# MATING DISRUPTION: WHAT IS IT AND HOW DOES IT WORK?

**Sheila Fitzpatrick**
**Pacific Agri-Food Research Centre**
**Agriculture & Agri-Food Canada**
**6947 # 7 Hwy, P.O. Box 1000, Agassiz**
**British Columbia, Canada VOM 1AO**

## Introduction

Mating disruption is an insect management technique that prevents male insects from finding females. The technique is constructed from the following “building blocks”: an understanding of communication and attraction between male and female insects; identification and synthesis of the sex attractant odor, called a pheromone, normally produced by female insects; and a way of releasing synthetic pheromone into the environment in quantities sufficient to prevent male insects from locating sexually receptive females.

I will first explain the pheromone-based mate-location behavior that is interrupted by mating disruption. I will then go on to explain how synthetic pheromone is released into an area, and how the pheromone interferes with mate location. From there, I’ll describe the implementation of mating disruption for control of the blackheaded fireworm in cranberries, and show some of the data from field tests. I hope to leave you with an understanding of this technique, and also to emphasize that this is “knowledge-based” technology.

## Finding a Mate: How Pheromones are Used by Moth Pests

When a female moth emerges from her pupal case, she has two or three predetermined missions. The first is to crawl away from the pupal case, climb onto something vertical, expand her wings and allow her soft body parts to harden. During this period of several hours, she is unable to fly and therefore quite vulnerable to predators. Once her wings are in working order, she may fly about in search of water and, in some cases, nectar for food. Her next task is to attract a mate, which she does by sending out a chemical signal.

The female’s chemical sex attractant signal is called a pheromone. It is produced in a gland at the end of her abdomen. To send the pheromone signal, the female extrudes her gland, allowing the volatile pheromone to evaporate from the gland surface. The pheromone signal drifts downwind away from the female, much the same way that smoke drifts away from a cigarette. The female may repeatedly extrude and withdraw her gland, so that the pheromone signal is puffed out in pulses. The pheromone is emitted in very tiny amounts, on the order of less than a billionth of a gram per hour.

The pheromone signal typically contains several chemicals in a ratio particular to the moth species. Thus the pheromone signal is a blend that carries the message, “There is a female of species ‘X’ seeking a mate of the same species”. Pheromones are kind of like personal ads.

A male moth detects the pheromone signal through sensory cells on the sensillae (tiny hairlike projections) of his antennae. The cells are so sensitive that they can detect tiny quantities -- molecules -- of pheromone chemicals. There is a different type of sensory cell for each chemical in the pheromone blend. Parts of the male’s brain are genetically programmed to decide if the chemical blend is coming from a female of his species.

Male moths spend much of their time “sniffing” for a pheromone signal. They sit on plants or other vertical structures with their antennae upraised, and fly around searching the air for pheromone. They frequently clean their antennae with their front legs, so that the delicate sensory cells are kept clean of dust and other odor molecules.

When a male moth detects a pheromone signal, he begins to fly upwind along the invisible, smoke-like “plume” of pheromone. He attempts to keep at least one antenna in the pheromone plume during upwind flight. If his right antenna loses the pheromone signal, he turns left across the wind, then continues upwind flight. If his left antenna loses the signal, he turns right. Thus his upwind track along the pheromone plume is full of zigzags. If he loses the plume altogether, he zigzags left and right in the air where he last sensed the pheromone. If he still can’t find it, he may land, clean his antennae, then take off and try again. Or he may fly downwind, then turn around and fly upwind in wide zigzags.

Like a smoke plume, the invisible pheromone plume gets narrower and less dispersed close to its source. As the male nears the female, his flight path gets straighter. He lands next to her, and does a series of courtship behaviors so rapid that the individual behaviors can’t be seen unless they are videotaped and played back slowly. Male courtship behaviors may include wing fanning, sound, touching and release of a male pheromone. All of these behaviors tell the female that the male is from the right species and the right side of the tracks. Females may reject males that don’t display all the elements of good courtship. When a male is accepted, he clasps the female’s genital armature with his own, and mating occurs. If the mating is successful, the female will begin to lay fertilized eggs, usually within 24 hours.

The mating disruption technique attempts to prevent males from finding and following a female’s pheromone signal. If mating disruption is successful, there will be many females “calling” (releasing pheromone), but no males answering.

## **How Mating Disruption Works**

The mating disruption technique takes a synthetic copy of the pheromone blend produced by female moths and broadcasts it over the area where male and female moths are found. To get synthetic pheromone for a given moth species, biologists rely on their friendly chemist colleagues to first identify the chemicals in the natural pheromone blend, then reproduce the blend. It was first thought that, to be an effective disruptant of mating, the synthetic pheromone blend had to be virtually indistinguishable from the natural phermone blend. However, we now know that it is also possible to disrupt mating by using only the main chemical component(s) in a pheromone blend.

The synthetic pheromone is injected into or enclosed in controlled-release devices, which are distributed in the field. These devices may be small plastic or polyvinyl chloride (PVC) tubes or spirals, flakes, "twist-tie ropes" ( small plastic tubes with a twist-tie wire inside), clips similar to bread ties, microscopic polyurea capsules, or timed-release spray canisters. The tubes and flakes are designed to be scattered by hand or from an airplane. Spirals, twist-tie ropes and clips are attached by hand to branches. Microscopic capsules can be sprayed from a helicopter or conventional sprayer, and timed-release spray canisters are attached to stakes and placed in the field by hand. All of these controlled-release devices allow small amounts of pheromone to escape gradually into the air around the crop.

For mating disruption of the blackheaded fireworm of cranberries, we began with PVC spiral dispensers, and are now testing microscopic capsules (Fitzpatrick) and timed-release spray canisters (Baker and Mafra-Neto).

Before going into more detail about the cranberry system, I would like to explain how synthetic pheromone, emanating from controlled-release devices, may disrupt mate location in moths. In the following discussion, you will notice that the words "may", "might", and "possibly', are often used. There are still many unsolved mysteries in our understanding of how mating disruption works.

**Reduced Responsiveness due to Sensory Adaptation or Habituation.** Male moths exposed to high, uniform concentrations of pheromone in the lab stop responding to females emitting pheromone. This may be because the sensory cells on their antennal sensillae become adapted and stop responding to the small amounts of pheromone emitted by females, or because the decision-making part of the brain gets habituated (overloaded) and no longer recognizes female pheromone. Adapted or habituated males are likely to stop searching. Sensory adaptation or habituation is similar to what happens to us when we walk into a kitchen filled with the aroma of baked goodies. At first the aroma is very strong but, after some time in the room, we cease to notice it.

**Camouflage of the Female's Pheromone Plume.** Synthetic pheromone in the air may camouflage the filamentous structure or the concentration of odor molecules in the female's natural pheromone plume. Camouflage renders the natural pheromone plume indistinguishable from the background of synthetic pheromone. Males continue to search, but cannot find the females.

**False Trail Following.** When there are many controlled-release devices in the field, each one emitting pheromone, there may be many false pheromone trails for males to follow. Males are able to locate the pheromone plumes, but their zigzagging flight takes them to controlled-release devices instead of females. Close to the device, the high concentration of pheromone may cause sensory adaptation or habituation, and the male may stop, clean his antennae and sit quietly for a while.

**Imbalance of Sensory Input.** When an incomplete pheromone blend is used to disrupt mating, some of the males' sensory cells may become adapted while the others continue to function. Thus the males may not be able to accurately perceive the natural pheromone blend released by a female. For example, the blackheaded fireworm pheromone contains at least three components, but we use only the main one as a mating disruptant. The sensory cells that are designed to perceive the main component may receive so many odor molecules that those cells become adapted and stop responding. A male downwind from a calling female would then perceive only part of her chemical signal, that is, the part containing the second and third pheromone components. He would not recognize this as a pheromone, and would not fly upwind toward the female. Another possibility is that all his sensory cells might continue to receive information, but he wouldn't recognize female pheromone because the ratio of odor molecules from the three components would be skewed.

## Species and Systems for which Mating Disruption is Likely to Work

Mating disruption is most likely to be a successful management technique for insects that have one kind of host plant. For such insects, if mating can be disrupted in fields containing their host plant, there is no chance that mated females will fly in from nearby fields containing other host plants. For example, if mating of blackheaded fireworm moths on a cranberry farm can be prevented, we can be sure that there will be no mated fireworm females in the pasture next door. However, if we were to attempt mating disruption of cranberry girdler on the same cranberry farm, mated girdler females from the pasture would probably fly onto the farm and lay eggs. Thus, even though mating of cranberry girdlers was disrupted on the farm, there would still be damage from cranberry girdler larvae.

Following the same reasoning, mating disruption of blackheaded fireworms on a farm is more likely to succeed if the neighbouring farms also use mating disruption or have very low fireworm populations. "Area-wide" mating disruption reduces the likelihood that mated females will fly into disrupted areas and lay eggs.

Mating disruption is most likely to work where populations of the target insect are low to moderate. Where populations are high, males and females are in close proximity and males may not have to follow pheromone plumes to find females. If they are very close, males may simply see or hear the females, approach and perform their courtship behaviors. Or, there may be so many females emitting pheromone that males have a good chance of following a pheromone plume to a female rather than to a controlled-release device.

Systems where crops are grown on even terrain and have little three-dimensional structure should allow pheromone from controlled-release devices to permeate most of the air above and around the plants. Crops on slopes are often subject to upslope winds in the morning and downslope winds late in the day. Winds can move the pheromone around and leave sections of the field or orchard unprotected. Similarly, the tops of trees may be left unprotected if controlled-release devices are placed in the lower part of the canopy.

The nature of the cranberry system and the monophagous (single host) lifestyle of the blackheaded fireworm lend themselves well to the mating disruption technique, so long as fireworm populations are low to moderate.

**Species for which Mating Disruption is Successful**

Mating disruption is being used to control pink bollworm on cotton in California, Arizona and Egypt; the oriental fruit moth in the United States, Europe, Australia and Brazil; tomato pinworm in the United States and Mexico; the lightbrown apple moth in Australia and New Zealand; the currant clear-wing moth in New Zealand; the European grape moth and grape vine moth in Europe; the grape berry moth in North America; codling moth in Europe and North America; and some leafrollers in orchards in Europe and North America. Mating disruption of other agricultural and forest pests is being tested in North America, Europe, South America and other parts of the world.

**Mating Disruption of Blackheaded Fireworm in Cranberries**

In 1992, my research team and I did the first, small-scale experiments on mating disruption of blackheaded fireworm in cranberries. We began by using PVC spirals releasing either the three-component blend of synthetic pheromone chemicals or the single, main component. Over the next three years we continued our tests in British Columbia, and came to three main conclusions. First, the spirals were great for small research plots but too labor-intensive for farms over five acres. Second, the single, main component disrupted mating as effectively as the three-component blend, which was good because formulating one component should be cheaper than formulating three. Finally, we concluded that mating disruption was a very promising technique for blackheaded fireworm in cranberries.

In 1996, a sprayable formulation of blackheaded fireworm pheromone became available, and we did tested it on a large scale in Wisconsin and Washington. This sprayable, microencapsulated formulation contains the single, main component of fireworm pheromone enclosed in microscopic, polyurea-based capsules. The formulation, called "MEC" for MicroEnCapsulated, can be applied by helicopter, fixed wing aircraft, mist blower or through the sprinklers. Alongside our tests of MEC, Drs. Baker and Mafra-Neto tested Metered Semiochemical Timed Release Systems (MSTRS): canisters of pheromone released at timed intervals. Both the MEC and the MSTRS gave very good results.

Before I discuss results of the MEC tests, I will explain how tests of mating disruption are evaluated. Earlier, I said that mating disruption is "knowledge-based technology". I've explained that we need to know the host range and population density of the target insect. We especially need to know how to tell if mating disruption is working.

**Evaluating Tests of Mating Disruption.**

**1. Caged Females.** After controlled-release devices have been applied to the field and synthetic pheromone is being released into the field, it would be great if we could capture wild females and see if they were mated. (Males transfer a spermatophore full of sperm during mating; this can be found in mated females.) However, to capture a representative number of wild females, we'd have to spend days walking all over the field during bloom and early fruit set. So the next best technique is to place virgin, lab-reared female moths in tiny cages that allow males to enter, and put the cages in the field for three or four days. The females are then brought back to the lab, killed quickly by freezing, and dissected to see if they contain spermatophores. We do this once or twice a week for the duration of the fireworm flight period, and compare the percentage of mated females in the pheromone-treated fields with the percentage in control fields (not treated with pheromone). If the pheromone treatment is effectively disrupting mating, the percentage of mated females in the treated field will be zero, or very low compared to the percentage in the control field.

The only loophole in this evaluation technique is that the females are mostly surrounded by a plastic cage. Much of the pheromone they release may stick to the plastic of the cage and their pheromone signal may be much weaker than that of wild females. Thus, this technique may be evaluating whether males can locate hard-to-find females in a pheromone-treated field. It may not be telling us much about whether males can find nearby females releasing normal amounts of pheromone.

**2. Pheromone Traps.** Another technique for evaluating mating disruption is to compare the number of males caught in sticky-bottomed pheromone traps in pheromone-treated field with the number caught in control field. If mating disruption is working, the number of males caught in the treated field will be zero, or very low (1-5%) compared to the number in the control field. The pheromone traps contain a rubber septum impregnated with a high concentration of the blend of three components of fireworm pheromone. In a sense, these traps are super-females. The assumption in this evaluation technique is that, if males can't find the pheromone lure in the trap, they can't find females either. However, if the pheromone lure is very concentrated, the trap's signal may be detectable over the background of synthetic pheromone even when a female's signal is not. Therefore, we take care to use pheromone lures that are about as readily located as caged females. We are also aware that, although the pheromone blend in the lure is our best copy of the natural blend, a female may be more attractive.

**3. Number of Larvae.** Another way of evaluating mating disruption is to compare the number of fireworm larvae in the pheromone-treated field with the number in a control field. This is a difficult thing to do on cranberry farms. A thorough evaluation of the number of larvae, or population density, in a field requires that samples be taken (visually or by sweep net) from all parts of the field. This involves much walking at the bloom and early fruit development stages. Also, insecticides are usually applied to kill larvae before they get very big. This is a good management practice, but makes it difficult to estimate the number of larvae in a field. One of Dan Mahr's students has shown that there is no correlation between the number of very young larvae picked up in a sweep-net sample and the actual number in a field. There is a correlation between the swept and actual numbers of older larvae (third, fourth and fifth instars).

**4. Combination of Methods.** The best evaluation method is to use techniques l-3, and use the same fields for about three years. If the synthetic pheromone treatment is effectively disrupting mating we should see a progressive decrease in, or continual low numbers of, mated caged females, males caught in pheromone traps, and larvae relative to the control field. Several fields should be treated with pheromone, and several should be left as controls. Management practices should be similar in each pair of treated and control fields.

**Results from Tests of Mating Disruption using "MEC" in Wisconsin. 1996.**

We tested MEC on three farms in Wisconsin in 1996. All were somewhat isolated, and had what we believed to be low to moderate populations of blackheaded fireworm. We had really excellent cooperation from all three growers. Drs. Baker and Mafra-Neto tested their MSTRS on the same farms, and will be discussing their results separately.

On one of the farms, it turned out that fireworm populations were a little too high for control by mating disruption. On another, we applied the MEC a little too early to give good control all the way through the first flight. On the third farm, populations were moderate and the timing of MEC applications was better. I will be discussing results from this third farm, which I will call Farm 1, because I think they illustrate many of the considerations important to the successful implementation of mating disruption.

On Farm 1, we chose four fields totalling 11.8 acres to receive MEC, and one 3.4-acre field as a control. The control field was upwind and across a road from the fields to be treated with MEC, to minimize the chance that pheromone would drift from the treated fields to the control. Our objective was to put on one application of MEC in the spring to disrupt males in the first flight of moths, and a second application to disrupt males in the second flight.

MEC was applied by helicopter on June 12, at a rate of 180 milliliters of product containing 36 grams of active ingredient (pheromone) per acre. We were a little early with the MEC application. Ideally, it should go on when the first males are caught in pheromone traps. The spring of 1996 was unseasonably cool, and moth flight began two to three weeks later than normal. We were hedging our bets by applying the material when we did, but we wanted to be early rather than late. As it was, larvae were still in the field at the time of pheromone application. An insecticide to control fireworm larvae was applied to all fields on the same day that pheromone was applied to the four chosen fields.

From June until September, we evaluated mating disruption by using pheromone traps and, in July and August, by sweeping for larvae. Now I've just finished telling you that it's best to combine three evaluation methods: caged females, pheromone traps and sweeps for larvae. What I didn't tell you is that to have enough female moths at the right time, a full-time technician is required to maintain the fireworm colony and provide a large number of females every week. So we compromised, and used pheromone traps and sweeps. I am confident that the data we obtained from pheromone traps was similar to data we would have obtained from caged females. And we needed a full-time technician anyway! Tony Bonanno was kept extremely busy checking and changing pheromone traps on all three farms, as well as doing sweeps on all three farms for four weeks.

The first moths were caught in traps on June 20 (Figure 1). This marked the beginning of the first flight. Trap catches in the control field increased to a peak on July 3. From June 27 until July 11, the numbers of moths caught in pheromone traps in the MEC-treated fields were about 85-93% lower than in the control field. On July 14, as bloom was just finishing, an insecticide was applied to all fields.

MEC was reapplied on July 15, four weeks after the first application. We were finding out from our field tests in British Columbia and Washington that MEC lasted

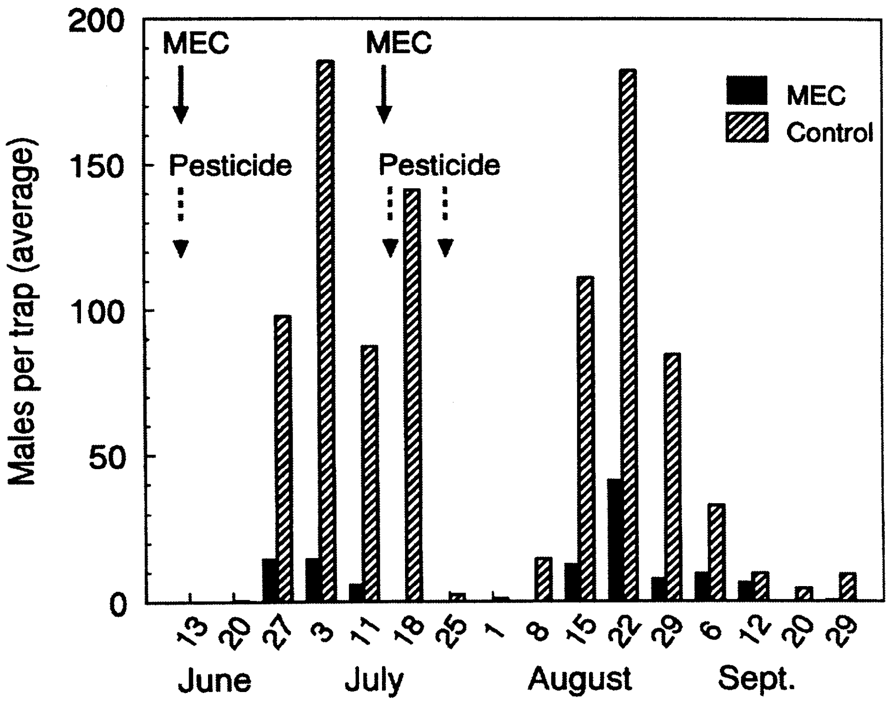


**Figure 1. Pheromone trap catches on Farm 1. Solid arrows show MEC applications; dotted arrows show insecticides.**

about four weeks in the field. Because there were still many moths coming to the pheromone traps in the control field, we knew that the first flight had not ended, and wanted to be sure that we had synthetic pheromone in the field for the entire duration of the first flight. On July 18, following the second application of MEC, trap catches in the MEC-treated fields plummeted to less than 1% of catches in the control field. This result is a very encouraging indication that the fresh MEC prevented males from finding pheromone traps and, we hope, from finding wild females in the field.

On July 25, larval populations in all fields were such that another application of insecticide was warranted. The insecticide brought the first flight of moths to an end. For the next three weeks, very few males were caught in any of the pheromone traps.

The second moth flight began on August 8 and continued until September 12. The second application of MEC probably lasted only until August 8 or 15, just as the second flight was climbing to a peak. Despite the MEC wearing off, pheromone-trap catches in the MEC-treated fields from August 22 until September 5 were 78-91% lower than catches in the control field. This reduction, while not enough to prevent subsequent fireworm populations from increasing, is still substantial. It suggests that MEC may have some residual activity beyond four weeks, and also suggests that there were fewer moths in the MIX-treated fields due to the disruption of mating during the first application of MEC in June and July.

It is interesting to note that the insecticide applications did not reduce the size of the second flight of moths in the control field relative to the first flight. I have no explanation for this, except to say that the insecticide does not seem to have done its job.

I would now like to discuss the pattern of moth captures in relation to the location of individual traps (Figure 2). In the control field, there were three traps: one at each end and one in the middle. The average number of moths caught per week was the same for each trap: 60. In the northernmost MEC-treated field, the average catch per week was 23 for the trap at the west end, 6 and 5 for the two traps in the middle, and 10 for the trap at the east end. In the MEC-treated field to the south of it, the average catch per week was 20 at the west end, 4 and 3 for the two traps in the middle, and 2 at the east end. In the other two MEC-treated fields, which were further east and south, the three traps caught an average of 1,3 and 4 moths per week.

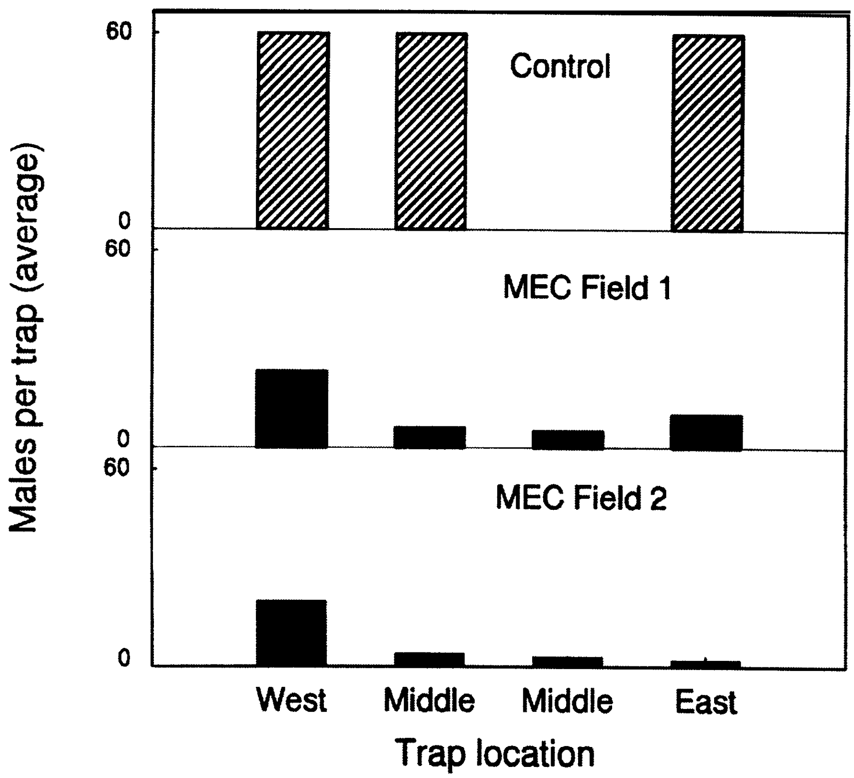


Figure 2. Pheromone trap catches according to trap location. Values are average totals for the season.

Of all the traps in the MEC-treated fields, those at the west end of the two northern- and westernmost MEC-treated fields caught the most moths. Why might that be?

The western ends of the MEC-treated bogs were next to a marsh. Therefore, it is probable that the helicopter pilot came in quite steeply to the west end of the cranberry fields in a effort to avoid spray drift to the marsh. If he did this with the MEC, he probably did it with insecticide as well, in 1996 and previous years. Thus there may have been a "hot spot" of fireworms at the west end of those two fields. Also, the prevailing wind in the area was usually westerly. Westerly winds would blow the MEC east, away from the west end of the field. Thus males at the west end may have been more able than those in other parts of the field to find the pheromone traps and the wild females.

Larvae showed up earlier in the control field than in the MEC-treated fields (Figure 3). The insecticide application on July 14 reduced the number of larvae in the control, but the number of larvae in the northernmost MEC-treated fields was relatively high, probably because the insecticide did not kill eggs that were ready to hatch. After the insecticide on July 25, almost no larvae were picked up in sweep nets in any of the fields. In the MEC-treated fields, larvae were swept from inner and edge sweeps. Visual inspection of the fields showed that there were "hot spots" of larvae under and around the pheromone traps. The most probable explanation for this is that the pheromone traps, which were covered with plastic bags during the insecticide and MEC application on June 12, acted as umbrellas over the cranberries and created small insecticide-free and MEC-free zones where larvae and moths could develop. In future, we will avoid having covered pheromone traps in the field during insecticide and MEC applications.

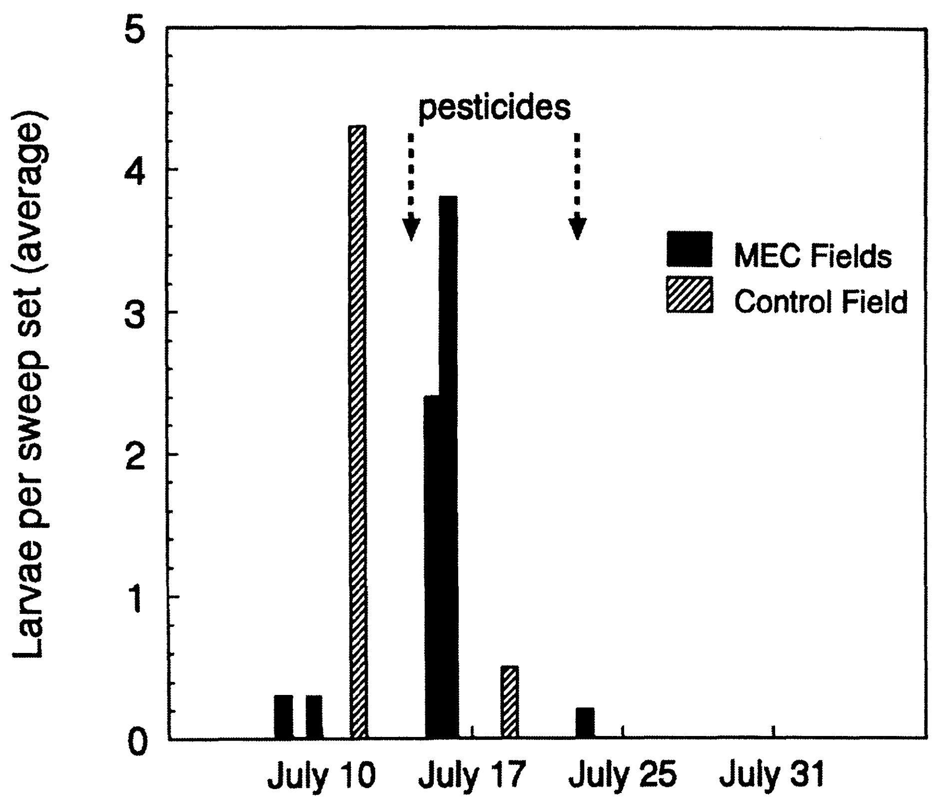


Figure 3. Average number of larvae per sweep set in MEC-treated fields versus the control field.

**Conclusions from MEC tests in Wisconsin.** In spring, MEC should be applied when pheromone traps catch the first fireworm moths. The second application of MEC should go on when pheromone traps catch the first moths of the second flight. I will be talking to the companies that make and sell the pheromone, to encourage them to extend MEC's "lifetime" beyond 4 weeks or to reduce the price of MEC so that it will be economical to use three applications if required. At upwind edges of fields, it may be most effective to use a combination of MEC and MSTRS (the timed-release canisters of pheromone tested by Drs. Baker and Mafra-Neto). Insecticides should be applied as usual in addition to MEC during the first year of mating disruption.

Mating of other moth pests, such as Sparganothis fruitworm, will not be disrupted by the MEC applied for blackheaded fireworm. Dr. Polaravarpu from Rutgers University, New Jersey, is working on mating disruption of Sparganothis and spotted fireworm. He and I will be coordinating our work so that management of the complex of moth pests in Wisconsin is achieved.

## Concluding Remarks

Mating disruption is a very promising non-insecticidal technique for management of blackheaded fireworm in cranberries, and is being used successfully on other species of moth pests in cotton, tomatoes, grapes and orchards. By attempting to prevent males from finding females, the technique disrupts behaviors that have evolved over millions of years and are vital to moth reproduction. For this reason, we know that implementation of mating disruption is not simply a matter of applying synthetic pheromone to a field and hoping it will work. We can expect that the moths will do everything they can to find each other through the miasma of synthetic pheromone.

We can improve the odds that mating disruption will work by eliminating "hot spots" of larvae and preventing high density patches of moths from occurring. We can ensure that MEC is applied to all areas of a field, and perhaps improve protection of upwind edges by using MSTRS (the timed-release canisters of pheromone tested by Drs. Baker and Mafra-Neto). Successful adoption and use of the mating disruption technique will ultimately depend on cooperation and sharing of knowledge between researchers, integrated pest management consultants, growers, pheromone companies and regulatory agencies.

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