CHEMISTRY AND FIELD EVALUATION OF THE SEX PHEROMONE OF WESTERN SPRUCE BUDWORM, Choristoneura occidentalis, FREEMAN

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Abstract—Chemical analyses and field bioassays showed a mixture of 92% (E)- and 8% (Z)-11-tetradecenal to be the sex attractant pheromone of the western spruce budworm, *Choristoneura occidentalis* Freeman. Females were also found to emit small amounts of the corresponding acetates and alcohols, but these components were not active in the bioassay. In contrast, in whole female tip extracts, (E)-11-tetradecenyl acetate predominates, and aldehyde pheromone components are present in lesser quantities, suggesting that final biosynthesis of pheromone takes place just prior to or during emission. At release rates approximating that of the female (2–4 ng/hr), a 92:8 E:Z blend of the synthetic aldehydes was at least as attractive as live females. Addition of the corresponding acetates or alcohols up to 50% of the aldehyde content did not significantly enhance or inhibit attraction. No major differences were apparent in pheromone production of females from a laboratory stock or from field collections from diverse geographic locations ranging from Colorado to British Columbia.

Key Words—Western spruce budworm, *Choristoneura occidentalis*, sex pheromone, attractants, moth behavior, chemical identification.

INTRODUCTION

The sex pheromone of the western spruce budworm, *Choristoneura* occidentalis Freeman, is known to be similar to that of the spruce budworm, *C. fumiferana* (Clem.) (Weatherston et al., 1971). The principal attractant component for males of both species is (E)-11-tetradecenal. Interspecific

attraction was strong between the two species, but there were sufficient differences in response of *C. occidentalis* and *C. fumiferana* males to suggest the presence of different "secondary" pheromone components (Sanders et al., 1977).

This study was undertaken to clarify the *C. occidentalis* pheromone system, with particular attention to potential effects of the secondary components which may optimize or inhibit attraction. Female moths from selected geographic locations were evaluated for their pheromone content. All bioassays for attractiveness were conducted under field conditions with baited traps.

METHODS AND MATERIALS

Our study approach was to collect late instar or pupal stage *C. occidentalis* from selected geographic locations, and subject them to standard pheromone extraction and analytical procedures. A nondiapause laboratory stock was also evaluated. Once determined chemically, blends of synthetic pheromone components were compared to live females for their relative attractiveness in field bioassays.

Pheromone Extraction. Initially, the method of pheromone extraction consisted of dipping the tip of the female abdomen in hexane for 10-20 sec or soaking excised abdominal tips in hexane for an extended period. Conditioning the moths for the dip or soak extractions consisted of holding them in a 16-hr light: 8-hr dark regime with the extractions taking place 13-15 hr following the onset of illumination. The specific techniques of removing abdominal tips for dipping in the solvent were basically those of Sower et al. (1973).

A system of air collection in Porapak-Q[®] was begun when a clear difference was revealed between the chemicals within or rinsed from the surface of the pheromone gland. Freshly emerged female moths in groups of 8-10 were placed in a 200-ml glass chamber fitted with an air-intake port at the base and channeled to a narrow outflow tube at the top. Compressed air was fed into the base of the chamber through a Porapak-Q column to ensure purity. Air moved through the chamber at a constant flow rate of 250 ml/min and was routed out the top through a second Porapak-Q column which trapped the pheromone. The glass chamber and glass columns were treated with silanizing reagent and thoroughly rinsed with solvent before usage. The amount of Porapak-Q used to absorb the pheromone was approximately 1 ml in bed volume. The moths were held under these conditions for 48 hr at a constant temperature of $21 \pm 2^{\circ}$ C and a light regime of 16 hr light: 8 hr dark. After 48 hr the moths were discarded, and the outflow Porapak-Q column was eluted with 1-2 ml of hexane to wash out the female moth effluvia. Prior to use for pheromone collections the Porapak-Q columns were washed thoroughly with methanol and hexane. All extracts were held in a freezer until processed for chemical analysis.

A slow-release polyvinyl chloride formulation containing 0.1% E-11tetradecenal was also checked for release rate using the same air collection technique. Pellets identical to those used for trap baits in field bioassays but longer in length (20 mm vs. 5 mm) were held in the collection apparatus for 48 hr at 26° C (three replications).

Insect Supply Sources. Six different sources of female C. occidentalis were subjected to the hexane-soak method of pheromone extraction. These included a nondiapause laboratory culture, reared on a wheatgerm-base artificial diet (Peterson, 1978), and field collections of pupae taken near Taos, New Mexico; McCall, Idaho; Missoula, Montana; Kamloops, British Columbia; and Warm Springs, Oregon.

The "dip" (gland wash) method of pheromone extraction was applied to two sources of insects, the nondiapause laboratory culture and the McCall, Idaho, collection.

Pheromone extracts by the air collection technique were limited to four populations: the nondiapause laboratory strain, and field collections from Twisp, Washington; Warm Springs, Oregon; and Fort Collins, Colorado.

Chemical Analyses. The sensitivity of two analytical techniques permitted the analysis of extracts derived from small numbers of insects (less than 20 females) without preparative treatment. The extracts were reduced in volume in Reactivials[®] by careful evaporation at room temperature under a slow stream of nitrogen to a volume of ca. 50 μ l, and analyzed as follows:

Glass capillary column gas chromatography enabled clear separations of E and Z stereoisomers of pheromone constituents at levels as low as 0.5 ng/injection. A Hewlett-Packard 5880A gas chromatograph equipped with an open tubular wall-coated glass capillary column (60 m \times 0.5 mm ID, J. & W. Scientific) with SP2340 liquid phase was used. The capillary injection system was operated in the splitless mode with inlet purging 30 sec after injection. Injector and detector temperatures were 200° C; the oven temperature was held at 90° C initially for 2 min, then temperature programed at a rate of 7° / min to a final value of 200° C. Carrier gas flow (helium) was 50 ml/min. An automated keyboard permitted the programing of parameters so the retention times of the compounds of interest were highly reproducible.

Our second technique combined the use of gas chromatography and mass spectrometry. A Varian Aerograph 2700 gas chromatograph equipped with a glass column (2 m \times 2 mm ID) packed with 1.5% OV-101 on 100-200 mesh Gas Chrom Q[®] was operated isothermally at 170°C. Injector and detector temperatures were 250°C. The instrument was interfaced with a DuPont 21-491B mass spectrometer equipped with a 4-channel DuPont SMID accessory for selected ion monitoring. The following characteristic fragment ions were monitored: m/e 192 (11-tetradecenal), m/e 194 (11-tetradecenyl acetate and 11-tetradecenol), and m/e 166 (ion common to all three compounds). Unlike the capillary GC system, the *E* and *Z* isomers were not separable on the packed column.

Bioassay Procedures. Following tentative identifications of pheromone components, synthetic blends of these materials were formulated into solidplastic controlled-release trap baits (Daterman, 1974). The relative attractiveness of these baits was evaluated in the field by comparing the numbers of male moths captured in traps baited with live females versus those baited with the different blends and strengths of synthetics. Traps used in these evaluations were fashioned from 2-liter paper milk cartons formed into a triangular cross-section. The traps were open at each end, lined with Tanglefoot® adhesive, and had a maximum capture capacity of about 130 males. Baits were all 5×3 -mm-diam pellets which contained 0.01, 0.1, or 3.0% total pheromone components by weight. Baits were suspended from the interior center of the trap on an insect pin. Female moths used as baits were 12-48 hr old when placed in the field and were confined singly within small cylinders (approximately 25×75 mm) of fiberglass screen. One female, caged in this way, was positioned in the center of each trap.

The first of four field bioassays reported here was conducted in 1976 and incorporated different proportions of E:Z 11-tetradecenals formulated in polyvinyl chloride bait pellets. All preparations contained 3% tetradecenals by weight, and the E:Z blends varied from 100% E isomer to a 60:40 E:Zratio. The baits were tested for attractiveness to C. occidentalis in south central British Columbia and in south central Oregon, and to C. Fumiferana in southern Ontario. Each treatment blend of the E:Z isomers was replicated in 10 baited traps for each location. The results of this test are included here to demonstrate a basic diference in the pheromone response of C. occidentalis and C. fumiferana.

Three additional field experiments were conducted in 1979 and 1980 to evaluate the addition of secondary components. The first of these compared only synthetic bait preparations as follows: >99% pure (E)-11-tetradecenal; a commercially available blend approximating the known pheromone for C. fumiferana (Sanders and Weatherston, 1976, reported 94:6 E:Z; Silk et al., 1980, reported 95:5 E:Z) of 97:3 E:Z 11-tetradecenal; a 92:8 blend of E:Z11-tetradecenal; and a 90:8:1.8:0.2 blend of (E)-11-tetradecenal:(Z)-11tetradecenal:(E)-11-tetradecenyl acetate:(Z)-11-tetradecenyl acetate. The >99% pure (E)-11-tetradecenal was prepared by progressive ether-in-hexane elution of a 97:3 blend of E:Z 11-tetradecenal through a silicic acid-silver nitrate column. Bait preparations were formulated in polyvinyl chloride and contained 0.1% by weight of the candidate pheromone components. Six sets or replications of the four baits were placed in the field for three days and nights, with the sequence of trap placement randomized within individual sets. All traps were hung on the ends of host tree branches 2-2.5 m above the ground, in such a way that the ends would remain open at all times. Within a replication, traps were placed 20-40 m apart, whereas the different trap lines or replicate sets were separated by 75 m or more. Host trees within the area were pole-sized and mature Douglas fir, *Pseudotsuga menziesii* (Mirb.) Franco, and grand fir, *Abies grandis* (Dougl.) Lindl. Population density of the insect was apparently similar within the trapping locations and the surrounding stands as judged by the uniformly light degree of defoliation.

The next series of baited traps was basically the same except that they included female-baited traps and the synthetic baits were formulated to contain 0.01% of the pheromone components. Again, six replications of each bait treatment were exposed in the field with the sequence of trap placement randomized within a set of the five treatments. The traps were collected after three days and nights of exposure in the field. We estimate that the 0.01% polyvinyl chloride preparations of (E)-11-tetradecenal approximated the release rate of this compound from females. This estimate was based on Porapak-Q collections from bait pellets and live females. In making this comparison we arbitrarily assumed the females each emitted pheromone for about 2 hr/day in the collection chambers. Polyvinyl chloride samples containing 0.1% (E)-11-tetradecenal and held in the same apparatus as the females, released the E-aldehyde at a rate of $2 \pm 0.6 \ \mu g/day$. Projecting downward to the 5×3 -mm-diam. bait size and 0.01% concentration, we get a release rate of 8 ng/hr at 26°C.

The final series of trap baits was designed to assess effects on male attraction when 11-tetradecenyl acetate and alcohol compounds were released in larger proportions in blends with the aldehyde. This was of interest because of the negative influences caused by the acetate and alcohol components associated with the C. fumiferana pheromone system (Sanders, et al., 1972; Sanders 1976; Weatherston and Maclean 1974). Also, the alcohols and acetates were expected to have lower release rates from the polyvinyl chloride baits, and field testing higher concentrations would partially compensate for this and provide greater opportunity to detect inhibiting or optimizing effects on attraction. Five combinations of synthetic aldehydes, acetates, and alcohols were tested and compared to one another and live female baits for attractiveness. Synthetic baits were formulated to contain 0.1% candidate pheromone components on a weight basis, and aldehyde, acetate and alcohol preparations were all 90-92% E and 8-10% Z isomer mixtures. The six treatments were as follows: live females (one per trap); 10 parts aldehyde only; 10 parts aldehyde plus 2 parts acetate; 10 parts aldehyde plus 2 parts acetate plus 2 parts alcohol; 10 parts aldehyde plus 5 parts acetate; and 10 parts aldehyde plus 5 parts acetate plus 5 parts alcohol. Traps were exposed in the field for three days and nights. Each bait treatment was replicated 10 times with the sequence of placement randomized within a replicate set of the six different treatments.

RESULTS AND DISCUSSION

Chemical Analyses. Table 1 lists the amounts of pheromone compounds measured in the effluvia and abdominal tip extracts of *C. occidentalis* obtained from a number of sources. Certain trends evident from the table can be summarized as follows:

The effluvia contained principally (E)-11-tetradecenal with lesser amounts of (Z)-11-tetradecenal, these isomers being present in the ratio 92:8. Small amounts of (E)-11-tetradecenyl acetate were present in some of the samples at about 2% of the amounts of 11-tetradecenal. No alcohols were detected in our air collection extracts. In contrast, 11-tetradecenyl acetate was the predominant pheromone-related constituent in all of the hexane-soak gland extracts, with smaller amounts of (E)-11-tetradecenal and 11-tetradecenol also present. The average value for the E-to-Z acetate stereoisomer ratio was 90:10 in these extracts.

Hexane-wash (dip extracts) of pheromone glands showed a clear difference in relative quantities of aldehydes and acetates found outside versus inside (soak extracts) the gland. Results were inconsistent, however, concerning the quantities of alcohols recovered in proportion to aldehydes. The proportion of 11-tetradecenol varied from 0 to 30% of the quantity of 11-tetradecenal (Table 1), and this caused us to consider the possibility that the dip technique was also extracting material from inside the gland. This skepticism was apparently unwarranted, however, since Silk and coworkers (1982) have shown that the alcohol is emitted in the *C. occidentalis* effluvia.

It is possible that some pheromone-related components in the effluvia, particularly small quantities of alcohols, were lost on the glass surfaces of our air collection apparatus. This factor could partially account for some of the discrepancies between our determination of acetate and alcohol content, and those of Silk et al. (1982).

As shown by the hexane-soak gland extracts, acetates are the major pheromone-related chemicals inside the gland, and they must be considered important precursors in biosynthesis. Conversion to aldehydes, the predominant chemicals released by females, apparently occurs at or very near the gland surface during the emission period.

Field Bioassays. Results of the 1976 experiment comparing the attraction of E: Z 11-tetradecenal blends to Oregon and British Columbia populations of C. occidentalis and an Ontario population of C. fumiferana showed a definite response difference between the species (Figure 1). The C. fumiferana

				Compound (ng	/female moth)		
				11-tetra	decenyl		
	Eamolae	11-teti	radecenal	ace	tate	11-tetra	decenol
	r entaces extracted ^a	E	(Z)	E	(Z)	E	(Z)
Effluvia							
Lab stock	30	10	(-)	I	(-)	ł	<u> </u>
Warm Springs, OR	32, 18, 24	20	(1.8)	0.4		ł	
Fort Collins, CO	32	20	(1.8)	0.5	Ĵ	ł	.
Twisp, WA	24	10	(0.9)	ц	(-)	ţ	(-)
Gland soak extract							
Lab stock	161, 158, 99, 40, 36	7	(-)	S	(0.5)	-	-
Idaho	73, 41, 29	2	(-)	10	(1)		-
Montana	58, 52, 41	7	(-)	9	(0.6)	ł	Ĵ
New Mexico	25	2	(-)	×	(0.8)	١	1
British Columbia	27, 31, 30	7	(-)	12	(1.2)	4	Î
Oregon	20, 30	7	<u>(</u>)	10	(1)		
Gland wash ("dip")							
Lab stock	286, 554	1	(-)	0.6	(-)	0.3	Ĵ
Idaho	117	-	(-)	0.5	(-)	I	()

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^aThe number of females extracted comprised the pool of specimens present in each analyzed sample.



FIG. 1. Response of *C. occidentalis* and *C. fumiferana* (Ontario) males to synthetic trap. baits containing different E:Z ratios of 11-tetradecenal.

males were more sensitive to changes in the E: Z isomer blends, displaying their strongest response and with no significant differences to blends composed of 92.5-99% E isomer. The Oregon C. occidentalis, in contrast, demonstrated maximum response with no significant differences to blends containing 80-97% E isomer. The British Columbia C. occidentalis were equally trapped by blends containing E isomer of 60-100%.

Subsequent testing of 0.1% synthetic baits to evaluate the attractant effects of (Z)-11-tetradecenal and 11-tetradecenyl acetate confirmed that the addition of (Z)-11-tetradecenal enhanced attraction (Table 2). The preparation from which all detectable (Z)-11-tetradecenal had been removed (>99%)

Males per trap
64.5a ^b
60.5a
51.7a

TABLE 2. ATTRACTION OF SYNTHETIC PHEROMONE BAITS FORC. occidentalis Formulated at 0.1% Concentration inPVC CONTROLLED-RELEASE PELLET

^aTDAL and TDAC refer to tetradecenals and tetradecenyl acetates.

^bMeans followed by same letter are not significantly different at P = 0.05.

pure) caught significantly fewer male moths than preparations where the chemical was present. The attractiveness of the other three preparations, however, did not significantly differ. Thus, 0.1% bait preparations showed no apparent differences in the attractiveness of the 97:3 and the 92:8 blends of (E)-11-tetradecenal:(Z)-11-tetradecenal, or the mixture of 90:8:1.8:0.2(E)-11-tetradecenal:(Z)-11-tetradecenal:(E)-11-tetradecenyl acetate:(Z)-11-tetradecenyl acetate.

The field test which compared live female attraction to various synthetic baits formulated at 0.01% concentration provided only a slightly different set of results (Table 3). Both synthetic preparations containing 8% of the (Z)-11tetradecenal were again superior ($P \le 0.05$) to the >99% pure (E)-11tetradecenal, but we did not show significant differences among any of the other four treatments. The average trap catches by the 97:3 blend of E: Z11-tetradecenal and the live female baits were only about half that of the traps baited with blends containing 8% of the Z isomer (Table 3), and it is possible that a more sensitive experimental design (more replication) would separate these groups. Addition of the 9:1, E: Z11-tetradecenyl acetate to the optimal aldehyde mixture did not significantly improve attraction. On the basis of these results we can say that (Z)-11-tetradecenal is a necessary component of the pheromone that clearly enhances attraction. It is also evident that the 0.01% baits composed of 90 or 92% (E)- and 8% (Z)-11-tetradecenal were at least as attractive as the authentic pheromone produced by live females. The quantities of pheromone recovered from live females in the air-collection device (Table 1) suggest the average female emits pheromone at a rate of about 2-5 ng/hr. Thus, the 0.01% bait pellets were approximating the female release rate at 26°C or below.

The final field test evaluating possible enhancing or inhibitory effects of

Trap baits	Males per trap ^b
90:8 E:Z 11-TDAL ^a	28.5a
+ 1.8:0.2 <i>E</i> ; <i>Z</i> 11-TDAC	
92:8 E:Z 11-TDAL	24.2a
97:3 <i>E</i> : <i>Z</i> 11-TDAL	13.7a,b
Live females (one/trap)	12.7a,b
100 (E)-11-TDAL (>99% pure)	6.2b

 TABLE 3. ATTRACTION OF LIVE FEMALES AND SYNTHETIC PHEROMONE

 BAITS FOR C. occidentalis Formulated at 0.01% Concentration in

 PVC CONTROLLED-RELEASE PELLET

^aTDAL and TDAC refer to tetradecenals and tetradecenyl acetates.

^bMeans followed by same letter are not significantly different at $P \le 0.05$.

11-tetradecenyl acetates and alcohols, when formulated at concentrations up to 50% of the aldehyde component, failed to show any such effects. Table 4 summarizes the results of this test which indicated no significant differences in attractiveness ($P \le 0.05$) among the synthetic preparations. Two of the synthetic preparations, one containing only the *E* and *Z* aldehyde pheromone components and the other containing these plus the corresponding acetates and alcohols at 20% of the aldehyde concentration, were significantly more attractive ($P \le 0.05$) than live females.

In a separate study of *C. occidentalis* pheromone chemistry, Silk et al. (1982) have reported larger quantities of the 11-tetradecenyl acetates plus 11-tetradecenyl alcohols in the female effluvia than we have reported here. The proportions of acetates and alcohols reported by Silk and coworkers are greater than the proportions used in any of our field bioassays, although the quantities used in this final evaluation begin to approach their ratios. Thus, if the acetates or alcohols were functional pheromone components and enhanced male attraction, such effects should have been evident in the results of this final bioassay. These results suggest that the acetates and alcohols are not necessary pheromone components for long-distance sex attraction.

Silk et al. (1980) have reported a parallel finding with *C. fumiferana*, where female emissions of acetate and alcohol components were apparently not active in the pheromone system. It is possible that the acohols or acetate play a role in short-distance communication between the sexes, and our trapping bioassay was not designed to detect this role. In any case, blends of 92% (*E*)-11-tetradecenal plus 8%(*Z*)-11-tetradecenal closely approximate the sex phreromone of *C. occidentalis*.

TABLE 4. EFFECTS ON ATTRACTION OF SYNTHETIC C. occidentalis SEX
PHEROMONE BAITS (FORMULATED AT 0.1% CONCENTRATION) BY
Addition of Large Quantities of Alcohols and Acetates
to the Optimum E and Z Blend of Aldehydes

Trap baits ^a	Males per trap ^b	
 10 TDAL + 2 TDAC + Z TDOH	62.7a	
10 parts TDAL	58.6a	
10 TDAL + 5 TDAC + 5 TDOH	51.4a,b	
10 TDAL + 2 TDAC	50.6a,b	
10 TDAL + 5 TDAC	45.0a,b	
Live females (one/trap)	29.7b	

^{*a*}TDAL, TDAC, and TDOH refer to tetradecenals, tetradecenyl acetates, and tetradecenyl alcohols, all of which were blended with 90-92% E and 8-10% Z isomers.

^bMeans followed by same letter are not significantly different at $P \le 0.05$.

CONCLUSIONS

Chemical analyses and field bioassays of the C. occidentalis pheromone system showed the active sex attractant components to be (E)- and (Z)-11tetradecenal in a ratio of ~92:8. Field tests with synthetic baits releasing these components at rates approximating that of female moths demonstrated they were at least as attractive as the females. Within the gland, these aldehyde components were present in relatively low quantities with the corresponding (E)-11-tetradecenyl acetate present in greater quantity. (Z)-11-Tetradecenyl acetate and (E)- and (Z)-11-tetradecenyl alcohols were also found within the gland in small quantities. In the effluvia, or material emitted by the females, we detected only the aldehyde and acetate. Female effluvia contained 90 parts (E)-11-tetradecenal:8 parts (Z)-11-tetradecenal:1.8 parts (E)-11-tetradecenyl acetate:0.2 parts (Z)-11-tetradecenyl acetate.

The (E)- and (Z)-11-tetradecenyl alcohols were recovered from some dip (gland-wash) extracts, and we cannot explain why air collection in Porapak-Q did not also detect alcohols in the female effluvia. In field bioassays we could not demonstrate any significant enhancing or inhibitory effects on attraction caused by either 11-tetradecenyl acetates or alcohols.

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