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# Pheromone Delivery System: Western Corn Rootworm (Coleoptera: Chrysomelidae) Pheromone Encapsulation in a Starch Borate Matrix

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**ABSTRACT** Western corn rootworm, *Diabrotica virgifera virgifera* LeConte, sex pheromone, racemic 8-methyl-2-decyl-propanoate (MDP) was successfully encapsulated in a starch borate (SB) matrix creating a controlled release granular formulation. The release rate of MDP from starch borate granules was attractive to male *D. virgifera* at high and low *D. virgifera* population levels in field corn. Male *D. virgifera* were attracted to the SB-MDP granules throughout the growing season, but efficacy declined during the period of peak female *D. virgifera* emergence and corn pollination. The SB-MDP delivery system was as effective as a septa-MDP delivery system, and SB-MDP formulations had a shelf life of at least 2 yr. Data suggest that the starch matrix concept may be useful as a delivery system for semiochemicals and may have potential as a tool that can be used in the development of new, more environmentally sound insect management systems.

**KEY WORDS** Insecta, semiochemicals, pheromones, *Diabrotica*

THE USE OF SEMIOCHEMICALS to monitor or facilitate the control of arthropod pests is a promising arthropod management strategy. Recently, there has been a growing interest in the general area of *Diabrotica* semiochemicals and their potential use in corn rootworm management programs. Research has been directed toward the identification of *Diabrotica* sex pheromones (Ball & Chaudhury 1973; Guss et al. 1982, 1983a,b, 1984; Chuman et al. 1987), attractants (Ladd et al. 1983, Ladd 1984, Andersen & Metcalf 1986, Lampman et al. 1987, Lampman & Metcalf 1987), and movement arrestant–feeding stimulants (Chambliss & Jones 1966, Metcalf et al. 1980, Metcalf 1986). Cotton wicks (Lampman & Metcalf 1987) and rubber septa (Guss et al. 1982, 1983a) commonly have been used to dispense *Diabrotica* semiochemicals in field experiments. However, few attempts have been made to incorporate *Diabrotica* semiochemicals into formulations or delivery systems that can be used to manage rootworm populations. Dry corn grit baits impregnated with insecticide and semiochemicals have been shown to kill western corn rootworm, *D. virgifera virgifera* LeConte and southern corn rootworm, *D. undecimpunctata howardi* Barber adults effectively (Metcalf et al. 1987), demonstrating the potential that semiochemical insecticide combinations may have in future corn rootworm management programs.

New controlled-release technology has been developed in which starch is used to encapsulate pesticides. Several encapsulation techniques have been reported. One process involves crosslinking starch with borate (Trimnell et al. 1982), calcium (Shasha et al. 1981), or xanthide (Shasha et al. 1976) to produce a polymeric matrix that traps the pesticide within small cells. A combination of starch and corn oil also has been used to develop a pH neutral macroencapsulation system (Dunkle & Shasha 1988). The starch matrix concept has been used successfully to develop controlled-release herbicide (Schreiber et al. 1978, Baur 1980), insecticide (Trimnell et al. 1982; ZBM., unpublished data) and *Bacillus thuringiensis* Berliner (Dunkle & Shasha 1988) granular formulations.

Our laboratory has been investigating the feasibility of using the starch matrix concept to create semiochemical controlled-release formulations. Because of recent *Diabrotica* semiochemical discoveries and the economic importance of corn rootworms, we selected *D. virgifera* and field corn as a model system in which to evaluate the starch matrix semiochemical concept. This paper presents the results of experiments that were designed to determine if a volatile semiochemical like *D. virgifera* sex pheromone could be encapsulated in the starch borate (SB) matrix, determine if the SB matrix could effectively be used as a pheromone delivery system in the field, and to determine the shelf life of the formulated product.

## Materials and Methods

**Starch Borate Matrix Formulation Process.** A modification of the technique described by Trimnell et al. (1982) was used to introduce *D. virgifera* sex pheromone, racemic 8-methyl-2-decyl-propanoate (MDP) into the SB matrix. The following procedure was followed to prepare a 454-g sample of SB-MDP granules. A slurry of water (607.2 ml)

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and corn flour (303.6 g, CCF 600 corn flour, Lauhoff Grain Company, Danville, Ill.) was made in a Hobart 200 mixer (Hobart Corporation, Troy, Ohio). MDP (839 mg) dissolved in 10 ml of hexane, calculated to give 1.85 mg MDP/g of final product, was then mixed into the slurry. A 6.6% sodium hydroxide solution (337.2 ml) was added to gelatinize the starch, followed by boric acid (33.7 g), which solidified the gel. Continuous mixing throughout the process subsequently broke up the starch borate mass into small particles with a rubbery texture. Additional corn flour (94.5 g) was added to coat the particles and minimize sticking. After air drying for 24 h, the particles were sifted through 20-mesh sieves to obtain uniform sized granules. The granules were then spread out in a thin layer until the drying process was complete. Granules made in 1985 were stored in 1-liter paper ice cream cartons; granules made in 1986 were stored in sealed glass jars. All granules were stored under dry, dark conditions at room temperature until used in field experiments.

All field experiments were conducted at the University of Nebraska Agricultural Research and Development Center, Mead, Nebr. The rate of 1.85 mg MDP/g of granule was used for all experiments.

*Experiment 1.* Four treatments were evaluated in Pherocon 1C traps (Trece, Salinas, Calif.) from 12 to 29 August 1985 to determine if SB-MDP granules would attract male *D. virgifera* and to compare the efficacy of a SB-MDP delivery system with a septa-MDP delivery system. Treatments included: an empty trap, 30 g of blank SB granules, one-30 g septum (yellow Pedigree eraser; Empire Pencil Corporation, Shelbyville, Tenn.) treated with 55.5 mg MDP in 0.66 ml of hexane to give 1.85 mg MDP/g of septum, and 30 g of SB-MDP granules. Treatments 2–4 were each placed in a 6-cm diameter watch glass and covered with a 20-mesh screen top. Each watch glass was then placed in the center of a trap bottom. All traps were hung at ear height on aluminum poles placed in irrigation line alleys (1.6 m wide) in a 3-ha pollinating trap crop corn field. Treatments were arranged in a randomized complete block design (five replications) with each block consisting of four traps spaced 6.1 m apart in a separate irrigation alley. Alleys were spaced 15.3–18.3 m apart. Traps were replaced every 3–4 d (each watch glass was transferred to a new trap) and the sex of each trapped *D. virgifera* was determined.

*Experiment 2.* During August 1986, MDP was again incorporated into the SB matrix and processed into 20-mesh granules. Five treatments (four rates of SB-MDP: 7.6 g, 3.8 g, 1.9 g, 0.95 g; blank SB granule: 7.6 g) were then placed in a 6.2-ha corn field to determine if beetle trap catch is significantly affected by the rate of MDP. Treatments were placed in Pherocon 1C traps as previously described, but the traps were tied directly to corn plants at ear height. Individual traps and blocks were spaced 15.25 m apart in a randomized complete block design (four replications). Traps were replaced every 4 d and trapped male *D. virgifera* were counted. Whole-plant beetle counts were taken in corn rows midway between treatments on 19 and 30 August 1986 to estimate *D. virgifera* population size in the field during the experiment. Whole-plant counts were taken at midday by quietly approaching 10 plants per block and counting all *D. virgifera* on each plant.

*Experiment 3.* Four treatments were evaluated in a 6.2-ha corn field from 7 August to 1 September 1986 to determine if 1-yr-old SB-MDP granules would attract male *D. virgifera* as well as newly formulated SB-MDP. Treatments included: an empty trap, 7.6 g blank SB granules, 7.6 g 1985 SB-MDP granules, and 7.6 g 1986 SB-MDP granules. A randomized complete block design (four replications) was used. Treatment placement within traps, trap and block spacing, trap replacement interval, sample processing procedure, and whole-plant count adult sampling dates were identical to those listed in Experiment 2.

*Experiment 4.* SB-MDP granules formulated in 1985 and 1986 were also evaluated in a 40-ha corn field from 11 July to 16 August 1987. The objectives of the experiment were to continue to monitor SB-MDP shelf life and to determine the efficacy of SB-MDP as a male *D. virgifera* attractant during several corn and beetle phenology periods. The experimental design and procedures were identical to those in Experiment 3 with the following exceptions. Individual traps and blocks were spaced 25 m apart. Each treatment consisted of 10 g SB granules and the empty trap treatment was deleted. The 1985 SB-MDP treatment was added to the experiment one week after the other treatments were placed in the field. Whole-plant beetle counts and beetle collections were taken seven times during the experiment to estimate *D. virgifera* population size and to determine sex ratios in the field. A sweep net was used to collect beetles by taking vertical sweeps up to the top of the canopy for 400 m in rows located midway between blocks (four rows per date).

The quantity of MDP encapsulated in the SB-MDP granules (formulated in August 1986) at the start and completion of Experiment 4 was analytically measured. Analyses were conducted at the USDA–ARS Northern Regional Research Center, Peoria, Ill. Weathered (1.00 g) and unweathered (200 mg) samples were placed in 1% Amizyme TX-8 solution in 0.1 *M* phosphate buffer at pH 7.0 (10 ml) and shaken with isooctane (10 ml) at 40–50°C for 30 min. The enzyme digested the starch and the isooctane extracted the released pheromone. The isooctane extracts were injected with the internal standard alachlor [2-chloro-2′,6′-diethyl-N-(methoxymethyl) acetanilide], at the rate of 2.0 mg-internal standard (Grob 1985, 33) equivalents per 10 ml extract. Standards for each determination were made by mixing 2.0 mg of MDP

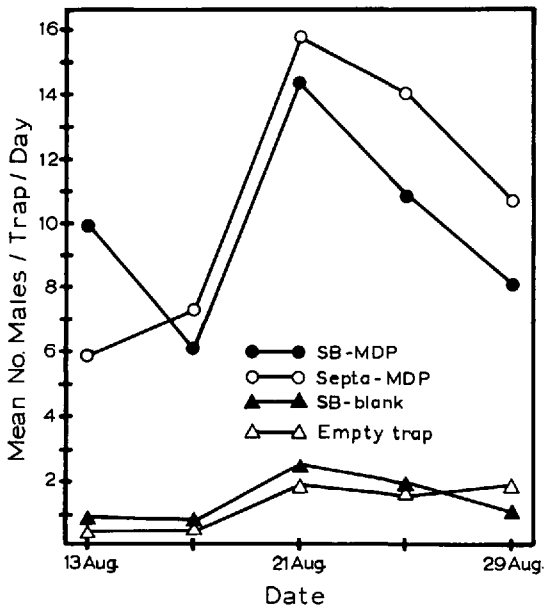


**Fig. 1.** Mean number of *D. virgifera* males collected in sticky traps per 24 h per period from each treatment during Experiment 1, 1985.

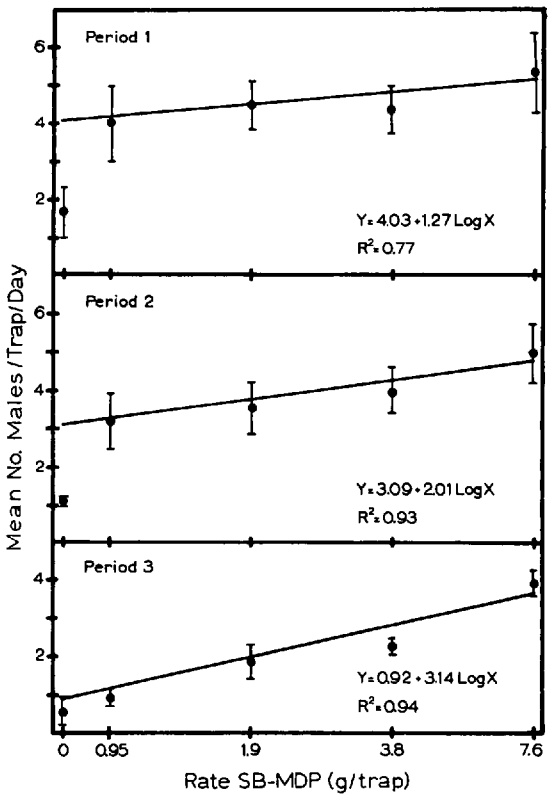


**Fig. 2.** Regression of mean number of *D. virgifera* males collected in sticky traps per 24 h on log MDP rate during three consecutive 4-d periods, 19–30 August 1986.

and 2.0 mg of internal standard in 10 ml of isooctane. The pheromone content of 2-μl samples was determined by gas liquid chromatography on a Tracor 560 with hydrogen flame ionization detection. The column consisted of nickel tubing (1.8 m by 3.2 mm [o.d.] packed with Chromosorb WHP, 150–175 μm, coated with 3% OV-1 silicone rubber. The method described by Grob (1985) was used to calculate actual weights of MDP extracted from the samples.

**Statistical Analyses.** Male *D. virgifera* trap counts were converted to mean number of males per 24 h before data analysis. Data from Experiments 1, 3, and 4 were subjected to analysis of variance for treatment effects over the entire experiment and within 4-d collection periods. Least significant difference tests were used for mean separation where statistical differences ($P < 0.05$) occurred within collection periods.

In Experiment 2, linear regression analysis was used to describe the relationship between rate of MDP and the number of male *D. virgifera* trapped per 24 h within each collection period. Only the four MDP treatments were used for each analysis.

## Results

**Experiment 1.** The mean number of *D. virgifera* males collected per period from each treatment are presented in Fig. 1. The analysis conducted on data from the entire experiment indicates that significant differences occurred among treatments ($F = 37.9$; df = 3, 12; $P < 0.01$), among collection periods ($F = 14.4$; df = 4, 48; $P < 0.01$), and, that a significant treatment × period interaction occurred ($F = 3.4$; df = 12, 48; $P < 0.01$). Within collection periods, there were no significant differences in the number of male *D. virgifera* collected among pheromone treatments except during the first period (13 August) when significantly more males were collected in SB-pheromone traps than in septa-pheromone traps (this caused the treatment × period interaction to be significant). There were also no significant differences in male *D. virgifera* trap catch among the two nonpheromone treatments within periods. Significantly more males were collected in pheromone treatments than nonpheromone treatments during each collection period.

**Experiment 2.** During each 4-d period, the mean number of *D. virgifera* males collected per trap increased with rate of SB-MDP (Fig. 2). The linear regression of SB-MDP rate upon males collected per trap was significant ($P < 0.05$) during two of the three periods (period 1: $F = 6.6$; df = 1, 3; $P = 0.12$; period 2: $F = 27.4$; df = 1, 3; $P = 0.03$; period 3: $F = 34.2$; df = 1, 3; $P = 0.028$). The *D. virgifera* population level remained fairly constant during the experiment (mean number of beetles per plant ± SEM: 19 August, 1.48 ± 0.22; 30 August, 1.15 ± 0.27).

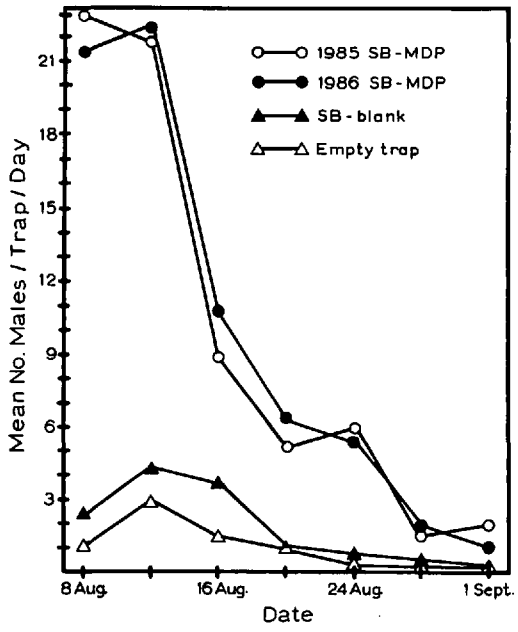


**Fig. 3.** Mean number of *D. virgifera* males collected in sticky traps per 24 h per period from each treatment during Experiment 3, 1986.

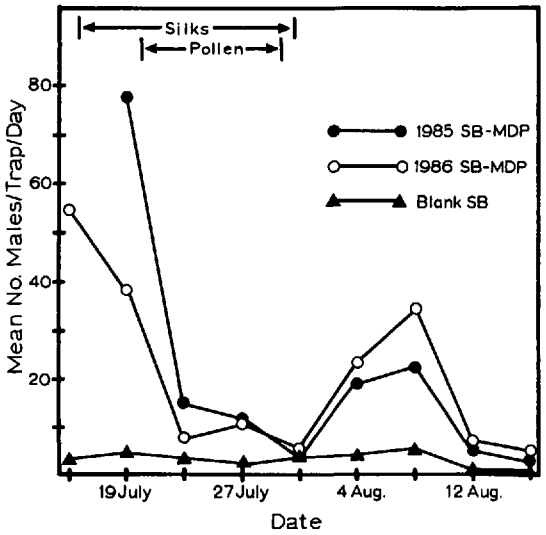


**Fig. 4.** Mean number of *D. virgifera* males collected in sticky traps per 24 h per period from each treatment during Experiment 4, 1987. The periods when green silks were present and pollination occurred in the corn field are indicated by silks and pollen, respectively.

**Experiment 3.** The mean number of *D. virgifera* collected per treatment declined during each successive collection period (Fig. 3) as *D. virgifera* population size declined in the experimental plots (mean number of beetles per plant ± SEM: 19 August, 1.7 ± 0.36; 30 August, 0.23 ± 0.07). This is reflected in the overall analysis as significant differences occurred among treatments ($F = 29.89$; df = 3, 9; $P < 0.01$), and among collection periods ($F = 29.86$; df = 6, 54; $P < 0.01$). There were no significant differences in the number of male *D. virgifera* collected among pheromone treatments and among nonpheromone treatments during each collection period. There were significantly more males collected in pheromone than nonpheromone baited traps during the first five collection periods. However, only one pheromone treatment was significantly different than both nonpheromone treatments during the last two collection periods (period 6: 1986 SB-MDP; period 7: 1985 SB-MDP), which caused the overall analysis treatment × collection period interaction to be significant ($F = 6.69$; df = 18, 54; $P < 0.01$).

**Experiment 4.** The levels of MDP encapsulated in the SB matrix before and after the experiment were 0.80 and 0.55 mg MDP/g of granule, respectively. This indicates that at least 43% of the MDP that was incorporated during the formulation process was actually encapsulated and only a 31% loss of MDP occurred during the 36-d trapping period.

The number of *D. virgifera* males caught per trap varied greatly within and among pheromone treatments during the experiment (Fig. 4). The overall analysis indicates that significant differences occurred among treatments ($F = 18.27$; df = 2, 6; $P < 0.01$), and among collection periods ($F = 41.51$; df = 8, 45; $P < 0.01$). A significant treatment × collection period interaction also occurred ($F = 18.33$; df = 15, 45; $P < 0.01$). Within each collection period, significantly more males were trapped in each pheromone treatment than in the unbaited treatment except for the period of 27–31 July. During that period, only the 1986 pheromone treatment was significantly different than the unbaited treatment.

Male *D. virgifera* trap catch was greatest during mid-July before corn began pollinating (Fig. 4). During this period, the *D. virgifera* population was very large (>9 beetles per plant) and males made up >50% of the population (Table 1). Male trap catch declined dramatically during the corn pollination period (20–30 July). *D. virgifera* population size remained fairly stable during this period (7–10 beetles per plant), but the sex ratio shifted so that females became more numerous than males in the test field. The number of *D. virgifera* in the test field steadily declined after the corn pollination period with males making up only 30–40% of the population (Table 1). However, the number of males collected in pheromone baited traps increased after pollination and peaked during early August (Fig. 4).

## Discussion

Gas liquid chromatography analyses of weathered and unweathered SB-MDP granules (Experiment 4) indicate that MDP was encapsulated in

**Table 1. Seasonal changes in *D. virgifera* population levels and sex ratios during Experiment 4**

| Date | Beetle collections[a] | | Mean beetles per plant ± SEM[b] |
|---|---|---|---|
| | *n* | ♀♀ : ♂♂ | |
| 13 July | 199 | 0.79 | 5.08 ± 0.41 |
| 17 July | 236 | 0.82 | 9.73 ± 0.83 |
| 21 July | 265 | 0.43 | 9.65 ± 0.72 |
| 25 July | 66 | 1.20 | 7.63 ± 0.78 |
| 29 July | 220 | 1.58 | 10.88 ± 0.94 |
| 2 Aug. | 219 | 1.70 | — |
| 5 Aug. | — | — | 7.68 ± 0.79 |
| 8 Aug. | 200 | 2.92 | — |
| 9 Aug. | — | — | 6.15 ± 0.79 |

[a] Beetle totals used for sex ratio calculations derived by pooling subsamples taken from four 400-m sweep net collections per date.
[b] Beetles counted on 10 plants per block, four blocks.

the starch borate matrix and that the MDP release rate from the matrix was fairly slow when granules were placed in Pherocon 1C traps. The amount of SB-MDP that was used and the subsequent release rate of MDP from the traps was attractive to *D. virgifera* males at low (Experiment 3) and very high (Experiment 4) *D. virgifera* population levels. The efficacy of the SB-MDP delivery system appeared to be as good as the septa-MDP delivery system (Experiment 1) and the linear relationship of SB-MDP rate to *D. virgifera* trap catch (Experiment 2) was similar to that reported by Guss et al. (1982) who used a cotton wick delivery system. Data from Experiments 3 and 4 also indicate that SB-MDP formulations can have a shelf life of at least 1–2 yr.

Various biotic and abiotic factors may have caused the significant treatment × collection period interactions that occurred in Experiments 1, 3, and 4. In Experiment 1, the significant difference in trap catch among MDP treatments during the first collection period (Fig. 1) may have been due to differences in initial MDP release rates from starch borate and septa delivery systems. In Experiment 3, low *D. virgifera* population levels may have contributed to the low number of beetles trapped and the variability of trap catch within and among treatments during collection periods 6 and 7 (Fig. 3). This may explain the inconsistencies in the data when periods 1–5 were compared with periods 6 and 7.

Corn and beetle phenological events that took place during late July may have caused a large part of the variation in trap catch observed in Experiment 4. In several continuous corn fields adjacent to Experiment 4 where *D. virgifera* phenology was monitored, the peak female emergence period occurred from 15 to 30 July (L.J.M., unpublished data). This, coupled with the increase in *D. virgifera* females in Experiment 4 during mid-July suggests that competition for *D. virgifera* males between SB-MDP baited traps and virgin *D. virgifera* females may have contributed to reduced SB-MDP efficacy.

The reduction in SB-MDP efficacy also coincided with the corn pollination period (Experiment 4). This suggests that male *D. virgifera* attraction to and feeding upon corn pollen and silks may have reduced male movement (and therefore encounters with MDP baited traps) or may have physiologically reduced the male response to MDP. Hummel & Andersen (1982) reported that pheromone communication of the southern corn rootworm, another *Diabrotica* species, is suppressed when males are associated with and feed on cucurbit plants.

Data reported in this paper suggest that the SB matrix may be useful as a delivery system for volatile *Diabrotica* pheromones to monitor corn rootworm populations. This delivery system also may be applicable to other insect semiochemicals and may have potential as a tool that could be used in the development of new, more environmentally sound insect management programs. Recently, we have successfully used the starch matrix concept to encapsulate plant derived *Diabrotica* semiochemicals and pesticides (T.J.W. & L.J.M., unpublished data). Additional research is needed to evaluate the potential of starch-based formulations for possible use in *Diabrotica* management programs.

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