

# Investigating the infection biology and ecology of *Gymnopus fusipes*, a fungal root rot pathogen

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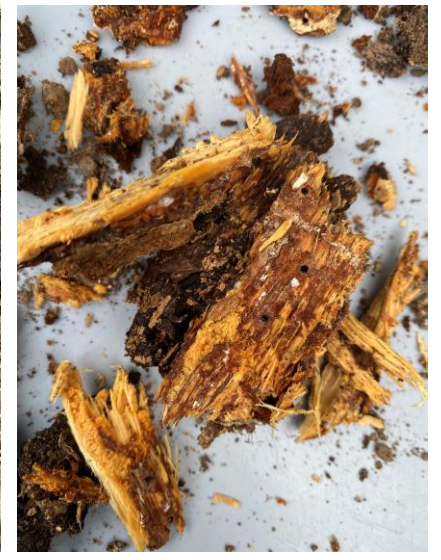


Forest Research



# *Gymnopus fusipes*

- AKA *Collybia fusipes* or Spindleshank
- Basidiomycete fungus
- Slow growing, primary pathogen
- Causal agent of *Collybia* root rot
- Linked to episodes of oak decline (UK and Europe)
- Numerous hosts (young and mature)
- **Above ground symptoms are not always correlated with status of below ground infection**



# A Systematic Review of the Literature

- Few focussed published works (96)
- Key knowledge gaps
- Infection biology and ecology
- Molecular information


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REVIEW ARTICLE

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## Using a systematic approach to synthesize existing knowledge on *Gymnopus fusipes* (syn. *Collybia fusipes*), the cause of *Collybia* root rot

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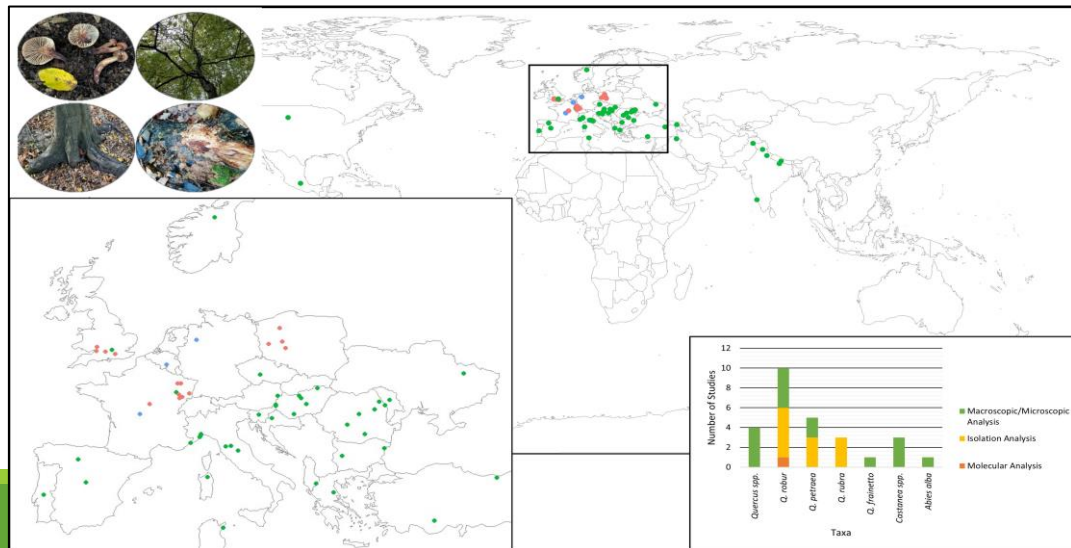
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### Abstract

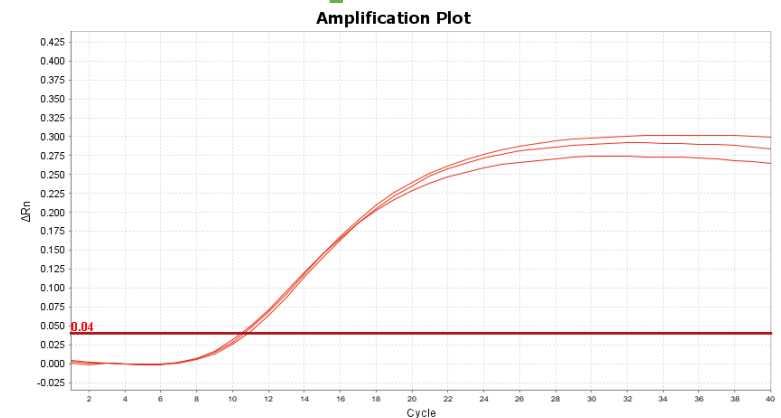
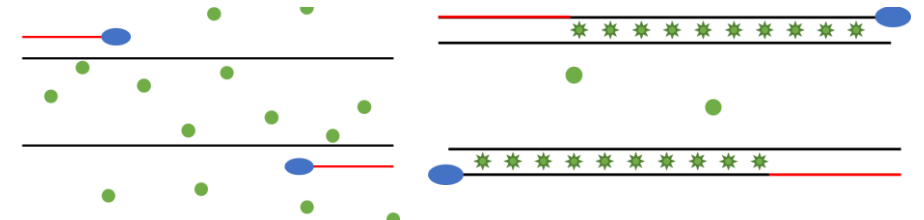
*Gymnopus fusipes* (syn. *Collybia fusipes*; syn. *Agaricus fusipes*) is an agaricomycete fungus known to cause root rot on a number of economically important tree species, including oak, where it has been linked to the development of chronic oak decline. Due to lack of correlation between above-ground decline symptoms and *G. fusipes* infection, its presence can often go undiagnosed until mortality. Although *G. fusipes* was first described over 200 years ago, there is still a paucity of information on the biology and ecology of this species, which represents a barrier to understanding its impacts on tree health. The aim of this review was to synthesize existing knowledge on the biology, ecology, host range and host interactions of *G. fusipes*. Using a systematic search, five online databases were used to obtain published literature resulting from the search terms '*Gymnopus fusipes*', '*Collybia fusipes*' and '*Agaricus fusipes*'.





# Developing a Rapid Diagnostic Tool

- *G. fusipes* specific quantitative PCR assay (qPCR)
  - Based on the fungal *18SrRNA* gene
  - Tested *in Silico* before being tested in the lab
- Measuring the accumulation of SYBR green fluorescence
- Thorough optimisation process to be highly specific
- **Positive results using pure cultures, fruiting body samples and woody tissue samples**



# Infection Biology and Mechanisms of Infection

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- Analyse *G. fusipes* infection at different stages
  - Early infection (Sapling infection trial)
  - Established infection (Field sampling)
  - Non-infective state (Plate cultures)



*G. fusipes* will express pathogenicity genes growing *in Planta* and will not when growing *in Vitro*

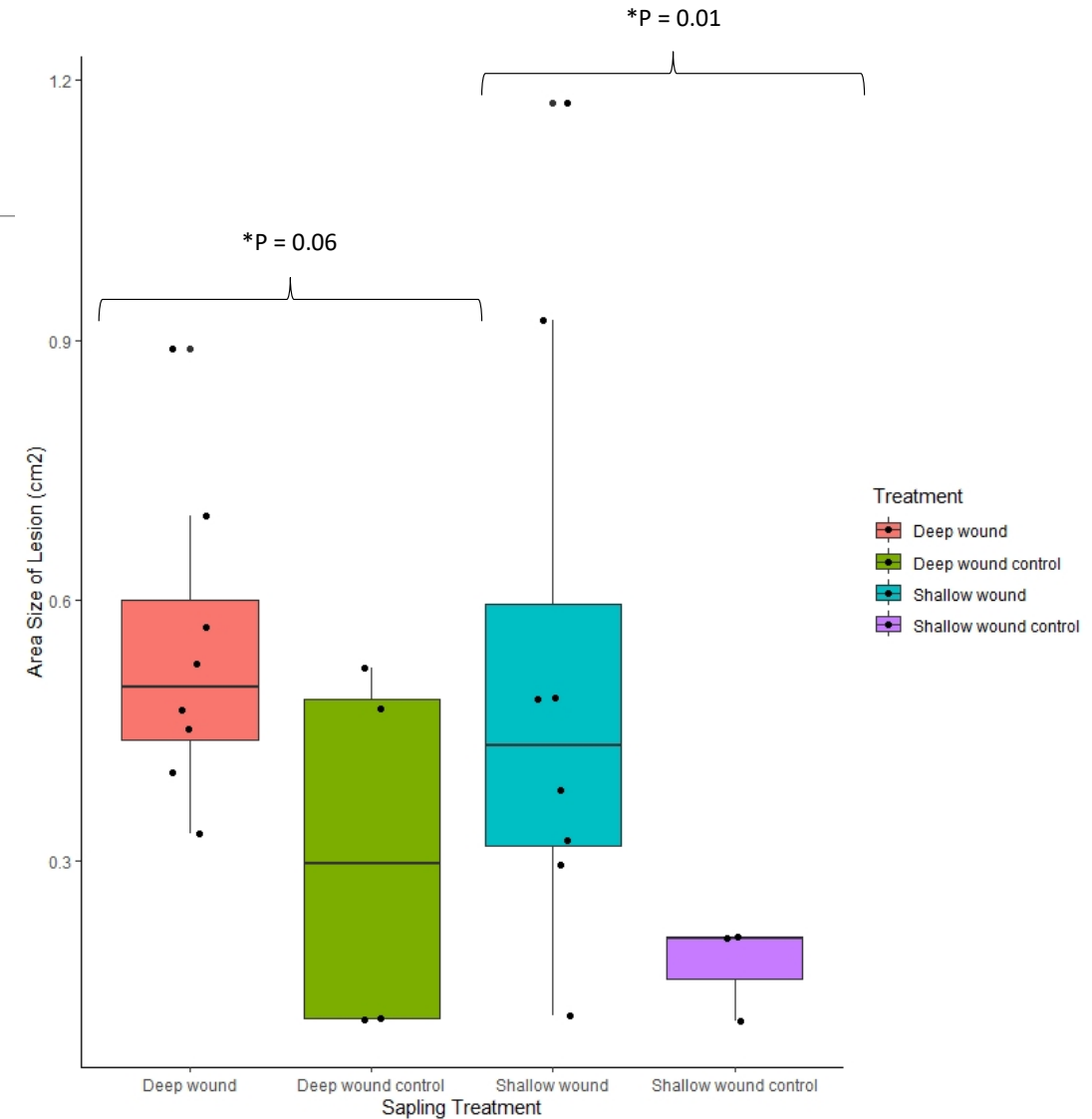
- **Aim to understand infection development at a molecular level**



*G. fusipes* transcriptomes will differ between an early infective state and an established infective state

# Sapling Infection Trial

- Saplings wounded (3 methods) and inoculated with *G. fusipes*
- Allowed to grow for ~ 6 months
- Lesion images analysed - similar to those observed in the field



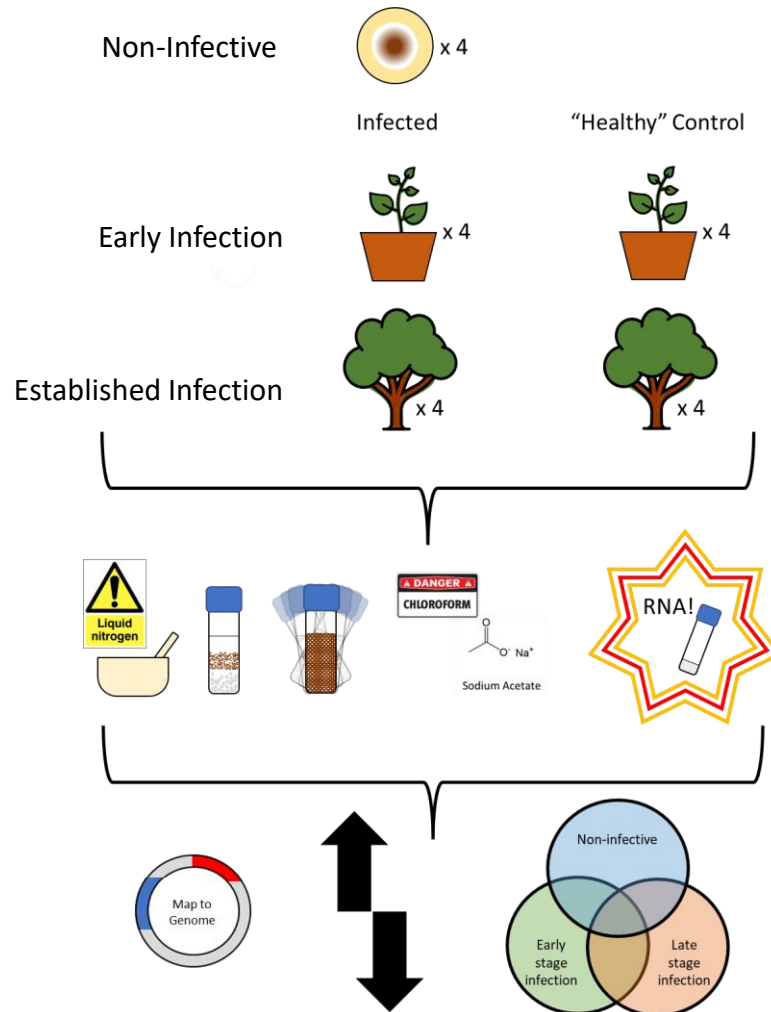


# Field Sampling of *G. fusipes*

- Heavily infected site in Hereford
- COD positive
- Abundance of *G. fusipes* fruiting bodies
  - Checked using the qPCR diagnostic
- 4 pairs of healthy/infected trees
- Lesions observed
- Samples of root tissue were taken for analysis



# Infection Biology with Gene Expression Analysis



- RNA-Seq Analysis (transcriptome/gene expression analysis)
  - RNA extracted from samples and sequenced
  - *Transcripts to be mapped to the *G. fusipes* genome*
- Sequencing the genome
  - DNA extracted from pure culture and sequenced (PacBio Hifi)
  - Assembly complete, *gene annotation in progress*
- Expectations
  - Early infection (sapling) – host ingress, overcoming defences, establishing infection
  - Established infection (field) – nutrient acquisition, propagation, reproduction



# Conclusions



## Systematic Literature Review

Lack of symptoms potentially led to a lack of research  
Incomplete and sometimes spurious data reporting  
Above ground symptoms do not always correlate with below ground status  
Could result in disruption to ecosystem services, damage to habitats and be a H&S risk



## Diagnostic Assay Development

*G. fusipes* specific  
Highly optimised  
Positive results for plate cultures, fruiting bodies and infected woody tissues  
Whole process takes < 48 hours



## Differential Gene Expression Analysis

Successful infection trial with lesions being similar to those in the field  
First instance of genome sequencing for *G. fusipes*  
First instance of RNA-seq for *G. fusipes*  
***Analysis to be finished for full RNA-seq data***

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