SEX PHEROMONE OF THE EASTERN SPRUCE BUDWORM (LEPIDOPTERA: TORTRICIDAE): OPTIMUM BLEND OF *TRANS-* AND *CIS*-11-TETRADECENAL

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Abstract

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A reinvestigation of the sex pheromone of the eastern spruce budworm, *Choristoneura fumiferana* (Clem.), showed that it contains 11-tetradecenal in the ratio 96% *trans-* : 4% *cis-*. Field trapping showed the pure *trans-* compound to be only slightly attractive. Maximum attraction occurred in the range 2% to 5% *cis-*.

Résumé

De nouvelles recherches sur la phéromone sexuelle de la Tordeuse des bourgeons de l'Epinette *Choristoneura fumiferana* (Clem.), ont démontré qu'elle comporte du 11-tétradécenal dans une proportion de 96% *trans-* : 4% *cis-*. Le piégeage sur le terrain a indiqué que le composé pur *trans-* était assez peu attractif. L'attraction maximum se produisit dans la gamme variant de 2% à 5% *cis-*.

Introduction

The main component of the sex pheromone of the eastern spruce budworm, *Choristoneura fumiferana* (Clem.), was identified by Weatherston *et al.* (1971) as *trans*-11-tetradecenal. However, inconsistent results with different commercial preparations of this compound in 1972 led to further investigations, reported here, which indicate that a small proportion of the *cis*-isomer together with the *trans-* compound is essential for attraction, and that the pheromone produced by female eastern spruce budworm, though predominantly *trans*-11-tetradecenal, also contains a small proportion of the *cis*-isomer, indicating that these two compounds are the main active components in the sex pheromone of this species.

Methods and Results

Laboratory Investigations

All *trans*-11-tetradecenal, and the *cis*-11-tetradecenal used in the 1975 field trials, were purchased from ChemSampCo, Columbus, Ohio, and met specifications of less than 3% impurities, and 0.0% alcohol or acetate. The *cis*-11-tetradecenal used in the 1974 field work was prepared by hydrolysis of *cis*-11-tetradecen-1-ol acetate obtained from Farchan Division, Story Chemical Products, Willoughby, Ohio. The resultant alcohol was converted into *cis*-11-tetradecenal by the method of Gunstone and Lie Ken Jie (1970).

Gas chromatography was performed on a Perkin-Elmer 990 gas chromatograph fitted with flame ionization detectors. The analytical conditions used for isomer preparation were 15.25 m × 0.05 cm stainless steel S.C.O.T. columns operated at 145°C with a helium flow rate of 3.3 ml/min. The liquid phases used were (a) DEGS and (b) PDEAS.

Column chromatography was carried out on a 50 cm × 2.5 cm column of Adsorbosil-CABN (60/100 mesh) containing 25% silver nitrate. Thin layer chromatography was performed on 20 cm × 20 cm glass plates coated with a 0.25 mm layer of Adsorbosil-1-ADN containing 5% silver nitrate. Detection of aldehydes was achieved by spraying the developed plates with a 0.1% solution of 2,4-dinitrophenylhydrazine in acidic ethanol. The adsorbosils were obtained from Applied Sciences Laboratories,

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Inc., State College, Pa. All solvents were distilled in glass and obtained from Caledon Laboratories Ltd., Georgetown, Ont.

Purification of trans-*11-tetradecenal*

A sample of commercial *trans*-11-tetradecenal was chromatographed on Adsorbosil-CABN. The column was made up in hexane, and after the introduction of the aldehyde a fore-run of 400 ml of hexane was collected. The column was then eluted with hexane/ether, 9/1, collecting 50 ml fractions. The fractions were monitored by thin layer chromatography on Adsorbosil-1-ADN eluting with hexane/ether, 9/1. The R_f values of the isomers were recorded as (*a*) *trans*-11-tetradecenal, 0.55 and (*b*) *cis*-isomer, 0.43. Fraction 1 from the column did not contain any aldehyde; fractions 2, 3, and 4 contained the bulk of the aldehyde; however, only fraction 4 contained any *cis*-isomer as evidenced by thin layer chromatography.

Determination of Isomer Composition of Synthetic Compounds

Using capillary gas chromatography the following retention times were obtained: (*a*) DEGS: *trans*-11-tetradecenal, 8.7 min; *cis*-isomer, 9.4 min; and (*b*) PDEAS: *trans*-11-tetradecenal, 13.65 min; *cis*-compound, 14.50 min. The isomer composition as analyzed by capillary gas chromatography is given in Table I.

Determination of the Isomer Composition in Washes from Female Budworm

A solution containing the natural pheromone was obtained by rinsing out jars, which had contained virgin females, with hexane as previously described (Weatherston *et al.* 1971). In this manner the wash from about 3000 female budworm was collected in 1700 ml of hexane. The solution was filtered to remove scales and other debris, dried over magnesium sulphate, and then reduced at room temperature to a volume of 100 μl. Aliquots (0.3 μl) of this solution were analyzed on the PDEAS column and gave reproducible results (three replicates) indicating that the 11-tetradecenal composition was *trans* 96% to 96.1% and *cis* 3.9% to 4.0%.

Field Trials

In the first series of experiments reported here (those carried out in 1974), the chemicals were placed in polyethylene vial stoppers (Kimble Glass, 2-dr size). Solutions of the chemicals were made up in hexane, and the appropriate quantities of the solutions containing the *trans*- and *cis*-isomers were pipetted into separate stoppers. The hexane was allowed to evaporate in a fume hood and the inserts were then placed in the stoppers. The stoppers were exposed for 10 days in the fume hood before starting the experiment to stabilize the release rates. They were then taped to the inside of 3-M brand SA-21 reloadable traps, which were hung at a height of approximately 2 m in a

Table I. Isomer composition of various synthetic compounds used in the field trials recorded in Tables II, III, and IV

Material	Column	Isomer composition	
		% *trans*	% *cis*
Commercial *trans*-11-tetradecenal (1974)	DEGS	97.1	2.9
cis-11-tetradecenal (prepared by J. Weatherston)	DEGS	2.1	97.9
Fraction 4 from column chromatography	DEGS	98.0	2.0
Fraction 3 " " "	DEGS	> 99	< 1
Fraction 2 " " "	DEGS	> 99.5	< 0.5
Commercial *trans*-11-tetradecenal (1975)	PDEAS	99.7	0.3
Commercial *cis*-11-tetradecenal (1975)	PDEAS	0.3	99.7

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Table II. Numbers of male *C. fumiferana* captured in traps baited with various combinations of *trans*- and *cis*-11-tetradecenal loaded in separate polyethylene vial stoppers (each treatment replicated 10 times)

Trans	*Cis*	Ratio	No. of males per trap
* 1 mg	0	100: 0	10.5[a]
† 1 mg	< 10 μg	100:< 1	32.9[ab]
† 1 mg	10 μg	100: 1	108.1[c]
† 1 mg	20 μg	100: 2	301.7[d]
† 1 mg	100 μg	100: 10	111.4[c]
† 1 mg	1 mg	100: 100	48.8[b]
	Virgin female		108.1[c]
	Check		7.7[a]

*,†Fraction 2 and fraction 3 (Table I), respectively.
NOTE: Numbers followed by different letters are significantly different at the 5% probability level (Duncan's New Multiple Range Test).

10-m grid pattern in a 45-year-old white spruce (*Picea glauca* (Moench) Voss) plantation near Sault Ste. Marie, Ont. Empty check traps and traps baited with virgin females were included in the array. There were 10 replicates of each treatment, placed out in random sequence. The experiment was run for 6 days. Traps were checked every 2 days when they were moved ahead to the next position in the grid to avoid "position effects." The results (Table II) clearly indicate that the pure *trans* is only slightly attractive but that catches are greatly increased by small quantities of the *cis*-isomer.

This test required amplification for two reasons. First, a more precise definition of the optimum blend was required, and second, polyethylene stoppers are not now the preferred method for dispensing the attractant operationally. A polyvinyl chloride (PVC) formulation (Fitzgerald *et al.* 1973) appears much better suited to the proposed use of the sex attractant in an extensive annual monitoring program (Sanders, unpub. data). Further trials were therefore carried out in 1975. To determine if male *C. fumiferana* showed optimum response to the same blend throughout their range, tests were conducted in New Brunswick, Pennsylvania, and Alberta, as well as in Ontario.

The PVC formulation was essentially as described by Fitzgerald *et al.* (1973) and Daterman (1974), except that the plasticizer (di-ethyl hexyl phthalate) and PVC resin powder were mixed in the ratio 60:40 rather than 50:50 to produce a less viscous liquid that facilitated the removal of air bubbles. Aliquots of the liquid plastic were then drawn off and mixed with appropriate quantities of *trans*- and *cis*-11-tetradecenal to give a variety of blends, including pure *trans*-, pure *cis*-, and mixtures of the two (see Table III). In all instances the combined aldehydes formed 3% of the final product by weight. The liquid plastic containing the aldehydes was then taken up in glass tubes of 4-mm inside diameter. After fusing, the plastic was removed from the tubes and cut into 10-mm or 2-mm lengths. These were mounted on insect pins and held in a fume hood for at least 10 days prior to the start of the experiments to ensure a stable release rate. The pins were then either taped to the inside of 3-M brand SA-21 reloadable traps, or stuck through the top of 3-M brand Sectar 1 traps, with the bait suspended inside the trap (Daterman 1974). All traps were hung at a height of 2 m.

In the Ontario trials SA-21 traps, 20 m apart, were used. They were set out in the same heavily budworm-infested, 45-year-old white spruce plantation that was used in 1974. Owing to the combination of high density populations in 1975 and the greater potency of the PVC over the vial stoppers, the adhesive surface of traps left out overnight was saturated with moths by morning. Traps were therefore checked every 1–2 h during daylight hours. In New Brunswick, Sectar 1 traps were placed out in

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Table III. Numbers of male *C. fumiferana* captured in traps baited with various combinations of *trans*- and *cis*- tetradecenal formulated 3% by weight in polyvinyl chloride. In Ontario exp. 2, each piece of plastic measured 2 mm long by 4 mm diam.; in all other trials each piece measured 10 mm long by 4 mm diam.

Ratio	Ontario				
trans *cis*	Exp. 1	Exp. 2	New Brunswick	Pennsylvania	Alberta
100: tr.	101.0^{a}	49.6^{abc}	10.6^{ab}	25.4^{a}	121.0^{ab}
99: 1	116.4^{a}	53.2^{bc}	11.6^{ab}	22.2^{a}	133.4^{ab}
98: 2	114.8^{a}	50.2^{abc}	14.0^{b}	26.0^{a}	136.4^{b}
97: 3	118.2^{a}	71.2^{e}	8.2^{abc}	25.6^{a}	124.4^{ab}
96: 4	117.6^{a}	63.0^{ce}	13.0^{b}	19.8^{a}	135.2^{b}
95: 5	113.8^{a}	45.4^{ab}	12.4^{ab}	26.6^{a}	131.0^{ab}
90: 10	83.0^{c}	37.8^{a}	13.8^{b}	69.Y^{a}	130.0^{ab}
80: 20	42.4^{d}	11.2^{d}	7.8^{abc}	23.0^{a}	129.8^{ab}
50: 50	9.6^{b}	1.2^{d}	4.0^{ac}	11.6^{c}	118.6^{ab}
5: 95	2.0^{b}	0.4^{d}	0.8^{c}	0.8^{b}	91.6^{c}
0:100	0^{b}	0.2^{d}	1.4^{c}	0.4^{b}	79.2^{c}
Virgin ♀	9.0^{b}	4.0^{d}	–	–	–
Check	1.4^{b}	1.4^{d}	0.2^{c}	0.2^{b}	34.6^{d}

NOTE: Numbers followed by different letters in each column are significantly different at the 5% probability level (Duncan's New Multiple Range Test).

balsam fir (*Abies balsamea* (L.) Mill.) – red spruce (*Picea rubens* Sarg.), and white spruce stands near Fredericton on 3 July, and were left out overnight. In Pennsylvania, Sectar 1 traps were placed 10 m apart in a hemlock (*Tsuga canadensis* (L.) Carr.) stand infested by sufficient budworm to cause barely visible defoliation. Traps were placed out on 11 June and collected on 1 July. In Alberta, Sectar 1 traps were placed out at 10-m intervals in a mixed spruce – aspen (*Populus* spp.) – balsam fir stand near Fort McMurray on 8 July, and were collected 9 July. The results (Table III) indicate that the pure *cis*-isomer is unattractive to male *C. fumiferana*.

Although the catches were high in both experiments in Ontario, the traps were emptied several times during the course of the experiment, and there was no evidence of the traps becoming saturated with moths. In the first experiment in Ontario, there were no significant differences among treatments in the range 100% through 95% *trans*, while in the second there were no significant differences in the range 100% through 90%, although the catch at 97% *trans* was almost twice that at 90%. In the other three areas there were no significant differences in the range 100% through 80% *trans*. In the Alberta trial this could have been due to saturation of the traps, which would tend to mask differences. In the other two areas, New Brunswick and Pennsylvania, catches were more variable. However, in all trials the highest catch occurred between 95% and 98% *trans*. The low catches by the virgin females in Ontario can be attributed to the fact that the experiments were run during the morning and early afternoon, when the females are not calling (Sanders and Lucuik 1972). The high catches obtained by the almost pure *trans*-isomer in all areas were attributed to the presence of a trace of the *cis*-isomer, estimated at less than 0.3%. This was confirmed by purifying a sample of the *trans* as described above, and comparing its attraction with the unpurified material using polyethylene stoppers. The pure *trans* was almost completely inactive (Table IV). The addition of a very small amount of the *cis*-isomer (ca. 0.15%) produced only slight attraction, but 1.5% *cis* produced a potent attractant. Dodecanal and dodecyl acetate were completely inactive, although, on the basis of the extensive publications by Roelofs and his co-workers, they might be expected to have synergistic properties.

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Table IV. Numbers of male *C. fumiferana* captured in traps baited with polyethylene stoppers containing commercial *trans*-11-tetradecenal (= A), or with the purified material (= B), or with A or B mixed with the *cis*-isomer and potential synergists dodecanal or dodecyl acetate

Chemical in stopper	No. of males per trap
1 mg commercial *trans* (1975) (= A)	45.6[c]
A + 5 mg dodecyl acetate	29.0[b]
A + 5 mg dodecanal	23.8[b]
2 mg pure *trans* (= B)	0.2[a]
B + 5 mg dodecyl acetate	0.4[a]
B + 5 mg dodecanal	0.2[a]
B + 30 μg *cis*-aldehyde (1.5%)	126.2[d]
B + 3 μg *cis*-aldehyde (0.15%)	1.6[a]
Check	0[a]

NOTE: Numbers followed by different letters are significantly different at the 5% probability level (Duncan's New Multiple Range Test).

Finally, the attractiveness of the *trans* : *cis* blend in a PVC formulation was compared with that of virgin females. Pieces of the plastic measuring 10 mm × 4 mm diam. and containing 3% by weight of a 97:3 blend of *trans* : *cis* were exposed in a fume hood for 30 days prior to the start of the experiment. They were then fastened to the inside of 3-M brand reloadable traps. Single 1-day-old virgin females were housed in small screen cages taped inside the traps. Traps were set out 10 m apart in a natural spruce–fir stand at Agawa, north of Sault Ste. Marie, where populations were light to moderate. Traps were checked daily and the screen cages were sprayed with water to provide the females with drinking water. The experiment was run for only 5 days to ensure that the females remained potent. The results were as follows: 30 females captured an average of 17.0 (± 1 S.E. = 2.1) males per trap; 10 pieces PVC averaged 177.1 (± 7.2) per trap; five empty checks averaged 1.2 (± 0.4), indicating the formulation of PVC used was about 10 times as potent as a single virgin female.

Discussion

Evidence is accumulating that many Tortricidae use sex pheromones incorporating more than one chemical. Diversity of chemical structure may be realized through differences in functional group, chain length, positional isomerism, or geometrical isomerism, mixed together in a variety of blends (Hill *et al.* 1975). In the case of the eastern spruce budworm, the original work (Weatherston *et al.* 1971) almost certainly ruled out all possibilities except positional and geometrical isomerism.

The *cis*-isomer was among chemicals previously assayed in a 1:1 ratio with *trans*-11-tetradecenal (Sanders *et al.* 1972). However, as is shown here, this combination is far from the optimum, and it did not differ significantly in attractiveness from the commercial preparation of *trans*-11-tetradecenal used at that time, which may have contained up to 3% of the *cis*-isomer.

The results in Table III could be interpreted as indicating that the catches are influenced more by the quantity of the *trans*-isomer than by the proportion of the *cis*. This interpretation is rejected because of the data in Table II, in which the quantity of *trans* remains constant in the presence of various quantities of *cis*. This point does illustrate, however, the danger of evaluating pheromone blends without also comparing the effects of holding the main component constant.

The optimum ratio from the field testing therefore appears to be between 2% and 5% *cis* throughout the range of *C. fumiferana*, corresponding well with the figure of 4% found in the extracts from virgin females. Suitable formulations of this blend can

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out-compete virgin females by a factor of at least 10. Thus the evidence suggests that this combination is very similar to the natural sex pheromone of this species. The apparent unconcern of the males for a more precise optimum chemical blend is of advantage in the design of an annual monitoring program using traps baited with the two chemicals, since it implies that small discrepancies in the formulations from year to year should not affect the catches significantly.

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References

Daterman, G. E. 1974. Synthetic sex pheromone for detection survey of European pine shoot moth. *Res. Pap. U.S. Dep. Agric. For. Serv.* PNW-180. 12 pp.

Fitzgerald, T. D., A. D. St. Clair, G. E. Daterman, and R. G. Smith. 1973. Slow release plastic formulation of the cabbage looper pheromone, *cis*-7-dodecenyl acetate: Release rate and biological activity. *Environ. Ent.* **2**: 607–610.

Gunstone, F. D. and M. Lie Ken Jie. 1970. TLC and GLC properties of some *cis*-octadecenyl compounds. *Chem. Phys. Lipids* **4**: 139–146.

Hill, A. S., R. T. Cardé, H. Kido, and W. L. Roelofs. 1975. Sex pheromones of the orange tortrix moth, *Argyrotaenia citrana* (Lepidoptera: Tortricidae). *J. Chem. Ecol.* **1**: 215–224.

Sanders, C. J., R. J. Bartell, and W. L. Roelofs. 1972. Field trials for synergism and inhibition of *trans*-11-tetradecenal, sex pheromone of the eastern spruce budworm. *Bi-mon. Res. Notes Can. Dep. Environ.* **28**: 9–10.

Sanders, C. J. and G. S. Lucuik. 1972. Factors affecting calling by female eastern spruce budworm, *Choristoneura fumiferana* (Lepidoptera: Tortricidae). *Can. Ent.* **104**: 1751–1762.

Weatherston, J., W. Roelofs, A. Comeau, and C. J. Sanders. 1971. Studies of physiologically active arthropod secretions. X. Sex pheromone of the eastern spruce budworm *Choristoneura fumiferana* (Lepidoptera: Tortricidae). *Can. Ent.* **103**: 1741–1747.

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