Response of soybean leaf water relations to tropospheric ozone

Edwin L. Fiscus, Fitzgerald L. Booker, Joseph E. Miller, and Chantal D. Reid

Abstract: Tropospheric O_3 and water stress cause significant reductions in crop growth and yield. The effects of chronic O_3 exposures on leaf water relations have been less thoroughly studied. Soybeans were grown in two years in open-top field chambers equipped to control O_3. The seasonal mean O_3 concentrations for the charcoal-filtered controls and supplemental O_3 treatments were 24 and 83 nL $\cdot$ L^{-1} for the first year (1990) and 20 and 99 nL $\cdot$ L^{-1} for the second year (1992). In 1990 leaves were sampled during four intervals of the 106-d growing season and subjected to potential$-$volume analysis. In 1992, leaves were sampled over a 3-week period, centered on 49 days after planting for potential$-$volume analysis as well as for midday xylem pressure potentials and leaf conductance. Ontogenetic changes in most of the parameters were large compared with treatment effects. O_3 treatment consistently caused decreased symplastic volume, specific leaf mass, and tissue elasticity. In 1992, these effects were accompanied by decreased leaf conductances with no discernable change in xylem pressure potential, although midday turgor increased by 32% and stomatal competency was maintained. Tissue elasticity decreases may be related to O_3-induced changes in cell wall structure during leaf expansion and may result in decreased symplastic volume.

Key words: *Glycine max*, ozone, leaf water relations, pressure$-$volume analysis, elasticity, elastic modulus.

Résumé : Les effets d'une application continuelle d'O_3 sur les relations hydriques des feuilles sont peu connus. Le soja fut cultivé en plein champs avec chambres à environnement permettant le contrôle d'O_3. Les moyennes saisonnières d'O_3 étaient 24 et 83 nL $\cdot$ L^{-1} pour les contrôles et pour l'O_3 additionel en 1990, et 20 et 99 nL $\cdot$ L^{-1} en 1992. En 1990, les feuilles furent échantillonnées 4 fois pendant la saison de croissance de 106 jours et furent analysées par courbe pression$-$volume. En 1992, les feuilles furent échantillonnées 3 semaines consécutives, et furent analysées par courbes pression-volume, potentiel hydrique du midi, et conductance foliaire. Pour la majorité des paramètres étudiés, les changements ontogénétiques étaient plus grands que l'effet d'O_3. Uniformément, l'O_3 réduisait le volume symplastique, la masse foliaire spécifique, et l'élasticité du tissu. En 1992, ces effets étaient accompagnés par une réduction de la conductance foliaire sans changement perceptible du potential hydrique du midi malgré que la turgescence du midi augmentait de 32% et la compétence des stomates était maintenue. La réduction de l'élasticité du tissu peut être reliée à des changements de la structure des parois cellulaires provoqués par l'O_3 pendant le développement foliaire, et il peut s'ensuivre un volume symplastique réduit.

Mots clés : *Glycine max*, ozone, relations hydriques des feuilles, analyse pression$-$volume, élasticité, module elastique.

Introduction

Tropospheric O_3 currently is recognized as the single most phytotoxic regional air pollutant and already may be responsible for a 15% decrease in production of some crops (Heck 1989). It therefore is important to determine as much as possible about the mode of action of this stressor and the secondary effects of chronic exposures.

It has been shown that concomitant water stress can moderate the effects of high levels of tropospheric O_3 (Miller et al. 1988), presumably by limiting access of the O_3 to the mesophyll via the stomata. Conversely, the stomatal response can also be affected by the presence of O_3. The most commonly reported stomatal response to O_3 fumigation in a wide variety of plants, as either a direct or an indirect effect, is a decrease in conductance (Chevone et al. 1990; Hill and Littlefield 1969; Olszyk and Tibbitts 1981; Reich et al. 1985; Reich 1987; Runeckles and Rosen 1974; Winner et al. 1988). Reich and Lassoie (1984) reported that the stomatal response to O_3 depended on leaf age and incident photon flux in *Populus* and concluded that O_3 exposure resulted in impaired stomatal function and the inability to control water loss. Barnes et al. (1990) and Maier-Maercker and Koch (1991) also concluded that chronic O_3 exposures resulted in a loss of stomatal control in *Picea abies*.

The effects of O_3 on the bulk leaf water relations have been studied much less than stomatal response. Hoffman et al. (1973) failed to find any influence of chronic O_3 exposures on leaf water relations of pinto bean. Likewise, Frost et al. (1991) found no effects of chronic O_3 fumigation (50 nL $\cdot$ L^{-1} for 16 h $\cdot$ d^{-1} for 27 d) on the total potential, solute potential, or yield turgor of expanding hybrid poplar leaves, even after additional episodic exposures of 120 nL $\cdot$ L^{-1}. In contrast, Dobson et al. (1990) reported large changes in leaf water relations in Sitka spruce, including a large decrease in elasticity, and Bender et al. (1991) reported that O_3 initially increased the osmotic potential in bush beans but decreased it later. In related work, Söber (1992) inferred decreased tissue elasticity in bean leaves, and Booker et al. (1991) reported decreased soybean leaf elasticity as a result of O_3 exposure. The purpose of this paper is to examine the osmotic and turgor relations of fully expanded soybean leaves in some detail to determine if O_3-induced changes in the bulk leaf water relations are significant in the context of the normal developmental sequence and the overall plant water balance. For this experiment we used a soybean cultivar that has been reported in preliminary studies to be sensitive to O_3.

Materials and methods

The overall experiment consisted of two parts. The first part was conducted in 1990 as a subset of a larger interaction study and the second part in 1992, also as a subset of a larger experiment.

1990

During 1990, soybeans (*Glycine max* (L.) Merr. cv. Essex) were grown in 15-L pots in a 2:1:1 (by volume) mixture of soil, sand, and Metromix 220 (Grace Horticultural Products, W.R. Grace and Co., Cambridge, Mass.) in open-top field chambers (Heagle et al. 1979) at Raleigh, North Carolina.

Plants were watered daily and fertilized biweekly with Peters Blossom Booster 10−30−20 (N−P−K) and three times during the season with Peters S.T.E.M. soluble trace elements and micronutrient mix (Peters Fertilizer Products, W.R. Grace and Co., Fogelsville, Pa.).

The experiment was a randomized design of three replicates, with two O_3 levels, CF control, and supplemental O_3, for a total of six chambers. All chambers were sampled four times throughout the experiment by taking three leaves from each chamber. Individual plants were sampled only once. Due to the time required for the potential−volume processing, each sampling period lasted several days and the day given for each sampling period is the midpoint. The sampling sequence among the chambers was randomized for each period. The seasonal data were analyzed by analysis of variance, and the period effects and treatments within each period were compared by LSD.

The O_3 treatments of CF air and O_3 additions to a final concentration of $1.7\times$ ambient were administered over a 132-d period starting at germination. The seasonal 12-h mean [O_3] in the CF and supplemental O_3 treatments were 24 and 83 nL $\cdot$ L^{-1}, respectively. Further details of experimental design, plant cultural conditions, growth, and exposure methodologies may be found in Miller et al. (1994).

1992

In 1992, the experiment was again a subset of a larger interaction study, with the same experimental design as 1990. During this experiment plants were cultured as described above except that they were grown in open-top chambers supplied with CF air for 34 d at which time half were transferred to chambers receiving supplemental O_3. In this case, exposures were conducted as constant additions for 7 h per day over a 30-d period. The 30-d, 7-h mean [O_3] in the CF and supplemental O_3 treatments were 20 and 99 nL $\cdot$ L^{-1}, respectively. This 30-d period spanned sampling periods 1 and 2 of the 1990 experiment, but potential−volume analysis was conducted throughout this experiment at 3- to 4-d intervals. Midday leaf conductances were measured with a model 1600 steady-state porometer (LI-COR Inc., Lincoln, Nebr.) and midday xylem pressure potentials (Soil Moisture Equipment Corp, Santa Barbara, Calif.) were measured. Parameters were again compared by analysis of variance.

Potential−volume analysis

In all experiments, leaves for potential−volume ($P-V$) analysis were taken from the fourth node from the apex of the main stem within 1.5 h after sunrise to assure a high water potential and to avoid the problems associated with rehydration (Meinzer et al. 1986). Leaves were enclosed in a plastic bag containing a moist paper towel and severed from the plant. Within 5 min after cutting they were placed in a pressure chamber lined with moist toweling. After the initial balance pressure was determined, the leaf was overpressurized for 15−20 min. Pressure was then brought below the previous balance pressure, and the expressed sap, which had been collected on absorbent material in contact with the cut petiole surface, was weighed. The new equilibrium balance pressure then was obtained. This sequence was repeated a sufficient number of times to obtain a complete $P-V$ curve as determined by on-line data plots. The BP$-V_E$

Table 1. Analysis of fitted and calculated $P-V$ parameters for ontogenetic changes in 1990.

Source	n	V_O (cm^3)	z (cm^{-3})	ε_{max} (MPa)	P_{max} (MPa)	N_S (mosmol)	TLP (MPa)	RSV$_{TLP}$	SLW[†] (mg·cm^{-2})
Period 1 (38 DAP)									
Filtered	3	1.3434a	−18.060a	23.57a	0.96ac	0.52a	−1.23ac	0.780a	3.43a
O$_3$	3	0.6238Ans	−22.718Ans	12.40Ans	0.86A*	0.21Ans	−1.15Ans	0.742Ans	3.00A***
Period 2 (58 DAP)									
Filtered	3	3.5093b	−6.999b	22.33a	0.93a	1.26b	−1.18a	0.792a	3.50a
O$_3$	3	2.5751Bns	−10.496Bns	28.83AB*	1.08B*	1.11Bns	−1.35Bns	0.803ABns	3.34ABns
Period 3 (72 DAP)									
Filtered	3	3.5390b	−6.276b	23.27a	1.08bc	1.54b	−1.38c	0.780a	4.21b
O$_3$	3	3.0973Bns	−13.921B*	42.17Bns	1.08Bns	1.36Bns	−1.28ABns	0.845B*	3.58B**
Period 4 (100 DAP)									
Filtered	3	3.6233b	−3.903b	15.17a	1.13b	1.63b	−1.53bc	0.733a	nd
O$_3$	3	4.3187Cns	−3.739Cns	18.90Ans	1.06Bns	1.87Cns	−1.41Bns	0.749Ans	nd
Ontogenetic	12	****	***	ns	***	****	***	**	***
Ontogenetic change		2.7×	4.6×	—	1.2×	3.1×	1.3×	1.1×	1.2×

NOTE: Days after planting (DAP; DAP$_0$ = 151) is the midpoint for each sampling period, each of which spanned 12 d. Significance of the O$_3$ treatment effect for each period is indicated as follows: ****, $P \leq 0.001$; ***, $P \leq 0.01$ **, $P \leq 0.05$; *, $P \leq 0.01$; ns, not significant ($P > 0.1$). Significant differences ($P \leq 0.05$) between sampling periods are indicated by different letters within the same column following the means (lower case for controls and upper case for O$_3$). Season-long ontogenetic changes for each parameter are calculated as the largest absolute value divided by the smallest. Statistical significance of ontogenetic changes is given for control plants only. n, number of samples; nd, no data.
[†]Estimated from leaf disks.

data pairs were then analyzed according to the following model:

$$[1] \quad \psi_{lx} = -BP = -\frac{R T N_S}{V_O - V_E} + P_{max} \exp(z V_E)$$

The first term on the right-hand side of the equation describes the osmotic relations of the tissue and was derived by Tyree and Hammel (1972). Note that the denominator is the symplastic volume at any point on the curve ($V = V_O - V_E$). The second term was used by Hellkvist et al. (1974) to describe the relationship between turgor and symplastic volume.

Data were fitted by a nonlinear least squares program (NLLSQ, CET Research Group Ltd., Norman, Okla.) that was modified to perform the following procedure. First, a least squares fit was performed on the data. Using the coefficients thus obtained, V_E was extrapolated backwards until the absolute value of the osmotic potential equalled P_{max}. This negative increment of V_E was paired with a zero value for ψ_{lx} and added to the data set, which then was subjected to a new least squares fit. The coefficients presented in this paper were obtained from the final data fit. Thus, P_{max} and V_O represent the fully hydrated condition of the leaf.

Following determination of the four coefficients in the equation, additional calculations were performed to find the TLP, or the leaf water potential at which the average turgor cannot be readily distinguished from zero. Three methods for determining TLP were compared and the results and a brief discussion of the comparison can be found in Appendix 1. Having established TLP, RSV$_{TLP}$ (= $V_O/(V_O - V_{ETLP})$) also was calculated.

In 1990, immediately after the final balance pressure was determined, two leaf disks (21 mm diameter) were cut from the blade of each trifoliolate leaflet, oven-dried at 50°C for 2 d, weighed, and the SLW determined. In 1992 SLW was

estimated from a random sampling of whole leaves taken from the appropriate node in all the chambers at the end of the sampling period. The leaves were dried and weighed the same as the disks.

Results and discussion

Before proceeding with the discussion of O$_3$ effects on the tissue water relations, it is necessary to clarify our use of elasticity terminology and the normal ontogenetic changes observed in these studies. In the remainder of this paper we shall use the term elasticity when discussing parameters z and ε_{max}. Although z is related, it is not the same as ε, and an explanation of the relationship between the two can be found in Appendix 2.

Developmental changes

Effects of treatments and developmental age of plants are shown in Table 1. In most cases the effects of age, as indicated by the change in the parameters from periods 1 to 4, were very large compared with any treatment effects and so will be discussed first. Examination of the effect of sampling period on the parameters reveals consistent and substantial trends in all but RSV$_{TLP}$. Substantial increases were noted in V_O, z, P_{max}, and N_S. TLP also dropped to significantly lower values through the season. RSV$_{TLP}$ showed a small but nonsignificant decline, especially during the last sampling period. The differences in TLP can be simply interpreted in terms of a combined effect of increased N_S and z, and although the absolute TLP decreased by 0.4 MPa over the season, the RSV$_{TLP}$ was stable, with no significant changes.

Since $P_{max} = |\psi_{\pi 100}|$, it can be expressed as $R T N_S/V_O$. The ontogenetic increases in P_{max} (Table 1) thus can be attributed to the slightly larger increase in N_S than in V_O. Although similar seasonal changes were observed by Rascio et al. (1988) in two wheat cultivars, it is unknown whether

Fig. 1. Normal ontogenetic changes in leaf pressure−volume relationships over the course of the 1990 experiments. Each line was calculated from eq. 1 using the relevant mean values from Table 2. ——, period 1; ——, period 2; ----, period 3; ....., period 4. Lines are terminated at $V_E \simeq V_O/3$, well below the TLPs, which are indicated by the crosses on the lines and for periods 1–4 are −1.28, −1.22, −1.43, and −1.62 MPa, respectively. The potential is expressed (A) as a function of the absolute quantity of water lost from the leaf, and (B) on the basis of relative symplastic volume.
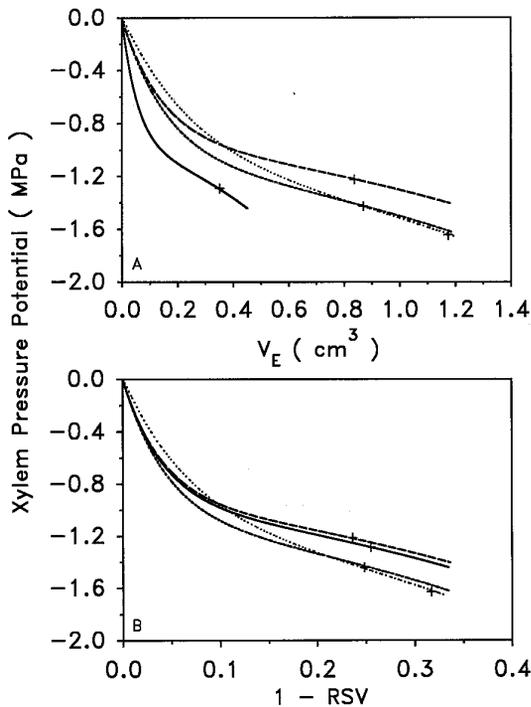
their observations could be traced to changes in N_S and (or) V_O.

Specific leaf masses (SLW) increased by 20% over the first three sampling periods in 1990. Whole leaf area and biomass sampling conducted throughout the season (Miller et al. 1994) indicated that increases in SLW continued through the final sampling period and that the final seasonal differential in SLW was probably about $1.3\times$.

Although ontogenetic differences among the relevant data in Table 1 are not always statistically significant, certain consistencies inherent in the data permit generalizations about the water relations parameters discussed thus far. First, the association between V_O and N_S suggests that the size of the symplast in these experiments is driven, and limited by accumulation of solutes. Second, consistent associations between SLW, V_O, and z indicate that smaller V_Os are associated with higher absolute values of z (less elastic tissue) and lower SLWs. Normal development proceeded from less elastic leaves with small symplastic volumes, osmotic potentials, and SLWs to more positive values with the exception of the osmotic potential, which declined.

An interesting aspect of the relationship between ε_{max} and z emerges from Table 1. Even though there are clear and major ontogenetic trends in both z and N_S, their combined

effect is to hold ε_{max} relatively stable through the first three sampling periods. Probably owing to approaching maturity and senescence, however, ε_{max} dropped by about 30% during the last sampling period.

The elasticity, as indicated by ε, is similar to the ontogenetic trends observed by Rascio et al. (1988). In one cultivar of wheat, ε held fairly constant until booting and then declined rapidly. However, in the other, more drought resistant cultivar, ε tended to decline more steadily through the season. Also, Tyree et al. (1978) noted a mechanical weakening of the cell wall prior to senescence in several tree species. These two sets of observations are consistent with the changes in elasticity reported here, suggesting an ontogenetic trend among all these species toward greater tissue flexibility.

The integrated effect of normal ontogenetic changes in parameters of leaf water relations is shown in Fig. 1, where the $P-V$ lines were calculated from the mean fitting coefficients in Table 1. Seasonal variations in the $P-V$ curves (Fig. 1A) appear to be dominated by z and ψ_π. Since ψ_π influences the expression of z, predicting the shape of the $P-V$ curve from these two parameters is not an obvious process. For example, the large change in z between periods 1 and 2 with little change in $\psi_{\pi 100}$ ($-P_{max}$) (Table 1) results in an early slope of the curve that is much steeper in period 1 than in period 2 (Fig. 1A), whereas little change occurs in TLP. In contrast, the further increase in z between periods 2 and 3 is accompanied by a decrease in $\psi_{\pi 100}$. This combination of changes results in an early slope of period 3 that is somewhat steeper than that of period 2, even though z is lower. In this instance, however, TLP decreases.

Even though there is a seasonal trend toward decreasing TLP, the large increase in absolute quantities of water loss necessary to reach TLP (Fig. 1A) can be directly attributed to larger leaf size and increased elasticity. When the water potential is plotted as a function of relative symplastic volume RSV (Fig. 1B), the apparent effects of leaf size are minimized. At the same time, the turgor relations expressed in terms of ε_{max}, and the influence of $\psi_{\pi 100}$, are emphasized. Thus, the curves from periods 1 and 2 are now nearly identical, since ε_{max} and P_{max} are virtually unchanged. In period 3, ε_{max} is still unchanged but the curve is shifted downwards as a result of the decrease in $\psi_{\pi 100}$. Finally, in the fourth period, ε_{max} decreases 35% from the previous period, whereas $\psi_{\pi 100}$ changes relatively little. The combined effect of the two parameters in the fourth period is to substantially decrease the initial slope but to cause convergence with the period 3 curve at lower RSVs and to shift the TLP to lower values. It is now in the context of these ontogenetic changes that we must interpret the O_3 effects.

O_3 exposure

Table 1 reveals that within any sampling period in 1990, there was little in the way of statistically significant effects. Both z and ε_{max} showed only occasional significant effects, but at a low level ($P \leq 0.1$). Only SLW showed an O_3 effect at the 0.05 level but only during the first and third sampling periods. The overall weakness of statistical significance of the O_3 effect during individual sampling periods was probably due to the small sample sizes. When the data were pooled over the four sampling periods (Table 2), the significance of the O_3 effect was much stronger.

Table 2. O_3 effects on the fitted and calculated $P-V$ parameters for 1990 (combined) and 1992.

Source	n	V_0 (cm^3)	z (cm^{-3})	ε_{max} (MPa)	P_{max} (MPa)	N_S (mosmol)	TLP (MPa)	RSV$_{TLP}$ (cm^3)	SLW[†] (mg · cm^{-2})
1990									
Filtered	12	3.0037	−8.809	21.08	1.02	1.24	−1.33	0.771	3.71
O_3	12	2.6537*	−12.719**	25.58*	1.02ns	1.14**	−1.30ns	0.785ns	3.31***
1992 (49 DAP)									
Filtered	6	1.7947	−15.538	22.67	0.85	0.62	−1.06	0.803	5.36
O_3	6	1.5634ns	−24.266**	35.76***	1.00***	0.62ns	−1.17**	0.833**	4.72***

NOTE: The midpoint DAP for the 1992 sampling period is indicated in parentheses after the year (DAP$_0$ = 165).
[†]Estimated from leaf disks in 1990 and from whole leaves in 1992.

O_3 additions resulted in significant ($P \leq 0.05$) seasonal decreases in z (44%) and SLW (11%) (Table 2). The case of V_0 is somewhat more complicated, with the filtered control rising to its maximum value by period 2. In the O_3 treatment, however, V_0 started out lower than the control but continued to increase throughout the season until at period 4, it exceeded the filtered control. The combined seasonal data still showed a 12% decline as a result of the O_3 treatment, but it is clear that for V_0 the perceived direction of change in response to O_3 treatment could depend on the developmental stage at sampling. Decreases in z and nonsignificant decreases in N_S combined to increase ε_{max} by about 21%, but at a low level of significance ($P < 0.1$). The increase would have been about 44%, had N_S remained constant.

The observed changes in z are in contrast with the lack of effect of O_3 on the elasticity of enlarging poplar leaves reported by Frost et al. (1991) using the Instron technique. However the relationship between the elastic properties indicated by the Instron and by $P-V$ analysis have never been well defined; thus the comparison of the two techniques may not be relevant. In addition, Frost et al. (1991) were studying effects of O_3 on cell growth and leaf expansion, whereas our leaves were chosen to represent the fully expanded condition. Given the large ontogenetic changes in z, the real significance of the observed changes due to O_3 exposure probably lies in the fact that they implicate disturbances in normal cell wall metabolism.

The combined seasonal effects of the O_3 treatments on the overall leaf $P-V$ curve did not appear to be very substantial, notwithstanding the 44% decline in z. It is clear from Fig. 2A that turgor loss will be more rapid in the O_3-treated plants and that, in combination with a smaller symplast, it will require a water loss of 30% more for the controls than for the O_3-treated plants to reach TLP. The difference in the symplastic water fraction required to reach TLP is, however, not significant (Fig. 2B; Table 2).

In 1992, sampling was more intense and the experiment confined to the time frame where the 1990 experiment indicated the plants would show the greatest differences in response to O_3. In addition, germination and early growth in CF-air prevented any acclimation to elevated O_3 which might occur. Based on comparison of Figs. 1 and 3 as well as Tables 1 and 2, it appears that the plants in 1992 were generally developmentally intermediate between periods 1 and 2 in 1990. The statistical significance of parameter differences is generally better in 1992, possibly for three reasons: (i) sample numbers were higher in 1992; (ii) average

Fig. 2. O_3 treatment effects for 1990. Each line was calculated from eq. 1 using the seasonal mean values from Table 2. ——, filtered air; ——, supplemental ozone. Lines are terminated at $V_E \simeq V_0/3$, well below the TLPs, which are indicated by the crosses on the lines, and are −1.39 and −1.35 MPa for the filtered air and ozone treatment, respectively.
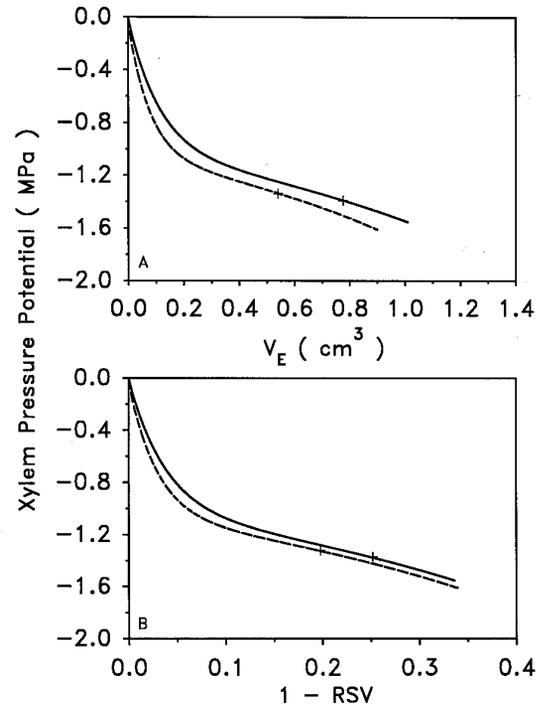

O_3 exposure levels were higher in 1992; and (iii) plant developmental stage for sampling in 1992 was chosen to emphasize the effects.

Although not significant in 1992, V_0 still showed a small decrease in the O_3 treatment. Both the decrease in V_0 and the lack of significance are consistent with the 1990 data from plants of similar age. All the other parameters in Table 2 except N_S showed significant effects at the $P \leq 0.05$ level or better. Even though the change in V_0 was not statistically significant, and there was no change in N_S in response to O_3 in 1992, they combined to yield a significant increase in P_{max}.

The decreases in elasticity noted for 1990 were confirmed

Fig. 3. O_3 treatment effects for 1992. Each line was calculated from eq. 1 using the mean values for the single sampling period from Table 2. ———, filtered air; ———, supplemental ozone. Lines are terminated at $V_E \simeq V_O/3$, well below the TLPs, which are indicated by the crosses on the lines, and are -1.08 and -1.19 MPa for the filtered air and ozone treatment, respectively.
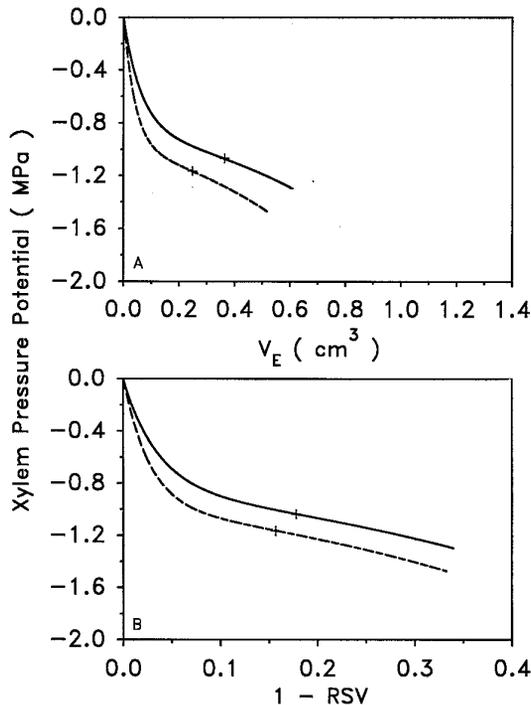
in 1992 and, combined with the increase in P_{max}, resulted in the general shape of the overall curves shown in Fig. 3. Here the effects of changes in these two parameters in response to O_3 are clear: the decrease in elasticity results in a much steeper initial slope, and the increase in P_{max} lowers the entire curve to more negative potentials. It should be noted that the effects are much more robust than might have been suspected from the combined data for 1990 (Fig. 2). However, the physiological significance of these shifts in the $P-V$ relationships to overall plant function is not obvious, even though, as in 1990, the charcoal-filtered plants could lose about 30% more water before reaching the TLP than the O_3-treated plants. However, leaves normally do not operate at or near the TLP. In fact, Tables 2 and 3 show that the midday leaf water potentials averaged only 42% of TLP and that the O_3 treatment reduced that further to 37% of TLP. Table 3 also shows that leaf conductance declined by 38% as a result of the O_3 treatment and that the adaxial conductance decreased relatively more than that on the abaxial surface. Midday leaf xylem pressure potential, however, remained unchanged by the O_3 exposure, although the turgor potential at the midday leaf water potential operating point increased by 32%. The lack of a change in the midday leaf water potential in response to O_3 resembles a typical initial response to water stress, a reduction in leaf conductance well before there is a measurable change in leaf water potential (Davies

and Zhang 1991). In the case of water stress, stomatal modulation is probably the result of an integration of plant hydraulic properties and root-generated growth regulators (ABA) being transported to the leaves (Tardieu and Davies 1993). However, decreases in water-use efficiency as a result of O_3 exposure, reported by Reich et al. (1985) for soybean cv Hodgson and in Essex soybean (J.E. Miller, unpublished data), indicate it is more likely that stomatal modulation results from damage to the photosynthetic capacity of the leaf leading to elevated internal $[CO_2]$ and subsequent stomatal closure. Similar data and interpretations were reported for Norway spruce by Wallin and Skärby (1992).

Decreased cell wall elasticity due to increased wall thickness and (or) lignification and increased leaf thickness are common responses to water stress (Cutler et al. 1977; Jones and Turner 1978; Kappen et al. 1975; Kaufmann 1981; Kramer 1969; Ladiges 1975; Levitt 1980; Sinclair and Venables 1983; Wilson et al. 1980). These responses to water stress, however, are not universal (Elston et al. 1976; Maier-Maercker and Koch 1991). Water stress also frequently is accompanied by decreases in solute potential due to osmotic adjustment. This association is interpreted as a mechanism to allow turgor, and normal physiological function, to be maintained to lower leaf water potentials (Hsiao 1973; Jones et al. 1981; Weatherley 1970). Temple (1990) has suggested that O_3, "and drought, should act to increase solute concentration in the foliage," so O_3 would, by enhancing the osmotic adjustment process, allow cotton to maintain leaf turgor at lower potentials. Our data show that in 1992 a decrease in osmotic potential, reminiscent of osmotic adjustment, occurred in soybean. In our case there was no change in the total quantity of solutes in the symplast (N_S) and the entire adjustment could be attributed to the decrease in V_O. However, Bender et al. (1991), in a study of the combined effects of water stress and O_3, reported that O_3 increased the osmotic potential in well-watered bush beans in the early part of the season but decreased it later on. The pattern in our 1990 data for each period, although not significant, is consistent with their observations. They also concluded that O_3 interfered with osmotic adjustment in drought-stressed plants. In another study of interactions between water stress and O_3, Dobson et al. (1990) reported large changes in leaf water relations of Sitka spruce in response to a single episodic O_3 exposure of 100 nL · L^{-1} for 3 h. These changes included increased ψ_π and what appears to be a very large decrease in elasticity. They attribute the former, and presumably the latter as well, to O_3-induced solute leakage from the symplast. The decreased elasticity shown in their data in response to a short-term exposure suggests that the changes they observed were due to immediate damage rather than an acclimation response. Barnes et al. (1990) also reported substantial reductions in both leaf conductance and tissue elastic modulus (ε) in Norway spruce as a result of chronic O_3 exposure. They attributed these changes in elasticity to cell wall loosening, as opposed to our observations in soybean. Although they did not specifically examine elasticity characteristics, Hoffman et al. (1973), in contrast, failed to find any influence of chronic O_3 exposures on leaf water potential or its components in pinto bean. Their observations are again consistent

Table 3. Midday leaf conductances (g_l), leaf xylem pressure potentials (ψ_{lx}), and turgor pressures (P_T) for 1992.

Treatment	g_l (cm · s^{-1})	Abaxial g_l/total	ψ_{lx} (MPa)	P_T (MPa)
Filtered	2.89 (30)	0.69 (30)	−0.45 (42)	0.42
O$_3$	1.78 (30)**	0.75 (30)**	−0.43 (42)ns	0.56

NOTE: Numbers are means of samples taken during 7 sampling days spread over a 20-d period centered around 49 DAP. The number of samples is given in parentheses. Significance as in Table 1.

with ours when taken in the context of ontogenetic changes. Combined data for 1990 showed little of the osmotic adjustment due to O$_3$ that our 1992 data clearly indicated. Also, in 1992 there was no measurable difference in the operating leaf water potential in response to O$_3$.

The changes we observed in elasticity would lead to turgor loss at a much lower level of total water loss. By promoting stomatal closure, more rapid turgor loss could result in water conservation and reduced gas exchange under much less demanding conditions than in the controls. The observed changes in elasticity without changes in $\psi_{\pi 100}$ over the 1990 season are consistent with reports of stomatal closure under conditions of chronic O$_3$ exposures (Chevone et al. 1990; Hill and Littlefield 1969; Olszyk and Tibbitts 1981; Reich 1987; Runeckles and Rosen 1974). Other observations that leaves tend to close stomata much more quickly in response to O$_3$ both under water stress conditions and at low humidities (Rich and Turner 1972) further support the role of decreased elasticity in the water relations of O$_3$-stressed plants. In addition, "premature and extensive stomatal closure" was reported by Maier-Maercker and Koch (1991) under conditions of low flux quotients (leaf uptake/transpiration) in O$_3$-damaged *Picea* needles. They felt that the combined response to O$_3$ and water stress was primarily due to a loss of stomatal control that reduced the ability of the leaf to achieve partial stomatal opening, as opposed to the fully open or fully closed states. This hypothesis is not supported by our 1992 data, which indicate adequate stomatal control even after several weeks of exposure to 100 nL · L^{-1} O$_3$. In 1992 we also observed no change in the midday water potential operating point, indicating, because of increased P_{max}, that the operating turgor was actually higher by about 0.15 MPa (Table 3). We can also estimate that in the O$_3$ treatment V_E at the operating point is reduced by 47% below the filtered air control. Given that the absolute quantities represented by the water loss at the operating point are very small to begin with (leaves were operating at 97−98% of V_O), this reduction, coupled with the increased steepness of the turgor-loss slope, may actually represent a much finer degree of stomatal control with more rapid modulation giving the appearance of "premature" closure. In any case, maintenance of the water potential operating point indicates continued stomatal competence under the circumstances of these experiments.

It is, however, difficult to see the connection between decreased tissue elasticity, as reported here, and the delignification of stomatal cells proposed by Maier-Maercker and Koch (1991) as part of the explanation for loss of stomatal sensitivity and control. There is, of course, no compelling

reason why these two processes could not proceed simultaneously, since at least part of the stomatal complex would be continuously exposed while the mesophyll cells would be partially protected from gaseous pollutants. Perhaps further research will clarify this particular point.

Implicit in observed changes in cell wall and leaf thickness due to water stress is increased SLW. Thus, the decreased SLW in response to O$_3$ stress reported here and elsewhere (Miller et al. 1988; Booker et al. 1992) adds to the dissimilarities in responses to water stress. We suggest, therefore, that although the direction of change in elasticity is the same in water and O$_3$ stresses, the changes likely occur by different mechanisms. Taken together, the decrease in SLW suggests that decreased elasticity is more a result of altered cell wall structure than of substantial increases in deposition of wall material. Booker (1993) reported some changes in cell wall composition in soybean leaves after O$_3$ treatment which may be related to decreased elasticity. He found that levels of lignothioglycolic acid (derivatives of phenolic polymers) and esterified hydroxycinnamic acids increased in cell wall material after O$_3$ treatment. In addition, blue autofluorescence of parenchyma cell walls, which is indicative of increased phenolic content, has also been observed in O$_3$-treated soybean leaves (Booker et al. 1991). Another possibility yet to be explored is oxidative cross-linking and insolubilization of structural proteins in the cell wall (Bradley et al. 1992), perhaps in covalent association with phenolic polymers (Liyama et al. 1994). Covalent cross-linking between cell wall polymers could contribute to wall strengthening and reduced extensibility, accounting for decreased elasticity and the smaller cell size implied by reductions in V_O and SLW. Such a response might also explain the speed of the O$_3$-induced changes noted by some workers.

Summary

Soybean undergoes major ontogenetic changes in all the $P-V$ parameters studied except for RSV$_{TLP}$. O$_3$ treatment effects must be considered in the context of these ontogenetic changes, which are generally larger. The combined effects of all the changes describe a normal developmental sequence going from relatively small, inelastic leaves with a low solute content to larger, thicker, more elastic leaves with higher solute content. In general, these associations between symplastic volume and the other parameters were consistent across treatments. Because of the magnitude of the ontogenetic changes, O$_3$ effects on P_{max}, TLP, and RSV$_{TLP}$ were difficult to detect on a season-long basis. Although unchanged throughout most of the season, ε_{max} declined during the last sampling period.

N_S was unaffected by O$_3$, but the reduction in V_O resulted in an apparent osmotic adjustment. Leaf conductance was reduced by O$_3$ with no detectable effect on midday ψ_{lx}. There was no apparent loss of stomatal control, but changes in elasticity and P_{max} shifted the midday water potential operating point from 42 to 37% of TLP. At the same time, however, the turgor potential at the operating point increased by 32% (Table 3). Associated decreases in SLW suggest that O$_3$-induced decreases in tissue elasticity are probably due to

changes in cell wall structure rather than depositions of additional cell wall material. In any case, these changes signal O_3-induced disturbances in cell wall metabolism.

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Appendix 1

Since the best criterion for establishing TLP is still somewhat controversial, it was estimated using the three most widely acknowledged approaches: (*i*) accepting an arbitrary value of 0.01 MPa; (*ii*) accepting an arbitrary value of 1% of P_{max} (Sinclair and Venables 1983); and (*iii*) calculating V_E at the inflection point (second derivative = 0) of eq. 1 (V_{ETLP}) (Jane and Green 1983).

The three methods for determining TLP and RSV_{TLP} are compared in Table A1. The means of the three methods for both calculated parameters are very close. The only notable difference among the methods is that the coefficient of variation for the second derivative is approximately half that of the other two methods. Because of this and since the inflection point is a more analytical method, we used the second derivative technique exclusively in analyzing the data in this paper. Although this method for determining TLP conforms to our interpretation of the approach that Jane and Green (1983) suggested as a way to overcome the inherent difficulty of assigning a TLP to an exponential turgor model, it must be noted that the TLP defined in this fashion (or by either of the other two methods) does not represent a precise symplastic volume where tissue turgor is zero. Further, the inflection point is not where turgor reaches zero, nor indeed, as suggested by Jane and Green (1983), where the slopes of the osmotic and turgor components are equal but only where the slope of the turgor component becomes small in relation to the slope of the osmotic potential.

Table A1. Means comparison of three methods of calculating the turgor loss potential (TLP) and the relative symplastic volume (RSV) at the TLP.

Variable	Method	Mean	Standard deviation	Standard deviation / mean
TLP (MPa)	Second derivative	−1.406	0.177	0.126
	1% of P_{max}	−1.402	0.284	0.202
	0.01 MPa	−1.404	0.285	0.203
RSV_{TLP}	Second derivative	0.755	0.052	0.069
	1% of P_{max}	0.767	0.106	0.138
	0.01 MPa	0.765	0.105	0.138

NOTE: $n = 72$ for all variables.

Appendix 2

The volumetric elastic modulus, usually defined as $\varepsilon = V (dP/dV)$ (Hellkvist et al. 1974), indicates the rate of change in turgor with tissue volume at any particular volume. On the other hand, z conveys the natural log of the "relative turgidity" at any particular V_E (i.e., $z = \ln (P/P_{max})/V_E$). In this sense,

z conveys a similar sort of information and if we expand ε according to the exponential turgor model of eq. 1, using the expressed volume terminology, we can write the volumetric elastic modulus in terms of z as

[A1] $\quad \varepsilon = -(V_O - V_E)\, z\, P_{max} \exp(z\, V_E)$

which is the same in principle as that derived by Sinclair and Venables (1983). With the tissue at full hydration ($V_E = 0$), eq. A1 simplifies to $\varepsilon_{max} = -V_O\, z\, P_{max}$. Further, we can replace P_{max} with the negative of the equivalent osmotic term and write $\varepsilon_{max} = -z\, R\, T\, N_S$, which expresses the dependence of the elastic modulus on both z and the solute content of the symplast. The conceptual relationship between these two coefficients (z and ε_{max}), which are based on similar physical considerations, becomes clear if we consider what might happen in a tissue with constant cell wall composition and structure. If we start with the tissue at full hydration, we can calculate particular values for z and ε_{max}. Consider what would happen if we then were able to increase N_S in the presence of an external source of water. Obviously, water would move into the symplast in response to the new osmotic gradient, and the tissue would expand slightly. The turgor would increase in proportion to the added solute, and the cell walls would be further strained to accommodate the increase, however slight, in V_O. The nature of ε_{max} is that it would increase in proportion to the added strain, whereas z would remain constant. Both coefficients provide informative and equally legitimate ways of interpreting the relationships between volume and turgor, and both are phenomenological in nature; thus, in the current context, either can be used in a diagnostic manner.