Biological Activity of Marmesin and Demethylsuberosin against a Generalist Herbivore, *Spodoptera exigua* (Lepidoptera: Noctuidae)

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No significant effects were observed in bioassays designed to measure impact of the furanocoumarin precursors demethylsuberosin and marmesin on typical physiological parameters (survival and development rate) of the generalist herbivore, *Spodoptera exigua* (Hübner). The linear furanocoumarin psoralen did increase developmental times and reduce survival. All of these compounds were demonstrated to have significant behavioral (feeding deterrence) effects against both first and third instars of *S. exigua* in diet-incorporation bioassays. In tests initiated with first instars, significantly ($P < 0.05$) more larvae preferred control diet to diet containing marmesin or psoralen on all five sample dates; control diets were significantly preferred to diets with demethylsuberosin on three of five sample dates. Similar results were observed for tests initiated with third instar larvae, but the avoidance of demethylsuberosin-containing diets was stronger. Therefore, assaying furanocoumarin precursors just for effects on growth and survival may not provide an accurate picture of the ecological importance of these compounds.

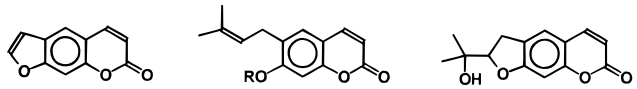
Keywords: *Furanocoumarin; psoralen; marmesin; demethylsuberosin; feeding deterrents; Spodoptera exigua; Apium graveolens*

INTRODUCTION

The linear furanocoumarins psoralen, 5-methoxypsoralen (bergapten), and 8-methoxypsoralen (xanthotoxin) have considerable biological activity against herbivores, including humans (Ashwood-Smith et al., 1985; Trumble et al., 1990; Beier et al., 1993). At low concentrations they have proven deadly to many insect species (Berenbaum, 1991), thus providing significant protection against insect herbivory. Even arthropods that have developed some tolerance to these compounds can show reduced performance upon ingestion, including delayed development (Diawara et al., 1993; Hadacek et al., 1994) that may be associated with antifeedant responses (Yajima et al., 1977; Muckensturm et al., 1981) and reduced survival (Diawara et al., 1994).

The highly polyphagous *Spodoptera* species are often used as test animals for studies of coumarins and furanocoumarins. However, antifeedant responses to these compounds are not always consistent across instars nor equivalent between species. Berenbaum (1978) reported that xanthotoxin acted as a feeding deterrent to sixth instar *S. eridania* (Cramer) but did not reduce feeding by first or second instars. Yajima et al. (1977) showed that coumarin, umbelliferone, two 7-hydroxycoumarins, and psoralen had little or no antifeeding activity against *S. litura* (F). In contrast, isopimpinellin and bergapten demonstrated strong antifeedant activity.

Little evidence is available on the biological activity of marmesin and demethylsuberosin, the immediate precursors to the furanocoumarins (Figure 1). According to Berenbaum (1991, and references therein), furanocoumarins are derived from umbelliferone via prenylation. In Apiaceae and Rutaceae, two plant families in which furanocoumarins are commonly found, umbelliferone is first converted to demethylsuberosin, which is subsequently transformed to marmesin (Brown, 1978). Experiments investigating the related coumarin derivatives ostruthin and osthol found effects on insect survival or growth (Hadacek et al., 1994). The experiments reported here were designed to examine potential physiological and behavioral effects of demethylsuberosin and marmesin on a generalist insect herbivore, *Spodoptera exigua* (Hübner).



Psoralen Demethylsuberosin Marmesin

Figure 1. Chemical structures of demethylsuberosin, marmesin, and the linear furanocoumarin psoralen.

MATERIALS AND METHODS

Synthesis of Demethylsuberosin and Marmesin. NMR spectra were recorded with a GE 300 instrument, at 300 MHz (protons) or 75 MHz (^{13}C). Mass spectra were recorded either with a Hewlett-Packard 5970B mass selective detector (electron impact, 70 eV) coupled to an H-P 5890 gas chromatograph equipped with a DB5-MS column (20 m × 0.2 mm i.d.; J&W Scientific, Folsom, CA) or with a VG 7070E instrument (Fisons International, Beverly, MA), using a direct insertion probe. The temperature program used for all GC-MS runs was 100 °C/0 min, 15 °C/min to 275 °C for 20 min. Melting points were measured with a Fisher-Johns melting point apparatus and are uncorrected.

The synthetic route to demethylsuberosin and marmesin is shown in Figure 2. Thus, a mixture of 7-hydroxycoumarin **1** (16.2 g, 100 mmol), benzyl bromide (19 g, 111 mmol), 50 g of K_2CO_3, and 600 mL of acetone was refluxed for 48 h. An additional 3 g of benzyl bromide was then added, and the mixture was refluxed for a further 24 h. The mixture was then cooled to 20 °C and filtered, and the filtrate was concentrated. The residue was added to water, and the aqueous mixture was extracted 3 times with $CHCl_3$. The combined $CHCl_3$ solutions were washed with water and brine, dried, and concentrated. The residue was recrystallized from EtOH (1 L), yielding pale yellow plates (23.4 g, 98%), mp 155−6 °C. NMR and mass spectra matched those previously reported (Trumble et al., 1992).

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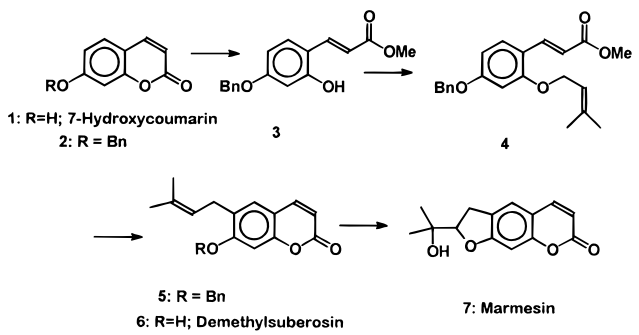

Figure 2. Schematic for synthesis of demethylsuberosin and marmesin.

Protected coumarin **2** (23 g, 97 mmol) was refluxed 20 h in a solution of NaOMe (prepared from 23 g of Na in 750 mL of dry MeOH). The solution was cooled to 20 °C, quenched with glacial acetic acid (65 mL), and poured into 2 L of cold 0.1 M HCl. The mixture was stirred thoroughly and filtered, and the filter cake was rinsed thoroughly with cold water. The solids were air dried overnight, then pumped under vacuum for 6 h, yielding 25 g (96%) of the ring-opened product **3** as a pale brown powder, which was used without further purification. An analytical sample was recrystallized from benzene, yielding **3** as a white solid, mp 185−6 °C. ^{1}H NMR: δ 7.89 (d, 1 H, J = 16.07 Hz), 7.48−7.3 (m, 6 H), 6.58 (dd, 1 H, J = 8.63, 2.25 Hz), 6.46 (d, 1 H, J = 16.46 Hz), 6.42 (d, 1 H, J = 2.64 Hz), 5.6 (br s, 1 H, OH), 5.07 (s, 2 H), 3.79 (s, 3 H). Compound **3** reverted to **2** upon attempted GC-MS analysis (see: Cairns et al., 1986c). MS (DIP, 50 eV): 284 (13), 253 (2.7), 133 (2.9), 91 (100), 65 (7.5).

Methyl ester **3** (13.5 g, 50 mmol) was refluxed with 1-bromo-3-methyl-2-butene (15 g, 100 mmol) and 40 g of K_2CO_3 in acetone (500 mL) for 2 h. The mixture was cooled and filtered, and the filtrate was concentrated. The residue was taken up in hexane (500 mL) at room temperature and filtered to remove unreacted starting material, and the filtrate was concentrated, yielding 12.53 g (74%) of ester **4** as a viscous yellow oil, which was used without further purification. NMR: δ 7.93 (d, 1 H, J =16.12 Hz), 7.48−7.3 (m, 6 H), 6.57 (m, 2 H), 6.44 (d, 1 H, J = 16.14 Hz), 5.49 (m, 1 H), 5.08 (s, 2 H), 4.55 (br d, 2 H, J = 6.48 Hz), 3.28 (s, 3 H), 1.80 (s, 3 H), 1.73 (s, 3 H). **4** rearranged to **5** and other isomeric products during attempted GC-MS analysis. MS (DIP, 50 eV): 352 (13), 284 (74), 253 (10), 224 (5), 193 (3), 162 (2), 134 (3), 91 (100), 69 (11).

Ester **4** (12.5 g, 37 mmol) was refluxed in diethylaniline (120 mL) under Ar for 3 h. The solution was cooled, poured into 1 L of 1.5 M HCl, and extracted 3 times with EtOAc. The combined EtOAc extracts were washed with 1.5 M HCl and brine, then dried, and concentrated. The off-white solid residue was purified in two batches by flash chromatography (25 cm x 5 cm i.d.), eluting with toluene:EtOAc, 95:5, yielding 8.43 g (71%) of substituted coumarin **5**. An analytical sample was recrystallized from EtOH (white needles, mp 108−9 °C). NMR: δ 7.63 (d, 1 H, J = 9.41 Hz), 7.48−7.3 (m, 5 H), 7.22 (s, 1 H), 6.84 (s, 1 H), 6.24 (d, 1 H, J = 9.48), 5.31 (m, 1 H), 5.16 (s, 2 H), 3.38 (br d, 2 H, J = 8.2 Hz), 1.78 (s, 3 H), 1.67 (s, 3 H). MS: 320 (M^+, 11), 229 (17), 187 (4), 175 (6), 92 (8), 91 (100), 65 (10).

Coumarin **5** (2.85 g, 8.4 mmol) in EtOH (25 mL) was added to an aqueous slurry of Raney nickel (10 g; Aldrich Chemical Co. no. 22-167-8), and the mixture was stirred for 28 min at room temperature under nitrogen. The mixture was then rapidly filtered through a plug of Celite and concentrated. The residue was taken up in $CHCl_3$, and the $CHCl_3$ solution was extracted twice with 0.2 M aqueous NaOH. The $CHCl_3$ solution was then washed with brine, dried, and concentrated, yielding 0.37 g (1.2 mmol) of unreacted starting material.

The combined NaOH solutions were acidified with 1 M HCl and extracted 3 times with EtOAc. The EtOAc extracts were washed with brine and dried, yielding 1.66 g (86%; quantitative based on recovered starting material) of crude demethylsuberosin **6**, contaminated with a small amount of overreduced material. A portion of the crude material was recrystallized from benzene, mp 131−2 °C (lit. 131−3 °C; Massanet et al., 1987). NMR: δ 7.63 (d, 1 H, J = 9.41 Hz), 7.20 (s, 1 H), 6.91 (s, 1 H), 6.25 (d, 1 H, J = 9.47 Hz), 5.32 (m, 1 H), 3.38 (br d, 2 H, J = 8.18 Hz), 1.80 (br s, 3 H), 1.77 (br s, 3 H). MS: 230 (M^+, 45), 215 (14), 187 (5), 176 (12), 175 (100), 159 (5), 147 (19), 115 (6), 91 (8), 77 (7), 69 (21), 51 (10), 41 (11).

Crude demethylsuberosin **6** (1.07 g, 4.7 mmol) in $CHCl_3$ (10 mL) was added dropwise to an ice-cooled slurry of *m*-chloroperbenzoic acid (3.36 g of 55% pure; 2 equiv) and $NaHCO_3$ (3.36 g, 40 mmol) in $CHCl_3$ (150 mL). The mixture was stirred for 3 h at 0 °C and then quenched by slow addition of a solution of 10 g $NaHSO_3$ in 150 mL water (Foams!). The mixture was stirred 20 min and then the layers were separated. The $CHCl_3$ layer was extracted twice with 0.2 M NaOH (to remove traces of overreduced demethylsuberosin from the previous step) and brine, then dried, and concentrated. The residue (1.01 g) was recrystallized from 30 mL of toluene, yielding pure marmesin **7**, mp 153 °C (lit. 150−152 °C, from benzene; Murray et al., 1971). ^{1}H NMR: δ 7.60 (d, 1 H, J = 9.46 Hz), 7.22 (s, 1 H), 6.76 (s, 1 H), 6.22 (d, 1 H, J = 9.56 Hz), 4.74 (t, 1 H, J = 8.84 Hz), 3.14−3.3 (m, 2 H), 1.76 (s, 1 H), 1.37 (s, 3 H), 1.23 (s, 3 H). ^{13}C NMR: δ 24.32, 26.08, 29.47, 71.62, 91.14, 97.87, 112.18, 112.73, 123.40, 125.10, 143.72, 155.60, 161.48, 163.18. MS: 246 (M^+, 47), 213 (23), 188 (72), 187 (100), 175 (14), 160 (28), 131 (20), 102 (8), 77 (15), 69 (14), 59 (61), 51 (16), 50 (10), 44 (14), 43 (34), 41 (12).

Bioassays for Survival, Weight Gain, and Developmental Rate. For diets containing psoralen (Aldrich Chemical Co., Milwaukee, WI), demethylsuberosin, or marmesin, the chemicals were dissolved in ethanol and adsorbed onto alphacel (ICN Biochemicals, Cleveland, OH), removing the ethanol by vacuum as described by Chan et al. (1978). The amount of alphacel constituted 5% of the entire diet media. The ethanol and alphacel procedure was used for all treatments including the control. Diet medium (Patana, 1969) then was added to the alphacel and blended for 5 min prior to dispensing into 30 mL plastic cups.

Diet cups were covered with Teflon FEP fluorocarbon film (E. I. DuPont de Nemours & Co., Wilmington, DE). This film is more transparent to ultraviolet light than glass. For treatments exposed to UV radiation, cups with larvae were placed beneath UV-producing fluorescent lamps (40W Sylvania 350 blacklight, Inland Lighting Supplies, Riverside, CA). UV lamps with a peak intensity at 350 nm were chosen because wavelengths between 300 and 400 nm are believed to be critical for activation of the psoralens (Musajo and Rodighiero, 1962). The lamps were adjusted in height such that the intensity of UV light beneath a layer of the Teflon film was 20 μmW/cm^2, a value equivalent to the irradiance observed beneath a celery leaf in coastal southern California at midmorning during the late summer (Trumble, personal observation). All UV measurements were made with a System 371 Optical Power Meter equipped with a Model 268 detector head with a UV optimization filter peaked at 365 nm (United Detector Technology, Hawthorne, CA). Our goal was neither to determine the extent of potential activation at varying levels of UV nor to prove that marmesin and demethylsuberosin are or are not activated by UV. Rather, we wanted to document if the level of UV occurring in foliage, in conjunction with the test compounds, would have significant developmental effects on *S. exigua*.

The potential influence of demethylsuberosin, marmesin, and psoralen on *S. exigua* larval survival, weight gain, and development rate was examined by using diet bioassay. Twenty neonate *S. exigua*/diet were placed in individual cups on diets containing demethylsuberosin (400 μg/g of diet), marmesin (100, 250, and 400 μg/g of diet), psoralen (375 μg/g of diet), and two control diets containing no furanocoumarins or precursors (one control diet was exposed to UV light, the other was not). Selection of relatively high test concentrations was based on LC_{50} values reported for psoralen (Diawara et al., 1993). Foliar levels of furanocoumarins in some celery plants have been found to exceed 400 μg/g of fresh weight (Trumble et al., 1990). Demethylsuberosin has been isolated from umbelliferous and rutaceous plants (Brown and Steck,

1972, and references therein), but in vivo concentrations were not reported. Because demethylsuberosin and marmesin must be produced before the furanocoumarins can accumulate, the levels of these compounds may be quite high. The speed of the biosynthetic transformation in *A. graveolens* from demethylsuberosin to marmesin to the linear furanocoumarins is not known. However, in *Ruta graveolens*, transformation from demethylsuberosin to marmesin appears to be fairly rapid (a matter of hours), while marmesin accumulates and only slowly transforms to furanocoumarins (Brown and Steck, 1972). In one of the few assays of marmesin in vivo (for *A. graveolens*), Afek et al. (1993) reported that 24 μg/g of fresh weight of marmesin were present concurrently with 10 μg/g of fresh weight of the linear furanocoumarins in the cultivar "Tender Crisp". Thus, the rates used in the experiments reported here represent the high end of concentrations, as might be found in resistant cultivars (Trumble et al., 1990; linear furanocoumarin levels >400 μg/g of fresh weight in *A. graveolens*) or stressed celery (Dercks et al., 1989; >165 μg/g of fresh weight in *A. graveolens*).

All insects except those on the second control diet were exposed to UV as previously described. A black cloth was used to separate the second control group from the UV lights but not the indirect room lighting controlling photoperiod (40-W Sylvania Cool Daylight). All insects were kept in an environmental chamber at 27 ± 2 °C and a 16:8 (L:D) photoperiod and examined daily. The entire test was replicated three times. For insects on each diet, larval weights at 9 days, pupal weights, time to pupation and adult eclosion, and survival to the pupal and adult stages were recorded.

Bioassays for Feeding Deterrence. Choice tests between control diet and either demethylsuberosin, marmesin, or psoralen were performed with neonates and third instar *S. exigua*, using arenas adapted from Gould et al. (1991). For neonates, arenas were constructed from 30 mL plastic cups lined with 4% agar (w/v) with two holes at opposite sides of the cup where 1.5 mL microcentrifuge cups were placed. One of these tubes contained control diet, and the other contained treated diet. Demethylsuberosin and marmesin were tested at 400 μg/g of diet, and psoralen was tested at 375 μg/g of diet. For each combination of treated diet and control diet, positions of larvae (5/arena) were monitored for 25 arenas for 5 days at approximately 24 h intervals. Throughout the test, larvae were held at 27 ± 2 °C and a 16:8 (L:D) photoperiod. This study was replicated 3 times.

Preferences of third instars for control or treated diets were tested at the same concentrations as in the neonate studies. The arenas consisted of 150 mL containers lined with 4% agar (w/v) with four holes in a cardinal arrangement with four, 1.5-mL microcentrifuge tubes. Opposite tubes contained the same treated or control diet. Two newly-molted third instars were placed in each of 25 arenas and monitored for position at 24-h intervals for 5 days. Throughout the test, larvae were held at 27 ± 2 °C and a 16:8 (L:D) photoperiod. This study was replicated 3 times.

Statistical Analyses. Because the data for the behavioral bioassays were not normally distributed, the Wilcoxon signed rank test was used (Lance, 1992). All other data were analyzed by using one-way ANOVA (SuperANOVA, 1989). Percent data were transformed with the arcsin square-root transformation prior to analysis. Means were separated at the 5% significance level using the Tukey-Kramer test or Fisher's protected LSD test (SuperANOVA, 1989).

RESULTS

Synthesis of Demethylsuberosin and Marmesin. Demethylsuberosin was synthesized essentially as outlined in a previous communication (Cairns et al., 1986b). More detailed descriptions of the syntheses of demethylsuberosin and marmesin are provided here to aid repetition (Figure 2). Thus, 7-hydroxycoumarin **1** was protected as its benzyl ether **2**, followed by opening of the pyranone ring with NaOMe, giving phenol **3**. Alkylation of phenol **3** with 1-bromo-3-methyl-2-butene did not go to completion. However, the product **4** was readily separated from unreacted **3** by trituration of the mixture with hexane and filtration to remove the hexane-insoluble **3**. Purified **4** was then refluxed in diethylaniline, producing a mixture of rearrangement products from which the desired demethylsuberosin benzyl ether **5** was separated by flash chromatography.

Clean removal of the benzyl protecting group from **5** proved problematic, as previously reported (Cairns et al., 1986a,b). Treatment of **5** with Raney nickel and hydrogen resulted in extensive overreduction of the prenyl side chain in addition to the desired removal of the benzyl ether. Monitoring the progress of the reaction was further complicated because **5** and the product **6** had very similar R_f values on TLC. However, it was found that careful treatment of **5** with an aqueous slurry of Raney nickel, terminating the reaction at approximately 75% conversion, minimized overreduction. The debenzylated product **6** was readily separated from unreacted **5** by extraction of a $CHCl_3$ solution of the crude mixture with aqueous base, with **6** being recovered from the aqueous phase after acidification and unreacted **5** being recovered for recycling from the $CHCl_3$ solution. Crude **6** was purified by recrystallization to remove traces of overreduced material, giving demethylsuberosin.

However, crude **6** could be used directly in the subsequent cyclization to marmesin, from which the overreduced material was readily removed. Thus, one-step epoxidation and cyclization under buffered conditions (Murray et al., 1971; Steck, 1971) produced marmesin in excellent yield. Overreduced material, containing a free phenolic functionality, was extracted into aqueous NaOH, and the marmesin product remaining was purified by recrystallization from benzene.

Bioassays for Survival, Weight Gain, and Developmental Rate. Even at high dosages (up to 400 μg/g of diet), demethylsuberosin and marmesin did not significantly affect larval or pupal survival (Figure 3). Psoralen, however, was toxic ($F_{6,12} = 6.08$, $P = 0.004$; Fisher's Protected LSD Test), allowing only 30% of the larvae to survive 9 days. Approximately 10% of the larvae successfully pupated, but only half of these emerged as adults (Figure 3c). These results are consistent with previous studies reporting no mortality from ingestion of other precursors (Hadacek et al., 1994) but substantial mortality from high concentrations of psoralen (Diawara et al., 1993).

Ingestion of the precursors did not increase developmental time in the larval or pupal stages or decrease larval or pupal weight as compared with control larvae exposed to UV (Table 1). Developmental times were longer for all larvae (including controls) exposed to UV than for larvae not subjected to UV radiation ($F_{6,19} = 3.253$, $P < 0.01$; Tukey-Kramer test). Similarly, larval weights were reduced for all larvae exposed to UV radiation, regardless of treatment ($F_{6,19} = 6.175$, $P < 0.01$; Tukey-Kramer test). Like previous studies, ingestion of psoralen significantly increased the number of days required to reach the pupal stage ($F_{6,19} = 3.253$, $P < 0.01$; Tukey-Kramer test) and the adult stage ($F_{6,19} = 2.242$, $P < 0.05$; Tukey-Kramer test) and significantly decreased larval weight ($F_{6,19} = 6.175$, $P < 0.01$; Tukey-Kramer test) (Table 1). Interestingly, pupal weights were not statistically different among any of the treatments, suggesting there is a critical weight which a larva must achieve in order to successfully pupate.

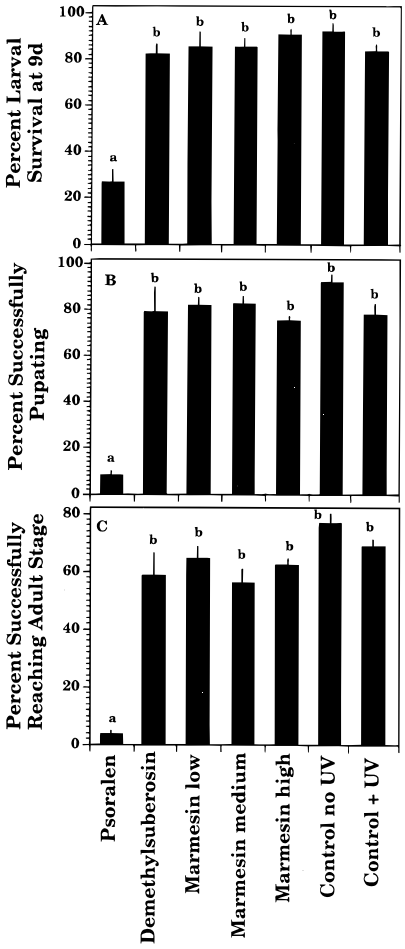

Figure 3. Survival, pupation, and adult eclosion of *S. exigua* exposed to psoralen, demethylsuberosin, or marmesin. (A) Percent survival of larvae at 9 days. (B) Percent of test population successfully pupating. (C) Percent of population successfully eclosing as adults. Rates for the compounds tested include demethylsuberosin (400 μg/g of diet), marmesin (low = 100, medium = 250, and high = 400 μg/g of diet), psoralen (375 μg/g of diet), and control diets containing no furanocoumarins or precursors. All treatments except one control group were exposed to UV light beneath a layer of Teflon film at 1.023 mW/cm^2. Lines above bars delineate standard errors of the mean. Different letters above bars within a test (A, B, or C) indicate significant differences (ANOVA, Fisher's protected LSD test).

Table 1. Effect of Ingestion of Linear Furanocoumarin Precursors and Psoralen on Developmental Rate and Weight Gain by *S. exigua*[a]

	developmental time, days		weight, mg	
treatment (rate, μg/g)	pupal	adult	larva[b]	pupa
psoralen (375)	22.8 c	30.0 c	1.9 a	101.2 a
marmesin (100)	16.6 ab	22.1 ab	29.6 b	125.8 a
marmesin (250)	16.2 ab	23.3 ab	23.3 ab	106.6 a
marmesin (400)	17.4 b	23.7 ab	26.6 ab	97.7 a
demethylsuberosin (400)	17.4 b	23.8 bc	28.5 ab	104.2 a
control plus (UV)	18.0 b	23.8 bc	37.9 b	106.4 a
control no (UV)	15.2 a	21.7 a	75.6 c	106.0 a

[a] Analyses conducted using Super ANOVA (Abacus Concepts, Berkeley, CA); different letters within columns indicate significant differences at the $P < 0.05$ level, Fishers protected LSD test. [b] Weights measured at 9 days.

Bioassays for Deterrency. Diets containing demethylsuberosin, marmesin, or psoralen were significantly less preferred by first instar *S. exigua* (Wilcoxon signed rank test, $P < 0.05$; see Figure 4 for specific sample dates). Avoidance by larvae of a demethylsuberosin-containing diet was statistically significant on three of five sample dates. For marmesin and psoralen, this trend was substantially stronger, with control diets strongly preferred to coumarin-containing diets on all sample dates. Fewer than 25% of the larvae were found on diets with marmesin or psoralen on any sample date.

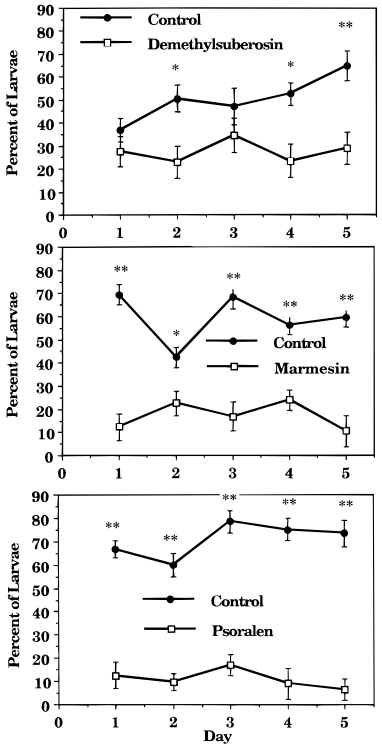

Figure 4. Percent of *S. exigua* choosing diets containing demethylsuberosin, marmesin, psoralen, or no coumarin-based compounds. Tests were initiated with first instars. For each sample date within each graph, asterisks above data points indicate significance at the $P < 0.05$ level (*) or the $P < 0.01$ level (**), Wilcoxon signed rank test.

Behavioral responses of third instar *S. exigua* to diets containing demethylsuberosin, marmesin, or psoralen were similar but more pronounced (Figure 5). For demethylsuberosin, significantly more larvae were found on control diets on four of five sample dates (Wilcoxon signed rank test, $P < 0.05$; see Figure 5 for specific sample dates). Control diets again were strongly preferred to coumarin-containing diets on every sample date. Less than 20% of the larvae were found on diets with marmesin or psoralen, with multiple sample dates showing 0−5% of the available population on diets containing these compounds.

DISCUSSION

The determination that demethylsuberosin and marmesin elicit a behavioral response has several interesting implications. Because the presence of the furanocoumarin precursors significantly affected feeding preferences, plants which produce these compounds could be afforded some protection from generalist insect herbivores. Thus, early in the evolution of the furanocoumarins, as well as at the present time, demethylsuberosin and marmesin could be expected to provide some defense against herbivores such as *S. exigua*. This result is important because it eliminates the need for two major biosynthetic steps to occur consecutively and without any apparent evolutionary driving force (production of demethylsuberosin and then marmesin) before the linear furanocoumarins can be synthesized by plants.

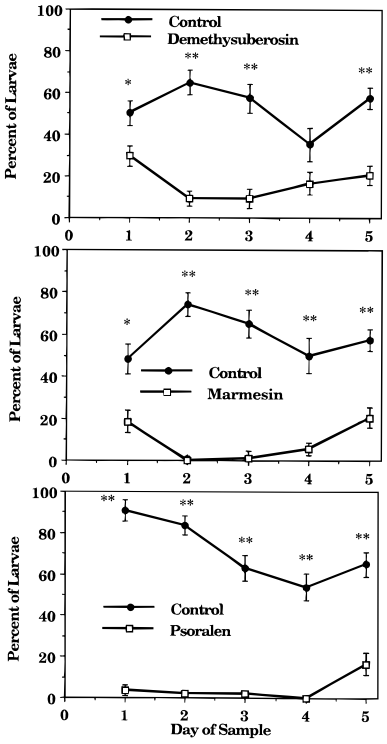

Figure 5. Percent of *S. exigua* choosing diets containing demethylsuberosin, marmesin, psoralen, or no coumarin-based compounds. Tests were initiated with third instars. For each sample date within each graph, asterisks above data points indicate significant differences between controls and treatments at the $P < 0.05$ level (*) or the $P < 0.01$ level (**), Wilcoxon signed rank test.

Spodoptera exigua, like many armyworms, is highly mobile in the latter larval stages and each individual usually feeds on multiple plants (Smits et al., 1987; Berdegue and Trumble, 1996). The consequences of leaving the host plant due to the presence of a feeding deterrent effect are probably substantial for the small first instars. Third instars, in contrast, are highly mobile and may feed on several plants a night (Smits et al., 1987). These larger instars can readily avoid plants containing feeding deterrents. Thus, the feeding deterrent effect is distinctly different for each life stage. Because food plants for the highly polyphagous *S. exigua* are common in both natural and agricultural habitats (Mitchell, 1979), host plant choice by larvae could be an important evolutionary force.

The results of our study indicate that assaying furanocoumarins and their precursors only for effects on growth and survival may not provide an accurate picture of the importance of these compounds. Feeding deterrents that are not toxic in the traditional sense have been shown to play significant ecological and evolutionary roles in herbivory (Bernays, 1990). Thus, it would be wise to assay for deterrence or other behavioral modifications before concluding that any linear furanocoumarin precursor does not have a significant effect on insect herbivores.

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