

## **A novel controlled-release device for disrupting sex pheromone communication in moths**

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**Abstract** - The results of experiments using a novel controlled release system, called the "Metered Semiochemical Timed Release System", or MSTRS™, for disrupting mating or pheromone source location by males of three lepidopterous pests, are described. In this system, pheromone is emitted at rates ca. 20 times higher than existing dispensers. Fewer dispensers are therefore needed for effective disruption. Unlike existing systems, MSTRS allows the user not only to choose how frequently pheromone is discharged but also to regulate the diel periodicity of this emission to correspond to the time of activity of the adults of the targeted pest insect. In addition, the pheromone is protected from oxidation and UV degradation since it is housed in pressurized canisters.

**Key words** - sex pheromone, mating disruption, controlled release dispensers, aerosol, *Ostrinia nubilalis*, *Cadra cautella*, *Rhopobota naevana*, Lepidoptera

### **Introduction**

There has been much progress over the past decade in improving the release-rate characteristics of some of the commercially most successful pheromone mating disruption formulations. However, none of the existing controlled-release technologies allow the user to actively alter the release rate. The existing systems are all passive systems that emit pheromone continuously according to ambient wind and temperature conditions.

We recently described a new system, called "Metered Semiochemical Timed Release Systems", or MSTRS™ (Mafra-Neto & Baker 1996a), in which an aerosol canister containing pheromone is placed in a machine and an aerosol spray-burst is

emitted onto a large pad on a timed basis (*e.g.*, every 15 minutes). Pheromone is then emitted from the pad at extremely high rates, ca. 20 times higher than most existing dispensers. Fewer dispensers are therefore needed for effective disruption. A similar system was reported by Shorey *et al.* (1996).

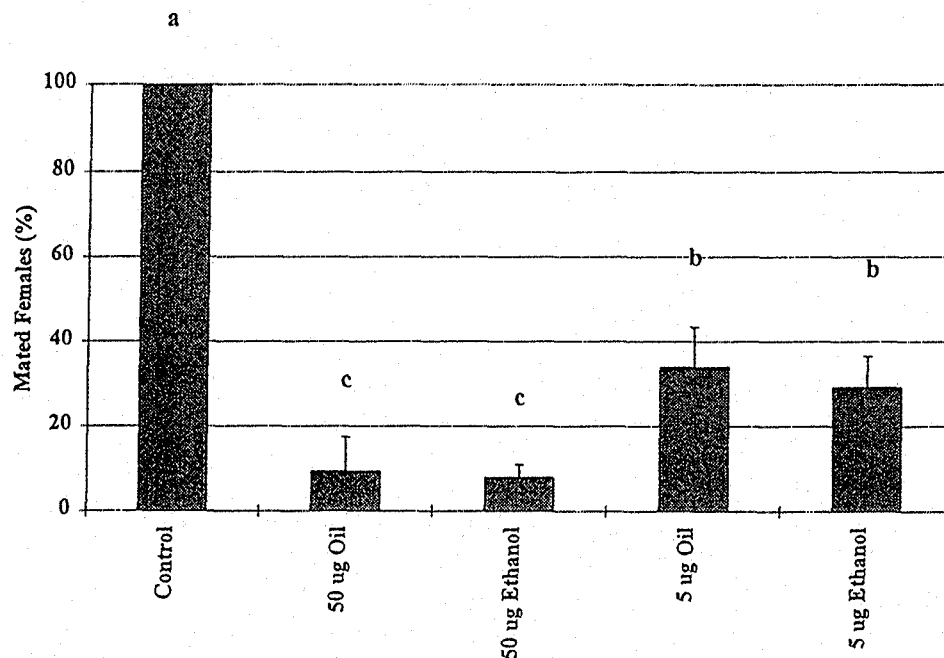
Unlike existing systems, ours allows the user not only to choose how frequently pheromone is discharged but also to regulate the diel periodicity of this emission to correspond to the time of activity of the adults of the targeted pest insect. Pheromone is not wasted by being passively emitted from the reservoir during periods of the day when the insects are inactive. In addition, the pheromone is protected from oxidation and UV degradation since it is housed in pressurized canisters. Although new to moth pheromone mating disruption research, aerosol dispensation of pheromone was examined in the early 1970s by bark beetle pheromone researchers for directing mass attacks of the southern pine beetle, *Dendroctonus frontalis*, onto targeted trees (Thomas *et al.* 1975). For unknown reasons, this work on bark beetles went completely unnoticed and the concept of using metered aerosols for moth mating disruption only emerged recently (Mafra-Neto & Baker 1996a; Shorey *et al.* 1996).

Here we summarize some of our results thus far for using MSTRS technology against the almond moth, *Cadra cautella*, which is a stored products pest, the European corn borer, *Ostrinia nubilalis*, a pest of corn, and the blackheaded fireworm, *Rhopobota naevana*, a serious pest of cranberries in North America.

### Almond moth, *Cadra cautella*

Laboratory-reared virgin males and females were released into 3 x 3 x 2.2 m rooms lined with white paper to make recovery of the moths easier. Two densities were tried, one low (15 females and 30 males) and the other one high (50 females and 150 males), the latter density for this sized room being above that for which successful disruption was achieved in previous studies (Sower & Whitmer 1977; Hodges *et al.* 1984; Hagstrum & Davies 1982). Depending on the experiment, either one or two MSTRS devices were placed in the corner(s) of the rooms, and the moths were allowed to fly freely. The machine-portion of the devices were purchased from Waterbury Companies, Inc. (Waterbury, Connecticut USA) and fitted with a spray-capturing pad 15 cm in diameter made of 1-cm-thick acrylic fiber. The machine sprayed pheromone from a pressurized canister containing either 0.4 g or 0.04 g of *C. cautella* pheromone, a blend of (*Z,E*)-9,12-tetradecadienyl acetate and (*Z*)-9-tetradecenyl acetate (Bedoukian Research Inc., Danbury, Connecticut) in a ratio of 10:0.9, onto the pad positioned 15 cm in front of the canister nozzle every 15 min, 24 hr a day. Two potential diluants were used in the cans, either ethanol or petroleum distillate. Treatments were replicated three times, with disruption being assessed by examination of females recovered from the rooms for the presence of spermatophores.

Significant disruption of mating, up to 100% for both the low-density and high-density populations of free-flying almond moths, was achieved over a 24-hr period with two MSTRS devices placed in opposite corners of the room at 1.3 m height, and emitting either 50  $\mu\text{g}$  or 5  $\mu\text{g}$  per spray-burst (Mafrá-Neto & Baker 1996a). Disruption was greater than 90%, even at the high moth density over a 72-hr period (Figure 1). The emission rate from the pads from the 50  $\mu\text{g}$ -per-spray treatment with ethanol as the diluant exceeded 0.6  $\mu\text{g}/\text{min}$  after receiving 22 sprays. We found that just one device per room emitting 50  $\mu\text{g}$  of pheromone per spray resulted in greater than 90% disruption of mating over 24 hr at the high moth density (Mafrá-Neto & Baker 1996a).



**Figure 1** Mean percentage of 50 free-flying female *C. cautella* that had mated after 72 hr in a 3 x 3 x 2.2 m room with 100 males ( $N = 3$ ). Rooms contained two MSTRS™ devices, one each in opposite corners, that every 15 min discharged either 50 or 5  $\mu\text{g}$  of pheromone, in solution with either ethanol or paraffin oil, onto a an acrylic pad

Our observations gave some insight into the mechanisms of disruption under these conditions. First, in the pheromone-treated rooms, males were completely inactive after 24 hr, and over 90% of the females were observed to be sitting on the walls, calling. In the control rooms, males were observed flying around, and many mating pairs were seen sitting on the walls. In the pheromone-treated rooms, interestingly, when the MSTRS device discharged, males were seen to begin wing-fanning and take flight, with many of the males orienting toward the pad, and some even touching the pad. After a few minutes, this activity would subside. These observations implied that

the habituation that was occurring was not absolute, and that the higher emission rate temporarily produced by a fresh spray on the pads could overcome that level of habituation.

These suppositions were confirmed in another study, in which males were pre-exposed to various levels of pheromone sprayed into a jar in which they were housed. The males pre-exposed to pheromone in this fashion were then flown in a wind tunnel to various dosages of pheromone from a point-source plume in clean air (Mafra-Neto & Baker 1996b). Males pre-exposed to higher amounts of pheromone exhibited elevated thresholds of response to subsequent exposure to pheromone plumes in the wind tunnel. The results showed that the optimum dosage for eliciting upwind flight and source location shifted to significantly higher levels for males pre-exposed to higher levels of pheromone, and that dosages that would otherwise be unacceptably high for unexposed males now could become the optimal dosages for males pre-exposed to high amounts of pheromone (Mafra-Neto & Baker 1996b). Thus habituation of *C. cautella* males during disruption is not absolute, and significant levels of misdirected flight (false trail-following) to the disruptant dispensers seemed to contribute directly to the disruption and perhaps to the males' elevated levels of habituation.

### European corn borer, *Ostrinia nubilalis*

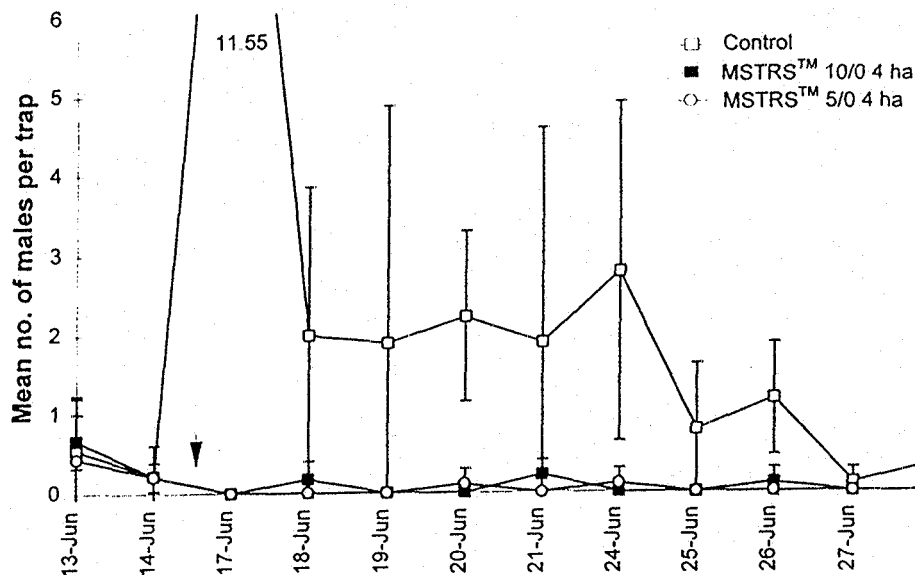
In a series of studies, Showers and colleagues (Showers & Reed 1976; DeRozari *et al.* 1977; Showers *et al.* 1980; Sappington & Showers 1983) reported that in Iowa, mating by *O. nubilalis* occurs mainly in grassy areas adjacent to corn fields, and not in the corn itself. They were able to demonstrate that treating the grass, either by mowing or with insecticide, resulted in significantly reduced damage to corn (Showers *et al.* 1980). Interestingly, there are no published reports of mating disruption for this species, and our objective was first of all to see whether we could disrupt mate location by males in small grassy plots. If this were to be successful, then in the ensuing years we would try to see whether we could prevent mating of feral females as well as reduce larval populations and damage to corn in neighboring fields.

We used MSTRS devices at two deployment densities in 0.4-ha grassy areas near Ames, Iowa, USA. Treatments were replicated three times, as were the 0.4-ha areas treated with twist-tie dispensers, also called "ropes" (Shin-Etsu Chemical Co. Ltd., Tokyo, Japan). The devices were identical to the ones used indoors for *C. cautella* (except for the pheromone blend employed) and were placed on stakes at a height of 1 m. The canisters contained either 4 or 8 g of the pheromone of the Iowa strain of this species (Klun *et al.* 1973), a blend of (*Z*)-11-tetradecenyl acetate (Z11-14Ac) and (*E*)-11-tetradecenyl acetate (E11-14Ac) (Bedoukian Research, Inc., Danbury, Connecticut) in a ratio of 97:3 *Z/E*. Devices containing the 4-g cans were placed at a density of 10/0.4 ha in 1-acre square-shaped grassy plots comprised of various

grasses including brome grass and foxtail grass. Machines containing the 8-g cans were deployed at a density of 5/0.4 ha in 3-acre plots of similar grass composition in a rectangular geometry. Machines discharged pheromone every 15 min, 24 hr/day onto the pad.

The Shin-Etsu ropes containing the Hamaki-con formulation (95:5 *Z/E*-ratio) were deployed in 0.4-ha, long, narrow plots, usually 5 to 10 m wide, situated in amongst the corn such as to form irregularly shaped islets. The grass was grabbed by hand to form a bunch, and a rope was twisted around the top of the bunch, with the researcher walking a prescribed number of meters between bunches to create a rope density of either 130, 400, or 1600 ropes/acre, making sure that the grassy strips received ropes along their edges.

Disruption was assessed by two wing-style sticky traps, (IPM Technologies Inc., Oregon) containing a 100-mg septum of the 97:3 blend used in the canisters, as well as by two wing traps containing five virgin females in a small cage plus a sucrose water source. The traps were placed at least 30 m from the nearest MSTRS device, and without regard to the location of the ropes. Release rates of Z11-14Ac from the pads of the 8-g machines after 14 days of emission was  $5.85 \pm 2.20 \mu\text{g}/\text{min}$  ( $N = 3$ ). Release rates from the pads from the 4-g machines after 14 days in the field were  $3.45 \pm 0.94 \mu\text{g}/\text{min}$ . ( $N = 3$ ). The Shin-Etsu ropes emitted pheromone at a rate of  $0.29 \mu\text{g}/\text{min}$ . after 6 days in the field, and  $0.30 \mu\text{g}/\text{min}$  after 30 days in the field ( $N = 3$ ).



**Figure 2** Mean capture of male *O. nubilalis* in wing traps containing 5 virgin females in grassy fields in which either 5 or 10 MSTRS™ devices/0.4 ha were deployed, each containing canisters loaded with either 8 or 4 g of pheromone, respectively. After two days of pre-treatment monitoring of all fields, the MSTRS devices were activated on June 15th (arrow). Bars indicate standard deviation ( $N = 3$ )

Disruption of males' ability to locate females or the synthetic pheromone dispensers averaged 98% for the 10/0.4 ha MSTRS treatment and 99.2% for the 5/0.4 ha MSTRS during the entire first flight (Figure 2). The capture rates of males as the flight began for the two days before the disruptant dispensers were deployed were equivalent in all plots. The capture levels and the levels of disruption caused by either the MSTRS or the Shin-Etsu ropes deployed at either 130 ropes/0.4 ha (94.9% disruption), 400 ropes/0.4 ha (95.5% disruption) or 1200 ropes/0.4 ha (98.8% disruption) were not significantly different ( $p > 0.05$ ; ANOVA,  $N = 3$ ).

The data show not only the potential for disrupting mating of this serious pest of corn using MSTRS technology, but also using Shin-Etsu twist-on dispensers. The geometry of the grassy areas varies considerably, and long narrow strips are not optimal for coverage by plumes from widely spaced, high-emission rate dispensers such as the MSTRS devices. Nevertheless, our data show that in large grassy plots having favorable geometry, a few high-emission-rate sources per ha can disrupt mate location by *O. nubilalis* males as effectively as hundreds of lower-emission-rate sources per ha having the same amount of pheromone on a per-ha basis.

### **Blackheaded fireworm, *Rhopobota naevana***

Significant work on disrupting mating of this serious pest of cranberries has been undertaken by Fitzpatrick *et al.* (1995), and has shown much promise for this technique, using Shin-Etsu ropes or Ecogen Spirals (Scentry/Ecogen, Billings, Montana) with a total application rate of ca. 70 g pheromone/acre. One problem with these dispensers, however, is that they must be retrieved at the end of the season due to the potential for the buildup of environmentally unacceptable levels of plastic in the cranberry marshes. The placement and retrieval of a high number of point sources on the cranberry beds would also result in unacceptably high foot traffic, which would damage the delicate, slow-growing plants. The use of MSTRS devices would be advantageous because only a few dispensers would be necessary per acre, mostly deployed around the perimeter of the beds where they could be fairly easily retrieved without incurring crop damage. Furthermore, the MSTRS can be stored for re-use in subsequent years.

We used MSTRS devices identical to those described above for *O. nubilalis*, except for the pheromone blend in the canisters, and affixed them to wooden stakes at a height of 20 cm above the cranberry plant canopy. The canisters contained either 8 or 20 g of *R. naevana* pheromone, which is a blend of Z11-14Ac, (Z)-11-tetradecenyl alcohol (Z11-14OH), and (Z)-9-dodecenyl acetate (Z9-12Ac) in a ratio of 9:3:1 (McDonough *et al.* 1987; Slessor *et al.* 1987). These components were purchased from Bedoukian Research Inc. Devices containing the 8-g cans were deployed at a density of 5/0.4 ha along and within cranberry beds or series of beds that averaged ca. 1.2 ha in total area. Two configurations were used for this density of

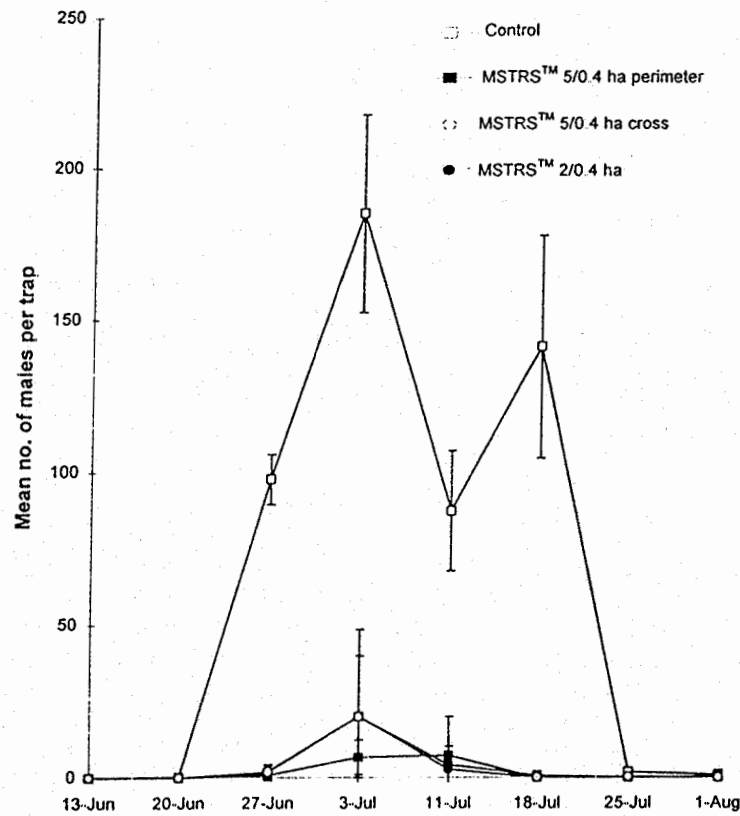
devices, one being a perimeter-only treatment with MSTRS spaced ca. every 30 m at the edges of the beds. The second consisted of the same density of devices and amount of pheromone per ha overall, but three devices were removed from the perimeter (remaining devices being more widely, but evenly spaced) and instead were deployed across the centers of the beds, bisecting them longitudinally. The 20-g cans were deployed at a density of 2/0.4 ha along the same sized beds, such that there were nine machines only around the perimeter of the 1.2-ha beds. Treatments as well as 1.2-ha control plots several hundred m from the treated beds were replicated three times in different locations within ca. 30 miles of each other in the cranberry growing region near Babcock, Wisconsin. Machines were programmed to discharge every 15 min, 24 hr/day.

Disruption was assessed by counting the number of males captured in wing traps baited with 10 µg of the above pheromone blend on a rubber septum, a lure that has been shown to be comparable in attractancy to females (Fitzpatrick *et al.* 1995). The wing traps were placed, three per 1.2-ha plot, at locations in the interior of the marsh, and not closer than 30 m from the nearest machine. The number of males captured was assessed weekly, the males removed, and trap bottoms replaced as needed.

Disruption averaged 99% in the first location, regardless of the MSTRS deployment pattern, and 95% in the second location throughout the first flight (detailed record for this location is shown in Figure 3), but averaged only 82%, 80%, and 57% for the 5/0.4 ha cross pattern, the 2/0.4 ha perimeter, and the 5/0.4 ha perimeter treatments, respectively, in the third location, which had a history of very high populations of fireworm and low yields compared to the industry average in the region. Captures in the control plots in the three locations averaged 52.3, 73.4, and 63.3 males/trap/week over the six-week flight period. Unlike the treated beds in the other two locations, the 1.2 ha comprising the treated areas for each of the three MSTRS deployment arrays in the poor disruption location were comprised of six, 0.2-ha beds separated by 1.5 m-high grassy banks functioning as dikes for water control. Thus, it is possible that the aerial transport of pheromone plumes from the MSTRS over the disruption areas could have been disturbed in these plots, resulting in lower efficacy of disruption. In all three locations, the MSTRS devices were deployed at the same time as a sprayable formulation of pheromone (Scentry/Ecogen) was applied directly to the cranberry beds; the MSTRS were as effective in disrupting pheromone source location as the sprayable formulation in all plots (Sheila Fitzpatrick, pers. comm.).

Our results show that a relatively few MSTRS per ha can effectively disrupt pheromone source location by *R. naevana* at levels of 98% disruption for an entire flight period on 1.2-ha cranberry beds. Again, as in the *O. nubilalis* studies, the geometry of deployment was important, and it must be considered that on smaller plots, there is a greater edge area to protect relative to the interior area of crop that is to be protected. In principle, the MSTRS technology should work better over a very large,

regularly shaped area where there will be fewer pheromone-plume-free holes along the edges.



**Figure 3** Mean capture of male *R. naevana* in wing traps containing 10  $\mu$ g of synthetic pheromone in 1.3-ha cranberry marshes at one of the three locations in Wisconsin in which either 2 or 5 MSTRS™ devices per 0.4 ha were deployed. The devices were activated before the first flight began and continued to release pheromone throughout the flight from either 20-g cans (2/0.4 ha) or 8-g cans (5/0.4 ha). Bars indicate standard deviation ( $N = 3$ )

Finally, it must be considered that the efficacy of widely-spaced dispensers such as these, whose plumes need to sweep for tens, and perhaps hundreds of meters horizontally over the crop canopy to both attract and habituate males sufficiently that they are prevented from mating, will likely be more dependent upon ambient meteorological conditions than will be numerous lower-emission-rate point sources spaced only meters apart throughout the crop. This vulnerability may be accentuated for species that mate during the daytime, when adiabatic lapse rates are highest, and unstable, rising air can carry plumes from disruptant dispensers up and away from the canopy.



## Acknowledgements

This work was supported by grants from the NRI competitive grants program of the USDA (Grant No.9402914; to TCB), from the Leopold Foundation for Sustainable Agriculture (No. 97-19; to TCB and MER), and from the Wisconsin Cranberry Association and Ocean Spray Cranberries, Inc. (both to TCB). We thank Sheila Fitzpatrick for the use of her unpublished results and collaboration in setting up and conducting the field studies on the blackheaded fireworm, Julie Todd, Allard Cossé, Simeon Wright and Jenny Remmers for their assistance with the cornborer and fireworm experiments, Jean Dyer for the supply of cornborer females, Eric Meador and Paul Shaputas for rearing the almond moths, and Allard Cossé for drawing Figures 2 and 3.

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