Building an index of alder canker pathogens in Alaska

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Thin-leaf alder (*Alnus incana* ssp. *tenuifolia*), has experienced widespread branch dieback and whole stem mortality from Alaska to the southern Rocky Mountains. Alaska's aerial detection survey has mapped active alder dieback and older whole stem mortality since 2010 (see the following section on alder dieback for more survey information). Most disease occurs on thin-leaf alder; however both Sitka alder (*Alnus viridis* ssp. *sinuata*) and Siberian alder (*Alnus viridis* ssp. *fruticosa*) are affected to lesser extents. Alder dieback on thin-leaf is usually due to girdling cankers (*Figure 30*) caused by the fungus Valsa melanodiscus. But we have frequently noticed several additional fungal species associated with very similar cankers on all three alder species in Alaska. Therefore, we are attempting to identify the major causal pathogens in the North American alder dieback epidemic with a focus on Alaska. In related studies, we are also examining the wood decay fungi which decompose dead alder stems, and water molds in the associated rhizosphere.

We began by reviewing the literature; however, national and regional disease records reflect limited investigation of alder diseases in Alaska. Our objective has been to isolate and identify fungi from diseased tissues and determine their respective roles in alder canker disease. Identifications are based upon DNA sequence similarity and standard morphology. The majority of fungi present in woody plants are referred to as endophytes. They exist benignly between plant cells and grow and metabolize especially slowly, utilizing the carbohydrates that are present to "glue" the plant cells together. Many endophytes are adapted to take advantage of senescing leaves or branches; similarly to pathogens, they will grow rapidly and reproduce on dying tissues. Some are latent pathogens that infect and then persist as endophytes for years. When the host begins to senesce, or is under enough stress, they become virulent, invading and killing healthy tissues. True pathogens are able to incite disease given a suitable host and environment conducive to infection. To determine pathogenicity we isolated each fungal species, and then performed inoculation trials on thin-leaf, Sitka, and Siberian alder in replicated plots in Southcentral and Interior Alaska. Inoculated stems were then monitored for symptom development and the inoculation site was measured (length and width of wound or resultant canker) approximately a year after inoculation. Because the annual window of opportunity for successful inoculation is short and weather dependent, this work will continue for an additional two years.

We have thus far isolated and identified 19 fungal species that are associated with cankers on the 3 alder species. Reported here are summaries for each of these fungi from the USDA Fungus-Host Distribution Database (http://nt.ars-grin.gov/fungaldatabases/fungushost/fungushost.cfm). We also note pathogens that have demonstrated significant virulence in our inoculation trials. Although results are preliminary, it is evident that several fungi are involved in alder canker disease in Alaska, rather than the more typical single-disease/single-pathogen scenario. These include *Valsa melanodiscus*, *Melanconis alni*, and *M. stilbostoma*. Surprisingly, although the following fungi have not previously been reported as causing disease, *Diatrype disciformis* and *Massarina corticola* were also found to be pathogenic.

Our studies on alder pathogens and endophytes, instigated by the widespread outbreak of alder dieback in Alaska, are providing significant contributions to plant pathology, fungal ecology, and mycological taxonomy. We have found unnamed species new to science, known species with expanded ecological roles, and a rather large and diverse community of visually similar canker pathogens on alder.

Figure 30: Alder canker caused by *Valsa melanodiscus*. A canker is a necrotic, often sunken lesion on a stem, branch, or twig which grows slowly, often over several years. Note distinct margin between healthy and dead (brown) tissue.

Figure 31: *Annulohypoxylon multiforme*. (Ascomycetes, Xylariales) The Fungus-Host Distribution Database lists 12 records of this species; all of which were from Poland, Russia, and Turkey. These records are from a diverse set of hardwoods, but one was from an unstated species of Alnus. Index Fungorum lists the synonymous *Hypoxylon multiforme* var. *alaskense* on corticated wood of *Alnus sitchensis* Alaska as reported in Ju, Y.M. and Rogers, J.D. 1996. A revision of the genus *Hypoxylon*. Mycologia Mem. 20: 365 pp.

Figure 32: *Cryptosphaeria ligniota*. (Ascomycetes, Xylariales) The Fungus-Host Distribution Database lists 34 records from north temperate regions, all but one (from dead limbs of *Salix viminalis*) were found upon species of *Populus*, including a record of canker from *P. tremuloides* from Alaska. This record came from Hinds, T.E., and Laurent, T.H. 1978. Common Aspen Diseases Found in Alaska. Pl. Dis. Reporter 62: 972-975.

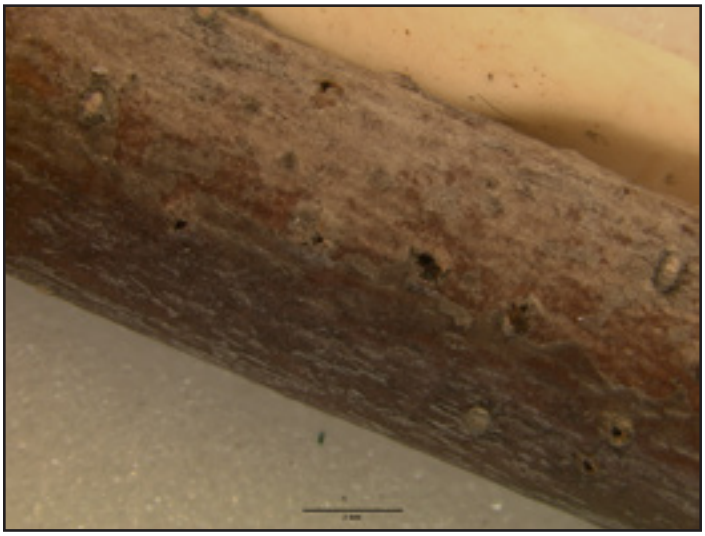

Figure 33: *Cryptosporella alni-sinuatae*. (Ascomycetes, Diaporthales) Only two records are found in the Fungus-Host Distribution Database, both are listed from *A. viridis* ssp. *sinuata*. One record is labeled as from USA and one from Washington state as reported in Mejia, L.C., Rossman, A.Y., Castlebury, L.A., and White Jr., J.F. 2011. New species, phylogeny, host-associations and geographic distribution of genus *Cryptosporella* (Gnomoniaceae, Diaporthales). Mycologia 103: 379-399.

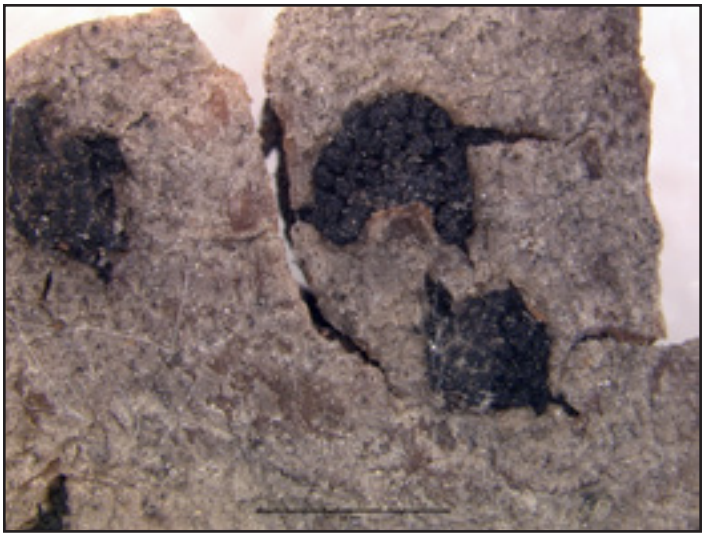

Figure 34: *Cryptosporella suffusa*. (Ascomycetes, Diaporthales) The Fungus-Host Distribution Database lists 59 records and cites this fungus as causing alder dieback in Europe. However, it also lists it from Canada (*A. incana* ssp. *rugosa*), Washington and Idaho (*A. viridis* ssp. *sinuata*, *A. incana* ssp. *tenuifolia*), and California (*A. rubra*). Although this fungus produced cankers with a larger mean size than the controls, statistically the canker size was not significantly different.

Figure 35: *Daldinia loculata*. (Ascomycetes, Xylariales) The Fungus-Host Distribution Database lists 8 records on hardwoods from North America, Asia, and southwestern Europe. The only *Alnus* species listed is from Germany.

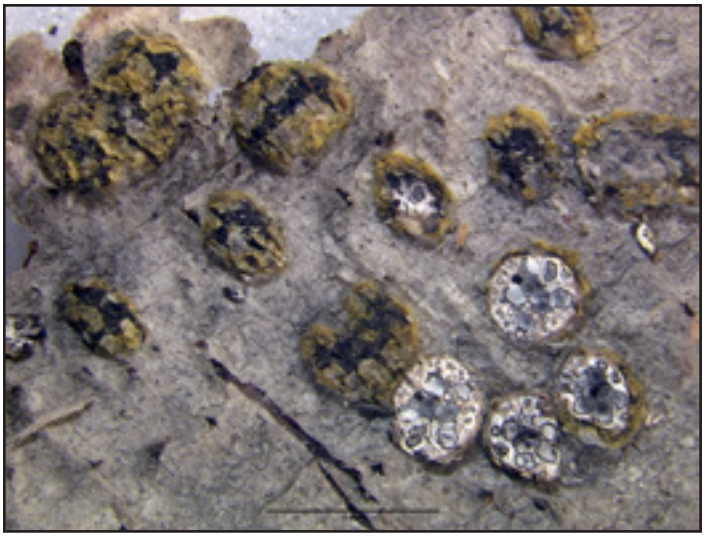

Figure 36: *Diatrype disciformis*. (Ascomycetes, Xylariales) The Fungus-Host Distribution Database lists 73 records mainly from hardwoods in temperate northern hemisphere, although India, Pakistan, and Turkey are also listed. The only reports from *Alnus* were from Alaska and Canada as recorded in Cash, E.K. 1953. A checklist of Alaskan fungi. Pl. Dis. Reporter Suppl. 219: 1-70. and Conners, I.L. 1967. An Annotated Index of Plant Diseases in Canada and Fungi Recorded on Plants in Alaska, Canada and Greenland. Res. Bra. Canada Dept. Agri. 1251: 1-381. Although not reported as pathogenic in the literature, this fungus was strongly pathogenic on all three alder species in the inoculation trials.

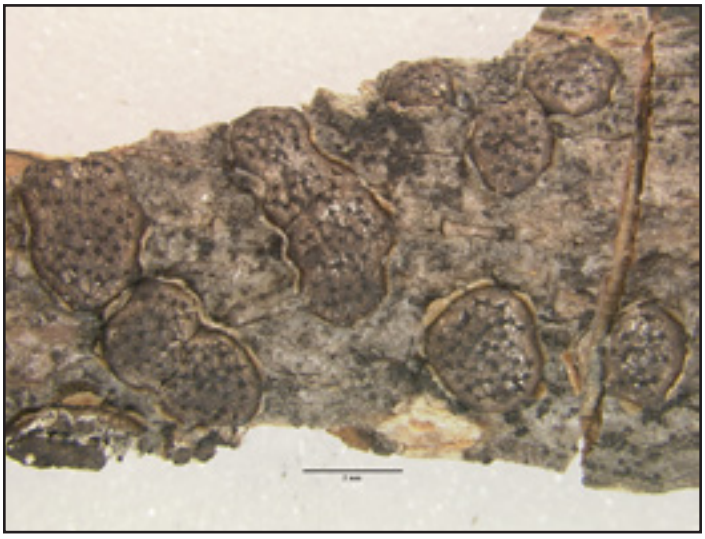

Figure 37: *Diatrype spilomea*. (Ascomycetes, Xylariales) The Fungus-Host Distribution Database only has three records of this fungus, all on Acer species from Europe.

Figure 38: *Diatrypella pulvinata*. (Ascomycetes, Xylariales) None of the 13 records in the Fungus-Host Distribution Database were found on Alnus, and all but one were from Europe.

Figure 39: *Hypoxylon fuscum*. (Ascomycetes, Xylariales) The Fungus-Host Distribution Database lists 175 records mainly on dead wood of the Betulaceae in the Northern hemisphere. Many were also found on various species of Alnus and one of these was listed as *Alnus* sp. in Cash, E.K. 1953. A checklist of Alaskan fungi. Pl. Dis. Reporter Suppl. 219: 1-70.

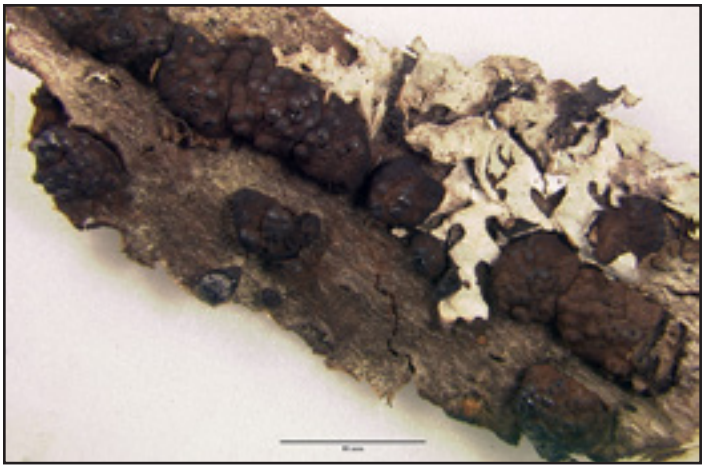

Figure 40: *Massarina corticola*. (Ascomycetes, Pleosporales) The Fungus-Host Distribution Database lists 18 records from temperate regions on numerous woody hosts. None of these 18 records are on *Alnus*. Although not reported as pathogenic in the literature, this fungus was strongly pathogenic on all three alder species in the inoculation trials.

Figure 41: *Melanconis stilbostoma*. (Ascomycetes, Diaporthales) The 98 host records are mainly from *Betula* species in temperate northern hemisphere. Only one from an *Alnus* species (*A. rhombfolia*). Kobayashi. 1970 Bull. Gov. Forest Exp. Sta. 226:13 associated it with branch dieback. This fungus is somewhat common on all three alder species and pathogenic in inoculation trials.

Figure 42: *Nectria dematiosa*. (Ascomycetes, Hypocreales) Only 12 records are recorded in the Fungus-Host Distribution Database that lists the fungus as widespread on deadwood of various hosts. Neither *Alnus* or Alaska were mentioned in the records, however Canada and Finland are prominent.

Figure 43: *Physalospora scirpi*. (Ascomycetes, Xylariales) *Physalospora scirpi* (current name in Index Fungorum) was previously known as *Anthrinium curvatum* a common inhabitant of leaves. The GenBank isolate that our Alaska fungus most closely matches was a CBS culture and phylogenetically it falls closest to *Anthrinium japonicum* and *Nigrospora* species. *Physalospora* spp. are common canker pathogens of apple and pear, but today many of those *Physalospora* have been transferred to *Botryosphaeria*. Apparently, *Physalospora scirpi* has not yet been transferred out of *Physalospora* into a suitable genus (it is not a *Botryosphaeria*).

Figure 44: *Plagiostoma samuelsii*. (Ascomycetes, Diaporthales) The Fungus-Host Distribution Database lists 6 records, all are on *Alnus* in California, Oregon, and Washington. Three of the records were on *A. incana* ssp. *tenuifolia*.

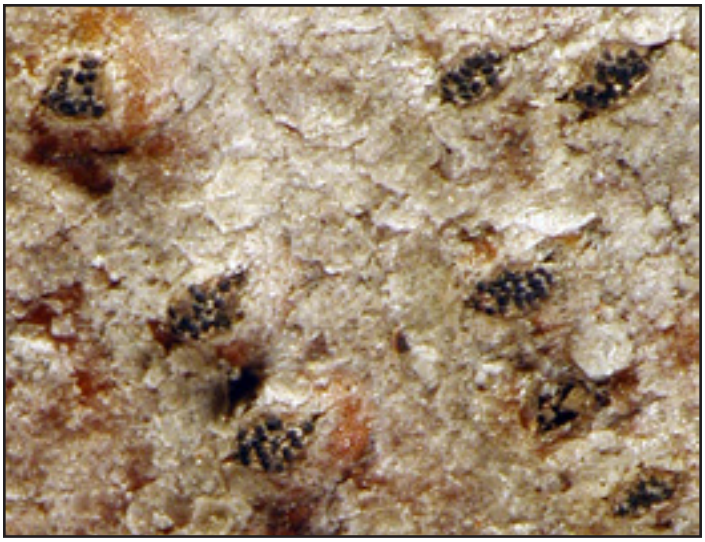

Figure 45: *Valsa diatrypoides*. (Ascomycetes, Diaporthales) The Fungus-Host Distribution Database lists only 3 records, all on *Alnus* from Canada and Poland. The Canadian records were reported in Conners, I.L. 1967. An Annotated Index of Plant Diseases in Canada and Fungi Recorded on Plants in Alaska, Canada and Greenland. Res. Bra. Canada Dept. Agri. 1251: 1-381.

Figure 46: *Valsa melanodiscus*. (Ascomycetes, Diaporthales) The Fungus-Host Distribution Database lists 13 records on species of *Alnus* in Europe and North America, including Alaska. These are reported in Spielman, L.J. 1985. A monograph of *Valsa* on hardwoods in North America. Can. J. Bot. 63: 1355-1378, Cash, E.K. 1953. A checklist of Alaskan fungi. Pl. Dis. Reporter Suppl. 219: 1-70, and Conners, I.L. 1967. An Annotated Index of Plant Diseases in Canada and Fungi Recorded on Plants in Alaska, Canada and Greenland. Res. Bra. Canada Dept. Agri. 1251: 1-381. this fungus is extremely common on cankered *A. tenuifolia* throughout south-central and interior Alaska. Although it caused lesions on all three alder species in our inoculation trials, it is much less commonly found on Sitka and Siberian alder.

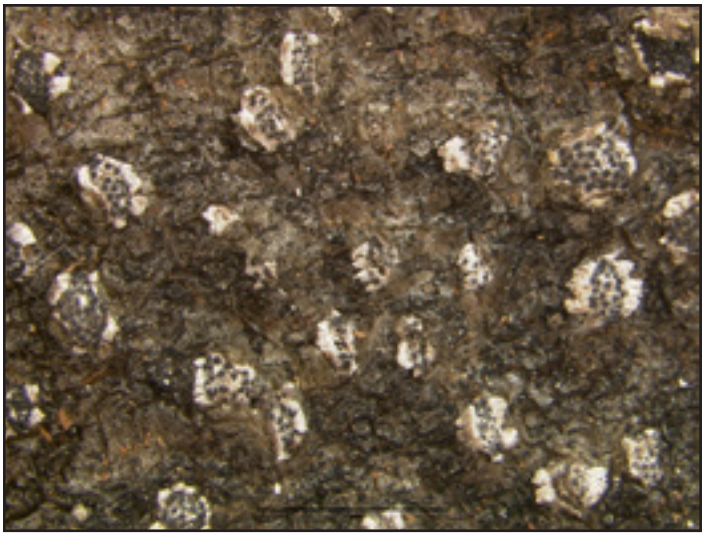

Figure 47: *Valsa* sp. (leucostomoid). (Ascomycetes, Diaporthales) *Valsa* sp. (leucostomoid) = *Leucostoma* sp. Our sample is morphologically a *Leucostoma* but phylogenetic work shows no support for the genus *Leucostoma* which instead falls within *Valsa*. This specimen has not yet been determined to species.

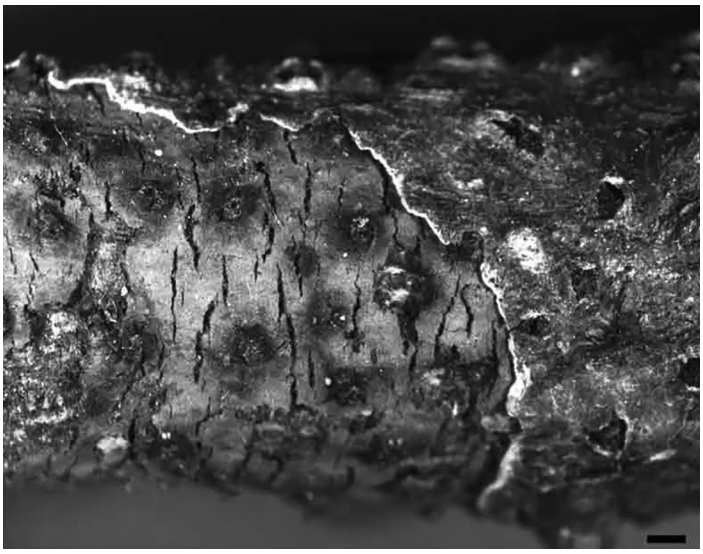

Figure 48: *Valsalnicola oxystoma*. (Ascomycetes, Diaporthales) The Fungus-Host Distribution Database lists 18 records, all on various species of *Alnus*. This fungus is described as causing alder dieback at high elevations in Europe and Alaska. The Alaskan record is from *A. incana* ssp. *tenuifolia* as recorded in Crous, P.W., Rossman, A.Y., Shivas, R.G., Summerell, B.A., Alves, J.L., Wingfield, M.J, Adams, G.C., Groenewald, J.Z., Bell, A., and Barreto, R.W. 2012. Fungal Planet description sheets: 128-153. Persoonia 29: 146-201. The record in Alaska and the new genus were a product of our study.