EFFECTS OF BLENDS OF SEX PHEROMONE ISOMERS ON MATING AND ELECTROANTENNOGRAMS OF SPRUCE BUDWORM (LEPIDOPTERA: TORTRICIDAE)

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Abstract

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Male and female laboratory reared spruce budworm moths, *Choristoneura fumiferana* (Clem.), were placed in cages in a conifer forest, and the surrounding air permeated by each of four blends of the *E* and *Z* isomers of the sex pheromone (11-tetradecenal) at two concentrations. Mating suppression of 53–83% was found for each blend tested. At one concentration the four suppressions were similar, while at the other only one was significantly different. Electroantennograms (EAGs) were obtained from male moths for each of six *E*:*Z* blends at four concentrations. EAGs were similar for most blends at a given concentration, but tended to be larger than at a blend of 0*E*:100*Z*.

These results were discussed using a current hypothesis on the ability of males to detect sex pheromone in air containing pockets of different isomeric blends.

Résumé

Des mâles et femelles de *Choristoneura fumiferana* (Clem.) élevées au laboratoire ont été mises en cage dans un boisé de conifères, et 4 mélanges des isomères *E* et *Z* de la phéromone sexuelle (11-tetradecenal) a 2 concentrations ont été incorporés à l'air ambiant. L'accouplement fut supprimé dans des proportions de 53–83% par les mélanges testés. A l'une des concentrations, les niveaux de suppression étaient similaires alors qu'à l'autre, l'un d'entre eux seulement était significativement différent. Des électroantennogrammes (EAG) de mâles ont été obtenus pour 6 mélanges *E*:*Z* à 4 concentrations. Les EAG se sont avérés similaires pour la plupart des mélanges à une concentration donnée, mais montraient une tendance à être plus élevés que pour un mélange 0*E*:100*Z*.

Ces résultats sont interprétés en rapport avec l'hypothèse voulant que les mâles puissent détecter la phéromone sexuelle dans de l'air contenant des poches de mélanges isomériques différents.

Introduction

Female spruce budworm moths, *Choristoneura fumiferana* (Clem.), release a sex pheromone to attract males. Manipulation of this chemical communication may provide a method for population control of this forest pest. The emphasis has been on widespread air permeation with synthetic sex pheromone released from many tiny emitters (Sanders 1979). Because of the high or constant level of sex pheromone in the air, the males may not be able to find a pheromone plume arising from a female (Shorey 1977; Seabrook 1978).

Research with chemicals structurally similar to the sex pheromone (analogues) may provide insight into the sensory and behavioural mechanisms involved, and may lead to a chemical better suited for actual control (Roelofs and Cardé 1977). The spruce budworm sex pheromone is 11-tetradecenal, and contains two geometrical isomers in the ratio of 96*E*:4*Z* (Sanders and Weatherston 1976) or 95*E*:5*Z* (Silk *et al.* 1980). There have been some experiments to assess the effects of different blends of these isomers. The number of male moths attracted to lures in traps varies with the blend of the sex pheromone components in the lure, being largest near or at the blend of the sex pheromone (Sanders

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1981*b*). Schmidt *et al.* (1980) and Sanders (1981*a*, *c*) conducted experiments to alter the attraction of male moths to virgin females or synthetic sex pheromone lures by permeating the air with analogues having different sex pheromone isomeric blends.

Palaniswamy *et al.* (in press) were able to suppress mating of laboratory reared spruce budworm moths held in cages in a forest by air permeation with synthetic sex pheromone. We tested the effects on mating using different isomeric blends of the sex pheromone components.

The electrical response of a male antenna to an airborne chemical is used to assay pheromone strength and to compare the response with sex pheromone with that to an analogue (Roelofs and Cardé 1977; Ross *et al.* 1979). We obtained electroantennograms (EAGs) for a range of sex pheromone isomeric blends.

Materials and Methods

Insects. Laboratory reared moths were used for both mating and electroantennogram (EAG) experiments. Larval, pupal, and adult spruce budworm rearing procedures were as described in Ross *et al.* (1979). Male and female moths were introduced into the field when 1–10 h old; males used for EAG studies were 48–71 h old.

Chemicals. Blends were made by combining locally made pure *E*-11-tetradecenal and *Z*-11-tetradecenal in different proportions. Their purity was determined using capillary column analysis with a Varian 3700 gas chromatograph utilizing an FID detector. Hydrogen was used as a carrier gas at an inlet pressure of 0.8 kg/cm^2. The capillary column was a 50 m, 0.25 mm ID SP1000 (G-SCOT). The column was kept at 170°C. Each isomer was 99% pure and contained no detectable amount of the other isomer. The method cannot detect less than 1% impurities.

Mating suppression. The method was based on that described in Palaniswamy *et al.* (in press). Experiments were performed in sites in a temperate conifer forest. There were eight treatment sites at least 500 m apart, and four control sites 500 m apart, but at least 3 km from the treatment sites. Four cages, each cylindrical with ends made of plywood and sides of nylon screening, were suspended in the centre of a site.

Treatment emitters were a 10% formulation of a particular *E*:*Z* blend of pheromone components with polyvinyl chloride, plasticizer, and antioxidant in a cylindrical shape (5 mm diam.). Control emitters were made in a similar manner, but without analogue or pheromone. In each site there were 20 emitter holders distributed among and around the cages such that wind from any direction would convey chemical through the four cages. An equal length of the appropriate emitter was placed on each holder at the start of an experiment and removed at the end. The length used depended on the concentration of chemical to be released in the site.

Just prior to an experiment the four cages of each site were lightly packed with five to six spruce and balsam fir branches. On day one 12 males were placed into cage *a* of each site. On day two 12 males were placed into cage *b* of each site, while 12 females were put into cage *a*. On day three, 12 males went into cage *c* and 12 females went into cage *b*; on day four 12 males went into cage *d* and 12 females went into cage *c*. On day five, 12 females went into cage *d* and all the moths were collected from cage *a*. Moths in cage *b* were collected on day six, in cage *c* on day seven, and in cage *d* on day eight. As moths were removed, they were counted, sexed and placed in 5% ethyl alcohol. Moths were sometimes missing or dead. Females were dissected to determine the presence of a spermatophore in the bursa copulatrix.

There were two groups of experiments. In each group there were four control sites, and two sites each with 95*E*:5*Z*, 80*E*:20*Z*, 50*E*:50*Z*, and 10*E*:90*Z* blends. The first group was conducted from 31 July to 7 August, 1979 with a release rate of 50–54 mg/h/ha. The second group was from 27 August to 3 September, 1979 with a release rate of 35–40 mg/

h/ha. The amount of analogue or pheromone released from a 2.5 cm length of emitter in a given time was measured before and after each experiment for each treatment and control. The average of these values was assumed to represent the release rate during the experiment. The stated release rates were calculated by considering the release rate from the total length of emitter in a site, and by assuming emission from the circular area (diam. = 5 m) in which the emitters were distributed.

Electroantennograms. A recording was made from a single male antenna on a half-head preparation as described in Ross *et al.* (1979). A Pasteur pipette containing a filter paper treated with a blend solution or solvent (hexane) was placed onto a 10 cc plastic syringe, and a puff injected into an air stream that passed over the antenna. For each antenna, in rapid succession, there was a puff from control and a puff from only one blend. At least 10 different antennae were used for each blend and for each concentration of the blend on the filter paper. There were six blends: 100*E*:0*Z*, 90*E*:10*Z*, 75*E*:25*Z*, 50*E*:50*Z*, 25*E*:75*Z*, 0*E*:100*Z*. Each blend was tested at four concentrations: 2×10^{-2}, 2×10^{-3}, 2×10^{-4}, and 2×10^{-5} mg on the filter paper.

Results

Mating suppression. There was no satisfactory statistical procedure with which to compare the effects of different treatments using the raw data. Expressing the number of females mated as a percentage and then comparing these computations with a parametric technique would misrepresent the data since the individual values of mated and retrieved females were discrete in nature and small in value. Because both the number of females mated and retrieved varied from cage to cage, some processing of the data was required before the application of a nonparametric procedure. The Chi-Square Contingency analysis was chosen because only addition of values was required. The fair way to compare the data from different treatments was to lump the values for each blend at each release rate (8 cages), and to lump the values from all control cages (16 cages) from the same experiment.

For each blend at each release rate, mating was significantly lower than control ($p < 0.05$). Table I shows the percentage mating and mating suppression. Comparing pairwise, the mating (and hence mating suppression) for the four treatments at 50–54 mg/h/ha were not significantly different. At the lower release rate mating suppression was only different between the 95*E*:5*Z* and 10*E*:90*Z* blends, the former being smaller than the latter ($p < 0.05$). Mating suppression for the same blend was only significantly different between the two release rates for the 10*E*:90*Z* blend.

Electroantennograms. For each blend at each concentration, the mean of the maximum amplitudes of the electroantennograms (EAGs) from blend puffs was significantly larger than that from the control puffs (*t*-test, $p < 0.05$). Figure 1 shows the mean response to different blends and indicates the results of applying a Student-Newman-Keuls analysis to individual responses (blend EAG less control EAG). In Fig. 1, means are arranged according to the ratio of *E*:*Z* in the blend (*x*-axis), and letters indicating similar subgroups of means are designated according to results of the statistical test; hence, means which are significantly different can be bracketed by means which are not significantly different. Because of the high dispersion about each mean, there were many subsets, and many means within each subset, of means; because of this, clear differences cannot be ascertained between many of the means. For a concentration of 2×10^{-2} mg, the response to a 90*E*:10*Z* blend was in the same subset of means as that to 100*E*:0*Z*, 75*E*:25*Z*, and 25*E*:75*Z*. But the latter were also in one or two other subsets. Such a shade of difference indicates that the response to the 90*E*:10*Z* blend was possibly different from and larger than these other responses. The responses for these blends were clearly greater than that for 0*E*:100*Z* since the former were in a different subset from the latter.

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Table I. Mating suppression (MS)[1] of laboratory reared moths in field cages by air permeation with different blends of sex pheromone components at two concentrations

Blend:	*E*:Z blend in 11-tetradecenal														
Release rate:	95:5			80:20			50:50			10:90			Control		
mg/h/ha	# ♀	% mated	% MS	# ♀	% mated	% MS	# ♀	% mated	% MS	# ♀	% mated	% MS	# ♀	% mated	% MS
50–54	88	14	68	87	17	59	88	15	65	99	18	57	161	42	–
35–40	79	19[2]	53	80	9	78	85	15	62	73	7[2]	83	165	41	–

[1]%MS = [(% mating in control − % mating in treatment) (% mating in control)] × 100.

[2]#♀ mated/#♀ retrieved.

95:5 —1/11, 1/10, 1/8, 2/11, 2/11, 2/11, 2/7, 4/10.

10:90—0/6, 0/8, 0/10, 0/10, 1/12, 1/11, 1/8, 2/8.

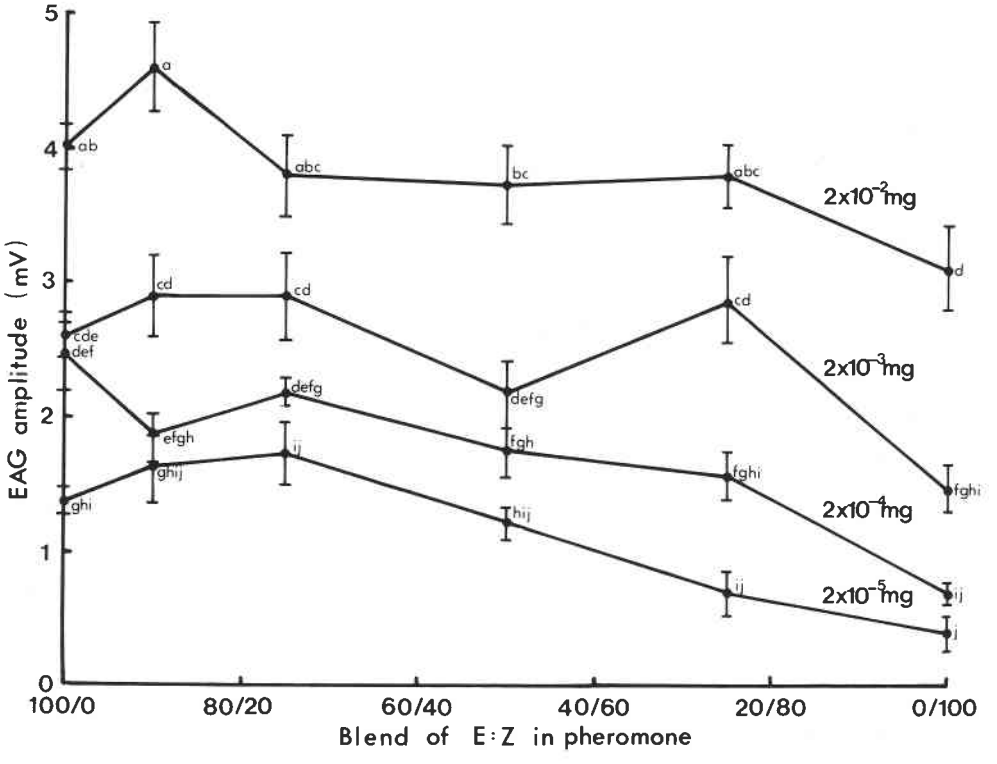

FIG. 1. Maximum EAG amplitude from different blends of *E*- and *Z*-11-tetradecenal (less control) at indicated concentrations. Means ± standard error of the mean ($n \geq 10$). Means with similar letters are not in significantly different subsets of means (Student-Newman-Keuls, $p < 0.05$). Temp.: 24°–26°C.

For a concentration of 2×10^{-3} mg, the response to the 0*E*:100*Z* blend was less than that to 100*E*:0*Z*, 90*E*:10*Z*, 75*E*:25*Z*, and 25*E*:75*Z*, and possibly to 50*E*:50*Z*. For 2×10^{-4} mg, the response to the 0*E*:100*Z* blend was less than that to 100*E*:0*Z*, 90*E*:10*Z*, 75*E*:25*Z*, and 50*E*:50*Z*, and possibly to 25*E*:75*Z*. For 2×10^{-5} mg, the response to the 0*E*:100*Z* blend was only less than that to 100*E*:0*Z*, and possibly that to the remaining blends.

The responses to the blends at 2×10^{-2} mg concentration were larger than that to 90*E*:10*Z*, 50*E*:50*Z*, 25*E*:75*Z*, and 0*E*:100*Z* at 2×10^{-4} mg, and possibly larger than that of 100*E*:0*Z* and 75*E*:25*Z*. The responses to the blends at 2×10^{-3} mg concentration are possibly larger than those at 2×10^{-5} mg.

Discussion

Mating was suppressed by all of the blends of sex pheromone components used to permeate the air around the caged moths. For 12 males and 12 females in the cage Palaniswamy *et al.* (in press) predicted mating suppressions of 64–68% for a release rate of 50–54 mg/h/ha and 50–55% for 35–40 mg/h/ha synthetic sex pheromone. For a release rate of 50–54 mg/h/ha we found mating suppressions were between 57–68% whatever the blend, and were not significantly different. These suppressions are within, or slightly lower than, the predicted range. Thus, at this release rate, the air may be permeated with any of a variety of blends to achieve the same mating disruption as sex pheromone. For a release rate of 35–40 mg/h/ha, we found mating suppressions for the 95*E*:5*Z*, 80*E*:20*Z*, and 50*E*:50*Z* blends were not significantly different, but found the mating suppression for

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95*E*:5*Z* was significantly lower than that for the 10*E*:90*Z* blend. The suppression for the 95*E*:5*Z* blend was within the predicted range, but that for the remainder was higher than the predicted range.

Sanders (1981*a*) found, when permeating the air with different blends of the sex pheromone components around virgin female or synthetic sex pheromone lures, that the number of males caught with the lures was greatly reduced when blends were similar to the pheromone blend, and that the number caught was much nearer control catches when blends contained a high proportion of the *Z* isomer. Sanders (1981*c*) believes that, as a male moves upwind to a source of pheromone, the male turns in response to perceived changes in the pheromone concentration rather than only turns after losing contact with the pheromone. He treats the case of releasing correct and incorrect blends into the same air space as a simple extension of this hypothesis. As long as the male can find pockets of the correct blend, the male can still move toward the source. When the incorrect blend is similar to the correct blend, the male has difficulty distinguishing between the different pockets. But as the incorrect blend contains more of the *Z* isomer, and becomes more dissimilar to the correct blend, the male has less difficulty distinguishing the different pockets.

Schmidt *et al.* (1980), however, found no difference in attraction by synthetic sex pheromone lures for a range of blends from 100*E*:0*Z* to 0*E*:100*Z* permeating the air. In our results, for a release rate of 50–54 mg/h/ha, there was no difference in mating suppression with a similar change in blend. The explanation suggested by Sanders (1981*c*) may still be applicable. The concentration of his permeants were probably lower than those of Schmidt *et al.* (1980) and of ours: Sanders (1981*a*) put out six emitters with a maximum of 0.03% blend by weight; Schmidt *et al.* (1980) put out nine emitters with 3% blend by weight; we put out 20 emitters with 10% blend by weight. At high concentrations of the 0*E*:100*Z* blend permeant, the number of pockets of the correct blend would be fewer than at lower concentrations of this blend, and the males would not be able to orient to the lures or females as well. The disorientation of males in blends with a high proportion of *E* isomer is very great even at low concentrations of the blend. As the blend concentration increases, the ability of males to orient in permeants of all blends would tend to become similar. In fact, this trend is apparent in fig. 3 of Sanders (1981*a*) where, as the percentage blend by weight in the emitters increases, the change in catch as the proportion of *Z* isomer increases becomes shallower and less distinct.

In our results, for a release rate of 35–40 mg/h/ha there was some evidence of a difference in mating suppression with a change in blend from 100*E*:0*Z* to 0*E*:100*Z*. For the 10*E*:90*Z* blend the suppression was significantly different from, and larger than, that for the 95*E*:5*Z* blend, but not significantly different from suppressions for the 80*E*:20*Z* and 50*E*:50*Z* blends. This suppression is opposite to what would be expected from the previous discussion. Before considering further explanations, or alternate hypotheses, on the basis of a single value, this data should be verified by additional experimental work.

The electroantennograms (EAGs) were similar for most blends at a given concentration, but tended to be larger than at a blend of 0*E*:100*Z*. These findings also fit the hypothesis of Sanders (1981*c*). The EAG represents a summation of the receptor potentials of responding antennal receptors, presumably the sensilla trichodea which are the sex pheromone receptors (Seabrook 1978). On the basis of antennal receptor response, in air permeated with pockets of sex pheromone and of the *Z* isomer, the male should receive more nervous input from a similar concentration of the former than the latter, and be more attracted to the sex pheromone than the *Z* isomer. With blends closer to that of the sex pheromone, the nervous input would be similar and differential attraction not expected.

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