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BEHAVIOR OF NEONATE DIAMONDBACK MOTH LARVAE [*Plutella xylostella* (L.)] ON LEAVES AND ON EXTRACTED LEAF WAXES OF RESISTANT AND SUSCEPTIBLE CABBAGES

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Abstract—Neonate *Plutella xylostella* moved more rapidly, spent more time walking, and engaged in searching behaviors more often on leaves of NY 8329, a resistant cabbage with glossy leaves, than on Round-Up, a susceptible variety with normal wax bloom. The neonates also spent significantly more time palpating and more time biting and spinning silk on the susceptible cabbage (although the latter two differences were not significant). Very similar differences in neonate behavior occurred on leaf surface wax extracts (hexane and dichloromethane) of the two cabbage genotypes. Leaf surface waxes are thus strongly implicated in eliciting reduced acceptance of the glossy cabbage by neonate *P. xylostella*. The chemical compositions of the leaf wax extracts were markedly different. Several compounds, including the triterpenols α- and β-amyrin, were found only in the glossy waxes. The percentages of some major wax constituents differed between wax extracts of the two cabbage types. These differences in wax composition may condition the plant resistance in glossy types.

Key Words—*Plutella xylostella*, Lepidoptera, Plutellidae, *Brassica oleracea*, plant epicuticular lipids, leaf surface waxes, insect movement, insect behavior, host-plant resistance.

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INTRODUCTION

The diamondback moth, *Plutella xylostella* (L.), a serious pest of crucifer crops, is extremely difficult to control because of resistance to insecticides in many populations (Talekar and Griggs, 1986; Shelton et al., 1991). Among the alternatives to pesticides are insect-resistant crop genotypes derived from the glossy-leafed cauliflower PI 234599 (Lin et al., 1983, 1984; Eigenbrode et al., 1990; Dickson et al., 1990). These genotypes have a glossy leaf surface wax, which differs from the normal whitish wax bloom (normal bloom) of cultivated *Brassica* (Eigenbrode and Shelton, 1990).

Larval survival on some glossy genotypes of cabbage is $< 1\%$ of survival on normal bloom susceptible cabbages, and first instars are most strongly affected (Lin et al., 1983; Eigenbrode et al., 1990). Neonates move several times faster, establish fewer feeding sites, and have much higher mortality on resistant glossy-leafed plants than on susceptible plants with normal bloom. Disruption or removal of the leaf waxes of NY 8329, a resistant glossy cabbage descended from PI 234599, eliminated differences in (Dickson et al., 1984) *P. xylostella* neonate movement rates between this line and Round-Up, a susceptible cabbage variety with normal bloom (Eigenbrode and Shelton, 1990). These results suggested that glossy leaf surface waxes conditioned resistance to *P. xylostella* by eliciting reduced acceptance of the plants by neonates.

In this paper we report quantitative descriptions of the behaviors of *P. xylostella* neonates on glossy-leafed resistant NY 8329 and normal bloom susceptible Round-Up, in order to define better the host acceptance behaviors of the larvae. The role of leaf waxes in host acceptance was examined by quantifying behaviors on leaf surface wax extracts of these two cabbage genotypes. The composition of the leaf wax extracts used in these assays was determined by GC-MS analysis and chemical differences examined as the possible basis of behavioral discrimination by the larvae.

METHODS AND MATERIALS

Plants and Insects. Seeds of glossy cabbage NY 8329 were provided by M.H. Dickson (Department of Horticulture, New York State Agricultural Experiment Station) and those of a susceptible hybrid cabbage, Round-Up, were obtained from Seedway (Hall, New York). Seedlings were started in the greenhouse on April 15, 1989, and transplanted to field plots on June 16, 1989. Bioassays and extractions were performed using plants six weeks after transplant. Neonate *P. xylostella* were obtained from a laboratory colony, established in the autumn preceding the bioassay studies and maintained through the winter in the laboratory. These insects were reared on the same meridic diet used to rear larvae used in determining resistance levels of the plants.

Bioassay. Before being used in bioassays, neonate larvae were subjected to a two-stage screening procedure to reduce variability in their level of activity. Batches of approximately 1000 eggs freshly laid on cabbage extract-treated aluminum foil sheets were incubated at 28°C. Soon after the larvae hatched, they descended from the foil sheet on a silken thread. The first stage of the screening accepted only those larvae descending 3 cm to 6 cm from the sheet in a 5-min period. This group then was placed in the center of a 10-cm circle on glass, and those larvae reaching the perimeter of the circle in an additional 5 min were used in the bioassays. Thus the larvae used were neonates that had not yet fed, were < 1 hr old, and were similar in activity levels.

Larvae were placed on the test substrate (plant material or wax extract) and their movement and behaviors were quantified for 5 min. Larval movement rates, orientation, and degrees of turn per centimeter of track, as well as time and number of occurrences of specific behaviors were recorded using a computer-assisted monitoring device (Eigenbrode et al., 1989). This device permits simultaneous recording of larval location and specific behaviors observed at 30 $\times$ magnification with a dissecting microscope. Larvae were confined for observation in a 3 $\times$ 5-cm arena by a barrier of silicone grease (Dow-Corning, Corning, New York). If a larva left the arena or became entrapped in the silicone grease barrier, the observation was terminated.

Average speed was defined as the average distance traveled per minute during the entire 5-min observation. Average speed is therefore determined by the speed of walking and amount of time spent walking. This variable is identical with movement rate reported in previous studies of *P. xylostella* on resistant plants (Eigenbrode and Shelton, 1990; Eigenbrode et al., 1991). Walking speed was defined as the average distance traveled per minute, calculated only while the insect was walking. The direction of travel was calculated every 0.25 cm of path. The change in the direction of travel from the previous determination (degrees of turn) then was calculated. The accumulated degrees of turn during a 5-min observation were divided by the total path length of that observation to determine the average degrees of turn per centimeter of path (turning). The specific behaviors recorded were biting, palpating, searching, spinning silk, and walking. Biting was defined as contraction of the mandibular muscles, visible through the larva's cuticle, while the mouthparts were in contact with the substrate. Palpating was defined as touching the mouthparts repeatedly to the substrate but not biting. Searching was defined as raising the front half of the body from the substrate and moving it from side to side. Spinning silk was defined as the deliberate side to side movement of the head while spinning a silken strand and anchoring the strand at the extremes of this movement. Walking was defined as forward movement by the larva.

Test Substrates. Tests were performed on fresh leaves or on leaf surface wax extracts deposited on glass. Leaves were cut from plants in the field and

the petioles were immediately placed in water-filled tubes. The leaves were brought to the lab within 15 min, and observations were made on the underside of the leaves [the leaf surface preferred by larvae in the field (personal observations; Salinas, 1984)]. Leaves selected for study were the sixth to eighth fully expanded leaves on the plant. Each leaf was used for recording for no more than 1 hr (approximately five larvae could be recorded during this time period).

Waxes were removed from the leaf surfaces by washing the leaves in either hexane or dichloromethane. Approximately 30 leaves (sixth to eighth fully expanded) of each of the two genotypes were washed for 10 sec in three consecutive baths of the respective solvent. The three washings were combined, dried over sodium sulfite, and filtered. The amount of wax in each pooled extract was determined by evaporating the samples and weighing the residues. The amount of wax/cm^2 of leaf surface of each cabbage genotype was calculated, using the combined area of the leaves used to prepare the extract. Volumes of extract were applied to clean glass slides and evaporated so that a film of waxes was deposited on the slide. The amount of extract applied was adjusted to produce a wax deposit of approximately 60 μg/cm^2, which is the wax load on the susceptible cultivar, Round-Up. Preliminary studies with different wax loads indicated that this was the best procedure to detect larval discrimination. Leaf waxes deposited on slides did not differ substantially between the two genotypes in morphology, as determined by SEM, unlike the waxes on intact leaves, which have extremely different crystal structures (Eigenbrode et al., 1991). The assays thus detect differences in larval response to wax qualities, independent of amount or morphology of wax on the leaves of the two cabbage genotypes. The wax coated slides were held at -20°C until they were used in the bioassays. Barriers were constructed and larval behaviors were recorded as on intact leaves.

Design of Tests and Analysis. For the fresh leaf tests, 10 leaves of NY 8329 and Round-Up were used and a total of 45 and 52 larvae were recorded on each of the two lines. The recordings took place over a four-day period using larvae from two consecutive cohorts of larvae. The cabbage genotype was alternated hourly during the recording.

The test design was similar on deposited wax extracts. For each solvent $\times$ genotype combination, 30 larvae were observed, five on each of six replicate slides. The treatments were alternated and the replicate slides were used in rotation during recording. The tests for each solvent were conducted and analyzed as separate experiments comparing cabbage varieties, and each experiment used larvae from a single cohort and was conducted over a three-day period.

The behavioral data described above were determined for each larva in the tests. Differences in the mean values of each behavior or category on the leaves or waxes of the two cabbage genotypes were evaluated with Student's t test.

GC-MS Analysis. Extracts were treated with *N,O*-bis(trimethylsilyl)-

acetamide at 110°C for 10 min. Samples were dried under a stream of N_2 and resuspended in hexane. Aliquots were analyzed by combined gas chromatography–mass spectrometry (Hewlett Packard 5890A/5970). The capillary column (25-m cross-linked methyl silicone) was held at 55°C for 3 min after injection (splitless), and the oven temperature was then raised to 305°C at a rate of 15°C/min and held at this temperature for 20 min. Individual peaks were identified by their mass spectra (Holloway et al., 1976; Heupel, 1985; Espelie and Bernays, 1989), which were recorded at 70 eV at intervals of 0.8 sec. Quantitation was based upon the integration of total ion chromatograms.

The trimethylsilyl ether derivatives of standard samples of α-amyrin (K&K Chemicals) and β-amyrin (Pfaltz & Bauer) were found to have retention times and mass spectra identical to those of the components recovered from NY 8329 cabbage leaves, and the mass spectra were matched by computer search with the National Bureau of Standards Mass Spectral Library.

RESULTS

Behaviors of neonate *P. xylostella* differed on fresh leaves of the two cabbage genotypes (Table 1). The larvae spent significantly more time walking, walked more frequently, and walked significantly faster on leaves of NY 8329 than on leaves of susceptible Round-Up. As a result, average speed and walking speed were both significantly greater on the glossy genotype. Neonates also accumulated more degrees of turn per centimeter of path length on Round-Up than on NY 8329, and this difference was marginally significant. The larvae searched significantly more often on resistant leaves and spent more time searching (although the latter difference is not significant at $\alpha = 0.05$). The larvae also spent less time palpating resistant leaves and palpated them less often than susceptible leaves (although the latter difference is not significant at $\alpha = 0.05$). Larvae spent less time biting and spinning and engaged in these two behaviors less often on the resistant leaves than on susceptible leaves (although these differences are also not significant at $\alpha = 0.05$).

Larval behaviors also differed on the extracted leaf surface waxes of the two cabbage genotypes (Table 1). Relative responses of the larvae to waxes of the two genotypes were very similar to the responses to intact leaves from these genotypes. The cabbage genotype eliciting the greater response (occurrences or time) in the intact leaf bioassay almost always also elicited a greater response in the bioassay on the wax extracts. Exceptions were palpations (on both wax extracts) and searching (on hexane extracts), in which there were no differences between the two genotypes. However, the size of the behavioral differences and statistical separations were not as great on leaf wax extracts as on fresh leaves. Statistical separation was greatest on leaves, less on hexane-extracted waxes,

TABLE 1. NEONATE *P. xylostella* BEHAVIORS ON INTACT LEAVES AND LEAF SURFACE WAX EXTRACTS OF NY 8329 AND ROUND-UP CABBAGE GENOTYPES.

Behavior	On Intact Leaf Surface			On Leaf Waves Extracted with Hexane			On Leaf Waxes Extracted with Dichloromethane		
	Round-up	NY 8329	P^a	Round-up	NY 8329	P	Round-up	NY 8329	P
Average time in seconds spent in each behavior (±SEM)									
Searching	45.55 ± 4.00	50.23 ± 2.74	0.3359	20.78 ± 2.92	20.28 ± 2.20	0.9146	14.42 ± 2.24	20.47 ± 2.62	0.1975
Walking	178.4 ± 10.32	214.9 ± 5.69	0.0032	154.43 ± 13.23	196.11 ± 9.68	0.0200	180.56 ± 12.08	200.13 ± 8.92	0.2899
Biting	10.57 ± 4.09	3.73 ± 1.42	0.1205	48.01 ± 8.85	18.66 ± 3.44	0.0001	35.18 ± 6.83	28.03 ± 5.18	0.3104
Palpating	44.69 ± 6.08	21.95 ± 3.02	0.0011	37.15 ± 3.66	28.92 ± 3.04	0.2853	37.25 ± 3.43	29.10 ± 2.76	0.0725
Spinning	9.61 ± 2.63	5.83 ± 1.87	0.2442	29.56 ± 6.70	15.79 ± 4.29	0.0658	27.19 ± 6.50	15.45 ± 3.89	0.1163
Average number of occurrences of each behavior (±SEM)									
Searches	8.73 ± 0.69	15.42 ± 0.66	0.0001	11.53 ± 1.51	13.06 ± 1.21	0.4939	8.80 ± 1.17	13.97 ± 1.50	0.0217
Walks	12.54 ± 0.63	17.62 ± 0.62	0.0001	23.53 ± 2.04	27.93 ± 1.56	0.2352	21.50 ± 1.72	27.37 ± 1.82	0.0315
Bites	0.82 ± 0.26	0.40 ± 0.13	0.1592	7.60 ± 1.00	5.66 ± 0.92	0.0630	6.07 ± 0.93	4.43 ± 0.58	0.1160
Palpations	5.38 ± 0.57	4.03 ± 0.42	0.0602	16.93 ± 1.21	17.33 ± 1.45	0.8250	17.23 ± 1.11	17.67 ± 1.30	0.8107
Spins	1.30 ± 0.28	0.89 ± 0.27	0.3005	6.33 ± 1.04	3.80 ± 0.91	0.0234	4.07 ± 0.68	3.40 ± 0.68	0.5493
Average speed and walking speed (cm/min) and turning (degrees/cm of path ±SEM)									
Average speed	0.54 ± 0.04	1.09 ± 0.06	0.0001	2.83 ± 0.38	3.98 ± 0.38	0.0323	2.92 ± 0.32	4.15 ± 0.45	0.0224
Walking speed	0.88 ± 0.05	1.49 ± 0.06	0.0001	5.37 ± 0.56	5.67 ± 0.51	0.6789	4.98 ± 0.46	5.93 ± 0.54	0.1860
Turning	384.6 ± 33.3	311.5 ± 16.3	0.0521	532.39 ± 95.33	440.52 ± 47.04	0.0523	483.04 ± 54.23	445.54 ± 68.57	0.1885
N	52	45		30	30		30	30	

[a] P = probability of a greater value of Student's t.

and least on dichloromethane-extracted waxes, both in the number of differences significant at $\alpha = 0.05$ (6 vs. 4 vs. 3) and the size of the P values in general. One exception to this trend was biting; on hexane extracts, biting was much greater on Round-Up relative to NY 8329 than on whole plants.

Average speed, but not walking speed, was significantly greater on waxes from resistant NY 8329 than on waxes from susceptible Round-Up. Average speed and walking speed are both more than four times greater on waxes than on leaves of both genotypes (average speed = 3.47 cm/min on waxes vs. 0.82 on leaves; walking speed = 5.46 cm/min on waxes vs. 1.19 on leaves). It is possible that this difference is due to the differences in the larval cohorts used in these experiments. This is unlikely, however, since variation between cohorts in other behaviors is minimal. The mean values for all other behavioral statistics are comparable on fresh leaves and wax extracts.

The wax extracts from the two genotypes were shown by GC-MS to be very different chemically (Table 2). The epicuticular lipids recovered from leaves of Round-Up were dominated by a few components: *n*-nonacosane, 14- and 15-nonacosanol, 15-nonacosanone, and *n*-hentriacontane. These compounds comprised 91% of the surface lipids in the dichloromethane extract of Round-Up leaves, but only 13% of the surface lipids in the dichloromethane extract of NY 8329 leaves (Figure 1). Primary fatty alcohols were the major components (37% in the dichloromethane extract and 45% in the hexane extract) of the NY 8329 leaf wax, with hexacosanol the dominant homolog (Table 3). The NY 8329 leaves had a much lower proportion of *n*-alkanes than did the Round-Up leaves, and within this class of compounds the NY 8329 surface lipids had a shorter average chain length (more C_{25} and C_{27}; no detectable C_{31}). The secondary alcohols, 13- and 14-heptacosanol, were found only in the extracts of NY 8329 leaves, while the secondary diol, 14,15-nonacosandiol, was more prominent in the Round-Up leaf wax extracts (Table 2). The triterpenols, α- and β-amyrin, were detected only in the surface lipids of the NY 8329 leaves.

The hexane and dichloromethane extracts of Round-Up leaves were similar in chemical composition. There were greater differences between the two extracts of NY 8329 leaves. The dichloromethane extracts had a larger proportion of fatty acids (10%) than did the hexane extracts (2%) and hexacosanoic acid was found only in the dichloromethane extract (Table 2). There was also a greater amount of triterpenols in the dichloromethane extract (9%) of NY 8329 leaves than in the hexane extract (3%).

DISCUSSION

The differences in neonate *P. xylostella* behavior on intact leaves of resistant glossy NY 8329 and susceptible normal bloom Round-Up cabbage support the hypothesis that larvae reject the resistant glossy plants. Increased larval

TABLE 2. COMPOSITION (%) OF CUTICULAR LIPIDS RECOVERED BY HEXANE OR DICHLOROMETHANE EXTRACTON OF ROUNDUP AND NY 8329 CABBAGE LEAVES

Peak	Component	Hexane		Dichloromethane	
		Round-up	NY 8329	Round-up	NY 8329
1	Tetradecanoic acid		0.5	0.1	1.1
2	Pentadecanoic acid				0.5
3	Hexadecanoic acid	0.3	0.9	0.2	3.4
4	Octadecenoic acid		0.2		0.8
5	Octadecanoic acid		0.3		2.1
6	*n*-Pentacosane		1.1		2.6
7	Docosanol		0.8		1.9
8	*n*-Heptacosane	3.0	5.9	0.9	4.7
9	Tetracosanol		10.7	0.2	4.3
10	Pentacosanol		5.9		3.8
11	13- and 14-Heptacosanol		3.5		5.9
12	*n*-Nonacosane	27.4	3.7	38.3	4.7
13	Hexacosanol	1.6	21.4	1.5	19.2
14	Heptacosanol	1.2	5.3	1.2	6.7
15	Hexacosanoic acid				1.7
16	14- and 15-Nonacosanol	14.7	2.4	19.4	3.7
17	15-Nonacosanone[a]	15.9	3.0	21.1	4.8
18	*n*-Hentriacontane	7.7		12.1	
19	Octacosanol		0.8		0.8
20	14, 15-Nonacosandiol	4.1		2.8	0.3
21	Triacontanol	1.5		0.8	0.4
22	β-Amyrin		0.8		2.5
23	α-Amyrin		2.0		6.2

[a] Elutes with 14- and 15-nonacosanol; estimated by integration of selected ion chromatograms.

movement rates, which had been interpreted as indicators of larval nonacceptance of glossy resistant *Brassica oleracea* (Eigenbrode and Shelton, 1990; Eigenbrode et al., 1991), are shown here to be associated with increased searching and reduced biting and silk spinning on these plants, as compared with the susceptible normal bloom type.

The epicuticular lipid composition found for the leaves of Round-Up was similar to that previously reported for *Brassica oleracea* (Netting et al., 1972; Baker, 1974). In each class of components, C_{29} was consistently the dominant homolog. NY 8329 lipids were not dominated by the C_{29} homologs and had a more diverse composition. The higher percentage of free fatty acids and primary alcohols and lower percentage in alkanes, secondary alcohols, and ketones in

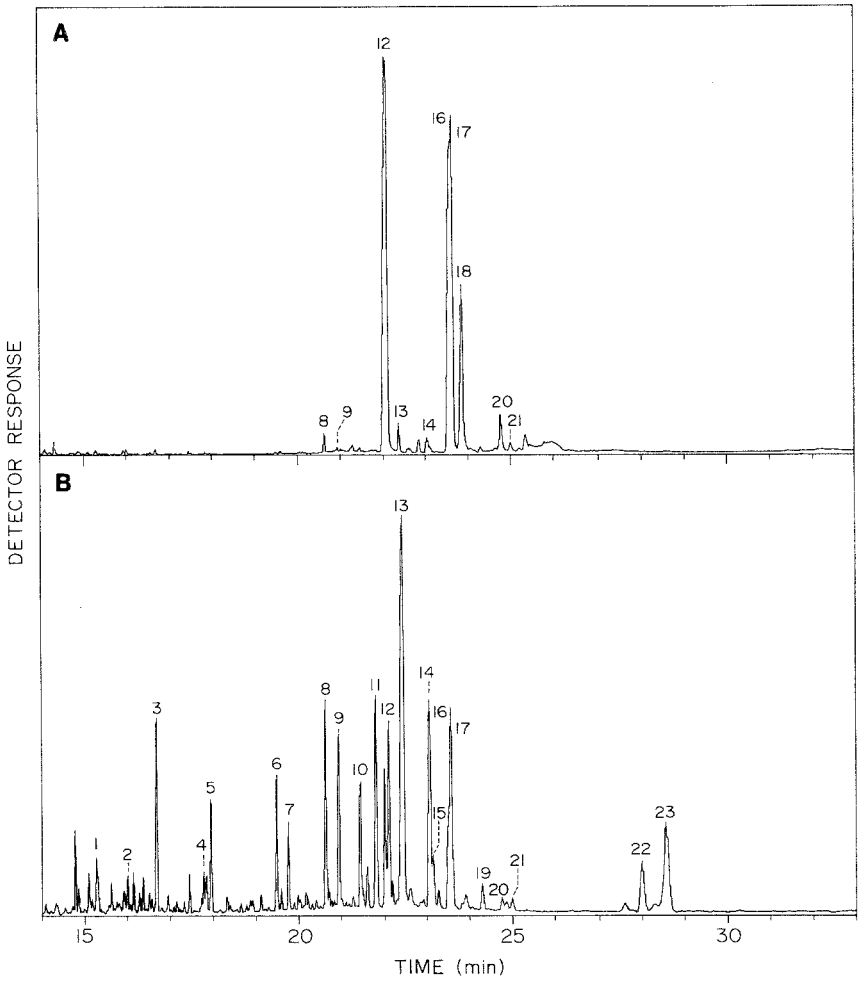

FIG. 1. Total ion chromatograms of the derivitized lipids recovered from the leaves of Round-Up (A) and NY 8329 (B) cabbage by dichloromethane extraction. Numbered peaks are identified in Table 2.

NY 8329 epicuticular lipids, as compared with the lipids of normal genotypes, resembles previously studied glossy mutants of *Brassica oleracea* (Baker, 1974).

The similarity of neonate *P. xylostella* behaviors on leaf wax extracts and intact leaves of NY 8329 and Round-Up implicate the leaf surface waxes in eliciting reduced acceptance of intact glossy plants. The substantial chemical differences between the wax extracts evidently produced the observed behavioral differences. It appears likely that discrimination by the larvae is a response

TABLE 3. CUTICULAR LIPID COMPOSITION (%) OF ROUNDUP AND NY 8329 CABBAGE LEAVES

Class of component	Hexane		Dichlormethane	
	Round-up	NY 8329	Round-up	NY 8329
n-Alkanes	38.1	10.7	51.3	12.0
Primary alcohols	4.3	44.9	3.7	37.1
Secondary alcohols	18.8	5.9	22.2	9.9
Ketones	15.9	3.0	21.1	4.8
Free fatty acids	0.3	1.9	0.3	9.6
Triterpenols	0.0	2.8	0.0	8.7

to a combination of stimulants and deterrents. Waxes from resistant and susceptible plants stimulate more biting and spinning than untreated glass or paraffin-treated glass substrates (unpublished data). In the experiments we report here, all larvae did some biting or spinning on resistant waxes and some searching on susceptible waxes. Those compounds comprising a lower percentage of the epicuticular waxes of NY 8329 than of Round-Up (Table 2, peaks 12, 16, 17, 20, and 21) are candidates as feeding stimulants or arrestants. This group contains the C_{29} *n*-alkane, alcohols, ketone, and a diol, as well as the C_{30} alcohol. Those components that comprise a higher percentage of (or are only present in) NY 8329 waxes (Table 2, peaks 1–11, 13–15, 22, and 23) may be deterrents. This group contains the free fatty acids, all the *n*-alkanes and fatty alcohols with C number <29, and α- and β-amyrin. Of particular interest are α- and β-amyrin, which together comprise 9% of the cuticular lipids of the NY 8329 leaves (Table 2) and had been found previously in the leaf wax of *Brassica napus* (Holloway et al., 1977). The triterpenols comprised only 0.4% of the total cuticular lipids in *B. napus*, but the α- and β-amyrin were found in the same ratio (2.5 : 1) as they were in the leaves of NY 8329. The palmitate ester of α-amyrin has been shown to inhibit growth of several lepidopteran species (Shankaranarayana et al., 1980). The structural similarity of α- and β-amyrin (which have the ursane and oleanane ring skeletons, respectively) to sterols may cause them to be toxic to some phytogphagous insects, and the presence of these compounds in the cuticle of NY 8329 leaves may play a role in the observed resistance to diamondback moth.

Hexane extracts of the cabbage leaves elicited greater behavioral differences with better statistical separation than did the dichloromethane extracts. Discrimination on the basis of biting was more clear between hexane extracts than intact plants. This suggests that the nonpolar constituents of the leaf waxes,

which are more dominant in the hexane extract, may be important in eliciting the behavioral differences. Those possible deterrents in higher relative concentrations in hexane extracts than in dichloromethane extracts (Table 2, peaks 1, 9, 10, and 13; tetradecanoic acid, tetracosanol, pentacosanol, and hexacosanol) and those possible stimulants in lower relative concentrations in hexane extracts (Table 2, peaks 16, 17, 20, 21; 14- and 15-nonacosanol, 15-nonacosanone, 14,15-nonacosandiol, and triacontanol) could be important allelochemics in the leaf waxes.

Although the physiological mechanisms by which insects detect nonpolar compounds with low volatility remain obscure, there is sufficient evidence that leaf surface wax components act as allelochemics. Plant surface wax extracts (Blaney and Chapman, 1970; Bernays et al., 1976, 1985; Albert and Parisella, 1983; Woodhead, 1983, 1987; Maloney et al., 1988; Woodhead and Padgham, 1988; Varela and Bernays, 1988; Chapman and Bernays, 1989), and specific compounds typically found in leaf waxes, including long chain alkanes (Klingauf et al., 1971, 1978), alcohols (Nayar and Fraenkel, 1962; Mori, 1982) esters (Woodhead, 1983; McKibben et al., 1985), and carboxylic acids (Greenway et al., 1978), can act as allelochemics affecting insect herbivore behavior. Thus, it is not unreasonable to conclude that specific compounds in *Brassica* waxes influence *P. xylostella* larval behaviors. Additional bioassays with chromatographic fractions of the crude wax extracts and with pure samples of the specific compounds listed above should determine the most active components.

The ability of *P. xylostella* larvae to discriminate between host-plant genotypes and leaf waxes is innate; the animals in these tests were neonates with no experience with potential food sources. In only a few other cases has innate discrimination on the basis of leaf surface lipids been demonstrated (Mori, 1982; Bernays et al., 1985; Varela and Bernays, 1988). In certain ecological settings, innate neonate discrimination at the leaf surface may permit larvae to locate the most suitable host plants, or host-plant tissues, before beginning to feed. Discrimination by neonates therefore can be adaptive and possibly occurs in many herbivores. However, increased searching and walking and reduced feeding behaviors on glossy cabbages in an agricultural monoculture apparently prevent timely establishment by *P. xylostella* larvae and result in the observed higher mortality on these plants.

The differences between neonate behaviors on the surface wax extracts of NY 8329 and Round-Up in this study were less distinct statistically than those on intact leaves of the resistant and susceptible cabbages. On intact leaves, the larvae apparently respond to other factors in addition to leaf wax composition. Our previous work has indicated that physical characteristics of the leaf surface waxes affect larval behavior and survival. Intact leaves of glossy resistant plants have reduced densities of wax crystallites and reduced amounts of wax/cm^2 (wax load) on their leaf surfaces, as compared with susceptible *Brassica* geno-

types (Eigenbrode et al., 1991). Crystallite density and wax load explained 69% of the variation in larval survival on a collection of 18 *Brassica* genotypes.

This statistical relationship between *P. xylostella* survival and movement and leaf wax physical characteristics may, however, only indicate a behavioral response to wax chemistry. This is possible because the morphology of leaf surface waxes on *Brassica* is largely determined by leaf wax chemistry (Jeffree et al., 1975; Holloway et al., 1977; Jeffree, 1986). Our data suggest, however, that chemical and physical attributes of leaf waxes act together, possibly with other leaf characteristics, to influence neonate *P. xylostella* behaviors. Differences in larval behavior occurred on pure wax extracts with identical morphology but were more pronounced on leaves with morphologically intact waxes. The much higher movement rates of larvae on wax extracts than on intact leaves, and the smaller differences in walking speed on extracts as compared with intact leaves (Table 1), suggest that wax morphology has particularly strong effects on larval locomotion.

This system represents one of the best examples of surface wax-mediated host-plant suitability, since survival of *P. xylostella* on glossy cabbages descended from PI 234599 is so much less than survival on normal bloom genotypes ($< 1\%$). It is therefore important to elucidate the mechanisms of this interaction. Better understanding of reduced acceptance and survival of *P. xylostella* on glossy resistant *Brassica* also will improve screening procedures for the development of cultivars resistant to this important pest.

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