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Variations in Stomatal Density, Stomatal Conductance and Leaf Water Potential Along an Altitudinal Gradient in Central Japan

By

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With 4 Figures

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Summary

TAKAHASHI K. & MIYAJIMA Y. 2008. Variations in stomatal density, stomatal conductance and leaf water potential along an altitudinal gradient in central Japan. – Phyton (Horn, Austria) 48 (1): 1–12, with 4 figures.

We examined water-related leaf physiological traits of two broad-leaved deciduous species (*Betula ermanii* and *Sorbus commixta*) and two evergreen conifer species (*Abies mariesii* and *A. veitchii*) along an altitudinal gradient in the subalpine

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forest zone on Mount Norikura, in central Japan. We tested whether water stress is greater at lower altitudes since temperature is higher and precipitation is lower at such altitudes. The examined altitudinal span of each species was several hundreds meter between 1600 m and 2400 m a.s.l. Stomatal density of the four species did not change with altitude. Although the daily maximum stomatal conductance of *B. ermanii* was highest at 2400 m a.s.l., that of the three other species did not show such a pattern. Predawn leaf water potential of the two *Abies* species did not change with altitude, while that of the two broad-leaved species was lower at the lowest altitude (1600 m a.s.l.). However, this reduction of the two broad-leaved species was not high enough to cause water stress. Therefore, this study suggested that water stress did not occur in the four species along the altitudinal gradient in the subalpine zone on Mount Norikura, at least, during the examined period.

Zusammenfassung

TAKAHASHI K. & MIYAJIMA Y. 2008. Variations in stomatal density, stomatal conductance and leaf water potential along an altitudinal gradient in central Japan. [Höhenabhängige Unterschiede der Spaltöffnungsdichte, der stomatären Leitfähigkeit und des Blattwasserpotentials anhand eines Höhenprofils in Zentraljapan]. – Phyton (Horn, Austria) 48 (1): 1–12, mit 4 Abbildungen.

Von zwei Laubbaumarten (*Betula ermanii* und *Sorbus commixta*) und zwei Nadelbäumen (*Abies mariesii* und *A. veitchii*) eines Höhenprofils in der subalpinen Waldzone am Berg Norikura in Zentraljapan wurden physiologische Eigenschaften bezüglich des Wasserhaushaltes untersucht. Es wurde geprüft, ob der Wasserstress in niedriger Seehöhe höher ist, da die Temperatur höher und die Niederschlagsmenge geringer ist. Die Höhenbestände betrugen einige 100 Meter zwischen 1600 und 2460 m a.s.l. Die Spaltöffnungsdichte aller 4 untersuchten Baumarten bleibt in allen Höhenlagen in etwa gleich. Bis auf *Betula ermanii*, die in einer Seehöhe von 2400 m a.s.l. die höchste stomatäre Leitfähigkeit zeigte, blieb die stomatäre Leitfähigkeit in allen Höhenlagen ebenfalls sehr ähnlich. Unmittelbar vor Sonnenaufgang ist das Wasserpotential der beiden Nadelbaumarten in allen Seehöhen annähernd gleich, während es bei den untersuchten Laubbaumarten in einer Seehöhe von 1600 m a.s.l. am geringsten war. Diese Abnahme des Wasserpotentials war aber nicht groß genug, um dies auf Wasserstress zurückführen zu können.

Insgesamt kann man schließen, dass Wasserstress bei den 4 untersuchten Baumarten auf Grund der unterschiedlichen Seehöhe in der subalpinen Zone des Norikura Berges während der Untersuchungszeitraumes nicht auftrat.

Introduction

Although vegetation changes with altitudes, each plant species distributes in vast altitudinal range, especially on high mountains in the temperate zone. For example, TAKAHASHI 1962 extensively studied altitudinal vegetation patterns in central Japan, and showed that many tree species distributed more than several hundreds meter in altitude. Such wide altitudinal distributions of plants are also found in Europe (KÖRNER & al. 1989). Many environmental factors change with altitude. Generally, precipitation increases with altitude, while temperature decreases. Solar UV radiation and wind velocity also increase with altitude (WOODWARD 1986, KÖRNER 1999). Thus, it is expected that each plant species is subject to different climatic conditions along altitudinal gradients.

Several studies have shown that tree species responded differently to climatic factors (i.e., precipitation, temperature and insulation) along altitudinal gradients. For example, BARTON 1993 and BARTON & TEERI 1993 suggested that low availability of soil moisture controlled the lower altitudinal distribution limit of pine species (*Pinus discolor*, *P. leiophylla* and *P. engelmannii*) in Arizona. Several researchers also reported that a major climatic factor enhancing the tree growth was summer precipitation at a low altitude, whereas summer temperature was important at a high altitude in boreal forests and in subalpine forests (ETTL & PETERSON 1995, BUCKLEY & al. 1997, PETERSON & PETERSON 2001, WILSON & HOPFMUELLER 2001, MÄKINEN & al. 2002, JUMP & al. 2006). Recently, TAKAHASHI & al. 2003, 2005 showed that radial growth of *Betula ermanii* was positively correlated with August precipitation and negatively correlated with August temperature at the lower distribution limit in central Japan, whereas growth was positively correlated with summer temperature and negatively correlated with summer precipitation at the upper distribution limit. A similar relationship was also found for *Abies veitchii* at the lower distribution limit in central Japan, i.e., the growth of this species was positively and negatively correlated with August precipitation and August temperature, respectively, as seen in *B. ermanii* at the lower distribution limit (TAKAHASHI & al. 2003). These findings suggest that tree growth may be determined more by water availability at lower altitudes. Accordingly, morphological and physiological characteristics of leaves should change with altitude corresponding to the climatic factors that influence tree growth. However, a few ecophysiolosical studies have examined how water stress changes along altitudinal gradients.

Loss of water by transpiration often exceeds water uptake by roots during daytime, but plants compensate the water loss during daytime with water uptake during nighttime. However, plants cannot take up enough water from soils during drought conditions even during the nighttime. In this case, predawn leaf water potential (Ψ_{pd}) often corresponds to soil water potential under drought conditions (BOWMAN & ROBERTS 1985, DAVIS & MOONEY 1986, BRÉDA & al. 1995). Therefore, the Ψ_{pd} is a good indicator of water stress of plants. In drought conditions, plants need to avoid water loss through stomata. Therefore, stomatal conductance and/or stomatal density of plants often decrease with increasing drought stress (KALLARACKAL & SOMEN 1997, MILLER & al. 1998, OREN & al. 1998, SCHOETTLE & ROCHELLE 2000, PANEK & GOLDSTEIN 2001), and should be also investigated for the examination of water stress along an altitudinal gradient.

The purpose of this study was to examine whether water stress is greater in lower altitudes. For this purpose, we selected four representative tree species (two deciduous broad-leaved species and two evergreen conifers) in the subalpine zone in central Japan, and the leaf traits such as stomatal density, stomatal conductance and Ψ_{pd} were examined along the altitudinal gradient.

Material and Methods

Study Site

This study was performed in the subalpine forest zone on the east slope of Mount Norikura (36°06′N, 137°33′E, 3026 m above sea level) in central Japan. Mean annual temperature recorded at Nagawa Weather Station (1068 m a.s.l., approximately 12 km in horizontal distance from the summit) was 8.4°C in 2003. Mean monthly temperatures in the coldest month of January and the hottest month of August were –4.2 and 20.3°C, respectively. Annual precipitation was 2206 mm.

Three major vegetation zones of tree species can be recognized between 800 m a.s.l. and the summit on Mount Norikura, i.e., a montane deciduous broad-leaved forest zone between 800 m and 1600 m a.s.l., a subalpine coniferous forest zone between 1600 m and 2500 m a.s.l. and an alpine dwarf pine (*Pinus pumila* REGEL) scrub zone between 2500 m and 3000 m a.s.l. near the summit (MIYAJIMA & al. 2007). The timberline is at about 2500 m a.s.l. on the examined east slope of Mount Norikura (TAKAHASHI 2003).

Dominant tree species were evergreen conifers *Abies veitchii* LINDL. and *Tsuga diversifolia* MAST. between 1600 m and 2000 m a.s.l., and evergreen conifer *Abies mariesii* MAST. and deciduous broad-leaved *Betula ermanii* CHAM., *Sorbus commixta* HEDL. and *Sorbus matsumurana* KOEHNE between 2200 m and 2500 m a.s.l. Anthropogenic effects on vegetation were negligible from 1600 m a.s.l. to the summit. Further details on vegetation and stand structure along this altitudinal gradient are given in MIYAJIMA & al. 2007 and MIYAJIMA & TAKAHASHI 2007.

Plant nomenclature followed EDITORIAL BOARD OF FLORA OF NAGANO PREFECTURE 1997.

Plant Material

In this study, the four dominant species in the subalpine zone were examined along the altitudinal gradient (1600 m to 2500 m a.s.l.), i.e., two evergreen conifers (*Abies mariesii* and *A. veitchii*) and two deciduous broad-leaved hardwood species (*Betula ermanii* and *Sorbus commixta*). According to MIYAJIMA & al. 2007 and this study, altitudinal distribution ranges of species were as follows; *B. ermanii*: 1600 m to 2500 m a.s.l., *S. commixta*: 1600 m to 2200 m a.s.l., *A. veitchii*: 1600 m to 2300 m a.s.l., and *A. mariesii*: 1900 to 2500 m a.s.l. The two broad-leaved species are shade-intolerant, and the two evergreen conifers are shade-tolerant. *S. commixta* is a small tree species, while the three other species are large canopy species. Altitudinal spans examined were as follows; *B. ermanii*: 1600 m to 2400 m a.s.l., *S. commixta* and A. veitchii: 1600 m to 2200 m a.s.l., *A. mariesii*: 2000 m to 2400 m a.s.l. Predawn leaf water potential (Ψ_{pd}), stomatal conductance and stomatal density were examined at 200 m altitudinal intervals for each species in 2003. The numbers of altitudes examined were five, four, four and three sites for *B. ermanii*, *S. commixta*, *A. veitchii* and *A. mariesii*, respectively. Altitudes examined were different according to leaf traits. Stomatal density was measured at all altitudes. However, time-consuming at the field such as stomatal conductance and its diurnal pattern were not measured at 1800 m and 2200 m a.s.l. Ψ_{pd} was also not measured at 1800 m a.s.l.

At each site, five trees were chosen for each species. We chose similar sized trees that were not shaded by neighboring trees to avoid confounding effects of light conditions and ontogeny on leaf traits. Diameter at breast height of trees selected ranged between ca. 3 and 6 cm.

Three leaves were collected from each of the five trees of each species at each altitude, and were brought to the laboratory. The stomatal frequency was estimated from nail varnish replicas of the abaxial surface at three points on each leaf. The number of stomata was counted at each point, using a binocular with a lattice micrometer.

Diurnal variation of stomatal conductance was measured for eight leaves of each broad-leaved species and eight current-year shoots of each *Abies* species at a height of 1.5 m aboveground. Measurements were performed between 7:00 and 17:00 at one hour intervals on sunny days in mid-summer using a LI-1600 (LI-Cor inc., Nebraska, USA). A conifer kit (1600-07) was used for the measurement of the evergreen conifers. Maximum stomatal conductance (g_{max}) of each leaf or shoot was determined as the highest value of stomatal conductance observed during the day.

From each tree, two sunlit current-year shoots at a height of ca. 1.5 m aboveground were collected for the measurement of Ψ_{pd}. Mature, fully expanded shoots were sampled; we avoided discolored and damaged shoots. Ψ_{pd} was measured using a PMS model 600 (PMS Instrument Co., Corvallis, USA).

Each of the water-related parameters (stomatal density, g_{max} and Ψ_{pd}) was compared among the altitudes for each species by Mann-Whitney U-test.

Results

Stomatal density was mostly constant, irrespective of altitude, and was not significantly different among the altitudes for each species (Mann-Whitney U-test, $P > 0.05$, Fig. 1). Mean stomatal density ($\pm$ SD, no. mm^{-2}) was 190 $\pm$ 30 for *B. ermanii*, 168 $\pm$ 19 for *S. commixta*, 114 $\pm$ 18 for *A. veitchii* and 98 $\pm$ 14 for *A. mariesii*. Diurnal measurements of stomatal conductance of the four species showed that stomatal conductance was generally high in the early morning, and then decreased until late afternoon (Fig. 2). However, daytime depression of stomatal conductance was observed in some cases; *B. ermanii* at 1600 m and 2000 m a.s.l., *S. commixta* at 2000 m a.s.l. and *A. mariesii* at 2400 m a.s.l. (Fig. 2). Maximum stomatal conductance (g_{max}) was different among the altitudes, except for *S. commixta* (Fig. 3). g_{max} was higher at the lower altitude for the two *Abies* species (Mann-Whitney U-test, $P < 0.05$, Fig. 3). On the contrary, g_{max} of *B. ermanii* was higher at 2400 m a.s.l. than at the two other lower altitudes (Mann-Whitney U-test, $P < 0.05$, Fig. 3).

Predawn leaf water potential (Ψ_{pd}) of *B. ermanii* and *S. commixta* was lower at the lowest altitude (1600 m a.s.l.) than at the higher altitudes

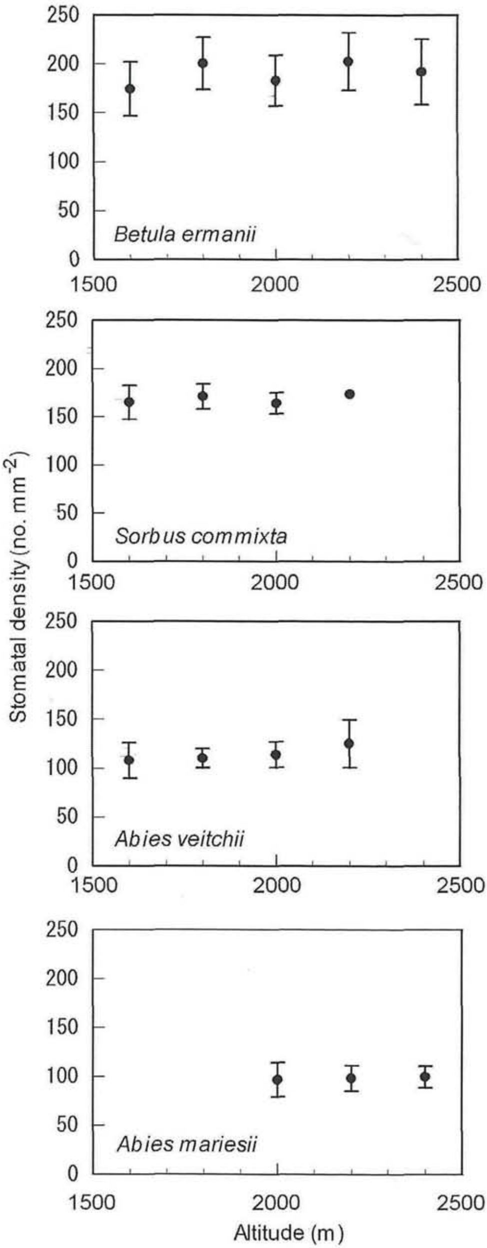

Fig. 1. Altitudinal changes of stomatal density for *Betula ermanii*, *Sorbus commixta*, *Abies veitchii* and *Abies mariesii* on Mount Norikura, central Japan. Stomatal density was not different among the altitudes for each species ($P > 0.05$, Mann-Whitney U-test). Mean values ± SD are shown in each panel.

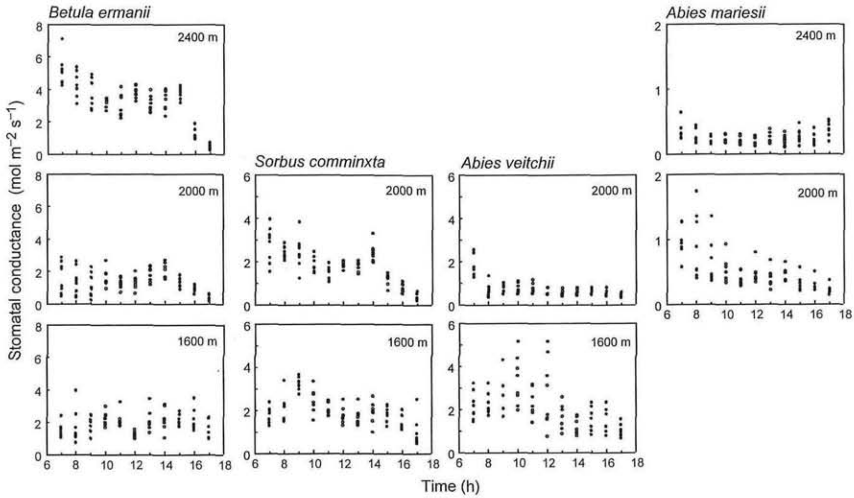

Fig. 2. Diurnal variation in stomatal conductance (g) of *Betula ermanii*, *Sorbus commixta*, *Abies veitchii* and *Abies mariesii* on Mount Norikura, central Japan. Each dot represents one measured leaf of the broad-leaved species or shoot of *Abies* species.

(Mann-Whitney U-test, $P < 0.05$), while that of the two *Abies* species did not differ among the examined altitudes (Fig. 4).

Discussion

Our previous study showed that the tree-ring width of the two species (*B. ermanii* and *A. veitchii*) was positively and negatively correlated with August precipitation and temperature, respectively, at the lower altitudinal distribution limit (TAKAHASHI & al. 2003). Whereas the relationship between tree growth and climatic factors was reversed at the upper distribution limit (TAKAHASHI & al. 2005). We therefore expected the reduction of stomatal density, predawn water potential and the maximal stomatal conductance at low altitudes. However, the occurrence of water stress was not observed in this study based on the water-related parameters.

Although predawn water potential of the two *Abies* species did not change along the altitudinal gradient, that of the two broad-leaved species was lower at the lowest site (1600 m a.s.l.). However, the leaf water potential of the two broad-leaved species was not low enough to cause water stress. The predawn water potential was ca. –0.6 and –0.45 MPa at 1600 m a.s.l. for *B. ermanii* and *S. commixta*, respectively. AUSSENAC & GRANIER 1978 showed that predawn water potential of conifer shoots was between –1.4 and –2.0 MPa under drought conditions. BRÉDA & al. 1993 compared the predawn water potential of *Quercus petraea* under natural drought

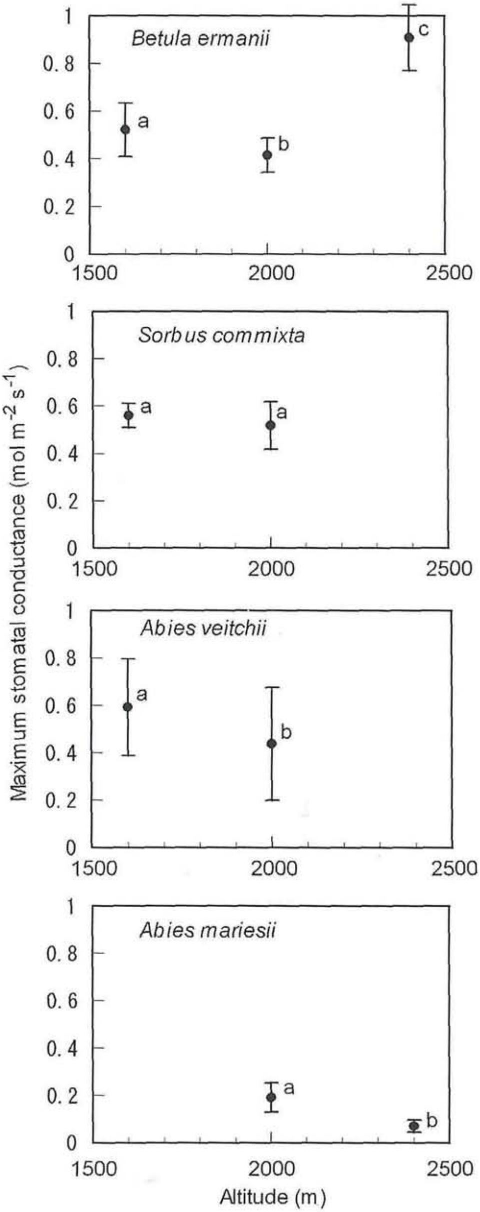

Fig. 3. Altitudinal changes of daily maximum stomatal conductance (g_{max}) for *Betula ermanii*, *Sorbus commixta*, *Abies veitchii* and *Abies mariesii* on Mount Norikura, central Japan. Different letters in each panel indicate significant difference ($P < 0.05$) evaluated by Mann-Whitney U-test. Mean values ± SD are shown in each panel.

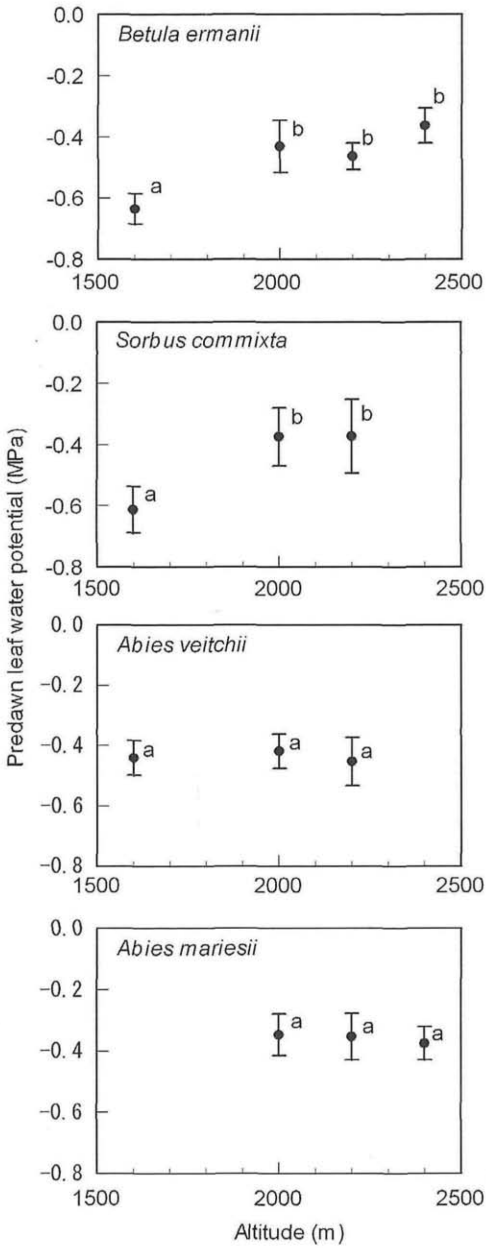

Fig. 4. Altitudinal changes of predawn water potential (Ψ_{pd}) for *Betula ermanii, Sorbus commixta*, *Abies veitchii* and *Abies mariesii* on Mount Norikura, central Japan. Different letters in each panel indicate significant difference ($P < 0.05$) evaluated by Mann-Whitney U-test. Mean values ± SD are shown in each panel.

conditions and irrigated conditions, and reported that the predawn water potential ranged between −0.3 and −0.8 MPa under the irrigated conditions and between −1.7 to −2.2 MPa under water deficit. STONEMAN & al. 1996 also observed that predawn shoot water potential of *Eucalyptus marginata* was about −0.4 MPa in wet season. The predawn water potential of the four species in this study was apparently less negative than the reported values under drought conditions and almost equivalent to those reported under irrigated conditions or in wet season.

This study concludes that water stress did not occur in the four species along the altitudinal gradient (1600 m to 2400 m a.s.l.) in the subalpine zone on Mount Norikura, even in the lowest altitudinal limits. Therefore, the results of this study did not support the prediction by the dendrochronological study of TAKAHASHI & al. 2003, i.e., the occurrence of water stress at lower altitudes. Annual tree-ring width would be positively correlated with the total amount of photosynthetic production. Photosynthetic rates often decrease under dry conditions (BABALOLA & al. 1968, HAVRANEK & BENECK 1978, PANEK & GOLDSTEIN 2001, TAKAHASHI & al. 2004). Interannual variations in precipitation and temperature during growth period are large. Actually, the narrow tree-ring widths of *B. ermanii* and *A. veitchii* at the lower distribution limit, observed in TAKAHASHI & al. 2003, were formed in the years with dry and hot summer. August precipitation of the examined year (2003) in this study was greater than the average, and therefore, water stress might not be observed. Thus, further studies are necessary to examine how environmental factors such as precipitation and temperature affect the photosynthetic production of the four species along the altitudinal gradient.

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