

## Mating Disruption of European Corn Borer, *Ostrinia nubilalis* by Using Two Types of Sex Pheromone Dispensers Deployed in Grassy Aggregation Sites in Iowa Cornfields

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**Abstract** – We investigated the potential of disrupting pheromone-mediated mating communication in European corn borer, *Ostrinia nubilalis* (Hübner). The female sex pheromone, a blend of (*Z*)-11-tetradecenyl acetate and (*E*)-11-tetradecenyl acetate in a ratio of ~97:3 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 controlled release system called the Metered Semiochemical Timed Release System (MSTRS™). Both dispensers were situated in grassy sites that constitute aggregation areas for *O. nubilalis* mating activity within and around cornfields at three different locations in Iowa. Pheromone-emission rate (after 7 days in the field) from the MSTRS™ (6.09 µg/min) was ca. 26 times greater than from the rope formulation (0.23 µg/min). Both dispensers during both first and second flights achieved a significant level of disruption of pheromone-source location (averaging 97%). 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 and 10% fewer females mated in the MSTRS™-treated and rope-treated fields, respectively. A similar level of disruption was also achieved during the second flight.

**Key Words** – *Ostrinia nubilalis*, European corn borer, Sex pheromone, Mating disruption, Controlled release dispensers

### Introduction

The use of sex pheromones to disrupt mating behavior of lepidopteran pest species, an idea first commercially realized in 1978, is continuing to be investigated in the 1990's, with increasing degrees of success (Witzgall and Arn, 1997). The majority of the currently available pheromone dispensers for mating disruption, including capillary tube evapora-

tors, microcapsules and ropes, however, release pheromone molecules passively and do not allow the user to control the duration and quantity of release (Weatherston, 1990). 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) described an efficient

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dispenser system that allows control over the timing and the amount of pheromone release. This controlled-release dispenser, the Metered Semiochemical Timed Release System (MSTRS™), is similar to the 'puffers' described by Shorey and Gerber (1996a, b). Both dispensers have shown considerable potential for pheromone-mating disruption of certain pest species (Mafra-Neto and Baker, 1996; Shorey and Gerber, 1996a, b; Baker *et al.*, 1997a, b; Fadamiro and Baker, 1999).

The European corn borer, *Ostrinia nubilalis* (Hübner), is generally regarded as 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). This reason and the needs for reduced insecticide use have necessitated research into alternative and environmentally safe pest control. As far as we know, no published reports by another group exist on the use of pheromone to disrupt mating of *O. nubilalis*, despite basic pheromone research findings on this species that are among the most complete of any moth species (Roelofs *et al.*, 1985, 1987).

In this paper, we report the second year results of a study that commenced in 1996 (Baker *et al.*, 1997a) on the mating disruption of *O. nubilalis* using its sex pheromone. 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% reduction in egg mass deposition, culminating in an overall 18% increase in yield. The results of Showers *et al.* (1980) showed that the biology and behavior of the 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% 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). The objective of this second year study was to test if we could actually prevent feral females from mating in treated grassy areas 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 (*Z*11-14:Ac) and (*E*)-11-tetradecenyl acetate (*E*11-14:Ac) 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. There was a minor difference in the ratio of the *Z/E* isomers of 11-tetradecenyl acetate in the two dispensers. Because the corn borer pheromone component ratio was unavailable in the ropes formulation, the Shin Etsu "Hamaki-con" ropes utilized in this study contained a 95:5 ratio of *Z*11-14:Ac and *E*11-14:Ac acetate (the pheromone component ratio of the lesser tea tortrix). The MSTRS™ formulation contained a 97.2:2.8 ratio of (*Z*)-11-tetradecenyl acetate and *E*11-14:Ac, similar to the ratio produced by female European corn borer. The ropes formulation, each containing 80-mg of pheromone 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  $\mu$ l 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 six thousand 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% 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.

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 p.m. and 6 a.m. 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 utilized by setting the MSTRS™ to

emit every 5 min between 8 p.m. and 2 a.m. Under the first regime, the MSTRS™ longevity would have been 230 days, and using the second regime would have been 83 days.

### Measurement of pheromone release rates from dispensers

Release rates of Z11-14:Ac from MSTRS™ pads after different days of emission in the field were measured by placing a circular cutout of the pad, contained within a frame (9.2 cm ID), on top of a glass funnel (10 cm ID). The stem of the funnel was connected with a Teflon connecting tube to a glass Pasteur pipette containing a 7-cm-long plug of packed glass wool. Air was drawn across the pad (2000 ml/min) and through the glass wool trap from the tip of the Pasteur pipette by means of a vacuum. After a 15-min collection time the vacuum was stopped, the pad removed from the funnel, and 100  $\mu$ g (1 mg/ml) of internal standard (Z11-tridecenyl acetate) was added to the glass wool. Funnel wall and glass wool plug were washed with 3 ml of redistilled HPLC-grade hexane. One microliter of this solution was analyzed for the amount of pheromone relative to the internal standard by capillary gas chromatography-mass spectrometry (GC-MS). A similar setup was used for Z11-14:Ac collection from Shin-Etsu ropes. Individual ropes were suspended in the glass funnel by means of a small hook attached to a pheromone-free acrylic pad.

Care was taken that neither pheromone pads nor ropes came in contact with the glass funnel surface. Trap breakthrough was checked and confirmed negative by analyzing collected material in a second, in-series-connected Pasteur pipette. Pad and rope collections were preformed in triplicate and collected amounts of Z11-14:Ac from the pheromone containing pads were calculated for the original pad diameter. All GC-MS analyses were performed by using a Hewlett-Packard 5890 GC with a direct interface to a Hewlett-Packard 5972 mass selective detector (30-m DB-225 capillary column, electron impact, 70 eV).

### Field locations and experimental design

Experiments, which were designed in 3  $\times$  3 model were conducted at three locations near the towns of Gilbert, Napier and Nevada 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 width to almost rectangular. Three grassy areas *ca.* 1–3 ha adjacent to cornfields were selected in each location. 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 minimize 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 are 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 1.5 m to account for increase in grass canopy height. Ropes were deployed at a density of 3000/hectare (*ca.* every 2 m) by twisting and tying a rope around the top of a bunch of grass.

At the Gilbert 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 Gilbert check plot was a grassy area of about 0.8

hectares that was situated about 4 km west of the treated plots.

The topography of the MSTRS™-treated plot in Napier 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 hectares of grassy terraces situated approximately 200 m north of the MSTRS™ plot. The Napier check plot was situated about 3 km away from the nearest treated plot and had a grassy area of approximately 0.8 hectares.

In Nevada, 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 hectare of grass in a cornfield located about 3 km away from the nearest treated field served as the check plot for the Nevada site.

#### 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 Intercept W 'wing' traps (IPM Technologies, Inc., Portland, OR) each baited with a rubber septum loaded with 100 µg of the 97.2 : 2.8

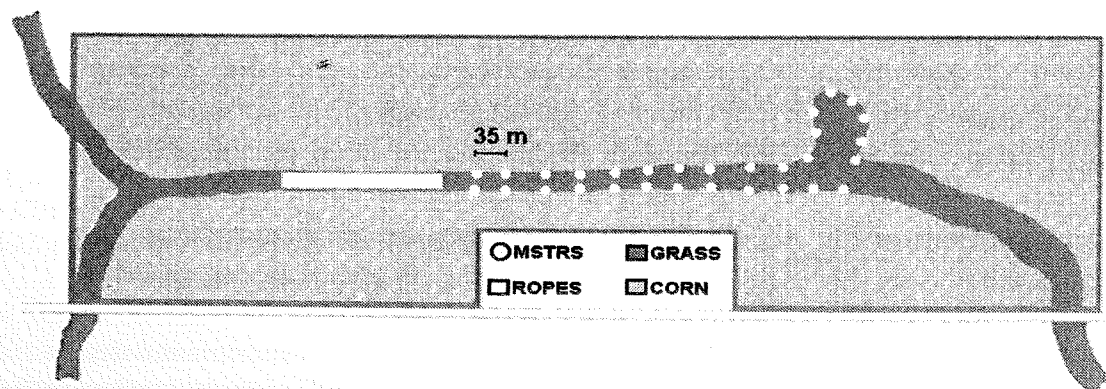


Fig. 1. Patterns of deployment of pheromone dispensers in treated fields in Gilbert, Iowa. MSTRS™ and rope dispensers were deployed along the edges of the grassy areas bordering and adjacent to cornfields.

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 2 to 3 days, and traps and bottoms were replaced as necessary. Pheromone lures were replaced weekly.

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 2 to 3 days. At the same time, the number of flying males was noted during the 15-min period. This was possible since *O. nubilalis* exhibits a remarkable degree of sexual dimorphism in which males have a much darker wing pigmentation. Throughout the study the same two student workers carried out moth collections and other field observations. In order to minimize the sampling of females possibly migrating between fields, moth collection was done near the middle grassy field. Females were preserved in appropriately labeled glass vials containing 70% ethanol, and were later dissected under the microscope at 10x, examining the bursa copulatrix for presence and number of spermatophores. Timing of daily visits to each field was randomized 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 analyzed with a two-way ANOVA (SAS 1985) with three locations (blocks) as replicates. 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 (block) 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 analyzed by considering each location as a replicate. Similarly, the data on number of spermatophores for each sampling date were analyzed 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

Both types of dispensers achieved significant disruption of pheromone source location in all three locations, during the first ( $P = 0.001$ ) and second flight ( $P = 0.005$ ) of adults (Fig. 2). Disruption of pheromone source location in the MSTRS™ plots averaged 97.3% and 96.7%, 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% during the first flight and 96.7% during the second flight (Table 1). 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).

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). The proportions of mated females were not significantly different ( $P > 0.05$ ) among the three locations. While approximately 97% of females captured in the untreated check plots during the first flight had mated at least once, about 20% of females captured in the MSTRS™ plots and 13% 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

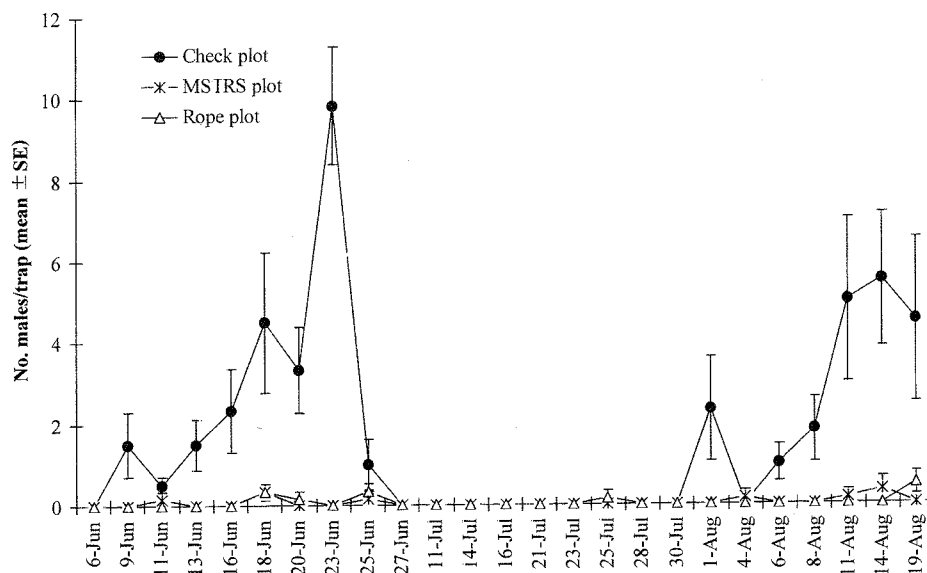


Fig. 2. Capture of male *Ostrinia nubilalis* in wing traps baited with 100 µg of synthetic sex pheromone. Traps were placed in grassy sites in which either MSTRS™ or rope pheromone dispensers were deployed. Values are means ( $\pm$ SE) of 3 locations per treatment.

Table 1. Seasonal number of *Ostrinia nubilalis* males captured per trap (mean  $\pm$  SE) and percentage disruption of pheromone source location during first and second flights in pheromone-treated and untreated plots in 1997

	Check plot	MSTRSTM plot	Rope plot	P
1st flight (6 Jun - 27 Jun, 1997) (% disruption)	2.45 $\pm$ 0.93 <sup>a</sup>	0.067 $\pm$ 0.04 <sup>b</sup> (97.3%)	0.083 $\pm$ 0.04 <sup>b</sup> (96.6%)	0.001*
2nd flight (21 Jul - 19 Aug, 1997) (% disruption)	1.68 $\pm$ 0.62 <sup>a</sup>	0.05 $\pm$ 0.03 <sup>b</sup> (96.7%)	0.05 $\pm$ 0.04 <sup>b</sup> (96.7%)	0.005*

Data were square-root-transformed and analyzed using a two-way ANOVA. Means from the same flight having no letters in common are significantly different at  $P < 0.05$  according to LSD test (SAS, 1985).

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%) 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 population 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% 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).

In addition to the approximately 15–20% reduction in the number 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 3). Mating fre-

Table 2. Percentage (mean  $\pm$  SE) of free-flying feral female *O. nubilalis* that were captured and found to be mated during the first and second flights in pheromone disruption and untreated plots in 1997

	Check plot	MSTRSTM plot	Rope plot	P
1st flight (6 Jun - 27 Jun, 1997)	97.10 $\pm$ 0.98 <sup>a</sup> (n = 327)	80.27 $\pm$ 5.11 <sup>b</sup> (n = 316)	86.77 $\pm$ 3.53 <sup>ab</sup> (n = 348)	0.03*
2nd flight (21 Jul - 19 Aug, 1997)	95.77 $\pm$ 1.13 (n = 274)	81.17 $\pm$ 9.66 (n = 264)	78.37 $\pm$ 10.93 (n = 193)	0.18

Data were analyzed using a two-way ANOVA. Means from the same flight having no letters in common are significantly different at  $P < 0.05$  according to LSD test (SAS, 1985). Total number (n) of females captured per flight in treated and check plots (3 locations per plot) is shown in bracket.

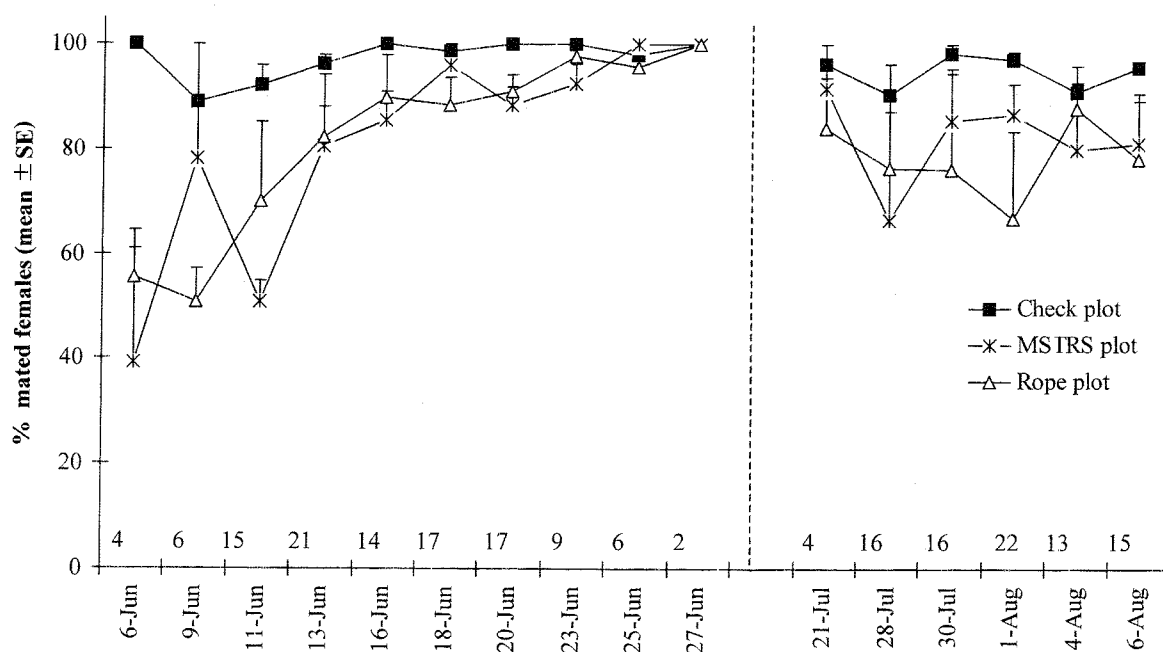


Fig. 3. Percentage of mated net-captured feral *O. nubilalis* females in pheromone-treated and check plots. Values are means ( $\pm$  SE) of 3 locations per treatment. Numbers immediately on top of the X-axis are average (n = 9 fields) numbers of females captured per 15 min. Broken lines represent the inter-flight period when no data were collected.

quency 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 3). This was significantly higher than the 1.5 and 1.57 average number of matings recorded in the MSTRS<sup>TM</sup> and ropes fields, respectively. The mean number of spermatophores recorded per first generation female captured in MSTRS<sup>TM</sup> fields was 1.33 and this was significantly lower than the mean per first generation female captured in rope-treated fields (Table 3). No significant difference in the percentage of mated first-

-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 females ( $P = 0.05$ ). A close look at the data suggests that both dispensers seemed to fair better in the Gilbert location than in the other two, especially during the second flight (Table 4). The reason for this better performance may be due to the unique landscape of 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).

The average number of spermatophores (ca. 2 per

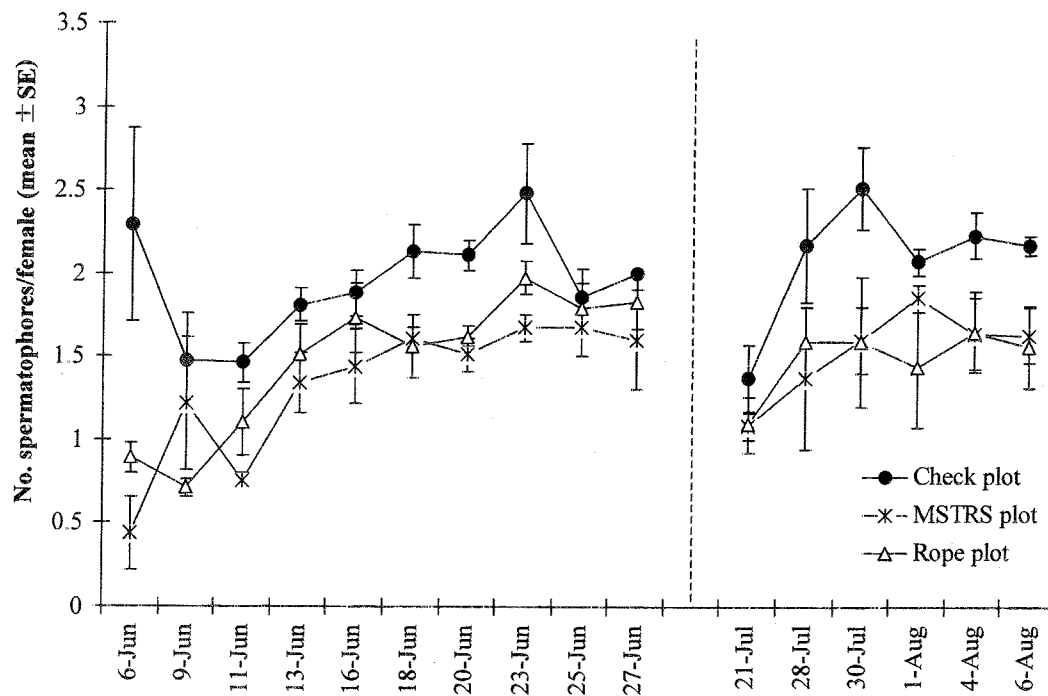


Fig. 4. Number of spermatophores in feral *O. nubilalis* females captured in pheromone-treated and check plots. Values are means ( $\pm$ SE) of 3 locations per treatment. Broken lines represent the inter-flight period when no data were collected.

Table 3. Mating frequency and mean number of spermatophores in net-captured feral *O. nubilalis* females during first and second flights in 1997

Plot	Flight	Total No. of females	% of Females with 0 to 4 spermatophores					Mean ( $\pm$ SE) No. spermatophores
			0	1	2	3	4	
Check	1st	327	3.5	26.3	49.7	19.3	1.3	1.88 $\pm$ 0.03 <sup>a</sup>
	2nd	274	5.1	19.5	39.7	27.9	7.7	2.17 $\pm$ 0.06 <sup>a</sup>
MSTRS <sup>TM</sup>	1st	316	20.3	34.5	38.6	6.6	0	1.33 $\pm$ 0.09 <sup>c</sup>
	2nd	264	17.7	20.8	41.5	18.9	1.1	1.63 $\pm$ 0.17 <sup>b</sup>
Rope	1st	348	13.8	26.7	48.3	11.2	0	1.58 $\pm$ 0.08 <sup>b</sup>
	2nd	193	21.2	22.3	34.7	21.2	0.5	1.56 $\pm$ 0.25 <sup>b</sup>

Means calculated for each flight were compared among treatments by using a two-way ANOVA. Means ( $\pm$ SE) from the same flight having no letters in common are significantly different at  $P < 0.05$  according to LSD test (SAS, 1985).

female), with multiple matings in the range of 70–75% in females collected in the check plot in this study (Table 3) seems to fall within the range recorded in other studies (Pesho, 1961; Drecktrah and Brindley, 1967; Loughner, 1971; Showers *et al.*, 1974; Elloit, 1977; Onokogu *et al.*, 1980). While Pesho (1961) reported that 8–43% of females from a feral population of *O. nubilalis* were mated more than once, Elliot (1977) recorded a maximum sper-

matophore count of four per female, with the percentage of multiple-mated females in the range of 15–50%. This proportion was similar to the 46.5% multiple matings recorded by Drecktrah and Brindley (1967). A slightly higher proportion of multiple matings was recorded by Onokogu *et al.* (1980), ranging between 31–70% for females reared in the laboratory on different diets.

It remains to be seen whether the significant, but



Table 4. Comparison of pheromone-mating disruption of feral females at three different locations in Iowa in 1997

Location	Flight	Check plot			MSTRS <sup>TM</sup> plot			Rope plot		
		No. females	% mated	No Spermato-phores	No. females	% mated	No. Spermato-phores	No. females	% mated	No. Spermato-phores
Gilbert	1st	173	95.4	1.93	127	78.1	1.27	143	83.9	1.52
	2nd	158	93.7	2.05	87	63.2	1.31	62	56.5	1.06
Napier	1st	69	97.1	1.86	88	89.8	1.51	96	93.8	1.72
	2nd	41	97.6	2.27	69	84.0	1.67	63	88.9	1.79
Nevada	1st	85	98.8	1.84	101	72.3	1.21	109	82.6	1.49
	2nd	75	96.0	2.19	108	96.3	1.91	68	89.7	1.84

Percentage mated females was calculated by comparing number of mated females with total number of females collected during each flight. Values for number of spermatothores are averages per female per location.

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, 1999). Also, the ~50% 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, 1999). 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% reduction in fecundity for *Cydia pomonella* females that experienced a two-day delay in mating.

We are encouraged by the level of mating disruption in our study, because 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 (e. g. 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.

Even though we had no control over the influx of females from the adjacent neighboring grassy strips in our relatively small disruption plots, our data still showed significant reductions in mating frequencies during both the first and second flight of the corn borers. To our knowledge there is no data available in the European corn borer literature to directly evaluate the significance of this reduction with respect to oviposition rates and larval damage. 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 can translate into some of the most successful and reliable disruption seen in any species (Rice and Kirsch, 1990; Barnes and Blomefield, 1997). For instance, in 1986, Rice and Kirsch (1990) found in their pooled samples from five disruption experiment sites within California's Central Valley, that 84.6%, 61.2%, 76.4%, 87.4% and 95.4% of the females captured in disruption plots had mated in generations 1 through 5, respectively. The percentages of females captured in the five untreated check plots that had mated in generations 1 through 5 were 90.2%, 91.6%, 95.6%, 97.4% and 95.0%,

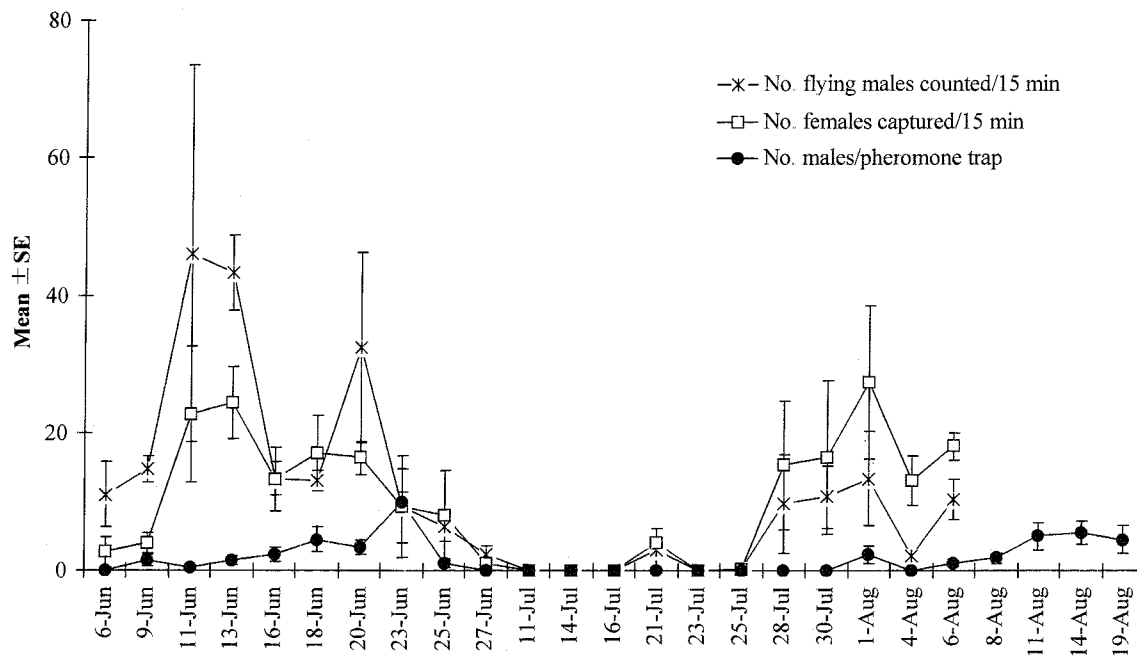


Fig. 5. Relationship between pheromone trap capture and abundance of *O. nubilalis* adults in untreated check plots. Values are means ( $\pm$ SE) of 3 locations. Only a small proportion of the male population is captured in 100  $\mu$ g synthetic pheromone-baited wing traps. Capturing of females and counting of flying males ended on the 6th of August.

Table 5. Pheromone emission rates (mean  $\pm$  SE) from MSTRS™ pads and Shin-Etsu ropes

Days in the field	Release rates of Z11-14: Ac in $\mu$ g/min (mean $\pm$ SE)	
	MSTRS™	Rope
1	5.29 $\pm$ 0.68	0.26 $\pm$ 0.01
7	6.09 $\pm$ 1.07	0.23 $\pm$ 0.02
26	—	0.22 $\pm$ 0.01
28	3.08 $\pm$ 0.35*	—
64	—	0.04 $\pm$ 0.01

Three replicates were conducted per dispenser.

— = no data collected.

\* MSTRS™ malfunction during the third week.

respectively. These data translate to only 5.6%, 30.4%, 19.2%, 10.0% and 0% mating disruption in generations 1 through 5, yet reductions of damage at the end of the season were 67% and 88% in the first two disruption sites, the only ones that had untreated checks (the others had insecticide treated checks) with which to compare damage.

Another interesting observation in this study is the relationship between male catch in pheromone traps and the number of free-flying males: only a

small number of flying males were caught in the pheromone traps relative to the number seen flying on any given date (Fig. 5). Low pheromone trap catches relative to adult abundance, as measured by traditional trapping methods such as blacklight traps have also been recorded for *O. nubilalis* in other studies (Roelofs *et al.*, 1972; Carde *et al.*, 1975; Oloumi-Sadeghi *et al.*, 1975; Fletcher-Howell *et al.*, 1983; DuRant *et al.*, 1986; Bartels *et al.*, 1997). The reason (s) for the low pheromone trap catches recorded in the current study remain unclear, since most of the identified variables that can influence pheromone trap catch, including blend composition (DuRant *et al.*, 1995), and trap placement (Fletcher-Howell *et al.*, 1983; Mason *et al.*, 1997) were taken into consideration in the design of the experiments. Although possible, the low trap catches recorded in the current study may not be related to trap design, since low catches in pheromone traps relative to black light traps have been recorded even in studies that utilized trap designs other than wing traps (Thompson *et al.*, 1987; Bartels *et al.*, 1997).

The current results have demonstrated the potential successful population control of this important

species by using pheromone-mating disruption. Despite the minor difference in pheromone component ratio, both types of dispensers showed similar levels of disruption efficacy. The mean emission rates from both dispensers are shown in Table 5. After 7 days in the field, release rate from the MSTRS™ was *ca.* 26 times (6.09 µg/min) higher than the release rate from ropes (0.23 µg/min). The MSTRS™ formulation, therefore, emitted pheromone at higher rates from fewer sources, but deployed lower overall amounts of pheromone per hectare than the rope formulation, which emitted pheromone at lower rates using more closely spaced point sources. The total amount of pheromone used per hectare of grass during the entire season was calculated as 32.4 g/h/y and 240 g/h/y, for the MSTRS™ and the rope dispensers, respectively. Since only the grassy areas, which constitute only about 5–15% of the total corn acreage, were treated in this study, the total amount of pheromone used per hectare of corn during the entire season would have amounted to about 1.62–4.86 g/h/y and 12–36 g/h/y, for the MSTRS™ and the rope dispensers, respectively. It may be possible to reduce the density of ropes and MSTRS™ substantially, especially if a more area-wide application were to be employed.

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