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Factors affecting capture of the white pine cone beetle, *Conophthorus coniperda* (Schwarz) (Col., Scolytidae) in pheromone traps

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Abstract: The white pine cone beetle, ***Conophthorus coniperda,*** is a serious pest of seed orchards. The sex pheromone (+)-*trans*-pityol, (2*R*,5*S*)-2-(1-hydroxy-1-methylethyl)-5-methyltetrahydrofuran, shows considerable promise to manage the cone beetle populations in seed orchards. Our work confirms that pityol is an effective attractant to capture male ***C. coniperdu.*** Traps need to be placed in the tree crown, preferably in the cone-bearing region, to trap out more insects. Japanese beetle traps were superior to the Lindgren funnel traps in capturing insects and trap colour had no significant effect. Commercially available bubble caps for dispensing pheromone were as effective as the experimental 'vial and wick' and the glass capillary tube units. Pityol released at about 0.1 mg . day-' (100 female equivalents) was effective, and higher (more expensive) rates did not significantly improve trap catch.

1 Introduction

The white pine cone beetle, ***Conophthorus coniperda*** (Schwarz), is found throughout the range of eastern white pine, ***Pinus strobus*** L. (Wood, 1982) and is a serious pest in seed orchards (DeBarr et al., 1982; De Groot, 1990). Current pest management is limited to burning infested cones while on the ground, but this technique requires adequate fuel and ideal burning conditions (Wade et al., 1989). The pheromones of this insect have been identified recently (Birgersson et al., 1995). The female-produced compound (+)-*trans*-pityol, (2*R*,5*S*)-2-(1-hydroxy-1-methylethyl)-5-methyltetrahydrofuran, holds considerable promise as an attractant to trap out males in an orchard, thus lowering subsequent populations (Birgersson et al., 1995; De Groot et al., 1997).

A number of factors can influence the success and rate of capture of insects in pheromone traps (Muirhead-Thompson, 1991). Pheromone lure dosage and release devices can influence how traps perform relative to virgin females (and/or males) and thus affect how well they attract insects. Insects may respond more to a trap of one colour over another, and certain trap designs can capture insects more efficiently. Similarly, the placement of the traps near, within, or away from the host plant(s) may or may not affect trap capture, depending on the link between host- and mate-finding. The objectives of our work reported herein were to examine (i) the attractiveness of pityol-baited traps of different colours and types, (ii) the location relative to the host plant, (iii) pheromone release devices, and (iv) pheromone release rates, in order to develop techniques for use in a trap out program for ***C. coniperda*** in seed orchards.

2 Materials and methods

2.1 Study sites

Field-trapping experiments were conducted from 1991 to 1995 in five locations in North America: two white pine seed orchards near Murphy (United States Forest Service, Beech Creek) and Morganton (North Carolina Forest Service), North Carolina, USA; a seed orchard (Ontario Ministry of Natural Resources) near Orono, Ontario, Canada; and two natural forests near Sault Ste. Marie, Ontario, Canada (Pancake Bay Provincial Park and Wells Township).

2.2 Synthetic pheromones and monoterpenes

The synthetic pheromone (±)-*trans*-pityol was used in all of the experiments and was 95.4% pure, contained 1.2% of the ***cis*** isomer and was prepared from (±)-sulcatol (97% optically pure, Phero Tech, Inc., Delta, British Columbia, Canada) by the method described in Pierce et al. (1995). Previous work by Birgersson et al. (1995) has shown that (±)-*trans*-pityol (hereafter referred simply as pityol) is as effective as the beetle-produced (+)-*trans*-pityol for capturing insects in traps. The monoterpene hydrocarbons, (±)-α-pinene (98% pure) and (1*S*)-(−)-β-pinene (99%), were purchased from Aldrich Chemical Co., Milwaukee, Wisconsin, USA; decane (99% +) and n-octane (99% +) were purchased from Sigma Chemical Co., St. Louis, Missouri, USA.

2.3 Chemical release devices and traps

Four release devices were used: glass capillary tubes, 2-ml glass vials with cotton wicks, plastic bubble caps (Phero Tech, Inc.) and 250-μl polyethylene centrifuge tubes. Glass capillary tubes were used for pityol only and were ≈ 2.5 cm long and heat-sealed at one end. Pityol (2–5 μl) was loaded into the capillary tubes with a microsyringe. The loaded capillary tubes were then placed inside 400-μl polypropylene centrifuge tubes,

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in-which four, equally spaced 0.5 mm holes were made to facilitate the release of volatiles and to protect the capillary tubes from moisture and dust. In all experiments where the capillary tubes were used. the inside diameter (I.D.) was 1 .04 mm, except in expt 1 (table 1), where a 0.70-mm-I.D. tube also was included. Three release rates of pityol from capillary tubes were tested at 24°C: (1) 0.108 ± 0.012 (S.D.) mg neat pityol. day-’ from a 1.06mm-I.D. tube, (2) 0.050 ± 0.001 mg neat pityol. day-’ from a 0.70-mm-I.D. tube, and (3) 0.021 ± 0.001 mg pityol · day-’ (diluted 1: 10 in decane) from a 1.04-mm-I.D. tube. For the 2-ml glass vial, a 5-cm length of 1.6-mm-I.D. Teflon@ tubing containing a cotton wick (‘vial and wick’) was inserted through a hole cut in the screw top to facilitate the release of the volatiles. Each vial contained 0.67 μl pityol, 100 μl a-pinene and 1900 μl n-octane, with a release rate of 2 ml · week-’ at 25°C in the laboratory. These

Table 1. Description of experiments designed to examine the effects of different release rates of pityol, trap colour, trap position in tree crown, pheromone release devices, and trap types on the response of C. coniperda ***to baited traps***

Experiment I
Objective: to determine the effect of release rate of pityol from glass capillary tubes.
Locution: Pancake Bay Provincial Park, ON, Canada.
Treatments: 3 μl of 10% pityol in decane, 1.04-mm-I.D. glass tube, 3 μl of 100% pityol in 0.70-mm-I.D. glass tube, 3 μl of 100% pityol in 1.04-mm-I.D. glass tube and unbaited trap; 12 replicates, 6 each collected weekly on 23 and 30 May, 1991.

Experiment 2
Objective: to determine the effect of release rate of pityol from bubble caps and glass capillary tube.
Locution: Orono Forest Station Seed Orchard, Orono, ON, Canada.
Treatments: 4 mg pityol in bubble cap, 14 mg pityol in bubble cap, 40 mg pityol in bubble cap, 135 mg pityol in bubble cap, 5 μl pityol in 1.04-mm-I.D. glass tube and unbaited trap; 16 replicates, 8 each collected weekly on 18 and 25 May, 1994.

Experiment 3
Objective: to determine the effect of release rate of pityol from bubble cap, and vial and wick lures each with a-pinene.
Location: Beech Creek Seed Orchard, Murphy, NC, USA.
Treatments: same four bubble cap lures as in expt 2, 2 μl pityol in 1.04-mm-I.D. glass tube, each of these five lures with 22.5 μl cl-pinene in 250 μl polyethylene centrifuge tube and 2 ml glass vial with 0.67 μl pityol and 100 μl a-pinene diluted in 1900 μl n-octane; 24 replicates, 8 each collected on 22 and 26 April, and 4 May, 1994.

Experiment 4
Objective: to determine the effect of release rate of pityol from bubble cap, and vial and wick lures each with a-pinene.
Location: Morganton Seed Orchard, Murphy, NC, USA.
Treatments: same four bubble cap lures as in expt 2, 2 μl pityol in 1.04-mm-I.D. glass tube, each of these five lures with 225 μl s-pinene in 250 μl polyethylene centrifuge tube and 2 ml glass vial with 0.67 μl pityol and 100 μl a-pinene diluted in 1900 μl n-octane; 72 replicates, 8 each collected on 14, 21, and 29 April, 5, 12, 19. and 26 May, and 2 and 13 June, 1994.

Experiment 5
Objective: to determine the effect of trap colour and a-pinene on trap catch.
Locution: Orono Forest Station Seed Orchard, Orono, ON, Canada.
Treatments: 2 μl pityol in 1.04-mm-I.D. glass tube and 225 μl a-pinene in 250 μl polyethylene centrifuge tube in red, green, blue, black, white or unpainted yellow Japanese beetle traps; 12 replicates, 6 each collected weekly on 9 and 16 June, 1993.

Experiment 6
Objective: to determine the effect of trap colour on trap catch.
Locution: Orono Forest Station Seed Orchard, Orono, ON, Canada.
Treatments: 40mg pityol in bubble cap in red, green, blue, black, white, yellow or unpainted yellow Japanese beetle traps; 28 replicates, 7 each collected weekly on 24 and 31 May, and 7 and 14 June, 1995.

Experiment 7
Objective: to determine the effect of trap height and trap type on trap catch.
Locution: Beech Creek Seed Orchard, Murphy, NC, USA.
Treatments: 2 ml glass vial with 0.67 μl pityol and 100 μl a-pinene diluted in 1900 μl n-octane in unpainted yellow Japanese beetle traps or 12-unit black Lindgren funnel traps placed 2 m above the ground (low) or in the upper third of the tree crown (high); 72 replicates, 9 each collected on 13, 22 and 29 April, 6, 13, 22 and 27 May, and 5 June, 1992.

Experiment 8
Objective: to determine the effect of trap type and pheromone release device on trap catch.
Location: Morganton Seed Orchard, Murphy, NC, USA.
Treatments: 2 ml glass vial with 0.67 μl pityol and 100 μl a-pinene diluted in 1900 μl n-octane or 2 μl pityol in 1 .04-mm-I.D. glass tube with 225 μl α-pinene in 250 μl polyethylene centrifuge tube in unpainted yellow Japanese beetle traps or 12-unit black Lindgren funnel traps placed in the upper third of the tree crown; 16 replicates for Japanese beetle traps and 24 replicates for Lindgren funnel traps, 2 or 3 each, respectively, collected on 15, 22 and 29 April, 5, 12, 19 and 26 May, and 3 June, 1992.

Experiment 9
Objective: To determine the effect of trap height and α- and β-pinene on trap catch.
Location: Wells Township, natural white pine stand, 40 km north of Thessalon, ON, Canada.
Treatments: 2 μl pityol in 1.04-mm-I.D. glass tube, 2 μl pityol in 1.04-mm-I.D. glass tube with 225 μl a-pinene and β-pinene in separate 250 μl polyethylene centrifuge tubes and unbaited Japanese beetle traps placed 2 m above the ground on tree trunk (low) or in the upper third of the tree crown (high); 28 replicates, 7 each collected on 28 May and 3 June, 1992.

vials were designed to release about 0.1 mg pityol . day-‘, equivalent to 100 females (BIRGERSSON et al., 1995) and similar to the 1.04-mm-I.D. capillary tube. Plastic bubble caps were loaded with 4, 14, 40 or 135mg pityol to release ≈ 0.02, 0.07, 0.2 and 0.67 mg · day-‘, respectively (Phero Tech, Inc.). Polyethylene (250 μl) centrifuge tubes were used to release α- and β-pinene. α-Pinene was released at 12.1 ± 0.3 mg . day-’ and β-pinene was released at 9.6 ± 0.3 mg . day-’ at 24°C in the laboratory, from centrifuge tubes with 225 μl of these monoterpenes.

Two types of trap were used in the experiments. Yellow Japanese beetle trap tops (Trécé, Inc., Salinas, California, USA) were fitted with plastic 500-ml Mason® jar bottoms and were used in all experiments. The jar bottoms contained about 50 ml ethylene glycol to decrease surface tension, as well as to kill and preserve trapped insects. In expts 5 and 6 (table 1), the Japanese beetle trap tops were painted with a water-based acrylic enamel (Canadian Tire, Inc., Toronto, Ontario); red, green, blue, black, white or yellow: spectral reflectance values are shown in DE GROOT and ZYLSTRA (1995). Black, 12-unit Lindgren multiple-funnel traps (Phero Tech, Inc.) were used in expts 7 and 8 (table 1). Chemical release devices were hung or taped to one of the vanes of the Japanese beetle traps or clipped onto one of the plastic rods between funnels 6 and 7 of the Lindgren trap. In all experiments, traps were hung in the upper third of the tree crown, with one trap per tree, spaced 12-30 m apart. In expts 7 and 8, traps were suspended also 2m above the ground from metal poles near a sample tree, and in expt 9, traps were attached to the trunk of the sample tree, 2 m above the ground (table 1).

2.4 Field experiments and data analysis

Nine experiments were conducted with specific objectives, site location, materials and experimental set-up as described in table 1. Experiments l-4 examined the effects of release rates of pityol from glass capillary tubes, ‘vial and wick’, and commercially available plastic bubble caps. Experiments 5 and 6 examined the effects of trap colour and expts 7-9 examined the effects of trap height, trap type, pheromone release device, and α- and β-pinene on trap catch. For each experiment, traps were deployed in a complete randomized block design. Beetles were collected from the traps at approximately weekly intervals (table 1) and were preserved in 70% ethanol. Japanese beetle trap bottoms were cleaned and refreshed with ethylene glycol. On each collection date, treatment locations were reassigned randomly within a block, except for expts 3, 4 and 8. In experiments using vial and wick baits, new lures were installed on each collection date. Fresh capillary tube baits were used in all experiments except in expt 2 where capillary tubes were compared with the bubble caps, which were not replaced as these devices are designed to remain in the field for the entire flight period of 4-6 weeks. In the laboratory, insects were counted and their sex determined by examination of the abdominal tergites (HERDY, 1959).

Trap catches of males were transformed by log (x + 1) and analyzed by ANOVA as a randomized block design. Treatment means were compared using the Tukey test with α = 0.05 (SYSTAT® 6.1, 1997). Counts of captured females in traps were at or near to zero and therefore were not used in the analyses.

3 Results

3.1 Release rates

There was a general but weak trend whereby increased release rates of pityol from glass capillary tubes and bubble caps resulted in increased trap catch of male C. ***coniperdu*** (expts l-4, table2). Undiluted pityol from 1.04-mm-I.D. glass tubes caught significantly more cone beetles than a 10% dilution of pityol (exptl). Although trap catches increased with increased pheromone load in the bubble caps, there were no significant differences among the bubble caps, except in expt 2 where the 40-mg load caught more beetles than the 4-mg load, and in expt 3 where the 135-mg load caught more than the 4- and 14-mg loads. Beetle responses to the 4-, 14-, 40-, and 135-mg load bubble caps were as good as to pityol emitted from 1.04-mm-I.D. glass tubes in all experiments. The performance of the bubble caps and the glass tubes was equal to the vial and wick device in expt 3, but not as good in expt 4.

3.2 Trap colour, trap type and trap height

Although yellow traps caught about twice as many beetles as the other colours, trap catch was not significantly different when traps were baited with pityol and α-pinene (expt 5, table 3). In traps where α-pinene lure was absent, green traps caught significantly fewer beetles than red, blue, black and yellow traps (expt 6, table 3). Unpainted yellow Japanese beetle traps (standard colour from Trécé, Inc.) did not differ from painted yellow traps.

The standard yellow Japanese beetle traps were significantly superior to the Lindgren funnel traps in catching cone beetles (expts 7 and 8, table4). Traps placed in the cone-bearing region caught significantly more beetles than traps placed 2m above the ground, suspended from metal poles adjacent to trees, or attached to the tree’s trunk (expts 7 and 9, table 4). In expt 8, the vial and wick device was as effective as the glass capillary tube, supporting the results from expts 3 and 4. The addition of α- and β-pinene did not improve significantly the attractiveness of traps baited with pityol when placed in the tree’s crown or 2 m above the ground (expt 9).

4 Discussion

The results from these studies, coupled with our previous work (BIRGERSSON et al., 1995; DE GROOT et al., 1998), provide the techniques for developing a trapping out programme for C. ***coniperdu*** in seed orchards. This study confirms our previous work ***(op cit.)*** that pityol is an effective attractant for male C. ***coniperdu.*** Clearly, traps need to be placed in the tree crown, preferably in the cone-bearing region, to trap out more insects. The importance of trap height has been shown for other cone and seed insects (HANULA et al., 1984; GRANT et al., 1989). Even for monitoring and detection purposes, traps should be placed at the crown as very few insects were caught when traps were located away from there. Traps baited with the female-produced sex pheromone pityol and the dominant host-produced compound α-pinene (BIRGERSSON et al., 1995) were insufficient to attract males when placed away from the tree crown. This also suggests that yet undisclosed attributes of tree crown and insect search behaviour are very important to mate-finding of male cone beetles.

The Japanese beetle traps were superior to the Lind-

Table 2. Response of male* Conophthorus coniperda *to traps baited with various release rates of pityol

Expt	Treatment	Males caught mean ± S.E.[1]
1	3 μl of 10% pityol in 1.04-mm-I.D. glass tube	2.2 ± 1.0 ab
	3 μl of 100% pityol in 0.70-mm-I.D. glass tube	4.0 ± 1.3 bc
	3 μl of 100% pityol in 1 .04-mm-I.D. glass tube	9.8 ± 4.1 c
	Unbaited trap	0.1 ± 0.1 a
2	4mg pityol in bubble cap	1.9 ± 0.7 ab
	14 mg pityol in bubble cap	3.6 ± 0.9 bc
	40 mg pityol in bubble cap	7.9 ± 2.3 c
	135 mg pityol in bubble cap	4.6 ± 1.1 bc
	5 μl pityol in 1.04-mm-I.D. glass tube	2.6 ± 0.6 bc
	Unbaited trap	0.0 ± 0.0 a
3	4mg pityol in bubble cap with α-pinene[2]	0.4 ± 0.2 a
	14 mg pityol in bubble cap with a-pinene	0.3 ± 0.2 a
	40 mg pityol in bubble cap with a-pinene	1.1 ± 0.3 ab
	135 mg pityol in bubble cap with a-pinene	2.6 ± 0.6 b
	2 μl pityol in 1.04-mm-I.D. glass tube with a-pinene	0.9 ± 0.3 a
	0.7 μl pityol with 100 μl a-pinene in 2-ml glass vial	1.1 ± 0.3 ab
4	4 mg pityol in bubble cap with α-pinene[2]	0.2 ± 0.1 a
	14 mg pityol in bubble cap with a-pinene	0.4 ± 0.1 a
	40 mg pityol in bubble cap with a-pinene	0.4 ± 0.1 a
	135 mg pityol in bubble cap with a-pinene	0.6 ± 0.3 a
	2 μl pityol in 1 .04-mm-I.D. glass tube a-pinene	0.5 ± 0.2 a
	0.7 μl pityol with 100 μl a-pinene in 2-ml glass vial	1.0 ± 0.2 b

[1] Means followed by the same letter within an experiment are not significantly different at P ⩽ 0.05, Tukey test statistic.
[2] 225 μl a-pinene was loaded into centrifuge tubes with the pityol bubble cap and glass tube baits.

Table3. Response of male* Conophthorus coniperda *to traps baited with pityol or pityol and u-pinene in different colours of Japanese beetle traps

Expt	Treatment	Males caught, mean ± S.E.[1]
5	Red trap'	9.4 ± 2.2 a
	Green trap.	9.1 ± 3.5 a
	Blue trap	5.4 ± 1.8 a
	White trap	9.3 ± 4.4 a
	Black trap	10.1 ± 4.4 a
	Unpainted yellow trap	21.0 ± 7.3 a
6	Red trap'	8.0 ± 3.7 bc
	Green trap	1.0 ± 0.3 a
	Blue trap	6.2 ± 2.3 bc
	White trap	3.8 ± 0.9 ab
	Black trap	6.0 ± 1.6 bc
	Yellow trap	8.4 ± 2.6 bc
	Unpainted yellow trap	16.0 ± 5.3 c

Means followed by the same letter within an experiment are not significantly different at P 6 0.05, Tukey test statistic.
[2] Each trap was baited with 2 μl pityol with 225 μl a-pinene.
[3] Each trap was baited with a 40 mg pityol bubble cap.

gren funnel traps, but why this is so is undetermined because we do not know how beetles approach these traps and how they are captured. Trap size and barrier style (flat vertical vane for the Japanese Beetle versus multiple funnels for the Lindgren trap) may have an effect on capture efficiency. Moreover, it may be that the liquid capture system employed with the Japanese beetle traps retains more beetles than the standard dry trap bottoms of the Lindgren traps. With the exception of the lower trap response of beetles to the green-coloured traps in one experiment, which is likely to have been an anomaly, trap colour had no effect. These results are in agreement with similar work on the red pine cone beetle, ***C. resinosae*** (DE GROOT and ZYLSTRA, 1995). The commercially available bubble caps for dispensing pheromone were as effective as the experimental 'vial and wick' and the glass capillary tube units. The bubble caps were easier to handle (no loading and exposure to chemicals was involved by using applicator) and remained active in the field as long as 8 weeks (expt 4). Pityol released at about 0.1 mg . day-' (100 female equivalents) was effective, and higher, more expensive rates did not improve trap catch significantly.

Studies to determine the effectiveness of a pheromone-based, trap out programme for C. ***coniperda*** may now be feasible. Based on this study, the-research programme should use the standard yellow Japanese beetle trap baited with 14–40 mg pityol loaded in a plastic bubble cap, with or without u-pinene, and placed in the cone-bearing region of a tree. The density of traps per unit area (e.g. hectares) should be examined under various densities of cone populations. The pityol-baited traps could be deployed to capture males within the orchard and plant-based deterrents such as green leaf volatiles (WILSON et al., 1996), 4-allylanisole (HAYES

Table 4. Response of male *Conophthorus coniperda* ***to traps baited with pityol from different release devices and trap styles, at 2m above the ground (low) and at the upper half of the tree's crown (high)***

Expt	Treatment	Males caught, mean ± S.E.[1]
7	Japanese beetle trap high[2]	8.9 ± 1.0 b
	Japanese beetle trap low	0.0 ± 0.0 a
	Lindgren funnel trap high	0.3 ± 0.1 a
	Lindgren funnel trap low	0.1 ± 0.1 a
8	Capillary tube lure in Japanese beetle trap[3]	15.8 ± 1.6 a
	Vial and wick lure in Japanese beetle trap	14.9 ± 2.1 a
	Capillary tube lure in Lindgren funnel beetle trap	5.9 ± 1.4 b
	Vial and wick lure in Lindgren funnel beetle trap	7.0 ± 1.5 b
9	2 µl pityol in glass tube high[4]	1.4 ± 0.2 a
	2 µl pityol in glass tube with α-pinene and β-pinene, high	1.9 ± 0.3 a
	Unbaited trap high	0.0 ± 0.0 b
	2 µl pityol in glass tube low	0.1 ± 0.1 b
	Unbaited trap low	0.0 ± 0.0 b

[1] Means followed by the same letter within an experiment are not significantly different at $P \leqslant 0.05$, Tukey test statistic.
[2] Each trap was baited with 0.7 µl pityol and 100 µl α-pinene.
[3] Capillary tube lure was 2 µl pityol in 1.04-mm-I.D. glass tube and 225 µl α-pinene in 250 µl polyethylene centrifuge tube; vial and wick lure was a 2-ml glass vial with 0.7 µl pityol and 100 µl α-pinene diluted in 1900 µl n-octane.
[4] Japanese beetle traps, 2 µl pityol in 1.04-mm-I.D. glass tube, 225 µl α-pinene and β-pinene in separate 250-µl polyethylene centrifuge tubes.

et al., 1994) or beetle-produced compounds such as conophthorin (DE GROOT, 1992; BIRGERSSON et al., 1995; DE GROOT et al., 1997) could be used around the perimeter of the orchard to deter insects from entering.

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