Growth-induced water potentials originate from wall yielding during growth

John S. Boyer

College of Marine Studies and College of Agriculture and Natural Resources, University of Delaware, 700 Pilottown Road, Lewes, DE 19958, USA

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Abstract

Multicellular plants display growth-induced water potentials that generate tensions on water in the apoplast and move water into the growing cells. The potentials are sometimes assumed to arise from wall yielding, keeping the turgor pressure below what otherwise would occur. There has been no direct test of this theory, and therefore whole plants or growing regions of stems (hypocotyls) of dark-grown soybean (Glycine max L. Merr.) seedlings were sealed in a pressure chamber, and wall yielding was decreased by applying external pressure. In whole plants, external pressure had little effect because the plants and water supply were uniformly exposed to the pressure. If pressure was applied to the stem while the roots were outside in water, stem elongation was markedly inhibited because the pressure raised the water potential of the growing region and decreased water entry, reducing wall yielding. Further increasing the pressure prevented water entry completely and measured the tensions in the apoplast in the same growing regions. Tensions were about 0.19 MPa at low external pressure, but diminished as wall yielding was inhibited. At external pressures of about 0.63 MPa, wall yielding was abolished and tensions approached zero. There was a linear relation between wall yielding and tension, supporting the theory that wall yielding lowers the turgor thus causing most of the growth-induced water potential.

Key words: Turgor pressure, osmotic potential, tension, growth, Glycine max L. Merr.

Introduction

In multicellular plants experiencing little transpiration, rapidly growing tissues have a water potential ($\Psi_w$) significantly lower than adjacent mature tissues (Cavalieri and Boyer, 1982; Fricke et al., 1997; Fricke and Flowers, 1998; Martre et al., 1999; Nonami and Boyer, 1993; Westgate and Boyer, 1985). The difference in $\Psi_w$ between these tissues is considered to be growth-induced (Molz and Boyer, 1978) and brings water into the cells for expansion of the cell volume as an essential part of the growth process (Boyer, 1968; Matyssek et al., 1991a, b). It was suggested that wall yielding during expansion creates the growth-induced $\Psi_w$ by preventing turgor pressure ($\Psi_p$) from being as high as it otherwise would be (Boyer, 1968; Nonami and Boyer, 1987).

So far, the wall yielding theory has received no direct test. As an alternative, it was suggested that the lower $\Psi_w$ were caused by large amounts of solute in the apoplast (Cosgrove and Cleland, 1983). When apoplastic solute was sought in growing regions of intact plants, however, the osmotic potentials ($\Psi_s$) were close to zero (Nonami and Boyer, 1987). Instead, tensions were detected (Nonami and Boyer, 1987). The tensions were on water in the apoplast and accounted for most of the growth-induced $\Psi_w$.

Because it is not known how growth-induced $\Psi_w$ form, this study was undertaken to determine whether wall yielding accounts for the low $\Psi_p$ and thus the growth-induced $\Psi_w$. External pressure ($P$) was applied to the growing tissue to prevent wall yielding (Boyer, 1968; Cosgrove, 1987; Meyer and Boyer, 1972). If wall yielding causes growth-induced water potentials, the lack of yielding should prevent tensions from forming in the apoplast.
apoplast. If wall yielding is not the cause of the growth-induced $\Psi_w$, the tensions should remain.

**Materials and methods**

**Plant material**

Soybean (*Glycine max* L. Merr. cv. Wayne) seeds were surface-sterilized in 1% NaOCl for 3 min, rinsed in tap water for 1 h, sown in vermiculite supplied with 0.1 mM CaCl$_2$ (5.0 ml g$^{-1}$ of dry vermiculite), and grown for 48 h. The seedlings were selected for uniformity, transplanted to identical medium, and used for the experiments after 24 h. Growth was at 29 °C in the dark in a water-saturated atmosphere. The seedling manipulations were carried out under a dim green safelight (maximum transmission at 525 nm and negligible transmission below 475 and above 575 nm).

**Pressure chamber measurements**

The seedlings were pressurized with air in a pressure chamber (Scholander et al., 1965). The chamber was lined with wet filter paper to prevent evaporation inside the chamber. In some experiments, the whole plant was enclosed in the pressure chamber with the roots in 0.1 mM CaCl$_2$ solution as a water supply (Fig. 1). The stem (hypocotyl) was marked with India ink prior to placement in the chamber. The plant was pressurized (Fig. 1A) and $P$ was released briefly every 3 h for a measurement of stem length from the ink mark to the top of the stem hook (Fig. 1B, C). Typically, experiments were conducted for 6 h but some continued for as long as 24 h.

In other experiments, the apical 32 mm of the stem was enclosed in the pressure chamber (Fig. 2). This tissue included all of the growing region (Matyssek et al., 1991b) with cotyledons usually attached (Fig. 2A). Mature tissue immediately below the growing region was placed in the seal for the chamber and extended outside for about 20 mm (Matyssek et al., 1991b). As the stem grew into the chamber, increasing amounts of mature tissue were present inside. The seal was adjustable and was tightened sufficiently to hold the stem against the $P$ to be used. The seal was not adjusted thereafter. This prevented solution from being released from the tissue under the seal when $P$ was applied. The pressure chamber was placed upside down so that the roots remained outside in 0.1 mM CaCl$_2$ solution as a water supply. The increased length of the stem was measured from the seal to the top of the stem hook after briefly releasing the $P$ every 3 h (Fig. 2B, C).

In order to measure the tension on water in the apoplast of the growing region, the seedling was set up as in Fig. 2, and the stem was pressurized for 6 h during which the stem elongated (Fig. 3B). Without releasing $P$, the chamber was inverted and the root system and mature stem were excised (Fig. 3C). The $P$ was adjusted ($\Delta P$) to prevent any solution from entering or leaving the cut surface of the stem for 10 min (Fig. 3D). The $P$ was then released and the stem length was measured in the same seedlings (Fig. 3E).

All the experiments were done in the growth chamber under the growth conditions in air saturated with water vapour. In addition, when measurements of $\Delta P$ were made, a cup lined with wet filter paper was inverted over the exposed ends of the cut stems to inhibit further evaporation.

**Results**

Stem elongation was generally steady when the whole seedling and root medium were pressurized together (Fig. 4A). In some instances, slight declines or increases in rate were seen after the $P$ had been applied, depending on the specific seedlings (Fig. 4A, inset), but were essentially steady on average for 24 h. All subsequent experiments were done for 6 h. High $P$ inhibited stem elongation slightly, and repeated experiments at 0.84 MPa showed elongation 75–98% of the rate in unpressurized controls (one of the experiments is shown in Fig. 4A). This experiment indicates that $P$ applied to the whole plant had only small effects on elongation.

By contrast, exposing the growing region of the stem to $P$ markedly inhibited elongation (Fig. 4B). Stems elongated at slower rates until they stopped growing at $P$ of 0.84 MPa. At each $P$, elongation was essentially steady. The stem elongation at each $P$ was considered to measure wall yielding.

In seedlings situated this way, the chamber was inverted and the roots and mature stem were excised. A thin film of solution was released from the cut cells of the stem. The film gradually disappeared as the solution...

**Fig. 1.** Experimental design for pressurizing whole soybean seedlings. Seedlings and their water source were placed in a completely sealed pressure chamber (A) and pressure $P$ was applied. Wall yielding subsequently occurred (B). Every 3 h, $P$ was released briefly and yielding was measured from the increase in stem length (C). Four seedlings were inside the chamber in each experiment.

**Fig. 2.** Experimental design for pressurizing the growing region of the stem of intact soybean seedlings. The apical 32 mm of the stem of a single seedling was enclosed in the pressure chamber while the remainder of the seedling extended outside with the roots in the water source. The stem was sealed in the pressure chamber and $P$ was applied (A). Wall yielding subsequently occurred (B). Every 3 h, $P$ was released briefly and yielding was measured from the increase in stem length (C).
Fig. 3. Experimental design for measuring apoplastic tension during wall yielding when the growing region of the stem was pressurized in intact soybean seedlings. The apical 32 mm of the stem of a single seedling was enclosed in the pressure chamber while the remainder of the seedling extended outside with the roots in the water source. The stem was scaled in the pressure chamber and $P$ was applied (A). Wall yielding subsequently occurred (B). After 6 h (B), the pressure chamber was inverted and the roots and mature stem tissue were excised (C), and the pressure was adjusted by $\Delta P$ to maintain the xylem solution at the cut surface of the stem for 10 min (D). After determining $\Delta P$, the pressure chamber was depressurized and the final stem length was measured (E). The $\Delta P$ measured apoplastic tension and the increase in stem length indicated wall yielding in the growing region.

Fig. 4. Increase in stem length when the whole seedling (A) or the growing region of the stem (B) was exposed to $P$. The experiment in (A) was conducted as in Fig. 1, and the experiment in (B) as in Fig. 2. The various $P$ were 0.00 (○), 0.21 (●), 0.42 (□), 0.63 (●), and 0.84 (■) MPa. Inset in (A) shows 24 h response. Data are means ± 1 SD of four seedlings.

was drawn into the stem because of elongation activity, and $P$ had to be increased to prevent the elongating tissues from absorbing the solution (Fig. 5). The adjustment of $P$ gave $\Delta P$. Figure 5 shows that, after the initial adjustment at 0 min, $\Delta P$ remained constant for at least 25 min. The constant $\Delta P$ indicates that the conditions were steady in the apoplast of the growing region, and dehydration of the tissue was negligible. The $\Delta P$ was about 0.18 MPa for the intact stems and 0.19 MPa if the cotyledons were removed from the same stems, indicating that the cotyledons had a negligible effect on $\Delta P$ (Fig. 5, inset table). The $\Delta P$ was considered to measure the tension on water in the apoplast (xylem and cell walls) of the growing region, as described earlier (Nonami and Boyer, 1987).

Because it was possible to vary wall yielding by varying $P$, and measure $\Delta P$ in the same growing region, the relation between yielding and $\Delta P$ was investigated. The stems elongated rapidly inside the pressure chamber when no external $P$ was applied (Fig. 6A). Rates inside the chamber and outside the chamber (undisturbed seedlings) were $0.55 \pm 0.11$ μm s$^{-1}$ and $0.53 \pm 0.07$ μm s$^{-1}$, respectively. When $P$ was increased, Fig. 6A shows that the rate decreased and became zero at $P$ of 0.63 MPa (slightly different from 0.84 MPa of Fig. 4B because a
new seed source was used). In the same seedlings, the $\Delta P$ became smaller as $P$ was increased. The $\Delta P$ approached zero when the elongation rate was slow at $P$ of about 0.50 MPa (Fig. 6B). At a $P$ sufficient to prevent elongation, $\Delta P$ became slightly negative (–0.06 MPa). Positive $\Delta P$ indicates that $P$ was increased for balance, and negative $\Delta P$ indicates that $P$ was decreased for balance. The relation between elongation rates and $\Delta P$ was essentially linear (Fig. 7).

**Discussion**

The data show that tension disappeared in the water in the cell walls when wall yielding was prevented in growing tissue. Because the tension arises from the growth-induced $\Psi_w$, the disappearance of tension indicates that the growth-induced $\Psi_w$ also disappeared. This gives strong evidence in support of the wall yielding theory for the origin of the growth-induced $\Psi_w$. Accordingly, when the walls begin to yield to $\Psi_p$ in multicellular tissues, the $\Psi_p$ is lowered and creates the growth-induced $\Psi_w$. The growth-induced $\Psi_w$ is transmitted to the apoplastic as a tension that brings water into the enlarging cells for the growth process. Cell size does not increase until water enters, and thus wall yielding has its effect until $\Psi_p$ is low enough for water to enter at the required rate.

Strictly speaking, applying $P$ to plant tissue does more than prevent wall yielding. It can cause elastic shrinkage and rapid displacement of wall polymers in addition to decreased irreversible expansion of the walls (decreased wall yielding) (Cosgrove, 1987; Nonami and Boyer, 1990). However, the elastic and rapid polymer changes are completed soon after a $P$ change (Nonami and Boyer, 1990; Prosise et al., 1999). After they are completed, the net tissue deformation becomes steady and reflects only expansion (Nonami and Boyer, 1990). Therefore, wall yielding was readily detected from measurements of steady length increases in the stems, and tension was detected in the same plants from the $\Delta P$ needed to maintain liquid at the cut surface of the stems after the roots were removed. The behaviour of the tension gave a direct test of the wall yielding theory.

It is noteworthy that $P$ did not directly inhibit stem elongation. When the whole plant was pressurized, the stems lengthened rapidly and showed only small responses. In order to inhibit wall yielding substantially, the roots had to be outside and exposed to atmospheric pressure. Under these conditions, $P$ raised the $\Psi_w$ of the tissue inside while $P$ outside remained unchanged. This decreased the force normally causing water to move into the shoot from the water outside. The $P$ inside did not dehydrate the tissue because water continued to be absorbed for stem elongation at all $P$ except the highest one, which just prevented elongation.

$P$ was used previously to prevent growth in leaves (Boyer, 1968) and stems (Cosgrove, 1987; Meyer and Boyer, 1972). Direct measurements of water uptake showed no uptake at $P$ high enough to prevent growth (Boyer, 1968). In the present work, the required $P$ were large (0.63–0.84 MPa, depending on the experiment) probably because the plants were intact and adjusted to the opposing force. Previous experiments showed that growth immediately ceased when water uptake was entirely prevented by excising the growing tissue from the water supply (Boyer et al., 1985; Matyssek et al., 1991a, b). Only small decreases in $\Psi_w$ and $\Psi_p$ occurred and were caused by wall relaxation in the absence of water entry. However, when mature tissue remained attached to the growing tissue, it acted as a water source.
Growth continued slowly and relaxation did not occur. The \( \Psi_w \) and \( \Psi_p \) eventually decreased by large amounts as the water source in the attached tissue was exhausted and/or the wall properties changed (Boyer et al., 1985; Matyssek et al., 1988, 1991a, b). There was a water source in the present experiments so that relaxation did not occur. The requirement for large \( P \) to prevent elongation suggests that wall properties adjusted in the intact plants as water entry became slower. Large \( P \) also were required in pressure block experiments (Cosgrove, 1987) and were attributed to continued wall loosening as growth was prevented.

In contrast, the \( \Delta P \) for measuring the tension was only 0.18 MPa at zero \( P \). Although the external water and roots had been removed, the remaining non-growing stem tissue acted as a water source that prevented relaxation. In the absence of relaxation, the measured \( \Delta P \) was stable. The \( \Delta P \) of 0.18 MPa compared favourably with the tension of about 0.20 MPa previously measured in similarly grown soybean stems (Nonami and Boyer, 1987). Therefore, the \( \Delta P \) was considered to measure the tension on water in the apoplast, and the effect of \( P \) was markedly different from \( \Delta P \). This difference was the key to measuring the tension, and \( \Delta P \) has been used extensively to measure xylem tensions with a pressure chamber (Scholander et al., 1965).

The xylem tensions extend into the apoplast because a thermocouple psychrometer operated at thermodynamic equilibrium measures apoplastic vapor pressures and showed that apoplastic tensions were close to those detected with a pressure chamber (isotopic psychrometer, Boyer, 1967a). When cells were killed so that only the apoplast controlled water uptake, tensions remained detectable with the pressure chamber and were similar to those in the xylem, further indicating that xylem tensions extend into the cell walls of the apoplast (Boyer, 1967b). Recently, xylem tensions were demonstrated with a pressure probe (Wei et al., 1999).

The \( \Delta P \) were large enough to account for most of the growth-induced \( \Psi_w \) of the growing region, which was about -0.20 MPa in similarly grown soybean seedlings (Nonami and Boyer, 1987). Small differences between various studies may be caused in part by root pressure that was absent when the root system was removed in the present work, but present in intact plants in other studies (Boyer et al., 1985; Cosgrove, 1987). In intact soybean seedlings, root pressures were about 0.03 MPa (Boyer et al., 1985) or 0.09 MPa (Cosgrove, 1987) inferred from changes in potential of mature tissue upon excision. In the present work, removal of root pressure by excision probably caused the \( \Delta P \) to be slightly negative when the wall yielding approached zero (Fig. 7).

Others observed similar growth-induced \( \Psi_w \) in various species (Barlow, 1986; Fricke et al., 1997; Fricke and Flowers, 1998; Martre et al., 1999). In many growing regions, the \( \Psi_p \) are lower than in the adjacent mature region (Fricke et al., 1997; Fricke and Flowers, 1998; Martre et al., 1999; Westgate and Boyer, 1985), but in soybean stems the \( \Psi_p \) is lower (Cavaleri and Boyer, 1982; Nonami and Boyer, 1993). In every instance, the \( \Psi_p \) were too low to balance \( \Psi_w \) and thus were lower than otherwise would have occurred. This contrast with the nearby mature tissue suggests that \( \Psi_p \) was kept low in the growing tissue, in support of the present findings.

From these results, it would be expected that any factor affecting growth rate would also alter the demand for water and thus the tension on water in the wall and the growth-induced \( \Psi_w \). The growth-induced \( \Psi_w \) diminished when growth was inhibited by low temperature (Boyer, 1993), low auxin supply (Maruyama and Boyer, 1994), low flux of inorganic nutrients (Fricke et al., 1997), and unfavourable auxin/cytokinin levels (Ikeda et al., 1999) in hydrated plants. Undifferentiated cells have been found next to the xylem that have a low diffusivity for water, and a significant tension had to be present to move water adequately for the growth demand (Nonami et al., 1997). As a result, a tension should be detectable in the apoplastic water as long as growth occurs. It would diminish when growth was inhibited by factors other than water availability. On the other hand, when plants were subjected to low \( \Psi_w \) around the roots, there was little initial change in the \( \Psi_w \) or \( \Psi_p \) of most of the cells in the growing region although growth nearly ceased (Nonami and Boyer, 1989). Instead, the xylem \( \Psi_w \) decreased, reversing the gradient in growth-induced \( \Psi_w \) next to the xylem. This blocked the ability of tension in the outlying tissues to move water out of the xylem, preventing water flow to the outlying tissues (Nonami et al., 1997). The rapid inhibition of growth thus resulted from a local change in the growth-induced \( \Psi_w \) gradient.

This support of the yielding theory indicates that the growth-induced \( \Psi_w \) is generated by the growth process itself and is an essential part of it. Because the growth-induced \( \Psi_w \) acts to bring water into the cells, which is the terminal step in the growth process, an inappropriate gradient in growth-induced \( \Psi_w \) can prevent growth and thus the final expression of all the earlier steps. The tensions in the apoplast that create most of the gradients are dynamic and can be rapidly altered (Nonami et al., 1997), which may explain many fluctuations in growth rates that are too rapid to be caused by altered gene expression or hormonal effects in multicellular plants.

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References


