

C-2.3 Studies on Dynamics of Heavy Metals in Woody Plants on Acid Soils

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Abstract

The effects of manganese (Mn) toxicity on photosynthesis of tree species in northern Japan representing different successional traits were examined. We sampled *Betula ermanii* and *Alnus hirsuta* as early successional species, *Ulmus davidiana* var. *japonica* as mid successional species, and *Acer mono* as late successional species. Seedlings were grown hydroponically in the solution containing nutrients and different concentrations of Mn (1, 10, 50, 100 mg L⁻¹) for 50 days. Based on the gas exchange measurements, Mn accumulation in leaves resulted in the decline of RuBP carboxylase activity and the rate of RuBP regeneration but effected less on the maximum efficiency of photochemistry in all plants. Based on these effects on photosynthesis, early successional species of *Betula ermanii* and *Alnus hirsuta* are suggested to have more tolerant capacity to excess Mn accumulation in leaves.

Abbreviations: PPF_D – photosynthetic photon flux density, Rubisco – ribulose-1,5-bisphosphate carboxylase/oxygenase, RuBP – ribulose-1,5-bisphosphate

INTRODUCTION

Manganese toxicity has one of the greatest adverse effects in plant growth in acid soils (Sumner et al., 1991). Soil acidification is considered as one of the main causes of forest decline due to "acid rain" (Haines & Carlson, 1989) since it increases leaching of basic cations such as Mg and Ca, and enhances solubility of toxic metals such as Mn and Al (Fernandez, 1989). Beech leaves were reported to have high Mn concentrations in southern Sweden where the soils were acidified by acid precipitation (Balsberg Pålsson, 1989). However, it's difficult to determine whether a given Mn concentration in a leaf is toxic or not because each species shows different tolerance and sensitivity for excess Mn (for review: Foy et al., 1978). T

herefore, we should establish what Mn concentration in the leaf of individual tree species constitutes toxicity.

The early successional species such as birch and willow were observed to grow in the acid sulfate soils with low pH levels below 5 which had poor vegetation (Sanada, 1986). Such adaptive capacity of early successional species observed in the acid soils could result from not only the high light environments due to poor vegetation but also from tolerant properties to Mn toxicity related to photosynthetic capacity. In our early study, white birch (*Betula platyphylla* var. *japonica*) known as early successional species was suggested to have higher tolerance to Mn toxicity when compared with herbaceous plants such as burley tobacco and wheat evaluated by net photosynthetic rate (Kitao et al., 1996).

Manganese toxicity is known to increase the activity of enzymes such as polyphenol oxidase, peroxidase and IAA oxidase, to interact other essential nutrients and to cause decrease in yield, (for review: Horst, 1988). Photosynthesis is among the most sensitive to Mn toxicity since the decline of photosynthesis preceded chlorophyll reduction and visible foliar symptoms (Nable et al., 1988).

In the northern broad-leaved forests of Japan, species of different successional traits exhibit different photosynthetic capacities, closely related to their leaf structures (Koike, 1988) and different shoot development patterns (Kikuzawa, 1988; Koike, 1990). Early successional species have higher mesophyll surface area per unit area (A_{mes}/A , Koike, 1988), which means lower resistance for CO₂ diffusion (Nobel, 1977), and higher photosynthetic capacity under high light while late successional species have lower A_{mes}/A and higher photosynthetic capacity under low light, and mid successional species has intermediate properties. As for shoot development, early successional species have higher rates of leaf turn-over, continuously producing the leaves with higher photosynthetic rates and shorter longevity (Koike, 1987).

We hypothesize that the photosynthetic properties of early successional species related to the leaf structure and the shoot development pattern should determine their tolerance capacity to Mn toxicity. Therefore, our aim was to examine how Mn toxicity affects the photosynthesis of each tree species by using gas exchange measurements, and to compare the tolerant capacity for each successional species with different photosynthetic properties. Based on these results, we discuss plausible mechanisms determining the tolerant capacity to Mn toxicity.

MATERIALS AND METHODS

Plant materials

The successional status of broad-leaved trees in northern Japan as described by Kikuzawa (1983) are *Alnus hirsuta* (Ah) and *Betula ermanii* (Be): early successional species, *Ulmus davidiana* var. *japonica* mid successional species and *Acer mona* late successional species. More than ten of two years-old seedlings of Be, Ah and Ud and 3 years-old seedlings of Am from the nursery (Oji Forestry & Landscaping Co., Ltd., Sapporo, Japan) were used. Seedlings of these 4 species were cultivated hydroponically in trays with adequate aeration and nutrient solution containing 40.0 (N), 10.8 (P), 27.2 (K), 6.1 (Mg), 5.0 (Fe) mg L⁻¹ and micronutrients in a glasshouse exposed to natural daylight. The initial size of seedlings

was from 20 cm to 30 cm in height. Four Mn concentrations were applied: 1 (control), 10, 50 and 100 mg L⁻¹ in the form of MnCl₂ · 2H₂O. The nutrient and Mn solution in the tanks were completely replaced every 10 days.

Measurement of photosynthetic gas exchange

The CO₂ assimilation rate was measured in two ways. First, net photosynthesis was made on attached, fully-expanded leaves (21 to 28 days-old) that had developed after the start of the Mn treatment. One leaf per several plants per treatment was measured by an open system infrared gas analyzer (Model H3, Analytical Development Company, UK). Light-saturated net photosynthesis at ambient CO₂ (35 Pa, Pn_{amb}), 13 Pa CO₂ and CO₂ free air were measured at 25°C, the optimum leaf temperature (Koike & Sakagami, 1985) and 1100 μmol m⁻² s⁻¹ PPFD provided by a cool halogen lamp (fiber optic light source, Nikon, Japan). Carboxylation efficiency (CE) was derived from the initial slope of CO₂ assimilation rate response to intercellular CO₂ pressures (Ci) (Farquhar & Sharkey, 1982; von Caemmerer & Farquhar, 1981) based on an assumption of uniform response of stomata (Terashima, 1992). As net photosynthesis at ambient CO₂ (35 Pa), 13 Pa CO₂ and CO₂ free air showed a linear response to Ci in this study, the initial slope was derived from the regression line.

Second, net photosynthesis at saturating CO₂ (5%, Pn_{sat}) was measured by oxygen evolution of leaf discs (3.14 cm²) in the gas phase with a Hansatech oxygen electrode in a thermostat-controlled chamber at 25°C, (Model LD2, Hansatech Instruments Ltd., King's Lynn, Norfolk, UK). A halogen lamp (Model LS2, Hansatech Instruments Ltd.) provided a light source of 900 and 50 μmol m⁻² s⁻¹ PPFD through neutral density filters (color glass filter, Toshiba, Japan). Dark respiration was measured by shutting off the light source. The maximum quantum yield of O₂ evolution (Φ_{O₂}), corrected for leaf absorptance, was derived from the slope of O₂ evolution rate at 0 (dark respiration rate) and 50 μmol m⁻² s⁻¹ PPFD. Leaf chlorophyll content was estimated using a SPAD chlorophyll meter (SPAD 502, Minolta, Osaka, Japan). The correction for leaf absorptance was made by the estimated leaf absorptance ratio from the function of SPAD value. Reflectance (R) and transmittance (Tr) were measured for the leaves for the wavelength 400–800 nm using a portable spectroradiometer (LI-1800C; Li-Cor Inc., Lincoln, NE, USA) with an external integrating sphere (LI-1800-12S; Li-Cor, Lei et al., 1996). Total absorptance was calculated as 1 - (R + Tr).

Manganese determination in leaves

Leaves used for gas exchange measurements were removed from the plants, rinsed with deionized water, then dried in an electric oven at 80°C for 3 days. Each sample was digested with a HClO₄ and HNO₃ mixture. The leaf Mn concentration was analyzed by atomic absorption spectrophotometer (Model 180-50, HITACHI, Japan).

RESULTS

Light-saturated net photosynthesis at ambient CO₂

For *Ulmus davidiana* var. *japonica* (*Ud*) in the treatment of 50 and 100 mg Mn L⁻¹, the plants were severely affected that there were no leaves suitable for measurements. The Pn_{amb} in control plants of *Betula ermanii* (*Be*), *Alnus hirsuta* (*Ah*), *Ulmus davidiana* var. *japonica* (*Ud*) and *Acer mono* (*Am*) were about 16, 13, 12 and 5 μmol m⁻² s⁻¹, respectively (Fig. 1). Among the species, *Am* showed significantly lower Pn_{amb} value, while the calculated intercellular CO₂ (Ci) was almost the same as other tree species (about 29 Pa). The decline of Pn was observed with increasing leaf Mn concentration in all plants.

At the leaf Mn level of 3000 μg g⁻¹ dw, the decline of Pn in *Be*, *Ah*, *Ud* and *Am* were 2.2 %, 5.6 %, 58 % and 40 % compared with control plants, respectively.

Carboxylation efficiency

Carboxylation efficiency (CE) decreased as leaf Mn concentration increasing in all plants (Fig. 2). Except for *Am*, the CE was about 0.6 μmol CO₂ m⁻² s⁻¹ Pa⁻¹ CO₂. The CE of *Am* was about 0.3 μmol CO₂ m⁻² s⁻¹ Pa⁻¹ CO₂. When leaf Mn concentration reached 3000 μg g⁻¹ dw, CE in *Be*, *Ah*, *Ud* and *Am* declined by 1.7 %, 3.7 %, 50 % and 31 %, respectively.

Net photosynthesis at saturating light and CO₂

The Pn_{sat} measured with oxygen electrode in saturated CO₂ and light was also affected by excess Mn in the leaves (Fig. 3). The Pn_{sat} in control plants of *Be*, *Ah*, *Ud* and *Am* were 14, 14, 16 and 14 μmol O₂ m⁻² s⁻¹, respectively. *Acer mono* and *Ud* showed considerably higher Pn_{sat} compared with Pn_{amb} in the control treatment. The Pn_{sat} declined with leaf Mn concentration increasing in all plants. The decline was significant in *Ud* and *Am*.

Quantum yield

Quantum yield of *Be*, *Ud* and *Am* in the control treatment was about 0.08 μmol O₂ μmol⁻¹ photon, while *Ah* lower value (about 0.07) (Fig. 4). Manganese accumulation had no effect on the Φ of *Be*. Quantum yield of *Ah* decreased with leaf Mn concentration increasing and the decline of Φ_{o2} was 0.7 % at leaf Mn concentration of 3000 μg g⁻¹ dw. Quantum yield of *Ud* and *Am* decreased more significantly with leaf Mn concentration increasing by 9.5 % and 8.3 % at 3000 μg g⁻¹ dw, respectively. However, compared with the decline in Pn_{amb}, Pn_{sat} and CE, that in Φ_{o2} was smaller.

DISCUSSION

The susceptibility to the Mn toxicity is known to be quite different among plant species (Foy et al., 1988). The present study also shows differential tolerant capacity among several tree species with different succ

essional traits grown in the northern Japan. The early successional species, *Betula ermanii* and *Alnus hirsuta* were tolerant while the mid and late successional species, *Ulmus davidiana* var. *japonica* and *Acer mono* were sensitive to excess Mn based on the gas exchange analysis.

Among the parameters of photosynthesis gas exchange, Pn_{amb} and CE were most affected by high Mn accumulation. The CE was derived from the initial slope of the relationship of CO_2 assimilation rate to intercellular CO_2 pressures. Since the Pn_{amb} was still on the initial slope, it showed the same trend as CE concerned with leaf Mn concentrations. The CE stands for the activity of photosynthetic key-enzyme, ribulose-1,5-bisphosphate carboxylase/oxygenase (rubisco, Sharkey, 1985; Farquhar & Sharkey, 1982). The carboxylation activity of rubisco decreased by the leaf Mn accumulations in all species (Fig. 2). The decline of the activity by rubisco might be caused by the change of its property. The activation of rubisco called carbamiration is known to need CO_2 and magnesium (Mg, Sharkey, 1990). When rubisco is activated by Mn^{2+} instead of Mg^{2+} , Mn^{2+} -rubisco has lower specificity of carboxylation to oxygenation by the factor of 20 than Mg^{2+} -rubisco (Jordan & Ogren, 1981).

The Pn_{sat} represents the rate of RuBP regeneration (Sharkey, 1985). The rate of RuBP regeneration is regulated by the rate of electron transport via the synthesis of NADPH and ATP (Sage & Reid, 1994).

Therefore, the decline of Pn_{sat} suggests that high leaf Mn reduced the electron transport activity under the saturating light ($900 \mu mol m^{-2} s^{-1}$ PPF). By means of the Chl fluorescence analysis, highly Mn accumulated leaves of white birch, *Betula platyphylla* var. *japonica* were observed to increase non-photochemical quenching after 15 min of 430 light exposure at ambient air (Kitao et al., 1996), indicating the increase in energy dissipation by photosystem II (PSII) as heat (Krause & Weis, 1991). It is possible that even when CO_2 was saturating as the substrate for rubisco the subsequent high DpH across the thylakoid readily turned the PSII in high Mn leaves to energy dissipating one.

At saturating CO_2 , Φ_{O_2} derived from the initial slope of photosynthetic light response curve stands for the maximum efficiency of photochemistry, as there are almost no effects from CO_2 diffusion resistance and photorespiration (Sage & Reid, 1994; Jarvis & Sandford, 1986; Björkman, 1981). Compared with the decline in the carbon reduction cycle activity, the maximum photochemical efficiency showed relatively high stability to leaf excess Mn accumulation.

The Pn_{sat} in control plants of early successional species of *Be* and *Ah* were almost the same as their Pn_{amb} , which the Pn_{sat} of *Ud* and *Am* were significantly higher than their Pn_{amb} . *acer mono* and *Ud* showed equivalent value of Pn_{sat} and Φ_{O_2} as compared to other species. These facts suggest that the capacity of photochemistry and the rate of RuBP regeneration of *Ud* and *Am* are equivalent to others, while their carboxylation capacity were considerably lower. During the measurement of Pn_{amb} , the calculated intercellular CO_2 pressure (C_i) was almost the same in all plants measured about 29 Pa. The difference of CE value in control plants may reflect the difference of CO_2 concentrations at the site of RuBP carboxylation caused by the different leaf structures (Syvertsen et al., 1995) since the enzymic properties of rubisco are almost the same among C_3 plants (Jordan & Ogren, 1983). The ratio of mesophyll area to leaf area is smallest in *Am* and increases with *Ud*, *Be*, *Ah* in order (Koike, 1988). As this ratio increases, CO_2 diffusion space increases and CO_2 concentration in the mesophyll may increase (Nobel, 1991; Sharkey, 1985) and show high CO_2 assimilation rate (Sage & Reid, 1994) if diffusion limitation is an important factor.

The different CO_2 concentrations expected from the variations of leaf structure among species may influence the differential susceptibility to high leaf Mn because the lower CO_2 concentrations decrease the rate of carboxylation and simultaneously increase that of oxygenation by rubisco (Ogren, 1984; Terashima et al., 1995.)

The alternative hypothesis which could explain the differential susceptibility is that the fractions of Mn^{2+} activated rubisco to total rubisco should be different among species. Manganese is known not to distribute homogeneously within the leaf. By means of autoradiographs using Mn^{54} , Mn was concentrated in marginal and interveinal tissue of leaves (Romney & Toth, 1954). Based on the X-ray microanalysis, Mn was observed to be highly concentrated in epidermis and mesophyll cells (Memon et al., 1981). Houtz et al. (1988) estimated that 31% of rubisco should have been activated by Mn^{2+} in the highly Mn concentrated ($6000 \mu\text{g g}^{-1} \text{dw}$) leaves of tobacco plants. Deposition of Mn in vacuoles is proposed to be a mechanism to compartmentalize potentially toxic Mn (Memon & Yatazawa, 1984). Rufty et al. (1979) suggested that higher tolerance of tobacco plant observed when grown in higher temperature resulted from higher deposition capacity of Mn in vacuoles in the expanding tissues. Therefore, the each successional species with different leaf structure including cell size, cuticle ratio, and leaf thickness (Koike, 1988) might have various capacity of vacuolar deposition of Mn.

Furthermore, the different shoot developing patterns might have influence on the sensitivity to Mn toxicity in the relation to plant growth. The higher rate of leaf turn-over observed in early successional species (Kikuzawa, 1988; Koike, 1990) may contribute to alleviate the effect of Mn toxicity on growth considered as the integration of photosynthesis via shedding of highly Mn-accumulated old leaves and continuous development of the less Mn-accumulated new leaves (Kitao 1996).

In conclusion, Mn accumulation in leaves affects the carboxylation efficiency and RuBP regeneration rate, while the maximum efficiency of photochemistry was affected to a lesser extent. Based on these results, *Be* and *Ah* are more tolerant for excess Mn accumulation in leaves than *Ud* and *Am*. The possibility is proposed that the early successional species should have greater tolerance than mid and late successional species.

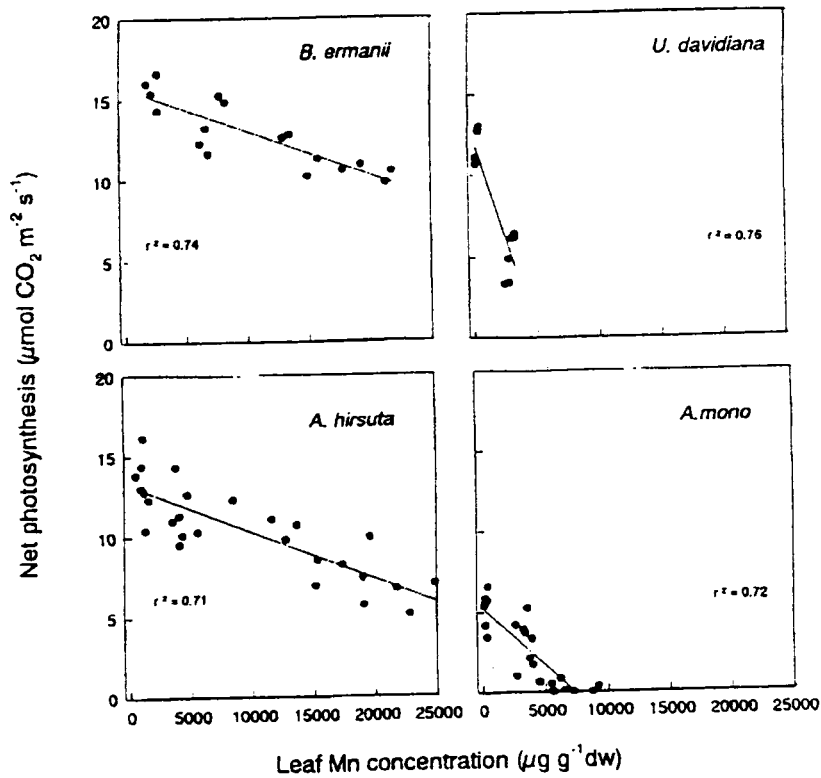


Fig. 1. Net photosynthesis of CO_2 uptake at ambient CO_2 (ca 35 Pa CO_2) and saturating light ($1100 \mu\text{mol m}^{-2} \text{ s}^{-1}$ PPF), as affected by leaf Mn accumulation. The measurements were taken on attached, fully-expanded leaves 21–28 days old at the leaf temperature of 25°C .

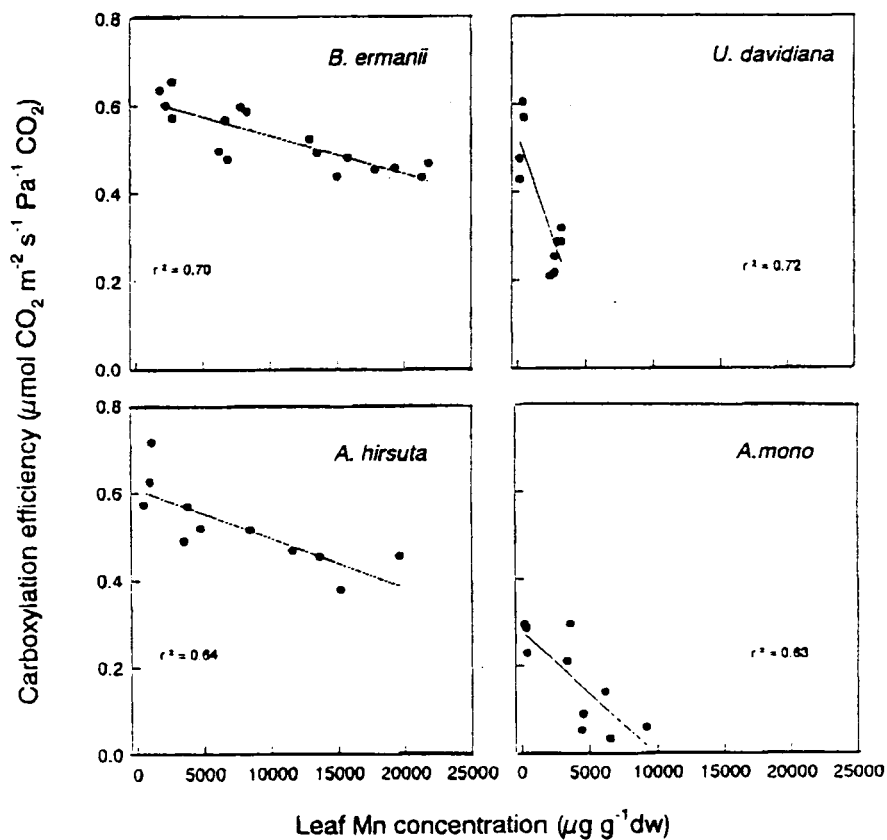


Fig. 2. Effect of leaf Mn accumulations on the carboxylation efficiency.

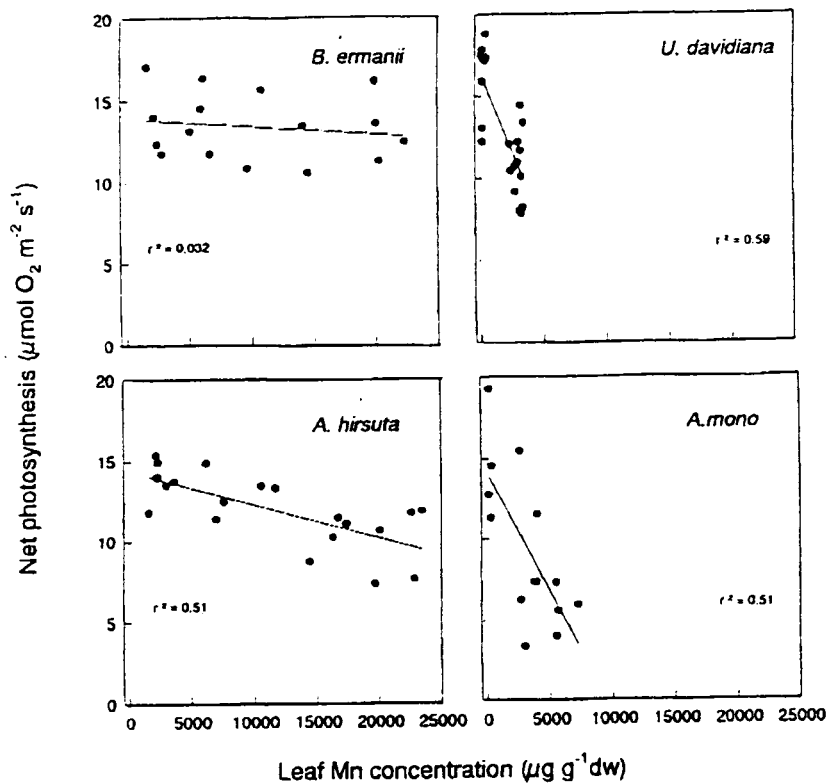


Fig. 3. Net photosynthesis of O_2 evolution at saturating CO_2 (5%) and saturation light ($900 \mu \text{ mol m}^{-2} \text{ s}^{-1}$ PPF), as affected by leaf Mn accumulation. The measurements were taken on leaf disks (3.14 cm^2) at 25°C .

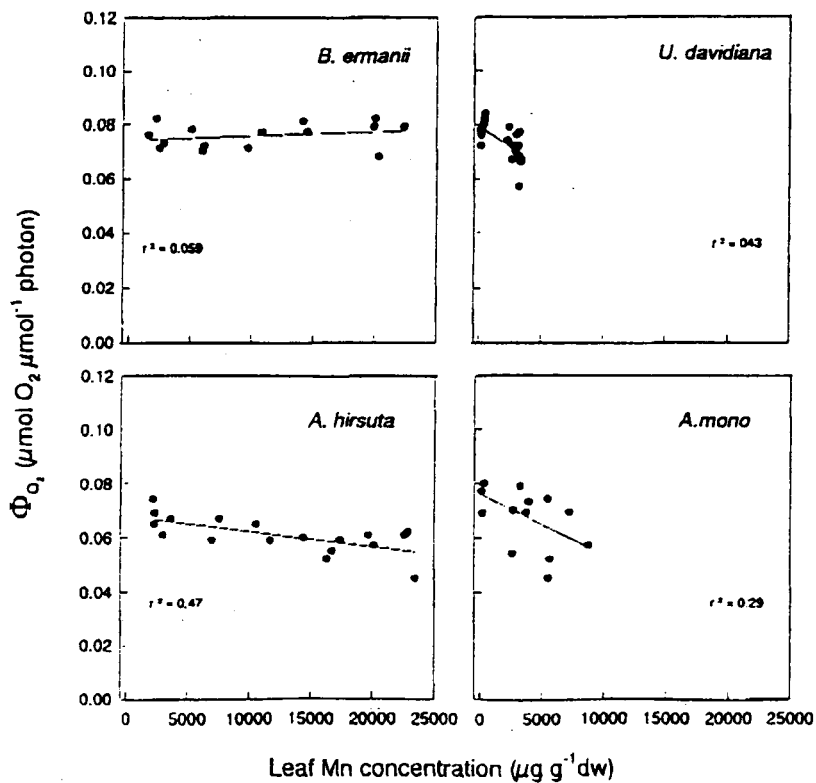


Fig. 4. Effect of leaf Mn accumulation on quantum yield of O_2 evolution (Φ_{O_2}). Φ_{O_2} was measured at saturating CO_2 (5%) and corrected for leaf absorbance.