MORPHOLOGY, LIFE HISTORY AND IDENTIFICATION OF SEX PHEROMONE COMPONENTS OF AN UNDESCRIBED SPECIES OF CHORISTONEURA (LEPIDOPTERA: TORTRICIDAE) ON SCOTS PINE IN BRITISH COLUMBIA

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ABSTRACT

The morphology and life history of a probable new species of tortricid on Scots pine in British Columbia is described. It differs from other Canadian pine feeding *Choristoneura*. Abdominal tip extracts of unmated females contained *Z*-11- and *E*-11-tetradecenyl acetates and alcohols. An equal mixture of these materials was an effective attractant for capturing males in delta traps and is recommended for the detection and monitoring of this insect.

INTRODUCTION

The conifer-feeding *Choristoneura* in North America are composed of three complexes or series (Powell 1980): (1) the Fumiferana complex, associated with *Picea* spp. and *Abies* spp., (2) the Lambertiana complex, generally feeding on *Pinus* spp. and (3) the Carnana complex which feed on *Pseudotsugata* spp. Harvey (1985) contends that there are only two groups, one associated with spruces, Douglas-fir (*Pseudotsuga menziesii* Mirb. Franco) and true firs (Abietoideae) and the other feeding on pines (Pinoideae). He includes the Carnana group in the Fumiferana group. The species described in this paper would, by Powell's classification, be included in the Lambertiana complex. In western North America this complex consists of three subspecies: *Choristoneura lambertiana lambertiana* (Busck) in northern California and southern Oregon, *C. l. subretiniana* Obraztsov in eastern California and *C. l. ponderosana* Obraztsov in Colorado, Wyoming and North Dakota. In addition, there are populations in Wyoming, Montana, Idaho, southeast British Columbia (Silver and Ross 1964) and Oregon which are intermediate between the three subspecies and vary in a clinal fashion across the range (Powell 1980). In eastern North America another species, *Choristoneura pinus pinus* Freeman, occurs, with a subspecies *C. p. maritima* indicated in the southern part of its range. No pine-feeding species has been previously identified in southwestern British Columbia or Washington State.

The sex pheromone for *C. pinus pinus* was identified in 1985, (Silk *et al.* 1985) and found to consist of *E*-11- and *Z*-11-tetradecenyl acetate (85:15) and *E*-11- and *Z*-11-tetradecen-1-ol (85:15); the acetate and alcohol components occurred at a ratio of 9:1. The isolation and identification of the sex pheromone components of *C. lambertiana* (Busck) remain to be investigated, although good attraction occurs in traps using either a blend attractive to *C. orae* (Gray *et al.* 1984) or to the attractive blend proposed for *Choristoneura* n. sp. described in this paper.

In June 1979, T.G.G. noticed *Choristoneura* larvae feeding on Scots pine (*Pinus sylvestris* L.) and assumed from their general appearance that they were *C. occidentalis*, although this species is not commonly found on pine. Therefore, to confirm this assumption, six delta traps baited with *C. occidentalis* pheromone were set out on 23 July and collected on 18 September 1979. There were no *Choristoneura* adults present in any of the traps.

The biology of some of the *C. lambertiana* subspecies has been described by McGregor (1968, 1970), Stevens *et al.* (1977), and Stark and Borden (1965). This paper is based on observations made from 1979 to 1982 on the biology and life history of a previously undescribed species on naturally infested Scots pine trees near George Massey Tunnel, Richmond, B.C., on laboratory rearings with pine cuttings, and on synthetic diet (Robertson 1979). The isolation and identification of the pheromone components used to monitor the populations are also discussed.

MORPHOLOGY

Egg Convex and ovate, 1.13 mm long x 0.6 mm wide, light green, darkening as eclosion approaches; laid in overlapping, shingle-like rows on the needles.

Larva

First instar: pale yellow with light reddish brown head, thoracic shield lighter than head, 2.07 mm long x 0.33 mm wide.

Second instar: yellow with dark brown head, thoracic shield brown but lighter than head, anal plate same color as thoracic shield, 2.00 mm long x 0.33 mm wide.

Third instar: creamy-brown with two rows of whitish dots visible with x10 magnification, dark brown head and thoracic shield, the latter with a white median line; thoracic legs same color as shield; light brown anal plate. 3.33 mm long x 0.50 mm wide.

Fourth instar: light brown; dorsum with two rows of paired whitish spots with black centers around setae; gonads visible in males; head dark brown, thoracic shield black with white leading edge; thoracic claws black; clypeus and antennae basal segments whitish, antennae black. 4.33 mm long x 0.66 mm wide.

Fifth instar: reddish brown with lighter sides, dorsum with two rows of paired yellowish spots, male gonads visible in third abdominal segment; head red brown; thoracic shield darker than head. 22.5 mm long x 2.25 mm wide.

Pupa Appendages light brown; thoracic segments reddish brown; abdominal segments light brown and finely textured; intersegmental regions reddish brown with coarser texturing; eight cremastal setae, two on each side and four on basal segment; exuviae pink. Males 11.0 to 12.5 mm, females 13.0 to 13.5 mm in length.

Adult This *Choristoneura* species resembles the coastal form of *C. occidentalis* (Dr. A. Mutuura, Biosystematics Research Centre, Ottawa, ON, personal communication). The head and thorax are grey-brown to reddish brown; the hind wings are darker grey than those of *C.lambertiana* (Busck); forewings are a grey ground color with brown to brownish orange markings and numerous black strigulae; abdomen grey; aedeagus lacks spicules unlike *C. pinus* which displays many spicules (Dang 1985); Voucher specimens are available for study from the Canadian National Collection, Biosystematics Research Centre, Ottawa, ON K1A 0C6 and from the Regional Collection, Pacific Forestry Centre, Victoria, B.C. V8Z 1M5 wingspread: males 17 to 19 mm, females 20 to 22 mm.

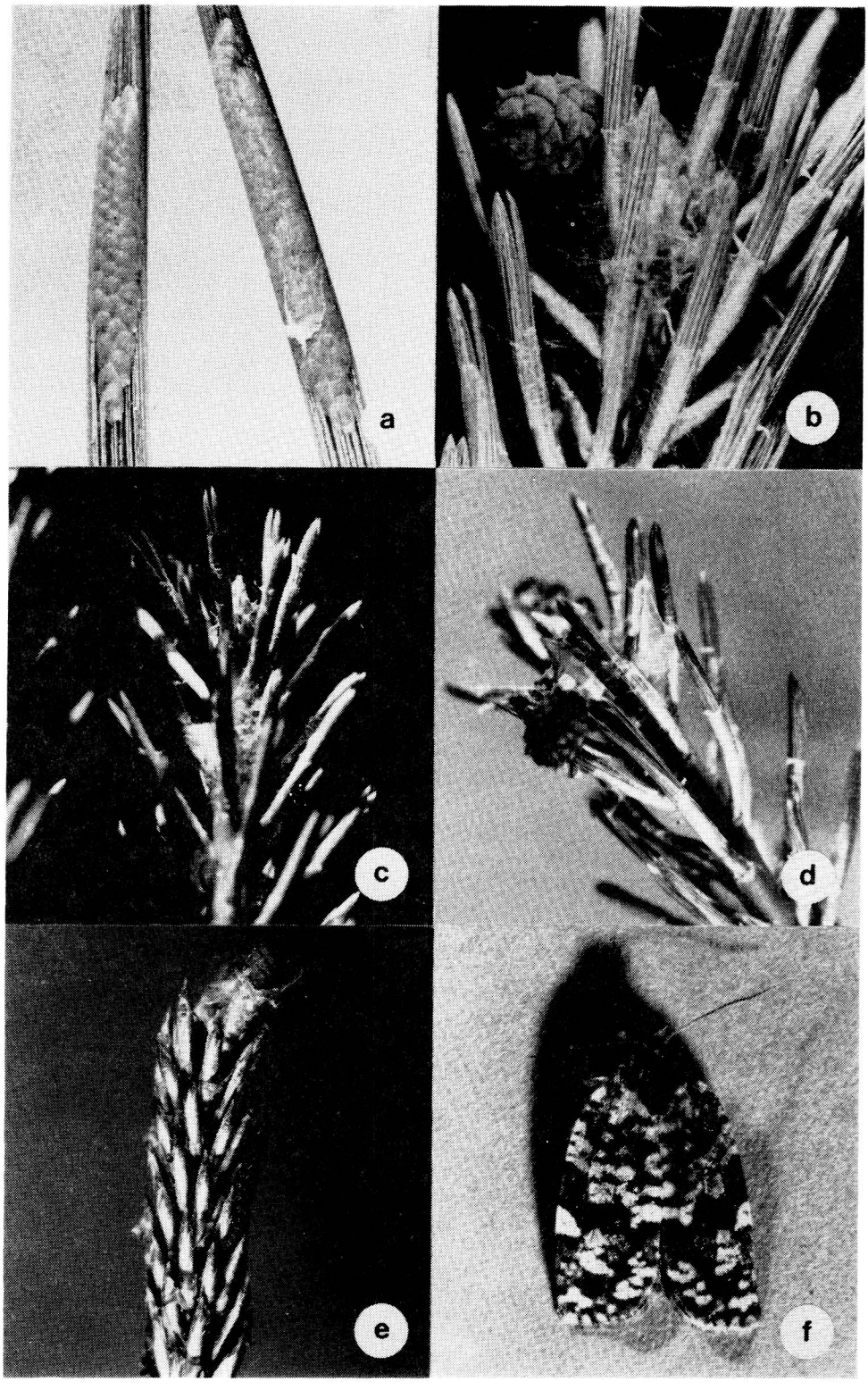

Fig. 3. Stages in the development of *Choristoneura* n. sp. on Scots pine at Richmond, B.C.: A. Egg masses on pine needles, B. Feeding site of second instar larva, C. Silk enclosure of third instar larva, D. Feeding site of fourth instar larva, E. Fifth instar, mature larva, F. Adult.

LIFE HISTORY

Clusters of eggs (Fig. 3A) were laid in a shingle-like fashion during the last week of July and the first week of August (Fig. 4) distally and on the top surface of pine needles, similar to other pine-feeding *Choristoneura*. In 1981, 32 egg masses were collected; of these 90% had two rows (mean number of eggs per mass was 23.5) and 10% had three rows (mean number of eggs per mass was 42.0). Field-collected egg masses hatched within 4 to 7 days at 20°C ± 2°C. The eggs slowly darkened and the black head capsules became visible through the chorions 48 h before eclosion.

The first instar larvae emerged from the eggs and dispersed to seek protected areas to spin overwintering hibernacula, often within the current year's pupal webbing on old foliage or old bud scales. They molted from first to second instar in the autumn and overwintered in the second instar. In 1982, 20 branches were cut from Scots pine and divided into three sections of current year's foliage, old foliage and bare twigs to determine the distribution of hibernacula. The sections were treated with hot NaOH solution, washed, filtered, and the larvae were counted (Miller *et al.* 1971). A total of 35 larvae were recovered of which 85% were found on the old foliage and bare branches. Even though two egg masses were found on the current foliage of two branches, only 12 larvae were recovered, indicating that larvae probably moved away from the light towards the bole of the tree to select an overwintering site. Terrell (1959) compared stem and branch samples for spruce budworm larvae on Douglas-fir and found, for an equivalent area, 2.9 larvae on the branches and 58 larvae on the bole.

In spring of 1981, young larvae first appeared on the tips of candles during the last week of May. Silk threads were visible between tips of needles, around candles and female cones (Fig. 3B) suggesting that larvae dispersed at this time. Larvae had spun silk enclosures at approximately 45° from the candle's main stem (Fig. 3C), but attached to it, about 25 mm from the tip. Feeding started at the base of needle sheaths of new growth. There was no evidence of needle mining, probably because the new needles were available when larvae emerged from their hibernacula. In 1982 larvae had spun silk enclosures by the second week of June and when feeding, only the top 1/4 to 1/3 of their bodies were exposed. Larvae fed with their heads outward and quickly retracted into their enclosures when disturbed.

Larvae feeding in the field molted from second to third and fourth instar and continued to feed at the needle bases (Fig. 3D). Those reared in the laboratory on artificial diet (Robertson 1979) stopped feeding at the end of the third instar and entered a second diapause (97%), even though rearing conditions simulated field conditions. Harvey (1967) similarly noted that *C.*

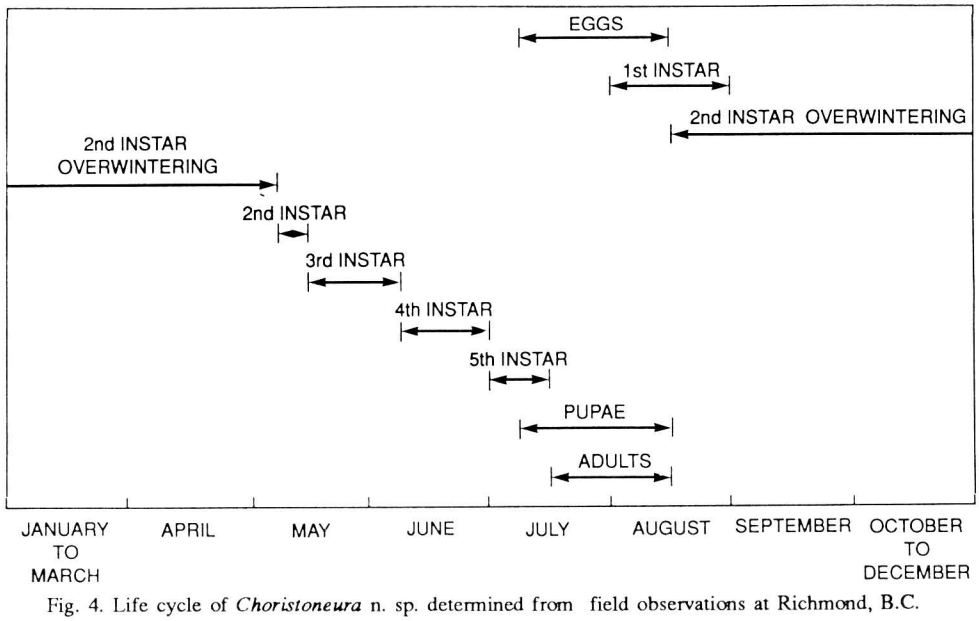

Fig. 4. Life cycle of *Choristoneura* n. sp. determined from field observations at Richmond, B.C.

orae, which we consider to be closely related to *Choristoneura* n. sp., tended to enter a second diapause (82%) when reared in the laboratory. When entering the second diapause, third instar larvae spun tight double silk enclosures and, once enclosed, shed the head capsule and integument at opposing ends of the hibernaculum. The larva reduced in size from 3.3 - 4.0 mm to 1.67 - 2.67 mm in length because they ceased feeding and used their food reserves to spin the hibernacula. There was no visible movement by the larvae unless subjected to high-intensity light or probing.

Fourth-instar larvae fed mostly on the south facing side of the host. Larvae did not attack the main stem or developing female cones but usually consumed one needle completely before chewing another. This behavior is unique to this species. Other *Choristoneura* species are wasteful feeders and often take one or two bites from a needle before moving to another; they thus cause very noticeable defoliation. Feeding sites had an average of 18 needles held to the developing candle with silk. Defoliation was therefore not detectable from a distance. Most larvae were in the fourth instar by the third week of June; there appeared to be more larvae present at that time than when observed as third instars, suggesting a second diapause in the field.

Fifth-instar larvae looked like those of *C. occidentalis*. They were more free roaming than previous instars (Fig. 3E), and they spun loose silk enclosures to secure developing side candles to the main candle.

Individual pupae were present on the foliage by the first week of July and were attached near the tips of candles by silk and dead needles. Often, pupae were found under curled immature cones. They were always oriented with the anterior end pointing distally along the axis of the candle. The pupal stage in the laboratory averaged 15 days at 19°C and the male/female ratio was close to 1.

The adults (Fig. 3F) were first evident in mid-July and were visible resting or laying eggs on the current year's foliage for approximately four weeks. The adults, which normally fly at dusk, flew only during daylight hours when branches were disturbed by the wind or physically moved.

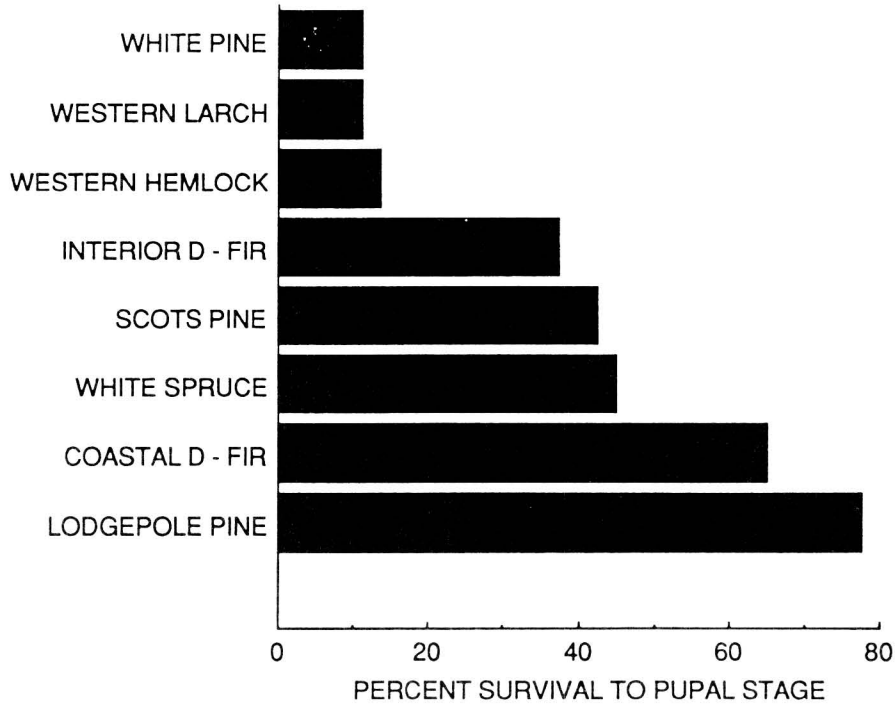

Fig. 1 Survival of *Choristoneura* n. sp. on different hosts maintained in an environmental chamber for 36 days. n=80

Host

Scots pine, *Pinus sylvestris* L. Laboratory rearings indicate that this insect can survive equally well on lodgepole pine (*Pinus contorta* Dougl.), coastal Douglas-fir and white spruce (*Picea glauca* (Moench) Voss), but it has not been found on these native species (Fig. 1). Scots pine is a non-native tree. These were planted by the Ministry of Transportation and Highways in 1959 as 18-inch seedlings. Increment cores taken in 1980 indicated that the trees were 21 years old.

Distribution

Living specimens occurred in Richmond, British Columbia. Pheromone trapping with equal amounts of the two acetates and two alcohols failed to catch any *Choristoneura* sp. on Scots pine in Norway, (Dr. A. Bakke, Norsk Institutt For Skogforskning, Postboks 61, Norway, personal communication). Similarly, traps baited with this blend failed to trap any *Choristoneura* sp. in Japan, (Dr. S. Suzuki, Hokkaido Forest Experiment Station, Hokkaido, Japan, personal communication). Monitoring with pheromone traps traced the origin of the Scots pine to a Richmond nursery (Fig. 2) which had imported the trees in the early 1950s, probably from Ontario or Washington State. This was confirmed by talking to the nursery owners but the trees' origin could not be verified due to the length of time that had lapsed.

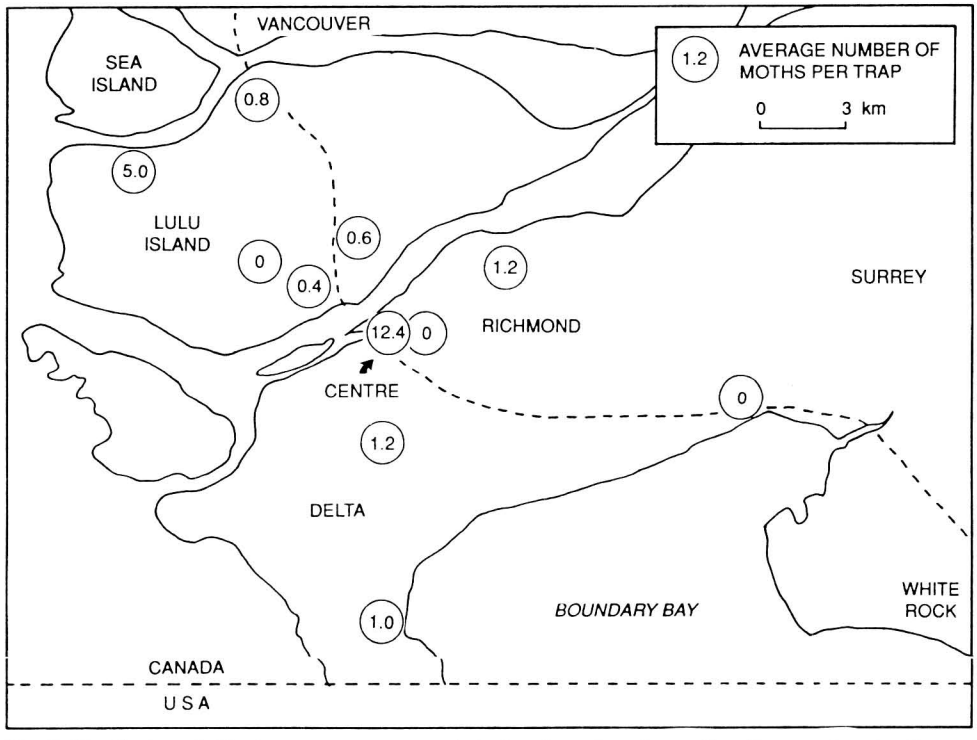

Fig. 2. Distribution of *Choristoneura* n. sp. around the location where it was first discovered at the George Massey Tunnel, Richmond, B.C.

Associated Insects

No parasitoids were encountered during this insect's life cycle, either in the 35 egg masses, or in more than 100 fourth- and fifth-instar larvae collected and reared. The most common lepidopteran present on the host trees was the European pine shoot moth, *Rhyacionia buoliana* (Schiffermüller), and in 1980 six out of ten new candles contained a shoot moth larva. The oblique-banded leaf roller, *Choristoneura rosaceana* (Harris), was also present in limited numbers, as was *Ditula* (*Batodes*) *angustiorana* (Haworth); both of these species are known to be polyphagous feeders. Silverspotted tiger moth larvae, *Lophocampa argentata* (Packard), were observed feeding on old foliage of several trees.

IDENTIFICATION OF PHEROMONE COMPONENTS

Methods

Late instar larvae were hand picked and reared to pupation on clipped branches in moist sand in a propagation box in a greenhouse at about 20°C. The pupae were sexed, separated and placed in petri dishes with moist filter paper. The female pupae were kept under a 16:8 L:D photoperiod and maintained at that regime after eclosion.

Abdominal tips were excised from unmated females 2 to 4 days old at 1 to 3 hours into the scotophase (Gray *et al.* 1984). Each tip was washed with 5 µl of redistilled hexane and the wash was injected into a Hewlett-Packard 5880A capillary gas chromatograph (CGC) in splitless mode, equipped with a flame ionization detector. The capillary column was 0.25 mm i.d. x 30 m methyl silicone (SE-30) (Hewlett-Packard Co., Palo Alto, CA), programmed at 80°C for 2 min, warming at 15°C/min to 180°C and isothermal at 180°C. Injector and detector temperatures were 275°C. Standards were run under identical conditions to enable the comparison of retention times.

A pooled sample of washes from five females was analyzed on a Hewlett-Packard 5985 capillary gas chromatograph/mass spectrometer (GC/MS) in the splitless mode. The 0.32 mm i.d. x 15 m SE-30 column (J & W Scientific, Folsom, CA) was programmed at 70°C for 1 min, warmed at 4°C/min to 210°C and isothermal at 210°C.

Field testing of candidate components was conducted in 1980 on Scots pine using delta traps (made from 2-L milk cartons) coated inside with Bird Tanglefoot (The Tanglefoot Co., Grand Rapids, MI 49504). The traps, with a trapping surface of 495 cm^2, were baited with candidate chemicals in polyvinylchloride (PVC) 5% w/w (Daterman 1974) which were impaled with a pin inside the delta traps. The lures were PVC rods 3 mm in diameter and 5 mm in length containing 1250 µg of the candidate chemical; they were aged for 5 days at 20°C prior to use to stabilize the release rate. The chemical lures were replicated four times while unmated females of *C. pinus pinus* and *C.* n. sp. were replicated twice.

Results and Discussion

Capillary gas chromatographs of individual tip washes indicated four pheromone compounds. No aldehyde component was detected, indicating that the species was more closely related to *C. orae* and the pine feeding *Choristoneura*, which lack an aldehyde component in their attractive blends (Gray *et al.* 1984; Harvey 1985; Silk *et al.* 1985). The four detected compounds had retention times coincident with *E*-11-tetradecen-1-ol (*E*-11-14:OH), *Z*-11-tetradecen-1-ol (*Z*-11-14:OH) (*E*/*Z*~ 2:1), *E*-11-tetradecenyl acetate (*E*-11-14:Ac) and *Z*-11-tetradecenyl acetate (*Z*-11-14:Ac) (*E*/*Z*~ 2.5:1). There was no indication of any saturated alcohols or acetates present in the single insect traces. Capillary GC/MS indicated identical retention times and fragmentation patterns for the four detected compounds and synthetic standards.

Field bioassays conducted in 1980 (Table 1) indicated that an equal mixture of *E*/*Z*-11-14:Ac and *E*/*Z*-11-14:OH was a better attractant than the individual compounds, although the means were not significantly different with the exception of the poor response to *Z*-11-14:Ac. Additional testing in 1981 (Table 2) again indicated that an equal mixture of *E*/*Z*-11-14:Ac and *E*/*Z*-11-14:OH was the most attractive blend and was able to attract more moths than did unmated females. The ability of unmated female *C. pinus pinus* to attract a considerable number of male *C.* n. sp. (Table 2) would suggest a taxonomic closeness, at least chemically if not morphologically (Dang 1985). An initial test in 1979 using a similar chemical blend as that proposed for *Choristoneura occidentalis* (Cory *et al.* 1982) containing *E*/*Z*-11-tetradecenals failed to attract any male *Choristoneura*. We therefore recommend as an effective sex attractant lure for detection and monitoring *Choristoneura* n. sp. *E*/*Z*-11-tetradecenyl acetates and *E*/*Z*-11-tetradecen-1-ols in equal amounts and a lure loading of 312 µg of each chemical.

CONCLUSIONS

This undescribed insect may be considered by some authorities as being a hybrid, or a host race, "a noninterbreeding sympatric population, which differs in biology but not, or scarcely,

Table 1. Number of Choristoneura n. sp. captured from 23 to 27 July 1980 at Richmond, B.C.

Lure	Composition %	Males caught	Average/ night/trap
E/Z-11-14:Ac+ E/Z-11-14:OH	25/25/25/25	54	3.38 a
Z-11-14:OH	100	38	2.38 a
E-11-14:Ac	100	37	2.31 a
E-11-14:OH	100	24	1.50 a
Z-11-14:Ac	100	1	0.06 b

Treatment totals followed by the same letter are not significantly different, Duncan's new multiple range test, $p < 0.05$.

Table 2. Number of males captured from 16 to 31 July 1981 at Richmond, B.C. when testing unmated females and synergism of isolated pheromone-like compounds.

Lure	Composition %	Males caught	Average/ night/trap
E/Z-11-14:Ac+ E/Z-11-14:OH	25/25/25/25	307	4.8 a
E/Z-11-14:Ac+ E-11-14:OH	33/33/33	274	4.3 a
E/Z-11-14:Ac	80/20	206	3.2 a
E/Z-11-14:Ac+ Z-11-14:OH	33/33/33	185	2.9 a
E-11-14:Ac+ E/Z-11-14:OH	33/33/33	12	0.2 b
E-11-14:Ac	100	6	0.1 b
Z-11-14:Ac+ E/Z-11-14:OH	33/33/33	3	0.05 b
Z-11-14:Ac	100	0	-- b
subtotal		993	
♀ Choristoneura n.sp.		158	3.4
♀ Choristoneura pinus pinus		108	2.5
Total		1259	

Treatment totals followed by the same letter are not significantly different. Duncan's new multiple range test, $p < 0.05$.

in morphology ... (and which are) prevented from interbreeding by preferences for different food plants or other hosts" (Mayr *et al.* 1953).

We believe that this insect is in fact a distinct species for several reasons: it possesses a unique pheromone, and is thus reproductively isolated; the ovipositing females display a distinct host preference, in this case Scots pine; the geographic distribution of the population, the ability of larvae to feed on Scots pine; and the feeding behavior of larvae, all these appear to be unique within this genus.

The origin of this species is unknown. The restricted distribution, proximity to international marine import terminals, exotic host, and the lack of parasites suggest that it may be an introduced species. However, taxonomically it appears closely related to *C. orae* and *C. pinus pinus*, both of which are Canadian species.

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