

PHEROMONES IN WHITE PINE CONE BEETLE,
Conophthorus coniperdu (SCHWARZ) (COLEOPTERA:
SCOLYTIDAE)

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Abstract—Female white pine cone beetles, *Conophthorus coniperda*, attacking second-year cones of eastern white pine, *Pinus strobus* L., produced a sex-specific pheromone that attracted conspecific males in laboratory bioassays and to field traps. Beetle response was enhanced by host monoterpenes. The female-produced compound was identified in volatiles collected on Porapak Q and in hindgut extracts as (+)-*trans*-pityol, (2*R*,5*S*)-(+)-2-(1-hydroxy-1-methylethyl)-5-methyltetrahydrofuran. Males and females produced and released the (*E*)-(-)-spiroacetal, (5*S*,7*S*)-(-)-7-methyl-1,6-dioxaspiro-[4.5]decane, which was not an attractant for either sex, but acted as a repellent

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for males. Porapak Q-trapped volatiles from both sexes contained (+)-*trans-pinocarveol* and (-)-*myrtenol*. In addition, **hindgut** extracts of females contained *trans-verbenol*, while males had pinocarvone and *verbenone*. Work in Georgia and Canada confirmed that the same isomers of **pityol** and spiroacetal are present in two distinct and widely separated populations of *C. coniperda*.

Key Words—*Conophthorus coniperda*, white pine cone beetle, Scolytidae, pheromone, pityol, 2-(1-hydroxy-1-methylethyl)-5-methyltetrahydrofuran, (*E*)-1-methyl-1,6-dioxaspiro[4.5]decane, spiroacetal, **chiral** analysis, walking bioassay, traps.

INTRODUCTION

The white pine cone beetle (WPCB), *Conophthorus coniperdu* (Schwarz) (Coleoptera: Scolytidae), occurs throughout the range of eastern white pine, *Pinus strobus* L., and is rarely found on other hosts (Wood, 1982). It is the most serious insect pest of eastern white pine cones in natural stands (Godwin and Odell, 1965; Hedlin et al., 1980) and seed orchards (DeBarr et al., 1982).

Semiochemicals are commonly used by scolytids for locating suitable hosts or mates (Borden, 1985). However, little is known about the responses of *Conophthorus* spp. to either pheromones or odors released by cones. Beetle-infested cones and their monoterpenes attracted male and female ponderosa pine cone beetles, *C. ponderosae* Hopkins, in laboratory bioassays (Kinzer et al., 1972; Kinzer and Reeves, 1976). However, Mattson et al. (1984) and Mattson and Strauss (1986) rejected the hypothesis of cone-directed alightment by the red pine cone beetle (RPCB), *C. resinosa* Hopkins, in response to cone-produced volatiles.

Recently, we reported evidence for a female-produced sex pheromone that attracts WPCB and RPCB males to their cone galleries (de Groot et al., 1991). Herein we describe the isolation and identification of both female- and male-produced compounds from airborne collections and **hindguts** of WPCB. Laboratory bioassays and field trapping were used to determine beetle response to host- and beetle-produced volatiles and to compounds identified as pheromone candidates. Volatiles from beetles and beetle-infested cones were analyzed independently in the United States and Canada to identify pheromone candidates. Analyses in Canada were also aimed at clarifying taxonomic relationships for three sympatric *Conophthorus* species (de Groot, 1992).

METHODS AND MATERIALS

Collection and Maintenance of Beetles. Eastern white pine cones containing overwintering WPCB were collected in February 1989 at the USDA Forest Service's Beech Creek seed orchard near Murphy, North Carolina. They were

kept in the dark at 5°C and 50–70% relative humidity until late March. Beetles were allowed to emerge naturally from the cones under a 16L: 8D photoperiod at 29°C and 70% relative humidity. They were sexed by examining the seventh and eighth abdominal tergites (Herdy, 1959) and held in darkness on moistened filter paper in Petri dishes at 4–5°C and 70% relative humidity.

WPCB were also collected during September and October 1988, near Chalk River, Ontario, and handled as described by de Groot et al. (1991). Materials and methods used in Canada are described in detail in Pierce et al. (1995).

Collection of Beetle and Host Volatiles. Fresh second-year cones (20–30 mm long) were picked from eastern white pines at Beech Creek, North Carolina; their petioles were sealed with melted paraffin, and they were stored at 5°C. Emerged WPCB were placed individually with one cone in a 10-ml plastic cup. To stimulate beetle attack, a basal cone scale was removed. Cones that were not successfully attacked within 2 hr were discarded. For cones with paired beetles, a male was added 24 hr after the female. After attacks were initiated, cones with beetles were placed in a small desiccator jar (600 ml; 10 cm ID). Charcoal-filtered air was introduced into the bottom of the desiccators and drawn over the attacked cones. Volatiles were collected on Porapak Q (Supelco, Inc. Bellefonte, Pennsylvania) columns (ID 50 mm \times 5 mm; 300 mg; 80–100 mesh) (Birgersson and Bergstrom, 1988; Dobson, 1991; Gries et al., 1988). In control aeration, undamaged cones were used to measure the turpentine background, while cones with a small hole (2 mm ID) drilled at the base were used to simulate beetle attack. Volatiles were collected for 24 hr at a flow rate of 500 ml/min. The Porapak Q columns were rinsed with 3 ml of redistilled diethyl ether (Baker p.a.) and the extracts were concentrated in 0.5-ml tip-vials prior to analysis.

To accumulate sufficient material for chiral analysis, several groups of ca. 100 single females attacking new cones were aerated for 24 hr, starting 24–48 hr after attack. The aeration extracts were combined and concentrated in a tip-vial to < 100 μ l. The concentrated extract was put on a short Sep-Pat (Millipore Corp., Bedford, Massachusetts) column filled with Florisil (Fisher Scientific Co., Pittsburgh, Pennsylvania) (8 mm \times 20 mm ID; 500 mg), and eluted with 3 ml pentane (redistilled) + 2 \times 1.5 ml pentane + 2 \times 1 ml diethyl ether + 0.5 ml ether (Figure 1). The last 0.5-ml ether fraction contained material sufficiently pure for chiral analysis.

Extracts of Beetle Hindguts. After the aerations, WPCB remaining in cones were excised and stored on Dry Ice. To analyze their volatiles, they were thawed, and pentane extracts were made of hindguts. Wings, elytra, and legs were removed from the degutted beetles, and a second pentane extract was made of the carcass. During dissection, the sex of the beetles was verified by the median lobe (de Groot, 1992) or spermatheca.

Synthetic Compounds. Syntheses of the racemate and the (5*S*,7*S*)-enantiomer of 7-methyl-1,6-dioxaspiro[4.5]decane followed the method of Jacobson

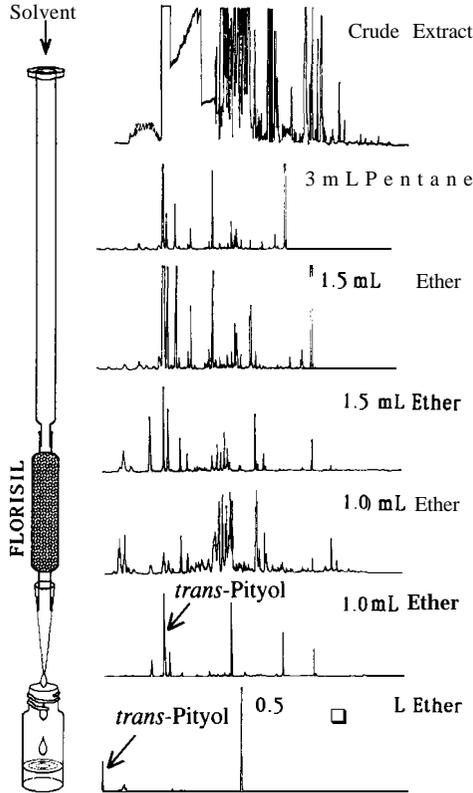


Fig. 1. Isolation of *trans*-pityol in Porapak Q-trapped volatiles from *C. coniperda* females in *P. strobus* cones.

et al. (1982). The enantiomers of 2-(1-hydroxy-1-methylethyl)-5-methyltetrahydrofuran (*trans*-pityol) were provided by K. Mori (for synthesis see Mori and Puapoomchareon, 1987). The (\pm)-*trans* and (+)-*trans*-pityol used in field bioassays were made by H.D. Pierce, Jr. (Pierce et al., 1995). The enantiomers of trans-pinocarveol were prepared from the enantiomers of β -pinene (Aldrich) according to Joshi (1968) by W. Francke; pinocarvone was obtained by oxidation of trans-pinocarveol. All other terpenes were commercially available (Aldrich, Milwaukee, Wisconsin).

Chemical Analyses. Porapak Q-trapped volatiles and hindgut extracts were analyzed on a Hewlett-Packard 5890-5970 coupled gas chromatograph and mass spectrometer (GC-MS). Each extract contained an internal standard of 250 ng heptyl acetate (C_7Ac) (Birgersson et al., 1984). The limit of reliable quantification was ca. 0.5 ng/beetle, and the limit of identification was <0.05 ng using

the extracted ion current profile (EICP). The fused silica capillary columns used were HP-1 (Hewlett-Packard Corp., Avondale, Pennsylvania) (25 m X 0.20 mm ID; 0.3 μm film thickness; temperature program: 50°C for 5 min then 8°/min to 200°C for 10 min) and HP-FFAP (Hewlett-Packard) (50 m X 0.20 mm; 0.3 μm film thickness; temperature program: 50°C for 5 min then 10°/min to 200°C for 10 min). In both columns the flow rate of the helium carrier gas was 25 cm/sec.

The absolute configuration of the spiroacetal in pentane extracts of male hindguts was determined on a glass capillary (40 m X 0.25 mm ID) coated with hexakis-per-O-hexyl- α -cyclodextrin (Tengö et al., 1990), programmed at 2°C/min from 50 to 100°C. The same column at 60°C was used to separate the enantiomers of trifluoroacetylated *trans*-pityol from aerations, while underivatized *trans*-pityol was resolved on octakis-(3-O-acetyl-2,6-di-O-pentyl)- γ -cyclodextrin at 80°C (Gregoire et al., 1992). Trifluoroacetates of monoterpene alcohols were separated on a glass capillary column (40 m X 0.25 mm ID) coated with heptakis-(3-O-methyl-2,6-di-O-pentyl)- β -cyclodextrin (König et al., 1989). Volatiles produced by *C. coniperda* collected in Chalk River, Ontario, were analyzed as described by Pierce et al. (1995).

Laboratory Bioassays of Beetle- and Host-Produced Volatiles and Candidate Pheromones. Responses by male and female WPCB to beetle- and host-produced volatiles were measured in laboratory bioassays (Payne et al., 1976; de Groot et al., 1991) at Athens, Georgia, during April 1989 and 1990, coinciding with emergence of feral WPCB at the Beech Creek seed orchard. For the bioassays, volatile concentrations were expressed as cone-minutes (CM) or beetle-minutes (BM) where 1 CM or BM is the quantity of volatiles collected during 1 min of aeration (Birgersson et al., 1995). Groups of five beetles of the same sex were exposed to diluted diethyl ether formulations containing 1 CM or BM/ μl equivalents dispensed from a 10- μl syringe at 1 $\mu\text{l}/\text{min}$ for 10 min. Beetles were placed onto the center of an 18.5-cm-diam. filter paper arena; positive responders were those that walked up the airstream to within 1 cm of the syringe tip. A minimum of 15 replications per sex were run per treatment in bioassay 1. Fewer replications were run for bioassays 2 and 3 because of low populations of feral beetles.

Bioassay 1 tested the responses of WPCB to: (1) control (air only); (2) diethyl ether; (3) diethyl ether extracts containing 10 CM of Porapak Q-captured volatiles from aerations of wounded uninfested cones; (4-6) diethyl ether extracts containing 10 BM of Porapak Q-captured volatiles, respectively, from males, females, or pairs of beetles feeding in cones; and (7 and 8) (*E*)-(*f*)-spiroacetal alone or in combination with volatiles from cones in 10 BM equivalents.

Bioassays 2 and 3 compared responses to candidate pheromones. Volatiles from aerations or synthetic chemicals were formulated in redistilled diethyl ether in 10 BM or 10 CM equivalents (Birgersson et al., 1995). In bioassay 2 the

effects of combining various beetle and host-produced compounds were compared in nine treatments (Table 1). In bioassay 3, the effects of excluding single components from four- or five-component mixtures were determined in 11 treatments (Table 1).

Field Experiments. Experiments 1-3 and 5 were conducted in the Beech Creek seed orchard and experiment 4 was in a natural white pine forest in Pancake Bay Provincial Park, 80 km north of Sault Ste. Marie, Ontario. Experiments 1 and 2 compared beetle catches in traps baited with: (1) *P. strobus*

TABLE 1. TREATMENTS TESTED FOR OLFACTORY RESPONSE BY MALE AND FEMALE *C. coniperda* TO COMBINATIONS (BIOASSAY 2) AND SINGLE DELETION (= SUBTRACTIVE; BIOASSAY 3) OF BEETLE OR HOST-PRODUCED VOLATILES IN WALKING BIOASSAYS, ATHENS, GEORGIA, 1990"

Treatment	Aeration of females in cones	Female compounds			Host volatiles			Male compound
		Pit ¹	Pit ²	tP Mt	CV	MT	BorAc	Spiroacetal
Bioassay 2								
CT	-	-	-	-	-	-	-	-
1	-	+	-	-	-	-	-	-
2	-	-	+	-	-	-	-	-
3	-	+	-	+	+	-	-	-
4	-	-	+	+	+	-	-	-
5			+	+	+	-	-	+
6			+	+	+	+	-	-
7	-	-	+	+	+	-	+	-
FV	+	-	-	-	-	-	-	-
Bioassay 3								
2			-	+	+	+	-	
3	-	-	+	+	-	+	-	-
4 (mixture 1)	-	-	+	+	+	+	-	-
5	-	-	+	+	+	-	+	-
6	-	-	+	+	+	-	-	+
7			-	+	+	-	+	+
8	-	-	+	-	+	-	+	+
9	-	-	+	+	-	-	+	+
10 (mixture 2)	-	-	+	+	+	-	+	+
FV	+	-	-	-	-	-	-	-

"Pit¹ = (\pm)-*trans*-pityol; Pit² = (+)-*trans*-pityol; tP = (+)-*trans*-pinocarveol; Mt = (-)-myrtenol; CV = volatiles from aerations of cones; MT = synthetic monoterpene hydrocarbons (mixture of α -pinene, β -pinene, myrcene, limonene 1: 1: 1; Aldrich); BorAc = bomyl acetate (Aldrich); Spiroacetal = (*E*)-(\pm)-7-methyl-1,6-dioxaspiro[4.5]-decane; CT = control (air only); FV = volatiles from aerations of cones infested with females.

cones that were artificially wounded; (2 and 3) cones infested with male or female WPCB (de Groot et al., 1991); (4 and 6) Porapak Q-trapped volatiles from wounded cones or cones infested with male or female beetles; or (7 and 8) (*E*)-(±)-spiroacetal alone or in combination with cone volatiles. Experiments 3 and 4 compared catches for (±)-*trans*-pityol, (+)-*trans*-pityol, and WP cone oils alone or in combinations. The volatile oil of *P. strobus* cones (WP cone oil) was prepared as described in Pierce et al. (1995) and dispensed from 2-ml polypropylene Eppendorf tubes (Brinkman Instruments, Rexdall, Ontario). No baits were placed in "unbaited" traps in Experiments 3 and 4. Experiment 5 compared catches to (±)-*trans*-pityol and *cr*-pinene with and without (*E*)-(±)-spiroacetal.

Plexiglas barrier traps (de Groot et al., 1991) were used in experiment 1, 12-unit multiple-funnel traps (Lindgren, 1983) were used in experiment 2, and yellow Japanese beetle trap tops (Trécé Inc., Salinas, California) fitted with plastic Mason jar bottoms containing 25 ml of propylene glycol were used in experiments 3-5. Traps were hung ca. 10-15 m high in the upper third of the tree crown, one trap per tree, and were spaced 12-30 m apart. For experiments 1-3 and 5, treatment locations were randomized at weekly intervals. For all experiments, beetles were removed from the traps weekly and the traps were rebaited. Captured beetles were preserved in 70% alcohol, identified, counted, and the sex determined.

Screw-cap glass vials (2 ml) served as releasers for volatiles in experiments 1, 2, and 5. Cotton wicks pulled through 5-cm lengths of 1.6-mm-ID Teflon tubing were inserted through holes drilled in the plastic caps. For experiment 1, a wire was used to suspend a vial in the center of a 10-cm hole cut in the Plexiglas vanes of each barrier trap. For experiment 2, a spring clip was used to attach a vial to one of the plastic rods between funnels 6 and 7 on the multiple-funnel traps. For experiment 5, the vial was clipped to one of the vanes of the Japanese beetle trap. Each vial contained volatiles formulated at a final volume of 2 ml of *n*-octane (Aldrich) and released at a rate of 2 ml/week at $25 \pm 2^\circ\text{C}$ in the laboratory. Volatiles collected on Porapak Q were eluted with redistilled diethyl ether and formulated in *n*-octane to release 20 cone equivalents (CE), female equivalents (FE), or male equivalents (ME), where 1 CE, FE, or ME is the quantity of volatiles collected during aerations of one cone or beetle-infested cone for 1 hr (Birgersson et al., 1995). Capillary tubes (1.04 mm ID) were used to dispense 3 μl neat pityol or spiroacetal in experiments 3 and 4. The release rate was 4.2 mg of pityol/week at 24°C in the laboratory.

Data Analyses. Percent response data from the bioassays were analyzed by the GLM procedure and count data from the field experiments (except experiment 5) were analyzed using the RANK and GLM procedures (SAS Institute, 1987). A completely randomized, factorial design was used for the pedestrian bioassays. Factor one, with two levels, was gender of the beetles bioassayed

for olfactory response. Factor two was mixtures of volatiles offered, with levels referred to as treatments. The treatments differed for each bioassay experiment and were various combinations of host-, female-, and male-produced volatiles. Within each experiment these treatments had a structure planned to provide tests of specific hypotheses, so all possible **pairwise** comparisons of treatments was not a goal. Within each experiment, treatment and gender main effects, and treatment-gender interaction were tested at a 95 % confidence level ($\alpha = 0.05$). In order to maintain this experiment-wise (α_E) confidence level and perform a group of planned, nonorthogonal contrasts of treatment-gender interaction effects using multiple *t* tests, Šidák's inequality was used to set a comparison-wise (α_C) confidence level used to declare significance of any specific comparison (Šidák, 1967; Games, 1977; Jones, 1984). Šidák's inequality relates α_E and α_C as $\alpha_C \leq 1 - (1 - \alpha_E)^{1/n}$, which sets an upper bound on the comparisonwise α_C for *n*, possibly dependent, comparisons that should be used to declare significance of any single comparison in a group and still maintain the experimentwise probability of a type-1 error. In the five field experiments, traps were deployed in a randomized block design. Two response variables were analyzed separately. One response was the number of males caught in baited traps, the other was the number of females caught. In these experiments, planned treatment contrasts were made using *t* tests with significance levels controlled with Šidák's inequality.

RESULTS

Volatiles from Attacked Cones. All *P. strobus* cones with or without *C. coniperda* released large amounts of host terpenes (Figure 2A). Attacked cones released the four major monoterpene hydrocarbons α -pinene, β -pinene, myrcene, and limonene (Mirov, 1961) and the oxygenated monoterpene bomyl acetate.

Both female and male beetles released (+)-*trans*-pinocarveol and (-)-myrtenol (Figure 2B and C). Volatiles from feeding males contained a beetle-produced peak (Figure 2C) with a mass spectrum dominated by two large fragments, *m/z* 84 and 87, and a molecular ion at *m/z* 156. The compound was identified as 7-methyl-1,6-dioxaspiro[4.5]decane by comparison of the mass spectral data to that reported by Francke et al. (1979b). This spiroacetal has been named MDOS by Kohnle et al. (1992) and conophthorin by de Groot (1992), after the genus. Analysis of Porapak Q-trapped volatiles from females feeding in second-year cones in Canada also revealed presence of the (*E*)-spiroacetal (96% ee) (Figure 3). After the female-specific compound **pityol** had been found in the **hindgut** extracts (see following), a reexamination of the Porapak Q-trapped volatiles using an extracted ion current profile (EICP) (Garland

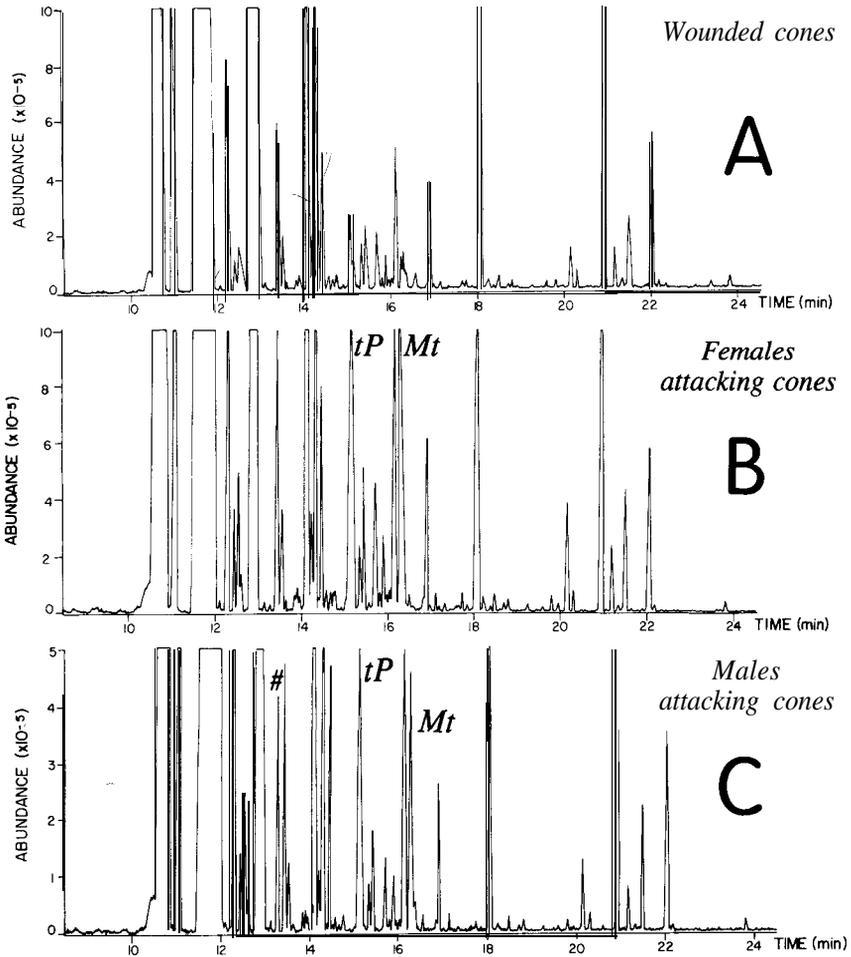


Fig. 2. Chromatograms from CC-MS analyses of Porapak Q-collected volatiles of *C. coniperda* attacking *P. strobus* cones in Georgia; fused silica column coated with HP-1. (A) Wounded cones, (B) single females in cones, (C) single males in cones. *tP* = *trans*-pinocarveol, *Mt* = myrtenol, # = spiroacetal.

and Powell, 1981) of *m/z* 59 revealed that it coeluted with, and was totally hidden by β -pinene in all aerations of female-infested cones (Figure 2B).

Analyses of Hindgut Extracts. In addition to the compounds identified in the aerations, analyses of hindguts showed that females produced *trans*-verbenol (Figure 4A) and males produced pinocarvone and verbenone (Figure 4B). Females also produced a female-specific compound (Figure 4A), with a mass

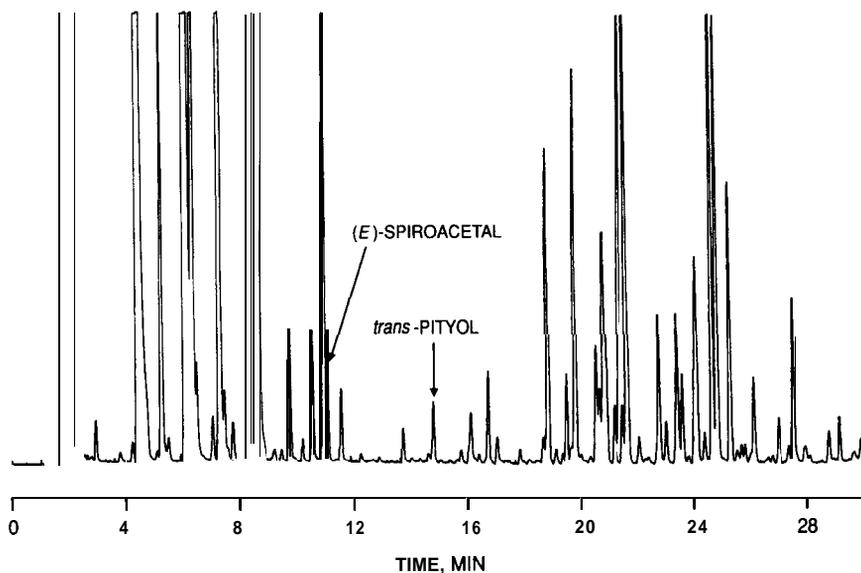


Fig. 3. Chromatogram of Porapak Q-collected volatiles of female *C. coniperda* attacking *P. strobus* cones in Ontario; glass capillary column coated with SP-1000 (30 m x 0.5 mm ID).

spectrum having a base peak of m/z 59 and a molecular ion at m/z 144, indicating a tertiary alcohol with the formula of either $C_8H_{16}O_2$ or $C_9H_{20}O$. The compound was identified as 2-(1-hydroxy-1-methylethyl)-5-methyltetrahydrofuran, first described as a pheromone of a *Pityophthorus* spp. and named **pityol** by Francke et al. (1987). Chiral analysis revealed the female-specific **pityol** to be the **2R,5S-isomer**, and 1% of a *cis* isomer was also present. The same isomer of **pityol** was also present in the Porapak Q-captured volatiles of feeding *C. coniperdu* from Canada (Figure 3).

The chromatogram for **hindguts** from males was dominated by a large peak of a spiroacetal. A smaller peak, with almost the same mass spectrum, eluted $1\frac{1}{2}$ min later (Figure 4B). Chiral analysis determined that the major peak was (**5S,7S**)-7-methyl-1,6-dioxaspiro[4.5]decane, and the minor peak, the **5R,7S**-isomer. Traces of other spiroacetals were detected, but not identified. **Pityol** was never detected in any male **hindgut** sample. No additional host- or beetle-produced compounds were found in pentane extracts of degutted beetles, besides those identified in **hindgut** extracts or aerations. Pentane extracts of both female and male *C. coniperdu* from Canada also contained both *E* and *Z* isomers of the spiroacetal (Figure 5).

Laboratory Bioassays. WPCB adults showed strong responses to Porapak

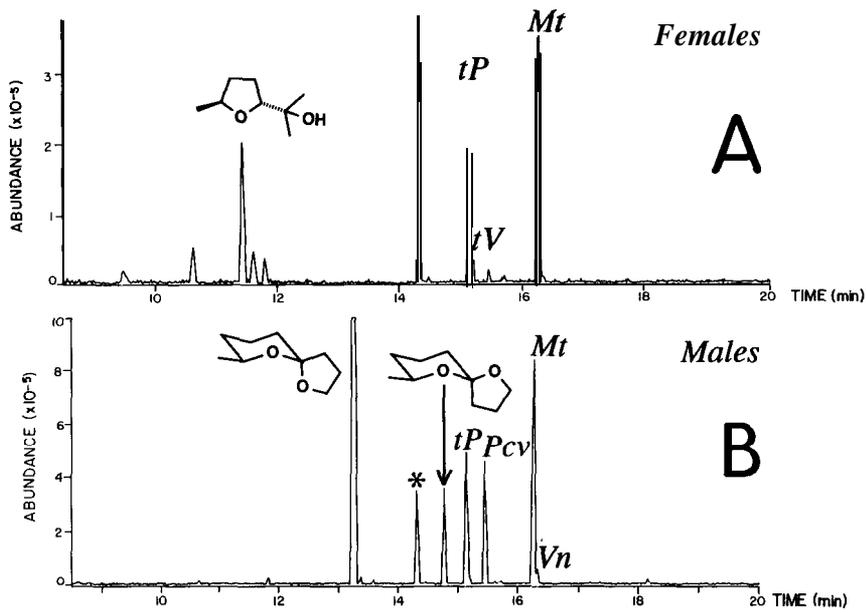


Fig. 4. Chromatograms from CC-MS analyses of volatiles of hindguts of *C. coniperda* attacking *P. strobus* cones in Georgia; fused silica column coated with HP-I. (A) Pityol from seventeen single females in cones 40 hr, (B) spiroacetal from 15 single males in cones 40 hr. *tP* = *trans*-pinocarveol; *tV* = *trans*-verbenol; *Mt* = myrtenol; *Vn* = verbenone; *Pcv* = pinocarvone; * 250 ng *C*₇Ac.

Q-trapped volatiles from infested cones in the walking bioassay (Figure 6, Table 2). Males responded more often than females. Significant response differences occurred among treatments ($F = 67.1$; $df = 7, 377$; $P = 0.0001$), between sexes ($F = 7.96$; $df = 1, 377$; $P = 0.0001$), and for the treatment-by-sex interaction ($F = 24.75$; $df = 7, 337$; $P = 0.0001$).

Only 4% of the beetles responded positively to air alone (Figure 6); adding ether to the airstream did not significantly increase the response (Table 2). However, both sexes responded significantly to Porapak Q-trapped volatiles containing host- and/or beetle-produced compounds, and males responded positively to cone volatiles. Significantly more beetles of both sexes responded to volatiles from males in cones than to cones alone. Over 90% of the test males responded to volatiles from cones with females, but females did not respond to this stimulus better than to cones alone. Females, but not males, responded significantly more to volatiles from cones containing pairs of beetles than to volatiles from cones containing either sex. Neither sex showed a significantly greater response to a combination of cone volatiles and spiroacetal than to cone

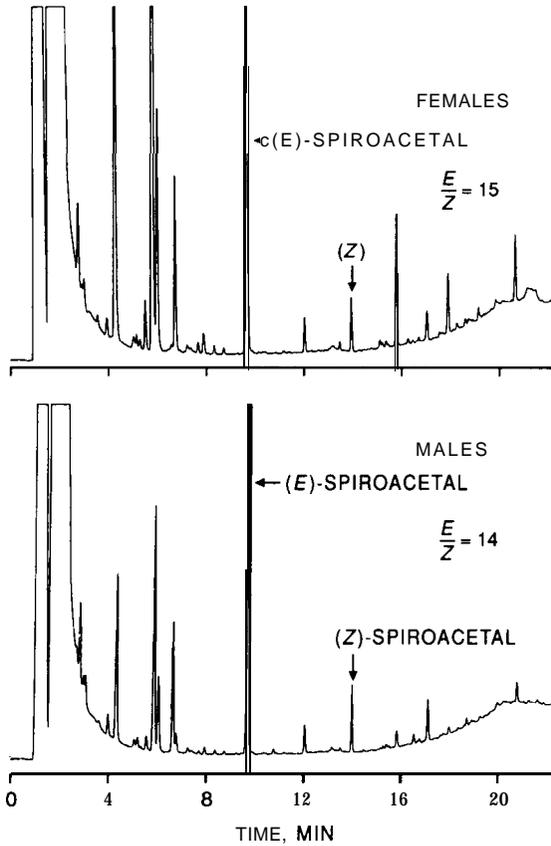


Fig. 5. Chromatograms of volatiles in pentane extracts of *C. coniperda*, excised from *P. strobus* cones in Ontario; glass capillary column coated with SP-1000.

volatiles or the spiroacetal alone. However, male response to a mixture of spiroacetal and cones volatiles was significantly less than their response to volatiles from male-infested cones.

Synthetic beetle and host-produced compounds also elicited strong positive responses by WPCB (Tables 3 and 4). Again, males responded more frequently than females. Responses differed significantly among treatments (bioassay 2: $F = 31.8$; $df = 8, 117$; $P = 0.0001$; bioassay 3: $F = 6.21$; $df = 10, 77$; $P = 0.001$) and between sexes ($F = 11.5$; $df = 1, 117$; $P = 0.0001$; bioassay 3: $F = 48.4$; $df = 1, 77$; $P = 0.0001$). Treatment-by-sex interactions were also significant (bioassay 2: $F = 5.46$; $df = 1, 117$; $P = 0.0001$; bioassay 3: $F = 2.96$; $df = 9, 77$; $P = 0.0045$).

Beetle responses to pityol alone or in combination with two other female-

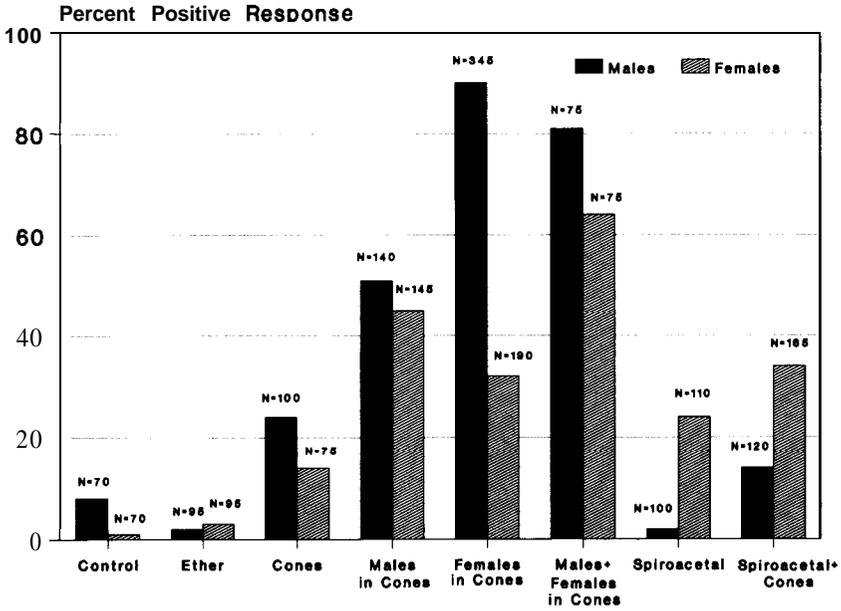


Fig. 6. Responses by female and male *C. coniperda* to volatiles in laboratory bioassay 1.

produced compounds, trans-pinocarveol and myrtenol, ranged from 18 to 33% (Table 3). Comparisons for the (±)-trans-pityol vs. control; (+)-trans-pityol vs. (f)-trans-pityol; and the (+)-trans-pityol, trans-pinocarveol, and myrtenol mixture vs. (+)-trans-pityol were not significant for either sex. Male response to (E)-(±)-spiroacetal plus the three-component mixture, was only 8%, while 33% responded to the mixture alone, but the differences were not significant for either sex.

Combining volatiles from cones with the three female compounds significantly increased male response to 91%, but did not increase female response (Table 3). Male response to a mixture of four host monoterpenes and the three female compounds was equal to their response to cone volatiles plus the three female compounds or Porapak Q-trapped volatiles from female-infested cones. In contrast, the female response of 82% to volatiles from females in cones was significantly greater than their 23% response to the three female compounds and monoterpenes.

Female responses were not significantly different for any component deletion from the complex mixtures of beetle- and host-produced compounds (Table 4). Male response was 91% for mixture 1 containing the three female-produced compounds plus cone volatiles and 81% for mixture 2 containing the three

TABLE 2. RESPONSE BY FEMALE AND MALE *C. coniperda* TO PORAPAK Q-TRAPPED VOLATILES FROM *P. strobus* CONES, CONES WITH BEETLES, AND (*E*)-(±)-SPIROACETAL IN WALKING BIOASSAY 1, ATHENS, GEORGIA 1989

Single df comparisons:	Females (%)			Males (%)		
	N	$\bar{X} \pm SE$	<i>P</i>	N	$\bar{X} \pm SE$	<i>P</i>
Air vs.	70	1 ± 1	0.8197	70	8 ± 8	0.4714 NS
ether	95	3 ± 2		95	2 ± 2	
All treatments except control vs.	760	36 ± 5	0.0001 ^b	880	44 ± 3	0.0001 ^b
ether	95	3 ± 2		95	2 ± 2	
Cones vs.	75	14 ± 3	0.1584 NS	100	24 ± 5	0.0018 ^b
ether	95	3 ± 2		95	2 ± 2	
Males in cones vs.	145	45 ± 4	0.0001 ^b	140	51 ± 3	0.0001 ^b
cones	75	14 ± 3		100	24 ± 5	
Females in cones vs.	190	32 ± 5	0.0052 NS	345	90 ± 2	0.0001 ^b
cones	75	14 ± 3		100	24 ± 5	
Males + females in cones vs.	75	64 ± 8	0.0001 ^b	75	81 ± 5	0.0762 NS
males or females in cones	335	39 ± 5		485	71 ± 3	
Cones + (<i>E</i>)-(±)-spiroacetal vs.	165	34 ± 5	0.0030	120	14 ± 3	0.1182 NS
cones	75	14 ± 3		100	24 ± 5	
Cones + (<i>E</i>)-(±)-spiroacetal vs.	165	34 ± 5	0.1177	120	14 ± 3	0.0774 NS
(<i>E</i>)-(±)-spiroacetal	110	24 ± 7		100	2 ± 1	
Cones + (<i>E</i>)-(±)-spiroacetal vs.	165	34 ± 5	0.0449 NS	120	14 ± 3	0.0001 ^b
males in cones	145	45 ± 4		140	51 ± 3	

^aNS = not significant.

^b $P \leq 0.0028$ is required for an experiment-wise error rate of $\alpha = 0.05$ (Šidák, 1967; Games, 1977) for each of the 18 comparisons.

female-produced compounds plus monoterpenes and bomyl acetate. These responses were significantly greater than to pityol alone. However, when pityol was removed from either mixture 1 or mixture 2, male response was significantly decreased to only 20%. Substituting bomyl acetate for the four monoterpene hydrocarbons significantly reduced male response.

Field Experiments. Catches of females in traps baited with beetle-infested cones or Porapak Q-trapped volatiles of infested cones were no greater than to uninfested cones, but significantly more males were attracted to female-infested cones than to uninfested cones or male-infested cones (Table 5, experiment 1). However, in experiment 2, more beetles of both sexes were both attracted to Porapak Q-trapped volatiles from female-infested cones than to volatiles from male-infested cones. The (*E*)-(±)-spiroacetal was not an attractant for either sex.

Females were not attracted to pityol in experiment 3, but in experiment 4 significantly more females were caught in traps baited with (±)-*trans*-pityol

TABLE 3. RESPONSE BY FEMALE AND MALE *C. coniperda* TO VARIOUS COMBINATIONS OF BEETLE- AND HOST-PRODUCED VOLATILES IN WALKING BIOASSAY 2, ATHENS, GEORGIA 1990

Single & comparisons	Females (%)			Males (%)		
	N	$\bar{X} \pm SE$	P	N	$\bar{X} \pm SE$	P
(±)- <i>trans</i> -Pityol vs. control ^a				29	22 ± 9	0.1237 NS ^b
(+)- <i>trans</i> -Pityol vs. (±)- <i>trans</i> -pityol	29	18 ± 9	0.5659 NS ^b	13	0 ± 0	0.8429 NS
(+)- <i>trans</i> -Pityol + warts-pinocarveol + myrtenol vs. (+)- <i>trans</i> -pityol	15	27 ± 18		28	24 ± 8	
(±)- <i>trans</i> -Pityol + <i>trans</i> -pinocarveol + myrtenol vs. (+)- <i>trans</i> -pityol + <i>trans</i> -pinocarveol + myrtenol	24	25 ± 7	0.9645 NS	29	22 ± 9	0.4347 NS
(+)- <i>trans</i> -Pityol + warts-pinocarveol + myrtenol + (<i>E</i>)-(±)-spiroacetal vs. (+)- <i>trans</i> -pityol + <i>trans</i> -pinocarveol + myrtenol	24	26 ± 9	0.9386 NS	26	33 ± 8	0.8328 NS
(+)- <i>trans</i> -Pityol + <i>trans</i> -pinocarveol + myrtenol + cone volatiles ^c vs. (+)- <i>trans</i> -pityol + <i>trans</i> -pinocarveol + myrtenol	25	28 ± 10	0.8172 NS	28	24 ± 8	
(+)- <i>trans</i> -Pityol + warts-pinocarveol + myrtenol + monoterpene ^c vs. (+)- <i>trans</i> -pityol + <i>trans</i> -pinocarveol + myrtenol + cone volatiles	24	25 ± 7	0.0344 NS	29	31 ± 8	0.0001 ^b
(+)- <i>trans</i> -Pityol + <i>trans</i> -pinocarveol + myrtenol + monoterpene ^c vs. (+)- <i>trans</i> -pityol	41	49 ± II	0.0093 NS	26	33 ± 8	0.4532 NS
(+)- <i>trans</i> -Pityol + <i>trans</i> -pinocarveol + myrtenol + monoterpene ^c vs. (+)- <i>trans</i> -pityol	24	25 ± 7	0.0047 NS	36	84 ± 5	0.0001 ^b
(+)- <i>trans</i> -Pityol + <i>trans</i> -pinocarveol + myrtenol + monoterpene ^c vs. volatiles from <i>C. coniperda</i> females in cones ^f	37	23 ± 12	0.0001 ^d	50	91 ± 5	0.1417 NS
	29	18 ± 9		36	84 ± 5	
	37	23 ± 12		28	24 ± 8	
	51	82 ± 7		36	84 ± 5	
				124	96 ± 2	

^aControl = air only; not tested for females.

^bNS = not significant.

^cVolatiles from aerations of cones.

^d $P \leq 0.003$ is required for an experiment-wise error rate of $\alpha = 0.05$ (Šidák, 1967; Games, 1977) for each of the 17 comparisons.

^eFour major monoterpenes from *P. strobus* cones (α -pinene, β -pinene, myrcene, limonene I : I : I : I).

^fVolatiles from aerations of cones infested with females.

TABLE 4. RESPONSE BY FEMALE AND MALE *C. coniperda* TO SINGLE DELETIONS FROM MIXTURES OF BEETLE OR HOST-PRODUCED VOLATILES IN BIOASSAY 3, ATHENS, GEORGIA 1990

Single <i>df</i> comparisons	Females (%)			Males (%)		
	N	$\bar{X} \pm SE$	P	N	$\bar{X} \pm SE$	P
Mixture 1 ^a – <i>trans</i> -Pityol vs. mixture 1	13 41	23 ± 2 49 ± 11	0.0878 NS ^b	10 50	20 ± 0 91 ± 5	0.0001 ^c
Mixture 1 – <i>trans</i> -pinocarveol vs. mixture 1	10 41	10 ± 10 49 ± 11	0.0288 NS	10 50	70 ± 3 91 ± 5	0.2335 NS
Mixture 1 – myrtenol vs. mixture 1	24 41	13 ± 8 49 ± 11	0.0051 NS	24 50	100 ± 0 91 ± 5	0.4591 NS
Mixture 2 ^d – bomyl acetate vs. mixture 1	37 41	23 ± 12 49 ± 11	0.0192 NS	36 50	84 ± 5 91 ± 5	0.4984 NS
Mixture 2 – monoterpenes vs. mixture 2 – bomyl acetate	19 37	5 ± 5 23 ± 12	0.1952 NS	23 36	38 ± 10 84 ± 5	0.0007 ^e
Mixture 2 – monoterpenes vs. mixture 2	19 19	5 ± 5 38 ± 18	0.0459 NS	23 23	38 ± 10 81 ± 15	0.0035 NS
Mixture 2 – <i>trans</i> -pityol vs. mixture 2	9 19	25 ± 25 38 ± 18	0.5259 NS	9 23	20 ± 20 81 ± 15	0.0019
Mixture 2 – <i>trans</i> -pinocarveol vs. mixture 2	13 19	30 ± 15 38 ± 18	0.6659 NS	10 23	100 ± 0 81 ± 15	0.2543 NS
Mixture 2 – myrtenol vs. mixture 2	8 19	43 ± 23 38 ± 18	0.7670 NS	10 23	50 ± 10 81 ± 15	0.1060 NS
Mixture 2 – bomyl acetate vs. mixture 2	37 19	23 ± 12 38 ± 18	0.3033 NS	36 23	84 ± 5 81 ± 15	0.8319 NS
Mixture 2 vs. volatiles from females in cones ^f				36 52	81 ± 15 100 ± 0	0.0645 NS

^aMixture 1 = *trans*-pityol + *trans*-pinocarveol + myrtenol + cone volatiles.

^bNS = not significant.

^c $P \leq 0.0024$ is required for an experiment-wise error rate of $\alpha = 0.05$ (Šidak, 1967; Games, 1977) for each of the 21 comparisons.

^dMixture 2 = *trans*-pityol + *trans*-pinocarveol + myrtenol + monoterpenes + bomyl acetate.

^eVolatiles from aeration of cones infested with females.

TABLE 5. MEAN NUMBERS OF *C. coniperda* CAPTURED IN TRAPS BAITED WITH CONES, BEETLE-INFESTED CONES, PORAPAK Q-TRAPPED HOST- OR BEETLE-PRODUCED VOLATILES OR SPIROACETAL (EXPERIMENT 1 + EXPERIMENT 2) AT USFS BEECH CREEK SEED ORCHARD, NORTH CAROLINA

Single <i>df</i> treatment comparisons	Number of beetles captured/trap ($\bar{X} \pm SE$) ^a			
	Experiment 1		Experiment 2	
	Females	Males	Females	Males
Treatments with beetle-produced compounds vs. cones ^b	2.5 ± 0.8 ^{NS}	3.0 ± 1.2 _d		
	1.4 ± 0.5	0.5 ± 0.2		
Males in cones' vs. cones	2.6 ± 0.9 ^{NS}	1.3 ± 0.6 ^{NS}		
	1.4 ± 0.5	0.5 ± 0.2		
Females in cones'' vs. cones	4.4 ± 1.3 ^{NS}	7.4 ± 2.9 _d		
	1.4 ± 0.5	0.5 ± 0.2		
Females in cones vs. males in cones	4.4 ± 1.3 ^{NS}	7.4 ± 2.9 _d		
	2.6 ± 0.9	1.3 ± 0.6		
Females + cone volatiles' vs. females in cones	1.9 ± 0.6 ^{NS}	3.8 ± 1.5 ^{NS}		
	4.4 ± 1.3	7.4 ± 2.9		
Males + cone volatiles' vs. males in cones	2.5 ± 0.9 ^{NS}	1.4 ± 0.5 ^{NS}		
	2.6 ± 0.9	1.3 ± 0.6		
Female + cone volatiles vs. male + cone volatiles	2.9 ± 0.9 ^{NS}	3.8 ± 1.5 ^{NS}	5.9 ± 4.3 _d	3.9 ± 2.3 _d
		1.4 ± 0.5	0.8 ± 0.5	0.3 ± 0.2
(<i>E</i>)-(±)-spiroacetal ^c vs. male + cone volatiles	1.0 ± 0.3 ^{NS}	1.1 ± 0.3 ^{NS}	0.5 ± 0.2 ^{NS}	0.4 ± 0.2 ^{NS}
	2.5 ± 0.9	1.4 ± 0.5	0.8 ± 0.5	0.3 ± 0.2
Treatment <i>F</i> test	4.92	8.70	50.1	20.01
<i>P</i>	0.0073	0.0005	0.0194	0.0062
<i>df</i>	5, 15	5, 15	2, 5	2, 5

^aSix Plexiglas barrier traps/treatment in experiment 1, Mar. 24-May 24, 1989; three 12-unit multiple-funnel traps/treatment in experiment 2. Apr. 19-May 24, 1989.

^bFive mechanically wounded *P. strobus* cones/trap.

^cNS = not significant.

^d*P* ≤ 0.0064 is required in experiment 1 for an experiment-wise error rate of α = 0.05 (Šidák, 1967; Games, 1977) for each of the two comparisons and *P* ≤ 0.025 is required in experiment 2 for each of the two comparisons.

^eFive *P. strobus* cones/trap, each with one male or female beetle.

^f20 Male or female beetle equivalents + 20 cone equivalents of volatiles/trap/hr.

^g10 ME of (*E*)-(±)-spiroacetal/trap/hr. (Birgersson et al., 1995).

combined with WP cone oil than in those with (±)-*trans*-pityol alone or (+)-*trans*-pityol with WP cones oil (Table 6). Males were attracted to (±)-*trans*-pityol or (+)-*trans*-pityol alone in experiment 4, but only to pityol combined with WP cone oil in experiment 3. Males did not discriminate between (+)-*trans*-pityol and (±)-*trans*-pityol in either experiment. The addition of *E*-(±)-

TABLE 6. MEAN NUMBERS OF *C. coniperda* CAPTURED IN BARRIER TRAPS BAITED WITH (+)-*trans*-PITYOL, (±)-*trans*-PITYOL OR *P. strobus* CONE OIL (WPCO) (EXPERIMENTS 3 + 4) AT BEECH CREEK SEED ORCHARD, NORTH CAROLINA, AND PANCAKE BAY PROVINCIAL PARK, ONTARIO

Single <i>df</i> treatment comparisons	Number of beetles captured/trap ($\bar{X} \pm SE$) ^a			
	Exp. 3 Beech Creek		Exp. 4 Pancake Bay	
	Female?	Males	Females	Males
(±)- <i>trans</i> -Pityol ^c vs. unbaited traps	0.0 ± 0.0	1.7 ± 0.8 ^{NSd}	0.1 ± 0.1 ^{NS}	5.9 ± 1.7,
(+)- <i>trans</i> -Pityol vs. unbaited traps	0.0 ± 0.0	0.2 ± 0.2 ^{NS}	0.1 ± 0.1 ^{NS}	6.1 ± 1.8,
(+)- <i>trans</i> -Pityol vs. (±)- <i>trans</i> -pityol	0.0 ± 0.0	0.0 ± 0.0 ^{NS}	0.0 ± 0.0 ^{NS}	0.0 ± 0.0
WP cone oil vs. unbaited traps	0.0 ± 0.0	0.2 ± 0.2 ^{NS}	0.1 ± 0.1 ^{NS}	6.1 ± 1.8 ^{NS}
(±)- <i>trans</i> -Pityol + WPCO ^e vs. (±)- <i>trans</i> -pityol	0.0 ± 0.0	1.7 ± 0.8 ^{NS}	0.1 ± 0.1 ^{NS}	5.9 ± 2.3 ^{NS}
(+)- <i>trans</i> -Pityol + WPCO vs. (+)- <i>trans</i> -pityol	0.2 ± 0.2	0.0 ± 0.0 ^{NS}	0.0 ± 0.0 ^{NS}	0.1 ± 0.1 ^{NS}
(+)- <i>trans</i> -Pityol + WPCO vs. (±)- <i>trans</i> -pityol + WPCO	0.0 ± 0.0	0.0 ± 0.0 ^{NS}	0.0 ± 0.0 ^{NS}	0.0 ± 0.0 ^{NS}
(±)- <i>trans</i> -Pityol + WPCO vs. unbaited traps	0.3 ± 0.2	4.5 ± 1.7,	0.4 ± 0.2,	10.1 ± 2.0 ^{NS}
Treatment <i>F</i> test	0.0 ± 0.0	1.7 ± 0.8,	0.1 ± 0.1,	5.9 ± 2.3 ^{NS}
<i>P</i>	0.3 ± 0.2	6.3 ± 2.7,	0.1 ± 0.1 ^{NS}	8.4 ± 2.1 ^{NS}
<i>df</i>	0.0 ± 0.0	0.2 ± 0.2	0.1 ± 0.1	6.1 ± 2.2 ^{NS}
	0.3 ± 0.2	6.3 ± 2.7 ^{NS}	0.1 ± 0.1 ^e	8.4 ± 2.1 ^{NS}
	0.3 ± 0.2	4.5 ± 1.7 ^{NS}	0.4 ± 0.2	10.1 ± 2.2 ^{NS}
	0.3 ± 0.2	4.5 ± 1.7,	0.4 ± 0.2,	10.1 ± 2.0,
	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	1.20	11.6	4.05	29.2
	0.3386	0.0001	0.0040	0.0001
	5, 25	5, 25	5, 45	5, 45

^aSix Japanese beetle traps/treatment at Beech Creek Apr. 23-May 10, 1990; 10 traps/treatment at Pancake Bay May 28-Jun. 4, 1990.

^b*F* test for treatments not significant.

^cPityol/trap 4.2 mg week from 1.04 mm ID capillary tube.

^dNS =not significant.

^e $P \leq 0.00639$ is required for an experimentwise error rate of $\alpha = 0.05$ (Šidák, 1967; Games, 1977) for each of the eight comparisons.

^fVolatile oil. 2 ml/week of *P. strobus* cones (Pierce et al., 1995).

spiroacetal to baits containing (±)-*trans*-pityol and a-pinene significantly reduced the trap catch of males but not females in experiment 5 (Table 7).

DISCUSSION

Identification of Pityol and Spiroacetal. The discovery of (+)-*trans*-pityol (1% *cis*) in female WPCB (Figure 7) constitutes the first record of occurrence in this genus. Pityol was first identified in, and named after, the European

TABLE 7. MEAN NUMBERS OF *C. coniperda* CAPTURED IN TRAPS BAITED WITH (\pm)-*trans*-PITYOL AND α -PINENE, AND (\pm)-*trans*-PITYOL, α -PINENE, AND (*E*)-(\pm)-SPIROACETAL AT BEECH CREEK SEED ORCHARD, NORTH CAROLINA

Treatment ^a	Number of beetles captured/trap ($\bar{X} \pm SE$)*	
	Females	Males
(\pm)- <i>trans</i> -Pityol and α -pinene	20.2 \pm 4.1 a	46.2 \pm 10.3 a
(\pm)- <i>trans</i> -Pityol, α -pinene and (<i>E</i>)-(\pm)-spiroacetal	20.0 \pm 5.2 a	12.2 \pm 3.1 b

^a(\pm)-*trans*-Pityol released at 100 FE/hr (0.7 mg/week), (\pm)- α -pinene at 100 mg/week and (*E*)-(\pm)-spiroacetal at 100 ME/hr (8 mg/week) (Birgersson et al., 1995).

^bSix Japanese beetle traps/treatment from April 30-June 3, 1990; means within a column followed by the same letter are not significantly different at $P < 0.05$; paired *t* test.

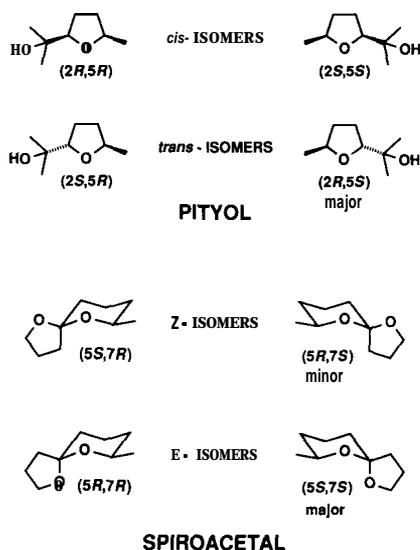


Fig. 7. Stereoisomers of *Conophthorus* pheromones

species, *Pityophthorus pityographus*, which also contained grandisol (Francke et al., 1987). Klimetzek et al. (1989) isolated *cis*-pityol from the elm bark beetle, *Pteleobius vittatus*, which also produced 3-methyl-3-buten-2-ol and *cis*-vittatol (*cis*-3-hydroxy-2,2,6-trimethyltetrahydropyran). In field tests, the combination of three compounds was far superior to any single compound or two-component mixture. The occurrence of pityol in *Conophthorus* in North America, and

Pityophthorus in Europe supports the hypothesis that these are closely related genera (Wood, 1982).

The spiroacetal produced by WPCB (Figure 7) was previously identified in abdominal glands of social wasps (Francke et al., 1979a). It also occurs in males of *Leperisinus vurius* (F.) and in frass of the fir beetle, *Cryphalus piceae*, where it acts as a strong inhibitor of aggregation for these two bark beetle species (Kohnle et al., 1992).

The first spiroacetal identified as a bark beetle-produced compound was isolated from *Pityogenes chalcographus* (Francke et al., 1977). This compound, chalcogran (2-ethyl-1,6-dioxaspiro[4.4]nonane), is weakly active by itself and is strongly synergized by methyl 2(*E*),4(*Z*)-decadienoate (Byers et al., 1988). Several alkyl-1,6-dioxaspiro[4.5]decanes occur in the social wasps, *Puruvespulu vulgaris* L., *P. germanica* (F.), and *Dolichovespulu saxonica* (F.), and in the ash bark beetle, *L. vurius* (F.) (Francke et al., 1979a).

Stereochemistry of Pityol and Spiroacetul. Chiral analyses showed that the *trans*-pityol produced by female WPCB is (2*R*,5*S*)-(+) (Figure 7). Based upon results of our bioassays and field experiments, (+)-*trans*-pityol, synergized by host monoterpene hydrocarbons, is a pheromone for WPCB. Since pityol exists in four different isomers (Figure 7), and only one is produced by the WPCB, it is possible that the stereochemistry is also a key to species separation. In field tests, (+)-*trans*-pityol and racemic grandisol acted synergistically as an aggregation pheromone for *P. pityographus*, whereas a mixture of (-)-*trans*-pityol and grandisol was inactive (Francke et al., 1987). Alone, (+)-*trans*-pityol exhibited only slight attraction for *P. pityographus*. A racemic mixture of *cis*-pityol was active for *P. vittatus*, but *cis*-pityol baits with (-)-*cis*-vittatol attracted more beetles than those with (+)-*cis*-vittatol (Klimetzek et al., 1989).

Isomers of the spiroacetal identified from WPCB were of the 5*S*,7*S* and 5*R*,7*S* configurations in a proportion of ca. 15 : 1 (Figure 7). However, spiroacetal produced from 1,8-dihydroxynonan-4-one in hydrochloric acid was mostly the more stable *E* isomer (*E/Z* ≥ 100). Under acidic conditions the spiroacetal may epimerize at the spirocenter. The 5*S*,7*S* isomer has the methyl group in an equatorial position and, due to the stabilizing anomeric effect, the second oxygen is attached axially at the spirocenter to the tetrahydropyran ring (Francke et al., 1981; Delongchamps et al., 1981). Thus, the natural mixture found in the WPCB is not in a thermodynamically stable equilibrium.

The instability of the bouquet opens the possibility that the "aging" (i.e., equilibration) of the proportion may function as a "built-in clock" providing information about the age of the signal. This effect is even more pronounced in the case of 2,8-dimethyl-1,7-dioxaspiro[5.5]undecane, which was identified from the cephalic secretion of the solitary bee *Andrenu wilkellu* (Bergstrom et al., 1982). The major component, the thermodynamically stable *E,E* isomer, has both methyl groups in equatorial positions and a diaxial coupling of the oxygens

at the spirocenter. It is accompanied by an *E,Z* product that also has two methyl groups in equatorial positions, but the oxygens at the spirocenter are in axial and equatorial positions. The ratio between these two compounds in the bees favors the thermodynamically less stable *E,Z* isomer. In *A. wilkella*, the more stable and highly attractive 2,8-dimethyl-1,7-dioxaspiro[5.5]undecane is of 2*S*,6*R*,8*S* configuration (Tengö et al. 1990); only this enantiomer is recognized by the bees.

Alkyl-1,6-dioxaspiro[4.4]nonanes, such as chalcogran (Francke et al., 1977), are much more sensitive to acidic epimerization at the spirocenter than the two other systems. In *Pityogenes chalcographus*, only (2*S*,5*R*)-2-ethyl-1,6-dioxaspiro[4.4]nonane showed strong activity (Byers et al., 1989). In nature this isomer is accompanied by the behaviorally inactive 2*S*,5*S* diastereomer, in a ratio of about 3 : 2 (Schurig and Weber, 1984), which almost represents the thermodynamically stable equilibrium.

Beetle Response. The strong male WPCB response to volatiles emanating from cones with females (Figure 6) agrees with the responses reported for male *C. ponderosae* (Kinzer et al., 1972) and *C. resinosae* (de Groot, 1992). However, WPCB males also responded strongly to volatiles from cones infested with pairs of beetles. The strongest response by female WPCB was to volatiles from cones with pairs or male beetles, as was reported for *C. ponderosae* females Kinzer et al., (1972). Few beetles were caught in experiment 1, perhaps because WPCB populations were sparse and the Plexiglas barrier traps were inefficient (Table 5).

Pityol alone elicited only weak WPCB responses in laboratory bioassays (Table 3) and in field experiment 3 (Table 6), but attracted males in field experiment 4. Neither *trans*-pinocarveol nor myrtenol, two other female-produced volatiles, enhanced WPCB responses in the laboratory (Tables 3 and 4). Because male WPCB did not distinguish between (±)-*trans*-pityol and the (+)-*trans*-pityol isomer (Tables 3 and 4), the more easily synthesized, and therefore less expensive, racemic *trans*-pityol may suffice for applied uses in the field.

Host monoterpenes often synergize scolytid pheromones (Borden, 1985). Volatiles collected from cones or a mixture of the four major monoterpene hydrocarbons found in cones, dramatically increased male response to pityol (Tables 3 and 4). Mixtures containing both pityol and monoterpenes produced male responses of about 80%, which were equal to those for volatiles from female-infested cones (Tables 3 and 4). Removing the host volatiles or the monoterpenes greatly decreased male response.

Female WPCB showed weak responses to cone volatiles or monoterpenes (Tables 2-4). Their response was much greater to a mixture of pityol, (+)-*trans*-pinocarveol, and monoterpenes, but was not as strong as to volatiles from female-infested cones (Table 3). Apparently other beetle- or host-produced compounds were emanating from female-infested cones. Bornyl acetate, a host vol-

atile released in prodigious amounts from wounded cones, did not affect beetle response.

The spiroacetal, the major male-produced volatile, did not attract male or female WPCB beetles (Figure 6 and Table 6). However, adding the spiroacetal to the three-compound female-produced mixture reduced male response from 30% to 8%, which was the lowest male response to any bioassay treatment containing pityol (Tables 3 and 4). Had the spiroacetal been added to a mixture of pityol combined with host volatiles, the potential for demonstrating a reduction in male response by the spiroacetal would obviously have been much greater. In the field, the spiroacetal inhibited the attraction of males (Table 7); Pierce et al. (1995) obtained similar field results with *C. resinosa*.

CONCLUSIONS

Independent and parallel chemical analyses undertaken in Georgia and Canada clearly confirmed that pityol and the spiroacetal are present in *C. coniperda*. Furthermore, the compounds do not differ in their chirality between two distinct and widely separated populations. Both pityol and the spiroacetal are potentially useful as behavior-modifying chemicals in an integrated pest management system for WPCB in *P. strobus* seed orchards. Pityol is also an effective attractant for male *C. resinosa* (Pierce et al., 1995), and we propose that it be tested in traps for monitoring WPCB and RPCB populations, or perhaps for mass-trapping populations. The spiroacetal could also have practical application as an inhibitor of normal cone beetle behavior.

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