

## Suppression of Mating by Blackheaded Fireworm (Lepidoptera: Tortricidae) in Wisconsin Cranberry Marshes by Using MSTRS™ Devices<sup>1</sup>

Henry Y. Fadamiro,<sup>2</sup> Allard A. Cossé,<sup>3</sup> Timothy Dittl,<sup>4</sup>  
and Thomas C. Baker

Department of Entomology,  
Iowa State University,  
Ames, Iowa 50011 USA

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**ABSTRACT** We conducted a study to optimize the deployment of our controlled pheromone release system called metered semiochemical timed release system (MSTRS™) and to measure its impact on male blackheaded fireworm, *Rhopobota naevana* (Hübner) (Lepidoptera: Tortricidae). The deployment pattern and pheromone emission rate of the MSTRS™ devices were adjusted to give optimal mating disruption in the widely dispersed cranberry (*Vaccinium macrocarpon* Aiton) beds from a perimeter-only pattern of deployment. During the first flight, disruption of pheromone source location averaged 98, 98, and 40% in the first, second, and third grower sites, respectively. During the second flight, disruption averaged 88% in the first grower site and 86% in the second and third grower sites. More importantly, high levels of mating disruption were achieved, as measured by the frequency of mating by captured free-flying females. About 24 and 15% fewer second-generation blackheaded fireworm females mated in the MSTRS™-treated beds in the first and second grower sites, respectively, compared with the check plots. In the first grower site, the mean number of matings, as measured by the numbers of deposited spermatophores, per female captured during the second flight was 0.75 in the MSTRS™ beds and 1.8 in untreated beds. Number of matings per female in the second grower site averaged 1.0 in the MSTRS™-treated beds and 1.48 in the untreated beds.

**KEY WORDS** *Rhopobota naevana*, blackheaded fireworm, Tortricidae, sex pheromone, mating disruption, spermatophores, controlled release

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The blackheaded fireworm, *Rhopobota naevana* (Hübner) (Lepidoptera: Tortricidae), is a major pest of cranberries (*Vaccinium macrocarpon* Aiton) in the United States (Sutherland 1978). Although a female-produced sex pheromone was identified for this species in the late 1980s (McDonough et al. 1987, Slessor et al. 1987), only recently has significant work been undertaken on the potential control of the blackheaded fireworm by using pheromone mating disruption (Fitzpatrick et al. 1995, Baker et al. 1997).

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<sup>2</sup>Department of Entomology, University of Minnesota, 219 Hodson Hall, 1980 Folwell Avenue, St. Paul, MN 55108-6125.

<sup>3</sup>Bioactive Agents Research, USDA-ARS, National Center for Agricultural Utilization Research, 1815 North University Street, Peoria, Illinois 61604.

<sup>4</sup>Ocean Spray Cranberries, Inc., P. O. Box 155, Babcock, Wisconsin 54413.

Fitzpatrick et al. (1995) first recorded significant reductions in incidence of mating of laboratory-reared females of *R. naevana* placed in plots treated with spiral polyvinyl chloride (PVC) dispensers (Scentry/Ecogen, Billings, Montana) loaded with the sex pheromone. The spiral PVC dispenser, however, like many available passive dispensers does not meet many of the requirements of a satisfactory dispenser technology, including fully controlled release of pheromone (Ogawa 1997).

A controlled release dispenser called the metered semiochemical timed release system, or MSTRS™, was described in Mafra-Neto & Baker (1996) and Baker et al. (1997). This dispenser allows the user control over the amount and timing of pheromone release. The use of MSTRS™ devices would be advantageous to cranberry growers because only a few dispensers would be necessary per acre, deployed only around the perimeter of the beds where they can be easily retrieved without incurring crop damage. Furthermore, research on optimizing MSTRS™ efficacy is needed even if treatments such as the PVC system are successful because aerial coverage near woods at the edge of beds is problematic, and MSTRS™ may be able to be used to supplement these formulations.

In 1996 we began testing the MSTRS™ devices against the blackheaded fireworm by using three different MSTRS™ densities and deployment patterns. These first tests were encouraging, in that we were able to reduce pheromone source location by blackheaded fireworm males by 98% in several plots (Baker et al. 1997), a level that was as good as that recorded with PVC dispensers (Fitzpatrick et al. 1995). The objective of this second-year study was to use just one deployment pattern and measure the impact of pheromone treatment in preventing mating of free-flying feral females, while improving the MSTRS™ pheromone mating disruption system for the blackheaded fireworm.

## Materials and Methods

**Pheromone formulation and dispenser.** The machine portion of the MSTRS™ (Waterbury Co., Waterbury, Connecticut) used in this study was a modified and updated version of the one described by Mafra-Neto & Baker (1996) and Baker et al. (1997). Briefly, the system consisted of a spray-bottle reservoir, a pump spray dispenser unit, and a timer mechanism to activate the spray discharge mechanism. A pad captured the spray and released the pheromone (Mafra-Neto & Baker 1996). Pads were formed by 0.5-cm-thick circular acrylic padding stretched on a needle point hoop (17.8 cm i.d.) and held in place 3 cm from the spray nozzle. The pump spray bottle contained a solution (maximum of 300 ml) with the desired concentration of pheromone in EtOH. 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 5- to 25-min intervals, delivering a replenishing spray onto the pad. The MSTRS™ devices could produce 6,000 recharging sprays of similar strength. The spray time interval ranged between 5 and 25 min, and 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 times when adult blackheaded fireworm is not sexually active.

All machines contained pump spray bottles filled with 250 ml of 100% EtOH containing 3.96 g of a blend of (*Z*)-11-tetradecenyl acetate (Z11-14:Ac), (*Z*)-11-tetradecenyl alcohol (Z11-14:OH), and (*Z*)-9-dodecenyl acetate (Z9-12:Ac) in a ratio of 9:3:1 (McDonough et al. 1987, Slessor et al. 1987). These components were purchased from Bedoukian Research, Inc., Connecticut, and each was  $\approx$ 95% pure. The MSTRS™ devices were activated prior to the emergence of first and second generation of blackheaded fireworm (as determined with pheromone traps). To achieve an early high release rate from the pads, the spray pads were primed with 0.5 g of pheromone at the time of deployment.

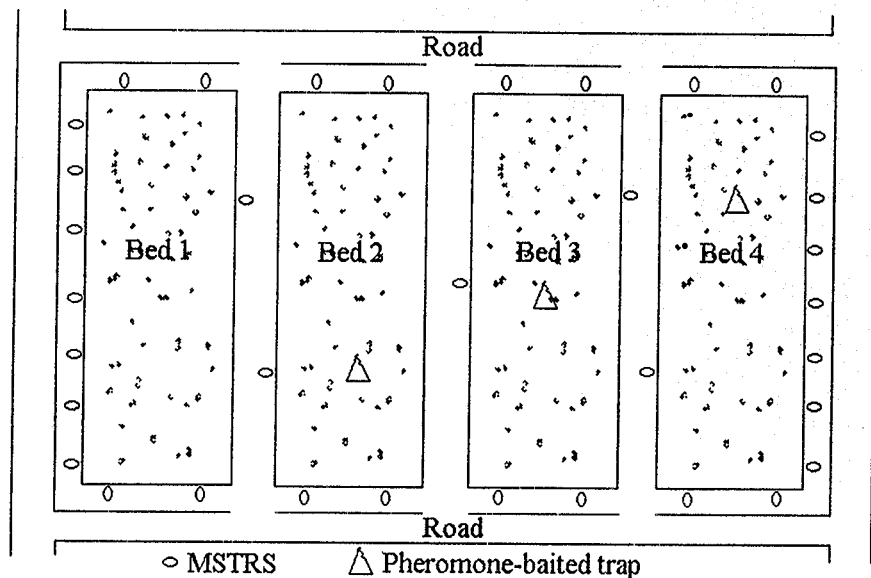
Release rates of the major pheromone component, Z11-14:Ac, from pads after different times of emission in the field were measured by placing a circular cutout of the pad, contained within a frame (9.2 cm i.d.), on top of a glass funnel (10 cm i.d.). The stem of the funnel was connected with a Teflon connecting tube to a glass pasteur pipette containing a 7-cm-long plug of packed glass wool. Air was drawn across the pad (2,000 ml/min) and through the glass wool trap from the tip of the pasteur pipette by using a vacuum. After a 15-min collection the vacuum was stopped, the pad removed from the funnel, and 100  $\mu$ g (1 mg/ml) of internal standard [(*Z*)-11-tridecenyl acetate] was added to the glass wool. The 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). 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).

Care was taken so that the pheromone pads did not come in contact with the glass funnel surface. Trap breakthrough was checked and confirmed to be negative by analyzing collected material in a second, in-series-connected pasteur pipette. Pad collections were performed in triplicate and collected amounts of Z11-14:Ac from the pads were calculated for the original pad diameter.

Blackheaded fireworm is known to mate during daylight hours in a broad period extending from early morning to dusk (S. Fitzpatrick, personal communication). MSTRS™ devices were programmed during the first flight (from 1 July to 1 August) to emit puffs of pheromone every 25 min for 12 h on a given day, between 0600 and 1800 hours (CST). During the second flight (from 5 August to 9 September), a shorter span of diel emission but with higher emission frequency per hour was used by setting the MSTRS™ devices to emit every 5 min between 0200 and 0800 hours. Timing of operation of the devices during the second flight was thereby aimed at loading the pad with the highest amount of pheromone at the start of the day, just before the mating period of adult blackheaded fireworm began.

**Experimental plot setup.** Treatments as well as untreated plots several hundred meters from treated beds were replicated three times in different grower sites within  $\approx$ 10 km of each other in the cranberry-growing region near Babcock, Wisconsin. Devices were deployed at a density of  $\approx$ 10 MSTRS™ per hectare (every 35 m) by using the perimeter-only pattern along cranberry beds

that we selected from the previous year's results. At the first grower site, 30 devices were deployed in three beds, totaling  $\approx 4$  ha. The untreated plot at this location consisted of two beds of 3 ha in total. At the second grower site, 35 devices were placed along the perimeter of four beds ( $\approx 5$  ha) (Fig. 1). The untreated plot was two beds of 1.6 ha in total area. The MSTRS<sup>TM</sup> plot at the third grower site during the first flight consisted of eight beds, totaling  $\approx 4.4$  ha. Thirty-one devices were placed at this site. The check plot consisted of three beds of  $\approx 1.6$  ha. Due to excessively high population pressure at this site that was uncharacteristic of normal commercial operations, work was discontinued at this third grower site at the end of the first flight. A new third site was selected for the second flight. At this new site, 30 devices, which were removed from the initial third site, were placed in two beds of  $\approx 2$  ha in total area. The untreated plot consisted of two beds, totaling  $\approx 1.2$  ha.



**Fig. 1.** Schematic representation of the pattern of deployment of MSTRS<sup>TM</sup> devices along the perimeter of four cranberry beds constituting the pheromone treatment at site 2. Each bed is  $\approx 1.25$  ha.

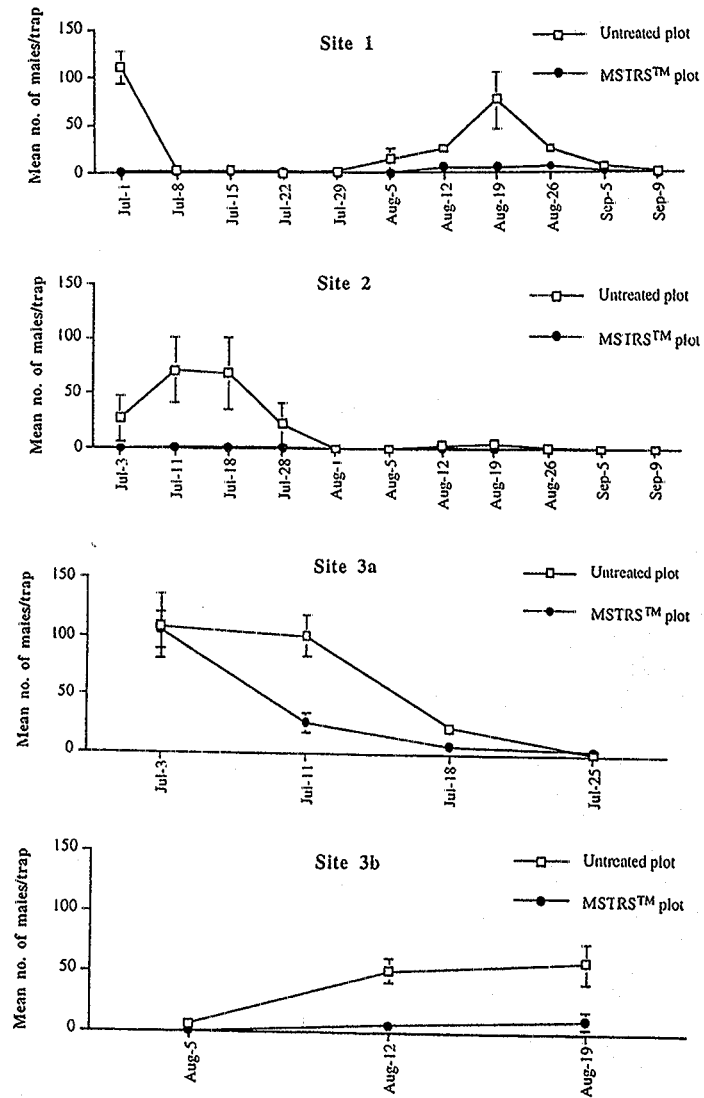
**Evaluation of disruption.** Mating disruption was assessed by using two parameters: numbers of males captured in Pherocon 1C sticky traps (Trécé, Salinas, California), and mating status and mating frequency of free-flying females captured in treated and untreated fields. Three Pherocon 1C white traps each baited with a rubber septum (Thomas Scientific, Swedesboro, New Jersey) loaded with 10 µg of the pheromone blend used in the MSTRS™ were placed in each treated plot, as well as in the untreated plots. Traps were placed in the interior of each bed and were not closer than 35 m from each machine. The number of males captured was assessed weekly, the males removed, and trap bottoms replaced as needed.

Free-flying, feral female blackheaded fireworm were collected by gently walking near the edges of each bed and capturing with a net any females that were seen flying, or by using a hand-held, battery-powered vacuum cleaner. We also used saturn-yellow Pherocon AM sticky traps baited with 100 mg each of four plant volatiles on a cotton dental wick in treated and untreated plots to try to increase the number of females captured. The components of the flower attractant were benzaldehyde, phenyl acetaldehyde, 2-phenyl ethanol, and  $\alpha$ -terpinyl acetate in the ratio of 1:1:1:1. Most of the adults captured in these volatile-baited traps were males, hence only females collected with net or hand-held vacuum cleaner were included in data analysis. Captured females were preserved in glass vials containing 70% EtOH and were later dissected under the microscope at 10 $\times$ , examining the bursa copulatrix for presence and number of spermatophores.

Mean weekly trap catch was calculated for each site. These means were used to calculate for each site the season-long mean trap catch for the MSTRS™ and untreated plots during first and second flights. Statistical analysis was with a two-way analysis of variance (ANOVA) testing the effect of treatment and site (each site was considered as a replicate) (SAS Institute 1985). Because of inclement weather, off-timing, and the use of insecticides by some growers, collection of feral female blackheaded fireworm was only possible during the second-generation flight, and only in two of the three sites. Even then, only few females were collected at these two sites. Number of mated females was considered as a proportion of the total number of females collected in each plot at each location. Data collected on the mating status of females collected in the treated and untreated plots at each location were analyzed by using the chi-square 2  $\times$  2 test of independence with Yates' correction of continuity (Parker 1979). For the data collected on the mating frequency of feral females, mean number of spermatophores per female was calculated for each plot at each site. These means were analyzed by using a *t*-test, examining differences between the means of two small samples (Parker 1979).

## Results and Discussion

A significant disruption of pheromone source location by males was achieved with the MSTRS™ during the first flight ( $P = 0.03$ ). The effect of location was also significant ( $P = 0.04$ ). Disruption of pheromone source location by first-generation male blackheaded fireworm, as measured by male trap catch, averaged 98% in both the first and second grower sites (Fig. 2; Table 1). Only 40%



**Fig. 2.** Mean ( $\pm$  SD) capture per trap of male blackheaded fireworm in traps containing 10  $\mu$ g of synthetic pheromone in cranberry marshes during first and second flights at the different grower sites (1, 2, 3a and 3b). Three traps were placed in each site. Site 3a was discontinued after the first flight and replaced with a new site (3b).

**Table 1. Number of male blackheaded fireworm captured per pheromone trap (mean  $\pm$  SD) and percentage of disruption of pheromone source location during first and second flights at three grower sites (cranberry plots).**

	Site 1	Site 2	Site 3 <sup>a</sup>	Mean of 3 sites
1st flight (1 July-1 Aug.)				
Untreated plot	23.20 $\pm$ 48.91	37.98 $\pm$ 21.23	59.08 $\pm$ 54.61	40.12 $\pm$ 17.98 a
MSTRS <sup>TM</sup> plot	0.53 $\pm$ 0.51	0.73 $\pm$ 0.76	35.68 $\pm$ 47.79	12.31 $\pm$ 24.0 b
Disruption (%)	97.7	98.1	39.6	69.3
<i>P</i>				0.03
2nd flight (5 Aug.-9 Sept.)				
Untreated plot	24.34 $\pm$ 27.13	1.56 $\pm$ 2.06	38.45 $\pm$ 28.92	21.45 $\pm$ 18.61
MSTRS <sup>TM</sup> plot	3.05 $\pm$ 2.67	0.22 $\pm$ 0.35	5.47 $\pm$ 5.35	2.91 $\pm$ 2.63
Disruption (%)	87.5	85.9	85.8	86.4
<i>P</i>				0.18

<sup>a</sup>The initial site 3 was discontinued after the first flight and a new site 3 was selected for the second flight. Means from the same flight having no letters in common are significantly different ( $P < 0.05$ ) according to a two-way ANOVA (SAS Institute 1985).

disruption, however, was achieved in the third grower site during this first flight (Fig. 2; Table 1), accounting for the significant effect of location recorded. As noted in Baker et al. (1997), this third grower site has a history of very high populations of fireworm and low yields compared with the industry average in the region. Thus, the use of this third grower site was discontinued at the end of the first flight. The degree of disruption of the ability of first-generation males to locate pheromone source, achieved in all three locations, was generally similar to that recorded during the 1996 study (Baker et al. 1997).

During the second flight, disruption of pheromone source location averaged 88, 86, and 86% in the first, second, and the new third grower sites, respectively (Fig. 2; Table 1), although these levels were not significant ( $P = 0.18$ ). Lower levels of disruption of pheromone source location by second-generation males compared with first-generation males also were recorded during the 1996 study (Baker et al. 1997). In the current study,  $\approx 30\%$  of the machines at each of the three locations broke down for at least 1 wk during the second flight. This also might have contributed to the lower levels of disruption recorded during the second flight.

Although not significant, a considerable reduction in the percentage of female blackheaded fireworm that had mated was recorded for females captured in the MSTRS<sup>TM</sup> plots compared with the untreated plots at the two sites (sites 1 and 2) in which females were captured (Table 2). At site 1, 91% of the females captured in the untreated plot had mated at least once compared with 68% matings in the MSTRS<sup>TM</sup>-treated beds ( $\chi^2 = 0.81$ ,  $df = 1$ ,  $P < 0.5$ ). Similarly,

**Table 2. Percentage of free-flying, second-generation female black-headed fireworm that were captured and found to be mated, and the mean number of spermatophores per female at the two sites (cranberry plots) where females were captured.**

	No. of females captured	Mated (%)	Mean ( $\pm$ SD) no. spermatophores per female
Site 1			
Untreated plot	11	90.9	1.82 $\pm$ 0.87a
MSTRS <sup>TM</sup> plot	12	66.7	0.75 $\pm$ 0.62b
Mating disruption (%)		24.2	58.8
<i>P</i>		< 0.5	< 0.01
Site 2			
Untreated plot	21	95.2	1.48 $\pm$ 0.68a
MSTRS <sup>TM</sup> plot	5	80.0	1.00 $\pm$ 0.71b
Mating disruption (%)		15.2	32.4
<i>P</i>		< 0.5	< 0.01

Means from the same location having no letters in common are significantly different ( $P < 0.05$ ) according to *t*-test for two small samples (Parker 1979).

95% of females captured in the untreated plot situated at site 2 had mated compared with 80% matings in the MSTRS<sup>TM</sup> plot ( $\chi^2 = 0.05$ ,  $df = 1$ ,  $P < 0.5$ ).

Significant reductions in the frequency of mating (number of spermatophores) per female also were recorded in the MSTRS<sup>TM</sup>-treated plot at both sites. At site 1, the mean number of spermatophores recorded per female captured in the untreated plot (1.82) was significantly greater than the mean (0.75) for females collected in the MSTRS<sup>TM</sup> beds ( $t = 3.44$ ,  $df = 21$ ,  $P < 0.01$ ). Similarly, the mean number of spermatophores recorded per female captured in the check plot (1.48) at site 2 was significantly greater than the mean number of spermatophores per female captured in the MSTRS<sup>TM</sup> plots (1.0) at this same location ( $t = 2.82$ ,  $df = 24$ ,  $P < 0.01$ ).

Even though very few blackheaded fireworm females were captured during this study, there is potential for the modest reduction in mating frequency of females in MSTRS<sup>TM</sup>-treated plots to translate into significant reductions in the reproductive success of the blackheaded fireworm and increased yield. Rice & Kirsch (1990) showed that modest reductions in the percentage of oriental fruit moth (*Grapholita molesta* Busck) females that mate in disruption plots can translate into some of the most successful and reliable disruption seen in any species. In a study on the effect of multiple mating on the reproductive performance of the European corn borer, *Ostrinia nubilalis* (Hübner), Fadamiro & Baker (1998) recorded a higher reproductive output for multiple-mated females compared with females that mated only once.



The mean emission rate from the MSTRS™ devices is shown in Table 3. After 7 d in the field, the release rate of Z11-14:Ac (the major pheromone component) was  $\approx 6$   $\mu\text{g}/\text{min}$  and after 28 d it was over 3  $\mu\text{g}/\text{min}$ , but this reduction was due to the malfunction of some of the machines due to worn-out dispenser belts in the peak of the summer heat. We have, however, been able to procure a stronger and more durable version of the belt that can withstand high heat. The total amount of pheromone used per hectare of marsh during both flight periods was calculated as 28 g/ha.

**Table 3. Pheromone emission rates (mean  $\pm$  SD) from MSTRS™ pads.**

Days in the field	Release rates of Z11-14:Ac in $\mu\text{g}/\text{min}$ (mean $\pm$ SD) <sup>a</sup>
1	5.29 $\pm$ 1.18
7	6.09 $\pm$ 1.85
26	-
28	3.08 $\pm$ 0.61*

<sup>a</sup>Three replicates were conducted per date. -, no data collected. \*, machine breakdown.

The current results demonstrated significant reductions in mating frequency of free-flying blackheaded fireworm females in cranberry beds by using MSTRS™ and confirmed the potential for population control of this important pest species by pheromone mating disruption. It now remains to be seen whether or not this technique can lead to significant reduction of damage by the blackheaded fireworm.

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