MOLECULAR CHARACTERIZATION OF *FUSARIUM* SPECIES ASSOCIATED WITH DAMPING-OFF OF CONIFER SEEDLINGS IN TREE NURSERIES

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Abstract

Fusarium can cause significant damage within conifer nurseries across the United States. Several species of *Fusarium*, which are morphologically similar to *F. oxysporum*, are known to cause damping-off, including *F. commune*, *F. fujikuroi*, *F. proliferatrum*, and *F. solani*. Isolates of *Fusarium* spp. (ca. 431) were collected from Idaho, Oregon, Nevada, Washington, Nebraska, Michigan, North Carolina, South Carolina, and Georgia. Initial species identification was conducted based on colony morphology. Because *F. oxysporum* and *F. commune* have almost identical colony morphology with the exception that *F. commune* can sporadically produce polyphialides, DNA sequencing of translation elongation factor 1-α (*tef1*) gene was conducted to identify *Fusarium* isolates. To date, *tef1* sequences have been obtained for 345 of the 431 isolates of *Fusarium*. Using NCBI Blast, 76.7% of the isolates were identified as *F. oxysporum*, 8.5% as *F. commune*, 4.4% as *F. redolens*, 1.5% as *F. proliferatum*, 3.2% as *F. fujikuroi*, 1.2% as *F. solani,* and < 1% of *F. proliferatum*. Several isolates were also labeled *Fusarium* spp. Further work will also be discussed, such as the development of microsatellite markers for population studies of *F. commune* associated with different hosts and diverse geographic areas.

Introduction

The genus *Fusarium* is ubiquitous in most container and bareroot nurseries on healthy and diseased conifer seedlings, in nursery soils, and on conifer seeds of several species, especially Douglas-fir (*Pseudotsuga menziesii*), western white pine (*Pinus monticola*), ponderosa pine (*P. ponderosa*), and loblolly pine (*P. taeda*) (James et al. 1990, Cram & Fraedrich 2009). Since the first report of Fusarium root rot in forest nurseries, the major pathogen was previously identified as *F. oxysporum* based on morphology (Bloomberg 1981). However, isolates of selected *Fusarium* spp. that had previously been characterized as pathogenic on Douglas-fir seedlings displayed a range of high, moderate, and low virulence (Stewart et al. 2006). Stewart et al. (2006) used DNA sequencing to determine that all the highly virulent isolates on Douglas-fir were *F. commune*, a recently named species (Skovgaard et al. 2003). Further, *F. proliferatum* was recently identified as a pathogen of sugar pine (*P. lambertiana*) in California (Stewart et al. 2016). DNA sequences from the mitochondrial small subunit (mtSSU) and translation elongation factor 1-alpha (*tef1*) regions are useful for distinguishing *Fusarium* species. Various *Fusarium* spp. have been reported as pathogens of conifer seedlings in tree nurseries. The objective of this study was to evaluate the occurrence and distribution of *Fusarium* species in conifer tree nurseries across the United States (Figure 1).

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Methods

A total of 345 isolates of *Fusarium* spp. were collected in forest nurseries throughout the western, mid-western, and southern United States (Table 1). Isolates from each state were collected from one to five forest nurseries, as well as from diverse sources of hosts/substrates including (1) diseased or healthy seedlings of Douglas-fir, western larch (*Larix occidentalis*), western redcedar (*Thuja plicata*), yew (*Taxus* sp.), lodgepole pine (*P. contorta*), western hemlock (*Tsuga heterophylla*), western white pine, ponderosa pine, grand fir (*Abies grandis*), rabbitbrush (*Chrysothamnus* sp.), sagebrush (*Artemisia tridentata*), Austrian pine (*P. nigra*), loblolly pine, blue spruce (*Picea pungens*), bitterbrush (*Purshia tridentata*), and eastern redcedar (*Juniperus virginiana*), (2) containers of various conifer seedlings, and (3) soil/growing medium. All 345 isolates were characterized using mtSSU and/or *tef1*. Template DNA was derived from scrapings of actively growing mycelial cultures (3-5 days old) or using chelex, a quick DNA extraction method. The PCR products were sequenced at the University of Wisconsin – Biotechnology Center (Madison, WI, USA) or at Eurofins (Centennial, CO, USA), and the sequences were Blasted to GenBank database (https://blast.ncbi.nlm.nih.gov/Blast).

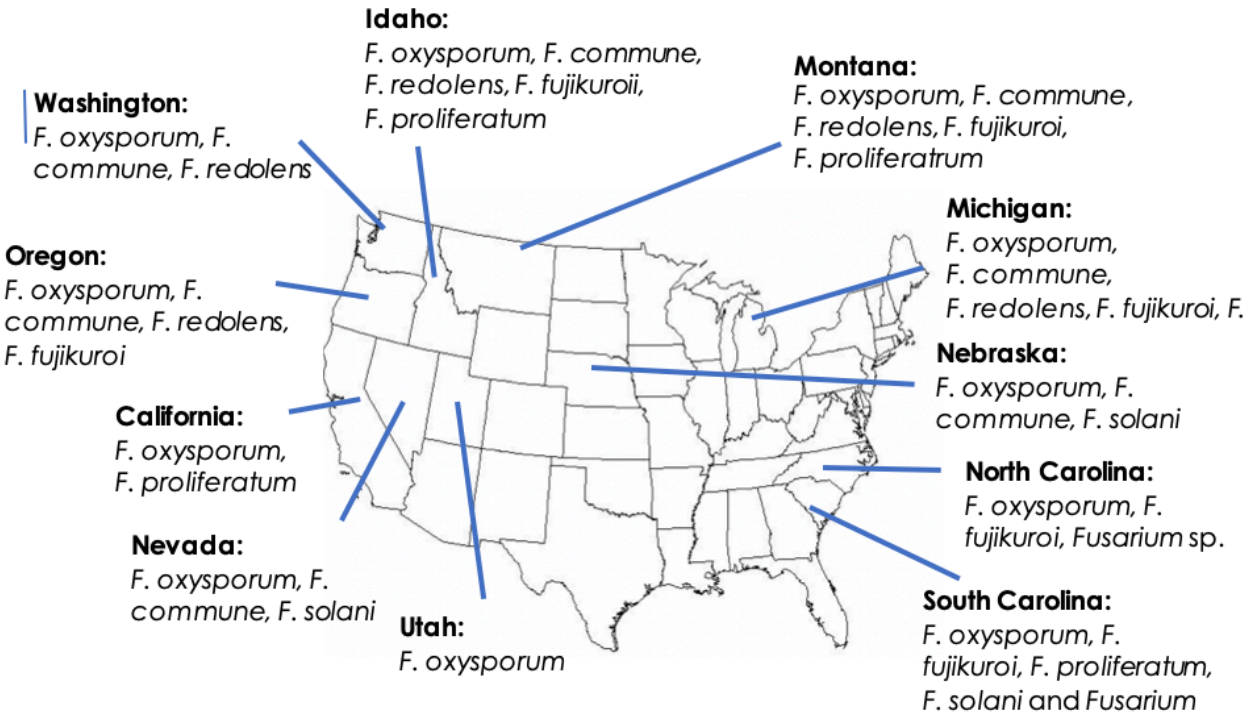

Figure 1: Fusarium *species found in each state.*

Results and Discussion

Seven species of *Fusarium* were identified within conifer nurseries in the western, mid-western, and southern states using genetic data from the mtSSU and *tef1*regions. The most diversity was observed in Idaho, Montana, Michigan, and South Carolina where at least five species were identified. The least diversity was observed in Utah, where *F. oxysporum* was identified from the one nursery sampled. Furthermore, although a total of 45 isolates were collected from five nurseries in California, 40 isolates were *F. oxysporum*, and only one was *F. proliferatum*. *Fusarium commune* was identified in Washington,

Oregon, Nevada, Idaho, Montana, Michigan, and Nebraska (Kim et al. 2012). *Fusarium redolens, F. fujikuroi,* and *F. solani* were also identified from most states. Interestingly, an unidentified *Fusarium* species was identified in North Carolina and South Carolina. Further sequencing of the RNA polymerase II (RPB2) region is warranted for identifying these isolates. Understanding the pathogenicity and host range of each *Fusarium* species is important to combat damping off in conifer tree nurseries, and more intensive studies are needed to better characterize the distribution of *Fusarium* species in these nurseries.

Table 1: *Count of* Fusarium *spp. isolates collected from each state (total n=350).*

State	Total number of isolates
CA	46
ID	56
MI	5
MT	14
NE	7
NV	13
OR	87
NC	42
SC	37
UT	12
WA	31

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