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Delphinella shoot blight and Grovesiella canker on *Abies lasiocarpa* in western USA

Gary A. Chastagner[a], Kathy Riley[a], Katie P. Coats[a], Håvard Eikemo[b] and Venche Talgø[b]

[a]Puyallup Research and Extension Center, Washington State University, Puyallup, WA, USA; [b]Biotechnology and Plant Health, Norwegian Institute of Bioeconomy Research (NIBIO), Ås, Norway

ABSTRACT

The impact of Delphinella shoot blight (*Delphinella abietis*) and Grovesiella canker (*Grovesiella abieticola*) on subalpine (*Abies lasiocarpa*) and corkbark fir (*A. lasiocarpa* var. *arizonica*) in a provenance trial in Idaho (ID) was evaluated in 2013. Both pathogens were previously reported from North America on fir species. *D. abietis* had been found on subalpine fir in USA, but not in ID, and *G. abieticola* on grand fir (*Abies grandis*) in ID, but not on subalpine or corkbark fir. *D. abietis* kills current-year needles and in severe cases buds and shoots, and *G. abieticola* results in dead shoots and branches and can eventually kill whole trees. Significant differences between provenances in susceptibility to *D. abietis* and *G. abieticola* were observed in the provenance trial in ID. In general, subalpine fir was more susceptible to both diseases than corkbark fir. In 2013, *D. abietis* was also found on subalpine fir in the Puget Sound area of Washington State and *G. abieticola* was seen on white fir (*Abies concolor*), but neither disease was detected in native stands of subalpine fir in Washington State. Morphological features of both fungi were described from samples collected in the provenance trial in ID in May 2016.

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Delphinella abietis; *Grovesiella abieticola*; subalpine fir; corkbark fir; *Abies lasiocarpa* var. *arizonica*; provenance trial; Idaho

Introduction

True firs (*Abies* spp.) play an important ecological role in their areas of origin, but they also have qualities making them desirable for other purposes. Many species have been collected and planted around the world. A number of true firs are commercially grown as Christmas trees and for bough production, used in landscaping, and grown for timber in the Pacific Northwest (PNW) states of Washington (WA), Oregon (OR), and Idaho (ID) in the United States (USA). Unfortunately, firs are susceptible in varying degrees to a number of fungal diseases, here represented by two recently found on subalpine fir [*Abies lasiocarpa* var. *lasiocarpa* (Hook.) Nutt.] and corkbark fir [*A. lasiocarpa* var. *arizonica* (Merriam) Lemmon] in a provenance trial in ID; Delphinella shoot blight caused by the fungus *Delphinella abietis* (O. Rostr.) E. Müll. and Grovesiella canker caused by the fungus *Grovesiella abieticola* (Zeller & Goodd.) M. Morelet & Gremmen. Initially, we visited the field trial in ID to look for the canker fungus *Neonectria neomacrospora*, which has recently caused severe damage to subalpine and other fir species in Scandinavia (Talgø et al. 2013). No *N. neomacrospora* was found in ID. However, it was found elsewhere in the PNW (WA and OR) (Chastagner et al. 2014).

The first description of *D. abietis* was as *Sphaerella abietis* O. Rostr. in Denmark nearly 115 years ago (Rostrup 1902). As indicated by the common name, the disease causes dead needles and shoots. In Canada, *D. abietis* was reported on subalpine fir (Funk 1985). However, the closely related species *Delphinella balsameae* (A. M. Waterman) E. Müller seems to be a more common pathogen on fir species there and has been reported on subalpine fir, white fir [*Abies concolor* (Gordon & Glend.) Lindl. ex Hildebr.], and balsam fir [*Abies balsamea* (L.) Mill.] in Vermont, USA (Merrill et al. 1997). In the USA, *D. abietis* has been reported on subalpine fir, but not from ID (Farr & Rossman 2016). In Europe, *D. abietis* is mainly a problem on fir species in Scandinavian Christmas tree and bough production, and especially in Norway (Solheim & Skage 2002; Talgø et al. 2016).

The first description of *G. abieticola* was from North America (Zeller & Goodding 1930). It was reported to damage noble fir (*Abies procera* Rehder), grand fir [*Abies grandis* (Douglas ex D. Don) Lindl.], white fir, and Shasta fir (*Abies magnifica* var. *shastensis* Lemmon) in the western USA (Chastagner & Staley 1985). The two latter hosts are especially susceptible (Chastagner et al. 2015a). Funk (1978) reported it on grand fir from Canada. *G. abieticola* has been reported on grand fir in ID (Farr & Rossman 2016), but not on corkbark or subalpine fir. It was reported on the latter host from Colorado (Farr & Rossman 2016). Attacks by *G. abieticola* result in dead shoots and branches and can eventually kill whole trees. According to Sieber & Kowalski (1993), *G. abieticola* is uncommon in Europe and not known to cause cankers. They described the conidial stages of the fungus, *Pitostroma abietinum* (macroconidia) and *Agyriellopsis caeruleo-atra* (microconidia), from European silver fir (*Abies alba* Mill.) in Poland. Furthermore, Sieber (1989) described *A. caeruleo-atra* as one of the most frequently occurring harmless endophytes in healthy twigs of *A. alba*.

The main aim of this work was to quantify the damage done by *D. abietis* and *G. abieticola* on subalpine and corkbark

CONTACT Gary A. Chastagner chastag@wsu.edu Puyallup Research and Extension Center, Washington State University, 2606 West Pioneer, Puyallup, WA 98371-4998, USA

fir in the provenance trial in ID in 2013. This is the first time *G. abieticola* and *D. abietis* have been reported from subalpine or corkbark fir in ID, and also the first time the two diseases have been reported on subalpine fir in western USA. In 2013, we also found *D. abietis* on subalpine fir in WA. Preliminary results from the survey were presented at an international conference (Chastagner et al. 2015b).

Material and methods

Background and layout of the provenance trial in Idaho

The provenance trial was established with three-year-old seedlings of subalpine and corkbark fir in 2001 at Sandpoint, ID (Barney et al. 2013), a field at the recently abandoned University of ID research station. The goal was selection of suitable trees for landscaping and Christmas tree production. The seed sources originated from the Rocky Mountain states of Colorado, Utah, and New Mexico and from the mountains of Arizona. Barney et al. (2013) indicated that the exact locations of the collection sites were proprietary and they only referred to the sources by the name of the national forests from which they were collected. Corkbark fir grows in native stands between 2438 and 3658 m toward the south, while subalpine fir is found between 610 and 3353 m northwards. A total of 960 trees were planted [3 replicates (blocks) of 16 randomly distributed subplots (seed sources) with 20 trees in each]. The 16 seed sources included six corkbark fir (Apache-Sitgreaves, Cibola, Coconino, Coronado, Gila, and Santa Fe) and 10 subalpine fir (Arapaho, Carson, Cibola, Dixie, Kaibab, Manti-La Sal, Rio Grande, San Isabel, San Juan, and Uncompahgre) (Barney et al. 2013).

Disease severity in the provenance trial in Idaho

A majority of the trees in the provenance trial did not look healthy in August 2013. Close examination revealed that they were suffering from Grovesiella canker and/or Delphinella shoot blight.

Disease estimates for both *G. abieticola* and *D. abietis* were carried out on a scale from 0 to 3, a modification of the scale used by Talgø et al. (2016):

- 0 = no damage
- 1 = minor damage
- 2 = medium damage
- 3 = severe damage

Identification

Samples were brought to the laboratory for confirmation of the field diagnosis in 2013. New samples were collected in May 2016. All samples were incubated in polyethylene bags serving as moist chambers (room temperature and saturated air for 5–7 days) for fruiting bodies to mature. Pseudothecia (*Delphinella*) and apothecia (*Grovesiella*) were studied in a dissecting microscope. Other morphological features, especially asci and ascospores, were examined in a light microcope from artificially colored (cotton blue) slides. Spore measurements were taken on 25 ascospores from each of the two fungi. Slides containing ascospores of *Delphinella* were made from needles that were infected in 2015.

Samples of *D. abietis* collected in OR in 2014 were identified by direct amplification (Harrington & Wingfield 1995) of fruiting body material and sequencing of the Internal Transcribed Spacer (ITS) regions of the ribosomal DNA.

ITS sequences from mature apothecia of *G. abieticola* collected from corkbark (seed source/provenance Coconino) and subalpine fir (seed source Manti-La Sal) in ID on May 21, 2016 were compared to sequences from mature apothecia collected from red fir (*A. magnifica* A. Murray) on 17 May 2016 in Puyallup, WA.

Statistic

The differences in resistance to *G. abieticola* and *D. abietis* between corkbark fir and subalpine fir was tested using a hierarchical generalized linear model in Minitab (REF) with provenance as a fixed effect nested under each of the two hosts.

Results

Symptoms, signs, and identification

In 2013, symptoms of both diseases were abundant at the Idaho site. By 2016, many of the trees had been taken out, leaving mostly the more disease resistant corkbark fir to serve as a future seed orchard. Very little infection by *D. abietis* was seen in 2016, but severe attacks by *G. abieticola* were still evident on many trees. Identification of the two pathogens was possible in the field based on the symptoms and signs they displayed.

Delphinella abietis

Needles infected by *D. abietis* in spring 2013 had become necrotic by August of the same year. The edges of the dead needles had curled toward the stomatal bands on the underside of the needles (*A. lasiocarpa* also has a stomatal band on the upper surface of the needles). In the most severe cases, buds and entire shoots were killed (Figure 1(A)). Dead needles were covered with numerous, black fruiting bodies (pseudothecia and pycnidia) (Figure 1(B)). Microscopy in 2013 showed that the fruiting bodies were either empty or not ripe. No ascospores were found. Most of the samples collected in 2016 had very limited disease symptoms on any growth since 2013 (Figure 2(A,B)). This may be due to several factors connected to the removal of the most susceptible trees: (1) less dense plantation and thereby unfavorable climatic conditions for the fungus, (2) reduction of inoculum/disease pressure, and (3) the most resistant trees remaining in the field. However, a few of the 2015 needles had pseudothecia with asci and ascospores (Figure 2(C–E)). The spores measured [11.5 – (15.7) – 18.6 × 3.2 – (5.0) – 6.1 µm] ($n = 25$).

ITS sequences from a fruiting body of *D. abietis* collected from noble fir in OR in 2014 (GenBank accession no. KX364384) were identical (only one base pair different) to a

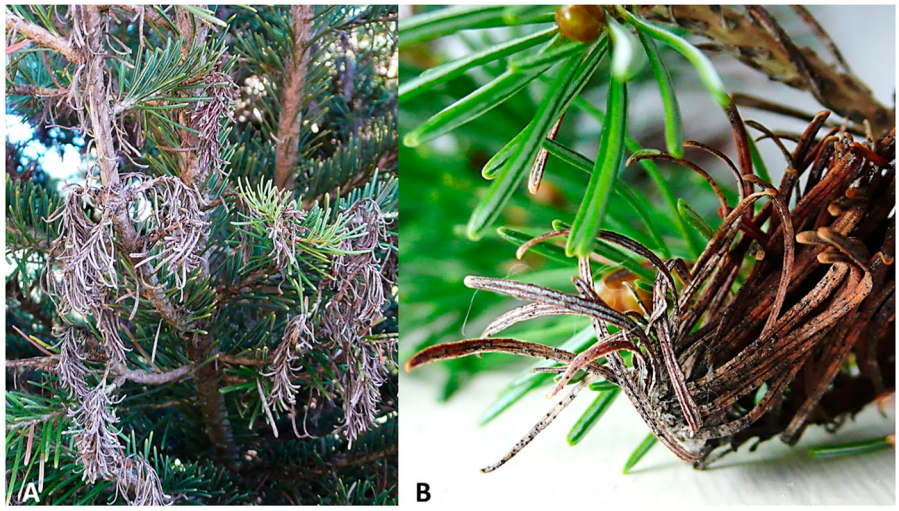

Figure 1. Delphinella shoot blight (*D. abietis*) on subalpine fir (*A. lasiocarpa*) at Sandpoint, Idaho in August 2013: (A) dead needles and shoots, (B) dead needles covered with small, black fruiting bodies. Photos: Venche Talgø

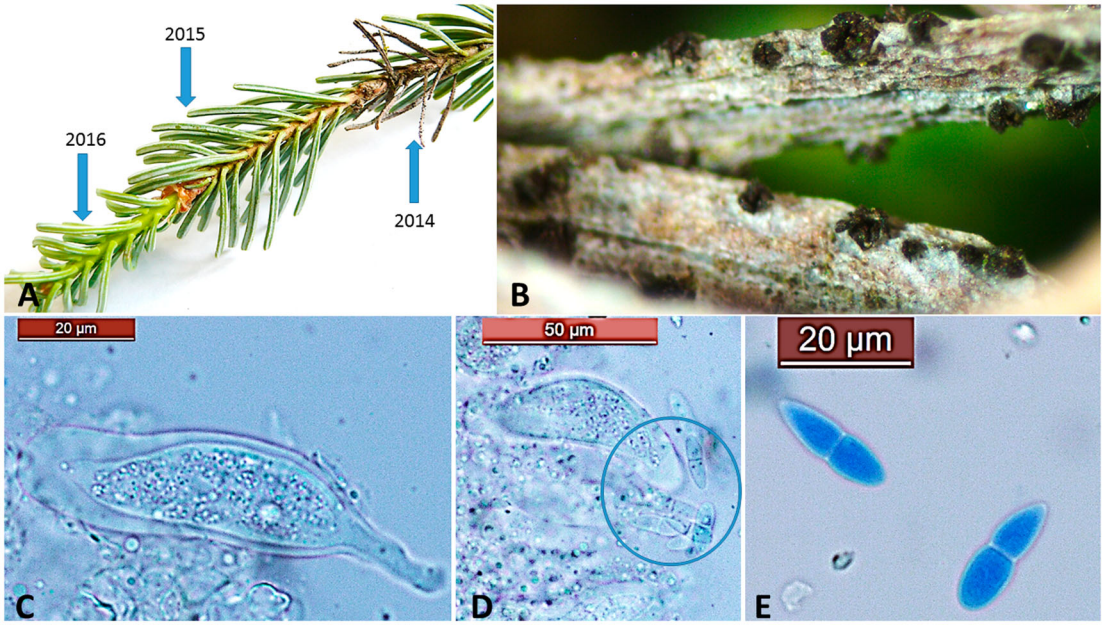

Figure 2. Symptoms, signs, and morphology of *D. abietis* on subalpine fir (*A. lasiocarpa*) samples collected at Sandpoint, Idaho in May 2016; (A) on this shoot needles from 2016 and 2015 had no symptoms, but some diseased needles from 2014 were still attached, (B) the fruiting bodies on the 2014 needles were cracked open and empty, (C) ascus, (D) ascus and ascospores (inside circle), (E) ascospores (scale bars in C and E = 20 µm, D = 50 µm). Photos: Venche Talgø.

Norwegian isolate of *D. abietis* reported in the GenBank in 2009 (GQ412731.1).

Grovesiella abieticola

Symptoms caused by *G. abieticola* consisted of dead shoots, twigs, and branches (flagging) (Figure 3(A,B)). Black fruiting bodies (apothecia) were present on the canker surfaces (Figure 3(C, D)). Microscopic examination of samples collected in the field in ID in August 2013 revealed that the fruiting bodies were immature. No apothecia had formed, but the contour of apothecia (primordia) were seen on incubated samples. In May 2016, mature apothecia were found (Figure 4(A–C)). They contained numerous asci, paraphyses, and ascospores (Figure 4(D,E)). The ascospores had up to nine septa (Figure 4(F)) and they measured [41.4 – (54.3) – 66.5 × 2.2 – (3.2) – 4.3 µm] ($n = 25$).

The sequences from apothecia from subalpine (GenBank accession no. KX358852) and corkbark (GenBank accession no. KX358853) fir in ID in 2016 were identical, and also identical to sequences from the apothecia collected from red fir in Puyallup, WA (GenBank accession no. KX358851). A single ITS sequence of *G. abieticola* submitted by Sieber and Kowalski (KU640383) is available for comparison in GenBank. The isolate, collected from *A. alba* (European silver fir) in Poland in 1991, is quite dissimilar to the Western USA ITS sequences with 83% similarity across 605 nucleotides.

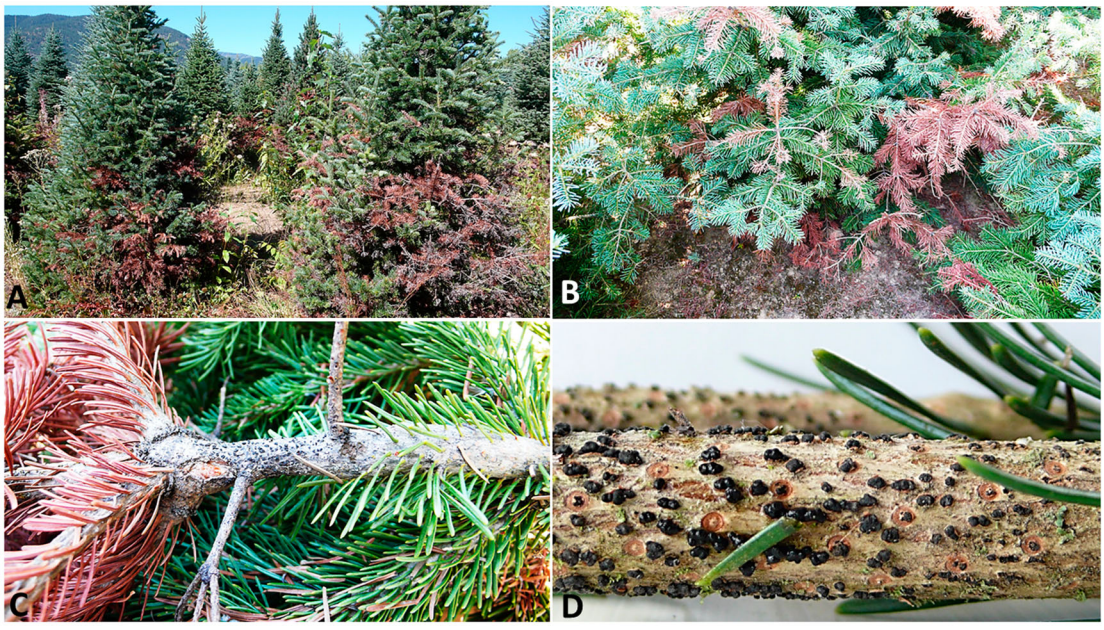

Figure 3. Grovesiella canker (*G. abieticola*) on subalpine fir (*A. lasiocarpa*) at Sandpoint, Idaho in August 2013; (A and B) dead twigs and branches (flagging), (C) canker covered by black fruiting bodies, (D) immature fruiting bodies. Photos: Venche Talgø.

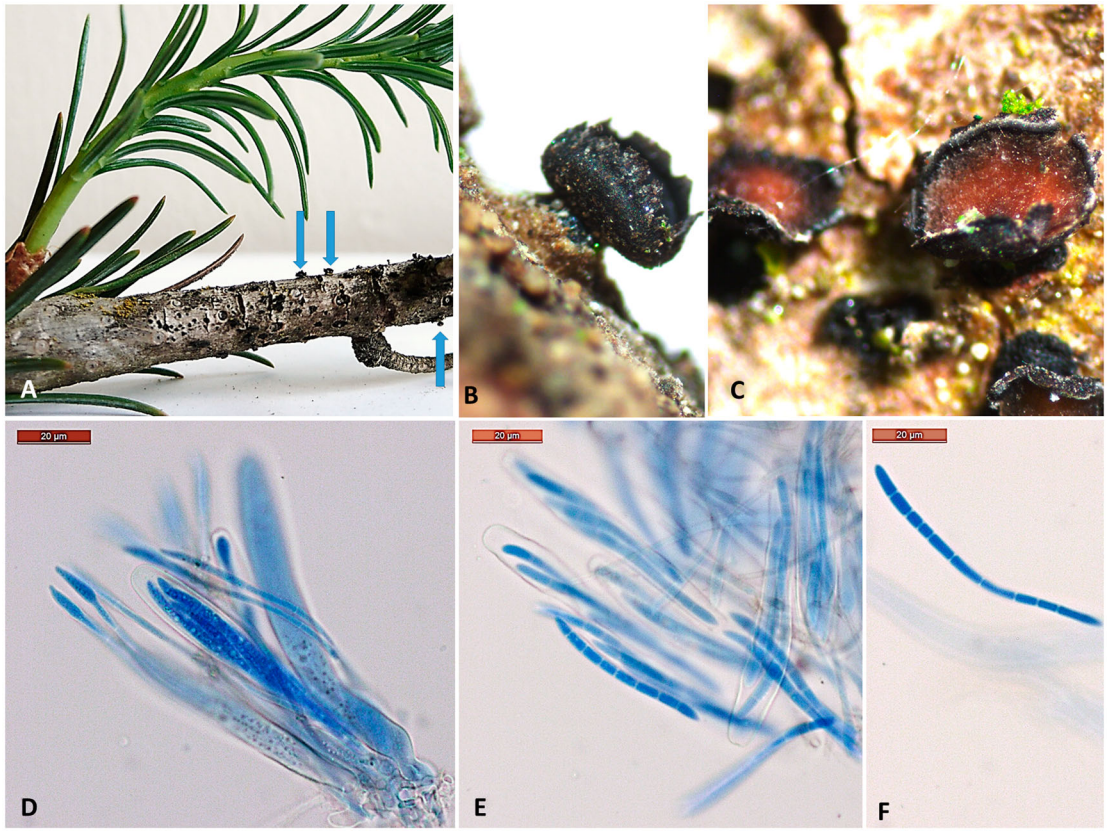

Figure 4. Symptoms, signs, and morphology of *G. abieticola* on subalpine fir (*A. lasiocarpa*) samples collected at Sandpoint, Idaho in May 2016; (A) mature apothecia (arrows) on dead shoot, (B and C) apothecia as seen in a dissecting microscope, (D) asci (with ascospores) and paraphyses, (E) asci and ascospores, (F) ascospore with nine septa (scale bars in D, E, and F = 20 µm). Photos: Venche Talgø

Disease severity

In general, subalpine firs were more susceptible to *D. abietis* than corkbark firs at the site in Sandpoint, ID (Figure 5). However, the most resistant seed source was the Carson subalpine fir.

Grovesiella abieticola susceptibility also varied significantly between provenances (Figure 6), and again, corkbark fir was

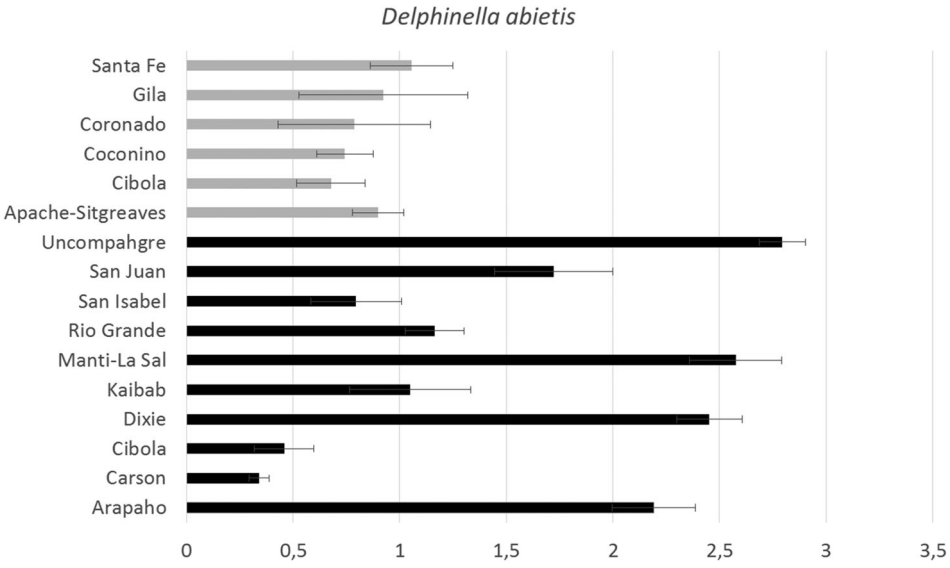

Figure 5. Disease rating (including standard error) of 6 seed sources of corkbark fir (*A. lasiocarpa var. arizonica*) (grey bars) and 10 of subalpine fir (*Abies lasiocarpa*) (black bars) attacked by *Delphinella abietis* (0 = no damage, 1 = minor damage, 2 = medium damage, and 3 = severely damaged).

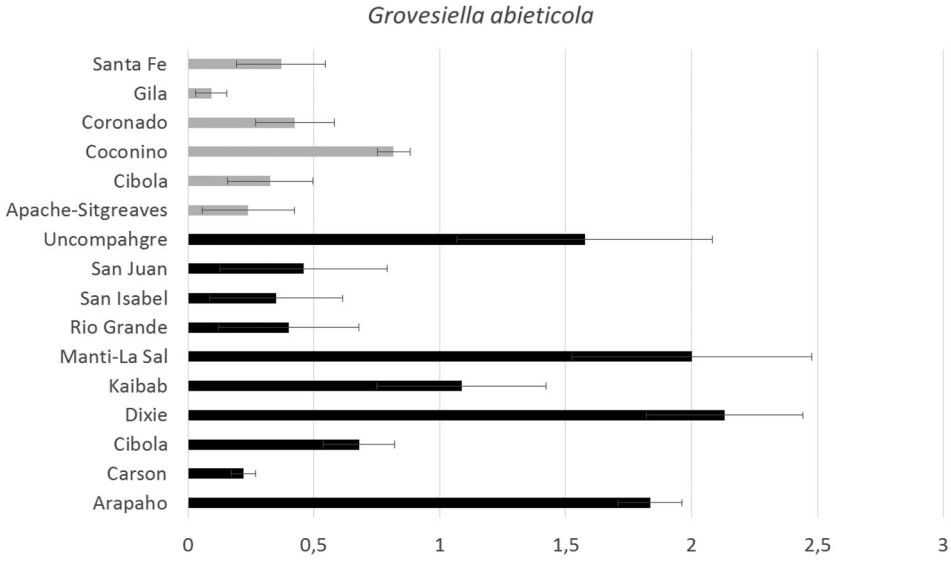

Figure 6. Disease rating (including standard error) of 6 seed sources of corkbark fir (*A. lasiocarpa var. arizonica*) (grey bars) and 10 of subalpine fir (*Abies lasiocarpa*) (black bars) attacked by *Grovesiella abieticola* (0 = no damage, 1 = minor damage, 2 = medium damage, and 3 = severely damaged).

generally less damaged than subalpine fir. Trees from four subalpine fir provenances (Uncompahgre, Manti-La Sal, Dixie and Arapaho) were severely damaged. They were also the most susceptible to *D. abietis*.

Statistical analysis showed that subalpine fir was significantly less resistant to both *G. abieticola* and *D. abietis* ($P < .001$).

Discussion

Infection by *D. abietis* occurs shortly after bud break (Talgø et al. 2016), but the critical time for infection by *G. abieticola* is unknown. Idaho has relatively warm and dry growing seasons. Irrigation used during the early years of plot establishment most likely made it possible for the two pathogens to establish in the trial and build up high inoculum pressure. By 2013, the plot had also become very dense, with restricted air movement and subsequently prolonged periods of wet foliage after precipitation, creating an ideal microclimate for the pathogens. During the survey for *N. neomacrospora* in 2013, neither *D. abietis* nor *G. abieticola* were detected in WA native stands of subalpine fir at Mt. Rainier, Mt. Spokane, Sherman Pass, or Frazer Creek. However, in the more humid and mild coastal climate west of the Cascades, in the lowland of WA, both diseases were found on non-irrigated trees; *G. abieticola* on white fir in Puyallup and *D. abietis* on subalpine fir in Federal Way. Recently, *D. abietis* has also been detected on noble fir in Christmas tree and bough production plantations in OR and high elevation bough production stands of noble fir in WA (unpublished data). Foresters managing the high elevation stands of noble fir refer to the disease as "purple needle eater" because of the frequent purple discoloration of the severely diseased foliage.

Needles killed by *D. abietis* typically stay attached to the twigs for several years, changing color from brown to grey (Talgø et al. 2016). This was also the case in ID. Thus, the attack in 2013 was obviously not the first year the disease occurred. Most likely, the disease described as a *Phoma*-type blight in previously published material from the provenance trial at Sandpoint (Barney et al. 2013) was *D. abietis*. *Grovesiella* was not previously reported from the provenance trial.

Our findings for *D. abietis* at Sandpoint were very similar to the findings reported by Barney et al. (2013), where corkbark fir was found to be more resistant than subalpine fir to the *Phoma*-type blight. This corresponds well with results from a provenance trial of corkbark and subalpine fir in Norway, where the general outcome was that susceptibility to *D. abietis* decreased with increasing altitude of the seed source and increased with the latitude (Talgø et al. 2016). Thus, the further south and higher up in the mountains the seeds are collected, that is, corkbark fir areas, the more resistant they are to *D. abietis*. Generally, corkbark fir has bluer foliage due to a thicker cuticle and wax layer, which may be a physical barrier for *D. abietis* in the infection process (Talgø et al. 2016).

G. abieticola typically produces characteristic dark apothecia on canker surfaces (Chastagner & Staley 1985). In August 2013, no mature apothecia were found at Sandpoint or Puyallup. While it is unclear when the initial infection of tissue by *G. abieticola* takes place, the presence of mature apothecia with spores during May 2016 suggests that infection by this pathogen may take place in the spring. With *G. abieticola*, resin flow and distal overgrowths often occur, and occasionally mortality when cankers completely girdle stems (Chastagner & Staket 1985). None of the two former symptoms were obvious in the provenance trial in ID in 2013, but were seen in 2016. In 2013, some trees were dead or missing. No attempt was made to identify the cause of mortality, since most of them already had been dead for a long period. However, of the two diseases in question, only Grovesiella canker causes mortality. No symptoms resembling root rot (*Phytophthora* spp.) were observed. There were no indications that any of the diseases predisposed the tree to one another.

The fact that *G. abieticola* is a severe pathogen on several fir species native to the PNW, and only a non-pathogenic symbiont or secondary pathogen of *A. alba* in Europe, may indicate that the fungus has a history of coevolution with *A. alba* and thus originates from Europe. Given the differences in the reported ITS sequence of *G. abieticola* from *A. alba* in Poland from the ITS sequences of isolates from Western USA, additional molecular studies with a larger collection of isolates would help clarify the relationship between these populations.

Management of *G. abieticola* is currently limited to cultural practices, such as sanitation. However in Norway, three fungicides (active ingredient dithianon, copper oxide, and tolylfluanid) applied during bud break and shoot elongation have been effective in controlling *D. abietis* (Talgø et al. 2016). Thus, protection of new shoots seems vital for managing this disease.

Since susceptibility to both pathogens varies considerably between provenances, the long-term solution to these particular disease problems may be selection and planting of the most resistant seed sources that are adapted to local production conditions.

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No potential conflict of interest was reported by the author(s).

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