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IMPACT OF ATMOSPHERIC POLLUTION ON LINEAR FURANOCOUMARIN CONTENT IN CELERY

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Abstract—In a series of laboratory studies, a single 4-hr acidic fog at pH levels associated with commercial celery (*Apium graveolens* L.) production near major population centers in California was found to stimulate development of psoralen, bergapten, xanthotoxin, and isopimpinellin within 24 hr and for at least 120 hr after exposure. At 120 hr posttreatment, the concentrations of phototoxin furanocoumarins (psoralen + bergapten + xanthotoxin) increased 540% in the leaves (to 135 μg/g fresh weight) and 440% in the petioles (to 55.56 μg/g fresh weight) of celery exposed to a pH 2.0 fog as compared to plants exposed to control fogs (pH 6.3–6.5). Concentrations of these compounds in test plants were 7.5 times higher than the amount known to produce contact dermatitis. The nonphototoxic isopimpinellin increased more than threefold in the leaves (to 39.23 μg/g fresh weight, 120 hr) and petioles (to 25.88 μg/g fresh weight) as compared to control plants. In contrast, a single ozone fumigation at 0.20 ppm for 2 hr generally reduced concentrations of phototoxin furanocoumarin in leaves of celery within 24 hr (ozone-treated plants = 37.9, controls = 69.5 μg/g fresh weight), but levels of these chemicals in leaves of ozone-fumigated plants increased rapidly and concentrations were not significantly different at 120 hr. Isopimpinellin concentrations in foliage followed a similar trend (at 24 hr, control = 25.11, ozone treated = 10.96 μg/g fresh weight, no difference at 120 h). In petioles, none of the linear furanocoumarin levels differed significantly at 120 hr posttreatment.

Key Words—Celery, *Apium graveolens*, atmospheric pollution, ozone, acidic fog, furanocoumarins, bergapten, xanthotoxin, psoralen, isopimpinellin.

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INTRODUCTION

Phototoxin dermatitis caused by contact with celery infected with a *Sclerotinia* fungi has been reported since the early 1900s (Austad and Kavli, 1983; Ashwood-Smith et al., 1985). Since the mid-1970s, the causal agents of the dermatitis have been definitively identified as linear furanocoumarins. When exposed to long-wave UV light, the phototoxic linear furanocoumarins (psoralen, xanthotoxin, and bergapten) rapidly alkylate DNA (Scott et al., 1976). These chemicals: (1) have proven lethal and carcinogenic in in vitro bioassays of bacterial and mammalian cells (Ashwood-Smith et al., 1980, 1982; Igali et al., 1970), (2) pose substantial toxicological risks for humans (Scott et al., 1976), and (3) have been recognized as causally related to skin cancer by the World Health Organization (IARC, 1983). The presence of high concentrations of psoralens in other *Apium* spp. and in related genera in the Apiaceae (Umbelliferae) also have been reported (Berenbaum, 1981b; Chaudhary et al., 1986).

Although the linear furanocoumarins were originally thought to be mycotoxins produced by *Sclerotinia*, Beier and Oertli (1983) demonstrated that the phytoalexin response also was initiated by general elictors including copper sulfate, UV light, and cold temperatures. Mechanical damage occurring during harvesting and storage also has been shown to increase concentrations from about 2 ppm to 95 ppm (Chaudhary et al., 1985). In addition, Berenbaum (1981a) and Zangerl and Berenbaum (1987) demonstrated that distribution of psoralens in wild parsnip was significantly correlated with nitrogen content. Thus, induction of psoralens appears to be stress-related while nitrogen levels influence within-plant distribution.

In coastal California, where well over 50% of the celery produced in the United States is grown (Ivey and Johnson, 1986), acidic fogs and ozone are significant stress factors that influence the content and form of nitrogen in plants. Acidic fogs with a pH of 3.6 or lower can cause visible foliar damage and deposit nitrogenous compounds directly on plant surfaces (Shriner, 1986). In the Los Angeles basin, fogs with a pH of 1.69 have been recorded (Hoffman, 1984). However, fogs between pH 2.0 and 3.0 are more common. Although urban encroachment has reduced the total acreage of farmland in Orange and Los Angeles counties, the areas most commonly exposed to high nitrogen acidic fogs, total value of field-grown celery exceeds $5 million dollars (U.S.) annually (Ivey and Johnson, 1986). Atmospheric ozone, which typically reaches concentrations exceeding 0.20 ppm on 20 or more days each year, visibly damages many plant species and significantly reduces total nitrogen content and increases soluble protein levels in tomatoes. Therefore, the purpose of our study was to investigate the psoralen concentrations occurring in a commercial celery variety exposed to stressful atmospheric conditions (ozone and acidic fog) associated with celery production in some areas of coastal California.

METHODS AND MATERIALS

Simulated Acidic Fogs. Simulated acidic fogs were prepared by adjusting distilled water to pH 2.0 and 3.0 with reagent-grade nitric and sulfuric acid mixed at a 2.5 : 1 (v/v) ratio. This acid ratio is typical of fogs in California, but lacked other ionic components of ambient moisture (Waldman et al., 1982). The pH levels also are consistent with fogs occurring near Los Angeles (Hoffman, 1984). Control fogs consisted of distilled water with a pH of 6.3–6.5. Fogs were created within a 1-m^3 chamber using a fogging apparatus designed by Musselman et al. (1985), which operated at 7.03 kg/cm^2 and produced droplets averaging 20 μm in diameter. Temperature at treatment averaged 22–26°C. Shade cloth was used to reproduce incident radiation levels consistent with coastal fogs of no more than 300 $\mu E/m^2/sec$. Three randomly selected celery plants (*Apium graveolens* L., var Tall Utah 52-70) each for control and test fogs were exposed for 4 hr, and then placed in a shaded location to dry for at least 4 hr (22–26°C). These plants were extracted and examined by HPLC (Beier et al., 1983a,b) at 0, 24, and 120 hr posttreatment. This experiment was replicated three times (nine test plants per pH and nine control plants each for 24- and 120-hr measurements, nine untreated plants were analyzed at time 0). To reduce chamber effects, the replicates were run sequentially on three different dates, alternating fogs at pH 2.0, 3.0, and 6.3–6.5. Linear furanocoumarin contents in plants were compared between treatments and controls at 24 and 120 hr postfumigation using Student's t tests.

Ozone Treatments. Eight celery plants were exposed to a single 2-hr ozone fumigation at 0.20 ppm in an 0.78-m^3 (interior space) environmental chamber equipped with a fan providing a complete air exchange every 45 sec. Ozone was added after input air was first scrubbed with a charcoal filter. Ozone was generated using an OREC OV35-O ozonator (Ozone Research & Equipment Corp., Phoenix, Arizona) and monitored within the chamber by a model 1008 ozone analyzer (Dasibi Environmental Corp., Glendale, California). Eight control plants also were exposed for 2 hr in the same chamber but without the ozone input. This experiment was replicated three times (24 test plants and 24 control plants). To reduce any chamber effects, the replicates were run sequentially, alternating control and ozone fumigations. Linear furanocoumarin contents in plants were compared between treatments and controls at 24 and 120 hr postfumigation using Student's t tests.

Extraction and Quantification. Extraction of the linear furanocoumarins psoralen, bergapten, xanthotoxin, and isopimpinellin was modified from a technique reported by Beier (Beier, 1985; Beier et al., 1983a,b). The frozen plant material was immersed in liquid nitrogen and crushed to a fine powder with a mortar and pestle. The powder was transferred into a 40-ml Ten Broeck homogenizer and further ground in 15 ml of deionized H_2O. The homogenate was

poured into centrifuge tubes, the volume brought up to 25 ml with deionized H_2O, and the samples centrifuged for 6 min at 570*g*. The supernatant was passed into separatory funnels through several layers of cheesecloth and the sediment discarded.

The aqueous phase was extracted once with 20 ml and three times with 10 ml of diethyl ether. Ether fractions were combined, centrifuged at 110*g* to break emulsions, and decanted into a round-bottomed flask. The samples then were reduced to dryness by rotary evaporation. The residue was dissolved in 0.6 ml acetonitrile and 0.4 ml H_2O was added; this flask washing step was repeated four times.

The combined flask rinses were passed through a C18 Sep-Pak cartridge (Waters Associates, Inc., Milford, Massachusetts) which was previously washed with 15 ml MeOH and rinsed with 15 ml H_2O), and the eluate collected. The Sep-Pak cartridge then was washed with 8 ml 60% acetonitrile in H_2O, and the eluates were combined. Fifteen milliliters of anhydrous EtOH was added to the sample to facilitate azeotropic distillation of the water, and the sample subsequently was reduced to approximately 1 ml by rotary evaporation. The remaining water was poured into a test tube and extracted twice with 5 ml ethyl acetate by vigorously shaking the tubes.

After phase separation, the ethyl acetate layers were drawn off with a Pasteur pipete, combined, and passed through a silica Sep-pak cartridge (previously washed with 15 ml of chloroform) to remove polar impurities. The Sep-Pak cartridge was then eluted with 8 ml of 7.5% ethyl acetate in chloroform and the combined eluates were concentrated to dryness by rotary evaporation. The samples were taken up to 1 ml in chloroform and stored at −70°C until analyzed. In all cases, extraction recoveries of standard solutions were equal to or greater than 83%.

Linear furanocoumarins were resolved on a 25-cm Ultrasphere 80 Å pore silica column, preceded by an Ultrasphere 5-μm guard column on a model 334 liquid chromatograph (Beckman, Fullerton, California). The solvent system consisted of HPLC grade chloroform (Aldrich, Milwaukee, Wisconsin) pumped at a flow rate 1.5 ml/min (modified from Beier, 1985). Eluting components were monitored with a Beckman 160 UV detector set at 254 nm; sample peaks were recorded and integrated on a model 3392A integrator (Hewlett Packard, Avondale, Pennsylvania). Recovery of standards always exceeded 83%. Elutants were identified using mass spectrometry performed on a 7070E mass spectrometer (VG Analytical, Ltd., Wythenshawe, U.K.). Samples were analyzed using a solid probe (200–250°C) under electron ionization conditions (70 eV, source temperature = 150°C). A mass range of 100–300 daltons was scanned. The standards used in this study were psoralen, bergapten (5-methoxypsoralen), and xanthotoxin (8-methoxypsoralen), and isopimpinellin (5,8-methoxypsoralen) (Figure 1). The first three were purchased from Aldrich, and the last was

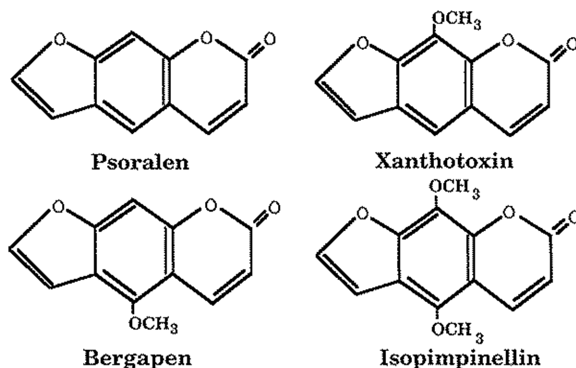

FIG. 1. Structures of the four linear furanocoumarins found in celery.

kindly provided by Dr. R.C. Beier of the Southern Plains Area Veterinary Toxicology and Entomology Research Laboratory in College Station, Texas.

RESULTS AND DISCUSSION

*Mass Spectrometry.*The most important fragment ions for psoralen included: *m/z* 187 (24%); 186 (71%, $[M]^{+\cdot}$); 159 (22%); 158 (100%); 130 (23%); 129 (45%); 103 (11%); 102 (53%); and 101 (27%). Key fragment ions for bergapten *m/z* 217 (26%); *m/z* 216 (72%, $[M]^{+\cdot}$); 201 (42%); 188 (27%); 187 (19%); 174 (22%); 173 (94%); 146 (21%); 145 (74%); 144 (20%); 102 (22%); and 100 (19%). Identifying fragment ions for xanthotoxin included: *m/z* 217 (26%); *m/z* 216 (72%, $[M]^{+\cdot}$); 201 (60%); 188 (45%); 174 (40%); 173 (100%); 158 (22%); 145 (92%); 129 (37%); 117 (21%); 116 (23%); 102 (25%); and 101 (21%). Key fragment ions for isopimpinellin included: *m/z* 246 (39%, $[M]^{+\cdot}$); 231 (100%); 203 (27%); 188 (39%); 175 (33%); 160 (30%); 147 (25%); 143 (23%); 104 (49%); and 103 (21%). No other linear furanocoumarins were detected.

Simulated Acidic Fogs. Leaves from plants exposed to acidic fogs with a pH of 2.0 had significantly higher concentrations of the phototoxic furanocoumarins than plants treated with control fogs within 24 hr posttreatment (Figure 1). At 120 hr after exposure, all linear furanocoumarins reached significantly higher levels after exposure to 2.0 pH fogs. In contrast, fogs of 3.0 pH did not increase the levels of any furanocoumarins at 24 hr. However, all compounds except psoralen increased significantly in leaves at 120 hr. Foliar and petiole lesions were observed on all plants exposed to fogs with a pH of 2.0, but not on plants treated with control fogs or with fogs of pH 3.0.

Leaves consistently had higher concentrations than petioles and the linear

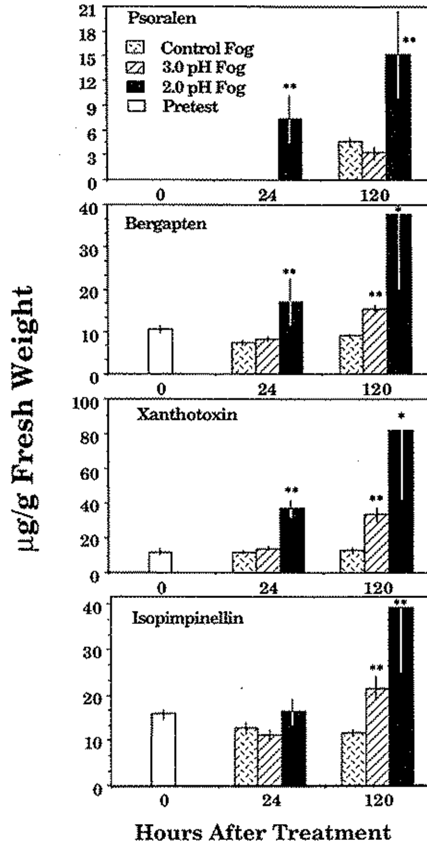

FIG. 2. Acute impact of acidic fogs of pH 2.0 and 3.0 and control fogs (pH 6.3–6.5) on linear furanocoumarin content in celery leaves. Bars delineate standard errors. Asterisk(s) next to points indicate significant differences from plants treated with a control fog (*$P \leq 0.05$, **$P \leq 0.01$, Student's t test, 16 df).

furanocoumarins psoralen, bergapten, xanthotoxin, and isopimpinellin (Figures 1 and 2). Although this trend has been reported previously, the levels of psoralen, bergapten, and xanthotoxin (phototoxic furanocoumarins) from control plants in our test were sixfold higher in leaves and stems than observed by Beier et al. (1983a) (mean from leaves or petioles for psoralen ≤ 0.15, bergapten ≤ 1.5, xanthotoxin 3.5 ppm), but were similar (ca. 1.4-fold greater) to the concentrations reported by Berkley et al. (1986) (phototoxic furanocoumarins = 11.2 ppm in petioles and = 20.4 ppm in leaves). Such increases may be due

to varietal effects (Berkley et al., 1986), but direct comparisons with previous studies were not possible because the cultivars tested were not reported.

The foliar concentrations of the phototoxic furanocoumarins in our study are of considerable concern because of a new trend in marketing intact celery plants (leaves not trimmed) (T. Batkin, manager, California Celery Research Advisory Board, Dinuba, California, personal communication) rather than the more common "topped" celery (most leaves removed). Even in our control plant foliage, levels of the phototoxic furanocoumarins were more than twice (ca. 30 μg/g fresh weight) the critical level of 18 μg/g fresh weight known to cause phototoxic dermatitis (Austad and Kavli, 1983). Foliage from plants exposed to an acidic fog with a pH of 3.0 exceeded 50 μg/g fresh weight. The foliar concentrations of these compounds in plants exposed to a single fog of pH 2.0 reached 140 μg/g fresh weight and should be considered hazardous.

The concentrations of phototoxic furanocoumarins in petioles (59.7 μg/g fresh weight) of plants exposed to fogs of pH 2.0 exceeded the critical level of 18 μg/g by over 300%. Thus, even celery marketed with the leaves trimmed may have considerable potential for human health concerns if exposed to highly acidic fogs. However, petioles from plants treated with a control fogs or a fog with a pH of 3.0 contained concentrations of these chemicals below the critical level.

Ozone Fumigations. In general, ozone fumigation had a markedly different effect on content of linear furanocoumarins than did acidic fogs. Ozone fumigation was conducted at 20 ppm, a level which is reached in excess of 20 or more days each year in agricultural areas near Los Angeles. Foliage of plants exposed to ozone had significantly reduced concentrations of psoralen, bergapten, and isopimpinellin; xanthotoxin levels were not significantly reduced (Figure 3). At 120 hr posttreatment, no significant differences were evident between treatments. Levels of these compounds in petioles at 24 hr followed similar trends but were not significant at the $P < 0.05$ level (Figure 4). No differences in concentration were detected at 120 hr following ozone fumigation. Several mechanisms for the observed reductions in furanocoumarin concentrations appear possible: (1) the ozonolysis of the olefinic bonds in the furan or lactone rings found on these compounds, (2) the induction of degradation enzymes is inhibited, and/or (3) the inhibition of biosynthetic enzymes is occurring. Additional research will be required to document the critical mechanism(s).

Although the plants examined in the acidic fog and ozone trials were all from the same seedlot, planted at the same time, and agronomically treated the same, the control plants in the ozone tests developed consistently higher quantities of the linear furanocoumarins. This could have been caused by a reduction in concentration following control fogs, or more likely, an increase in furanocoumarin content occurring as a result of plant size (Austad and Kavli, 1983).

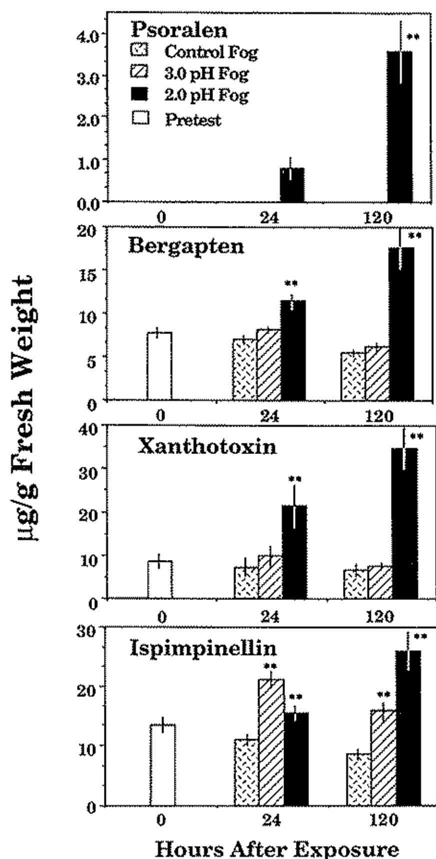

FIG. 3. Acute impact of acidic fogs of pH 2.0 and 3.0 and control fogs (pH 6.3–6.5) on linear furanocoumarin content in celery petioles. Bars delineate standard errors. Asterisk(s) next to points indicate significant differences from plants treated with a control fog (*$P \leq 0.05$, **$P \leq 0.01$, Student's *t* test, 16 *df*).

The plants used in the ozone trial were about eight weeks older than those used in the acidic fog tests and had reached the 36/carton stage. Nonetheless, the foliar contents of these chemicals were above the levels required to cause contact dermatitis. Because contact dermatitis in celery handlers is common [19 cases in 48 handlers in the most recent study (Seligman et al., 1987)], we suspect that the concentrations of phototoxic furanocoumarins found in our study are not unusual. At the least, the effects of atmospheric pollutants will help

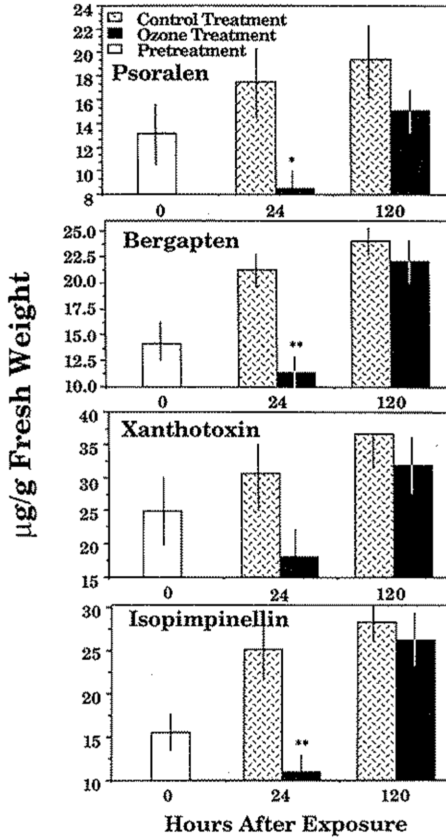

FIG. 4. Acute effects of 2-hr ozone fumigation of celery at 20 ppm on linear furanocoumarin content in foliage of a commercial celery variety. Bars delineate standard errors. Asterisk(s) next to points indicate significant differences from plants not treated with ozone ($*P \leq 0.05$, $**P \leq 0.01$, Student's t test, 16 df).

account for some of the variability in furanocoumarin contents reported in the literature.

The concentrations of linear furanocoumarins found in our study may be conservative. None of the plants used in any portion of this study exhibited symptoms of disease or were subjected to mechanical damage or cold temperatures, which could increase furanocoumarin content (Austad and Kavli, 1983; Ashwood-Smith et al., 1985; Beier and Oertli, 1983; Chaudhary et al., 1985). In addition, plants in our study were subjected to only acute exposure to atmo-

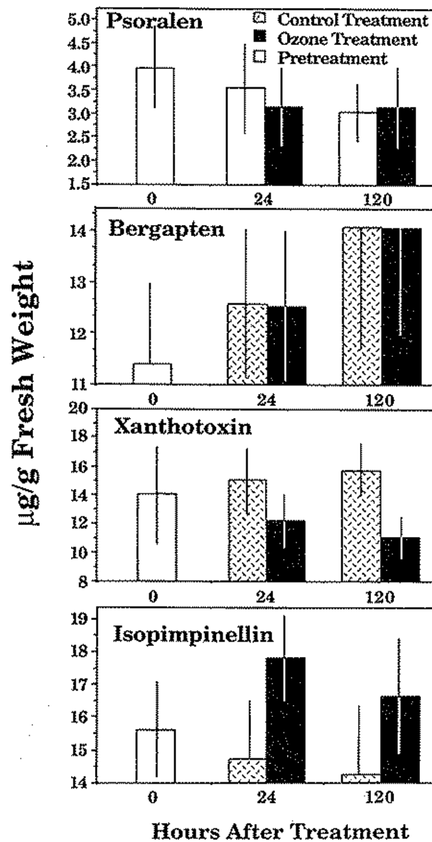

FIG. 5. Acute effects of 2-hr ozone fumigation of celery at 20 ppm on linear furanocoumarin content in petioles of a commercial celery variety. Bars delineate standard errors. Asterisk(s) next to points indicate significant differences from plants not treated with ozone (* $P \leq 0.05$, ** $P \leq 0.01$, Student's t test, 16 *df*).

spheric pollutants. The impact of longer or chronic exposures to acidic fogs or ozone in combination with other environmental stresses on the production of linear furanocoumarins is worthy of additional study. To our knowledge this is the first report of anthropogenic air pollutants inducing production of plant metabolites which can be injurious to human health. These results raise the intriguing possibility that continuing production of atmospheric air pollutants could be having deleterious effects on our food supply beyond simply decreasing yields (Leung et al., 1982) or increasing insect damage (Trumble et al., 1987). This, in turn, leads to the possibility that certain geographic areas subject

to acidic fogs may be less desirable for production of celery for human consumption.

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