

The role of autoxidation of α -pinene in the production of pheromones of *Dendroctonus ponderosae* (Coleoptera: Scolytidae)

D. W. A. HUNT¹ AND J. H. BORDEN

Centre for Pest Management, Department of Biological Sciences, Simon Fraser University, Burnaby, B.C., Canada V5A 1S6

B. S. LINDGREN

Phero Tech Incorporated, 1140 Clark Drive, Vancouver, B.C., Canada V5L 3K3

AND

G. GRIES

Centre for Pest Management, Department of Biological Sciences, Simon Fraser University, Burnaby, B.C., Canada V5A 1S6

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The monoterpene α -pinene, a major component of the terpene composition of *Pinus* spp., has been reported to act as a host-produced kairomone for a variety of bark beetle species, including the mountain pine beetle, *Dendroctonus ponderosae* Hopkins. However, our experiments indicate that α -pinene autoxidizes under normal temperature and atmospheric conditions to form significant quantities of *trans*-verbenol, an aggregation pheromone for many species of bark beetles. The quantities of α -pinene present in the resin that can flow from small wounds in pine trees appear to be sufficient to produce *trans*-verbenol at rates similar to those by female beetles that are actively synthesizing the compound. *trans*-Verbenol can then autoxidize rapidly to form verbenone, with the content of this compound reaching 8% within 13 weeks of exposure to air. Verbenone is often used by scolytids as an antiaggregation pheromone. Approximately 1.9% of the *trans*-verbenol and 2.7% of the verbenone found in Porapak Q aerations of phloem with boring spruce beetle, *Dendroctonus rufipennis* (Kirby), females, as well as 0.8% of the *trans*-verbenol and 0.8% of the verbenone found in aerations of phloem with boring *D. ponderosae* females, was due to the autoxidation of α -pinene and (or) the release of oxygenated compounds found in the phloem before bark beetle attack. The natural interconversion of α -pinene, *trans*-verbenol, and verbenone under ambient conditions suggests that many experiments involving the behavioral activity of these compounds require re-evaluation.

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On a rapporté que le monoterpène α -pinène, le principal terpène de *Pinus* spp., agissait comme une kairomone produite par l'hôte pour une variété d'insectes de l'écorce, incluant le *Dendroctonus* du pin ponderosa, *Dendroctonus ponderosae* Hopkins. Cependant, nos expériences montrent que le α -pinène s'auto-oxide dans les conditions atmosphériques et de température normales pour former des quantités importantes de *trans*-verbénol, une phéromone d'aggrégation pour plusieurs espèces d'insectes de l'écorce. La quantité de α -pinène présente dans la résine qui exude des petites blessures sur les pins semble suffisante pour produire le *trans*-verbénol à un rythme semblable à celui des insectes femelles qui synthétisent activement ce composé. Le *trans*-verbénol peut alors s'auto-oxider rapidement pour former la verbénone, dont la concentration atteint 8% après 13 semaines d'exposition à l'air. La verbénone est souvent utilisée par les scolytes comme phéromone d'anti-aggrégation. Environ 1,9% du *trans*-verbénol et 2,7% de la verbénone détectés avec le Porapak Q dans les émanations provenant du phloème suite à une attaque de dendroctones femelles de l'épinette, *Dendroctonus rufipennis* (Kirby), de même que 0,8% du *trans*-verbénol et 0,8% de la verbénone détectés dans les émanations provenant du phloème suite à une attaque de *D. ponderosae* femelles, étaient dus à l'auto-oxidation du α -pinène et (ou) l'émission de composés oxygénés présents dans le phloème avant l'attaque par des insectes de l'écorce. La transformation naturelle du α -pinène, du *trans*-verbénol et de la verbénone dans les conditions ambiantes suggère que plusieurs expériences impliquant l'effet de ces composés sur le comportement devraient être réévaluées.

[Traduit par la revue]

Introduction

The monoterpene α -pinene is a major constituent of the resin of *Pinus* spp., as well as many other conifers. Female mountain pine beetles, *Dendroctonus ponderosae* Hopkins, convert α -pinene into *trans*-verbenol (Hughes 1973), an aggregation pheromone for this species (Pitman 1971), as well as verbenone, an antiaggregation pheromone (Ryker and Yandell 1983), and other oxygenated products (Pierce et al. 1987). On exposure to α -pinene, beetles in a variety

of other scolytid species also produce verbenols (Hughes 1973, 1975; Renwick et al. 1973, 1976; Klimetzek and Francke 1980) and verbenone (Renwick and Vité 1970; Rudinsky 1973; Rudinsky et al. 1974a), which they often use as pheromones. In addition, α -pinene has been reported to act as a host-produced kairomone for numerous scolytid species, including *D. ponderosae* (Pitman 1971), *Dendroctonus pseudotsugae* Hopkins (Furniss and Schmitz 1971), *Dendroctonus frontalis* Zimmermann (Renwick and Vité 1970), *Dendroctonus rufipennis* (Kirby) (Furniss et al. 1976), *Gnathotrichus retusus* (LeConte) (Borden et al. 1980), *Gnathotrichus sulcatus* (LeConte) (Borden et al. 1980), and *Trypodendron lineatum* (Olivier) (Vité and Bakke 1979).

¹Author to whom all correspondence should be addressed. Present address: Agriculture Canada Research Station, Harrow, Ont., Canada N0R 1G0.

At elevated temperatures in the presence of high levels of oxygen, α -pinene autoxidizes to *trans*-verbenol, verbenone, and other products (Moore et al. 1956). The possibility that α -pinene may autoxidize under ambient conditions in nature appears to have been largely overlooked by researchers studying bark beetle behavior. Since many species that use α -pinene as a kairomone also use *trans*- or *cis*-verbenol as an aggregation pheromone and verbenone as an antiaggregation pheromone, we hypothesized that some of the activity that has been attributed to α -pinene is due to its autoxidation products. Through autoxidation of α -pinene, some of the reported bark beetle pheromones could be wholly or in part products of the host tree. Certain phenomena, such as the increased incidence of bark beetle attack on lightning-struck trees (Coulson et al. 1983, 1986; Krawielitzki et al. 1983) and wounded trees, may be attributed in part to attractants produced by the autoxidation of α -pinene. Finally, if α -pinene were found to autoxidize at a biologically significant rate, many experiments on the chemical ecology of bark beetles that have involved α -pinene would have to be re-evaluated.

Our objective was to study the autoxidation of α -pinene to *cis*- and *trans*-verbenol and verbenone under normal temperature and oxygen conditions, and to evaluate whether autoxidation products could affect *D. ponderosae* behavior in nature.

Material and methods

Autoxidation of α -pinene

Samples were taken from three 1-kg jars of α -pinene (>99% pure, Aldrich Chemical Co., Milwaukee, WI) that had been stored at various temperatures (Table 1) for 2.5 years, and these were analysed by gas chromatography (GC) to quantify the presence of oxygenated products. Two of the jars had been opened periodically and some of the α -pinene removed.

α -Pinene (>99% pure) was distilled with lithium aluminum hydride at reduced pressure such that no oxygenated products were detectable using GC, and this material was stored well sealed at -9 or -20°C . To assess the production of autoxidation products, aliquots of this recently distilled α -pinene (0.2 mL each) were added to empty 1.8-mL vials, and these uncapped vials were exposed to air at 20 – 22°C . After various durations, samples of the contents in the vials were extracted in distilled pentane for GC analysis.

Simulated phloem experiment

A sheet of fiber-glass screen (16 mesh) was stapled onto a sheet of Saran Fabric (30 mesh; Chicopee Manufacturing Co., Cornelia, GA), shaped into cylinders of the same height as 500-mL jars, and fitted inside the jars to allow approximately 4 mm clearance around the full circumference of the screen. In five of the jars, the 4-mm space was tightly filled with 40 g of a medium composed of powdered cellulose and distilled water, or powdered cellulose, distilled water, and ground phloem of lodgepole pine, *Pinus contorta* var. *latifolia* Engelm. The cellulose-based medium was used to stimulate the phloem tissue in which bark beetle galleries are constructed and to induce feeding *D. ponderosae* to release volatiles from their guts. In three of the jars, 20 or 50 wild or axenically reared (Whitney and Spanier 1982; Hunt and Borden 1989), adult female *D. ponderosae* were allowed to bore in the medium, whereas three control jars did not contain beetles. A 1.8-mL vial containing 0.2 mL of recently distilled α -pinene was placed in each jar, whereas in one jar, a vial containing *trans*-verbenol was substituted, and the jars were tightly sealed. Before the experiment, three of the jars containing medium were autoclaved once at approximately 115°C for 30 min.

The atmosphere in the jars was sampled at 24- or 48-h intervals by inserting the needle of a 10-mL, gas-tight syringe (Hamilton

Co., Reno, NA) through a rubber septum in the lid. For each sample, the syringe was pumped 4 times to allow the contents of the atmosphere in the jar to coat the plunger and the interior surface of the syringe. Three millilitres of the headspace was injected directly into the GC.

To quantify compounds produced by the beetles and (or) by α -pinene autoxidation, and which adhered to the medium in the jars, medium from each jar was steam-distilled (Godefoot et al. 1981, 1982) at the end of an experiment. Samples of medium with known amounts of α -pinene or *trans*-verbenol added were similarly steam-distilled to assess any oxidation due to the heat and aeration involved in this process.

Five each of the wild and axenically reared *D. ponderosae* that had been boring in the autoclaved medium contained in the jars were individually extracted at the end of the experiment (168 h), and analysed using GC.

Autoxidation in slow-release devices

trans-Verbenol (150 mg) containing 0.4% verbenone and 11.9% *cis*-verbenol, was added to 1.8 mL polyethylene Eppendorf® centrifugation tubes. Equal numbers of the tubes contained a dental cotton ball substrate as used in commercial slow-release devices for pheromones (Phero Tech Inc., Vancouver, Canada) or no substrate, and one-half of each group contained 0.1% antioxidant 330 (Ethyl Corp.). The tubes were left open and placed at 20 – 22°C in a Plexiglas chamber, with a constant airflow generated by a small centrifugal sucking fan to simulate field conditions. Weekly for 6 weeks the *trans*-verbenol was extracted from one tube of each of the treatments and analysed by GC; during the final 7 weeks, the analysis was conducted every 3 or 4 weeks.

Aeration of phloem sandwiches and shredded phloem

Galleries under construction by single female *D. ponderosae* or *D. rufipennis* in phloem sandwiched between sheets of Plexiglas were aerated using the method of Gries et al. (1988). Volatiles released from the entrance hole and from frass expelled from the gallery were continuously collected and trapped on Porapak Q (Applied Science Laboratories Inc., State College, PA). To compare the products of autoxidation of α -pinene with the production of the beetles, shredded spruce, *Picea engelmannii* Parry, phloem was placed into a 30-mL vial and the volatiles trapped as described above. This host species was chosen because of its high α -pinene content (Drew and Pylant 1966). The ratios of α -pinene to *trans*-verbenol and verbenone in volatiles from shredded phloem were used to estimate the proportion of *trans*-verbenol and verbenone in beetle galleries that could be attributed to autoxidation from α -pinene.

Gas chromatographic analyses

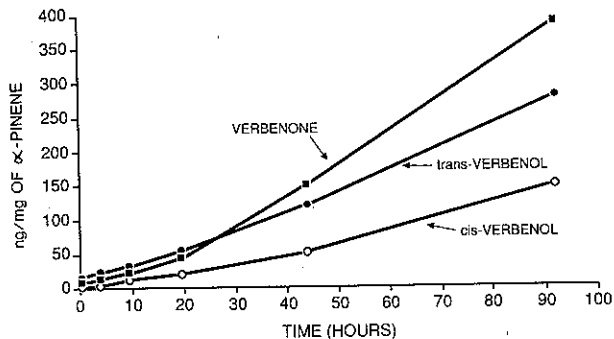
α -Pinene samples, extracts of cellulose and ground phloem media, and gas samples were analysed on a Hewlett Packard 5880A GC equipped with a glass capillary column (30 m \times 0.66 mm internal diameter (ID)) coated with SP-1000 (Supelco, Inc., Bellefonte, PA).

The abdomens of five each of the wild and axenically reared *D. ponderosae* that had been boring in the autoclaved medium were removed and immediately immersed in 100 μL of double-distilled pentane in individual 1.8-mL glass vials over dry ice. After macerating in pentane the abdomen, which included the hindgut, Malpighian tubules, a large portion of the midgut, and other tissues, the vial was sealed and allowed to sit at room temperature for approximately 15 min, and the pentane extract was then drawn off with a syringe and put into a clean vial. The macerated tissue was rinsed twice with 25 μL of double-distilled pentane at room temperature, and the rinses were added to the clean vial, which was then closed with a teflon-lined screw cap and stored at -20°C . These extracts were also analysed on a Hewlett Packard 5880A GC equipped with a glass capillary column (30 \times 0.66 mm ID) coated with SP-1000.

For the slow-release device experiment, samples were extracted by adding 1 mL of ether to each Eppendorf® tube, stirring the solu-

TABLE 1. Accumulation of α -pinene oxidation products in 1-kg jars of α -pinene stored under various conditions for 2.5 years

Storage conditions	Percent composition			
	<i>trans</i> -Verbenol	Verbenone	<i>cis</i> -Verbenol	Myrtenol
Unopened, 0–4°C	0.08	0.05	0.02	0.02
Opened periodically, 0–4°C	0.10	0.09	0.04	0.02
Opened periodically, room temp.	0.19	0.16	0.05	0.04

FIG. 1. Accumulation of α -pinene oxidation products in 0.2-mL samples of distilled α -pinene exposed to air in a 2-mL vial.

tion with a glass rod for 1 min, and then shaking the closed tube for 1 min. Five microlitres of this solution was diluted in 1 mL of ether in a clean glass vial, which was shaken for another 1 min. One microlitre of ether and 1 μ L of the sample solution was drawn into a syringe, and the sample was analysed on a Hewlett Packard 5890A, with a 30-m DB1701 megabore column.

For the phloem aerations, trapped volatiles from 30 h of aeration of beetle galleries or shredded phloem were desorbed with 1 mL of double-distilled pentane:ether (80:20), and concentrated to 10% of former volume. These samples were analysed by coupled GS – mass spectrometry, using a 60 m \times 0.32 mm ID DB-1 column.

Results

Autoxidation of α -pinene

cis- And *trans*-verbenol, myrtenol, and verbenone were all found in 1-kg jars of α -pinene stored under various conditions (Table 1). Figure 1 shows that α -pinene autoxidizes to *cis*- and *trans*-verbenol and verbenone when exposed to air at room temperature. Myrtenol was also produced (Table 1), as well as several other unidentified products. For approximately the first 2 days, *trans*-verbenol was found in larger amounts than verbenone, whereas verbenone predominated thereafter (Fig. 1). Although the α -pinene used in these experiments had been distilled several weeks prior to its use and stored tightly sealed at -9°C , it contained detectable amounts of the oxygenated products before being exposed to air (Fig. 1). As a result, subsequent experiments used more recently distilled α -pinene that was stored at -20°C .

Simulated phloem experiment

trans-Verbenol and verbenone were the predominant autoxidation products of α -pinene in a 500-mL jar containing 0.2 mL of distilled α -pinene (Table 2, expt. I); *cis*-verbenol and myrtenol were present at levels too low to be quantified. *trans*-Verbenol and verbenone accumulated at approximately 2.7 and 1.0 ng/mg of α -pinene/day over 8 days, equivalent to 0.67 and 0.25 ng/mL of air, respectively. When the cellulose medium was added to the

jar, the accumulation of products in the atmosphere was reduced, particularly when beetles were not added (Table 2, expt. II).

Steam distillations established that large quantities of oxygenated products of α -pinene were adhering to the medium lining the 500-mL glass jars (Table 3). Verbenone predominated when the jars with medium were not autoclaved (Table 3, expt. I). When 50 wild female *D. ponderosae* were added to the nonautoclaved medium, the level of verbenone in the medium was approximately sevenfold higher than in the jars without beetles. Owing to the presence of ground phloem in the medium in expt. I, it was not known how much verbenone was formed from vapors from the vial of α -pinene and how much was formed from α -pinene in the phloem.

When ground phloem was eliminated from the medium and the jars of medium were autoclaved before the experiment, moderate quantities of *cis*- and *trans*-verbenol, as well as verbenone, were found in the steam distillates (Table 3, expt. II). Medium in the jar containing wild *D. ponderosae* contained 23 and 74 times the amounts of *trans*-verbenol and verbenone, respectively, than in the medium with no beetles. Medium in the jar containing axenically reared *D. ponderosae* contained even more *trans*-verbenol, but only half as much verbenone as the medium with wild *D. ponderosae*. The 147 ng of *trans*-verbenol and 35 ng of verbenone/mg of α -pinene in the autoclaved medium without added beetles (Table 3, expt. II) should be due entirely to autoxidation of the α -pinene in the vial.

Distillation of medium spiked with a known amount of α -pinene or *trans*-verbenol confirmed that the heat and aeration involved in steam distillation did not cause detectable oxidation. This absence of oxidation was probably due to the atmosphere inside the distillation apparatus being saturated with pentane vapor.

Each of the five axenically reared and wild beetles that were removed from the autoclaved medium at the termination of the experiment contained *trans*- and *cis*-verbenol within the range normally found using this extraction technique (Hunt and Borden 1989), indicating that the beetles were producing these compounds at normal levels during the experiment.

Autoxidation in slow-release devices

trans-Verbenol exposed to air for 13 weeks in Eppendorf[®] tubes contained approximately 8% verbenone when a cotton ball was added to the tubes and 6% verbenone without substrate added (Fig. 2). *cis*-Verbenol underwent autoxidation more quickly than the *trans* isomer, and addition of the antioxidant effectively stopped the autoxidation of verbenols to verbenone (Fig. 2).

Aeration of spruce phloem sandwiches and shredded phloem
Ratios of α -pinene to *trans*-verbenol and verbenone in the

TABLE 2. Accumulation of α -pinene oxidation products in the atmosphere within a 500-mL jar containing 0.2 mL of distilled α -pinene in an open 2-mL vial

Expt. no.	Treatment	Time after exposure to air (h)	Amount of oxidation products (ng/mg of α -pinene)	
			<i>trans</i> -Verbenol	Verbenone
I	α -Pinene	24	0.4	<0.2
		48	1.4	0.3
		72	2.8	1.4
		120	7.9	3.8
		192	21.9	8.1
II	α -Pinene + 40 g of cellulose-based medium	24	<0.2	<0.2
		48	<0.2	<0.2
		96	0.3	0.3
III	α -Pinene + 40 g of cellulose-based medium + 50 <i>D. ponderosae</i>	24	2.2	0.2
		48	2.6	0.7
		96	14.1	3.8
		168	14.0	2.7

NOTE: The cellulose-based medium was composed of powdered cellulose, ground *P. contorta* phloem, and distilled water.

TABLE 3. Accumulation of α -pinene oxidation products in steam distillates of cellulose-based medium from 500-mL jars containing 0.2 mL of distilled α -pinene for 192 h

Expt. No.	Treatment	Amount of oxidation products (ng/mg of α -pinene)			
		<i>cis</i> -Verbenol	<i>trans</i> -Verbenol	Verbenone	Myrtenol
I	Cellulose-based medium, ^a not autoclaved	<7	<7	7 920	7 400
	50 wild <i>D. ponderosae</i> in medium, not autoclaved	<7	<7	55 320	19 293
II	Cellulose-based autoclaved medium ^b	16	147	35	10
	20 wild <i>D. ponderosae</i> in autoclaved medium	57	3 442	2 603	40
	20 axenically-reared <i>D. ponderosae</i> in autoclaved medium	1 481	8 893	1 526	1 456

^aMedium composed of powdered cellulose, ground *P. contorta* phloem, and distilled water.

^bMedium composed of powdered cellulose and distilled water.

volatiles trapped on Porapak Q aerations of shredded spruce phloem were compared with ratios from aerations of individual female *D. rufipennis* or *D. ponderosae* boring in, respectively, spruce, *P. engelmannii*, or pine, *P. contorta*, phloem that was sandwiched between sheets of Plexiglas. These ratios indicated that approximately 1.9% of the *trans*-verbenol and 2.7% of the verbenone found in aerations of phloem with boring *D. rufipennis* females, as well as 0.8% of the *trans*-verbenol and 0.8% of the verbenone found in aerations of phloem with boring *D. ponderosae* females, were due to the autoxidation of α -pinene and (or) the release of oxygenated compounds found in the phloem before bark beetle attack (Table 4).

Discussion

The accumulations of *cis*- and *trans*-verbenol and verbenone in vials of distilled α -pinene exposed to air (Fig. 1) demonstrate that α -pinene autoxidizes under normal temperature and oxygen conditions. This suggests that α -pinene stored at room temperature for extended periods before being used in experiments would contain significant

quantities of *cis*- and *trans*-verbenol, and verbenone, even if the container had not previously been opened (Table 1). The more rapid accumulation of *trans*-verbenol than verbenone in the atmosphere above a source of α -pinene (Table 2, expt. I) is in opposition to that within the liquid (Fig. 1) and may reflect different evaporation rates of *trans*-verbenol and verbenone. The delay in accumulation of oxygenated products in the atmosphere during the first few days of the experiment (Table 2, expt. I) was probably due to these compounds adhering to and finally saturating the surface of the glass jar.

Although *D. ponderosae* produce large quantities of *trans*-verbenol and other oxygenated compounds when exposed to α -pinene vapors, these products are probably not released from their guts in large quantities unless the insects are feeding. Thus, it was necessary to include the powdered cellulose medium in the jars to induce feeding *D. ponderosae* to release oxygenated compounds that had been formed from α -pinene vapors taken in through their spiracles.

The minimal accumulation of *trans*-verbenol in the atmosphere in jars containing unautoclaved cellulose medium,

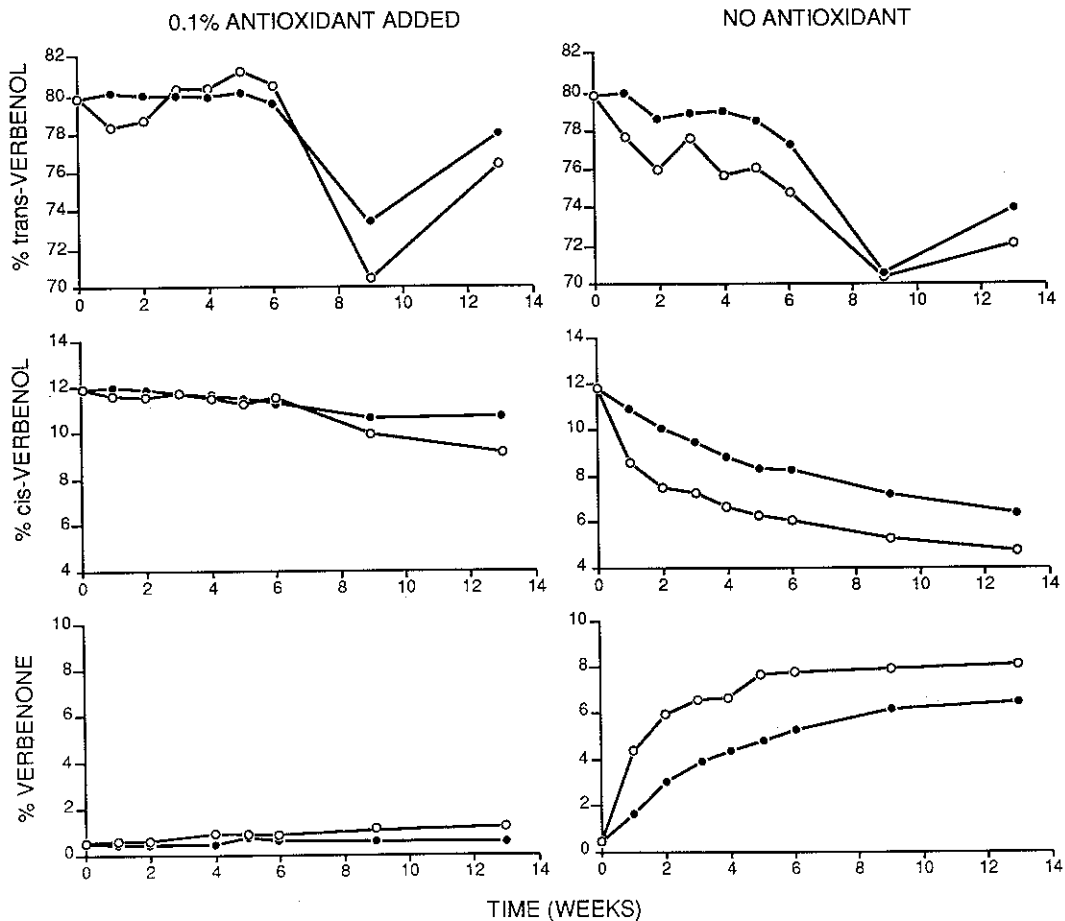


FIG. 2. Autoxidation occurring over time in Eppendorf® tubes containing *trans*-verbenol (contaminated with 0.4% verbenone and 11.9% *cis*-verbenol), with or without an antioxidant added. Open symbols denote tubes containing a cotton substrate, whereas solid symbols denote tubes that did not contain a substrate.

particularly when beetles were not present (Table 2), was not due to the *trans*-verbenol adhering to the medium, since the steam distillate of the medium contained virtually no verbenols but large quantities of verbenone (Table 3, expt. I). Also, the distillate from *trans*-verbenol spiked medium was found to contain only *trans*-verbenol; thus, the heat and aeration involved in steam-distilling the medium had not caused the *trans*-verbenol to oxidize into verbenone.

GC analysis of vials containing 0.2 mL of *trans*-verbenol instead of α -pinene revealed that after 16 days the vial contained approximately 85% *trans*-verbenol, 14% *cis*-verbenol, and 1% verbenone, whereas the steam distillate of the powdered cellulose medium contained 79% *trans*-verbenol, 6% *cis*-verbenol, and 15% verbenone. This indicated that conversion to verbenone had occurred in the medium. In the nonautoclaved medium (Table 3, expt. I), *trans*-verbenol was probably converted to verbenone by microorganisms. The degree of conversion of both *cis*- and *trans*-verbenol to verbenone in the nonautoclaved medium is similar to that for the conversion of verbenols by yeasts associated with *D. ponderosae* (Hunt and Borden 1990), and *Ips typographus* L. (Leufvén et al. 1984).

The complete conversion of verbenols to verbenone in nonautoclaved medium with or without wild female *D. ponderosae* (Table 3, expt. I) suggests that the metabolic capacity for efficiently converting verbenols to verbenone is present in free-living microorganisms as well as those associated with *D. ponderosae*. Thus, the results support

the hypothesis that the ability to perform such conversions is common among many microorganisms (Hunt and Borden 1990).

The much higher ratio of *trans*-verbenol to verbenone in medium from the jar with axenically reared *D. ponderosae* than in medium from the jar with wild *D. ponderosae* (Table 3, expt. II) is consistent with the finding that yeasts that are closely associated with *D. ponderosae* can convert verbenols into verbenone (Hunt and Borden 1990). Yeasts such as *Hansenula capsulata* Wickerham and *Pichia pinus* (Holst) Phaff likely were transported into the jar by the wild beetles, particularly in their mycangia (Whitney and Farris 1970), and once established in the medium, converted *trans*-verbenol into verbenone.

The higher levels of oxygenated products of α -pinene in medium with axenically reared than wild *D. ponderosae* (Table 3, expt. II) are consistent with the observation that axenically reared *D. ponderosae* produce higher levels of these compounds than beetles with their full complement of microorganisms (Hunt and Borden 1989). However, the presence of verbenone in the medium with axenically reared beetles (Table 3, expt. II) contradicts the finding that axenically reared beetles do not produce verbenone (Hunt and Borden 1989). It is possible that microorganisms not eliminated by autoclaving produced this verbenone, although it is more likely that it was produced by autoxidation of beetle-produced *trans*-verbenol. This hypothesis is supported by our finding that *trans*-verbenol exposed to air

TABLE 4. Quantities of volatiles captured on Porapak Q during 30-h aerations of shredded phloem alone or a phloem sandwich containing a boring bark beetle

Source	Sample No.	α -pinene (μ g)	Total <i>trans</i> -verbenol (μ g)	Autoxidized <i>trans</i> -verbenol		Total verbenone (μ g)	Autoxidized verbenone	
				μ g	%		μ g	%
Shredded								
<i>P. engelmannii</i>								
phloem								
	1	639.0	0.0766	0.0766	100	0.6390	0.6390	100
	2	709.8	0.0993	0.0993	100	0.4259	0.4259	100
	3	687.2	0.0824	0.0824	100	0.2803	0.2803	100
	Mean	678.6	0.0861	0.0861	100	0.4484	0.4484	100
<i>P. contorta</i>								
phloem and \varnothing								
<i>D. ponderosae</i>								
	1	13.0	0.3752	0.0015	0.4	1.423	0.0085	0.6
	2	71.2	0.7347	0.0088	1.2	4.770	0.0429	0.9
	3	34.1	0.5310	0.0042	0.8	1.947	0.0214	1.1
	Mean	39.4	0.5469	0.0048	0.8	2.713	0.0242	0.8
<i>P. engelmannii</i>								
phloem and \varnothing								
<i>D. rufipennis</i>								
	1	235.6	1.719	0.0292	1.7	5.890	0.1531	2.6
	2	288.3	2.191	0.0416	1.9	6.920	0.1868	2.7
	3	278.4	1.492	0.0343	2.3	6.125	0.1837	3.0
	Mean	267.4	1.801	0.0350	1.9	6.312	0.1745	2.7

will autoxidize to form large quantities of verbenone, particularly when exposed on a large surface area (Fig. 2). Field studies also indicate that *trans*-verbenol will autoxidize to verbenone very rapidly under some conditions. Eppendorf[®] tubes containing 30 μ L of *trans*-verbenol on dental cotton balls contained up to 62% verbenone after 2 months in the field (B. S. Lindgren, unpublished data). Since these release devices are used in baits for inducing mountain pine beetle attacks on host trees, such autoxidation can be a serious problem. Our data show that the addition of an antioxidant will eliminate autoxidation almost entirely (Fig. 2), making these simple release devices reliable for *trans*-verbenol.

The 147 ng of *trans*-verbenol and 35 ng of verbenone/mg of α -pinene present in the autoclaved medium without added beetles (Table 3, expt. II) is presumed to be due entirely to the autoxidation of α -pinene. When compared with the 3442 ng of *trans*-verbenol and 2603 ng of verbenone/mg of α -pinene in the autoclaved medium with 20 wild *D. ponderosae* (Table 3, expt. II), autoxidation is responsible for approximately 4.3% of the *trans*-verbenol and 1.3% of the verbenone produced. This translates into α -pinene autoxidation producing approximately 0.9 female equivalents of *trans*-verbenol and 0.3 female equivalents of verbenone in this experiment.

It is also possible to relate the levels of *trans*-verbenol and verbenone in the atmosphere of jars containing α -pinene (Table 2), or in the cellulose medium in these jars (Table 3, expt. II), with the rate of resin exudation from wounds in living *P. contorta* and the proportion of α -pinene present in that resin. For example, the 147 ng of *trans*-verbenol formed by autoxidation of α -pinene in expt. II (Table 3) is equal to a rate of approximately 0.8 ng/mg of α -pinene/h. If an average female *D. ponderosae* produces 200–300 ng of *trans*-verbenol in 24 h of exposure to α -pinene (Borden et al. 1986), the production rate is approximately 10 ng/h. Thus, about 12.5 mg of α -pinene exudation/h would produce *trans*-verbenol at approximately the same rate as an average female beetle. At a content of 5–7% α -pinene in

the monoterpenes of *P. contorta* xylem resin (Smith 1983), and with approximately 14% of lodgepole pine xylem resin collected in British Columbia being composed of turpentine (Mirov 1961), then approximately 1360 mg/h of exuded resin would be required to produce one female equivalent of *trans*-verbenol from autoxidation. Raffa and Berryman (1983) reported that *P. contorta* that sustained high *D. ponderosae* attack densities exhibited a mean preattack resin flow of 0.719 mL/h from a single 11 mm diameter hole drilled into the cambium. This rate of resin exudation should produce approximately 0.5 female equivalents of *trans*-verbenol from α -pinene autoxidation. Thus, the *trans*-verbenol formed from autoxidation of α -pinene present in resin may be responsible for the increased incidence of *D. ponderosae* attack on wounded trees, as well as contributing to aggregation on already attacked trees.

Although α -pinene constitutes only 5–7% of the resin monoterpenes in *Pinus ponderosa* Laws. and *P. contorta*, other hosts of *D. ponderosae* contain much higher levels of α -pinene. In *Pinus edulis* Engelm., *Pinus flexilis* James, *Pinus lambertiana* Dougl., *Pinus monophylla* Torr. and Frém., and *Pinus monticola* Dougl., α -pinene is present as 60–85% of the monoterpenes (Mirov 1961). Other *Pinus* spp. that are hosts of bark beetles that use verbenols as pheromones also contain high levels of α -pinene. For example, of the 10 hosts of *D. frontalis* (Wood 1982), *P. ponderosa* is the only one in which α -pinene is not the major monoterpene (Mirov 1961). This prevalence of α -pinene in *Pinus* spp. suggests that autoxidation of α -pinene in wound exudate may be a significant factor in attracting bark beetles to host pines.

One key to the role of α -pinene autoxidation in modifying the natural attack behavior of *D. ponderosae*, as well as the response to α -pinene deployed by man to attract beetles, is the ratio of production of *trans*-verbenol to verbenone. Ryker and Yandell (1983) found that verbenone significantly reduced the numbers of *D. ponderosae* caught in traps baited with *trans*-verbenol when the release rate of the verbenone

was 15% of that of the *trans*-verbenol. As the atmosphere above a source of α -pinene contains verbenone produced by autoxidation at approximately 25–30% of the level of *trans*-verbenol (Table 2), the net effect of the autoxidation of α -pinene may be partially antiaggregative for *D. ponderosae* under these conditions. Further research is needed to determine how much verbenone can be present in a sample of *trans*-verbenol before the aggregative effect for *D. ponderosae* is significantly reduced. In contrast, bark beetles such as *D. frontalis*, for which verbenone is a multifunctional pheromone (Rudinsky 1973; Rudinsky et al. 1974a, 1974b) and *trans*-verbenol is attractive (Renwick and Vité 1969), would be attracted to the products of autoxidation unless the concentration of verbenone reached an extremely high level.

Our data indicate that the quantities of *trans*-verbenol and verbenone released from the autoxidation of α -pinene in phloem may be significant in relation to the total rate of release of these compounds from bark beetle galleries (Table 4). Conversion of *trans*-verbenol to verbenone by microorganisms (Leufvén et al. 1984; Hunt and Borden 1990) may also account for some of the production of verbenone in the shredded phloem samples (Table 4).

It is possible that scolytids initially used terpene alcohols that were produced naturally through autoxidation of host monoterpenes to locate prospective host trees, particularly wounded hosts. Subsequent to this, the scolytids, and (or) their symbiotic microorganisms (Brand et al. 1975; Byers and Wood 1981; Hunt and Borden 1989), would have developed the enzymatic capacity to produce large quantities of terpene alcohol aggregation pheromones.

When the composition of resin from *Pinus* spp. is analysed, oxygenated compounds are seldom reported (e.g., Smith 1977, 1983). It is not clear if this is because oxygenated compounds are not present, or simply that they are not noted. Lu et al. (1975) reported that 2.4% of the neutral fraction of the oleoresin of *Pinus taiwanensis* Hayata was composed of oxygenated compounds, with *trans*-verbenol and verbenone prominent among these. Assuming that these oxygenated terpenes were not formed from autoxidation after the resin was collected, then the resin that exudes from wounded trees may contain significant quantities of *trans*-verbenol even before it is exposed to air. It should be noted that the α -pinene content in *P. taiwanensis* turpentine is approximately 73% (Mirov 1961), which is much higher than in hosts of *D. ponderosae* such as *P. contorta* and *P. ponderosa* (Smith 1977, 1983).

The autoxidation of α -pinene to *trans*- and *cis*-verbenol and verbenone at the levels that we have reported necessitates the re-evaluation of many experiments involving α -pinene. Many bark beetle species that use *trans*- or *cis*-verbenol as a pheromone have also been reported to use α -pinene as a kairomone. It is possible that for some species, the activity of α -pinene is partly or entirely due to the formation of verbenols or verbenone through autoxidation. The finding that *trans*-verbenol can substitute for α -pinene as a synergist for frontalin in attracting *D. frontalis* (Payne et al. 1978; Renwick and Vité 1969) could indicate that the activity of α -pinene for this beetle is due to the formation of *trans*-verbenol through autoxidation. Similarly, although α -pinene is thought to be a synergist for lineatin in attracting *T. lineatum*, both *cis*- and *trans*-verbenol also act as synergists (Klimetzek 1984). The observation that 1-day-old

oleoresin from *P. ponderosa* becomes ineffective as a synergist for *trans*-verbenol in attracting *D. ponderosae* (Billings et al. 1976) could be due to a buildup of verbenone through autoxidation of α -pinene in the resin. Certain phenomena, such as the increased incidence of bark beetle attack on lightning-struck trees (Coulson et al. 1983, 1986; Krawielitzki et al. 1983) and wounded trees, may be explained by α -pinene autoxidation. In addition, our detection of significant quantities of verbenone apparently produced by autoxidation in semiochemical baits containing *trans*-verbenol necessitates the re-evaluation of many field experiments involving *trans*-verbenol.

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