# EFFECT OF PHEROMONE PERMEATION ON SUSTAINED FLIGHT OF MALE SPRUCE BUDWORM

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## Abstract

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Flight durations of male spruce budworm moths, *Choristoneura fumiferana* (Clem.), that were locked-on to pheromone plumes from female moths were measured in a wind tunnel. Flight was sustained by use of a movable patterned ceiling. The longest sustained flight was 53 min. The effects of different background concentrations of synthetic pheromone were tested by surrounding the female moths with rubber septa loaded with synthetic pheromone. The duration of sustained flights decreased as the concentration of background synthetic pheromone increased, but at all concentrations some males persisted in orientated flight for 10 min or longer.

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## Résumé

La durée du vol a été mesurée chez des mâles de la Tordeuse des bourgeons de l'épinette, *Choristoneura fumiferana* (Clem.), exposés à des effluves de phéromone de femelles dans une soufflerie. Le vol a été maintenu au moyen d'un plafond mobile à motifs. La période la plus longue de vol soutenu a été de 53 minutes. Les effets de différentes concentrations ambiantes de phéromone synthétique ont été examinés par l'installation de septums de caoutchouc imbibés de phéromone synthétique autour des femelles. La durée des vols soutenus diminuait en raison inverse de la concentration de phéromone synthétique ambiante, mais, à toutes les concentrations, des mâles ont maintenu leur vol directionnel pendant au moins 10 minutes.

[Traduit par la Rédaction]

## Introduction

The major component of the sex pheromone of the spruce budworm, *Choristoneura fumiferana* (Clem.), has been identified as a 95:5 blend of *E*:*Z*-11-tetradecenal (Sanders and Weatherston 1976; Silk et al. 1980). Subsequent experiments showed that an additional biologically active component is missing from this blend but, despite further investigations (Silk and Kuenen 1988), no additional components have been identified. Field experiments have shown that the binary blend of *E*:*Z*-11-tetradecenal reduces the ability of male moths to locate female moths, although attempts to show reductions in population densities have been inconclusive (Silk and Kuenen 1984). Experiments have therefore been carried out with the binary blend in a wind tunnel to evaluate the effectiveness of the binary blend for disrupting male orientation (Sanders 1982, 1995, 1996).

With no background of pheromone, 99% of the males flew directly upwind to caged "target" female moths (Sanders 1995). However, when the females were surrounded by rubber septa loaded with the binary blend of the synthetic pheromone, the percentages of males reaching the females after they had been exposed to the ambient pheromone concentrations in the tunnel were reduced to 70% at a loading of 1 μg/septum,

33% at 10 μg/septum, 13% at 100 μg/septum, and 3% at 1000 μg/septum (Sanders 1996).

However, because of the limited size of the wind tunnel the males had to fly only 1.5 m in these experiments to reach the females, which usually took less than 10 s. Conditions in the field would be much more demanding. Not only would female moths be farther away in many instances, but also wind directions are far more variable, requiring the males to persist in oriented flight for much longer periods of time (David et al. 1983; Murlis and Jones 1981). An additional experiment was therefore carried out to determine the length of time males would persist in flying towards calling female moths in the presence of various background concentrations of the synthetic pheromone. This was carried out by use of moving optomotor cues provided by a moving ceiling pattern in the wind tunnel. This is effective in restricting the upwind progress of flying male moths that have locked-on so that they remain flying in the centre of the tunnel (Miller and Roelofs 1978; Sanders 1985).

It is also possible that preexposure to pheromone could affect the activity levels of the moths in holding cages, and that such activity prior to their release in the wind tunnel could affect the duration of their subsequent flights. The small holding cages used in previous experiments provide little opportunity for the moths to fly. Therefore flight durations of males held in larger cages were compared with those of males held in the smaller cages.

## Materials

**Insects.** The insects used in this experiment were from rearing stock maintained by the Insect Production Unit of the Canadian Forest Service, Great Lakes Forestry Centre. They were reared on synthetic diet (Grisdale 1970) at 21°C and approximately 70% relative humidity. Male and female insects were segregated as pupae. Moths were collected each morning and kept in 30 cm × 20 cm × 30 cm high screen cages on 17L:7D cycles. For the males, the scotophase was 2030–0330 hours. For the females the scotophase was 1400–2100 hours to ensure that the females were calling when the bioassays began at 1400 hours (Sanders and Lucuik 1972). The cages were sprayed each morning with water to provide moisture for the moths.

**Chemicals.** E- and Z-11-Tetradecenal (Farchan Chemicals Ltd., Willoughby, Ohio) were > 98% pure, with no detectable traces of the opposite isomers or of the corresponding acetates or alcohols. They were mixed to provide a 95:5 blend of *E*:*Z*-11-tetradecenal in a stock solution in cyclohexane which was then serially diluted to provide concentrations of 1 mg/mL, 100 μg/mL, 10 μg/mL, and 1.0 μg/mL. From these, 100-μL aliquots were pipetted into rubber septa (catalogue No. 1780J07, A.H. Thomas, Philadelphia, Pennsylvania) to provide the required loadings of 100, 10, 1.0, and 0.1 μg/septum.

**Wind Tunnel.** The wind tunnel is the same as that used in previous experiments on spruce budworm moth orientation (Sanders 1982, 1996); it is constructed of plate glass, 90 cm × 90 cm in cross section and 2 m long. Lighting was maintained on a 17L:7D light cycle, scotophase 2030–0330 hours, with light intensity approximately 35 lx. Wind speed was approximately 50 cm/s. A sheet of plate glass was mounted horizontally in the tunnel as an artificial floor (Sanders 1996), separating the tunnel vertically into two equal halves. A wire frame was placed in the top half adjacent to the screen at the upwind end of the tunnel, with 14 locations marked on it for mounting the rubber septa. These were positioned in three rows, 24 cm apart, with the first row 150 cm from the downwind end of the tunnel. The first and third rows had six septa

in an evenly spaced 2 × 3 grid, the second row had two septa, positioned at mid-height of the half tunnel, 30 cm in from opposites sides.

## Methods

Freshly emerged male moths were divided into three groups, A, B, and C, of approximately 10 moths each. Groups A and B were housed individually in small screen cages, 3 cm in diameter and 5 cm in length. Group C was placed together in a 29 × 29 × 29 cm metal screen cage which allowed the moths more freedom to fly. All three groups were then placed in the pheromone-free lower half of the tunnel to acclimate them to the conditions in the tunnel. The following day at 1100 hours, two 1-day-old virgin female moths in individual screen cages, 3 cm in diameter and 2.5 cm long, were set up in the centre of the top half of the tunnel 10 cm from the upwind screen. Bioassays started at 1400 hours. One at a time the small cages, each containing an individual male, were moved downwind of the two target female moths, the lid of the cage was removed and the subsequent behaviour of the moth observed. Males from group C, which was held in the large cage, were first captured individually in small cages and then moved to the release position. For all three groups of moths the duration of casting flight following release and the start of upwind casting flight (locking-on) were recorded. If after 1 min a moth remained in the cage, it was shaken out to ensure that it was capable of flight; if it immediately fell to the floor on two successive releases, it was rejected as incapable of flight; and if it was capable of flight, it was recorded as quiet. If after taking flight and before locking-on a male landed on the inside surface of the tunnel and remained there for 10 s, it was recorded as going to the side.

The individual groups of males were then treated as follows.

Once a group A male locked-on and started to move upwind, the patterned ceiling of the tunnel was kept stationary and the male was allowed to proceed upwind unchecked. Records were kept of whether it passed a point 50 cm from the upwind end of the tunnel, reached the target females or a septum, or flew to the inside surface of the tunnel, landed, and remained quiet for 10 s.

Group B males were handled as for those of group A, but as soon as a male locked-on to a pheromone source, movement of the patterned ceiling was begun to sustain its flight in the middle of the tunnel and prevent it from reaching a pheromone source. Times were recorded when it locked-on, and when it ceased orientated flight by veering off to the side, the floor, or the ceiling of the tunnel and did not recover its former orientated flight within 10 s. In the first few runs males were allowed to fly until they veered off to floor or sides, regardless of how long this took. However, some males flew for 30 min or longer which limited the number of males that could be assayed during an afternoon. Therefore, for the remainder of the experiments, flights were terminated after 10 min.

Group C males, which were kept together in the large cage, were captured one at a time in small cages, moved to the upper half of the tunnel downwind of the females, and then treated in the same manner as the males in group B.

The experiments were continued until at least 40 males from each group had locked-on in each treatment.

## Results and Discussion

The number of males in each group and their subsequent behaviour after release are shown in Table 1. All those males of group A (those that were allowed to fly without the restraining influence of the moving ceiling) that locked-on in the absence

TABLE 1. Numbers ($n$) of male spruce budworm moths in each experimental group, showing the percentages remaining quiet in the release cages (Q), flying to the inside surface of the tunnel without locking-on (S), locking-on (L), and the percentages of moths that locked-on in group A which flew to either the target females (FE) or a rubber septum (SP)

| | Group A (small cages, normal flight) | | | | | | Group B (small cages, sustained flight) | | | | Group C (large cages, sustained flight) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Loading in septa (μg) | | % of $n$ | | | % of L | | | % of $n$ | | | | % of $n$ | | |
| | $n$ | Q | S | L | FE | SP | $n$ | Q | S | L | $n$ | Q | S | L |
| 0 | 42 | 0 | 0 | 100 | 100 | 0 | 42 | 0 | 0 | 100 | 42 | 0 | 0 | 100 |
| 0.1 | 42 | 0 | 2 | 98 | 76 | 24 | 47 | 0 | 0 | 100 | 46 | 2 | 2 | 96 |
| 1.0 | 51 | 6 | 0 | 94 | 29 | 63 | 50 | 2 | 0 | 98 | 53 | 8 | 2 | 90 |
| 10 | 65 | 5 | 6 | 89 | 20 | 68 | 63 | 3 | 6 | 91 | 64 | 3 | 7 | 90 |
| 100 | 91 | 10 | 24 | 66 | 3 | 55 | 100 | 22 | 18 | 60 | 90 | 11 | 19 | 60 |

of background pheromone (zero pheromone loading, Table 1) flew directly to the females. This, together with the fact that some males locked-on to the female pheromone plume with all treatments, is confirmation that the females were calling in all cases. However, as in previous experiments (Sanders 1996), the proportion of males that reached the females became progressively less as the concentration of background pheromone increased, declining to only 3% at the highest pheromone loading (100 μg/septum). As the loading in the septa increased, so did the numbers of males flying to the septa instead of the females, but at the 100-μg loading there was a decline in the numbers flying to the septa, with many males veering off to the sides of the tunnel or not flying at all (Table 1).

Durations of sustained flights of the males whose upwind flight was restrained by the moving ceiling are shown in Figure 1. Among the males that were allowed unlimited time, the longest flight was 53.01 min. This was a male moth that locked-on to the target females in the presence of the 14 septa each loaded with 1 μg of synthetic pheromone. The second longest was 43.74 min by a male that locked-on to the females with no background pheromone.

With no background of synthetic pheromone, more than half the males flew for 4 min or longer, and 25% of them flew for more than 10 min. As the background of synthetic pheromone increased, the duration of sustained flight tended to decrease. This reduction in the ability of males to sustain flight should be taken into account when evaluating the disruptive effect of synthetic dispensers in a wind tunnel. For example, with no background pheromone, 100% of males that locked-on were successful in reaching the target females (Table 1), and 65% flew for longer than 4 min (Fig. 1). In the presence of septa loaded with 10 μg, 25% of the males that locked-on still reached the females, but of those only 19% flew for longer than 4 min. If 4 min is the minimum time required for a male to reach a female, then with no background pheromone 65% of the males could have reached a female, but in the presence of septa loaded with 10 μg of synthetic pheromone, less than 5% could have reached a female. Therefore, evaluation of disruption in a wind tunnel by the numbers of males able to reach calling female moths could be misleading without consideration of the persistence of the male moths.

Durations of flight times were, however, very variable and in all the concentrations tested a few males were able to sustain flight for more than 10 min. The longest flight of 53 min was in the presence of septa loaded with 1 μg of pheromone, which equals a concentration of background pheromone in the order of 20 pg/m$^3$ (Sanders 1996). This suggests that complete disruption of a population will be very hard to achieve with the binary blend of the synthetic pheromone. Even if the majority of male

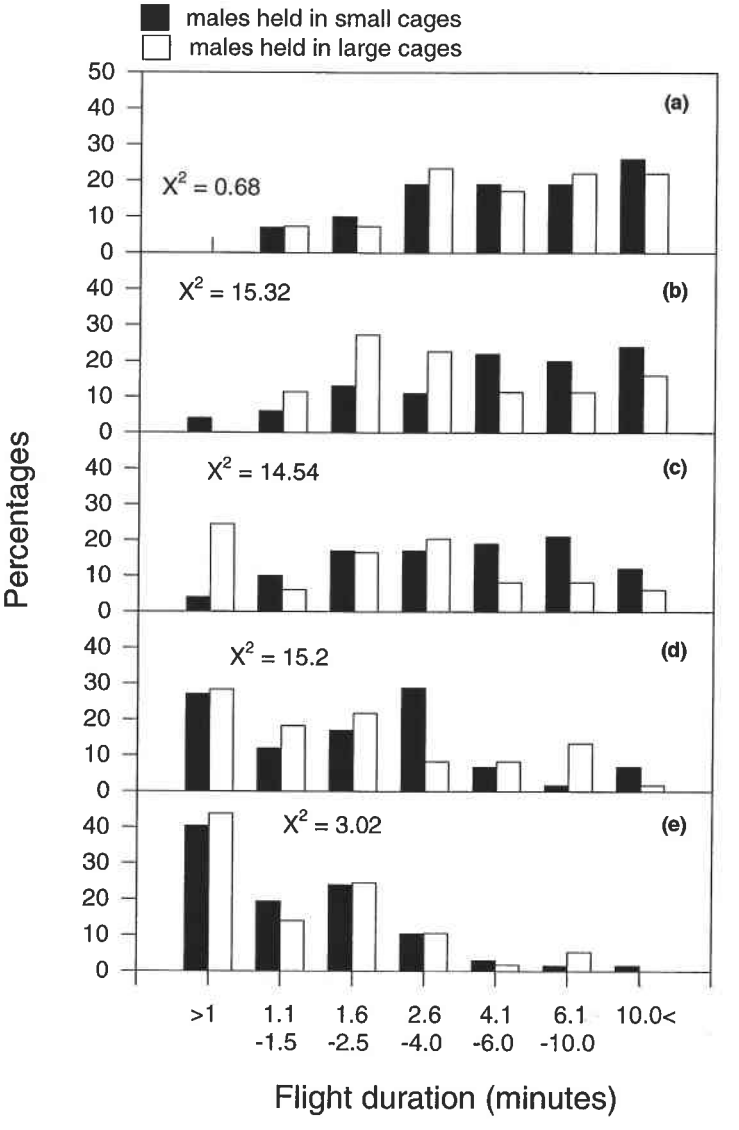


FIG. 1. Frequency distributions of flight durations of male spruce budworm moths orientating in a wind tunnel to caged female moths. Flight was sustained by a moving patterned ceiling. (*a*) Female moths alone with no other pheromone sources ($n = 42$ for males held individually in small cages prior to assays, and $n = 41$ for males held together in a large cage,). (*b–e*) Females surrounded by 14 septa loaded with synthetic pheromone as follows: (*b*) 0.1 µg/septum ($n = 46$ and 44), (*c*) 1.0 µg/septum ($n = 48$ and 49), (*d*) 10 µg/septum ($n = 59$ and 60), and (*e*) 100 µg/septum ($n = 67$ and 57). $\chi^2$ values are for comparisons between distributions of males held in small cages versus males held in a large cage.

moths have become habituated, a few will still remain locked-on and will be able to locate a female.

Differences also occurred in flight durations between males that were held in the small cages versus those held in the larger cages (Fig. 1). Differences were not significant in the absence of background pheromone, or at the highest loadings of synthetic pheromone, 100 µg ($\chi^2 = 0.68$ and 3.02, respectively; df = 5), but in the presence of

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septa loaded with 0.1 and 1 μg there were significant tendencies for males in the larger cages to fly for shorter periods ($\chi^2$ = 15.32 and 14.54, respectively; df = 5). Possibly this effect was associated with the greater opportunity for flight among the males held in the larger cages. Despite this, some males flew for 10 min or longer. It is therefore concluded that activity before release does not greatly affect the rate at which habituation takes place.

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