T.E. Bakes

Pest Management — Future Challenges

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Widely-spaced, high-emission-rate pheromone sources suppress mating of European corn borer females

Thomas C Baker, Henry Y Fadamiro and Allard A Cossé

Department of Entomology, Iowa State University, 411 Science II Building, Ames, IA 50011, USA

Abstract

The female sex pheromone of *Ostrinia nubilalis* (Hübner) (Lepidoptera: Crambidae) was released from two dispenser types and in two deployment patterns, a Shin-Etsu rope formulation spaced 2 m apart and a widely-spaced (35 m) pattern using a high-emission-rate system called the Metered Semiochemical Timed Release System (MSTRS™). Both dispensers were deployed in grassy sites that constitute aggregation areas for *O. nubilalis* mating activity within and around cornfields at three different locations in Iowa. A significant level of disruption of pheromone-source location (averaging 97 percent) was achieved by both dispensers during both first and second flights. More importantly, a significant level of mating disruption was achieved, as measured by the frequency of mating by free-flying feral females. The mean number of matings, as measured by spermatophores, per first generation female was 1.33 in the MSTRS™ plots and 1.58 in the rope plots, compared with 1.88 in untreated check plots. During the second flight, the number of matings per female averaged 1.63 in the MSTRS™ plots, 1.56 in the rope plots and 2.17 in untreated check plots. There was also a significant reduction in the proportion of females that mated at least once during both flights in MSTRS™ plots. During the first flight, 17 percent and 10 percent fewer females mated in the MSTRS™-treated and rope-treated fields, respectively. A similar level of disruption of mating was also achieved during the second flight. An overlooked, potential advantage of the area-wide use of mating in the context of increasing use of transgenic corn by growers is that pheromone disruption should differentially reduce mating of any rare, low density, Bt-resistant individuals in grassy areas compared with that of susceptible individuals emerging at higher densities from non-Bt corn fields. Indeed, the delaying of the onset of resistance by using pheromone mating disruption where possible in all transgenic crops may be a general integrated pest management benefit beyond crop damage reduction.

Introduction

Pheromone mating disruption of Lepidopteran pest species, an idea first commercially realised in 1978, is continuing to be investigated in the 1990s, with increasing degrees of success (Witzgall and Arn 1997). One key factor determining the success of this novel technique is the availability of a satisfactory dispenser system (Witzgall and Arn 1997). In addition to protecting the active ingredients from degradation, a satisfactory pheromone dispenser should be a temperature-independent and economically efficient release system that emits sufficient amounts of pheromone only when needed (Ogawa 1997). Mafra-Neto and Baker (1996) and Baker *et al.* (1997b) described such a controlled release dispenser system, the Metered Semiochemical Timed Release System (MSTRS™). Apart from providing high pheromone-emission-rates, the MSTRS™ dispenser technology allows control over the timing and the frequency of pheromone release. Due to sufficient pheromone release, the MSTRS™ technology allows the use of only a few widely-spaced dispensers per acre, deployed in the action sites of the pest species. Therefore, problems associated with the deployment and retrieval of traditional dispensers, as common with formulations such as the microcapsules and ropes (Weatherston 1990) are averted.

The European corn borer, *Ostrinia nubilalis* (Hübner), is one of the most important major pests of corn, *Zea mays* L., in the United States. In Iowa alone, annual crop losses approaching $100 million have been attributed to this pest (Steffey 1995). However, *O.*

nubilalis is considered the most underscouted and undertreated insect pest of corn (Steffey 1995). Methods available for its control, including the use of chemical and microbial insecticides, have not proved very satisfactory (Showers *et al.* 1989; Steffey 1995). Basic sex pheromone research on this species is amongst the most complete of any moth species (Klun *et al.* 1973; Roelofs *et al.* 1985). However, virtually no research has been conducted on the possible use of sex pheromone to control the European corn borer.

In 1996 we commenced a study on the pheromone mating disruption of *O. nubilalis* (Baker *et al.* 1997a). We tested the efficacy of two pheromone dispensers, the Shin Etsu ropes (Pacific Biocontrol, Ltd.) and the MSTRS™ a controlled release system (Mafra-Neto and Baker 1996). The dispensers were deployed in the grassy areas within and adjacent to cornfields in Iowa. These grassy areas serve as aggregation and mating sites for adult moths, out of which mated females fly to nearby cornfields to lay eggs at dusk (Showers *et al.* 1980; Sappington and Showers 1983). Showers *et al.* (1980) sprayed these grassy sites with an insecticide, carbaryl, and recorded a 94 percent reduction in egg mass deposition, culminating in an overall 18 percent increase in yield. The results of Showers *et al.* (1980) showed that the biology and behaviour of European corn borer therefore may make mating disruption in these grassy areas a potentially suitable control technique for this species. We reasoned that if, in these small-plot disruption experiments, we could demonstrate that we can reduce the ability of females to mate in these grassy areas, then we would have a chance at reducing egg laying and damage in nearby cornfields. If effective, this technology may prove cost-effective, because only the grassy areas, which constitute about 5-15 percent of the total corn area protected, might need to be treated.

Our first-year (1996) results indicate that we could disrupt pheromone trap location by males in small 0.4-ha grassy areas (Baker *et al.* 1997a). This second year study was to test whether our pheromone treatments could actually prevent feral females from mating in treated grassy areas with the dispensers deployed on a larger scale.

Materials and methods

Insects and pheromone formulations

The sex pheromone of the Iowa strain of European corn borer consists of a blend of (*Z*)-11-tetradecenyl acetate and (*E*)-11-tetradecenyl acetate in a ratio of 97:3 (Klun *et al.* 1973). Two pheromone dispensers, the Shin Etsu ropes (Pacific Biocontrol Ltd.) and the MSTRS™ were employed in this study. The Shin-Etsu 'Hamaki-con' formulation ropes, each containing 80 mg of a 95:5 ratio of (*Z*)-11-tetradecenyl acetate and (*E*)-11-tetradecenyl acetate (the pheromone component ratio of the lesser tea tortrix, because the corn borer pheromone was unavailable in the rope formulation) were deployed in grassy areas within cornfields spaced 2 m apart (a density of 3000/ha). The machine portion of the MSTRS™ (Waterbury Co., Waterbury, CT) used in this study was a modified and updated version of the one described by Mafra-Neto and Baker (1996). Briefly, the system consisted of a spray-bottle reservoir, a non-aerosol pump spray dispenser unit, and a timer mechanism to activate the spray discharge mechanism. To this machine was added a pad to capture the spray and release the pheromone (Mafra-Neto and Baker 1996). Pads were formed by 0.5 cm thick circular acrylic padding stretched on a needle-point hoop (17.8 cm ID) and held in place 3 cm from the spray nozzle. The pump spray bottle contained a solution (max 300 ml) with the desired concentration of pheromone in an ethanol solution. A valve on top of the bottle delivered 50 µl of material per spray, and the bottle was housed in the spray dispenser unit with the valve positioned under a lever controlled by a battery-powered timer mechanism. The timer mechanism pressed the valve, which could be set at any 5 min time interval, delivering a

replenishing spray onto the pad. The MSTRS™ devices could produce 6,000 recharging sprays of similar strength. The spray time interval, ranging between 5 and 25 min, could be varied and in addition, the timer mechanism allowed us to program the MSTRS™ to spray pheromone only during a particular time of day, such as during the moths' active period. Therefore, we could prevent pheromone from being wasted by sprays being discharged during the daytime when *O. nubilalis* is not sexually active.

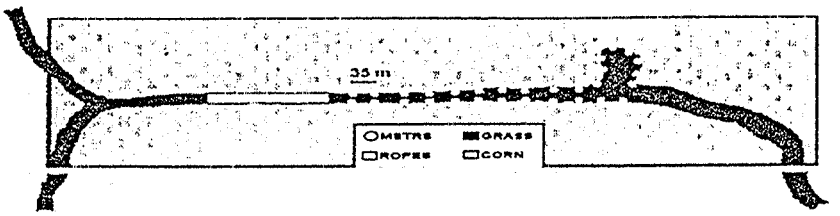
All machines contained pump spray bottles filled with 250 ml of a solution of 100 percent ethanol containing 2.74 g of a blend of Z11-14:Ac and E11-14:Ac in a ratio of 97.2/2.8 Z/E. (Bedoukian Research Inc., Connecticut). The MSTRS™ machines were activated a few days prior to the emergence of first and second generation adult corn borers (as determined by pheromone traps, sweep samples, and black-light traps). In order to achieve an early high release rate from the MSTRS™ spray pads, the pads were primed with 0.5 g of pheromone at the time of deployment. Release rates of Z11-14:Ac from MSTRS™ pads, as well as from the rope formulation after different days of emission in the field were measured (Fadamiro et al., unpublished data). After seven days in the field, release rate from the MSTRS™ device was ca. 26 times (6.09 (g/min) higher than the release rate from ropes (0.23 (g/min). The MSTRS™ devices were programmed during the first flight to emit puffs of pheromone every 25 min for 12 hours on a given 24 h day, between the hours of 6 pm. and 6 am. Timing of operation of the MSTRS™ was thereby set to correspond to the time of activity of adult *O. nubilalis* (Showers *et al.* 1976). During the second flight, a shorter span of daily emission but with higher emission frequency per hour was utilised by setting the MSTRS™ to emit every 5 min between 8 pm and 2 am. Under the first regime, the MSTRS™ longevity would have been 230 days, and using the second regime would have been 83 days.

Field locations and experimental design

Experiments, which were designed in 3 x 3 model, were conducted at three locations in Iowa. In Iowa, grassy areas typically occur along fencerows, waterways and soil conservation lanes within and around cornfields borders. The dimensions of these grassy areas, which are comprised of various grass types including brome grass and foxtail grass, ranged from narrow, long islands of grass of about 10 m in width to almost rectangular. Three grassy areas ca. 1-3 ha adjacent to cornfields were selected in each of three locations. One plot at each location was used for the MSTRS™ and rope treatment, respectively, and the third served as the check plot. In each location, the check plot was located at least 3 km away from the treated plots to minimise potential drift effects from the pheromone plots. The rope and MSTRS™ were either contiguous or located no more than 1 km from each other. Pheromone dispensers (ropes or MSTRS™) were deployed in these grassy areas that both bordered or were insinuated within cornfields. In all locations, MSTRS™ deployed at a density of 12/ha (every 35 m) were attached on stakes at a height of 1 m above the ground. This height was increased during the second flight to about 1.5m to account for increase in grass canopy height. Ropes were deployed at a density of 3000/ha (ca. every 2 m) by twisting and tying a rope around the top of a bunch of grass.

At the first location, ropes were deployed on a total of about 0.5 ha of grass within a ~32 ha cornfield (Fig. 1). The MSTRS™ devices were deployed on ca. 2 ha on the same long strip of grass and contiguous with the rope treatment. Twenty-nine MSTRS™ were placed in this area (Fig. 1). The check plot was a grassy area of about 0.8 ha that was situated about 4 km west of the treated plots.

Figure 1. Patterns of deployment of pheromone dispensers in treated fields at Location 1.



The topography of the MSTRS™-treated plot at location 2 was different in that a creek ran through the middle section of the 2.5 ha grassy area site where 28 MSTRS™ devices were deployed. Ropes were deployed in a total of about 0.8 ha of grassy terraces situated approximately 200 m north of the MSTRS™ plot. Location 2 check plot was situated about 3 km away from the nearest treated plot and had a grassy area of approximately 0.8 ha.
At location 3, 29 MSTRS™ were deployed every 35 m in the grassy border (10 m wide) surrounding a 4.6-ha cornfield. The field was intersected by three long narrow grassy terraces (ca. 10 m wide), and 3 MSTRS™ were placed on the terraces themselves (1 per terrace). The grassy areas around and within comprised ca. 2 ha of grass, total. The rope-treated plot was located about 3 km east of the MSTRS™ plot. Approximately 0.4 ha of grassy island within a 20 ha seed corn field was treated with about 1200 ropes. A ca. 0.4 ha of grass in a cornfield located about 3 km away from the nearest treated field served as the check plot for this third location.

Assessment of mating disruption
Mating disruption was assessed by using two parameters: numbers of males captured in wing traps, and mating status and mating frequency of free-flying females captured in treated versus untreated fields. Two wing traps (IPM Technologies, Portland, OR) each baited with a rubber septum loaded with 100 µg of the 97.2:2.8 Z/E pheromone blend used in the MSTRS™ were deployed in each treated plot, as well as in the check plots. Traps were deployed in the middle section of each field. The distance between two traps was at least 200 m, and a trap was located at least 40 m away from the nearest MSTRS™. Traps were attached to stakes at the level of the grass canopy (Mason et al. 1997). Trap catches were counted every two to three days, and traps and bottoms were replaced as necessary.
After checking for male trap catch on a given sampling-day, workers captured free-flying *O. nubilalis* females by walking through the grass and capturing with a net any flying females that were disturbed. Collection of females was done for a period of 15 min per plot every two days or so. Throughout the study the same two student workers carried out moth collections and other field observations. In order to minimise the sampling of females possibly migrating between fields, moth collection was done near the middle grassy field. Females were preserved in appropriately labelled glass vials containing 70 percent ethanol, and were later dissected under the microscope at 10x, examining the bursa copulatrices for presence and number of spermatophores. Timing of daily visits to each field was randomised such that no particular field was consistently visited during a particular time of day.

Statistical analysis
Data obtained from all evaluations were transformed using the square root method and analysed with a two-way ANOVA (SAS 1985) testing for the effect of treatment, location (each location was considered a replicate of each treatment), and any interactions. Means were compared by using the LSD test (SAS 1985). Mean trap catch on each sampling date

was calculated for each location and these means were used to calculate a treatment mean for each date, considering each location as a replicate. Means calculated for each date were then used to calculate the season-long mean trap catch for each treatment during the first and second flights.

Data collected on mating status of females for each sampling date were presented as percentages and analysed by considering each location as a replicate. Similarly, the data on number of spermatophores for each sampling date were analysed by considering each location as a replicate. A first-flight and second-flight season-long average for percentage of mated females was calculated for each location by considering the number of mated females as a proportion of the total number of females collected during an entire flight period. Means of three locations (replicates) were then calculated for each treatment. A similar approach was also used to calculate for each treatment a first-flight and second-flight season-long average mean spermatophore count.

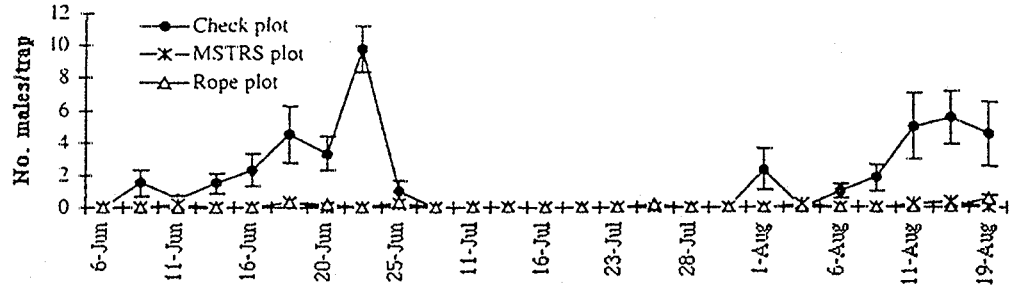
Results and discussion

A significant disruption of pheromone source location was achieved by both dispensers at all three locations, during the first ($p = 0.001$) and second flight ($p = 0.005$) of adults (Table 1, Fig. 2). Disruption of pheromone source location in the MSTRS™ plots averaged 97.3 percent and 96.7 percent, during the first and second flights, respectively. This level of disruption was not different from that achieved in the rope-treated plots, averaging 96.6 percent during the first flight and 96.7 percent during the second flight. Levels of disruption of trap catch were not significantly different ($p > 0.05$) among the locations. The degree of disruption of pheromone source location achieved in the current study was similar to that recorded during our 1996 study (Baker *et al.* 1997a).

Table 1: Seasonal number of European corn borer males captured per trap (mean ± SE) and percentage disruption of pheromone source location during first and second flights in pheromone-treated and untreated plots. In this and Table 2, means from the same flight having no letters in common are significantly different at $p < 0.05$.

	Check plot	MSRTR™ plot	Rope plot
1st flight (6 June - 27 June 1997) (% disruption)	2.45 ±0.931a	0.067±0.04 b (97.3%)	0.083±0.04b (96.6%)
2nd flight (21 Jul - 19 Aug 1997) (% disruption)	1.68±0.62 a	0.05±0.03 b (96.7%)	0.05 (96.7%)

Figure 2. Mean number (± SE, n = 3 per plot) of male European corn borer captured in wing traps deployed in pheromone-treated and check plots. Traps were baited with 100 µg of synthetic pheromone.

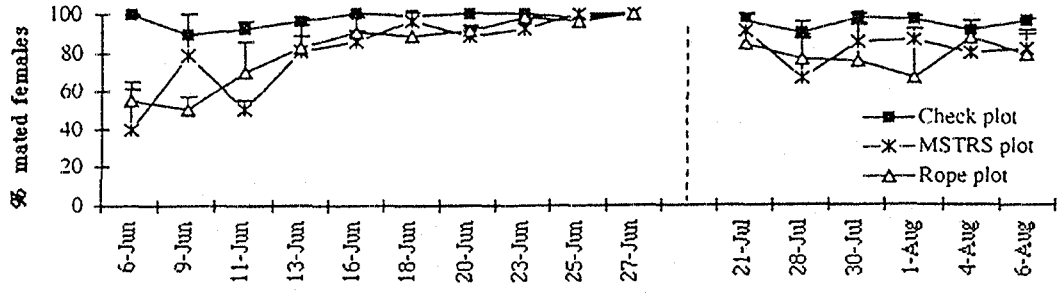


A significant reduction in the percentage of females that had mated was recorded for females captured in the MSTRS™ plots during the first flight ($p = 0.03$; Table 2). No significant effect of location was recorded ($p > 0.05$). While approximately 97 percent of females captured in the untreated check plots during the first flight had mated at least once, about 20 percent of females captured in the MSTRS™ plots and 13 percent of females in the ropes plots remained unmated throughout the first flight season. The same trends of reduction in the percentage of mated females in pheromone-treated fields were recorded during the second flight, although these were not significant ($p > 0.05$; Table 2). Analysis of the data collected on a day-by-day basis shows that a very high (up to 50 percent) level of mating disruption was achieved in pheromone-treated fields during the beginning of the first flight. However, as the season progressed, the proportion of mated females in these treated plots increased (Fig. 3). Not surprisingly, the increase in the proportion of mated females recorded as the season progressed coincided with increase in field populations during this period (Fig. 3). There was not as much evidence for delayed mating in females captured in disruptant-treated fields during the second flight and no significant differences in the proportion of unmated females were recorded. A ca. 10-15 percent reduction in the proportion of mated females in the treated fields was recorded at the beginning of the second flight, and this level of disruption was maintained throughout the season (Fig. 3).

Table 2: Percentage (mean ± SE) of free-flying feral female European corn borer that were captured and found to be mated and the mean number (±SE) of spermatophores per female during the first and second flights in pheromone disruption and untreated plots.

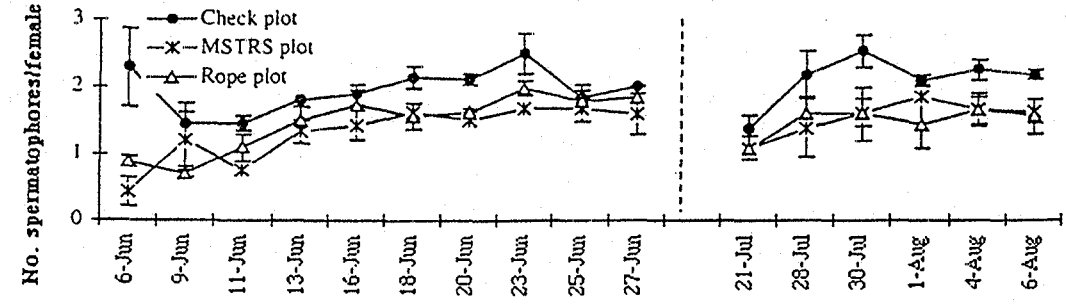
	Disruption parameter	Check plot	MSTRS plot	Rope plot
1st flight (6 Jun - 27 Jun 1997)	No. females	327	316	348
	% mated	97.10±0.98 a	80.27±5.11 b	86.77±3.53 ab
	No. spermatophores	1.88±0.03 a	1.33±0.09 c	1.58±0.08 b
2nd flight (21 Jul - 19 Aug 1997)	No. females	274	264	193
	% mated	95.77±1.13 a	81.17±9.66 a	78.37±10.93 a
	No. spermatophores	2.17±0.06 a	1.63±0.17 b	1.56±0.25 b

Figure 3. Mean percentage (±SE) of feral female European corn borer that were captured and found to be mated in pheromone-treated and check plots. Broken lines represent the inter-flight period.



In addition to the approximately 10-20 percent reduction in the proportion of mated females in both MSTRS™ and rope plots recorded during this study, significant reductions in the frequency of mating (number of spermatophores) per female were also recorded for first- ($p = 0.005$) and second- ($p = 0.03$) generation *O. nubilalis* females captured in pheromone-treated fields (Table 2). Mating frequency was reduced early in disruptant plots in the flights and seemed to stay reduced throughout the flights (Fig. 4). On average, each female captured in untreated check plots during the entire study had mated twice (Table 2). This was significantly higher than the 1.5 and 1.57 average number of matings recorded in the MSTRS™ and ropes fields, respectively. The mean number of spermatophores recorded per first generation female captured in MSTRS™ fields was 1.33 and this was significantly lower than the mean per first generation female captured in rope-treated fields (Table 2). No significant difference in the percentage of mated first- and second-generation females was recorded among the three locations ($p > 0.05$). However, a significant effect of location was recorded for the mean number of spermatophores per second-generation female ($p = 0.05$). A close look at the data suggests that both dispensers seemed to fare better in the first location than in the other two, especially during the second flight. The reason for this better performance may be due to the unique landscape of the pheromone in this location. Also, this is the one location in which both types of dispensers were deployed next to each other along the same long strip of grass (Fig. 1). It is interesting to note that both the rope and MSTRS™ dispensers achieved similar efficacy in the current study, despite the subtle differences in the ratio of the Z/E isomers in both dispensers.

Figure 4. Mean number (±SE) of spermatophores per female European corn borer captured in pheromone-treated and check plots. Broken lines represent the inter-flight period.



It remains to be seen whether the significant, but modest reduction in mating frequency of females in pheromone-treated plots in the current study could translate into significant reduction in oviposition of *O. nubilalis* and reduced damage. Studies in our laboratory have recorded a higher reproductive output (fecundity and fertility) for multiple-mated female European corn borer, compared with single-mated individuals (Fadamiro and Baker, in press). Also, the ~ 50 percent reduction in the number of mated females recorded during the first week of the first flight may lead to a further reduction in the size of progeny produced by females in the pheromone-treated plots. Data from another experiment suggest that a week delay in first mating may lead to a near zeroing of fecundity and fertility of female *O. nubilalis* (Fadamiro and Baker, in press). Delayed mating, rather than prevention of mating, has been cited as potentially being responsible for the success of codling moth area-wide mating disruption in reducing oviposition and damage in the Pacific Northwest (Knight 1997). The author had recorded a 40 percent reduction in fecundity for *Cydia pomonella* females that experienced a two-day delay in mating.

The level of mating disruption achieved in the current study is encouraging since it was achieved in small plots requiring only short-distances for mated females to move from the untreated to treated portions of the grass, especially within the strips that were contiguous with the treated portion (Fig. 1). We propose that the likelihood of migration of females from untreated fields adjacent to and usually contiguous with pheromone-treated fields will have made the level of mating disruption appear to be lower than what was actually being achieved. An unknown proportion of the females net-captured in the treated fields will have migrated in from nearby untreated grassy strips.

There is very little data for any species that relates actual mating frequency reduction for free-flying females to subsequent crop damage or population reduction. In one of the only other studies to examine the mating frequencies of feral, free-flying female moths in disruption plots, Rice and Kirsch (1990) showed that very modest reductions in the percentage of *Grapholita molesta* females that mate in disruption plots translate into some of the most successful and reliable disruption seen in any species (Rice and Kirsch 1990; Barnes and Blomefield 1997).

One key consideration when using widely-spaced, strong sources is that the geometry of deployment of such a low number of devices per hectare is important. It must be considered that the smaller the plot, the greater the amount of edge there is to protect relative to the interior area of crop. In principle, the strong, widely spaced dispenser technologies should work better over a very large, regularly shaped area where there will be fewer pheromone-plume-free holes along the edges. Thus, when smaller plots are used having a much higher edge-to-area ratio, a greater number of high-emission rate dispensers should be used to fill holes that will occur along the edges. It is likely that for hectarages that are very small, the traditional low-emission-rate closely spaced arrays of dispensers will be the optimal choice. There should be an 'angular advantage' gained by using strong, widely spaced point sources because a small angular shift in wind direction will translate far downwind into extremely large linear increases in coverage of the crop with active ingredient compared to low-emission-rate sources, whose effects will be more local. However, when the plumes need to sweep for perhaps hundreds of meters horizontally over the crop canopy to both attract and habituate males sufficiently so that they are prevented from mating, high-emission-rate sources will be more dependent upon ambient meteorological conditions than will be numerous lower-emission-rate point sources spaced only metres apart throughout the crop. Thus, the angular advantage that occurs for horizontal wind shifts becomes an angular

disadvantage when the vertical movement of air is considered. This disadvantage may be worst when trying to disrupt species that mate during the daytime, when adiabatic lapse rates are highest and unstable, rising air can potentially carry plumes from disruptant dispensers up and away from the crop canopy.

Use of the correct pheromone blend should be even more important with widely spaced, high-emission-rate dispensers, which will more likely depend on the attraction of males from far downwind in order to also habituate them optimally. Another consideration will be whether the plumes need to travel long distances below canopy level (as in orchards) compared to above the canopy, as in field crops. The within-canopy movement could dissipate the plume strength through adsorption on the foliage as well as by shearing the originally emitted pheromone strands into finer, less-concentrated strands.

If pheromone mating disruption involving traditional technologies as well as MSTRS™ or puffers or even other new types of high-emission rate, widely space dispensers is to continue to gain increased usage as an IPM tactic, it is essential that an assessment of their efficacy include actually measuring the mating frequency of freely flying females in addition to the amount of egg-mass deposition or crop damage assessments. It also will be crucial to account for other factors affecting our ability to assess efficacy, such as whether area-wide or smaller-plot, local applications were used.

With the increasing use of transgenic, 'Bt' corn in the Midwestern United States, there are opportunities for pheromone mating disruption to play an important role in *O. nubilalis* pest management. Area-wide use of widely spaced retrievable mating disruptant dispensers such as MSTRS™ could provide a way to not only suppress populations of European corn borer on non-Bt corn hectarage, but could help preserve susceptibility of corn borers to Bt corn by reducing mating between any resistant adults that may emerge from Bt corn. Concurrent use of mating disruption therefore could complement Bt corn plantings by slowing the development of resistance, thereby providing an extra advantage to growers and industry alike. If significant reduction of mating can be achieved in grassy areas where both susceptible and resistant adults may aggregate, as should be the case especially, among the rare resistant individuals, then benefits beyond crop damage reduction in resistance management may be significant. Indeed, in other transgenic crops such as cotton, pheromone mating disruption should be considered as a possible co-treatment for slowing the onset of resistance.

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