Increased Ovipositional Attractancy to Surfactant-Treated Broccoli by the Diamondback Moth (Lepidoptera: Plutellidae): Tests of Potential Mechanisms[1]

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J. Entomol. Sci. 33(3): 261-269 (July 1998)

Abstract Studies were conducted to investigate potential mechanisms by which treatment of broccoli leaves with the surfactant Latron CS-7® causes increased diamondback moth, *Plutella xylostella* (L.), oviposition. The importance of vision on oviposition was investigated by use of choice and no-choice tests conducted in the presence of light and in complete darkness. Females oviposited 10.7 and 12.8 times as many eggs on treated plants relative to nontreated plants in the presence and absence of light, respectively, indicating that females do not prefer treated plants based solely on visual cues. Greenhouse studies showed that moths continue to lay significantly more eggs on surfactant-treated plants up to 3 d after initial treatment of plants with Latron CS-7. No difference was observed in larval development or survival on treated versus nontreated plants. Scanning electron microscopy indicated a dramatic difference in wax crystallite morphology of surfactant-treated plants relative to nontreated plants. Ovipositional attractancy of surfactant-treated leaves to diamondback moths could be due to a change in mechanoreceptor cues on the leaf surface or volatile compounds released from the leaf surface as a result of altered plant wax micromorphology.

Key Words *Plutella xylostella,* oviposition, spray adjuvants, behavior, *Brassica*

The diamondback moth, *Plutella xylostella* (L.), is a serious world-wide threat to plants in the Family Cruciferae (Talekar and Talekar 1986). Because of high cosmetic standards of cruciferous vegetables, effective control of diamondback moth is essential (Talekar and Shelton 1993). Control of the diamondback moth has relied extensively on synthetic insecticides. Due to the waxy surface of cruciferous plants, insecticides are often used in conjunction with surfactants, or spreader-stickers, which increase leaf coverage thereby enhancing efficacy. Many studies have demonstrated avoidance of leaf surfaces treated with insecticides such as pyrethroids (Tan 1981, Kumar and Chapman 1984, Dobrin and Hammond 1985, Iftner et al. 1986, Hoy et al. 1990, Hoy and Hall 1993). In contrast, a previous study conducted by Riggin-Bucci and Gould (1996) indicated that diamondback moth females prefer to oviposit on plants treated with certain surfactants relative to nontreated plants. The reason for this attractancy has not been determined.

[1]Received 19 December 1996; accepted for publication 08 June 1997.

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Tabashnik (1985) has shown that diamondback moths use visual and chemoreceptor cues to locate their host plants. We observed during preliminary laboratory tests that broccoli leaves treated with the surfactant Latron CS-7 appeared darker green than nontreated leaves and hypothesized that vision could be involved in differential egg laying. Surfactant-treated plants may also have a different surface texture than normal plants due to surfactant-induced changes in micromorphology of the leaf surface (Noga et al. 1987). It is also possible that surfactants change the epicuticular wax layer in a way that enhances the release of volatiles such as mustard oils which have been shown to stimulate diamondback moth oviposition (Gupta and Thorsteinson 1960).

We conducted a study to better understand the mechanism by which ovipositing diamondback moths distinguish between surfactant-treated plants and nontreated plants.

Materials and Methods

Choice tests were conducted in complete darkness to ascertain if there is a visual component to the ovipositional attractancy of treated plants. Duration of the effect of surfactant treatment on diamondback moth ovipositional behavior and the effect of treatment on larval growth also were examined. Scanning electron microscopy was conducted on treated and nontreated broccoli leaves to investigate the effect of the surfactant on the morphology of the host plant leaf surface.

Insects. Diamondback moths were obtained from a laboratory colony maintained at North Carolina State Univ., Raleigh, NC. The colony was initiated from pupae received from the PeeDee Coastal Experiment Station, Clemson Univ., Clemson, SC, in 1991. Approximately 75 moths (10% of colony) were collected from Johnston and Sampson cos., NC, and introduced into the colony each year. Diamondback moth larvae were fed a wheat-germ based diet modified from Biever and Boldt (1971) and Shelton et al. (1991) excluding aueromycin, tenox and formalin. Collard extract (300 ml/4 liters diet) also was added to the diet. Collard extract was obtained by grinding 65 g of fresh collard leaves in a blender, adding 500 ml water and boiling. Adults were given a solution of 35% honey: 35% beer: 30% water and housed in 30 × 30 × 30 cm Plexiglas® cages at 14:10 (L:D). The colony was maintained in continuous culture with at least 1000 individuals present at any given time.

Plants. Broccoli plants, *Brassica oleracea* var. *italica* 'Green Comet', were grown from seed in the greenhouse in 10-cm clay pots using Metro Mix 220. Plants were fertilized at 2-wk intervals with Peters Plant Fertilizer (20-20-20, 1 tsp/0.5 liter). Tests were conducted with 6 to 8-wk-old plants comparable in size and leaf number.

Visual cues. To investigate the relative importance of visual cues in ovipositional preference for treated plants, laboratory experiments were conducted using choice and no-choice tests with continuous light or continuous darkness. Plant treatments consisted of a surfactant and a water-treated control. The surfactant used was Latron CS-7® (2.5 ml/liter) (Rohm and Haas, Philadelphia, PA), a nonionic spreader-binder designed for use with insecticides (Thomson 1986). The surfactant was applied to the plants using a handheld mister. Plants were sprayed until run-off and allowed to dry for 4 to 6 h before experiments were conducted.

In choice tests, one treated and one nontreated plant were placed into a 30 × 30 × 30 cm Plexiglas® cage where one side of the cage had a 20-cm diam mesh-covered opening and the opposite side had a 20-cm diam opening covered with a sock sleeve.

The cages were positioned such that the mesh opening was on the bottom and the sleeve at the top. The cages were elevated 10 cm using pots at each of 4 corners to facilitate air flow.

One previously mated 2 to 3-d old female diamondback moth was introduced into each Plexiglas cage each night the experiment was conducted. One cage was maintained in continuous darkness and the other in continuous light for 10 h beginning at 1700 h. Plants were then removed and the number of eggs was recorded for each plant. The experiment was conducted for 3 and 4 nights in the light and dark, respectively.

In no-choice tests, either one treated or one nontreated plant was placed into a Plexiglas cage designed the same as described above. One previously mated 2 to 3-d-old female moth was introduced into each cage (1 treated and 1 nontreated) and left in continuous darkness or continuous light for 10 h, beginning at 1700 h. Light and dark treatments were replicated five times on different nights.

In choice and no-choice tests, a paired *t*-test was used to determine if the mean number of eggs on treated and nontreated plants differed within a light or dark treatment. Light versus dark treatments were not compared statistically.

Durational effects. Greenhouse studies were initiated to determine the duration of the effect of the surfactant Latron CS-7 on diamondback moth oviposition. Plants were treated as described in the visual cues experiments. Plants were held for 2 and 3 d before placing them into the greenhouse arenas. After a 19-h exposure period (1600-1100 h), plants and diamondback moth adults were removed, and plants were inspected for number of eggs deposited. Plant treatments were rotated clockwise by 1 position in each arena. New plants were used for each replicate. For 2-d delay tests, 11 replications were conducted over 2 nights (6 in one night, 5 in the other). For 3-d delay tests, 24 replications were made over 4 nights (6 each night).

Differences among treatment means were determined by a three-way ANOVA (P = 0.05, SAS Institute 1987) with day, arena and treatment as the main variables.

Larval survival and development. To determine whether surfactant treatment of plants had a broad effect on larval growth as well as ovipositional preference, 4 plants were treated with the surfactant Latron CS-7 (rate given above) and 4 nontreated plants were infested with six second-instar diamondback moth larvae. Weights and survival were recorded 5 d later. In a second experiment, 8 plants of each treatment were infested with six second instars and weights and survival were noted after 7 d. Results were analyzed using *t*-tests. Mean weight of larvae on each plant was considered the experimental unit.

Scanning electron microscopy. Scanning electron microscopy was conducted on broccoli leaves treated with the surfactant Latron CS-7 and on nontreated leaves. Preparation of broccoli leaves was modified from Eigenbrode et al. (1991). Treatments were applied to 8-wk-old plants as described for the previous laboratory and greenhouse studies. Plants were healthy and turgid when treated. Treated plants were allowed to dry completely before 2 leaves from 2 plants of each treatment (8 leaves total) were excised, immersed in liquid nitrogen, and placed into a desiccator. Leaves were lyophilized for 24 h using a Virtis bench top 6 model freeze dryer at −60°C (Virtis Co., Inc., Gardiner, NY).

Lyophilized samples were coated in a Technics Hummer V with gold palladium source. Thickness was monitored with a digital thickness monitor to approximately 30 nm. Leaf waxes of *Brassica* are deposited in microscopic crystallites (Jeffree 1986).

Wax crystallites on upper and lower surfaces of each sample were viewed with a Phillips 505T electron microscope and photographed at a magnification of 4000×.

Results

Visual cues. Choice and no-choice tests conducted in the presence of light showed that significantly more ($P < 0.05$) diamondback moth eggs were laid on treated plants relative to nontreated plants (Table 1). In choice tests, mean number of eggs on treated plants was 10.7 times higher than the number of eggs laid on non-treated plants ($t = 2.6767$; n = 7; $P = 0.0202$). Moths in no-choice tests in the presence of light laid 5.5 times as many eggs on treated plants ($t = 2.3198$; n = 5; $P = 0.0489$).

Choice and no-choice tests conducted in complete darkness also resulted in significantly more eggs ($P < 0.05$) on treated plants relative to nontreated plants (Table 1). A 12.8-fold increase in mean number of eggs on treated plants was observed in choice tests ($t = 2.5795$; n = 5; $P = 0.0326$), and a 4.7-fold difference was observed in no-choice tests ($t = 2.4597$; n = 5; $P = 0.0393$).

Durational effects. Choice tests conducted in the greenhouse 2 and 3 d after treatment of broccoli plants with surfactant or water indicated that significantly ($P < 0.05$) more eggs were laid on plants treated with surfactant (Table 2). Experiments conducted 2 d post-treatment showed that 3.6 times as many eggs were laid on surfactant-treated plants as on water-treated plants ($F = 30.82$; df = 1, 11; $P = 0.0014$). When moths were exposed to plants 3 d after treatment, 1.7 times as many eggs were laid on plants treated with surfactant ($F = 10.64$; df = 1, 24; $P = 0.0052$) (Table 2). These results indicate that the effect of surfactant on broccoli plants remains present 2 d after treatment, but that the effect may be decreasing after 3 d.

Larval survival and development. There was 100% survival of larvae in both treatments of both experiments. There was no significant difference ($P > 0.05$) in mean weight of larvae from surfactant-treated versus nontreated plants in either

Table 1. Mean number (±SE) of diamondback moth eggs laid on broccoli plants treated with either the surfactant Latron CS-7 (tr) or nontreated (nt) in choice and no-choice tests. Plants were either maintained in the presence of light or in complete darkness

		Light			Dark		
	Treatment	n	Mean (±SE)	P*	n	Mean (±SE)	P*
Choice Tests							
	tr	7	15.0 ± 5.0		5	10.2 ± 3.6	
	nt	7	1.4 ± 0.9	0.0202	5	0.8 ± 0.4	0.0326
No-choice Tests							
	tr	5	15.4 ± 5.0		5	8.4 ± 2.2	
	nt	5	2.8 ± 2.1	0.0489	5	1.8 ± 1.0	0.0393

* Indicates results from paired *t*-test. $P < 0.05$ is considered statistically significant.

Table 2. Mean number (±SE) of diamondback moth eggs laid on broccoli plants 2 d and 3 d post-treatment with surfactant (Latron CS-7®) or water in greenhouse choice tests (2d: F = 30.82; df = 1, 6; P = 0.0014; 3d: F = 10.64; df = 1, 15; P = 0.0052, SAS Institute 1987)

Trt	n	Mean (±SE)
2 Days Post-Treatment		
Latron CS-7	11	7.57 ± 0.91
Control	11	2.09 ± 1.41
3 Days Post-Treatment		
Latron CS-7	24	8.31 ± 1.12
Control	24	4.79 ± 1.14

experiment. Five days after treatment mean weights of larvae were 1.62 (±0.51) and 1.33 (±1.40) on surfactant-treated and nontreated plants, respectively. Seven days after treatment larvae weighed 3.80 (±0.31) and 3.51 (±0.59) on surfactant-treated and nontreated plants, respectively.

Scanning electron microscopy. Scanning electron microscopy (4000×) revealed that the nontreated broccoli leaf surface was covered with dendritic wax crystallites (Fig. 1C and D). There seemed to be no difference in the amount of wax or in the shape of the wax crystallites on the upper leaf surface (Fig. 1C) versus the lower leaf surface (Fig. 1D).

Wax crystallites on leaves treated with the surfactant, Latron CS-7, appeared polygonal-shaped (Fig. 1A and B) compared to the dendritic shapes seen on non-treated leaves (Fig. 1C and D). There appeared to be no difference in crystallite morphology on the upper (Fig. 1A) and lower leaf surface (Fig. 1B) of treated leaves. Although amount of wax was not measured, visual observation under scanning electron microscopy indicated that there was a difference in number of wax crystallites on treated versus nontreated leaves.

Discussion

Scanning electron micrographs indicated that wax crystallite morphology of broccoli leaves is dramatically altered when treated with the surfactant Latron CS-7. Crystallites from treated leaves appeared polygonal compared to nontreated leaves which were dendritic (Fig. 1). Changes in leaf micromorphology as a result of treatment with surfactants has been found previously (Noga et al. 1987, Wolter et al. 1988, Neinhuis et al. 1992). Treatment of *B. oleracea* var. 'gongylodes' with Triton X-110 or Triton X-114 has been shown to cause solubilization of the three-dimensional network of wax resulting in clumping of polygonal crystallites (Noga et al. 1987).

Similarly, Eigenbrode and Shelton (1992) have shown that cabbage, *B. oleracea* var. 'capitata', treated with the herbicide S-ethyl dipropylthiocarbamate (EPTC) has reduced amounts of epicuticular wax. Scanning electron micrographs from that study showed that nontreated cabbage leaves exhibited dendritic crystallites similar to those observed on broccoli, and EPTC-treated leaves assumed polygonal arrange-

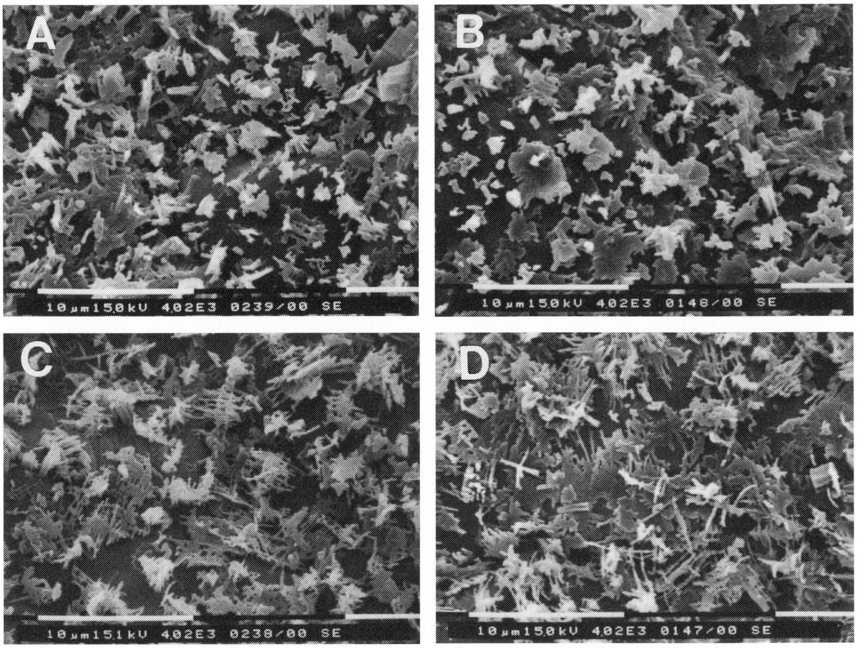

Fig. 1. Scanning electron micrographs of (A) upper leaf surface of broccoli ('Green Comet') treated with the surfactant Latron CS-7®, and (B) lower leaf surface of broccoli treated with the surfactant Latron CS-7®, (C) upper leaf surface of normal, nontreated broccoli ('Green Comet'), (D) lower leaf surface of normal, nontreated broccoli. Bars = 10 µm. (Only one micrograph from each treatment is shown because variation within a treatment was insignificant).

ments much like our surfactant-treated broccoli. Our results also show that broccoli wax crystallites are dendritic in the natural state and assume the polygonal appearance after surfactant treatment. Eigenbrode and Shelton (1992) found that survival of diamondback moth larvae was significantly lower on EPTC-treated plants than on nontreated plants. We found no difference in larval survival or weight on broccoli plants when treated with surfactant relative to survival on nontreated plants (Table 3).

Research has shown that glossy genotypes of *B. oleracea* tend to exhibit resistance to diamondback moth larvae (Dickson and Eckenrode 1980, Stoner 1990, Eigenbrode and Shelton 1992). Glossy cabbage cultivars have reduced amounts of epicuticular wax relative to normal susceptible genotypes (Eigenbrode and Espelie 1995). When the wax from glossy and normal cabbage is removed or mechanically disrupted, diamondback moth larvae do not discriminate between glossy and nonglossy genotypes (Eigenbrode and Shelton 1990). The wax crystallite morphology of glossy genotypes is similar in structure to that of our surfactant-treated broccoli leaf (var. 'Green Comet'), whereas normal wax-bloom cabbages have crystallites similar to nontreated broccoli leaves (Eigenbrode et al. 1991).

Eckenrode et al. (1986) conducted an experiment where epicuticular wax of broc-

Table 3. Mean weight (±SE) of diamondback moth larvae 5 and 7 d after feeding on broccoli plants treated with either Latron CS-7® alone or non-treated (control)

	5 d		7 d	
	N	*Mean (±SE)	N	**Mean (±SE)
Latron CS-7	4	1.62 ± 0.51	8	3.80 ± 0.31
Control	4	1.33 ± 1.40	8	3.51 ± 0.59

* $t = 0.5613$, $P = 0.5949$
** $t = 0.4433$, $P = 0.6643$

coli and cabbage leaves was altered by either dipping leaves in ether or by brushing. They observed that leaves with altered wax morphology were darker green than nonaltered leaves. Their results showed that oviposition by diamondback moths was significantly enhanced when wax morphology of their host plant was altered. They attributed an increase in oviposition to either the physical change in wax crystallites due to ether dips or brushing, a change in color of treated leaves from light to dark green, or a change in the release of mustard oils which could have influenced oviposition (Gupta and Thorsteinson 1960, Eckenrode et al. 1986). Tabashnik (1985) has shown that diamondback moths use visual, olfactory and mechanoreceptor cues to locate their hosts for oviposition. Results from our laboratory studies indicate that diamondback moth females do not respond to surfactant-treated plants based solely on visual cues because choice and no-choice tests conducted in complete darkness continued to result in increased oviposition on treated plants.

A study conducted on the role of wax bloom in suppression of diamondback moth oviposition has shown that diamondback moth females prefer to lay eggs on cabbage leaves when the leaf wax is removed by treatment with synthetic detergent (Uematsu and Sakanoshita 1989). These researchers found that eggs laid on nonwaxy surfaces, such as glass, polyvinyl or Japanese radish leaf, were strongly adhered to the substrate, and egg shape was relatively flat. Eggs deposited on leaves of normal cabbage or broccoli were loosely attached and sometimes clung to each other causing abnormal egg masses. They concluded that wax bloom may be ovipositionally suppressive. Although we did not measure the amount of wax on treated versus nontreated leaves, the number of wax crystallites did not appear different between treated and nontreated leaves (Fig. 1).

Our study focused on the effect of a surfactant on greenhouse-grown plants. It is important to note that epicuticular lipids of plants grown in the greenhouse may differ from those of field-grown plants (Yang et al. 1993). Riggin-Bucci and Gould (1996) have found that surfactants play a role in ovipositional attractancy in the field. Scanning electron micrographs of field-grown plants (not shown) indicate that the effect of Latron CS-7 on wax morphology is evident but scattered among areas of a given leaf. It appears that the effect is spatially inconsistent because of the field application method. A boom-delivered application in the field would not be expected to completely cover a leaf surface.

Although scanning electron microscopy was not conducted to determine the duration of the effect of the surfactant on wax morphology, greenhouse studies showed an effect of surfactant treatment on moth ovipositional behavior 2 and 3 days post-application (Table 2). We observed that leaves treated with the surfactant Latron CS-7 still appeared darker green and less shiny than nontreated leaves. A study conducted by Wolter et al. (1988) indicated that wax crystallites of *B. oleracea* var. *gongylodes* had not regenerated 7 d after treatment with the surfactant Triton X-100. However, Wortmann (1965) observed reformation of rape, *B. napus* L., crystallites within 7 d. Therefore, additional studies are needed to determine the longevity of the effect of Latron CS-7 on leaf surface.

Acknowledgments

We thank V. Knowlton for scanning electron microscopy assistance. Our appreciation is also extended to P. Temple and J. Sykes for technical support. Sanford Eigenbrode provided a valuable review of this manuscript. This work received financial support from the USDA Southern Regional Integrated Pest Management Program.

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