Entomologia Experimentalis et Applicata **94:** 243–248, 2000.

Application of synthetic sex pheromone for management of diamondback moth, *Plutella xylostella,* in cabbage

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Accepted: November 16, 1999

Key words: diamondback moth, *Plutella xylostella*, sex pheromones, mating disruption

Abstract

Over a 2-year period field trials were conducted to assess the potential to disrupt mating of*Plutella xylostella* (L.) using a commercial rope formulation of a 70:30 mixture of (*Z*)-11-hexadecenal and (*Z*)-11-hexadecenyl acetate, two components of the sex pheromone of the female. Screened field cages were placed into blocks of cabbage which were either treated with the pheromone or left untreated. Different densities of *P. xylostella* pupae were placed into each cage and then larval and pupal counts were made of the subsequent generation. In addition, sentinel females at mating stations were placed in each cage to assess the influence of the pheromone on the ability of males to locate and mate with females. Likewise, we used pheromone traps to assess whether the pheromone treatment influenced the ability of males to locate a pheromone source. In both years larval and pupal populations, produced as a result of the original inoculation, did not differ between pheromone-treated and untreated fields. The effect of pheromone treatment on larval and pupal numbers did not change with changes in inoculated *P. xylostella* density, however, the density of *P. xylostella* released caused significant differences in the density of the subsequent generation. No significant differences were detected between the number of sentinel female adult *P. xylostella* that successfully mated in pheromone-treated fields compared with untreated fields. Significant differences in the numbers of male *P. xylostella* caught in pheromone-baited traps occurred between pheromone-treated and untreated fields in the first trial of 1993, and in the first trial in 1994 but not in the second trial. Such differences are often thought of as indications of mating disruption, although our other data presented in this study and reports from other studies indicate this is not always the case. Previous studies on mating disruption of *P. xylostella* in larger scale field tests have been performed but the results have been variable and often ambiguous. Overall, our results indicate that mating disruption of *P. xylostella* with the present technology does not appear to work even under the very controlled situations which we utilized to eliminate insect movement between plots.

Introduction

The diamondback moth (DBM), *Plutella xylostella* (L.), occurs wherever cruciferous crops are grown and is considered the most important insect pest of crucifers worldwide with an annual cost of management of $1 billion (Talekar & Shelton, 1993). Several factors contribute to management problems of this pest: high reproductive potential, wide range of alternative weed hosts, its dispersal ability and its demonstrated capacity to develop resistance to a wide range of insecticides. The increasing failure of insecticides to control *P. xylostella* has stimulated the development of alternative tactics including inoculative release of parasitoids, conservation of natural enemies, mating disruption using pheromones, and various cultural practices (Talekar & Shelton, 1993).

Studies in Japan (Ohbayashi et al., 1992; Ohno et al., 1992), Taiwan (Chow, 1992; Nemoto et al., 1992), Canada (Chisholm et al., 1984) have indi-

cated variable control with mating disrupton using synthetic sex pheromones. In a more recent report from Florida (McLaughlin et al., 1994) the authors suggest that a rope formation of a 70:30 mixture of (*Z*)-11-hexadecenal and (*Z*)-11-hexadecenyl acetate, two components of the sex pheromone, may provide some level of control in larger commercial fields.

Herein we report the results of field studies utilizing *P. xylostella* in field cages to assess mating disruption and population levels in the subsequent generation.

Materials and methods

Field studies were conducted on the New York State Agricultural Experiment Station (NYSAES) Fruit and Vegetable Research Farm in Geneva, New York. The *P. xylostella* used in these studies were collected in 1988 from an NYSAES farm and maintained in our laboratory (Shelton et al., 1991).

1993 Study. On 14 and 16 June, four 0.2 hectare fields (50 rows each 45 m long), each separated by ca. 0.4 km, were planted with cabbage, cv. 'Bravo'. Plants were transplanted as bareroot seedlings (2–3 leaves) spaced 0.9 m apart between rows and 46 cm apart within rows. When plants had 7–8 leaves, a polyethylene 'rope' dispenser (Shin-Etsu Chemical Co., Tokyo, Japan) containing a sex pheromone blend of the diamondback moth – a 70:30 mixture of (Z)-11-hexadecenal and (Z)-11-hexadecenyl acetate – was strung 36 cm above ground level along every tenth row (9.2 m apart) in two of the fields. The application rate of the rope for each field was 275 g A.I./ha., the standard commercial rate. The other two fields were left untreated.

The 1993 study was conducted in two successive trials with four fields per trial, two fields being treated with the pheromone rope and the other two serving as controls. Trial 1 ran between 6 July and 9 August and Trial 2 ran between 10 August and 15 September. In each trial, four polypropylene screened (5 threads per cm) cages (3.7 m $\times$ 3.7 m $\times$ 1.9 m high) supported by a frame were set up in each field. Cages prevented *P. xylostella* immigration or emigration. Cages were 20 rows apart within each field and, in the pheromone treated fields, each cage was centered on a rope line which ran through the cage. Cages enclosed 30 plants and cages were moved to new locations within each field between trials. Plants were inspected and any insects were removed prior to setting the cages in place.

In order to assess the potential for the pheromone to suppress mating and thereby subsequent larval populations, *P. xylostella* were added to each cage. On the first day of each trial, cages were infested with *P. xylostella* by placing pupae (just prior to emergence of adults) into each cage. Each of the cages was infested at a density of 0 (control), 1, 5, or 25 *P. xylostella* pupae per plant. Different densities were used to assess whether mating disruption and the subsequent generation would be influenced by the initial population density. This range of density can be encountered in commercial plantings.

Another measure of mating disruption within the cages involved using sentinel females at mating stations placed inside the cage. The day after peak adult emergence, 10 July (Trial 1) and 13 August (Trial 2), 17 mating stations each containing a 24-h-old virgin female *P. xylostella* were placed into each cage. Females had their wings clipped and were placed into clear plastic food containers (21 $\times$ 14 $\times$ 2.5 cm) coated with Teflon (TFE-Fluocarbon Resin Dispersion, DuPont, Washington, WV) to prevent their escape, and the containers were placed within the crop canopy. Female moths were gathered the next morning and individually placed into 30 ml plastic cups containing a 1 cm square aluminum sheet for an ovipositional substrate (Shelton et al., 1991) and a cotton wick soaked with 10% sucrose solution as a food source. Cups were then stored in an environmental chamber (21 °C, 60% r.h., L8:D16) and checked for viable eggs after 4 d. Female moths that laid viable eggs were considered as mated.

In order to assess the potential for the pheromone to suppress the larval and pupal populations, approximately 3 weeks after *P. xylostella* pupae were released in cages *P. xylostella* larvae and pupae were counted on 10 randomly selected plants in each cage. As a further measure of the ability of the pheromone rope to disrupt communication, once the larval and pupal counts were completed a 1-C wing trap (Trécé, Inc., Salinas, CA) baited with a rubber septum loaded with a synthetic sex pheromone (compositon and release rate are proprietary information) of *P. xylostella* (Pherocon^{TM}, Trécé, Inc., Salinas, CA) was placed inside each cage. After 3 days, the number of male *P. xylostella* caught in each trap was recorded.

The effect of pheromone application (main plot effect) and *P. xylostella* density (subplot effect) on mating success, numbers log $(x + 1)$ of *P. xylostella*

larvae and pupae on plants and pheromone-baited trap catches within the cages was analyzed using an analysis of variance (ANOVA) procedure with cages nested within fields. Trials were analyzed separately.

1994 Study. Between 15 June and 5 July, six 0.2 ha fields ca 0.4 km apart were planted with cabbage, cv. 'Cheers' in a fashion identical to the 1993 study. When plants had 7–8 leaves, pheromone rope was strung in three of the fields in an identical fashion as in 1993. Two cages were set up in each field. Cages were 20 rows apart within each field and, in pheromone treated fields, centered on a rope line. Each cage enclosed 30 plants and into each cage we placed either 1 or 25 *P. xylostella* pupae per plant.

Six days after the cages were infested, 12 mating stations were placed in each cage as in 1993. Female moths were collected the following day and checked for mated status as in 1993. Approximately 3 weeks after the *P. xylostella* release, *P. xylostella* larvae and pupae were counted on 10 randomly selected plants in each cage. Immediately following the larval and pupal counts, four 1-C wing traps, each baited as in the 1993 study, were placed in each field (not within the cages as in the 1993 study). Traps were placed outside the cages to assess whether the pheromone rope would affect catches of native insects. Each of the four traps in a field was positioned so that each trap was located in a cardinal direction and approximately 20–30 m from the center of the field. After 3 days, the number of male *P. xylostella* caught in each trap was recorded.

The effect of pheromone application (main plot effect) and *P. xylostella* density (subplot effect) on mating success, number log $(x + 1)$ of *P. xylostella* larvae and pupae on plants and pheromone-baited trap catches outside the cages was analyzed using an ANOVA procedure with cages nested within fields.

Results

Larval and pupal counts. Counts did not differ between pheromone-treated and untreated fields in either the 1993 (both trials) or 1994 studies (Table 1). Release rates in 1993 were 0, 1, 5 and 25 pupae per plant, and in 1994 release rates were 1 and 25. To visually illustrate the populations which resulted from these pupal infestations in the cages the data are presented in Figure 1, but as categories of low (1 and 5) and high (25) initial pupal inoculations. Figure 1, combined with the lack of significant interaction between *P. xylostella* density and pheromone treatment shown in Table 1, indicates that the pheromone treatment had no effect on larval and pupal numbers regardless of *P. xylostella* density. However, the density of *P. xylostella* pupae released caused significant differences in the subsequent generation in both the 1993 (both trials) and 1994 studies (Table 1). Greater densities of *P. xylostella* pupae released resulted in more *P. xylostella* larvae and pupae on the plants in the next generation. There were differences in the number of larvae and pupae generated from the high release rate (25) between 1993 and 1994 trials, but in neither year did the pheromone significantly decrease the population.

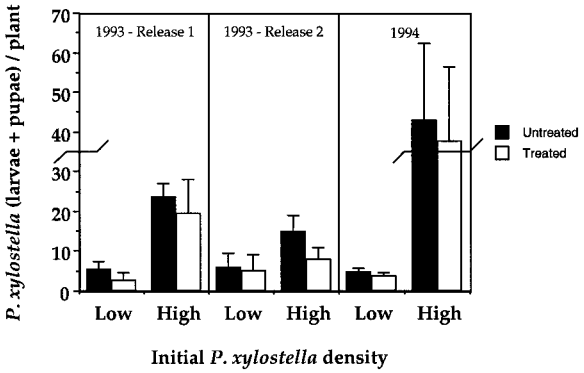

Figure 1. Mean number of *P. xylostella* larvae and pupae on plants in cages containing low and high (see text) augmented densities of *P. xylostella* in treated (white columns) and untreated (black columns) fields. Vertical lines are standard error bars.

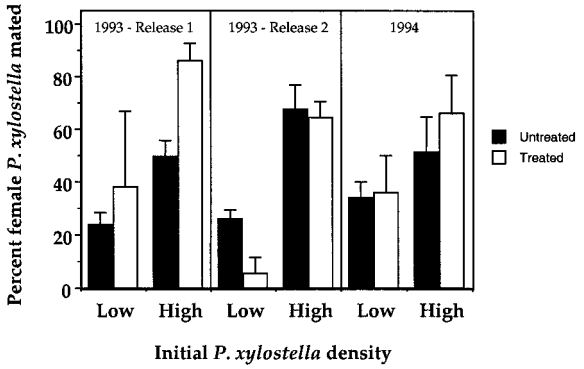

Figure 2. Mean percent of sentinel female *P. xylostella* mated in cages containing low and high (see text) augmented densities of *P. xylostella* in treated (white columns) and untreated (black columns) fields. Vertical lines are standard error bars.

Mating disruption. No significant differences were detected between the number of sentinel female adult *P. xylostella* that successfully mated in pheromone-treated compared with untreated fields in either the

Table 1. Analysis of variance for the effect of pheromone rope (Rope) and *P. xylostella* density (Density) on numbers of *P. xylostella* larvae and pupae recorded on cabbage plants

Source	1993 (release 1)			1993 (release 2)			1994		
	df	F		df	F		df	F	
Rope	1	2.29	NS	1	0.30	NS	1	1.96	NS
Error A	2			2			4		
Density	2	16.54	**	3	10.18	**	1	68.06	**
Density × Rope	2	0.29	NS	3	0.78	NS	1	0.02	NS
Error B	4			6			4		

**, significant at P = 0.01. *, significant at P = 0.05; NS, not significant at P = 0.05.

Table 2. Analysis of variance for the effect of pheromone rope (Rope) and *P. xylostella* density (Density) on mating success of *P. xylostella*

Source	1993 (release 1)			1993 (release 2)			1994		
	df	F		df	F		df	F	
Rope	1	0.31	NS	1	6.77	NS	1	0.36	NS
Error A	2			2			4		
Density	2	5.27	NS	2	14.10	*	1	5.40	NS
Density × Rope	2	3.07	NS	2	1.74	NS	1	0.39	NS
Error B	4			4			4		

**, significant at P = 0.01. *, significant at P = 0.05; NS, not significant at P = 0.05.

1993 (both trials) or 1994 studies (Table 2 and Figure 2 in which the data are again presented as 'low' (0 release rate excluded) and 'high' initial infestations). Density of *P. xylostella* released caused a significant difference between the mating success of female moths only during the second trial in 1993, where greater densities of *P. xylostella* caused a greater percentage of the stationed females to be mated. The ANOVA revealed no significant interaction between *P. xylostella* density and pheromone treatment, suggesting that the effect (if any) of pheromone treatment on mating success did not change with changes in *P. xylostella* density.

Trap catches. Significant differences in the numbers of male *P. xylostella* caught in pheromone-baited traps occurred between pheromone-treated and untreated fields in the first trial of 1993, and in the first trial in 1994 but not the second trial (Table 3 and Figure 3 in which the data are again presented as 'low' (0 release rate excluded) and 'high' initial infestations). In the first trial of 1993 (pheromone traps placed inside the cage) and in 1994 (pheromone traps placed outside the cage) the numbers of male *P. xylostella* trapped were substantially greater in untreated fields than in pheromone-treated fields. In the 1993 trials no significant interaction between *P. xylostella* density and pheromone treatment was detected, suggesting that the effect of pheromone treatment on male *P. xylostella* trap catches did not change with changes in *P. xylostella* density. In 1994 the pheromone traps were placed outside the cage so this interaction was not tested.

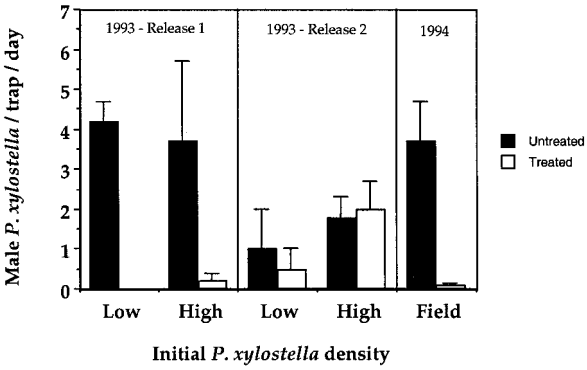

Figure 3. Mean numbers of male *P. xylostella* caught per trap per day in cages containing low and high (see text) augmented densities of *P. xylostella* in treated (white columns) and untreated (black columns) fields. Traps were placed outside the cages in 1994. Vertical lines are standard error bars.

Table 3. Analysis of variance for the effect of pheromone rope (Rope) and *P. xylostella* density (Density) on numbers of male *P. xylostella* caught in 1-C wing traps baited with a commercial synthetic pheromone

Source	1993 (release 1)			1993 (release 2)			1994		
	df	F		df	F		df	F	
Rope	1	21.01	*	1	0.53	NS	1	8.46	*
Error A	2			2			4		
Density[a]	3	0.68	NS	3	4.22	NS	–	–	
Density × Rope[a]	3	0.22	NS	3	0.51	NS	–	–	
Error B	6			6			–		

**, significant at P = 0.01. *, significant at P = 0.05; NS, not significant at P = 0.05.
[a]The effect of density and the interaction between *P. xylostella* density and pheromone treatment could not be tested in 1994 because traps were placed outside the cages.

Discussion

It is difficult to evaluate mating disruption in field trials since the pheromone and insects may move freely between plots and this often limits the ability to have replications and untreated controls. In our study we used cages to control several variables in order to assess whether pheromone disruption would inhibit mating and thus decrease the size of the subsequent generation. Under these 'best case' conditions in our trials we were not able to detect any mating suppression or decrease in the population of the subsequent generation. This occurred regardless of the density of the insects within the cage. In our study there may be some question about the use of a laboratory colony of *P. xylostella* to assess pheromone disruption. However, we argue that these insects were fit enough to mate in the field cages and produce a subsequent generation and that they probably used pheromone communication to locate their mates.

In probably the largest trial to assess pheromone disruption of *P. xylostella*, McLauglin et al. (1994) conducted a nonreplicated trial on a commercial farm in Florida. From this study they suggest their data indicate that marketable cabbage could be grown using mating disruption technology, but there are also indications that such a suggestion may be premature. In their trials, as in our trials, male captures in pheromone-baited traps were reduced, but because of the inefficiency of the traps they are not always a good indicator of absolute mating disruption (Roelofs et al., 1979). Also at times there was considerable mating of sentinel females in their trials as well as the occurrence of mated females in the pheromone block, a further indication that mating may not have been suppressed to the degree required. Additionally, natural enemies may have played a role in pest suppression in the pheromone treated plot since it received only three insecticide applications compared with the control plot which received 13 applications. At least one of the materials used in that study, permethrin, has been shown to greatly reduce predator populations and thereby increase survival of another lepidopteran pest of cabbage, *Pieris rapae* (M. Schmaedick & A.M. Shelton, unpubl.). Furthermore, the authors note that it is often necessary to protect the outer 'wrapper' leaves since cabbage is often marketed with them, and that the pheromone treatment did not protect the wrapper leaves to the extent required.

The results in Florida are evidence of the difficulty in conducting pheromone disruption trials for highly mobile pests. While it is necessary to conduct such tests in real world settings, they are often difficult to interpret. The tests reported in our trials were much smaller in scale but provide some evidence that the present technology may not provide sufficient control of *P. xylostella* in the field.

Acknowledgements

We thank H. Dann, L. Ferris, D. Hoobler, J. Weyant, W. Wilsey for technical assistance; the Plant Genetics Resource Unit, NYSAES, Geneva, NY, for loaning us the cages; the Shin-Etsu Chemical Co., Tokyo, Japan, for donating the synthesized *P. xylostella* pheromone and 'rope' dispensers, and; W. Roelofs, C. Linn, M. Schmaedick and J. Tang for their helpful critique during the design of this study. We also thank C. Linn, M. Schmaedick, J. Tang, and

R. Vickers for reviewing earlier versions of this manuscript and J. Barnard for his statistical help. This project was funded by Northeast Regional Integrated Pest Management Grant #93-34103-8425.

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