

# RESPONSES OF MALE SPRUCE BUDWORM (LEPIDOPTERA: TORTRICIDAE) TO DIFFERENT CONCENTRATIONS OF SEX PHEROMONE AS MEASURED IN A SUSTAINED-FLIGHT WIND TUNNEL

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## Abstract

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Responses were recorded of male spruce budworm (*Choristoneura fumiferana* (Clem.)) exposed to natural pheromone and a wide range of concentrations of synthetic pheromone in an all-glass wind tunnel equipped with a moving, patterned ceiling. The numbers of males wing-fanning and plume-following decreased with decreasing concentration of the pheromone plume. Speed of upwind flight with the ceiling stationary increased with decreasing concentration, but the durations of flight sustained by moving the ceiling were not significantly different under the different concentrations.

Males exposed to pheromone produced by calling females showed a higher incidence of plume-following, and flew significantly faster, than males exposed to a similar concentration of synthetic pheromone.

## Résumé

On a enregistré les réactions de mâles de la tordeuse des bourgeons de l'épinette (*Choristoneura fumiferana* (Clem.)) exposés à un phéromone naturel et à une vaste gamme de concentrations de phéromone synthétique dans un tunnel à soufflerie tout en verre équipé d'un modèle de plafond mobile. Les nombres de mâles battant des ailes et suivant un sillage décroissaient en fonctions directe de la concentration du sillage de la phéromone. La vitesse du vol à contre-courant avec le plafond immobile augmentait en fonction inverse de la concentration, alors que les durées du vol soutenu au moyen du déplacement du plafond ne différaient pas significativement selon les différentes concentrations.

Les mâles exposés à la phéromone produite par des femelles en rut manifestaient une plus forte incidence de poursuite du sillage, et volaient significativement plus vite que les mâles exposés à une concentration similaire de phéromone synthétique.

## Introduction

Kennedy and Marsh (1974) established that pheromone-stimulated moths regulate their upwind flight towards a pheromone source by means of visual cues from their surroundings. They were able to demonstrate this by moving a patterned floor underneath the flying moths: the moths' forward motion along the wind line was governed by the speed at which the floor was moved back underneath the flying moths.

Using this technique, Miller and Roelofs (1978*a,b*) were able to sustain the flight of gypsy moths (*Lymantria dispar* L.) and redbanded leafrollers (*Argyrotaenia velutinana* (Walker)) for periods of over 1 h and demonstrated the usefulness of the technique by analyzing the response of male gypsy moths to various enantiomeric blends of the synthetic attractant. Cardé and Hagan (1979) have also used such a sustained-flight wind tunnel for analyzing the responses of male gypsy moths to various concentrations and isomeric relationships of the synthetic pheromone and to a variety of wind speeds.

The utility of such sustained-flight, moving-floor wind tunnels for analysis of moth behavior in response to pheromone plumes has led us to develop a similar tunnel for evaluating the behavior of the spruce budworm (*Choristoneura fumiferana* (Clem.)) in response to its pheromone, primarily with a view to determining the

optimum dosages and methods of dispensing synthetic attractant for disrupting male orientation to calling females.

Our initial experiments, reported here, were designed to determine a suitable protocol for the bioassays, and appropriate concentrations of pheromone for providing the longest durations of sustained flight.

### Materials

**Wind tunnel.** The tunnel is essentially similar to that described by Kennedy and Marsh (1974), Miller and Roelofs (1978*a*), and Cardé and Hagaman (1979) with a pusher fan blowing air into a tunnel, and an exhaust hood at the downwind end to evacuate the pheromone from the laboratory. The internal square cross-section of the tunnel is 90 cm with a length of 190 cm. The upwind end of the tunnel consists of four layers of cheesecloth, dyed a dark grey to reduce contrast with the sides. The downwind end is covered by a sliding aluminum mesh-screen to prevent escape of the moths. Since experiments on disruption necessarily involve permeating the entire tunnel with pheromone, there is a constant problem of contamination of the tunnel surfaces. To minimize this, and to facilitate cleaning, the floor, ceiling and sides are all of plate glass. The moving optomotor cues necessary for sustaining flight are therefore outside the tunnel, in contrast with previously described sustained-flight tunnels in which the floor of the tunnel is moved.

In our initial experiments we attempted to sustain flights by using a moving floor with black and white stripes 25 cm wide under fluorescent overhead lighting diffused through 'pearl' plexiglass, which gave a lighting intensity of 195 lux at floor level. However, this did not prove very satisfactory. Flights were sustained at the most for 1 or 2 min, and usually ended with the male progressing upwind to the pheromone source even as the floor was moved faster and faster.

It was apparent that reflections of the overhead lights from the glass floor of the tunnel largely obscured the moving floor pattern, and to avoid this we installed a moving ceiling pattern. A matt black board was placed under the glass floor so that the moving ceiling was clearly reflected, thereby increasing the illusion of movement. Two widths of the black and white stripes were used, 25 cm and 40 cm.

The striped cloth rested on the tunnel roof and a 'pearl' plexiglass sheet was placed immediately above this with two 120 cm fluorescent lamps mounted above the plexiglass so that plexiglass and lamps were between the upper and lower loops of the striped cloth. As a result light from the lamps was diffused through the plexiglass and then through the white areas of the cloth, giving a very pronounced light and dark pattern, with an average light intensity of 75 lux at floor level. Throughout the experiments the wind speed was maintained at 40 cm/sec as measured with an Alnor velometer, type 3002, and by timing the movement of a puff of smoke in the tunnel.

**Insects.** The insects were reared from laboratory stock on artificial diet as described by Grisdale (1970). They were separated by sex as pupae and the emerged moths were collected each morning and kept in screened cages until required for use. Male and female moths were held in the same room, which was maintained at 70% R.H., 21°C on a 17/7 L/D cycle, with the onset of dark at 2030. Females to be used in the tunnel as lures were transferred during the 24 h following their emergence to an L/D cycle advanced 6 h to entrain them to start calling by mid-day (Sanders and Lucuik 1972). They were then housed individually in screen cages, 3 cm diam × 2.5 cm long, which were taped to a wire stand that held them in the center of the upwind end of the tunnel for bioassaying male response. Two females were used together for each bioassay in case one was abnormal.

**Chemicals.** The synthetic pheromone of the spruce budworm, a 96:4, E:Z blend of  $\Delta$ -11-tetradecenal (Sanders and Weatherston 1976), was incorporated into PVC, as previously described (Fitzgerald *et al.* 1973; Daterman 1974; Sanders 1978) to give a range of concentrations differing by factors of 10, from 3% attractant by weight down to .00003%. All formulations were cut into cylindrical pellets 4 mm diam  $\times$  10 mm long. At the time of formulation the pellets contained from 4.2 mg pheromone at the 3% concentration down to 42 ng at the 0.0003% concentration.

The pellets were between 10 and 52 days old when used. Measurements of weight loss indicate that a pellet containing 4.2 mg pheromone of this age is releasing the pheromone at about 1  $\mu$ g/h (at 21°C) and that each 10-fold reduction in concentration produces a 10-fold reduction in release rate (Sanders 1981). A pellet containing 42  $\mu$ g is therefore releasing 10 ng/h, which is close to the hourly rate of release from a virgin female spruce budworm (Silk *et al.* 1980). When not being used in the experiment, the PVC pellets were kept in a fume hood, shielded from direct room lights to avoid breakdown of the pheromone.

For use in the tunnel, pellets were mounted on insect pins and pinned in the center of a piece of tape, 2.5 cm square, stuck onto the upwind cheesecloth screen. Tests with cigarette smoke showed that the air flow in the tunnel was close to lamina and without the 2.5 cm square tape the smoke plume remained pencil-thin for half the length of the tunnel. However, with the tape in place sufficient turbulence was created to provide a well structured plume, widening out to 25 cm in cross-section at the downwind end.

### Methods

**Experimental protocol.** All experiments were carried out with 2-day-old males and were conducted for convenience between 1330 and 1630 h each day, 4-7 h before the onset of darkness. The males required for experimentation were transferred each morning individually to small cylindrical, aluminum screened cages, 6 cm long  $\times$  3 cm diam, which were kept under a desk lamp close to the tunnel at a light intensity of 1100 lux. Exposure of the males to the pheromone, and release into the pheromone plume, was carried out as follows. A cage containing an individual male was transferred to the tunnel and held for .50 min in the air stream at one side of the tunnel well away from the pheromone plume to allow the moth to acclimate to the conditions in the tunnel. At the end of the .50 min the cage was moved over into the center of the plume, held there for .25 min (even if the male was wing-fanning or flying), after which the cover was removed from the screened cage. If the moth did not fly during the following 1.0 min, it was recorded as unresponsive.

The following events were recorded: (a) whether or not the moth responded by wing-fanning, (b) whether it flew in the characteristic zig-zag fashion of a male moth responding to a pheromone plume (Kennedy 1977), a process we shall refer to here as 'locking on', (c) either the time taken for the moth to fly upwind 1 m with stationary floor and ceiling (from a point 50 cm upwind from the release point to 150 cm), or the duration of flight sustained by the moving ceiling or the moving floor.

Protocols for sustaining flight were as follows. When a moth which had locked on to the pheromone plume passed the half-way mark along the tunnel, the floor or ceiling was started moving slowly. The speed of movement was then constantly adjusted so that the moth remained in flight approximately half way along the tunnel. Duration of flight was recorded from the moment the moth locked on until it veered off and hit the side of the tunnel or alighted on the floor.

Flights in which males rose and hit the tunnel roof were not included in the calculations, since this could be due to the attraction of the lights overriding response to the pheromone, and giving an abbreviated flight.

### Results and Discussion

**Comparison between moving floor and moving ceiling.** The moths were found to be far more sensitive to movement of the ceiling than to movement of the floor in the tunnel described here. With the moving ceiling males could be allowed to approach to within a few cm of the source and yet they still reduced flight speed and so were carried back down the tunnel when the ceiling was moved. With the moving floor, once a male approached to within 20 or 30 cm of the source it continued upwind flight to the source, apparently indifferent to the movement of the floor. Furthermore, to sustain flight for 1.0 min the floor had to be moved at an average speed for 10 moths of  $2,988 \pm 432$  ( $= 1$  S.E.) cm/min, while the necessary speed for the ceiling was  $865 \pm 69$  cm/min. Not only did the floor have to be moved much faster, but as indicated by the standard error, speeds were far more variable than with the ceiling. This was largely because the floor speed had to be increased the closer the males came to the source.

It therefore appears that when the floor was moving the males were able to use cues other than the floor, possibly the sharp visual cues where ceiling and front screen meet, as a gauge of their forward movement. The other possibility is that they were able to utilize the structure or concentration of the plume close to the source, but this theory must be rejected since the same behavior did not occur with the moving ceiling, although the plume remained the same.

The fact that the speed of the moving surface necessary to sustain flight differed, depending upon whether the moving surface was ceiling or floor, indicates that the rate of movement of the surface should not be used as a measure of the insect's ground speed, or of the distance the insect is flying.

The evident superiority of the moving ceiling over the moving floor may be attributable to reflection of the overhead lights which obscured the floor pattern moving below the glass. The use of a moving ceiling with the lighting above it enhanced the pattern. However, it is possible that the flight attitude of a male moth locked on to a pheromone plume exposes more of the eye surface to cues from above than below, and that a moving ceiling will always be superior to a moving floor. An attempt was made to resolve this by placing the lighting under the floor, and so removing the reflection. However, of 20 males which were assayed, 18 on taking flight immediately went to the floor while the other two went to the side, and the attempt was abandoned.

**Comparisons among responses to different concentrations of synthetic pheromone.** The responses of males and the times taken to fly upwind 1 m are shown in Table I. Responses and duration of flight sustained by moving the ceiling are shown in Table II. Response, as measured by the percentage of males wing-fanning and locking on to the synthetic pheromone declined with the concentration of pheromone. The trends in both Tables I and II are quite similar even though they refer to different groups of males.

Although times taken to fly 1 m (Table I) were not significantly different with the different concentrations, there was a significant trend ( $F = 8.4$ ), with longest times (slowest flight) at the higher concentrations, confirming the observations of Cardé and Hagaman (1979) on gypsy moths.

No significant differences or trends were detectable, however, in the duration of sustained flights in response to the different concentrations (Table II). Possibly the high variability masked any differences, but flights of over 9 min were recorded at all concentrations except the lowest, while the longest flight, 60.51 min, was at the highest concentration, and the second longest, 29.98 min, was at the next to lowest.

This is in contrast with the data presented for the gypsy moth by Cardé and Hagaman (1979). They also found wide variation in the duration of sustained flight of individual males. Nevertheless, they were able to show that the lowest dosage used produced a significantly shorter duration of flight. The range of concentrations used in the present experiments with the spruce budworm (from  $10^2$  down to  $10^{-3}$  that of a calling female) should have detected differences if they did occur. We therefore conclude that duration of sustained flight is independent of concentration, and this implies that the persistence of anemomenotactic flight behavior in male spruce budworm is governed by perception of pheromone in the air space and that concentration *per se* does not influence this persistence.

**Comparison between responses to virgin females and synthetic pheromone.** According to Silk *et al.* (1980), female spruce budworm release a maximum of 20-40 ng of pheromone/night, which, allowing for an 8 h calling

Table I. Responses of male spruce budworm when introduced into a plume of pheromone produced by two virgin females or by different source concentrations of synthetic pheromone, and times taken for males to fly upwind 1 m along the pheromone plume. Release rate from concentration  $10^{-2}$  approximately 10 ng/h, the same as from a calling female

	2 ♀ ♀	Relative concentration of synthetic pheromone						
		1	$10^{-1}$	$10^{-2}$	$10^{-3}$	$10^{-4}$	$10^{-5}$	0
<i>Response:</i>								
n	52	71	77	88	110	105	90	45
% fanning	79	94	75	70	46	25	14	0
% locking on	75	75	65	51	35	20	18	0
% reaching 150 cm	73	61	44	39	30	19	16	0
<i>Flights over 1 m:</i>								
n	38	44	34	34	34	21	14	0
Flight time*	.084 <sup>a</sup>	.137 <sup>b</sup>	.136 <sup>b</sup>	.131 <sup>b</sup>	.117 <sup>ab</sup>	.093 <sup>ab</sup>	.107 <sup>ab</sup>	—
(min ± 1 S.E.)	±.005	±.011	±.011	±.010	±.009	±.006	±.007	—

\*Times followed by same letter not significantly different ( $P = .05$ ).

Table II. Responses of male spruce budworm when introduced into a plume of sex pheromone produced by two virgin females or by different source concentrations of synthetic pheromone, and duration of flights sustained by moving patterned ceiling. Release rate from concentration  $10^{-2}$  approximately 10 ng/h, the same as from a calling female

	2 ♀ ♀	Relative concentration of synthetic pheromone					
		1	$10^{-1}$	$10^{-2}$	$10^{-3}$	$10^{-4}$	$10^{-5}$
<i>Response:</i>							
n	29	47	70	75	69	70	45
% fanning	79	87	69	73	23	0	0
% locking on	76	81	54	52	14	6	7
% sustaining flight	76	74	40	32	14	6	7
<i>Sustained flights:</i>							
n	21	35	29	23	10	4	3
Duration	15.25	4.59	2.16	3.04	4.38	10.69	4.03
(min ± 1 S.E.)	±1.28 <sup>a</sup>	±1.81	±0.40	±0.58	±1.23	±6.50	±1.23

<sup>a</sup>No significant differences among times.



period, translates into a maximum of 5 ng/h, the same order of magnitude as the release rate from a PVC pellet loaded with .03% pheromone (Sanders 1981), the  $10^{-2}$  concentration in Tables I and II.

It is therefore disturbing that the responses of males to this concentration differed widely from their response to virgin females in percent locking on, percent reaching 150 cm, percent sustaining flight, and in speed of flight. This raises the possibility that the synthetic pheromone used here is not precisely the same as that produced by a virgin female. Possibly the addition of small amounts of the congeneric acetate and the saturated aldehyde which were found by Silk *et al.* (1980) in the effluvia of calling females are necessary.

### References

- Cardé, R. T. and T. E. Hagaman. 1979. Behavioral responses of the gypsy moth in a wind tunnel to airborne enantiomers of disparlure. *Environ. Ent.* **8**: 475-484.
- Daterman, G. E. 1974. Synthetic sex pheromone for detection survey of European pine shoot moth. *U.S. Dep. Agric. For. Serv. Res. Pap.* 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-dodeceny acetate: Release rate and biological activity. *Environ. Ent.* **2**: 607-610.
- Grisdale, D. 1970. An improved laboratory method for rearing large numbers of spruce budworm, *Choristoneura fumiferana* (Lepidoptera: Tortricidae). *Can. Ent.* **102**: 1111-1117.
- Kennedy, J. S. 1977. Olfactory response to distant plants and other odor sources. In H. H. Shorey and J. J. McKelvey, *Eds.*, *Chemical Control of Insect Behavior*. Wiley.
- Kennedy, J. S. and D. Marsh. 1974. Pheromone regulated anemotaxis in flying moths. *Science* **184**: 999-1001.
- Miller, J. R. and W. L. Roelofs. 1978a. Sustained flight wind tunnel for measuring insect responses to wind-borne sex pheromones. *J. Chem. Ecol.* **4**: 187-198.
- 1978b. Gypsy moth responses to pheromone enantiomers as evaluated in a sustained-flight tunnel. *Environ. Ent.* **7**: 42-44.
- Sanders, C. J. 1978. Evaluation of sex attractant traps for monitoring spruce budworm populations (Lepidoptera: Tortricidae). *Can. Ent.* **110**: 43-50.
- 1981. Release rates and attraction of PVC lures containing synthetic sex attractant of the spruce budworm, *Choristoneura fumiferana* (Lepidoptera: Tortricidae). *Can. Ent.* **113**: 103-111.
- 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.
- Sanders, C. J. and J. Weatherston. 1976. Sex pheromone of the eastern spruce budworm: Optimum blend of *trans*- and *cis*-11-tetradecenal. *Can. Ent.* **108**: 1285-1290.
- Silk, P. J., S. H. Tan, C. J. Wiesner, R. J. Ross, and G. C. Lonergan. 1980. Sex pheromone chemistry of the eastern spruce budworm, *Choristoneura fumiferana*. *Environ. Ent.* **9**: 640-644.

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