Distribution and Severity of Alder *Phytophthora* in Alaska[1]

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Abstract

In Alaska, an unprecedented dieback and mortality of *Alnus incana* ssp. *tenuifolia* has occurred which stimulated an effort to determine causal agents of the disease. In Europe, similar dieback and mortality of *Alnus incana* and *Alnus glutinosa* has been attributed to root rot by a spectrum of newly emergent strains in the hybrid species *Phytophthora alni.* The variable hybrids of *P. alni* were grouped into three subspecies: *P. alni* ssp. *alni* (PAA), *P. alni* ssp. *multiformis* (PAM), and *P. alni* ssp. *uniformis* (PAU). From 2007 to 2008, we conducted a survey of *Phytophthora* species at 30 locations with stream baiting as used in the 2007 national *Phytophthora ramorum* Early Detection Survey for Forests in the United States. Additionally, *Phytophthora* species from saturated rhizosphere soil beneath alder stands were baited *in situ* using rhododendron leaves. We discovered PAU in rhizosphere soils in 2007 at two sample locations in unmanaged stands hundreds of miles apart, on the Kenai Peninsula and near Denali National Park. PAA was reported to be the most aggressive and pathogenic to alders and PAM and PAU were significantly less aggressive than PAA, though still pathogenic. To ascertain whether PAU was of restricted distribution due to recent introduction, or widespread distribution, we extended the survey in 2008 to 81 locations. Intensive sampling was conducted at five alder stands exhibiting dieback and 10 alder genets per location were excavated to expose nearly the entire root system for evaluation of the severity of root rot, ELISA detection of *Phytophthora* in diseased roots, and isolation of *Phytophthora* species. At intensive sites, four bowls each containing 500 ml samples of saturated rhizosphere soil were baited by floating three detached leaves of *Rhododendron* spp. for a 2-week period. Leaves were rinsed and sealed in plastic bags and shipped to the laboratory where leaf tissues were placed in PARPH-V8 agar selective for *Phytophthora* spp. *Phytophthora* spp. were identified from DNA sequence of the ITS-rDNA region. The survey yielded some species newly reported for the U.S., including *P. aff. gallica,*and an undescribed species in Clade 8C closely related to *P. ramorum* and *P. foliorum*, and other undescribed species. The Clade 8C species was of restricted range in our isolations, and all 20 isolates were from one location. The species was of interest to researchers developing systems for detection of *P. ramorum*. Thirty-three isolates of PAU were identified out of approximately 600 isolated and sequenced *Phytophthora* spp. PAU was collected from 11 geographically distributed stands. Only one isolate was obtained from bait floating in a water course (the Tanana River) out of 81 watercourses sampled. Soil isolates were from four plots in southcentral Alaska along the Kenai and Russian Rivers, and seven plots in the interior, including a plot in Fairbanks, three plots between Delta Junction and Fairbanks along Highway 2, two between Slana and Tok along Highway 1, and one near Denali National Park on Highway 3. PAU was widely distributed and difficult to isolate. Severity of root rot was low, with less than one diseased root discovered per genet, on average. Root rot does not appear to be a significant contributor to the dieback and mortality of alder in Alaska.

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Introduction

Species of alder (*Alnus*) were exhibiting a dieback and mortality more severe and extensive than has been recorded in history in Europe and North America. Study of the mortality in Europe was well underway by 1993 (Gibbs and others 1999) and it was usually described as exhibiting long linear cankers on stems caused by *Valsa oxystoma* Rehm 1875, and root rot caused by *Phytophthora* species. *Valsa oxystoma* is prominent in the pathology literature in Europe, having a long association with, and periodic and extensive mortality of *Alnus* (Tabeuf 1895). The stem canker, though dramatic, has been in recent times discounted as being a primary pathogen in the disease etiology of alder mortality in Europe. No clear explanation for discounting the stem canker, however, has been given. Dieback was often recorded associated with drainages having ephemeral or variable water flow which suggested water stress was a predisposing environmental factor (Webber and others 2004). Various additional episodes of dieback and mortality of alders have been documented over the years, especially in Europe (Cech and Hendry 2003). These include damage due to hydrological extremes.

In Europe, the common alder, *A. glutinosa,* and other alders, primarily *A. incana* ssp. *incana*, were exhibiting *Phytophthora* collar rot, root rot of fine roots, and tar spots on stems with dieback. Collar rot and tar spots were above ground symptoms associated commonly with *Phytophthora* diseases of trees. Tar spots were locations where a break in bark overlying infected cambium exudes a black liquid, like those observed in sudden oak death (SOD), caused by *Phytophthora ramoum*. These symptoms have been associated with the newly emergent species of *Phytophthora*, *P. alni* Brasier and S. A. Kirk 2004, which was the causal agent of the lethal root and collar disease of alder species in Europe. In Europe, alder *Phytophthora* was well documented as a lethal root and collar disease of alder in the United Kingdom (U.K.), France, Germany, Austria, Hungary, Italy, and the Netherlands. Considerable research has followed the discovery of the newly emergent alder *Phytophthora* because it was found to have arisen from hybridizations between other *Phytophthora* species (Brasier and Kirk 2001, Brasier and others 2004). *P. alni* has three variants which vary in their virulence and pathogenicity. *P. alni* ssp. *alni* (PAA) appears to be the most aggressive and pathogenic to *Alnus* species. The other two, *P. alni* ssp. *uniformis* (PAU) and *P. alni* ssp. *multiformis* (PAM), appear to be significantly less aggressive than PAA, though still considered pathogenic. The PAA variant was typically considered the primary agent killing alders in Europe. PAU and PAM variants were not well understood for their role in causing alder mortality. PAU has been detected across Europe and now was detected in Alaska. PAU was often found in soil, asymptomatic plants, and areas where PAA does not occur. Ioos and others (2006) suggested that PAU and PAM might have existed for a long time on or in the vicinity of alder trees before the recent emergence of large-scale death of alder in Europe. Thus the occurrence of PAU or PAM in the past might not have been noticed because of the lack of conspicuous symptoms or death of whole trees.

Of particular interest was that the parent species in the hybridizations were not pathogenic on alder. For hybridizations to occur in nature and result in a jump to a new host was of great interest to plant pathologists studying plant epidemics caused by newly emergent or recently introduced plant pathogens, such as *P. ramorum*.

Beginning in 2000 in North America, widespread and serious branch dieback and mortality of thinleaf alder, *Alnus incana* ssp. *tenuifolia*, was reported by land managers and others in the southern Rocky Mountains and, beginning 2003, in Alaska (Worrall 2009). Thinleaf alder ranges from the Arctic south to Arizona, and from the Pacific Coast east to central Alaska and the Rocky Mountains (Furlow 1979). In Alaska it may occur near sea level, while in the southern Rocky Mountains it was limited to higher elevations (approximately 3000 m) and riparian areas. Its primary value was in stabilizing soils and in shading and cooling streams, thereby improving fish habitat. It was a keystone nitrogen fixation species with the *Frankia* symbiont. In Alaska, less serious canker and dieback was observed in *A. sinuata* and *A. crispa,* species that more commonly occur in highlands and in the interior.

The widespread and serious branch dieback and mortality increasingly raised concerns about the future of the alder riparian ecosystem. We became involved as collaborators in studies to quantify the extent and severity of dieback and mortality in Alaska and the southern Rocky Mountains from southern Wyoming to northern New Mexico, to identify which pathogens might be potential causal agents of disease, while others assessed the remaining potential direct and indirect causal factors of the epidemic. A major impetuous throughout the study was the concern whether an introduced pathogen was spreading and becoming established in North America.

Figure 1—Typical canker on stem of *A. incana* spp. *tenuifolia*, common in stands with dieback and mortality (photo by J.J. Worrall).

The broad range goals of our 3 years of research were to improve our understanding of the cause of alder mortality in Alaska by documenting symptoms, symptom severity, and signs of disease on alder suffering dieback and mortality; compare them to those in healthy stands; isolate and identify plant pathogens; and participate in completing Koch's postulates with select pathogens. Throughout the widespread mortality, stems of the trees have exhibited narrow linear cankers, approximately 100 cm long by 2 to 7 cm wide (fig. 1). The cankers have been correlated with the dieback and mortality (Worrall 2009) and the canker surfaces usually have been entirely covered with densely aggregated ascostromata or, less often, conidiomata.

Therefore, one objective was to isolate the pathogenic fungi growing at the advancing margins of cankers on *A. incana*, isolate from stromata formed on the cankered tissues, and identify the isolated ascomycetes using morphology and molecular sequence homology. This objective necessitated a re-evaluation of the *Valsa* spp. (anamorphs *Cytospora* spp.) pathogenic on *Alnus* spp. using phylogenetic analyses and virulence among the characterized species compared on the host.

Initially, the project was funded by local U.S. Department of Agriculture, Forest Service (USDA FS), Forest Health Protection (FHP) contracts, but as the possibility increased that an exotic pathogen of concern may have been introduced, funding came from the USDA FS Forest Health Technology Enterprise Team (FHTET). The FHTET had just completed the modeling of risk factors for introduction, spread, and establishment of *P. alni* (lead by Marla Downing and posted on the Internet at http://www.fs.fed.us/foresthealth/technology/invasives_phytophthoraalni_riskmaps.shtml). Similarly, the Europeans had established a website on the potential risk of spread of *P. alni* into non-infested regions of Europe. The better studied epidemic in Europe was used as reference in modeling the risk to North American forests, and experts consulted included T. Jung (Germany). In Europe, *Alnus* species were utilized extensively in habitat restoration where nursery stock was routinely out-planted along river banks. Therefore, the introduction and spread of *P. alni* in North America was proposed as likely originating in nurseries and spreading by interstate trade in nursery stock with establishment of the pathogen occurring following out-planting, or resulting from propagules carried by watercourses from epicenters such as production nurseries. At the time we initiated our studies, *P. alni* had not been previously found in North America, although rumors had been heard of the occurrence of an isolate tentatively referred to as *P. alni* that had been found in a survey of nurseries in Minnesota. This incident was later reported by Schwingle and others (2007), but the species could not be confirmed as *P. alni*.

During these studies we collected data on edaphic factors, stem and genet size and density, and detailed landscape features. However, these latter measurements were for use in modeling and were not reported herein. The publication of Worrall (2009) thoroughly covers investigations of many ecological factors that have or have not been correlated with the epidemic disease in the southern Rocky Mountains, and the publication by the team of Ruess and others (2009) covers some ecological features of the Alaskan epidemic.

A second objective was to isolate *Phytophthora* spp. from roots, rhizosphere soils, crowns, and tar spots of alder as well as from adjacent watercourses and wetlands. Due to growing concern among pathologists and state and federal regulatory officials that cryptic invasion by the *Phytophthora* that devastates alder in Europe may be damaging Alaskan riparian forests, a more extensive survey for alder *Phytophthora* was conducted in 2007 to 2008. *Phytophthora* species were baited and trapped from a total of 81 sites across south central and interior Alaska (fig. 2). The objectives necessitated the identification and virulence testing of *Phytophthora* spp. reported as pathogenic on alder.

Particular interest in a need to know whether particular rivers or streams might be carrying the propagules of PAU and spreading the pathogen downstream was expressed by government agents. Therefore, once PAU had been discovered, it became essential to determine its location, distribution, and whether it caused serious damage to alder. The data generated from the study should address some questions concerning the biology and ecology of the newly discovered PAU. Providing this needed information to USDA Animal and Plant Health Inspection Service (APHIS) and Alaska Division of Agriculture personnel should aid them in determining whether they should pursue specific actions in regard to the finding of PAU in Alaska. The project was designed to provide some answers to the major concerns listed below:

1. What pathogen(s) are causing the stem cankers?
2. Are the stem canker pathogens native or introduced?
3. Can Koch's postulates be reproduced with select canker pathogens on thinleaf alder?

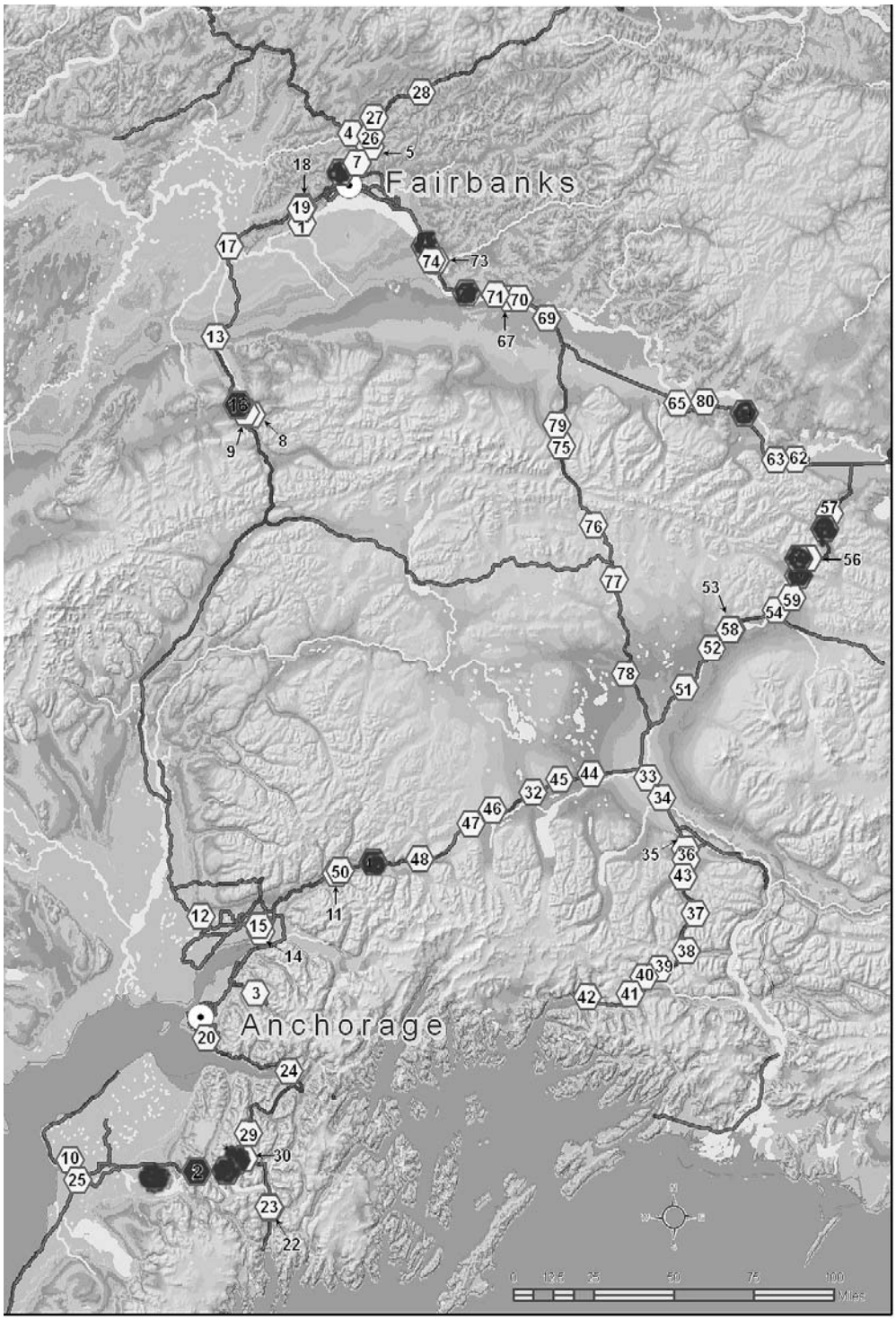

Figure 2—Map showing the distribution of extensive plots in alder stands and baited watercourses adjacent to alder stands in Alaska.

4. What was the incidence and severity of *Phytophthora* crown and root rot in alders in Alaska?
5. Is PAU causing disease on alder in riparian ecosystems in Alaska?
6. What was the distribution of PAU among alders in Alaska?
7. What streams and rivers yield PAU inoculum and may be transporting the pathogen?
8. Is PAU a recently introduced pathogen or a long established member of the Alaskan riparian ecosystem?
9. Can root rot be reproduced with PAU, a new species related to *P. ramorum, P.* aff. *gallica,* and *P. pseudosyringae* on thinleaf alder, and Koch's postulates completed?

Answering these questions concerning the biology of pathogens present in alder stands with dieback and mortality will improve decision making in the management of alder in Alaska.

Methods and Materials

Field Procedures

Extensive plots were selected from GPS identified locations established by the Alaskan USDA FS FHP team of L. Trummer. Efforts were made to target plots with a rating of "high" mortality from drive-by observations, and to target plots that would provide a well-spaced distribution. Individual sites were selected on the criteria of ease of visual location from roads once GPS coordinates were identified.

Landscape Parameters–

Once in a suitable stand, alder density was examined and an area that had sufficient alders to encompass three square subplots of 13 by 13 m [any orientation] was located using a chain. Thirty alder stands (2007 extensive plots) were evaluated for age composition by counting the number of stems greater than 12.5 cm diameter at the ground line, and those of less than 12.5 cm diameter in a central plot of 0.081 hectare ($1/20^{th}$ acre) and two microplots of 0.021 hectare ($1/300^{th}$ acre) oriented along a compass line. The slope, aspect, and form of the landscape at the central plot was measured with compass and a rangefinder/hypsometer or estimated visually. Whether the site was of a riparian, flooded, or non-flooded type; dry, wet, or seasonally wet and vertical or horizontal in proximity to the relevant waterway were measured and recorded. In the central subplot of each of the 30 extensive plots one tree was chosen (if possible, a tree partially alive that exhibited recent dieback) for soil and root studies. One pit (approximately, 60 by 60 cm) was dug to a depth of 60 cm with a shovel and a standard soil probe was used for greater penetration below 60 cm. Soil structure was evaluated, the depth of soil profile change was noted based on color and mottling, and presence of ice, gravel, or hard pan barriers to root penetration were recorded. Soil texture percent and stratification were estimated tactically and visually, and soil organic matter was estimated. Plot data was recorded in the attached Excel datalog files on the forms supplied by FHTET with some additional fields added.

Trapping *Phytophthora* Species

Phytophthora and *Pythium* species were trapped from roots, soils, and water sources using rhododendron leaves and nucleopore filtering. All references to leaves herein refer to *Rhododendron catawbiense* leaves.

Baiting Waterways

Sixteen to 20 leaves were placed in a nylon screen [window screen] envelope, 30 by 30 cm. The leaves were spaced in four rows of four to five leaves using staples to create four long pockets in the screen envelope. One edge of the envelope was wrapped around a 2.5 cm diameter PVC pipe for anchoring, and a strip of bubble wrap [about 8 by 30 cm] was stapled to the opposite end for floatation. A rope was passed through the PVC pipe and tied in a circle. Then about 10 m of 286 kg fishing line was attached to the rope and anchored to a tree trunk on shore. The envelope was tossed into the water to float. Two such envelopes of leaves, henceforth called baits, were left at each alder plot in the nearby waterway. Usually one screen of bait was positioned midstream anchored by a 2.2 kg fish weight on a 2 m tether, and the second bait floated at the shoreline without an anchor. Following about 2 weeks in the waterway, the baits were retrieved; leaves were removed and washed, packaged in sterile plastic sample bags, and periodically mailed to the laboratory triple-wrapped with a copy of permit papers (APHIS permit P526-070620-002). In instances when water temperatures were cold or incubation time was less than 2 weeks, scissors were used to wound each leaf in the envelopes to accelerate colonization by *Phytophthora* species. This methodology was described on the USDA FS SOD website (http://fhm.fs.fed.us/sp/sod/sod.shtm). The screen baits were deployed in rivers and nearby watercourses that had considerable numbers of *A. incana* ssp. *tenuifolia, Alnus sinuata* and/or *Alnus crispa* present. When suitable sites were located where dieback occurred in an alder stand, the site was established using a string chain and compass to outline a minimum of one central plot of 0.081 hectare ($1/20^{th}$ acre) and two microplots of 0.012 hectare ($1/300^{th}$ acre). The site was named and numbered. The location was logged using global positioning (GPS) in coordinates of the WGS 84 system and elevation.

In 2007, baits were floated in 30 watercourses. In 2008, baits were floated in 51 additional water courses for a total of 81 sampled locations. Baiting materials were diversified in 2008 with the addition of bearberry leaves (*Arctostaphylos uva-ursi*), alder twigs (*A. incana* ssp. *tenuifolia*) and a reduced number of rhododendron leaves in a screen.

Filtering Water From Waterways

Over 2 l of clear water from each of 30 watercourses were collected in 2007 and double bagged at each extensive plot, then placed in an ice chest for transport. That evening, the water was filtered through a 3 to 5 µm mesh polypropylene microbial filter (47 mm diameter nucleopore membrane filters). Using a 60 ml syringe, 300 ml of water was pressed through each filter and three filtrations per plot were collected for about1 l. When water-borne sediments occluded filter pores, more than three filters were employed to filter about 1 l of water. The filters were laid face down on the surface of a leaf and sandwiched between leaves in a stack. The sandwiched leaves and filters were placed tightly together in a sterile bag with distilled water and incubated at ambient temperatures. After about 2 weeks the leaves were removed and washed to remove the membrane, soil, and debris, then packaged in sterile plastic sample bags and periodically mailed to the laboratory triple bagged with a copy of

APHIS permit papers. A similar methodology is described on the USDA FS SOD website.

Baiting Rhizosphere Soil

At each extensive plot, soil was excavated from one tree for soil structure studies as described above. Soil and roots collected from the excavation were placed in plastic Ziploc® bags. The collected soil was obtained from that intermingled with roots and clinging to roots and usually included root fragments, for this reason we refer to it as rhizosphere soil. In the subsequent evening, a portion of the soil was placed in two styrofoam bowls and watered with distilled water to saturation. Roots were treated as described below. Three leaves, some wounded and some intact, were placed on the soil surface and the unit was sealed in a water-tight Ziploc® bag. The bowls were maintained at ambient temperature (from car interior to motel rooms). After about 2 weeks the leaves were removed, washed, packaged in sterile plastic sample bags, and periodically mailed to the laboratory triple bagged with a copy of APHIS permit papers.

Baiting Roots

In Alaska, collected roots were washed carefully, cut into 1 cm long segments and 6 pieces sandwiched between two leaves. This was repeated for a minimum of 24 root pieces/plot. The sandwiched leaves and roots were placed tightly together in a sterile bag with distilled water and incubated at ambient temperatures. After about 1 week the leaves were separated and washed to remove root pieces and soils, packaged in sterile plastic sample bags, and periodically mailed to the laboratory triple bagged with a copy of APHIS permit papers.

Excavation of Entire Root Systems and Intensive Plot Sampling

In 2008, five plots were chosen in the Kenai Peninsula for intensive sampling. These plots included one location where *P. alni* had been discovered in 2007, three plots along the watercourse, the Russian River, common to the *P. alni* infested plot, and a fifth plot several miles from the river near Kenai City. At these intensive plots, five live alder genets (multi-stemmed alder clumps sharing a common crown and root system) with recent dieback were chosen. The circumference of the crown was visually divided into quadrants, and four workers excavated the root system of a quadrant by removing soil to expose roots for examination (see fig. 3). For each quadrant, 10 flare roots and 10 pencil-diameter sized roots were visually assessed for disease and decay using a pen knife. The number and condition of roots exhibiting rot were recorded. Root systems with rot were assayed using recently diseased root tissues prepared for the *Phytophthora* detection ELISA diagnostic kit (Agdia Inc., Elkhart, IN 46514) and processed in the field (Timmer and others 1996). Per genet, the total number of stems > 2.5 cm, living stems, dead stems, and cankered stems (dead and alive) were recorded. A walk-by survey of every genet in the intensive plot was made in search of tar spots. All tar spots were assayed with the ELISA kit in the field. Soil from the rhizosphere of each quadrant was placed in a separate styrofoam bowl and sealed in a plastic bag until evening. In the evening, distilled water and rhododendron leaves were added to each bowl and incubated at ambient temperature for about 2 weeks. Leaves were harvested, washed, packaged, and mailed as described above. Everett M. Hansen from Oregon State University joined us during some of the excavations and searches for tar spots (below) as a mentor and collaborator.

Figure 3—Excavation of four quadrants of the root system of a multi-stemmed alder genet for examination of roots for root rot, and for sampling rotted roots for ELISA *Phytophthora*-detection assays, *in situ*.

Sampling Tar Spots and Collar Rots

Three to four observers walked through five intensive and three extensive plots searching for tar spots and above ground visual evidence of collar rot. At the same time, alders exhibiting recent dieback were examined for little leaves and yellowing foliage. To facilitate recognition of these disease symptoms in the field, this survey of symptoms was conducted using the disease leaflet of Jung and Blaschke (2001). Affected bark from tar spots and suspect collar rots were excised from the trees, and the cambium examined for discoloration. Samples with discolored cambium were tested with the *Phytophthora* detection ELISA assay. The remaining material was wrapped in rhododendron leaves and placed in a sterile plastic bag with some distilled water to encourage sporulation and infection.

Laboratory Procedures

All laboratory procedures were performed at Michigan State University (MSU).

Isolations from Leaves

Upon arrival at the lab, sample bags were placed in a locked 13 ^{o}C incubator until the investigator could open the packages and sample bags in the certified, and APHIS-approved ,microbiological biosafety level 2 hood/cabinet. A number four cork borer was used to aseptically cut disks of leaf material along the length of each leaf. The disks were pushed down into agar plates containing PARPH-V8 *Phytophthora*-selective medium. These plates were incubated at 19 ^{o}C in the dark in a locked incubator and examined at 1-week and 2-week intervals. This methodology is described in Hwang and others (2008). Colonies were categorized by appearance,

picked from the plates, and transferred individually to 5 cm Petri plates of PARPH agar overlain with a membrane of cellophane.

Canker Pathogens

From all extensive plots and a majority of the 81 watercourse sites, ascostromata on bark pieces of less than 2.5 by 2.5 cm were collected from active cankers or dead branches of *Alnus* spp. from 2006 to 2008 (APHIS permit P526P-07-07216). Cultures were isolated from the transition between necrotic and white cambium at the margins of cankers using standard methods. These included misting 95 percent ethanol on the external surfaces, brief ignition (flaming), aseptic cutting to expose the canker margin, and isolation of infected tissue pieces. Acidified malt extract agar (1.25 percent malt extract, 100 ppm streptomycin sulfate, pH 5) was used to culture the fungi. Additionally, axenic cultures were obtained by cutting fruiting bodies in half horizontally and applying a drop of water to an exposed locule. Following swelling of the gelatinous matrix, some of the spore mass was lifted and streaked across the surface of the agar medium. Actively growing colonies were purified by excising a terminal cell from an individual hypha. A terminal cell was transferred to an agar plate and subsequent cultures were derived from the hyphal tip cell. Other cultures were obtained from culture collections. The origin for collected specimens was recorded as GPS coordinates.

DNA Preparation, Sequencing, and Analysis

Hyphae were harvested from the cellophane surface for grinding and DNA extraction. Dilutions of DNA extracts were used in PCR amplification reactions of the ITS region of the ribosomal DNA operon. Standard PCR amplification methodology was employed with pairs of primers. Primers included ITS1, ITS1f, ITS5, and ITS4 for the ITS-rDNA (Gardes and Bruns 1993; White and others 1990); Bt-UP4 and Bt-1b for a portion of the β-tubulin gene (Lavésque, personal communication; Glass and Donaldson 1995); and FM75, FM77-80, FM84, FM84-85 for the COXI-COXII region (Martin and Tooley 2003). The cycling reactions were performed in a DNA Thermal Cycler (Perkin-Elmer Corp., Norwalk, CT, U.S.) using standard protocols. PCR products were sorted by size on electrophoretic gels. Appropriate PCR products were purified and submitted for autosequencing at the MSU Research Technology Support Facility. Twenty µl of each PCR product were purified by using the DNA binding resin and protocol of Wizard PCR Preps DNA purification system (Promega Corp., Madison, WI, U.S.) and used in sequencing reactions. Sequencing was performed using a *Taq* DyeDeoxi Terminator™ cycle system, the ABI Catalyst 800, and the ABI Prism 373A or 377 fluorescence sequencer (PE Applied Biosystems, Foster City, CA, U.S.) using the Big Dye fluorescent labeling sequencing kit (PE Applied Biosystems). Amplified double-stranded PCR products were sequenced independently along both strands with the primers listed above. Final sequences were compared for homology to the NCBI GenBank database using BLASTn software. Identification of *Phytophthora* and *Pythium* isolates were preliminarily based on about 760 bp of sequence homology at about 98 percent match (standard procedures). Final identification of distinct species was based on homology to two or more different gene sequences and morphological examination. The RAS-Ypt gene sequence was used as the second homologous match in confirmation of identification of isolates of *P. alni* subspecies (Ioos and others 2006). The identification to subspecies *uniformis* was by amplification and restriction enzyme digestion of sequence characterized markers, as described in the SCARS protocol of Ioos and others (2005).

Results and Discussion

During July 2007, three isolates of *P. alni* ssp. *uniformis* Brasier & S. A. Kirk 2004 (PAU) were isolated from soil beneath *A. incana* ssp. *tenuifolia* in Alaska in two of the extensive plots (Adams and others 2008a; Trummer and others 2007). The two extensive plots were in remote, unmanaged stands hundreds of miles apart: on the Kenai Peninsula and near Denali National Park. Both of these locations, however, appeared to receive common traffic from international tourists, particularly tourists seeking fishing opportunities in Alaskan rivers. The alders in the plots exhibited the dieback common to a widespread epidemic disease in Alaska and the southern Rocky Mountains, including stem cankers with abundant ascostromata. PAU were trapped from saturated rhizosphere soil baited with rhododendron leaves. Species identification of the three isolates was based on DNA sequence homology of ITS and RAS-Ypt molecules (Ioos and others 2006), subspecies identification by SCAR profiles (Ioos and others 2005), and morphology (GenBank EU371544-371553). Caducous sporangia were observed in the Alaskan isolates by Hansen and Reeser (Oregon State University); this characteristic had not been previously observed in *P. alni*. Labs of USDA FS and APHIS (S. Diehl and Z.G. Abad, respectively) have additionally confirmed the identification. *P. alni* was not recovered from the other 28 sample sites (extensive plots) in 2007.

The pathogenic fungi isolated from the advancing margins of the cankers and from most stromata have been species of *Valsa* (anamorph *Cytospora*). Symptoms of Phytophthora root rot were not evident on examination and collection of roots during excavations of the tree in each extensive plot in 2007 (Worrall 2009). Therefore, in 2008 further efforts were undertaken to observe root condition in stands that had yielded PAU.

In 2008, we discovered 30 more isolates (total = 33) of PAU. All but one isolate was trapped from baited soils, while one was trapped by alder twig bait in the Tanana River. The range of known occurrence of this oomycete has been expanded to include 11 geographically distributed alder stands or adjacent watercourses out of 81 watercourses sampled. Soil isolates were from four plots in south central Alaska along the Kenai and Russian Rivers, and seven plots in the interior, including a plot in Fairbanks, three plots between Delta Junction and Fairbanks along Highway 2, two between Slana and Tok along Highway 1, and one near Denali National Park on Highway 3. PAU was widely distributed and difficult to isolate. Discovery of the putatively exotic and invasive species, PAU, led directly to a need to confirm this species' virulence on the native *Alnus* spp. in Alaska. The PAA variant has not been found in North America. We stress that only the *P. alni* ssp. *uniformis* variant has been found in Alaska. Pathogenicity tests on *A. incana* ssp. *tenuifolia* are underway in 2009 at Oregon State University under the direction of Hansen.

Isolates of PAU were present in four out of five intensive plots, and in all the intensive plots that were within the tributaries and drainage basin of the Russian River corridor in the Kenai Peninsula. The one intensive plot several km from the Russian River drainage did not yield any isolates of PAU. The frequency of isolation of PAU among the *Phytophthora* trapped in the four PAU-positive intensive plots was low and ranged from 0.6 to 14 percent (0.6, 10, 11, and 14 percent). This distribution along a river corridor in the south central state, and the isolation of PAU directly from one river in the interior, supported the supposition that a low level of

PAU inoculum may be spread in the rivers of south central and interior Alaska where the dieback and mortality has been observed.

Results of evaluation of the condition of stems and roots of alder genets in the intensive plots are summarized in table 1. Excavations of total root systems in 2008 revealed that symptoms of root rot, collar rot, and tar spot were rarely present at the intensive plots and examined extensive plots. Severity of root rot was low with less than one diseased root discovered per genet, on average (table 1). Armillaria root rot was encountered on four genets in the intensive plots and more frequently at one extensive plot (Potter Marsh). Rotted roots occasionally tested positive with ELISA for *Phytophthora*, but positive assays were limited generally to approximately one root (about 1 cm diameter) per genet with one to three positive assays per intensive plot (10 genets/plot). The scarcity of root rot in flare roots, pencil diameter roots, and *Phytophthora* isolations from fine rootlets provided evidence that *Phytophthora* root rot was unlikely to be a significant contributor to the dieback and mortality of alder in Alaska.

Table 1—Disease symptoms in intensive plots (mean of 10 genets/plot)

Intensive Plot	% Dieback	% Valsa	% Root Rot	% Collar Rot	% Tar Spot	% Sprouts	% Little Leaf
Cooper Landing	31	50	0.5	0	0	80	10
Quart Creek	40	73	2.5	20	30	80	0
Daves Creek	48	72	0.5	0	20	90	0
Kenai City	64	31	1	0	0	100	0
Hidden Lake	48	71	1.5	10	0	100	0
Average	46	59	1.2	6	10	90	2

Tar spots were rare and difficult to find in all plots. Crown rots were also rare. ELISA assays did not indicate that *Phytophthora* was likely present in cambium underlying tar spots or crown rots. Isolations from tar spots yielded a variety of ascomycetes, including *Cadophora* spp., *Cryptosporella suffusa,* and *Hypocrea* spp. Crown rots yielded a variety of ascomycetes, including *Diatrypella* sp. and *Phoma* spp., but not oomycetous *Phytophthora* spp.

The *Phytophthora* spp. and *Pythium* spp. isolated during the riparian surveys in Alaska and identified by sequencing the ITS region of the nuclear ribosome repeat unit are tallied in table 2 (Adams and others 2008b). The four methods of trapping the plant pathogenic water molds each yielded more propagules of the species of *P. gonapodyides* than any other species. The greatest diversity of species was trapped by baiting the saturated rhizosphere soil, and the least by incubating rootlets with bait leaves. The nucleopore filtering method did not yield the quality of results expected from literature reports (Hwang and others 2008). In part, this latter result may be due to the cold temperatures of the watercourses in the Alaskan north from late June through July. After July, salmon runs entice bears to move toward the watercourses and among the alder stands, so field work is suspended.

Table 2—Number of isolates of *Phytophthora* and *Pythium* species from Alaskan surveys

Alaskan Species Beneath Alder	2007	2008	Total
Phytophthora gonapodyides	90	83	173
Phytophthora sp. "hungarica" (isol. UASWS032)	5	29	34
Phytophthora alni subsp. *uniformis*	3	30	33
Phytophthora cactorum	1	3	4
Phytophthora pseudosyringae	0	11	11
Phytophthora sp. "near-ramorum" n. sp.	0	15	15
Phytophthora aff. *gallica*?	0	2	2
Phytophthora inundata	1	0	1
Phytophthora (no known relatives) n. sp.	0	2	2
Phytophthora sp. in other unnamed groups	2	14	16
Phytophthora rosacearum	4	12	16
Phytophthora megasperma sensu stricto (isol. 97-104)	15	48	63
Phytophthora megasperma sensu lato			
Phytophthora sp. "Missaukee" (isol. P47)	0	10	10
Phytophthora sp. "Missaukee" (isol. P79)	0	10	10
Phytophthora sp. "Missaukee" (isol. P61)	0	3	3
Phytophthora sp. 4, "Missaukee" FFL-2008	0	20	20
Phytophthora sp. raspberry group	0	1	1
Phytophthora sp. “SalixSoil” (isol. WD54a,b)	36	15	51
			465
	2007	2008	Total
Pythium sterilum	17	8	25
Pythium macrosporum	5	41	46
Pythium undulatum	12	7	19
Pythium pachycaule	1	6	7
Pythium anandrum	0	5	5
Pythium lutarium	0	5	5
Pythium delawari	0	3	3
Pythium boreale	1	0	1
Pythium sp. in other unnamed groups	48	8	56
			170
		Grand total	636

A considerable number of isolates in Alaska were identified as belonging to unnamed species (table 2). Most of these belonged to related groups of *P. megasperma s. l.* (Hansen and others 1986, 2009). The lack of an assignable name was the result of a paucity of distinctive morphological characteristics available for taxonomists to use in differentiating among them. Many of the *Pythium* species were new reports for the Americas, and several have just recently been described beneath alders in European studies (table 2).

The unnamed groups of *Phytophthora* species were referred herein to the isolate number accessioned in the NCBI GenBank database to which they most closely match in DNA sequence homology based on BLAST searches. Similarly, the four unnamed *Pythium* spp. were best matched to isolates P15845, 93-70P, UASWS018, 824b, or B07. *Pythium* spp. were similar to those we trapped using the same methods in 2007 in the southern Rocky Mountains (data not shown).

A putative new species of *Phytophthora* previously unknown to science related to *P. ramorum* was found in Alaska during riparian *Phytophthora* surveys and confirmed in November 2008 (Trummer and others 2008). The Alaska *Phytophthora* was sent to the USDA APHIS Plant Protection and Quarantine (PPQ) Plant Safeguarding and Pest Identification, National Identification Services Molecular Diagnostics Laboratory and also to Hansen, Oregon State University. Both labs have analyzed DNA sequences of the ITS-rDNA and COX I-II regions and reached the conclusion that the unique sequence identifies this isolate as a member of Clade 8C, the *P. ramorum/P. lateralis* clade, but as a new member. The discovery of a new *Phytophthora* species at the Quartz Creek site is especially interesting because its sequence aligns closest to several other tree pathogens of importance, including *P. lateralis*, a root pathogen of Port-Orford-cedar; *P. hibernalis*, a citrus pathogen that also can cause cankers on Port-Orford-cedar; *P. foliorum*, a new species of unknown virulence and host range; and *P. ramorum*, an oak pathogen. It was agreed that the new *Phytophthora* isolates were unique and worthy of pursing formal description. Since the new species was in the group (Clade 8C) which contains *P. ramorum*, it may be useful in improving the accuracy of detection assays for *P. ramorum*. Testing of the original nested primers for detecting *P. ramorum* (Garbellotto 2003) revealed that an amplified PCR product of the appropriate size occurred with the Phyto1-Phyto2 primer pair and the Phyto-3-Phyto-4 primer pair (fig. 4).

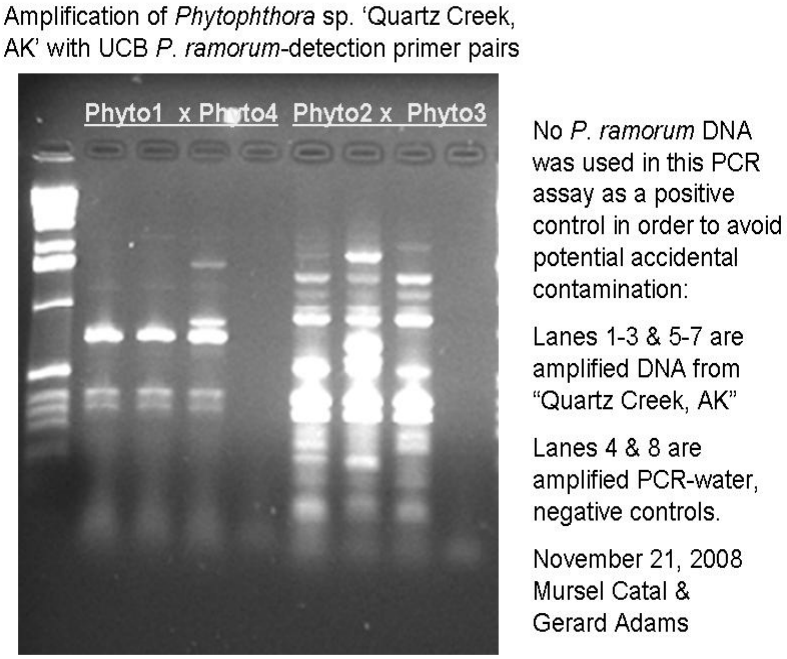

Figure 4—The original primer pairs designed to be species-specific in detection (amplification) of *P. ramorum* amplified the DNA of the new species and, in the past, might have given a false-positive identification which would not be clarified well by sequencing homology comparisons.

Further testing of the new species with DNA methodology developed for species-specific detection of *P. ramorum* revealed that the TaqMan real-time qPCR protocol in current use (Bilodeau and others 2007) did not give false-positive recognition of the new species. Despite the DNA amplification of the new species by the primer pair, the probe oligonucleotide did not anneal to the amplified sequence of the new species (fig. 5). We tentatively refer to this new species as *P.* "near-ramorum," herein.

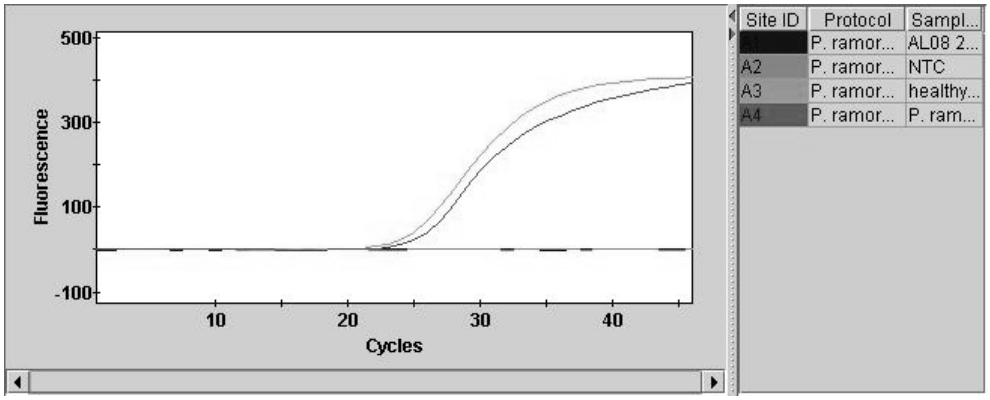

Figure 5—Michigan State University (MSU) Diagnostic Services sample 20084515. Results of real-time PCR for detection of *P. ramorum* using a TaqMan system on the Cepheid SmartCycler per APHIS work instruction WI-B-T-1-6. The new species did not show a false-positive determination in the current *P. ramorum* detection protocol because the internal probe did not anneal to the amplified product (Assay by Jan Byrne, MSU).

Isolates of *P.* "near-ramorum" came from the intensive plot along Quartz Creek, Kenai Peninsula, Alaska, where we had excavated nearly the entire root systems of genets while looking for root rot in 2008. An example of the condition of a genet that yielded soil isolates of *P.* "near-ramorum" at this location follows. Genet 3 had 40 percent top dieback and had *V. melanodiscus* cankers. It also had six roots with rot, four with Armillaria root rot and decay, and two with rot of unknown cause and no decay. While ELISA test kits for detection of *Phytophthora* were not used on genet 3, several other genets at the Quartz Creek site were positive for *Phytophthora* spp. The unusual feature of this discovery is that 15 isolates of *P.* "near-ramorum" from several genets were readily recovered at this site, but not one isolate was found in any other of the 81 widely distributed sites containing alder stands. The restricted distribution of *P.* "near-ramorum" might be suggestive of a recent introduction of an exotic species. The origin of this species might be an important keystone in unraveling the geographic origin and evolution of *P. ramorum.*

More than 50 canker isolates from Alaska were selected for DNA sequence comparisons. Alaskan isolates were referred to by the name of the river, creek, or marsh and a strain number, for example, LittleSusitnaRiver5 (often abbreviated). Sequences of additional isolates from *A. incana* from other geographic regions in North America were included in the analyses. Sequences of *Valsa* spp./*Cytospora* spp. from other parts of the world, culture collections, and from other hosts were included in phylogenetic analyses. Many of the latter have been characterized in previous studies (Adams and others 2005, 2006).

Alder mortality and dieback of *A. incana* ssp. *tenuifolia* was often associated with extensive cankers with *V. melanodiscus* G.H. Otth 1870, *Cryptosporella suffusa* (Fr.:Fr.) L.C. Mejia & Castl. 2008, *Melanoconis alni* (primarily seen in *A. sinuata* and *A. crispa* stands), or other ascomycetes on stems. Most canker isolates from Alaska fell into two distinct clades in each of the phylogenetic analyses of individual gene data sets. The majority of isolates were identified as *V. melanodiscus* and the remainder as *V. diatrypoides*. In contrast, Michigan isolates from *A. incana* ssp. *rugosa*, and a reference culture obtained from cankers on *A. incana* ssp. *tenuifolia* in Oregon, by Filip and others (1992), clustered with the species-complex represented primarily by *Valsa nivea* (Hoffm.) Fr. (= *Leucostoma niveum* (Hoffm.) Höhn.). However, some Michigan isolates clustered within the sister taxon, *Valsa leucostoma* (Pers.) Fr. (= *Leucostoma persoonii* (Nitschke) Höhn.). Serious dieback and mortality are not present in alder stands in Michigan and Oregon. In virulence trails on bolts of *A. incana* ssp. *rugosa*, only *V. melanodiscus* and *V. diatrypoides* were virulent (Adams, unpublished data). Cytospora cankers were also prevalent in the European disease situation, but in Alaska *Cytospora* may be the primary pathogenic agent involved in the dieback following predisposition of the host by unknown environmental stresses. Cankers that resulted from inoculations with *V. melanodiscus* isolates (Stanosz and others 2008) resembled naturally occurring cankers in the stands exhibiting dieback and mortality. Cankers were longitudinally elongated with sunken and necrotic bark, the underlying cambium was discolored, and margins between diseased and healthy tissues were discrete. Fruiting occurred occasionally in the necrotic bark. Analysis of variance of log transformed data revealed strong support for effect of location ($P = 0.04$), but not that of isolate ($P = 0.12$) or interaction ($P = 0.20$) on canker length (Stanosz and others 2008). Re-isolation of the inoculated pathogen yielded colonies consistent with *V. melanodiscus* from a majority of chips from margins of cankers on inoculated stems, but not from control stems. The fungus was isolated in pure culture from each canker and Koch's postulates completed (Stanosz and others 2008).

The finding of more isolates of *P. alni* ssp. *uniformis* remains perplexing. Thorough excavations of 50 alder root systems in Alaska in summer 2008 (fig. 3) revealed little evidence of root disease. Diseased roots seldom tested positive for *Phytophthora* spp. by ELISA assays. Examination of plants for other symptoms of *Phytophthora* disease, such as collar rot and tar spot on stems, has yielded scarce symptoms.

There were many unanswered questions, particularly on the origin of these organisms, their ability to cause disease, and the corresponding host ranges. Further study was needed to determine whether these organisms were introduced, how they might have been introduced, and if so whether these organisms have been causing disease on alders or other plant species in Alaska. Current research should provide a detailed estimation of the population genetics of the pathogen, *V. melanodiscus*, that was causing disease and mortality of alders in Alaska riverine forests. Similarly, we have begun studies of the population genetics of the pathogen *P. alni* ssp. *uniformis*. The study will identify variation in genetic markers among the populations and verify whether the populations show high diversity characteristic of native populations or low diversity characteristic of recent invasive introductions. These results will support or refute the hypotheses concerning whether the alder mortality was caused by an invasive species, or by predisposing environmental conditions and an Alaskan native pathogen. The project should also verify whether a *P. alni* has been introduced

from Europe. Additionally, current research spearheaded by E.M. Hansen, P. Reeser and laboratory colleagues at Oregon State University will demonstrate whether PAU or other *Phytophthora* species are capable of causing root rot of alder. The results of the study have improved our understanding of the cause of alder mortality.

We do not know the host range of this new *Phytophthora* species in Alaska other than the fact that it can infect rhododendron leaves. Rhododendrons do not occur in the native environment in Alaska, though several other ericaceous hosts were present. Perhaps the new *Phytophthor*a sp. and PAU have co-existed benignly in Alaska beneath alder and have not been noted due to the lack of surveys or the lack of conspicuous symptoms or death of alder or other associated plant species. Conversely, further analysis of the isolates may reveal that the organisms could have been introduced into America on wading boots or other equipment of European fishing tourists or other travelers. Risk models of invasive species apparently have not considered this potential route to introduction of forest pathogens.

Research conclusions on these topics should improve decision-making in the management of alder in Alaska. Data from this study can be used to refine models of decline and mortality used in the National Forest Risk Map project.

Conclusions

1. Are the stem canker pathogens native or introduced?
   Preliminary AFLP analysis shows high diversity and sexual reproduction.
2. Can Koch's postulates be reproduced with the canker pathogens?
   Yes, three labs have demonstrated pathogenicity.
3. What was the incidence and severity of root rot in alders in Alaska?
   Less than 0.6 percent of roots per genet (excluding *Armillaria* spp.).
4. What was the distribution of PAU among alders in Alaska?
   Select locations along most major highway routes.
5. What streams and rivers may be transporting PAU?
   The Russian, Kenai, and Tanana Rivers.
6. Is PAU a recently introduced or a long-established pathogen?
   Yet uncertain, but it was not introduced from nurseries, as predicted.
7. Is PAU causing disease on alder in riparian ecosystems in Alaska?
   Probably not significant.
8. Can alder root rot be reproduced with PAU, *P.* aff. *gallica,* and *P. pseudosyringae*?
   We may know soon (via E.M. Hansen).
9. Should we continue to be concerned about *P. alni*?
   Vigilance is needed concerning introduction of *P. alni* ssp. *alni.*

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Literature Cited

Adams, G.C.; Catal, M.; Trummer, L.; Hansen, E.M.; Reeser, P. and Worrall, J.J. 2008a. *Phytophthora alni* ssp. *uniformis* found in Alaska beneath thinleaf alders. Online. Plant Health Progress doi: 10.1094/PHP-2008-1212-02-BR. http://www.fs.fed.us/r10/spf/fhp/phytophthora/Adams_PlantHealthProgress_CNIPM.pdf (June 16, 2009).

Adams, G.C.; Catal, M.; Trummer, L.M. and Worrall, J.J. 2008b. *Phytophthora* and *Pythium* species new to North America, Alaska, or Colorado, from beneath declining *Alnus* spp. Inoculum. 59(4): 17.

Adams, G.C.; Roux, J. and Wingfield, M.J. 2006. *Cytospora* species (Ascomycota, Diaporthales, Valsaeceae): introduced and native pathogens of trees in South Africa. Australasian Plant Pathology 35: 521–548.

Adams, G.C.; Wingfield, M.J.; Common, R. and Roux, J. 2005. Phylogenetic relationships and morphology of *Cytospora* species and related teleomorphs (Ascomyota, Diaporthales, Valsaceae) from *Eucalyptus*. Studies in Mycology. 52: 1–147.

Bilodeau, G.J.; Levésque, C.A.; de Cock, A.W.A.M.; Duchaine, C.; Brière, S.; Uribe, P.; Martin, F.N. and Hamelin, R.C. 2007. Molecular detection of *Phytophthora ramorum* by real-time Polymerase Chain Reaction using TaqMan, SYBR green and molecular beacons. Phytopathology. 97: 632–642.

Brasier, C.M. and Kirk, S.A. 2001. Comparative aggressiveness of standard and variant hybrid alder phytophthoras, *Phytophthora cambivora* and other *Phytophthora* species on bark of *Alnus*, *Quercus,* and other woody hosts. Plant Pathology. 50: 218–229.

Brasier, C.M.; Kirk, S.A.; Delcan, J.; Cooke, D.E.L.; Jung, T. and Man in't Veld, W.A. 2004. *Phytophthora alni* sp. nov. and its variants: designation of emerging heteroploid hybrid pathogens spreading on *Alnus* trees. Mycological Research. 108(10): 1172–1184.

Carbone, I. and Kohn, L.M. 1999. A method for designing primer sets for speciation studies in filamentous ascomycetes. Mycologia. 91(3): 553–556.

Cech, T. and Hendry, S. 2003. A review of diebacks and declines of alder (*Alnus* spp.) in Europe. In: Gibbs J.; Dijk van, C.; and Webber, J. eds Phytophthora disease of alder in Europe. Forestry Commission Bulletin 126. UK Forestry Commission, United Kingdom. 15–24.

Filip, G.M.; Parks, C.A. and Starr, G.L. 1992. Incidence of wound-associated infection by *Cytospora* sp. in mountain alder, red-osier dogwood, and black hawthorn in Oregon. Northwest Science. 66: 194–198.

Furlow, J.J. 1979. The systematics of the American species of *Alnus* (Betulaceae). Part 2. Rhodora. 81: 151–248.

Gardes, M. and Bruns, T. 1993. ITS primers with enhanced specificity for basidiomycetes: application to the identification of mycorrhizae and rusts. Molecular Ecology. 2: 113–118.

Garbelotto, M. 2003. Molecular diagnostics of *Phytophthora ramorum*, causal agent of Sudden Oak Death. Sudden Oak Death Online Symposium. www.apsnet.org/online/SOD doi: 10.1094/SOD-2003-MG (June 16, 2009).

Gibbs, J.N.; Lipscombe, M.A. and Peace, A.J. 1999. The impact of *Phytophthora* disease on riparian populations of common alder (*Alnus glutinosa*) in southern Britain. European Journal of Forest Pathology. 29: 39–50.

Glass, N.L. and Donaldson, G.C. 1995. Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous fungi. Applied Environemental Microbiology. 61(4): 1323–1330.

Hansen, E.M.; Wilcox, W.F.; Reeser, P.W. and Sutton, W. 2009. *Phytophthora rosacearum* and *P. sansomeana*, new species segregated from the *Phytophthora megasperma* "complex". Mycologia. 101(1): 129–135.

Hansen, E.M.; Brasier, C.M.; Shaw, D.S. and Hamm, P.B. 1986. The taxonomic structure of *Phytophthora megasperma*: evidence for emerging biological species groups. Transactions of the British Mycological Society. 78: 557–573.

Hwang, J.; Oak, S.W. and Jeffers, S. 2008. Detecting *Phytophthora ramorum* and other species of *Phytophthora* in streams in natural ecosystems using baiting and filtration methods. In: Frankel, S.J.; Kliejunas, J.T. and Palmieri, K.M., tech. coords. Proceedings of the sudden oak death third science symposium. Gen. Tech. Rep. PSW-GTR-214. Albany, CA: U.S. Department of Agriculture, Forest Service, Pacific Southwest Research Station. 55–58 p.

Ioos, R.; Andrieuz, A.; Marçais, B. and Frey, P. 2006. Genetic characterization of the natural hybrid species *Phytophthora alni* as inferred from nuclear and mitochondrial DNA analyses. Fungal Genetics and Biology. 43: 511–529.

Ioos, R.; Husson, C.; Andrieux, A. and Frey, P. 2005. SCAR–based PCR primers to detect the hybrid pathogen *Phytophthora alni* and its species causing alder disease in Europe. European Journal of Plant Pathology. 112: 323–335.

Jung, T. and Blaschke, M. 2001. Phytophthora – Wurzelhalsfäule der Erlen (Phytophthora Collar Rot of Alders). LWF Merkblatt (LWF Leaflet) No. 6. Freising, Germany: Bavarian State Institute of Forestry (LWF). (http://www.waldwissen.net/themen/waldschutz/pilze_nematoden/lwf_merkblatt_06_2001.pdf. (June 16, 2009).

Jung, T. and Blaschke, M. 2004. *Phytophthora* root and collar rot of alders in Bavaria: distribution, modes of spread, and possible management strategies. Plant Pathology. 53: 197–208.

Jung, T. and Nechwatal, J. 2008. *Phytophthora gallica* sp. nov. a new species from rhizosphere soil of declining oak and reed stands in France and Germany. Mycological Research. 112 (10): 1195–1205.

Martin, F.N. and Tooley, P.W. 2003. Phylogenetic relationships among *Phytophthora* species inferred from sequence analysis of mitochondrially encoded cytochrome oxidase I and II genes. Mycologia. 95(2): 269–284.

Ruess, R.W.; McFarland, J.M.; Trummer, L.M. and Rohrs-Richey, J.K. 2009. Disease-mediated declines in N-fixation inputs by *Alnus tenuifolia* to early-successional floodplains in interior and south-central Alaska. Ecosystems. doi: 10.1007/s10021-009-9237-5.

Schwingle, B.W.; Smith, J.A. and Blanchette, R.A. 2007. *Phytophthora* species associated with diseased woody ornamentals in Minnesota nurseries. Plant Disease. 91: 97–102.

Stanosz, G.R.; Trummer, L.M.; Rohrs-Richey, J.K.; Adams, G.C. and Worrall, J.J. 2008. Response of *Alnus tenuifolia* to inoculation with *Valsa melanodiscus*. Phytopathology. 98: S150.

Trummer, L.M.; Adams, G.C. and Catal, M. 2008. Briefing paper: 2008 findings from Alaska. A new species of *Phytophthora* related to *P. ramorum* and more isolates of *P. alni* ssp. *uniformis*. http://www.fs.fed.us/r10/spf/fhp/phytophthora/2008_Alaska_*Phytophthora*_Briefing.pdf. http://www.fs.fed.us/r10/spf/fhp/phytophthora/phytophthora08v4.pdf. (June 16, 2009).

Trummer, L.M.; Worrall, J.J. and Adams, G.C. 2007. Briefing paper: *Phytophthora alni* ssp. *uniformis*, a first finding in North America. USDA Forest Service.

http://www.fs.fed.us/r10/spf/fhp/phytophthora/Alaska_Briefing_Alder_Phytophthora_Nov18.pdf. (June 16, 2009).

Timmer, L.W.; Menge, J.A.; Zitko, S.E.; Pond, E.; Miller, S.A. and Johnson, V. 1993. Comparison of ELISA techniques and standard isolation methods for *Phytophthora* detection in citrus orchards in Florida and California. Plant Disease. 77(8): 791–796.

Tabeuf von, K.F. 1895. Pflanzenkranheiten durch kryptogame parasiten verursacht. Verlag von J. Springer, Berlin. 239–240.

Webber, J.; Gibbs, J. and Hendry, S. 2004. *Phytophthora* disease of alder. Information Note FCIN6 (REV). Forestry Commission, United Kingdom. 6 p.

White, T.J.; Bruns, T.D.; Lee, S.B. and Taylor, J.W. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis, M.A.; Gelfand, D.H.; Sninsky, J.J. and White, T.J. eds. PCR protocols, a guide to methods and applications. San Diego, California: Academic Press: 315–322.

Worrall, J.J. 2009. Dieback and mortality of *Alnus* in the southern Rocky Mountains, USA. Plant Disease. 93(3): 293–298.