

from the puparia. Torgersen (1970) also had difficulty in obtaining adult emergence even though he tried various temperatures and photoperiods to break what appears to be a parasite pupal diapause. There was considerable variation between the hymenopterous species recovered between outbreaks and also between locations (Table 1.). Of particular interest are: *Charmon* (= *Eubadizon*) *extensor*, *Mesochorus pictilis* and *M. sylvarum*, *Mesopolobus* sp. (possibly n. sp.), and *Meteorus* sp. (possibly n. sp.), which previously had not been reported attacking this host in British Columbia or Alaska. These species may be capable of changing hosts as the opportunity arises. Of the sixteen species identified during this outbreak six species were first records for British Columbia.

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Sex pheromone components of an undescribed *Choristoneura* species (Lepidoptera: Tortricidae) on lodgepole pine in British Columbia

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ABSTRACT

E11-tetradecenyl acetate (E11-14 OAc) and Z11-tetradecenyl acetate (Z11-14 OAc) are sex pheromone components of an undescribed, pine-feeding *Choristoneura* (C. n. sp. CPG=Prince George) in British Columbia. Compounds were identified by coupled gas chromatographic-electroantennographic (GC-EAD) and coupled gas chromatographic-mass spectroscopic (GC-

MS) analyses, and were field tested near Prince George, B.C. A 65:35 blend of E11-14 OAc and Z11-14 OAc attracted as many male *C. n. sp.* CPG as did the most attractive virgin females, suggesting that the natural sex pheromone has only two significant components. This two-component blend is suggested for detecting and monitoring *C. n. sp.* CPG populations.

INTRODUCTION

There is a large complex of *Choristoneura* species feeding on a variety of coniferous and deciduous trees in British Columbia (Freeman 1967; Powell 1980). The taxonomic relationships of these tortricid moths are not clear and new entities are still being discovered. Comparative morphology and ecology indicate that an undescribed *Choristoneura* species (*C. n. sp.* CPG = Prince George) occurs on lodgepole pine (*Pinus contorta* var. *latifolia*) in an area 200 km north to 40 km south of Prince George with concentrations near Bear and McLeod Lakes (T.G. Gray, unpublished observations). Approximately 35% of the larvae were found feeding in and around the staminate cones. They were difficult to detect unless disturbed. This feeding behaviour resembles that of *C. lambertiana* on lodgepole pine near Yahk, B.C. (T.G. Gray, unpublished observations) and that of another undescribed species (*C. n. sp.* CR = Richmond) on Scots pine (*Pinus sylvestris*) (Gray and Slessor 1989).

Because sex pheromones provide important information on the taxonomic relationships of moths (Roelofs and Comeau 1969; Roelofs and Brown 1982), we have conducted laboratory analyses and field experiments to characterize the sex pheromone components of this *C. n. sp.* CPG.

METHODS AND MATERIALS

Insect Rearing. In June 1989, three hundred larvae in the penultimate instar were collected from immature fringe lodgepole pine between Woodpecker and Carswell, B.C., for rearing and isozyme analysis. Twenty-five larvae were reared in each of twelve containers with ten cubes of artificial diet (Robertson 1979) at 23°C, 50% RH, and a photoperiod of 16:8 (L:D). Pupae were kept either in cages with potted lodgepole pines or in kraft paper bags with waxed paper strips to provide emerging females with oviposition sites. Eggs were transferred to petri dishes in black plastic bags (Stehr 1954) to induce first instar larvae to spin hibernacula. After 3 weeks at 20°C, the larvae were kept at 0°C for 3 months to satisfy diapause requirements. Following cold treatment, they were reared as above to pupation. Large larvae were subjected to electrophoretic analyses. Adults were used for pheromone identification. Dead males were checked for the presence of spicules on their aedeagi (Dang 1985).

Pheromone analysis. Sexed pupae were placed in petri dishes with moist filter paper at 17°C, 40-50% RH and a photoperiod of 16:8 (L:D) until adult eclosion. After 1.0, 1.5, 2.0 and 2.5 hrs into the scotophase, the last 3-4 abdominal segments of 2- to 5-day-old virgin females were removed. The abdominal tips were individually extracted for about 30 sec in 5 µl of redistilled hexane, rinsed with an additional 5 µl of hexane and discarded. Each gland extract was tested individually. Four µl were analyzed in a Hewlett Packard 5890 gas chromatograph, equipped with a 0.25 mm x 30 m DB-1 column and 2 µl of the same extract in a Hewlett-Packard 5890 gas chromatograph coupled to a Finnigan MAT 700 ion trap detector (GC-MS) which was equipped with a 0.25 mm x 20 m Supelcowax column. The oven temperature program for both chromatographs were: constant at 55°C for 1 min, heated at 25°C/min to 175°C, constant at 175°C for 1 min, and then heated at 6°C/min to 250°C. Injector and detector temperatures were 180°C and 295°C, respectively. Additional gland extracts were subjected to gas chromatographic analyses on a DB-210 column (0.25 mm x 30 m) utilizing both flame ionization (FID) and electroantennographic detection (EAD) (Arn et al. 1975) (Fig. 1).

Field bioassay of candidate pheromone components. Field experiments were conducted in a mature lodgepole pine stand 70 km north of Prince George. Each five-replicate experiment was set up in a randomized complete block with traps at 40 m intervals. The delta 2-litre milk-carton traps were suspended 2 m above ground and baited with polyvinylchloride dispensers (Daterman 1974) impregnated with candidate pheromone components in HPLC grade hexane. The 855 cm² trap surface was covered with the adhesive Tangle-Trap (Tanglefoot Company,

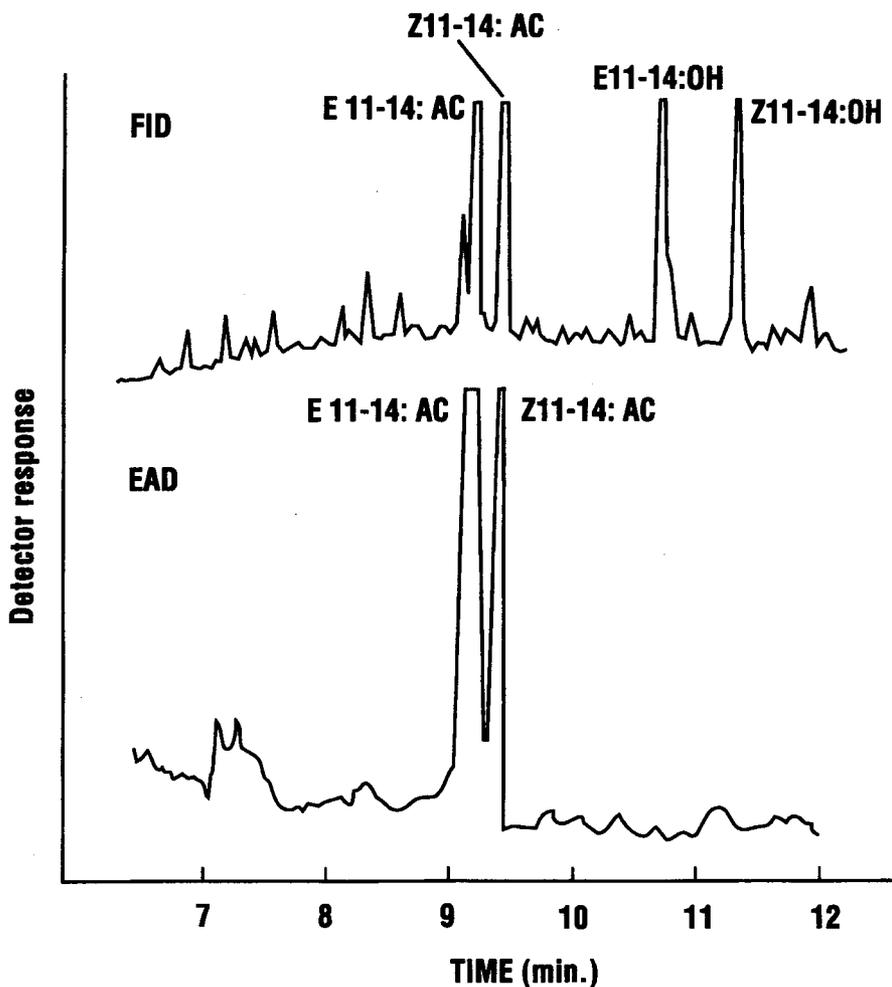


Figure 1: Detector responses to one female equivalent of pheromone extract chromatographed on a Hewlett Packard 5859A instrument (DB-210 column, 0.25 mm x 30 m I.D., 1 min at 100°C, 20°C/min to 180°C, 1°C/min to 220°C). The antennal recording was carried out with a single antenna of male *C. n. sp.* CPG (FID = flame ionization detector, EAD = electroantennographic detector).

Grand Rapids, MI 49504) to immobilize moths entering the trap. Traps were checked and advanced one position daily and those containing 20 or more males were replaced with a new trap using the same bait. The first experiment tested the attraction of *E11*-tetradecenyl acetate (*E11*-14 OAc), *Z11*-tetradecenyl acetate (*Z11*-14 OAc), *E11*-tetradecenol alcohol (*E11*-14 OH) and *Z11*-tetradecenol alcohol (*Z11*-14 OH) alone and in combinations (Table 1). The second five-replicate experiment tested the attraction of various doses of *E11*-14 OAc and *Z11*-14 OAc at the natural 65:35 ratio (Table 2).

RESULTS AND DISCUSSION

GC-EAD analyses of female gland extracts revealed four compounds two of which elicited antennal responses (Fig. 1). Retention indices and GC-MS analyses indicated *E11*-14 OAc, *Z11*-14 OAc, *E11*-14 OH and *Z11*-14 OH. Synthetic *E11*-14 OAc and *Z11*-14 OAc elicited strong electrophysiological responses, while the corresponding alcohols were hardly EAD-ac-

Table 1

Comparison of attractiveness of two acetate isomers and two alcohol isomers against *Choristoneura n. sp.* (CPG) at a loading of 5% w/w of chemicals polymerized in PVC, at Bear Lake, BC in August 1990. n = 45

Lure	Dose (μg)	Average No. males/ night/trap \pm S.D.
<i>E/Z</i> -11-14 OAc	(750/500)	51.4 a* \pm 21.0
<i>E/Z</i> -11-14 OAc + <i>E</i> -11-14 OH	(725/325/200)	45.4 a \pm 16.3
<i>E/Z</i> -11-14 OAc + <i>E/Z</i> -11-14 OH	(725/325/100/100)	26.2 b \pm 7.3
Female <i>Choristoneura n. sp.</i> (CPG)		10.2 c** \pm 6.9
<i>E</i> -11-14 OAc	(1250)	2.8 c \pm 3.8
<i>Z</i> 11-14 OAc	(1250)	0.2 c \pm 0.4
<i>E</i> -11-14 OH	(1250)	0.8 c \pm 1.0
<i>Z</i> -11-14 OH	(1250)	0
Unbaited control trap		0

* Means followed by the same letter are not significantly different at $P < 0.05$ (Duncan's New Multiple Range Test).

** Average of five unmated females.

Table 2

Catches of male undescribed pine feeding *Choristoneura* (*C. n. sp.* CPG) in sticky traps baited with various doses of a 65:35 blend of *E*11-14 OAc and *Z*11-14 OAc, Bear Lake, British Columbia, August 1991. N=35.

Lure	Dose (μg)	Average No. trap/night	Males caught/ CI
<i>E/Z</i> -11-14:Ac	(750)	14.8 a*	8.15
<i>E/Z</i> -11-14:Ac	(2000)	14.6 ab	8.55
<i>E/Z</i> -11-14:Ac	(2500)	14.0 ab	12.95
<i>E/Z</i> -11-14:Ac	(1250)	9.0 abc	10.55
<i>E/Z</i> -11-14:Ac	(250)	6.4 bc	14.35
<i>E/Z</i> -11-14:Ac	(125)	0	

* Means followed by the same letter are not significantly different ($P < 0.05$) Tukey's W Procedure.

tive, suggesting that they may not be part of the pheromone blend of female *C. n. sp.* CPG. Identical retention times on three columns with different retention characteristics (DB-1, DB-210, Supelcowax) and identical mass spectroscopic characteristics of female-produced and authentic compounds confirmed our structural assignments.

*E*11-14 OAc, *E*11-14 OH, *Z*11-14 OAc and *Z*11-14 OH tested individually at 1250 μg each did not attract male *C. n. sp.* CPG in the field test (Table 1). However, a binary combination of *E*11-14 OAc and *Z*11-14 OAc at the 65:35 ratio found in gland extracts, attracted as many males as did the most attractive virgin females. Five of the latter attracted an average of 10.2 males each. Addition of 200 μg of *E*11-14 OH to the acetate blend did not affect the trap catches, while addition of 100 μg of both *E*11-14 OH and *Z*11-14 OH significantly decreased attraction (Table 1). Significant effects on trap catches of the alcohols, comprising 5-40% of the chemical lure, were not confirmed in subsequent experiments.

Pheromone quantity in gland extracts of female *C. n. sp.* CPG peaked 1.5 hours into the scotophase. With 80 ng per female it exceeded those of other *Choristoneura* up to four times. Only

C. orae produces similar large amounts of pheromone (Cory et al. 1982; Gray et al. 1984). A dose response test confirmed that large amounts of the acetate pheromone components are more attractive than lower concentrations (Table 2). In contrast, only 2.5 µg of *E*11-tetradecenal aldehyde (*E*11-14 Ald) was sufficient to attract large numbers of *C. occidentalis* and *C. biennis* (Cory et al. 1982). Lack of *E*11-14 Ald in the pheromone blend of *C. n. sp.* CPG contrasts with the pheromone blend of female *C. orae*, which do produce small amounts of *E*11-14 Ald in addition to large quantities of *E*11-14 OAc and *Z*11-14 OAc.

Isozymes and spicule numbers on the aedeagi of *C. n. sp.* CPG resembled those of other *Choristoneura* species in B.C., but differed from eastern species (G.T. Harvey, Forestry Canada, Sault Ste. Marie (retired) personal communication). Adults of *C. n. sp.* CPG are similar to *C. n. sp.* CR but the forewings have a lighter background colour and the black strigulae are absent. *C. n. sp.* CPG is close in size to *C. lambertiana* except that the forewings are not so light and creamy in colour but ochreous with distinctive orange-brown markings. The males are darker in wing colour than females, as observed in some other *Choristoneura* (Harvey, personal communication).

The two-component blend of *E*11-14 OAc and *Z*11-14 OAc in a 65:35 ratio with the corresponding alcohols being benign, and corresponding aldehydes being absent, differs from that of other western conifer-feeding *Choristoneura*. Occurrence on lodgepole pine and sexual dimorphism further indicate that *C. n. sp.* CPG may be a new species.

CONCLUSION

A population of *Choristoneura* was detected near Prince George, British Columbia. Morphological, ecological and pheromone characteristics are distinct from other *Choristoneura* species in British Columbia. Larvae feed on staminate flowers of lodgepole pine. Male moths are distinctly darker than female moths. The sex pheromone is comprised of *E*-11-tetradecenyl acetate and *Z*-11-tetradecenyl acetate at a unique 65:35 ratio. Corresponding aldehydes are not produced and corresponding alcohols are not behaviourally active. A lure containing 750 µg *E/Z*-11-14 OAc at a 65:35 ratio is recommended for detection and monitoring of *C. n. sp.* CPG.

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Response of *Trichogramma* sp. nr. *sibericum* (Hymenoptera: Trichogrammatidae) to age and density of its natural hosts, the eggs of *Rhopobota naevana* (Lepidoptera: Tortricidae)¹

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ABSTRACT

Responses of an indigenous *Trichogramma* sp. nr. *sibericum* (Hymenoptera: Trichogrammatidae) to the age and density of eggs of the blackheaded fireworm, *Rhopobota naevana* (Hübner) (Lepidoptera: Tortricidae) were determined in the laboratory. The parasitoid wasp showed a significant ($P < 0.05$) preference for eggs 1-7-day-old over those 21-day-old. No significant differences ($P > 0.05$) in percentages of parasitized eggs, however, were found among groups of eggs below 7-day-old. At host egg densities below 20 per wasp, the number of eggs parasitized significantly ($P < 0.05$) increased with egg density, and tended to stabilize at densities above 30. The rate of parasitism decreased significantly ($P < 0.05$) with increased host egg density. Superparasitization occurred at densities of 5-10 host eggs, but was rarely observed at densities above 20 eggs. The mean number of progeny per wasp significantly ($P < 0.05$) increased with host density, whereas the clutch size (the number of parasitoid offspring per parasitized host) significantly ($P < 0.05$) decreased with an increase in host density.

INTRODUCTION

Although egg parasitic *Trichogramma* (Hymenoptera: Trichogrammatidae) species head the list of beneficial insects as biological control agents (Stinner 1977), no studies have been reported on using *Trichogramma* to control the blackheaded fireworm, *Rhopobota naevana* (Lepidoptera: Tortricidae), a major pest on cranberry in North America. However, the use of *Trichogramma* to control this pest may be realistic and possible because two species of *Trichogramma* have been discovered recently from natural fireworm populations in cranberry fields in British Columbia (Li et al. unpublished data). One of the two indigenous species, *Trichogramma* sp. nr. *sibericum*, showed a high affinity for fireworm eggs in the laboratory (Li et al. unpublished data.). If this fireworm-attacking *Trichogramma* can be successfully mass reared under laboratory conditions, field release for control of the fireworm may be realized.

Host age preference of *Trichogramma* towards a host is fundamental to a release program and is a critical factor in selection of an effective *Trichogramma* as a biological control agent (Marston and Ertle 1969; Schmidt 1970) because timing of a release is one of the most important factors influencing efficacy in the field. Thus, host age preference by *Trichogramma* must be determined before using the wasp in a biological control program. Knowledge of the relationship between host density and parasitism is also critical for both inundative releases in the field and mass rearing in the laboratory. In the present study, we report the effects under labo-