Emerging Diseases in Europe
-
At the example of Sooty Bark Disease and Canker Stain Disease

Cryptostroma corticale – causal agent of the Sooty Bark Disease

History, biology, symptoms, diagnosis, current research

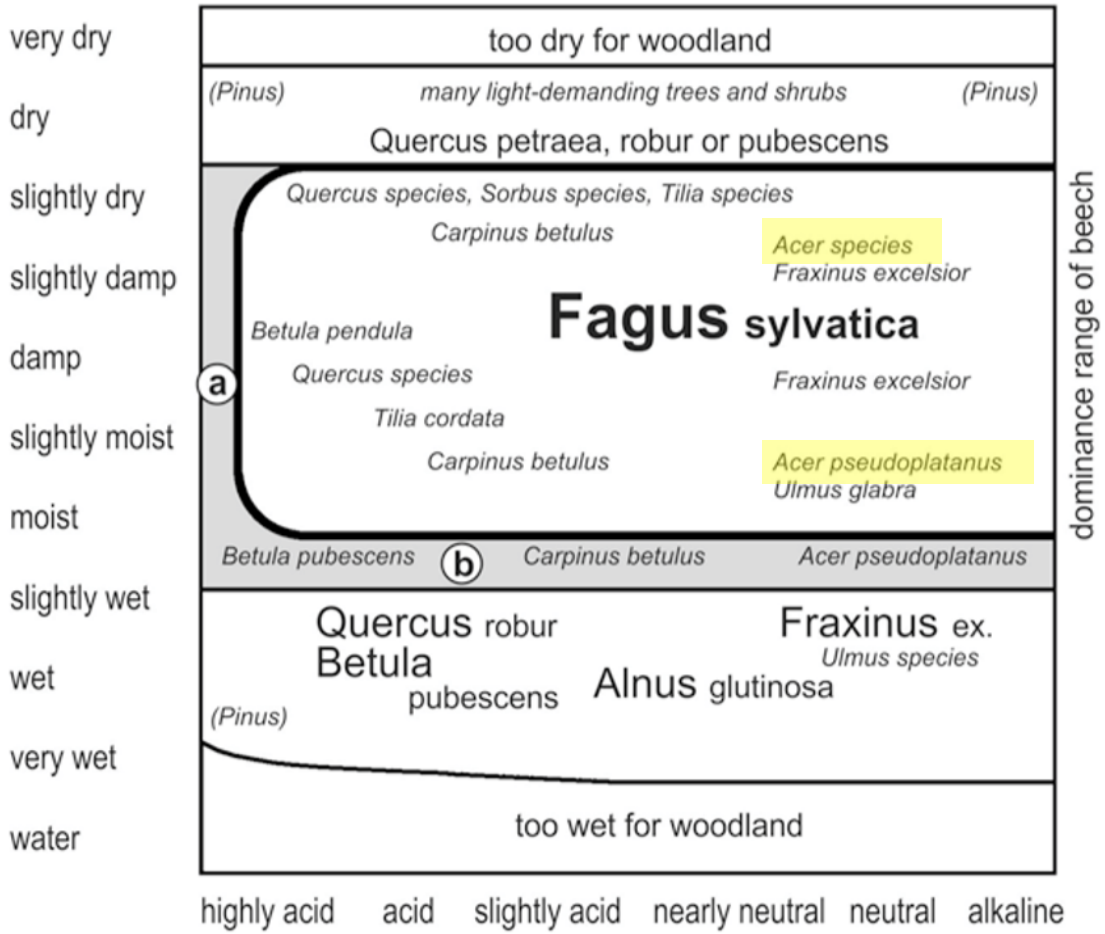
Acer pseudoplatanus L.

-grows on nutritious soil with good water availability

-valuable timber

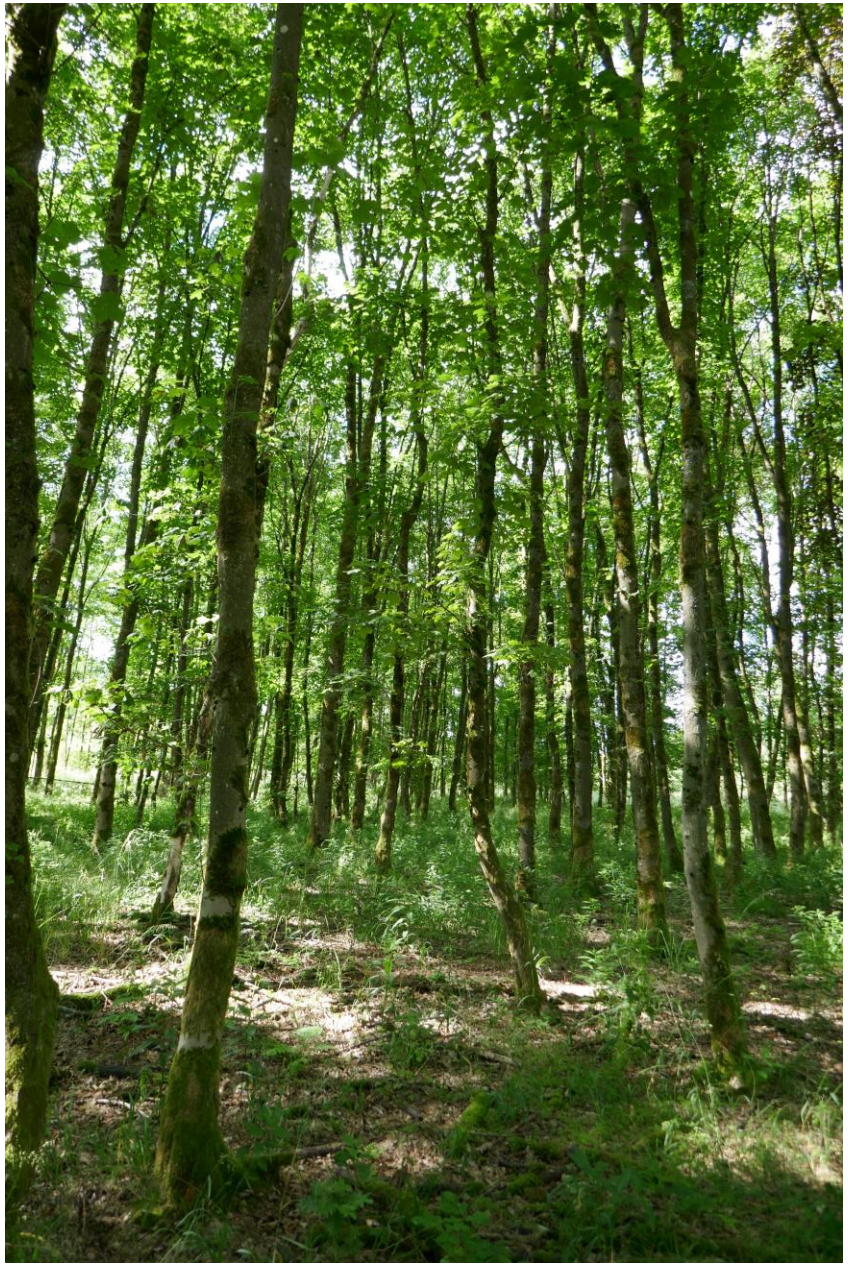
-often mixed with beech and European ash

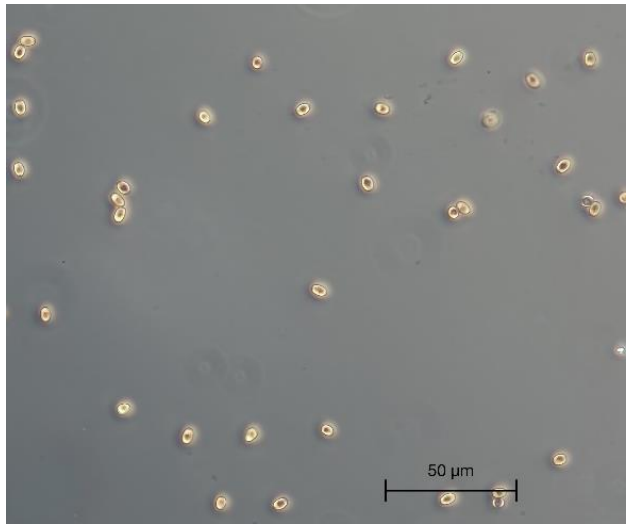
-used for *e.g.* building instruments

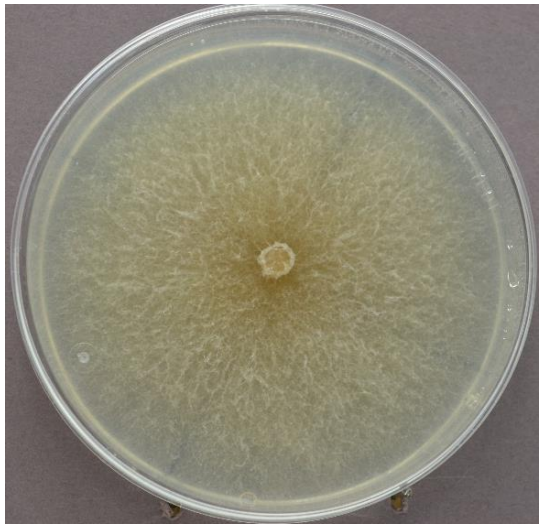


LEUSCHNER and ELLENBERG 2017 p. 186

Acer pseudoplatanus L.







History of *Cryptostroma corticale*

-first described from dead wood in 1889 as *Coniothyrium corticale* by Ellis and Everhard in Canada

-first report in Europe from Wanstead Park in London, UK in 1945 (Gregory and Waller 1951)

-renamed by Gregory and Waller in 1951

-belongs to the Ascomycota, Graphostromataceae, most likely to the Xylariales

-closely related to *Biscogniauxia* sp. and *Graphostroma* sp.

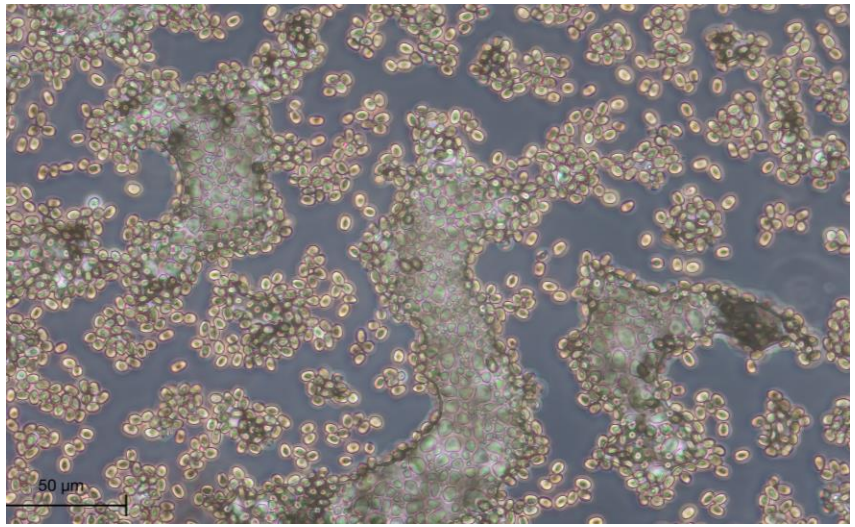
History of *Cryptostroma corticale*

-periodic but rare appearance of symptoms after exceptionally warm years

-relatively constant appearance in literature since 2003/2005

-a lot of new publications since 2020

-know to have a saprophytic, pathogenic and endophytic stage



Biology

-hyaline mycelium, first white, later turning brownish

-mycelium plug discolors dark after a week (differential diagnosis to *B. nummularia*)

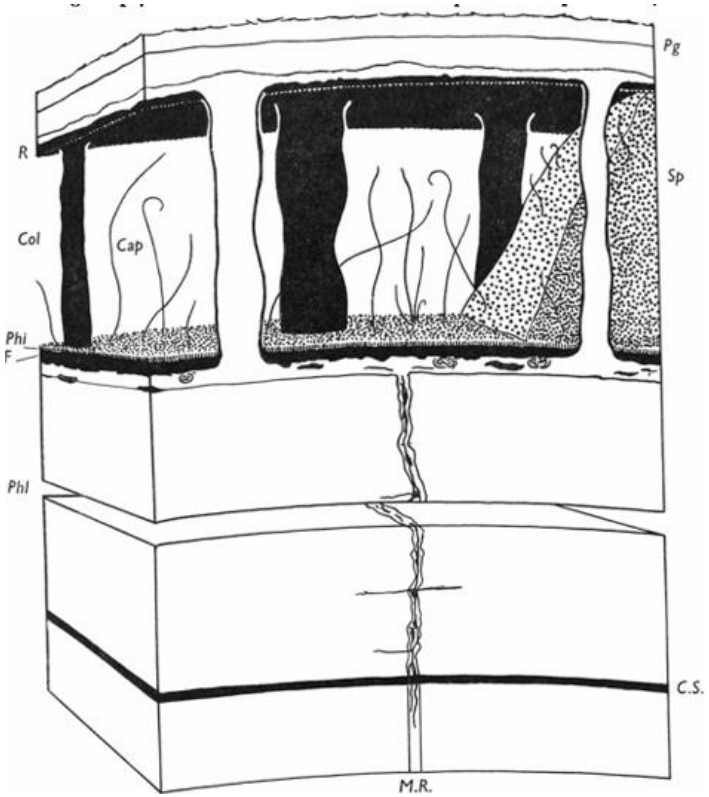
-spores

-ovoid

-dark due to melanin-inforced cell-wall,

-about 5 x 3.6 µm

Biology



Pg, phellogen; R, roof stroma; Col, columns; Cap, capillitium; Sp, spore mass; Phi, phialide layer; P, floor stroma; Phi, phloem ; C.S. cambial stroma; M .R. medullary ray.

Symptoms

-wilting / crown dieback

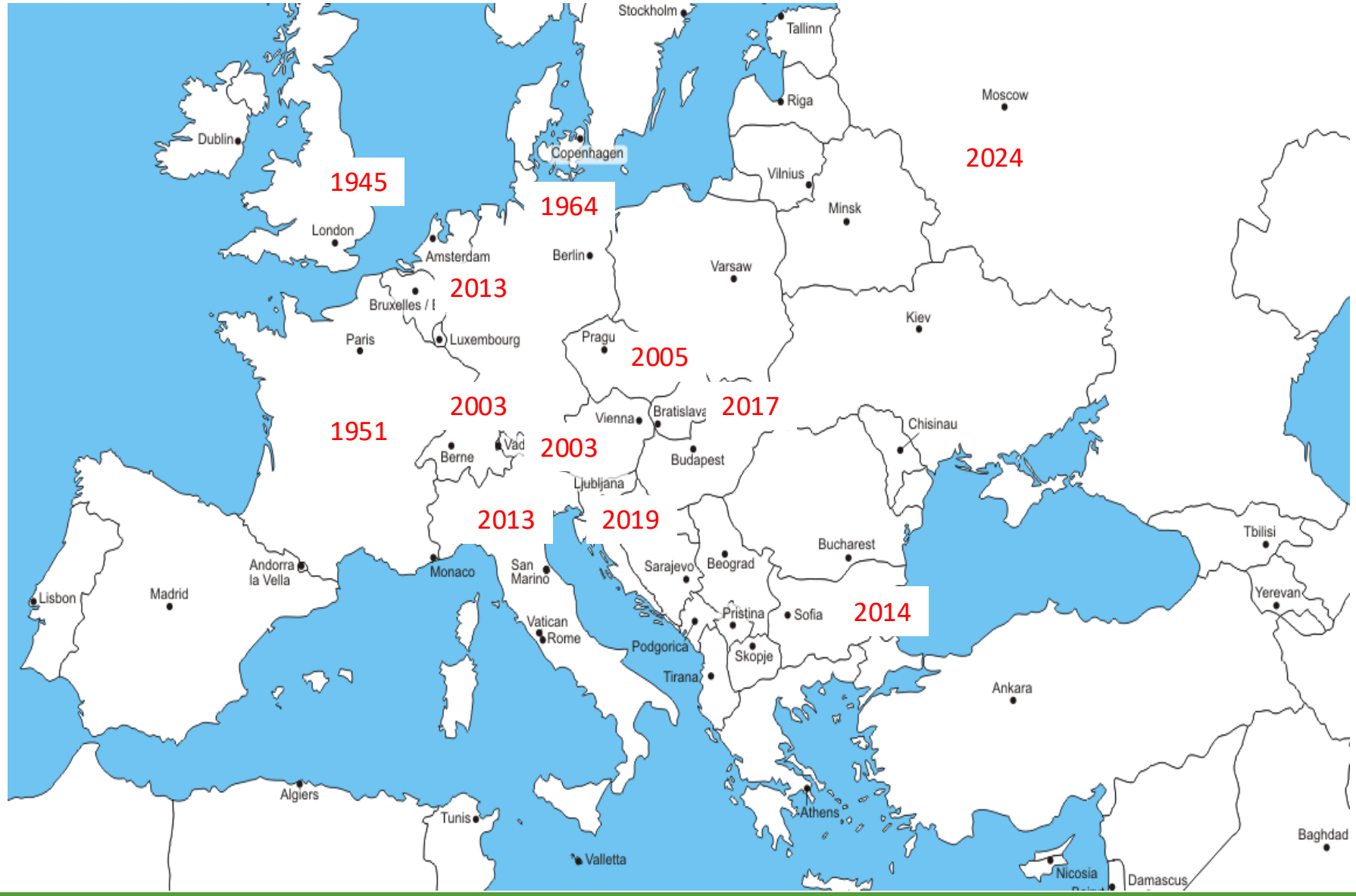
-discoloration of the wood

-blistering of the bark

-sporulation

Symptoms

First reports of SBD in Europe



Susceptibility of different *Acer* species based on Dickenson (1980)

TABLE 4 : Susceptibility of *Acer* spp. to infection by *C. corticale*.

Species	Stain	Reisolation	Confirmation[c]
EXPERIMENT 1[a]			
A. nigrum (1)	> 26.0	59	59
A. saccharum	19.0	21	20
A hervii	20.0	17	17
A. rubrum	8.5	8	3
A. negundo	15.0	7	7
A. palmatum	3.0	6	6
A. cappadocicum	8.0	6	4
A. saccharinum	6.5	5	5
A. japonicum	5.5	5	5
A. pseudoplatanus (1)	5.0	5	4
A. campestre	5.0	5	3
A. platanoides	3.0	3	1
A. palmatum dissectum atropurpureum	1.0	2	2

-infection of one living plant per species

-*A. nigrum* seems to be most susceptible
-even *A. saccherum*, which is from the natural host range, seems quite susceptible in this experiment

Sporulation tests of Dickenson (1980)

-inoculation of autoclaved wood blocks with *C. cortical*

-sporulation documented eventually (after 5 months latest) on all samples tested

-Acer campestre, *A. cappadoicum*, *A. hcrsii*, *A. japonicum*, *A. palmatum*, *A. rubrum*, *A. saccharinum*, *A. saccharum*

-Aesculus hippocastanum L. (horse chestnut)

-Euonymus europeaus

-Fagus sylvatica L. (common beech)

-Ligustrum vulgare

-Platanus x *hispanica*, *Platanus orientalis* L. (oriental plane)

-Populus nigra

-Prunus spinosa

-Quercus robur

-Salix caprea, *S. viminalis*,

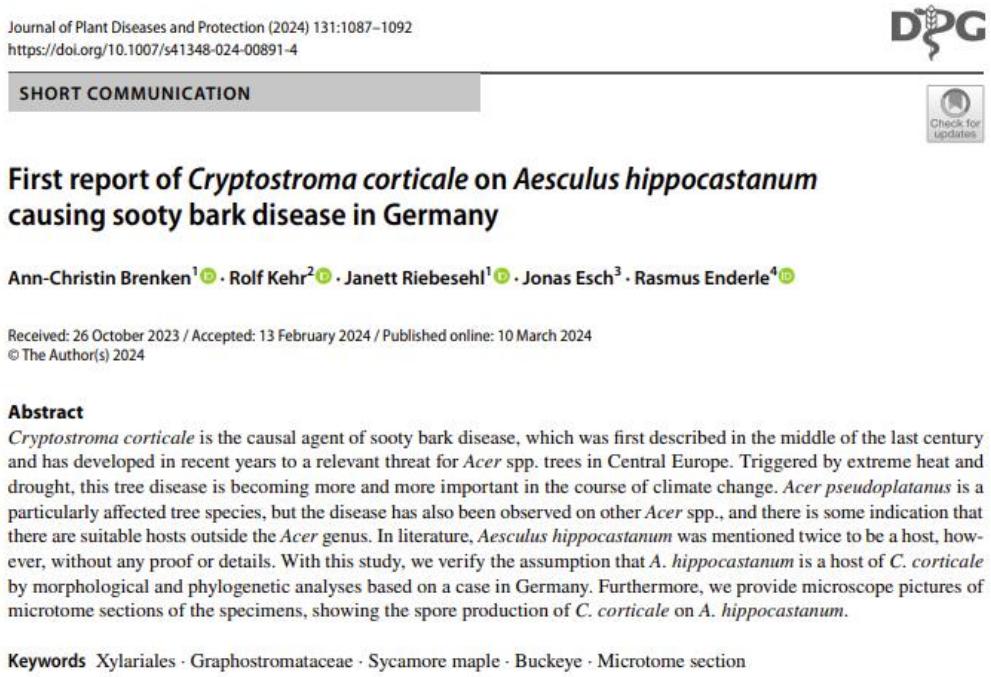
-Taxus baccata L. (couuuon yew)

-Tilia x *europaea* L. (common lime).

Reports from other tree species

-*Aesculus hippocastanum* in Germany by Brenken et al. (2024)

-Reported from *Tilia* and *Carya* in Wisconsin by Gregory and Waller (1951)



Journal of Plant Diseases and Protection (2024) 131:1087–1092
https://doi.org/10.1007/s41348-024-00891-4

SHORT COMMUNICATION

First report of *Cryptostroma corticale* on *Aesculus hippocastanum* causing sooty bark disease in Germany

Ann-Christin Brenken[1] · Rolf Kehr[2] · Janett Riebesehl[1] · Jonas Esch[3] · Rasmus Enderle[4]

Received: 26 October 2023 / Accepted: 13 February 2024 / Published online: 10 March 2024

Abstract
Cryptostroma corticale is the causal agent of sooty bark disease, which was first described in the middle of the last century and has developed in recent years to a relevant threat for *Acer* spp. trees in Central Europe. Triggered by extreme heat and drought, this tree disease is becoming more and more important in the course of climate change. *Acer pseudoplatanus* is a particularly affected tree species, but the disease has also been observed on other *Acer* spp., and there is some indication that there are suitable hosts outside the *Acer* genus. In literature, *Aesculus hippocastanum* was mentioned twice to be a host, however, without any proof or details. With this study, we verify the assumption that *A. hippocastanum* is a host of *C. corticale* by morphological and phylogenetic analyses based on a case in Germany. Furthermore, we provide microscope pictures of microtome sections of the specimens, showing the spore production of *C. corticale* on *A. hippocastanum*.

Keywords Xylariales · Graphostromataceae · Sycamore maple · Buckeye · Microtome section

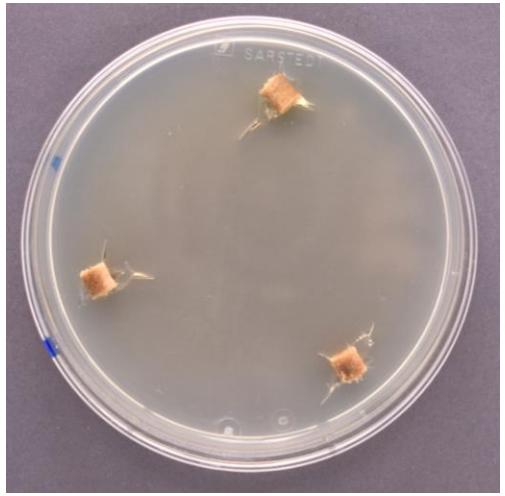
Methods of Diagnosis

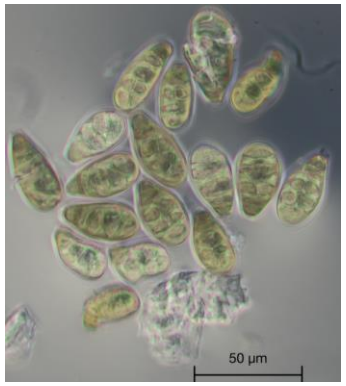
-examination under microscope

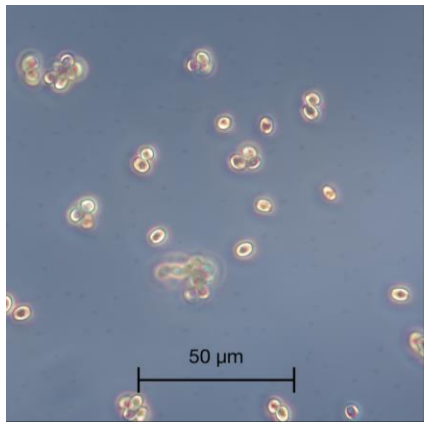
-plating out spore/woody material

-PCR of mycelium with subsequent BLASTn analysis

-qPCR with species specific primers from plant sample







Differential Diagnosis

-*C. Corticale* is easy to confuse with *Stegonsporium pyriforme* s.l.

-> both produce black spores on sycamore

-*C. corticale* produces a continous layer of spores underneath the bark

-*S. pyriforme* produces little black spots that break through the bark

Current research – storage of infected wood

stacks of wood with symptoms without cover

stacks of wood with symptoms covered with soil

stacks of wood with symptoms covered with foil

stacks of wood without SBD outbreak from diseased trees

Current research – storage of infected wood

packs of 3 logs with symptoms covered in foil

single logs with symptoms without cover

single logs covered with soil

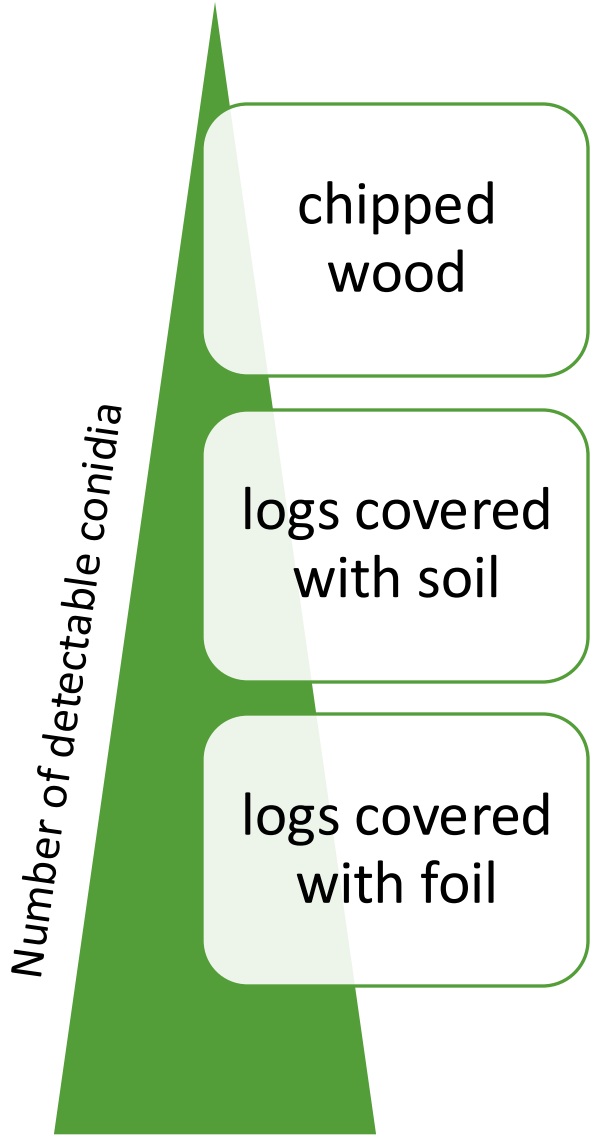
Current research – storage of infected wood

chipped wood dispersed in a larger area

heap of chipped wood

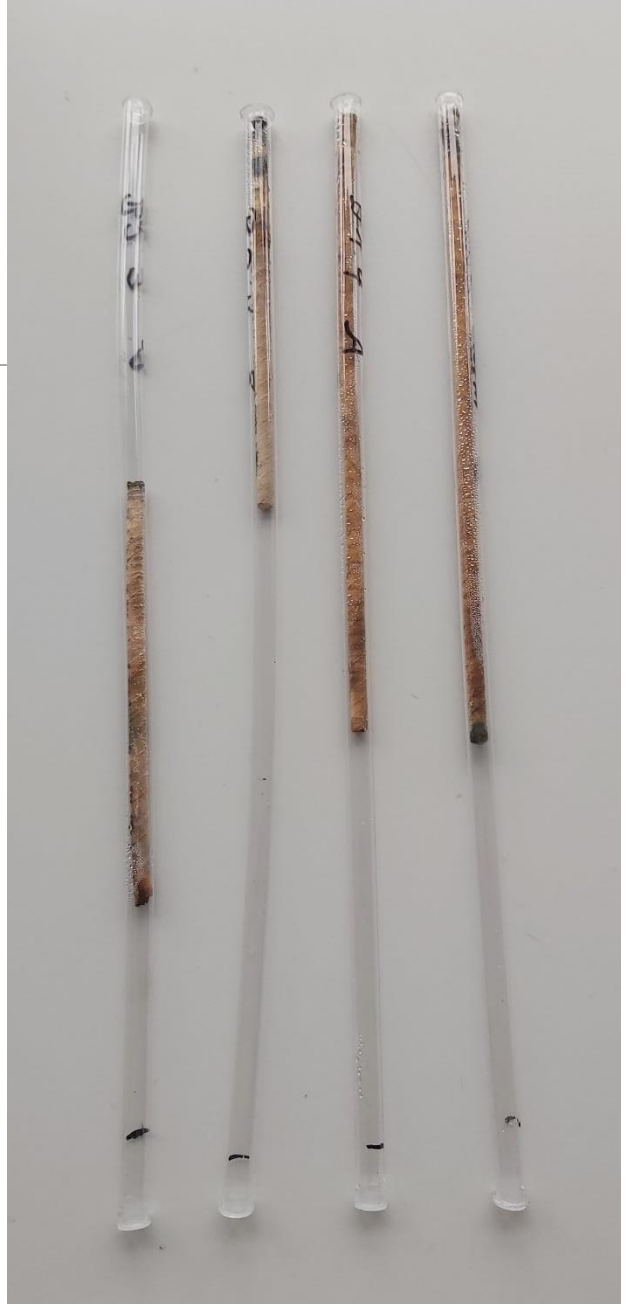
Persistence of the conidia

- study started in August 2020 and ended in September 2022
- at every sampling conidia could be found
- not all conidia found germinated, especially not from the shredded wood
- throughout sampling time fresh conidia could be found in different capacities every time
- in the beginning many conidia, over time less and less but always some to be found



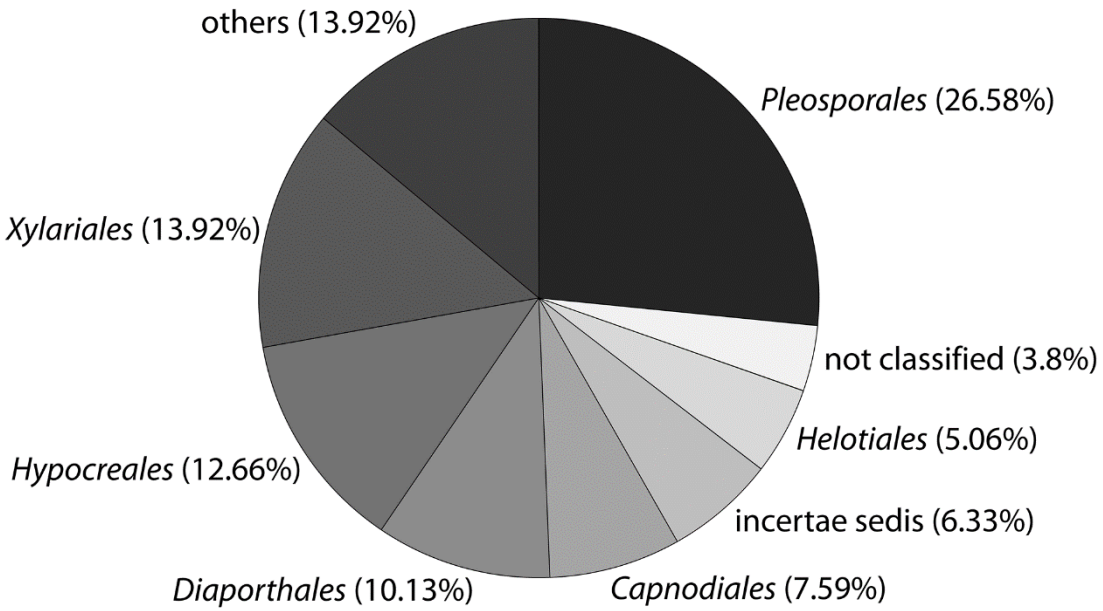
Current research – Analysis of fungal endophytes

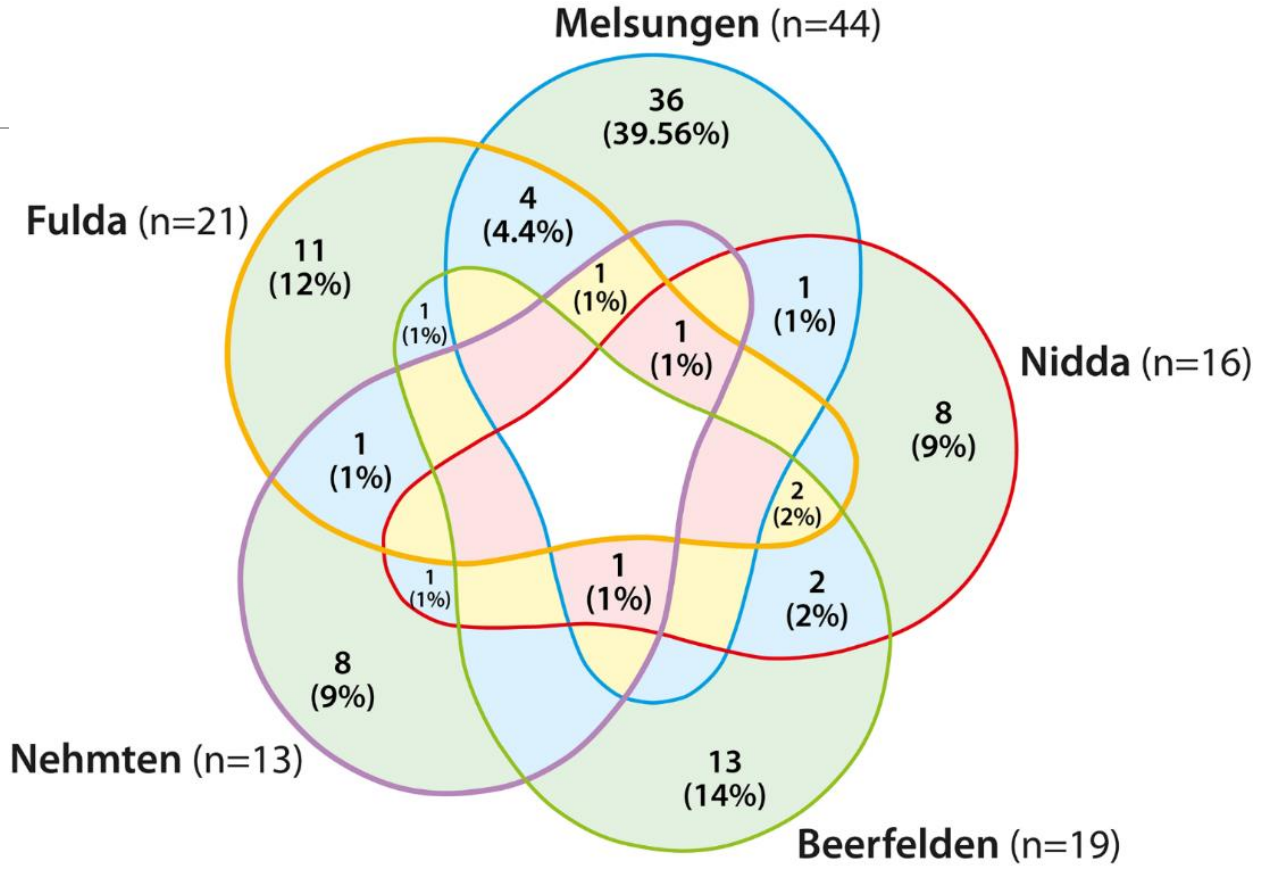
- sampling of visibly healthy trees
- determination of fungi through ITS
- *C. corticale* detected in visibly healthy trees
- in total nine Basidiomycota were found (out of 91 morphotypes)



Fungal endophytes from woody tissue of sycamore

Isolated orders of the Ascomycota
(n = 79 taxa)





n = number of isolated morphotypes;
only at one site, **morphotype at 2 sites**, **morphotype at 3 sites, morphotype at 4 sites**

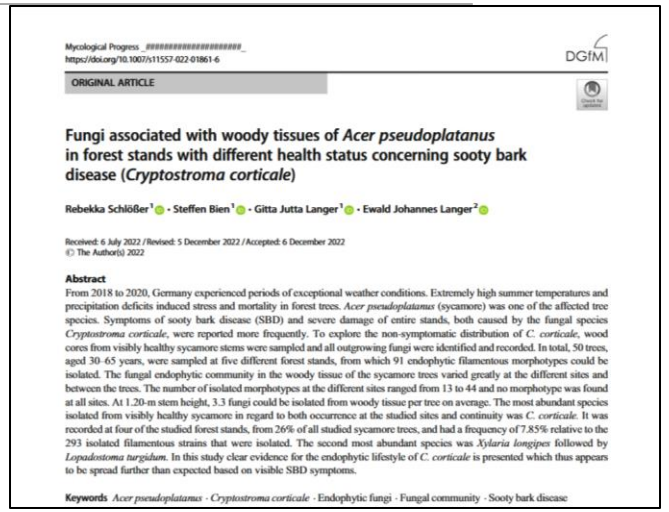
Most important results of the endophyte analysis

-fungal composition and number of isolated fungi varied distinctly between sites

-significantly more Ascomycota isolated than Basidiomycota

-isolates dominated by *Pleosporales* and *Xylariales* unlike described by Sieber (2007)[1]

-C. corticale isolated from site in Schleswig-Holstein



Mycological Progress _____
https://doi.org/10.1007/s11557-022-01861-6

DGfM

ORIGINAL ARTICLE

Fungi associated with woody tissues of *Acer pseudoplatanus* in forest stands with different health status concerning sooty bark disease (*Cryptostroma corticale*)

Rebekka Schlößer[1] · Steffen Bien[1] · Gitta Jutta Langer[1] · Ewald Johannes Langer[2]

Received: 6 July 2022 / Revised: 5 December 2022 / Accepted: 6 December 2022
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Abstract

From 2018 to 2020, Germany experienced periods of exceptional weather conditions. Extremely high summer temperatures and precipitation deficits induced stress and mortality in forest trees. *Acer pseudoplatanus* (sycamore) was one of the affected tree species. Symptoms of sooty bark disease (SBD) and severe damage of entire stands, both caused by the fungal species *Cryptostroma corticale*, were reported more frequently. To explore the non-symptomatic distribution of *C. corticale*, wood cores from visibly healthy sycamore stems were sampled and all outgrowing fungi were identified and recorded. In total, 50 trees, aged 30–65 years, were sampled at five different forest stands, from which 91 endophytic filamentous morphotypes could be isolated. The fungal endophytic community in the woody tissue of the sycamore trees varied greatly at the different sites and between the trees. The number of isolated morphotypes at the different sites ranged from 13 to 44 and no morphotype was found at all sites. At 1.20-m stem height, 3.3 fungi could be isolated from woody tissue per tree on average. The most abundant species isolated from visibly healthy sycamore in regard to both occurrence at the studied sites and continuity was *C. corticale*. It was recorded at four of the studied forest stands, from 26% of all studied sycamore trees, and had a frequency of 7.85% relative to the 293 isolated filamentous strains that were isolated. The second most abundant species was *Xylaria longipes* followed by *Lopadostoma turgidum*. In this study clear evidence for the endophytic lifestyle of *C. corticale* is presented which thus appears to be spread further than expected based on visible SBD symptoms.

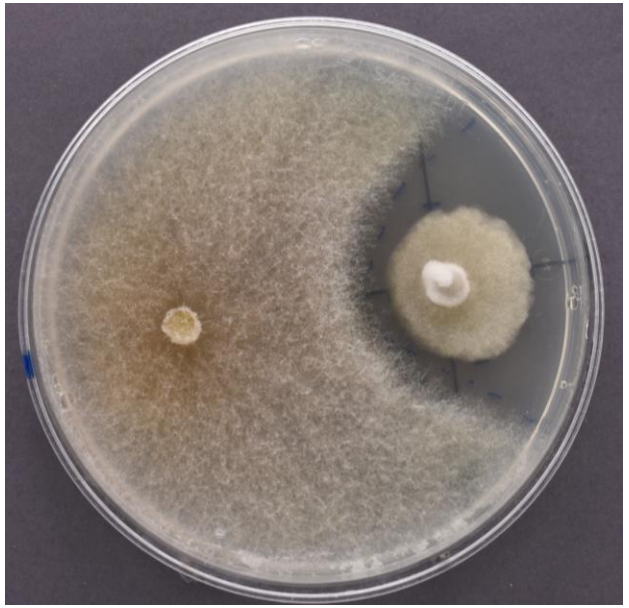
Keywords *Acer pseudoplatanus* · *Cryptostroma corticale* · Endophytic fungi · Fungal community · Sooty bark disease

SCHLÖßER et al. 2023, Mycological Progress

[1] SIEBER (2007) Endophytic fungi in forest trees: are they mutualists? Fungal Biol Rev 21:75–89. https://doi.org/10.1016/j.fbr.2007.05.004

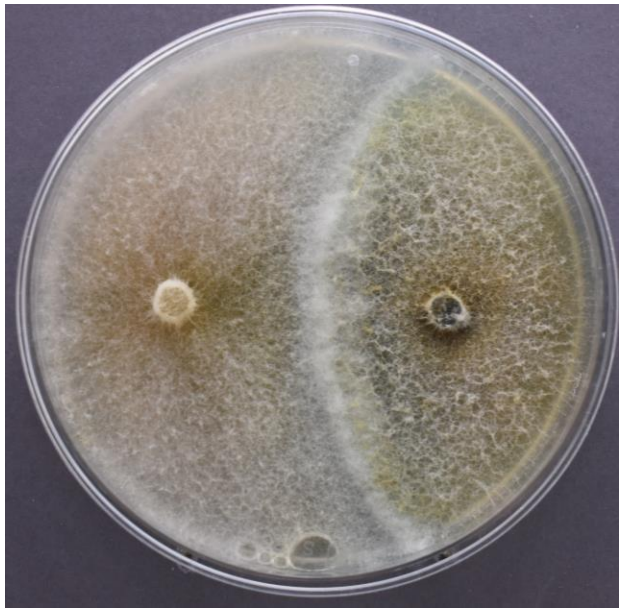
Current research

-search for a potential antagonist to be used as biological control agent in the future



Preussia cf. *aemulans*

Diaporthe pustulata



Arthrinium cf. *marii*

Schlößer et al. unpublished

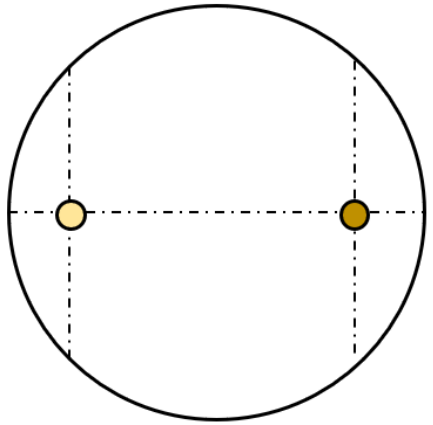
Current research

-five potential antagonists identified through different tests

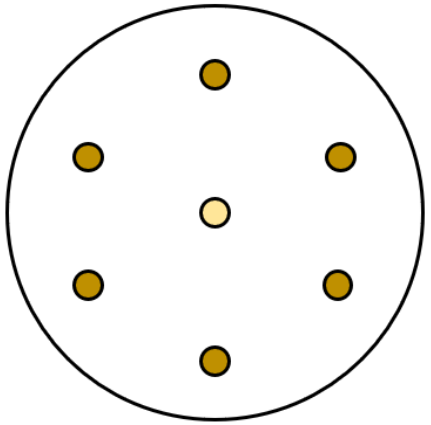
-results need to be followed by *in planta* experiments

-possibly a analysis of secondary metabolites might be beneficial

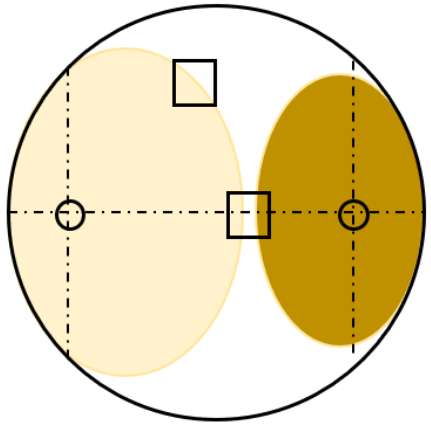
Dual-culture Antagonism assay



Growth challenge against six plugs



Test of vitality



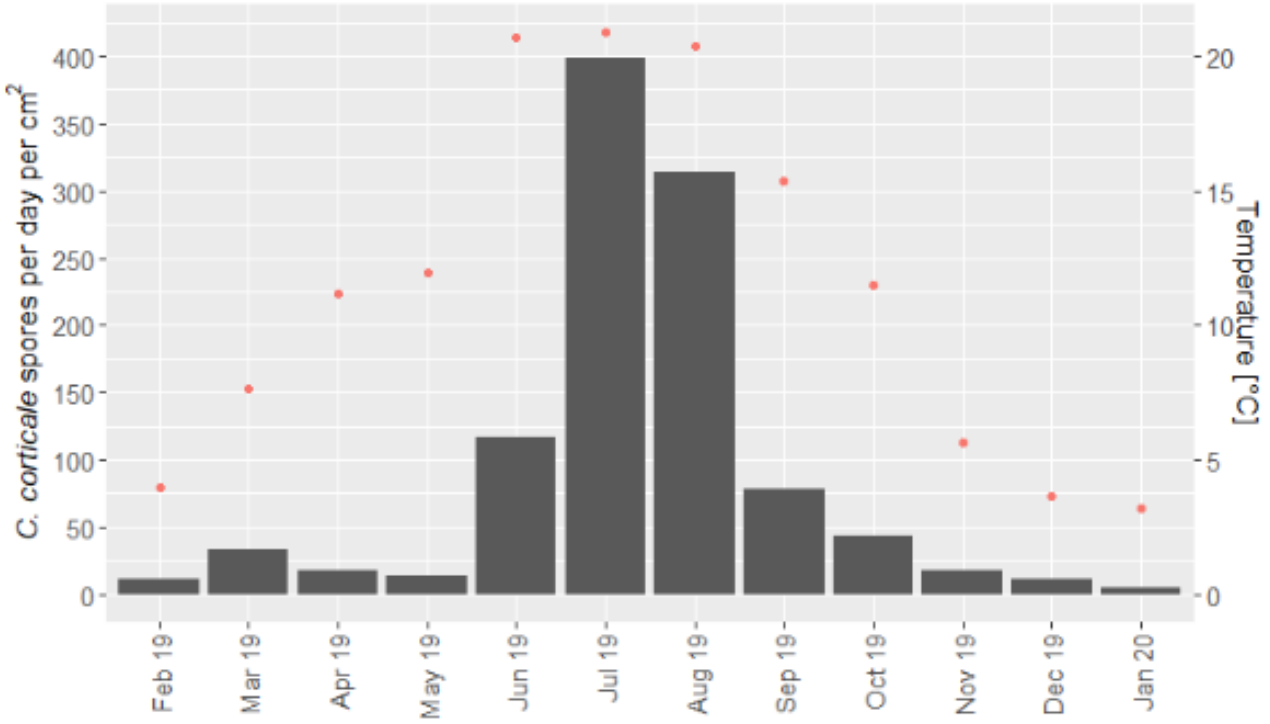


Figure 3. Seasonal abundance of spores of *C. corticale* per day per cm^2 (grey columns) from February 2019 to January 2020 in spore traps in the forest site close to Wuerzburg, Germany; n = 2–3; right y-axis: mean monthly temperatures 2019 in the weather station Wuerzburg (red dots) of the German Meteorological Service.

Current research

-spores can travel a distance of up to 300 km in the air (Muller et al. 2023)

-sporulation seems to be the strongest during the summer months (Burgdorf et al. 2022)

Current research

-recently a prick-test for testing for allergic reaction to *C. corticale* has been established

-at risk occupations can thus be tested beforehand to be better prepared and protected

› Pneumologie. 2024 Sep 3. doi: 10.1055/a-2369-8458. Online ahead of print.

[Diagnosis and Treatment of Hypersensitivity Pneumonitis – S2k Guideline of the German Respiratory Society and the German Society for Allergology and Clinical Immunology]

[Article in German]
Dirk Koschel [1 2 3], Jürgen Behr [4 5], Melanie Berger [6 7], Francesco Bonella [8], Okka Hamer [9 10], Marcus Joest [11], Danny Jonigk [5 12], Michael Kreuter [13], Gabriela Leuschner [4 5], Dennis Nowak [14], Monika Raulf [15], Beate Rehbock [16], Jens Schreiber [17], Helmut Sitter [18], Dirk Theegarten [19], Ulrich Costabel [8];
Deutsche Gesellschaft für Pneumologie und Beatmungsmedizin e.V. (DGP) (federführende Fachgesellschaft) undDeutsche Gesellschaft für Allergologie und klinische Immunologie e.V. (DGAKI)Deutsche Gesellschaft für Pathologie e.V. (DGP)Deutsche Gesellschaft für Arbeitsmedizin und Umweltmedizin e.V. (DGAUM)Deutsche Röntgengesellschaft e.V. (DRG)Bundesverband Deutscher Pathologen e.V. (BDP)

Affiliations + expand

PMID: 39227017 DOI: 10.1055/a-2369-8458

Abstract in English, German

Hypersensitivity pneumonitis (HP) is an immune-mediated interstitial lung disease (ILD) in sensitized individuals caused by a large variety of inhaled antigens. The clinical form of acute HP is often misdiagnosed, while the chronic form, especially the chronic fibrotic HP, is difficult to differentiate from other fibrotic ILDs. The present guideline for the diagnosis and treatment of HP replaces the former German recommendations for the diagnosis of HP from 2007 and is amended explicitly by the issue of the chronic fibrotic form, as well as by treatment recommendations for the first time. The evidence was discussed by a multidisciplinary committee of experts. Then, recommendations were formulated for twelve questions on important issues of diagnosis and treatment strategies. Recently published national and international guidelines for ILDs and HP were considered. Detailed background information on HP is useful for a deeper insight into HP and the handling of the guideline.

Protecion measures for workers (and citizens)

-protective clothing, including especially an appropriate mask during works

-felling of infected trees preferrably under wet conditions

-choosing seasons with lower sporecount (winter months)

-security parameter around the felling site to protect citizens

-avoid using wood from infected stands as firewood

Summary

-emerging pathogen

-already widely spread (newly to Russia and new reports from western America and Canada)

-hard to detect because of endophytic stage

-potentially broad host range, for now primarily on *A. pseudoplatanus* and *A. campestre*

-differences in spore pressure throughout seasons

Literature

Brenken A-C, Kehr R, Riebesehl J, et al (2024) First report of *Cryptostroma corticale* on *Aesculus hippocastanum* causing sooty bark disease in Germany. J Plant Dis Prot 131:1087–1092. https://doi.org/10.1007/s41348-024-00891-4

Brooks RK, Omdal D, Brown S, et al (2023) *Cryptostroma corticale*, the causal agent of sooty bark disease of maple, appears widespread in western Washington State, USA. Forest Pathology 53:. https://doi.org/10.1111/efp.12835

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Ceratocystis platani

–

Canker Stain Disease

HISTORY, SYMTOMS, QUARANTINE ORGANISMS, MONITORING, MANAGEMENT

Platanus orientalis L.

-natural distribution from Greece on eastwards

-found as an ornamental tree all over central Europe

-grows best on rich soils with a lot of light

-drought tolerant

Ceratocystis platani (J.M.Walter) Engelbr. & T.C.Harr.

-fungus of the Sordariomycetes

-wound pathogen causing tracheomycosis

-optimal growth temperature 25 °C

-asexual spores can survive for around 100 days in the soil

-spores not known to be airborne but can disperse through air by sawdust

History of the Canker Stain Disease

-*Ceratocystis platani* is native to North America

  -American sycamore: fairly resistant (co-evolution)

  -Oriental and London plane: very susceptible

-usually fatal within 1-5 years after infection

-unintentionally introduced by US army in Italy and France on infected plane wood packaging during WWII

-most likely one-event introduction

-dispersal via contaminated pruning tools, road construction machinery, rivers, roots

-quarantine pathogen

  -stringent and costly eradication measures are implemented

History of the Disease

- the disease is devastating in Greece, infecting very old oriental planes of great aesthetic value

-2011 first report from Istanbul, Turkey, confirmed in 2016

-2014 first report from Albania

-2016 Canal du midi (southern France, 260 km long and built in 1667), 42 000 plane trees, UNESCO world heritage site

-> more than 26 000 trees felled between 2006 and 2020

-several outbreaks in the northern parts of France (Nantes, Paris region) in 2019 and 2020

-potential spread to northern Europe not limited by climate

1972

1973

1978

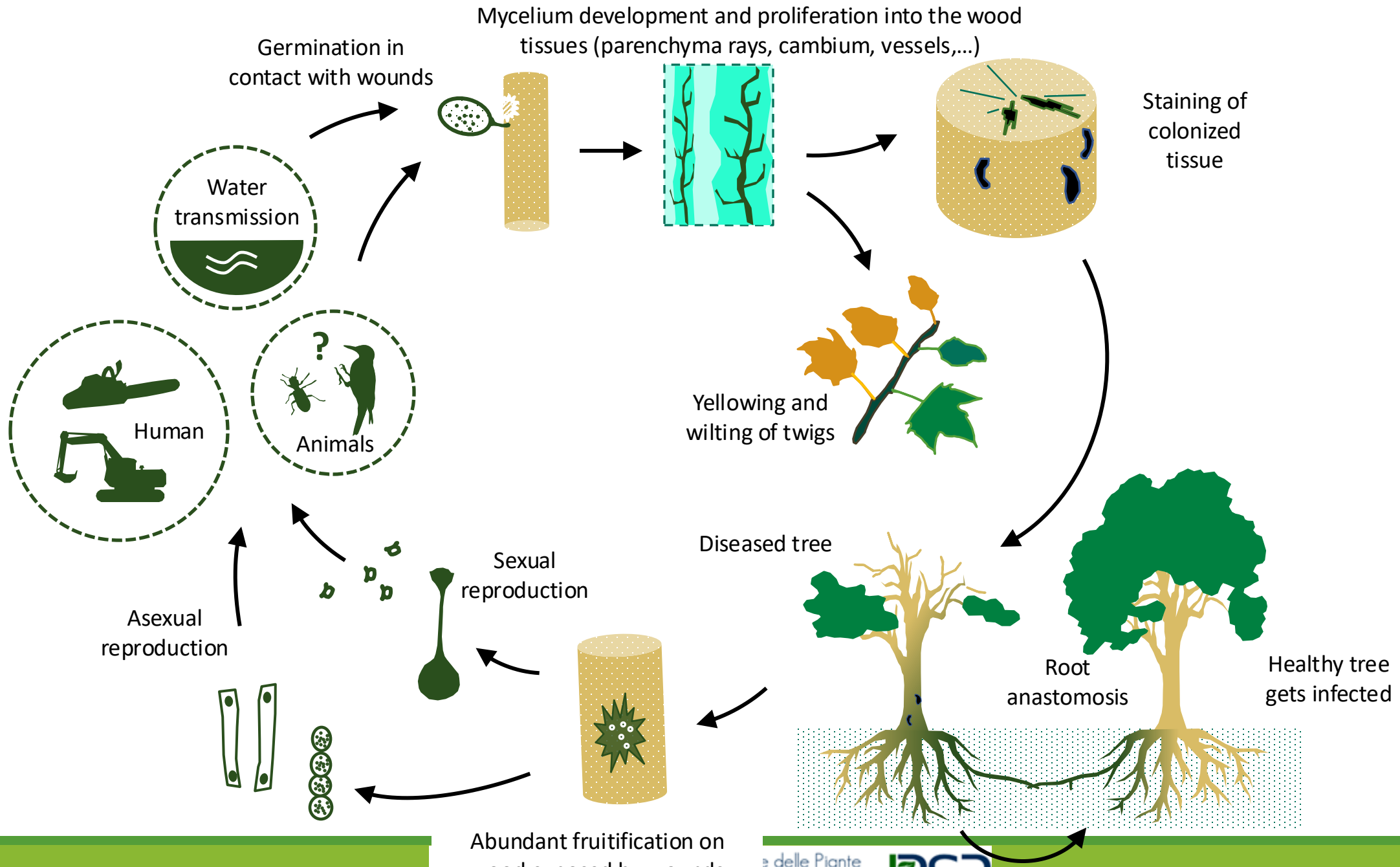
1983

1988

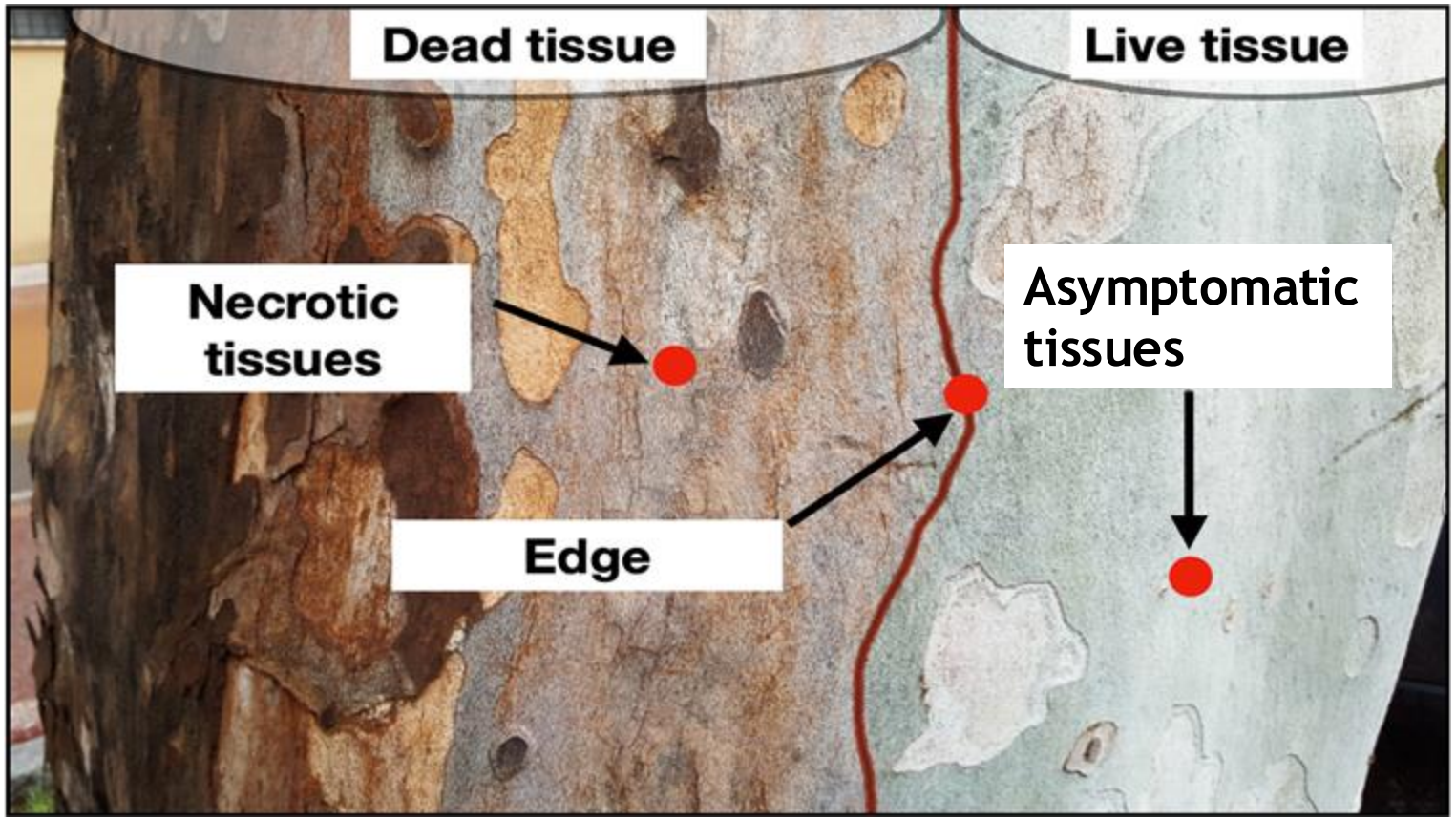
1993

1998

2003


Germination in contact with wounds
Mycelium development and proliferation into the wood tissues (parenchyma rays, cambium, vessels,…)
Staining of colonized tissue
Water transmission
Human
Animals
?
Yellowing and wilting of twigs
Diseased tree
Root anastomosis
Healthy tree gets infected
Sexual reproduction
Asexual reproduction
Abundant fruitification on wood exposed by wounds

Symptoms of CSD



Symptoms of CSD

CSD as a Quarantine Organism

What is a "Quarantine Organism"?

Quarantine organisms are plant pathogens that are not yet present in the EU, or are present but not widely distributed, and deemed by EU legislation as being harmful to plant health.

-> This results in frequent monitoring for already present pests and ones with high risk of introduction

-selective controls of 'dangerous goods' at entry points like ports and airports

-control of endangered areas (*e.g.* close to entry points)

CSD as a Quarantine Organism

-on EPPO A2 list since 2019

-lists of quarantine pests recommended for regulation by EPPO

-A1 list = pests absent from EPPO region

-A2 list = pests which are present in EPPO region

https://www.eppo.int/ACTIVITIES/quarantine_activities

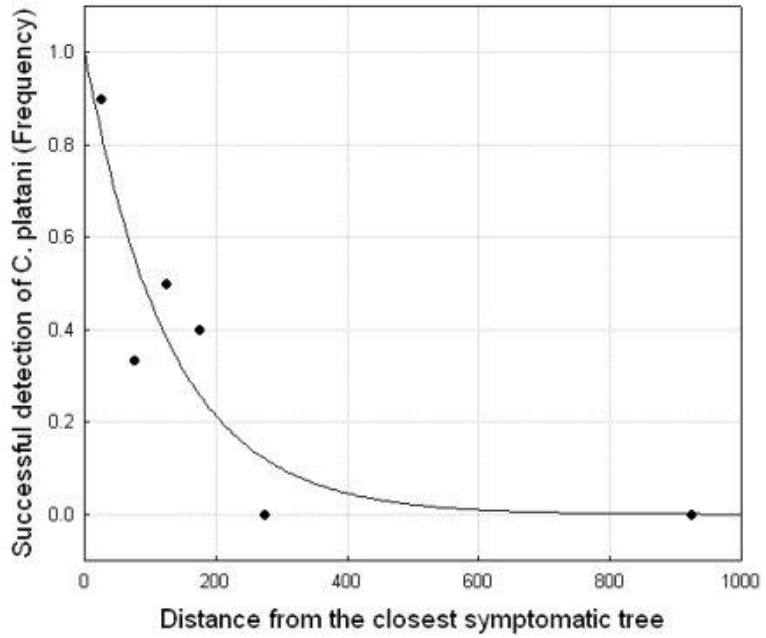
Monitoring based on EU regulation 2022/1629 and the pest-survey card

-annual risk based serveys for the presence of *C. platani* at potential infection points, preferrably between may and september

-serveys include: - visual examination
- sampling and testing of samples

-where infection is identified phytosanitary measures should be taken for erradication

Monitoring - spore traps installed during felling of diseased trees



-the inoculum dispersal during the sanitation cuts was evaluated less than 200 m from the closest symptomatic tree

Diagnosis

-baiting of the fungus

-use of assumably infected plant material

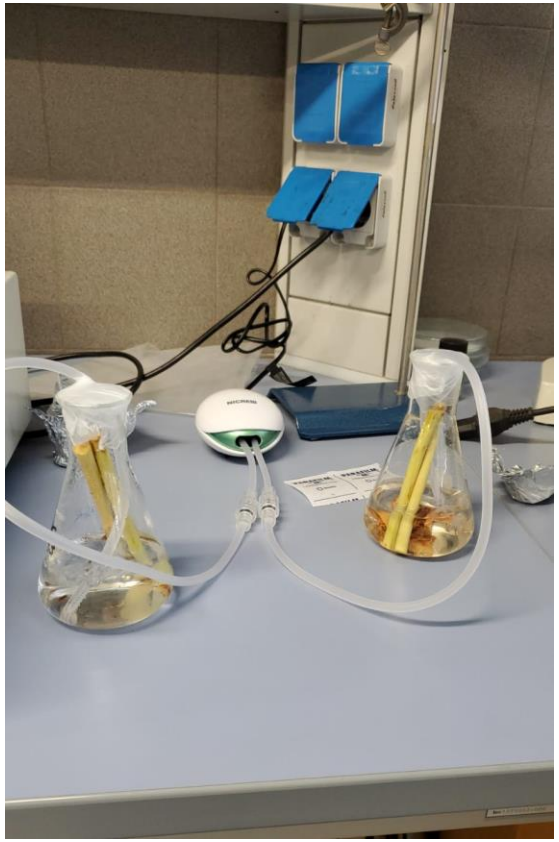
-asymptomatic plane tree sticks or carrot is placed in a dish or water with the piece of infected wood

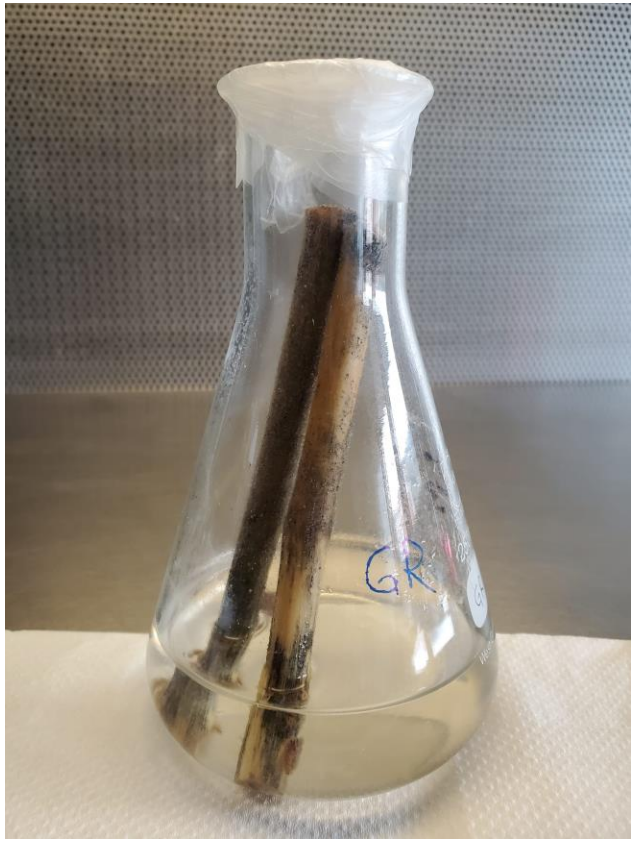
-water is aerilised by fish tank air pump

-after 2-3 days mycelium can be seen on the bait material

-molecular diagnostics (in the field)

Baiting





Diagnosis of *Ceratocystis platani*

Early detection of the disease

- Speed
- Specificity
- Sensitivity

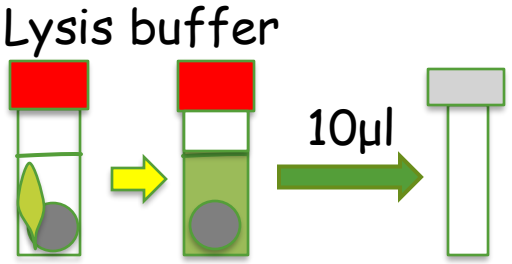
Classical isolation methods | **Molecular methods**

Imaged by Dr. P. Tsopelas)

1. Sampling

[1-3 minutes]

2. DNA Extraction



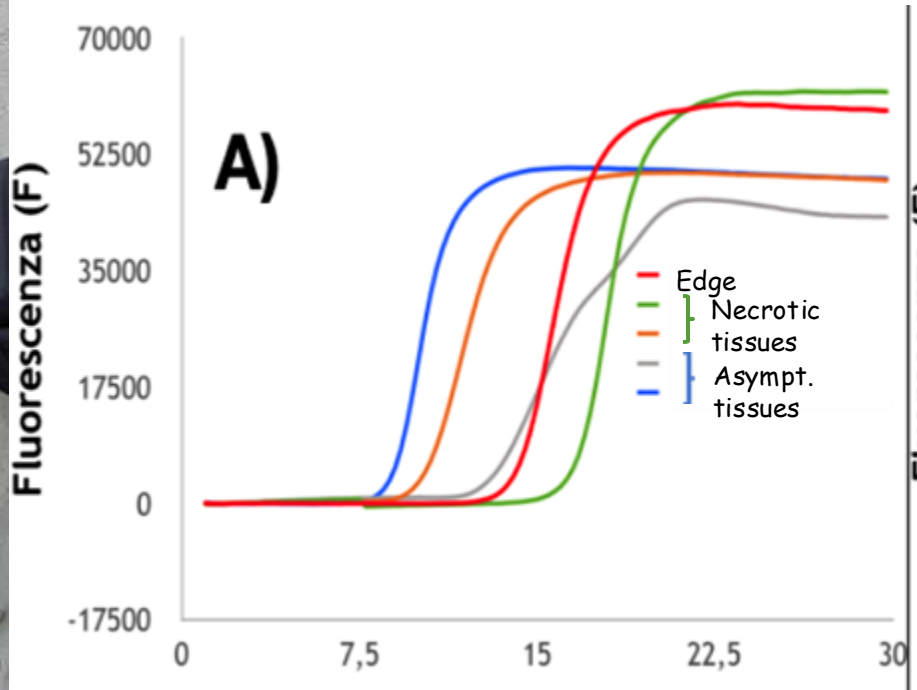
[2-3 minutes]

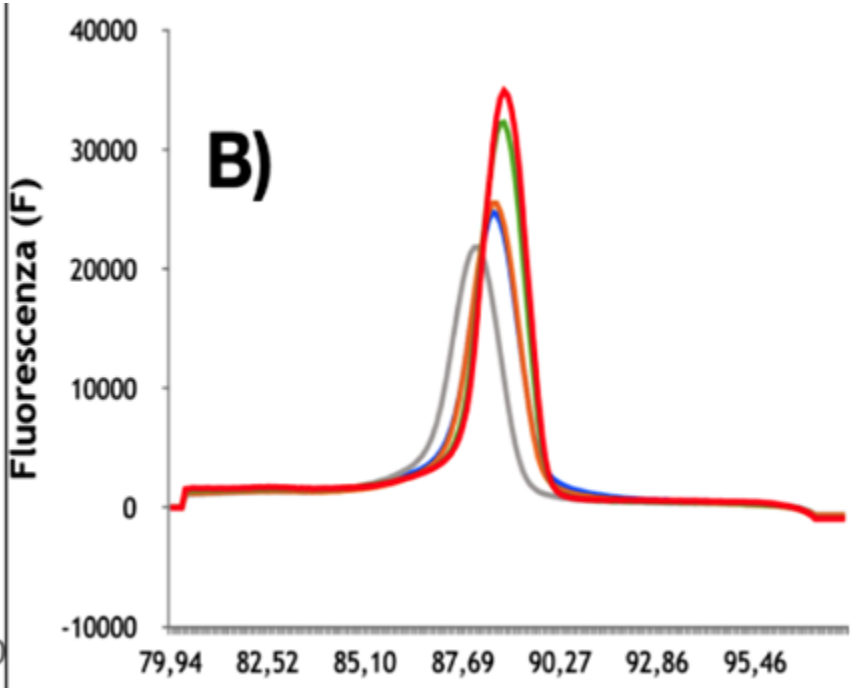
Elution buffer

3µl

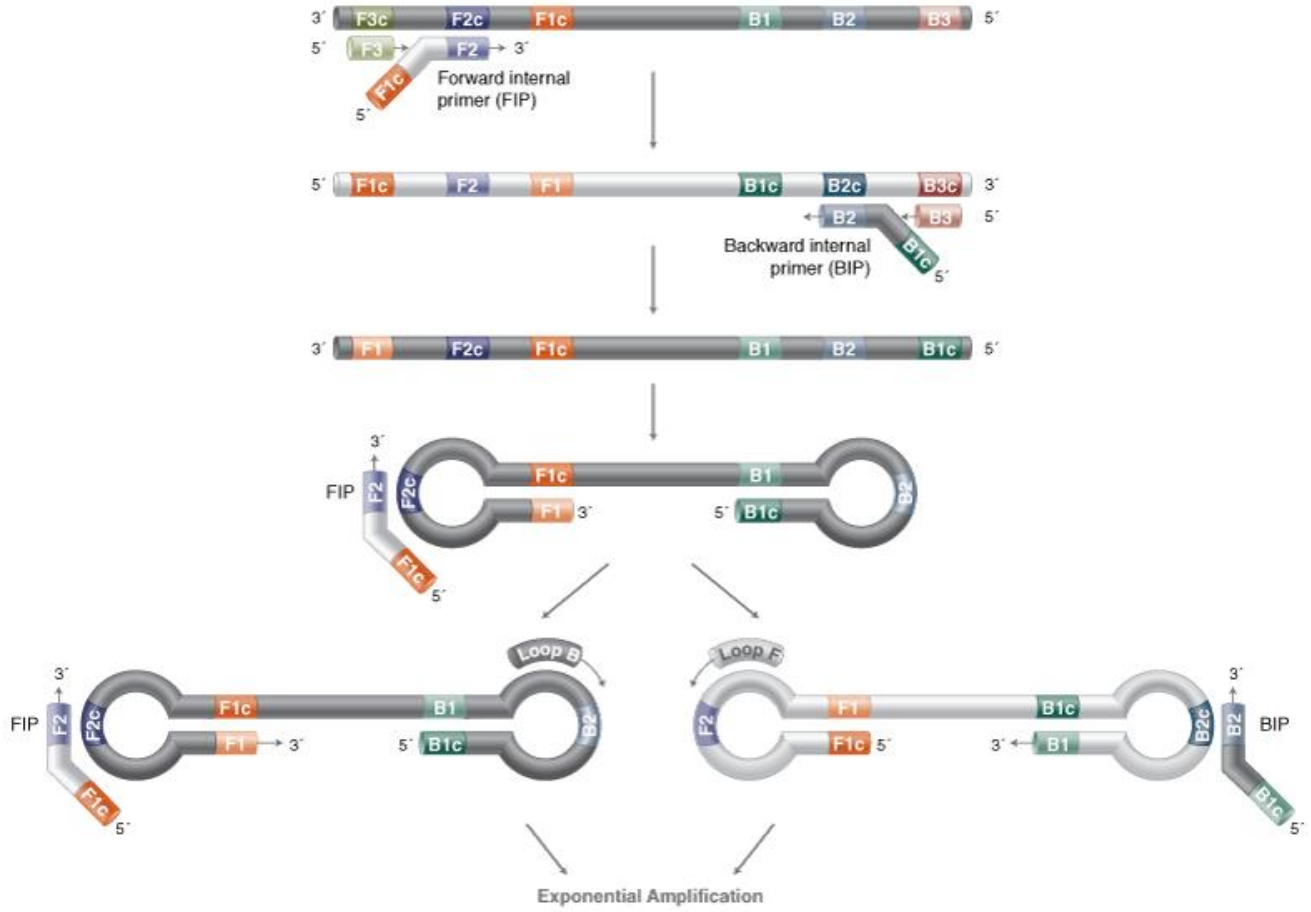
3. LAMP

[≈30 minutes] 16 samples





LAMP


F3c
F2c
F1c
B1
B2
B3
F3
F2
Forward internal primer (FIP)
F1c
B1c
B2c
B3c
Backward internal primer (BIP)
F1
FIP
Loop B
Loop F
BIP
Exponential Amplification

LAMP vs. classic PCR

Loop-Mediated Isothermal Amplified

-LAMP much faster in amplifying
  -isothermal = entire reaction works at one temperature

-4-6 primers needed

-2 primers bind to strand with a 5` overhang which is the reverse complement to the third primer

-these later bind together and you recieve a dumbbell structure that amplifies in a loop

-result shows whether specific target was in sample or not

-also possible to quantify amout of target in sample

-LAMP is less sensitive to inhibitors

Polymerase Chain Reaction

-PCR is based cycles with different temperatures

-2 primers needed

-sample must be clean

-primers usually not specific (in contrast to qPCR)

Management based on EU regulation 2022/1629

-establishment of buffer zone including infested area with a 1 km buffer

-removal of infected plants within infected zones

-applying appropriate measures to prevent spread

-plane wood cannot be moved outside the infected zone (other than to bring to appropriate treatment facility)

-not allowed to plant new plane trees in infected zone

-infected soil cannot be transported outside the zone, unless previously appropriately treatred

-pruning tools need to be cleaned and disinfected

-pruning wounds need to be treated to prohibit infection through the fresh wound

Management

-affected trees should be cut immediately

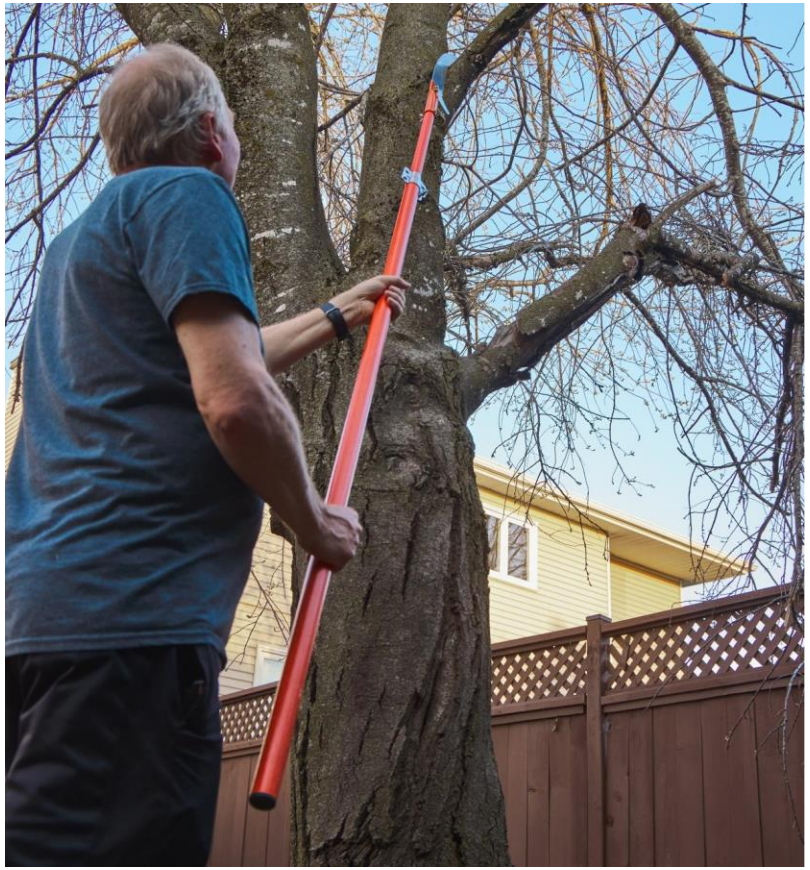
-at least the neighbouring trees should also be cut

-debris and sawdust should be sprayed with fungicide after

-soil around stem base should be removed

-monitor site to make sure not more trees are infected

Management



- spread mainly through human activity, also through roots

-source plants locally

-sterilize pruning tools after every tree

Interesting research – VOC in CSD

-four different *C. platani* cultures were tested

-six related species and five non-related species were also tested

-5 plant trees infected with *C. platani*, 5 as mock-control

-pure *C. platani* culture emitted mix of six specific VOCs, unique to this species

-same VOC were detected in infected plants as early as 4 dpi

-> possibility to detect early infection with CSD by VOC analysis of bark

Summary

-*C. platani* is an invasive species spreading further throughout Europe

-monitoring and management regulations by EU in place

-spread by water, root anastomosis and pruning tools

-possibility of early detection by VOC

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Thank you for your attenion!

Thank you to the SG3 of the department of Forest Protection of NW-FVA!

Thank you to the Forest Pathology group of the Institute of Sustainable Plant Protection of CNR!