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Survival and behavior of *Plutella xylostella* larvae on cabbages with leaf waxes altered by treatment with S-ethyl dipropylthiocarbamate

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Abstract

Cabbages (*Brassica oleracea* L.) treated with S-ethyl dipropylthiocarbamate (EPTC) herbicide had reduced amounts of leaf surface waxes (40.6% of controls) and reduced densities of leaf surface wax crystallites (20.8% of controls). Leaf waxes of EPTC-treated plants chemically and morphologically resembled leaf waxes of genetically glossy cabbages resistant to the diamondback moth *Plutella xylostella* (L.) (Lepidoptera: Plutellidae). Survival of larvae was significantly reduced on EPTC-treated cabbage plants in three out of four experiments (62.0–15.3% of survival on controls). *P. xylostella* neonates also moved more rapidly on EPTC-treated plants than on untreated controls (1.84 ± 0.16 cm/min on controls *vs.* 3.94 ± 0.24 cm/min on treated plants; $P = 0.0001$). These results support the hypotheses that reduction in leaf waxes is the basis of resistance to *P. xylostella* in genetically glossy plants and that reduced acceptance by larvae is associated with this resistance. Modification of leaf surface waxes with EPTC or similar compounds may have potential as an economic control for *P. xylostella* in *Brassica* crops.

Introduction

The most promising sources of host plant resistance to the diamondback moth are glossy leaf genotypes of *Brassica oleracea* L. (Dickson & Eckenrode, 1980; Lin *et al.*, 1983; Eigenbrode *et al.*, 1990; Eigenbrode & Shelton, 1990; Stoner, 1990; Eigenbrode *et al.*, 1991a). The resistance is due to greatly reduced larval survival on the glossy plants (Lin *et al.*, 1983; Eigenbrode & Shelton, 1990). Neonate *P. xylostella* also move more rapidly on the glossy resistant leaves, relative to movement rates on susceptible leaves with normal wax, and this behavior has been interpreted as indicative of reduced larval acceptance of these genotypes (Eigenbrode & Shelton, 1990; Eigenbrode *et al.*, 1991a, b).

Reduced survival of *P. xylostella* larvae on glossy genotypes is associated with reduced amounts of surface wax and lower densities of wax crystallites on leaves of these plants, as compared with normal bloom susceptible genotypes (Eigenbrode *et al.*, 1991a). However, it has not been demonstrated conclusively that these leaf

wax characteristics cause the resistance in glossy plants.

Wax load and crystallite density can be artificially reduced in normal wax *Brassica* by treating plants with the thiocarbamate herbicide, S-ethyl dipropylthiocarbamate (EPTC) (Flore & Bukovac, 1974; 1976; 1978) or with trichloracetic acid (TCA) (Macey, 1974) and resemble genetic glossy plants. Reduction of wax load and crystallite density on susceptible normal-wax host plants can provide the basis for more conclusive tests of the role of reduced leaf waxes in resistance to *P. xylostella*. EPTC or TCA have been used previously to study wax-mediated interactions between insects (Klingauf *et al.*, 1978) and pathogens (Blakeman & Sztejnberg, 1973) and their host plants. In this paper we document *P. xylostella* larval survival and neonate movement rates on susceptible normal wax cabbage genotypes treated with EPTC to produce glossy leaf surface waxes.

Materials and methods

Four experiments were conducted in 1989; two (*P1* and *P2*) using potted cabbage, and two (*F1* and *F2*) using cabbage in the field.

Culture and treatment of potted plants

Susceptible, normal wax 'Market Prize' cabbage was grown in the greenhouse in 16 cm diameter pots with Cornell Mix (Boodley & Sheldrake, 1977). When plants were 5 weeks old the pots were placed on outdoor gravel beds. After the plants had acclimated for one week, the soil in each pot was treated with 10 ml of aqueous solution containing EPTC at a rate equivalent to 2.24 kg AI (active ingredient)/ha (10.4 mg AI/pot). The method of treating plants with EPTC was adapted from Flore and Bukovak (1974). Experiment *P1* included 20 treated plants and 20 untreated controls, and the plants were placed outdoors on 5 July. Experiment *P2* included 27 treated plants and 27 untreated controls, and the plants were placed outdoors on 15 July. A third group of 5 treated plants and 5 controls was kept in the greenhouse and used for behavioral studies of the larvae.

Culture and treatment of field-grown plants

Eight-week-old seedlings of susceptible, normal wax 'Round-Up' cabbage were transplanted into field plots at the Vegetable Research Farm of the New York State Agricultural Experiment Station (NYSAES) at Geneva, N.Y. Plants were spaced 45 cm apart within rows and 90 cm apart between rows. Experiment *F1* was transplanted on 13 July, and 40 plants were treated with 20 ml of the aqueous solution of EPTC (20.8 mg AI/plant) on 15 August. The solution was poured onto the soil at the base of each plant. An equal number of plants were treated with water only, as controls. Experiment *F2* was transplanted on 2 August, and 43 plants were treated with 40 ml of the aqueous solution of EPTC (41.6 mg AI/plant) on 15 September. Thirty-nine plants were treated with water as controls. When leaves on treated plants in each experiment appeared glossy (about one week after treatment) they were infested with *P. xylostella* or analyzed as described below.

In Experiment *F1*, the outer frame leaves of treated plants did not respond to the EPTC and retained the normal waxy bloom, but the inner leaves did become glossy. The procedure was therefore modified in Experiment *F2*; when inner leaves turned glossy, all the outer, nonglossy leaves were removed so that the entire remaining plant was glossy. This treatment removed about one third of the leaf area of the plants. Controls in *F2* were defoliated in a similar manner.

Survival of P. xylostella

In all four experiments, when EPTC-treated plants turned glossy, treated and control plants were infested with *P. xylostella* eggs as described elsewhere (Eigenbrode *et al.*, 1990). Potted plants were infested with 200 eggs and field-grown plants were infested with 300 eggs. When larvae reached

fourth instar, the infested plants were censused for the number of larvae alive on the plants. Larvae not recovered were assumed to have moved off the plants and died in the soil (Eigenbrode & Shelton, 1990). The percentage of larvae surviving on each plant was calculated and larval survival on treated and control plants was compared with Student's *t*-test.

Movement rates of neonate P. xylostella

The sixth fully-expanded leaf was excised from each of the five potted EPTC-treated and five control plants that were grown in the greenhouse. The cut end of the leaf petioles were immediately placed in a water-filled tube. Within 20 min of excision, the movements of nine larvae were simultaneously recorded on each leaf for five minutes, using video (Eigenbrode & Shelton, 1990). Larval movement rates were measured during review of the video recordings. Movement rate was defined as the average distance (cm) traveled per minute by a larva during the five-minute observation. The larval movement rates on treated and control plants were compared with Student's *t*-test.

Leaf surface wax characteristics

The amount of leaf surface wax was determined on treated plants and controls in Experiments *P1*, *P2*, and *F2*. The determinations were made within one day of infestation of the plants with eggs. Waxes from approximately ten leaves per treatment were removed with 10-second dips in three consecutive 200 ml baths of dichloromethane. The three washings were combined. The area of the extracted leaves was measured. Three samples (50 ml) of each extract were evaporated to dryness, the wax residue was weighed, and the areas of the extracted leaves were used to calculate μg wax/cm^2 of leaf surface (wax load).

The leaf wax extracts from EPTC-treated and untreated plants in Experiment *P2* were also analyzed with thin layer chromatography (TLC) as described previously (Eigenbrode *et al.*, 1991a). For comparison, the TLC plates also included similarly prepared wax extracts from a genetically resistant glossy cabbage, NY 8329. Extracts were concentrated and the plates were spotted with aliquots containing 150 μg of leaf surface wax of each of the three types of wax. Identification of spots, visualized by charring after treatment with HCL, was by comparison with standards.

Leaf surfaces of EPTC-treated with untreated plants in *P2* were examined with SEM (Eigenbrode *et al.*, 1991a). At the SEM console, the number of crystallites were counted in 20 fields of view (4000 $\times$) on leaf surfaces of treated and untreated plants. Individual crystallites were readily distinguished on treated leaves. On the untreated leaves, only clearly visible individual rod or tube-like structures were considered crystallites (Fig. 1).

Insecticidal activity of EPTC

This test was conducted to determine if the reduction in larval survival on the EPTC-treated plants was due to toxicity of EPTC residues in the plants.

On the day of infestation, the residues in treated plants (*F2*) were determined by the Analytical Laboratories at the Department of Food Science and Technology at NYSAES to be 0.003 ppm EPTC. Artificial diet, normally used for rearing *P. xylostella* in the laboratory, was prepared with 0.001, 0.003, 0.010, 0.050, and 0.500 ppm EPTC. Larvae were reared on these diets and the percentage surviving was determined at pupation. Each EPTC treatment was replicated 15 times. A replicate consisted of one 20 ml plastic cup containing 2 g of diet, inoculated with 10 larvae. Percentages of larvae surviving were angularly transformed and the effect of treatment on survival was examined using analysis of variance.

Results

Survival and movement rates of P. xylostella *larvae*

EPTC treatment of plants in both potted experiments significantly reduced larval survival as

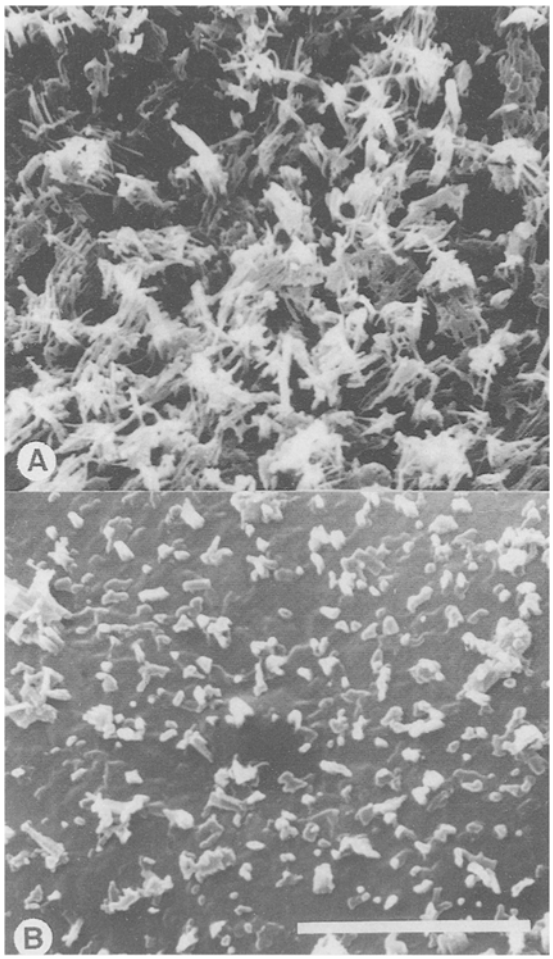

Fig. 1. Scanning electron micrographs of the leaf surface of 'Market Prize' cabbage: (a) untreated control, and (b) treated with EPTC (experiment *F2*). Bar = 10 μm.

compared with untreated controls (Table 1). Average survival on potted plants was low, which is typical of small plants (personal observations). In *F1* no differences in larval survival occurred but, as noted previously, all the leaves of the treated plants in this experiment did not become glossy. In *F2*, in which only glossy leaves were left on the treated plants and the treatment rate of EPTC was doubled, survival of diamondback larvae was significantly reduced on EPTC-treated plants. The percent reduction in larval survival was not as great in *F2* as in the potted plant studies. Larvae also moved approximately twice as fast on the potted EPTC-treated plants as on untreated controls (1.84 ± 0.16 cm/min on controls *vs.* 3.94 ± 0.24 cm/min on treated plants; $P = 0.0001$).

Table 1. Percentage of *P. xylostella* larvae surviving on EPTC-treated cabbage plants and untreated control plants

Experiment[a]	EPTC treated[b]	Control[b]	P value for *t*-test	Percent reduction
P1	2.2 ± 0.3	14.3 ± 1.4	0.0001	84.7
P2	4.4 ± 0.52	12.2 ± 0.4	0.0001	63.9
F1	23.1 ± 2.52	24.8 ± 2.3	ns	(6.9)
F2	25.1 ± 3.8	40.3 ± 3.1	0.003	38.0

[a] *P* = experiment using potted plant; *F* = experiment using field-grown plant.
[b] $x \pm$ SEM.

Surface wax characteristics

Wax loads were reduced on EPTC-treated plants in both experiments with potted plants and in *F2* (Table 2). The percent reduction of wax load was greater on the potted plants than on the field-grown plants. Due to the incomplete production of glossy leaves in *F1*, wax loads were not determined in that experiment. Wax crystallite densities were also reduced on EPTC-treated plants (*F2*) as compared with untreated controls (Fig. 1). The average density of leaf surface wax crystallites, in 20 SEM fields of view on treated plants, was 211/1000 μm^2. Crystallite density on untreated fields was 1014/1000 μm^2. Crystallites on

Table 2. Leaf wax loads in $\mu g/cm^2$ of leaf surface on EPTC-treated cabbage plants and untreated control plants

Experiment[a]	EPTC treated	Control	Percent reduction
P1	18.1	54.8	67.1
P2	20.5	63.5	67.7
F1	not determined	not determined	–
F2	38.4	84.5	54.6
$\bar{x}$	27.5	67.6	59.4

[a] *P* = experiment conducted using potted plants; *F* = experiment conducted using field-grown plants.

EPTC-treated plants were either short rods or polygon-like formations. Crystallites on controls were predominately longer rods, often in dendritic arrangements.

The TLC patterns of epicuticular waxes from EPTC-treated cabbage differed markedly from those of untreated plants and resembled those from the *P. xylostella*-resistant genetic glossy cabbage NY 8329 (Fig. 2). Both glossy waxes have markedly enhanced alkyl ester bands, and reduced ketone and secondary alcohol bands, as compared with wax from untreated 'Market Prize'. The free fatty acid band appears more complex, and there is unidentified material between bands 2 and 3 in both glossy waxes, which does not occur in the waxes of the untreated control. However, the genetic and EPTC glossy waxes also differ from one another in several ways. The primary alcohol band is markedly enhanced in waxes from genetic glossies but only slightly enhanced on the EPTC glossy waxes. The aldehyde band appears to be slightly enhanced in genetic glossy wax, but not in EPTC glossy wax, as compared with waxes from the untreated control.

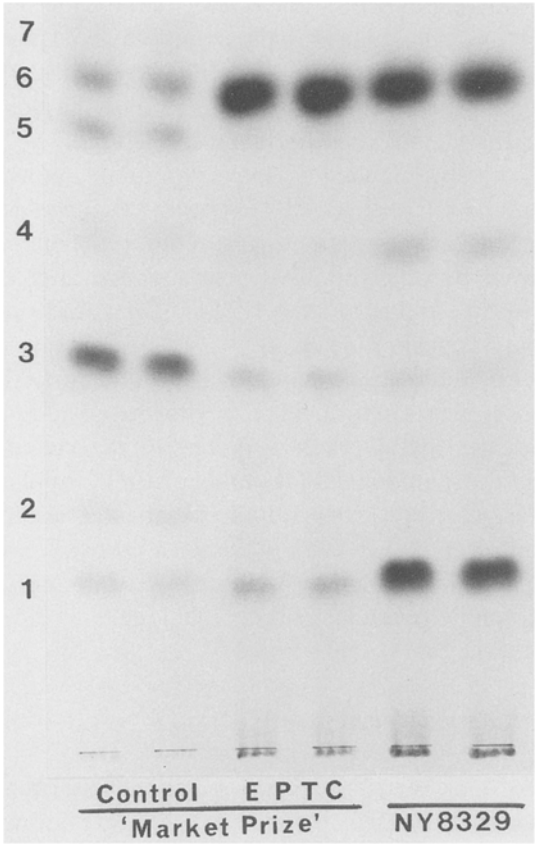

Fig. 2. Thin layer chromatograms of waxes from EPTC-treated and untreated 'Market Prize' cabbage, and NY 8329 glossy cabbage. The bands are: 1 primary alcohols, 2 free fatty acids, 3 secondary alcohols, 4 aldehydes, 5 ketones, 6 alkyl esters, 7 alkanes.

Insecticidal activity of EPTC in diet

There was no toxicity due to EPTC in artificial diet at any of the concentrations tested (Table 3). The range of concentrations included the low level detected in plants from *F2* (0.003 ppm) and a concentration (0.500 ppm) 167 times as great as that detected in the plants.

Discussion

The reduced wax load and crystallite density, as well as altered composition of surface waxes on EPTC-treated plants are similar to those on genetic glossies. Wax load on EPTC-treated plants in this study averaged 25.6 $\mu g/cm^2$ and those on untreated plants averaged 67.6 $\mu g/cm^2$. These are comparable with the average wax load of 27.5 $\mu g/cm^2$ on 10 glossy resistant genotypes vs 61.3 $\mu g/$

Table 3. Percentage *P. xylostella* larvae surviving on artificial diet with EPTC added

EPTC concentration ppm	Percentage surviving x ± SEM
0.000	94.00 ± 2.14
0.001	93.33 ± 2.11
0.003*	92.00 ± 2.00
0.010	88.67 ± 2.74
0.050	93.75 ± 2.56
0.500	91.33 ± 1.92

ANOVA $F = 0.780$, $P = 0.5667$, df = 5, 85. There are no significant differences among the treatment means.

* Concentration in EPTC-treated plants (*F2*) on the day of infestation.

cm^2 on 8 normal wax susceptible genotypes of *Brassica oleracea* reported by Eigenbrode *et al.* (1991a). Wax crystallite densities on EPTC-treated 'Market Prize' and untreated controls in this study (211/1000 μm^2 and 1014/1000 μm^2) are also comparable with average crystallite densities on glossy and normal wax genotypes (88.8/1000 μm^2 and 817.6/1000 μm^2) reported by Eigenbrode *et al.* (1991a). The composition of EPTC glossy waxes, as determined qualitatively with TLC, more closely resembles that of genetic glossy NY 8329 than untreated 'Market Prize' (Fig. 1).

Changes in leaf surface waxes are the principal characteristics which can account for reduced survival and increased movement rates of *P. xylostella* on EPTC-treated plants. Although EPTC has several minor effects on plant metabolism, its main effects are reduction in wax load and changes in the composition and morphology of leaf surface waxes (Corbett, 1984). The lack of toxicity of EPTC to *P. xylostella* at concentrations two orders of magnitude greater than found in treated plants (Table 3) eliminates the possibility that larval survival was reduced in the present study by EPTC residues in the treated plants. Chemical or physical attributes of glossy waxes, or both, may be responsible for resistance to *P. xylostella*. Our research with genetic glossies has implicated both (Eigenbrode *et al.*, 1991a, b). Modification of leaf waxes with EPTC does not help to separate these possibilities, because it alters both chemical and physical characteristics. However, unique aspects of the TLC patterns common to genetic and EPTC-generated glossy waxes may indicate the most important chemical attributes for producing the resistance. Since morphological attributes are determined at least in part by wax chemistry (Jeffree *et al.*, 1975), there are methodological barriers to determining the specific mode of action of leaf wax-based resistance to *P. xylostella*.

The substantial reduction in larval survival on treated plants suggests that EPTC and similar herbicides may be useful in agriculture to induce resistance to *P. xylostella* in *Brassica* crops. If practical, this new application of thiocarbamate herbicides would provide a timely alternative to the insecticides which are currently losing efficacy against *P. xylostella*. The method is also attractive from the standpoint of human health because EPTC residues are metabolized rapidly but the effect on leaf waxes, and thus presumably the induction of resistance to the insect, lasts as long as 30 days (unpublished data). Depending on conditions, the treatments may be used to provide simultaneous insect and weed control. Finally, since the basis of the resistance is apparently larval nonacceptance, development of adapted biotypes of *P. xylostella* may be slower than development of resistance to chemical controls.

Although genetically resistant glossy cultivars are also promising alternatives to chemical insecticides, EPTC-induced resistance to *P. xylostella* in existing cultivars could short circuit the difficult and time-consuming breeding efforts required to produce such cultivars. EPTC-treated plants might have a further advantage because the glossy trait is viewed as a horticultural defect by some consumers. Under certain conditions, control may be effected by EPTC-generated glossy leaf for the early stages of the crop development, followed by other controls near harvest, after the crop has outgrown the EPTC effects and once again appears normal.

The difficulties encountered producing EPTC glossy plants in the field (Experiments *F1* and *F2*), indicate that work is required to determine if methods, amounts, and timing of EPTC applications can be developed for practical economic control of *P. xylostella* in *Brassica* crops. We are currently conducting research to determine the economic potential of this technique.

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