

# RESPONSE OF CONE AND TWIG BEETLES (COLEOPTERA: SCOLYTIDAE) AND A PREDATOR (COLEOPTERA: CLERIDAE) TO PITYOL, CONOPHTHORIN, AND VERBENONE

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## Abstract

*The Canadian Entomologist* 132: 843 – 851 (2000)

Field studies were conducted in the United States and Canada to determine the response of the white pine cone beetle, *Conophthorus coniperda* (Schwarz), and the red pine cone beetle, *Conophthorus resinosae* Hopkins, to two potential inhibitors, conophthorin and verbenone, of pheromone communication. Trap catches of male *C. coniperda* and *C. resinosae* were significantly reduced and generally declined with increasing concentrations of conophthorin in traps baited with the pityol, a female-produced pheromone. Verbenone did not significantly reduce trap catches of *C. coniperda*. Conophthorin, but not verbenone, significantly reduced cone attacks by *C. coniperda* when placed near cone clusters. The twig beetles, *Pityophthorus cariniceps* LeConte and *Pityophthorus puberulus* (LeConte), responded to traps with pityol and  $\alpha$ -pinene baits alone or with conophthorin. *Thanasimus dubius* (F.) (Coleoptera: Cleridae) was attracted to the pityol and  $\alpha$ -pinene, but conophthorin had no effect on attraction of this generalist bark beetle predator. Verbenone significantly reduced trap catches of *T. dubius* in pityol-baited traps.

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de Groot P, DeBarr GL. 2000. Réactions des scolytes des cônes et des scolytes des rameaux (Coleoptera : Scolytidae) et d'un prédateur (Coleoptera : Cleridae) au pityol, à la conophthorine et à la verbénone. *The Canadian Entomologist* 132 : 843-851.

## Résumé

Des études sur le terrain ont été entreprises aux États-unis et au Canada pour déterminer la réaction du Scolyte des cônes du pin blanc, *Conophthorus coniperda* (Schwarz) et du Scolyte des cônes du pin rouge, *C. resinosae* Hopkins, à deux inhibiteurs potentiels de la communication par les phéromones, la conophthorine et la verbénone. Le nombre de captures de mâles de *C. coniperda* et de *C. resinosae* a diminué significativement et de façon générale lorsqu'augmentait la concentration de conophthorine dans des pièges garnis de pityol, une phéromone femelle. La verbénone n'a pas réduit significativement le nombre de captures de *C. coniperda*. La conophthorine, mais pas la verbénone, a réduit significativement le nombre d'infestations de cônes par des *C. coniperda* placés au voisinage de groupes de cônes. Les scolytes des rameaux *Pityophthorus cariniceps* LeConte et *Pityophthorus puberulus* (LeConte) ont eu des réactions positives aux pièges garnis de pityol ou d' $\alpha$ -pinène, seuls ou en combinaison avec de la conophthorine. *Thanasimus dubius* (F.) (Coleoptera : Cleridae) était attiré par les pièges garnis de pityol ou d' $\alpha$ -pinène, mais la conophthorine n'avait pas de pouvoir d'attraction pour ce prédateur de scolytes. La verbénone a eu pour effet de diminuer le nombre de *T. dubius* capturés dans les pièges garnis de pityol.

[Traduit par la Rédaction]

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## Introduction

In North America, the white pine cone beetle, *Conophthorus coniperda* (Schwarz) (Coleoptera: Scolytidae), is found throughout the range of eastern white pine, *Pinus strobus* L. (Pinaceae). The red pine cone beetle, *Conophthorus resinosae* Hopkins, is similarly distributed on red pine, *Pinus resinosa* Ait. (Wood 1982). Both insects are serious pests in seed orchards (Hedlin *et al.* 1980). Female cone beetles produce the sex pheromone (+)-*trans*-pityol, (2*R*,5*S*)-2-(1-hydroxy-1-methylethyl)-5-methyltetrahydrofuran to attract males (Birgersson *et al.* 1995; Pierce *et al.* 1995). Pityol was first identified in, and named after, the European bark beetle, *Pityophthorus pityographus* Ratz. (Coleoptera: Scolytidae) (Francke *et al.* 1987). Males and females also produce a spiroacetal, (5*S*,7*S*)-(-)-7-methyl-1,6-dioxaspiro[4.5]decane. This compound was first discovered in the social wasps, *Paravespula vulgaris* (L.), *Paravespula germanica* (F.), and *Dolichovespula saxonica* (F.) (Hymenoptera: Vespidae) and later found in *Cryphalus piceae* (Ratz.) and *Leperisinus fraxini* Panzer (Coleoptera: Scolytidae) (Francke 1981; Francke *et al.* 1978, 1979; Kohnle *et al.* 1992). This spiroacetal is known as "Conophthorin" (de Groot 1992; Pierce *et al.* 1995) and "MDOS" (Kohnle *et al.* 1992).

Since 1990, we have undertaken a series of experiments to develop a pheromone-based pest management system for cone beetles (de Groot *et al.* 1991, 1998; Birgersson *et al.* 1995; Pierce *et al.* 1995; de Groot and Zylstra 1995; de Groot and DeBarr 1998). Our efforts have focused primarily on the development of ( $\pm$ )-*trans*-pityol to attract males to traps and thus remove them from the breeding population in a seed orchard. This tactic is often referred to as trap out. Borden (1993) and Lindgren and Borden (1993) described a "push-pull" pest management tactic that combines an attractant to "pull" insects into traps and a repellent to "push" insects away from their hosts. One potential compound for the development of the push tactic is verbenone, 4,6,6-trimethylbicyclo[3.1.1]hept-3-en-2-one, which is an antiaggregation pheromone for several species of Scolytidae (Borden 1996). Another push compound is conophthorin, as it reduces the number of males captured in pityol-baited traps (Birgersson *et al.* 1995; Pierce *et al.* 1995; de Groot *et al.* 1998).

In this study, our objectives were to determine effective release rates and release devices for conophthorin and to determine the efficacy of verbenone as an inhibitor. We hypothesized that if these two compounds were inhibitory, then we would expect to see a reduction in trap catch when they were placed in traps baited with ( $\pm$ )-*trans*-pityol, and a reduction in cone attack when they were placed near cones. In the course of these studies, we also collected substantial numbers of *Pityophthorus cariniceps* Lec., *Pityophthorus puberulus* (Lec.), and *Thanasimus dubius* (F.) (Coleoptera: Cleridae) in some of our experiments and included these in our analyses. The twig beetles, *Pityophthorus* spp. Eichhoff, are closely related to *Conophthorus* spp. Hopkins, but usually attack weakened or dying twigs (Bright 1981; Wood 1982). The clerid *T. dubius* is a predator of numerous species of Scolytidae (Hopkins 1899) and responds to beetle pheromones (Vité and Williams 1970; Billings and Cameron 1984).

## Methods and Materials

**Study Sites.** During 1995–1998, we conducted seven experiments. The experiments for *C. coniperda* were conducted in two white pine seed orchards in North Carolina, United States, one located in Morganton (North Carolina Forest Service) (35°45'N, 81°45'W), and the other near Murphy (United States Forest Service, Beech Creek) (35°00'N, 84°00'W). Experiments for *C. resinosae* were conducted in a red pine plantation located 10 km north of Thessalon, Ontario, Canada (46°15'N, 83°30'W).

**Treatments.** Four experiments were conducted to test release rates of ( $\pm$ )-*E*-conophthorin in traps baited with ( $\pm$ )-*trans*-pityol. In Experiment 1, we compared three release rates of conophthorin. Conophthorin was released from experimental vials as described below. In Experiments 2–4, we tested commercially available release devices for three release rates of conophthorin. For Experiment 2, population levels of *C. coniperda* were sparse in 1996 and the experiment was repeated in 1997 (Exp. 3) at another location. Experiments 1–3 evaluated conophthorin in traps for *C. coniperda*, and Experiment 4 tested conophthorin against *C. resinosa*. Previous work has demonstrated that ( $\pm$ )- $\alpha$ -pinene enhanced trap catches of *C. coniperda* but not of *C. resinosa* in traps baited with ( $\pm$ )-*trans*-pityol, and therefore ( $\pm$ )- $\alpha$ -pinene was included only in our experiments for *C. coniperda* (de Groot and Zylstra 1995; de Groot and DeBarr 1998; de Groot *et al.* 1998). Experiment 5 tested ( $\pm$ )-*E*-conophthorin and (*S*)-(-)-verbenone alone or together in traps baited with ( $\pm$ )-*trans*-pityol and ( $\pm$ )- $\alpha$ -pinene. Experiment 6 tested the effect of conophthorin released at white pine cone clusters on *C. coniperda* attacks, and Experiment 7 examined the effects of two release rates of (*S*)-(-)-verbenone on cone attacks.

Traps (treatments) were laid out in a complete randomized block design (spatially distinct lines or groups of traps) with five to eight replicates of each treatment. Traps were hung in the upper third of tree crowns, one trap per tree, and spaced 15–30 m apart. Beetles were removed from the traps at weekly intervals and the treatments within each block were randomly reassigned (traps moved with lures) to reduce potential trap position bias. Captured insects were preserved in 70% ethanol until they were identified and counted. Cone beetles were sexed by examination of the abdominal tergites (Herdy 1959). Voucher specimens were deposited in the collection at the Great Lakes Forestry Centre, Canadian Forest Service, Sault Ste. Marie, Ontario, Canada. In Experiment 6, 14 clusters of white pine cones were tagged in the upper third of the tree and randomly assigned to either the conophthorin treatment or the untreated control (seven each). In Experiment 7, 24 clusters of white pine cones were located and randomly assigned to the two verbenone treatments or the untreated control (eight each). In Experiments 6 and 7, treatments were installed on 8 April 1998 and cones were examined for damage by cone beetles on 8 June when the attack period was completed.

**Chemicals.** Synthetic pheromones ( $\pm$ )-*trans*-pityol (85% pure), ( $\pm$ )-*E*-conophthorin (89% pure), and (*S*)-(-)-verbenone [87% (-); 13% (+)] were obtained from Phero Tech, Inc. (Delta, British Columbia). We have demonstrated previously that ( $\pm$ )-*trans*-pityol and ( $\pm$ )-*E*-conophthorin were as effective as (+)-*trans*-pityol and (-)-*E*-conophthorin, respectively (Birgersson *et al.* 1995; Pierce *et al.* 1995). Hereafter these pheromone compounds are referred to simply as pityol, conophthorin, and verbenone. The ( $\pm$ )- $\alpha$ -pinene (98% pure) was purchased from Aldrich Chemical Co. (Milwaukee, Wisconsin, United States), and *n*-octane (99% pure) was purchased from Sigma Chemical Co. (St. Louis, Missouri, United States).

**Chemical Release Devices and Traps.** In Experiment 1, volatiles were released from 2-mL screw-cap glass vials. Each vial had a 5-cm-long cotton wick pulled through a piece of 1.6 mm i.d. Teflon<sup>®</sup> tubing that was inserted through a hole drilled in the vial cap. Synthetic compounds were formulated in a final volume of 2 mL of *n*-octane per vial. For the control, octane was emitted from each vial at about 200 mg/day (24°C). For the remaining treatments, each vial contained octane plus 0.7 mg of pityol released at about 0.1 mg/day and 100 mg of  $\alpha$ -pinene released at about 14.3 mg/day. Three of the four treatments also had 1.6, 8, or 40 mg of conophthorin added per vial to achieve release rates of about 0.23, 1.14, and 5.71 mg/day, respectively.

Commercial release devices for pityol, conophthorin, and verbenone were deployed for Experiments 2–7. Pityol (40 mg/cap) was released at about 0.2 mg/day (22°C) from a polyethylene “bubble cap” (Phero Tech Inc.). Conophthorin was released from 3.2 mm o.d. polyvinyl chloride “rope” containing 2.2 mg conophthorin/cm (Phero Tech Inc.). For Experiments 2–4, the rope was cut into 2-, 6-, and 18-cm lengths to provide release rates of about 0.07, 0.2, and 0.6 mg/day, respectively, for the first 10 d at 20°C (Phero Tech Inc.). After 10 days, the release rates stabilized to about 0.02, 0.07, and 0.2 mg/day for another 70 d (Phero Tech Inc.). A short wire, run through a hole punched in the middle of each piece of rope, was used to attach the rope to the trap. A 6-cm length of conophthorin rope was used in Experiment 5. Verbenone was released at about 0.6 mg/day (22°C) from a polyethylene bubble cap (Phero Tech Inc.). A polyethylene, 250- $\mu$ L Eppendorf centrifuge tube (Brinkman Instruments, Rexdall, Ontario) was used to release  $\alpha$ -pinene (225  $\mu$ L per tube) at about 12 mg/day at 22°C.

Yellow Japanese beetle trap tops (Trécé Inc., Salinas, California, United States) fitted with plastic 500-mL Mason<sup>®</sup> jars were used for Experiments 1–5. Each jar contained about 50 mL of propylene glycol to kill and preserve trapped insects. Trap jars were cleaned and refreshed with clean propylene glycol on each collection date. Chemical release devices were attached to the top of the trap.

**Data Analysis.** Numbers of beetles for each species caught were pooled by replicate and transformed by  $\log(x + 1)$  to satisfy assumptions of normality and homogeneity of variance (Zar 1999). Transformed data were analyzed by ANOVA by the General Linear Model for randomized block designs of SYSTAT<sup>®</sup> 9.0 (SPSS Inc., Chicago, Illinois) followed by Tukey’s test at  $\alpha = 0.05$ . There were too few trap catches of females in experiments 4 and 5 for analysis. Data for Experiments 6 and 7 were analyzed by one-way ANOVA (Zar 1999).

## Results

In all experiments, the addition of conophthorin to traps baited with pityol or pityol plus  $\alpha$ -pinene significantly reduced trap catches of male cone beetles (Exps. 1, 3–5; Tables 1–4). Trap catches of male *C. coniperda* and *C. resinosae* generally declined with increasing concentration of conophthorin (Exps. 1, 3, 4). In Experiments 1 and 4, there was a significant reduction in numbers of males caught from the lowest to the highest release rates, but not in Experiment 3. The trap catch of female *C. coniperda* was not significantly reduced by conophthorin when it was added to pityol-baited traps (Exps. 1, 3; Tables 1, 2). In Experiment 3, the highest trap catch of females occurred when traps were baited with pityol and a medium release rate of conophthorin (0.07 mg/day). Unlike conophthorin, verbenone did not significantly reduce the trap catch of male *C. coniperda* in pityol-baited traps (Exp. 5). Furthermore, there was no apparent synergy when verbenone was added with conophthorin in the reduction of the trap of males (Exp. 5). Both conophthorin alone and verbenone alone were no more attractive than control traps releasing octane alone (Exp. 5). Conophthorin placed near living cones significantly reduced cone attacks by *C. coniperda*, but when verbenone was placed near cones neither release rate significantly reduced attacks (Exps. 6, 7; Table 5).

In Experiment 1, significantly more *P. cariniceps* were caught in traps baited with pityol and  $\alpha$ -pinene than in the control traps baited only with octane (Table 1). Similarly, significantly more *P. puberulus* were caught in traps baited with pityol than in unbaited traps (Exp. 4; Table 3). A release rate of conophthorin of 0.23 mg/day did not reduce the catch of *P. cariniceps* in pityol-baited traps, but higher release rates did (Exp. 1). When release rates of conophthorin below 0.2 mg/day were tested (Exp. 2), release rates of conophthorin at 0.02–0.2 mg/day significantly increased the trap catch

TABLE 1. Response of *Conophthorus coniperda*, *Pityophthorus cariniceps*, and *Thanasimus dubius* to three release rates of conophthorin in traps baited with pityol and  $\alpha$ -pinene, Morganton, North Carolina (Exp. 1).

Treatment*	<i>C. coniperda</i>		<i>P. cariniceps</i>	<i>T. dubius</i>
	Males	Females		
( $\pm$ )- <i>trans</i> -Pityol, $\alpha$ -pinene	78.4 $\pm$ 11.4a	8.0 $\pm$ 5.2a	40.8 $\pm$ 8.8a	34.5 $\pm$ 1.7a
( $\pm$ )- <i>trans</i> -Pityol, $\alpha$ -pinene, low <i>E</i> -( $\pm$ )-conophthorin	33.6 $\pm$ 7.6b	15.8 $\pm$ 2.7a	25.1 $\pm$ 2.5a	34.0 $\pm$ 4.7a
( $\pm$ )- <i>trans</i> -Pityol, $\alpha$ -pinene, medium <i>E</i> -( $\pm$ )-conophthorin	16.1 $\pm$ 2.5bc	18.4 $\pm$ 2.0a	11.8 $\pm$ 2.1b	30.9 $\pm$ 3.6a
( $\pm$ )- <i>trans</i> -Pityol, $\alpha$ -pinene, high <i>E</i> -( $\pm$ )-conophthorin	12.9 $\pm$ 2.5c	11.2 $\pm$ 1.8a	9.0 $\pm$ 1.1b	34.0 $\pm$ 5.5a
Octane control	0.0 $\pm$ 0.0d	0.1 $\pm$ 0.1b	0.1 $\pm$ 0.1c	1.8 $\pm$ 0.7b

NOTE: Values in the table are mean ( $\pm$  SE) number of individuals caught per trap. Means within a column followed by the same letter are not significantly different (Tukey's test,  $P > 0.05$ ). There were eight traps per treatment, and the experiment was conducted from 4 April to 18 May 1995.

\* Low, medium, and high release rates of conophthorin are about 0.23, 1.14, and 5.71 mg/day, respectively, at 24°C.

TABLE 2. Response of *Conophthorus coniperda*, *Pityophthorus cariniceps*, and *Thanasimus dubius* to three release rates of conophthorin in traps baited with pityol and  $\alpha$ -pinene, Morganton (Exp. 2) and Murphy (Exp. 3), North Carolina.

Treatment*	<i>C. coniperda</i>		<i>P. cariniceps</i>	<i>T. dubius</i>
	Males	Females		
Experiment 2 <sup>†</sup>				
( $\pm$ )- <i>trans</i> -Pityol, $\alpha$ -pinene	0.3 $\pm$ 0.2	0.0 $\pm$ 0.0	14.0 $\pm$ 3.6b	10.5 $\pm$ 1.8a
( $\pm$ )- <i>trans</i> -Pityol, $\alpha$ -pinene, low <i>E</i> -( $\pm$ )-conophthorin	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	125.0 $\pm$ 15.2a	19.8 $\pm$ 4.4a
( $\pm$ )- <i>trans</i> -Pityol, $\alpha$ -pinene, medium <i>E</i> -( $\pm$ )-conophthorin	0.5 $\pm$ 0.3	0.0 $\pm$ 0.0	94.7 $\pm$ 15.2a	17.7 $\pm$ 3.5a
( $\pm$ )- <i>trans</i> -Pityol, $\alpha$ -pinene, high <i>E</i> -( $\pm$ )-conophthorin	0.2 $\pm$ 0.2	0.0 $\pm$ 0.0	85.3 $\pm$ 16.9a	16.3 $\pm$ 3.1a
Experiment 3 <sup>‡</sup>				
( $\pm$ )- <i>trans</i> -Pityol, $\alpha$ -pinene	37.8 $\pm$ 13.6a	6.2 $\pm$ 2.5ab	1.0 $\pm$ 0.3	2.6 $\pm$ 0.7a
( $\pm$ )- <i>trans</i> -Pityol, $\alpha$ -pinene, low <i>E</i> -( $\pm$ )-conophthorin	9.0 $\pm$ 3.5b	6.2 $\pm$ 1.6ab	1.6 $\pm$ 0.7	3.4 $\pm$ 1.7a
( $\pm$ )- <i>trans</i> -Pityol, $\alpha$ -pinene, medium <i>E</i> -( $\pm$ )-conophthorin	9.8 $\pm$ 4.5b	11.4 $\pm$ 5.7a	2.6 $\pm$ 1.2	5.0 $\pm$ 1.3a
( $\pm$ )- <i>trans</i> -Pityol, $\alpha$ -pinene, high <i>E</i> -( $\pm$ )-conophthorin	3.8 $\pm$ 2.1b	1.4 $\pm$ 0.5b	1.6 $\pm$ 1.0	2.6 $\pm$ 1.2a

NOTE: Values in the table are mean ( $\pm$  SE) number of individuals caught per trap. Means within a column and experiment followed by the same letter are not significantly different (Tukey's test,  $P > 0.05$ ). There were six traps per treatment in experiment 2 (29 March – 14 May 1996) and five traps per treatment in experiment 3 (1 April – 12 June 1997).

\* Low, medium, and high release rates of conophthorin are about 0.02, 0.07, and 0.2 mg/day, respectively, after 10 days at 20°C.

<sup>†</sup> The number of captured *C. coniperda* was too low for analysis.

<sup>‡</sup> The number of captured *P. cariniceps* was too low for analysis.

in traps baited with pityol and  $\alpha$ -pinene (Table 2). These same release rates of conophthorin did not result in an increase in trap catch of *P. puberulus* compared with traps baited with pityol only (Exp. 4).

Significantly more *T. dubius* were caught in traps baited with pityol and  $\alpha$ -pinene than in the traps baited with octane only (Exp. 1; Table 1) or unbaited traps (Exp. 5; Table 4). When conophthorin was added to traps baited with pityol and  $\alpha$ -pinene, it did not reduce the trap catch of *T. dubius* for the release rates tested (0.02–5.71 mg/day) (Exps. 1–3; Tables 1, 2). Verbenone significantly reduced trap catches of *T. dubius* when it was added to traps baited with the pityol and  $\alpha$ -pinene, or the pityol,  $\alpha$ -pinene, and conophthorin combination (Exp. 5). Removing verbenone from the quaternary treatment (leaving only pityol,  $\alpha$ -pinene, and conophthorin) resulted in a trap catch the same as that for traps baited only with pityol and  $\alpha$ -pinene. There was no difference in

TABLE 3. Response of *Conophthorus resinosae* males and *Pityophthorus puberulus* to three release rates of conophthorin in traps baited with pityol, Thessalon, Ontario (Exp. 4).

Treatment*	<i>C. resinosae</i>	<i>P. puberulus</i>
(±)- <i>trans</i> -Pityol	125.3±5.5a	8.2±2.2a
(±)- <i>trans</i> -Pityol, low <i>E</i> -(±)-conophthorin	9.3±1.8b	3.9±1.3a
(±)- <i>trans</i> -Pityol, medium <i>E</i> -(±)-conophthorin	4.4±1.1bc	3.6±1.3a
(±)- <i>trans</i> -Pityol, high <i>E</i> -(±)-conophthorin	2.5±0.7c	6.1±1.9a
Unbaited control	0.8±0.4c	0.3±0.1b

NOTE: Values in the table are mean (± SE) number of individuals caught per trap. Means within a column followed by the same letter are not significantly different (Tukey's test,  $P > 0.05$ ). There were eight traps per treatment for both *C. resinosae* (29 May – 26 June 1997) and *P. puberulus* (29 May – 12 June).

\* Low, medium, and high release rates of conophthorin are about 0.02, 0.07, and 0.2 mg/day, respectively, after 10 days at 20°C.

TABLE 4. Response of *Conophthorus coniperda* males and *Thanasimus dubius* (both sexes combined) to conophthorin and verbenone in traps baited with pityol and α-pinene, Morganton, North Carolina (Exp. 5).

Treatment	<i>C. coniperda</i>	<i>T. dubius</i>
(±)- <i>trans</i> -Pityol, α-pinene	28.2±15.0a	4.6±1.2a
(±)- <i>trans</i> -Pityol, α-pinene, <i>S</i> -(-)-verbenone	13.0±4.2a	0.6±0.4b
(±)- <i>trans</i> -Pityol, α-pinene, <i>S</i> -(-)-verbenone, <i>E</i> -(±)-conophthorin	10.6±6.1a	0.6±0.4b
(±)- <i>trans</i> -Pityol, α-pinene, <i>E</i> -(±)-conophthorin	3.8±1.5b	4.8±2.3a
<i>S</i> -(-)-Verbenone	0.2±0.2b	0.0±0.0b
<i>E</i> -(±)-Conophthorin	0.0±0.0b	0.2±0.2b
Unbaited trap	0.0±0.0b	0.2±0.2b

NOTE: Values in the table are mean (± SE) number of individuals caught per trap. Means within a column followed by the same letter are not significantly different (Tukey's test,  $P > 0.05$ ). There were five traps per treatment (1 April – 13 June 1997).

trap catch among traps baited with verbenone only or conophthorin only or traps without baits (Exp. 5).

## Discussion

The results from this study are consistent with our previous studies showing conophthorin inhibits attraction by male cone beetles to the sex pheromone pityol (Birgersson *et al.* 1995; Pierce *et al.* 1995; de Groot *et al.* 1998). Although conophthorin reduced traps catch significantly, some males were not deterred and entered the pityol-baited traps. This suggests that conophthorin alone is insufficient to completely block males from seeking females. We hypothesize that males guard their mates and produce conophthorin as an inhibitor to announce their presence to rival males. A male enters the cone through the entrance constructed by the female and follows behind her while she lays eggs (Godwin and Odell 1965). In laboratory bioassays where groups of males were released together to determine response to volatiles, male aggressive behaviour was common, particularly when the volatiles from females were tested (Birgersson *et al.* 1995).

Conophthorin was found in male *Pityophthorus carmeli* Swaine and male *Pityophthorus nitidulus* (Mannerheim) (Dallara *et al.* 2000). In field bioassays,

TABLE 5. Effect of conophthorin released from PVC rope (Exp. 6) and verbenone released from bubble caps (Exp. 7) on cone attacks by *Conophthorus coniperda*, Murphy, North Carolina.

Treatment	Mean ( $\pm$ SE) number of beetle-infested cones per cone cluster
Experiment 6	
E-( $\pm$ )-Conophthorin	0.7 $\pm$ 0.3*
Control	1.7 $\pm$ 0.4
Experiment 7	
Low S(-)-verbenone	1.5 $\pm$ 0.6 <sup>†</sup>
High S(-)-verbenone	1.1 $\pm$ 0.4
Control	1.4 $\pm$ 0.5

NOTE: In Experiment 6 there was one cone cluster per treatment on seven trees (treated 8 April, counted 8 June 1998); in Experiment 7 there was one cone cluster per treatment on eight trees (treated 8 April 1998, counted 8 June 1998), and low and high release rates of verbenone are one and two bubble caps, respectively, at about 0.6 mg/day at 22°C.

\* Number of infested cones different between treatments ( $F_{1,12} = 5.50$ ,  $P = 0.0370$ ).

<sup>†</sup> Number of infested cones not different among treatments ( $F_{1,21} = 0.14$ ,  $P > 0.05$ ).

conophthorin alone did not attract *Pityophthorus* spp., but significantly reduced catches of *Pityophthorus setosus* Blackman (predominately males) to (E)-(+)-pityol. *Pityophthorus carmeli* (predominately females) responded only to a combination of (E)-(-)-conophthorin and (E)-(+)-pityol. Dallara *et al.* (2000) suggest that conophthorin may function as a pheromone component for *P. carmeli* and as a multispecies synomone that decreases competition of *P. carmeli* and *P. nitidulus* with *P. setosus*.

Typically, white pine cones occur in clusters of two or more cones. We have observed that cones within each cluster are attacked sequentially by a single female, unless other females arrive because cones are sparse and beetles abundant. Males do not initiate cone attacks (Godwin and Odell 1965). Our trap catch results indicate that conophthorin is neither attractive nor inhibitory to female *C. coniperda*, but when conophthorin was placed near cone clusters it caused a reduction in cone attacks. We hypothesize that the reduction in cone attacks is due to the inability of a female to attract a male to the cone (because of the presence of conophthorin) and flies off to another cone cluster, leaving behind unattacked cones. Godwin and Odell (1965) observed that when a female did not attract a mate she left the cone early and that this pattern was consistent and characteristic of non-inseminated females.

Conophthorin appears to be a good candidate to "push" male beetles away from cones and thus lower their mating success, whereas verbenone is not. Green leaf volatiles have also been shown to interrupt the response of *C. resinosae* to pityol (de Groot and MacDonald 1999) and in combination with conophthorin may be more effective than either compound alone; this remains to be determined. If attractants can be found for females, then the success of a "push-pull" tactic would be greatly enhanced.

We captured substantial numbers of female *C. coniperda* in some of our experiments. This is in contrast to our previous studies where we seldom caught females (Pierce *et al.* 1995; de Groot and Zylstra 1995; de Groot and DeBarr 1998; de Groot *et al.* 1998; de Groot and MacDonald 1999). We speculated that females recognize pityol as a signal to indicate the occupancy of other females in cones and thus avoid those

cones. Hence, we should expect to catch few females in pityol-baited traps. However, in light of our results from the current study, we hypothesize that when competition for cones is severe, females use pityol as an aggregation pheromone to locate scarce but potentially available cones in a cone cluster. We have observed that as the number of available cones decline in a seed orchard, the number of females caught in our traps increases. An alternative explanation for this observation is that as females age they become less choosy, regardless of the abundance of cones. Either way, this apparent behavioral plasticity or ontogeny would be interesting to pursue.

Our study provides evidence that pityol is attractive to *P. cariniceps* and *P. puberulus*. Studies will be needed to determine if these species produce pityol. Dallara *et al.* (2000) have identified pityol from male *P. carmeli*, female *P. nitidulus*, and female *P. setosus*. Field bioassays suggested that (*E*)-(+)-pityol is an aggregation pheromone component for *P. carmeli* and *P. setosus* (Dallara *et al.* 2000).

Our studies demonstrated for the first time that the clerid predator *T. dubius* was attracted to traps containing pityol and  $\alpha$ -pinene with or without conophthorin. Additional work will be needed to determine if the response was to pityol,  $\alpha$ -pinene, or both. Semiochemical-baited traps are used to monitor changes in the ratio of *T. dubius* to its prey, the southern pine beetle, *Dendroctonus frontalis* (Zimmerman) (Coleoptera: Scolytidae), in the southern United States and predict an increase or decrease in population levels (Billings 1988). Research is needed to determine if this technique can also be used to predict population trends for *C. coniperda* in seed orchards. Richerson and Payne (1979) concluded that the use of inhibitors such as brevicomin and verbenone did not affect the numbers or distribution of *T. dubius* attracted to the aggregation pheromone frontalin released by *D. frontalis*. In Experiment 5, verbenone significantly reduced trap catches of *T. dubius* when added to traps with pityol and  $\alpha$ -pinene, or pityol,  $\alpha$ -pinene, and conophthorin. Because catches of *C. coniperda* in traps baited with pityol and  $\alpha$ -pinene were not significantly reduced by verbenone, it might be added to the traps used in a trap-out strategy to reduce unwanted catches of *T. dubius*. Otherwise, such traps would remove clerid predators from the area.

### Acknowledgments

This paper is dedicated to John H Borden. We have had the privilege of working with John over several years to understand the chemical ecology of cone beetles and have benefited greatly from his wisdom, insight, and clear thinking. For this study, we appreciate the assistance of L Barber, M Cody, C Crowe, C Logan, D Grooms, B Zylstra, R Nott, and N Parker. We thank G Beaver, USDA Forest Service, and D Rogers, North Carolina Forest Service, for assistance in their orchards, DE Bright Jr for identifying the species of *Pityophthorus* spp., and DR Miller and two anonymous reviewers for their helpful comments on an earlier draft of the manuscript. The research was funded by the Canadian Forest Service and the USDA Forest Service.

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