Eurasian J. For. Res. 22: 23-27,2022 DOI:10.14943/EJFR.22.23

Responses of ectomycorrhizal diversity of larch and its hybrid seedlings and saplings to elevated CO_2, O_3, and high nitrogen loading

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Abstract

The genus *Larix* (larch) is broadly distributed in northern Eurasia, including Japan and Korea. Larch species are a typical afforestation tree in northeastern China, Russia, Korea, and Japan. Larch and red pine seedlings inoculated with *Pisolithus arhizus* (earth-ball fungus) have increased photosynthetic rates as well as being acid-soil tolerant. Infecting *P. arhizus* with a variety of ectomycorrhizae (ECM) accelerated these functions. Under elevated CO_2 (about 700 ppm) conditions, larch and pine seedlings infected with ECM showed no down-regulation in photosynthesis and displayed higher growth rates than those grown in ambient CO_2 (380 ppm) conditions. Simulated nitrogen (N) deposition (50 kg N $hr^{-1}yr^{-1}$) increased photosynthesis and growth of F_1 hybrid larch for the initial two years. There were no significant effects for the next three to eight years on Brown Forest Soil. Among the three species (Japanese, Dahurian and F_1 hybrid larch), the dominant ECM species preferred N. Elevated O_3 decreased growth, ECM infection rates, and ECM species diversity; however, elevated CO_2 moderated or increased them in hybrid larch F_1. After elevated CO_2 and O_3 fumigation on F_1 seedlings, we found *Suillus* sp., a larch specialist ECM in F_1, which means these ECM will be effective materials for larch plantations.

Key words: Elevated CO_2, Ozone, nitrogen deposition, larch, hybrid larch, ectomycorrhiza (ECM).

Introduction

The larch is a typical ectomycorrhizal (ECM) tree species and dominant deciduous conifer in the northern hemisphere, especially dominating permafrost ecosystems (Osawa *et al.* 2010, Qu *et al.* 2010, 2014; Figure 1). Some larch species are representative afforestation species in northeast China, Russian Far East, Korea, and Japan. Most larches can tolerate cold and late frost (Kurahashi 1988), thus attaining significant biomass with a high growth rate in cold regions.

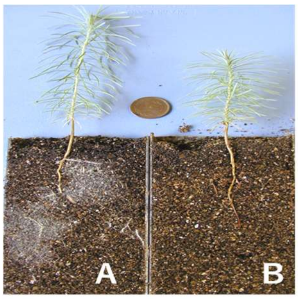

Figure 1. Japanese larch seedlings inoculated with Ectomycorriza; *Pisolithus arhizus* (A) and No-inoculated as Control (B). Diameter of the coin in photo is 2.3mm. (Qu L,

Due to these good growth traits, larch (Japanese larch) was exported to Europe as a pollen resource. This high growth results from its high photosynthetic rate and unique arrangement of two different types of needles, i.e., short-shoot and long-shoot needles (Kitaoka *et al.* 2020). For breeding practices, paternal inheritance in chloroplast DNA in larch species (Szmidt *et al.* 1987) has surely been contributing to improving photosynthesis via pollen father.

From 1999, the Chinese government decided to reforest farmland and employed larch in northeast China to increase forests (Zhang *et al.* 2000). In northern Japan, hybrid larch F_1 (*Larix gemelinii* var. *japonica* × *L. kaempferi*; hereafter F_1) was produced to improve tolerance to grazing damage by voles and stem straightness, thus enhancing timber quality (Hokkaido 2007; Ryu *et al.* 2009). Nowadays propagation methods have improved, i.e., stem cutting, so the F_1 is becoming a principal afforestation tree species in Japan (Kita *et al.* 2018).

Since the 1960s, with the rapid economic development, air pollutants (NOx, SOx, Ozone: O_3) and increasing CO_2 have impacted forest health and vigor. SOx pollutants were reduced by desulfurization equipment during the 1970s; however, NOx has hardly decreased because it is mainly produced by diesel cars (Izuta 2017). NO_2 is transformed to O_3 and NO under UV radiation (Gregg *et al.* 2003). In addition,

(Received; Oct. 31, 2021: Accepted; Nov. 11, 2021)
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atmospheric CO_2 concentration [CO_2] has increased since the Industrial Revolution and has reached around 418 ppm at the current rate of 2.2 ppm yr^{-1}. The photosynthetic rate is usually reduced by elevated CO_2 under root restricting conditions. In addition, lockdowns applied amid the Covid-19 pandemic may decrease the rate to about 7 ppm yr^{-1} between 2019 and 2020.

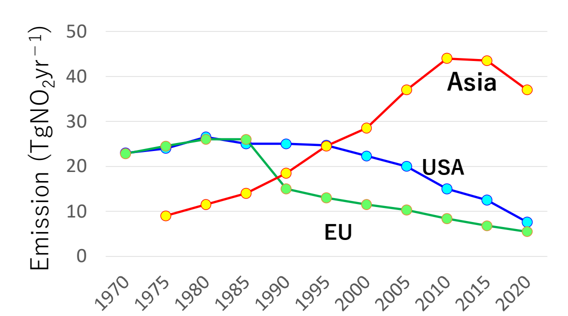

Figure 2. Yearly trend of NO_2 emission of 3 regions. Data are cited from Akimoto (2003) and Kurokawa and Ohara (2020). 1990~: EU-LRTAP convention; USA-EPA Air Pollutant Emission and NIES.

Sufficient CO_2 and adequate N are regarded as the productive atmospheric environment for forest trees. We summarize the effects of changing environment (CO_2, N deposition, and O_3) on the growth of larch and larch-ECM interactions.

Elevated CO_2

In many cases, we found down-regulation of photosynthesis, even in a FACE system (Free Air CO_2 Environment; Koike *et al.* 2015). We expected ECM to act as a carbon sink and moderate down-regulation in photosynthesis, Japanese red pine seedlings inoculated with a kind of commercial ECM (*Pisolithus arhizus*) showed no down-regulation (Choi *et al.* 2005). The same trend was expected in larch species (Qu *et al.* 2004). Hybrid larch F_1 was planted in the FACE for three years and its aboveground tended to fall down by wind because of increased above-ground biomass (Watanabe *et al.* 2013). After five years of CO_2 fumigation in FACE, Japanese larch decreased biomass allocation to branches and increased it by about 20% in the stem compared with ambient CO_2 (Watanabe *et al.* 2016). Birch (*Betula platyphylla* var. *japonica*) and kalopanax (*Kalopanax septemlobus*) allocate about 10% less biomass to their stems. However, no clear anatomical structure of them changed with elevated CO_2.

Elevated O_3

Ozone levels have been increasing around the northern hemisphere in the past several decades (Sitch *et al.* 2007). With the GIS method and OTC experiment, Watanabe *et al*. (2010) predicted that the growth of Japanese larch would be reduced by elevated O_3 around the Kanto plain, however, it will be not as large in northern Japan. As mentioned above, O_3 concentration is generally high in the suburbs due to the reaction of NO_2 with UV radiation (exhaust gas: NO from diesel cars plus UV=NO_2) (Gregg *et al.* 2003). This cycling of NOx under UV can lead to O_3 in suburban green areas around big cities.

According to recent NO_2 trend in Asia (Kurokawa and Ohara 2020), we should pay attention to rapid an increase of NO_2 emission as a precursor of O_3. Based on the statistics of EU (LRTAP) and US (EPA), the NO_2 emission from Asia reached to about 43 Tg NO_2 yr^{-1} which is 4 times larger than that from EU or UAS (Figure 2). Therefore, we should know this evidence and try to give O_3 tolerance to larch plantation as suggested by Watanabe *et al.* (2006).

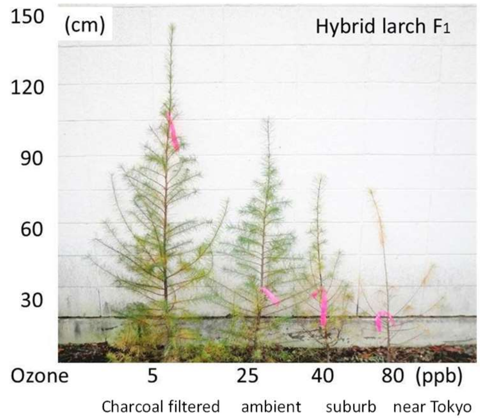

Figure 3. Growth o F_1 under different O_3 (Modified from Kita *et al.* 2018)

Based on screening experiments using open top chambers (OTCs), Yamaguchi *et al*. (2011) summarized the O_3 sensitivity of 18 tree species with use of OTC and potted seedlings in Japan, and among them, the Japanese larch showed a moderate sensitivity. What about the F_1 under elevated O_3?

The specific difference in O_3 sensitivity was examined between Japanese and F_1 seedlings planted on the ground of OTCs (<5, 25, 45, and 80 ppb). The growth of both larches was significantly suppressed by 80 ppb (Figure 3; Sugai *et al.* 2017). The biomass of F_1 seedlings decreased under 25 ppb, compared to <5 ppb, but due to its heterosis it maintained a similar biomass with Japanese larch seedlings in elevated O_3 treatments. The biomass of Japanese larch decreased at 80 ppb, but it was lower at low O_3 (<5 ppb) compared to 25 ppb. This phenomenon is regarded as "hormesis" (Agathokleous 2018). Namely, mild stresses often accelerate growth and some growth function. As almost all practical production of larch seedlings is done in the suburbs, we tried to use ethylenediurea (EDU) to moderate the adverse effects of elevated O_3 on larch seedlings (Agathokleous *et al.* 2020, 2021). An effective concentration of EDU is

400 mg EDU L^{-1} applied as soil drench it protects both Japanese and F_1 seedlings against toxicities induced by exposure to elevated O_3 for up to 3-4 years.

Nitrogen deposition and elevated O_3

The combined effects of N and elevated O_3 were studied in seedlings of two broadly distributed species, Siebold's beech and Japanese larch, in OTCs. The beech is classified as highly sensitive to O_3（Yamaguchi *et al.* 2011）. With increasing N (NH_4NO_3), O_3 sensitivity of the beech increased in terms of Accumulated exposure Over Threshold of 40 ppb O_3 (AOT40) (Yamaguchi *et al.* 2007). In contrast, O_3 sensitivity of Japanese larch decreased with increasing N up to 50 kg N ha^{-1} yr^{-1} (Watanabe *et al.* 2006) as suggested in above-section. However, F_1 had slightly increased O_3 sensitivity with 50 kg N $ha^{-1}yr^{-1}$ under free-air O_3 exposure (Dong-Gyu *et al.* 2016), which may be attributed to decreased (or too much accelerated) leaf life-span with N application (Sugai *et al.* 2019).

In general, phosphorous (P) is the second most important nutrient after N; for the growth of F_1 an adequate supply P and N is required. Magnesium (Mg) was the limiting element in the nursery of Hokkaido University (Mao *et al.* 2014). In this edaphic condition, we examined the effects of N deposition (NH_4NO_3) on the growth of hybrid larch F_1 for eight years. However, except for N application to F_1 by the 2nd year, almost no difference in growth of F_1 was found between the N treatment and the control (=no N application). Based on DNA analysis of the ITS region in symbiotic ECM (Wang *et al.* 2015), most of them infecting Japanese larch, Dahurian larch, and F_1 were nitrogenous species (Wang *et al.* 2018).

Elevated CO_2 and O_3

Plants usually close stomata under elevated CO_2, and consequently, plants can reduce the absorption of O_3. We examined the effects of elevated O_3 (80 ppb）on the growth and ECM infection and diversity of F_1 seedings under elevated CO_2 in OTCs（Wang *et al.* 2015). Under elevated O_3, ECM infection rate and species diversity were reduced; however, these trends were moderated by elevated CO_2 (600 ppm) (Figure 4). Early successional types of ECMs were only found at ambient and elevated CO_2. However, larch specialist *Suillus* sp. dominated at elevated O_3. This evidence suggests that *Suillus* sp. may support the growth of the host plant: F_1.

As Qu *et al.* (2004) suggested the photosynthetic rate in larch species infected with multiple ECM species was higher than that with a single ECM species (Figure 2). This phenomenon is recognized as follow: most ECM activity depends on soil pH; some ECM prefer low pH

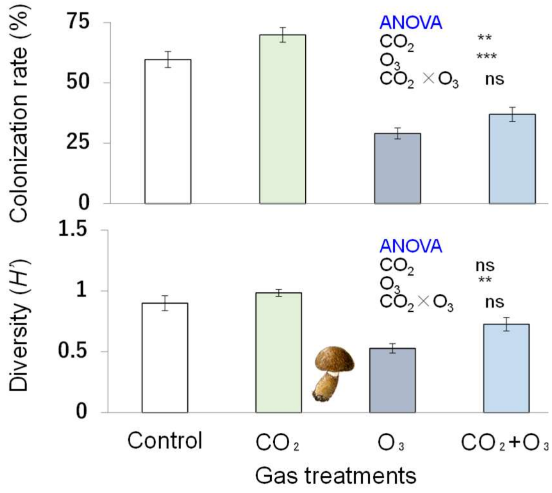

Figure 4. Combination effect of elevated O_3 and CO_2 on ECM infection and diversity (*H'*).
Modified from Wang *et al.* (2015) and the *H'* is the Shannon index showing biodiversity. The larger *H'*, the higher biodiversity is. Illustration of fruit body indicates *Suillus* sp.

but some require neutral or high pH (6~8), such as *Rhizopogon rubescens* (Furusawa *et al.* 2020). Aluminum (Al) is released below pH<4.5 and inhibits root growth. These are species-specific traits, and multiple ECM infections may benefit the host plants.

Conclusion

Larch trees planted in China in 1999 to return farmland to forests should be thinned. In northern Japan, Japanese larch is intensively used after considerable improvements in timber utilization. After harvest, we should make plantations with container-grown seedlings to save labor and attain high plantation efficiency. We also expect a new CO_2 sink when planting a new plantation. If we make new plantations with hybrid larch F_1, we should ensure they do not increase N deposition under elevated O_3. To make planting stocks, we should inoculate larch seedlings with ECM (*Suillus* sp.); larch specialist for increased tolerance against environmental stress.

Acknowledgements:

We deeply appreciate the staff of Hokkaido Forestry Research Institute for their continuous support of our researches. Financial support in part by JST (No. JPMJSC18HB: representative researcher, M. Watanabe of TUAT and T. Watanabe of HU) and by the National Key Research and Development Program of China (2017YFE0127700; LY. Qu) are acknowledged.

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