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Short Communication

USDA Forest Service, Rocky Mountain Research Station, Moscow, Idaho, USA

Occurrence of the Root Rot Pathogen, *Fusarium commune*, in Forest Nurseries of the Midwestern and Western United States

Mee-Sook Kim[1], Jane E. Stewart[2,3], R. Kasten Dumroese[2] and Ned B. Klopfenstein[2]

Authors' addresses: [1]Department of Forestry, Environment, and Systems, Kookmin University, Seoul 136-702, Korea; [2]USDA Forest Service, Rocky Mountain Research Station, Moscow, ID 83843, USA; [3]Present address: Horticultural Crops Research Laboratory, USDA ARS, 3420 NW Orchard Avenue, Corvallis, OR 97330, USA (correspondence to Ned B. Klopfenstein. E-mail: nklopfenstein@fs.fed.us)

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Abstract

Fusarium commune can cause damping-off and root rot of conifer seedlings in forest nurseries, and this pathogen has been previously reported from Oregon, Idaho, and Washington, USA. We collected *Fusarium* isolates from additional nurseries in the midwestern and western USA to more fully determine occurrence of this pathogen. We used DNA sequences of the mitochondrial small subunit gene to identify *F. commune*. In addition to confirming the occurrence of *F. commune* in Oregon, Idaho, and Washington, USA, we also discovered that *F. commune* is even more widespread with this first report of *F. commune* occurring in Nevada, Montana, Nebraska, and Michigan, USA.

Introduction

In most container and bareroot nurseries, the genus *Fusarium* is ubiquitous in nursery soils, on seeds of several conifer species, and on healthy and diseased conifer seedlings, especially Douglas-fir (*Pseudotsuga menziesii*), western white pine (*Pinus monticola*), and ponderosa pine (*Pinus ponderosa*) (James et al. 1990). The first report of Fusarium root rot in forest nurseries identified *Fusarium oxysporum* as the major pathogen, based on morphology (Bloomberg 1981). However, selected *Fusarium* spp. isolates, previously characterized as pathogenic on Douglas-fir seedlings, displayed a range of high, moderate, and low virulence (Stewart et al. 2006). On the basis of DNA sequences (mtSSU: mitochondrial small subunit and EF-1α: nuclear translation elongation factor 1-alpha), Stewart et al. (2006) identified all the highly virulent isolates as *F. commune*, a recently named species (Skovgaard et al. 2003). DNA sequences from the mtSSU or EF-1α regions were useful to distinguish *F. commune* from *F. oxysporum*.

Currently, *F. commune* is only reported in Oregon (Skovgaard et al. 2003; Leon 2009), Idaho (Stewart et al. 2006), and Washington (Leon 2009), although Fusarium root rot is widely reported from forest nurseries throughout western North America. Presumably, *F. commune* is one of the major pathogens in this disease; yet, little is known about its occurrence in forest nurseries across the midwestern and western USA. Thus, our study objective was to evaluate the occurrence of *F. commune* in the midwestern and western USA.

Materials and Methods

We collected 260 isolates of *Fusarium* spp. from forest nurseries in the midwestern and western USA, including Oregon (79 isolates), Idaho (56), California (43), Washington (31), Montana (14), Nevada (13), Utah (12), Nebraska (7), and Michigan (5). These isolates, collected from one to five nurseries in each state, represented diverse sources of host/substrate: (i) diseased or healthy seedlings of Douglas-fir, western white pine, ponderosa pine, grand fir (*Abies grandis*), sagebrush (*Artemisia tridentata*), rabbitbrush (*Chrysothamnus* sp.), eastern redcedar (*Juniperus virginiana*), western larch (*Larix occidentalis*), blue spruce (*Picea pungens*), lodgepole pine (*Pinus contorta*), Austrian pine (*Pinus nigra*), bitterbrush (*Purshia tridentata*), Pacific yew (*Taxus brevifolia*), western redcedar (*Thuja plicata*), and western hemlock (*Tsuga heterophylla*), (ii) containers used to grow various conifer seedlings, and (iii) soil/growing media. All 260 isolates were previously classified as *F. oxysporum* based on morphological similarities (Bloomberg 1981), but were not identified based on DNA sequence data. The fungal cultures used in this study were deposited at the USDA Forest Service, Rocky Mountain Research Station, Forestry Sciences Laboratory, Moscow, Idaho, USA, and living subcultures are available upon request.

Table 1
Isolates of *Fusarium commune* derived from the midwestern and western USA

Collection no.	Isolate no.	Host/Substrate	Location	GenBank accession no.
9806H	Q247	*Taxus brevifolia* (diseased)	Washington	JF264721
0131A	Q301	*Pseudotsuga menziesii* (healthy)	Oregon	JF264722
9246D	Q210	*Pinus monticola* (diseased – container)	Idaho	JF264723
0437G	Q321	*Pseudotsuga menziesii* (container)	Nevada	JF264724
9209C	Q211	*Pinus monticola* (healthy – container)	Montana	JF264725
9922A	Q153	*Juniperus virginiana* (diseased)	Nebraska	JF264727
0631C	Q329	Soil	Michigan	JF264726

For molecular species identification, we characterized all isolates using mtSSU sequences. We used the Stewart et al. (2006) protocol for fungal culture and PCR amplification of the region. Because template DNA was derived from scrapings of actively growing mycelial cultures (3–5 days old), we did not extract DNA. PCR amplicons from the mtSSU region were sequenced with an ABI 3700 DNA Sequencer (Life Technologies Corp., Carlsbad, CA, USA) at the University of Wisconsin – Biotechnology Center (Madison, WI, USA). Resulting sequences were blasted to GenBank database. To confirm species identification, we subsequently performed phylogenetic analyses (Parsimony and Bayesian), using the methods of Stewart et al. (2006), on 29 *F. commune* isolates identified with mtSSU sequences. For the analyses, we obtained from GenBank mtSSU sequences of previously identified *F. oxysporum* (*Fo*-N1, DQ016170; NRRL 26442, AF250562; NRRL 31073, AF362290; NRRL 25603, AF008453), *F. commune* (*Fc*-H14, DQ016150; NRRL 22900, AF362288; NRRL 28058, AF324293), *F. subglutinans* (NRRL 22016, AF160289), and *F. proliferatum* (NRRL 22057, AF060376). The mtSSU sequence of *F. redolens* (NRRL 31255) was obtained from a TreeBASE (http://www.treebase.org) matrix (M143) to serve as the outgroup. Phylogenetic analyses were conducted using PAUP*4.0b10 (Swofford 2003) and MrBayes v.3.0b4 (Huelsenbeck and Ronquist 2001).

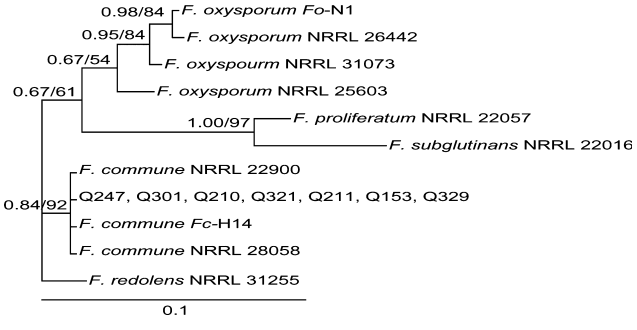

Fig. 1 Bayesian phylogeny generated from the mitochondrial small subunit rDNA sequences. Posterior probabilities/parsimony bootstrap values are listed for each clade. *Fusarium* isolates from this study (Q247, Q301, Q210, Q321, Q211, Q153 and Q329) are described in Table 1. The phylogeny was rooted with *Fusarium redolens* (NRRL 31255) and reference strains of *F. oxysporum* (*Fo*-N1, NRRL 26442, NRRL 31073, NRRL 25603), *F. commune* (*Fc*-H14, NRRL 22900, NRRL 28058), *F. proliferatum* (NRRL 22057) and *F. subglutinans* (NRRL 22016) are included from previous studies (Skovgaard et al. 2003; Stewart et al. 2006)

Results and Discussion

The 29 *Fusarium commune* isolates we identified, based on GenBank BLAST searches using mtSSU sequences, had 100% sequence identity with *F. commune* isolates described by Skovgaard et al. (2003) (AF362277-AF362283; AF362285-AF362288; AF250560; AF324293; U34509) and identified by Stewart et al. (2006) (DQ016142-DQ016146; DQ016156; DQ016157; DQ016159). We found *F. commune* in Oregon (nine of 79 isolates), Idaho (eight of 56), Washington (four of 31), Montana (one of 14), Nevada (three of 13), Nebraska (two of 7), and Michigan (two of 5). Sequences of mtSSU among the 29 *F. commune* isolates were identical therefore only one *F. commune* isolate from each state was deposited into GenBank (Table 1). No *F. commune* isolates were found among our samples from California (43 isolates representing three nurseries) or Utah (12 isolates representing one nursery). We identified most (ca. 80%) *Fusarium* isolates derived from the midwestern and western USA as *F. oxysporum* based on the mtSSU sequences, although we observed a few isolates of *F. redolens*, *F. solani*, and *Fusarium* spp. (data not shown).

Phylogenetic analyses based on mtSSU region showed that all the *F. commune* isolates from this study grouped with the previously identified *F. commune* (Skovgaard et al. 2003; Stewart et al. 2006) that is genetically distinct from *F. oxysporum* (Fig. 1). The mtSSU data set consisted of 650 characters, and of these, 599 were constant. Of the 51 variable characters, 26 (4% of the total characters) were parsimony informative. The cladogram produced by Bayesian analysis displayed two distinct and well-supported (posterior probability $< 95\%$) clades, which separated *F. commune* and *F. oxysporum* on the basis of the mtSSU data set (Fig. 1). Parsimony analysis of the data set showed similar results.

Fusarium commune is a recently identified species (Skovgaard et al. 2003). It is distinct from, but genetically close to, the *F. oxysporum* and *Gibberella fujikuroi* species complex. Although subtle morphological differences may be observed between *F. commune* and *F. oxysporum* (Skovgaard et al. 2003), these differences are not always a reliable method for distinguishing the two species (Stewart et al. 2006). Based on morphological analyses, *F. oxysporum* was historically identified as the main cause for Fusarium root rot and damping-off of conifer seedlings in forest nurseries (James et al.

1990). Stewart et al. (2006) first showed, however, that *F. commune* is a cause of Fusarium root disease on Douglas-fir. Our study results indicate that *F. commune* occurs widely throughout the midwestern and western USA. More intensive surveys are needed to better characterize the distribution and host range of *F. commune* in forest nurseries worldwide.

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