Behavior of Neonate Imported Cabbageworm Larvae (Lepidoptera: Pieridae) Under Laboratory Conditions on Collard Leaves with Glossy or Normal Wax[1]

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ABSTRACT Movement and feeding of newly-hatched imported cabbageworms, *Pieris rapae* (L.), on the upper and lower leaf surfaces of collard plants with glossy and normal waxes were monitored using a videocamera. During the 50-min observation period, more of the larvae fed when placed on the lower surface of leaves with normal wax (50 to 68%) than on the upper surface (23 to 32%), or on either upper (27%) or lower (16%) surfaces of leaves with glossy wax. A larger portion of the larvae moved at least 1 mm on glossy leaves than on normal leaves, and they moved farther during the observation period on glossy than on normal leaves, and on the upper leaf surface compared to the lower leaf surface. The failure of a large proportion of the larvae to begin feeding on either surface of leaves with glossy wax is likely to be an important factor in the resistance to imported cabbageworms observed in the field.

Key Words *Pieris rapae, Brassica oleracea,* imported cabbageworm, epicuticular wax, plant resistance to insects, antixenosis, insect behavior

Glossy lines of various cole crops, including broccoli, cauliflower, cabbage, kale, and collards (all *Brassica oleracea* L.), have been shown to reduce population densities of cabbage looper [*Trichoplusia ni* (Hübner)], diamondback moth [*Plutella xylostella* (L.)], and imported cabbageworm [*Pieris rapae* (L.)] in the field (Dickson and Eckenrode 1980, Shelton et al. 1988, Stoner 1990, 1992). The glossy trait is a visible difference in the color and texture of the leaf surface resulting from differences in the microscopic structure, quantity, and chemical composition of the epicuticular wax (Eigenbrode et al. 1991b).

Although there is some variation in insect resistance among lines, glossy plants in general have fewer imported cabbageworm eggs in the field (Stoner 1990/1992) and are less favorable for survival by imported cabbageworm larvae

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when artificially infested with eggs in the field (Stoner 1992). In seven field trials, the glossy parent used in this experiment, 'Green Glaze,' reduced the abundance of imported cabbageworm larvae under natural infestation in the field by 49 to 96% compared to the normal "Vates' collards (Stoner 1992).

The mechanisms by which glossy leaf waxes confer resistance to imported cabbageworm have not been examined, but the effects on diamondback larvae have been studied in considerable detail. First-instar diamondback larvae move more rapidly, bite less, and establish fewer leaf mines on resistant plants with glossy wax than on normal, susceptible plants (Eigenbrode and Shelton 1990, Eigenbrode et al. 1990, Eigenbrode et al. 1991a, b). In addition, when caged with first-instar diamondback larvae, each of three generalist predators [*Chrysoperla carnea* (Stephens), *Orius insidiosus* (Say), and *Hippodamia convergens* Guerin-Meneville] killed more of the larvae on glossy plants than on plants with normal wax, both in the greenhouse and in the field (Eigenbrode et al. 1995). The increased effectiveness of these predators was attributed to a combination of greater mobility of the predators and altered behavior of the diamondback larvae on glossy plants.

The distribution of imported cabbageworm eggs and larvae on cauliflower plants with normal leaf wax was described by Stewart and Sears (1988). Throughout the season, imported cabbageworm eggs and early-instar (I-III) larvae were found predominately on the lower surfaces of the leaves. From transplanting to heading, both were mainly on the lower surface of older leaves, but as the plants aged, more eggs and early instars were found on the lower surfaces of the middle leaves. On normal cabbage and cauliflower plants, later instars move upward to feed on younger leaves and on cabbage or cauliflower heads, increasing the cosmetic effects and economic importance of the damage (Hoy and Shelton 1987, Stewart and Sears 1988, Hoy et al. 1990).

Thus, the critical time for plant resistance to act against imported cabbageworms is in the early instars, before the economic damage becomes important. The purpose of this study was to evaluate how differences in leaf wax affect the movement and feeding behavior of neonate imported cabbageworms on the upper and lower surfaces of the leaf.

Materials and Methods

Glossy and normal phenotypes segregating 3:1 from greenhouse mass pollination among plants of the glossy, open-pollinated collard variety 'Green Glaze' (Alf Christianson Seed Co., Mount Vernon, WA) were separated into two populations, called 'Green Glaze F_1 glossy' and 'Green Glaze F_1 normal'. The purpose of using the F_1 progeny was to create two populations that would share a gene pool except for the gene for glossiness and linked genes. These two populations, and the collard 'Vates' (Stokes Seed Company, Buffalo, NY), an additional standard variety with normal wax, were used for all experiments. These plants were grown in the greenhouse, in 20-cm pots of Pro-Mix BX soil mix (Premier Horticulture Ltée Rivière du Loup, Québec) under a 16:8 L:D regime, using either metal halide or sodium vapor lamps. Mature, fully-expanded, but not senescent leaves were used.

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Insects were from a laboratory colony, started from 20 adults of *P. rapae* collected at Lockwood Farm in Hamden, CT on 17 September 1992, and maintained until June 1993. Eggs were collected by covering a beaker with a collard leaf, then wrapping a Parafilm strip (American Can Company, Greenwich, CT) around the sides of the beaker. The female butterfly would land on the leaf, then bend her abdomen around to lay her eggs on the Parafilm. Adults were maintained on a 10% sugar solution treated with yellow food coloring, and fed through dental wick. Larvae in the colony were maintained on Brussels sprout plants, 'Jade Cross E.'

Larvae were used in experiments on the same day that they hatched. Because they were hatching on a Parafiilm sheet, they had not previously made contact with a host plant. Three or four larvae per trial were carefully transferred to the leaf surface with an 00 paintbrush. Before the larvae were placed on the leaf, a rectangular arena 10 cm × 10 cm, with the midrib of the leaf running down the center, was marked on the leaf with white laboratory tape. This allowed us to quantify distances traveled on the videotape. The leaf was attached to a foam board in a vertical position, with either the upper or lower surface exposed for testing. Each leaf and each larva was used in only one trial.

Movements of the larvae were recorded with a Panasonic AG-180U VHS Reporter video camera on a time-lapse setting, so that 1 s of activity was recorded each minute. Each trial was recorded for 50 min, and at the end of the trial it was noted whether any of the larvae had fed on the leaf or had left the arena. The video recording was played back on a Panasonic CT1081-Y color monitor, measuring 41 cm × 33 cm, and the path of each larva was traced onto a sheet of clear plastic held up to the monitor, and marked off in 10-min segments. These maps were then transferred to tracing paper for measurement and analysis. Only 3 of 143 larvae left the arena during the test period, all three on leaves with glossy wax. Data for these 3 larvae were removed from the analysis of distance traveled.

The number of larvae feeding and the number of larvae that walked (defined as larvae that moved at least 1 mm during the 50-min test) were analyzed by plant type using a Pearson χ^2 test and the interaction of plant type and leaf surface (upper or lower) affecting these variables was analyzed with a Mantel-Haenszel χ^2 test (Wilkinson et al. 1992). The distance traveled by walking larvae was analyzed using ANOVA on the log-transformed data with plant type and leaf surface as main factors and also testing for a plant type by leaf surface interaction. An index of the straightness of the path of each larva was calculated by dividing the distance between the starting and ending points over the whole 50-min interval by the sum of the distances traveled in each 10 min interval.

Results and Discussion

Neonate imported cabbageworm larvae were more likely to feed during the 50-min observation period on normal leaves than on glossy leaves (Table 1). This difference was entirely due to more feeding on the lower surface of normal leaves (χ^2 = 14.1, df = 2, P = 0.001), with only 16% of the larvae feeding on the

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Table 1. Proportion of imported cabbageworm neonates feeding and walking at least 1 mm in 50 min on upper and lower leaves of collard lines differing in wax characteristics.

Plant type	Leaf surface	No. larvae	Proportion feeding*	Proportion moving > 1 mm**
Green Glaze F_1 glossy	upper	22	0.27	0.86
	lower	25	0.16	0.96
Green Glaze F_1 normal	upper	22	0.23	0.59
	lower	24	0.50	0.54
Vates (normal wax)	upper	25	0.32	0.80
	lower	25	0.68	0.60

*Effect of plant type on larval feeding, Pearson $\chi^2 = 8.648$, df = 2, $P = 0.013$. Interaction of plant type and leaf surface on larval feeding, Mantel-Haenszel $\chi^2 = 5.026$, $P = 0.025$.

**Defined as moving at least 1 mm during the observation period. Effect of plant type on walking, Pearson $\chi^2 = 14.619$, df = 2, $P = 0.001$. Neither effect of leaf surface, nor interaction of plant type and leaf surface on walking were significant.

lower surface of the glossy leaves, compared to 50% to 68% on the lower surface of normal leaves. There was no significant difference in the proportion feeding on the upper surface in all three lines.

Nearly all the larvae (86 to 96%) walked on the glossy leaves, whether placed on the upper or lower surface (Table 1). On both normal lines, a substantial proportion of the larvae (20 to 46%) moved less than 1 mm in the 50-min observation period.

Those larvae that did move traveled farther in 50 min on plants with glossy leaf wax than on plants with normal leaf wax (Table 2). They also traveled farther on the upper surface of the leaf than on the lower surface. Larvae traveling farther than 2 cm generally doubled back or traveled in circles rather than in a straight line. This is reflected in the fact that the distance from the position at the start to the position at the end of the 50-min period is only 64% (mean ± 29% SD) of the sum of the five distances traveled over each 10-min period. Thus, the small fraction (17%) of the larvae that traveled at least 2 cm did not have an obvious direction, but appeared to be wandering.

Overall results of these experiments with neonate imported cabbageworms are similar to those reported for neonate diamondback larvae (Eigenbrode and Shelton 1990), because both species walk more and feed less on glossy leaves than on normal leaves. In this study, the leaf surface tested also played an important role. The imported cabbageworms always traveled farther on the upper surface than the lower surface, and fed less on the upper than the lower leaf surface on plants with normal wax.

This suggests that some chemical or physical cue on the lower surface of leaves with normal wax causes the larva to stop moving and begin feeding.

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Table 2. Distance traveled in 50 min by walking imported cabbageworm neonates on the upper and lower surfaces of collard leaves differing in wax characteristics.

			Distance Traveled* (cm)	
Plant type	Leaf surface	No.	Mean	± SD
Green Glaze F_1 glossy	upper	19	2.2	1.9
	lower	24	1.8	1.2
Gree Glaze F_1 normal	upper	13	1.6	1.3
	lower	13	0.6	0.4
Vates (normal wax)	upper	20	1.1	1.1
	lower	15	0.7	0.5

*Effect of plant type on distance traveled, transformed as ln (x), df = 2,95; $F = 7.183$; $P = 0.001$. Effect of leaf surface, df = 1,95; $F = 6.558$; $P = 0.012$. Interaction of plant type and leaf surface was not significant.

Regardless of the surface being tested, the leaf was in the same vertical position, with the same lighting, so differences in behavior on the different surfaces must be attributed to characteristics of the leaf.

Neonate imported cabbageworms, even on glossy leaves, moved very slowly in this experiment. The distances traveled in 50 min in this experiment are comparable to the rates traveled per minute by neonate diamondback larvae when observed for 1 hr in the field (Eigenbrode and Shelton 1990). Although I have never done the same experiment in the field, I have found that there is also very little movement of early-instar imported cabbageworm larvae from leaf to leaf on intact plants with leaves in a natural position in the greenhouse (K. Stoner, observations). Thus, an imported cabbageworm hatching on the upper side of a leaf might move to the lower side before beginning to feed, but normally would choose an initial feeding site within a few centimeters from where it hatched. The lower leaf surface may be a more favorable environment for survival of early instars because it is more sheltered from sun and rain, and particularly from the hazard of drowning in rainwater that pools on the upper leaf surface.

Given the very minimal rates of travel of even the fastest moving larvae on glossy leaves, it is difficult to see how differing rates of movement of neonates could play a major role in the observed resistance of glossy plants to imported cabbageworm. These neonate larvae are not likely to move off the plant or onto a neighboring plant, and they are probably only marginally more likely though their own movement to encounter pathogens, predators, spray deposits or other hazards. On the other hand, the more rapid movement and more effective

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searching of predators on the glossy plants (Eigenbrode et al. 1995) may be important.

The failure of a large proportion of the larvae to begin feeding is likely to be an important factor in the observed resistance of glossy plants in the field. Newly-hatched larvae that fail to feed would soon be subject to desiccation and starvation. In an insect stage with poor mobility, delay in accepting the host can only increase the natural rate of mortality.

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