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NEONATE *Plutella xylostella* RESPONSES TO SURFACE WAX COMPONENTS OF A RESISTANT CABBAGE (*Brassica oleracea*)

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Abstract—Behavior of neonate *Plutella xylostella* was observed and quantified during the first 5 min of contact with cabbage surface waxes and surface wax components deposited as a film (60 $\mu g/cm^2$) on glass. The time larvae spent biting was greater and the time walking was less on waxes extracted from the susceptible cabbage variety, Round-Up, than on an insect-resistant glossy-wax breeding line, NY 9472. The waxes of both cabbage types were characterized and some of the compounds present at higher concentrations in the glossy waxes were tested for their deterrent effects on larvae by adding them to the susceptible waxes. Adding a mixture of four *n*-alkane-1-ols or a mixture of α- and β-amyrins to wax from susceptible cabbage reduced the number of insects biting and, among those biting, reduced the time biting and increased the time walking in a dose-dependent manner. Among individual *n*-alkane-1-ols, adding C_{24} or C_{25} alcohols reduced the number of insects biting but only adding C_{25} alcohol reduced the time spent biting among those insects that initiated biting. Adding a mixture of five *n*-alkanoic acids did not affect biting, but increased the time spent palpating and decreased walking time. Among individual *n*-alkanoic acids, only adding C_{14} significantly increased the time palpating. If the observed responses were gustatory, the results indicate that some primary wax components, including specific long-chain alkyl components, have allelochemical activity influencing host acceptance behavior by a lepidopteran larva.

Key Words—Surface waxes, triterpenoids, amyrins, alcohols, fatty acids, diamondback moth, glossy wax, deterrent, host selection, insect-plant interactions, host plant resistance.

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INTRODUCTION

From an ecological and evolutionary perspective, plant surface attributes are expected to be important factors influencing host selection by phytophagous insects (Chapman, 1977). Plant surface waxes may play a role in this process. Several studies show that they influence herbivore behavior (reviewed in Woodhead and Chapman, 1986; Juniper, 1994; Eigenbrode and Espelie, 1995), but it is not always clear whether the primary wax components, or plant secondary compounds associated with the waxes, are the active factors. Some reports show that wax components themselves have allelochemical activity (Klingauf et al., 1971; Bernays et al., 1976; Greenway et al., 1978; Sherwood et al., 1981; Mori, 1982; Phelan and Miller, 1982; Woodhead, 1983; Herbach, 1987; Varanda, 1992; Udayagiri and Mason, 1997), but rarely have these studies included wax components known to vary among host plants that elicit different behavior by an insect herbivore (Bernays et al., 1976; Woodhead, 1983). More studies are needed to examine the responses of insects to typical surface wax components with potential relevance in host selection.

Brassica oleracea with glossy surface waxes are resistant to *Plutella xylostella* L. (Lepidoptera: Plutellidae), and this resistance is associated with different neonate larval behavior on the plant surfaces (Eigenbrode et al., 1991a). *P. xylostella* larvae spent more time walking and searching and less time biting and palpating on surface waxes extracted from an insect-resistant cabbage that has a glossy wax caused by the mutation g_a (Dickson and Eckenrode, 1980; Dickson et al., 1984) than they do on waxes extracted from a susceptible cabbage with a typical waxy bloom (Eigenbrode et al., 1991b). This effect appears to be allelochemical because the waxes as bioassayed did not differ in surface wax morphology. Primary wax components appear to be the active compounds, rather than volatiles or polar compounds that might be on the plant surface, because the extracts were obtained with hexane and subjected to evaporation under reduced pressure at 40°C. The glossy waxes had smaller proportions of *n*-alkanes, secondary alcohols, and ketones and greater proportions of *n*-alkanoic acids (fatty acids), *n*-alkane-1-ols (primary alcohols), and triterpenols as compared with typical *B. oleracea* waxes (Eigenbrode et al., 1991b). The components comprising a greater proportion in waxes from the susceptible cabbage may include stimulants that increase biting, palpating, and spinning and decrease walking, while those comprising a greater proportion in waxes from resistant glossy plants may include deterrents that reduce acceptance behavior by the larvae. The present study was designed to test the deterrent effects of some of the surface wax components elevated in a glossy wax cabbage with the gl_a gene.

METHODS AND MATERIALS

Plants and Insects. Surface waxes were obtained from two cabbage genotypes grown in the field with irrigation in March and April 1994 in Tucson, Arizona. The susceptible typical-wax cabbage was the commercial hybrid Round-Up (Fery Morris), and the resistant glossy-wax cabbage was breeding line NY 9472 expressing gl_a, obtained from Dr. M. H. Dickson, Cornell University.

P. xylostella neonates were obtained from a colony maintained on artificial diet at the University of Arizona and derived from the Geneva 88 colony (A. M. Shelton, Cornell University). Eggs were collected on aluminum foil and allowed to hatch at room temperature. Within approximately 1 hr of hatch, insects descending from the foil sheet on silk strands were used in behavioral bioassay.

Bioassay Procedure. The bioassay procedure was similar to the one used by Eigenbrode et al. (1991a). Wax mixtures were deposited as an amorphous film (60 $\mu g/cm^2$ unless otherwise noted) on glass slides (4-cm × 4-cm) by rapid evaporation of a hexane solution. Neonate *P. xylostella* were confined on this film within a 4-cm-diam. circular arena bounded by a bead of silicone grease (Dow-Corning). Individual neonates were observed with a dissecting microscope during their first 5 min of contact with each test mixture, and the total times in behavioral categories (Table 1) were recorded on a computer (Arena program, Eigenbrode and McInnis, unpublished; Eigenbrode et al., 1989). Observations

TABLE 1. CATEGORIES USED TO QUANTIFY NEONATE *P. xylostella* BEHAVIOR IN BIOASSAYS

Behavioral category	Defnition
Biting	Contracting mandibular muscles, visible through cuticle, while mouthparts are in contact with substrate
Spinning	Deliberate side-to-side movement of head while spinning a strand and anchoring the strand of silk at the extremes of this movement
Palpating	Touching mouthparts repeatedly to the substrate but not biting
Searching	Raising the front half of the body from the substrate and moving the body from side to side
Walking	Forward movement
Other	Unidentifiable; stationary; or in contact with barrier on observational arena; together accounting for less than 5% of total time

were conducted in a controlled temperature cabinet (28°C). Illumination was omnidirectional from a fiberoptic ring light attached to the microscope objective.

Response of neonate larvae to wax extracts of NY 9472 and Round-Up was quantified to confirm previously reported relatively reduced biting and increased walking on waxes from the resistant plant (Eigenbrode et al., 1991a). The wax extracts were analyzed (methods below), and representatives of primary alcohols, fatty acids, and triterpenoids (three classes of components found in higher concentrations in the NY 9472 waxes as compared with Round-Up waxes) were bioassayed by adding them as purified compounds to Round-Up waxes. The procedure was designed to detect any deterrent effects of these components in the context of a surface wax mixture as they would be encountered on the plant. The waxes from susceptible cabbage stimulate biting, palpating, and spinning silk, providing a baseline response against which deterrence of tested components can be detected.

Two types of tests were conducted. In the first, susceptible waxes were augmented with mixtures of the components within each of the three classes. The mixtures of primary alcohols and fatty acids were in equal proportions by weight, and the mixture of amyrins was the 1:8 α:β ratio obtained from a natural source (see below). Each mixture was added to susceptible wax extracts in a range of amounts to approximate the concentration of the respective class in NY 9472 waxes. In the second type of test, individual primary alcohols and fatty acids were used to augment Round-Up waxes by 10%. None of the treatments mimics the exact quantitative composition of the resistant NY 9472 waxes, nor were the amounts of wax adjusted to reflect the lower amounts of waxes found on NY 9472 versus Round-Up (Eigenbrode et al., 1991b). The primary objective was to identify components that act as deterrents in a wax mixture.

The control in all bioassays was the susceptible wax extract alone (control I). In some experiments, a second control (control II) was used to measure the effect of dilution of susceptible waxes by the test mixtures. Control II consisted of the susceptible waxes applied to the glass plate in an amount reduced proportional to the maximum dilution by the test mixture.

Test Compounds and Mixtures. Individual (>98% purity) primary alcohols and fatty acids were obtained commercially (Sigma, St. Louis). Amyrins were extracted and purified from germinating *Pisum sativum*, following the methods of Takayuki and Tsuyoshi (1975). Peas (450 g, three days after imbibition) were ground in a mortar and pestle and soaked in 2 liters of methanol for 24 hr. The methanolic extract was filtered and partitioned two times against equal volumes of hexane. The hexane fraction was concentrated and separated on silica gel G columns (2 cm × 10 cm) with a 9:1 hexane–ether mobile phase. Fractions were monitored with GC-MS to isolate amyrins to 98% purity. The α- and

β-amyrin ratio was 1 : 8 in the purified mixture, and the contaminants appeared to be structurally related based on their mass spectra.

Wax Extracts and Analysis. Plant waxes were extracted from NY 9472 and Round-Up cabbage at the preheading stage with 20-sec hexane washes of mature leaves at room temperature. To avoid extracting internal plant constituents, cut petioles and damaged leaves were not immersed in the solvent. Waxes of all leaves of 10 plants of each type were pooled. For analysis, each wax extract was derivatized with *N,O*-bis(trimethylsilyl)acetamide to generate trimethylsilyl ethers and esters of alcohols and acids. The derivatized mixture was dissolved in hexane and analyzed by gas chromatography (GC). For quantification, the derivatized lipids were injected along with an *n*-hexadecane internal standard onto a fused silica capillary column (100% methylpolysiloxane, HP Ultra-1, 15 m, 0.2 mm ID, 0.250 μm film) on a Hewlett Packard 5890 series II gas chromatograph equipped with a flame ionization detector. The oven temperature program was 80°C for 1 min, 15°/min to 260°C, hold 10 min, then 5°C/min to 320°C and hold 15 min. Response factors were determined from known standards (*n*-pentacosane, *n*-heptacosane, *n*-nonacosane, *n*-triacontane, and trimethyl-silyl esters and ethers of 1-tetradecanoic acid, 1-hexadecanoic acid, 1-octadecanoic acid, 1-hexacosanoic acid, 1-docosanol, 1-octacosanol, and 1-triacontanol), or approximated for components for which standards were not obtained. Identification of the components was on the basis of mass spectra of representative samples run on a Hewlett Packard 5890 gas chromatograph equipped with a HP 5973 quadrapole mass selective detector and on the retention times of standards. Relative composition of the wax components was calculated on a percent basis.

Experimental Design and Statistical Analysis. Thirty insects were observed on the wax extracts from glossy and normal-wax cabbage. Forty insects were observed on treatments involving Round-Up waxes augmented with pure compounds. Three to five insects were observed on each of several individual preparations of the treatments within an experiment. Because all wax treatments appeared similar, it was possible to conduct the experiments using a single-blind design in which the observer was not aware of the treatment. Biting was the most variable behavioral category, and some insects did not bite at all during a 5-min observation. Therefore, a two-step analysis was used. First, the proportion of insects biting during the 5-min observation was compared among treatments using χ^2. Second, only the subset of insects biting in 5 min was included in a multivariate analysis of variance (MANOVA) (Harris, 1985) with times (standardized to seconds per minute) in each behavior as dependent variables and wax treatment as the independent variable. Wilk's lambda MANOVA statistic assessed the significance of treatment effect on all behavioral categories simultaneously and individual ANOVA *F* statistics were used to assess the influence

of each behavior. Times in each behavior were transformed to $\log(x)$ to normalize distributions and stabilize variances before analysis.

RESULTS

A smaller proportion of insects initiated biting during 5 min on waxes from glossy cabbage NY 9472 than on waxes from susceptible Round-Up cabbage (Table 2). Among those insects biting within 5 min, time allocated to behavioral categories differed on the two waxes, largely because of reduced biting and increased walking on glossy waxes (Figure 1).

The waxes on the two types of cabbage differed in composition. The concentrations of primary alcohols, fatty acids, and amyrins were elevated in the glossy cabbage waxes (Table 3) as reported for another NY line expressing gl_a (Eigenbrode et al., 1991a). In addition, this analysis showed esters, alkenes, and some unidentified components also are elevated in NY 9472 glossy waxes. Individual wax components selected for subsequent bioassay are indicated in Table 3.

The proportion of insects biting on waxes from susceptible Round-Up was reduced when these waxes were augmented with a mixture of primary alcohols (Table 2). Among those insects biting within 5 min, time allocated to the behavioral categories differed significantly; specifically, biting was reduced and walking was increased on waxes augmented with primary alcohols (Figure 2). There was an approximate dose-related response to primary alcohol concentration in total time spent biting and walking.

The number of insects biting differed significantly among Round-Up waxes augmented with the individual primary alcohols and the control (Table 2). The number of insects biting on waxes augmented with C_{24} and C_{25} alcohols was lower compared to the control and waxes augmented with C_{26} and C_{27} alcohols. Among those insects biting during 5 min, time allocated to behavioral categories differed significantly in response to adding different alcohols (Figure 3); specifically, biting was greatly reduced on waxes with added C_{25} alcohol, and searching was reduced on waxes with added C_{24} alcohol. There was a tendency towards reduced biting, spinning, and palpating in response to adding alcohols with odd-numbered chain lengths (C_{25} and C_{27}) as compared with the even-numbered chain-length alcohols (Figure 3).

The proportion of insects biting on susceptible waxes was not significantly reduced by augmenting with a mixture of fatty acids (Table 2). Among insects biting during 5 min, time allocated to behavioral categories differed significantly; specifically, spinning and palpating were increased and walking was decreased in a dose-dependent manner in response to augmenting with the mixture of fatty acids (Figure 4).

TABLE 2. PROPORTION OF *P. xylotella* NEONATES BITING DURING 5 MINUTES ON EXTRACTED SURFACE WAXES OF RESISTANT AND SUSCEPTIBLE CABBAGE AND ON WAXES OF SUSCEPTIBLE CABBAGE AUGMENTED WITH PURIFIED WAX COMPONENTS

Experiment and treatments	Test mixture ($\mu g/cm^2$)[a]	% of test compounds in mixture[b]	N	Prop. biting	P[c]
Cabbage wax extracts					
Waxes of susceptible Round-Up	60		30	0.97	
Waxes of resistant glossy NY 9472	60		30	0.83	0.047
Susceptible wax with added mixture of primary alcohols					
Control I	60	0	40	0.93	
Control II	36	0	40	0.76	
Primary alcohols C_{24}, C_{25}, C_{26}, C_{27}	60	5	40	0.83	
Primary alcohols C_{24}, C_{25}, C_{26}, C_{27}	60	10	40	0.50	
Primary alcohols C_{24}, C_{25}, C_{26}, C_{27}	60	20	40	0.43	
Primary alcohols C_{24}, C_{25}, C_{26}, C_{27}	60	40	40	0.53	<0.001
Susceptible wax with added individual primary alcohols					
Control I	60	0	40	0.83	
C_{24}	60	10	40	0.70	
C_{25}	60	10	40	0.57	
C_{26}	60	10	40	0.83	
C_{27}	60	10	40	0.93	0.008
Susceptible wax with added mixture of fatty acids					
Control I	60	0	40	0.76	
Control II	48	0	40	0.76	
Fatty acids C_{14}, C_{16}, C_{18}, $C_{18:1}$, C_{26}	60	1	40	0.73	
Fatty acids C_{14}, C_{16}, C_{18}, $C_{18:1}$, C_{26}	60	5	40	0.57	
Fatty acids C_{14}, C_{16}, C_{18}, $C_{18:1}$, C_{26}	60	10	40	0.50	
Fatty acids C_{14}, C_{16}, C_{18}, $C_{18:1}$, C_{26}	60	20	40	0.60	0.119
Susceptible wax with added individual fatty acids					
Control I	60	0	40	0.73	
Control II	54	0	40	0.85	
C_{14}	60	10	40	0.58	
C_{16}	60	10	40	0.64	
C_{18}	60	10	40	0.75	
$C_{18:1}$	60	10	40	0.44	
C_{26}	60	10	40	0.75	0.034
Susceptible wax with added amyrins					
Control I	60	0	40	0.90	
α- and β-amyrin (8:1)	60	1	40	0.51	
α- and β-amyrin (8:1)	60	3	40	0.56	
α- and β- amyrin (8:1)	60	5	40	0.37	<0.001

[a] Amount of test mixture applied to glass for bioassay.
[b] % by weight by which susceptible waxes were augmented with the test compound or test mixture.
[c] P value for χ^2 based on expected equal proportion biting on all treatments within an experiment.

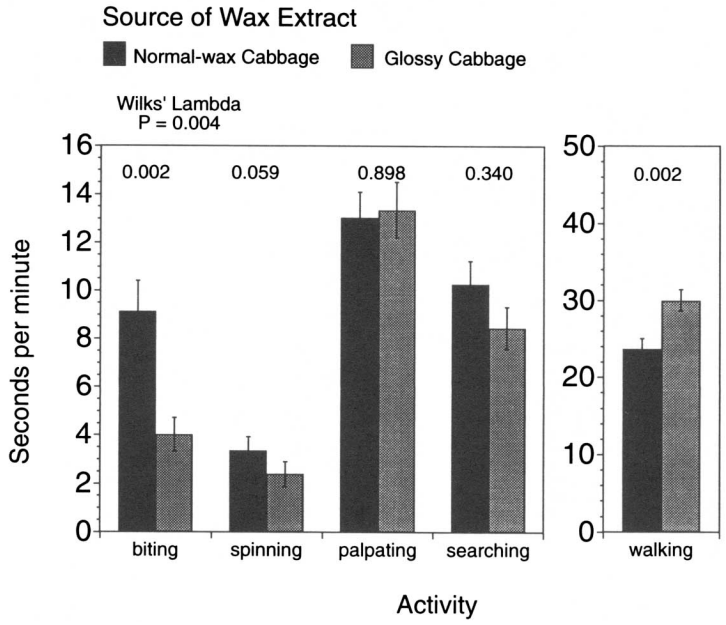

FIG. 1. Proportion of time (seconds per min averaged over 5-min observation) spent by neonate *P. xylostella* in different activities on waxes extracted from resistant, glossy NY 9427 and susceptible, normal-wax Round-Up cabbage. The Wilk's lambda *P* value was used to test for significance of all behavioral categories as response variables to the treatments. Individual *P* values are for ANOVAs for each behavioral category. Of the 30 insects, those biting during the assay were included in the analysis: normal-wax, 29; glossy, 25.

TABLE 3. RELATIVE COMPOSITION (% BY WEIGHT) OF NY 9472 AND ROUND-UP CABBAGE SURFACE WAXES AND COMPOUNDS TESTED FOR DETERRENT EFFECTS ON *P. xylostella* LARVAE

Component class and individual component (order of elution within component class)	Carbon chain length	Round-Up normal-wax (%)	NY 9472 glossy wax (%)	Tested for deterrent effect
Aldehydes				
Tetracosanal	24		0.49	
Hexacosanal	26	0.33		
Octacosanal	28	0.23		

TABLE 3. CONTINUED

Component class and individual component (order of elution within component class)	Carbon chain length	Round-Up normal-wax (%)	NY 9472 glossy wax (%)	Tested for deterrent effect
Alkanes				
Heneicosane	21		0.15	
Tetracosane	24		0.38	
Pentacosane	25	0.17	0.98	
Heptacosane	27	0.65	1.94	
Nonacosane	29	45.18	1.61	
Triacontane	30	0.10	0.29	
Hentriacontane	31	4.26		
Alkenes				
Nonacosene	29	0.17	1.74	
Amyrins				
α-amyrin		0.00	0.59	*
β-amyrin		0.20	0.98	*
Fatty acids				
Tetradecanoic acid	14	0.08	0.19	*
Pentadecanoic	15	0.09	0.11	
Hexadecanoic acid	16	0.07	0.65	*
Octadecenoic acid	18:1	0.03	0.24	*
Octadecanoic acid	18	0.09	0.49	*
Eicosanoic acid	20		0.13	
Docosanoic acid	22	0.07	0.38	
Tetracosanoic acid	24	0.08	1.35	
Hexacosanoic acid	26	0.75	6.23	*
Ketones				
Nonacosanone	29	25.05	2.55	
Primary alcohols				
Docosanol	22	0.04	0.97	
Tricosanol	23	0.12	0.24	
Tetracosanol	24	0.16	2.97	*
Pentacosanol	25	0.18	0.79	*
Hexacosanol	26	0.76	20.66	*
Heptacosanol	27	0.97	1.98	*
Octacosanol	28	0.31	0.57	
Secondary alcohol				
13- and 14-Heptacosanol	27	0.10	0.74	
14- and 15-Nonacosanol	29	13.41		
Secondary diol				
14,15-Nonacosandiol	29	0.63		
Wax esters		1.91	38.02	
Unidentified		3.70	11.58	
Total		100.00	100.00	

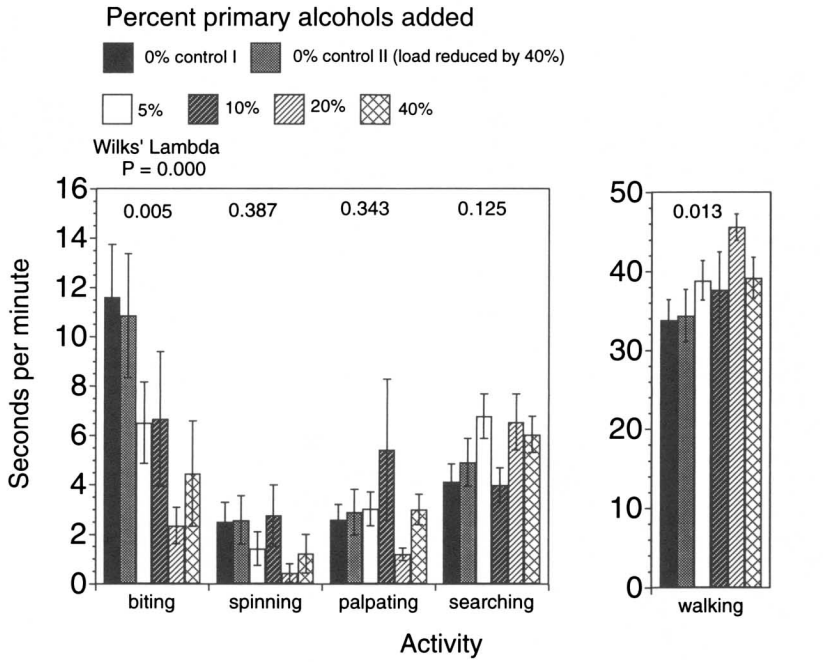

FIG. 2. Proportion of time (seconds per min averaged over 5-min observation) spent by neonate *P. xylostella* in different activities on waxes of susceptible cabbage augmented with a mixture of primary alcohols (C_{24}, C_{25}, C_{26}, C_{27}) at a range of concentrations. The Wilk's lambda *P* value was used to test for significance of all behavioral categories as response variables to the treatments. Individual *P* values are for ANOVAs for each behavioral category. Of a sample of 40 insects, those biting during the assay were included in the analysis: control I, 37; control II, 23; 5%, 33; 10%, 20; 20%, 17; 40%, 21.

The proportion of insects biting on waxes augmented with individual fatty acids differed significantly, largely due to a reduced proportion biting on waxes with added $C_{18:1}$ fatty acid (oleic) (Table 2). Among those insects biting during 5 min, time allocated to behavioral categories differed. Only palpation differed among the treatments, and the greatest effect was increased palpating in response to added C_{14} fatty acid (Figure 5). There were no other significant effects of individual fatty acids on larval behavior.

The proportion of insects biting was significantly reduced on waxes from susceptible Round-Up augmented with the mixture of α- and β-amyrins (Table 2). Among those insects biting during 5 min, time allocated to behavioral categories differed significantly; biting and spinning were reduced, and walking

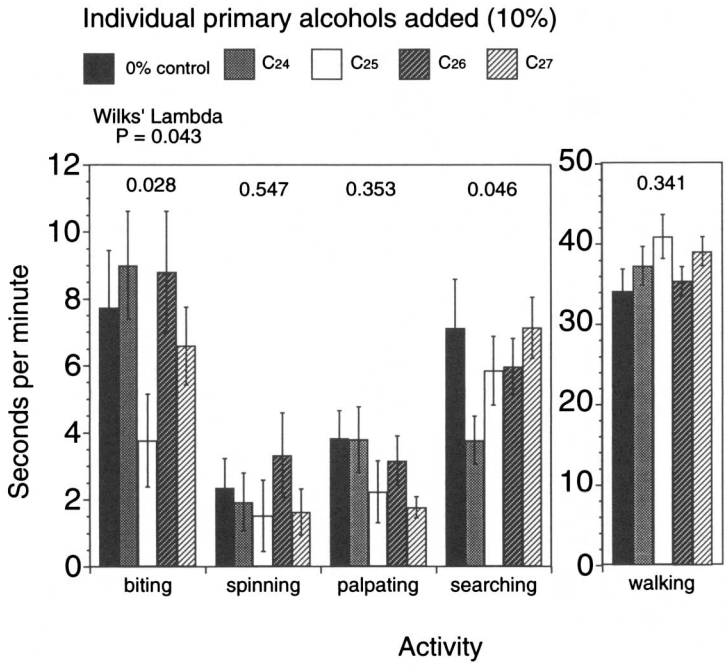

FIG. 3. Proportion of time (seconds per min averaged over 5-min observation) spent by neonate *P. xylostella* in different activities on waxes of susceptible cabbage augmented by 10% with individual primary alcohols. The Wilk's lambda *P* value was used to test for significance of all behavioral categories as response variables to the treatments. Individual *P* values are for ANOVAs for each behavioral category. Of a sample of 40 insects, those biting during the assay were included in the analysis: control, 33; C_{24}, 28; C_{25}, 33; C_{26}, 33; C_{27}, 37.

was increased on waxes with added amyrins (Figure 6). A dose–response effect is apparent for all three behavioral categories.

Control II, which measured the effect of dilution of the susceptible waxes with tested components was not substantially different from control I, except in the alcohol mixture experiment. In that experiment, control II consisted of susceptible waxes reduced by 40%, resulting in a film of only 36 $\mu g/cm^2$, which reduced the number of insects biting (Table 2). Therefore, with the exception of the waxes augmented by 40% with primary alcohols, the effects detected in these bioassays can be attributed to allelochemical activity rather than to dilution of compounds in the susceptible waxes.

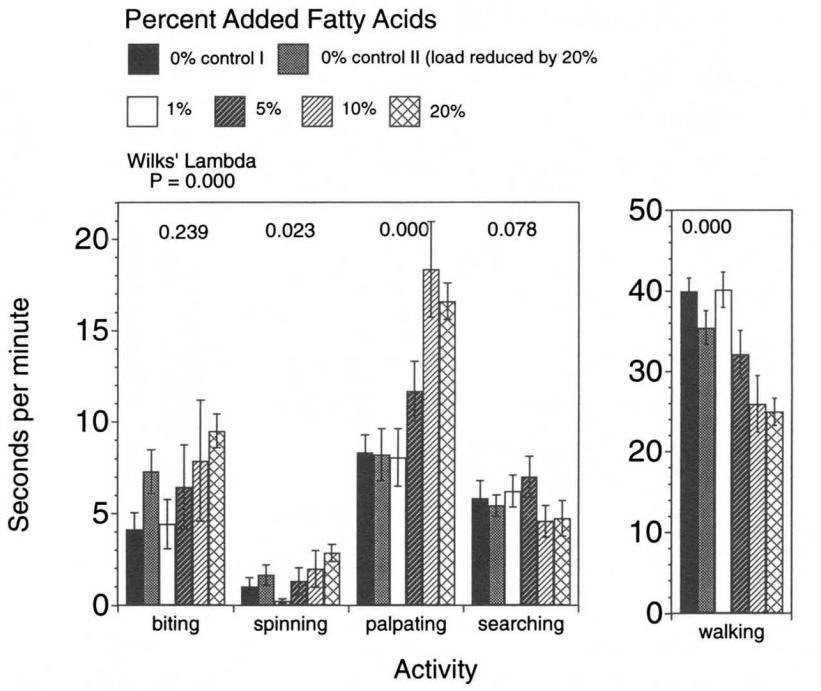

FIG. 4. Proportion of time (seconds per min averaged over 5-min observation) spent by neonate *P. xylostella* in different activities on waxes of susceptible cabbage augmented with a mixture of fatty acids (C_{14}, C_{16}, C_{18}, $C_{18:1}$, C_{26}) at a range of concentrations. The Wilk's lambda *P* value was used to test for significance of all behavioral categories as response variables to the treatments. Individual *P* values are for ANOVAs for each behavioral category. Of a sample of 40 insects, those biting during the assay were included in the analysis: control I, 30; control II, 30; 1%, 29; 5%, 20; 10%, 20; 20%, 24.

DISCUSSION

Waxes of resistant glossy cabbages and susceptible cabbages differ allelochemically, as indicated by reducing biting and increased walking by *P. xylostella* neonates on glossy waxes (Eigenbrode et al., 1991a). The higher concentrations of amyrins and primary alcohols in glossy waxes must contribute to this larval response. Augmenting waxes from susceptible cabbage with mixtures of amyrins or primary alcohols reduced larval biting and increased walking on these waxes in an approximately dose-dependent manner. On waxes from susceptible cabbage augmented with primary alcohols or amyrins to concentrations

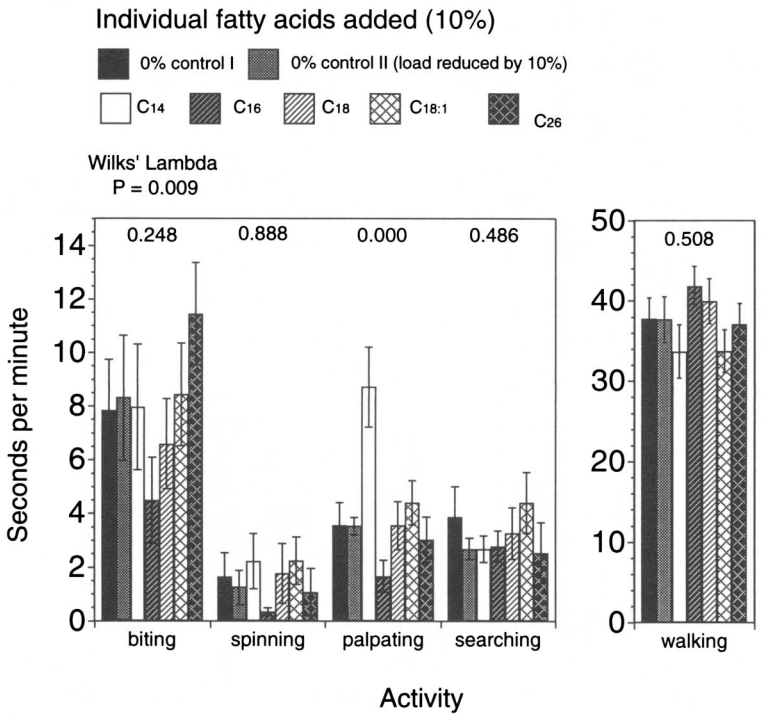

FIG. 5. Proportion of time (seconds per min averaged over 5-min observation) spent by neonate *P. xylostella* in different activities on waxes of susceptible cabbage augmented by 10% with individual fatty acids. The Wilk's lambda *P* value was used to test for significance of all behavioral categories as response variables to the treatments. Individual *P* values are for ANOVAs for each behavioral category. Of a sample of 40 insects, those biting during the assay were included in the analysis: control I, 29; control II, 34; C_{14}, 23; C_{16}, 26; C_{18}, 30; $C_{18:1}$, 18; C_{26}, 30.

near those occurring in glossy waxes, larval biting and walking rates were similar to those on waxes from glossy plants.

In contrast to the primary alcohols and amyrins, the greater concentration of fatty acids in glossy waxes may not contribute to reduced biting and increased walking on these waxes. Adding a test mixture of five of these fatty acids to waxes from susceptible cabbage increased palpation and spinning and decreased walking by *P. xylostella*, indicating these compounds could be stimulants or arrestants.

Among individual primary alcohols, only C_{25} alcohol substantially deterred

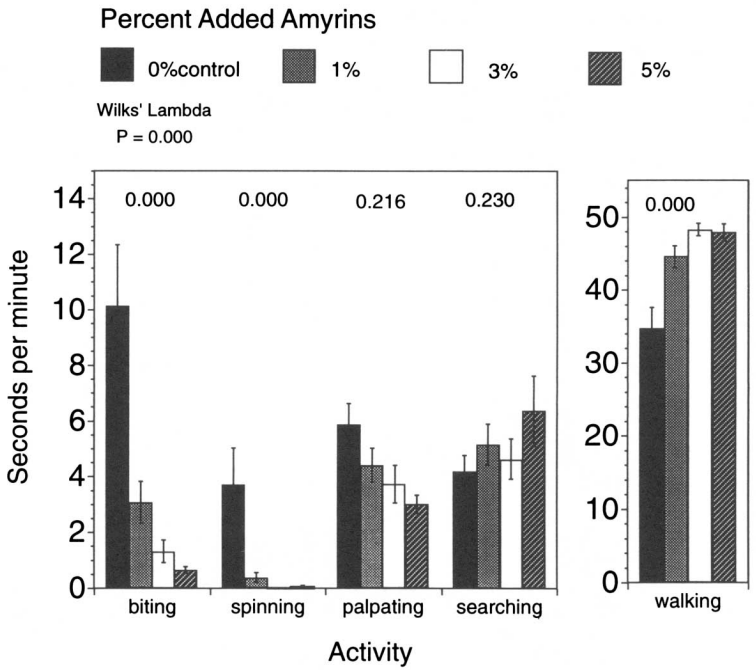

FIG. 6. Proportion of time (seconds per min averaged over 5-min observation) spent by neonate *P. xylostella* in different activities on waxes of susceptible cabbage augmented with a mixture of α- and β-amyrin (8 : 1) at a range of concentrations. The Wilk's lambda *P* value was used to test for significance of all behavioral categories as response variables to the treatments. Individual *P* values are for ANOVAs for each behavioral category. Of a sample of 40 insects, those biting during the assay were included in the analysis: control I, 36, 1%, 20; 3%, 22; 5%, 15.

biting when added to wax from susceptible cabbage and may have contributed most to the effect of the mixture. Individual amyrins were not tested, and it remains to be determined whether their activities differ. Among individual fatty acids tested, only C_{14} fatty acid individually increased palpation, so this component likely accounts for increased palpation in response to the mixture. None of the individual fatty acids affected walking, so the effect of their mixture on walking remains unexplained. Finally, $C_{18:1}$ fatty acid reduced initiation of biting but had no effect on time allocation among those insects biting. Thus, the effects of fatty acids on *P. xylostella* larvae are complex and not simply related to responses of the insects to intact glossy waxes.

The data do not fully explain the larval response to waxes from glossy cabbages because the treatments employed do not mimic glossy waxes. First,

not all potential deterrents (those in higher concentrations in NY 9472 waxes), including some fatty acids and primary alcohols, were tested. Second, the concentrations in augmented mixtures differed from those in glossy waxes. C_{24}, C_{25}, and C_{27} alcohols occur in lower proportions than the C_{26} alcohol in glossy waxes, and the ratio of amyrins is 3:1 α:β, not the 8:1 ratio used in these experiments. Last, potential deterrents were tested by augmentation, but potential stimulants were not tested by removal (e.g., ketones that are in lower concentrations in glossy waxes). Nonetheless, the data indicate that cabbage surface waxes with elevated amyrins and primary alcohols, especially C_{25}, should elicit reduced acceptance by neonate *P. xylostella* and contribute to resistance to this insect.

Responses of *P. xylostella* to individual primary alcohols are consistent with a general hypothesis that atypical or rare wax components should deter herbivores, while more common ones should stimulate them (Woodhead and Chapman, 1986). Primary alcohols occur widely in plant waxes, but those with odd-numbered chain lengths always occur in lower concentrations than those with even-numbered chain lengths (Walton, 1990). In the present study, the odd-numbered chain-length alcohols, C_{25} and C_{27}, generally reduced biting, spinning, and palpating more than the even-numbered chain-length alcohols.

Responses of *P. xylostella* to individual fatty acids are not consistent with the hypothesis that rare, shorter-chain, fatty acids should be more deterrent, as has been found for other insects (e.g., Sherwood et al., 1981). Rather, effects of fatty acids tested were not clearly related to chain length.

The deterrent effects of amyrins towards *P. xylostella* are consistent with evidence that these and other saponins are deterrent or toxic to insects (Gershenzon and Croteau, 1991). Amyrins inhibit feeding by *Locusta migratoria* (Bernays and Chapman, 1976), and the structurally related ursolic acid is deterrent to *Schizaphis graminum* (Varanda, 1992). Concentrations of amyrins in surface waxes are correlated with resistance to *Stephanitis pyrioides* in *Rhododendron* (*Azalea*) (Balsdon et al., 1995) and with aphid resistance in *Sorghum* and *Rubus occidentalis* (Heupel, 1985; Robertson et al., 1991). Amyrins are major wax components in waxes of some plant species (e.g., Baas and Figdor, 1978; Smith and Severson, 1992), but are absent or minor components of waxes of the crucifer species examined so far (Eigenbrode et al., 1991a; Jenks et al., 1995). A deterrent effect of amyrins above certain concentrations in surface waxes might help *P. xylostella* larvae avoid nonhost plants in a mixed canopy.

P. xylostella responses in these bioassays were not necessarily mediated by gustation and could have been affected by physical properties of the mixtures tested. For example, shorter chain-length or unsaturated alkyl compounds have lower melting points than longer-chain or saturated homologs, and this could influence the physical properties of the mixtures containing them at the bioassay temperature (28°C). Physical characteristics of the mixtures were not measured,

however, nor is it obvious how to measure subtle differences in physical properties that might be detected by neonate insects.

Whether discrimination is by gustation or some other means, the data demonstrate behavioral activity of plant surface wax components and show that some of these components can deter insect feeding behavior. This adds to the growing evidence that plant surface wax components can mediate insect-plant interactions.

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