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Synthesis and assessment of attractiveness and mating disruption efficacy of sex pheromone microcapsules for the diamondback moth, *Plutella xylostella* (L.)

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Microcapsules of sex pheromone of the diamondback moth (DBM), *Plutella xylostella* (Lepidoptera: Plutellidae) were synthesized through complex coacervation using gelatin and gum arabic as wall-forming materials. The encapsulated pheromone released from these microcapsules into the air was monitored over six weeks in the field. Results of a field trial show that the attractiveness of microcapsules was superior to that of rubber septa loaded with the same amount of pheromone. The mating disruption efficiency of the pheromone treatment was estimated through comparing numbers of moths captured in pheromone-baited traps placed in pheromone treatment fields and pesticide treatment fields. The estimated mating disruption efficiency of the pheromone treatments ranged from 76.94% to 98.67% during the season. This study shows that pheromone microcapsules might provide a new method for *P. xylostella* control.

Plutella xylostella, sex pheromone microcapsules, synthesis, attractiveness, mating disruption

The diamondback moth (DBM), *Plutella xylostella* (Lepidoptera: Plutellidae), is the most destructive insect of cruciferous plants throughout of the world[1]. Synthetic sex pheromones of the diamondback moth are proving to be a viable alternative to conventional pesticides for management of this pest in cruciferous vegetable protection programs. For example, sex pheromones are effective for population monitoring[2−5], mass trapping[1,6−9], and mating disruption[10−15]. The major components of *P. xylostella* sex pheromones have been identified as a mixture of (*Z*)-11-hexadecenal (Z11-16:Ald), (*Z*)-11-hexadecenyl acetate (Z11-16:Ac), and (Z)-11-hecadecen-1-ol (Z11-16:OH)[16−20]. Among these components, (*Z*)-11-hexadecenal is unstable in air[21]. Hollow fibers, rubber septa and wax emulsions are some of the devices commonly used to deliver the pheromone throughout the mating period of the insect[22]. Several studies have used microcapsules to encapsulate insect mate attractant pheromones, and this method has been evaluated for efficacy of control of *Lymantria dispar*[23], *Choristoneura fumiferana*[24], and *Platyedra gassypiella*[25], among other species. The microencapsulated formulation of synthetic sex pheromone for *P. xylostella* has not been developed or evaluated as an attractant. To date, the most common methods of microencapsulation for insect pheromones have involved coacervation processes and interfacial polycondensation. However, aldehydes cannot be encapsulated satisfactorily without some loss of active ingredient by interfacial polycondensation processes involving aliphatic polyamines, since the latter reacts with the aldehydes during the encapsulation process and during storage. In addition, alcohols cannot be encapsulated by existing interfacial

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methods because they react rapidly with the eletrophilic monomers[26]. Thus, coacervation processes were chosen as the preferred method of microencapsulation for the sex pheromones of *P. xylostella*.

Here we report the synthetic process of microencapsulation of *P. xylostella* sex pheromone through complex coacervation with gelatin and gum arabic used as wall-forming materials. We evaluated the attractiveness to diamondback moths of pheromone microcapsules compared to that of rubber septa by capture success in field monitoring traps. We also estimated mating disruption efficiency of the pheromone microcapsule treatment.

1 Materials and methods

1.1 Materials

Gelatin (isoelectric point at pH 8.0) and gum arabic were purchased from ACROS Organics, New Jersey, USA. Tannic acid was purchased from Tianjin Fuchen Chemical Reagent Factory, China. 25% glutaraldehyde solution ($C_5H_8O_2$), polyvinyl alcohol (PVA, 87%, hydrolyzed, 100000 Da) and isopropyl alcohol were purchased from Beijing Gaili Fine Chemicals Co. Ltd., China. Aqueous sodium hydroxide (0.05 N) and acetic acid (0.05 N) were purchased from Beijing Beihua Chemical Co. Ltd., China. Z11-16:Ald (98%), and Z11-16:Ac (98%) and Z11-16:OH (97%) were purchased from Shin-Etsu Chemical Co., Ltd., Japan. Talcum powder (< 30 μm) and rubber septa (green, 1.5 cm×0.8 cm) were obtained from the Institute of Zoology, Chinese Academy of Sciences.

1.2 Encapsulation procedure

The primary encapsulating solutions were made by dissolving 2.5 g of gum arabic and 2.5 g of gelatin separately in 100 mL of distilled water at (20 ± 1)℃ and allowing the solutions to stand for 30 min in a 200-mL beaker. The aqueous solutions were warmed in a water bath at (50 ± 1)℃ and gently stirred until complete dissolution. Gum arabic solution was then poured into a 500-mL flask, and 4 mL (5 mg/mL) of *P. xylostella* synthetic sex pheromone (Z11-16:Ald, Z11-16:Ac and Z11-16:OH 50∶50∶1) dissolved in hexane, 20 mL polyvinyl alcohol solution (1%) and the gelatin solution were added sequentially. The mixture was homogenized by stirring with an electromagnetic beater at 500 r/min for 20 min. The pH of the mixture was adjusted to 4.3 by the dropwise addition of 10% acetic acid solution while stirring at 500 r/min for 20 min; this procedure caused coacervate droplets to form and encapsulate the emulsified oil. Two drops of Tween-20 were added to aid emulsification. Two grams of talcum powder were added 30 min later (to act as a solid dispersant), then 10 mL 25% aqueous gluteraldehyde solution was added to induce the process of cross-linking and solidification at 200 r/min for 1 h. Next, the mixture was cooled to 5℃ by immersion in an ice bath. The final pH was then adjusted to 9.0 by the dropwise addition of 10% sodium hydroxide solution. The mixture was warmed up to 25℃ in a water bath and 10% (*w*/*w*) tannic acid was added to further cross-link and solidify. The resulting dispersion of microcapsules was stirred at 200 r/min for a further 30 min, then diluted to 400 mL with distilled water and filtered through filter paper into a Buchner funnel (100 mm diameter). The precipitate was washed with water and isopropyl alcohol two times, respectively. The wet mass was dried in a vacuum oven at 20℃－25℃ for 24 h, after which the microcapsules (5.95 g) were graded using a series of sieves for uniformity of size in the range of 100－150 μm diameter. These microcapsules were then stored in a glass bottle at 0℃－5℃.

1.3 Encapsulation efficiency

10 mg microcapsules were crushed by sonication[22] and extracted with 2 mL hexane (with 20 μg E10-16:Ald as internal standard). The encapsulated pheromone was then dissolved in hexane and the quantities of the pheromone components remaining in the samples were measured by gas chromatography (GC) on a Hewlett Packard 5890 series II gas chromatograph using a capillary column (DB-WAX, 30 m×0.25 mm×0.2 μm) with a flame ionization detector (FID) and a splitless injector using nitrogen as the carrier gas. The GC conditions were as follows: the column temperature started at 80℃ (holding for 5 min), rose to 210℃ at 10℃/min, then held at 210℃ for 15 min. The encapsulation efficiency was calculated by the formula:

Encapsulation efficiency=(pheromone entrapped with microcapsules) × total weight of microcapsules×100/(initial weight of pheromone)[27].

There were 5 replicates of the treatment.

1.4 Release measurement

Sixty centrifuge tubes (1.5 mL, with a hole of 1 cm di-

ameter in the cap) filled with 10 mg microcapsules each were placed in a field 1 km away from the field in which trapping experiments were conducted, i.e., these tubes were exposed to the same conditions (temperature, wind speed and humidity) experienced in the field trapping experiments. At 4 d intervals (initial 2 d interval), 5 centrifuge tubes were randomly selected (5 replicates), crushed by ultrasonic disintegrator (Soniprep 150, pulse time, 15 s) for 5 min, and extracted with 2 mL hexane (with 20 μg E10-16:Ald as internal standard). The residual encapsulated pheromone was then dissolved in hexane and the quantities of the pheromone components remaining in the samples were measured by gas chromatography (as described above).

The amounts of residual active ingredients in microcapsules were calculated over a period of 6 weeks (from 14 August to 25 September in 2005). The percentage retention of all analytes was calculated by the formula: Retention = analyte remainning at 'X' release time×100/ analyte at zero release time[28].

1.5 Attractiveness assessment

Field tests were conducted in a broccoli (*Brassica oleracea* L. var. *italica* Plenck) cultivation area between 14 August and 17 September 2005 and in a cabbage (*Brassica oleracea* L. var. *capitata* L.) cultivation area between 6 June and 6 July 2006 in Pinggu County, Beijing, China.

The dispensers used were plastic centrifuge tubes filled with microcapsules (30 mg, equal to one rubber seprum). Rubber septa (Z11-16:Ald, Z11-16:Ac and Z11-16:OH 50∶50∶1 μg) and blank untreated traps were used as control treatments.

In 2005, 15 water traps (diameter 10 cm) were filled with a dilute detergent solution and fitted with a plastic roof. Treated dispensers were hung from the roof above the surface of the water. The traps were placed in the fields 30 cm above the crops and approximately 17 m apart. The number of male moths in the traps was counted every 2 d from 1 June to 1 July after each pheromone application. There were 5 replicates of each treatment.

In order to assess the shelf life of microcapsules and rubber septa, these dispensers were tested after one year of storage at 0℃－5℃. Aged dispensers were compared with newly formulated microcapsules and rubber septa. Twenty water traps were set up and fitted with either rubber septa control dispensers or plastic centrifuge tubes filled with 30 mg microcapsules (equal to one rubber septum) in a fashion identical to the 2005 study. The number of male moths in the traps was assessed every 2 d for 1 month after each pheromone application. There were 5 replicates of each treatment.

1.6 Mating disruption assessment

Mating disruption trials with microencapsulated formulations were carried out in Pinggu County, Beijing, China from 2 June to 2 July 2006 in a cabbage (*Brassica oleracea* L. var. *capitata* L.) cultivation area. Pheromone dispensers (30 mg microcapsules/centrifuge tube) were attached to a bamboo pole approximately 1.0 m from the ground at intervals of 4－5 m at the rate of 18 g a.i./ha 7 d after the first spring emergence of *P. xylostella* (28 May, 2004; 31 May, 2005). For suppression of *P. xylostella* population density, *Bt* (0.6 *l* a.i./ha) was used one time after the first peak of *P. xylostella* (9 June) in the mating disruption fields. Plots sprayed with chlorfluazuron (0.6 kg a.i./ha) 4 times (9 June, 14 June, 20 June, 27 June) were used as controls. The trial was laid out in a randomized complete block design with five replications (0.02 ha) of each treatment. The distance between the mating disruption field and the chemical insecticide control field was over 1 km. A plastic water trap baited with synthetic pheromone was positioned at the center of each plot. The effectiveness of mating disruption was measured as the reduction in capture of male moths in the traps in the treated plots relative to capture in similar traps in untreated, control plots.

1.7 Statistical analysis

Data were analyzed in SPSS 11.01 and differences between means were tested for significance at the 5% level by Tukey's test or Paired-samples *t* test.

2 Results

2.1 Encapsulation efficiency

The encapsulation efficiency of Z11-16:Ald, Z11-16:Ac and Z11-16:OH were 95.76%, 89.60% and 95.21% respectively (Table 1). This shows that the three components of sex pheromones of *P. xylostella* were encapsulated efficiently through this microencapsulation process.

2.2 Persistence assessment

The percentage of active ingredients remaining in microcapsules after 6 weeks of direct exposure in the field (Table 2) is shown in Figure 1. These data show

Table 1 Encapsulation efficiency of synthetic pheromone of *P. xylostella*

Pheromone	Weight in sample microcapsules (10 mg) ± SE (μg)	Weight in total microcapsules (5.95 g) ± SE (mg)	Weight of initial (mg)	Encapsulation efficiency ± SE (%)
Z11-16:Ald	15.94 ± 0.03	9.48 ± 0.01	9.90	95.76 ± 0.13
Z11-16:Ac	14.9 ± 0.02	8.87 ± 0.01	9.90	89.60 ± 0.08
Z11-16:OH	0.32 ± 0.01	0.19 ± 0.01	0.20	95.21 ± 0.01

Table 2 Temperature and relative humidity during the experiment in 2005[a)]

Date	Temperature (℃)			Relative humidity (%)		
	maximum	minimum	average	maximum	minimum	average
14－21 Aug	35.0	24.0	28.8	100.0	61.0	79.5
22－28 Aug	33.0	23.0	26.5	97.0	54.0	78.0
29 Aug－4 Sep	33.0	21.0	25.7	94.0	47.0	76.0
5－11 Sep	32.0	19.0	23.4	96.0	57.0	75.0
12－18 Sep	30.0	14.0	21.6	91.0	52.0	73.0
19－26 Sep	29.0	13.0	20.3	89.0	40.0	68.5

a) Only light rain in the evening of 14 Aug, the other days were generally sunny during the experiment.

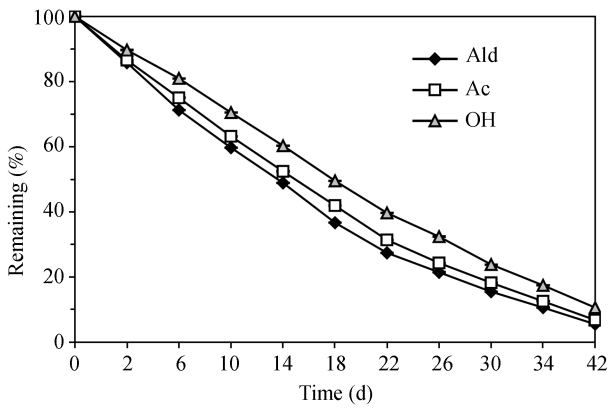

Figure 1 Persistence of pheromone components of *P. xylostella* entrapped in microcapsules.

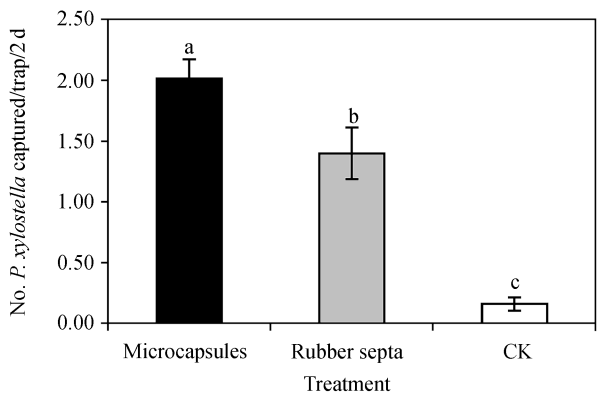

Figure 2 Means of *P. xylostella* captured with different treatments. Numbers in columns followed by the same letters do not differ significantly at $P<0.05$ (one-way ANOVA followed by Tukey's test, SPSS 11.01).

that after a very high initial pheromone release rate on days 0－2, the microcapsules had a fairly constant rate of pheromone release later in the season.

2.3 Attractiveness assessment

In the 2005 and 2006 trials, with different *P. xylostella* density in different seasons, traps baited with the microcapsules dispenser captured more moths than did rubber septa (Figures 2 and 3), and the microcapsules remained attractive throughout the entire season (Figures 4 and 5).

In the 2006 trial, the average number of captured moths was significantly different at $P<0.05$ not only between the 'new' microcapsules and the 'old' microcapsules but also between the 'old' microcapsules and the 'new' rubber septa (Figure 3). It appears that protection of pheromone is superior in the former dispenser type during long-term storage.

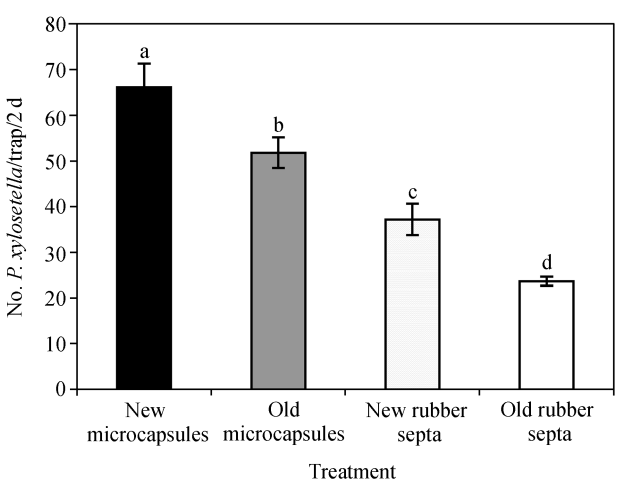

Figure 3 *P. xylostella* caught in traps baited with two types of 'new' and 'old' dispensers. Numbers in columns followed by the same letters do not differ significantly at $P<0.05$ (one-way ANOVA followed by Tukey's test, SPSS11.01).

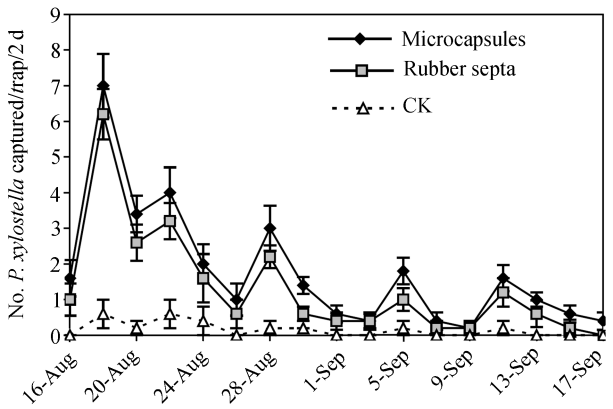

Figure 4 Season-long capture in traps with different treatments in 2005. CK, blank untreated traps.

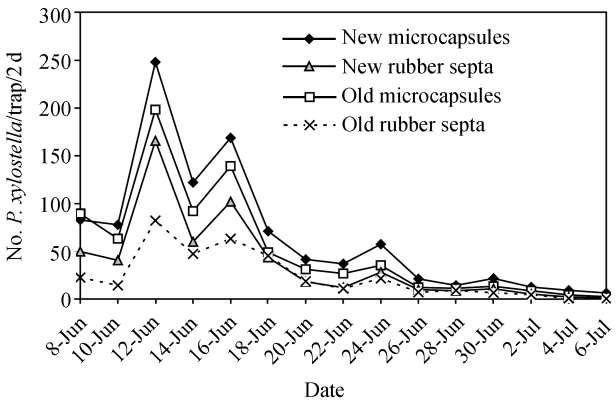

Figure 5 Season-long capture in traps with different treatments in 2006.

2.4 Mating disruption assessment

No significant differences were detected between the number of male *P. xylostella* captured in pheromone-treated fields compared to control fields on 2 June, suggesting that the density of *P. xylostella* in all fields was equal before the experiment. There were significant differences in the numbers of male *P. xylostella* caught in pheromone-baited traps between pheromone-treated and untreated (control) fields (Table 3). Reduction in capture of male *P. xylostella* lasted for at least four weeks when the formulation was applied at 18 g a.i./ha (Figure 6), and the estimated mating disruption efficiency of the pheromone treatments ranged from 76.94% to 98.67% during the season (Table 3).

3 Discussion

Synthetic sex pheromone of *P. xylostella* was encapsulated efficiently through a complex coacervation process using gelatin and gum arabic as wall-forming materials.

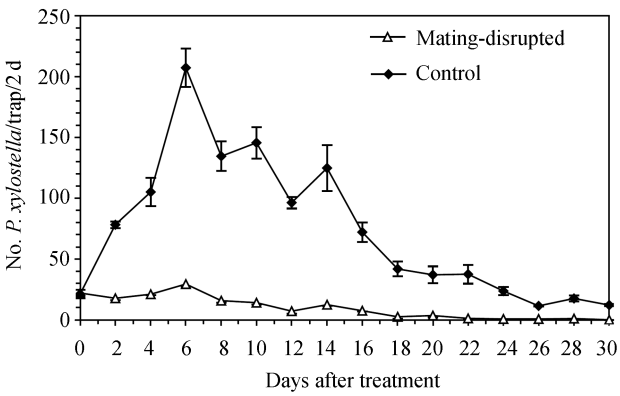

Figure 6 Mating disruption of *P. xylostella* in 0.02 ha plots with microcapsules applied at 18 g a.i./ha (2006).

Table 3 Number of *P. xylostella* moths caught in traps baited with synthetic sex pheromone in the pheromone-treated and untreated fields (2006)

Date	Microcapsules (mean ± SE)	Insecticide (mean ± SE)	Reduction (%) (mean ± SE)
2-Jun[a)]	22.20 ± 2.23 a	21.30 ± 3.46 a	——
4-Jun	17.80 ± 1.66 a	78.20 ± 2.75 b	76.94 ± 2.68
6-Jun	21.20 ± 0.97 a	105.20 ± 11.56 b	78.52 ± 3.15
8-Jun	29.60 ± 2.04 a	207.20 ± 15.66 b	85.41 ± 1.39
10-Jun	15.80 ± 1.53 a	134.60 ± 12.20 b	87.52 ± 2.25
12-Jun	14.20 ± 1.50 a	145.60 ± 12.93 b	89.86 ± 1.69
14-Jun	7.20 ± 1.88 a	96.40 ± 4.74 b	92.81 ± 3.02
16-Jun	12.60 ± 1.08 a	124.80 ± 18.85 b	89.02 ± 1.60
18-Jun	7.60 ± 0.68 a	72.20 ± 8.05 b	88.67 ± 2.23
20-Jun	2.60 ± 1.60 a	42.00 ± 6.03 b	94.62 ± 3.49
22-Jun	3.60 ± 1.50 a	37.20 ± 6.92 b	87.23 ± 5.96
24-Jun	1.40 ± 0.98 a	37.60 ± 7.67 b	96.54 ± 2.12
26-Jun	0.80 ± 0.37 a	23.80 ± 3.51 b	96.81 ± 1.67
28-Jun	0.80 ± 0.58 a	11.60 ± 0.52 b	93.46 ± 4.85
30-Jun	1.20 ± 0.37 a	17.60 ± 2.48 b	94.39 ± 3.34
2-Jul	0.20 ± 0.20 a	12.20 ± 0.86 b	98.67 ± 1.33

a) Pretreatment. Means followed by the different letters in the same range are significantly different at $P<0.05$ (Paired-samples t test).

It is widely recognized that to optimize the technique, the performance of pheromone formulations should be evaluated in the field or forest rather than in the laboratory. Only by field examination of the emission rates of the formulations, their comparative longevity, the effects of weather, and the spatial distribution of the particles can a rational choice of formulation be made[23]. In our study, the microcapsules had a fairly constant release rate when they were directly exposed for 6 weeks in field during 2005. More moths were caught in traps baited with microcapsules than in either those baited with rubber septa with the same pheromone content or the blank control treatments. Microcapsules remained attractive to male moths throughout the season and more moths were caught in traps baited with aged or new microcapsules than with aged or new rubber septa.

During the mating disruption experiment, signifycantly fewer male *P. xylostella* were caught in pheromone-baited traps in pheromone-treated compared to untreated fields. Although *Bt* (0.6 *l* a.i./ha) was applied in pheromone-treated plots on 9 June 2006 to suppress the population density of *P. xylostella* for commercial purposes, greater than eight moths per trap per night were captured in pheromone traps (Table 3), the threshold level for insecticide treatment[5]. The numbers of male *P. xylostella* were substantially greater in untreated fields than in pheromone-treated fields, suggesting that the microcapsules effectively disrupted pheromone-mediated communication of *P. xylostella*.

Microcapsules are generally applied over large areas with conventional spray equipment and numerous variables can be manipulated to control the release characteristics, e.g. capsule wall thickness, capsule size, capsule wall composition and internal composition[29]. In our study microcapsules were applied with centrifuge tubes, but the results still provide some evidence that the *P. xylostella* sex pheromone microcapsules have favorable release characteristics and biological efficacy. Because they were easily prepared, protected the pheromone from oxidation and irradiation during storage and release, and controlled the rate of release of the pheromone active ingredient, microcapsules show promise as efficient delivery devices for synthetic pheromone of *P. xylostella*.

Microcapsules of *P. xylostella* sex pheromone in our study were small particles with in the 100－150 μm diameter range. If some emplastic material could be bound to the microcapsules and adhered to male *P. xylostella*, microcapsules might serve as flying dispensers (mobile point sources) of pheromone, causing a confusion effect similar to the effect of electrostatic Entostat™ powder (http://www.exosect.com/solutions/products/exosex.asp). More extensive research is needed to further optimize microcapsules of *P. xylostella* sex pheromone for use in the control applications of male trapping and mating disruption.

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