with ethanol and α -pinene for a given year.
^cCaptures differ significantly from those of α -pinene alone.

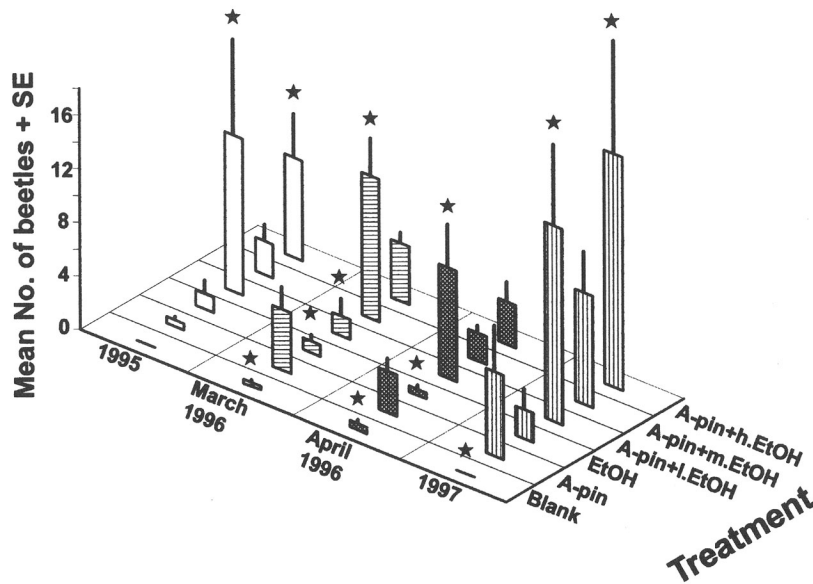


FIG. 1. Mean number of *Tomicus piniperda* caught in Lindgren funnel traps baited with α -pinene (A-pin, 30 mg/day at 21°C), ethanol (EtOH, 28 mg/day at 21°C), A-pin + l.EtOH, A-pin + m.EtOH, and A-pin + h. EtOH α -pinene (30 mg/day at 21°C) with ethanol in the release rate proportions 1:0.1, 1:0.9 and 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 (\pm 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)

^aProportions among release rates of α -pinene and three release rates of ethanol are not modified by changes in ambient temperature ($P = 0.99$).

^bFor 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

	<i>df</i> effect	<i>MS</i> effect	<i>df</i> error	<i>MS</i> error	<i>F</i>	<i>P</i> 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. \times 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

	<i>df</i> effect	<i>MS</i> effect	<i>df</i> error	<i>MS</i> error	<i>F</i>	<i>P</i> 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 together 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 (Castrovilho 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 sensitivity 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 -pinene. 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.

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