Phytophthora alni

Scientific Name
Phytophthora alni subsp. *alni* Brasier & S.A. Kirk
Phytophthora alni subsp. *multiformis* Brasier & S.A. Kirk
Phytophthora alni subsp. *uniformis* Brasier & S.A. Kirk

Synonyms:
None

Common Name(s)
Phytophthora disease of alder

Type of Pest
Fungal-like organism

Taxonomic Position
Class: Oomycetes, **Order:** Pythiales, **Family:** Pythiaceae

Reason for Inclusion in Manual
CAPS Target: AHP Prioritized Pest List – 2010 to 2016

Background Information
Phytophthora alni is a hybrid pathogen of alder (*Alnus* spp.). Because this *Phytophthora* hybrid does not consist of a single entity but comprises a range of phenotypically diverse allopolyploid genotypes, *P. alni* was split into three subspecies: *P. alni* subsp. *alni* (*Paa*), *P. alni* subsp. *uniformis* (*Pau*), and *P. alni* subsp. *multiformis* (*Pam*) (Brasier et al., 2004). An allopolyploid has more than two sets of chromosomes that are derived from two or more different species. The variants appear to range in their virulence and pathogenicity on European alders. *Phytophthora alni* subspecies *alni* appears to be the most aggressive and pathogenic to European alder species (Brasier and Kirk, 2001). The two other variants, *Pau* and *Pam*, appear to be significantly less aggressive than *Paa*, though still considered pathogenic. The *Paa* variant is considered the primary agent killing alders in Europe and is the most frequent subspecies observed in Europe at this time (Brasier, 2003).

In 1993, a previously unknown and lethal disease of alder (Fig. 1) was described in southern Britain (Gibbs, 1995). Initially it was thought to be caused by *Phytophthora cambivora*, a fungus well-known as a pathogen of broad-leaved trees but not previously reported from alder. Even though the alder pathogen exhibited female gametangia (oogonia) with distinctive surface ornamentation and two-celled amphigynous (collar-like) male gametangia (antheridia), it quickly became clear that the pathogen was an entirely new species. The pathogen had several unusual properties and was suggested to be a new species hybrid, which had probably originated relatively recently (Brasier et

al., 1995). The pathogen was self-fertile rather than outcrossing, had submerged instead of an aerial colony type, and had markedly lower cardinal temperatures for growth. It also exhibited an unusually high level of zygotic abortion (Brasier et al., 1995).

Figure 1. Alder plantation on former agricultural land; note infected grey alder (*A. incana*) with sparse, chlorotic and small-sized foliage. Photo courtesy of T. Jung. www.forestryimages.org.

Brasier et al. (1999) used molecular analysis to show that the alder fungus was a hybrid most likely between *P. cambivora* and a fungus close to *P. fragariae* (a pathogen of strawberry). Researchers originally thought that *Pam* and *Pau* were genetic breakdowns of *Paa* (Brasier et al., 1999). More recently, Ioos et al. (2006) demonstrated that *P. cambivora* and *P. fragariae* are not the progenitors of *P. alni*. They showed that *Paa* is actually a hybrid of *Pam* and *Pau* (Ioos et al., 2006; Ioos et al., 2007a, b; Bakonyi et al., 2007; Ersek and Nagy, 2008).

Pest Description

From Brasier et al. (2004):

Phytophthora alni subsp. *alni:*

Colonies: Colonies on carrot agar (CA) (10 days at 25°C (77°F) in darkness) usually, appressed-felty with no or very sparse aerial mycelium; sometimes appressed with a little uniform wooly overgrowth close to the colony surface. Colonies are often irregular in outline, sometimes faster or slower growing areas. Optimum temperature for growth on CA is 23-25°C (73-77°F). Upper limit for growth on CA is approximately 29°C (84°F). Radial growth at 25°C on CA of 10 isolates) is 4.1-7.5 mm per day (mean 5.9 mm per day).

Sexual Reproduction: Homothallic; gametangia usually frequent to abundant (some cultures gradually lose fertility). A proportion of oogonia are larger or more mature. These oogonia with tapered stalks, variably warty with bullate protuberances are similar to those of *P. cambivora*. The range of isolate means (5 isolates) for diameter of mature oogonia is 42.8-50 µm; overall range is 37-55 µm. A further proportion of oogonia are rather small with diameters of approximately 25-35 µm, others only partially developed; some distinctly comma-shaped; some distorted or with beak-like or tube-like protuberances. A high proportion of oogonia, common range approximately 36-70% with either fully aborted or with thin-walled oospores. Viability (not germinability) of normal-looking oospores (tetrazolium method) usually low (31-36%). Oospores are plerotic. Diameters in larger oogonia: range of isolate means (5 isolates) 33.3-43.5 µm; overall range 27.5-50 µm. Antheridia predominately two-celled and amphigynous. A small proportion may be one-celled, or with a basal septum that is difficult to observe. Antheridial lengths: range of isolate means (mature oogonia only, 5 isolates) 23.5-27µm; overall range 20-30 µm. Antheridial widths, range of means 18.5-19.5 µm; overall range 15-20 µm.

Asexual Reproduction: Sporangia not seen on CA. Sporangia are produced sparsely in pea broth or when plugs from margins of actively growing cultures on CA or oatmeal agar are partially submerged in Petri's solution, fresh, unsterile pond water, or unsterile soil leachate. Borne singly on long sporangiophores, ellipsoid, non-papillate, non-caduceus, with a broad exit pore. Sporangial length: range of means (10 isolates) 48-59 µm; overall range approximately 30-70 µm. Sporangial width: range of means 31.3-42.8 µm; overall range approximately 27.5-50 µm. Sporangial length x width ratio: range of means (10 isolates) 1.32-1.62. After zoospore release, showing nested and extended proliferation (Fig. 2). No chlamydospores observed. Hyphal swellings not reported.

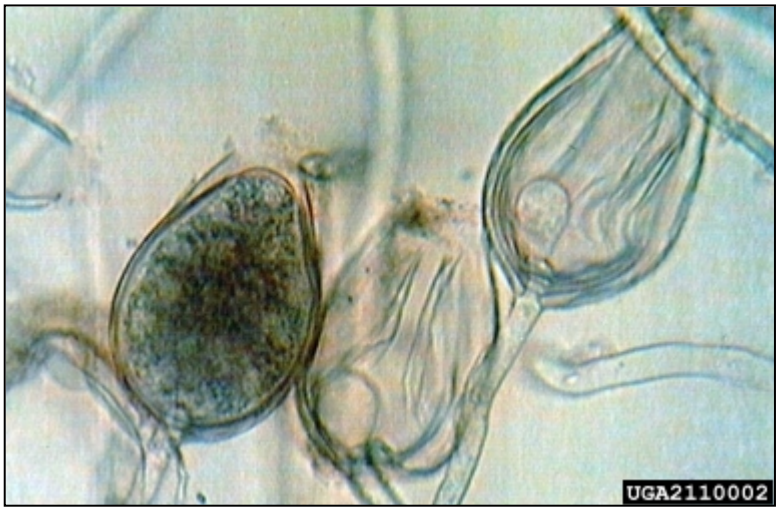

Figure 2. Non-papillate sporangia of *P. alni* showing nested proliferation. Photo courtesy of T. Jung. www.forestryimages.org.

Phytophthora alni subsp. *uniformis:* (previously known as the Swedish variant) Colonies: Colonies on CA have an irregular appressed colony, often with a little wooly aerial mycelium in the colony center but submerged growth at the edge.

Sexual Reproduction: Gametangia generally frequent. Some cultures are highly unstable and chimeric, with gametangia produced only beneath patches of aerial mycelium. Oogonia mostly smooth-walled (Fig. 3), but some slightly wavy edged to verrucose. Occasionally oogonia have large, distorted beak-like protuberances. Oogonial diameter: range of isolate (2 isolates) is 39.8-49 µm; overall range is 37.5-55 µm. A high percentage (>90%) of oogonia usually with normal-looking oospores. Oospore diameter: range of isolate means (2 isolates) 33.3-43.4 µm; overall range approximately 30-47.5 µm. Antheridia consistently two-celled and amphigynous. NB: the antheridial cross wall is often close to the base of the antheridium and could be overlooked. Antheridial length: range of isolate means (2 isolates) 19.3-23.5 µm; overall range approximately 17.5-27.5 µm.

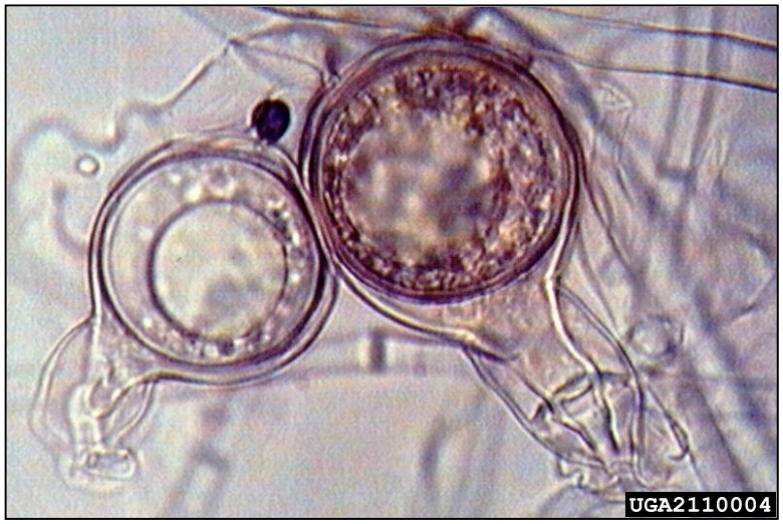

Figure 3. Smooth-walled oogonium of *P. alni* (Swedish variant) with oospore and amphigynous antheridium. Photo courtesy of T. Jung. www.forestryimages.org.

Asexual Reproduction: Sporangia similar to those of suspecies *alni*.

Phytophthora alni subsp. *multiformis:* (previously known as the Dutch, UK, and German variants). *P. alni* subsp. *multiformis* comprises a range of forms with rather different phenotypes, yet with close molecular affinities.

Colonies: Colonies on CA irregular, often with moderate to dense aerial mycelium. Among different subpopulations upper temperature limits for growth on CA vary from approximately 27 to 32°C (81-90°F).

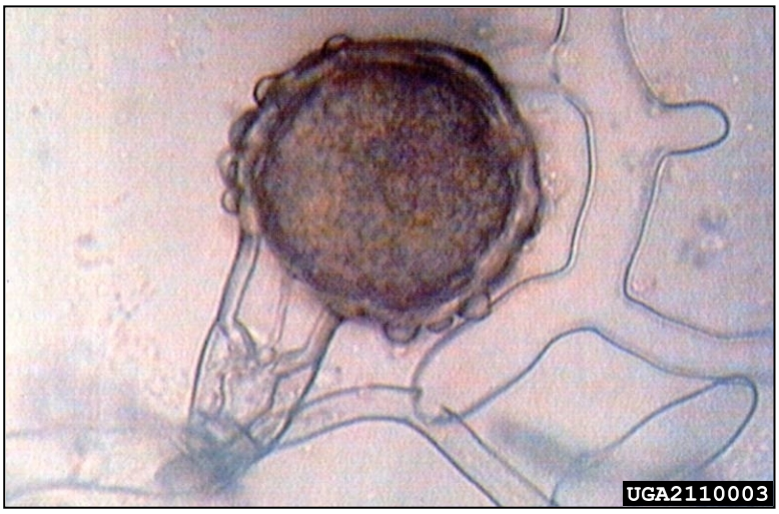

Figure 4. Bullate (puckered/blistered) oogonium of *P. alni* (German variant) with oospore and amphigynous antheridium. Photo courtesy of T. Jung. www.forestryimages.org.

Sexual Reproduction: Homothallic. A wide variety of different oogonial forms occurred, from near smooth to extremely ornamented (Fig. 4). Oogonial diameters commonly 50-60 µm, range approximately 45-65 µm. A wide variety of antheridial types also occur, from single-celled to two-celled amphigynous and occasionally or sometimes predominately paragynous.

Asexual Reproduction: Sporangia similar to those of susp. *alni*.

See Brasier et al. (2004) for detailed descriptions of the Dutch, UK, and German variants of *P. alni* susp. *multiformis*.

Biology and Ecology

Little is known about the biology of the pathogen under field conditions. Most species of *Phytophthora* infect their hosts mainly by motile spores (zoospores) that are dispersed through water and in the soil. Infection of alder via zoospores in water could explain the high incidence of the disease on alder in riparian zones, although the disease may also occur in sites remote from waterways (orchard shelter belts and woodland plantations). According to Brasier (2003), it is rare to isolate the pathogen from river water or from soil around infected alder trees. Ioos et al. (2005), however, detected *P. alni* in a river using a PCR test, which confirms that *P. alni* can spread naturally in surface water.

Figure 5. Mature common alder with collar rot caused by *P. alni* showing sparse, chlorotic and small-sized foliage. Photo courtesy of T. Jung. www.forestryimages.org.

Strnadova et al. (2010) showed that *P. alni* causes more significant damage to alders that are stressed by flooding than to unstressed plants. Flooding clearly induces a decrease in host resistance (reduced uptake of nitrogen and other nutrients, investment to rebuilding of the root system, etc.) and accelerates the development of disease caused by *P. alni*. Alder stands planted on sufficiently high banks or hills were only rarely infected, because they were less affected by increases in water level (Schumacher et al., 2006).

Zoospores of the pathogen have been shown to be attracted to fine roots of alder, but it is not known whether such roots are infected in the field. On the contrary, it appears that infection may take place through the bark near the root collar (Webber et al., 2004). Mechanical injury may not be necessary for infection. Foliar and crown symptoms do

not occur until the root collar has been largely girdled. Thus, many years may elapse between infection and the appearance of visible disease in the crown of affected trees (Cree, 2006).

Figure 6. Left: Common alder (*A. glutinosa*) in a non-flooded forest plantation with root and collar rot (tarry spots) caused by *P. alni*. Right: Grey alder (*A. incana*) with collar rot caused by *P. alni*; note the typical tarry spots at the outer bark and the tongue-shaped orange-brown necrosis of the inner bark. Photos courtesy of T. Jung. www.forestryimages.org.

Baiting tests in Germany showed that the alder *Phytophthora* was present in rootstocks of alders from three out of four nurseries that regularly brought in alder plants for re-sale, but not in rootstocks from four nurseries that grew their own alder from seed (Jung and Blaschke, 2004). This suggests that one avenue of spread for *P. alni* is via the planting of nursery stock. Schumacher et al. (2006) inoculated alder fruits in the laboratory and found that all isolates of *P. alni* were able to infect alder seeds from the water and tree harvests via zoospores. The authors also showed, however, that the fruits after drying the air or deep freezing were no longer infectious. The pathogen could survive in the fruits in a water medium for about 7 days.

Little is known about the role of oospores in the biology of the pathogen. The alder *Phytophthora* is homothallic, and produces oospores in culture, but viability as determined by the tetrazolium bromide method, is very low (26 to 31%) and no

germination was observed in more than 4,000 oospores analyzed (Delcan and Brasier, 2001).

Optimum temperature of the pathogen in culture is 22.5-25.0°C (73-77°F) with the upper temperature limit about 30°C (86°F) (Brasier et al.,1995; Santini, 2001). At a temperature of -15°C (5°F), all isolates died within three days; while at -5°C (23°F) all isolates died within 30 days (Schumacher et al., 2006). In contrast, 100% of the isolates survived at a temperature of 0°C (32°F) after a period of 30 days. Schumacher et al. (2006) also noted intra-annual variation in the activity of *P. alni* in Germany. In the frost-rich months from December to March the development of *P. alni* was largely suppressed. In the spring pathogen activity started again and restricted repeatedly in May and June. Starting in July, the pathogen could be isolated continually with high rates until severe frost at the end of the year.

The optimal pH range for production of sporangia was shown experimentally to be between 6 and 7 (Schumacher et al., 2006). Within a pH range 3.5 to 5.5, sporangial formation was absent.

Symptoms/Signs

P. alni causes a serious disease of alder (*Alnus* spp.), including lower stem bark lesions, root and collar necrosis, and crown dieback typical of other *Phytophthora* diseases. Diseased trees do not show crown symptoms until most of the bark at the base of the tree has been killed.

From a distance, diseased alders attract attention in mid-to-late summer, because the leaves are abnormally small, yellow, and sparse (Fig. 1, 5). They often fall prematurely, leaving the branches bare. In a tree with severe crown symptoms, the lower part of the stem is often marked with a black or rusty colored exudate known as ‘tarry spots’ (Fig. 6) that can occur up to 2-3 meters from the ground. The spots indicate that the underlying bark is necrotic or dead (Thorain et al., 2007). Tongue-shaped orange-brown necrosis of the inner bark is common (Fig. 6). Over the next few years, the fine twig structure, the bark, and eventually the trunk will break up. It is quite common for narrow strips of bark to remain alive and to support a limited growth of new shoots from the trunk and major branches (Webber et al., 2004). Adventitous roots may be seen on the stems of trees as a result of prolonged flooding of the root system, in response to death of the bark, or as a result of *Phytophthora* disease

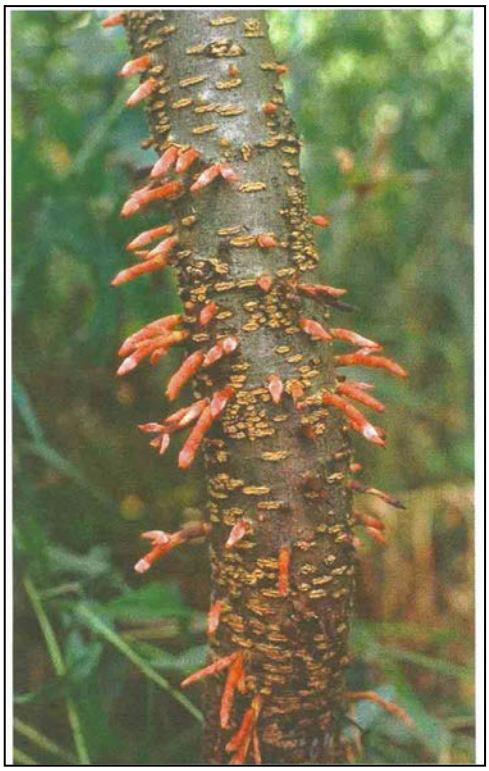

Figure 7. Adventitious roots on lower stem of young *Alnus glutinosa* tree. Photo from Gibbs et al. (2003).

(Gibbs et al., 2003) (Fig. 7). Although not a specific symptom, the development of adventitious roots can be a useful indication of the presence of a bark lesion further down the stem. Early and often excessive fructification with unusually small cones is also observed (Jung and Blaschke, 2004).

Pest Importance

There are four alder species native to Europe: common alder (*Alnus glutinosa*), gray alder (*A. incana*), Italian alder (*A. cordata*), and green alder (*A. viridis*). In general, members of the genus *Alnus* are pioneer species, able to colonize bare, open ground rapidly and with a great ability to tolerate wet sites (Webber et al., 2004). The roots have specialized nodules that fix atmospheric nitrogen as a result of a symbiotic association with the actinomycete *Frankia*. Common alder in particular has considerable landscape value along waterways; it plays a vital role in riparian ecosystems and the root system helps to stabilize riverbanks (Webber et al., 2004).

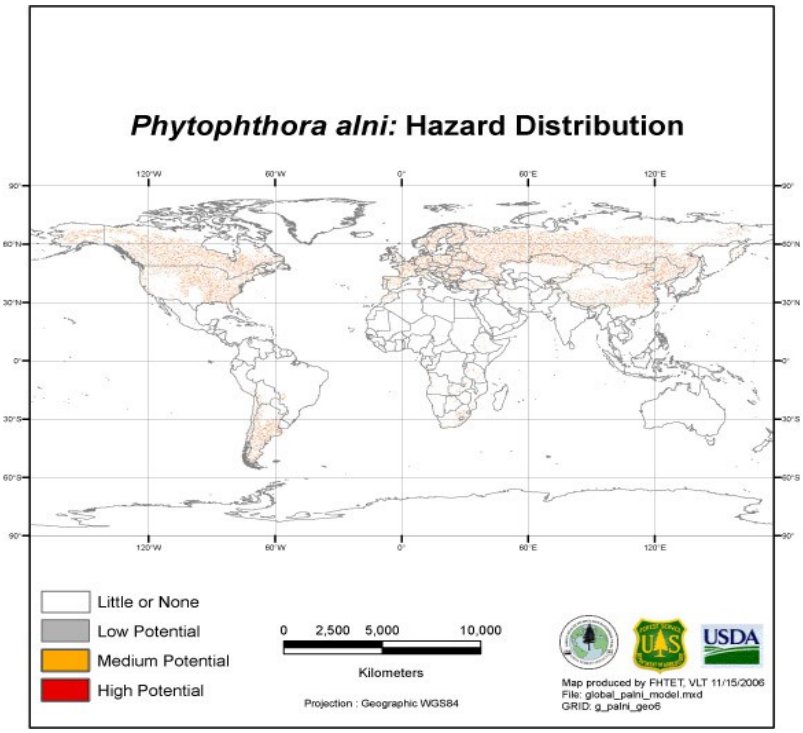

Figure 8. Hazard Distribution showing the regions that with susceptibility to *Phytophthora alni*. Image from Downing et al. (2008). http://www.forestencyclopedia.net/p/p5/p3389/p3328/p3331

Very high losses have occurred in some localities (parts of France and Germany); while in others the disease impact has been relatively small (Webber et al., 2004). The susceptibility of North American *Alnus* species is currently unknown, so it is difficult to assess the economic and ecological range of *P. alni*. Cech (1998) reports that strains of the alder *Phytophthora* were pathogenic to *Alnus rubra* (red alder), which is present in the United States and is one of the few commercial hardwood species in the western United States (Cree, 2006). Heavy loss of alders due to *Phytophthora* infection could result in significant ecological effects including changes in forest composition, wildlife food and habitat, increased soil erosion, and changes in soil composition (Cree, 2006).

Jung et al. (2007) and Downing et al. (2008) developed a model to predict the potential distribution of *P. alni* in Bavaria, Germany in order to have a tool for assessing the potential hazard posted by *P. alni* to forests in other regions of the globe. Preliminary

results of an application of the multi-criteria (drainage, streams, climate, distribution of alder species, distribution of wholesale and retail nurseries, and urban settlements) model to the world regions (Fig.8), indicates that there are regions of the United States with susceptible alder forests.).

Known Hosts

Phytophthora alni causes disease of alder [*Alnus cordata* (Italian alder), *A. glutinosa* (European, black alder), *A. incana* (gray alder), *Alnus rubra* (red alder), and *A. viridis* (green alder)].

In greenhouse inoculation trials, *Castanea sativa* (chestnut), *Juglans regia* (walnut), and *Prunus avium* (sweet cherry) were shown to be experimental hosts (Santini et al., 2003; Santini et al, 2006).

Known Vectors (or associated insects)

P. alni is not a known vector, is not known to be vectored, and does not have any associated organisms.

Known Distribution

The disease is widespread in southern England and in Europe in general. The disease has been reported from 18 countries: Austria, Belgium, Czech Republic, England, Estonia, France, Germany, Hungary, Ireland, Italy, Lithuania, The Netherlands, Poland, Scotland, Slovakia, Slovenia, Spain, and Sweden (Gibbs, 1995; Sazbo, 2000; Santini, 2001; Streito et al., 2002b; Brasier et al., 2004; Webber et al., 2004; Oszako and Orlikowsi, 2005; Cerny et al., 2008; Trzewik et al., 2008; Solla et al., 2009; Pintos Varela et al., 2010). Reports from Denmark do not now appear to have involved the alder *Phytophthora* (Gibbs et al., 2003).

Phytophthora alni subsp. *uniformis* has also been reported in Alaska and Oregon (Adams et al., 2008; Sims et al., 2012). The other subspecies are exotic to the United States.

Potential Distribution within the United States

Phytophthora alni has spread throughout Europe via planting of infested nursery stock and irrigation of fields with infested river water. Once introduced, the pathogen spreads naturally with streams, floods, and other drainage water. *P. alni* can also be passively transported with bare-root nursery stock, as it is able to adhere to and infect fine roots of visually symptomless plants of alder and other tree species exposed to the pathogen (Downing et al., 2008).

Until 2006, the alder *Phytophthora* had not been found outside of Europe. A report suggests that a similar pathogen may have been found in a nursery in Minnesota, infecting woody ornamental species (Schwingle et al., 2007; Schwingle and Blanchette, 2008). This report, however, has not been confirmed by USDA APHIS. Additionally, *Phytophthora alni* subsp. *uniformis* was found in Alaska on *Alnus incana* ssp. *tenuifolia*

in 2007 (Adams et al., 2008; Trummer, 2009; Adams et al., 2010). *Phytophthora alni* subsp. *uniformis* has also been found on root tissue of symptomatic alder in Oregon (Sims et al., 2012).

A recent risk map (susceptibility potential) developed by the Forest Health Technology Enterprise Team (FHTET) (Fig. 9) shows that areas along the west coast (Oregon, Washington, and California) and most of the eastern United States are at medium to high risk for *Phytophthora alni* subsp. *alni* (the most widespread and pathogenic subspecies). This map combines the introduction potential (nurseries and municipalities that receive bare root sock from countries where *P. alni* is present) and establishment potential (presence of the alder host, flood-prone areas, and slopes less than 11%).

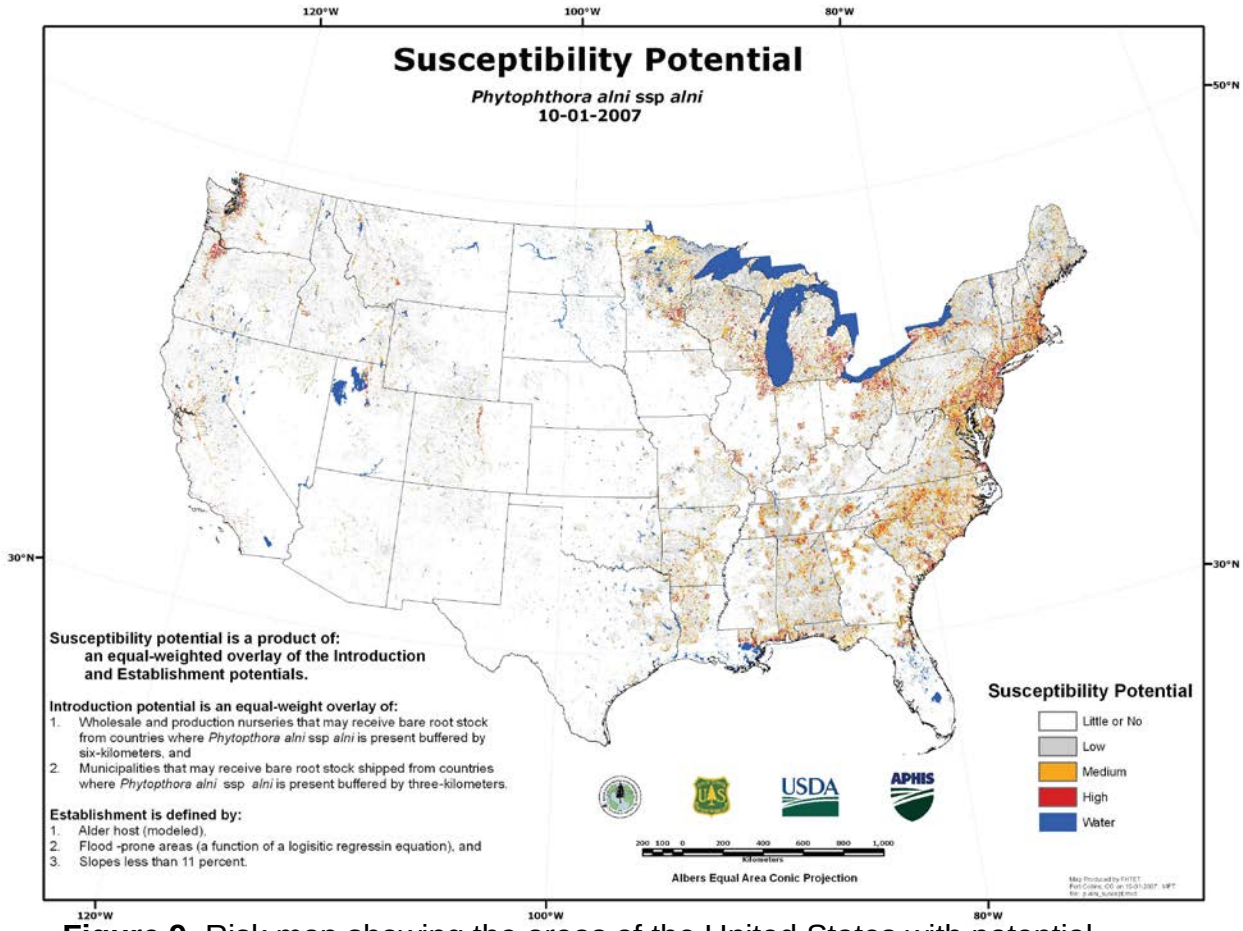

Figure 9. Risk map showing the areas of the United States with potential susceptibility to *Phytophthora alni*. Image from FHTET. http://www.fs.fed.us/foresthealth/technology/invasives_phytophthoraalni_riskmaps.shtml

Survey

CAPS-Approved Method:

1. *In situ* water sampling with rhododendron leaf baits: Use rhododendron leaves as bait by cutting the leaves in a herringbone pattern. Place 3-4 cut leaves into a mesh bag. Place the mesh bag into the water source for a minimum of 48 hours to 1 week (preferable).

   Alder twigs, apple, eucalyptus, and oak leaflets have also been used as baits for *P. alni*.

   A baiting technique using rhododendron leaves has been used in Alaska (Adams, 2007; Adams et al., 2010).

2. Collect bark lesions or soil from symptomatic trees.

Literature-Based Methods:

Worrall (2009) and Adams et al. (2010) provide basic methodologies for locating alder trees and for conducting disease surveys. Dieback occurred in both areas due to *P. alni* subsp. *uniformis* (Adams et al., 2010) and/or other tree pathogens (Worrall, 2009; Adams et al., 2010). Worrall (2009) noted that riparian areas are long and narrow and that the alder within those habitats is patchy. Therefore, a strict, systematic sampling scheme was considered inefficient and unproductive. Transects were placed in accessible areas that had alder according to the following hierarchical criteria: i) accurately represent the proportion of dieback and mortality of alder in the area, ii) include as much alder as possible, and iii) otherwise represent the vegetation and site conditions in the riparian area. All the sampling within the survey was completed by a single crew. Line-intercept transects were 30-m long, except where obstacles (streams, bluffs, etc.) prevented complete transects. Adams et al. (2010) targeted areas with 'high' mortality from drive-by observations and with a well-spaced distribution. Once a suitable stand was identified, 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. See each paper for specific information on data collected on site, etc.

Riparian and forest stands of alder are visually surveyed for symptoms of root and crown rot associated with *P. alni* (Jung and Blaschke, 2004). The inner bark of active lesions is orange-brown and mottled; while old, inactive lesions are uniform dark brown. Koltay (2007) specifically looked for the presence/absence of tarry spots on the stem and root collar in a survey of Hungary.

Jung and Blaschke (2004) collected rhizosphere soil samples from around symptomatic trees and bark samples (including the cambium) from the upper 20 cm of active lesions. The bark samples were placed in distilled water and transported to the laboratory in cool boxes. Over 2-5 days, the water was replaced four times per days in order to remove excess polyphenols. Small pieces (~8 x 3 x 3 mm) were cut from different parts and depths of the lesion, blotted on filter paper and placed onto selective PARPNH agar (~150 pieces per tree in 7 to 8 Petri dishes). Some pieces of tissue from old inactive lesions were shredded, flooded with distilled water, and baited with oak leaflets. The water was replaced daily to remove excess polyphenols and to decrease bacterial

populations. Isolations from soil samples were carried out using 2 – to 7-day old leaflets of *Quercus robur* seedlings as baits floated over flooded soils. Infected brownish leaflets, which normally appeared after 3-7 days, were blotted dry, cut into small segments, and plated onto selective PARPNH agar. Petri dishes were incubated at 20°C (68°F) in the dark and examined for *Phytophthora*-like hyphae (Jung and Blaschke, 2004). Morphological and physiological characters and ITS DNA sequences were used to assign the isolates to *P. alni*. Solla et al. (2009) used a similar isolation protocol but used pieces that were 4 x 4 x 2 mm and plated onto V8-PARPH agar.

Phloem tissue from the outer edges of necrotic lesions may be washed in running water and plated directly onto a selective medium (Tsao and Guy, 1977) and incubated at 20°C (68°F) (Brasier et al., 1995). The pathogen has been cultured using the apple method (Campbell, 1949; Brasier and Strouts, 1976). Isolation from soil samples was attempted via the apple method and eucalyptus baiting (Brasier et al., 1995). Schumacher et al. (2006), however, noted that the apple method failed for baiting *P. alni* in Germany. The authors found little difference between juvenile oak and alder leaves for baiting, but noted that living freshly cut alder bark was the most successful method for baiting *P. alni*. The bark was cut from cleaned 2- to 3-year-old alder shoots in thin strips (1-2 cm x 0.5 cm) using a scalpel.

Streito et al. (2002a) used corn meal agar and a selective medium derived from PARBHy to culture *P. alni* from active bark lesions and baits. *P. alni* was also isolated from the water of two rivers with a baiting method using alder twigs (1-year old, 1 cm diameter, 10 cm in length), but the efficiency of the method was quite low.

Key Diagnostics/Identification

CAPS-Approved Method:

1. Serological: An ELISA test is available for *Phytophthora* at the genus level for primary screening. A positive does not indicate *P. alni*.

ID must be confirmed by other methods.

2. Morphological: Samples of inner bark (phloem) tissue from outer edges of necrotic lesions may be washed in running water and plated directly onto a selective medium and incubated at 20°C. Letting samples soak for 2-5 days and replacing the water four times per days has been used to remove excess polyphenols and bacteria, which can be inhibitory to *P. alni*.

The pathogen can be isolated by direct plating of necrotic bark tissue and from water using alder twigs as bait, but the efficiency of this method is low (Streito et al., 2002a).

Literature-Based Methods:

General information on detecting *Phytophthora* spp. is available in O'Brien et al (2009).

Characteristic symptoms: Crown decline and the tarry spot symptoms occurring together reliably indicate the presence of a basal stem necrosis produced by

Phytophthora. After removing the outer bark layers around the tarry spots a red-brown to black discolored necrotic area is exposed. It is mostly tongue-shaped, growing upwards as well as in a periclinal direction (Cech, 1998).

Morphological: Morphologically the gametangia of *P. alni* are similar to *P. cambivora*, but mycologists can distinguish these pathogens in a laboratory through colony type, their ability to self-fertilize (homothallic), oogonia shape, lower optimal growth temperature, and other characteristics (Brasier et al., 1999; Brasier et al., 2004; Jung and Blaschke, 2004). Brasier et al. (2004) gives the complete species descriptions for each subspecies of *P. alni*.

Serological: Lane et al. (2007) discuss the development of a rapid on-site antibody-based testing device (lateral flow device) by Forsite Diagnostics (York, UK) for the detection of *Phytophthora* spp. in plant tissue samples in the field. The test would only be useful for identification at the genus level for primary screening similar to the ELISA test discussed above.

According to Olsson (1999), an improved DAS-ELISA technique developed for *Phytophthora* spp. from strawberry and raspberry are suitable for mass testing of plant material and for diagnosing the alder *Phytophthora* in Sweden. The sensitivity of this method was found to be comparable to that of DNA-based methods using PCR, although specific data are not given

Molecular: De Merlier et al. (2005) developed a PCR test to identify *P. alni* subsp. *alni* and *P. alni* subsp. *uniformis*. However, this test did not detect *P. alni* subsp. *multiformis* (Ioos et al., 2005). Ioos et al. (2005) developed a PCR-based test that detects and differentiates the various subspecies of *P. alni*. Where the De Merlier et al. (2005) method used DNA extracted from laboratory-grown cultures, the Ioos et al. (2005) method extracted DNA directly from soil, water, or wood. Additionally, Bakonyl et al. (2006) used SAP and SWAP PCR primers to identify all three subspecies of *P. alni* from pure culture and from plant tissue. This test can detect a minimum of 20 pg of DNA from pure cultures or DNA extracted from as few as 10 zoospores. Oszako et al. (2007) discuss the development and evaluation of a set of real-time PCR assays for *P. alni* based on the sequence characterized amplified regions (SCARs) previously developed by Ioos et al. (2005) in their abstract, however specific information about this assay is not currently available.

Note: Single-strand conformation polymorphism (SSCP) analysis of PCR-amplified ribosomal DNA internal transcribed region I has now been used to identify and provide molecular fingerprints for 59 *Phytophthora* species, including *P. alni* subsp. *alni* (Kong et al., 2003; Kong et al., 2004; Gallegly and Hong, 2008).

Easily Confused Pests

Reliable diagnosis of *P. alni* in the field is not possible; symptoms are similar to other root and collar rot *Phytophthora* species. Other *Phytophthora* that are commonly recorded on alder in Europe include: *P. citricola*, *P. cactorum*, and *P. gonapodyides*

(Streito, 2003). *Phytophthora syringae* and *P. megasperma* have also been isolated from *Alnus glutinosa* in Europe (Streito, 2003). Multiple *Pythium* spp. (another Oomycete) were also recorded from alder in Europe.

Similar results were obtained in the Alaskan surveys from 2007 and 2008 (Adams et al., 2010). *Phytophthora gonapodyides*, *Phytophthora* sp.'*hungarica*', *P. cactorum*, *P. pseudosyringae*, *P. gallica*, *P. inundata*, *P. rosacearum*, *P. megasperma*, *Phytophthora* sp. '*Missauke*', *Phytophthora* sp. '*SalixSoil*', *Phytophthora* sp. 'near *ramorum*' n. sp., and two unnamed *Phytophthora* species were isolated from Alaska. *Pythium sterilum*, *P. macrosporum*, *P. undulatum*, *P. pachycaule*, *P. anandrum*, *P. lutarium*, *P. delawari*, *P. boreale*, and '*Pythium* species in unnamed groups' were also isolated from Alaska. A common fungal pathogen, *Valsa melanodisca* (anamorph *Cytospora umbrina*) has been observed on alder in the southern Rocky Mountains, Alaska, Michigan, and Oregon (Worrall, 2009; Adams et al., 2010).

Morphologically the gametangia of *P. alni* are similar to *P. cambivora*, but mycologists can distinguish these pathogens in a laboratory through colony type, their ability to self-fertilize (homothallic), oogonia shape, lower optimal growth temperature, and other characteristics.

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