Development of Insecticide-Resistant and -Susceptible *Spodoptera exigua* (Lepidoptera: Noctuidae) Exposed to Furanocoumarins Found in Celery

M. J. BREWER,[1] T. MEADE,[2] AND J. T. TRUMBLE[3]

Environ. Entomol. 24(2): 392–401 (1995)

ABSTRACT When fed linear furanocoumarins throughout larval development, *Spodoptera exigua* (Hübner) life history was affected as exposure to psoralen, xanthotoxin, and bergapten increased from control levels to those found in commercial 52-70HK celery and insect-resistant 87A 147-2 celery (0 to 0.0022 to 0.029% fresh weight of diet, respectively). As measured by life-history traits, the furanocoumarins were more biologically active in mixture than in isolation. Insecticide resistance status mediated the effect of furanocoumarins on some but not all life-history traits measured, and the type of resistance (methomyl and fenvalerate resistance) did not result in the same response in all measures. As compared with the methomyl- and fenvalerate-resistant colonies, larval development was extended in the insecticide-susceptible colony when larvae were exposed to the highest furanocoumarin concentration. As measured by survival, the insecticide-susceptible and fenvalerate-resistant colonies were more sensitive to furanocoumarin exposure than the methomyl-resistant colony. Pupal weight and egg production of those individuals surviving exposure did not indicate a furanocoumarin and insecticide resistance status interaction. Differences in monooxygenase activity, as measured by *O*-demethylation, were not detected in methomyl-resistant or insecticide-susceptible *S. exigua* larvae exposed or not exposed to furanocoumarins. These results suggest that differential selection for furanocoumarin tolerance among these populations may occur because of the diversity of *S. exigua* exposure to insecticides and host plants varying in linear furanocoumarin content.

KEY WORDS *Spodoptera exigua*, linear furanocoumarin, insecticide resistance

Spodoptera exigua (HÜBNER), BEET ARMYWORM, is exposed to a broad array of insecticides (Trumble 1990) and host plants containing allelochemicals with bioactivity as demonstrated with *S. exigua* and other lepidopterans (e.g., Brattsten et al. 1977, Yu 1987, Calvin & Rodriguez 1988, Meade et al. 1994). There is a high degree of variation in *S. exigua* exposure to insecticides and allelochemicals because adults are highly mobile and larvae are polyphagous, occurring on cultivated (e.g., asparagus, beans, tomato, potato, cotton, beets, pepper, celery, lettuce, onions, and alfalfa) and noncultivated (e.g., nightshade, pigweed, amaranth, and malva) plants in the families Liliaceae, Fabaceae, Solanaceae, Malvaceae, Chenopodiaceae, Apiaceae, Asteraceae, and Amaranthaceae (Metcalf & Flint 1962, Peterson 1962). Use of pyrethroid and carbamate insecticides is common among the cultivated host plants (Brewer et al. 1990, Trumble 1990) and has resulted in *S. exigua* resistance to methomyl, deltamethrin, and fenvalerate (Meinke & Ware 1978, Chaufaux & Ferron 1986, Brewer et al. 1990). Consequently, interest in breeding plants resistant to *S. exigua* has increased (Trumble et al. 1990). Although not a desirable trait in plant breeding because of adverse human effects (e.g., Musajo & Rodighiero 1962), linear furanocoumarins are common in cultivated and uncultivated Apiaceae that have been part of a breeding program (Trumble et al. 1990) and may be utilized by *S. exigua*. They also are present within other Apiaceae and Fabaceae, Solanaceae, and Asteraceae (Murray 1982). Noncultivated *Apium leptophyllum* (Pers.) occurs throughout coastal California (Munz & Keck 1959).

Plant allelochemicals and insecticides can be degraded by similar enzyme systems (Brattsten 1988). The enzyme action of monooxygenases affords protection from linear furanocoumarins (e.g., Ivie et al. 1983, Berenbaum & Neal 1985) as well as pyrethroid and carbamate insecticides (Kuhr 1970, Soderlund et al. 1983). Induction of monooxygenases by host-plant feeding reduces the toxicity of carbamate and pyrethroid insecticides to *Spodoptera* spp. and insects in related genera (Yu et al. 1979; Berry et al. 1980; Yu 1982, 1984). Further, insecticide-susceptible insects with unexpressed resistance mechanisms, protected from insecticides by induction, may increase in frequency within a population in an environment with heavy use of insecticides (Brattsten 1988).

[1] Department of Plant, Soil, and Insect Sciences, P.O. Box 3354, University of Wyoming, Laramie, WY 82071.
[2] Department of Entomology, 321 Agriculture Building, University of Arkansas, Fayetteville, AR 72701.
[3] Department of Entomology, University of California, Riverside, CA 92521.

0046-225X/95/0392–0401$02.00/0 © 1995 Entomological Society of America

Possibly less understood is whether an insecticide-resistant population may affect susceptibility to allelochemicals. With regard to assumed insecticide-susceptible populations, increased insect stress resulting from feeding on host plants with allelochemical-based resistance may be responsible for increased insecticide susceptibility (van Emden 1991). Yu (1984) showed that allelochemical induction of detoxification enzymes occurred in insecticide-susceptible and resistant *S. frugiperda* (J. E. Smith). May an insecticide-resistant population of *S. exigua* be less susceptible to allelochemicals? Such a relationship may provide a preadaptation for *S. exigua* to utilize host plants containing high levels of allelochemicals. Linear furanocoumarin concentrations found in *Apium* that are a part of a breeding program and are found in natural or agricultural ecosystems may be useful to address this question. Celery, *Apium graveolens* L., hosts large populations of *S. exigua* in the coastal region of California, and methomyl and fenvalerate commonly are used (Brewer et al. 1990). Linear furanocoumarin content and larval utilization differ among celery genotypes (Trumble et al. 1990, Diawara et al. 1992). Our objectives were to determine if insecticide resistance results in reduced *S. exigua* susceptibility to linear furanocoumarins found in celery genotypes and if monooxygenases play a role in this interaction.

Materials and Methods

Spodoptera exigua larvae, with various levels of resistance to methomyl (carbamate) and fenvalerate (pyrethroid), were exposed to the linear furanocoumarins (Aldrich Chemical, Milwaukee, WI) psoralen, bergapten (5-methoxypsoralen), and xanthotoxin (8-methoxypsoralen) at levels found in a commercial celery cultivar, 'Tall Utah' 52-70HK (*A. graveolens* var. *dulce*), and in a breeding line, University of California, Davis, entry number 87A 147-2 (clone of backcross *A. graveolens* × [*A. graveolens* × *A. chilense* Hook & Arn.]). These two celeries represented the low and high range of concentrations found in leaves of six celery genotypes that were commercially available or were part of a selection program for developing celery resistant to insects (Trumble et al. 1990). Concentrations found in leaves were chosen, because leaves generally contain the highest concentrations within the plant and leaves are an important feeding site of *S. exigua* (Trumble et al. 1990).

The *S. exigua* colonies were exposed to diets containing the three furanocoumarins in mixture or each furanocoumarin in isolation. The furanocoumarins were tested in mixture on the same fresh-weight basis as found in 52-70HK and 87A 147-2 celery leaves. Similarly, they were tested in isolation at concentrations found in the two celeries and at concentrations representing the sum concentrations of the three furanocoumarins found in the celeries. A control diet containing no furanocoumarins was prepared for each test. Following the procedure of Diawara et al. (1993), the furanocoumarins were dissolved in acetone, adsorbed onto cellulose (Alphacel, ICN Biomedicals, Costa Mesa, CA), and incorporated into diet containing lima beans (Patana 1969). Therefore, larvae were exposed to psoralen at concentrations of 0 (control and psoralen concentration found in 52-70HK), 22.27 (sum of furanocoumarin concentrations found in 52-70HK), 34.09 (psoralen concentration found in 87A 147-2), and 292.92 (sum of furanocoumarin concentrations found in 87A 147-2) μg/g diet. Similarly, larvae were exposed to bergapten at 0, 9.49 (bergapten concentration found in 52-70HK), 22.27, 168.98 (bergapten concentration found in 87A 147-2), and 292.92 μg/g diet. Larvae were exposed to xanthotoxin at 0, 12.78 (xanthotoxin concentration found in 52-70HK), 22.27, 89.85 (xanthotoxin concentration found in 87A 147-2), and 292.92 μg/g diet. Larvae were exposed to the three furanocoumarins in mixture at 0, 22.27 (composed of 0 μg psoralen, 9.49 μg bergapten, and 12.78 μg xanthotoxin per g diet), and 292.92 (composed of 34.09 μg psoralen, 168.98 μg bergapten, and 89.85 μg xanthotoxin per g diet) μg/g diet. These concentrations allowed assessment of *S. exigua* life history as affected by the range of furanocoumarin exposure anticipated to occur when *S. exigua* feeds on these celeries.

The insecticide-resistant and -susceptible colonies were fed diets containing the furanocoumarin mixtures, and the insecticide-resistant colonies were fed diets containing the furanocoumarins in isolation. The insecticide-susceptible colony was not exposed to furanocoumarins in isolation because the test using the furanocoumarin mixtures indicated differences in survival between the two insecticide-resistant colonies, but no differences in survival were detected between the insecticide-susceptible and fenvalerate-resistant colonies (see *Results and Discussion* for data). We chose to explore the differences in the insecticide-resistant colonies in more detail. The insecticide-susceptible colony was established in 1982 from insects collected in Orange County, California, and was maintained in culture without exposure to insecticides through completion of these experiments. This colony was used as the insecticide-susceptible test source. The fenvalerate-resistant and methomyl-resistant colonies were established in 1987 from insects collected in the state of Baja California Norte, Mexico, and Kern County, California, respectively. The three collection localities were similar in that a large diversity of *S. exigua* host plants were in cultivation and uncultivated hosts also occurred. The localities were separated by a minimum of 240 km.

At the time of colony establishment, the fenvalerate-resistant colony was 11.8-fold less susceptible to fenvalerate than the insecticide-susceptible colony, and the methomyl-resistant colony was 19.0-fold less susceptible to methomyl than the insec-

ticide-susceptible colony. To enhance resistance, these colonies were exposed to fenvalerate and methomyl, respectively, for 19 generations. At generation 20, the fenvalerate-resistant colony was 64.2-fold less susceptible to fenvalerate than the susceptible colony, and the methomyl-resistant colony was 22.7-fold less susceptible to methomyl than the susceptible colony (Brewer et al. 1990). These colonies, after laboratory selection, were used as the resistant test colonies. It was assumed that the differences in number of years in culture were not relevant to potential differences in insecticide or allelochemical susceptibility and monooxygenase activity. The susceptibility of the insecticide-susceptible colony was similar to that of a >20-yr-old culture (Brewer & Trumble 1989). Wheeler et al. (1992) reported that long-term rearing of another lepidopteran, *Anticarsia gemmatalis* Hübner (Lepidoptera: Noctuidae), on artificial diet without periodic introduction of field individuals did not affect the activity of monooxygenases. All source colonies were maintained at 27 ± 1°C and a photoperiod of 16:8 (L:D) h on the semidefined diet used in the tests.

Insect Development. Growth, survival, and reproduction of the insecticide-resistant and -susceptible colonies fed the diets were measured. Thirty neonate larvae were placed in separate 30-ml diet cups filled with ≈10 ml of diet containing the appropriate furanocoumarin concentration. Cups were covered with Teflon FEP Fluorocarbon film (E. I. DuPont Nemours, Wilmington, DE), a covering that effectively transmits light in the UV range. All treatments were exposed to incandescent light in a 16:8 h photoperiod and UV light (40-W Sylvania 350 Blacklight) for 6 h during the light cycle. The blacklight source was used because wavelengths between 320 and 360 nm are important in the light-mediated bioactivity of linear furanocoumarins (Berenbaum 1978). Insects are affected by furanocoumarins independent of UV light (Berenbaum 1978), but this effect was not considered here because *S. exigua* is exposed to UV light when feeding on celery leaves. UV lights were adjusted in height from the surface of the diet such that the penetrance beneath the Teflon film was ≈1.00 mW/cm^2 (measured with a System 371 Optical Power Meter [United Detector Technology, Hawthorne, CA]). This light represented conditions slightly elevated from those measured at the celery plant canopy on a clear spring day in coastal southern California (Trumble et al. 1991). Larvae were held at 27 ± 1°C under these light conditions until pupation. Pupae and adults were held at this temperature under incandescent light in a 16:8-h photoperiod.

Larvae were examined daily. Mortality and larval development time (i.e., days from egg hatch to pupation) were recorded. Pupae were removed from the diet cups, weighed, and examined for visible deformities. After surface sterilization with a 5% bleach solution, the pupae were separated by sex and weighed. Weights of pupae not advancing to the adult stage were discarded. Pupae of the same sex and treatment were placed in emergence containers (i.e., 0.5-liter ice cream cartons with plastic-wrap lids, supplied with a 5% honey solution). As emergence occurred, moths with twisted wings or sections of the pupal case adhered to the body were discarded and not counted as successfully emerged. Male-female pairs for each furanocoumarin–*S. exigua* colony treatment combination were chosen randomly and placed in ovipositional containers (i.e., emergence chambers with green blotter paper inserted into the side of the container). The blotter paper was replaced every second day, and eggs were counted. Females laid eggs in clusters covered with scale; therefore a camel's-hair brush was used to remove the scales and each layer of eggs in the cluster to obtain a reliable count. Examinations were terminated when death of both adults in a rearing container was observed.

Larval development time, pupal weight, and egg production were analyzed as n (number of concentrations of a furanocoumarin or furanocoumarin mixture) by m (number of *S. exigua* colonies) factorials with i replications of each furanocoumarin–*S. exigua* treatment combination. Furanocoumarin concentration and insecticide resistance status were considered random effects; therefore the error term for the furanocoumarin and colony main effects was the interaction. The error term for the interaction was the residual (Steel & Torrie 1980). Number of observations differed because mortality and the number of male-female pairs available per treatment combination differed. For proportion successfully emerged, one observation per treatment combination was used, based on number of adults emerged of the original number of larvae exposed to each treatment combination. Survival proportion means were separated by Ryan's (1960) multiple-comparison test for proportions. Analyses by sex were conducted for the development time and pupal weight measures. The experiment exposing the *S. exigua* colonies to the furanocoumarins in mixture was conducted twice. The experiments exposing the *S. exigua* resistant colonies to furanocoumarins in isolation were conducted once.

Monooxygenase Activity. Monooxygenase activity of the insecticide-susceptible and methomyl-resistant *S. exigua* colonies reared on diets with or without furanocoumarins was assayed using a model substrate reaction, the *O*-demethylation of *p*-nitroanisole to *p*-nitrophenol (Hodgson & Casida 1961, Hansen & Hodgson 1971). Bovine serum albumin, glucose-6-phosphate, glucose-6-phosphate dehydrogenase, NADP, *p*-nitrophenol, phenylmethylsulfonyl fluoride, and Tris were purchased from Sigma Chemical (St. Louis, MO). Glycerol, magnesium chloride, sodium hydroxide, and sucrose were purchased from Fisher Scientific (Tustin, CA), and *p*-nitroanisole was purchased from Eastman Kodak (Rochester, NY).

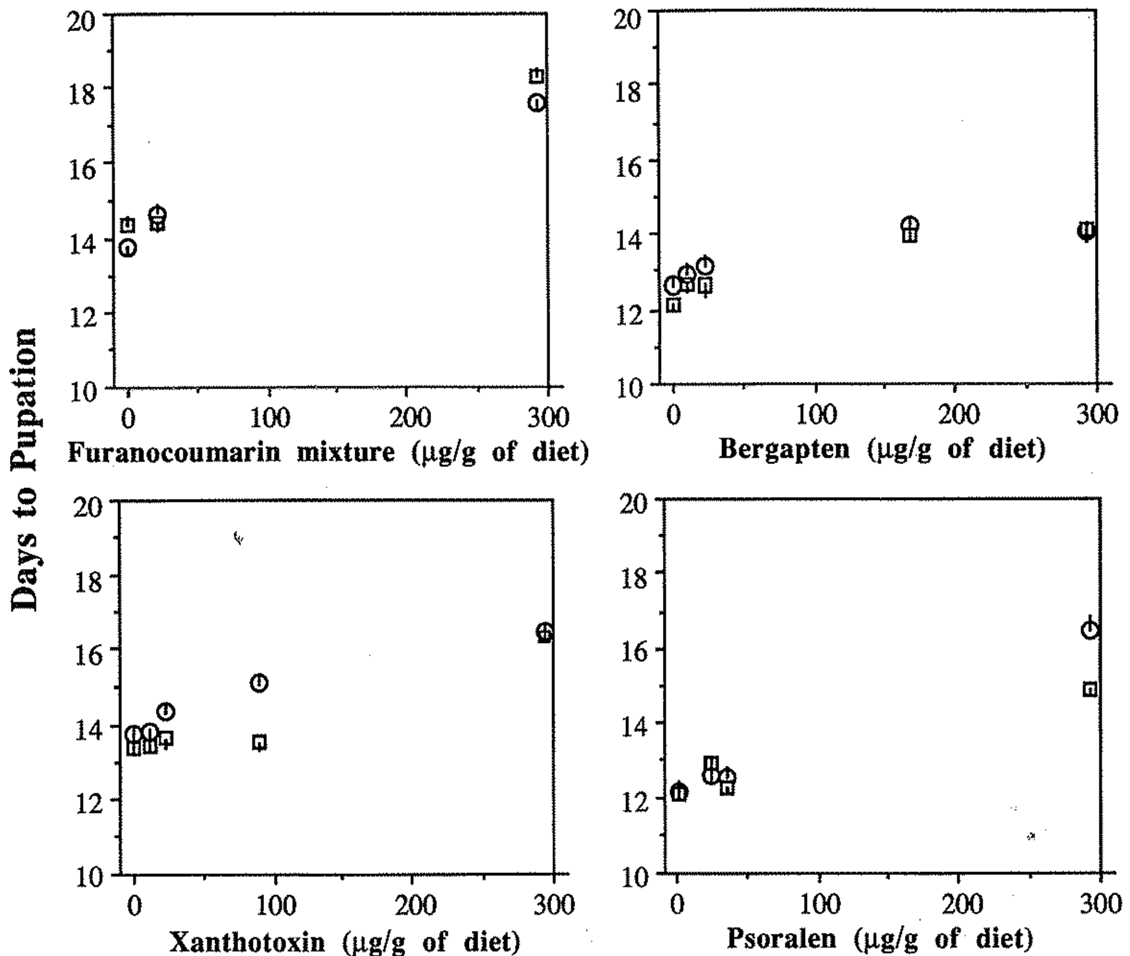

Fig. 1. Larval development time of female (circle) and male (square) *S. exigua* after exposure to different concentrations of psoralen, bergapten, xanthotoxin, and the furanocoumarins in mixture. Means are averaged among colonies tested, and bars are standard error of the means.

Larvae were reared on diet without furanocoumarins or with the high concentration of the furanocoumarin mixture found in the insect-resistant celery 87A 147-2. Insects were reared under UV and incandescent light under the same conditions as previously described.

Midguts were dissected from 15–20 fifth instars, 2–4 d old, of each colony and rearing regime. Gut contents were removed, and the remaining midgut tissue was rinsed with sucrose medium (0.24 *M* sucrose; 1 m*M* EDTA; 1% polyvinylpyrrolidone) containing 2 m*M* phenylmethylsulfonyl fluoride (Crankshaw et al. 1979). Midguts were stored in this medium until homogenized (≤1 h). They were homogenized in sucrose medium containing 5 m*M* phenylmethylsulfonyl fluoride using a Potter-Elvehjem tissue grinder fitted with a Teflon pestle. Homogenates were centrifuged at 10,000 × g_{max} for 10 min, and the resulting supernatants were centrifuged at 100,000 × g_{max} for 1 h. The resulting microsomal pellet was suspended in 3 ml of a potassium phosphate buffer (0.15 *M*, pH 7.8) containing 1 m*M* EDTA and 50% glycerol. The protein suspension was flash frozen in a dry-ice acetone bath and stored at −70°C until used in the determination of monooxygenase activity (≈2 wk). The procedure was conducted at temperatures below 4°C.

The *O*-demethylation of *p*-nitroanisole to *p*-nitrophenol was used as an indicator of monooxygenase activity. The protein concentration of each microsomal preparation was determined by the Bradford assay (Bio-rad Chemical Division, Richmond, CA) using bovine serum albumin as the standard. Microsomal protein (≈0.8 mg) was incubated for 30 min at 31°C in 10-ml Erlenmeyer flasks with an NADPH regenerating system (i.e., 0.5 m*M* NADP, 2.5 m*M* glucose-6-phosphate, 0.4 units glucose-6-phosphate dehydrogenase, and 7.5 m*M* magnesium chloride) in a 50 m*M* Tris buffer, pH 7.8. Microsomal protein was incubated in Tris buffer without the regenerating system to serve as

Table 1. Results of analyses of variance comparing larval development time and pupal weights among colonies differing in insecticide resistance and exposure to psoralen, bergapten, xanthotoxin, and these furanocoumarins in mixture

Parameter Effect	Psoralen			Bergapten			Xanthotoxin			Mixture		
	df	*F*	*P*	df	*F*	*P*	df	*F*	*P*	df	*F*	*P*
Larval development time: females												
Colony	1	10.8	0.046	1	5.58	0.0088	1	4.91	0.09	2	0.67	0.56
Furanocoumarin	3	64.3	0.0032	4	55.3	0.0009	4	30.3	0.0021	2	57.4	0.0011
Interaction	3	0.48	0.70	4	0.98	0.80	4	0.25	0.91	4	3.32	0.011
Residual	96	SS = 279		129	SS = 76.8		129	SS = 431		243	SS = 1,598	
Larval development time: males												
Colony	1	0.0	0.98	1	0.76	0.43	1	0.01	0.93	2	0.02	0.98
Furanocoumarin	3	43.1	0.006	4	10.3	0.022	4	12.07	0.017	2	58.18	0.0011
Interaction	3	0.61	0.61	4	1.30	0.275	4	1.54	0.195	4	2.83	0.025
Residual	100	SS = 169		129	SS = 195		117	SS = 310		226	SS = 507	
Pupal weight: females												
Colony	1	2.76	0.20	1	19.8	0.011	1	62.3	0.0014	2	38.6	0.002
Furanocoumarin	3	0.96	0.51	4	3.16	0.15	4	3.50	0.13	2	1.93	0.26
Interaction	3	1.95	0.13	4	1.01	0.40	4	41.0	0.80	4	0.41	0.80
Residual	96	SS = 25,727		129	SS = 20,348		129	SS = 45,048		243	SS = 81,213	
Pupal weight: males												
Colony	1	13.4	0.035	1	3.53	0.13	1	19.9	0.011	2	18.2	0.0098
Furanocoumarin	3	0.17	0.91	4	0.46	0.76	4	0.69	0.63	2	3.54	0.13
Interaction	3	1.52	0.22	4	1.94	0.11	4	1.83	0.13	4	0.71	0.59
Residual	100	SS = 22,698		129	SS = 21,258		117	SS = 20,392		226	SS = 41,810	

Insecticide-susceptible, fenvalerate-resistant, and methomyl-resistant colonies were exposed to the furanocoumarin mixture and resistant colonies were exposed to the furanocoumarins in isolation. Analyses were conducted separately for females and males. Error term for the colony and compound effect is the interaction, error term for the interaction is the residual. SS is the residual sum of squares.

a control. The reaction was initiated by adding 20 μl of 10 m*M* *p*-nitroanisole (in ethanol). The final volume of the reaction mixture (including *p*-nitroanisole) was 1.0 ml. The reaction was terminated by adding 0.5 ml 1 *M* sodium hydroxide. Reaction mixtures were centrifuged for 10 min at $10{,}000 \times g_{max}$, and absorbance of the supernatant was determined at 400 nm. Results were expressed as nmoles *p*-nitrophenol produced per mg microsomal protein per minute. All assays were run in duplicate and were repeated two to three times for each *S. exigua* colony. The analysis to detect differences in *p*-nitrophenol production was a two (methomyl-resistant and -susceptible colonies) by two (exposed or not exposed to furanocoumarins) factorial (Steel & Torrie 1980). The error terms were set as in the life history analyses.

Results and Discussion

Insect Development. The experimental replication effect did not indicate differences among the duplicate furanocoumarin mixture experiments ($P > 0.20$) and was not considered further. All furanocoumarins tested were bioactive, resulting in adverse growth, survival, and reproduction of *S. exigua*. Larval development time of all colonies increased as furanocoumarin concentration increased from control levels to those found in commercial 52-70HK celery and insect-resistant 87A 147-2 celery (0 to 0.0022 to 0.029% fresh weight of diet, respectively) ($P < 0.05$ for furanocoumarin effect [Table 1; Fig. 1]). When each furanocoumarin was fed separately to the insecticide-resistant colonies, larval development time also was extended ($P < 0.05$ for furanocoumarin effect [Table 1; Fig. 1]). Diawara et al. (1993) also reported development delays when an insecticide-susceptible *S. exigua* population was exposed to these furanocoumarins in isolation at similar and higher concentrations. Using higher concentrations, xanthotoxin fed to *S. eridania* (Cramer) from egg hatch at 0.1 and 1.0% of diet extended the time period of the first stadium (Berenbaum 1978).

The status of insecticide resistance of the three colonies differentially affected larval development time as concentration of the furanocoumarin mixture changed. This interaction was small but detectable ($P < 0.05$ [Table 1]) and for males was seen as an ≈9% longer development time for the insecticide-susceptible colony as compared with the methomyl-resistant colony when each was exposed to the high furanocoumarin mixture (Fig. 2B). Larval development time of the fenvalerate-resistant colony was intermediate. There was little variation in development time when the colonies were exposed to the low concentration or not exposed. When the furanocoumarins were exposed in isolation to the two resistant colonies, there were no detectable differences in larval development time among the colonies as furanocoumarin concentration increased ($P > 0.15$ for interaction [Table 1]).

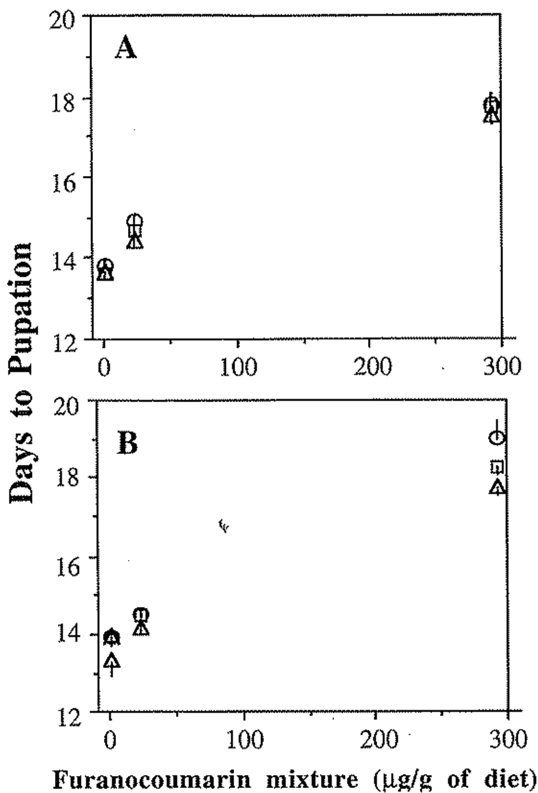

Fig. 2. Larval development time of female (A) and male (B) *S. exigua* of the insecticide-susceptible (circle), fenvalerate-resistant (square), and methomyl-resistant (triangle) colonies after exposure to different concentrations of the furanocoumarin mixture. Bars are standard error of the means.

Pupal weights were less affected by exposure to furanocoumarins. There were no detectable changes in pupal weights when larvae were exposed to the different concentrations of the furanocoumarin mixture or furanocoumarins in isolation ($P > 0.10$ for furanocoumarin effect [Table 1]). There were pupal weight differences among colonies ($P < 0.05$ for colony effect [Table 1]). Averaged across concentrations of the furanocoumarin mixture, female mean weights for the insecticide-susceptible, fenvalerate-resistant, and methomyl-resistant colonies were 131, 119, and 130 mg, respectively. The SEMs were 2–4 mg in all cases. But for this life-history trait, resistance status did not affect response to changing concentrations of furanocoumarins as judged by the nonsignificant interaction terms ($P > 0.10$ for interactions [Table 1]).

Differences in survival to the adult stage were detected. This measure generally allowed better discrimination among treatments than the larval development time measure. Mortality was detected in the larval stage, particularly for those individuals exposed to the higher furanocoumarin concentrations, but there were no peaks of mortality at a particular instar. Mortality also was detected in the pupal and emerging adult stages (i.e., dead misshapen pupae and adults considered moribund at emergence, with twisted wings or parts of the pupal case remaining attached to the body). Berenbaum (1978) found that *S. eridania* did not survive past the second stadium when exposed to xanthotoxin beginning at egg hatch at higher concentrations than used here. Reduction in survival was greatest when larvae were exposed to the mixture than when larvae were exposed to furanocoumarins separately at the same overall concentration (Table 2). In contrast, Diawara et al. (1993) reported survival rates of an insecticide-susceptible population greater than expected when psoralen, xanthotoxin, and bergapten were combined and exposed to *S. exigúa,* as compared with exposure of these compounds separately. They used combined concentrations ≈1.4-fold higher than those used in our study, and psoralen was used as the predominant component. Our assay was limited to lower concentrations found in selected *Apium* genotypes that may be encountered by *S. exigua.* Psoralen generally had less effect on *S. exigua* measures than the other furanocoumarins (psoralen affected larval development time but had little effect on other measures [Tables 1–3]) and was the smallest component in the furanocoumarin mixtures. Both concentrations and proportions of the individual constituents of the mixture may affect *S. exigua* response to the furanocoumarins.

The status of insecticide resistance of the three colonies did result in differential survival as concentration of the furanocoumarin mixture changed. There was a reduction in survival as furanocoumarins concentration increased when larvae of the insecticide-susceptible and fenvalerate-resistant colonies were exposed. The methomyl-resistant colony was much less affected (Table 2).

Of the *S. exigua* surviving to adults, differences in egg productivity of the three colonies were not detected ($P > 0.05$ for colony effect [Table 3]). Reduction in egg production was detected when larvae of the three colonies were exposed to increasing concentrations of the furanocoumarin mixture ($P < 0.05$ for furanocoumarin effect [Table 3; Fig. 3]), but these changes across furanocoumarin concentration were unaffected by insecticide resistance status as indicated by the nonsignificant interaction term (Table 3).

The furanocoumarin and insecticide resistance status dual effect detected in survival and to a lesser extent in larval development time suggested that the responses of the *S. exigua* populations were sufficiently variable that selection for furanocoumarin tolerance can occur. The methomyl-resistant colony appeared to have the greatest preadaptation to survive furanocoumarin exposure. Because these colonies were not derived from the same base population, there was the possibility that there was an underlying cause of the differential

Table 2. Survival (proportion successfully emerged) among colonies exposed to psoralen, bergapten, xanthotoxin, and these furanocoumarins in mixture

Colony[a]	Psoralen		Bergapten		Xanthotoxin		Mixture	
	Conc[b]	Survival	Conc	Survival	Conc	Survival	Conc	Survival
Sus	—		—		—		0	0.88a
Fen	0	0.83ab	0	0.8a	0	0.97ab	0	0.77abc
Met	0	0.9a	0	0.7a	0	0.73ab	0	0.85ab
Sus	—		—		—		—	
Fen	—		9.49	0.7a	12.78	0.9ab	—	
Met	—		9.49	0.8a	12.78	0.73ab	—	
Sus	—		—		—		22.27	0.68bcd
Fen	22.27	0.8ab	22.27	0.63a	22.27	0.97a	22.27	0.57cd
Met	22.27	0.9a	22.27	0.77b	22.27	0.9ab	22.27	0.9a
Sus	—		—		—		—	
Fen	34.09	0.77ab	168.98	0.5a	89.85	0.9ab	—	
Met	34.09	0.93a	168.98	0.73a	89.85	0.9ab	—	
Sus	—		—		—		292.92	0.28ef
Fen	292.92	0.73ab	292.92	0.47a	292.92	0.67b	292.92	0.18f
Met	292.92	0.57b	292.92	0.8a	292.92	0.8ab	292.92	0.5de

Insecticide-susceptible, fenvalerate-resistant, and methomyl-resistant colonies were exposed to the furanocoumarin mixture and the resistant colonies were exposed to the furanocoumarins in isolation. Survival proportions in the same column followed by the same letter are not significantly different ($P > 0.05$; Ryan's [1960] multiple-comparison test for proportions), based on 30 individuals per colony and concentration for the psoralen, bergapten, and xanthotoxin tests and 60 individuals per colony and concentration for the furanocoumarin mixture test.

[a] Sus, insecticide-susceptible colony. Fen, fenvalerate-resistant colony. Met, methomyl-resistant colony.

[b] Conc, concentration in μg per g of diet; compositions of the mixture are given in text.

effects among the colonies seen here that was not related to insecticide resistance status. As revealed in crossing experiments of the fenvalerate-resistant and insecticide-susceptible colonies (Brewer & Trumble 1991), fenvalerate resistance was determined to be a heritable trait and was linked to heritable differences in development time and egg production but not in pupal weight and adult emergence. Similar to that past work, furanocoumarin exposure in this study affected development time differentially in the fenvalerate-resistant and insecticide-susceptible colonies, but pupal weight and adult emergence in response to furanocoumarin exposure behaved similarly in the two colonies. When an interaction was detected, reference to the controls (no exposure to furanocoumarins) provided evidence that the colonies reacted differently to furanocoumarin exposure and that the differences were not a result of simple colony differences. Therefore, insecticide resistance status seems to be the most plausible explanation of the differential effect of the three colonies when exposed to furanocoumarins.

Monooxygenase Activity. When *S. exigua* larvae were exposed to the furanocoumarin diet, *O*-demethylation was numerically lower in the methomyl-resistant colony than in the susceptible colony, whereas *O*-demethylation was numerically higher in the methomyl-resistant colony than in the susceptible colony when both colonies were fed diet without furanocoumarins (Fig. 4). But the data analysis did not indicate an insecticide resistance status and furanocoumarin interaction ($F = 2.72$; df = 1, 11; $P = 0.12$). The furanocoumarin concentration and colony main effects also were not significant ($P > 0.20$) as can occur given the switch in monooxygenase activity of the colonies when exposed and not exposed to the furanocoumarin mixture. These trends showed similarities and differences from past work. Using the synergist piperonyl butoxide, monooxygenases were not implicated as a mechanism of fenvalerate or methomyl resistance in *S. exigua*, although both insecticide-resistant and -susceptible populations were synergized at approximately equal rates with piperonyl butoxide (Brewer & Trumble 1994). In

Table 3. Results of analyses of variance comparing egg production among colonies differing in insecticide resistance and exposure to psoralen, bergapten, xanthotoxin, and these furanocoumarins in mixture

Effect	Psoralen			Bergapten			Xanthotoxin			Mixture		
	df	*F*	*P*	df	*F*	*P*	df	*F*	*P*	df	*F*	*P*
Colony	1	5.97	0.09	1	2.20	0.21	1	9.91	0.035	2	1.97	0.25
Furanocoumarin	3	1.41	0.39	4	0.13	0.96	4	4.25	0.095	2	8.44	0.037
Interaction	3	0.72	0.54	4	1.76	0.147	4	0.39	0.81	4	0.49	0.745
Residual	39	SS = 3,621,672		66	SS = 14,552,235		50	SS = 1,228,238		103	SS = 17,059,007	

Insecticide-susceptible, fenvalerate-resistant, and methomyl-resistant colonies were exposed to the furanocoumarin mixture, and the resistant colonies were exposed to the furanocoumarins in isolation. Error term for the colony and compound effect is the interaction, error term for the interaction is the residual. SS is the residual sum of squares.

Fig. 3. Egg production of *S. exigua* surviving to adults after exposure as larvae to different concentrations of psoralen, bergapten, xanthotoxin, and the furanocoumarin mixture. Means are averaged among colonies tested, and bars are standard error of the means.

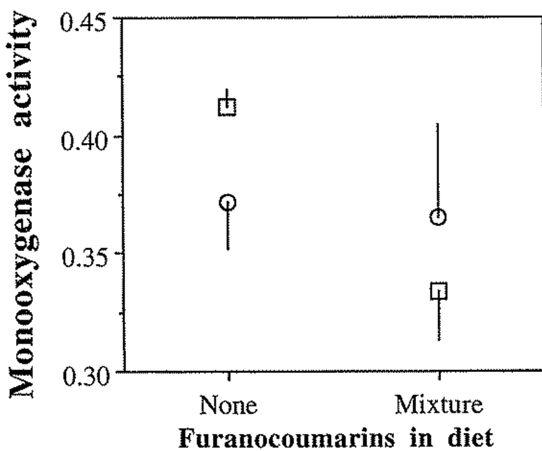

Fig. 4. Monooxygenase activity expressed in nmoles *p*-nitrophenol per mg microsomal protein per min, as measured by *O*-demethylation of *p*-nitroanisole, of methomyl-susceptible (circle) and -resistant (square) *S. exigua* fed diet containing furanocoumarins in mixture at levels found in insect-resistant celery (mixture) or fed diet not containing furanocoumarins (none).

contrast, *S. frugiperda* resistant to carbaryl had elevated levels of monooxygenases compared with a susceptible colony when sixth instars were fed control diet, and monooxygenases were induced by allelochemicals including xanthotoxin, as measured by heptachlor epoxidase (Yu 1984). *S. exigua,* in our study, was exposed to furanocoumarins throughout larval development, and such long-term exposure may result in monooxygenase suppression. Alternatively, monooxygenase activity of *Spodoptera* spp. may be substrate specific, even though monooxygenases are considered general purpose defenses (Brattsten 1988). Yu (1984) found that linear furanocoumarin exposure to *S. frugiperda* increased cytochrome P450 content and induced heptachlor epoxidase activity, while inhibiting aldrin epoxidase, biphenyl 4-hydroxylene, and *p*-chloro-*N*-methylaniline *N*-demethylene activity. Short- and long-term exposure and use of multiple substrates appear necessary to clarify the action of *S. exigua* monooxygenases on furanocoumarins.

Overall, *S. exigua* live history was affected as furanocoumarin exposure increased from control lev-

els to those found in commercial 52-70HK celery and insect-resistant 87A 147-2 celery (0 to 0.0022 to 0.029% fresh weight, respectively). The furanocoumarins were more biologically active in mixture than in isolation. Insecticide resistance status mediated the effect of furanocoumarins on survival and to a lesser extent on length of larval development time. The effect may result in differential selection for furanocoumarin tolerance among these populations. In particular, the decreased survival of the insecticide-susceptible and fenvalerate-resistant colonies when exposed to linear furanocoumarins may place these populations at a competitive disadvantage with methomyl-resistant populations when these populations feed on cultivated and noncultivated *Apium*. Host plants with higher linear furanocoumarin content may further differentiate among *S. exigua* populations, although linear extrapolation of these data is not suggested. Additional studies using a variety of insects differing in host plant range and insecticide and allelochemical susceptibility are suggested to evaluate the extent to which the interaction of insecticide resistance and allelochemicals may result in changes in host–plant utilization.

Acknowledgments

We thank W. Carson, A. Jones, K. White, E. Younce (University of California, Riverside), M. Diawara (University of Southern Colorado), and S. Eigenbrode (University of Arizona) for assistance in the laboratory. We thank J. D. Hare (UCR) for use of laboratory facilities. Thanks to M. Diawara and two anonymous reviewers for comments. Support was provided by the California Celery Research Advisory Board.

References Cited

Berenbaum, M. 1978. Toxicity of a furanocoumarin to armyworms: a case of biosynthetic escape from insect herbivores. Science (Washington, DC) 201: 532–534.

Berenbaum, M. & J. J. Neal. 1985. Synergism between myristicin and xanthotoxin, a naturally cooccurring plant toxicant. J. Chem. Ecol. 11: 1349–1358.

Berry, R. E., S. J. Yu & L. C. Terriere. 1980. Influence of host plants on insecticide metabolism and management of variegated cutworm. J. Econ. Entomol. 73: 771–774.

Brattsten, L. B. 1988. Potential role of plant allelochemicals in the development of insecticide resistance, pp. 313–348. *In* P. Barbosa & D. K. Letourneau [eds.], Novel aspects of insect–plant interactions. Wiley, New York.

Brattsten, L. B., C. F. Wilkinson & T. Eisner. 1977. Herbivore–plant interactions: mixed-function oxidases and secondary plant substances. Science (Washington, DC) 196: 1349–1352.

Brewer, M. J. & J. T. Trumble. 1989. Field monitoring for insecticide resistance in beet armyworm (Lepidoptera: Noctuidae). J. Econ. Entomol. 82: 1520–1526.

1991. Inheritance and fitness consequences of resistance to fenvalerate in beet armyworm (Lepidoptera: Noctuidae). J. Econ. Entomol. 84: 1638–1644.

1994. Beet armyworm resistance to fenvalerate and methomyl: resistance variation and insecticide synergism. J. Agric. Entomol. 11: 291–300.

Brewer, M. J., J. T. Trumble, B. Alvarado-Rodriguez & W. E. Chaney. 1990. Beet armyworm (Lepidoptera: Noctuidae) adult and larval susceptibility to three insecticides in managed habitats and relationship to laboratory selection for resistance. J. Econ. Entomol. 83: 2136–2146.

Calvin, J. C. & E. Rodriguez. 1988. The influence of dietary B-Carboline alkaloids on growth rate, food consumption, and food utilization of larvae of *Spodoptera exigua* (Hübner). J. Chem. Ecol. 14: 475–484.

Chaufaux, J. & P. Ferron. 1986. Sensibilite differente de deux populations de *Spodoptera exigua* Hüb. (Lepid. Noctuidae) aux baculovirus et aux pyrethrinoides de synthese. Agronomie (Paris) 6: 99–104.

Crankshaw, D. L., H. K. Hetnarski & C. F. Wilkinson. 1979. Microsomal NADPH-cytochrome c reductase form the midgut to the southern armyworm (*Spodoptera eridania*). Insect Biochem. 9: 43–48.

Diawara, M. M., J. T. Trumble, C. F. Quiros & J. G. Millar. 1992. Resistance to *Spodoptera exigua* in *Apium prostratum*. Entomol. Exp. Appl. 64: 125–133.

Diawara, M. M., J. T. Trumble, K. K. White, W. G. Carson & L. A. Martinez. 1993. Toxicity of linear furanocoumarins to *Spodoptera exigua*: evidence for antagonistic interactions. J. Chem. Ecol. 19: 2473–2484.

Hansen, L. G. & E. Hodgson. 1971. Biochemical characteristics of insect microsomes: N- and O-demethylation. Biochem. Pharmacol. 20: 1569–1578.

Hodgson, E. & J. E. Casida. 1961. Metabolism of N: N-dialkyl carbamates and related compounds by rat liver. Biochem. Pharmacol. 8: 179–191.

Ivie, G. W., D. Bull, R. C. Beier, N. W. Pryor & E. H. Oertli. 1983. Metabolic detoxification: mechanism of insect resistance to plant psoralens. Science (Washington, DC) 221: 374–376.

Kuhr, R. J. 1970. Metabolism of carbamate insecticide chemicals in plants and insects. J. Agric. Food Chem. 18: 1023–1030.

Meade, T., J. D. Hare, S. L. Midland, J. G. Millar & J. J. Sims. 1994. Phthalide-based host-plant resistance to *Spodoptera exigua* and *Trichoplusia ni* in *Apium graveolens*. J. Chem. Ecol. 20: 709–726.

Meinke, L. J. & G. W. Ware. 1978. Tolerance of three beet armyworm strains in Arizona to methomyl. J. Econ. Entomol. 71: 645–646.

Metcalf, C. L. & W. P. Flint. 1962. Destructive and useful insects, their habits and control, 4th ed. McGraw-Hill, San Francisco.

Munz, P. A. & D. D. Keck. 1959. A California flora. University of California Press, Berkeley.

Murray, R.D.H. 1982. The natural coumarins. Wiley, New York.

Musajo, L. & G. Rodighiero. 1962. The skin-photosensitizing furanocoumarins. Experientia (Basel) 18: 153–200.

Patana, R. 1969. Rearing cotton insects in the laboratory. U.S. Dep. Agric. Prod. Res. Rep. 108.

Peterson, A. 1962. Larvae of insects, an introduction to nearctic species, Part I: Lepidoptera and plant-infesting Hymenoptera. Edwards Brothers, Ann Arbor, MI.

Ryan, T. A. 1960. Significance tests for multiple comparison of proportions, variances and other statistics. Psychol. Bull. 57: 318–328.

Soderlund, D. M., J. R. Sanborn & P. W. Lee. 1983. Metabolism of pyrethrins and pyrethroids in insects, pp. 401–435. *In* D. H. Hutson & T. R. Roberts [eds.], Progress in pesticide biochemistry and toxicology, vol. 3. Wiley, New York.

Steel, R.G.D. & J. H. Torrie. 1980. Principles and procedures of statistics, a biometrical approach, 2nd ed. McGraw-Hill, New York.

Trumble, J. T. 1990. Vegetable insect control with minimal use of insecticides. HortScience 25: 159–164.

Trumble, J. T., W. Dercks, C. F. Quiros & R. C. Beier. 1990. Host plant resistance and linear furanocoumarin content of *Apium* accessions. J. Econ. Entomol. 83: 519–525.

Trumble, J. T., W. J. Moar, M. J. Brewer & W. G. Carson. 1991. Impact of UV radiation on activity of linear furanocoumarins and *Bacillus thuringiensis* var. *kurstaki* against *Spodoptera exigua:* implications for tritrophic interactions. J. Chem. Ecol. 17: 973–987.

Van Emden, H. F. 1991. The role of host plant resistance in insect pest mis-management. Bull. Entomol. Res. 81: 123–126.

Wheeler, G. S., F. Slansky, Jr., & S. J. Yu. 1992. Laboratory colonization has not reduced constitutive or induced polysubstrate monooxygenase activity in velvetbean caterpillars. J. Chem. Ecol. 18: 1313–1325.

Yu, S. J. 1982. Induction of microsomal oxidases by host plants in the fall armyworm, *Spodoptera frugiperda* (J. E. Smith). Pestic. Biochem. Physiol. 17: 59–67.

1984. Interactions of allelochemicals with detoxification enzymes of insecticide-susceptible and resistant fall armyworms. Pestic. Biochem. Physiol. 22: 60–68.

1987. Microsomal oxidation of allelochemicals in generalist (*Spodoptera frugiperda*) and semispecialist (*Anticarsia gemmatalis*) insects J. Chem. Ecol. 13: 423–436.

Yu, S. J., R. E. Berry & L. C. Terriere. 1979. Host plant stimulation of detoxifying enzymes in a phytophagous insect. Pestic. Biochem. Physiol. 12: 280–284.

Received for publication 11 August 1994; accepted 22 November 1994.

Reprinted from JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY, 1995, *43*.
Copyright ©1995 by the American Chemical Society and reprinted by permission of the copyright owner.

Implications of Distribution of Linear Furanocoumarins within Celery

Moussa M. Diawara,*,† John T. Trumble,‡ Carlos F. Quiros,§ and Richard Hansen||

Departments of Biology and Art, University of Southern Colorado, Pueblo, Colorado 81001, Department of Entomology, University of California, Riverside, California 92521, and Department of Vegetable Crops, University of California, Davis, California 95616

Celery (*Apium graveolens*) has occasionally been reported to have hazardous concentrations of the carcinogenic linear furanocoumarins; therefore, fresh, healthy plant parts of the celery variety Tall Utah 52-70R, which forms the basis of all celery varieties in the market, and the new breeding line UC-08 were analyzed for linear furanocoumarin composition and concentrations to determine the relative safety of each plant part for human consumption. The older, outer celery leaves (44.9 μg/g of fresh weight) and the leaves on mature inner petioles (9.9 μg/g) contained significantly more linear furanocoumarins than the leaves on the innermost immature "heart" petioles (3.6 μg/g), heart petioles (1.5 μg/g), outer petioles (1.4 μg/g), inner mature petioles (1.0 μg/g), or the root (0.9 μg/g). Except for the leaves on mature outer or inner petioles, which had levels of furanocoumarins high enough to threaten human and animal health, all other plant parts tested had levels that are reportedly safe for handling and consumption. Implications of these findings for food safety, breeding for pest resistance, and evolutionary biology are discussed.

Keywords: *Apium graveolens; psoralen; bergapten; xanthotoxin; carcinogens; food safety; evolutionary biology*

INTRODUCTION

In an attempt to reduce cancer incidence in the United States by 50% by the year 2000, public health efforts are directed toward changing the ratio of fat to fiber in the human diet by increasing fiber intake (Smolin and Gosvenor, 1994). Therefore, consumption of vegetables as a component of the human diet will likely increase because these constitute an important source of fiber. However, some of the biosynthetic chemicals that vegetable crops produce for protection against insect herbivores, pathogens, or adverse environmental conditions can be hazardous to humans; these include the linear furanocoumarins. The linear furanocoumarins are bioactive compounds that have been isolated from species in a number of plant families including Rutaceae, Apiaceae (Umbelliferae), Asteraceae, Fabaceae, Moraceae, Pittosporaceae, Solanaceae, Brassicaceae, Amaranthaceae, Rosaceae, Cyperaceae, and Thymeleaceae (Scott et al., 1976; Murray et al., 1982; Berenbaum, 1991); these plant taxa include many of the most commonly consumed grocery vegetables.

The linear furanocoumarins are photoactivated (Zangerl and Berenbaum, 1987; Trumble et al., 1991) plant secondary metabolites that have been used since ancient times to treat human skin disorders such as skin depigmentation (vitiligo), psoriasis, mycosis fungoides, polymorphous photodermatitis, and eczema (Musajo and Rodighiero, 1962; Van Scott et al., 1975; Scott et al., 1976). However, the use of these furanocoumarins in medicine has been associated with higher incidence of skin cancer (Musajo and Rodighiero, 1962; Stern et al., 1979; Grekin and Epstein, 1981; Berenbaum, 1991).

* Author to whom correspondence should be addressed [telephone (719) 549-2813; fax (719) 549-2732; e-mail moussa@starburst.uscolo.edu].

† Department of Biology.

‡ Department of Entomology.

§ Department of Vegetable Crops.

|| Department of Art.

A number of studies have demonstrated that the furanocoumarins are carcinogenic, mutagenic, and photodermatitic (Roelandts, 1984; Berkley et al., 1986; Koch, 1986; Berenbaum, 1991). Oral administration of 8-methoxypsoralen to patients for treatment of psoriasis resulted in basal-cell and squamous-cell carcinomas (Stern et al., 1979). Young (1990) reported photocarcinogenicity of psoralens to mice and humans. Celery (*Apium graveolens* L. var. *dulce*) has been among the most extensively studied vegetables for linear furanocoumarin content because of the potential for high concentrations of these compounds in the plant (Chaudhary et al., 1985; Trumble et al., 1990; Heath-Pagliuso et al., 1992; Diawara et al., 1992, 1993) and risks associated with handling or ingestion of celery (Ljunggren, 1990). A team of specialists from the Special Pathogens Branch of the Center for Disease Control and Prevention and the National Institute for Occupational Safety and Health conducted a study designed to determine furanocoumarin-related dermatitis among grocery workers (Fleming, 1990). The study revealed that 24% (30 of 127) of the workers handling celery developed contact dermatitis.

A cross-sectional epidemiological study of workers in two Oregon grocery stores revealed that handling healthy-looking celery caused photodermatitis, and these conditions worsened with ultraviolet A (UVA) light exposure (Berkley et al., 1986). The study also reported that, in addition to celery, handling potato, parsnip, carrot, citrus, parsley, and spinach also induced contact photodermatitis. In-vitro bioassays with bacterial and mammalian cells proved the furanocoumarins to be lethal and carcinogenic (Ashwood et al., 1980). The World Health Organization recognized these psoralens as causal agents of skin cancer in humans (International Agency for Research on Skin Cancer, 1983). These chemicals can reach the skin not only by contact but also through oral ingestion with the diet. Ljunggren (1990) diagnosed a serious generalized phototoxic burn in a human patient following celeriac ingestion. Finkel-

0021-8561/95/1443-0723$09.00/0 © 1995 American Chemical Society

Table 1. Distribution of Linear Furanocoumarins (Micrograms per Gram of Fresh Weight) within the Commercial Celery Variety Tall Utah 5270R (*A. graveolens* L. Var. *dulce*)[a]

plant part	psoralen		bergapten		xanthotoxin		total	
	mean	SE	mean	SE	mean	SE	mean	SE
outer leaf	3.936 b	1.214	28.002 c	4.549	17.899 c	3.660	49.836 c	8.496
inner leaf	0.168 a	0.128	4.791 b	0.571	2.988 b	0.552	7.947 b	1.163
heart leaf	0.020 a	0.017	2.419 ab	0.432	1.289 ab	0.240	3.729 ab	0.657
outer petiole	0.008 a	0.004	0.633 a	0.117	0.351 a	0.075	0.993 a	0.191
inner petiole	0.001 a	0.001	0.483 a	0.092	0.315 a	0.077	0.800 a	0.168
heart petiole	0.002 a	0.002	0.520 a	0.100	0.391 a	0.101	0.914 a	0.200
root	0.036 a	0.009	0.567 a	0.118	0.490 a	0.115	1.093 a	0.232
P	0.0001		0.0001		0.0001		0.0001	
$F_{6,54}$	26.622		61.878		46.020		58.174	

[a] Means within a column not followed by the same letter are statistically different at the 5% level using the Tukey–Kramer test (Super ANOVA, 1989). Means represent actual data; analyses based on square root transformations.

stein et al. (1994) recently reported an outbreak of photodermatitis among workers due to handling of celery containing levels of linear furanocoumarins as high as 84 μg/g of fresh weight. These furanocoumarin-related health hazards are even more serious when plants are infected with disease-causing pathogens (Scheel et al., 1963; Surico et al., 1987).

The causal agents of the dermatitis due to celery have been known since the mid 1970s as the linear furanocoumarins psoralen, 5-methoxypsoralen (bergapten), and 8-methoxypsoralen (xanthotoxin) (Austad and Kavli, 1983; Ashwood-Smith et al., 1985). These three compounds were also the major linear furanocoumarins isolated from other common grocery vegetables (Berenbaum, 1981a,b; Ivie et al., 1981; Beier, 1983; Seligman et al., 1987; Trumble et al., 1990; Diawara et al., 1992). There has been a great deal of research on the production of linear furanocoumarins in celery and their mammalian toxicity over the past 30 years (Scheel et al., 1963; Finkelstein et al., 1994). However, despite all of the fear surrounding celery handling and consumption, to our knowledge, there is no literature on the specific distribution of linear furanocoumarins within celery other than that leaves generally have higher concentrations than petioles (Berkley et al., 1986; Trumble et al., 1990; Diawara et al., 1993). Consequently, analysis of the different structures and locations within the celery plant to characterize their furanocoumarin composition and concentration for determination of the safest portions for human consumption was the major goal of this study.

MATERIALS AND METHODS

The commercial celery variety Tall Utah 52-70R and the breeding line UC-08 were obtained from germplasm resources held at the University of California—Davis, Department of Vegetable Crops. UC-08 originated from UC1, a *Fusarium*-resistant line derived from celeriac (*A. graveolens* var. *rapaceum*) and Tall Utah 52-70R (Orton et al., 1984). These two test entries were selected because Tall Utah 52-70R forms the basis of all celery varieties in the market and UC-08 is a new variety developed for disease resistance.

The two celery genotypes were seeded on February 7, 1992, at Fuji Seed Co. in Ventura, CA, and transplanted into the field on August 5, 1992. All plants were transplanted in single rows of 8 m × 76.2 cm on sandy loam soil at the University of California's Agricultural Operations field in Riverside, CA, in randomized complete blocks, with four replicates. Plots were furrow-irrigated to maintain adequate soil moisture, and local standard cultural practices were followed.

Plant Sample Collection. Sample collections for determination of furanocoumarin composition and concentration were made using fresh, healthy (nondiseased) celery at the marketable growth stage. Samples were pooled within celery genotype in each replicate. A total of 10 whole plants (including the leaves, petioles, and roots) per celery genotype were collected and transported to the laboratory (two to three plants per replicate were used). For each plant, the outermost senescing leaves and associated petioles were discarded because these were no longer fresh, and the following seven parts were separated: roots (including the underground plant base), outer healthy looking (potentially marketable) petioles and leaves, mature inner leaves and petioles, and the immature heart leaves and petioles. For the purpose of this study, the "heart" included the hidden non-fully-expanded innermost leaves and associated petioles. All samples were immediately stored at −65 °C until time of chemical analysis.

Linear Furanocoumarin Analyses. All plants were analyzed for the three major linear furanocoumarins found in *Apium* spp.: psoralen, 5-methoxypsoralen (bergapten), and 8-methoxypsoralen (xanthotoxin) (Trumble et al., 1990; Diawara et al., 1992, 1993). Extraction of linear furanocoumarins was conducted as previously described (Diawara et al., 1992; Trumble et al., 1992). Briefly, sample tubes were spiked with 5 μg of a synthetic internal standard, 7-benzyloxycoumarin [synthesized from commercially available 7-hydroxycoumarin (Aldrich Chemical, Milwaukee, WI] (Trumble *et al.*, unpublished methods). Plant samples were homogenized in distilled H_2O, extracted with toluene, and the crude extract was partially purified by passage through an Extract Clean solid phase extraction cartridge tube (Alltech Associates, Inc., Deerfield, IL) and eluted with acetone in chloroform (95:5). The purified extracts were concentrated to dryness and then reconstitued in 250 μL of hexane. HPLC analyses were carried out with a Hewlett-Packard 1040 HPLC pump and an HP 1050A diode array detector with a Chemstation data system (Hewlett-Packard, Avondale, PA). Peaks were monitored and quantified at 280 nm. An Alltech Econosil silica column (25 cm × 4.6 mm, 5 μm particle size) with a 10 mm × 4.6 mm guard column filled with the same packing material was used, eluted isocratically with hexane:tetrahydrofuran (81:19). The tetrahydrofuran (HPLC grade) from Aldrich Chemical Co. gave markedly better resolution than THF from Fisher Scientific.

Statistical Analyses. All data were analyzed as a randomized complete block design using ANOVA (Super ANOVA, 1989); the plants were the blocks, and the parts were the treatments. Statistically different means were separated at the 5% significance level using the Tukey–Kramer test (Super ANOVA, 1989).

RESULTS

Composition and concentrations of linear furanocoumarins in the commercial celery 52-70R are reported in Table 1. Psoralen, bergapten, and xanthotoxin were all present in this celery genotype. For each chemical, leaves on the outer petioles (Figure 1) generally had significantly higher levels of individual linear furanocoumarins than any other portion of 52-70R. The total concentration of all three compounds per plant part showed the same trend; the outer plant leaves had the highest amount, followed by mature inner leaves. The total concentration of linear furanocoumarins in the