

2-001-2

Molecular detections of soil inocula of an ectomycorrhizal fungus, *Rhizopogon togasawariana*, in Japanese Douglas-fir forests

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Purpose: Endangered Japanese Douglas-fir, *Pseudotsuga japonica*, relies on root associating ectomycorrhizal fungi for its nutrient absorption. Among the fungi, an ectomycorrhizal fungus, *Rhizopogon togasawariana*, was detected only from Japanese Douglas-fir seedlings grown in soils under both in vitro and in situ. This infers that spores rather than hyphae of the species can play an important role for the growth and survival of the host tree. The goal of this study was to clarify spatial distribution of the soil inocula of *R. togasawariana* in Japanese Douglas-fir forests. To achieve this, we developed specific primer pairs for *R. togasawariana*, and examined soil samples collected in the field to detect the fungus.

Methods: We established three study sites at where Japanese Douglas-fir populations were remained. In each site, soil samples were collected at the border between Japanese Douglas-fir forests and Japanese cedar (*Cryptomeria japonica*) and/or Japanese cypress (*Chamaecyparis obtusa*) plantations. Specific primer pairs for *R. togasawariana* focusing on the ITS region were designed based on its holotype sequence, and their effectiveness was assessed by using *R. togasawariana* colonized roots. Candidate specific primer pairs were applied for genomic DNA extracted directly from the soils. Successfully amplified PCR products were sequenced and compared by the BLAST search to estimate the identity of fungal species.

Results and Conclusions: In total, 80 pairs of primers were designed in silico, of which 5 pairs were specific and successfully amplified derived from in vitro *R. togasawariana* roots. Based on the obtained data, we discuss the availability for the primer pairs to detect the fungal inocula and possible distribution pattern of this fungus.