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Ozone-Induced Changes in Soybean Cell Wall Physiology

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INTRODUCTION

One effect of ozone (O_3) on plants is a stimulation of phenylpropanoid metabolism similar to that induced by microbial attack (10). Defense responses involving phenylpropanoids include synthesis of isoflavonoid phytoalexins and reinforcement of cell walls by deposition of lignin and related wall-bound phenolics (1). Many phenolics autofluoresce, and this can be used to localize lignin, suberin, or other phenolic compounds histologically. Lignification, suberization, and the binding of phenolics to cell walls has been correlated with peroxidase activity (2, 8, 9). Increased peroxidase activity, which typically occurs in response to O_3 or microbial infection, may thus affect cell wall composition. These responses are important to understand because they cause major changes in cell wall structure and physical characteristics. One physical property of the cell wall, the volume elasticity, can be estimated from water potential-volume data obtained with a pressure bomb. The objective of this research was to examine the chronic effects of O_3 on soybean cell wall physiology using parallel physical, biochemical, and histological methods.

METHODS AND MATERIALS

Soybeans (*Glycine max* L. Merr. cv Essex) were grown in pots in open-top field chambers and treated with either charcoal-filtered air (CF) or 1.5x ambient O_3 12 h daily. Essex soybeans were also grown in the greenhouse for 35 d and then transferred to continuously-stirred tank reactors (CSTRs) where they were treated with either CF air or CF air plus 0.10 $\mu L\ L^{-1}$ O_3 6 h daily.

Leaf pressure-volume curves obtained from plants grown in open-top chambers were analyzed according to the following equation:

$$\Psi_{lx} = \frac{R\,T\,N_s}{V_o - V_e} + P_{max}\,EXP\,(z\,V_e) \qquad (4, 11)$$

where ψ_{lx} is the leaf xylem pressure potential, N_s is the number of osmoles of solute contained in the symplast, V_o is the symplastic volume at full hydration, P_{max} is the turgor at full hydration, V_e is the volume of sap expressed from the leaf, and z is a coefficient defining the rate of change of turgor with volume.

The activity of soluble *p*-phenylenediamine-pyrocatechol oxidase (PPD-PC) and syringaldazine oxidase (SYR) was measured in leaf extracts. *p*-Phenylenediamine-pyrocatechol oxidase is a nonspecific peroxidase, but SYR activity has been histochemically located in lignifying tissues (3). Activities of PPD-PC and SYR were assayed with modifications according to Goldberg *et al.* (3) and Imberty *et al.* (5).

In addition, leaf tissues were periodically examined using UV-fluorescence microscopy to detect changes in phenolic composition (2). Fresh sections were examined using differential interference contrast and epifluorescence optics (UV330-380 excitation filter and 420 nm barrier filter).

RESULTS

The pressure-volume analysis showed that cell wall elasticity decreased in response to O_3. We found that the coefficient z was 31% lower in O_3-treated plants compared with CF air plants ($p<0.03$), suggesting less tissue flexibility.

For plants grown in open-top chambers, soluble peroxidase activity (both PPD-PC and SYR) increased 19 d after planting and thereafter in O_3-treated leaves. The activity of SYR in the O_3 treatment was 17 times greater than that in controls by 75 d after planting. Increased peroxidase activity was also observed in plants treated 7 d with O_3 in a CSTR.

Ultraviolet-fluorescence microscopy revealed vivid cell wall autofluorescence in O_3-treated leaves (Fig. 1). Epidermal and mesophyll cell walls autofluoresced

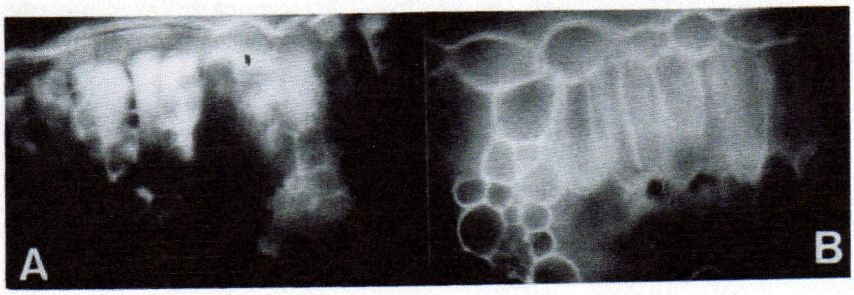

Figure 1. Ultraviolet-fluorescence of transverse fresh sections of mature soybean leaves from plants treated 13 d in CSTRs with either CF air (A) or CF air plus 0.10 $\mu L\ L^{-1}$ O_3 (B). Note that epidermal and mesophyll cell walls in only the O_3-treated tissue autofluoresced blue. The autofluorescence in the O_3-treated tissue indicates that cell walls were impregnated with phenolic compounds (2). Phenolics in the xylem vessels autofluoresced blue in tissues from both treatments.

blue in O_3-treated plants. In contrast, epidermal and mesophyll cell walls in CF-treated plants did not autofluoresce.

DISCUSSION

Results from our experiments indicate that treatment of soybean plants with O_3 caused physical and chemical changes in cell walls. These results are consistent with a hypothesis that O_3 induces plant defense responses commonly associated with pathogenesis and oxidative stress (7). Impregnation of the cell wall with phenolic esters, suberization, and perhaps lignification appear to be elicited defense responses for stabilizing the cell wall architecture against microbial degradation (1). Autofluorescence and the accumulation of cinnamic esters and suberin at infection sites in plants have been observed (6, 9). As shown by our parallel physical, biochemical, and histochemical methods, cell walls in O_3-treated soybean leaves share several features of these elicited plant defense responses.

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