Furanocoumarins in Florida Celery Varieties Increased by Fungicide Treatment†

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Florida celery cultivars Florida 2-14, Florimart, M9, Florida 296, M68, and Junebelle contained total psoralen + bergapten + xanthotoxin + isopimpinellin ranging from about 12 to 50 μg/g. Treatment of the commercial Florida cultivar 2-14 with Bravo 500, Manzate-D, or Kocide 101 fungicides increased bergapten 2−4 times in leaves and stalk, xanthotoxin 2−3 times in stalk, and isopimpinellin about 2−3 times in leaves but did not increase psoralen levels. These increases represent increased calculated biological activity toward human skin. This is the first report of fungicide induction of furanocoumarins in celery.

Keywords: *Apium graveolens; plant breeding; induction; fungicide; allelochemical; phototoxin; dermatitis; maneb; chlorothalonil; cupric hydroxide; phytoalexin; food safety*

INTRODUCTION

Linear furanocoumarins (LFCs) were identified in the late 1940s as the cause of the photosensitization properties of the plants that contain them (Fahmy et al., 1947; Fahmy and Abu-Shady, 1948). LFCs are found in a wide variety of plants in the Apiaceae, Rutaeae, and Moraceae (Gray and Waterman, 1978; Berenbaum, 1981; Murray et al., 1982; Matern et al., 1988; Zobel and Brown, 1990). The biological importance and activities of these compounds have been reviewed (Beier and Nigg, 1992). Briefly, LFCs are known to intercalate into DNA and form covalent bonds in the presence of UVA light (320−400 nm). Psoralens have been used to determine the structure and function of DNAs and RNAs because of their reactivity with these compounds (Cimino et al., 1985). LFCs have been used clinically to treat skin disorders such as vitiligo and prurigo nodularis and have been proposed as a treatment for jet lag. About 40 mg of psoralen administered orally, combined with UVA light, is referred to as PUVA therapy. PUVA therapy has caused papillomas, keratoanthomas, and squamous cell carcinomas in mice and has been linked to genital cancer in men (Beier and Nigg, 1992).

Austad and Kavli (1983) showed that 12.5 μg/g of bergapten plus xanthotoxin in infected celery caused phototoxic dermatitis in celery harvesters. Phytophotodermatitis in grocery store workers was linked to 9.8 μg/g of psoralen + bergapten + xanthotoxin in a disease resistant celery variety (Berkley et al., 1986). A level of 8 μg/g of the same compounds in the same disease-resistant variety was linked to celery handler photodermatitis (Seligman et al., 1987). Psoralen, bergapten, xanthotoxin, and isopimpinellin (Figure 1) have been quantified in celery cultivars that are both resistant and susceptible to the celery leafminer, *Liriomyza trifolii* (Burgess) (Trumble et al., 1990), although isopimpinellin is not photoactive in cell culture (Ashwood-Smith et al., 1983, 1992; Hudson et al., 1987) nor in a chick skin bioassay (Ivie and Beier, 1996).

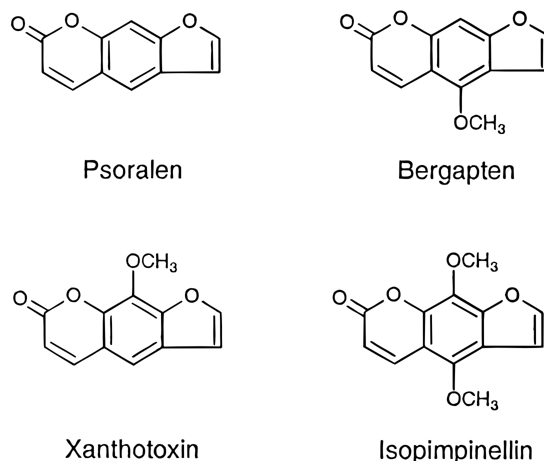

Figure 1. Structural formulas of psoralen, bergapten, xanthotoxin, and isopimpinellin.

Atmospheric pollution has increased furanocoumarin content of celery up to 540% of normal levels (Dercks et al., 1990), and infection of celery roots and stems by the fungus, *Fusarium oxysporum* f. sp. *apii*, has been shown to increase the LFC content of celery (Heath-Pagliuso et al., 1992). Seasonal effects on furanocoumarin content of celery have also been reported (Trumble et al., 1992). Generally, pesticides have had little effect on raising furanocoumarin levels in celery (Trumble et al., 1992).

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The LFC content of celery was quantified in three breeding lines (Diawara et al., 1993a) and was investigated as a potential integrated pest management tool for beet armyworm (*Spodotera exigua* Hübner) (Diawara et al., 1993b).

It is well-known that celery handlers and field workers are frequently affected with photosensitization of the fingers, hands, and forearms (Birmingham et al., 1961; Legrain et al., 1926). These skin disorders were correlated with handling celery infected with the "pink-rot" disease organism (*Sclerotinia sclerotiorum*) (Henry et al., 1933; Austad and Kavli, 1983), are referred to as celery dermatitis, celery itch, or celery blisters, and are known to be caused by linear furanocoumarins in diseased celery (Scheel et al., 1963). There are a number of reports of the effects of linear furanocoumarins in diseased celery on celery workers and handlers (Henry et al., 1933; Birmingham et al., 1961; Marasas and van Rensburg, 1979). Prior to 1981, compounds in diseased celery that caused skin disorders were thought to be mycotoxins (Richards, 1972; Busby and Wogan, 1981). It was then shown that psoralen and other linear furanocoumarins were phytoalexins in celery (Beier and Oertli, 1983). Linear furanocoumarins also have been shown to be present in healthy celery (Beier et al., 1983). Studies were conducted by the CDC in 1984 (Berkley et al., 1986) and by NIOSH in 1986 (Seligman et al., 1987) concerning cases of photophytodermatitis of grocery store workers. The results showed that a new celery cultivar that had approximately 10 times the level of linear furanocoumarins than other commercial cultivars on the market was responsible for these phototoxic reactions in grocery store workers. It has been known for many years that celery field workers and handlers commonly contracted photophytodermatitis; the findings by the CDC and NIOSH were the first to document cases of photophytodermatitis in grocery store workers as a result of celery exposure.

The purposes of this investigation were to quantify the linear furanocoumarins that may be present in Florida commercial and breeding celery cultivars at harvest, and to investigate potential increases of celery LFC content induced by fungicide treatments commonly used in celery production.

MATERIALS AND METHODS

Linear furanocoumarins (Figure 1) used as standards in this study were obtained as follows: psoralen (purity 100% by our HPLC, Interchem Corp., Paramus, NJ), xanthotoxin (listed purity 99%, Aldrich Chemical Co., Inc., Milwaukee, WI), bergapten (listed purity 99%, Aldrich Chemical Co., Inc., Milwaukee, WI), and isopimpinellin (100% by company HPLC, Indofine Chemical Co., Somerville, NJ). The fungicides used were fixed cupric hydroxide ($Cu(OH)_2$) (Kocide 101, Kocide Chemical Corp., Houston, TX), tetrachloroisophthalonitrile (chlorothalonil) (Bravo 500, ISK Biotech Corp., Painesville, OH), and manganese ethylenebisdithiocarbamate (Manzate D) (Dupont, Wilmington, DE). All chromatography solvents were from Omni-Solv, EM Science, Cherry Hill, NJ, or HPLC grade (Fisher Scientific, Fairlawn, NJ).

Cultivar Field Experiment. Celery seedlings were grown from seed in a peat-vermiculite based growth medium (Terra-Lite vegetable plug mix, W. R. Grace Co., Cambridge, MA) in a greenhouse at Sanford, FL. Seedlings were transferred at 3 weeks into styrofoam flats with 4 cm^2 cells containing the same growth medium and watered as needed. A dilute Hoagland's solution (Tuite, 1969) was applied twice weekly. When approximately 10 cm tall, plants were transplanted into experimental breeding plots, 16 plants per plot, and 4 plots per cultivar. Our field breeding plots of celery varieties were located on organic soil within 200 m of commercial celery operations at the same location. The commercial celery was harvested one week before our field plots were sampled. The cultivars Florida 2-14, M9, M68, Junebelle, Florida 296, and Florimart were used. Four plants from each field plot were harvested 80 days after transplanting by cutting at the base, transported on ice, held at 4 °C, and processed at random over 3 days.

Analyses of plant extracts were essentially by the method of Beier (1985). Plants from cultivar field plots were processed by removing the upper leaves, similar to kitchen preparation, mincing them on a cutting board, and weighing out 2 g subsamples. These samples contained the stalk and leaves on the smaller petioles. Four subsamples per sample were extracted immediately and analyzed. Each celery subsample (2 g) was placed in a 50 mL screw-capped glass tube and glass distilled, and deionized water (15 mL) and ethyl ether (15 mL) were added to the tube. The glass sample tube was immersed in an ice-water bath, and the sample was macerated for 1 min with a SDT-1810 Tissumizer (Tekmar, Cincinnati, OH). The resulting emulsion was centrifuged for 5 min at 2000*g*. The ether layer was removed and placed in a 250 mL round-bottomed flask. A similar extraction procedure was repeated twice more with 10 mL of ether. The ether fractions were consolidated in the round-bottomed flask and the combined extractions were taken to dryness at 40 °C on a vacuum rotary evaporator. At dryness, the residue was removed from the round-bottomed flask with two, 2 mL aliquots of 60% acetonitrile in water, transferred to a 5 mL Leuerlock glass syringe, and passed through a C_{18} Sep-Pak cartridge (Waters Associates, Inc., Milford, MA), which had been pre-eluted with MeOH (15 mL) followed by H_2O (15 mL), and collected in a 125 mL round-bottomed flask. The C_{18} Sep-Pak was then further eluted with 8 mL of 60% acetonitrile in water; the eluate was collected into the same 125 mL flask. Methanol (15 mL) was added to the 125 mL flask, and the residue was taken to dryness at 40 °C on a vacuum rotary evaporator. The residue in the flask was transferred by rinsing with three 1 mL aliquots of chloroform, consolidated in a 5 mL Leuerlock glass syringe, and passed through a pre-eluted (15 mL of chloroform) silica Sep-Pak into a 125 mL round-bottomed flask. The silica Sep-Pak was further eluted with 8 mL of 7.5% ethyl acetate in chloroform into the same flask. These combined fractions were taken to dryness as described above. The residue was transferred to a glass scintillation vial with three 3 mL rinses of chloroform and taken to dryness on a nitrogen evaporator at 40 °C. HPLC analyses were performed on the same day as extractions. Just before HPLC analysis, methanol (10 mL) was added to the vial, and the vial was shaken by hand for 1 min to dissolve the sample.

An Adsorbosphere phenyl 5 μm, 150 mm × 4.6 mm i.d. column was used (Alltech Assoc., Inc., Deerfield, IL) with a Waters CN guard column for HPLC. The mobile phase was 25/75, acetonitrile/water at 1 mL/min with detection at 225 nm with a Waters model 490E variable wavelength detector. Injections (15 μL) were made with a Waters Wisp autoinjector in line with a Waters dual pump and Waters 600E controller. A mixture of psoralen, xanthotoxin, bergapten, and isopimpinellin standards at five concentrations was injected after every fourth sample to produce a five-point standard curve for each day of analysis.

The recovery efficiency of LFCs in the assayed samples was assessed by analyzing a fourth ether extraction of four subsamples of the Junebelle cultivar. Fourth extractions contained no detectable LFCs. The residual celery tissue was then fortified with 300 ng of psoralen, xanthotoxin, bergapten, and isopimpinellin (0.15 ppm) and re-extracted with the procedure described above ($n = 4$ per concentration). Recoveries were 85.4% ± 0.4% (psoralen), 86.7% ± 1.1% (xanthotoxin), 94.9% ± 1.8% (bergapten), and 88.0% ± 2% (isopimpinellin). Quantities below 0.1 ppm are reported as trace.

ANOVA (SAS Inst., Inc., 1990) and Tukey's Honestly Significant Difference (Hochberg and Tamhane, 1987) tests were used to examine statistical differences between mean compound levels for the *cultivar* field data (Table 1). Repli-

Table 1. Cultivar Field Experiment: Furanocoumarins in Florida Celery Cultivar Kitchen Preparations (μg/g)[a]

cultivar	psoralen	bergapten	xanthotoxin	isopimpinellin	TFC[b]
1. Florida 296	4.18 ± 2.93[a]	28.51 ± 9.03[a]	16.86 ± 8.27[a]	14.96 ± 4.19[a]	49.55 ± 9.35[a]
2. Florimart	1.25 ± 0.26[b]	7.65 ± 2.04[b]	8.74 ± 1.77[a,b]	6.88 ± 1.28[b]	17.64 ± 3.30[b]
3. M9(1)	0.73 ± 0.42[b]	5.57 ± 1.03[b]	9.72 ± 2.26[a]	5.85 ± 1.10[b]	16.02 ± 3.67[b]
4. M9(2)	0.01 ± 0.00[c]	8.74 ± 1.48[b]	6.42 ± 2.94[b]	3.43 ± 0.54[b]	15.17 ± 3.69[b]
5. Florida 2-14	0.72 ± 0.35[2b]	9.31 ± 2.55[b]	4.49 ± 1.54[b]	4.80 ± 1.41[b]	14.52 ± 3.52[b]
6. M68	0.11 ± 0.08[b]	7.92 ± 0.93[b]	5.08 ± 3.00[b]	5.38 ± 1.11[b]	13.11 ± 3.23[b]
7. Junebelle	0.01 ± 0.00[c]	9.94 ± 2.91[b]	1.71 ± 0.64[b]	3.17 ± 0.85[b]	11.66 ± 4.34[b]

[a] Mean ± SD, $n = 4$; means followed by the same roman letter are not different at $\alpha = 0.05$ by Tukey's HSD test. [b] TFC = sum of psoralen, bergapten, and xanthotoxin.

cates through subsamples within a variety were compared for subsample differences.

Cultivar Florida 2-14 Fungicide Experiment. Seedlings approximately 10 cm tall were transplanted into 5 × 8 × 0.25 m concrete soil tanks containing the same organic soil (Lauderhill muck, pH 5.8) as the field plots. Plants received no supplemental fertilizer thereafter. After 2 months of growth when plants were approximately two-thirds mature and petioles had developed that appeared to be marketable (which are criteria to begin fungicide application in commercial fields), fungicides were applied four times to each plot at 7-day intervals. Treatments were applied with a compressed CO_2 powered sprayer to plants in an 8-m row for each plot. The sprayer was calibrated at the beginning and end of the treatments by timing deposition into a 1000 mL graduated cylinder. Treatment 1 was chlorothalonil (Bravo 500) at a rate equivalent to 1.2 kg/ha of formulation. Treatment 2 was manganese ethylenebisdithiocarbamate (Manzate D) at a rate of 1.68 kg/ha formulation. Treatment 3 was Kocide 101 (a cupric hydroxide-based fungicide containing 50% Cu^{2+}) at a rate of 3.36 kg/ha, and treatment 4 was a control that received 467 L/ha of water. These treatments were made according to label instructions for the rate and gallons of water per unit area.

Celery plants were sampled 2 h after the last fungicide application and 2, 4, 6, 8, and 10 days thereafter. On each sampling day, one plant was selected at random from each treatment plot. Since the plants were large (40−48 cm tall and 2−3 kg in weight), each plant was vertically divided into quarters with two radial cuts. One quarter of each plant was randomly selected as the sample. For each sample, leaves and petioles were separated and finely and separately chopped in a food processor. The chopped tissue was slowly stirred while small aliquots (approximately 50 mg) were removed and bulked until separate leaf and petiole 5.0 g samples had been obtained. Samples were immediately frozen and kept frozen at −20 °C until extraction and analysis.

Methods of sample extraction, cleanup, and HPLC for the fungicide experiment, were similar to those of Beier et al. (1983) with one modification; prior to separation and quantification by HPLC, the final leaf sample residues were dissolved in 900 μL of chloroform. Cultivar FL2-14 samples fortified with standards at 0.3 ppm had recoveries in all cases equal to or greater than 88%.

For the *fungicide* experiment, an ANOVA was used to examine treatment, day, and treatment by day interactions, followed by comparison of least square means using the t-test procedures for the General Linear Model (SAS Inst. Inc., 1990). For stalks, isopimpinellin was not analyzed due to discarded isopimpinellin control samples. Linear regression was also used to examine differences in treatment and control slopes (SAS Inst. Inc., 1990). For total furanocoumarin content (TFC) in the fungicide experiment, a one-way ANOVA for untransformed TFC was run for leaves and for stalks as a function of treatment. Variation and outliers were examined with the Univariate Procedure (SAS Inst. Inc., 1990). Finally, the change in biological activity toward human skin was estimated. The individual values were divided by 5; multiplied by 100 (psoralen), 37.5 (xanthotoxin), and 27.5 (bergapten); and totaled (Musajo and Rodighiero, 1962; Musajo et al., 1974).

RESULTS

Cultivar Field Experiment. The statistical analyses comparing replicates within a variety through analyses of subsamples showed no difference for any of the compounds examined. Means were calculated from the subsamples and were used for statistical analyses. Variety Florida 296 was higher than other varieties for psoralen, bergapten, isopimpinellin, and total furanocoumarin content, but not for xanthotoxin in Florimart and M9(1) (Table 1). Varieties M9(2) and Junebelle had very low psoralen content. The psoralen content of the remaining varieties did not differ. Xanthotoxin, bergapten, and isopimpinellin contents were not different among the other varieties (excluding Florida 296) except that variety M9(1) was higher than Junebelle, M68, M9-(2), and FL2-14 for xanthotoxin. Florida 296 contained higher total furanocoumarins than the other varieties which were not different from one another (Table 1).

Fungicide Experiment (Cultivar Florida 2-14). There were statistically significant differences between treatments, but only a few differences between days. There was no significant time−treatment interaction at the 0.05 level. That is, the difference between any two treatments was consistent from day to day even though daily means may be different within each treatment. The relationship between treatments was the same using linear regression analysis (data not reported). Treatments exhibited more variation in individual furanocoumarin content than controls with the Manzate-D treatment being the most variable.

For psoralen, there was no effect of treatment for leaves and stalks (Tables 2 and 6). Bravo 500, Manzate-D, and Kocide 101 increased the bergapten level in leaves and stalk about 2−3× overall (Tables 3 and 6), but Bravo 500 treatment resulted in a higher overall average of bergapten than Manzate-D and Kocide 101 which did not differ from one another. Xanthotoxin was increased 2−3× in stalks by each of the three compounds, but not in leaves, and was decreased by Manzate-D in the leaf (Table 4). Isopimpinellin levels in leaves were increased 2× by Bravo 500 (Table 5).

Because isopimpinellin is not biologically active on human skin, Table 6 presents biologically active total furanocoumarin content (TFC) for leaf and stalk for psoralen, bergapten, and xanthotoxin. There were no significant day or treatment by day effects for either leaf or stalk. Stalk TFC was increased about 2× in the Bravo 500 treatment. Manzate-D and Kocide 101 stalk TFC were not different from the control (Table 6). For leaves, the Bravo 500 treatment showed more TFC than the Manzate-D and control treatments, but the Manzate-D and Kocide 101 treatments were not different than the control. These changes resulted in increased potential biological activity toward human skin for the Bravo 500 treatment overall for stalk and leaf (Table 7).

Table 2. Fungicide Experiment: Psoralen in Florida Cultivar 2-14 over 10 Days (μg/g)[a]

organ	trt/day	2 h	2 days	4 days	6 days	8 days	10 days	overall mean ± SEM
leaf	Bravo 500	5.46 ± 0.59[a]	6.28 ± 0.52[a]	5.89 ± 0.92[a]	4.32 ± 0.34[a]	7.36 ± 1.56[a]	5.60 ± 0.78[a]	5.63 ± 0.27[a]
leaf	Manzate-D	3.39 ± 0.99[a]	5.11 ± 1.65[a]	4.29 ± 1.41[a]	4.56 ± 1.97[a]	5.22 ± 1.64[a]	6.23 ± 1.54[a]	4.76 ± 1.31[a]
leaf	Kocide 101	5.44 ± 0.67[a]	5.64 ± 0.20[a]	5.71 ± 0.24[a]	3.49 ± 0.34[a]	5.87 ± 0.38[a]	8.11 ± 0.89[a]	5.73 ± 0.23[a]
leaf	control	4.58 ± 1.50[a]	5.29 ± 0.45[a]	6.06 ± 0.82[a]	5.51 ± 0.95[a]	5.43 ± 1.09[a]	4.81 ± 0.48[a]	5.36 ± 0.73[a]
stalk	Bravo 500	0.11 ± 0.01[a]	0.09 ± 0.02[a]	0.12 ± 0.01[a]	0.11 ± 0.00[a]	0.21 ± 0.04[a]	0.10 ± 0.01[a]	0.12 ± 0.01[a]
stalk	Manzate-D	0.12 ± 0.03[a]	0.10 ± 0.01[a]	0.09 ± 0.01[a]	0.15 ± 0.04[a]	0.14 ± 0.04[ab]	0.14 ± 0.02[a]	0.12 ± 0.02[a]
stalk	Kocide 101	0.11 ± 0.01[a]	0.12 ± 0.01[a]	0.09 ± 0.01[a]	0.11 ± 0.02[a]	0.11 ± 0.01[b]	0.19 ± 0.07[a]	0.12 ± 0.02[a]
stalk	control	0.11 ± 0.01[a]	0.10 ± 0.00[a]	0.09 ± 0.01[a]	0.19 ± 0.08[a]	0.10 ± 0.01[b]	0.14 ± 0.01[a]	0.12 ± 0.01[a]

[a] Mean ± SEM, means within an organ for a given day followed by the same roman letter are not different at $\alpha = 0.05$ by Fisher's protected least significant difference.

Table 3. Fungicide Experiment: Bergapten in Florida Cultivar 2-14 over 10 Days (μg/g)

organ	trt/day	2 h	2 days	4 days	6 days	8 days	10 days	overall mean ± SEM
leaf	Bravo 500	3.70 ± 0.53[a]	5.48 ± 1.51[a]	3.89 ± 1.19[a]	3.28 ± 0.63[a]	5.45 ± 1.57[a]	1.42 ± 0.22[a]	3.66 ± 0.57[a]
leaf	Manzate-D	1.13 ± 0.17[c]	3.58 ± 1.75[ab]	2.63 ± 1.05[ab]	2.75 ± 1.45[ab]	3.52 ± 1.28[a]	1.60 ± 0.39[a]	2.52 ± 0.93[b]
leaf	Kocide 101	2.35 ± 0.20[b]	2.47 ± 0.20[ab]	2.41 ± 0.45[ab]	0.70 ± 0.24[b]	4.12 ± 0.84[a]	1.69 ± 0.06[a]	2.28 ± 0.07[b]
leaf	control	2.75 ± 0.45[b]	1.45 ± 0.24[b]	1.02 ± 0.23[b]	0.81 ± 0.36[b]	0.77 ± 0.17[b]	2.16 ± 0.61[a]	1.46 ± 0.02[c]
stalk	Bravo 500	0.28 ± 0.07[a]	0.39 ± 0.19[a]	0.36 ± 0.03[a]	0.33 ± 0.11[a]	0.47 ± 0.05[a]	0.37 ± 0.09[a]	0.36 ± 0.06[a]
stalk	Manzate-D	0.20 ± 0.08[a]	0.31 ± 0.13[ab]	0.18 ± 0.04[b]	0.40 ± 0.19[a]	0.28 ± 0.11[b]	0.12 ± 0.04[b]	0.24 ± 0.08[b]
stalk	Kocide 101	0.14 ± 0.01[a]	0.31 ± 0.07[ab]	0.14 ± 0.02[b]	0.20 ± 0.07[a]	0.23 ± 0.08[bc]	0.18 ± 0.11[b]	0.19 ± 0.04[b]
stalk	control	0.11 ± 0.04[a]	0.06 ± 0.00[b]	0.09 ± 0.01[c]	0.19 ± 0.08[a]	0.11 ± 0.01[c]	0.08 ± 0.02[b]	0.10 ± 0.01[c]

[a] Mean ± SEM, means within an organ for a given day followed by the same roman letter are not different at $\alpha = 0.05$ by Fisher's protected least significant difference.

Table 4. Fungicide Experiment: Xanthotoxin in Florida Cultivar 2-14 over 10 Days (μg/g)[a]

organ	trt/day	2 h	2 days	4 days	6 days	8 days	10 days	overall mean ± SEM
leaf	Bravo 500	5.44 ± 0.02[a]	6.17 ± 0.94[a]	5.70 ± 0.88[a]	5.03 ± 0.40[a]	8.26 ± 1.78[a]	5.02 ± 0.96[ab]	5.77 ± 0.30[a]
leaf	Manzate-D	2.10 ± 0.74[b]	4.11 ± 2.00[a]	2.17 ± 1.55[b]	3.96 ± 2.27[a]	3.14 ± 1.53[b]	2.95 ± 1.13[b]	3.01 ± 1.28[b]
leaf	Kocide 101	4.89 ± 0.75[a]	5.15 ± 0.39[a]	5.03 ± 0.21[a]	2.43 ± 0.28[a]	7.05 ± 0.56[a]	6.27 ± 1.07[a]	5.15 ± 0.25[a]
leaf	control	6.23 ± 0.30[a]	4.40 ± 0.70[a]	4.64 ± 0.83[a]	3.69 ± 1.00[a]	4.81 ± 0.85[ab]	6.56 ± 0.67[a]	5.01 ± 0.38[a]
stalk	Bravo 500	0.35 ± 0.09[a]	0.41 ± 0.18[a]	0.49 ± 0.04[a]	0.44 ± 0.09[a]	0.38 ± 0.04[a]	0.26 ± 0.02[a]	0.39 ± 0.03[a]
stalk	Manzate-D	0.23 ± 0.07[ab]	0.30 ± 0.14[a]	0.18 ± 0.07[b]	0.33 ± 0.17[a]	0.22 ± 0.10[a]	0.36 ± 0.23[a]	0.26 ± 0.11[b]
stalk	Kocide 101	0.24 ± 0.04[ab]	0.44 ± 0.05[a]	0.25 ± 0.04[b]	0.25 ± 0.07[a]	0.29 ± 0.09[a]	0.39 ± 0.24[a]	0.31 ± 0.04[ab]
stalk	control	0.09 ± 0.03[b]	0.12 ± 0.02[a]	0.19 ± 0.06[b]	0.41 ± 0.18[a]	0.21 ± 0.05[a]	0.19 ± 0.04[a]	0.22 ± 0.05[b]

[a] Mean ± SEM, means within an organ for a given day followed by the same roman letter are not different at $\alpha = 0.05$ by Fisher's protected least significant difference.

Table 5. Fungicide Experiment: Isopimpinellin in Florida Cultivar 2-14 over 10 Days (μg/g)[a]

organ	trt/day	2 h	2 days	4 days	6 days	8 days	10 days	overall mean ± SEM
leaf	Bravo 500	4.28 ± 0.52[a]	4.69 ± 1.13[a]	6.02 ± 0.56[a]	3.98 ± 0.21[a]	8.30 ± 2.78[a]	5.40 ± 0.98[a]	5.28 ± 0.55[a]
leaf	Manzate-D	2.80 ± 0.60[a]	3.35 ± 0.82[a]	3.67 ± 0.72[b]	3.30 ± 0.92[a]	3.42 ± 1.37[a]	6.69 ± 0.60[a]	3.88 ± 0.46[b]
leaf	Kocide 101	2.92 ± 1.22[a]	3.11 ± 0.83[a]	2.55 ± 0.38[b]	2.14 ± 0.80[a]	5.21 ± 1.66[a]	2.52 ± 0.58[b]	3.03 ± 0.78[b]
leaf	control	3.58 ± 0.13[a]	3.70 ± 1.09[a]	2.66 ± 1.01[b]	2.09 ± 1.65[a]	5.62 ± 1.39[a]	1.60 ± 0.64[b]	2.50 ± 0.82[b]

[a] Mean ± SEM, means within an organ for a given day followed by the same roman letter are not different at $\alpha = 0.05$ by Fisher's protected least significant difference.

Table 6. Experiment: Total Furanocoumarin (Psoralen + Bergapten + Xanthotoxin) Content of Florida Cultivar 2-14 over 10 Days (μg/g)[a]

organ	trt/day	2 h	2 days	4 days	6 days	8 days	10 days	overall mean ± SEM
leaf	Bravo 500	14.60 ± 0.64[a]	17.93 ± 2.97[a]	15.48 ± 2.96[a]	12.64 ± 1.28[a]	21.07 ± 4.46[a]	12.04 ± 1.58[a]	15.07 ± 1.11[a]
leaf	Manzate-D	6.63 ± 1.87[b]	12.79 ± 5.37[a]	9.10 ± 3.18[a]	11.27 ± 5.67[a]	11.88 ± 4.38[a]	10.78 ± 3.01[a]	10.29 ± 3.50[b]
leaf	Kocide 101	12.68 ± 1.56[a]	13.27 ± 0.51[a]	13.15 ± 0.72[a]	6.63 ± 0.64[a]	17.05 ± 1.61[a]	16.07 ± 1.94[a]	13.16 ± 0.47[ab]
leaf	control	13.56 ± 1.49[a]	11.14 ± 1.12[a]	11.73 ± 1.84[a]	10.02 ± 2.28[a]	11.01 ± 1.94[a]	13.53 ± 1.27[a]	11.83 ± 1.09[b]
stalk	Bravo 500	0.74 ± 0.17[a]	1.11 ± 0.42[a]	0.97 ± 0.04[a]	0.88 ± 0.18[a]	1.06 ± 0.10[a]	0.73 ± 0.10[a]	0.90 ± 0.08[a]
stalk	Manzate-D	0.55 ± 0.11[ab]	0.70 ± 0.28[ab]	0.45 ± 0.11[b]	0.88 ± 0.40[a]	0.64 ± 0.25[b]	0.62 ± 0.23[a]	0.63 ± 0.20[b]
stalk	Kocide 101	0.49 ± 0.06[ab]	0.86 ± 0.11[ab]	0.42 ± 0.01[b]	0.56 ± 0.15[a]	0.63 ± 0.17[b]	0.76 ± 0.43[a]	0.64 ± 0.11[b]
stalk	control	0.31 ± 0.02[b]	0.28 ± 0.03[b]	0.45 ± 0.01[b]	0.81 ± 0.41[a]	0.45 ± 0.08[b]	0.36 ± 0.03[a]	0.40 ± 0.07[b]

[a] Mean ± SEM, means within an organ for a given day followed by the same roman letter are not different at $\alpha = 0.05$ by Fisher's protected least significant difference.

DISCUSSION

Furanocoumarin levels in celery as determined in previously published studies (Table 8) and those levels we found in Florida cultivars (Table 1) are likely to cause chronic dermatitis in handlers and these effects may be exacerbated with fungicide treatment (Table 7). Austad and Kavli (1983) proposed a total furanocoumarin level of 12.5 μg/g for a contact dermatitis response. Junebelle, M68, and M9(2) from Florida field celery [M68 and M9(2)] were at or above the 12.5 μg/g contact dermatitis level. The furanocoumarin levels we report (Tables 1 and 6) are generally comparable to

Table 7. Estimated Biological Activity toward Human Skin; Psoralen + Bergapten + Isopimpinellin[a]

	treatment	relative reactivity						overall mean
		2 h	2 days	4 days	6 days	8 days	10 days	
leaf	Bravo 500	$170 \pm 11^{a(+)}$	202 ± 26^{a}	182 ± 31^{a}	142 ± 12^{a}	239 ± 50^{a}	157 ± 22^{a}	$180 \pm 12^{a(++)}$
	Manzate-D	90 ± 26^{b}	153 ± 57^{a}	117 ± 38^{a}	136 ± 64^{a}	147 ± 51^{b}	156 ± 41^{a}	135 ± 18^{b}
	Kocide 101	158 ± 20^{b}	165 ± 6^{a}	165 ± 7^{a}	92 ± 8^{a}	193 ± 14^{ab}	218 ± 26^{a}	166 ± 10^{ab}
	control	154 ± 30^{b}	147 ± 13^{a}	162 ± 24^{a}	142 ± 28^{a}	149 ± 28^{b}	157 ± 12^{a}	152 ± 9^{ab}
stalk	Bravo 500	6.4 ± 1.2^{a}	8.5 ± 2.9^{a}	8.1 ± 0.3^{a}	7.2 ± 1.1^{a}	9.6 ± 1.0^{a}	5.9 ± 0.6^{a}	7.6 ± 0.5^{a}
	Manzate-D	5.2 ± 0.9^{ab}	5.9 ± 1.9^{ab}	4.2 ± 0.8^{b}	7.6 ± 3.1^{a}	6.0 ± 2.1^{b}	6.2 ± 2.0^{a}	5.9 ± 0.8^{b}
	Kocide 101	4.7 ± 0.6^{ab}	7.4 ± 0.6^{a}	4.0 ± 0.1^{b}	5.1 ± 1.3^{b}	5.7 ± 1.2^{b}	7.6 ± 3.9^{a}	5.9 ± 0.7^{b}
	control	3.5 ± 0.2^{b}	3.2 ± 0.2^{b}	4.3 ± 0.2^{b}	8.2 ± 4.0^{a}	4.4 ± 0.8^{b}	4.2 ± 0.01^{a}	4.43 ± 0.6^{b}

[a] Mean ± SEM, means followed by the same roman letter are not different at $\alpha = 0.05$ by Fisher's protected least significant difference. (+) Significantly different from control at $\alpha = 0.07$. (++) Significantly different from the control at $\alpha = 0.13$.

Table 8. Celery Furanocoumarin Content Reported in the Literature (μg/g)

plant part	psoralen	xanthotoxin	bergapten	isopimpinellin	treatment	reference
stalk	0.1−3.0				store celery	Berkeley et al., 1986
leaf	2.6−8.5	(total LFC range: 7.2−20.4)			store celery	Berkeley et al., 1986
stalk	1.0−4.9	0.7−7.2	0.2−0.9		store celery	Seligman et al., 1987
leafy end	0.7−4.2	1.0−18.5	0.6−3.7		store celery	Seligman et al., 1987
stalk	35	33	17	25	120 h, pH 2.0, fog	Dercks et al., 1990
stalk		8	7	13	control	Dercks et al., 1990
leaf	16	82	38	38	120 h, pH 2.0, fog	Dercks et al., 1990
stalk		2	4.5	NA	field plants	Diawara et al., 1993a
leaf	>1	16	30	NA	field plants	Diawara et al., 1993a
stalk	2.7	47.6	135.5	111	celery accession	Trumble et al., 1990
leaf	34.1	89.9	169	115.6	celery accession	Trumble et al., 1990
stalk		0.13	0.78		Tall Utah 5270-R, 1990 season	Trumble et al., 1992
leaf	0.1	6	9		Tall Utah 5270-R, 1990 season	Trumble et al., 1992

those reported by Berkley et al. (1986) and Seligman et al. (1987) as levels associated with human dermatitis. The levels found in Tall Utah (California) and in the petioles of California field plants (Diawara et al., 1993a) are lower than those expected to cause contact dermatitis. Our results indicate that significant levels of furanocoumarins can occur in Florida-grown celery and that such levels vary significantly between cultivars. Interestingly, the origin of Florida 296 included germplasm introduced from wild-type celery for the purpose of disease resistance. Most older celery cultivars were selected using Utah types or from other long-cultivated celery types. Present celery improvement efforts often include wild-type celery as sources of disease and insect resistance.

The increased leaf and stalk levels of bergapten, xanthotoxin, and isopimpinellin we observed is the first report of a change in individual furanocoumarin content in celery after fungicide treatment. These changes appear to result from increased psoralen synthesis followed by conversion of psoralen to bergapten or xanthotoxin since psoralen levels were essentially unchanged over the course of this experiment. The increase in isopimpinellin appeared to lag behind the increase in bergapten and xanthotoxin in leaves (Tables 2−5). The induced increase in bergapten, xanthotoxin, and isopimpinellin may be cyclic (Beier and Oertli, 1983), but our data do not support a cyclic phenomenon (Table 6). Since the total bergapten + psoralen + xanthotoxin did not differ from controls on day 10 (Table 7), the biological activity of altered LFC content will be determined by the biological activity of individual compounds. The biological activity in leaves did not differ from control at $\alpha = 0.05$. Bravo 500 treatment increased the biological activity of the stalk (Table 7). It should be noted that bergapten content in carefully "deleafed" stalks was about 1.0 ppm (Table 3). In the "table preparation," stalk bergapten contents were lower than leaves but were above 5.0 ppm (Table 1). Diawara et al. (1995) showed that in two celery cultivars LFC in celery was in the order outer leaf > inner leaf > heart leaf > outer petiole > inner petiole. The heart petiole and root LFC contents were generally less than the outer petiole. Diawara et al. (1995) suggested an allelochemical function for the LFCs as an explanation for varied levels within the plant. Isopimpinellin can act as an insect antifeedant, similar to bergapten and xanthotoxin, depending on the insect (Muckensturm et al., 1981; Yajima and Munkata, 1979; Yajima et al., 1977). It is possible that a thorough understanding of allelochemical response, including relaxation times, could lead to "nonpesticidal" management of crop pests, management which would also reduce human exposure to biologically active plant products.

Increases in psoralen, bergapten, and xanthotoxin were noted in celery initially treated with copper sulfate after 3 days at 4 °C (Beier and Oertli, 1983). Rahe and Arnold (1975) showed increases in phaseolin in *Phaseolus vulgaris* about 26 h after touching with dry ice. Celery samples were held at 4 °C and randomly processed in our cultivar experiment. The cultivar comparisons we made here differ in design and object from the previous experiments. The linear furanocoumarin content shown in Table 1 can be expected in home preparation.

Because of their biological activity, higher levels of celery psoralen, bergapten, or xanthotoxin levels in plants treated with pesticide(s) should be of concern (Nigg et al., 1993). The linear furanocoumarins serve as an example of the double-edged sword effect of breeding for "natural" chemicals to produce resistance to herbivores. That is, resistance may be achieved but there could be a health cost to humankind.

Considering the variety of substances present in celery and the variables which increase, decrease, or alter the quality of linear furanocoumarins (Beier and Nigg, 1992), new celery varieties and sources of celery germplasm should be assessed for their LFC content under "normal" and "induced" conditions prior to incorporation and cultivar release, a suggestion which has

been made before (Beier and Nigg, 1992; Heath-Pagliuso et al., 1992; Trumble et al., 1992; Diawara et al., 1993a,b).

Regardless of the fact that our results show some fungicides may increase the *estimated* biological activity of phytoalexins towards human skin, a balanced management practice is advisable on this issue. For instance, in assessing the utility of plant toxins for breeding resistance, Diawara et al. (1993b) showed that combinations of psoralen with bergapten and xanthotoxin resulted in antagonistic effects on mortality of *Spodoptera exigua* (beet armyworm) mortality. Trumble et al. (1990) failed to link furanocoumarins with resistance of celery to *Liriomyza trifolii* (leafminer), so breeding plants for toxic compounds to achieve resistance is not a panacea for pest problems. In regard to human food safety, acidic fogs raised the psoralen + bergapten + xanthotoxin levels in leaves by 540% to 135 μg/g fresh weight; in petioles by 440% to 55.56 μg/g (Dercks et al., 1990). *Fusarium oxysporum*, a celery pathogen, raised the celery petiole level from about 5 μg/g to about 50 μg/g (Heath-Pagliuso et al., 1992). Furanocoumarin levels after Bravo treatment (Table 6) were only about 15 μg/g. In a balanced investigation of food safety, a pest control agent may thus increase the safety of food and this consideration should be part of any program investigating phytoalexin induction and food safety.

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