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TOXICITY OF LINEAR FURANOCOUMARINS TO *Spodoptera exigua*: EVIDENCE FOR ANTAGONISTIC INTERACTIONS

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Abstract—The linear furanocoumarins psoralen, bergapten, and xanthotoxin were tested for toxicity to the beet armyworm *Spodoptera exigua* (Hübner) under short ultraviolet (UVB) radiation. Increased dietary concentrations of each furanocoumarin significantly decreased insect larval weight, extended generation time, and induced higher mortality. Xanthotoxin was the most toxic, followed by psoralen and bergapten. Combining psoralen with bergapten, xanthotoxin, or both resulted in significantly antagonistic effects on insect mortality. The combination of bergapten and xanthotoxin, however, produced additive effects. The implications of these observations for *S. exigua* resistance in the wild plant accession of *Apium prostratum* and the enigma the findings represent for plant–insect relationships are discussed.

Key Words—*Spodoptera exigua*, Lepidoptera, Noctuidae, psoralen, 5-methoxypsoralen, 8-methoxypsoralen, furanocoumarins, antagonistic toxicity, plant–insect interactions, *Apium prostratum*.

INTRODUCTION

The linear furanocoumarins are plant secondary compounds that have been isolated from members in a number of plant families including Rutaceae, Apiaceae, Compositae, Leguminosae, Moraceae, Pittosporaceae, Solanaceae, and Thy-

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meaceae (Scott et al., 1976; Murray et al., 1982). These compounds are photosensitizers (Igali et al., 1970; Zangerl and Berenbaum, 1987) and have shown toxicity against a broad spectrum of animals (Berenbaum, 1978; Berenbaum and Neal, 1985; Trumble et al., 1991). Because of this toxicity, it has been suggested that the linear furanocoumarins can play an important role in plant–herbivore interactions (Berenbaum, 1978; Berenbaum and Feeny, 1981; Trumble et al., 1991).

The major linear furanocoumarins isolated from *Apium* species are psoralen, bergapten (5-methoxypsoralen), and xanthotoxin (8-methoxypsoralen) (Trumble et al., 1990). These compounds were toxic to the beet armyworm *Spodoptera exigua* (Hübner) when combined in artificial diet (psoralen 15 μg/ml diet + bergapten 38 μg/ml diet + xanthotoxin 82 μg/ml diet) during assays exposed to 350 $\mu W/cm^2$ UVB radiation (Trumble et al., 1991). Diawara et al. (1992) reported higher concentrations of these furanocoumarins in the *S. exigua*-resistant wild *Apium prostratum* ssp. *prostratum* than the susceptible celery *A. graveolens*. However, *A. prostratum* resistance was primarily nonpreference based, and additional studies designed to test furanocoumarin-free plant extracts for insect preference suggested that the resistance was not only furanocoumarin-induced. Subsequent studies found no significant correlation between *S. exigua* resistance in celery breeding lines and linear furanocoumarin concentrations (Diawara et al., 1993). Therefore, the potential role of these linear furanocoumarins in the *A. prostratum–S. exigua* relationship is not clearly defined. We initiated this study to compare three linear furanocoumarins (psoralen, bergapten, and xanthotoxin) for toxicity against *S. exigua* and to determine the additive, synergistic, or antagonistic effect of their combinations. Information on the relative toxicity of these different linear furanocoumarins to herbivores, when ingested alone or in combination, will improve our understanding of the role of these compounds in plant–herbivore interactions.

METHODS AND MATERIALS

The three linear furanocoumarins psoralen, bergapten, and xanthotoxin were obtained from Aldrich Chemical Company (Milwaukee, Wisconsin). *S. exigua* larvae were from a colony less than 1 year old maintained on artificial diet (Shorey and Hale, 1965) at 27 $\pm$ 2°C and 16:8 hr light–dark photoperiod. Activity of the three linear furanocoumarins against *S. exigua* was determined in a two-phase experiment conducted between April 1991 and May 1992.

Experiment I: Toxicity of Individual Furanocoumarins. Seven concentrations of each of the three linear furanocoumarins were tested: 0 (control), 62.5, 125, 250, 375, 500, and 750 μg/g diet. This range, which was chosen following pilot studies of toxicity, includes the total concentration range of 0 to 406

μg/g fresh leaf found in *Apium* accessions for the three furanocoumarins (Trumble et al., 1990; Diawara et al., 1992). Procedures for incorporating the chemicals in insect diet were modified from Chan et al. (1978). For each concentration, the furanocoumarin was dissolved in 10 g of acetone, which was removed by vacuum after adsorbtion of the furanocoumarin onto 3 g of alphacel. Approximately 82 g of diet medium was then added to the alphacel, which had been diluted in 15 g of warm dH_2O, to bring the total weight to 100 g (alphacel constituted 3% of the diet medium). The mixture was blended for 5 min and then dispensed into 30-ml clear plastic cups.

Diets were allowed to cool at ambient temperature and one neonate *S. exigua* was placed in each cup. Cups were then covered with Teflon FEP Fluorocarbon film (E.I. DuPont de Nemours & Co., Wilmington, Delaware). A total of 15 larvae per chemical per concentration were evaluated. Cups were arranged in a randomized complete block design in an environmental chamber at 27 ± 2°C, 75% relative humidity, and 16:8 hr light–dark photoperiod. A 300 $\mu W/cm^2$ UVB radiation, produced by fluorescent lamps (40-W Sylvania 350 Black Light, burn in >50 hr, Inland Lighting Supplies, Riverside, California), was maintained beneath the Teflon film by adjusting the height of the lamps. This value was chosen on the basis of UV radiation readings taken during February 1991 under canopy of celery grown at the University of California Agricultural Operations fields at Riverside, California, using a System 371 Optical Power Meter equipped with a model 268 detector head (United Detector Technology, Hawthorne, California). To approximate daylight conditions, UV radiation was maintained for 6 hr/day from 1000 hr to 1600 hr within the daytime of the 16:8 hr light–dark photoperiod of the environmental chamber. For each diet treatment, weight of larvae at nine days, weight of pupae, generation time from egg to pupa and from egg to adult, and survival to pupa and to adult were recorded. The experiment was replicated four times.

For statistical analysis, data for larval and pupal weight, generation time, and larval stage mortality were analyzed using ANOVA and overall mortality data were analyzed using the Proc Probit procedures (SAS Institute, 1990). Control mortality was <2.3% and Abbott's (1925) formula was used to correct overall survival data for control mortality whenever less than 100% survival was recorded on the control treatment.

Experiment II: Toxicity of Furanocoumarin Combinations. Joint effects were determined by testing all combinations of LC_{25}s (estimated from the probit lines generated in experiment I) of the three compounds for toxicity to *S. exigua*. The treatments were control, psoralen, bergapten, xanthotoxin, psoralen + bergapten, psoralen + xanthotoxin, bergapten + xanthotoxin, and psoralen + bergapten + xanthotoxin. Dietary treatment preparations and bioassay procedures as well as insect growth and/or survival variables recorded were the same as for the phase I experiment. This experiment also was replicated four times.

For statistical analysis, a χ^2 test (Gomez and Gomez, 1984) was used to analyze the joint effects of 1:1 (LC_{25}:LC_{25}) combinations of the three chemicals. Expected mortality was determined using the formula $E = O_a + O_b(1 - O_a)$, where E is the expected percent mortality, O_a is the expected percent mortality due to chemical a alone, and O_b is the expected percent mortality due to chemical b alone (Finney, 1971; Salama et al., 1984). Finney's method (1971) was modified as reported by Salama et al. (1984) and Moar et al. (1990) to test the joints effects of 1:1:1 (LC_{25}:LC_{25}:LC_{25}) combinations of the three chemicals. The formula used to determine expected mortality was $E = O_a + O_b(1 - O_a) + O_c(1 - O_a)(1 - O_b)$, where E is the expected percent mortality, O_a is the expected percent mortality due to chemical a alone, O_b is the expected percent mortality due to chemical b alone, and O_c is the expected percent mortality due to chemical c alone. Calculated χ^2 values were compared with χ^2 tabular values ($df = 3$, $P < 0.05$). Depending on whether the calculated χ^2 value was significantly greater or smaller than the tabular value, we concluded that there were additive, synergistic, or antagonistic reactions among the different chemicals involved.

RESULTS AND DISCUSSION

Experiment I: Toxicity of Individual Furanocoumarins. Overall, increased dietary concentrations of all three furanocoumarins significantly decreased *S. exigua* larval weight as compared with the control (for psoralen $P < 0.001$, $F = 92.55$, $df = 6, 284$; for bergapten $P < 0.001$, $F = 16.26$, $df = 6, 305$; for xanthotoxin $P < 0.001$, $F = 48.26$, $df = 6, 290$) (Figure 1a). However, no significant differences were found between concentrations exceeding 125 μg/g diet for any of the individual test chemicals. Significant differences were seen among the three furanocoumarins for larval weight ($P < 0.001$, $F = 16.26$, $df = 2, 879$). This differential larval growth, however, varied within concentration, as significant furanocoumarin × concentration interactions occurred ($P < 0.001$, $F = 9.15$, $df = 12, 879$). At the lowest rate of 62.5 μg/g of furanocoumarin in diet, larvae ingesting bergapten had the lowest weight (Figure 1a). However, at rates of 250 μg/g diet and above, a trend of decreasing larval size was evident where psoralen > xanthotoxin > bergapten.

Pupal weights also were significantly reduced as psoralen ($P = 0.0001$, $F = 13.94$, $df = 6, 225$) or xanthotoxin ($P < 0.001$, $F = 6.57$, $df = 6, 222$) concentrations increased in the diet (Figure 1b). However, increasing concentrations of bergapten in the diet had no effects on insect pupal weight ($P = 0.231$, $F = 1.36$, $df = 6, 287$). The three compounds differed in their influence on pupal weight ($P < 0.001$, $F = 9.93$, $df = 2, 766$), but no consistent patterns were observed in the ranking of the three chemicals for toxicity as their dietary

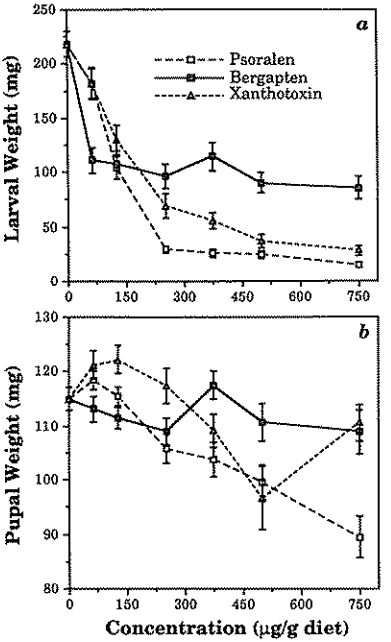

FIG. 1. Differential influence of increased concentrations of psoralen, bergapten, and xanthotoxin on *Spodoptera exigua* larval weight (a) and pupal weight (b). Each data point represents at least 50 observations. Bars represent standard errors.

concentrations increased (Figure 1b) due to furanocoumarin by concentration interactions, i.e., differential response to furanocoumarins ($P = 0.0001$, $F = 4.46$, $df = 12, 766$).

Insect generation time from egg to pupa was significantly extended as dietary furanocoumarin concentrations increased (for psoralen $P < 0.001$, $F = 58.47$, $df = 6, 254$; for bergapten $P < 0.001$, $F = 11.47$, $df = 6, 288$; for xanthotoxin $P < 0.001$, $F = 23.37$, $df = 6, 225$) (Figure 2a). Generation time from egg to adult emergence also significantly increased as dietary individual furanocoumarin concentrations increased (for psoralen $P = 0.001$, $F = 22.72$, $df = 6, 192$; for bergapten $P = 0.0001$, $F = 5.65$, $df = 6, 212$; for xanthotoxin $P = 0.0001$, $F = 15.11$, $df = 6, 167$) (Figure 2b). However, similar to trends found with larval weight, concentrations of bergapten above 62.5 μg/g diet or concentrations of psoralen or xanthotoxin above 125 μg/g diet did not produce significantly different generation times. Overall, larvae reared on diets containing psoralen took significantly longer to pupate ($P < 0.001$, $F = 42.14$, $df = 2, 769$) and to emerge as adult ($P < 0.001$, $F = 22.31$, $df = 2, 573$) compared with larvae exposed to xanthotoxin or bergapten (Figure 2). Like the trend seen

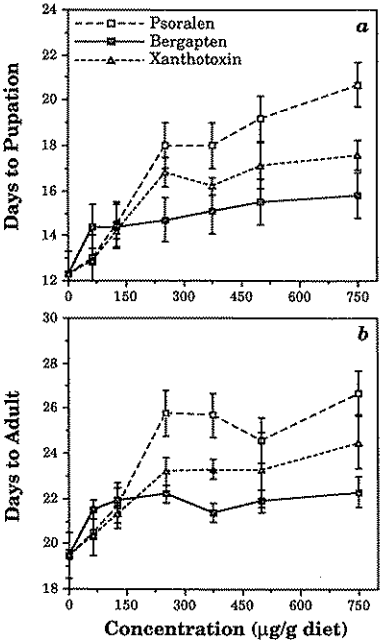

FIG. 2. Differential influence of increased concentrations of psoralen, bergapten, and xanthotoxin on *Spodoptera exigua* generation time from egg to pupa (a) and from egg to adult (b). Each data point represents 5–50 observations. Bars represent standard errors.

for the growth variables of larval and pupal weight, this differential developmental time was not consistent across concentrations as evidenced by significant furanocoumarin by concentration interactions for both days to pupation ($P < 0.001$, $F = 9.02$, $df = 12, 769$) and days to adults ($P < 0.001$, $F = 5.44$, $df = 12, 573$). Feeding on psoralen and xanthotoxin significantly extended insect generation times at high concentrations, but not at concentrations lower than 150 μg/g diet (Figure 2).

Higher rates of three furanocoumarins significantly increased larval stage mortality ($P = 0.001$, $F = 4.67$, $df = 6, 60$) (Figure 3). No furanocoumarin × concentration interactions occurred for this variable ($P = 0.250$, $F = 1.31$, $df = 12, 60$); xanthotoxin induced a significantly higher larval mortality than the other compounds ($P = 0.0037$, $F = 6.47$, $df = 2, 60$). Although psoralen numerically caused more mortality than bergapten at most rates, these furancoumarins were not significantly different in their toxicity (Figure 3).

Based on LC values, xanthotoxin was the most toxic of all three linear furanocoumarins to *S. exigua*; the LC_{50}s for xanthotoxin, psoralen, and bergapten were 245.89, 385.16, and 449.09 μg/g diet, respectively (Table 1). In

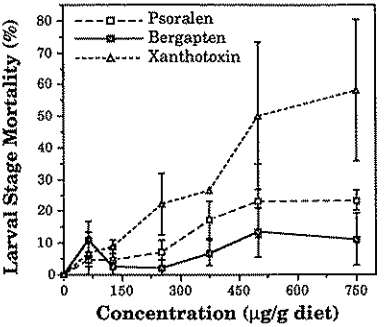

FIG. 3. Differential influence of increased concentrations of psoralen, bergapten, and xanthotoxin on *Spodoptera exigua* mortality during the larval stage (from egg hatch to pupation). Each data point represents 5–50 observations. Bars represent standard errors.

TABLE 1. TOXICITY OF PSORALEN, BERGAPTEN, AND XANTHOTOXIN AGAINST *Spodoptera exigua* WHEN INCORPORATED INTO ARTIFICIAL DIET

Treatment	N^a	Slope ± SEM[b]	LC_{25} (95% FL)[b]	LC_{50} (95% FL)[b]
Psoralen	60	1.671 ± 0.263	152.1 (104.4–196.3)	385.2 (303.5–526.5)
Bergapten	60	1.423 ± 0.363	137.8 (86.8–250.9)	449.9 (310.2–679.0)
Xanthotoxin	60	1.857 ± 0.247	106.6 (72.4–138.4)	245.9 (197.5–305.1)

[a] Number of larvae assayed.
[b] Micrograms furanocoumarin per gram diet. FL = Fiducial limit.

general, larvae were more adversely affected by increased dietary concentrations of xanthotoxin and psoralen than bergapten. Psoralen was more detrimental to insect growth (Table 3 below). Xanthotoxin has also been reported to be toxic to other *Spodoptera* species including the southern armyworm *S. eridania* (Cramer) (Berenbaum, 1978) and the fall armyworm *S. frugiperda* (J.E. Smith) (Ivie et al., 1983).

Experiment II: Joint Action Using LC_{25}s of Psoralen, Bergapten, and Xanthotoxin. Combining psoralen with either bergapten, xanthotoxin, or both resulted in a significantly antagonistic effect on insect mortality (Table 2). The combination of bergapten and xanthotoxin, however, produced an additive effect. Developmental data presented in Table 3 provide an insight into the potential mechanisms of these antagonistic effects. As suggested by the heavier 9-day weight, we suspect the larvae ate at a significantly faster rate when reared on the control diet or LC_{25}s of the three furanocoumarins alone than when reared on chemical combinations. As a result they ingested more allelochemicals than

TABLE 2. COMBINED TOXICITY OF PSORALEN, BERGAPTEN, AND XANTHOTOXIN AGAINST *Spodoptera exigua* WHEN INCORPORATED INTO ARTIFICIAL DIET

Mortality (%) using LC_{25}[a]			Expected % mortality	Observed % mortality	χ^2 value	Effect interpretation
Psoralen	Bergapten	Xanthotoxin				
23.9	11.8		32.9	23.4	36.524	Antagonism[ab]
23.9		22.4	40.9	13.5	77.237	Antagonism[a]
	11.8	22.4	31.5	28.2	1.241	Additivity[b]
23.9	11.8	22.4	47.9	37.9	29.662	Antagonism[a]

[a]Actual percent mortality observed using estimated lethal concentrations needed to kill 25% of the test population at adult emergence.
[ba]$\alpha = 0.001$, $df = 3$; [b]$\alpha = 0.1$, $df = 3$.

TABLE 3. DEVELOPMENTAL VARIABLES OF *Spodoptera exigua* REARED ON DIETS CONTAINING COMBINATIONS OF PSORALEN, BERGAPTEN, AND XANTHOTOXIN

Treatment	Weight (mg)		Survival to pupation (%)[c]	Developmental time (days)	
	9-day larvae[a]	Pupae[b]		Egg–pupa[d]	Egg–adult[e]
Control	261.3 a[f]	114.6 a	100.0 a	12.9 e	19.3 d
Psoralen	148.4 c	115.2 a	93.4 a	16.1 c	23.2 b
Bergapten	203.5 b	118.6 a	93.3 a	14.2 d	20.7 c
Xanthotoxin	226.0 b	115.4 a	94.8 a	13.8 de	20.6 c
Psoralen + bergapten	40.1 d	116.6 a	91.7 a	17.2 b	24.2 a
Psoralen + xanthotoxin	52.2 d	135.7 a	95.0 a	17.4 b	24.4 a
Bergapten + xanthotoxin	104.1 c	118.8 a	86.7 a	15.8 c	22.4 b
Psoralen + bergapten + xanthotoxin	26.4 d	117.0 a	86.7 a	18.6 a	24.6 a

[a]$P = 0.0001$; $F = 125.33$; $df = 7,468$
[b]$P = 0.3978$; $F = 1.05$; $df = 7,441$
[c]$P = 0.3852$; $F = 1.12$; $df = 7,31$
[d]$P = 0.0001$; $F = 74.22$; $df = 7,440$
[e]$P = 0.0001$; $F = 80.48$; $df = 7,364$
[f]Means within a column followed by the same letter are not significantly different at the 5% level (SAS, 1990).

could be metabolized; these larvae developed quickly to the pupal stage, but many could not survive to adulthood. On the other hand, combining the different furanocoumarins could reduce rate of food intake by larvae through increased feeding deterrency. Antifeedant activity against insects has been reported for bergapten (Muckensturm et al., 1981) and xanthotoxin (Yajima and Munakata,

1979). Combining these chemicals with psoralen increased furanocoumarin concentrations in the diet and apparently reduced the feeding rate (larvae did not starve themselves because fecal pellets were observed in the test cups during the experiment). A reduced feeding rate might allow larvae to metabolize the furanocoumarins better by either detoxifying them in the midgut prior to absorption (Ivie et al., 1983; Bull et al., 1984; Nitao, 1990) or by excreting them efficiently (Nitao, 1990). Larvae in our tests reared on chemical combinations usually took significantly longer to pupate, but were able to survive to the adult stage at a higher percentage than insects reared on the single compounds (Tables 2 and 3).

A second possible mechanism would occur if combining the linear furanocoumarins increased mixed function oxidase (MFO) activities in *S. exigua* larvae resulting in less biological effect with higher concentrations of combinations than lower concentrations of individual compounds. Increased enzymatic activity following ingestion of xanthotoxin has been reported in the Lepidoptera larvae *Trichoplusia ni* (Hübner) (Lee and Berenbaum, 1989) and *Depressaria pastinacella* (Duponchel) (Nitao, 1989). Although there is limited literature available on enzymatic induction in *S. exigua*, secondary compounds have been shown to increase MFOs in larvae of *S. eridania* (Brattsten et al., 1977). Bull et al. (1984) reported that both the xanthotoxin-tolerant *Papilio polyxenes* (Stoll) and the xanthotoxin-susceptible *S. frugiperda* metabolized this chemical by oxidative cleavage of the furan ring, but the rate of the metabolism is much higher in *P. polyxenes*.

At the LC_{25} concentrations used in our study, the furanocoumarins acted on insect growth primarily during the pupal stage; percentages of larvae surviving to pupation were not significantly different and were relatively high for all treatments (Table 3). Although insects reared on diets containing chemical combinations took significantly longer to emerge as adults compared with larvae feeding on single compounds, these differences resulted only from the extended feeding time during the larval stage (from egg to pupa) because the average number of days from pupa to adult was comparable for all treatments, varying between six and seven days (Table 3). Therefore, the different dietary treatments did not differ much in their effect on insect developmental time during the pupal stage.

These findings have implications for *S. exigua* resistance in the wild plant accession *Apium prostratum* (Diawara et al., 1992). Diawara et al. (1992) reported 100% *S. exigua* larval stage mortality on diet containing *A. prostratum* with a total concentration of linear furanocoumarins less than 250 μg/g diet. However, in the study reported here, furanocoumarins singly or in combination with one or two others did not induce $>60\%$ larval mortality even at dietary concentrations as high as 750 μg/g diet. Overall mortality was $<50\%$ when LC_{25}s of all three chemicals were mixed in the diet. Therefore, in spite of the

fact that these furanocoumarins may be deterrent or toxic to larvae, the findings reported here support suggestions by Diawara et al. (1992) that other factors may be involved in the strong *S. exigua* nonpreference feeding resistance observed in *A. prostratum*.

Finally, this study raises broader questions. If generalists are usually affected most strongly by the presence or absence of deterrents, and specialists by the presence of attractants (Jermy and Szentesi, 1978; Berenbaum, 1981a,b), why would the generalist *S. exigua* feed readily on a diet with high concentrations of potentially feeding-deterrent furanocoumarins? Further, why would plants produce a complex of furanocoumarins when feeding on individual furanocoumarins causes greater mortality for *S. exigua*? Perhaps the most conservative hypothesis is that evolution of this defense occurred under selection pressure from other mortality factors including pathogens (Karasawa et al., 1990; McCloud et al., 1992), other herbivores (Berenbaum, 1981a,b; Trumble et al., 1990), or as an allelopathic protection against other plants (Friedman et al., 1982). Thus, combinations of chemicals may have greater effects on these "driving" mortality components, and any negative impact on *S. exigua* would be fortuitously beneficial. Certainly, the extended developmental time observed for *S. exigua* larvae feeding on combinations of furanocoumarins could help protect the plant by enhancing potential mortality from natural enemies or environmental factors. Although some of these latter, specific questions may be addressed rapidly by additional experimentation, the broader questions of why and how the generalist *S. exigua* evolved an effective detoxification system for the furanocoumarins will require a much better knowledge of plant–insect associational history than is currently available.

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