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SHORT COMMUNICATION



The pine pathogen Diplodia sapinea is associated with the death of large Douglas fir trees

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Pseudotsuga menziesii (Douglas fir)

- Native to North America
- One of the most important non-native tree species in European forestry
- Promising alternative to native conifers
- High growth rates
- Drought-resistant
- Less affected by pests and pathogens • (but this may change over time)

Thomas et al. (2022)

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Diplodia sapinea (Syn. Sphaeropsis sapinea)

- Endophytic in symptomless healthy trees
- Isolated from many different broadleaf and conifer

SPECIES (Zlatkovic et al., 2017, Bußkamp et al., 2021)

- Becomes pathogenic in drought-stressed trees
- "Winner" of climate change (Blumenstein et al., 2021)
 - Spread to the North
 - Scots pine increasingly affected
 - Severe epidemics in many parts of Europe



© Landscape: Diplodia Blight | Center for Agriculture, Food, and the Environment at UMass Amherst

Diplodia sapinea (Syn. Sphaeropsis sapinea)

- Diverse symptoms on main hosts (*Pinus spp.*)
 - Shoot and crown dieback
 - Intensive resin flow •
 - Blue-staining of sapwood ۲
 - Death of trees •
 - Black pycnidia containing brown conidia ٠
- Differences in virulence among strains (Bußkamp et al., 2021)
- Host switch increases virulence

(Blumenstein et al., 2022)







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Background

- Previous observations in Austria (Steyrer et al., 2020)
- Observations of symptoms on large Douglas fir trees in Schönberg am Kamp in May 2022
- Unexpectedly no bark beetle infestation
- Two other Austrian sites in Eastern Austria:
 - Hafnerbach
 - Bad Sauerbrunn
- A total of 13 recently dead Douglas fir trees were examined
- What was the cause of the death of large Douglas fir?

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Material and Methods – Fungal isolation

- Fungal isolation from blue-stained sapwood
- Cultivation on 2 % malt extract agar (MEA), incl. streptomycin sulfate
- Identification based on
 - Colony morphology
 - DNA sequencing of ITS region
 - Diplodia sapinea-specific primers



Results – Field observations and isolations

- Symptoms (in 7 out of 13 Douglas fir trees)
 - Trees (dbh 21-41cm) died in 2022 (reddish foliage)
 - Intensive resin flow on the bole ٠
 - Blue-staining of the sapwood ۲
- *Diplodia sapinea* isolated from seven trees at three sites
- Isolation frequency 93% (141 out of 152 sapwood slivers)
- Identity of isolates (one per tree) confirmed by sequencing of ITS region and species-specific primers
- Six other dead trees affected by root rot, but no resin flow or blue-stained sapwood

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Inoculation Experiment

- Greenhouse experiment: duration 54 days
- Two-year-old seedlings of *Pinus sylvestris* and *Pseudotsuga menziesii*
- Ten individuals/ isolate/ tree species
- Random wound-inoculation of each seedling with
 - One of the seven isolates
 - Or control (sterile MEA)
 - Ten seedlings/ tree species remained untreated
- Re-isolation from inoculated trees





Results – Greenhouse experiment

- All *D. sapinea* inoculated seedlings developed symptoms
 - Browning of needles
 - Shoot dieback •
 - Phloem necrosis and blue-staining of sapwood
 - Death ullet
- Minor necrosis in control plants treated with MEA ●
- No symptoms in untreated plants lacksquare
- Re-isolation rate of D. sapinea: 96% (Ps. menziesii), • 94% (*P. sylvestris*); not isolated from control plants



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Results – Symptoms





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Results – Survival of seedlings

- First symptoms two weeks after inoculation
- First dead seedlings in both species 22 days after inoculation
- Faster disease progression in Douglas fir
- Survival after 54 days:

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- Ps. menziesii 20 % survival
- *P. sylvestris* 81 % survival
- Significant differences in virulence among isolates



50%

0

100%

75%

survival probabilities



Results – Growth rate lesion length and blue-staining

- Higher growth rates of lesion length and blue-staining in Douglas fir \bullet
- Some isolates also showed higher growth rates \bullet
- Growth rate: length of necrosis/ blue-staining divided by day of measurement (mm/day) \bullet



Growth rate (GR) of proximal bark lesion length

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Growth rate (GR) of blue-staining in the sapwood

Discussion

- Experimental evidence that *D. sapinea* kills large Douglas fir
 - Confirms previous observations (Steyrer et al., 2020)
- Novel serious threat to Douglas fir?
- High temperature and drought scenarios may increase severity
- Higher mortality on non-native host -> does host switch increase fungal virulence? (cf. Blumenstein et al. 2022)
- Higher risk in mixed stands with pines?
- Can *D. sapinea* occur as an endophyte in Douglas fir?





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