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EVIDENCE FOR A MULTICOMPONENT SEX PHEROMONE IN THE YELLOWHEADED SPRUCE SAWFLY

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Abstract—The existence of a female-produced sex pheromone in the yellowheaded spruce sawfly, *Pikonema alaskensis* (Rohwer) (Hymenoptera: Tenthredinidae) was demonstrated by field and greenhouse bioassays. Virgin females, their empty cocoons (with which they were confined during handling procedures), and the hexane extract of these cocoons were attractive in the field. The only Florisil fraction of this extract consistently attractive by itself was that eluted with hexane, but three, more polar fractions (eluted with 5%, 25%, and 50% ether in hexane) each synergized the hexane fraction, increasing bioassay responses 10–30 times. Fractions derived directly from virgin females yielded comparable results. The greenhouse data corroborated the field data, except that the 5% ether–hexane fraction, while very synergistic in the field, was consistently inactive in the greenhouse.

Key Words—Sex pheromone, bioassay, synergism, sawfly, Hymenoptera Tenthredinidae, *Pikonema alaskensis*, experimental design.

INTRODUCTION

The pheromones of various diprionid sawflies in the genera *Diprion* and *Neodiprion* have been studied in depth (e.g., Coppel et al., 1960; Casida et al., 1963, Jewett et al., 1976; Matsumura et al., 1979), but relatively little has been published on pheromones of sawflies in other families. Borden et al. (1978) presented evidence for a sex pheromone in the pamphiliid, *Cephalcia lariciphila* Wachtl. In the Tenthredinidae, Longhurst and Baker (1980) demonstrated the existence of a pheromone in *Nematus ribesii* (Scop.), and Forbes and Daviault (1964) described male behavior in *Pristiphora geniculata*

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(Htg.) which was typical of responses to sex pheromones. No chemical identifications have been reported for these three species. This paper and the following (Bartelt et al., 1982) describe biological and chemical aspects of pheromonal communication in the yellowheaded spruce sawfly, *Pikonema alaskensis* (Rohwer) (Hymenoptera:Tenthredinidae).

P. alaskensis is a significant defoliator of young and/or open-grown trees of various spruce (*Picea*) species in Canada and the northern United States. Its biology has been described by Nash (1939). In Minnesota, the insect is a particular problem in young plantations of white spruce, *P. glauca* (Moench) Voss. It has one generation/year, adults emerging in late May or early June. The flight season coincides with the opening of the spruce buds, and the eggs are inserted in needles of the expanding shoots. The eggs hatch in about a week, and the larvae feed until early July, when they drop to the ground and form cocoons just under the soil surface. They overwinter as prepupal larvae and pupate about 2 weeks prior to adult emergence the following May.

Mating behavior in *P. alaskensis* has not been reported, but it was readily observed in a bright, greenhouse environment when males and virgin females were placed among potted spruce trees. Males hovered around the trees, landed near females, and scrambled over the foliage until locating them. Mating was as in other sawflies, end-to-end with both individuals dorsal side up, and lasted 10–15 sec. The cocoons from which females had emerged, and with which they had been confined in the laboratory handling procedure, were similarly attractive to males when placed on the spruce foliage. Males attempted to mate with the empty cocoons or with other males visiting the same cocoons. Similar hovering and mating behaviors were seen in the field. These observations, and the fact that the species is arrhenotokous (Houseweart and Kulman, 1976), suggested that a female-produced sex pheromone exists and plays a part in the normal life cycle.

METHODS AND MATERIALS

Handling of Insects, Extraction, and Chromatography. The insects used for pheromone collection and laboratory bioassay were collected in late June 1977–1979 as 4th–6th instar larvae in white spruce plantations near Grand Rapids, Minnesota. They were reared on spruce foliage to the cocoon stage and then stored at 0° C. Each winter and spring the previous summer's cocoons were warmed to 15° at the rate of 1000–3000/week, giving a supply of adults from early January into June 1978–1980. During the 4–5 weeks at 15° before adult emergence, the cocoons were placed individually in 1 × 2.5-cm (size 000) gelatin capsules. The isolation prevented mating and also prevented the females, which are rather aggressive, from killing each other.

The males were used within 2 days of emergence for laboratory bioassay. In late spring, some of the females were used for field bioassay; otherwise they were killed by freezing when 3–5 days old, and the insects, cocoons, and capsules were separated and washed with hexane. The extracts were concentrated under vacuum and stored at −70° until used for bioassay or purified. Counts were kept so that the number of "female equivalents" (FE) or "cocoon equivalents" (CE) per milliliter could be calculated.

Crude extracts were subjected to column chromatography on Florisil (2.5% water by weight). The columns were 20 × 3.0 cm, with 2000–6000 equivalents/run. Each column was eluted consecutively with 200-ml volumes of 10 solvents: hexane; then 2.5%, 5%, 7.5%, 10%, 25%, and 50% ether in hexane (by volume); then pure ether; acetone; and finally, methanol. The volume of each solvent was about twice the void volume of the column. The effluent was collected as 10 fractions of 200 ml each. The fractions were stored at −70° until used for bioassay or chemical work.

Field Bioassay. Pheromone preparations were evaluated in the field by catches of males on "sticky" traps. The traps were made from cylindrical, white cardboard ice cream cartons, 9 × 9 cm, open on both ends, and coated on the inside with Tack Trap®. The traps were usually secured to branches of 3 to 5-m tall white spruce trees at a height of 1–2 m. If necessary, foliage was removed from near the trap openings to allow the males clear access. Trees with traps were normally separated by at least 5 m. The studies were done in infested white spruce plantations near Grand Rapids, Minnesota.

Extracts or chromatographic fractions were placed on new 5.0-cm plastic Petri plate bottoms (1978) or 4.0 cm watch glasses (1979–1980) and the solvent allowed to evaporate before setting them in traps. The plates were prepared just before use. Single virgin females used to bait traps were held in brass screen cages 4 cm long × 2 cm in diameter. The studies used 5 FE, 5 CE, or 1 virgin female/trap. The traps were normally baited in early to mid morning, when male sawflies were becoming active.

These studies usually employed two treatment traps/tree. The traps of a pair were separated by ca. 10 cm. In some 1978 studies, an additional, control trap was inserted between the traps of a pair with pheromone treatments. Assignment of treatment pairs to trees and treatments to locations within trees were randomized. Trap catches were recorded at the end of the day. All counts were transformed to $\log (n + 1)$ before analysis to stabilize variance. Trees were treated as "blocks" in statistical analysis, removing the often considerable tree-to-tree variability from residual error. The final study of 1978 (Table 3) employed the balanced incomplete block design, with the five treatments compared in pairs, in all possible combinations. Analysis was done according to Yates (1940).

A serious limitation of the field bioassay was the short duration of the

flight season. In 1978 adult emergence in the test plot was monitored with 12 emergence traps (Thompson and Kulman, 1976). Of the 115 adults captured, 94% emerged within a span of 8 days and 63% within 3 days. This, coupled with the short male life-span, limited the acquisition of meaningful field data to little more than 1 week/year.

Greenhouse Bioassay. Pheromone bioassays during winter and early spring were conducted in a greenhouse cage, 1.5 × 1.5 m at the base and 2 m high. It was screened on front and back and fitted with glass elsewhere. Twelve potted spruce trees ca. 0.5 m tall were placed inside. Air was circulated through the cage by an electric fan set 3 m from the front. About 100 male sawflies were added each day that bioassays were run. (Males are short-lived and rarely lasted more than 2 days in the cage). Pheromone preparations were placed on 6.0-cm watch glasses, at the rate of 2.5 CE or FE/plate. After the solvent evaporated, the plates were positioned in the upwind end of the cage, 10 cm apart, in a row perpendicular to the air flow, and just above the spruce trees. The treated surfaces of the plates were downwind.

The essence of the bioassay is that males ignored clean plates but landed frequently on those treated with pheromone. The visits were usually brief (about 15 sec), and the bioassay was quantified by recording the number of males on each plate every 15 sec for an 8-min period, after which the plates were rotated to different positions and the whole process repeated. The number of runs and the plate placements were chosen, a priori, so that each treatment was assayed once at each cage position. The total counts were then analyzed as a Latin square, the factors being treatment, run, and plate position in the cage. The transformation, $\sqrt{n+1}$, was found to stabilize variance. Data were taken only when at least one male was responding in the cage at virtually every 15-sec check.

Generally, a "low" control and a "high" control were included in each test. The low one was most often a clean plate and the high one an appropriate treatment which the sawflies visited readily in previous tests. The other treatments were then evaluated according to the range of responses shown toward the "controls." Consistent responses toward the high control ensured that inactivity toward any other treatment was due to the treatment itself, rather than the unsuitability of bioassay conditions or other factors. This scheme allowed a clear interpretation of negative results for test treatments.

The intensity of the response varied with environmental factors. Activity was greatest on bright days. Most bioassays were run when the temperature was 20–25°; when the sky was heavily overcast, raising the temperature further would sometimes initiate activity. Bioassays could be run during the morning or afternoon, but the intensity of response usually fell off after 1–3 hr of continuous testing. After several hours of rest the males would usually respond again. The spruce trees in the cage and the air movement caused by the fan were both essential for a reliable, consistent response.

RESULTS AND DISCUSSION

Field Studies. Except where otherwise stated, the studies were performed in late May 1978. As in the greenhouse, the empty cocoons with which females had been confined were attractive to males in the field, compared to controls (Table 1). The hexane extract of these cocoons was also attractive. Of the 10 Florisil fractions, only that eluted with hexane was similar in activity to the crude extract, indicating the pheromone to be quite nonpolar in nature. The recorded cumulative emergence increased from 12% to 84% over the 3 days of the study; thus it was run during the population peak.

In a concurrent test, single, 1 to 2-day-old virgin females were similar in attractiveness to 5 CE of the hexane–Florisil fraction. The mean ($N = 5$) 1-day catches of males (and ranges) were: 81.4 (28–119) for females, 5.4 (2–11) for controls (inserted between the baited traps), and 93.0 (65–152) for the hexane fraction. The low control catches indicated that the two treatments were not acting as a single large trap. Most males flew precisely enough to enter only the attractive traps, even though there was less than 1 cm between trap openings.

Of the remaining Florisil fractions (Table 1), the 5% and 25% ether-hexane fractions (denoted below as just the 5% and 25% fractions) each appeared slightly attractive in one of the six replications, the catches being 11

TABLE 1. MEAN 1-DAY TRAP CATCHES OF *P. alaskenis* MALES FOR EMPTY COCOONS FROM FEMALES, HEXANE EXTRACT OF THESE COCOONS, AND FLORISIL FRACTIONS OF THIS EXTRACT

	Treatment[a]	Control
Cocoons (5/trap)	51.0 ***	2.0
Hexane extract (5 CE/trap)	28.0 ***	1.2
Florisil fractions (5 CE/trap)		
Hexane	62.3 ***	1.2
2.5% ether in hexane	0.5	1.3
5.0% ether in hexane	3.3	2.2
7.5% ether in hexane	0.7	0.3
10.% ether in hexane	0.5	0.3
25.% ether in hexane	2.5 *	0.2
50.% ether in hexane	1.3	0.8
Ether	0.3	0.8
Acetone	0.2	0.7
Methanol	0.3	1.2

[a]Each treatment trap paired with a control; 2 reps/day on 3 consecutive days; * and *** imply significant differences from controls at the 0.05 and 0.001 levels (t tests).

and 14, and their controls, 1 and 0, respectively. These catches accounted for the slightly higher means for these fractions in Table 1.

To test for synergistic effects, the 5% and 25% fractions, alone and in combination with the hexane fraction, were compared to the hexane fraction (Table 2). The combinations of the 5% and/or 25% fractions with the hexane fraction greatly exceeded just the hexane fraction in attractiveness, by factors of 13 to 29. Equally striking, there was no synergism when the hexane fraction and the 5% or 25% fractions were in separate traps ca. 10 cm apart (Table 2, lines 1, 2, and 3). Trap catches on the hexane fraction alone were lower than in previous experiments, probably because the population was past its peak and was declining; the recorded cumulative emergence increased from 84% to 90% during the study. In 1979, three treatments of the study were repeated, using chemicals extracted directly from females rather than their empty cocoons. As in 1978, the 5% and 25% fractions significantly synergized the hexane fraction (Table 2).

The final field study in 1978 was conducted to compare virgin females; the hexane extract; the hexane–Florisil fraction; the combination of 5%, 25%, and hexane fractions; and controls (Table 3). The study was run at the end of the flight season when the population of males was very low (the recorded cumulative emergence increased from 97% to 100% during the study), and the virgin females and the hexane–Florisil fraction were no longer significantly more attractive than controls. The combination of 5%, 25%, and hexane

TABLE 2. MEAN 1-DAY TRAP CATCHES OF *P. alaskenis* MALES ON VARIOUS COMBINATIONS OF 5%, 25%, AND HEXANE FRACTIONS, TESTED AGAINST HEXANE FRACTION (ONE TEST PAIR/TREE, WITH CENTRAL CONTROL IN 1978)

	Test fraction(s)[a]	Control	Hexane fraction	Observations/mean
1978 (5 cocoon equivalents/trap)				
5%:	2.3	1.0	0.7	3
25%:	0.3 *	1.0	5.7	3
5% + 25%:	0.5	1.5	3.5	2
Hexane + 5%:	63.0 ***	1.7	4.7	3
Hexane + 25%:	28.7 ***	0.7	1.0	3
Hexane + 5% + 25%:	98.0 ***	3.3	5.7	3
1979 (5 female equivalents/trap)				
Hexane + 5%:	13.7 **		0.7	3
Hexane + 25%:	39.7 *		4.0	3
Hexane + 5% + 25%:	97.0 ***		4.3	3

[a]Significant differences (t tests) between treatments and the hexane fraction, at the 0.05, 0.01, and 0.001 levels, indicated by *, **, and ***, respectively.

TABLE 3. MEAN 1-DAY CATCHES OF *P. alaskenis* MALES FOR A PAIRED COMPARISON OF 5 TREATMENTS (BALANCED INCOMPLETE BLOCK DESIGN).

Treatment	Mean catch ($N = 8$)[a]
Control	0.63 a
Virgin females (1/trap)	1.13 a
Hexane Florisil fraction (5 CE/trap)	1.25 a
Hexane extract (5 CE/trap)	4.75 b
Hexane + 5% + 25% fractions (5 CE/trap)	39.80 c

[a]Different letters denote significant differences at the 0.05 level by the least significant difference (LSD) method.

fractions, however, was still quite active. The crude hexane extract was between the hexane fraction and the 3-fraction combination in attractiveness. While this extract contained the same chemicals as the more active combination, it apparently had additional masking or inhibitory chemicals as well.

Other Field Results and Observations. Qualitatively, the males were most active around noon, and fewer were caught in the cooler, early morning hours or toward evening. This was the same trend reported by Casida et al. (1963) for *Diprion similis*, the introduced pine sawfly. Males were less active on overcast or cool days than on sunny, warm days. When traps were left in the field overnight, there was no evidence of males being caught during the hours of darkness. There was no indication of females being attracted to the pheromone treatments.

The host tree seems to play an important role in sex attraction. In a 1980 experiment, three traps placed in spruce trees and baited with 2 FE of a potent, purified form of the hexane fraction (the "HPLC fraction," Bartelt et al., 1982) caught 25, 106, and 188 males, while three identical traps attached to poles ca. 2 m from other spruce trees (trap height 1.5 m) caught 0, 0, and 2. On the second day, fresh traps set at the same six locations (but with trap positions, on poles or in trees, reversed) caught 77, 78, and 94 in trees and 0, 1, and 1 on poles. In a subsequent, similar test, traps baited with the purified hexane fraction plus the 5% and 25% fractions gave comparable results: a total of 182 males in three traps in spruce trees and 0 on three poles. Traps in other kinds of trees in the plantation (balsam firs, willows, poplars) caught 0–10 males/day, far below catches in spruce trees. While *Diprion similis* has been reported to fly 30 m or more from the host plant in response to virgin females or pheromone baits (Coppel et al., 1960), male *P. alaskensis* seemed reluctant to respond to the female scent anywhere but in spruce trees.

Greenhouse Bioassay—Florisil Fractions. The active Florisil fractions were used to develop the greenhouse bioassay, the tool to be used to monitor

pheromone purification when adults were not present in the field. The following results illustrate the properties of the bioassay, parallels between field and greenhouse results, and some additional conclusions about the Florisil fractions.

As in the field, hexane-Florisil fractions derived from the empty cocoons of females were active (Table 4), although two such preparations differed somewhat in activity. A hexane-Florisil fraction derived directly from virgin females was superior to the cocoon-derived material, however (Table 4). The hexane fractions were usually in the bioassay cage about an hour before the male response began. The attractiveness of empty cocoons was probably an artifact of the insect-handling procedures and had no biological significance. Extracts of the females' gelatin capsules were also found to be attractive.

Table 4 also shows the variability typically seen between two replications of a treatment (in no case were such differences significant), and it also shows that a "real" difference between treatments (e.g., the 1978 cocoon material and controls) could be obscured by the presence of an even more active treatment (the female-derived fraction). Absolute scores for a treatment tended to vary from experiment to experiment, depending on what other treatments were present, and therefore, only comparisons of scores within an experiment are meaningful.

Also as in the field, the 25% fraction strongly synergized the hexane fraction (Table 5). One hour after the plates were prepared, the combination

TABLE 4. GREENHOUSE BIOASSAY: MEAN SCORES FOR HEXANE-FLORISIL FRACTIONS FROM SEVERAL SOURCES (2.5 CE OR FE/PLATE)[a]

Treatment		Mean ($N = 5$)[b]
Cocoons, 1978	#1	62.3 c
	#2	60.7 c
Control		2.3 a
Cocoons, 1979	#1	40.3 bc
	#2	30.2 b
Cocoons, 1978	#1	4.3 a
	#2	11.4 a
Control		1.5 a
Females, 1979	#1	59.3 b
	#2	76.2 b

[a]There were 5 runs/plate and 2 plates for each treatment except controls.
[b]Plates followed by different letters were significantly different (LSD, 0.05).

TABLE 5. GREENHOUSE BIOASSAY: TEST FOR SYNERGISM BY 5% and 25% FLORISIL FRACTIONS DERIVED FROM COCOONS (2.5 CE/PLATE) AND CHANGES OVER TIME

	1 hour old		4 hours old		1 day old	
Treatment	Mean[a] ($N = 5$)	Ratio to hexane fraction	Mean ($N = 5$)	Ratio to hexane fraction	Mean ($N = 5$)	Ratio to hexane fraction
Hexane	5.6 a	1	4.9 ab	1	40.3 bc	1
Control	0.9 a	0.16	0.2 a	0.04	1.9 a	0.05
Hexane + 5%	7.3 a	1.3	10.6 b	2.2	60.3 c	1.5
Hexane + 25%	122.9 b	22	33.2 c	6.8	33.0 b	0.82
Hexane + 5% + 25%	130.1 b	23	34.7 c	7.1	25.8 b	0.64

[a]In each test, different letters indicate significant differences (LSD, 0.05).

of hexane and 25% fractions had a score about 20 times that of the hexane fraction alone, similar to the ratio of trap catches in the field. After 4 hr, however, the combination exceeded the hexane fraction by only sevenfold, and after one day (males having been added to the cage) there was no difference between these treatments. The effect of the 25% fraction was relatively short-lived. The hexane fraction, by itself, was not significantly more active than the control on the first day but was clearly active on the second day. The 1- and 4-hr data again showed that the activity of the hexane fraction could be obscured by the presence of an even more active treatment (the combination). Curiously, the 5% fraction never did exhibit the ca. 15-fold synergism in the greenhouse that was seen in the field, and the combination of all three fractions behaved much as the combination of 25% and hexane fractions.

In another test of the cocoon-derived Florisil fractions (Table 6), the 25% fraction again strongly synergized the hexane fraction, and again, the 5% fraction did not. In addition, the 50% fraction was similar in synergism to the 25% fraction. (The 50% and hexane fractions had not been tested together in the initial field studies. It is possible for a single compound to occur in two consecutive fractions, but this issue has yet to be resolved for the 25% and 50% fractions). No other fractions gave comparable synergism, although more subtle effects could not be ruled out.

Table 7 shows the results of a test for synergism in female-derived Florisil fractions. The 25% and 50% fractions again showed strong synergism and, as before, the hexane fraction alone had a low score when competing with these combinations. Here too, the 5% fraction did not synergize the hexane fraction, although the same preparation later gave positive synergistic results

TABLE 6. GREENHOUSE BIOASSAY: TESTS FOR SYNERGISM IN FLORISIL FRACTIONS DERIVED FROM COCOONS (2.5 CE/PLATE)[a]

Treatment	Mean ($N = 3$)[b]	Treatment	Mean ($N = 3$)
Hexane + 25%	138.7 b	Hexane + 25%	95.0 b
Hexane	2.7 a	Hexane + 50%	105.3 b
Hexane + 5%	3.3 a	Hexane + ether	2.7 a
Hexane + 25%	125.5 b	Hexane + 25%	106.0 c
Hexane + 2.5%	4.5 a	Hexane + methanol	1.0 a
Hexane + 10%	2.0 a	Hexane + 7.5%	9.7 b

[a] A subsequent, qualitative test found the hexane + 25% fractions to be considerably more active than the hexane + acetone fractions.
[b] In each test, different letters indicate significant differences (LSD, 0.05).

in the field (Table 2). No other fractions showed strong synergism, although some appeared repellent, the combinations with the hexane fraction behaving as controls.

Other Observations. Qualitatively, males responding to the hexane fraction in the greenhouse approached the plates from downwind, hovering and moving toward the plates very slowly and precisely. Upon landing they

TABLE 7. GREENHOUSE BIOASSAY: TESTS FOR SYNERGISM IN FEMALE-DERIVED FLORISIL FRACTIONS (2.5 FE/PLATE)

Treatment	Mean[a]	Ratio to hexane fraction
Hexane	41.4 bc	1
Control	1.9 a	0.05
Hexane + 2.5%	30.6 b	0.74
Hexane + 5.0%	15.8 b	0.38
Hexane + 7.5%	66.2 c	1.6
Hexane + 10%	3.5 a	0.08
Hexane	7.4 a	1
Control	0.5 a	0.07
Hexane + 25%	201.5 b	27
Hexane + 50%	148.8 b	20
Hexane	50.9 c	1
Control	2.2 a	0.04
Hexane + ether	13.6 b	0.27
Hexane + acetone	74.8 c	1.5
Hexane + methanol	5.5 ab	0.11

[a] In each test, treatments followed by different letters were significantly different (LSD, 0.05).

TABLE 8. BIOASSAY CHARACTERISTICS OF VARIOUS FLORISIL FRACTIONS AND COMBINATIONS

Treatment	Field bioassay	Greenhouse bioassay
Hexane	Catches above control levels when population high.	Upwind hovering, alighting on plate, occasional mating attempts.
Hexane + 25% (or + 50%)	Catches far above those for the hexane fraction alone.	As with the hexane fraction, except mating attempts more numerous and vigorous. Also "branch-swarming" behavior.
Hexane + 5%	Catches far above those for hexane fraction alone.	Activity pretty much as with the hexane fraction alone; 5% fraction has little effect.

walked about, investigating the plates with their antennae. Males contacting others on a plate frequently attempted to mate, backing toward each other to engage the claspers while beating their wings. Less often, single males spontaneously showed the same behavior toward the treated plate. Visits typically lasted about 15 sec.

When the 25% (or 50%) fraction was mixed with the hexane fraction, the mating attempts were much more intense and frequent (perhaps because of the larger number of males crowding onto the plates), and in addition, other males congregated on the spruce shoots immediately downwind of the treated plate. These also actively attempted to mate with each other. This "branch swarming" behavior was rarely seen when only the hexane fraction was present. The bioassay characteristics of the active Florisil fractions in the field and greenhouse are summarized in Table 8.

An extract of male sawflies, and empty cocoons and gelatin capsules from males, were tested in the greenhouse but were not attractive to males.

To gauge the polarity of the chemicals in the active fractions, standards were chromatographed under the same conditions. The hydrocarbons eluted with hexane; esters, aldehydes, and ketones of 14–18 carbons eluted with 5% ether in hexane; and alcohols of 12–14 carbons eluted with 25% ether in hexane. The low polarity of the primary component suggested a difference from the diprionid sawflies, which use esters for sex attraction (Jewett et al., 1976). In addition, the existence of powerful synergists and the drastically reduced activity of pheromone away from the host tree are features not reported for diprionid species. Bartelt et al. (1982) deals with the identification of the active material in the hexane fraction. Work on the synergists is still in progress.

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REFERENCES

BARTELT, R.J., JONES, R.L., and KULMAN, H.M. 1982. Hydrocarbon components of the yellow-headed spruce sawfly sex pheromone: A series of (*Z*,*Z*)-9,19 dienes. *J. Chem. Ecol.* 8(1):95–114.

BORDEN, J.H., BILLANY, D.J., BRADSHAW, J.W.S., EDWARDS, M., BAKER, R., and EVANS, D.A. 1978. Pheromone response and sexual behaviour of *Cephalcia lariciphila* Wachtl (Hymenoptera: Pamphiliidae). *Ecol. Entomol.* 3:13–23.

CASIDA, J.E., COPPEL, H.C., and WANTANABE, T. 1963. Purification and potency of the sex attractant from the introduced pine sawfly, *Diprion similis*. *J. Econ. Entomol.* 56:18–24.

COPPEL, H.C., CASIDA, J.E., and DAUTERMAN, W.C. 1960. Evidence for a potent sex attractant in the introduced pine sawfly *Diprion similis* (Hymenoptera: Diprionidae). *Ann. Entomol. Soc. Am.* 53:510–512.

FORBES, R.S., and DAVIAULT, L. 1964. The biology of the mountain-ash sawfly, *Pristiphora geniculata* (Htg.) (Hymenoptera: Tenthredinidae), in eastern Canada. *Can. Entomol.* 26:1117–1133.

HOUSEWEART, M.W., and KULMAN, H.M. 1976. Fecundity and parthenogenesis of the yellowheaded spruce sawfly, *Pikonema alaskensis*. *Ann. Entomol. Soc. Am.* 69:748–750.

JEWETT, D.M., MATSUMURA, F., and COPPEL, H.C. 1976. Sex pheromone specificity in the pine sawflies: Interchange of acid moieties in an ester. *Science* 192:51–53.

LONGHURST, C., and BAKER, R. 1980. Sex pheromones in the gooseberry sawfly *Nematus ribesii*. *Naturwissenschaften* 67:146.

MATSUMURA, F., TAI, A., COPPEL, H.C., and IMAIDA, M. 1979. Chiral specificity of the sex pheromone of the red-headed pine sawfly, *Neodiprion lecontei*. *J. Chem. Ecol.* 5:237–249.

NASH, R.W. 1939. The yellow-headed spruce sawfly in Maine. *J. Econ. Entomol.* 32:330–334.

THOMPSON, L.C., and KULMAN, H.M. 1976. Parasite complex of the larch sawfly in Minnesota. *Environ. Entomol.* 5:1121–1127.

YATES, F. 1940. The recovery of interblock information in balanced incomplete block designs. *Ann. Eugen.* 10:317–325.