J For Res (2003) 8:153–161
DOI 10.1007/s10310-002-0021-z

ORIGINAL ARTICLE

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Leaf water relations in *Pinus densiflora* Sieb. et Zucc. on different soil moisture conditions

Received: September 11, 2001 / Accepted: December 17, 2002

Abstract To elucidate the differences in the leaf water relations of *Pinus densiflora* Sieb. et Zucc. growing in different soil moisture conditions, we examined the pressure-volume curve and the diurnal changes in the stomatal conductance, the transpiration rate, and the leaf water potential. The leaf water relations were compared using field-grown 40-year-old pine trees growing on the upper and lower parts of a slope. We also compared the leaf water relations of potted 4-year-old saplings growing at pF 4.2 and pF 1.8 soil moisture levels for almost 1 year. The values of the ratio of symplasmic water at turgor loss point to symplasmic water at saturated point (V_p/V_o) and bulk modulus of elasticity (ε) of both the adult trees on the upper part of the slope and the potted saplings growing on pF 4.2 soil moisture were higher than those values of both the adult trees on the lower part of the slope and the potted saplings growing on pF 1.8 soil moisture, respectively. The field-grown adult tree and the potted saplings growing under long-term water stress tended to reduce their stomatal conductance in response to the acute soil drying. It is suggested that *P. densiflora* growing under long-term water stress rapidly closed its stomata in response to soil drying and avoided losing water, and could also rapidly absorb water with reducing water loss because of the decrease in the leaf pressure potential derived from the high ε values.

Key words Drought resistance · Long-term water stress · *Pinus densiflora* · Pressure-volume curve · Stomatal conductance

Introduction

Pinus densiflora Sieb. et Zucc. is an evergreen conifer, widely distributed in Japan (Yoshioka 1958). Especially in the warm-temperature zone of Japan, *P. densiflora* is a major component of secondary forest and is found growing in various environmental conditions from the ridge to the lower part of mountain slopes. The physiological characteristics of trees at different site conditions of the slope, such as the differences in leaf water relations, have been studied on an eroded slope for *Chamaecyparis obtusa* S. et Z. (Mizunaga 1986), and differences in leaf water relations (Yoshikawa et al. 1992) and water dynamics (Kobayashi et al. 1993) were examined on the sand dune slopes of the semiarid region for *Salix matsudana* Koidz. For *Cryptomeria japonica* (L.f.) D. Don, the xylem pressure potential was compared (Sato and Morikawa 1976), and the differences in height growth were found to be associated with the transpiration rate and the physical and chemical properties of soil (Tange 1987; Tange et al. 1989). However, the physiological differences in *P. densiflora* at the different site conditions of the slope have not been sufficiently well researched. The soil moisture condition is greatly influenced by the part on the slope (Kubota et al. 1987; Enoki et al. 1996; Yanagisawa and Fujita 1999). The physiological differences among potted *P. densiflora* saplings growing under the different soil moisture conditions for a period of over 1 year have not been investigated and compared with those of *P. densiflora* growing on the different site conditions of a slope. Moreover, in respect of pine wilt disease, which causes severe damage in pine forests, it was pointed out that the differences in damage at different sites on a slope may be related to the differences in drought resistance influenced by the growing soil moisture conditions (Miki et al. 2001a, b). Therefore, it was necessary to elucidate the

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physiological differences of pines growing in different soil moisture conditions.

The objective of the present study was to clarify the leaf water relations in *P. densiflora* growing in different soil moisture conditions through the measurement of the pressure-volume curve, the stomatal conductance, the transpiration rate, and the leaf water potential. We used adult *P. densiflora* trees at different site conditions on the slope and potted *P. densiflora* saplings growing under different soil moisture conditions; at the pF 4.2 and 1.8 soil moisture levels, as samples.

Materials and methods

Plant materials

Japanese red pine trees (*Pinus densiflora*) approximately 40 years of age were selected in secondary pine forests. Four-year-old Japanese red pine saplings raised in pots were used for the comparison measurements.

The adult pine trees were components of pine forests on a south-facing slope in the Okayama Prefectural Nature Conservation Center (34°50′N, 134°3′E), which is located in the southern part of Okayama prefecture, western Japan. We selected three *P. densiflora* trees as sample trees in two site conditions, i.e. the upper part and the lower part of a slope. The altitude of the upper part was 279 m and of the lower part was 264 m. The soil of these pine forests was derived from granite parent material. The drainage area (Hada et al. 1994; Miki et al. 2001a, b), which is the index of the soil moisture conditions in the soil of a slope, was calculated to be 34 m^2 for the upper part and 135 m^2 for the lower part, on a topographical map of 1 : 1000. It has already been confirmed that soil moisture condition with a smaller drainage area tends to be drier than that with a larger drainage area (Miki et al. 2001a, b). The range of the diameters at breast height (dbh) of sampled trees was 12–18 cm, and that of the heights was 11–12 m, in the upper part of the slope. The range of dbh of sampled trees was 13–21 cm, and that of the heights was 14–15 m, in the lower part of the slope.

The pine saplings were transplanted in the pots in April 2000 (Fig. 1). The height of the saplings ranged from 63 cm to 81 cm. The pots were 18 cm in diameter and 26.5 cm deep. Weathered granite soil was used. These saplings were nursed in the experimental field of Okayama University (34°41′N, 133°53′E) until July 6, 2000. All saplings were well watered every other day and maintained at field capacity soil moisture until the start of the soil moisture treatment. The pots were moved into the greenhouse on July 6, 2000. Two kinds of soil moisture treatment were applied to the pine saplings. Soil moisture was regulated to fluctuate slightly around the constant moisture contents representing pF 1.8 and pF 4.2, based on the pF–soil moisture curve. The pF–soil moisture curve was constructed using the centrifuging method. Pots were weighed and watered twice a week during July 2000 to April 2001 and three or four times a week during May and June 2001. The number of saplings available for measurement at each soil moisture level was three.

Pressure-volume curve

Water potential (ψ_w^{tlp}), symplasmic water (V_p), and relative water content (RWC^{tlp}) at turgor loss point, osmotic potential (ψ_s^{sat}), and symplasmic water (V_o) at saturated point and apoplastic water (V_a) were determined by the pressure-volume curve (Tyree and Hammel 1972; Maruyama and Morikawa 1983), using a pressure chamber (model 600; PMS Instrument Corvallis, OR, USA). Bulk modulus of elasticity (ε) was calculated by the experienced equation,

$$\psi_p = \varepsilon\left(\frac{V_o - V_e - V_p}{V_p}\right)^n$$

where ψ_p is leaf pressure potential, V_e is volume of water expressed from the sample, n is a constant (Tyree and Hammel 1972). Symplasmic water at saturated point per dry weight of the leaf (V_o DW^{-1}) and the number of osmoles per dry weight of the leaf (N_s DW^{-1}) were calculated from the results of the pressure-volume curve. The diurnal leaf pressure potentials were estimated by the pressure-volume curves and the values of the diurnal leaf water potentials.

For the field-grown pine trees, leaf materials for determining the seasonal course of leaf water relations were obtained by collecting leaf samples of similar exposure and height on each sample tree. A branch with several leaves was cut from the sample trees. The base of each sample branch was cut off in the water immediately after sampling. Sampled branches were bagged while keeping the cut end of the branch in the water to minimize dehydration during transport. Bagged samples from the field were taken to the laboratory and the base of each branch was cut in the water again. Sample branches were bagged, with the black plastic bag covered with aluminum foil overnight in the laboratory for a sufficient uptake of water before analysis. The measurements were conducted during May to November 2000

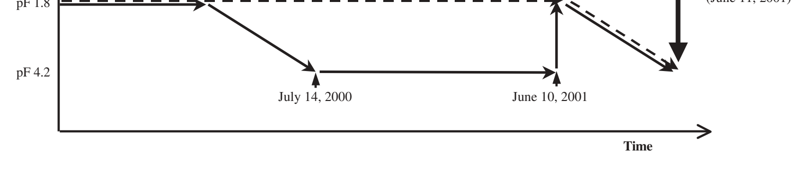


Fig. 1. Soil moisture condition of each treatment. *Solid* and *broken lines* represent pF 4.2 and pF 1.8 treatment saplings, respectively. Saplings were stored in the outside and inside of a greenhouse during the period represented by *white* and *shaded bars* at the top of the figure

for adult pine trees. The number of measurements was one per individual each month, because the reappearance of the pressure-volume curve was superior (Maruyama and Morikawa 1984). The number of measured individuals was 1–3 per month on both the upper and the lower parts of the slope. The measurements were conducted on a needle fascicle of a 1-year-old leaf.

The leaf materials of the potted saplings were sampled using the same sampling method as for the field-grown adult trees. The number of samples was three in the pF 4.2 treatment and seven in the pF 1.8 treatment. The measurements were conducted on a needle fascicle of a 1-year-old leaf for the potted saplings in June, August, and September 2001.

Diurnal measurements

Diurnal measurements were conducted in adult pine trees of Okayama Prefectural Nature Conservation Center and in potted saplings. The transpiration rate, stomatal conductance, relative humidity, leaf temperature, cuvette temperature, and photon flux density (PFD) were measured from predawn to sunset using a steady state porometer (LI-1600; Li-Cor Lincoln, NE, USA). Leaf water potential was measured using a pressure chamber (model 600; PMS Instrument Co). Each measurement was conducted on a needle fascicle of a 1-year-old leaf. The intervals between measurements were approximately 2 h. The number of trees and saplings for the diurnal measurement was one on each site of the slope and three on each pF treatment, respectively. Three leaves were measured on each sample individual and three data points were logged on each leaf. The measurement for the 40-year-old trees was conducted on September 14 and October 16, 2000. The September date was 1 day after rainfall and the soil moisture conditions on the upper and the lower parts of the slope were at pF 1.4 and pF 0.7 soil moisture, respectively (Table 1). The October date was 6 days after rainfall and the soil moisture conditions on the upper and the lower parts of the slope were at pF 2.6 and pF 2.5 soil moisture, respectively (Table 1). The soil was relatively moist on September 14, but relatively dry on October 16.

Table 1. Soil moisture conditions[a] at a depth of 40 cm (unit: pF)

Part of the slope	Sep. 14		Oct. 16	
	Average	SE	Average	SE
Upper part (34 m^2)[b]	1.4	±0.082	2.6	±0.004
Lower part (135 m^2)	0.7	±0.059	2.5	±0.008

[a] Averages from 06:00 to 16:00 at intervals of 2 hours
[b] Size of drainage area

Table 2. Soil moisture conditions[a] in potted saplings (unit: pF)

Treatment	Jun. 11		Jun. 17	
	Average	SE	Average	SE
pF 4.2	1.5	±0.109	4.3	±0.315
pF 1.8	1.5	±0.046	4.2	±0.304

[a] Averages from 04:00 to 20:00 at intervals of 2 hours

The measurement for 4-year-old saplings was conducted on June 11 and 17, 2001. Both the pF 4.2 treatment and pF 1.8 treatment saplings were well watered on June 10 and were at pF 1.5 soil moisture on June 11, 2001 (Fig. 1, Table 2). Watering was stopped for potted saplings in both the pF 4.2 and pF 1.8 treatments after the irrigation on June 10. The soil moisture was at almost pF 4.2 for both treatments on June 17, 2001 (Fig. 1, Table 2). The soil was relatively moist on June 11, whereas it was relatively dry on June 17.

Results

Leaf water relations in field-grown adult pine trees

The seasonal trends in each parameter derived from the pressure-volume curve are shown in Fig. 2. There were significant correlations for the seasonal change patterns of each parameter between the upper and the lower parts (coefficient of rank correlation, $P < 0.05$). In the leaves of pine trees on both the upper and the lower parts of the slope, the ratio of symplasmic water at turgor loss point to symplasmic water at saturated point (V_p/V_o), apoplastic water (V_a), bulk modulus of elasticity (ε), osmotic potential at saturated point (ψ_s^{sat}), and water potential at turgor loss point (ψ_w^{tlp}) showed great seasonal fluctuation, whereas relative water content at turgor loss point (RWC^{tlp}) was almost constant in the range of 0.65–0.86. The values of V_a, ψ_s^{sat}, and ψ_w^{tlp} in June were lower than those in May. During June to September, V_p/V_o and ε values were higher than those values for other months, and the values of V_a, ψ_s^{sat}, and ψ_w^{tlp} were lower than those values for other months.

The values of V_a and ψ_w^{tlp} were almost 0.4 and −2.5 MPa, respectively, in trees on both the upper and the lower parts of the slope during June to September. There were no differences in V_a and ψ_w^{tlp} between pine trees on the upper and lower slopes for the growing season not including November. The values of V_p/V_o, ε, and RWC^{tlp} for pine trees on the upper slope were higher than those for pine trees on the lower slope from August to September. The values of ψ_s^{sat} in pine trees on the upper slope were lower than those in pine trees on the lower slope from August to September.

Symplasmic water at saturated point per dry weight of the leaf ($V_o\ DW^{-1}$) and the number of osmoles per dry weight of the leaf ($N_s\ DW^{-1}$) are related to ψ_s^{sat} (Tyree and Hammel 1972). $V_o\ DW^{-1}$ and $N_s\ DW^{-1}$ were compared with the sample trees of the upper slope and those of the lower slope (Fig. 3). $N_s\ DW^{-1}$ and $V_o\ DW^{-1}$ increased from July to September in both the upper and the lower parts (coefficient of rank correlation, $P < 0.05$). The differences in $N_s\ DW^{-1}$ were not observed between the upper part and the lower part. The osmotic adjustment in the summer was thought to occur in both the upper and the lower parts. However, a difference in the osmotic adjustment between the upper part and the lower part was not detected.

The stomatal conductance, transpiration rate, and leaf water potential were compared between the pine trees on the upper and lower parts of the slope (Fig. 4). At 1 day

Fig. 2. Seasonal changes in the leaf water relation parameters in the field-grown adult pine trees. *Vertical bars* indicate SE

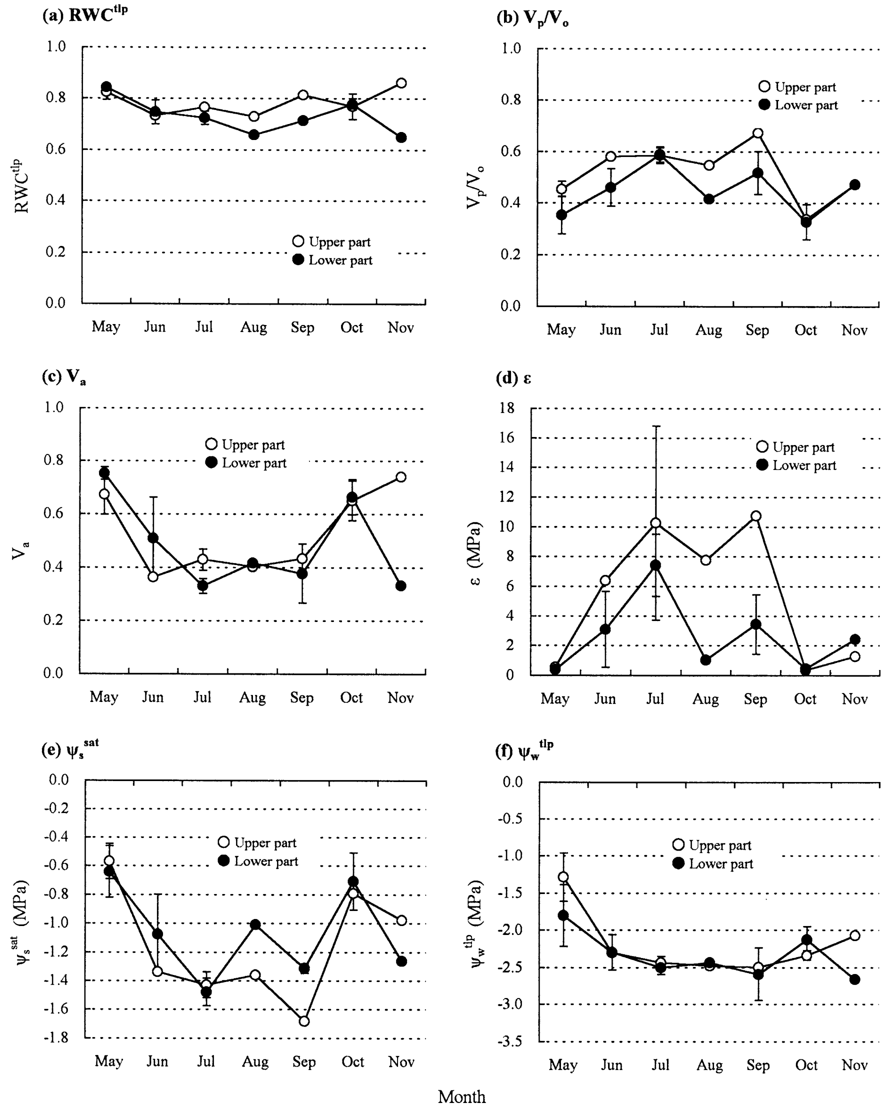
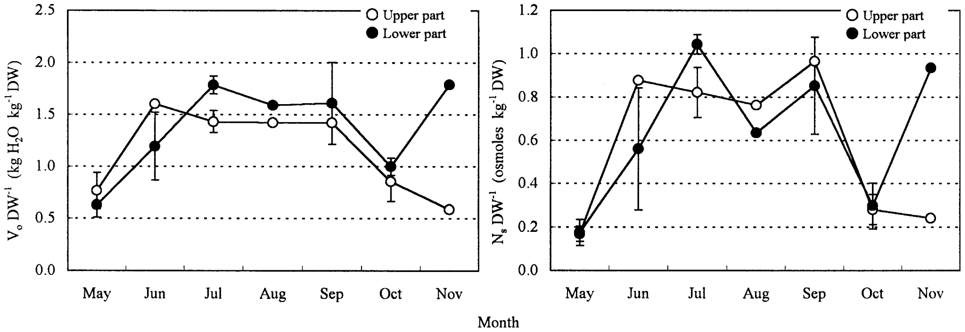


Fig. 3. Seasonal changes in the volume of symplasmic water at saturated point per dry weight (V_o DW^{-1}) and the number of osmoles per dry weight (N_s DW^{-1}) in the field-grown adult pine trees. *Vertical bars* indicate SE



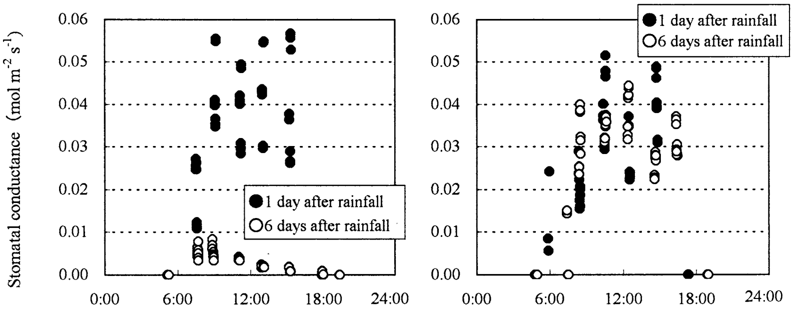
after rainfall (closed circles in Fig. 4), there were no differences for the maximum values of the stomatal conductance and the transpiration rate between the pine trees on the upper and the lower part of the slope. In the pine tree on the lower part of the slope, the diurnal changes in the stomatal conductance, the transpiration rate, and the leaf water potential at 6 days after rainfall (open circles in Fig. 4) were similar to those at 1 day after rainfall. On the other hand, in the pine tree on the upper part of the slope, the stomatal conductance, transpiration rate, and leaf water potential at 6 days after rainfall were lower than those values at 1 day after rainfall.

The leaf water potential decreased after the dawn, and showed the minimum values around noon in the trees on both the upper and the lower parts of the slope whether the soil moisture conditions were dry or moist. The predawn

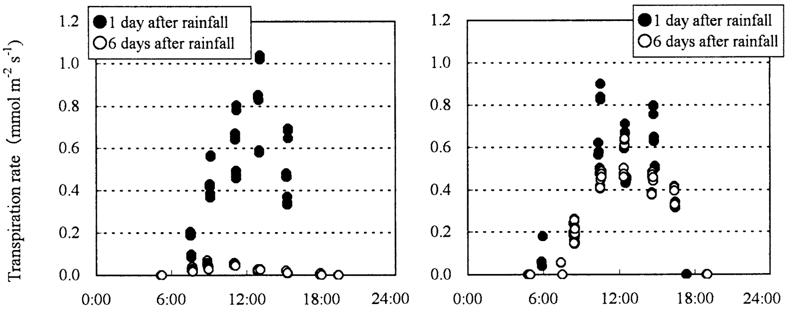
Fig. 4. Diurnal changes of the stomatal conductance, transpiration rate, and leaf water potential in the field-grown adult pine trees. One day after rainfall (●, September 14, 2000, the soil moisture level on the upper part and the lower part of the slope was pF 1.4 and pF 0.7, respectively), and 6 days after rainfall (○, October 16, 2000, the soil moisture level on the upper part and the lower part of the slope was pF 2.6 and pF 2.5, respectively)

Pine tree on the upper part of the slope **Pine tree on the lower part of the slope**

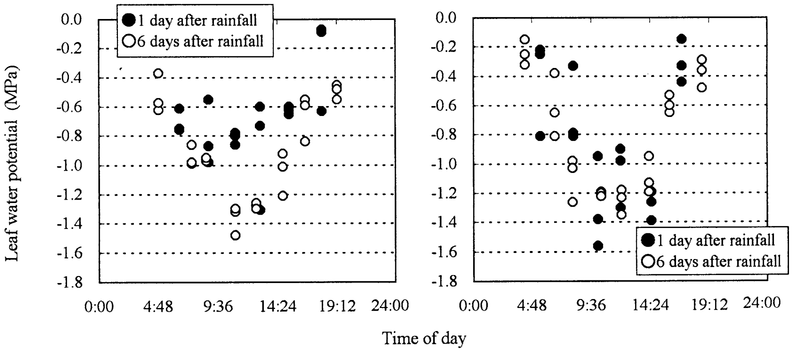
(a) Stomatal conductance



(b) Transpiration rate



(c) Leaf water potential



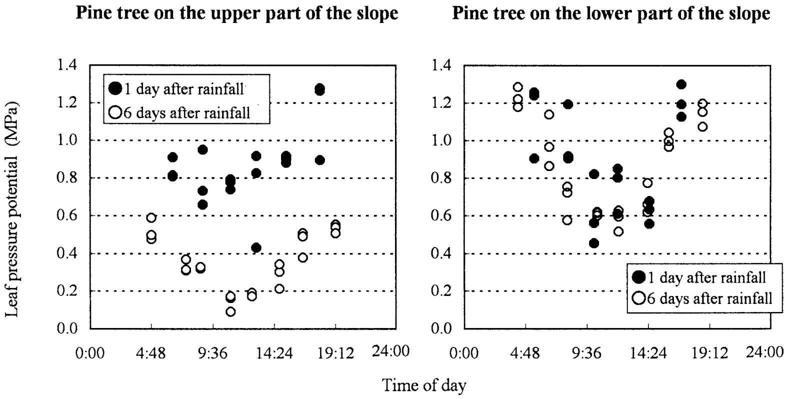
leaf water potential in the pine tree on the upper part of the slope was lower than that in the pine tree on the lower part of the slope whether the soil moisture conditions were dry or moist. However, there were no differences for the predawn leaf water potential between 1 day and 6 days after rainfall in the pine trees on both parts of the slope. In the pine tree on the lower part of the slope, there were no differences for the minimum value of the leaf water potential between on 1 day and 6 days after rainfall. In the pine tree on the upper part of the slope, the minimum values of the leaf water potential at 6 days after rainfall were lower than those at 1 day after rainfall. However, no differences in the minimum values of the leaf water potential at 6 days after rainfall were observed between the different growing sites on the slope.

The diurnal changes in leaf pressure potentials were compared between the pine trees on the lower and the upper parts of the slope on 1 day and 6 days after rainfall (Fig. 5). In the pine tree on the lower part, there were no differences for the leaf pressure potential between 1 day and 6 days after rainfall. In the pine tree on the upper part, the leaf pressure potential at 6 days after rainfall was lower than that at 1 day after rainfall. Moreover, at 6 days after rainfall, it was obvious that the leaf pressure potentials in the pine tree on the upper part were lower than those in the pine tree on the lower part.

Leaf water relations in potted pine saplings

In the second year after the soil moisture treatment, relative water content at turgor loss point (RWC$^{\mathrm{tlp}}$), apoplastic water (V_a), ratio of symplasmic water at turgor loss point to symplasmic water at saturated point (V_p/V_o), bulk modulus of elasticity (ε), osmotic potential at saturated point (ψ_s^{sat}), and water potential at turgor loss point (ψ_w^{tlp}), were obtained with pressure-volume curves (Table 3). V_p/V_o and ε of pF 4.2 treatment saplings were significantly higher than those

Fig. 5. Diurnal changes in the leaf pressure potential in the field-grown adult pine trees, 1 day (●) and 6 days (○) after rainfall. See Fig. 4 for details


Pine tree on the upper part of the slope

Pine tree on the lower part of the slope

Table 3. Leaf water relation parameters in potted saplings

	pF4.2 saplings	pF1.8 saplings	*t* test
RWC$^{\mathrm{tlp}\,a}$	0.89	0.83	n.s.[i]
SD	±0.052	±0.065	
V_a[b]	0.80	0.76	n.s.
SD	±0.093	±0.08	
V_p/V_o[c]	0.46	0.30	*
SD	±0.015	±0.117	
ε[d] (MPa)	1.35	0.52	*
SD	±0.558	±0.456	
$\psi_s^{\mathrm{sat}\,e}$ (MPa)	−1.13	−0.74	n.s.
SD	±0.098	±0.317	
$\psi_w^{\mathrm{tlp}\,f}$ (MPa)	−2.44	−2.52	n.s.
SD	±0.133	±0.539	
V_o DW$^{-1\,g}$ (kg H_2O kg^{-1} DW)	0.454	0.601	n.s.
SD	±0.138	±0.216	
N_s DW$^{-1\,h}$ (osmoles kg^{-1} DW)	0.267	0.185	n.s.
SD	±0.094	±0.129	

[a] RWC$^{\mathrm{tlp}}$, Relative water content at turgor loss point
[b] V_a, Apoplastic water
[c] V_p/V_o, Symplasmic water at turgor loss point/symplasmic water at saturated point
[d] ε, Bulk modulus of elasticity
[e] ψ_s^{sat}, Osmotic potential at saturated point
[f] ψ_w^{tlp}, Water potential at turgor loss point
[g] V_o DW^{-1}, Volume of symplasmic water at saturated point per dry weight
[h] N_s DW^{-1}, Number of osmoles per dry weight
[i] n.s., non significant
*$P < 0.05$

values for the pF 1.8 treatment saplings (*t* test, $P < 0.05$). Significant differences were not detected in RWC$^{\mathrm{tlp}}$, V_a, ψ_s^{sat}, and ψ_w^{tlp} (*t* test, $P > 0.05$). Symplasmic water at saturated point per dry weight of the leaf (V_o DW^{-1}) and the number of osmoles per dry weight of the leaf (N_s DW^{-1}) were compared between the pF 4.2 and pF 1.8 treatment saplings (Table 3). There were no significant differences in V_o DW^{-1} and N_s DW^{-1} between pF 4.2 treatment and pF 1.8 treatment saplings (*t* test, $P > 0.05$). A difference in osmotic adjustment was not detected between the soil moisture treatments.

In the diurnal changes in the pF 1.8 treatment saplings, the maximum value of the stomatal conductance (g_s^{max}) at 1 day after watering was not significantly different from that at 7 days after watering (Table 4). In pF 4.2 treatment saplings, g_s^{max} at 7 days after watering was significantly lower than that at 1 day after watering (Tukey's HSD test, $P < 0.05$). The maximum values of the transpiration rate (E^{max}) and the minimum values of the leaf water potential (ψ_w^{min}) on 1 day after watering were not significantly different between the pF 4.2 treatment and the pF 1.8 treatment saplings (Tukey's HSD test, $P > 0.05$). E^{max} and ψ_w^{min} of pF 1.8 treatment saplings at 7 days after watering were not significantly different from that at 1 day after watering (Tukey's HSD test, $P > 0.05$). E^{max} and ψ_w^{min} of pF 4.2 treatment saplings at 7 days after watering were significantly lower than those at 1 day after watering (Tukey's HSD test, $P < 0.05$). However, E^{max} and ψ_w^{min} of pF 4.2 treatment at 7 days after watering were not significantly lower than those values of pF 1.8 treatment at 7 days after watering (Tukey's HSD test, $P > 0.05$).

There were no significant differences among the leaf water potential at predawn ($\psi_w^{\mathrm{predawn}}$) of any treatments and soil moisture conditions (Tukey's HSD test, $P > 0.05$). The leaf predawn pressure potential ($\psi_p^{\mathrm{predawn}}$) also did not vary by the treatments and the soil moisture conditions (Tukey's

Table 4. Maximum and/or minimum of the stomatal conductance, the transpiration rate, the leaf water potential, and the leaf pressure potential in potted saplings

	pF4.2 saplings		pF1.8 saplings	
	1 day after watering[g]	7 days after watering[h]	1 day after watering	7 days after watering
$g_s^{\max a}$ (mol m^{-2} s^{-1})	0.030	0.020	0.031	0.039
SD	±0.0028	±0.003	±0.0025	±0.0071
Tukey's test	**a**	**b**	**a**	**a**
$E^{\max b}$ (mmol m^{-2} s^{-1})	0.972	0.658	1.091	1.012
SD	±0.1403	±0.133	±0.1808	±0.2194
Tukey's test	**a**	**b**	**a**	**ab**
$\psi_w^{\text{predawn c}}$ (MPa)	−0.22	−0.24	−0.23	−0.31
SD	±0.115	±0.084	±0.041	±0.045
Tukey's test	**a**	**a**	**a**	**a**
$\psi_w^{\min d}$ (MPa)	−0.85	−1.50	−0.79	−1.16
SD	±0.227	±0.261	±0.087	±0.011
Tukey's test	**a**	**b**	**a**	**ab**
$\psi_p^{\text{predawn e}}$ (MPa)	0.97	0.96	1.08	1.04
SD	±0.066	±0.048	±0.024	±0.026
Tukey's test	**a**	**a**	**a**	**a**
$\psi_p^{\min f}$ (MPa)	0.61	0.24	0.78	0.54
SD	±0.129	±0.149	±0.032	±0.006
Tukey's test	**a**	**b**	**a**	**a**

Different bold letters indicate significant differences (Tukey's HSD test, $P < 0.05$)

[a] $g_s^{\max}$, Maximum stomatal conductance

[b] $E^{\max}$, Maximum transpiration rate

[c] ψ_w^{predawn}, Leaf water potential at predawn

[d] $\psi_w^{\min}$, Minimum leaf water potential

[e] ψ_p^{predawn}, Leaf pressure potential at predawn

[f] $\psi_p^{\min}$, Minimum leaf pressure potential

[g] June 11, 2001 (The soil moisture level of pF 4.2 and pF 1.8 treatment saplings was pF 1.5)

[h] June 17, 2001 (The soil moisture level of pF 4.2 and pF 1.8 treatment saplings was pF 4.3 and pF 4.2, respectively)

HSD test, $P > 0.05$). However, the minimum value of the leaf pressure potential ($\psi_p^{\min}$) of pF 4.2 treatment saplings at 7 days after watering was significantly lower than that of pF 4.2 treatment saplings at 1 day after watering and pF 1.8 treatment saplings on 1 and 7 days after watering (Tukey's HSD test, $P < 0.05$).

The pF 4.2 treatment saplings tended to represent the low leaf pressure potential and close stomata in the dry conditions, and the pF 1.8 treatment saplings tended to represent the high leaf pressure potential and maintain opening the stomata even in dry conditions. It was confirmed that the leaf water relations and the stomatal response to soil drying in potted saplings growing in different soil moisture conditions were similar to those in adult pine trees growing on different parts of the slope.

Discussion

The values of ψ_s^{sat} and ψ_w^{tlp} seasonally fluctuate through the effect of soil moisture condition, temperature, and growth (Ritchie and Shula 1984; Doi et al. 1986), and were indicators of the osmotic adjustment in conifer leaves (Choi 1992). Seasonal changes in ψ_s^{sat} and ψ_w^{tlp} occur during the growth of leaves due in part to the changing dynamic balance of the water content and the solute concentration of conifer leaves (Tyree et al. 1978; Maruyama et al. 1996). The number of osmoles per dry weight (N_s DW^{-1}), which represents the solute concentration of leaf tissue, increased during the summer in trees on both parts of the slope in this study. Thus, the decrease in ψ_s^{sat} for the summer period, when the soil and the air become dry, occurred not because of the decrease in the leaf water content by the growth of leaves, but because of the osmotic adjustment to the drought.

Water in leaves is distributed in the symplast and the apoplast, and the relative apportioning of water into the symplast and apoplast may constitute a mechanism for drought resistance (Choi 1992). The values of relative water content at turgor loss point (RWC$^{\text{tlp}}$) were almost constant from May to November in trees on both the upper and the lower parts of the slope. However, for the summer period, the values of apoplastic water (V_a) decrease, while the values of the ratio of symplasmic water at turgor loss point to symplasmic water at saturated point (V_p/V_o) increased. Therefore, it was thought that water flowed into the symplast from the apoplast during the summer period because of the increase in N_s DW^{-1} from osmotic adjustment, and that pressure potential was maintained. It was reported that the increase in bulk modulus of elasticity (ε) was caused by the drought stress (Wilson and Ludlow 1983; DeLucia and Heckathorn 1989). The increase in ε was observed for the summer period in trees on both parts of the slope. It was thought that trees on both parts of the slope rapidly closed their stomata and prevented a water loss to drought during the summer. These results suggest that adult trees growing on both parts of the slope adapted to summer drought by osmotic adjustment and by the less elastic cell wall, and that these adaptations to drought stress were common in *P. densiflora* under different soil moisture conditions.

We compared the leaf water relations in the field-grown adult trees and potted saplings growing under different soil moisture conditions. The field-grown adult trees and the potted saplings growing under long-term water stress had higher values of V_p/V_o and ε than those growing under moist conditions. Large values of ε can be considered as advantageous to drought stress, because less elastic cell walls allow a large and rapid drop in the leaf potential and cause stomata to close. On the other hand, there were no differences in N_s DW^{-1} between the growing soil moisture conditions in both the field-grown adult trees and the potted saplings. It was suggested that *P. densiflora* under long-term water stress avoids the water loss not by osmotic adjustment, which is directly energy-dependent, but by the acquisition of mechanical stomatal closure from the less-elastic cell walls.

The maximum daily leaf conductance is correlated with the leaf water potential at predawn for *Pseudotsuga menziesii* (Mirb). Franco. (Running 1976). However, in this study, no differences in the predawn leaf water potential were observed between the moist and the dry condition in both the adult tree on the upper part of the slope and the potted saplings on the pF 4.2 treatment, which showed a decrease in stomatal conductance in response to soil drying. In both the adult tree on the upper part of the slope and the potted saplings on the pF 4.2 treatment, the minimum daily leaf pressure potential in the dry condition was lower than that in the moist condition and that in the adult tree on the lower part of the slope and potted saplings on the pF 1.8 treatment in the dry condition, respectively. A decrease in leaf pressure potential brings about a drop in pressure potential in guard cells and causes stomatal closure (Mohr and Schopfer 1992). A decrease in leaf pressure potential triggers an increase in abscisic acid, which causes the stomatal closure (Pierce and Raschke 1980). It was inferred that the stomatal closure to soil drying in the adult tree on the upper part of the slope and the potted saplings on the pF 4.2 treatment was highly correlated with the decrease in leaf pressure potential.

These results suggest that *P. densiflora* growing under chronic water stress could close their stomata in response to acute water stress, such as little rain, through the decrease in leaf pressure potential because they accommodated to the drought by the adjustment of the leaf water relations, which were high values of V_p/V_o and ε. The decrease in leaf pressure potential brings about not only stomatal closure but also an increased water pressure gradient between soil and plant. It was suggested that *P. densiflora* growing under long-term water stress not only decreases its water loss but also improves its absorbency in response to acute water stress. However, in the *P. densiflora* growing under moist conditions stomatal closure in response to drought was not observed. It was suggested that *P. densiflora* growing under moist conditions would tend to be stressed if there was a serious shortage of soil water, because they could not recover the water condition at the whole plant level.

Pine trees attacked by the pinewood nematode [*Bursaphelenchus xylophilus* (Steiner et Buhrer) Nickle] die from extensive water deficit due to tracheid cavitations (Ikeda and Suzaki 1984; Kuroda et al. 1991). It was reported that acute water stress, such as little rain, tended to accelerate the progress of pine wilt disease (Ooyama et al. 1975, 1976; Mineo 1976; Kobayashi 1978, 1979; Suzuki 1984; Ikeda 1996; Iwasaki et al. 1999). Therefore, it may be assumed that *P. densiflora* growing under long-term water stress can resist the influence of the extensive water deficit caused by the shortage of precipitation and by the decrease in the hydraulic conductivity resulting from pine wilt disease because of its capacity for drought resistance.

Acknowledgments We express our gratitude to staff at the Okayama Prefectural Nature Conservation Center for their support, and Ms. Akiyo Hirai of the Laboratory of Ecophysiology, Okayama University, for her assistance throughout this study.

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