Feeding Deterrence of *Spodoptera exigua* (Lepidoptera: Noctuidae) Larvae by Low Concentrations of Linear Furanocoumarins

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ABSTRACT We conducted choice tests and behavioral observations of *Spodoptera exigua* (Hübner) larvae to study the effect of linear furanocoumarins (bergapten, xanthotoxin, and psoralen) individually or in combination at concentrations commonly found in *Apium graveolens* L. and UV-A light on larval preference and feeding behavior. Linear furanocoumarins had a negative and concentration-dependent effect on diet preference by *S. exigua* larvae. Behavioral observations showed that preference of control diet is the result of feeding deterrence by these compounds. The larvae leave the furanocoumarin-containing diet more frequently by becoming more mobile after ingestion of the treated diet, as measured by time spent questing (in which larvae walk or lift their thoraxes, and move from side to side in a searching motion) and number of questing occurrences. The UV-A effect was not significant. We conclude that linear furanocoumarins (at the concentrations found in *A. graveolens*) can play an important role as behavioral modifiers rather than as toxic defenses against larvae of the generalist herbivore *S. exigua*.

KEY WORDS beet armyworm, insect behavior, allelochemicals, psoralen, xanthotoxin, bergapten

FURANOCOUMARINS ARE BENZ-2-PYRONE compounds with a furan ring fused at the 6,7 (linear) or 7,8 (angular) positions. They are activated by ultraviolet light (photoactivated) to form highly reactive excited states (Musajo and Rodighiero 1962, Berenbaum 1990). They are restricted in distribution among plants, and can play a defensive role against herbivores (Berenbaum 1990, Diawara and Trumble 1997). A review of the furanocoumarin literature suggests that furanocoumarins evoke widely varying responses from different species of insects and at different concentrations (Berenbaum 1990). However, research concerning the effects of furanocoumarins on insects has been conducted for <20 insect species, with the bulk of the studies concentrating on metabolic effects on only 2 or 3 species.

The effect of specific linear furanocoumarins on insect behavior has been reported for a few other insects such as *Psila rosae* (F.) (Stätdler and Buser 1984), *Spodoptera litura* F. (Yajima and Munakata 1979), *Trichoplusia ni* (Hübner) (Berenbaum 1990), *Heliothis virescens* (F.) (Klocke et al. 1989), and *Helicoverpa (=Heliothis) zea* (Boddie) (Berenbaum et al. 1991). However, there is variation among insect species in their response to these compounds. Furthermore, what is known is mostly based on consumption rate measurements in no-choice bioassays; as Gould and Anderson (1991) demonstrated, results obtained in choice tests can be significantly different from results obtained in no-choice bioassays.

The generalist herbivore *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae) is an important pest of >35 crops around the world. In California, *S. exigua* is a major pest of *Apium graveolens* L. (celery) and other vegetable crops (Van Steenwyk and Toscano 1981, Griswold and Trumble 1982, Trumble et al. 1990). Previous studies indicate that *S. exigua* larvae are highly mobile, have a sophisticated host selection behavior, and are capable of detecting and avoiding pesticidal toxins like CryIC from *Bacillus thuringiensis* (Smits et al. 1987, Berdegué and Trumble 1996, Berdegué et al. 1996). Development and survival of *S. exigua* were increasingly and negatively affected as concentrations of bergapten, psoralen, and xanthotoxin also increased (Brewer et al. 1995).

Linear furanocoumarin concentration varies by location in *A. graveolens*. Bergapten had the highest concentrations in both leaves and petioles (5–30 and 0.5–1.0 μg/g, respectively), followed by xanthotoxin (2–18 and 0.1–0.5 μg/g, respectively) and psoralen (0.05–4 and <0.05 μg/g, respectively) (Trumble et al. 1990, 1992; Diawara et al. 1995).

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In this study we tested whether the behavior of *S. exigua* larvae is affected by low concentrations of linear furanocoumarins (bergapten, xanthotoxin, and psoralen) commonly found in *A. graveolens*. Because linear furanocoumarins are photoactivated by UV light between 320 and 360 nm (UV-A)(Musajo and Rodighiero 1962), we also tested whether that UV-A has an effect on furanocoumarin-containing diet preference and consumption by the larvae.

Materials and Methods

Insects. The *S. exigua* colony was originally collected in 1982 from Orange County, California, and has been maintained on artificial diet (modified from Patana 1969) in incubators at 28 ± 2°C and a photoperiod of 14:10 (L:D) h. New genetic material has been added from the same area every year since 1983, with the last addition 6 mo before the study.

Choice Tests. To examine the possible effect of linear furanocoumarins as behavioral elicitors for *S. exigua* larvae, we conducted choice tests with 1st (neonates) and 3rd–4th instars (late instar) between artificial diet (control diet) and artificial diet containing linear furanocoumarins. The methods for these tests were adapted from Gould et al. (1991). The test arenas consisted of 30- and 150-ml plastic cups with 4% agar (wt:vol) in the bottom with 2 and 4 holes in opposite sides for tests with neonates and late instars, respectively. Polyethylene microcentrifuge tubes (1.5 ml) were placed in the holes. Half of the tubes contained control diet and the remaining tubes contained diet with linear furanocoumarins (Berdegué et al. 1996).

We tested psoralen, bergapten, and xanthotoxin (Sigma, St. Louis, MO) independently and in combination at concentrations commonly found in *A. graveolens* ("Tall Utah 5270-R') (Trumble et al. 1990, Diawara et al. 1995). Bergapten was tested at concentrations of 0.50, 0.65, 1.00, and 5.00 μg/g with neonates and 0.50, 1.00, and 5.00 μg/g with late instars. Xanthotoxin was tested at concentrations of 0.10, 2.00, and 5.00 μg/g with neonates and 2.00, 5.00, 10.00, and 18.00 μg/g with late instars. Psoralen was tested at concentrations of 0.05, 1.00, and 5.00 μg/g with neonates and 1.00 and 5.00 μg/g with late instars. We examined the effect of combinations of linear furanocoumarins at levels commonly found in petioles ([0.50 μg of bergapten + 0.10 μg of xanthotoxin + 0.05 μg of psoralen]/g diet) on both neonates and late instars. Finally, we tested the effect of combined furanocoumarins at the level commonly found in inner ([5.00 μg of bergapten + 2.00 μg of xanthotoxin + 0.05 μg of psoralen]/g diet) and outer ([28.00 μg of bergapten + 18.00 μg of xanthotoxin + 4 μg of psoralen]/g diet) *A. graveolens* leaves on late instars (Trumble et al. 1990, Diawara et al. 1995).

We incorporated linear furanocoumarins into artificial diet by dissolving the compounds in acetone and mixing the solution with 3 g of a non-nutritive fiber (Alphacel, ICN, Costa Mesa, CA). The acetone was evaporated and the mixture was resuspended in 27 ml of distilled water. Finally, 70 g of warm artificial diet were added and the mixture was stirred for 3 min before pouring.

We tested the effect of UV-A (350 nm) on larval preference by placing half of the arenas under fluorescent lights (40-W Sylvania Blacklight, General Electric, Cleveland, OH) with an emmitance peak of 350 nm. The arenas were covered with plastic wrap (Smart and Final Plastic, Los Angeles, CA) to permit penetration of ultraviolet radiation. The intensity of the UV-A radiation was measured with a System 371 Optical Power meter with a 268 detector head calibrated at 350 nm (United Technologies, Hawthorne, CA). We regulated the distance between the arenas and the lights to obtain an intensity of ≈400 $\mu W/cm^2$ inside the cups covered with plastic wrap. This level of ultraviolet radiation approximates the intensity found in the canopy of celery fields in southern California (M.B. and J.T.T., personal observation). Controls had UV-A levels <2 $\mu W/cm^2$. We exposed the UV-A treated arenas to UV-A light for 6 h/d during the photophase.

To test diet preference by neonates, we placed 5 neonates (2–12 h old) inside each test arena. For diet preference experiments with late instars, we used two 3rd instars (2–12 h old) per arena. We then placed the arenas in a walk-in chamber at 27 ± 3°C and a photoperiod of 14:10 (L:D) h. We recorded the position of the larvae twice daily at 0900 and 1600 hours for 4 d. Because of differences in number of neonates per arena (caused primarily by mortality), we calculated a preference ratio for each arena with the formula: y = (number of larvae on control diet − number of larvae on linear furanocoumarin-containing diet)/total number of larvae. Because the number of late instars per arena was consistent, preference was calculated by subtracting the number of larvae on linear furanocoumarin-containing diet from the number of larvae on control diet.

Diet consumption by late instars was estimated as follows. An uncorrected diet consumption estimate for each microcentrifuge tube was obtained by subtracting the final from the initial microcentrifuge tube weights. Average water loss for each type of diet was calculated by averaging the differences between initial and final weights of tubes in 3 arenas without larvae and maintained under the same experimental conditions. Consumption was estimated by subtracting the average water loss for each type of diet from the uncorrected diet consumption estimate for each tube. The consumption estimates for the 2 tubes with control diet per arena were added to obtain the total amount of consumed control diet for each arena. The total amount of consumed furanocoumarin-containing diet per arena was obtained following the same method. The difference in consumption

between control and furanocoumarin-containing diet for each arena was estimated by subtracting the consumed furanocoumarin-containing diet from the control diet.

The experiment used a nested design with UV-A and OBSERVATION as main effects and REPLICATIONS nested within the UV-A level. The UV-A effect had 2 levels (present or absent), and the OBSERVATION effect had 8 levels (2 observations per day for 4 d). Each arena was considered a replication, and we replicated each furanocoumarin-UV-A concentration combination 25–27 times.

Behavior Tests. The following 3 distinct feeding behaviors have been described for *S. exigua* (Berdegué and Trumble 1996): (1) eating (i.e., chewing with the mouthparts in contact with the diet surface); (2) questing (i.e., when larvae walk or lift their thoraxes and move from side to side in a searching motion); and (3) swallowing (i.e., which commonly occurs after eating when larvae repeatedly contract esophageal muscles and move their mandibles as if chewing, but mandibles are not in contact with the diet surface). For this test we followed the methods described by Berdegué et al. (1996) and studied the behavior of 1-d-old 4th instars (starved for 12–18 h) by observing the larvae individually on 1.1-cm^3 cylinders of diet during a 9-min period. We tested 3 types of diet—diet containing a combination of linear furanocoumarins at concentrations similar to those found in inner *A. graveolens* leaves ([5.00 μg of bergapten + 2.00 μg of xanthotoxin + 0.05 μg of psoralen]/g diet), diet containing a combination of linear furanocoumarins at concentrations similar to those found in outer *A. graveolens* leaves ([28.00 μg of bergapten + 18.00 μg of xanthotoxin + 4.00 μg of psoralen]/g diet), and a control diet.

A single larva was placed on the cylinder, and the observations began as soon as the larva showed any activity. The observations were recorded using a computer-assisted monitoring device (Eigenbrode et al. 1989); time spent and number of occurrences associated with specific behaviors were recorded. We also recorded the time spent resting (when no activity was observed) and number of resting occurrences. Observations of the larvae for each linear furanocoumarin-containing and control diet were done concurrently. The experiment was repeated 30 times for each furanocoumarin combination and 60 times for control diet (30 times for each furanocoumarin-containing diet).

Data Analyses. The assumption of normality for all data sets was tested with the UNIVARIATE procedure (SAS Institute 1990). Preference data from tests with neonates were analyzed using the GLM procedure. The UV-A and OBSERVATION effects for tests with neonates were analyzed using the UV-A (REPLICATION) and OBSERVATION × UV-A interactions as their respective error terms. We used categorical models (CATMOD procedure) to analyze the OBSERVATION and UV-A effects and their interaction on preference data for late instars. Differences in consumption and preference data for late instars were examined using GLM and MEANS procedures (SAS Institute 1990).

Data for pairwise comparisons between time spent in a particular behavior on control diet and linear furanocoumarin-containing diet and number of occurrences of a particular behavior were analyzed using chi square and *t*-tests (NPAR1WAY and TTEST procedures) (SAS Institute 1990).

Results

Choice Tests. The UV-A effect was not significant (GLM: $P > 0.10$) for any of the tests. Therefore, the UV-A effect is not considered further. All linear furanocoumarins tested had an effect on diet preference by *S. exigua* larvae at levels commonly found in *A. graveolens* (Tables 1 and 2). The highest bergapten and psoralen concentrations and the 2 highest xanthotoxin concentrations caused preference for control diet by neonates. The intermediate psoralen concentration (1.00 μg/g) caused preference for treated diet. The linear furanocoumarin combination at levels similar to those commonly found in *A. graveolens* petioles caused preference for control diet by neonates, although the effect of the individual components at the same concentrations was not significant (Table 1).

Tests with late instars indicated a preference for bergapten-containing diet at the intermediate concentration (1.00 μg/g). The highest bergapten concentration tested (5.00 μg/g) caused preference for control diet. Xanthotoxin and psoralen caused a substantial preference for control diet by late instars at concentrations similar those found in outer *A. graveolens* leaves (18.00 and 5.00 μg/g, respectively). Tests with late instars showed no effect of the linear furanocoumarin combinations at concentrations found in *A. graveolens* petioles and inner leaves. Late instars avoided diet with a linear furanocoumarin combination at concentrations found in outer leaves (Table 2).

A nonsignificant observation effect in preference tests with neonates and bergapten, xanthotoxin (with the exception of the lowest xanthotoxin concentration tested), the lowest psoralen concentration, and the combination of linear furanocoumarins similar to the concentration found in *A. graveolens* petioles indicated that diet preference did not change with time. Conversely, diet preference changed with time for tests with diet containing the lowest xanthotoxin concentration and the intermediate and highest psoralen concentrations (Table 1). Diet preference by late instars did not change with time (CATMOD: $P > 0.10$).

To test a possible concentration-dependent effect of bergapten on larval preference, we performed linear regressions with the ratio of neonates on treated diet as the dependent variable. As bergapten concentrations increased, preference for

Table 1. Effect of linear furanocoumarins on diet preference by *S. exigua* neonates

Furanocoumarin	Concn, μg/g	Preference ratio[a]	Effect[b]	df	*F* ratio	*P*
Bergapten	0.50	+0.03 ± 0.01	Preference	1	0.12	0.7252
			Observation	1	1.40	0.4458
	0.65	+0.07 ± 0.03	Preference	1	2.62	0.1061
			Observation	1	54.59	0.0856
	1.00	+0.06 ± 0.01	Preference	1	2.23	0.1362
			Observation	1	0.09	0.8117
	5.00	+0.33 ± 0.03	Preference	1	54.07	0.0001
			Observation	1	1.01	0.4985
Xanthotoxin	0.10	−0.01 ± 0.03	Preference	1	0.15	0.6958
			Observation	1	4.79	0.0297
	2.00	+0.04 ± 0.03	Preference	1	11.65	0.0007
			Observation	1	5.62	0.2541
	5.00	+0.22 ± 0.03	Preference	1	78.75	0.0001
			Observation	1	4.52	0.0947
Psoralen	0.05	+0.08 ± 0.02	Preference	1	0.30	0.5856
			Observation	1	0.61	0.4355
	1.00	−0.04 ± 0.03	Preference	1	5.12	0.0243
			Observation	1	749.98	0.0232
	5.00	+0.10 ± 0.03	Preference	1	27.65	0.0001
			Observation	1	9.22	0.0027
Combination	Petioles[c]	+0.08 ± 0.02	Preference	1	12.31	0.0005
			Observation	1	8.24	0.2135

[a] Number of neonates on control diet − number of neonates on treated diet/total number of neonates. A positive value indicates a greater number of neonates on control diet.
[b] A significant observation effect denotes an increase or decrease in preference for a particular type of diet as the experiment progressed.
[c] Petiole concentration was (0.50 μg of bergapten + 0.10 μg of xanthotoxin + 0.05 μg of psoralen)/g.

control diet by neonates also increased [REG: $y = 0.4812 - 0.0299x$ ($r = 0.15$, $P < 0.0001$, $n = 100$)]. There was no relationship between bergapten concentration and late-instar preference (REG: $P > 0.10$).

Consumption and preference data for late instars showed similar results (Tables 2 and 3). The larvae consumed more bergapten-containing diet at concentrations similar to those found in *A. graveolens* petioles (0.50 and 1.00 μg/g). More control diet was eaten than bergapten-containing diet when the concentration was increased to the levels found in inner leaves (5.00 μg/g). Although there was no significant difference in preference for the lowest xanthotoxin concentration tested (Table 2), the larvae consumed significantly more treated than control diet (Table 3). Higher xanthotoxin concentrations (18.00 μg/g) resulted in significantly greater consumption of the control diet relative to the treated diet. There were no differences in consumption between control and psoralen-containing diet at the concentrations tested. Although preference tests with late instars and mixed linear furanocoumarins incorporated at concentrations found in petioles indicated no differences, results from consumption data showed greater consump-

Table 2. Effect of linear furanocoumarins on diet preference by late instar *S. exigua*

Furanocoumarin	Concn, μg/g	Preference[a]	df	*t*-test	*P*
Bergapten	0.50	−2.56 ± 1.57	16	−1.63	0.1236
	1.00	−3.25 ± 1.49	16	−2.18	0.0452
	5.00	5.06 ± 1.99	16	−2.54	0.0227
Xanthotoxin	2.00	−4.75 ± 3.10	8	−1.53	0.1698
	5.00	−0.44 ± 1.67	16	−0.26	0.7967
	10.00	−2.75 ± 2.47	8	−1.11	0.3035
	18.00	4.13 ± 1.88	8	2.20	0.0637
Psoralen	1.00	−3.63 ± 1.83	8	−1.98	0.0883
	5.00	3.93 ± 1.39	15	2.83	0.0135
Combination	Petioles[b]	−0.56 ± 1.98	16	−0.28	0.7801
	Inner leaves[c]	2.27 ± 1.23	15	1.85	0.0863
	Outer leaves[d]	20.5 ± 4.04	8	5.06	0.0015

[a] Number of larvae on control diet − number of larvae on treated diet. A positive value denotes a greater number of larvae on the control diet.
[b] Concentration of linear furanocoumarins in *A. graveolens* petioles, (0.50 μg of bergapten + 0.10 μg of xanthotoxin + 0.05 μg of psoralen)/g diet.
[c] Linear furanocoumarin concentration in *A. graveolens* inner leaves, (5.00 μg of bergapten + 2.00 μg of xanthotoxin + 0.05 μg of psoralen/g diet.
[d] Linear furanocoumarin concentration in *A. graveolens* outer leaves (28.00 μg of bergapten + 18.00 μg of xanthotoxin + 4 μg of psoralen)g diet.

Table 3. Effect of linear furanocoumarins on diet consumption by late instar *S. exigua*

Furanocoumarin	Concn, μg/g	Consumption (g), mean ± SE[a]	df	*t* value	*P*
Bergapten	0.00	1.0185 ± 0.08	46	−2.17	0.0352
	0.50	1.1350 ± 0.07			
	0.00	0.9347 ± 0.08	50	−4.16	0.0001
	1.00	1.1796 ± 0.08			
	0.00	1.2006 ± 0.07	50	2.85	0.0063
	5.00	1.0108 ± 0.07			
Xanthotoxin	0.00	0.1898 ± 0.03	23	−3.36	0.0028
	2.00	0.3321 ± 0.03			
	0.00	1.1528 ± 0.08	50	1.66	0.1030
	5.00	1.0482 ± 0.07			
	0.00	1.1428 ± 0.06	26	0.78	0.4441
	10.00	1.0798 ± 0.06			
	0.00	1.5056 ± 0.08	25	2.87	0.0084
	18.00	1.2001 ± 0.07			
Psoralen	0.00	1.2065 ± 0.06	26	−1.31	0.2004
	1.00	1.3266 ± 0.07			
	0.00	1.0936 ± 0.06	50	0.80	0.4288
	5.00	1.0464 ± 0.05			
Combination	0.00	0.9220 ± 0.08	50	3.17	0.0026
	Petioles[b]	0.7852 ± 0.08			
	0.00	1.0629 ± 0.07	50	1.72	0.0926
	Inner leaves[c]	0.9767 ± 0.06			
	0.00	2.6690 ± 0.11	25	15.13	0.0001
	Outer leaves[d]	0.5050 ± 0.08			

[a] Grams of consumed control or furanocoumarin-containing diet.
[b] Concentration of linear furanocourmarins in *A. graveolens* petioles was (0.50 μg of bergapten + 0.10 μg of xanthotoxin + 0.05 μg of psoralen)/g diet.
[c] Linear furanocoumarin concentration in *A. graveolens* inner leaves was (5.00 μg of bergapten + 2.00 μg of xanthotoxin + 0.05 μg of psoralen)/g diet.
[d] Linear furanocoumarin concentration in *A. graveolens* outer leaves was (28.00 μg of bergapten + 18.00 μg of xanthotoxin + 4 μg of psoralen)/g diet.

tion of control diet. The combination of linear furanocoumarins at concentrations found in outer *A. graveolens* leaves also caused greater consumption of control diet by late instars.

Behavioral Tests. For the linear furanocoumarin combination found in inner *A. graveolens* leaves, the larvae had significantly more eating (NPAR1WAY: df = 1, $\chi 2 = 6.01$, $P < 0.05$) and questing (NPAR1WAY: df = 1, $\chi 2 = 13.68$, $P < 0.001$) occurrences on furanocoumarin-containing diet (4.17 ± 0.60 eating occurrences and 4.23 ± 0.44 questing occurrences) than on control diet (2.17 ± 0.28 eating occurrences and 2.20 ± 0.25 questing occurrences). There were no significant differences in time spent eating, swallowing, questing, and resting and number of occurrences of swallowing and resting between control and treated diet ($P > 0.05$).

Figure 1 shows the results of the behavioral observations on diet containing the furanocoumarin combination found in outer *A. graveolens* leaves and control diet. For these tests, the 4th instars spent significantly more time eating (TTEST: df = 58, $T = 2.63$, $P = 0.01$) and less time questing (TTEST: df = 58, $T = -2.37$, $P < 0.05$) on control than on treated diet (Fig. 1A). Also, the larvae had significantly more eating (NPAR1WAY: df = 1, $\chi 2 = 8.23$, $P < 0.005$) and questing (NPAR1WAY: df = 1, $\chi 2 = 19.30$, $P = 0.0001$) occurrences on treated than on control diet (Fig. 1B). There were no significant differences in time spent swallowing and resting and number of occurrences of swallowing and resting between control and treated diet ($P > 0.05$).

Discussion

The low concentrations of linear furanocoumarins found in *A. graveolens* (≤50 μg/g) had substantial effects on the behavior of both early- and late instar *S. exigua*. These compounds produced a negative and concentration-dependent effect on diet preference by *S. exigua* larvae; higher concentrations resulted in greater preference for control diet. Our results support Brewer et al. (1995) in their conclusion that linear furanocoumarins are more biologically active in combination than individually.

Our results on diet preference are analogous to previous reports on related *Spodoptera* species, which indicated that antifeedant responses to furanocoumarins were not always consistent across instars and that quantitative differences in linear furanocoumarins (or combinations of these chemicals) cause qualitative changes in behavioral responses. Late instar *S. exigua* were more sensitive to bergapten than neonates, as indicated by the preference for 1.00 μg of bergapten per gram of diet by late instars (a concentration which had no significant effect on diet preference by neonates). Conversely, *S. exigua* neonates were deterred by xanthotoxin concentrations lower than

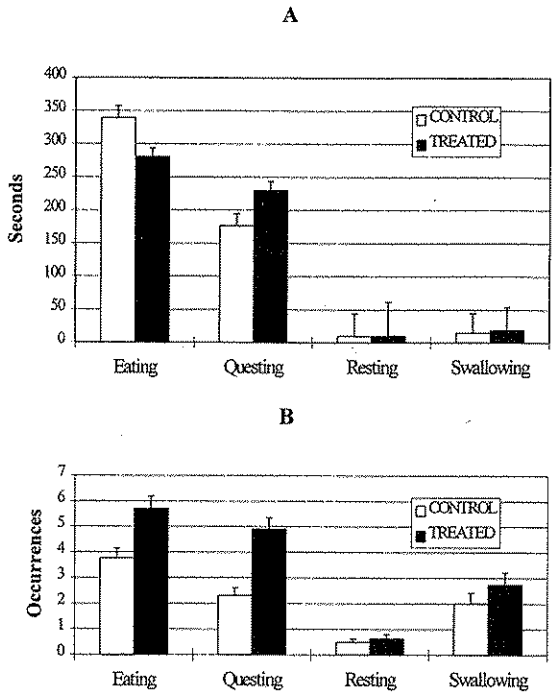

Fig. 1. Results of 9-min observations of the behavior of late instars when confronted with either control or linear furanocoumarin-containing diet (linear furanocoumarins found in outer *A. graveolens* leaves [28.00 μg of bergapten +18.00 μg of xanthotoxin + 4.00 μg of psoralen]/g diet). Bars above each individual behavior indicate total time spent (*s*) (A) and number of occurrences (B) of that particular behavior. Lines above each bar indicate standard errors.

those causing late-instar deterrence (Tables 1 and 2). Berenbaum (1978) reported that xanthotoxin acted as a feeding deterrent to 6th instar *S. eridania* (Cramer) but did not reduce feeding by 1st or 2nd instars.

Behavioral observations of larvae on diet incorporating the combination of linear furanocoumarins at the concentration found in outer *A. graveolens* leaves (Fig. 1) demonstrate that preference for control diet by the larvae is the result of feeding deterrence because the larvae fed on the diet before being stimulated to leave. Upon ingesting the furanocoumarin-containing diet, the larvae became more mobile as measured by time spent questing and number of questing occurrences. These results indicate the important role linear furanocoumarins may play in deterring herbivory by naive insects. As noted by Bernays and Chapman (1994), this type of deterrence may be more important for highly mobile insects such as *S. exigua,* because those unable to move to a new food source must adapt to the chemicals to survive.

Deterrence of *S. exigua* by linear furanocoumarins is not surprising because linear furanocoumarins have been shown to act as feeding deterrents–repellents to other generalist insect species. What is surprising from our results is the low concentrations at which these compounds elicited deterrence to *S. exigua*. For example, bergapten reportedly reduces diet consumption in *H. virescens* (Klocke et al. 1989). *Leptinotarsa decemlineata* (Say) and *Mythimna unipuncta* (Haworth) avoid filter paper disks treated with xanthotoxin and bergapten at concentrations ranging from 2,000 to 8,000 μg/ml (Muckensturm et al. 1981). *H. zea* neonates exhibited a greater avoidance of diet with xanthotoxin (≈60 μg/g diet) alone than to a diet with a furanocoumarin extract (at an equimolar concentration) from *Pastinaca sativa* L., even though the extract was more toxic than xanthotoxin alone (Berenbaum et al. 1991). Yajima and Munakata (1979) showed that coumarin, umbelliferone, two 7-hydroxycoumarins, and psoralen had little or no antifeedant activity against 3rd-instar *S. litura* (F). In contrast, isopimpinellin and bergapten demonstrated strong antifeedant activity at concentrations >10 μg/g diet (Yajima and Munakata 1979). These authors also found that *S. litura* 3rd instars preferred diet treated with bergapten at a concentration of 5 μg/g diet.

In 2 of the 3 experiments with a significant time effect, preference for control diet decreased with time (Table 1). In the case of the intermediate psoralen concentration, preference for diet containing 1.00 μg of psoralen per gram of diet tended to increase with time. Deterrence by the highest psoralen concentration tested also decreased with time. These results suggest that *S. exigua* larvae become habituated or increasingly able to process psoralen (sensu Bernays and Weiss 1996). Conversely, the deterrent effect of bergapten and the 2 highest xanthotoxin concentrations did not change significantly as the experiments progressed. Furthermore, deterrence by the lowest xanthotoxin concentration tested increased with time. Hence, differences in the effect of time between these linear furanocoumarins may be caused by differences in larval habituation or ability to process psoralen and the 2 other compounds. Linear furanocoumarins have been shown to induce production of substrate-specific monooxygenase in oligophagous and polyphagous insect herbivores (Cohen et al. 1989, Nitao 1989, Cohen et al. 1992, but see Brewer et al. 1995). Therefore, the observed changes in neonate behavior by diet containing psoralen as the experiments progressed may indicate differences between psoralen and both xanthotoxin and bergapten in their ability to induce monooxygenase production in *S. exigua* neonates.

The effect of UV-A was not significant for any of the tests in this study, supporting the hypothesis that UV-A does not modify the behavioral response of *S. exigua* larvae to linear furanocoumarins. This result is similar to that of Berenbaum et al. (1991), where no significant UV (at 280 nm) × treatment interaction was found on *H. zea* survival. There are 2 possible explanations for our result. The first is that the intensity of UV-A was not high enough.

Higher intensities of UV-A cause negative direct effects on *S. exigua* as well as increased toxicity of linear furanocoumarins (Trumble et al. 1991). However, we believe that these results provide biologically meaningful data because they represent UV-A levels commonly found within a plant canopy. An alternative explanation, which we favor, is that linear furanocoumarins may not need to be photoactivated to act as behavioral elicitors. This suggestion is in agreement with the conclusion reached by Berenbaum (1978)—that photoactivation was not required for xanthotoxin to act as a feeding repellent against *S. eridania*.

Spodoptera exigua larvae on *A. graveolens* change their feeding site as they develop. First–third instars feed on leaves, whereas 4th and 5th instars prefer the lower petioles; nutritional differences in plant parts do not account for this change in feeding preference (Griswold and Trumble 1985a). Fifth instars (feeding without protection from the web that is characteristic of early instars) are strongly photonegative; hence these authors hypothesized that the tendency to feed on the lower petioles during the day may protect late instars from linear furanocoumarin photoactivation (Griswold and Trumble 1985b). Our results indicating a deterrent effect on late instars by concentrations of linear furanocoumarins similar to those found in outer *A. graveolens* leaves, coupled with the lack of deterrence by concentrations similar to those found in *A. graveolens* petioles and inner leaves, are consistent with this hypothesis.

The concentrations of linear furanocoumarins needed to cause direct mortality to insects are high. Bergapten and xanthotoxin caused no mortality to *S. litura* at concentrations as high as 500 μg/g (Yajima and Munakata 1979). For *S. exigua*, the LC_{50}s for psoralen, bergapten, and xanthotoxin are 152.1, 137.8, and 106.6 μg/g of diet, respectively (Diawara et al. 1993). These concentrations are 5–3,000 times greater than those commonly found in *A. graveolens* (Trumble et al. 1990, 1992; Diawara et al. 1995). In addition, low concentrations of furanocoumarins (similar to those in this study) caused little variation in survival or developmental time of *S. exigua* (Brewer et al. 1995). Berenbaum (1990) suggested that generalist herbivores may avoid plant chemical defenses such as furanocoumarins through behavioral adaptations. Furthermore, Jermy (1984), Bernays and Graham (1988), and others postulate that the function of secondary compounds may be to modify behavior rather than to function as toxic defenses. Our studies showing that larvae of the generalist herbivore *S. exigua* are deterred in a concentration-dependent manner by low and nontoxic linear furanocoumarin concentrations commonly found in *A. graveolens* support this hypothesis.

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