# SHORT COMMUNICATION

# Photosynthetic and stomatal responses of *Larix kaempferi* seedlings to short-term waterlogging

Kazuhiko Terazawa,[1] Yutaka Maruyama[2] and Yasushi Morikawa[2]

[1] *Hokkaido Forestry Research Institute, Donan Branch, Kikyo, Hakodate, Hokkaido, 041 and* [2] *Forestry and Forest Products Research Institute, Ibaraki, 305 Japan*

The effects of short-term waterlogging on net photosynthesis, stomatal conductance and shoot water status of 2 year old seedlings of *Larix kaempferi* (Lamb.) Carr. were studied under controlled environmental conditions. Waterlogging for 8 days induced significant stomatal closure and reduced net photosynthesis. After 3 days of waterlogging, stomatal conductance was reduced to 35% of that of non-waterlogged plants, and net photosynthesis was reduced to 25% of the pre-waterlogged level. At the beginning of waterlogging, slight shoot dehydration was observed. However during the latter stage of the waterlogging shoot dehydration disappeared and stomatal conductance and net photosynthesis increased. No adaptive morphological changes to waterlogging were observed in the stems and roots. Recovery of stomatal conductance and net photosynthesis after drainage was not observed within 11 days.

Key words: *Larix kaempferi*; net photosynthesis; stomatal conductance; waterlogging; water relations.

## INTRODUCTION

Japanese larch, *Larix kaempferi* (Lamb.) Carr., a deciduous conifer, is native to the subalpine forests in central Japan. As a pioneer tree species, it invades the bare ground on disturbed mountain slopes or gullies and often forms pure stands (Tatewaki *et al*. 1965; Nakamura 1985). These habitats are characterized by well-drained soil conditions because the soil surfaces are generally covered with volcanic or fluvial coarse deposits. This species is rarely distributed on poorly drained flat sites, except for a sprinkling of dwarfish individuals that are found on the tussocks of the moor in the Nikko district (Tatewaki *et al*. 1965). This species may not be able to invade poorly drained sites because of a lack of resistance to flooding stress. This hypothesis is supported by the following facts observed in plantations of this species; the growth of Japanese larch planted on pseudogley soil, when subject to occasional standing water, was reduced markedly (Yamane *et al*. 1990) and seedling death occurred under ex-

tremely wet soil conditions that were caused by a combination of impermeable subsoil and continuous rainfall (Usui *et al*. 1990).

A few experimental studies have been carried out to examine the effects of anaerobic or flooded soil environments on the growth of Japanese larch seedlings. The high oxygen demand of Japanese larch roots in culture solution (Tsutsumi 1962), and the remarkable reduction in the growth rates of Japanese larch seedlings when flooded for 60 days (Tsukahara & Kozlowski 1984) indicate the plant's intolerance to flooded soil. However the physiological responses of this species to flooded soil have never been studied. In the present study the authors examined the effects of waterlogging on net photosynthesis, stomatal conductance and shoot water status of Japanese larch seedlings and reconfirmed the sensitivity of this species to soil inundation.

## METHODS

Two year old seedlings of Japanese larch were obtained from the nursery at the Hokkaido For-

estry Research Institute (Bibai, Hokkaido) and transported to the Forestry and Forest Products Research Institute (Kukisaki, Ibaraki) in April 1988. The seedlings were transplanted into plastic pots (16 cm in diameter and 27 cm in height, one plant per pot) containing a pumiceous soil for horticultural use and were grown under conditions of natural light with fertilizer and water added as needed. Plants averaged 45 cm in height.

In July 1988 five uniform seedlings were transferred to a growth chamber. Photosynthetically active radiation at plant height in the chamber was 450 $\mu$mol m$^{-2}$ s$^{-1}$, day length was 14 h, day and night temperatures were 25°C and 20°C, respectively, and relative humidity was kept near 80%. After 7 days of acclimation to the chamber conditions, three seedlings were immersed in large pots (25 cm in diameter and 29 cm in height) filled with tapwater. The level of the water was maintained just at the soil surface and the water was not changed. The two remaining seedlings were maintained in freely drained conditions and were watered daily. After 8 days of waterlogging, the waterlogged seedlings were drained and then were treated similarly to the non-waterlogged plants during the following 11 days. Prior to the main experiment, a preliminary experiment was performed on two waterlogged and two non-waterlogged seedlings in order to examine the propriety of the following experimental procedure, particularly the method of net photosynthesis measurement.

Measurements of net photosynthetic rate (Pn) and transpiration rate (Tr) were started 2 days before waterlogging and were made daily during waterlogging and at intervals of 1 or 2 days after drainage. Net photosynthetic rate was measured with an open system using an infra-red gas analyzer (Model ZFD, Fuji Electric Co. Ltd). One branch about 15 cm long containing several short and long shoots was selected from each seedling and placed in a cylindrical assimilation chamber (5 cm in diameter and 17 cm long). Air kept at 25°C was supplied to the chamber at a flow rate of 1–2 L min$^{-1}$. Net photosynthetic rate measurements were made on the same branches throughout the study at the 5th–6th hour of the light period. Transpiration rate was measured using a steady-state porometer (LI-1600, Li-Cor Inc.). Measurements were taken from three or four long shoots (about 5 cm long) with fully expanded leaves for each seedling. The same shoots were used

throughout the study. Transpiration rate measurements were made at the 4th–5th hour of the light period. At the end of the experiment, the foliage used for Tr measurements was removed and total leaf area (one sided) of each shoot was estimated using a leaf area meter (LI-3050, Li-Cor Inc.) in order to calculate stomatal conductances ($g_w$). Xylem pressure potential (XPP) of long shoots was determined using a pressure chamber (Model 600, PMS Instrument Co.). Xylem pressure potential was measured on one or two shoots of each plant at the 12th hour of the light period on the day before waterlogging, on the 2nd, 6th and 7th day after initiation of waterlogging, and on the 3rd, 8th and 11th day after drainage.

## RESULTS AND DISCUSSION

In the preliminary experiment, the authors checked the method of measurement of Pn by comparing the Pn value with that described by Crane *et al.* (1983). Net photosynthetic rate before the initiation of waterlogging in the preliminary experiment was 0.0549 $\pm$ 0.0103 $\mu$mol CO$_2$ g$^{-1}$s$^{-1}$ ($\bar{x} \pm$ SD). According to the PPFD (photosynthetic photon flux density)–Pn curves described by Crane *et al.* (1983), the Pn of young and mature foliage of Japanese larch seedlings at a PPFD of 450 $\mu$mol m$^{-2}$ s$^{-1}$ was approximately 3.4 $\mu$g CO$_2$ g$^{-1}$s$^{-1}$, corresponding to 0.077 $\mu$mol CO$_2$ g$^{-1}$ s$^{-1}$. The temperature of 25°C at which the Pn measurements for this study were taken was higher than the optimum of 17–20°C for net photosynthesis reported by Crane *et al.* (1983). Thus, values of Pn measured in this study appear to be reasonable, even though a simplified assimilation chamber without humidity control or internal air circulating fan was used.

The waterlogging induced rapid reductions of $g_w$ and Pn in Japanese larch seedlings (Fig. 1). Three days following the initiation of waterlogging, $g_w$ was reduced to 35% of that of the non-waterlogged seedlings and Pn was reduced to 25% of the pre-waterlogged level. Thereafter, $g_w$ and Pn tended to increase in waterlogged seedlings, although both $g_w$ and Pn did not reach the level of the non-waterlogged plants. The increase in Pn following a rapid reduction after the initiation of waterlogging was also observed in the preliminary experiment under the same experimental procedures (Fig. 1).
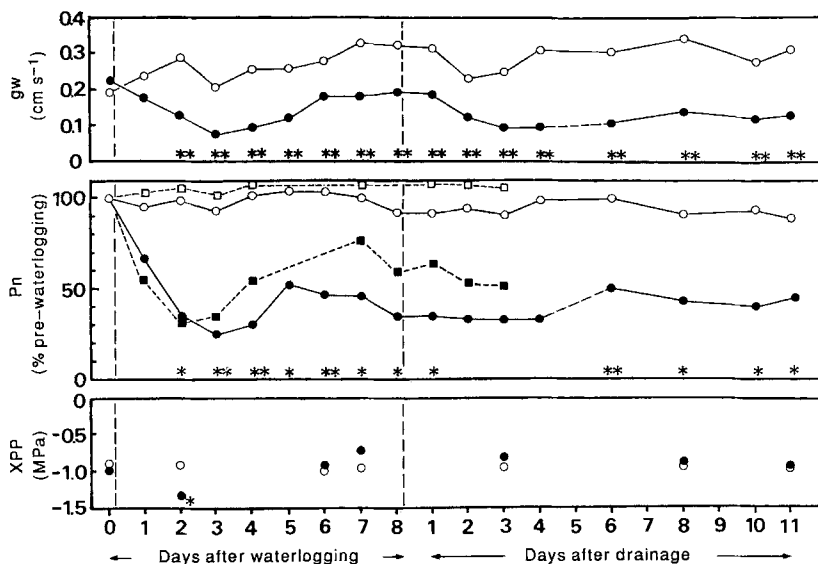
**Fig. 1.** Effects of waterlogging on $g_w$, Pn and shoot XPP of *Larix kaempferi* seedlings. Each point represents the mean of three plants for waterlogged (●) and of two plants for non-waterlogged (○) treatments, respectively. Data of waterlogged plants after the 6th day of drainage represent the mean of two plants, because one waterlogged seedling shed leaves severely. Data of the preliminary experiment under the same experimental procedure were also included for Pn, where squares (■) represents the mean of two waterlogged plants and (□) represents the mean of two non-waterlogged plants. Statistical differences between treatments are denoted by asterisks: $*P < 0.05$; $**P < 0.01$.

Rapid reductions in $g_w$ and Pn in waterlogged seedlings during the first 3 days of the treatment were associated with a slight decrease in XPP. Xylem pressure potential in waterlogged seedlings decreased to $- 1.3$ MPa on the 2nd day of waterlogging, while in the non-waterlogged plants XPP showed constant values between $- 0.9$ MPa and $- 1.0$ MPa throughout the experiment (Fig. 1). During the latter period of waterlogging, however, XPP of the waterlogged seedlings increased to values similar to those of the non-waterlogged plants.

Flooding or waterlogging induces rapid stomatal closure and a reduction in photosynthesis in various woody species (Regehr *et al*. 1975; Zaerr 1983; Kozlowski 1984; Pezeshki & Chambers 1985, 1986). Therefore the rapid reductions in $g_w$ and Pn after the initiation of waterlogging in this study are common responses for waterlogged plants. However the subsequent increases in $g_w$ and Pn that were observed during the latter stage of continuous waterlogging have been reported rarely. Although stomatal re-openings during a longer flooding period were reported in seedlings of *Fraxinus pennsylvanica* (Sena Gomes & Koz-

lowski 1980), *Gmelina arborea* and *Tectona grandis* (Osonubi & Osundina 1987) and *Eucalyptus camaldulensis* (Van Der Moezel *et al*. 1989), they were associated with the formation and growth of adventitious roots on submerged portions of stems. In this study, neither adventitious roots nor hypertrophy of the lenticel were formed or developed on the stems, both considered to be important morphological adaptations to flooding (Hook 1984). Furthermore, no newly formed roots, as reported by Hook and Brown (1973), were observed in waterlogged plants at the end of the experiment. Thus, the increases in $g_w$ and Pn at the latter period of the waterlogging were probably due to some cause other than adaptive morphological changes of stem and root.

The responses of $g_w$ and Pn in waterlogged Japanese larch were similar to the pattern of transpiration in actively growing *Picea sitchensis* seedlings that were waterlogged (Coutts 1981). In that study, transpiration increased temporarily following a rapid reduction after the initiation of waterlogging. Coutts also reported an initial reduction followed by an increase in leaf water potential, similar to that observed in this study.

Coutts suggested that this complex response in the transpiration and water relations of waterlogged *Picea sitchensis* seedlings might involve changes in hormone balance.

The tips of some leaves in one waterlogged seedling became chlorotic 3 days after the termination of waterlogging. Thereafter, the chlorosis extended to whole leaves and the leaves began to shed 6 days after drainage. At the end of the experiment this plant had lost almost all its leaves. Neither chlorosis nor shedding of leaves occurred in the remaining two seedlings from the waterlogged treatment during the 11 days of the recovery period. However recoveries of $g_w$ and Pn were not observed in those seedlings after drainage. Even 11 days after the termination of waterlogging, $g_w$ remained at 42% of that of the non-waterlogged plants and Pn was still 46% of the pre-waterlogged level.

The influence of 8 days of waterlogging on $g_w$ and Pn in Japanese larch was prolonged after the termination of the waterlogging. As XPP in the waterlogged seedlings was equal to that of the non-waterlogged plants at this time, the prolonged stomatal closure after drainage may not have been caused by loss of leaf turgor, but possibly by permanent injury of the stomata (Kozlowski & Pallardy 1979) or growth regulator imbalance (Reid & Bradford 1984).

The results of the present study suggest that the net production of Japanese larch seedlings may be severely reduced by soil inundation of even a short duration due to its prolonged influence after drainage. Tsukahara and Kozlowski (1984) also emphasized the extreme sensitivity of Japanese larch seedlings to flooding from the results of their 60 day flooding experiment, where the dry weight of roots was already reduced 20 days after flooding. The lack of tolerance to waterlogging may be an important factor that restricts this species in its natural habitats to well-drained sites.

## ACKNOWLEDGEMENTS

## REFERENCES

Coutts M. P. (1981) Effects of waterlogging on water relations of actively-growing and dormant Sitka spruce seedlings. *Ann. Bot.* 47: 747–53.

Crane J. L. Jr, Dickmann D. I. & Flore J. A. (1983) Photosynthesis and transpiration by young *Larix kaempferi* trees: C₃ responses to light and temperature. *Physiol. Plant.* 59: 635–40.

Hook D. D. (1984) Adaptations to flooding with fresh water. In: *Flooding and Plant Growth* (ed. T. T. Kozlowski) pp. 265–94. Academic Press, Orlando.

Hook D. D. & Brown C. L. (1973) Root adaptations and relative flood tolerance of five hardwood species. *For. Sci.* 19: 225–9.

Kozlowski T. T. (1984) Responses of woody plants to flooding. In: *Flooding and Plant Growth* (ed. T. T. Kozlowski) pp. 129–63. Academic Press, Orlando.

Kozlowski T. T. & Pallardy S. G. (1979) Stomatal responses of *Fraxinus pennsylvanica* seedlings during and after flooding. *Physiol. Plant.* 46: 155–8.

Nakamura T. (1985) Forest succession in the subalpine region of Mt. Fuji, Japan. *Vegetatio* 64: 15–27.

Osonubi O. & Osundina M. A. (1987) Stomatal responses of woody seedlings to flooding in relation to nutrient status in leaves. *J. Exp. Bot.* 38: 1166–73.

Pezeshki S. R. & Chambers J. L. (1985) Responses of cherrybark oak seedlings to short-term flooding. *For. Sci.* 31: 760–71.

Pezeshki S. R. & Chambers J. L. (1986) Variation in flood-induced stomatal and photosynthetic responses of three bottomland tree species. *For. Sci.* 32: 914–23.

Regehr D. L., Bazzaz F. A. & Boggess W. R. (1975) Photosynthesis, transpiration, and leaf conductance of *Populus deltoides* in relation to flooding and drought. *Photosynthetica* 9: 52–61.

Reid D. M. & Bradford K. J. (1984) Effects of flooding on hormone relations. In: *Flooding and Plant Growth* (ed. T. T. Kozlowski) pp. 195–219. Academic Press, Orlando.

Sena Gomes A. R. & Kozlowski T. T. (1980) Growth responses and adaptations of *Fraxinus pennsylvanica* seedlings to flooding. *Physiol. Plant.* 66: 267–71.

Tatewaki M., Ito K. & Toyama M. (1965) Phytosociological Study on the Forests of Japanese Larch (*Larix leptolepis* Gordon). Nihon Shinrin Shokusei Kenkyukai, Sapporo (in Japanese with English summary).

Tsukahara H. & Kozlowski T. T. (1984) Effect of flooding on *Larix leptolepis* seedlings. *J. Jpn. For. Soc.* 66: 333–6.

Tsutsumi T. (1962) Studies on nutrition and fertilization of some important Japanese conifers. *Bull. Govt For. Exp. Stn* 137: 1–158 (in Japanese with English summary).

Usui G., Terazawa K. & Goshu K. (1990) The growth depression of afforested young *Larix kaempferi* under moist conditions. *Trans. 101th Mtg Jpn. For. Soc.* 101: 293–4 (in Japanese).

Van Der Moezel P. G., Watson L. E. & Bell D. T. (1989) Gas exchange responses of two *Eucalyptus*

species to salinity and waterlogging. *Tree Physiology* 5: 251–7.

YAMANE G., USUI G., GOSHU K., KIKUCHI K. & TERAZAWA K. (1990) Multivariate analysis of site factors on afforested *Larix leptolepis* Gordon. *Bull.* *Hokkaido. For. Res. Inst.* 28: 54–63 (in Japanese).

ZAERR J. B. (1983) Short-term flooding and net photosynthesis in seedlings of three conifers. *For. Sci.* 29: 71–8.