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A comparison of photosynthetic capacity between *Myrica gale* var. *tomentosa*, a nitrogen-fixing shrub, and co-occurring species in an oligotrophic moor

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Abstract

Photosynthetic and transpiration (*E*) rates, stomatal conductance, and leaf nitrogen content were surveyed for *Myrica gale* var. *tomentosa*, a N_2-fixing wetland shrub, *Betula platyphylla* var. *japonica*, and *Rhododendron japonicum* in Ozegahara moor, an oligotrophic moor in Central Japan. Net photosynthetic rate saturated with irradiance (P_{max}) of *M. gale* was 15.2-16.5 μmol(CO_2) m^{-2} s^{-1}, higher than those of the other species throughout the growing season. P_{max} was positively correlated with leaf N content among the three species. The large leaf N content in *M. gale* was due to N_2-fixation in root nodules. In a comparison of *M. gale* in two habitats, P_{max}, leaf N content, and root nodule development were larger in the wetter habitat. *M. gale* showed high *E* and no midday depression of P_{max} even under high irradiance and large vapour pressure deficit between leaves and ambient air on a midsummer day. These traits of photosynthesis and water relations were associated with the dominance of this shrub in wetter sites such as stream sides and hollows.

Additional key words: areal leaf mass; *Betula platyphylla* var. *japonica*; irradiance; diurnal course; intercellular CO_2 concentration; leaf nitrogen content; net photosynthetic rate; *Rhododendron japonicum*; stomatal conductance; transpiration rate.

Introduction

Myrica gale with its two geographic varieties, var. *gale* and *tomentosa*, is an actinorhizal N_2-fixing shrub growing on wet and infertile soils. In Europe and northern North America, *M. gale* var. *gale* is one of pioneer plants of wetlands

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(Dansereau and Segadas-Vianna 1952) and a dominant species in several plant associations on open peatlands (Gates 1942, Dansereau and Segadas-Vianna 1952, Schwintzer 1979). This plant can obtain a substantial amount of nitrogen from the atmosphere by N_2 fixation in root nodules (Bond 1951, Sprent *et al.* 1978, Schwintzer 1979). Its luxuriant growth and dominance in infertile soils depend primarily on its ability to fix N_2 (Sprent *et al.* 1978, Schwintzer *et al.* 1982).

M. gale var. *tomentosa* is distributed from north-east Asia to north-west North America. Maruyama *et al.* (1982) suggest that this shrub is an important source of N in an oligotrophic wetland, Ozegahara, which is a typical highland moor in Japan. The habitat and biomass of this shrub in the Ozegahara moor have increased during last decades (Hogetsu *et al.* 1982).

In general, net photosynthetic rate (P_N) is positively related to leaf N content (*e.g.*, Mooney *et al.* 1981, Hirose and Werger 1987, Evans 1989, Reich *et al.* 1992, Anten *et al.* 1996, Mohammad *et al.* 1997). The P_{max} of *M. gale* inhabiting on infertile soils may be higher than that of non-N_2-fixing plants occurring in the same habitat. However, photosynthesis of this shrub growing in fields has not been measured yet.

We compared in the field P_{max} of *M. gale* var. *tomentosa* and other co-occurring woody species having no N_2-fixing capacity and analyzed the effect of stomatal conductance (g_s) and leaf N content on the photosynthesis.

Materials and methods

Plants: Field surveys were made in the Ozegahara moor, which is a part of Nikko National Park in Central Japan and which is protected as a special natural monument (36°55'N, 139°15'E). This moor is flat throughout, 7.6 km^2 in area and 1400 m in altitude, and is surrounded by mountains. For the past decade, annual mean temperature was 4.6 °C, and maximum and minimum monthly means were 17.4 °C in August and -7.6 °C in January, respectively. Annual precipitation was 1966 mm and seasonal maximum snow depth was usually more than 3 m.

Content of available nutrients in the soil such as inorganic nitrogen compounds is very low (Hogetsu *et al.* 1954). *M. gale* var. *tomentosa* (further on *M. gale*) occurs in a wide range of the Ozegahara moor and dominates mainly in periodically flooded sites such as stream sides and hollows. This shrub flowers in late June after the thawing of snow and then it develops leafy shoots. Leaves are senescent in late September and are shed in October. The period available for photosynthesis of *M. gale* is about 3 months.

Ecophysiological surveys were made in a hollow surrounded by a grassy area 20 cm higher than the hollow, and in a peat moss high moor. The *M. gale* dominated and grew up to 50 cm in height at the hollow site, while it was more sparse and less than 30 cm high at the peat moss site. The soil water-table was higher in the hollow site than in the peat moss site. Soil pH was higher than 5.0 in the hollow site and lower than 5.0 in the peat moss site throughout the growing season. *B. platyphylla* var. *japonica* and *R. japonicum* growing beside the *M. gale* population in the hollow site were surveyed for comparison.

Measurements of ecophysiological traits and microclimate: P_N, E, g_s to water vapour, and intercellular CO_2 concentration (C_i) were monitored using a portable photosynthesis system (model *6200*, *LI-COR*, Lincoln, NE, USA). Measurements on fully expanded leaves in upper shoots were carried out on 13 June (early summer) and 5 September (late summer) in 1995 and on 30 July (midsummer) in 1996 at the hollow site, and on 1 August in 1996 at the peat moss site, throughout daytime at approximately one-hour intervals. Two or three measurements were made for each species at each time.

On the same days, microclimate and leaf temperature were monitored from sunrise to sunset. Photosynthetic photon flux density (PPFD, 400-700 nm) was measured with quantum sensors (*IKS-25*, *Koito*, Yokohama, Japan). Air and leaf temperatures were measured with fine-wire copper-constantan thermocouples (type *K*, *Hayashi-Denko*, Tokyo, Japan). Wind speed was measured with a hot-wire anemometer (*V-01-AND*, *I-Denshi*, Tokyo, Japan) and relative air humidity with a humidity sensor (*HMD 30Y*, *Vaisala*, Finland) at 1.5 m above the ground. These values were stored at one-minute intervals in a data-logger (*THERMODAC-E*, *Eto-Denki*, Tokyo, Japan).

Leaf-to-air vapour pressure deficit (VPD) was calculated as the difference between saturated vapour pressure at the leaf temperature and the vapour pressure in ambient air (Jones 1983).

Dry matter and nitrogen contents in leaves: Leaves were collected from basal parts (leaf order 1 to 5) of upper shoots, six times from July to October in the three species at the hollow site and only in *M. gale* at the peat moss site. The area of lamina in each leaf sample was measured. Then the laminae were dried at 70 °C to a constant mass and weighed. Nitrogen content was determined with an automatic nitrogen-carbon analyzer (*NC-90A*, *SCAS*, Osaka, Japan) for these laminae. The determination of N content was also made for the leaves for which photosynthesis was measured on midsummer days in 1996.

Statistics: The differences among means were analyzed by the one-way ANOVA.

Results

Photosynthetic capacity and leaf nitrogen contents: The relationship of P_N and PPFD, measured at the hollow site in midsummer, showed that both the P_{max} and PPFD for this value were highest in *M. gale* and lowest in *R. japonicum* (Fig. 1). Dark respiration rate (R_D) and compensation irradiance were also greater in *M. gale* than in other species. These relationships between the three species with respect to carbon fixation were also found in early and late summer.

P_{max} of *M. gale* was significantly higher than that of other species throughout the growing season ($p<0.001$), and P_{max} of *B. platyphylla* was significantly higher than

Table 1. Maximum net photosynthetic rate, P_{max} [μmol(CO_2) m^{-2} s^{-1}], in different seasons and sites, and leaf dry mass per unit leaf area (LMA) [g m^{-2}], nitrogen concentration per unit dry mass [% d.m.] or leaf area [g m^{-2}] in summer in the tree plant species. Means and SDs are shown. - = not determined. Values followed by the same letters are not significantly different ($p>0.05$).

		Hollow site *M. gale*	*B. platyphylla*	*R. japonicum*	Peat-moss site *M. gale*
P_{max}	13 July	15.60±0.87[a]	9.00±2.21[c]	8.82±0.95[c]	-
	30 July	16.50±0.70[a]	13.60±0.69[b]	8.67±0.94[c]	-
	1 August	-	-	-	14.10±1.28[b]
	5 September	15.20±1.85[ab]	10.50±1.44[c]	7.56±0.74[d]	-
LMA		62.8±7.6[a]	70.0±3.8[a]	58.2±10.3[a]	68.6±5.8[a]
N per d.m.		3.12±0.41[a]	2.07±0.10[b]	2.08±0.42[b]	2.48±0.51[b]
N per area		1.94±0.06[a]	1.43±0.08[c]	1.21±0.10[d]	1.68±0.24[b]

that of *R. japonicum* in midsummer and early autumn ($p<0.01$) (Table 1). P_{max} of *M. gale* in the hollow site was significantly higher than that in the peat moss site ($p<0.01$).

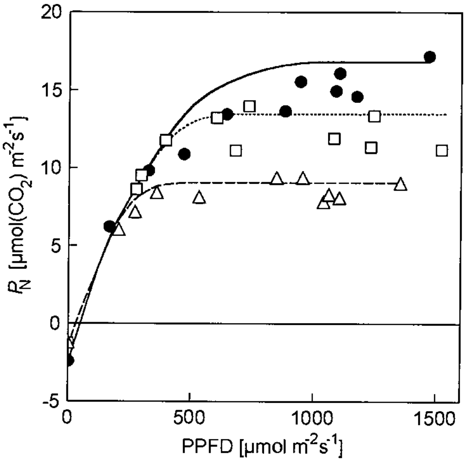

Fig. 1. Irradiance (PPFD) response curves of net photosynthetic rate (P_N) of *M. gale* var. *tomentosa* (●), *Betula platyphylla* var. *japonica* (□), and *Rhododendron japonicum* (Δ), constructed from values obtained at one-hour intervals during daytime on a clear day in midsummer (30 July).

The seasonal maximum leaf dry mass per unit leaf area (LMA) was not different among the three species, although it increased during summer in *M. gale* and *R. japonicum,* and was almost constant throughout the growing seasons in *B. platyphylla* (Fig. 2). The N content of *M. gale* was higher than those of the other species throughout the growing season (Fig. 2). The leaf N content in each species was nearly constant during summer and decreased with senescence of the leaves in autumn, indicating the transport of N from dying leaves to woody parts. At the last sampling in mid-October, an abscission layer had fully developed in *M. gale* and *B. platyphylla* but not in *R. japonicum,* and more N remained in *M. gale* leaves than in the leaves of other species.

The values of leaf N in mid- to late-summer were pooled and compared among species and between sites, because the number of samples was too small for statistical tests (Table 1). Leaf N concentration [% of dry mass] of *M. gale* in the hollow site was significantly higher in the peat moss site ($p<0.05$), and was higher

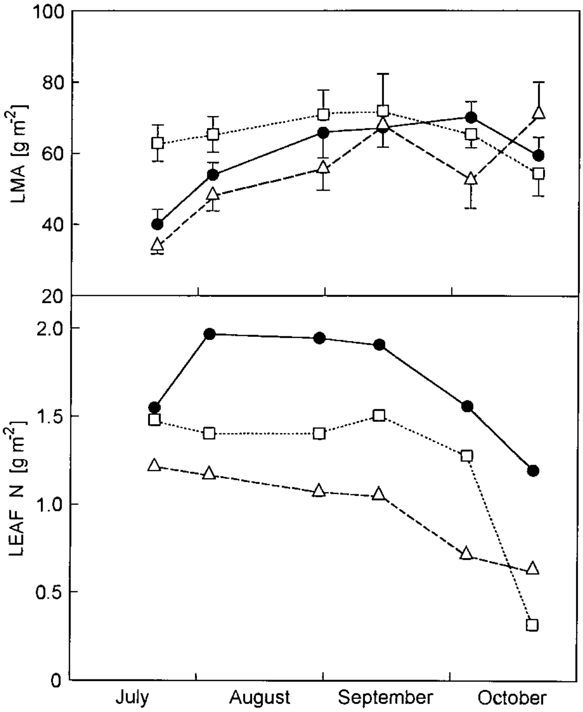

Fig. 2. Seasonal changes in leaf dry mass per unit leaf area (LMA) and leaf N content per unit leaf area. *M. gale* var. *tomentosa* (●), *Betula platyphylla* var. *japonica* (□), and *Rhododendron japonicum* (Δ).

than those of *B. platyphylla* and *R. japonicum* ($p<0.001$). Similar relationships among species and between sites were found in N content per unit leaf area.

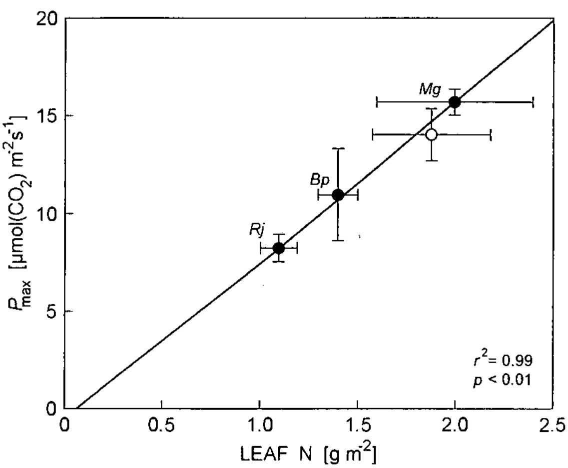

Fig. 3. Dependence of maximum net photosynthetic rates (P_{max}) on leaf N contents among the three species, *M. gale* var. *tomentosa* (*Mg*), *Betula platyphylla* var. *japonica* (*Bp*), and *Rhododendron japonicum* (*Rj*) on midsummer days (30 July and 1 August). Solid circles show the three species at the hollow site, and an open circle shows *M. gale* at the peat moss site. Means and SD (*bars*) are shown.

P_{max} was linearly related to leaf N content among the three species (Fig. 3; $p<0.01$, $r^2=0.999$). The ratios of P_{max} to the leaf N content in each species were similar.

Diurnal course of photosynthesis: The date in midsummer (30 July 1996) was most suitable for comparison of the diurnal course of P_N between species, because leaves were functionally mature and meteorological conditions were fairly good. On this date, it was clear before 14:00 h, and cloudy or rainy thereafter. Diurnal changes in PPFD, air temperature, wind speed, air humidity, leaf temperature, and VPD are shown in Fig. 4. PPFD was greater than 1000 μmol m^{-2} s^{-1} for about five hours around midday. Wind speed was lower than 1 m s^{-1} throughout the day. Air temperature was 23-25 °C around midday and the mean during daytime was 21.2 °C. Leaf temperatures were 2-4 °C higher than the air temperature under the rather high PPFD. The leaf temperature of *B. platyphylla* was highest among the three species. The relative humidity of ambient air was 60-70 % around midday. VPD for each species increased until 14:00 h and then decreased.

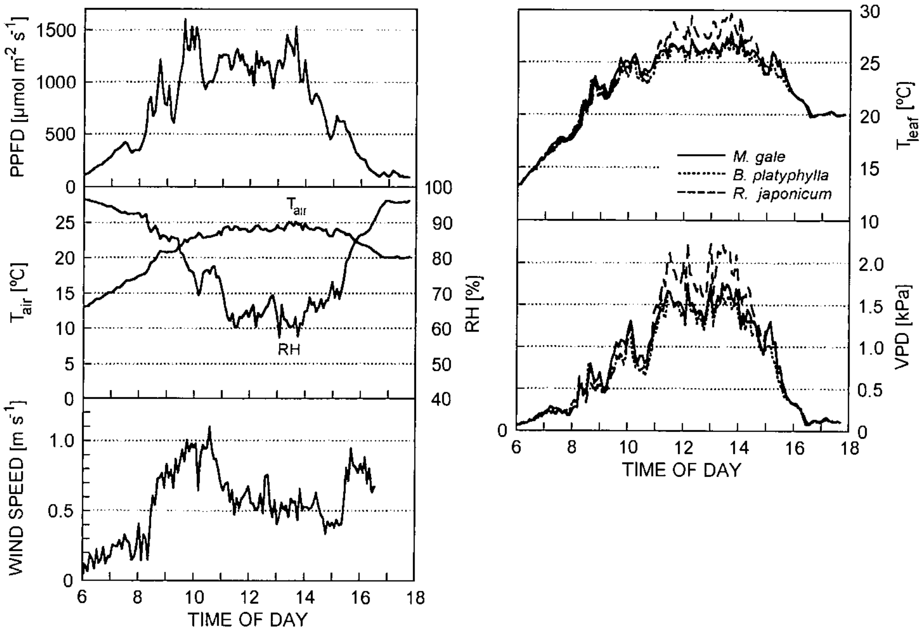

Fig. 4. Diurnal changes in photosynthetic photon flux density (PPFD), air temperature (T_{air}), ambient relative humidity (RH), wind speed, leaf temperature (T_{leaf}), and vapour pressure deficit (VPD) on 30 July, 1996. Wind speed was not measured after 16:30 h because of rainfall. T_{leaf} and VPD are shown for each plant species.

Of the three species, *M. gale* had the highest P_N under high irradiance (Fig. 5). The P_N of *M. gale* was around 15 μmol(CO_2) m^{-2} s^{-1} from 09:00 to 14:00 h with a maximum rate of 17.5 μmol m^{-2} s^{-1}, and no midday depression was found. The P_N of *B. platyphylla* peaked (*ca.* 14 μmol m^{-2} s^{-1}) before midday and thereafter significantly decreased (p<0.001). It did not recover in the afternoon, probably because of the decrease in PPFD. The P_N of *R. japonicum* was the lowest among the species throughout the day, and was almost constant at about 9 μmol(CO_2) m^{-2} s^{-1} under high irradiance.

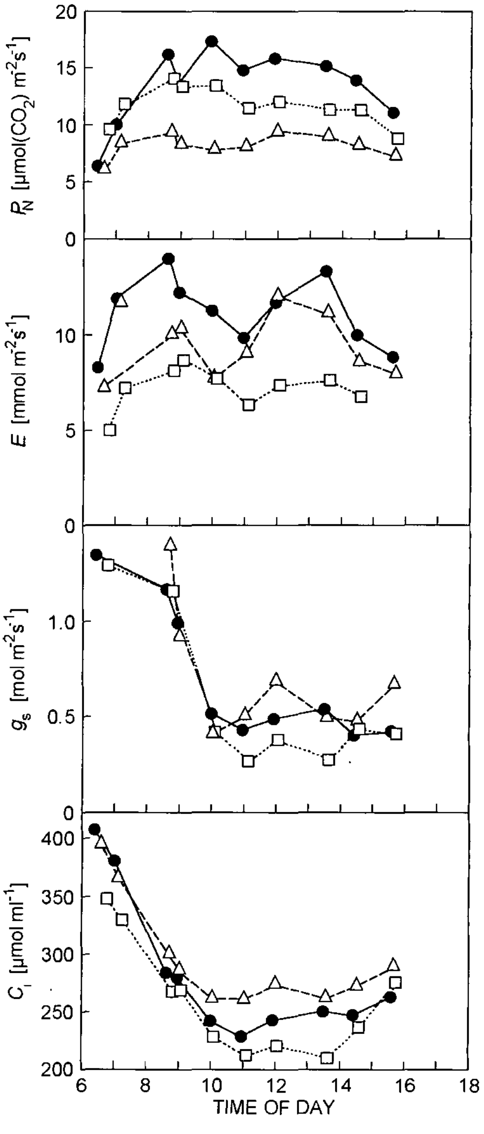

Fig. 5. Diurnal courses in net photosynthetic rate (P_N), transpiration rate (E), stomatal conductance (g_s), and intercellular CO_2 concentration (C_i) on 30 July, 1996. *M. gale* var. *tomentosa* (●), *Betula platyphylla* var. *japonica* (□), and *Rhododendron japonicum* (Δ).

The E of *M. gale* was the highest and that of *B. platyphylla* was the lowest among the three species throughout the day (Fig. 5). A midday decrease in E was found for *M. gale*. In each species, g_s was highest at the first measurement in the early morning, decreased rapidly with the increase in VPD, and was maintained at a low level after 10:00 h (Fig. 5). The minimum g_s under high irradiance around midday was lower in *B. platyphylla* than in the other species. The C_i also decreased rapidly in the morning (Fig. 5). The C_i in *M. gale,* although depressed, was higher than that in *B. platyphylla,* even though P_N was higher in *M. gale*.

Discussion

The P_{max} for *M. gale* [16.5 μmol(CO_2) m^{-2} s^{-1} in midsummer] was higher than it was for the other co-occurring species. In an extensive survey, average maximum P_N ranged from 10 to 15 μmol m^{-2} s^{-1} in deciduous broad-leaved trees, and from 6 to 10 μmol m^{-2} s^{-1} in deciduous dwarf shrubs of heath and tundra (Larcher 1995). Early successional species showed higher P_N than mid- and late-successional ones among deciduous broad-leaved trees in northern Japan, where P_{max} of the early successional trees, *Populus maximowiczii* and *B. platyphylla,* were about 13 and 10 μmol(CO_2) m^{-2} s^{-1}, respectively (Koike 1988). In the present study, P_{max} of *M. gale* was considerably higher than the reported photosynthetic capacities of deciduous shrubs and trees from temperate to boreal regions.

The P_{max} of *B. platyphylla* in Ozegahara moor was also higher than the above values measured at lower altitude (140 m a.s.l, Koike 1988). Körner (1989) showed that higher altitude samples had higher N content per unit leaf area in the same or related species. The high P_{max} of *M. gale* in the Ozegahara moor may be in part attributable to the effect of altitude.

The P_{max} depended clearly on the leaf N content in both inter-specific and site-to-site relationships (Fig. 3). The nitrogen use efficiency, defined as the ratio of P_N to leaf N content (Hirose and Werger 1987), was almost equal among the different species and sites. The high P_{max} of *M. gale* on the N-deficient moor depended on the actinorhizal N_2-fixation in root nodules.

Midday depression of P_N must be the result of a combination of stresses (photoinhibition, water stress, heat stress, *etc.*) and accumulation of assimilates in chloroplasts (Schulze and Hall 1982, Larcher 1995). The midday depression of P_N occurred in *B. platyphylla* but not in *M. gale* on a clear day in midsummer (Fig. 4). This difference between species in the diurnal course of P_N was connected to a greater depression of g_s in *B. platyphylla*. Among several subarctic wetland shrubs, *M. gale* var. *gale* was relatively less sensitive in stomatal response to large VPD and showed a smaller decrease in leaf water potential under high *E* (Blanken and Rouse 1996). This shows that soil-to-leaf hydraulic resistance of this plant species is relatively small. These traits in water relations are advantageous in a habitat where water is not limiting, and may be one of the reasons for dominance of *M. gale* in wetter sites such as stream sides and hollows where soil water-table is constantly high.

Both the development of root nodules and plant growth are negatively related to soil water-table depth in *M. gale* var. *gale* seedlings (Schwintzer and Lancelle 1983). The optimal pH for the development of root nodule ranges from 5.4 to 6.3 (Bond 1951). In the peat moss site, which had a deeper soil water-table and lower soil pH, poor development of root nodules was observed. Lower leaf N content in this site seems to result from lower N_2-fixing activity. The dependence of N_2-fixing activity on the soil water conditions may be another reason for the higher dominance of *M. gale* on the wetter sites.

Abundant N is supplied to litter and soil in plant communities containing N_2-fixing plants (Maron *et al.* 1996, Simpson *et al.* 1996). *Alnus maximowiczii*, an N_2-fixing tree growing on nutrient-poor volcanic soils, had a high amount of N in the leaves and most of the N was not transported to the plant body at the shedding of leaves and thus ended up in the litter (Sakio and Masuzawa 1992). After the development of an abscisssion layer in autumn, more N remained in the dead leaves of *M. gale* than in the dead leaves of *B. platyphylla*. Assuming that all of the autumnal decrease in leaf N resulted from the transport from dying leaves to the living plant body, the transported N amounted to 38 % of the seasonal maximal value of leaf N in *M. gale* and to 71 % in *B. platyphylla*. The increase in biomass of *M. gale* in Ozegahara moor (Hogetsu *et al.* 1982) probably affects the structure of the plant communities by changing the soil nutrient conditions.

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