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The incidence, pathogenicity and management of raspberry *Phytophthora* root rot in the UK

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The raspberry root rot complex

Root rot of the European red raspberry (*Rubus idaeus*), caused by a consortium of *Phytophthora* and other oomycete species, is a recurring and destructive disease of this commodity fruit. Through large scale surveying of UK grower sites, direct isolation from symptomatic plants and subsequent disease studies, this project has shown more diverse Peronosporales species associated with raspberry root rot in the UK than previously thought.

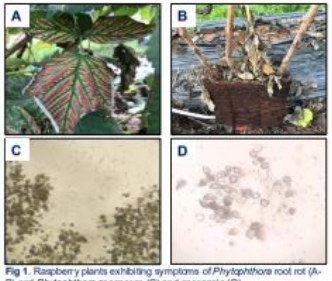

Fig 1. Raspberry plants exhibiting symptoms of *Phytophthora* root rot (A-B) and *Phytophthora* zoospores (C) and sporangia (D)

Species identification workflow

1. DNA extraction from 186 diseased tissue samples
2. PCR with *Phytophthora*-specific primers and ITS 4&5
3. Sanger sequencing and metagenomic amplicon sequencing for species identification via Illumina metabarcoding
4. Determine the influence of location, growing techniques, variety on *Phytophthora* species abundance and diversity

Species present on selected UK grower farms

Species	Reported on *Rubus*	Reported in the UK
Phytophthora citrophthora	✓	✓
Phytophthora plurivora	AHDB SF198 report only	✓
Phytophthora idaei	✓	✓
Phytophthora bisheria	✓	✓
Phytophthora citricola	✓	✓
Phytophthora rubi	✓	✓
Phytophthora pseudocryptogea	✓	✓
Phytophthora hedriandra	X	✓
Phytophthora meadii	X	X
Phytophthora ilicis	X	✓
Phytophthora nicotinaea	X	X
Phytopythium litorale	X	✓
Phytopythium vexans	X	✓

Pathogenicity testing - detached leaf assay

Sterile raspberry leaves were floated on 100 mL nonsterile soil extract infested with Peronosporales species. After 7 days, leaf area, lesion area and percentage disease were recorded via APS Assess 2.0 software. Koch postulates were confirmed for all species.

Fig 2. Leaf assay (A) and example of leaf lesions (B)

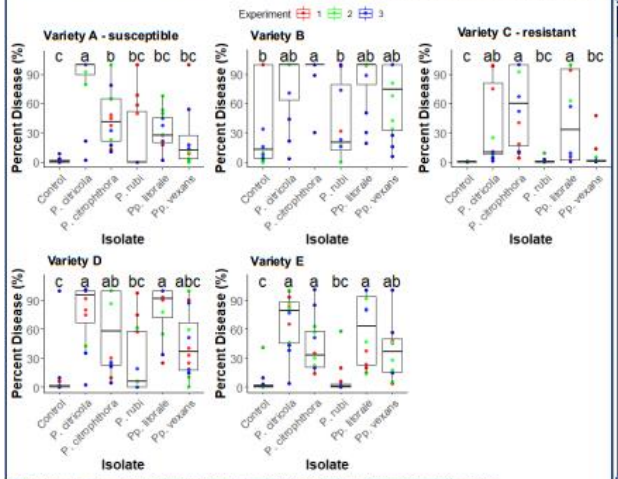

Fig 3. Leaf pathogenicity assay of five raspberry varieties floated on soil infested with one of five Peronosporales species (n=12).

Pathogenicity testing – whole plant assay (ongoing)

To investigate if these species could infect whole plants, 4-month-old primocanes of the same five raspberry varieties were inoculated with zoospores from each species and observed over 12 weeks, after which the root systems of each were assessed, scored and the diseased material was isolated on *Phytophthora*-specific media.

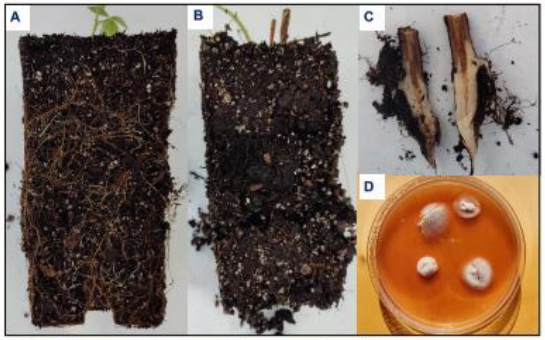

Fig 4. Healthy (A) and diseased (B) raspberry root ball, raspberry stem with lesion (C) and resultant colony from plating on V8-PARP media (D)

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There is more to raspberry *Phytopthora* than *P. rubi*, emergent pathogens such as *Phytopythium* can infect *Phytophthora*-resistant varieties and some varieties are more pathogenic than *Phytophthora.*

These findings highlight the importance of the inclusion of other Phytophthora and Phytopythium species into raspberry breeding programme resistance screens to produce varieties which are more resilient to changes in species diversity in raspberry root rot, ensuring clean and robust genotypes for the UK growers.

Understanding the genetic basis of Ramularia disease resistance in barley

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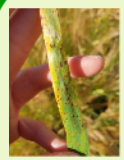
Background

Ramularia Leaf Spot in barley



- Causative agent, the Dothideomycete *Ramularia collo-cygni*
- Outbreaks in all temperate regions worldwide
- Affects grain quality and can cause yield losses ranging between 20% to 70%

Control

- Control mostly via foliar fungicide applications (QoI, SDHI, DMI)
- Resistance against QoI fungicides has quickly evolved in R. collo-cygni
- No known source of plant genetic resistance to RLS

Project Aims

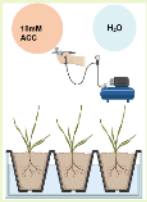
1. Characterising genetic regions against resistance to RLS
2. Investigating the link between senescence and Ramularia Leaf Spot
3. Analysing the ethylene-responsiveness in spring barley cultivars varying in their susceptibility to RLS

Methods

Phenotypic Dataset:
263 spring barley varieties scored for RLS in 2013

GWAS

Genotypic Dataset:
50 k iSelect Chip (Bayer et al., 2017)



Impact of ACC on *Rcc*-growth and development:

- Inoculation of 14 days of barley cv. Fairing seedlings
- Treatment with ACC 7-days post inoculation
- Monitoring symptom development, *Rcc*-DNA & Leaf Nitrogen Content [%]

Results

Impact of ACC on *Rcc*-growth and development:

Disease symptoms

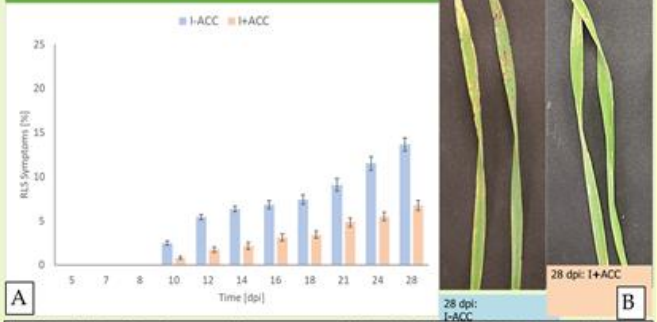


Fig. 2: Ramularia Leaf Spot Symptoms (RLS) post treatment with 10 mM ACC/$H_2$0. I-ACC: Rcc-Inoculated plants treated with H20 as a control; I+ACC: Rcc-Inoculated plants treated with 10 mM 1-aminocyclopropane-1-carboxylic acid (ACC). A: Bar chart showing average RLS-disease symptoms in percent per prophyll leaf area of 12 leaves and standard error.
B: Pictures showing disease symptoms 28-days post inoculation.

Rcc-DNA *in planta*

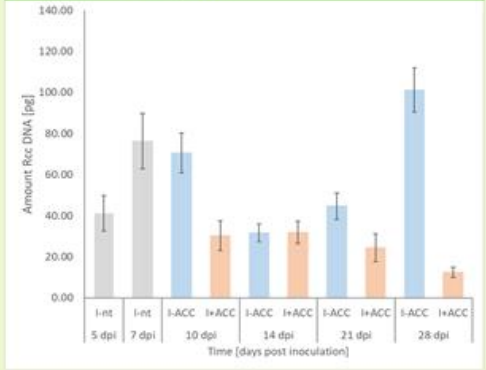


Fig. 3: *R. collo-cygni* DNA levels post treatment with 10mM ACC/H_2O. I-ACC: Rcc-Inoculated plants treated with H_2O as a control; I+ACC: Rcc-Inoculated plants treated with 10mM 1-aminocyclopropane-1-carboxylic acid (ACC). Graph shows the average amount of Rcc-DNA in picogrammes in six biological replicates. Each biological replicate consists of two prophyll leaves.

Results

Genome Wide Association Study:

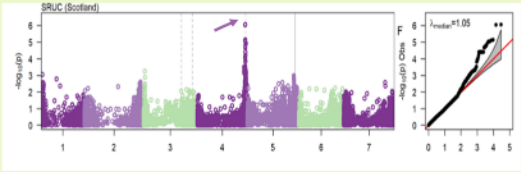


Fig. 1: Manhattan and Q-Q Plot of GWAS Study of 238 spring barley varieties.

- One major QTL identified at the end of chromosome 4H in the Scottish field trial
- Barley lines carrying the A allele are associated with increased resistance to RLS, while those carrying the G allele with increased susceptibility to RLS

Discussion

- Exogenous application of ACC reduces symptom development of RLS and appears to reduce the colonisation by the fungus after 21dpi.
- The Identification of ethylene-responsive genes (ERFs) at the end of chromosome 4H lead to the hypothesis that a differential expression of those ERFS may be responsible for the quantitative resistance s to RLS in spring barley cultivars observed in field trials (data not shown).

Future Work

- Characterising the gene expression of three identified ethylene-responsive genes in a subset of spring barley varieties identified from the GWAS in response to 10 mM ACC treatment
  → Objectives:
  - Treatment with 10 mM of ACC/control of spring barley cv. Fairing Chieftain, Hydra, RGT Planet, Tocada, and Heather
  - RT-qPCR of three *HvERFs* after 0-, 6-, 12-, 24-, and 48 hours post treatment

Acknowledgements

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Leading the way in Agriculture and Rural Research, Education and Consulting

Rotting Raspberries: Determining the Susceptibility and Origins of *Cladosporium* Infections of Raspberries

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Plant Science into Practice

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The Problem:

- *Cladosporium* has been noted to cause dark lesions on fruit, making them unmarketable.

One study revealed that over 50% of fruit were infected with *Cladosporium* post-harvest at one farm in Kent[1].

This PhD aims to understand biotic and abiotic factors that impact *Cladosporium* inoculum and development on raspberries and elucidate potential control measures for growers.

When are Raspberries Susceptible to *Cladosporium*?

Knowledge of raspberry fruit age and relative susceptibility to *Cladosporium* will allow us to determine when to apply control measures.

Raspberries inoculated with *Cladosporium* inoculum or control at different developmental stages (Fig. 1).

All the fruit from branches either sterilised or left unsterilised, then incubated for 4 days.

Fruit scored for severity of skin lesions and stigmata infections on a scale from 0 (no infection) to 5 (most drupelets/stigmata infected).

Fig 1. The stages of development inoculated and what type of infection they are susceptible to.

When are raspberries susceptible to Cladosporium skin lesions?

- **Green fruit are not susceptible. Ripe fruit are more susceptible to skin lesions than ripening fruit (odds ratio 2.04, S.E. 0.283).**

Susceptibility to stigmata infections across development stages and difference in number of stigmata infected after sterilisation:

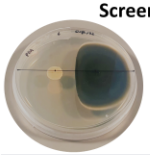
- **Infection scores on stigmata were significantly higher on the unsterilised stigmata than sterilised ones, indicating that *Cladosporium* colonisation occurs predominantly on the surface of stigmata (p<0.00, df=1). There is no significant difference in infection across developmental stages (p = 0.069,df= 3).**

***Cladosporium* can colonise the stigma early on in fruit development and subsequently cause skin lesions as the fruit ripens when there is an increase in susceptibility.**

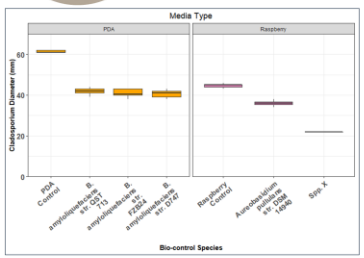
Can Bio-controls Provide Preventative Protection Against *Cladosporium*?

Bio-controls that can outcompete the colonisation of *Cladosporium* on the surface of raspberries may provide some protection against *Cladosporium* skin lesions.

Screening Bio-controls on Petri Dishes



Plates Inoculated with *Cladosporium* 3 days prior to bio-controls. The inhibited diameter of *Cladosporium* and bio-control growth (mm) was recorded at 3 time points (N= 65, 9 Blocks, 1 repeat in time).



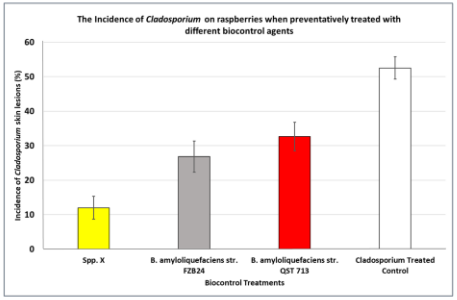
All bio-control products in the plate screening showed reductions in the *Cladosporium* colony size vs their respective controls.

Fig. 2. The diameter of *Cladosporium* colonies 9 days post inoculation with a biocontrol colony.

Field Testing of Bio-controls

Ripening raspberries sprayed with a bio-control treatment, then inoculated with *Cladosporium* inoculum (N= 1463, 6 blocks, 1 repeat in time).

Assessed for absence or presence of *Cladosporium* after 4 days incubation.



All bio-control products in the field screening had reductions in the incidence of *Cladosporium* vs the control.

Fig. 3. The incidence (% of fruit with skin lesions) of *Cladosporium* on raspberries treated with different bio-controls. All bio-control treatments received the same inoculum as the *Cladosporium* treated control.

There are bio-control products that provide protection against Cladosporium skin lesions. More work is needed to optimise the application of bio-control products to provide maximum protection to raspberries.

References: 1. O'Neill, T. et al. 2012. Agronomy for Sustainable Development 32: 673-682.

Statistical analyses of these experiments are in progress

Early Detection and Spread of Tomato Powdery Mildew (TPM) in Commercial Glasshouses

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Prof. John Clarkson, Dr. Phil Morley, Dr. Jon West

ROTHAMSTED RESEARCH

Introduction

- The UK produces approximately 90,000 T of tomatoes a year[1].
- *Erysiphe neolycopersici* (TPM) and *Botrytis cinerea* are the main diseases.
- The diseases lead to reduced yields, decreased quality of tomatoes and, if left unattended, plant death.
- Growers spray the crop prophylactically to avoid TPM.
- For better disease management we aim to detect pathogens before they infect the plant and reduce unnecessary spraying.

Objectives

- Explore the patterns of distribution of TPM in commercial glasshouses
- Determine the optimal position for spore traps for early detection of *E. neolycopersici* & *B. cinerea* under UK glasshouse conditions

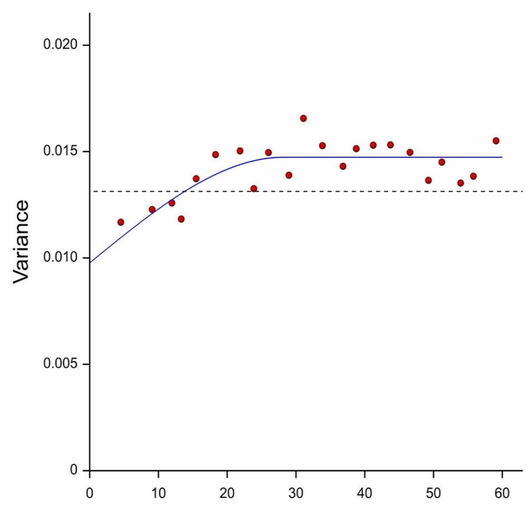
Methods

- Monitored TPM in four commercial glasshouses (each 1Ha) over the season.
- Disease scored visually on the IPGRI scale (1-9) every two weeks between June – November 2021.
- Mapped disease by kriging.
- Sampled spores using rotor rods and 7-day Burkard spore traps over the season.
- Analysed daily spore counts using molecular techniques for *B. cinerea and E. neolycopersici*

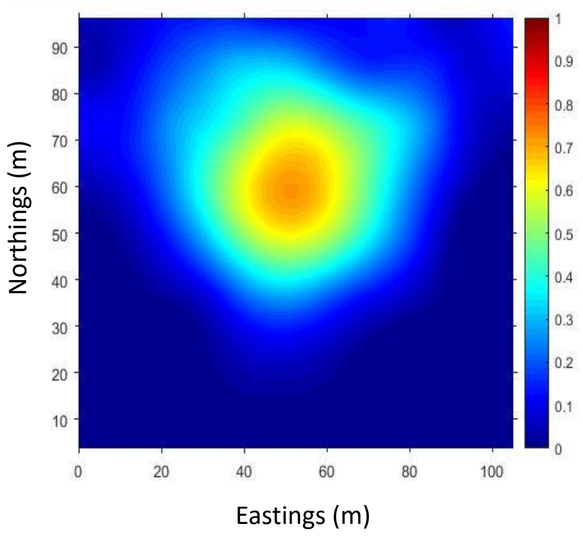
Results

TPM was modelled based on the visual symptoms monitored in the glasshouses. The variograms represent the short-range variation patterns, and the kriged maps are the final representation of the disease with long-range and short-range variation factored in.

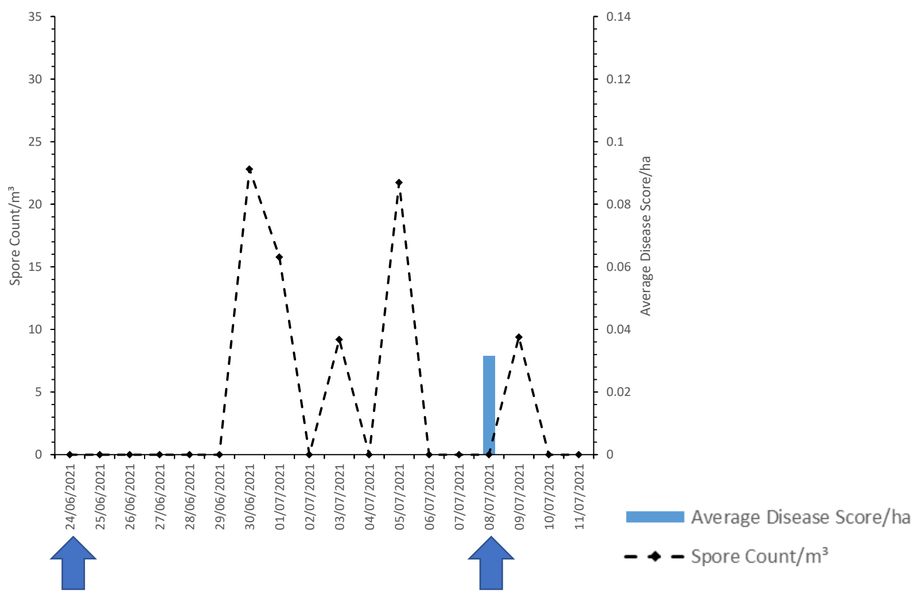
Variogram



Kriged Map



Graphs below show two incidents in 2021 where the 7-day Burkard spore trap detected disease before it was visually recorded. This happened 9 and 8 days before disease incidence was recorded. The arrows point at when the disease was monitored for.



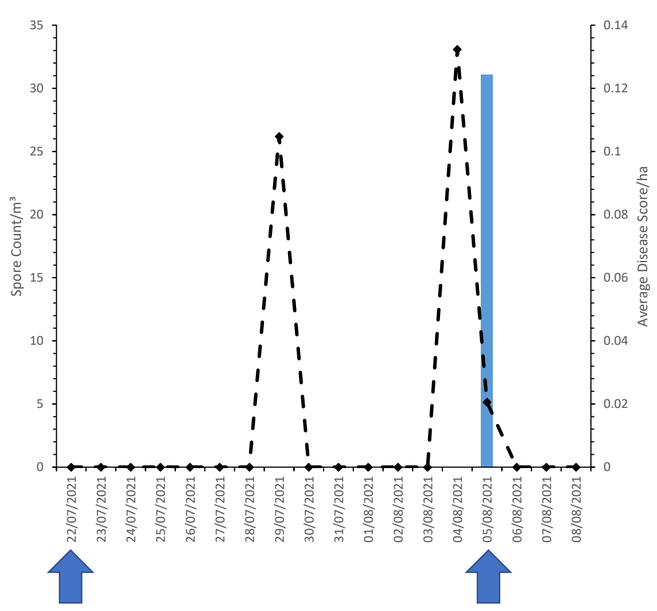
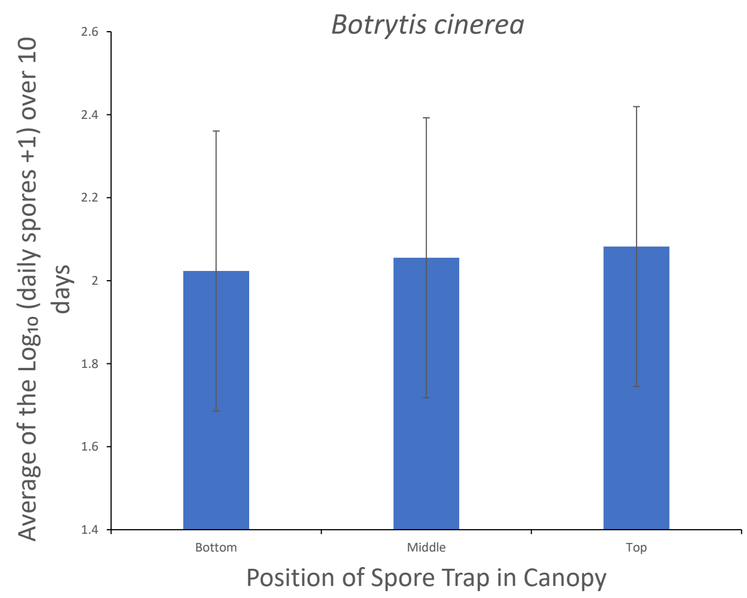


Table shows average spore counts from rotor rods for *B. cinerea* over 10 days in each block within two glasshouses and replications of the experiment in time.

	Replication		
Block	1	2	3
1	6.9	3928.4	0.0
2	9.5	573.3	0.0
3	0.7	237.7	0.0
4	0.6	680.4	0.0

B. cinerea results showing that height is not an important factor when sampling in glasshouses.



Conclusion

- Short-range variation extends between 20-60m
- Disease is introduced through the main pathways used by personnel and machinery
- Position and time of spore trapping is more important than the height at which the spore trap is positioned
- Spore trapping can detect *E neolycopersici* up to 9 days before visible symptoms

Future Work

- Model the progression of *Erysiphe neolycopersici* overtime in commercial glasshouses.
- Confirm whether proximity to the disease foci are the main causes of differences in spore counts.

Acknowledgments and References

[1] Food and Agriculture Organization of the United Nations., (2019). FAOSTAT statistics database [online]. FAO. [Viewed 18 Febuary 2019]. Available from: http://www.fao.org/faostat/en/#data/QC

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