

Phytophthora austrocedrae

Scientific Name

Phytophthora austrocedrae Greslebin et al., 2007

Synonyms

Phytophthora austrocedri Greslebin et. al., 2007

Common Name(s)

'Mal del ciprés' (MDC, cypress sickness), cypress wither/mortality, Austrocedrus Root Disease (ARD), 'secamiento del ciprés' (cypress drying)

Type of Pest

Fungal-like organism

Taxonomic Position

Class: Oomycetes, **Order:** Peronosporales, **Family:** Peronosporaceae

Reason for Inclusion in Manual

CAPS Target: AHP Prioritized Pest List – 2014-2015

CAPS Pests of Additional Concern list since 2016

Background Information

Austrocedrus chilensis (Cordilleran cypress) is an endemic tree in the Cupressaceae family found in southern Argentina and Chile. It forms pure and mixed stands with *Nothofagus* spp. and, among the few conifers inhabiting the Patagonian Andes in southern Argentina, it has the largest distribution, covering approximately 160,000 hectares (395,000 ac.). Mortality of *Austrocedrus chilensis*, termed 'Mal del Ciprés' or 'cypress wither/mortality', was first detected in 1948 in Argentina. Greslebin et al. (2007) isolated a new *Phytophthora* species, *P. austrocedrae*, from necrotic lesions of stem and roots of *Austrocedrus chilensis*. *Austrocedrus chilensis* is not present in the United States, but other hosts, such as *Juniperus communis*, are present in the United States.

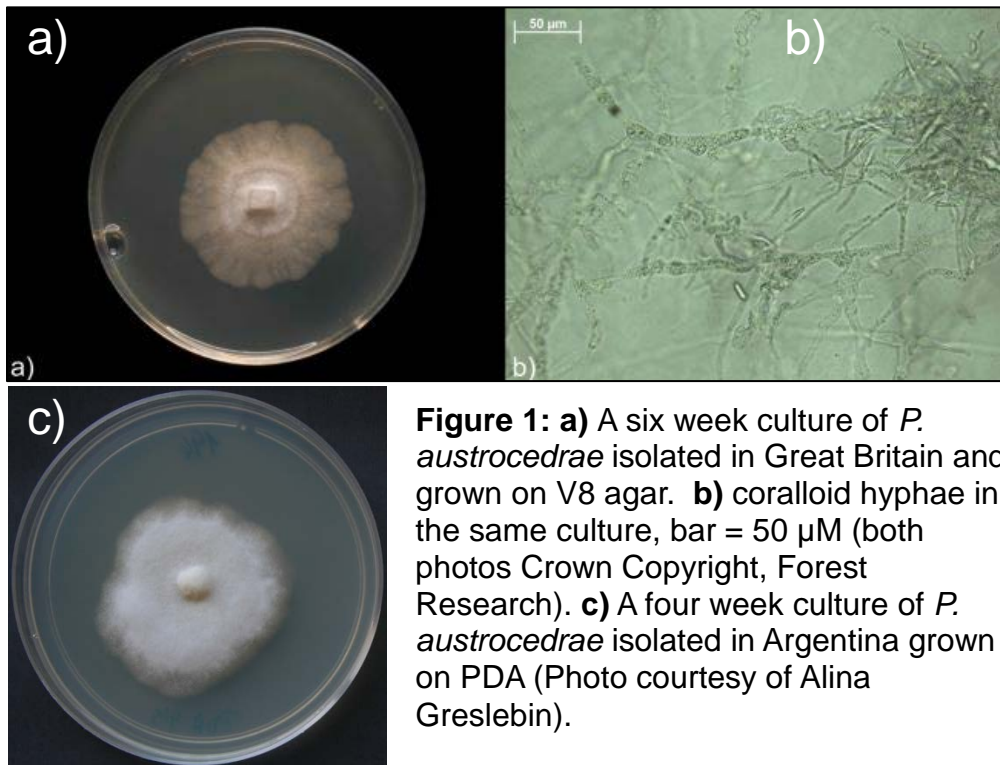


Figure 1: a) A six week culture of *P. austrocedrae* isolated in Great Britain and grown on V8 agar. **b)** coralloid hyphae in the same culture, bar = 50 μM (both photos Crown Copyright, Forest Research). **c)** A four week culture of *P. austrocedrae* isolated in Argentina grown on PDA (Photo courtesy of Alina Greslebin).

Pest Description

Phytophthora austrocedrae is a pathogen that is known to infect trees in the plant family Cupressaceae. *P. austrocedrae* is located in clade 8 of the Cooke et al. (2000) molecular phylogeny of the *Phytophthora* genus, which includes *P. syringae* and *P. lateralis* (the latter is another important pathogen of plants in Cupressaceae Goheen et al., 2000)). Phylogenetic analysis of internal transcribed spacer (ITS) ribosomal DNA (rDNA) of *P. austrocedrae* indicates that *P. syringae* and *P. obscura*, both known to be located in Europe and America, are its closest relatives (Grünwald et al., 2011a). ITS rDNA is commonly used for phylogenetic analysis because it is easy to amplify from small quantities of DNA and has a high degree of variation between closely related species (Iwen et al., 2002).

Phytophthora austrocedrae is a homothallic species characterized by semi-papillate sporangia, oogonia with amphigynous antheridia, and in culture has a very slow growth rate (1-2 mm per day on V8 juice agar at optimal temperature for growth (17.5°C (63.5°F)) (Greslebin et al., 2007). After six weeks in culture, dense white mycelia (Fig. 1a) with coralloid hyphae (Fig.1b) form.

Morphological characteristics (as described in Greslebin et al., 2007): “Hyphal swellings usually form in solid and liquid media, but are more abundant in the former. Swellings are globose to subglobose and catennulated, sometimes with distorted shapes. Sporangioophores were mostly simple, 3 to 11 μm in diameter, frequently with hyphal swellings. Sporangia are borne terminally on mostly



Figure 2: *Phytophthora austrocedrae* infected trees. Reddening and browning of foliage over most of the crown in **a)** *Juniperus communis* (Common juniper), **b)** *Chamaecyparis nootkatensis* (Nootka cypress), and **c)** *Austrocedrus chilensis* (Chilean cedar). **d)** Orange brown lesions in the phloem at the stem collar and upper roots in an infected juniper. (Figures a, b, and d: Crown Copyright, Forest Research. Figure c courtesy of Alina Greslebin).

unbranched sporangiophores. They are ovoid, obpiriform, limoniform or ellipsoid; semi-papillate, papilla 1 to 3(-5) μm thick, non-papillate sporeangia are infrequently observed. Sporeangia measure on average $50 \pm 12 \times 36 \pm 7 \mu\text{m}$ (range 22 to 83 \times 15 to 58 μm) length:breadth ratio average 1.4 ± 0.2 (range 1.1 to 2.0) and infrequently have distorted shapes. Sporeangia with hyphal projections and lateral attachment of the sporangiophore are frequently observed in all isolates. The abundance of sporeangia in water culture (soil extract or river water) is variable. Sporeangia are not observed in solid media. Oogonia form in single-strain culture in: V8 juice agar (V8A), tomato juice agar (TA), corn meal agar (CMA), CMA β , and potato dextrose agar (PDA β). In addition, oogonia form in CMA amended with NAR (25mg/l nystatin, 200mg/L ampicillin, and 10mg/l rifampicin) or PAR (10mg/L pimarcin, 200mg/L ampicillin, and 10mg/L rifampicin). Oogonia are usually formed in selective media after about 20 days. They usually form more quickly and are more abundant on selective media than on media without antibiotics. Oogonia are globose or nearly so, on average 38.5

$\pm 7 \times 39 \pm 6 \mu\text{m}$ in diameter (range 22 to 56 μm), with hyaline to light brown, smooth walls. Oospores are globose, on average 31.6 μm in diameter (range 17 to 48 μm), hyaline, with smooth walls 1 to 2(–3) μm thick. Antheridia are amphigynous, hyaline, one-celled, and average $18 \pm 3.5 \times 14 \pm 2 \mu\text{m}$ (range 10–30 \times 8–20 μm).”

Biology and Ecology

Phytophthora austrocedrae is an oomycete ‘water mold’ fungal-like pathogen of Cupressaceae plants (Greslebin et al., 2007). *Phytophthora* spp. form sporangia which produce zoospores that can move through water and swim towards new hosts (Zentmyer, 1961). When *P. austrocedrae* comes into contact with a susceptible host, it penetrates the bark and enters the phloem and xylem tissues of the plant (Vélez et al., 2012). The pathogen grows both up and down from the lesion, killing the tissue to cause a dark necrotic spot (Fig 2b) (Green et al., 2015). *Phytophthora austrocedrae* grows down and kills the root tissue, and the lesions can expand at up to a rate of 11.5 cm per month (Greslebin and Hansen, 2010). When *P. austrocedrae* has sufficiently girdled the tree, the foliage of the tree will turn red or brown and foliar dieback is visible (Greslebin and Hansen, 2010).

There can sometimes be branch lesions that are thought to be caused when *P. austrocedrae* propagules are splashed from an infection or the soil onto susceptible branch tissue. This has only been reported so far in *Juniperus communis* (Green et al., 2015; Ristaino and Gumpertz, 2000), but this may affect other susceptible trees.

Phytophthora austrocedrae is a homothallic organism, which means that it can sexually reproduce to form oospores by itself without a partner. Oospores for *Phytophthora* spp. are often a survival propagule that have the ability to survive for years inertly, although there has been no research to determine the mechanism of survival for *P. austrocedrae* (Crone et al., 2013; Ristaino and Gumpertz, 2000). The relative importance of sexual and asexual reproduction in naturally occurring infections in the environment for *P. austrocedrae* has not been explored, and epidemics in Argentina and Great Britain are thought to be caused by clonal populations, and so it is thought that the pathogen has been introduced to these areas (Henricot et al., 2017).

Phytophthora austrocedrae is temperature sensitive, and can grow best in culture between 10 and 20°C (Greslebin et al., 2007). However, it has been shown that necrotic stem lesions expand at a faster rate when inoculations were done during the summer as opposed to winter, which suggests that there may be specific environmental conditions that promote infection (Greslebin and Hansen, 2010). In addition, there are highly variable rates for lesion expansion as well as foliar symptom development (Green et al., 2015; Greslebin and Hansen, 2010).

The disease is favored by environments with lower temperatures and with higher precipitation (Havrylenko et al., 1989). In addition, 'mal de ciprés' is associated with poor soil drainage and saturated soils (La Manna and Rajchenberg, 2004a; La Manna and Rajchenberg, 2004b). The disease clusters in stands (Rosso et al., 1994).

Phytophthora spp. can be spread long distances by either water or contaminated soil. Similar *Phytophthora* diseases of forests are managed by reducing spread by contaminated shoes, livestock, equipment, and vehicles (Goheen et al., 2000). Similarly, proximity to a stream can be important for the spread of the pathogen to new areas (Goheen et al., 2000; Reeser et al., 2011).

Phytophthora austrocedrae was first identified in the Patagonia region of Argentina (Greslebin et al., 2007), but its geographic origin remains unknown.

Symptoms/Signs

Symptoms are similar in appearance in all known hosts. Above ground symptoms of infected trees include foliage reddening or browning over all or most of the crown (Green et al., 2012) (Fig. 2a through c), a progressive withering and defoliation, crown thinning, loss of radial growth, dieback, decay of main roots, and death of the tree, which remains standing or falls because of the wind (La Manna and Rajchenberg, 2004a). Trees may die rapidly, within months, or this process may take up to 75 years (Filip and Rosso, 1999; Greslebin and Hansen, 2010). In the case of rapid decline, foliage changes from chlorotic (yellow) to red, or slowly, with chlorosis followed by progressive defoliation leading to tree death after several years (Filip and Rosso, 1999).

Phytophthora austrocedrae produces necrotic lesions that affect the entire thickness of the phloem, evidenced by discoloration of the tissue and superficial staining of the sapwood, and there are often resin pockets within the phloem (Greslebin and Hansen, 2010; Greslebin et al., 2007) (Fig. 2d). There is often a yellowing in advance of the lesion margin (Green et al., 2015).

Pest Importance

In the United Kingdom (UK), *P. austrocedrae* is an emerging threat and is already having a considerable environmental and social impact on juniper forests (Green et al., 2015). *Phytophthora austrocedrae* infected juniper has now been identified at more than 19 upland woodland sites in northern Britain, some of them in nature reserves (Green et al., 2015). *Juniperus communis* was already recognized as vulnerable in the UK before the discovery of *P. austrocedrae* (Anon., 2007; Fraser, 2015; McBride, 2005). Any further contributing factor in juniper decline, including infection by the pathogen *P. austrocedrae*, could be highly significant to the survival of this species there (Webber et al., 2012). In the uplands of the UK today, the main importance of juniper is for the related aspects of nature conservation and game management. Juniper is a long-lived shrub component of many semi-natural habitats and has a unique and specialized

group of associated insects, fungi and lichens. It is also an important food plant for a wide range of invertebrates (McBride, 2005).

The economic impact of *P. austrocedrae* has not been well documented. In Argentina, the high quality wood of *Austrocedrus chilensis* is used in construction and woodworking (Diaz Vaz, 1985). *Austrocedrus chilensis* is also valued for its scenic beauty, but specific economic data is currently unavailable and the environmental impact has not been quantified. In the UK, *C. lawsoniana* is a valued ornamental species and accounts for a 'significant portion' of the £29 million (~\$44.68 million) in garden center sales of conifers each year (Webber et al., 2012).

In the United States, known *P. austrocedrae* hosts in the Cupressaceae family are common in most states (Fig. 3) (NRCS, 2018). The potential for this pathogen to have environmental impact is high based on host presence.

Phytophthora at the genus level is on the harmful organism lists of Canada, French Polynesia, Mexico, Namibia, Seychelles, South Africa, and Venezuela (PExD, 2018 [queried July 23, 2018]). If this pest were found in the United States, there are potential trade implications with these countries.

Known Hosts

Major Hosts

Austrocedrus chilensis (Chilean cedar, Cordilleran cypress). *Juniperus communis* (common juniper) (Green et al., 2012; Greslebin et al., 2007).

Minor Hosts

Chamaecyparis lawsoniana (Lawson cypress), *Chamaecyparis nootkatensis* (Nookta cypress), and *Cupressus sempervirens* (Mediterranean cypress), *Juniperus horizontalis* (creeping juniper) (Green et al., 2013; Green et al., 2016; Henricot et al., 2017; Mahdikhani et al., 2017).

Known Vectors (or associated insects)

P. austrocedrae is not known to act as a vector to any other organism, nor is it known to have a vector.

Known Distribution

South America: Argentina (Patagonia). **Europe:** Great Britain (England, Scotland, and Wales) and Germany. **Asia:** Iran (EPPO, 2011; Green et al., 2012; Greslebin et al., 2007; Henricot et al., 2017; Mahdikhani et al., 2017).

Although there is no official identification, there is some evidence that the disease may also occur in South-Eastern Chile (Filip and Rosso, 1999).

Pathway

About half of all known species of *Phytophthora* are not present in the United States (Cline et al., 2008). These exotic species may present a threat to U.S. agriculture and natural resources. Some *Phytophthora* species can have large host ranges, and the risk of introducing new *Phytophthora* species on imported nursery stock is high (Brasier, 2008). The genus *Phytophthora* is listed as reportable at the port of entry (PestID, [queried July 20, 2018]). Long incubation and latent infections (time between infection and symptom development/production of new inoculum) are common with this genus (Elliott et al., 1966), and reliance upon visual inspection at ports-of-entry is unlikely to restrict the movement of this important group of plant pathogens.

In 2011, *P. austrocedrae* was first detected in Europe (Green et al., 2012). Geographically, this pest was only known to occur in South America prior to this find. In 2017, it was reported in Iran (Mahdikhani et al., 2017). While its natural spread is likely to be slow, there is significant potential for *P. austrocedrae* to quickly spread into the wider environment via the plant trade (Webber et al., 2012). Movement of this pest across multiple continents and oceans shows that the pest is spreading geographically, most likely by human-assisted means.

Juniperus spp. (all propagules except seeds) are prohibited from Europe (USDA, 2018). Since we do not know if *P. austrocedrae* is seedborne, seed could constitute an open pathway. No regulations were found for *Chamaecyparis* spp., and this host constitutes a potential pathway as well (USDA, 2018).

Potential Distribution within the United States

Chamaecyparis nootkatensis is present in the western United States from Alaska to California, and *C. lawsoniana* is present in Oregon and California. *Juniperus communis* is present in over 40 continental U.S. states (NRCS, 2018). Given the wide distribution of known *P. austrocedrae* hosts, particularly *Juniperus* spp., in the United States, (Fig. 3), the potential for *P. austrocedrae* to spread if it becomes established is likely. Distribution may be limited, however, by the cool temperature (50-68.5°F) requirements for *P. austrocedrae* for growth and reproduction (Greslebin et al., 2007).

Survey

CAPS-Approved Method*: The CAPS-approved survey method is to collect symptomatic plant tissue by visual survey.

*For the most up-to-date methods for survey and identification, see Approved Methods on the CAPS Resource and Collaboration Site, at <http://caps.ceris.purdue.edu/>.

The survey methods are the same for each known host. The following is a detailed survey method for infected juniper as described in Forest Research (2012).

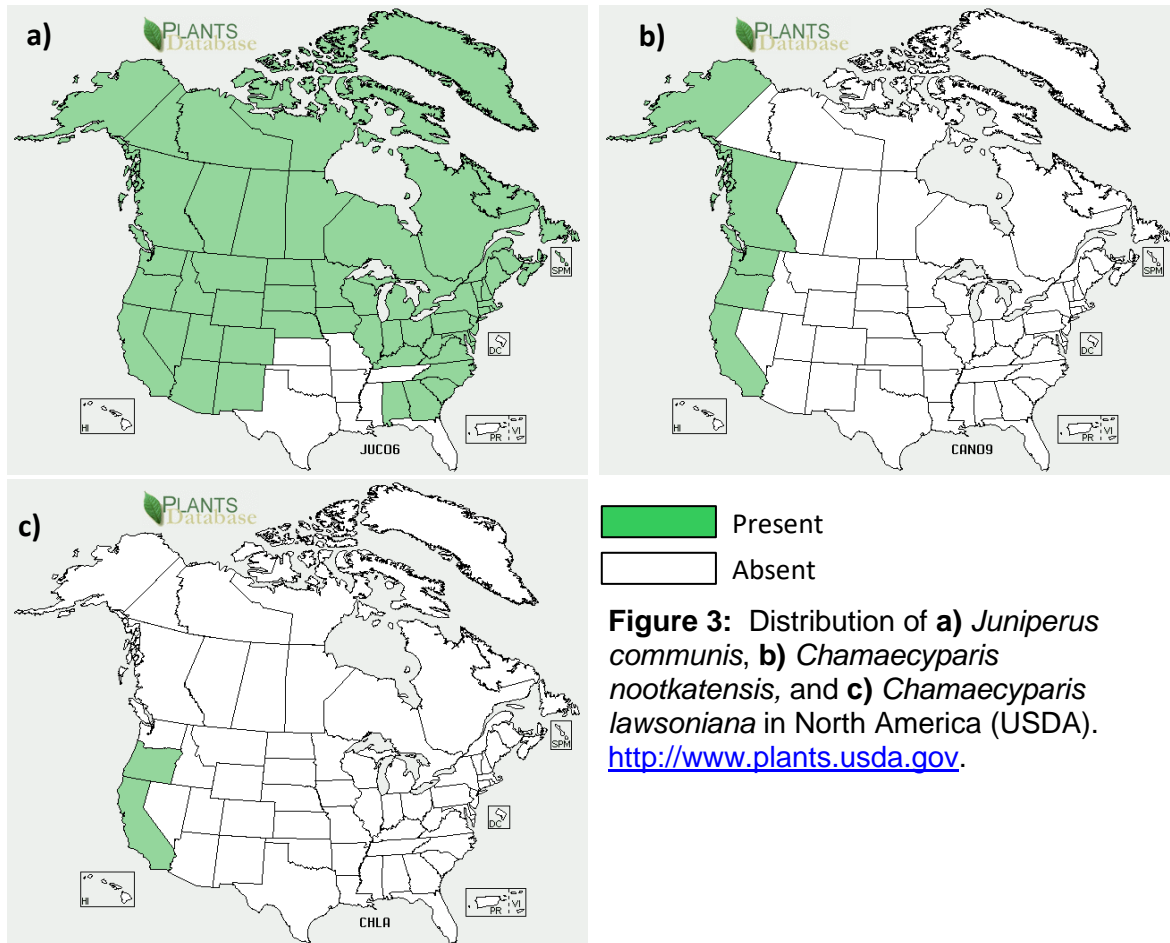


Figure 3: Distribution of **a) *Juniperus communis***, **b) *Chamaecyparis nootkatensis***, and **c) *Chamaecyparis lawsoniana*** in North America (USDA). <http://www.plants.usda.gov>.

Phytophthora austrocedrae primarily attacks the roots and stem bases of juniper. Infections may extend to 50 cm (19.7 in) or more up diseased stems. Infected trees have foliage reddening and browning over all or most of the crown (Fig. 4a). When the outer bark is cut away from the base of infected trees, discolored phloem (inner bark) is revealed (Fig. 4b, 4c). It is usually orange-brown in color (Fig. 4b, 4c); whereas healthy phloem is white. Thus, a distinct color difference between infected and healthy tissue at the extending margin of the lesion is visible. Often, infected phloem has resin pockets, and a diffuse yellow coloration is sometimes visible in the healthy phloem in advance of the lesion margin.

Collecting samples of juniper for diagnosis:

- Phloem (inner bark) samples are used for diagnosis purposes. Take samples from trees that are in the early-to-mid stages of decline. Trees that are already dead with dead, bronzed foliage do not make suitable samples as the inner bark is invariably too dry to yield *P. austrocedrae* on isolation.
- Use a sharp knife to cut away the outer bark at the base of the main stem and upper roots of an affected tree, exposing the phloem (inner

bark). Look for signs of browning in the phloem, which indicates phloem killing and possible infection by *P. austrocedrae*.

- If an aerial infection is suspected, cut away the outer bark at the base of affected branches to look for diseased, discolored phloem.
- Live, healthy phloem is white in color, whereas diseased phloem is dull orange-brown (Fig. 4b, 4c). If the phloem is infected, then work outwards gradually removing bark until revealing the transition between infected and healthy phloem, *i.e.* where the orange brown phloem meets the healthy white phloem. This is known as the live-dead junction (Fig. 4b, 4c). With *P. austrocedrae* the live-dead junction will often be seen as an area of healthy phloem with 'tongues' or strips of infected tissue extending into it.
- If diseased phloem is found, cut away several sections of phloem, each about 5 to 10 cm² (0.8 to 1.5in²), cutting down to the wood underneath the phloem. Make sure the sample contains tissue from the live-dead junction.
- Always sterilize cutting tools after working on each individual tree.
- Put samples in a sealable plastic bag (e.g., a freezer bag) and label with location, date and contact details.

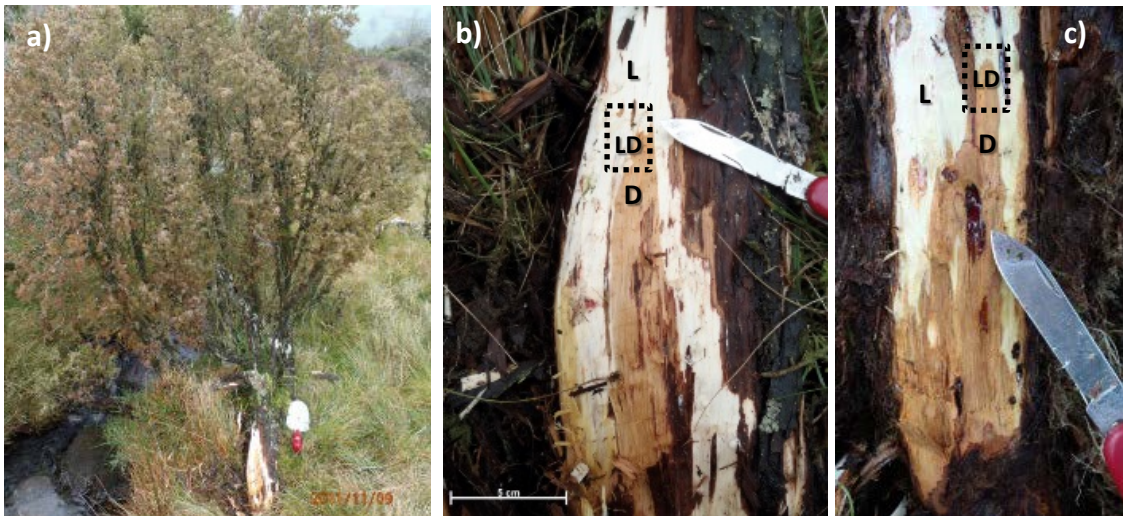


Figure 4: a) Juniper infected with *P. austrocedrae* at the stem base. b, c) Basal lesions on juniper infected with *P. austrocedrae*. Outer bark has been cut away to reveal diseased phloem. L indicates healthy phloem; D indicates infected tissue; LD indicates live-dead junction with tongue of infection extending into the healthy tissue. Box indicates good section for sampling (taken from Forestry Commission, 2012, Crown Copyright, Forest Research).

Key Diagnostics

CAPS-Approved Method:

1. **Serological:** An Enzyme-Linked ImmunoSorbent Assay (ELISA) Reagent Set for *Phytophthora* (AGDIA, Cat# SRA 92600/1000) at the genus level for primary screening. A positive does not indicate *P. austrocedrae*.

ID must be confirmed morphologically.

2. **Morphological:** Samples of inner bark (phloem) tissue from lesion margins may be directly plated on a variety of selective media (PARNBP, PAR, NAR, BARP with a corn meal agar base and SMA + MRP¹) immediately after collection or after washing necrotic tissue with running tap water for 24 to 48 hours (Green et al., 2012; Greslebin et al., 2007). After initial isolation, colonies are transferred to a non-selective medium such as clarified V8 juice agar or tomato juice agar and stored for about six weeks at 16 to 17°C (60.8 to 62.5°F) in the dark until final identification can be made (Green et al., 2013; Greslebin et al., 2007).

*For the most up-to-date methods for survey and identification, see Approved Methods on the CAPS Resource and Collaboration Site, at <http://caps.ceris.purdue.edu/>.

Literature-Based Methods:

Green et al. (Green et al., 2012) isolated the pathogen using SMA + MRP *Phytophthora* selective medium of Elliott et al. (Elliott et al., 1966)¹ amended before autoclaving with 0.5 ml of a 4% MBC (benomyl hydrochloride) solution and incubated at room temperature (15-24°C (59-75°F)) in the dark. After transfer to V8 agar, colonies are very slow growing (<0.5 mm per day) at 17°C (62.5°F), forming dense, white mycelia (Fig. 1a) with coralloid hyphae (Fig. 1b) (Green et al., 2012). However, isolation from *Juniperus communis* lesions in Great Britain yielded a low rate of success (<10%), perhaps due to the slow growth rate (Green et al., 2015).

¹SMA+MRP medium contains a basal *Phytophthora* selective medium composed of sucrose, 10.0 g; L-asparagine, 1.0 g; KH₂PO₄, 0.5 g; MgSO₄ · 7H₂O, 0.25 g; trace element solution, 1.0 ml; thiamine hydrochloride, 1.0 mg; Difco Bacto-Agar, 10.0 g; water, 1L. The trace element solution contains: Na₂B₄O₇ · 10H₂O, 88 mg; CuSO₄ · 5H₂O, 393 mg; Fe₂(SO₄)₃ · 6H₂O, 910 mg; MnCl₂ · 4H₂O, 72 mg; Na₂MoO₄ · 2H₂O, 50 mg; ZnSO₄ · 7H₂O, 4403 mg; EDTA, 5 g; water 1L (Elliott et al., 1966). The basal medium is amended before autoclaving with 0.5 ml of a 4% MBC (benomyl hydrochloride) solution as in Brasier et al. (Brasier et al., 2005). The pH was adjusted to 6.5 with 1 M NaOH. After autoclaving at 121°C for 15 min the agar was cooled then further amended with 0.4 ml of a 2.5% suspension of pimaricin and 3 ml of a 1% w/v solution of rifamycin SV.

Based on morphological characteristics and sequencing of the ITS and *coxII* regions (GenBank Accession Nos. JQ346527 and JQ346528), isolates can be identified as *P. austrocedrae* (Green et al., 2012; Greslebin and Hansen, 2010; Greslebin et al., 2007). Direct PCR and sequencing of diseased phloem from

basal and branch lesions on juniper trees from which no *Phytophthora* is obtained can yield the same result (Green et al., 2012). There are free curated online sequence databases, Phytophthora Database and *Phytophthora*-ID, for identification of *Phytophthora* spp. using ITS, *coxI* and *coxII* loci to aid in molecular diagnosis (Grünwald et al., 2011b; Park et al., 2013),.

A TaqMan real-time PCR method for *P. austrocedrae* identification has recently been published that specifically amplifies *P. austrocedrae* ITS rDNA. This was validated with samples of infected bark, and thus this assay could potentially be used for diagnosis of field samples (Mulholland et al., 2013).

Easily Confused Pests

Disease symptoms caused by *P. austrocedrae* can be confused with other pathogens including other *Phytophthora* species. *Phytophthora cinnamomi*, a pathogen which is already present on a range of host plants in the UK and in the United States, can cause similar symptoms to *P. austrocedrae* on ornamental hosts. *Phytophthora lateralis* has been found to infect *C. lawsoniana* in the UK with similar host symptoms (Green et al., 2013). There is a similar symptomatology to 'mal de ciprés' caused by *P. austrocedrae* for a disease in the same region where no causal agent has been reported, which may be due to physical damage (Greslebin and Hansen, 2010).

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Reviewers

Dr. Sarah Green, Centre for Ecosystems and Biosecurity, UK.

Dr. Alina Greslebin, Protección Forestal, Centro de Investigación y Extensión Forestal Andino Patagónico (CIEFAP), CC 14, 9200 Esquel, Chubut, Argentina

Revisions

May 2015: Added Mulholland et al. (2015) reference for the new real time PCR method.

May 2018

- 1) Comprehensive literature review and update completed
- 2) Added Iran to **Known Distribution**.
- 3) Added Mediterranean cypress to list of **Known Hosts** and revised list.
- 4) Updated format of references.