Seasonal Patterns and Pesticidal Effects on the Phototoxic Linear Furanocoumarins in Celery, *Apium graveolens* L.

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Seasonal trends and pesticidal effects on the phototoxic linear furanocoumarins in petioles and leaves of celery (*Apium graveolens* L.) were documented. Total linear furanocoumarins in petioles from untreated plants (range = 0.34–1.84 μg/g of fresh weight) did not reach levels known to cause contact dermatitis in either 1989 or 1990. In leaf samples, total linear furanocoumarin concentrations in untreated plants at harvest (1989, 2.95 μg/g; 1990, 5.90 μg/g) were low but exceeded levels known to produce dermatitis for at least 6 weeks in 1990 (maximum = 15.85 μg/g). Similar concentrations were recorded only once in 1989 (11.52 μg/g). Bergapten showed the highest seasonal and weekly concentrations in leaves and petioles during both years, followed by xanthotoxin and then psoralen. The concentration of bergapten in petioles declined significantly as plants matured. Concentrations of furanocoumarins in the leaves did not correlate with concentrations in the petioles in either year. In general, pesticides had relatively little effect on linear furanocoumarin induction.

INTRODUCTION

Over the past 15 years, concern about worker safety and consumer health has stimulated a variety of studies on the linear furanocoumarin composition and content of celery, *Apium graveolens* L. (Apiaceae). These compounds, which are produced by a wide variety of food plants in the families Apiaceae, Rutaceae, and Moraceae (Berenbaum, 1981; Zobel and Brown, 1990), are believed to cause mutagenesis, carcinogenesis (Koch, 1986; Roelandts, 1984; Young, 1990), and photodermatitis (Ljunggren, 1990). The mode of action for these effects appears primarily to be DNA binding or cross-linking and enhanced RNA degradation, all of which occur through a cycloaddition reaction with pyrimidine (Gasparro, 1988). However, binding to other cellular components including cytoplasm and fatty acids also may cause significant detrimental effects (Frederiksen et al., 1989; Specht et al., 1988).

Several studies have focused on the incidence of photodermatitis in grocery store workers following contact with celery. In a national survey of grocery store workers, Berkley et al. (1986) found that 26% of the respondents (59 of 224) met the case definition for contact photodermatitis. In a similar survey investigating an "outbreak" of contact dermatitis, 30 active cases of 126 workers were identified (Fleming, 1990). Although celery was implicated as the causal factor, concurrent exposure to other furanocoumarin-containing plants confounded the results.

Information on exposure of celery harvesters, consumers, and pest control advisors to linear furanocoumarins is quite limited. Although anecdotal reports of contact dermatitis in celery harvesters are common (Trumble, personal observation), documentation in the scientific literature is rare and often associated with increased levels of furanocoumarins occurring in celery infected with *Sclerotinia sclerotiorum* (Austad and Kavli, 1983; Karasawa et al., 1990). Austad and Kavli (1983) determined that an acute exposure to celery containing 18 μg/g of fresh weight of the linear furanocoumarins was enough to cause the contact dermatitis response. Little or no information is currently available on the levels of furanocoumarins occurring in heathly celery at the time of harvest. Surprisingly, no studies are available on the seasonal changes in furanocoumarin concentrations in celery. Thus, the potential risk for celery pest control advisors, which is associated with the plant contact necessary when sampling for insect and pathogen occurrence (Genung et al., 1979; Trumble and Nakakihara, 1983), has not been determined. Therefore, a primary objective of this study was to document seasonal variations in the phototoxic furanocoumarin concentrations in a commercial celery variety.

A variety of stress-inducing factors (UV light, cold temperatures, mechanical damage, etc.) have been shown to increase furanocoumarin concentrations in celery (Berenbaum, 1981; Beier and Oertli, 1983). Dercks et al. (1990) demonstrated that stress and nitrogen addition related to exposure to acidic fogs, which occur in the Los Angeles Basin, caused substantial increases in linear furanocoumarin contents of celery. Therefore, a second objective was to determine if some of the common pesticides applied in celery would induce or reduce furanocoumarin production. Some chemicals, notably the postplanting herbicide prometryn, cause an apparent stress in celery, resulting in yellowing of the foliage (Trumble, personal observation). The insecticide naled has been shown to cause physiological stress in other plant systems (Trumble et al., 1988). The other insecticides selected provide variable suppression of insect damage; insect damage has been shown to induce linear furanocoumarin production in another plant species in the Apiaceae (Zangerl, 1990).

MATERIALS AND METHODS

Horticultural Practices. Celery (Tall Utah 5270-R) was transplanted in a sandy loam soil on August 11, 1989, and August 15, 1990, at the University of California's South Coast Research and Extension Center in Santa Ana, CA. The plants were sprinkler irrigated for 3 weeks and drip irrigated (water pH 7.2–7.5) thereafter. Plant spacings and fertilization schedules were consistent with local practices. Experimental plots were four beds wide (two rows per bed on 1-m centers) by 15.25 m and separated by a 1.5-m buffer with four replicates of each treatment in a randomized complete block design. Pesticide treatments, suppliers, and rates applied are listed in Table I. All treatments except the herbicide prometryn received spreader-sticker and antifoam surfactant. Weekly application dates for insecticides in 1989 included September 22 and 28, October 5, 12, 19, and 26, and November 3, 10, and 16; prometryn was applied once on

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Table I. Pesticides, Rates, Classes, Formulations, and Manufacturers Examined for Impact on Linear Furanocoumarin Production in *A. graveolens*

chemical treatment	chemical class	application rate[a]	formulation type	manufacturer
untreated control				
prometryn	substituted triazine	3.74	emulsion (4E)	Ciba Geigy (Greensboro, NC)
B. thuringiensis	bacterial insect pathogen	1.12	wettable powder	Abbott Labs (Chicago, IL)
naled	organophosphate	1.12	emulsion	Valent (Walnut Creek, CA)
methomyl	carbamate	1.00	soluble liquid	Du Pont (Wilmington, DE)

[a] kg of ai/ha, except for prometryn, which is in L/ha.

September 1. Weekly application dates in 1990 included September 26, October 2, 12, 16, 23, and 30, and November 6 and 13; prometryn was applied once on September 3. All chemicals were applied by a tractor-mounted boom sprayer operated at 7.03 kg/cm^2. Four nozzles were used per bed, and carrier (H_2O) was at 934.6 L/ha. Disk-type cone nozzles incorporated D3 orifice disks, No. 23 or 25 cores, and 50-mesh screens.

Petioles were collected five and six times during the growing season in 1989 and 1990, respectively. Collections were made the week of the first chemical application and repeated every other week until harvest. In 1989, plant samples were taken on September 20 and 27, October 9 and 23, November 7 and 20. In 1990, plant samples were collected on September 17, October 1, 15, and 19, and November 15. On each date in 1989, tissue samples from each of four randomly selected plants in each replicate were collected and analyzed. On the basis of this experience, samples were taken and analyzed from only one plant per replicate in 1990. Field samples were standardized to the extent possible by collecting the largest petioles available on each sampling date. All samples were placed on ice for transport to the laboratory and subsequently stored at −65 °C until analysis.

Fully expanded (mature) leaf tissue associated with the aforementioned petioles provided a less complete data set. Due to a miscommunication, leaf samples collected in 1989 were analyzed for four and eight plants per treatment from two to four replicates per treatment. In 1990, only two to eight plants from two to four replicates per treatment were analyzed. On the last two sampling dates, only two plants from a single replicate were examined. Thus, an ANOVA of linear furanocoumarin contents of the leaf samples was not conducted, but means and standard errors were calculated.

Insect Damage and Population Assessment. The number of plants damaged by the most common lepidopterous insects *Spodoptera exigua* (Hübner) and *Agrotis ipsilon* (Rottemburg) (Griswold and Trumble, 1985) in 25 plants per replicate four replicates/treatment) were recorded at harvest. Populations of an important dipteran pest of celery, *Liriomyza trifolii* (Burgess) (California Celery Research Advisory Board, 1984), were monitored using four 14 × 28 cm styrofoam trays inserted between celery rows. Numbers of larvae and puparia in four trays per replicate were counted weekly until harvest from September 27 and October 1 in 1989 and 1990, respectively.

Procedures for Linear Furanocoumarin Analyses. A rapid method for analysis of linear furanocoumarins similar to that reported by Diawara et al. (1992) was utilized. Unless otherwise specified, all solvents used were of Fisher Scientific Optima grade. This internal standard was chosen to avoid any chance of the standard being present as a celery component. Several derivatives of commercially available 7-hydroxycoumarin were synthesized and evaluated, including the benzyl ether and the benzoate and *p*-nitrobenzoate esters. 7-Benzyloxycoumarin was determined to have the best overall combination of chemical stability, UV profile, and retention time, similar to those of the analytes of interest. The benzyl ether was synthesized (Greene and Wuts, 1991) by refluxing 7-hydroxycoumarin (1.62 g, 10 mmol, Aldrich Chemical Co.), Na_2CO_3 (5 g), and benzyl bromide (2 g, 11.7 mmol) in 100 mL of acetone for 60 h under N_2 in a fumehood. The mixture was then cooled and concentrated by rotary evaporation to dryness. The residue was taken up in water and extracted three times with chloroform. The combined chloroform extracts were backwashed with brine, dried over Na_2SO_4, and concentrated to dryness. The residue was recrystallized from hot ethanol. The resulting cream-colored solid (1.8 g, 71%) gave one peak on HPLC analysis: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.64 (d, 1 H, J = 9.7 Hz, H4), 7.35–7.5 (m, 6 H, H5, and 5 aromatic H of benzyl group), 7.02 (dd, 1 H, J = 8.4, 2.3 Hz, H6), 6.99 (d, 1 H, J = 2.3 Hz, H8), 6.26 (d, 1 H, J = 9.7 Hz, H2), 5.14 (s, 2 H, benzylic H); MS, m/z (relative abundance) 252 (9, M$^+$), 161 (1), 133 (1), 105 (2), 91 (100), 65 (12), 51 (7). Mass spectral data were recorded with a Hewlett-Packard (Avondale, PA) 5970 mass selective detector in electron impact mode (70 eV) interfaced to a Hewlett-Packard 5890 gas chromatograph. An Ultra-2 column (25 m × 0.2 mm, Hewlett-Packard) was used with He carrier gas, programming from 50 to 250 °C.

Samples of leaf (2 g) or petiole (4 g) tissue were spiked with 5.0 μg of ISTD and homogenized in heavy-walled round-bottom sample tubes (28 × 116 mm) in 10 mL of deionized H_2O for 2.5 min with a Polytron-R tissue homogenizer. Toluene (10 mL) was added to each tube, and the tubes were capped with aluminum foil held in place with a rubber band and vortexed for 1.5 min; the resulting thick emulsions were centrifuged for 40 min to separate the layers. The upper toluene layers were quantitatively transferred to clean 13 × 100 mm culture tubes and concentrated to dryness with a Jouan RC 10.10 centrifugal evaporator (Jouan Inc., Winchester, VA) at 60 °C with a vacuum of approximately 100 mmHg. A further 0.5 mL of toluene was added to each tube, and the contents were concentrated to dryness again to remove any traces of water by azeotropic distillation. The samples were reconstituted in 100 μL of toluene and loaded onto Extract Clean solid-phase extraction tubes (500 mg of silica, Alltech Associates, Inc., Deerfield, IL) which had been preconditioned with 4 × 2 mL toluene. The sample was rinsed twice onto the cartridge with 0.1 mL of toluene, discarding the eluate. The cartridge was then eluted by gravity flow with 5% acetone in chloroform (3 × 0.5 mL), discarding the first 0.5 mL of eluate. The eluate containing the furanocoumarin fraction was collected in 5-mL centrifuge tubes and concentrated to dryness as before. The residue was vortexed for 30 s in HPLC mobile phase (vide infra) and centrifuged briefly to pelletize any particulate matter; a 20-μL aliquot was analyzed by HPLC. If not used immediately, the dried-down tubes were capped and stored at −20 °C.

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 290 nm. This wavelength was chosen because all three compounds and the internal standard had strong and similar absorbances at this wavelength and interference from other compounds was minimized. Relative response factors at this wavelength were as follows: ISTD, 1.00; psoralen, 1.23; 5-methoxypsoralen, 1.51; 8-methoxypsoralen, 1.34.

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, mixed by HPLC pump). Tetrahydrofuran (HPLC grade) from Aldrich gave markedly better resolution than THF from Fisher Scientific. Relative retention times were as follows: ISTD, 1.00; psoralen, 1.15; 5-methoxypsoralen, 1.19; 8-methoxypsoralen, 1.38.

Extraction efficiencies were calculated as follows. A pooled sample of plant material was split into three portions. One portion was spiked with ISTD only (5 μg), and the second and third with 1- and 20-μg quanities of each of the linear furanocoumarins, respectively. These were replicated three times. All samples were run through the extraction and cleanup steps, reconstituted in acetonitrile, spiked with a second internal standard (the benzyl ester of 7-hydroxycoumarin, ISTD2), and quantitated vs ISTD2 by HPLC. The spiked samples were corrected for the amounts of linear furanocoumarins found in the unspiked controls. The extraction efficiencies were then calculated by comparison of these values with a calibration curve prepared from linear

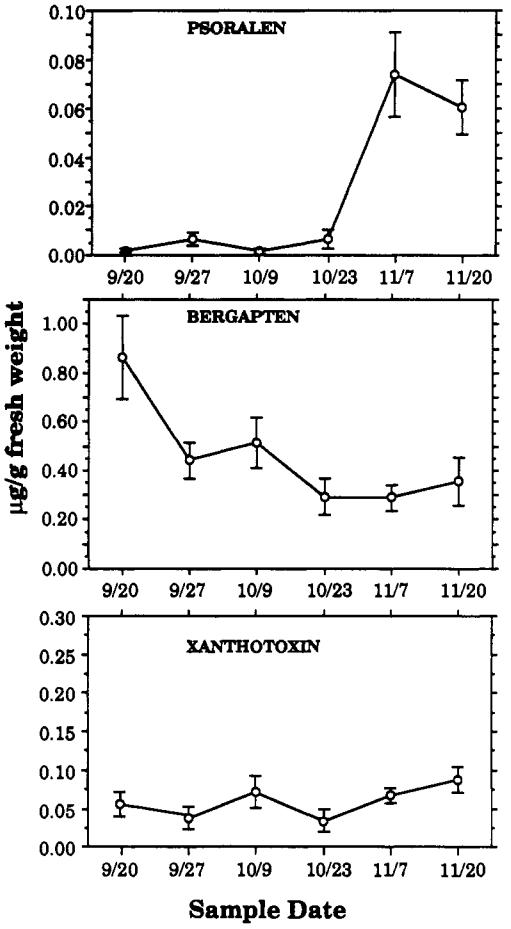

Figure 1. Seasonal variation during 1989 of the concentrations of the phototoxic linear furanocoumarins psoralen, bergapten, and xanthotoxin in petioles of *A. graveolens* not treated with pesticides. Each data point represents 16 plants. Bars delineate standard errors.

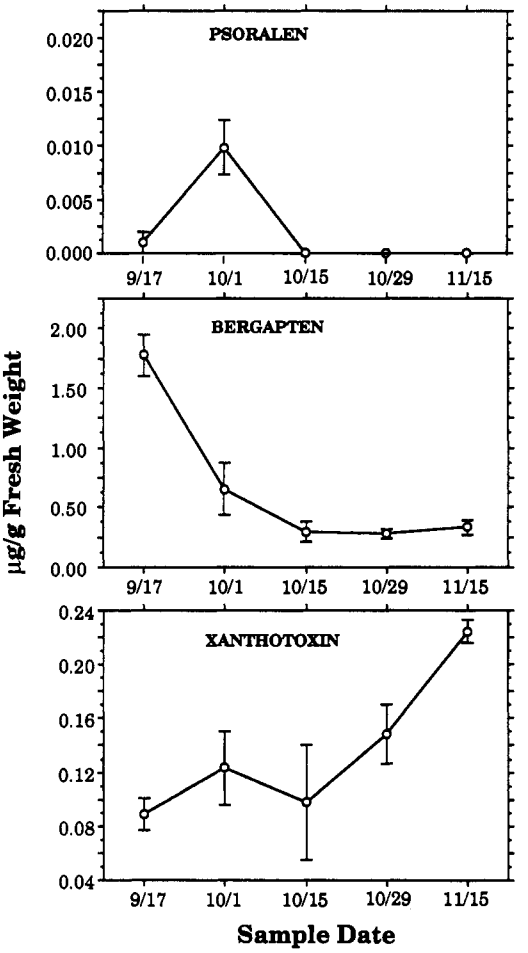

Figure 2. Seasonal variation during 1990 of the concentrations of the phototoxic linear furanocoumarins psoralen, bergapten, and xanthotoxin in petioles of *A. graveolens* not treated with pesticides. Each data point represents 8–16 plants. Bars delineate standard errors.

furanocoumarin standards (with ISTD2) dissolved in acetonitrile. Extraction efficiencies at the 1- and 20-µg levels were as follows: psoralen, 90.7 ± 5.7 and 95.6 ± 0.5%; 5-methoxypsoralen, 87.7 ± 5.4 and 96.9 ± 1.0%; 8-methoxypsoralen, 90.2 ± 6.6 and 95.2 ± 3.0%.

Calibration lines were generated using at least three concentrations of standards at concentrations spanning the range of interest. Calibration lines for the three compounds were linear, with r^2 values of 0.99 or better. Minimum detectability was conservatively estimated as 5 ng/g for each compound.

Statistical Analyses. The psoralen, bergapten, and xanthotoxin contents of petioles were compared using an ANOVA for randomized complete block design with repeated measures (samples were collected at 2-week intervals from the same blocks) (Steel and Torrie, 1980). Fisher's protected LSD (PLSD) test (Fisher, 1949) then was used to rank concentrations among weeks and treatments. For weeks showing significant treatment differences, a separate ANOVA was conducted to document within-week variation in treatments. A PLSD test was used to rank treatments. These analyses were conducted using Super-ANOVA software (Abacus Concepts, Inc., Berkeley, CA). Correlation analyses of concentrations of the linear furanocoumarins in leaves vs petioles were accomplished using StatWorks software (Cricket Software, Inc., Philadelphia, PA).

RESULTS AND DISCUSSION

Furanocoumarin Levels in Untreated Plants. Total concentrations of linear furanocoumarins in petioles from untreated plants did not reach levels believed to cause chronic (7 µg/g of fresh weight; Austad and Kavli, 1983) or acute (18 µg/g; Seligman et al., 1987) dermatitis in either 1989 (maximum = 1.05 µg/g) or 1990 (maximum = 1.86 µg/g) (Figures 1 and 2). Thus, either the celery petioles produced in the Los Angeles Basin of southern California were not exposed to furanocoumarin-inducing acidic fogs [see Dercks et al. (1990)] during the autumns of 1989 and 1990 or acidic fogs did not induce furanocoumarin production in field plantings.

In leaf samples, total linear furanocoumarin concentrations from untreated plants at harvest (1989, 2.95 µg/g; 1990, 5.90 µg/g) were higher than in petioles and exceeded 7 µg/g for a period of at least 6 weeks during the 1990 season (maximum = 15.85 µg/g) (Figures 3 and 4). The observation that higher quantities of the furanocoumarins occur in leaves than in petioles has been made by several researchers (Berkley et al., 1986; Trumble et al., 1990; Diawara et al., 1992). Concentrations above 7 µg/g were recorded during only one sampling period in 1989 (11.52 µg/g, 10 weeks prior to harvest). Thus, risk of contact dermatitis in celery harvesters due to furanocoumarin exposure would have been minimal. However, pest control advisors repeatedly sampling fields for disease occurrence or insect infestation should wear protective clothing.

Bergapten consistently showed the highest seasonal (1989, 0.46 µg/g; 1990, 0.56 µg/g) concentrations in leaves and petioles of untreated plants during both years, followed by xanthotoxin (1989, 0.08 µg/g; 1990, 0.13 µg/g) and then psoralen (1989, 0.03 µg/g; 1990, trace) (Table II). No other consistent trends across years were observed for either xanthotoxin or psoralen in leaves or petioles. The concentration of bergapten in petioles declined by 55–75% as plants matured in both years (Figures 1 and 2).

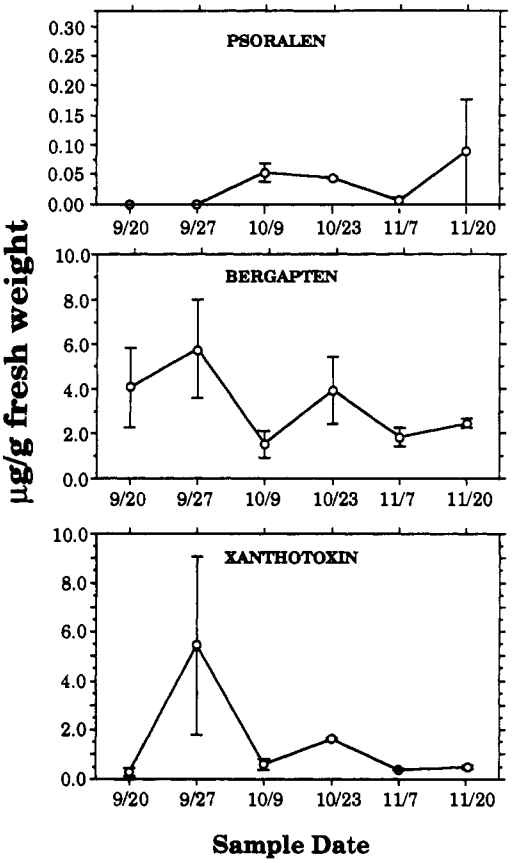

Figure 3. Seasonal variation during 1989 of the concentrations of the phototoxic linear furanocoumarins psoralen, bergapten, and xanthotoxin in leaves of *A. graveolens* not treated with pesticides. Each data point represents 2–8 plants. Bars delineate standard errors.

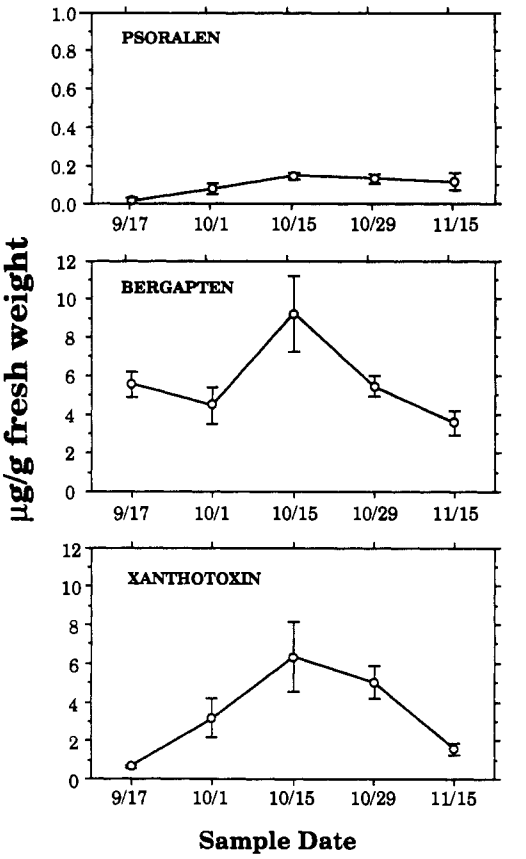

Figure 4. Seasonal variation during 1990 of the concentrations of the phototoxic linear furanocoumarins psoralen, bergapten, and xanthotoxin in leaves of *A. graveolens* not treated with pesticides. Each data point represents 2–8 plants. Bars delineate standard errors.

Concentrations in the leaves did not correlate with concentrations in the petioles in either year (1989, psoralen $P = 0.696$, bergapten $P = 0.661$, xanthotoxin $P = 0.099$; 1990, psoralen $P = 0.736$, bergapten $P = 0.491$, xanthotoxin $P = 0.843$). Therefore, both plant parts may need to be sampled to determine potential problems for harvesters or pest control advisors exposed to both leaves and petioles as compared to consumers, who primarily have contact with the petioles.

Furanocoumarin Levels in Pesticide-Treated Plants. In 1990, a single application of the herbicide prometryn and multiple applications of the insecticides *Bacillus thuringiensis*, naled, and methomyl did not significantly affect seasonal concentrations of the phototoxic linear furanocoumarins in petioles (psoralen $P = 0.507$, $F = 0.891$; bergapten $P = 0.534$, $F = 0.845$; xanthotoxin $P = 0.712$, $F = 0.527$; df = 4, 9). In 1989, significant seasonal differences were observed in furanocoumarin concentrations among pesticide treatments (psoralen $P = 0.011$, $F_{4,12} = 5.31$; bergapten $P = 0.021$, $F_{4,12} = 4.33$; xanthotoxin $P = 0.25$, $F = 1.544$). In general, methomyl treatments had the lowest quantity of furanocoumarins and the untreated plots showed the highest, or among the highest, concentrations.

The differences in furanocoumarin contents between untreated and methomyl-treated plants in 1989 might have been caused by insect-induced furanocoumarin production such as that noted for wild parsnip by Zangerl (1990). However, our data cannot prove or disprove this hypothesis for several reasons: (1) In 1990, no treatment differences in furanocoumarin concentrations were observed despite higher levels of lepidopteran damage at harvest (control, 26%; methomyl, 4%) than occurred in 1989 (control, 11%; methomyl, 0%). (2) Differences in leafminer (*L. trifolii*) populations were much greater in 1990 (maximum weekly mean, control, 1/tray, methomyl, 128/tray) than in 1989 (maximum weekly mean, control, 1/tray, methomyl, 4/tray). (3) The potential was high for selecting various combinations of damaged and undamaged plants in the random collection of tissue samples from only one to four plants per replicate, which would obscure insect effects. Thus, determination of insect effects on furanocoumarin production would best be documented on a plant by plant basis, where insect damage or populations could be monitored.

Some "week" main effects were significant. Bergapten was the only furanocoumarin to show a clear trend in petioles during both years, with a rapid decline followed by maintenance of a low but relatively stable concentration in the 6–8 weeks prior to harvest (1989, $P < 0.01$, $F_{5,15} = 5.604$; 1990, $P < 0.01$, $F_{4,9} = 14.545$). Psoralen concentrations in 1989 fluctuated significantly by week ($P < 0.01$, $F_{5,15} = 7.096$) but did not show a similar trend in 1990, when the week effect was not significant ($P = 0.263$). Xanthotoxin concentrations across time were not significantly different in either year (1989, $P = 0.178$; 1990, $P = 0.522$).

Treatment by week interactions were significant in 1989 for psoralen ($P < 0.01$, $F_{20,60} = 2.525$), bergapten ($P < 0.01$, $F_{20,60} = 6.786$), and xanthotoxin ($P < 0.01$, $F_{20,60} = 2.734$) in petioles. In 1990, treatment by week interactions were significant only for bergapten ($P < 0.01$, $F_{19,48} = 379.46$; psoralen $P = 0.434$; xanthotoxin $P = 0.485$). However, no clear trends were discernible in either year. The relatively low concentrations of these compounds, coupled with the

Table II. Effect of Pesticide Application on Phototoxic Furanocoumarins in Petioles of Field-Grown *A. graveolens*

chemical treatment	year	mean seasonal linear furanocoumarin concn[a]			
		psoralen	bergapten	xanthotoxin	total
untreated control	1989	0.03 c	0.46 bc	0.08 a	0.56
prometryn		0.03 ab	0.45 bc	0.08 a	0.54
B. thuringiensis		0.03 a	0.48 c	0.05 a	0.53
naled		0.02 bc	0.34 ab	0.07 a	0.43
methomyl		0.01 a	0.26 a	0.05 a	0.32
untreated control	1990	–[b]	0.56 a	0.13 a	0.70
prometryn		–	0.54 a	0.12 a	0.66
B. thuringiensis		–	0.78 a	0.13 a	0.91
naled		–	0.66 a	0.15 a	0.81
methomyl		–	0.77 a	0.13 a	0.89

[a] Values in μg/g of fresh weight; means followed by the same letter do not differ significantly at the $P < 0.05$ level; Fishers protected LSD test. [b] Values were below 0.01 μg/g.

inconsistent patterns within and between years, did not support any firm conclusions from these interaction analyses.

The relatively high levels of furanocoumarins found in celery 6–10 weeks prior to harvest may put pest control advisors at substantial risk from contact photodermatitis. Such work requires cutting, collecting, and handling of foliage and extensive contact with cut surfaces of the plant. To our knowledge, individuals employed for this purpose have not been surveyed for a history of photosensitive reactions. In addition, because concentrations of bergapten decline significantly with increasing plant maturity, researchers investigating furanocoumarin contents of new accessions should specify plant age to allow appropriate and comparative interpretations. Finally, although the celery produced during this study did not contain hazardous levels of furanocoumarins at harvest, postharvest practices can dramatically increase furanocoumarin concentrations (Chaudhary et al., 1985). Therefore, an analysis resulting in a putatively acceptable furanocoumarin content at harvest does not ensure a risk-free commodity in grocery stores.

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