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Biochemical Plant Responses to Ozone[1]

IV. Cross-Induction of Defensive Pathways in Parsley (*Petroselinum crispum* L.) Plants

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Parsley (*Petroselinum crispum* L.) is known to respond to ultraviolet irradiation by the synthesis of flavone glycosides, whereas fungal or elicitor stress leads to the synthesis of furanocoumarin phytoalexins. We tested how these defensive pathways are affected by a single ozone treatment (200 nL L^{-1}; 10 h). Assays were performed at the levels of transcripts, for enzyme activities, and for secondary products. The most rapid transcript accumulation was maximal at 3 h, whereas flavone glycosides and furanocoumarins were maximally induced at 12 and 24 h, respectively, after the start of ozone treatment. Ozone acted as a cross-inducer because the two distinct pathways were simultaneously induced. These results are consistent with the previously observed ozone induction of fungal and viral defense reactions in tobacco, spruce, and pine.

The phytotoxicity of the air pollutant ozone in plants is well documented (Guderian, 1985; Treshow and Anderson, 1989). The emphasis is usually placed on detrimental effects on primary metabolism, such as photosynthesis, carbon allocation, and growth. Our previous studies in different plant species have shown that secondary metabolism provides even more strongly affected targets, because single, subacute ozone pulses were sufficient to induce genes (Sandermann et al., 1990; Ernst et al., 1992b), enzymes (Sandermann et al., 1990; Galliano et al., 1993), and biosynthetic products (Guderian, 1985; Sandermann et al., 1989). These responses included the induction of stilbene phytoalexins (Rosemann et al., 1991) and of putative plant defense proteins such as β-1,3-glucanase, chitinase, and PR proteins (Eckey, 1992; Schraudner et al., 1992). The results have supported the hypothesis that a major effect of ozone is to change the phytopathological disposition of plants (Sandermann et al., 1989).

To further extend these studies, parsley (*Petroselinum crispum* L.) seedlings have now been exposed to ozone. Parsley represents a well-studied phytopathological model system in which two separate defensive pathways are present (Hahlbrock et al., 1976). UV irradiation induces the flavone glycoside pathway (Hahlbrock et al., 1976; Hahlbrock and Wellmann, 1970; Hahlbrock and Scheel, 1989), whereas fungal spores or fungal elicitor induce the furanocoumarin phytoalexin pathway (Hahlbrock et al., 1976; Hauffe et al., 1986). Both phenylpropanoid pathways are induced from very low background levels in parsley cell-suspension cultures. In contrast, these pathways are also present constitutively in certain tissues of intact parsley plants (Knogge et al., 1987). Flavone glycosides are localized mainly in the leaf epidermal cell layer, and furanocoumarins are localized in the leaf oil ducts. UV irradiation induced the CHS mRNA in total leaf extracts about 4- to 8-fold compared to etiolated seedlings (Schmelzer et al., 1988). The high constitutive level of the furanocoumarin pathway has prevented detection of zoospore induction in total leaf extracts of parsley (Knogge et al., 1987). Histochemical methods including in situ hybridization have more clearly revealed the induction of the two distinct pathways in intact parsley leaves. UV treatment resulted in an increase of the flavone glycoside pathway in epidermal cells (Hahlbrock et al., 1976; Schmelzer et al., 1988). Fungal infection led to furanocoumarin accumulation near the infection sites and to secretion of the metabolites into infection droplets (Hahlbrock et al., 1976; Tietjen et al., 1983; Scheel et al., 1986).

It will be reported in this paper that ozone acts as a cross-inducer because parameters of both defensive pathways were simultaneously induced in intact parsley plants. This study could not be performed with parsley cell-suspension cultures because of their insensitivity to ozone (Eckey, 1992). Some of the results have been communicated in abstract form (Ernst et al., 1992a).

MATERIALS AND METHODS

Chemicals

L-[2,6-^{3}H]Phe and S-adenosyl-L-[*methyl*-^{14}C]Met were purchased from Amersham Buchler (Braunschweig, Germany). Alkaline phosphatase-coupled anti-rabbit IgG from goat was obtained from Gibco BRL Life Sciences (Eggenstein, Germany). Antisera for CHS and MAT-3 were kindly supplied

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Abbreviations: CAD, cinnamyl alcohol dehydrogenase; CHS, chalcone synthase; 4CL, 4-coumaroyl-CoA ligase; Eli, elicitor; HRGP, hydroxyproline-rich glycoprotein precursor; IWF, intercellular wash fluid; MAT-3, malonyl-CoA:flavonol-3-O-glucoside malonyltransferase; PAL, phenylalanine ammonia-lyase; POD, peroxidase; PR, pathogenesis-related; XMT, S-adenosyl-methionine:xanthotoxol-O-methyltransferase.

by Professor K. Hahlbrock (Max-Planck-Institut für Züchtungsforschung, Köln, Germany) and by Professor U. Matern (Universität Freiburg, Germany), respectively.

A molecular mass marker protein set (14.4–94 kD) was purchased from Pharmacia-LKB (Freiburg, Germany). 5-Bromo-4-chloroindolyl-3-phosphate and nitroblue tetrazolium were obtained from Sigma Chemie (Deisenhofen, Germany). The commercially available natural substances (apiin, coniferyl alcohol, xanthotoxol, bergapten, psoralen, isopimpinellin, xanthotoxin, and umbelliferone) were purchased from Roth (Karlsruhe, Germany).

The cDNA probes for PAL (Eli 4), CHS, 4CL, Eli 16, HRGP (Eli 9), POD (Eli 11), PR-1-1, and PR-2 from parsley were kindly supplied by Dr. I. Somssich (Max-Planck-Institut für Züchtungsforschung). These clones were described by Somssich et al. (1989). A β-1,3-glucanase clone from tobacco (Mohnen et al., 1985), a superoxide dismutase clone from tobacco (Bowler et al., 1989), and a carrot extensin clone (Chen and Varner, 1985) were donated by the respective authors. Actin-cDNA from soybean was a gift of Dr. R. B. Meagher (Shah et al., 1982).

Plant Material

Seeds of *Petroselinum crispum* L. (Hamburger Schnitt) were obtained from a local garden center. Seeds were germinated on a 2:1 mixture of standard substrate (type T; Fruhstorfer, Lauterbach, Germany) and perlite in a dark environmental growth chamber. Growth conditions were 24°C and 78% RH. After 10 d in the dark, seedlings were transferred to continuous white light (150 μE m^{-2} s^{-1} PPFD) for 2.5 weeks. Biochemical analyses were carried out with the pooled excised leaves (Eckey-Kaltenbach et al., 1993).

Ozone Exposure

If not noted otherwise, the standard ozone treatment was performed with 200 nL L^{-1} of ozone for 10 h under the conditions of precultivation. Ventilation was applied at 0.5 m s^{-1}. Ozone was generated by electrical discharge in dry O_2. The desired ozone concentration was maintained as described by Galliano et al. (1993).

Metabolite Analysis

Furanocoumarins

Frozen parsley leaves (100–150 mg) were ground in liquid N_2 to a powder using a Microdismembrator instrument (Braun, Melsungen, Germany). This step was followed by four consecutive extractions at 4°C with a total volume of 10 mL of methanol. After centrifugation (15,000*g*, 5 min, 4°C), the methanolic extracts were concentrated to 2 mL, and 10-μL aliquots were subjected to reversed-phase HPLC using a Spherisorb ODSII column (5 μm; 250 × 4.6 mm; Bischoff, Leonberg, Germany) at a flow rate of 1 mL min^{-1} with H_2O:tetrahydrofurane (7:3, v/v) containing 1% H_3PO_4. The HPLC method was adopted from Knogge et al. (1987). The eluates were monitored at 300 nm with a UV-visible analyzer (absorption detector 163; Beckman, München, Germany). Peak areas were integrated and related to known amounts of reference substances. For additional identification, a diode array detector was used.

Flavone Glycosides

The extraction and quantification of flavone glycosides by A_{340} measurement were performed as described by Hahlbrock et al. (1976). Where noted, separation of flavone glycosides was performed with a special HPLC system adopted from Zielke and Sonnenbichler (1990). For routine identification and quantification of 6''-*O*-malonylapiin and apiin, the above HPLC system for determination of furanocoumarins was used.

Enzyme Assays

Extraction and determination of PAL activity in parsley plant material was done according to the method of Rosemann et al. (1991). The assay used ^{3}H-labeled Phe as substrate, with the ^{3}H-labeled product cinnamic acid being extracted with ethylacetate. Extraction and determination of XMT enzyme activity were performed as described by Hauffe et al. (1986). Measurement of CAD activity was carried out by the previous assay procedure (Galliano et al., 1993).

Blotting Procedures

Electrophoresis of protein extracts prior to western blot analysis was performed in the presence of SDS according to the method of Laemmli (1970). A commercially available protein marker set (Pharmacia-LKB, Freiburg, Germany) was used for molecular mass determination. Electroblotting onto a membrane (Immobilon PVDF, Millipore, Eschborn, Germany) was performed according to the manufacturer's instructions. Transfer of proteins was monitored by staining with Ponceau S. After destaining and pretreatment with BSA, the membrane was incubated with the primary antibody (rabbit IgG) for 2 h. CHS antiserum was diluted by a factor of 1:4000, and MAT-3 antiserum was diluted by a factor of 1:5000. After incubation with an alkaline phosphatase-coupled secondary antibody (goat anti-rabbit IgG alkaline phosphatase conjugate; Gibco BRL Life Sciences) in a dilution of 1:3000, the antibodies were detected by addition of 5-bromo-4-chloroindolyl-3-phosphate and nitroblue tetrazolium (Eckey, 1992). The blots were scanned at 600 nm for quantification.

Total RNA was extracted as previously described (Kuhn et al., 1984). RNA blotting and hybridization (northern and dot blot analyses) using Hybond N membranes (Amersham Buchler) were performed as described by Sambrock et al. (1989). For hybridization the cDNA probes were ^{32}P-labeled with a random-primed DNA-labeling kit (Hexa Prime I; Appligene, Heidelberg, Germany). Autoradiograms were scanned with a densitometer (Elscript 400; Hirschmann, Unterhaching, Germany) and quantified relative to the reprobing signal obtained with an actin-cDNA probe.

Microscopy and Preparation of IWF

The preparation of leaf sections, aniline blue staining for callose, and general microscopic methods were carried out as

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described by Jahnen and Hahlbrock (1988) and Eckey (1992). Excised parsley leaves were vacuum infiltrated (260 mbars) in sterile water for five 1-min periods. IWF was then collected by centrifugation (1000g; 10 min; 4°C) as described by Eckey-Kaltenbach et al. (1993).

RESULTS

Ozone Effects on Parsley Plants

Parsley seedlings were exposed to ozone at high RH to reduce stomatal effects. After a 10-h treatment with 200 nL L^{-1} of ozone, the first visible symptoms appeared (Fig. 1A). Parsley leaves were scored for percentage of necrotic area of total leaves (Eckey-Kaltenbach et al., 1993). After the standard ozone treatment (200 nL L^{-1}, 10 h), the percentage of necrosis reached 40% at 24 h and 50% at 48 to 72 h (Fig. 1B). Leaf damage then progressed further. The onset of ozone exposure is defined as time zero, and postincubation in ozone-free air begins at 10 h. After the seedlings were fumigated with only 100 nL L^{-1} of ozone for 10 h, the formation of lesions was delayed but then reached the same extent of necrosis (40% at 84 h) as in the standard fumigation. The necrotic area contained large amounts of callose as detected by staining with aniline blue reagent (Fig. 1C). Under UV light (340–380 nm), the necrotic area showed a strong bluish fluorescence, probably due to the presence of phenolic compounds (Fig. 1D).

Accumulation of Secondary Metabolites

The leaf content of flavone glycosides and of furanocoumarins was followed during the standard ozone exposure and the postincubation period. The HPLC method used was adopted from Knogge et al. (1987) for direct comparison with pathogen-induced secondary compounds. It yielded a good resolution of the furanocoumarins with the following retention times: umbelliferone, 7.1 min; xanthotoxin, 10.3 min; bergapten, 15.5 min; psoralen, 12.0 min; and isopimpinellin, 12.3 min. Flavone glycosides appeared at 7.8 min (6''-*O*-malonylapiin) and 5.5 min (apiin). The healthy plants exhibited high constitutive levels of total furanocoumarins (about

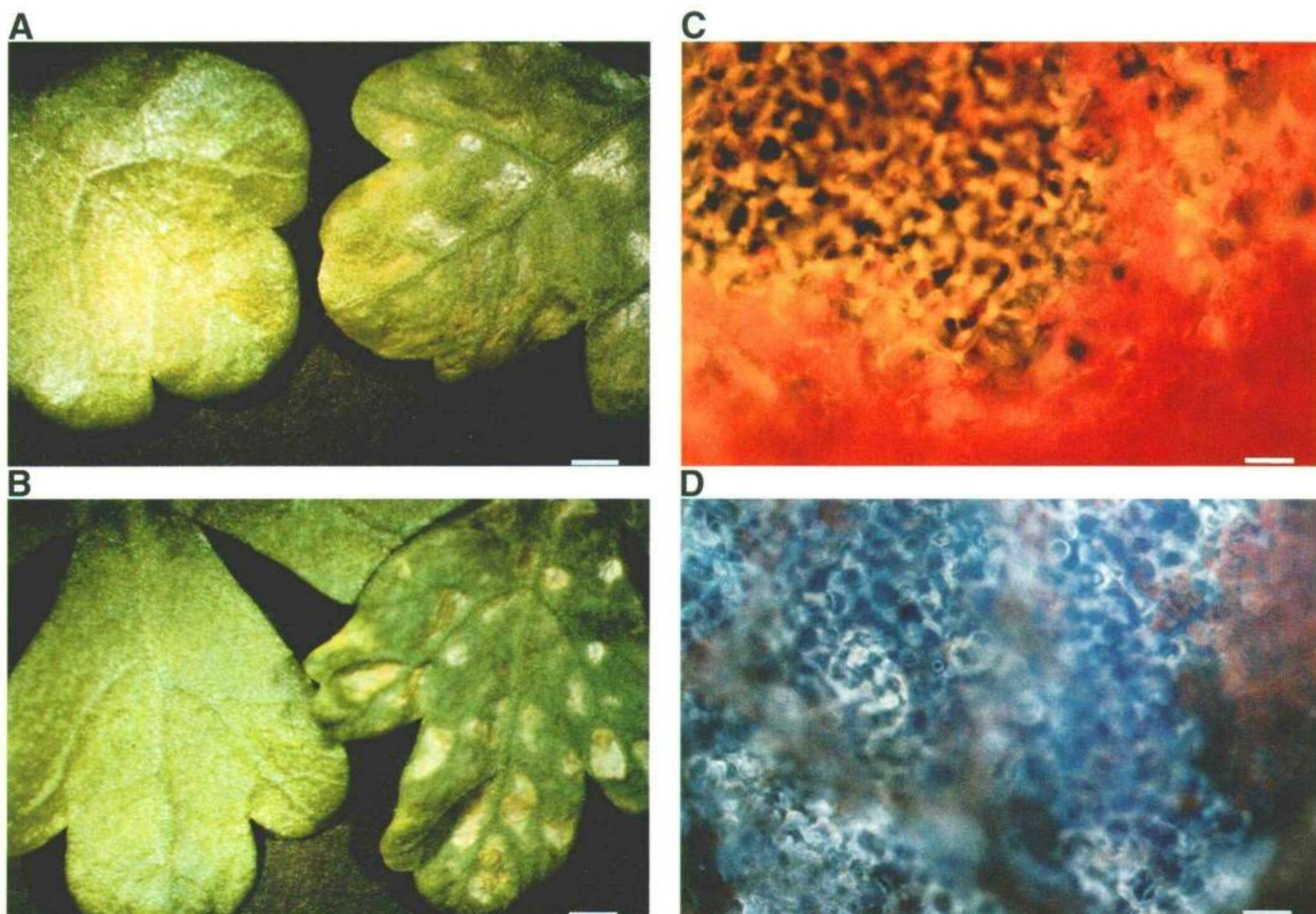

Figure 1. Ozone effects on parsley plants. A, The first visible symptoms at the end of the 10-h treatment with 200 nL L^{-1} of ozone in comparison with a control leaf. B, Formation of lesions and necrosis after 30 h of standard ozone exposure and postincubation in comparison with a control leaf. C, Detection of callose with aniline blue reagent. D, Detection of phenolic compounds by their bluish fluorescence under UV light (340–380 nm). Panels C and D were obtained after 30 h of standard ozone exposure and postincubation. The bars correspond to lengths of 1.5 μm (A and B) and 10 μm (C and D).

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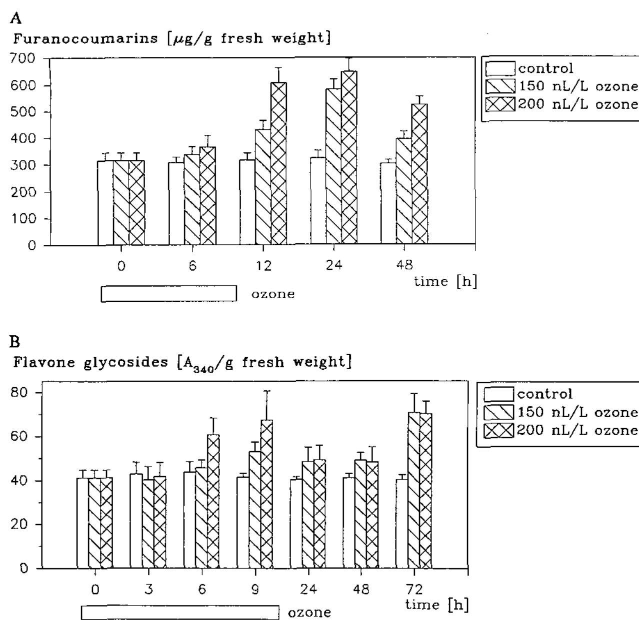

Figure 2. Effect of ozone treatment on furanocoumarins (A) and flavone glycosides (B) of parsley plants. The secondary metabolites were determined at several time points in parsley plants kept in the absence of ozone (open bars) or treated during the indicated 10-h interval with 150 nL L^{-1} (dashed bars) or 200 nL L^{-1} of ozone (crossed bars) (mean values ± SE, n = 5 or 6).

400 $\mu g\ g^{-1}$ fresh weight). In contrast, parsley cell-suspension cultures showed only about 400 ng g^{-1} fresh weight as the basal total content after 7 d of growth (Eckey, 1992). As shown in Figure 2A, there was a 2-fold increase of total leaf furanocoumarins after the standard ozone treatment (200 nL L^{-1}, 10 h). Maximum accumulation was reached at 12 to 24 h. The individual furanocoumarins listed above were also quantified. They showed the same induction kinetics and the same extent of accumulation as the total furanocoumarins. The induction factors (1.3- to 2.7-fold) increased linearly with ozone concentration (100–250 nL L^{-1}; 10 h). The basal amounts of furanocoumarins increased slightly with plant age, but the induction factors were independent of age.

The amount of total flavones and flavonoid glycosides was measured by A_{340} (Hahlbrock et al., 1976) after the standard ozone fumigation. During the first 12 h, there was a transient 1.6-fold accumulation, as shown in Figure 2B. There was a second increase 2 d after ozone treatment (at 72 h). More than 10 different but unidentified flavone compounds were detected by a special HPLC system (Zielke and Sonnenbichler, 1990) in the methanolic parsley plant extracts.

Attempts were made to study the leaf apoplastic compartment, which is assumed to be the first reaction site of ozone (Heath, 1989). The IWF was collected by centrifugation after a vacuum infiltration step. Xanthotoxin and bergapten were most prominent and accumulated 7- and 2-fold at 12 h.

Induction of Biosynthetic Enzymes

The enzyme PAL is involved in both the furanocoumarin and flavone glycoside biosynthetic pathways. The ozone response of this enzyme activity is shown in Figure 3A. A 3-fold increase was reached at the end of the ozone exposure period, followed by a decrease to the initial level. A second less pronounced increase in activity occurred during lesion development at 72 h.

XMT was tested as a specific enzyme of furanocoumarin biosynthesis. The standard ozone treatment led to a 2-fold increase (Fig. 3B) at 12 and 24 h.

CHS, a specific enzyme of the flavone glycoside pathway, could not be reliably determined because of high endogenous hydrolase activities. However, western analysis showed a single protein band with an apparent molecular mass of 44 kD (Eckey, 1992). Scanning of the alkaline phosphatase indicator stain revealed a 1.2-fold increase at 6 and 12 h (data not shown).

Western blotting was also performed for MAT-3. In this case a single protein band with a molecular mass of 40 kD was detected (Eckey, 1992). A 1.2-fold increase was found at 12 h (data not shown). Because of the known malonylesterase activity in 2.5-week-old parsley plants (Matern, 1983), malonyltransferase activity could not be determined reliably. The lignin biosynthetic enzyme CAD showed statistically significant maxima (1.5-fold) at 12 h as well as at 72 h. The basal activity of this enzyme was 125 µkat/kg of protein (Eckey, 1992).

Induction of Transcript Accumulation

Northern blotting of total RNA from ozone-treated and control parsley leaves revealed that transcript amounts of

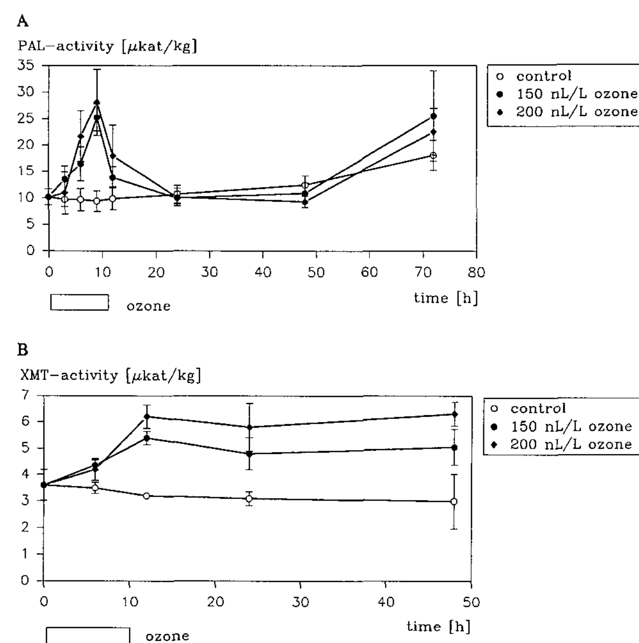

Figure 3. Effect of ozone treatment on PAL activity (A) and XMT activity (B) of parsley plants compared to control plants. The enzyme activities were determined at several time points in parsley plants kept in the absence of ozone (O) or after ozone treatment (10 h) with 150 nL L^{-1} (●) or 200 nL L^{-1} (♦) (mean values ± SE, n = 3 or 6).

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PAL as well as 4CL (not shown) increased about 10-fold at 6 h (Fig. 4A). A comparative study of PAL transcript amount and PAL enzyme activity showed the expected difference in the time course of the induction processes (Fig. 4B). The same time course of transcript induction was found for 4CL as well as for CHS, which showed a 13- and 2-fold increase, respectively.

Transcripts not associated with phenylpropanoid metabolism were also examined using homologous stress-induced cDNA probes. The transcripts of the two PR proteins PR-1 and PR-2, as well as an elicitor-induced cDNA probe with unknown function (Eli 16; Somssich et al., 1989), showed maximal amounts (5- to 7-fold) at 3 h. Transcripts measured with POD (Eli 11) and HRGP (Eli 9) cDNA probes were induced about 2.4- and 6.7-fold, respectively, with a maximum at 12 h. One set of original data for each group is shown in Figure 5.

No transcript accumulation could be detected using the cDNA probes available for the basic isoform of β-1,3-glucanase, Mn-dependent superoxide dismutase, and carrot extensin.

Time Sequence of Ozone Effects

In the standard ozone treatment (200 nL L^{-1}; 10 h) the onset times of various parameters measured ranged from 3 to 72 h as summarized in Table I. At 3 h the first transcript increases were measured for PR-1, PR-2, and Eli 16 (early genes). In phenylpropanoid metabolism, the first effects at 6 h concerned the transcript amounts of PAL, 4CL, and CHS (intermediate genes). The maxima of the corresponding enzyme activities were reached between 9 and 12 h when the first visible symptoms also appeared. The pathway products showed maximal accumulation at 12 and 24 h. At this time, transcript amounts of the late genes (POD, HRGP) also reached their maxima. There were secondary increases at 48 to 72 h for transcript amounts (PAL, 4CL, and CHS), enzyme activity (PAL), and product accumulation (flavone glycosides). All of these increases coincided with complete leaf necrosis.

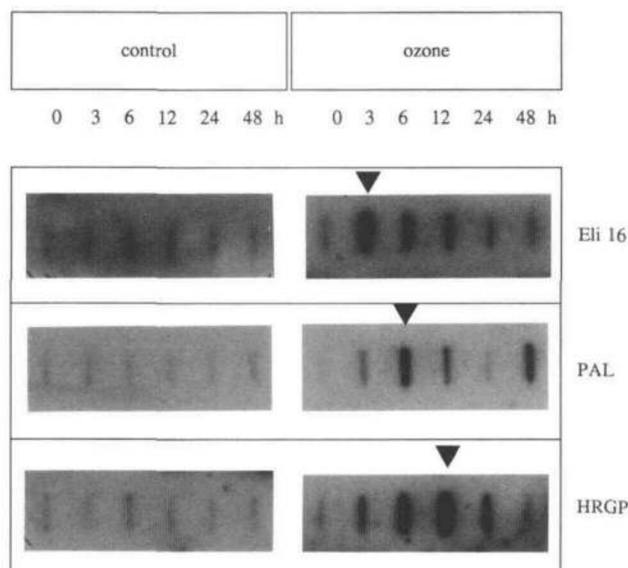

Figure 5. Time course of transcript accumulation for an early (Eli 16), an intermediate (PAL), and a late (HRGP) stress gene. Total RNA (1 μg) from control parsley plants (left) or parsley plants subjected to the standard ozone treatment (right) was analyzed by slot blot hybridization. Maximal transcript accumulation was at 3, 6, and 12 h, respectively, as marked by arrowheads.

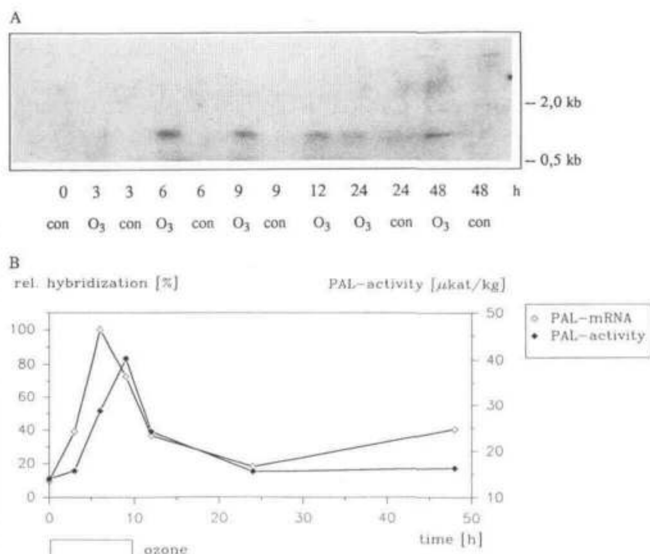

Figure 4. Induction of transcripts in parsley plants. A, Northern blotting of total RNA (5 μg) in ozone-treated (O_3) and control (con) parsley leaves. Total RNA from control and treated plants (200 nL L^{-1}; 10 h) was separated electrophoretically and then hybridized to a PAL cDNA probe. The marker bands are indicated at the right. The slot for 48-h ozone shows an additional background spot. B, Time courses of transcript accumulation (◇) and enzyme activity (◆) of PAL. Transcript amounts ($n = 4$) and enzyme activity ($n = 6$) were determined as described in "Materials and Methods." The standard ozone treatment was applied and mean values are shown.

DISCUSSION

An exposure of parsley plants to 100 nL L^{-1} of ozone for 10 h was sufficient to induce subsequent lesions and necrosis formation. The ozone symptoms observed (Fig. 1) were in agreement with older reports (Hill et al., 1961; Oshima et al., 1978) classifying parsley as an ozone-sensitive plant on the basis of growth and assimilate-partitioning measurements. The occurrence of callose and of fluorescent material during late symptom development provides a good analogy to ozone effects on tobacco leaves (Kerner, 1990; Schraudner et al., 1992). It is interesting that callose and fluorescent phenolic compounds have also been described for necrotic development caused by *Phytophthora megasperma* f. sp. *glycinea* in parsley (Jahnen and Hahlbrock, 1988). The detection of furanocoumarins in the IWF was reminiscent of pathogen-infected parsley leaves, which secreted furanocoumarins into infection droplets (Tietjen et al., 1983; Scheel et al., 1986). Apiin has previously been reported to increase about 10-fold

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Table I. *Time sequence of ozone-induced stress reactions in parsley*

The onset of ozone treatment is defined as time zero. Postincubation in ozone-free air started after 10 h of ozone exposure.

Time	Reaction
h	
3	Transcript accumulation of early genes (PR-1, PR-2, Eli 16)
6	Transcript accumulation of intermediate genes (PAL, 4CL, CHS)
9–10	Maximum of PAL activity
	Leaf spots as first visible damage
	Accumulation of flavone glycosides
12–14	Transcript accumulation of late genes (POD, HRGP)
	Maximum accumulation of flavone glycosides
	Accumulation of furanocoumarins
	Maximum activity of XMT and CAD
	Reduction of total ascorbate, induction of apoplastic ascorbate (Eckey-Kaltenbach et al., 1993)
	Apoplastic accumulation of apiin (Eckey-Kaltenbach et al., 1993) and of furanocoumarins
24	Maximum accumulation of furanocoumarins
	Start of leaf necrotization
48	Accumulation of callose, necrotic fluorescence
	Renewed transcript accumulation of PAL, 4CL, and CHS
72	Renewed increase of PAL and CAD activities and of flavone glycosides
	Complete necrosis and death of leaves

in the IWFs of ozone-treated parsley plants (Eckey-Kaltenbach et al., 1993).

The present results revealed an approximate 2-fold increase of flavone glycosides as well as furanocoumarins after a standard ozone fumigation (200 nL L^{-1}; 10 h). The ozone-induced accumulation of total flavone glycosides showed an early transient increase as well as a late secondary one. This induction pattern appeared to be due to a biosynthesis of flavone glycosides followed by metabolism or degradation and a renewed biosynthesis. This type of induction kinetics was also observed for enzyme activities and transcript amounts. The early increase in flavone glycosides can be compared to the UV response of intact parsley plants (Schmelzer et al., 1988). The later secondary increase appeared to be connected to complete necrosis development. The plants were kept under constant light to minimize diurnal rhythms and stomatal limitations for ozone uptake. However, the constant light regime leads to the possibility that ozone merely increases the induction potential of the applied irradiance rather than being a direct inducer. A similar explanation could apply for the report concerning the 1.8-fold apiin induction by a jasmonate precursor in light-grown parsley cells (Dittrich et al., 1992).

In the present study, there was a simultaneous induction of the furanocoumarins, which generally are not light induced (Hahlbrock and Scheel, 1989). The furanocoumarins showed high constitutive levels in healthy parsley plants. On this elevated background, the ozone-dependent 2-fold accumulation in total leaf extracts was highly significant. Previous attempts to demonstrate the induction of the furanocoumarin pathway in total leaf extracts after infection with *P. megasperma* f. sp. *glycinea* zoospores had failed (Knogge et al., 1987). An approximate 2-fold increase after ozone treatment was found for the total amount of furanocoumarins as well as for the individual components.

On the basis of the time course of transcript accumulation, early, intermediate, and late stress genes could be defined. The three early genes for PR-1, PR-2, and Eli 16 were strongly induced at 3 h. The induction of intracellular PR proteins in parsley has also been described as one of the earliest effects after fungal infection (Somssich et al., 1989). In addition, β-1,3-glucanase, chitinase, and PR protein 1b have been shown to be ozone induced in tobacco (Ernst et al., 1992b). POD and HRGP were determined to be late stress genes. These genes are known also to be induced by fungi and other stressors (Somssich et al., 1989; Sauer et al., 1990; Sutherland, 1991). In the phenylpropanoid pathway, ozone-induced transcript accumulation preceded the increase of enzyme activity as well as product accumulation.

In conclusion, all pathogen-responsive cDNA probes tested (PR-1, PR-2, Eli 16, PAL, 4CL, HRGP, POD) are shown here to be induced also by ozone treatment. However, ozone was an inducer that differed from the previous stressors in that the two distinct defensive pathways of parsley were induced simultaneously as shown in Figure 6. Ozone is thus termed a cross-inducer. In addition, parsley cDNA libraries have been constructed with poly(A)$^{+}$ RNA isolated from ozone-treated leaves. A number of ozone-induced, as well as ozone-

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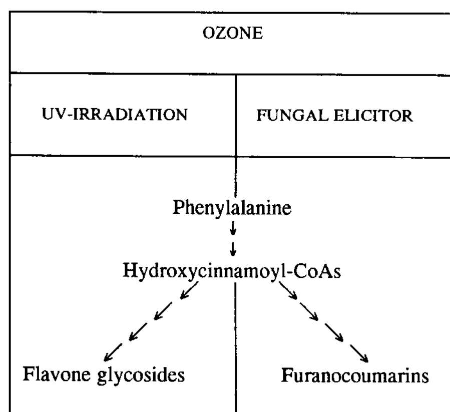

Figure 6. Model for the effects of ozone, UV irradiation, and fungal elicitor on the defensive pathways in parsley.

repressed, cDNA clones were selected. First, sequence analysis resulted in the identification of clones for PR 1–1, PR 1–3, and a small heat-shock protein (Ernst et al., 1992a, and our unpublished results).

An ozone induction of defense reactions was observed previously in pine, in which ozone treatment led to an increase of stilbene phytoalexins and stilbene synthase in the needles (Rosemann et al., 1991). Stilbenes are normally present constitutively in heartwood and are induced by fungi as phytoalexins in pine softwood. In spruce, ozone led to the induction of polyamines (Sandermann et al., 1990) and CAD (Galliano et al., 1993). Both responses are well-known plant defensive reactions. In tobacco, fungal and viral defense reactions (polyamines, ethylene, tyramine, stress proteins) were also induced by ozone (Sandermann et al., 1989; Schraudner et al., 1992). Cross-induction of plant defense pathways by ozone may, therefore, be a fairly general phenomenon.

All of these results lead to a number of scientific as well as practical questions. For example, it has to be clarified how the signal chains for flavone glycoside and furanocoumarin induction can be activated at the same time. How similar are the defense programs induced in plants by different stressors? It is interesting that induction of the two known defense pathways was not additive in cultured parsley cells upon simultaneous treatment with UV light and elicitor, which completely blocked the light-induction of flavone glycosides (Lozoya et al., 1991). Does the ozone induction of flavone glycosides and furanocoumarins occur in their known histological areas (epidermis and oil ducts, respectively) or in the areas of developing visible ozone lesions? A practical question is whether cross-induction of defensive pathways by ozone will also occur with crop plants and weeds in the field. Recent experiments with tobacco have indeed shown that β-1,3-glucanase (Schraudner et al., 1992) and ethylene metabolism (our unpublished results) react strongly to present ambient summer ozone concentrations. Changes in disposition for plant diseases are, therefore, expected (Sandermann et al., 1989), but this important possibility remains to be examined in further experiments.

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