2014, 1
ISSN 2191-1398
DOI 10.5073/jkidspdd.2014.001

Julius Kühn-Institut
Bundesforschungsinstitut für Kulturpflanzen
Federal Research Centre for Cultivated Plants

JKI Data Sheets

Plant Diseases and Diagnosis

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Phytophthora austrocedrae Gresl. & E. M. Hansen

Julius Kühn-Institut, Federal Research Centre for Cultivated Plants

Imprint

The open access series „JKI Data Sheets – Plant Diseases and Diagnosis“ is a publication that publishes original papers, pathogen descriptions, findings and reports on biotic and abiotic causes of crop diseases and crop damage.

All manuscripts submitted for publication in the JKI Data Sheets are peer-reviewed by at least two independent referees while the anonymity of author(s) is preserved.

Publisher/Editor-in-Chief:
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Submission of manuscripts:
Please go to the journal's website at
http://pub.jki.bund.de/

ISSN: 2191-1398

DOI: 10.5073/jkidspdd.2014.001

Status in the EU

Not listed on any of the EPPO lists http://www.eppo.int/QUARANTINE/Alert_List/alert_list.htm.

History

Phytophthora austrocedrae Gresl. & E.M. Hansen is a *Phytophthora* species that up to now has only attacked needle trees and shrubs. It was first detected in Argentina on *Austrocedrus chilensis* (Chilean incense cedar, *Cupressaceae*). This conifer is naturally distributed in Argentina and in Chile. It is the most important conifer species of the Andean Patagonian forest. Disease symptoms had been observed since 1948 (Varsavsky *et al.*, 1975 cited from Havrylenko *et al.*,1989; summary of the history of *Austrocedrus chilensis* decline in Filip & Rosso 1999, and La Manna & Matteucci 2012) but the causal agent could not be identified before 2007.

In Europe *P. austrocedrae* had first been reported in UK in spring 2011 in a public park where it could be isolated from diseased *Chamaecyparis nootkatensis* (Nootka cypress, *Cupressaceae*) and from *C. lawsoniana* (Lawson's cypress, *Cupressaceae*). The first cases of *P. austrocedrae* in the natural environment in the UK were discovered on *Juniperus communis* (common juniper, *Cupressaceae*) in the North Pennines, England in 2011. A recently made sequence analysis of an unknown *Phytophthora* isolate stored in the JKI culture collection showed that this isolate is a *P. austrocedrae* (Wagner and Werres, unpublishded data). It was isolated in 2001 from *Juniperus horizontalis* 'Glauca' (R. Ulrich, personal communication) and originated from a German nursery that imported plants. So it seems that *P. austrocedrae* has been present in Europe earlier than 2011.

Geographical distribution

Europa: - Germany 2001 (nursery, only a single detection; determination of the species in 2013)

- UK 2011 (public park: *Chamaecyparis nootkatensis, C. lawsoniana*; natural environment, private garden, nursery: *Juniperus communis*);
2012 (natural environement, private garden, nurseries: *Juniperus communis*) (Forestry Commission, 2013; Green *et al.*, 2012; EPPO Reporting Service 2011/135, 2012/057)
Status: "Present in some areas, subject to official control" (EPPO Reporting Service 2012/140)

South America: Argentina and Chile (Greslebin & Vélez, unpublished data)

Host range

- *Austrocedrus chilensis*
- *Chamaecyparis lawsoniana, Chamaecyparis nootkatensis*
- *Juniperus communis, Juniperus horizontalis* 'Glauca'

Disease symptoms (Fig.1)

In Argentina *P. austrocedrae* is the causal agent of "Mal del ciprés de la cordillera" (Cordilleran cypress sickness) or 'Secamiento del ciprés' (cypress drying) on *Austrocedrus chilensis* (Greslebin & Hansen, 2010). The crown of infected trees show first discolored leaves (color can switch from green to yellow and finally to red) and can result in defoliation. At the stem base it can be seen necrotic lesion extending from killed roots up to 1 m up the tree bole. The necrosis affected the entire thickness of the phloem and, at least, the closest millimeters of sapwood. Lesions could be active or inactive. When active, they are bright chestnut brown, moist and flexible. When inactive, they are dark brown, dry and hard, and it is very difficult to distinguish them from the outer bark. Trees are able to wall off old inactive lesions with callus tissues. Wood rottings in roots and in the stem sapwood, caused by saprotrophic Basidiomycetes, are frequently associated to the necrotic lesions. On the outer bark, "bleeding resination" occurs. Resin flow usually emerges from a resin pocket in the phloem near the active margin of a necrotic lesion. Plants can be killed by *P. austrocedrae* within short time.

The infected *Chamaecyparis* in UK gardens showed similar diseases symptoms; die-back, browning foliage and stem lesions. Infection was initially thought to be *P. lateralis* due to the similarity of symptoms (Werres & Wagner, 2012; Robin, 2013) and host but *P. austrocedrae* was eventually isolated.

The *P. austrocedrae* infection of *Juniperus communis* in the natural environment in the UK is severe in places (Green, *et al*., 2012). Trees of all ages are affected. Infected trees often fail to flush during the spring; they then become discoloured from green to yellow to red. This process appears to happen within a short period of time, perhaps within one growing season. Upon the removal of the outer bark from the base of the trunk, necrotic phloem can be observed. There is often a clear dead/live junction with cinnamon coloured necrosis (Forestry Commission, 2013). Resinosis can occasionally be observed. Infected trees are typically found growing on wet, boggy ground.

Attention: Disease symptoms are not specific only for *P. austrocedrae*! To specify the cause of the disease samples must be examined in the laboratory.

Genetics / Biology / Morphology (Tab. 1, Fig. 2)

First description by Greslebin *et al*. (2007).

P. austrocedrae belongs to morphological group IV (Greslebin *et al*., 2007) and genetically to the new subclade 8d (Grunwald *et al*., 2012). It is genetically closely related to *P. syringae and P. obscura* (Grunwald *et al*., 2012).

Tab. 1: Morphological characteristics of *Phytophthora austrocedrae* (Fig. 2)

	According to Greslebin *et al*. (2007)	**According to Grünwaldt *et al*. (2012)** (on Carrot Piece Agar)
Vegetative growth:		
Temperature (°C):		
Minimum	-	5-10
Optimum	17.5	15-20
Maximum	<25	20
Growth rate at optimum temperature (mm/24 h):	1-1.8	0.8
Sporangia	semipapillate non caducous ovoid, obpyriform limoniform or ellipsoid	semipapillate non caducous ovoid, obpyriform limoniform or ellipsoid
Length x width (range)	22-83 x 15-58	40-82 x 28-44
Length x width (average)	38-62 x 29-43	59 x 36.9
Length : width (range)	1.1-2 : 1	1.1-2.2 : 1
Length : width (average)	1.2-1.6 : 1	1.6 : 1
Sporangiphores	mostly simple, frequently with hyphal swellings	-
Chlamydospores	none	none
Gametangia (usually observed on agar media)	homothallic	homothallic
Oogonia	globose or nearly so wall hyaline to light brown	globose or nearly so
Diameter (range, µm)	22-56	30-64
(average, µm)	39 ± 6	49.6
Antheridia	amphigynous one-celled	amphigynous
Length x width (range, µm)	10-30 x 8-20	10-35 x 10-20
Length x width (average, µm)	18 ± 3.5 x 14 ± 2	19.1 x 15.3
Oospores	globose, aplerotic to plerotic	globose, aplerotic
Diameter (range, µm)	17-48	20-62
(average, µm)	32	44.4
Wall thickness (µm)	1-2(3), smooth walls	-

- = no data available

Diagnosis

Direct isolation

Best samples are pieces from the tissue closest to the necrotic phloem (inner bark) or xylem. Discolored phloem occurs first at the trunk or stem base or at the root collar. Cut out little pieces at the life-dead junction between discolored and healthy (white) looking tissue. Pieces cut from the wood are not favorable because the wood is too dry. In general isolation is difficult.

In Argentina best season for isolation from *Austrocedrus* are spring and autumn but spring is the best. Usually no positive isolation is obtained in summer or hot days (>25°C) because *P. austrocedrae* is very sensitive towards high temperatures. In the UK spring and autumn also seem to be the best season for isolation from *Juniperus*. Keeping the samples and Petri dishes in a portable cooler can improve isolation during seasons with high temperatures.

Place the tissue pieces directly onto agar medium and incubate at 15-17.5°C (*P. austrocedrae* prefers low temperatures). The following recipes have been shown to be best for direct isolation:

V8 medium (proven for isolation from *Juniperus*)

5g of calcium carbonate is added to 354 ml of V8 tomato juice and centrifuged for 4 minutes at 4500 rpm. The supernatant is collected and made up to 1 litre with distilled water. It is then autoclaved for 15 minutes at 121°C.

CMA-PAR-β (proven for isolation from *Austrocedrus*)

Corn Meal Agar (Sigma, 17 g L^{-1}) amended with 10 mg pimaricin (50%), 200 mg ampicillin (100%) and 10 mg rifampicin (70%) and 30 µg β-sitosterol g L^{-1} (60%). The medium can also be used without antibiotics.

Dissolve the β-sitosterol in 96% ethanol before you mix it with the agar and the water. Then autoclave for 15 min at 121°C. After sterilization add the antibiotics. They can also be dissolved in 96% ethanol (check the instructions for use, whether they can also be dissolved in destilled water).

In many media amendment of β-sitosterol to any culture increases growth rate and gametangia development. That was shown for vegetable media (V8 agar, tomato agar and PDA media). Using CMA media adding β-sitosterol is mandatory for.

Further details for isolation from *Juniperus* see http://www.forestry.gov.uk/pdf/phytophthora_austrocedrae_juniper_factsheet.pdf/$file/phytophthora_austrocedrae_juniper_factsheet.pdf

Bait test for soil and water

- ***Austrocedrus chilensis* seedlings** gave the best results as bait because they were very selective. Fifteen to thirty days old seedlings, grown on sterile sand, were effective to isolate *P. austrocedrae* from soil. Other less selective baits (as *A. chilensis* leaves and small branches and leaves and petiols of other plants) can also work but, as *P. austrocedrae* grows very slowly, isolates are usually overgrown by other phytiaceous species that can also colonize the bait and grow faster than *P. austrocedrae*.
- Testing ***Juniperus* spp**. and **Rhododendron leaves** as baits are in progress

Serological methods

There is no serological test that is specific for *P. austrocedrae*. But commercially available ELISA test systems that are specific for *Phytophthora* on the genus level can give first indications on a *P. austrocedrae* infection (Greslebin & Hansen, 2010). If the test kits give a positive result further tests or direct isolation must help to determine whether it is *P. austrocedrae* or another *Phytophthora* species.

PCR

Primer specific for *P. austrocedrae*	Cross reaction with according to the literature
Paus-481-F, Paus-554-R, Paus-507-TM[1] (TaqMan real-time PCR)	none with *P. obscura, P. syringae, P. ramorum, P. kernoviae* (and others, see reference)

[1] Mulholland *et al*. (2013) - = no information

Recommendation

- Examination of samples with at least two different diagnostic techniques, e.g. for plant samples: direct isolation and PCR. Commercially available ELISA kits for *Phytophthora* detection are genus specific and can give a first indication on a *Phytophthora* infection.
- Determination of the *Phytophthora* species in pure agar cultures by morphology, by sequence analysis and/or PCR.
  For differences between the closely related *P. austrocedrae P. syringae* and *P. obscura* see Grunwaldt *et al.* (2012).

Help with diagnosis

In Argentina: Alina GRESLEBIN, agreslebin@unpata.edu.ar
In Germany: Sabine WERRES, sabine.werres@jki.bund.de
In UK: Matthew ELLIOT, matthew.elliot@forestry.gsi.gov.uk

What to do in case plants are suspected to be infected?

Please contact your national authorities for help with diagnosis.

In **Argentina**: Alina GRESLEBIN, agreslebin@unpata.edu.ar. Please, contact for instructions on material collection and treatment.

Examples for **European contact addresses**: addresses.pdf
In **Germany** please contact first your plant protection services; address list see
http://www.jki.bund.de/de/startseite/unser-service/linksammlung.html
In **UK** please contact Forest Research via the Tree Health Diagnostic and Advisory Service at
http://www.forestry.gov.uk/fr/ddas

Risk analysis

UK: "Rapid assessment of the need for a detailed Pest Risk Analysis for *Phytophthora austrocedrae*" (http://www.fera.defra.gov.uk/plants/plantHealth/pestsDiseases/documents/phytophthoraAustrocedrae.pdf)

Literature

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http://forestphytophthoras.org/species/austrocedrae

http://www.q-bank.eu/

http://phytophthora-id.org/

http://www.forestry.gov.uk/pdf/phytophthora_austrocedrae_juniper_factsheet.pdf/$file/phytophthora_austrocedrae_juniper_factsheet.pdf

Acknowledgement

We thank Everett Hansen for the reference isolates of *P. austrocedrae*.

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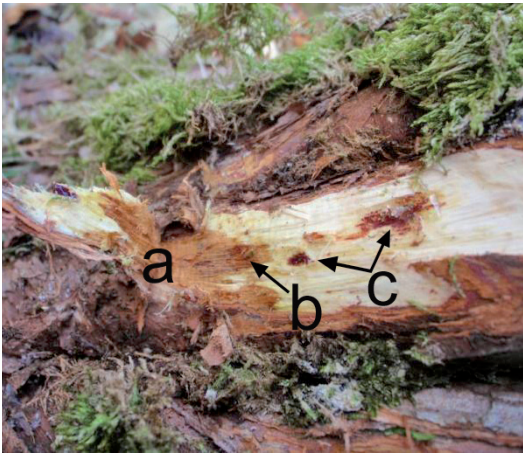
Fig. 1: Disease symptoms of *P. austrocedrae*

Diseases symptoms on *Austrocedrus chilensis*: Discolored needles (left) and dead trees (right) (1)

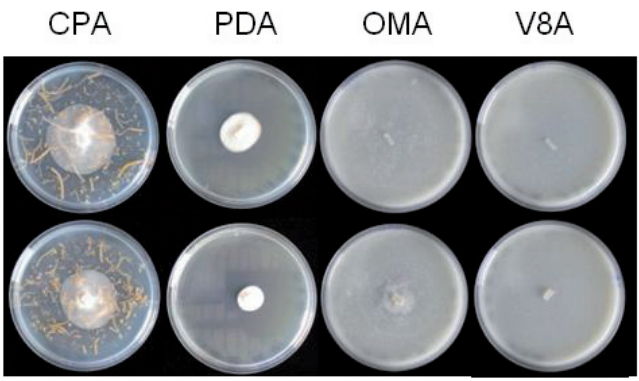
Cambium necrosis at the stem base of *Austrocedrus chilensis* (1)



A failing *Juniperus communis* (left) with light green foliage and a number of bronzed branches. A *P. austrocedrae* lesion on a *J. communis* trunk, note the cinnamon coloured lesion (a) with a yellow growing front (b) and the presence of resin pockets ahead of the lesion (c) (2)

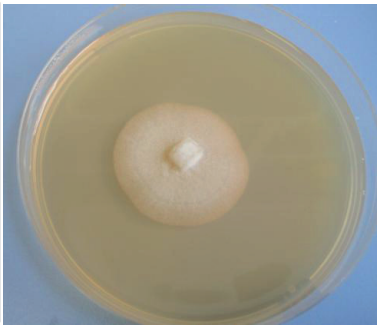
A lesion on *J. horizontalis* from a UK garden caused by *P. austrocedrae* (2)

Fig. 2: *Phytophthora austrocedrae*

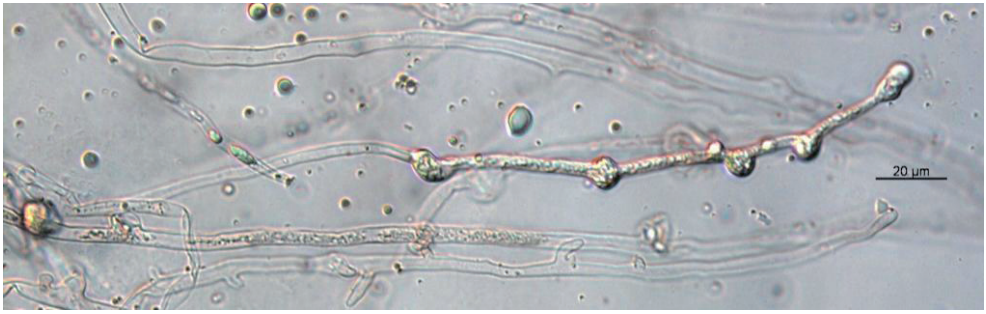


Colony pattern on different media (incubation for 18 d, 20°C) (3)
Upper row: *P. austrocedrae* JKI-018-09-00-00-00-00 (=PAU-09-001 = AG195)
Lower row: *P. austrocedrae* JKI-019-09-00-00-00-00 (=PAU-09-002 = AG203)

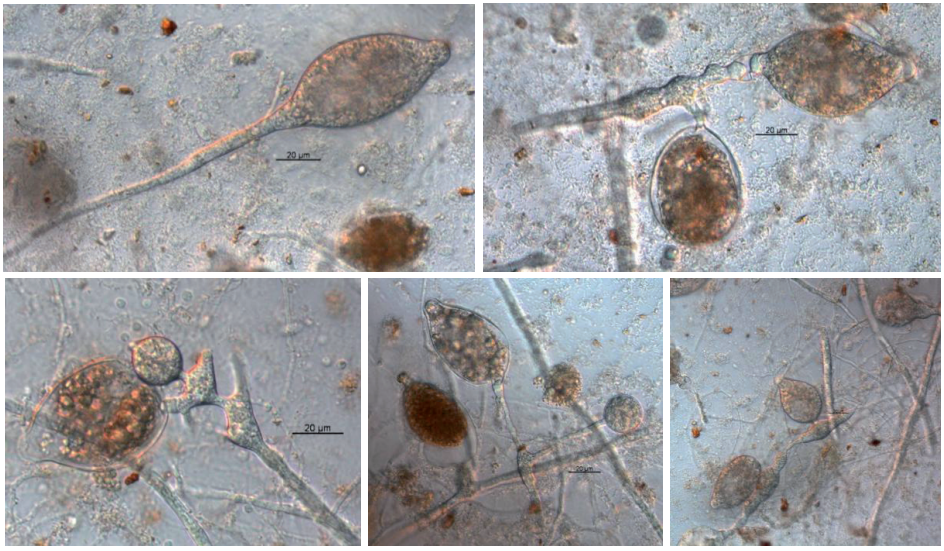
CPA = Carrot Piece Agar
PDA = Dextrose Potatoe Agar
OMA = Oat Meal Agar
V8A = Vegetable Juice Agar



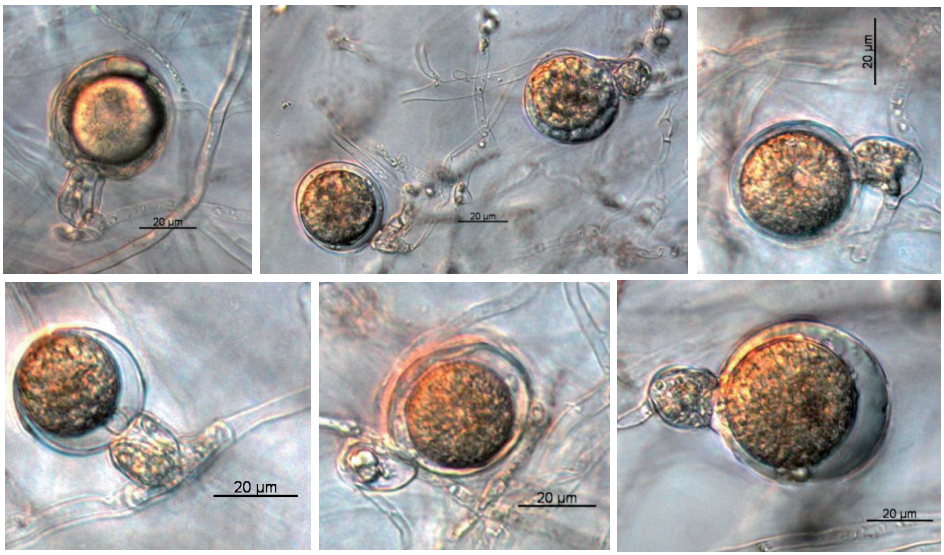
UK isolate TDJ3 growing on V8 media
(at 17.5°C after 3 weeks) (2)



Hyphae of *P. austrocedrae* JKI-018-09-00-00-00-00 (on Carrot Piece Agar) (3)



Sporangia and sporangiophores of *P. austrocedrae* JKI-18-09-00-00-00-00
(incubation in an unsterile extract prepared from white peat with addition of $CaCO_3$, pH ca. 4.0, 20°C) (3)



Oogonia, antheridia and oospores of *P. austrocedrae* JKI-19-09-00-00-00-00 (on Carrot Piece Agar) (3)

Photos: (1) = A. Greslebin, (2) = M. Elliot, (3) = S. Werres