EFFECTS OF OZONE AND/OR EXCESS SOIL NITROGEN ON GROWTH, NEEDLE GAS EXCHANGE RATES AND RUBISCO CONTENTS OF Pinus densiflora SEEDLINGS

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Abstract. The effects of ozone (O\(_3\)) and excess soil nitrogen (N), singly and in combination, on growth, needle gas exchange rates and ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) contents of Pinus densiflora seedlings were investigated. One-year-old seedlings were grown in 1.5-L pots filled with brown forest soil with 3 levels of N supply (0, 100 or 300 mg N·L\(^{-1}\) fresh soil volume). The seedlings were exposed to charcoal-filtered air or 60±5 nL·L\(^{-1}\) O\(_3\) (8 hours a day) in naturally-lit phytotrons for 173 days from 22 May to 11 November.

The exposure to O\(_3\) or high N supply to the soil caused a significant reduction in the dry weights of the seedlings. Although no significant interactive effects of O\(_3\) and excess soil N were detected on the dry weight growth of the seedlings, the whole-plant dry weight of the O\(_3\)-exposed seedlings grown in the soil treated with 300 mg N·L\(^{-1}\) was greatly reduced compared with the control value. Ozone reduced net photosynthetic rate at 350 μmol·mol\(^{-1}\) CO\(_2\) (A\(_{350}\)), carboxylation efficiency (CE) of photosynthesis and Rubisco content without a significant change in the gaseous phase diffusive conductance to CO\(_2\) (gs) of the needles. The excess soil N reduced the A\(_{350}\), CE, gs and Rubisco content of the needles. These results suggest that the reduction in the dry weight growth of Pinus densiflora seedlings induced by the exposure to O\(_3\) and/or excess soil N was caused by reduction in the net photosynthetic rate mainly due to the decrease of Rubisco quantity in the chloroplasts.

Keywords: ozone, excess soil nitrogen, Pinus densiflora Sieb. et Zucc., growth, photosynthesis

1. Introduction

Ozone (O\(_3\)) is considered to be a major phytotoxic air pollutant and has been associated with forest decline observed in Europe and North America (Skärby et al., 1998). In Japan, relatively high concentrations of O\(_3\) above 100 nL·L\(^{-1}\) (ppb) are detected in several mountainous areas where forest decline or tree dieback have been observed (Totsuka et al., 1997).

In general, soil nitrogen (N) is one of the most important limiting factors in forest ecosystems when N load to the soil is relatively low. However, elevated N deposition to forest ecosystems is considered to be one of the environmental stresses adversely affecting forest tree species (Nihlgård, 1985; Aber et al., 1989). When N supply to forest ecosystems is much more than its demand, excess soil N may lead to a reduction in the growth and physiological functions of forest tree species as a result of soil acidification and/or imbalance of plant nutrient status (Skeffington and Wilson, 1988; Nilsson et al., 1988). Therefore, there is the possibility that forest tree species will be adversely affected by elevated O\(_3\) and excess soil N, singly and in combination, in the near future.
However, there is no information about the combined effects of \( \text{O}_3 \) and excess soil N on Japanese forest tree species.

In the present study, we investigated the effects of \( \text{O}_3 \) and excess soil N, singly and in combination, on dry weight growth, needle gas exchange rates and ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) content of \text{Pinus densiflora} seedlings. We used \text{Pinus densiflora} as the plant material because this tree is one of the most representative Japanese coniferous tree species, and forest decline and dieback of this tree are observed mainly in the western parts of Japan.

2. Materials and Methods

Nitrogen was added as \( \text{NH}_4\text{NO}_3 \) to brown forest soil collected from the A-horizon below coniferous tree stands (Kusaki, Gunma Prefecture, Japan) at 0, 100 or 300 mg N-L\(^{-1}\) fresh soil volume, which corresponds to 0, 135 or 405 kg N-ha\(^{-1}\), respectively. These soil N treatments were designated as N-0, N-100 or N-300, respectively. On 28 April, one-year-old seedlings of \text{Pinus densiflora} Sieb. et Zucc. were transplanted into 1.5-L pots filled with the soil of these three different N contents. All the seedlings were grown in a naturally-lit greenhouse for 24 days, and then were grown in four naturally-lit phytotrons (Koito Industry Co.) for 173 days from 22 May to 11 November. In the phytotrons, air temperature and relative air humidity were maintained at 25/18 °C (6:00-18:00/18:00-6:00) and 70±5%, respectively. During the experimental period, the potted soil was irrigated daily with deionized water. From 22 May to 11 November, the seedlings were exposed to charcoal-filtered (CF) air (<5 nL-L\(^{-1}\) \( \text{O}_3 \)) or 60±5 nL-L\(^{-1}\) \( \text{O}_3 \), 8 hours a day, from 9:00 to 17:00 in the phytotrons. Two replicated chambers were randomly assigned to each gas treatment. A mixture of charcoal-filtered air and that with \( \text{O}_3 \), which was generated with a silent electrical discharge \( \text{O}_3 \) generator (MO-5, Nihon Ozone Co.), was introduced into the phytotrons through a water trap to remove nitrogen by-products produced by the \( \text{O}_3 \) generator (Brown and Roberts, 1988). The concentrations of \( \text{O}_3 \) in the phytotrons were continuously monitored with an UV absorption \( \text{O}_3 \) analyzer (Model 1100, Dylec Inc.). On 11 November, all the seedlings were harvested for measuring the dry weights of plant organs.

On 8 September, measurements of gas exchange rates of current-year needles were made using an infrared gas analyzer system with a 12 cm\(^3\) leaf chamber (LCA-4, ADC Co. Ltd.). During the measurements of needle gas exchange rates, air temperature, relative air humidity and photosynthetic photon flux density in the leaf chamber were maintained at 25.0±0.5 °C, 65±5% and 1500±50 \( \mu \text{mol-m}^{-2}\cdot\text{s}^{-1} \), respectively. Charcoal-filtered air was introduced into the leaf chamber at a rate of 273 \( \mu \text{mol-s}^{-1} \). Net photosynthetic rate at 350 \( \mu \text{mol-mol}^{-1} \) \( \text{CO}_2 \) \( (A_{350}) \) and gaseous phase diffusive conductance to \( \text{CO}_2 \) \( (g_s) \) were determined on the basis of needle dry weight. The intercellular \( \text{CO}_2 \) concentration \( (C_i) \)-response curves of net photosynthetic rate \( (A) \) were generated by measuring the \( A \) at 8 different atmospheric \( \text{CO}_2 \) concentrations. The carboxylation efficiency \( (CE) \) of photosynthesis was determined as the initial slope of \( A/C_i \) curve.