In many temperate evergreen plant species, reductions in turgor loss point of leaves ($\Psi_{tlp}$) and leaf osmotic potential at full turgor ($\pi_{sat}$) occur from late summer to winter.

To test the hypothesis that this seasonal change in leaf water relations is driven by root temperature, we manipulated the temperature of the roots and shoots of *Cryptomeria japonica* D. Don seedlings separately. Whole-plant warming diminished the seasonal changes in shoot water relations observed in the control plants, whereas shoot warming did not. Compared with the controls, root warming diminished the change in $\Psi_{tlp}$ but not in $\pi_{sat}$, whereas cooling accelerated the seasonal changes in shoot water relations. These results indicate that: (1) temperature responses of roots are involved in the seasonal changes in $\Psi_{tlp}$ from late summer to winter; and (2) root temperature is partly responsible for the simultaneous changes in $\pi_{sat}$. Whole-plant cooling caused increased root hydraulic resistance, suggesting that seasonal changes in shoot water relations represent adaptive responses to increased root hydraulic resistance at low root temperatures.

Keywords: osmotic potential at full turgor, soil cooling, soil warming, turgor loss point.

Introduction

Reductions in turgor loss point of leaves ($\Psi_{tlp}$), the water potential at which the leaf loses turgor, and leaf osmotic potential at full turgor ($\pi_{sat}$) from late summer to winter are well-known phenomena that have been observed in a wide range of temperate evergreen plant species (e.g., Tyree et al. 1978, Colombo and Teng 1992, Norisada et al. 1996). These phenomena can be considered separately from the lowering of $\pi_{sat}$ in response to freezing temperatures in winter, which has been interpreted as a mechanism to increase plant tolerance to freezing. Because reductions in both $\Psi_{tlp}$ and $\pi_{sat}$ help plants maintain turgor under conditions of limited water supply, the seasonal decrease in both parameters could be interpreted as responses to water limitations.

It is well known that water uptake by roots is decreased at low root temperatures because of increased root hydraulic resistance (Kramer 1983), which can be attributed to lowered water permeability of cells, as well as to increased viscosity of water (Bolger et al. 1992, Wan et al. 2001, Bloom et al. 2004, Melkonian et al. 2004). Decreased root water uptake caused by low temperatures can delay transport of water to the shoot, which can in turn induce responses in shoot water relations that are appropriate for conditions of limited water supply. If the seasonal change in water relations results from decreased water uptake in response to lower root temperatures, soil temperature would be a major factor regulating this change in water relations. Although much research has been conducted on the effects of root temperature on leaf gas exchange and of plant water relations on seedling growth (e.g., DeLucia 1986, Wan et al. 1999), we found no reports in the literature on the involvement of root temperature in changes in leaf water relations from late summer to winter.

We tested the hypothesis that a decrease in soil temperature contributes to the seasonal change in shoot water relations from late summer to winter because of decreased water uptake at low temperatures. To verify this hypothesis, we first subjected rooted cuttings of *Cryptomeria japonica* D. Don, a warm-temperate conifer endemic to Japan, to a range of temperatures that seedlings experience from late summer to winter, under controlled conditions and examined the effects of low temperature on root hydraulic resistance. Then we experimentally warmed or cooled the roots of potted *C. japonica* seedlings in the field and compared shoot water relations with those of seedlings growing under natural conditions. Additionally, we warmed the whole plant or a part of the shoot and examined the effects of the treatments on shoot water relations.

Materials and methods

Temperature effects on root hydraulic resistance

Rooted cuttings of *C. japonica* cv. ‘Ayasugi’ were individually transplanted to 1/10,000-a Wagner’s pots (100 cm$^2$ surface area)
area and 18.5 cm depth) containing vermiculite and raised under natural conditions for 1 year, with the occasional supply of 5:10:5 N,P,K nutrient solution (1000-fold diluted HYPONEX, Osaka, Japan). Temperature treatments were applied to rooted cuttings with fully developed current-year leaves by placing 5–7 cuttings in a growth chamber set at 5, 11, 18, 24, or 33 °C for 1 week. At the time of the measurements, the root system was washed free of soil, excised above the root collar and immersed in water at the treatment temperature inside a pressure chamber (Model 3005, Soil and Moisture, Santa Barbara, CA), with the cut end protruding through the chamber lid. The temperature of the water in the chamber was maintained by a controller (Model CTR-240, Komatsu Electronics, Hira-tsuika, Japan), which circulated temperature-controlled water with the cut end protruding through the chamber lid. The temperature of the water in the chamber was maintained by a controller (Model CTR-240, Komatsu Electronics, Hira-tsuika, Japan), which circulated temperature-controlled water

**Plant materials and temperature treatments**

In June 1997, 3-year-old seedlings were transplanted to 7.7-l clay pots containing loamy subsoil, which were then buried in the ground at the nursery of the Experimental Station at Tanashi, University Forests, the University of Tokyo, so that the top of each pot was level with the ground surface. Four treatments were established: warming part of the shoot (shoot warming); warming the whole seedling (whole-plant warming); cooling the seedling (root cooling); and warming the whole seedling (root warming). In addition, a control receiving no treatment was established. Seedling height was about 1 m at the beginning of the experiment. Treatments, except for the whole-plant warming treatment, were applied in the field. For shoot warming, the apical 10 cm of a branch, including current-year and some older shoots of each of two individuals, was enclosed in a portable temperature-controlled chamber (MC-A, Koito, Tokyo, Japan). For the whole-plant warming treatment, the potted seedlings were transferred to a growth chamber (Koitotron S-180A, Koito, Tokyo, Japan) maintained at 25 °C. The root cooling treatment was applied by circulating antifreeze, with its temperature controlled by a refrigerated circulator (CAP-300, Tokyorika, Tokyo, Japan), through a tube coiled around the pot. The root warming treatment involved warming the soil by running heating cables through water-filled pipes surrounding the buried pots. Five seedlings were subjected to each treatment. The treatment schedules are summarized in Table 1.

**Measurement of shoot water relations**

Because *C. japonica* needles are arranged spirally and cover the branchlets completely, we examined shoots instead of isolated leaves. Shoot water relations of the current-year shoots were measured in a pressure chamber (Model 3005, Soil and Moisture) every 2 to 4 weeks from September 1997 to March 1998 and from September 1998 to January 1999 by the pressure-volume (P–V) method (Tyree and Hammel 1972). Sunlit branches were excised and recut immediately under water, and then rehydrated overnight before measurement. One branch was excised from each plant and measurements were made on two current-year shoots shorter than 10 cm from each branch. Shoot water potential at the Pmax, Pin and maximum bulk modulus of elasticity (Ebulk) were obtained from each P–V curve.

**Sugar concentrations of current-year shoots**

We determined total sugar concentrations of the current-year shoots at the time of the water relations measurements in the first year by a modification of the anthrone method (Watanabe et al. 2000). Current-year shoots were excised before dawn and immediately placed in polyvinyl bags and kept on ice until analysis. Half of each sample was analyzed for sugar and the rest was dried at 80 °C for 10 h to determine the dry mass ratio, which was subsequently used to express sugar concentrations as percentages of shoot water content and shoot dry mass. To measure sugar concentrations, we rinsed a sample weighing 50 to 300 mg (fresh mass) with reverse osmosis water, dried the sample surface with a paper towel, then crushed the sample with a mortar and pestle in liquid N2 before adding 10 ml of hot 80% (v/v) ethanol. The homogenate was centrifuged at 1870 g at 5 °C for 5 min and the supernatant decanted. The pellet was resuspended in 12 ml of hot ethanol and the suspension centrifuged. The supernatant from the second extraction was combined with that of the first and kept at –20 °C until analyzed.

After evaporating the ethanol from 250 µl of extract in a microtube, we added 300 µl of Milli-Q water, 50 µl of 0.3 N BaOH and 50 µl of 5% ZnSO4, and centrifuged the mixture at 18,500 g at 5 °C for 10 min. Next, we added 100 µl of 0.2% (w/v) anthrone in H2SO4 to the supernatant, followed by incubation at 100 °C for 8 min. The tube was then placed in ice water. We measured absorbance at a wavelength of 630 nm (A630) with a microplate reader (Model 450, BIO-RAD, Hercules, California) at 5 °C until analyzed.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Duration of treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>September 1997–March 1998</td>
</tr>
<tr>
<td>Whole-plant warming</td>
<td>December 1997–March 1998</td>
</tr>
<tr>
<td>Root warming</td>
<td>October 1997–February 1998</td>
</tr>
<tr>
<td></td>
<td>December 1997–March 1998</td>
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</tbody>
</table>
USA). We used glucose as the standard and expressed the total sugar concentrations as glucose equivalents.

**Water potential and transpiration of current-year shoots**

Transpiration rates of current-year shoots of seedlings in the control, root cooling and root warming treatments were measured with a steady-state porometer (LI-1600, Li-Cor, Lincoln, NE) for 1 to 3 consecutive days at an interval of 2 to 4 weeks from September to December 1998. We measured the water potentials of the current-year shoots with a pressure chamber before dawn and at midday at the end of November and in early December 1998.

**Temperature measurements**

Air and soil temperatures for each treatment were measured with platinum resistance thermometers (Chino, Tokyo, Japan) set 30 cm above ground and at the midpoint of the pot. Data were logged every 10 min by a data logger (SQ1250, Grant, Cambridge, U.K.).

**Statistical analysis**

Treatment effects on shoot water potential at the $\Psi_{tlp}$ and $\pi_{sat}$ were evaluated by a $t$ test at each measurement time. Treatment effects on shoot sugar concentrations were evaluated by the Mann-Whitney’s test or Dunnett’s test. Treatment effects on transpiration rates and in situ shoot water potentials were evaluated by Steel’s test.

**Results**

**Effects of low temperature on root hydraulic resistance**

The hydraulic resistance of the root system of the *C. japonica* rooted cuttings increased as the temperature decreased (Figure 1). The change in root hydraulic resistance with decreasing temperature closely paralleled the curve for the viscosity of water, suggesting that the hydraulic resistance of the root increased with decreasing temperatures, primarily as a result of the increasing viscosity of water (Figure 1).

**Treatment effects on the water relations of current-year shoots**

The $\Psi_{tlp}$ and $\pi_{sat}$ values for current-year shoots of the control plants both decreased throughout the fall (Figure 2). The decreases in $\Psi_{tlp}$ and $\pi_{sat}$ paralleled the decrease in soil temperature (Figure 3).

The whole-plant warming treatment began at the end of December 1997, when the daily mean soil temperature of the control plants was less than 5 °C (Figure 4). Values of $\Psi_{tlp}$ and $\pi_{sat}$ for the current-year shoots of the control plants maintained under natural conditions decreased further during the period that the whole-plant warming treatment was administered to the treatment plants (Figure 4). In contrast, in plants subjected to the whole-plant warming treatment, $\Psi_{tlp}$ and $\pi_{sat}$ increased by about 0.5 MPa during the 1-month treatment (Figure 4). By early January, all differences between the treatment and the control were statistically significant ($P < 0.05$, $t$ test).

In contrast to the whole-plant warming treatment, the shoot warming treatment, which began at the same time as the whole-plant warming treatment, resulted in no significant differences in $\Psi_{tlp}$ and $\pi_{sat}$ compared with shoots of the same plants maintained under natural conditions. In early January, $\Psi_{tlp}$ of the warmed and untreated shoots were $-2.72 \pm 0.08$ and $-2.86 \pm 0.03$ MPa (mean ± SD), respectively, and the corresponding values for $\pi_{sat}$ were $-2.05 \pm 0.00$ and $-2.02 \pm 0.02$ MPa (mean ± SD). By the end of the 1-month treatment, the warmed shoots showed similar reductions in $\Psi_{tlp}$ and $\pi_{sat}$ as the untreated shoots, with warmed and untreated shoot values for $\Psi_{tlp}$ of $-3.13 \pm 0.01$ and $-3.20 \pm 0.18$ MPa, and values for $\pi_{sat}$ of $-2.15 \pm 0.04$ and $-2.20 \pm 0.26$ MPa, respectively.

**Figure 1.** Temperature response of root hydraulic resistance in *Cryp-

tomeria japonica* cuttings. Error bars denote standard deviations. The
curve shows the temperature dependence of the viscosity of water ($y =
-1.13 \times 10^{-5}x^3 + 1.26 \times 10^{-3}x^2 - 6.45 \times 10^{-2}x + 2.00$, where
$y$ = relative viscosity and $x$ = temperature). Both hydraulic resistance and the viscosity of water are expressed in relative values, with the value at 25 °C set at 1.

**Figure 2.** Seasonal changes in turgor loss point of leaves ($\Psi_{tlp}$) (○) and leaf osmotic potential at full turgor ($\pi_{sat}$) (▲) of current-year shoots of *Cryptomeria japonica* seedlings under natural conditions from late summer to winter. Error bars denote standard deviations.
When soil temperatures remained above 5 °C, the $\Psi_{tlp}$ and $\pi_{sat}$ of the current-year shoots in the root warming treatment showed decreases similar to those of the control (Figure 5). However, when the mean daily soil temperature decreased to less than 5 °C as the season progressed, the $\Psi_{tlp}$ of current-year shoots in the root warming treatment decreased marginally less than the corresponding control value ($P < 0.10$, t test). By January, the difference was highly significant ($P < 0.01$, t test) (Figure 5). In contrast, the root warming treatment had no apparent effect on $\pi_{sat}$, despite a marginally significant difference in early January (Figure 5). A smaller decrease in $\Psi_{tlp}$ of current-year shoots in the root warming treatment was also observed when the treatment began in early December (data not shown).

The $\Psi_{tlp}$ and $\pi_{sat}$ values for the current-year shoots in the root cooling treatment showed decreases similar to those in the control at soil temperatures greater than 10 °C (Figure 6). However, when the mean daily soil temperature in the root
cooling treatment decreased to less than 5 °C as the season progressed, the $\Psi_{\text{tlp}}$ and $\pi_{\text{sat}}$ values of current-year shoots in the root cooling treatment decreased more than those of the control (Figure 6). This difference was generally either marginally significant ($P < 0.10$, $t$ test) or significant ($P < 0.05$, $t$ test) from the beginning of November onward.

Under natural conditions, $\pi_{\text{sat}}$ was highly inversely correlated with shoot sugar concentration, expressed as a percentage of shoot water content ($r = -0.84$; Figure 7A), and with sugar concentration expressed per unit dry mass ($r = -0.70$; Figure 7B). Both correlations were highly significant ($P < 0.001$). Current-year shoots contained less sugar per unit dry mass in the whole-plant warming treatment than under natural conditions, whereas mass-based sugar concentration of current-year shoots in the root warming treatment showed no clear difference from the controls (Table 2).

Under natural conditions, $\varepsilon_{\text{max}}$ increased as the season progressed (data not shown). Changes in $\Psi_{\text{tlp}}$ and $\varepsilon_{\text{max}}$ were calculated by subtracting the mean value of the control from the measured values of the temperature-treated seedlings at each measurement time. Compared with the control, changes in $\Psi_{\text{tlp}}$ of shoots in the temperature treatments were highly inversely correlated with changes in $\varepsilon_{\text{max}}$ ($r = -0.70$; Figure 8).

Transpiration rates per unit of shoot mass decreased in plants growing under natural conditions as the season progressed (Figure 9), and the decrease was accompanied by a decrease in stomatal conductance (data not shown). Transpiration rates were reduced by the root cooling treatment, with the magnitude of the change varying over the course of the season (Figure 9). During the first month of treatment (September), transpiration rates were generally lower in the root cooling treatment than in control treatment, but the difference was not significant (Figure 9). Transpiration in shoots in the root cooling treatment became significantly less than that of control shoots until the end of November (Mann-Whitney’s test or Steel’s test, $P < 0.05$), but no significant difference was seen in December. The root warming treatment had no significant effect on transpiration until the end of November, but became significant in December (Figure 9).

The root cooling and root warming treatments had no significant effects on predawn shoot water potential at the end of November or the beginning of December (data not shown). The only significant difference in daytime shoot water potential was a decrease in the root warming treatment in late November, but this difference was not consistent across the three measurement periods (data not shown).

Figure 6. Effects of the root cooling treatment on seasonal changes in the turgor loss point of leaves ($\Psi_{\text{tlp}}$) (●, ○) and on leaf osmotic potential at full turgor ($\pi_{\text{sat}}$) (▲, △) of the current-year shoots of Cryptomeria japonica seedlings. The root cooling treatment began in September 1998. Error bars denote standard deviations. Significant differences between plants in the control and in the root cooling treatment at each measurement time (based on the $t$ test) are indicated as * ($P < 0.10$), ** ($P < 0.05$), or *** ($P < 0.01$). Daily mean soil temperatures in each treatment are shown in the upper panel.

Figure 7. Relationship between shoot sugar concentration and leaf osmotic potential at full turgor ($\pi_{\text{sat}}$) in current-year shoots of Cryptomeria japonica seedlings. Sugar concentrations were calculated as (a) percentage of shoot water content (SWC), or (b) percentage of shoot dry mass (DM).
Discussion

Increase in root hydraulic resistance at low temperatures

Root hydraulic resistance in *C. japonica* rooted cuttings increased 1 week after subjecting the whole plant to the low temperature treatment (Figure 1). Increases in root hydraulic resistance in response to non-freezing low temperatures have been reported for tree species such as aspen (*Populus tremuloides* Michx.) (Wan et al. 2001) and olive (*Olea europaea* L. cv. Picual) (Pavel and Fereres 1998). These increases can be attributed to the physical properties of both water and the plant (Bolger et al. 1992, Wan et al. 2001, Bloom et al. 2004, Melkonian et al. 2004). The viscosity of water increases as temperature decreases, which increases hydraulic resistance irrespective of any changes in the plant. Simultaneously, however, the plant contributes to the measured hydraulic resistance by a decrease in cell permeability to water (Lee et al. 2004). Because the hydraulic resistance of the roots of the *C. japonica* rooted cuttings increased as temperature decreased, closely paralleling the increase in viscosity of water, we conclude that the increased viscosity of water with the decrease in temperature was the primary cause of the increase in hydraulic resistance.

Wan et al. (2001) reported that aspen showed a larger increase in hydraulic resistance than was predicted based solely on the increase in viscosity of water when the temperature of the solution surrounding the roots was decreased rapidly over a range of temperatures similar to those tested in our study. The smaller response by the roots of *C. japonica* may reflect acclimation during the 1-week low-temperature treatment. Rapid acclimation of root hydraulic conductivity to low temperatures has been reported in several species (e.g., Fennell and Markhart 1998, Vernieri et al. 2001). Moreover, a lower sensitivity of roots to low temperatures would be an advantage for evergreen species in temperate zones, because it would decrease the risk of daytime water deficits during the winter or early spring, when soil temperatures change more slowly than air temperatures.

### Table 2. Effects of root warming and whole-plant warming treatments on sugar concentrations of current-year shoots of *Cryptomeria japonica* seedlings.

<table>
<thead>
<tr>
<th>Date</th>
<th>Sugar concentration (% dry mass)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Root warming</td>
</tr>
<tr>
<td>October 17</td>
<td>9.5 (2.1)</td>
</tr>
<tr>
<td>December 5</td>
<td>10.4 (0.1)</td>
</tr>
<tr>
<td>December 26</td>
<td>17.1 (2.9)</td>
</tr>
<tr>
<td>January 8</td>
<td>14.1 (3.9)</td>
</tr>
<tr>
<td>January 20</td>
<td>16.6 (2.6)</td>
</tr>
<tr>
<td>February 5</td>
<td>12.1 (1.8)</td>
</tr>
</tbody>
</table>

Figure 8. Relationship between changes in leaf cell elasticity ($\varepsilon_{\text{max}}$) and changes in turgor loss point of leaves ($\Psi_{\text{tlp}}$) of current-year shoots of *Cryptomeria japonica* seedlings. Bars denote standard errors. Change values represent the value for the treated plants minus the value for the control plants.

Figure 9. Effects of temperature treatments on seasonal changes in transpiration rates of current-year shoots of *Cryptomeria japonica* seedlings. The root cooling and root warming treatments began in September and October 1998, respectively. Error bars denote standard deviations. Means for a given measurement time followed by asterisks (**) differ significantly from the control (Mann-Whitney’s test or Steel’s test, *P* < 0.05).

### Root temperatures and the acclimation of shoot water relations

The $\Psi_{\text{tlp}}$ and $\pi_{\text{sat}}$ values of current-year shoots decreased as the
season progressed from late summer to winter (Figure 2), confirming previous observations on temperate evergreen plant species (e.g., Tyree et al. 1978, Colombo and Teng 1992, Norisada et al. 1996). Decreases in $\Psi_{t lp}$ and $\pi_{sat}$ during the autumn would help maintain shoot activity during a water deficit caused by increasing root hydraulic resistance. The whole-plant warming treatment diminished the seasonal change in shoot water relations observed under natural conditions (Figure 4). Warming the whole plant by putting seedlings in a chamber would have changed air humidity and light quality as well as temperature when compared with the natural condition. Higher vapor pressure deficit could affect leaf characteristics, thereby increasing tolerance to higher evaporative demand (e.g., Roberts and Zwiazek 2001). However, because the effects of the whole-plant warming treatment diminished the seasonal change in shoot water relations, which was the opposite of that predicted, we consider that the observed change is solely the result of increased temperature. The UV radiation in the chamber would be less compared with the natural condition. Although morphological changes in leaves in response to increased UV radiation have been reported (e.g., Nogues et al. 1998), we think it is unlikely that changes in UV radiation on fully developed leaves caused the observed seasonal change in shoot water relations.

Seasonal changes in the shoot water relations of *C. japonica* seedlings as winter approached were diminished by warming the roots (Figure 5) and accelerated by cooling the roots (Figure 6), suggesting that root temperature is involved in the acclimation of shoot water relations to winter conditions. The finding that the seasonal change in shoot water relations was diminished by whole-plant warming, but not by shoot warming corroborates these results. Because the root cooling treatment encompassed temperatures that cause increases in root hydraulic resistance of *C. japonica* rooted cuttings (Figure 1), shoot water relations could have been altered. Transpiration was reduced by the root cooling treatment and increased by the root warming treatment (Figure 9), indicating that water supply was limited during the fall. The changes in transpiration were accompanied by changes in stomatal conductance (data not shown). Because leaf development in *C. japonica* seedlings ceases during the months covered by our study, changes in transpiration caused by the root temperature treatments were likely caused by changes in stomatal openness or in epidermal water conductivity. However, cell wall composition can change quickly in response to a low-temperature treatment (e.g., Zabotin et al. 1998), so we cannot rule out the possibility of changes in the composition of the epidermal cell wall, such as wax deposition. Nevertheless, we consider it more likely that changes in stomatal openness were primarily responsible for the changes in transpiration brought about by the root temperature treatments. Similar reductions in transpiration or stomatal conductance in response to low soil temperatures have been reported in many tree species (Kramer 1983, Teskey et al. 1984, Delucia 1986, Day et al. 1991, Ryyppö et al. 1998, Bergh and Linder 1999, Wan et al. 1999, Landhäusser et al. 2001, Lahti et al. 2002, Strand et al. 2002, Mellander et al. 2004).

In contrast to $\Psi_{t lp}$, $\pi_{sat}$ was unaffected by the root warming treatment (Figure 5), but was affected by the root cooling (Figure 6) and the whole-plant warming treatments (Figure 4). These results suggest that the temperatures of both shoots and roots are responsible for determining changes in $\pi_{sat}$.

**Temperature and cell wall elasticity**

Changes in $\Psi_{t lp}$ in response to the temperature treatments were accompanied by changes in cell wall elasticity (Figure 8). The $\varepsilon_{max}$ of cells in the shoots increased in the control seedlings during the fall (data not shown), and this change was affected by the root temperature treatments (Figure 8). An increase in $\varepsilon_{max}$ reflects an increase in the rigidity of the cell wall. Cell wall thickness is known to increase at low temperatures (e.g., Stefanowska et al. 1999), which makes the cell wall more rigid. Changes in $\varepsilon_{max}$ caused by the root temperature treatments may have resulted in part from changes in cell wall thickness.

It has been proposed that the chemical properties of cell walls affect their mechanical properties, although a detailed relationship between these two sets of properties has yet to be defined. Changes in cell wall elements that are known to be related to cell wall rigidity have been reported within 24 h of low temperature treatment (Zabotin et al. 1998, Janas et al. 2000, Nakamura et al. 2003, Solecka and Kacperska 2003). The mechanism of such short-term responses to low temperatures in the cell walls of leaves or roots is unknown. Thus far, changes in the cell wall composition of remote organs in response to a local temperature treatment have not been reported. Investigations on changes in cell wall composition that contribute to changes in their mechanical properties as well as on the effects of low root temperatures on the cell wall composition of leaves would shed light on the mechanism responsible for seasonal changes in the water relations of shoots.

**Origin of the sugars contributing to the lowering of $\pi_{sat}$**

Changes in $\pi_{sat}$ under natural conditions were accompanied by changes in shoot sugar concentrations (Figure 7). Because we measured only total sugar concentration, expressed as glucose equivalents, the contribution of the changes in sugar concentration to the changes in $\pi_{sat}$ could not be evaluated. The correlation between $\pi_{sat}$ and sugar concentration was significant, but relatively low (Figure 7), a result that could be attributed to simultaneous changes in shoot water content (data not shown). An increase in sugar concentrations under non-freezing low temperatures has been well documented (e.g., Equiza et al. 2001, Kontunen-Soppela et al. 2002). It could be the result of some combination of (1) inhibition of translocation of photoassimilates when sink activity decreases, (2) inhibition of translocation of photoassimilates when export activity decreases (Leonardos et al. 2003), and (3) osmotic adjustment to protect the plant against possible water deficits (Premachandra et al. 1992a, 1992b, Gebre et al. 1994). Root temperature could affect sink activity of *C. japonica* seedlings (Negishi 1966) as well as water deficits through effects on root hydraulic conductivity (Figure 1). Shoot temperature could affect source activity. Because shoot sugar concentrations were unaf-
fected by the root warming treatment (Table 2), it seems reasonable to hypothesize that both root and shoot temperatures are responsible for the observed increase in shoot sugar concentrations. The whole-plant warming treatment would have released the stresses of low temperature on both parts of the plant, resulting in the observed decrease in shoot sugar concentration (Table 2).

In conclusion, seasonal changes in shoot water relations from late summer to winter were caused by decreased soil temperatures rather than decreased air temperatures. Changes in shoot water relations caused by changes in root temperature were accompanied by changes in cell wall elasticity and shoot sugar concentrations. The mechanism by which these changes occur in response to changing root temperature requires additional investigation.

Acknowledgments

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Colombo, S.J and Y. Teng. 1992. Seasonal variation in the tissue sugar concentrations. The mechanism by which these changes occur in response to changing root temperature requires additional investigation.


