Use of pheromones and other semiochemicals in integrated production
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Can wind tunnels help improve the efficacy of mating disruption?

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Abstract: Wind tunnels can provide useful information for improving mating disruption in the field. However, certain precautions must be taken. Levels of disruption determined in the tunnel may be an overestimate unless allowances are made for males remaining active for several days and locating females in subsequent flights. On the other hand disruption might be underestimated because many males that locate females in the tunnel may not be able to sustain orientated flight long enough in the field to reach calling females. Levels of disruption for spruce budworm and Oriental fruit moth were similar at similar concentrations of atmospheric pheromone with a significant level at 20-30ng synthetic pheromone/m^3. However, disruption in the spruce budworm was primarily due to males flying to the wrong source, but in the Oriental fruit moth disruption was primarily due to sensory fatigue. This suggests that different mechanisms of disruption will occur for different species and that different formulations may be appropriate for different species.

Key words: mating disruption, wind tunnel, behaviour, spruce budworm, *Choristoneura fumiferana*, Oriental fruit moth, *Grapholita molesta*, Tortricidae, Lepidoptera

Introduction

The year 2000 marks the jubilee, not only of the IOBC-WPRS Working Group "Use of Pheromones and Other Semiochemicals in Integrated Control", but also the discovery in 1975 that the sex pheromone of the spruce budworm, that had been previously identified as E-11-tetradecenal, consisted in fact of a blend, the E-11-tetradecenal plus the isomer, Z-11-tetradecenal, in a ratio of 97E:3Z (Sanders and Weatherston 1976). That discovery marked the start of work on the application of the pheromone in the integrated control of spruce budworm.

Research covered two fronts, the use of pheromone-baited traps for monitoring population densities, and the use of the pheromone for controlling populations by mating disruption. The first application, population monitoring, is now in operational use throughout North America, and recent developments in this program are presented later in this symposium (Lyons et al.). In addition to the trapping program, research was also carried out to assess the potential of controlling budworm populations by using synthetic pheromone components or their analogues for mating disruption.

Small-scale field trials showed promise, and trials were therefore scaled up to the semi-operational stage with the aerial application of pheromone in various formulations. However, these all showed limited success, possibly due to shortcomings in the formulations or to the ever-present possibility of female moth migration into the treated plots. Such field trials are costly, and, because spruce budworm are univoltine, only one trial can be carried out each year, limiting the rate of progress. Therefore attention was turned to the possibility of using a wind tunnel to refine the technology.

The characteristics of the tunnel have been described elsewhere (Sanders et al. 1981). It differs from most other tunnels used in pheromone research in that all the surfaces are glass, for easy cleaning, and the moving background pattern that can be used to prolong moth flight is in the ceiling and not the floor. The standard protocol used in all experiments was to house virgin female moths in small cages at the upwind end of the tunnel and then to release male moths at the downwind end of the tunnel and monitor their responses. With appropriate conditioning at least 90% of the male moths regularly took flight, locked on to the pheromone plume from the females, and flew upwind to the caged females. To assess the impact of synthetic pheromone components and pheromone analogues on male behaviour and their success in locating the female moths, the candidate chemicals were incorporated into rubber septa which were then arranged in a 3-dimensional array surrounding the caged females. Using this technique it was simple to show that pheromone homologues, the 14-C acetates or alcohols which are components of a sibling species, the jack pine budworm, had no effect as disruptants. Furthermore it was established that disruption of the males was greatest with pheromone closest to the natural E:Z ratio of around 95:5 (Sanders 1995). Therefore all subsequent experiments concentrated on this blend.

One of the key questions in field trials, not only for the spruce budworm, but for mating disruption of most pests, is what concentration of pheromone is necessary to achieve a given level of disruption. Accordingly, the candidate chemical that was being tested for disruption was loaded into rubber septa, and these were then pinned to a wire frame in 4 rows. The first and third rows contained 6 evenly spaced septa, the second and fourth had 2 septa positioned in the gaps of the other two rows. Each septum was backed by a 2.5 x 2.5 cm tape to create turbulence. The result was a 3-dimensional array of 16 septa around the caged-females. In order to approximate conditions in the field, where males would be exposed to the ambient pheromone during the day before becoming active in the evening, the male moths were left in the ambient pheromone conditions to acclimatize for several hours. Their behaviour was then compared to that of moths kept in clean air. To achieve this, the tunnel was divided horizontally into two halves, with clean air in the bottom half and the pheromone treatment in the top half. This ensured that the two groups of males were subjected to the same conditions of airflow and light intensity. Each day the male moths for assay were put individually into small cages, and one half were kept in the top half, one in the bottom. They were then moved into position, one at a time, directly down-wind of the caged females and the lid of the cage was removed. In the following presentation the results are summarised under 4 categories: a) flying to the caged females; b) flying to a septum; c) arrestmant (ceasing upwind flight and veering off to the surface of the tunnel); d) inactivity (not taking flight although shown to be capable of it).

Results

As the loading of pheromone in the septa increased so the number of males reaching the females decreased and the number reaching the septa instead increased (Figure 1). An important point to note here is that there was virtually no arrestment of activity among the naïve males (those that had been kept in clean air with no previous experience of pheromone). Among the experienced males (those that had been held for 3 hours in the ambient pheromone) the percentage of males reaching the females or the septa was very similar to that for the naïve males, but a significant number of males became incapacitated, presumably because they were much more active while kept in the ambient pheromone conditions which resulted in damage to their wings. Again, as with the naïve males, there was very little arrestment of activity. This implies very little sensory fatigue (adaptation or habituation). This same pattern was found even when males were held in the ambient pheromone for four days (Sanders 1996).

It can argued that demonstrating disruption with rubber septa in a wind tunnel has little relevance to field conditions where different types of formulation are used. One way of making the data more relevant is to determine the atmospheric concentration of pheromone necessary for disruption. Once the atmospheric concentration necessary for a satisfactory level of disruption has been determined in the tunnel, then formulations can be designed to achieve this concentration in the field. Ideally atmospheric concentrations of pheromone should be determined by direct measurement. However, so far attempts to do this have failed due to lack of sensitivity of the techniques at the lowest concentrations. Possibly this could be achieved by using the technique described by Koch later in this symposium. In the absence of a direct method, estimates of ambient concentration were made by using the formulae of (Butler and McDonough, 1979) and adjusting these for wind speed and the cross section of the tunnel. There is no clear threshold figure above which disruption is successful, however, the calculations suggest that an atmospheric concentration of ca 20 ng/m^3 is necessary to produce a significant amount of disruption (Figure 1).

A weakness of these experiments is that observations on each male were terminated when the males reached a pheromone source or when they stopped flying,

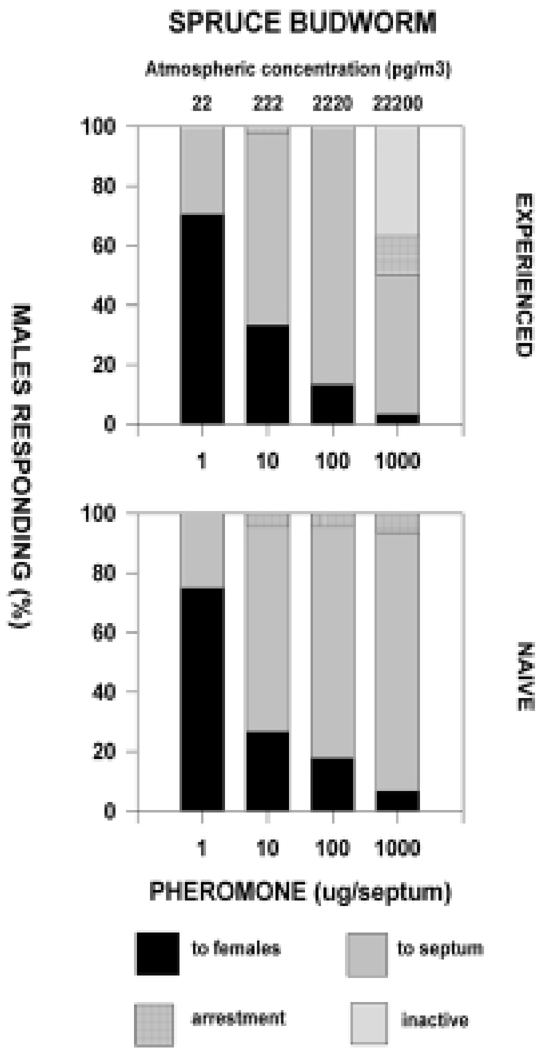

Figure 1. Responses of male spruce budworm to caged virgin females surrounded by septa loaded with synthetic pheromone. From Sanders and Lucuik (1996)

whereas in the field they remain available and may make repeated attempts to locate the pheromone source. The importance of this can be assessed by determining the behaviour of males subsequent to the end of normal observations. Do they remain quiet or do they become active again and resume the search? To answer this the activity of males was recorded over a 24 hour period using a video-recorder. The numbers of re-visits by the males to the pheromone sources was then recorded by replaying the video tapes. The results (Sanders 1995) showed that from groups of 12 males left in the tunnel there were on average over 40 subsequent visits to the caged females with a background of septa loaded with 1µg of synthetic pheromone and 15 with a loading of 10µg.

Another shortcoming of the tunnel is that the distance males had to fly before they reached the females or a septum was less than 2 m and took only a few seconds.

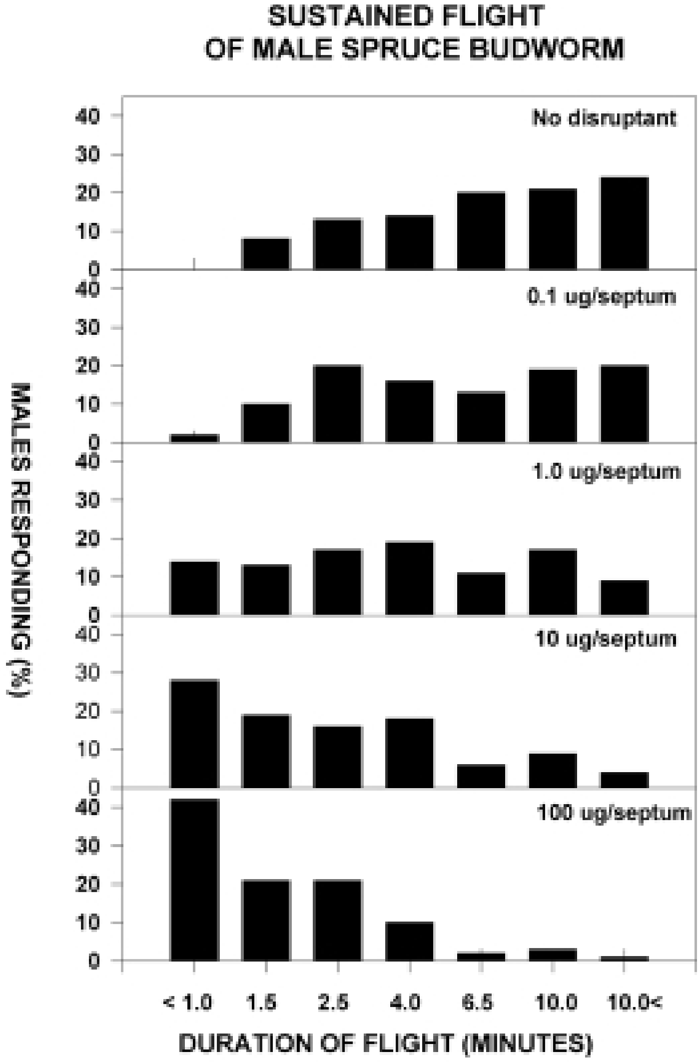

Figure 2. Duration of sustained flights of male spruce budworm to caged females surrounded by septa loaded with synthetic pheromone in a wind tunnel. Flights sustained by a moving ceiling pattern. From Sanders (1998)

In the field they may have to fly many metres, and flights may last for a minute or more. To determine the significance of this, the moving ceiling pattern of the wind tunnel was utilised. Males were released as before in different concentrations of ambient pheromone, but when the moths showed orientated flight (zig-zagging) the ceiling was set in motion and the duration of sustained flight before the moths landed on the tunnel surfaces was recorded.

In clean air (no septa present) males flew for several minutes with many flights longer than 10 min, and one 65 min (Figure 2). As the concentrations of synthetic pheromone released into the air space increased so the duration of flights decreased (Figure 2), i.e. arrestment occurred much sooner. Therefore, in addition to disorienta-

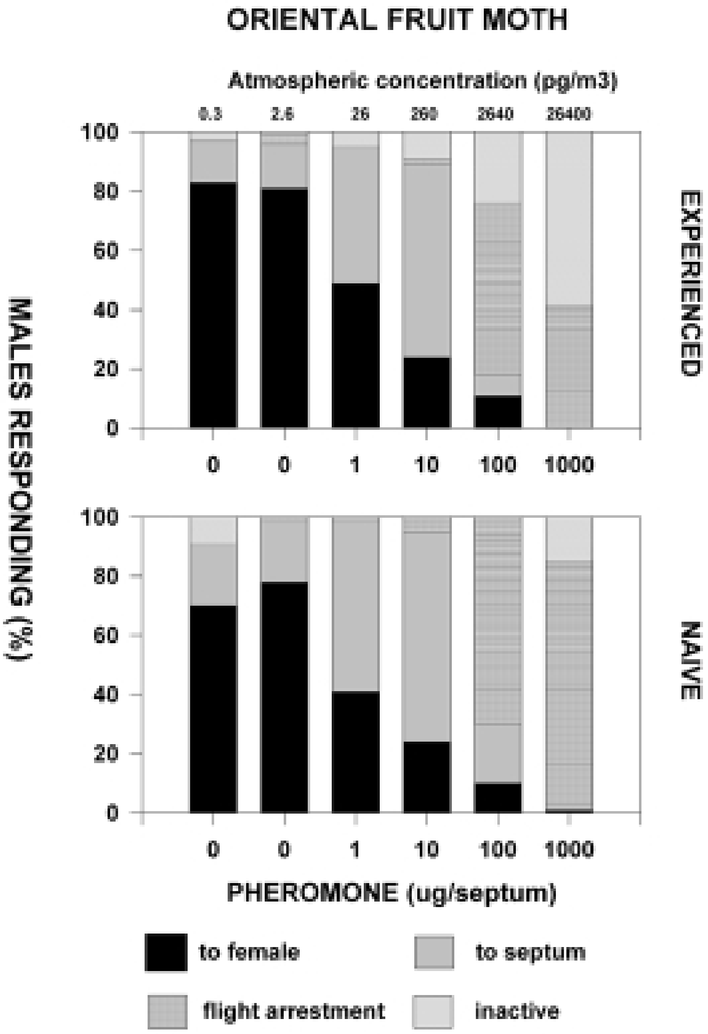

Figure 3. Responses of male Oriental fruit moth to caged virgin females surrounded by septa loaded with synthetic pheromone. From Sanders and Lucuik (1996)

tion, the chances of many males that lock-on and reach the females in the wind tunnel may be reduced under field conditions because of sensory fatigue during the longer flight times.

A further question is how do these results compare to those that might occur with other species. Therefore for comparative purposes, similar experiments were carried out with the Oriental fruit moth. The insects for these experiments were kindly provided by R.M. Trimble, AgCanada, Vineland, ON. They were treated in a similar manner to that described above for the spruce budworm. The results (Figure 3) show a similar level of disruption to that found for the spruce budworm, as measured by the numbers of males successfully locating the caged females. However, when comparisons of the mechanisms of disruption, it can be seen that disruption of Oriental fruit moth was due to much higher levels of inactivity and flight arrestment than were found in the spruce budworm (Figure 4).

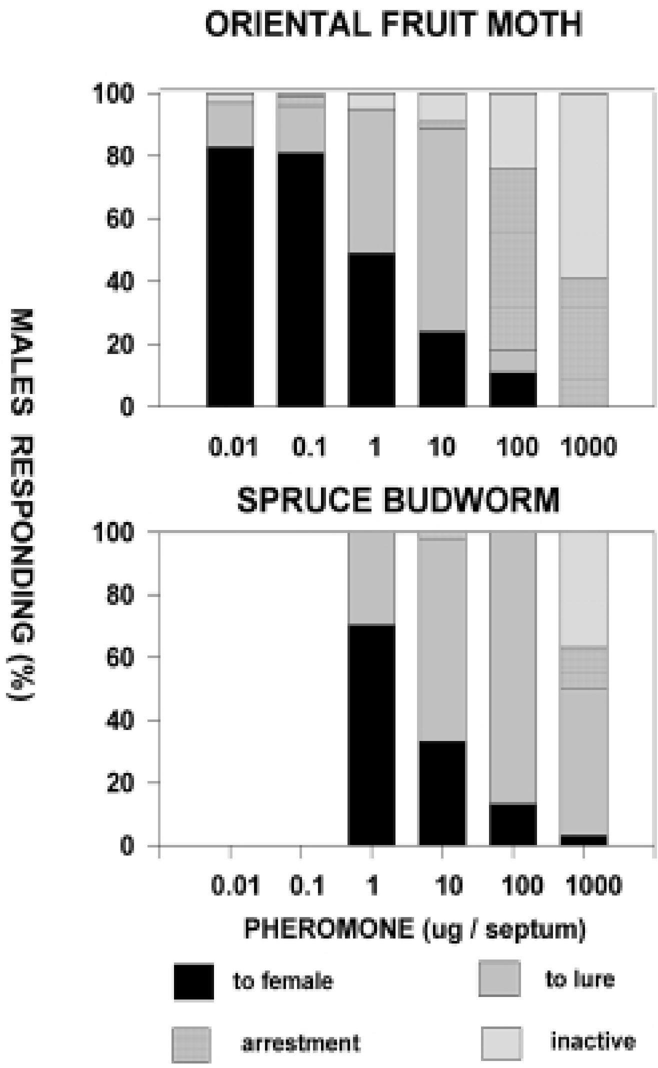

Figure 4. Comparison of male spruce budworm and Oriental fruit moth responses in a wind tunnel, taken from 'experienced' males from Figures 1 and 2

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