RESPONSES OF *CONOPHTHORUS* SPP. (COLEOPTERA: SCOLYTIDAE) TO BEHAVIORAL CHEMICALS IN FIELD TRIALS: A TRANSCONTINENTAL PERSPECTIVE

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Abstract

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We tested six behavioral chemicals, pityol, conophthorin, 4-allylanisole, verbenone, 2-hexenol, and α-pinene, in a series of field trials directed at six combinations of *Conophthorus* Hopkins – *Pinus* L. spp. (Pinaceae) in sites distributed across North America. Beetle – host tree combinations included *Conophthorus ponderosae* Hopkins on *Pinus ponderosa* Laws., *C. ponderosae* on *Pinus monticola* Dougl., *Conophthorus conicolens* Wood on *Pinus pseudostrobus* Lindl., *Conophthorus teocotum* Wood on *Pinus teocote* Schl. & Cham., *Conophthorus coniperda* (Schwarz) on *Pinus strobus* L., and *Conophthorus resinosae* Hopkins on *Pinus resinosa* Ait. *trans*-Verbenol was tested only on *C. resinosae* on *P. resinosa*. Traps baited with pityol caught more beetles than unbaited traps in nearly all of the assays, and conophthorin consistently inhibited male beetle response to pityol for all species tested. Behavioral responses of species of *Conophthorus* to α-pinene appeared to parallel host phylogeny, inasmuch as beetles using Haploxylon pines as hosts utilized α-pinene as a synergist for the beetle-produced pityol, whereas beetles using Diploxylon pines as hosts did not. α-Pinene was a synergist for pityol in *C. ponderosae* on *P. monticola* and *C. coniperda* on *P. strobus*, but not for species of *Conophthorus* on any other pines tested. *Conophthorus ponderosae* on *P. ponderosa* was the only beetle–host combination tested where verbenone was a synergist for pityol, but this effect was not consistent in all years of testing. It was also the only beetle–host combination in which 4-allylanisole was a repellent. For all other

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beetle–host combinations, verbenone was neutral to slightly repellent and 4-allylanisole was either synergistic or neutral in pityol-baited traps. Promising synergists and interruptants/repellents were identified for implementation in pest-management regimes, including conophthorin as an interruptant for all species of *Conophthorus* tested, 4-allylanisole as an interruptant for *C. ponderosae* on *P. ponderosa*, α-pinene as a synergist for pityol in all species tested on Haploxylon pines, and 4-allylanisole as a synergist for pityol in *C. conicolens* and *C. coniperda*.

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Résumé

Nous avons testé six substances chimiques affectant le comportement, le pityol, la conophthorine, le 4-allylanisole, la verbénone, le 2-hexenol, et l'α-pinène, au cours d'une série de tests en nature sur six combinaisons de *Conophthorus* Hopkins – *Pinus* spp. (Pinaceae) en plusieurs endroits répartis dans toute l'Amérique du Nord. Les combinaisons coléoptère – arbre-hôte ont été les suivantes: *Conophthorus ponderosae* Hopkins sur *Pinus ponderosa* Laws., *C. ponderosae* sur *Pinus monticola* Dougl., *Conophthorus conicolens* Wood sur *Pinus pseudostrobus* Lindl., *Conophthorus teocotum* Wood sur *Pinus teocote* Schl. et Cham., *Conophthorus coniperda* (Schwarz) sur *Pinus strobus* L. et *Conophthorus resinosae* Hopkins sur *Pinus resinosa* Alt. Le *trans*-verbénol a été testé seulement sur *C. resinosae* sur *P. resinosa*. Les pièges garnis de pityol ont capturé plus d'insectes que les pièges non garnis dans presque tous les tests et la conophthorine inhibait systématiquement la réponse des mâles au pityol chez toutes les espèces testées. La réponse de toutes les espèces de *Conophthorus* à l'α-pinène semble fonction de la phylogénie de l'hôte puisque les scolytes qui utilisent les pins Haploxylon comme hôtes utilisent l'α-pinène comme synergiste du pityol, alors que les scolytes qui utilisent les pins Diploxylon comme hôtes n'utilisent pas l'α-pinène. L'α-pinène a été synergiste du pityol chez *C. ponderosae* sur *P. monticola* et chez *C. coniperda* sur *P. strobus*, mais pas chez les espèces de *Conophthorus* testées sur d'autres espèces de pins. La combinaison de *C. ponderosae* sur *P. ponderosa* a été la seule où la verbénone a servi de synergiste au pityol, mais cet effet a varié d'une année à l'autre. Il s'agit aussi de la seule combinaison pour laquelle le 4-allylanisole avait des propriétés repoussantes. Pour toutes les autres combinaisons, la verbénone était neutre ou légèrement repoussante, et le 4-allylanisole était synergiste ou neutre dans les pièges à pityol. Les meilleurs synergistes et interrupteurs/repousseurs ont été identifiés pour être intégrés dans des programmes d'aménagement : la conophthorine, qui inhibe toutes les espèces de *Conophthorus*, le 4-allylanisol qui inhibe *C. ponderosae* sur *P. ponderosa*, l'α-pinène qui sert de synergiste du pityol chez toutes les espèces testées sur des pins Haploxylon, et le 4-allylanisole le synergiste du pityol chez *C. conicolens* et *C. coniperda*.

[Traduit par la Rédaction]

Introduction

Cone beetles in the genus *Conophthorus* Hopkins (Coleoptera: Scolytidae) destroy upwards of 90% of cone crops in managed pine seed orchards and seed-production areas across North America (Hedlin *et al.* 1980; Cibrian Tovar *et al.* 1986; de Groot 1986) and are a primary vector of pitch canker, *Fusarium circinatum* (Nirenberg and O'Donnell) in *Pinus radiata* D. Don (Hoover *et al.* 1995). The life cycle of *Conophthorus* spp. is largely cryptic, with all life stages spent inside the cone except for the adults during the brief period from emergence to initiation of attack on

developing cones (Hedlin *et al.* 1980; de Groot 1986). This cryptic habit makes control with insecticides problematic (DeBarr *et al.* 1982; Shea *et al.* 1984), so there is an urgent need for alternatives such as behavior-modifying chemicals.

Pheromones are important in detection, monitoring, and suppression (mass-trapping or mating disruption) of pest populations (Cardé and Minks 1997). Other behavioral chemicals, such as interruptants or repellents, can reduce damage without being directed at pest population levels *per se* (Munakata 1977). Pest suppression does not always reduce damage, because compensatory mechanisms (increased immigration or reduced competition) can maintain damage levels even when large numbers of insects are trapped. The male trap-out strategy to disrupt mating and thereby reduce damage has promise, but it may reduce cone beetle populations and yet fail to protect cones. Female cone beetles initiate cone attack as the first step in courtship (Hedlin *et al.* 1980), so even unmated females kill cones. For these reasons, one of our primary goals is the development of female-specific cone beetle repellents.

Much progress has been made in elucidating the chemical ecology of *Conophthorus* spp. in the last decade (de Groot *et al.* 1991; Birgersson *et al.* 1995; Pierce *et al.* 1995). Kinzer *et al.* (1972) demonstrated a response to infested cones by male *Conophthorus ponderosae* Wood (= *C. monticolae* Hopkins) in laboratory bioassays, and de Groot *et al.* (1991) provided evidence for the existence of a female-produced attractant pheromone in *Conophthorus coniperda* (Schwarz) and *Conophthorus resinosae* Hopkins. Birgersson *et al.* (1995) and Pierce *et al.* (1995) identified this semiochemical as (+)-*trans*-pityol [(2*R*,5*S*)-(+)-2-(1-hydroxy-1-methylethyl)-5-methyltetrahydrofuran; hereinafter referred to as pityol]. Both Birgersson *et al.* (1995) and Pierce *et al.* (1995) identified an inhibitory compound, conophthorin [(5*S*,7*S*)-(–)-7-methyl-1,6-dioxaspiro-[4,5]decane] from *Conophthorus* spp. Miller *et al.* (2000) found (2*R*,5*S*)-(+)-*trans*-pityol in female *C. ponderosae* and (5*S*,7*S*)-(–)-conophthorin in males from *Pinus monticola* Dougl. on Texada Island, British Columbia, Canada; they also found that α-pinene synergizes pityol in *C. ponderosae* on *P. monticola*. Birgersson *et al.* (1995) and de Groot *et al.* (1998) showed that racemic α-pinene (2,6,6-trimethylbicyclo[3.1.1]hept-2-ene; hereinafter referred to as α-pinene) is also a synergist for the attraction of male *C. coniperda* by pityol. In contrast, α-pinene does not function as a synergist in *C. resinosae* in Ontario (de Groot and Zylstra 1995) nor in *C. ponderosae* in California (JD Stein and NG Rappaport, unpublished data). Rappaport *et al.* (unpublished data) identified (+)-*trans*-pityol, but not conophthorin, from Porapak-Q collections of female *C. ponderosae* volatiles.

Three other behavioral chemicals may have promise for cone beetle population management. One of these, verbenone (4,6,6-trimethylbicyclo[3.1.1]hept-3-en-2-one) has been identified in hindgut extracts of *C. coniperda* (Birgersson *et al.* 1995) and in Porapak-Q collections from *C. ponderosae* (NG Rappaport, unpublished data). Another is the phenylpropanoid compound 4-allylanisole (also known as 4-allylanisol, estragol, and methyl chavicol), an interruptant for Scolytidae (Coleoptera) in the genera *Ips* DeGeer and *Dendroctonus* Erichson (Hayes and Strom 1994). Finally, short-chain aliphatics called "green leaf volatiles" are nearly ubiquitous in the plant kingdom and interrupt the response to attractants in a number of Scolytidae, including *Dendroctonus ponderosae* Hopkins, *Dendroctonus frontalis* Zimm., *Ips avulsus* (Eichhoff), *Ips grandicollis* (Eichhoff), and *Trypodendron lineatum* (Olivier) (Dickens *et al.* 1992; Wilson *et al.* 1996; Borden *et al.* 1997). (*E*)-2-Hexen-1-ol inhibits male *C. resinosae* response to pityol and was the most effective of a range of green leaf aldehydes and alcohols tested (de Groot and MacDonald 1999).

The objectives of this study were to (*i*) characterize the responses of different *Conophthorus* spp. to several promising behavioral chemicals under field conditions and (*ii*) identify behavioral chemicals with promise for cone protection. To accomplish

these objectives, we baited traps with the sex pheromone pityol, in combination with various putative interruptants, repellents, and synergists, and then assessed the effects on male beetle response.

Materials and Methods

Behavioral Chemicals and Release Devices. Polyethylene bubblecaps, Eppendorf tubes, or vials were used as release devices (Phero Tech, Inc., Delta, British Columbia) (Table 1). Unless otherwise stated, all treatments used a single release device per trap. In 1998, we tested three release rates of verbenone (one bubblecap, two bubblecaps, and three bubblecaps) and 4-allylanisole (one vial, three vials, and five vials), to determine whether the release rates of the putative synergist and inhibitor affected trap catch by pityol. Our earlier tests suggested that both might act as multifunctional behavioral chemicals within the genus *Conophthorus*, so we tested a range of release rates to determine (*i*) what the optimum release rates were and (*ii*) whether the function of either of these two chemicals was dose dependent. In 1999, we tested three release rates of conophthorin (100, 10, and 5% in octanol), to determine whether a lower release rate was as effective as the standard neat formulation in inhibiting male beetle response. Octanol was chosen as a diluent because of its favorable physical properties; we know of no evidence that it is behaviorally active for *Conophthorus* spp. or other scolytids.

Locations, Host Species, and Beetles Species. *Conophthorus ponderosae* assays were conducted in a *Pinus ponderosa* clone bank managed by the U.S. Department of Agriculture (USDA) Forest Service, Tahoe National Forest, near Foresthill, California; the nominal species *C. ponderosae* (= *C. monticolae* Wood) (Wood 1977) was tested in a *P. monticola* seed orchard managed by the USDA Forest Service, Idaho Panhandles National Forests, Coeur d'Alene, Idaho; *Conophthorus conicolens* Wood on *Pinus pseudostrobus* Lindl. was studied in a seed production area managed by the *ejido* (commune) of Nuevo San Juan Parangaricutiro in the state of Michoacán, Mexico; *Conophthorus teocotum* Wood was assayed in 1997 and 1998 in a sparse stand of *Pinus teocote* Schl. & Cham. near the town of Paracho, Michoacán, Mexico; trials for *C. coniperda* on *Pinus strobus* L. were conducted at Beech Creek seed orchard (USDA Forest Service, Nantahala National Forest) in Murphy, North Carolina; and trials of *C. resinosae* in *Pinus resinosa* Ait. were conducted in 1997 and 1998 in a seed orchard in Thessalon, Ontario (Table 2).

Beetle Trapping. Release devices for the behavioral chemicals were attached to the vanes of yellow Japanese beetle traps (Trécé, Inc., Salinas, California) fitted with either Mason jars filled with about 25 mL of propylene glycol (de Groot *et al.* 1991; de Groot and DeBarr 1998) or 250-mL polypropylene collecting jars (Nalge Nunc International, Rochester, New York) containing 2.4-cm^2 pieces of 2,2-dichlorovinyldimethylphosphate (Pest Strip, Loveland Industries, Inc., Greeley, Colorado) as killing agent. Baited traps were suspended in the upper one-third of the crown of the trees (de Groot and Zylstra 1995; de Groot and DeBarr 1998). Numbers of replicates ranged from 6 to 10, and the minimum distance between traps was 15 m. For all tests conducted in California, Idaho, and Mexico, controls were replicated five times regardless of the number of treatment replicates, because power analyses demonstrated that this number of replications was sufficient to detect meaningful differences between treatments and controls. In North Carolina and Ontario, traps were rotated randomly each week, to avoid potential position effects. In California, Idaho and Mexico, traps were left in place for the entire exposure period, because earlier work (JD Stein, unpublished data) showed that tree

TABLE 1. Description of semiochemical-releasing devices.

Semiochemical*	Release device	Release rate (mg/day)
(±)-*trans*-Pityol	Polyethylene bubblecap	0.14
Conophthorin (neat)	Eppendorf tube, 1.5 mL	0.25
Conophthorin (10%)	Eppendorf tube, 1.5 mL	0.025
Conophthorin (5%)	Eppendorf tube, 1.5 mL	0.0125
4-Allylanisole	Polyethylene vial	75
trans-Verbenol	Polyethylene bubblecap	2.9
S-(–)-Verbenone	Polyethylene bubblecap	4.5
(*E*)-2-Hexen-1-ol	Polyethylene bubblecap	3.0
(–)-α-Pinene	Polyethylene bubblecap	120

*Chemical purities were >97%, except for 4-allylanisole, which was 80%. Enantiomeric purity of chiral compounds was as follows: *trans*-pityol, racemic; conophthorin, racemic; *trans*-verbenol, 80:20 (–):(+); verbenone, 85:15 (–):(+); α-pinene, 95:5 (–):(+).

position had no effect on beetle response. Traps were deployed as soon as sites were accessible in the spring, which according to preliminary tests was roughly coincident with the start of beetle flight. Traps were monitored on a biweekly basis and were removed after two successive samples yielded no beetle catches (mid- to late summer). Beetles were collected from the jars, identified, tallied, and their sex determined (Herdy 1959). Voucher specimens were deposited in the Essig Museum of Entomology, University of California, Berkeley, California.

Experimental Design and Statistical Analyses. For the California, Michoacán, and Idaho studies, treatments were replicated from 5 to 10 times in a completely randomized design, with one treatment per tree. Trap counts were analyzed with generalized linear models for over-dispersed Poisson distributed responses (counts) (McCullough and Nelder 1989). Results are tabulated as estimated means with 95% confidence intervals of the true means as a measure of the spread around the estimates of the means. The asymmetry of the confidence intervals about the means reflects the skewness of the data distributions. The 95% confidence intervals were not used for multiple comparisons, because the model is not a regular linear model. Rather, we used the maximum likelihood ratio test with the Bonferroni approach for multiple comparisons (experimentwise $\alpha = 0.05$). The North Carolina and Ontario studies were designed as randomized complete block designs, with treatments replicated one time per block. The blocks were replicated from 6 to 10 times. SAS GENMOD procedures (SAS Institute Inc. 1997) were used for the data analysis.

Results

Trap counts (Table 2) are expressed as mean numbers of male beetles per trap, because in all experiments the absolute numbers of females caught was low. The number of beetles caught in unbaited traps in all tests was not different from zero ($\alpha = 0.05$).

Experiment 1: *Conophthorus ponderosae* on *P. ponderosa*. 4-Allylanisole, conophthorin, and verbenone caught virtually no *C. ponderosae* beetles when deployed solo in the *P. ponderosa* stand in California. Pityol-baited traps caught more beetles than control traps and, when verbenone was added to pityol, trap catch was enhanced.

TABLE 2. Response of male *Conophthorus* spp. to traps baited with semiochemicals.

Experiment	No. of male beetles/trap*	95% confidence interval
1. *C. ponderosae* on *P. ponderosa* (10†)—Foresthill, California (39°05′N, 120°45′W), 1996		
Control (unbaited)	0.2*ab*	0.0–8.0
Pityol	11.8*c*	8.4–16.6
4-Allylanisole	0.2*a*	0.0–2.7
Conophthorin	1.6*ab*	0.6–4.0
Verbenone	0.0*a*	0.0–0.0
Pityol + 4-allylanisole	4.9*bc*	2.9–8.3
Pityol + conophthorin	1.0*ab*	0.3–3.2
Pityol + verbenone	23.8*d*	18.7–30.2
Pityol + 4-allylanisole + verbenone	5.9 *bc*	3.5–9.8
2. *C. ponderosae* on *P. ponderosa* (10†)—Foresthill, 1997		
Control (unbaited)	0.5*a*	0.1–3.8
Pityol	32.7*b*	25.1–42.6
4-Allylanisole	1.1*a*	0.3–4.3
Conophthorin	2.5*a*	1.0–6.2
Verbenone	0.4*a*	0.0–3.9
Pityol + 4-allylanisole	11.1*c*	7.2–17.1
Pityol + conophthorin	1.4*a*	0.4–4.7
Pityol + verbenone	26.5*b*	20.0–35.0
Pityol + 4-allylanisole + conophthorin + verbenone	0.6*a*	0.1–3.8
Pityol + 2× verbenone	71.2*d*	56.0–90.6
3. *C. ponderosae* on *P. ponderosa* (10†)—Foresthill, 1998		
Control (unbaited)	1.9*a*	0.6–6.1
Pityol	17.9*bcd*	2.2–26.2
Pityol + 1× 4-allylanisole	7.9*abc*	4.5–14.0
Pityol + 3× 4-allylanisole	5.4*a*	2.7–10.8
Pityol + 5× 4-allylanisole	7.3*ab*	4.0–13.3
Pityol + 1× verbenone	20.8*cd*	14.6–29.6
Pityol + 2× verbenone	25.5*d*	18.5–35.1
Pityol + 4× verbenone	20.6*cd*	14.4–29.4
4. *C. ponderosae* on *P. ponderosa* (10†)—Foresthill, 1999		
Control (unbaited)	1.6*abc*	0.2–14.8
Pityol	51.3*d*	38.6–68.3
Hexenol	3.1*a*	1.0–9.9
Pityol + conophthorin	2.8*b*	0.8–9.5
Pityol + conophthorin10	18.4*ce*	11.4–29.6
Pityol + conophthorin5	30.8*de*	21.3–44.5
Pityol + hexenol	27.9*de*	18.9–41.1
Pityol + verbenone	42.1*de*	30.7–57.7
Pityol + 2× verbenone	42.4*de*	31.0–58.1
Pityol + conophthorin + 2× verbenone	5.4*abc*	2.2–13.0

TABLE 2 (*continued*).

Experiment	No. of male beetles/trap*	95% confidence interval
5. *C. ponderosae* on *P. monticola* (10†)—Coeur d'Alene, Idaho (47°40′N, 116°45′W), 1998		
Control (unbaited)	2.6*a*	0.3–26.3
Pityol	69.3*b*	45.2–106.3
Pityol + 4-allylanisole	37.3*bc*	20.2–68.8
Pityol + conophthorin	5.0*ac*	1.0–26.3
Pityol + verbenone	67.2*b*	41.6–108.7
Pityol + 2× verbenone	79.4*b*	52.2–120.8
Pityol + α-pinene	208.2*d*	160.7–269.7
6. *C. conicolens* on *P. pseudostrobus* (10†)—Parangaricutiro, Mexico (19°25′N, 102°15′W), 1997		
Control (unbaited)	0.0*a*	0.0–0.0
Pityol	1.0*a*	0.5–2.0
4-Allylanisole	0.0*a*	0.0–0.0
Conophthorin	0.0*a*	0.0–0.0
Verbenone	0.1*a*	0.0–0.9
Pityol + 4-allylanisole	5.6*b*	4.2–7.5
Pityol + conophthorin	0.2*a*	0.0–0.9
Pityol + verbenone	0.9*a*	0.4–1.9
Pityol + 4-allylanisole + conophthorin + verbenone	1.5*a*	0.9–2.6
7. *C. conicolens* on *P. pseudostrobus* (10†)—Parangaricutiro, 1998		
Control (unbaited)	0.0*a*	0.0–0.0
Pityol + 4-allylanisole	10.6*b*	7.3–15.5
Pityol + 4-allylanisole + conophthorin	0.9*ac*	0.2–3.3
Pityol + 4-allylanisole + verbenone	4.5*bc*	2.5–8.0
Pityol + 4-allylanisole + conophthorin + verbenone	1.1*ac*	0.3–3.6
8. *C. teocotum* on *P. teocote* (5†)—Paracho, Mexico (19°39′N, 102°05′W), 1997		
Control (unbaited)	0.0*a*	0.0–0.0
Pityol	4.0*a*	1.7–9.2
Pityol + 4-allylanisole	5.4*a*	2.5–11.9
Pityol + conophthorin	0.2*a*	0.0–15.1
Pityol + verbenone	4.0*a*	1.6–10.0
9. *C. teocotum* on *P. teocote* (10†)—Paracho, 1998		
Control (unbaited)	0.0*a*	0.0–0.0
Pityol	2.7*ab*	1.5–4.9
Pityol + 4-allylanisole	5.2*b*	3.4–8.1
Pityol + conophthorin	0.2*a*	0.0–1.8
Pityol + verbenone	0.6*a*	0.1–2.1
Pityol + 4-allylanisole + verbenone	2.1*ab*	1.1–4.2
Pityol + 4-allylanisole + conophthorin + verbenone	1.6*ab*	0.7–3.4
10. *C. coniperda* on *P. strobus* (6†)—Murphy, North Carolina (35°10′N, 84°15′W), 1997		
Control (unbaited)	0.2*a*	0.1–5.8
Conophthorin	0.2*a*	0.1–5.8
Verbenone	0.3*a*	0.2–3.5

TABLE 2 (*concluded*).

Experiment	No. of male beetles/trap*	95% confidence interval
Pityol + α-pinene	26.0*b*	15.4–45.2
Pityol + α-pinene + conophthorin	5.7*ac*	2.5–13.3
Pityol + α-pinene + verbenone	12.5*bc*	6.6–24.2
Pityol + α-pinene + conophthorin + verbenone	13.5*bc*	7.2–25.7
11. *C. coniperda* on *P. strobus* (6†)—Murphy, 1997		
Control (unbaited)	0.2*a*	0.2–0.2
4-Allylanisole	1.3*a*	0.2–11.4
Pityol + α-pinene	45.7*b*	26.8–79.8
Pityol + α-pinene + 4-allylanisole	135.7*c*	88.8–214.7
12. *C. coniperda* on *P. strobus* (10†)—Murphy, 1998		
4-Allylanisole	0.1*a*	0.0–0.7
Pityol + α-pinene	4.8*b*	1.7–14.7
Pityol + α-pinene + 1× 4-allylanisole	8.3*b*	3.4–22.6
Pityol + α-pinene + 2× 4-allylanisole	11.6*b*	5.0–30.0
Pityol + α-pinene + 4× 4-allylanisole	5.9*b*	2.2–17.2
13. *C. resinosae* on *P. resinosa* (6†)—Thessalon, Ontario (46°20′N, 83°35′W), 1997		
Control (unbaited)	0.8*a*	0.2–3.4
Pityol	136.0*b*	112.0–165.2
Pityol + *trans*-verbenol	126.7*b*	104.1–154.4
Pityol + verbenone	137.9*b*	113.6–167.4
Pityol + *trans*-verbenol + verbenone	114.6*b*	93.8–140.1
14. *C. resinosae* on *P. resinosa* (8†)—Thessalon, 1997		
Control (unbaited)	0.0*a*	0.0–0.0
Pityol	15.4*b*	13.5–17.7
Pityol + 4-allylanisole	19.9*c*	17.4–22.7
4-Allylanisole	0.1*a*	0.1–0.1
15. *C. resinosae* on *P. resinosa* (10†)—Thessalon, 1998		
Pityol	117.3*a*	89.6–153.7
Pityol + 4-allylanisole	119.9*a*	91.7–156.9
Pityol + 2× 4-allylanisole	110.5*a*	84.2–145.1
Pityol + 4× 4-allylanisole	102.0*a*	77.5–134.4

NOTE: Treatments: 3× 4-allylanisole, triple dose of 4-allylanisole; 4× 4-allylanisole, quadruple dose of 4-allylanisole; 2× verbenone, double dose of verbenone; 4× verbenone, quadruple dose of verbenone; conophthorin10, 10% conophthorin in octanol; conophthorin5, 5% conophthorin in octanol.

*Means within an experiment followed by the same letter are not significantly different (likelihood ratio test, $\alpha = 0.05$). The Bonferroni approach was used to interpret the significance of multiple comparisons.

†Number of replicates.

Adding 4-allylanisole to pityol reduced trap catch. The combination pityol + 4-allylanisole + verbenone was not different from pityol + 4-allylanisole without verbenone.

Experiment 2: *Conophthorus ponderosae* on *P. ponderosa*. The number of beetles was higher in pityol-baited traps than in unbaited traps. No cone beetles were caught in traps baited with 4-allylanisole, conophthorin, or verbenone. Adding conophthorin or 4-

allylanisole to pityol significantly reduced trap catch. The addition of a single bubblecap of verbenone to pityol did not increase trap catch as it did in 1996, but adding two verbenone bubblecaps did increase trap catch by pityol.

Experiment 3: *Conophthorus ponderosae* on *P. ponderosa*. Pityol-baited traps caught more beetles than unbaited control traps. There was no difference in trap catches using one-, three-, or five-vial release devices of 4-allylanisole with pityol, and only the triple-vial treatment was significantly lower than pityol alone. There was no difference between the single, double, and quadruple dosages of verbenone added to pityol. None of the trap catches for the combinations of pityol and verbenone was significantly higher than those for pityol alone.

Experiment 4: *Conophthorus ponderosae* on *P. ponderosa*. Traps baited with pityol caught more beetles than unbaited control traps. Both 100% conophthorin and 10% conophthorin combined with pityol reduced beetle trap catch below the level of pityol alone, but 5% conophthorin did not. The number of beetles trapped in treatments using both pityol and hexenol was not significantly different from traps baited with pityol alone and adding verbenone, in either single or double dosages, did not increase trap catch by pityol.

Experiment 5: *Conophthorus ponderosae* on *P. monticola*. Pityol-baited traps caught more beetles than unbaited traps. Adding verbenone, at either single or double dosages, did not affect trap catch by pityol. The addition of 4-allylanisole did not reduce trap catch. Adding α-pinene to pityol increased trap catch by pityol, whereas adding conophthorin reduced trap catch to the level of unbaited control traps.

Experiment 6: *Conophthorus conicolens* on *P. pseudostrobus*. Pityol-baited traps did not catch significantly more *C. conicolens* than unbaited traps, but adding 4-allylanisole to pityol increased the response significantly. Adding either conophthorin or verbenone to pityol did not affect trap catch when compared with pityol alone, but adding both to pityol + 4-allylanisole (the most attractive combination) did reduce beetle trap catch.

Experiment 7: *Conophthorus conicolens* on *P. pseudostrobus*. Based on our 1997 results, pityol combined with 4-allylanisole was used as the standard attractive blend. As in 1997, traps baited with a combination of pityol + 4-allylanisole attracted more beetles than unbaited traps. The addition of verbenone did not affect the trap catch of pityol + 4-allylanisole significantly, whereas the addition of either conophthorin alone or conophthorin + verbenone reduced trap catch by pityol + 4-allylanisole.

Experiment 8: *Conophthorus teocotum* on *P. teocote*. There were no significant differences between any of the 1997 treatments. The stand is heterogeneous and the number of replicates (five) was the lowest of any of our experiments, thus there may not have been sufficient power to detect meaningful differences. Results are reported here because the trends, while lacking sufficient power for testing hypotheses, are consistent with those of Experiment 9, which was carried out at the same site.

Experiment 9: *Conophthorus teocotum* on *P. teocote*. Traps baited with pityol + 4-allylanisole caught more *C. teocotum* than unbaited traps. Traps baited with only pityol did not trap significantly more beetles than unbaited traps. Adding either verbenone or verbenone + conophthorin to traps baited with 4-allylanisole + pityol did not significantly reduce trap catches.

Experiments 10 and 11: *Conophthorus coniperda* on *P. strobus*. In Experiment 10, traps baited with either verbenone or conophthorin caught no more *C. coniperda* than did unbaited traps. Traps baited with pityol + α-pinene caught more beetles than did unbaited traps. Adding conophthorin to the pityol + α-pinene blend reduced trap catch by 75%, but this difference was not significant. Paradoxically, adding verbenone to the pityol + α-pinene blend did not significantly reduce the numbers of beetles trapped, whereas the addition of verbenone to the pityol + α-pinene + conophthorin blend was no different from the blend without conophthorin (de Groot and DeBarr 2000). In Experiment 11, traps baited with 4-allylanisole caught no more beetles than unbaited traps, whereas traps baited with pityol + α-pinene caught more beetles than did unbaited traps. Adding 4-allylanisole to the pityol + α-pinene blend resulted in an almost threefold increase in the number of beetles caught.

Experiment 12: *Conophthorus coniperda* on *P. strobus*. Traps baited with either pityol + α-pinene or pityol + α-pinene + 4-allylanisole caught more beetles than did unbaited traps. Unlike in 1997, there was no difference between traps baited with pityol + α-pinene and those baited with pityol + α-pinene + 4-allylanisol. There was also no difference between catches for traps baited with one-, two-, or four-vial release devices of allylanisole. Beetle response was much lower in 1998 than in 1997 however, as indicated by the numbers trapped by the same lures in the 2 years, which suggests that beetle populations were also much smaller.

Experiments 13 and 14: *Conophthorus resinosae* on *P. resinosa*. Pityol-baited traps caught significantly more beetles than unbaited traps. Adding verbenone, *trans*-verbenol, or both did not reduce trap catch by pityol. Traps baited with only 4-allylanisole did not trap more beetles than unbaited traps. Adding 4-allylanisole to traps baited with pityol significantly increased beetle trap catch, but the absolute amount of the increase was not large.

Experiment 15: *Conophthorus resinosae* on *P. resinosa*. Mean numbers of beetles in traps baited with pityol plus 1-, 2-, or 4-vial release devices of 4-allylanisole did not differ from pityol-baited traps.

Discussion

For every *Conophthorus* species studied, conophthorin consistently reduced trap catches of males by the attractive blend (either pityol or pityol + synergist). Conophthorin, verbenone, and 4-allylanisole never attracted significant numbers of cone beetles when presented solo. Pityol deployed alone attracted more beetles than did unbaited traps for all species tested except for the Mexican species *C. conicolens* and *C. teocotum*. 4-Allylanisole synergized male response to pityol in *C. conicolens* and *C. coniperda*, whereas α-pinene synergized male response to pityol in both *C. ponderosae* on *P. monticola* (Miller *et al.* 2000) and *C. coniperda* on *P. strobus*.

One, two, or four verbenone bubblecaps added to traps baited with pityol were equally effective in attracting *C. ponderosae* on *P. ponderosa* in California. One, three, or five vials of 4-allylanisole were likewise equally effective in reducing trap catches of *C. ponderosae* in pityol-baited traps in California. These results suggest that the most cost-effective strategy for using either of these semiochemicals is the deployment of a single release device per trap. Although we did not test octanol by itself for behavioral activity, the results (Exp. 4) suggest that octanol is behaviorally neutral (*i.e.*, the responses

appear to reflect simply the range of conophthorin release rates rather than any effects of the diluent).

The response of *Conophthorus* spp. to α-pinene appears to follow a pattern that reflects host (*Pinus*) phylogeny, at least among cone beetles tested to date. The major divisions of the genus are the subgenus *Pinus* (also known as Diploxylon, yellow, or hard pines) and the subgenus *Strobus* (also known as Haploxylon, white, or soft pines) (Perry 1991; Price *et al.* 1998). It has not yet been determined which subgenus of *Pinus* is ancestral, *Pinus* or *Strobus* (Millar and Kinloch 1991). There is a divergence of beetle behavior that appears to track the divergence of the subgenera of *Pinus*. Both *C. coniperda* on *P. strobus* and the nominal species *C. ponderosae* on *P. monticola* infest Haploxylon pines, and both use α-pinene as a synergist for pityol. Mitochondrial DNA sequences suggest that *C. ponderosae* on *P. monticola* in Idaho is a different species from *C. ponderosae* on *P. ponderosa* in California (NG Rappaport, unpublished data). In contrast, *C. ponderosae* on *P. ponderosa* and *C. resinosae* on *P. resinosa* (de Groot and Zylstra 1995) infest Diploxylon pines, and their response is not synergized by α-pinene. This trend does not appear to be related to the production of α-pinene by the host trees, because *P. resinosa* produces α-pinene as the major constituent (90%), by far, of its xylem resins, whereas *P. ponderosa* (in California) produces extremely small amounts of α-pinene (1–6%) (Mirov 1961; Smith 1977). Southern pines are also Diploxylon pines, and cone beetles are notably absent in these species, with the single exception of *Conophthorus echinatae* Wood on *Pinus echinata* Mill., which is restricted to a small part of the range of *P. echinata* in southern Missouri (Hedlin *et al.* 1980). We have not yet tested α-pinene as a synergist for pityol in the Mexican (Diploxylon) pines, but such tests are planned. Further assays of *Conophthorus* spp. in other Haploxylon and Diploxylon pines may shed further light on this trend.

The unique response of *C. ponderosae* on *P. ponderosa* to 4-allylanisole and verbenone is also of interest. This beetle–host combination was the only one studied in which 4-allylanisole repelled beetles. Beetles on all other hosts tested responded to 4-allyanisole either neutrally or as a synergist to the female-produced pheromone pityol. This result may stem from (*i*) interspecific genotypic variation, assuming that the populations of *C. ponderosae* on *P. ponderosa* and *P. monticola* are separate species; (*ii*) intraspecific genotypic variation in cone beetle response, assuming that the beetles on *P. ponderosa* and *P. monticola* are conspecific; or (*iii*) differing host volatiles that are an inadvertent part of the semiochemical bouquet. *Pinus ponderosa* is the only species of pine included in our study that was shown to contain 4-allylanisole as a measurable constituent of xylem resins (Mirov 1961), and its xylem resins are uniquely high in the monoterpene Δ-3-carene (Smith 1977). Further investigation is needed to understand the relationship between host monoterpene release and beetle response.

The response by *C. ponderosae* on *P. ponderosa* to verbenone as a synergist for pityol, although not consistent, was also unique in our studies. Tests in North Carolina (de Groot and DeBarr 2000) suggest that verbenone may interrupt the response of *C. coniperda* to pityol, although not as effectively as does conophthorin. The pattern of response of *C. ponderosae* on *P. ponderosa* to traps baited with both pityol and verbenone appears to be correlated with beetle population densities. Populations of *C. ponderosae* at Foresthill Clone Bank have gradually increased in the absence of control measures, so we have the opportunity to compare the effect of verbenone on the attractiveness of pityol over a range of cone beetle population densities (as measured by response to pityol-baited traps). During the 4 years when both pityol and pityol + verbenone were tested at Foresthill Clone Bank, there was an inverse exponential relationship between beetle populations and synergism by verbenone. Thus, when beetle populations were low, verbenone served as a synergist. As beetle populations increased, this synergistic effect diminished to zero. This phenomenon could be an adaptation to

enhance mate location when populations are low. We did not have sufficient replication within each year (only a single replicate) to test the fit of the model for this hypothetical explanation.

Trap catches of beetles in all the Mexican pines studied were low, despite apparently high levels of cone beetle attack in these stands. It appears likely that there are important pheromone components for these species that we have not yet identified.

The responses by *Conophthorus* spp. seen in this study were frequently counterintuitive, either when viewed against the background of the behavioral chemistry of other scolytids or when examined for consistency within the genus *Conophthorus*. For example, both verbenone and 4-allylanisole have been generally described as interruptants for other scolytids (Hayes and Strom 1994; Borden 1997). In our study, however, 4-allylanisole was shown to function variably as a synergist for the female attractant pityol, an interruptant to the response of male beetles to pityol, or as behaviorally neutral. Verbenone served as a synergist for pityol in at least some of our tests with *C. ponderosae*. These results underscore the need for caution in attempting to predict behavioral responses of *Conophthorus* spp. based on past results with congeners or other scolytids.

Pest Management Implications. Our studies have identified three potential synergists for use in trap-out strategies: (*i*) α-pinene for *C. ponderosae* attacking *P. monticola*; (*ii*) 4-allylanisole for *C. coniperda* attacking *P. strobus*, *C. conicolens* attacking *P. pseudostrobus* and, perhaps, for *C. teocotum* attacking *P. teocote*; and (*iii*) possibly verbenone for *C. ponderosae* attacking *P. ponderosa*. Such synergists should prove helpful in designing successful trap-out, mating-disruption, or "push-pull" pest control strategies. 4-Allylanisole shows promise as an interruptant/repellent for cone protection in at least some *Conophthorus* spp. tested, as does conophthorin for all species tested in the genus.

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