EFFECTS OF PROLONGED EXPOSURE TO DIFFERENT CONCENTRATIONS OF SYNTHETIC PHEROMONE ON MATING DISRUPTION OF SPRUCE BUDWORM MOTHS IN A WIND TUNNEL

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Abstract

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Male spruce budworm moths were kept in a wind tunnel for 4 days and assayed each day to determine their ability to locate calling females that were surrounded by rubber septa loaded with synthetic pheromone, a 95:5 blend of *E*:*Z*-11-tetradecenal. As the concentration of synthetic pheromone increased, the numbers of males successfully locating females decreased, the latency of response increased, and the speed of flight decreased. At release rates from the septa close to those of a calling female, 6–60 ng/h per septum, most disrupted males flew to a septum instead of the females. At the highest release rate tested, 600 ng/h, many males were inactive or flew to the sides of the tunnel, which indicates sensory fatigue. This effect was more pronounced among males that were continuously exposed to the synthetic pheromone for the 4 days than among males kept in pheromone-free air between assays. About a quarter of the males died or became unable to fly after the 4 days, but there was no change in the proportions of the different responses of males, or in their response times, with increasing age, nor was there evidence that males were conditioned by their experience on previous days. The results led to the conclusion that time-averaged atmospheric concentrations of the synthetic pheromone as high as 20 ng/m^3 are necessary to achieve effective disruption of the orientation of male spruce budworm moths to calling female moths.

Sanders, C.J. 1996. Effets d'une exposition prolongée à différentes concentrations d'une phéromone synthétique sur les accouplements de Tordeuses de bourgeons de l'épinette dans un tunnel d'essai. *The Canadian Entomologist* **128**: 57–66.

Résumé

Des mâles de la Tordeuse des bourgeons de l'épinette ont été gardés dans un tunnel d'essaie pendant 4 jours et observés chaque jour pour déterminer leur capacité de localiser des femelles à la recherche de mâles et entourées de septums de caoutchouc garnis d'une phéromone synthétique, un mélange 95:5 de *E*:*Z*-11-tétradécénal. À mesure qu'augmentait la concentration de phéromone synthétique, le nombre de mâles capables de localiser les femelles a diminué, leur temps de latence a augmenté et leur vitesse de vol a diminué. Aux taux de libération de phéromone voisins de ceux d'une femelle à la recherche d'un mâle, 6–60 ng/h par septum, la plupart des mâles perturbés ont volé vers un septum plutôt que vers une femelle. Au plus haut taux de libération utilisé, 600 ng/h, plusieurs mâles sont devenus inactifs ou ont volé vers les côtés du tunnel, ce qui dénote un fatigue sensorielle. Cet effet s'est avéré plus marqué chez les mâles exposés à la phéromone synthétique durant les 4 jours que chez les mâles exposés à de l'air sans phéromone entre les expériences. Environ un quart des mâles sont morts ou sont devenus incapables de voler après les 4 jours, mais l'âge n'influençait pas les proportions des différentes réactions des mâles, ou leur temps de latence, et les mâles ne semblaient pas conditionnés par leur expérience des jours précédents. Ces résultats permettent de croire que des concentrations de phéromone synthétique d'au moins 20 ng/m^3 dans l'atmosphère (en moyenne sur une période de temps) sont nécessaires pour qu'il y ait bouleversement de l'orientation des mâles de la Tordeuse des bourgeons de l'epinette vers des femelles à leur recherche.

[Traduit par la Rédaction]

Introduction

The use of synthetic pheromones to control lepidopterous pests by disrupting normal mating behavior ('mating disruption') is now operational for a number of species (Cardé

and Minks 1995). The principal objective in mating disruption of Lepidoptera is to reduce the ability of the male moths to perceive and respond to the female-emitted pheromone. The possible mechanisms involved in the disruption of pheromone-mediated behavior in the control of lepidopterous pests have been reviewed by Bartell (1982) and Cardé (1988). The two principal mechanisms are disorientation of male flight behavior, in which a male is unable to discern the female-emitted plume from the background of synthetic pheromone, and sensory fatigue which causes the male to cease its response.

The orientation of male spruce budworm moths, *Choristoneura fumiferana* (Clemens) (Lepidoptera: Tortricidae), to calling female moths can be suppressed in a wind tunnel by surrounding the female moths with an array of dispensers emitting synthetic pheromone (Sanders 1982, 1985, 1995). Of the compounds that have been tested, a binary blend of the two main pheromone components, *E*- and *Z*-11-tetradecenal in the natural ratio 95*E*:5*Z* (Sanders and Weatherston 1976), is the most effective disruptant (Sanders 1995). Both field and laboratory bioassays have shown that there are additional minor components that remain unidentified (Sanders 1984), but it is not known how effective the complete pheromone blend would be as a disruptant. Dispensers that emit 95:5 *E*:*Z*-11-tetradecenal at rates close to, or one or two orders of magnitude greater than, the release rate of a female moth caused males to become disoriented to the female plume and orientate instead to synthetic pheromone plumes. However, at the release rates of the synthetic pheromone that caused this behavior there was no evidence of sensory fatigue, and when males were left for several hours in the wind tunnel they continued to search and showed a high probability of eventually locating the female moths (Sanders 1995). By itself, therefore, flight disorientation is not likely to be effective in preventing mating. At higher concentrations of the synthetic pheromone, sensory fatigue became more pronounced and was effective in preventing males from locating the calling females. However, sensory fatigue did not occur until release rates of the synthetic pheromone were well in excess of that produced by a calling female moth (Sanders 1985, 1995).

In earlier wind tunnel experiments on mating disruption (Sanders 1982, 1985), the sources of synthetic pheromone were presented in a 2-dimensional array. The methodology was improved in later experiments by surrounding the target females with a 3-dimensional array of dispensers (Sanders 1995), which better simulated conditions in a field disruption trial. In all of these experiments the male spruce budworm moths were kept in the ambient pheromone concentrations in the wind tunnel for 3 h before the bioassays were started. This is considerably shorter than the lengths of time males would be exposed in an operational disruption program in the field, where they are subjected to synthetic pheromone all day, and possibly several days, before they encounter a pheromone plume from a calling female. Moreover, the later experiments (Sanders 1995) made no comparison between males kept in the ambient pheromone and those kept in pheromone-free air. In the experiments reported here, the tunnel was divided into two halves. In one half, males were kept exposed to synthetic pheromone for 4 days and their responses were compared with those of males that were kept in pheromone-free air in the other half of the tunnel.

Materials

Insects. Insects used in these studies were from rearing stock maintained by the Insect Production Unit of the Forest Pest Management Institute, Canadian Forest Service, Sault Ste. Marie, Ontario (FPMI). They were reared on a synthetic diet following the techniques described by 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 by 20 by 30 cm high screen cages on 17:7 L:D cycles. For the males, the scotophase was 2030–0330 hours; for the females it 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.

Wind Tunnel. The wind tunnel has been described previously (Sanders 1982). It is constructed of plate glass and is 90 by 90 cm in cross section and 2 m long. Lighting was maintained on a 17:7 L:D cycle, scotophase 2030–0330 hours, with light intensity during the photophase of ca. 35 lx. Wind speed was ca. 50 cm/s. For these experiments, a sheet of plate glass was mounted horizontally in the tunnel as an artificial floor, separating the tunnel vertically into two equal halves. A wire frame was placed in the top half with 16 locations marked on it for mounting the rubber septa. These were positioned in four rows, A, B, C, and D, 24 cm apart, with the first row 100 cm from the downwind end of the tunnel. The first and third rows, A and C, had six septa in an evenly spaced 2 × 3 grid, the second and fourth rows, B and D, had two septa each, positioned at mid-height of the half tunnel, 30 cm in from opposites sides.

Chemicals. Rubber septa (A.H. Thomas, Philadelphia, Pennsylvania. Catalogue No. 1780J07) that had previously been extracted with ethanol by the technique of Steck et al. (1979) were used as sources of the disruptant chemicals. All experiments were carried out with a 95:5 blend of the two major pheromone components, *E*-and *Z*-11-tetradecenal (*E*- and *Z*-11-14:Ald). These were obtained from Farchan Chemicals Ltd., Willoughby, Ohio, and 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*-11-14:Ald. Stock solutions were made up in cyclohexane and then serially diluted to provide concentrations of 10 mg/mL, 1 mg/mL, 100 μg/mL, and 10 μg/mL. From these, 100-μL aliquots were pipetted into the rubber septa to provide the required loadings.

Release rates from these septa can be calculated using the formulae $R = M * {}^{1}/_{t}{}^{1}/_{2} * \log_{n}2$, and $M = M_0 e^{(-t*{}^{1}/_{t}{}^{1}/_{2}*\log_{n}2)}$ (Butler and McDonough 1979), where R = the release rate, M_0 = the amount of pheromone loaded into the septa, M = the amount of pheromone remaining in the septa at the start of the observation period, and $t^1/_2$ = the half life of the active ingredient, which in the case of a 14-carbon chain aldehyde is 43 days at around 20°C (Heath et al. 1986). From this it can be calculated that the release rates after 5 days for septa loaded with 1, 10, 100, and 1000 μg of *E*:*Z*-11-14:Ald were 0.6, 6.0, 60, and 600 ng/h, respectively. Given that the cross section of one half of the tunnel is 0.4 m^2 and that the wind speed was 50 cm/s, then the time-averaged concentrations of *E*:*Z*-11-14:Ald produced by septa loaded with 1, 10, 100, and 1000 μg were 0.02, 0.2, 2.2, and 22.2 ng/m^3, respectively.

Methods

On the 1st day of each experiment, Day 1, rubber septa loaded with the appropriate concentration of the *E*:*Z*-11-14:Ald blend were pinned to each of the 16 locations on the wire frame in the upper half of the tunnel. Each septum was backed by a piece of black tape, approximately 2.5 by 2.5 cm. This created a turbulent plume, as could be seen by releasing smoke from the location of a septum.

At 1100 hours on Day 1, two 1-day-old virgin females that had been kept for their first night on an advanced 17:7 L:D cycle, scotophase 1400–2100 hours, were placed individually in small screen cages, 3 cm in diameter and 2.5 cm long. These were taped to a stand that was positioned in the upper half of the tunnel within the array of rubber septa, so that the cages were 30 cm from the upwind screen, halfway between the artificial floor and the roof of the tunnel, and 30 cm in from one side of the tunnel. Also, at 1100 hours on Day 1, ca. 30 male spruce budworm moths that had been kept on a 17:7 L:D cycle were placed individually in small screen cages. One half of these were placed on a rack in the lower half of the tunnel, the other half on a rack in the upper half of the tunnel. The males in the upper half of the tunnel were placed on the opposite side of the tunnel to the target females. This

ensured that they were not exposed to the plume from the target females, although they were continually exposed to synthetic pheromone from the septa.

Starting at 1400 hours on Day 1, the cages containing the males were moved one at a time directly downwind of the target females, 30 cm in from the end of the tunnel. The lid was removed from the cage and the subsequent behaviors of each male were recorded and timed as follows: (*i*) wing-fanning, (*ii*) taking-flight, (*iii*) locking-on to the female plume and passing a point 50 cm from the downwind end of the tunnel, (*iv*) proceeding upwind to pass a point 100 cm from the downwind end (row A of the septa), and (*v*) landing on a septum or the cages housing the female moths. Each male was allowed 1 min to respond, and each assay was terminated if the male landed on an inside surface of the tunnel and remained inactive for 10 s. After each male was assayed it was recaptured and returned to the rack in the half of the tunnel that it had come from. Each day after the assays were completed, the target females were removed, the male cages were sprayed with water, and left in situ on the racks until the next day (Day 2). At 1100 hours on Day 2 fresh 1-day-old target females were placed in the tunnel and the males were assayed again. This was repeated on Days 3 and 4. The cages containing each male were marked and were kept in the same location on the racks so that a record could be kept of the behavior of each male throughout the 4 days.

Analyses. Comparisons of numbers of males responding were carried out by the χ^2 test for homogeneity (Sokal and Rolf 1981). Comparisons among response times were carried out by first transforming the data to logarithms, which normalized the distributions, and then subjecting the transformed data to multivariate analyses, using the Minitab GLM procedure (Minitab Inc., State College, PA). Standard deviations were calculated from the untransformed data.

Results

Incapacitated Moths. Some of the male moths became incapacitated during the 4 days of each experimental run. That included those that died, and those that had damaged wings and were unable to fly normally—usually manifested by a reluctance to fly, and when forced to fly immediately hitting the floor and being unable to lift off again. Overall, out of the total of 328 moths assayed, none were incapacitated on the 1st day, 4.9% on the 2nd day, a further 11.0% on the 3rd day, and a further 11.9% on the 4th day, for a total of 27.8%. The numbers incapacitated on different days among the different treatments were very varied. Overall, the number was lowest (10.0%) among those continually exposed to the highest concentration, but differences among the different concentrations and exposures were not significant ($\chi^2 = 10.0$, df = 7, $p^{0.05} = 14.1$).

Wing-fanning. Almost all of the naive males (i.e. those that were kept in the lower half of the wind tunnel unexposed to the synthetic pheromone) wing-fanned immediately when moved to the upper half of the tunnel downwind of the two target females (Table 1). However, among the males that were exposed continually to pheromone in the upper half of the tunnel, there was a decline in the percentage wing-fanning as the concentration of *E*:*Z*-11-14:Ald increased. There was also a tendency for the level of response among these males to decline as they aged.

Disruption of Orientation. Disrupted males included both those that followed false trails to a rubber septum, and those that failed to fly or that went to the sides or roof of the tunnel after taking flight. Levels of disruption did not differ significantly between the exposed and naive males for any of the four loadings of *E*:*Z*-11-14:Ald (Fig. 1). Furthermore, there were no significant differences in levels of disruption on successive days within each experiment, except in the case of the naive males at the lowest loading of *E*:*Z*-11-14:Ald (1 μg), for which disruption increased over time, with values of 25%, 42.5%, 54.1%, and 51.5% on days 1 through 4, respectively (χ^2 for the 4 days data = 8.64, df = 3, $p^{0.05} = 7.81$).

TABLE 1. Percentages of male spruce budworm that wing-fanned during a 1-min exposure period downwind of two virgin females that were surrounded by rubber septa loaded with different concentrations of 95:5 *E*:*Z*-11-tetradecenal

		Percentage of males wing-fanning			
	Age (days)	1-μg loading (*n*)	10-μg loading (*n*)	100-μg loading (*n*)	1000-μg loading (*n*)
Exposed	1	95.5^{a} (44)	80.0^{abcd} (45)	66.7^{bc} (45)	20.0^{b} (30)
	2	95.2^{a} (42)	64.4^{bcd} (45)	63.6^{bc} (44)	16.7^{b} (30)
	3	81.1^{a} (37)	57.8^{cd} (45)	47.5^{bc} (40)	34.5^{b} (29)
	4	93.3^{a}(30)	51.5^{d} (33)	28.6^{c} (35)	7.7^{b} (26)
Naive	1	100^{a} (44)	95.6^{ab} (45)	100^{a} (45)	100^{a} (30)
	2	100^{a} (41)	91.1^{b} (45)	100^{a} (41)	93.3^{a} (30)
	3	100^{a} (37)	87.2^{abc} (39)	100^{a} (35)	100^{a} (26)
	4	94.3^{a} (35)	94.0^{a} (33)	95.8^{ab} (24)	100^{a} (24)

Percentages in the same column followed by the same letter are not significantly different (G-test for homogeneity, $p = 0.05$).

Levels of disruption did differ significantly among the four concentrations. The combined data for exposed and naive males were 27.3%, 63.0%, 84.4%, and 95.0% disruption for the 1-, 10-, 100-, and 1000-μg loadings, respectively ($X^2 = 97.0$, df = 3, $p^{0.05} = 7.81$). At the three lowest pheromone loadings, by far the larger proportion of disrupted moths went to septa, with no differences among the exposed or naive males (91.8, 95.8, and 89.9%, respectively, for the combined exposed and naive moths at 1, 10, and 100 μg). At the highest loading (1000 μg), 95.2% of the disrupted naive males went to the septa, but among the exposed males the figure was 51.8% (X^2 values for differences between exposed and naive males for each of the 4 days were 13.5, 12.1, 6.9, and 17.3, df = 3, $p^{0.05} = 7.81$). Among the remaining 48.2%, i.e. those that did not fly to a septum, most (77.8%) did not take flight at all during the allocated 1-min exposure period.

Results were analyzed for evidence that the behavior of the males was influenced by their experience in previous bioassays. In only one of the 10 treatments in which enough males responded for an analysis to be carried out was there any evidence for conditioning or learning (Table 4). This was among males exposed continuously to the 10-μg loadings. Out of 45 males that flew to females or septa on Day 1, 15 (33%) chose the target females over the septa. Of these 15 males, six (40%) again chose females on Day 2. Of the six, one was incapacitated the following day and four of the remaining five (80%) again chose females. On Day 4 all four males again chose the females. In the other nine instances (Table 4) the percentages remained relatively unchanged, which indicates that the males were not influenced by their decisions on previous days.

Response Times. Time of take-off. Exposed males were significantly slower to take flight when moved downwind of the target females than were naive males (Table 2, $F = 308.5$, df = 1, $p > 0.001$). Also, both exposed and naive males were significantly slower to take flight as the concentration of *E*:*Z*-11-14:Ald increased ($F = 27.3$, df = 3, $p > 0.001$). There was also a significant interaction between concentration and exposure (concentration × exposure, $F = 18.6$, df = 3, $p > 0.001$), i.e. the delays in response among the different concentrations and the different ages were not uniform in both exposed and naive groups, and an examination of the data in Table 2 indicates that the delays caused by concentration were more pronounced among the exposed males than the naive.

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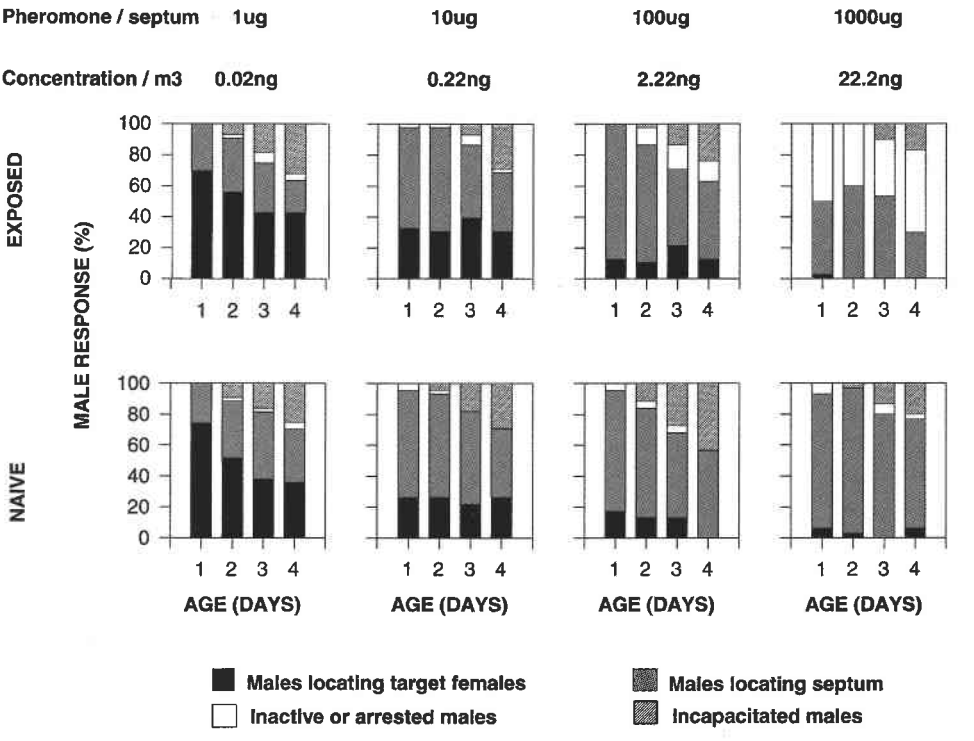

FIG. 1. Percentages of male spruce budworm moths (*a*) flying to target females, (*b*) flying to rubber septa, (*c*) remaining inactive or flying to inside of tunnel, (*d*) incapacitated or dead when released in a wind tunnel on 4 successive days downwind of two target females surrounded by rubber septa loaded with different concentrations of synthetic pheromone, 95:5 *E*:*Z*-11-tetradecenal. Top row of graphs shows responses of males that were kept exposed to the synthetic pheromone throughout the 4 days of the experiment, bottom row males that were kept in pheromone-free air between assays. Time-averaged concentrations of synthetic pheromone/m^3 are based on calculations of release rates from the septa (see text).

Response times also slowed with increasing age ($F = 11.7$, df = 3, $p > 0.001$) and, again, the tendency was more pronounced among the exposed males (exposure × age, $F = 7.6$, df = 3, $p > 0.001$).

Time to fly 50 cm. In the presence of the lower concentrations of *E*:*Z*-11-14:Ald, males that took flight locked-on to either the target females or a rubber septum, and immediately proceeded upwind to the pheromone source. However, as the concentration of *E*:*Z*-11-14:Ald increased, males tended to engage in crosswind casting before proceeding upwind, and in some instances they did not proceed upwind at all, but flew to the sides of the tunnel. Because it was impossible to determine objectively when crosswind casting ceased and upwind progress began, these behaviors were difficult to quantify. They are described here by recording the times after taking flight that males proceeded past a point 50 cm upwind from the release point. Those males that engaged in crosswind casting obviously took longer to reach this point.

Times taken by males to proceed upwind past the 50-cm mark increased significantly as the concentration of synthetic *E*:*Z*-11-14:Ald increased (Table 3, $F = 62.1$, df = 3, $p > 0.001$), with the effect evidently slightly greater among the exposed than the naive males (concentration × exposure, $F = 2.86$, df = 3, $p = 0.036$). Overall, there was a tendency for

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TABLE 2. Times (s±1 SD) taken by male spruce budworm to take flight after exposure to the plume from two target virgin female moths which were surrounded by rubber septa loaded with 95:5 *E*:*Z*-11-tetradecenal

	Age (days)	Times after exposure to female plume when males took flight (s)			
		1-μg loading	10-μg loading	100-μg loading	1000-μg loading
Exposed	1	3.9±2.9	5.6±4.7	7.8±2.9	9.3±10.0
	2	5.4±4.3	7.9±16.9	6.7±8.6	12.4±15.0
	3	6.7±7.6	10.2±17.1	9.7±9.9	20.4±16.1
	4	4.5±2.9	8.2±12.4	11.4±19.6	16.3±15.8
Naive	1	3.6±1.1	3.8±1.5	3.6±1.8	4.8±3.2
	2	4.0±3.8	3.8±2.9	3.7±2.1	4.1±2.4
	3	4.2±2.0	4.2±10.6	4.1±7.8	3.4±2.8
	4	4.3±2.3	4.9±3.7	3.6±2.7	6.4±11.2

Multivariate analysis of times when males took flight after being moved to a position downwind of target females against a background of different concentrations of synthetic pheromone. Analyses carried out on logarithmically transformed data.

Source	df	Mean square	*F*	*p*
Concentration	3	1.952	27.3	>0.001
Exposure	1	22.082	308.5	>0.001
Age	3	0.836	11.7	>0.001
Conc×Expo	3	1.332	18.6	>0.001
Conc×Age	9	0.074	1.0	0.407
Expo×Age	3	0.543	7.6	0.001
Conc×Expo×Age	9	0.172	2.4	0.011
Error	995	0.072		

the exposed males to proceed upwind more slowly than the naive males ($F = 7.0$, df = 1, $p = 0.008$). Older males proceeded upwind more slowly ($F = 10.0$, df = 3, $p > 0.001$), with no differences in patterns between the different concentrations (concentration × age, $F = 1.78$, df = 9, $p = 0.068$), and only a slight, but significant difference in pattern between exposed and naive males with the older exposed males taking longer to reach the 50-cm mark than the equivalent naive males (exposure × ages, $F = 5.8$, df = 3, $p > 0.001$).

Discussion

Disruption in this study was measured by the reduction in numbers of male moths able to locate two caged calling female moths. This included males that flew to the septa or to the sides of the tunnel after, and also those that did not fly after 1-min exposure to the pheromone plume from the caged females. Previous experiments have shown that very few males that remain inactive for 1 min respond after longer exposure, and when forced to fly these males rarely flew to the females (Sanders 1982). It is assumed therefore that these males had become habituated.

Levels of disruption were similar to those recorded for comparable release rates of *E*:*Z*-11-14:Ald in previous experiments (Sanders 1985, 1995). Release rates from a calling female spruce budworm moth are estimated to be 20–40 ng per night by Silk et al. (1980), or 60 ± 50 ng per night by Morse et al. (1982). Under laboratory conditions and the standard scotophase of 7 h, peak calling lasts about 7 h (Sanders and Lucuik 1972), therefore average release rates are 5–10 ng/h. However, rates of release by female spruce budworm moths are

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TABLE 3. Times (s±1 SD) taken by male spruce budworm to fly 50 cm upwind in the presence of two target virgin female moths that were surrounded by rubber septa loaded with 95:5 *E*:*Z*-11-tetradecenal

	Age (days)	Times taken for males to fly 50 cm (s)			
		1-μg loading	10-μg loading	100-μg loading	1000-μg loading
Exposed	1	3.9±2.3	3.9±1.3	2.6±4.2	7.8±4.1
	2	4.9±2.3	4.7±1.7	6.1±6.4	8.9±6.5
	3	5.4±2.6	5.1±3.9	5.0±1.8	9.2±5.6
	4	4.4±3.1	5.4±6.1	5.7±2.8	9.3±4.6
Naive	1	3.5±1.3	3.7±2.3	4.8±2.9	8.2±7.3
	2	3.8±1.8	3.6±2.1	4.8±3.2	7.9±6.6
	3	4.4±2.8	4.5±2.8	5.0±4.8	7.9±10.1
	4	4.4±2.5	4.1±2.2	5.0±2.2	7.4±6.3

Multivariate analysis of times taken for males to proceed upwind 50 cm when released downwind of target females against a background of different concentrations of synthetic pheromone (Table 3). Analyses carried out on logarithmically transformed data.

Source	df	Mean square	*F*	*p*
Concentration	3	3.307	62.1	>0.001
Exposure	1	0.372	7.0	0.008
Age	3	0.533	10.0	>0.001
Conc×Expo	3	0.152	2.9	0.036
Conc×Age	9	0.095	1.8	0.068
Expo×Age	3	0.311	5.8	>0.001
Conc×Expo×Age	9	0.172	2.1	0.023
Error	1048	0.053		

very variable, both among females and during the calling period of individual females, with maximum rates up to 50 ng/h (Morse et al. 1982). Septa loaded with 10 or 100 μg *E*:*Z*-11-14:Ald have release rates of 6 or 60 ng/h, respectively, and therefore represent the low and high ends of the range of release rates from female moths.

At release rates of *E*:*Z*-11-14:Ald below those of a calling female (1-μg loadings), most males successfully located the target females regardless of whether they were continuously exposed to the synthetic pheromone or not. At release rates equivalent to calling females (the 10- or 100-μg loadings), more males went to the septa than to the females, i.e. false trail following became a significant factor. However, in previous experiments (Sanders 1995) males that followed false trails remained active for at least 3 h and eventually located the target females. Therefore, without some additional factors contributing to disruption, false trail following in response to the binary blend of *E*:*Z*-11-14:Ald by itself is unlikely to reduce mating of spruce budworm sufficiently for effective control. There is strong evidence that the binary blend is not the complete pheromone, and it is possible that false trail following would be more effective with the complete blend. Males kept continuously in the atmosphere of *E*:*Z*-11-14:Ald responded more slowly to the target females and flew more slowly as the concentration of *E*:*Z*-11-14:Ald increased. This, in itself, would probably reduce the chances of males successfully locating females in a field situation but, again, would not prevent persistent males from locating females. A further factor, such as sensory fatigue, would therefore appear to be essential for effective disruption. At the 1- and 10-μg loadings there was no evidence of sensory fatigue. At the 100-μg loading, some of the males that were

TABLE 4. Percentages of individual males making the same choice (between either two caged females or a septum loaded with *E*:*Z*-11-tetradecenal) on successive days. For instance at the 1-μg loading of *E*:*Z*-11-tetradecenal, 70% of the exposed males went to the females on Day 1. Of those same males, 59% chose the females again on Day 2, and of those 64% again chose the females on Day 3, etc. For further explanation see text. An asterisk denotes insufficient data (less than six males)

		Percentages					
		1-μg loading		10-μg loading		100-μg loading	
	Age (days)	♀♀	Septum	♀♀	Septum	♀♀	Septum
Exposed	1	70	30	33	24	*	87
	2	59	31	40	18	*	89
	3	64	33	80	*	*	70
	4	44	*	100	*	*	92
Naive	1	75	25	67	71	*	78
	2	68	60	70	66	*	83
	3	45	50	69	65	*	90
	4	67	*	89	70	*	100

continuously exposed to *E*:*Z*-11-14:Ald for 2 or more days showed sensory fatigue by failing to make upwind progress to either septa or target females. At the highest loading (1000 μg), which gave a release rate approximately 10-fold that of the most potent female and generated an atmospheric concentration of about 20 ng/m^3, about half the males that were exposed to the pheromone continuously for 3 h or longer failed to make upwind progress, which is strong evidence of sensory fatigue, presumably habituation. Among the males that were kept in air permeated by pheromone there was no increase over the 4 days of each experiment in the numbers of males failing to make upwind progress, even at the highest concentration. Furthermore, there was no firm evidence of any carry-over effect from day to day among these males which would indicate sensitization or conditioning, such as was recorded by Figueredo and Baker (1992) in oriental fruit moth, *Grapholita molesta* (Busck) (Lepidoptera: Tortricidae), among males that were given a series of exposures to pheromone over a period of several days.

The experiments reported here indicate that exposure of male spruce budworm moths to the synthetic pheromone, *E*:*Z*-11-14:Ald, does cause habituation, and that an atmospheric concentration of about 20 ng/m^3 is necessary for an effective level of disruption. Furthermore, the results show that exposure need occur for only a few hours; prolonged exposure over several days did not appreciably increase the level of habituation. It is important to note that pieces of tape were placed behind each septum to create turbulence. Not only does this simulate conditions in the field, but it may also affect the level of habituation because it has been shown that pulsed exposure to synthetic pheromone is more effective in achieving habituation than constant exposure (Kuenen and Baker 1981), possibly because it avoids adaptation of the receptors.

Conditions in the wind tunnel are only an approximation of those in the field. However, the results from wind tunnel experiments can be used as a guide to the concentrations of pheromone that are necessary to cause effective disruption of male spruce budworm moths in the field. Dispensers that release *E*:*Z*-11-14:Ald at, or slightly above, the release rates from female moths produced a high incidence of false trail following in the wind tunnel. But, because males remain active in the presence of septa loaded with *E*:*Z*-11-14:Ald and continue to search, there is a high probability that they will eventually find a female moth even if they respond later and fly more slowly. Therefore, disruption of the spruce budworm is not likely

to be effective unless the males can be prevented from continuously searching. It is possible that a more complete pheromone blend would make false trail following more effective, but it is probable that it would be inadequate to prevent mating without an additional mechansim such as habituation. The evidence from the wind tunnel experiments described indicates that continuous exposure to a time-averaged concentration of around 20 ng/m^3 is necessary to achieve effective disruption.

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