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# The BCPC 7th Disease Review 2021

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## Exploring alternatives to enhance plant health – arrive with an open mind

**10.00 Chair Introduction** – *Kate Storer*, ADAS
**10.20 The ins and outs of endophytes** – *Matevs Papp-Rupar,* East Malling Research
**10.55 Modern plant breeding mycorrhizal interactions –** *Tim Mauchline*, Rothamsted Research
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**13.15 How can we help growers get the most out of bio fungicides? The AHDB AMBER Project –** *David Chandler,* Warwick University
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**14.25 Discussion & Chair Summary**
**15.00 End**

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# Chemical Warfare – The Fungal Quest To Conquer Oilseed Rape

Fortune J.A., Baker D., Stanley J., Bingol E., Karandeni-Dewage C.S., Ritchie F., Fitt B.D.L. & Huang Y

## Introduction

- Phoma stem canker is caused by two similar co-existing pathogens – *Leptosphaeria maculans* (Lm) and *L. biglobosa* (Lb).
- They cause yield losses worth >£73M to oilseed rape growers in England and Wales despite the use of fungicides and resistant cultivars.
- Their lesions have a different appearances on the leaves and stem of plants.
- Lm produces a secondary metabolite called Sirodesmin PL (Fig. 1), that has an inhibitory effect on Lb.
- Lb does not produce Sirodesmin PL.
- Previous studies showed that Lb ascospores were released later than Lm. However, recent studies that have used qPCR analysis have reported the Lm and Lb ascospores are frequently released at similar times (Fig.2).

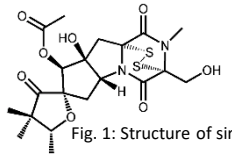


Fig. 1: Structure of sirodesmin PL

## Aims

- Investigate the interactions between Lm and Lb *in vitro* and *in planta*
- Identify changes in phytotoxin production as result of increased interspecific competition

## Methods

1. Fungal plugs of Lm or Lb were individually ('Lm only' and 'Lb only') or simultaneously inoculated ('Lm&Lb) into liquid culture (Fig 3)..
2. They were placed in a rotary shaker.
3. After 14 days, the culture filtrate was separated from the fungal mycelial mass for each treatment.
4. Secondary metabolites were extracted using ethyl acetate to create three "Extracts" - 'Lm only' , 'Lb only' and 'Lm&Lb'.
5. Agar plates were inoculated with fungal plugs of either Lm or Lb, then they were inoculated with one of the "Extracts" or ethyl acetate (EtOAc) as a control.
6. After 7 days post inoculation the colony area was measured using standardized images
7. Extracts were analysed using HPLC and LC-MS to identify the composition of the 'Extracts' and quantified using a gliotoxin standard curve.

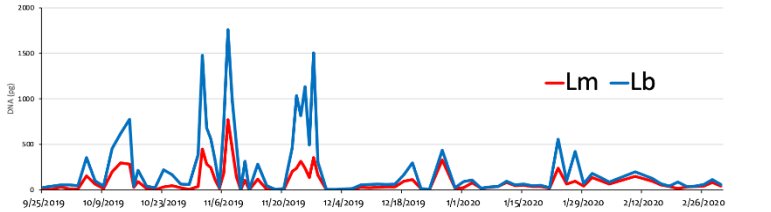


Fig. 2: Ascospore release pattern of *L. maculans* (Lm) and *L. biglobosa* (Lb) between Sept 2019 – March 2020 at Terrington St Clement, Norfolk detected using qPCR analysis.

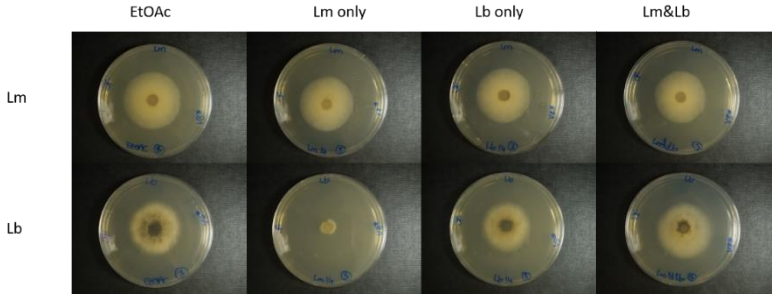


Fig. 4: Colony morphology when secondary metabolites were applied to fungal plugs of L. maculans (Lm) and *L. biglobosa* (Lb).

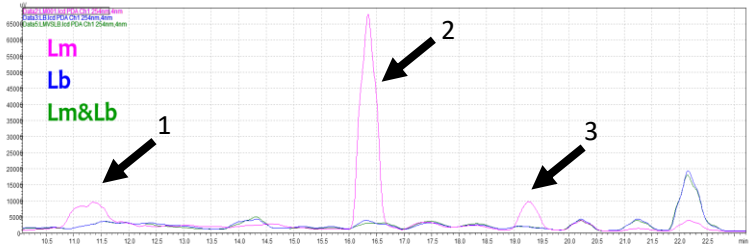


Fig. 3: HPLC chromatograph of secondary metabolite extracts from liquid media inoculated with either L. maculans (Lm only) or *L. biglobosa* (Lb only), or co-inoculated with both (Lm&Lb).

## Results – Composition

- Using HPLC, three maxima were identified that were unique to the 'Lm only' extract (Fig. 3).
- These peaks were not identified in the 'Lm&Lb' extract.
- Using LC-MS, maximum 1 was identified to contain de-acetylsirodesmin and phomamide, maximum 2 as Sirodesmin PL, and maximum 3 was unknown.

## Results – *in vitro*

- When the secondary metabolite extracts were applied to fungal plugs of *L. maculans*, there were no inhibition of colony area compared to the EtOAc control (Fig. 4)
- However, when the extracts were applied to fungal plugs of *L. biglobosa* , there was an inhibition of growth when the 'Lm only' extract was applied compared to the EtOAc control.
- There was no inhibition of growth when the 'Lb only extract was applied compared to the EtOAc control.
- There was no inhibition of growth when the 'Lm&Lb' extract was applied compared to the EtOAc control

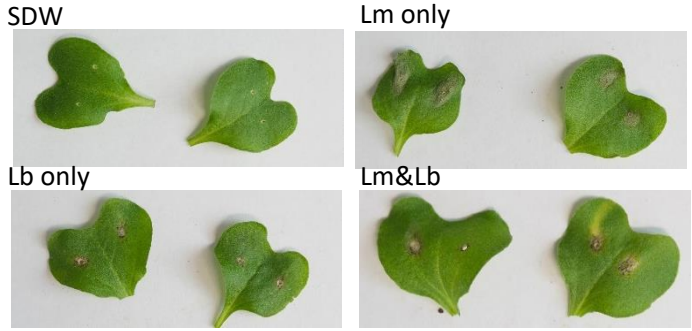


Fig. 5: Lesion phenotype when cotyledons were inoculatied with either L. maculans (Lm only) or *L. biglobosa* (Lb only), or co-inoculated with both pathogens (Lm&Lb).

## Results – *in planta*

- When SDW was applied, no lesion was formed (Fig.5).
- When Lm inoculum was applied the lesion was large, pale lesion, characteristic of Lm.
- When Lb inoculum was applied the lesion was small, dark and defined, characteristic of Lb.
- When Lm and Lb was inoculated simulataneously the lesion was like that of Lb only.

## Conclusions and agricultural relevance

- The extract that resulted in inhibition of Lb growth in vitro was the one that contained Sirodesmin PL and its precursors.
- When Lm and Lb were simultaneously inoculated Sirodesmin PL and its precursors were not produced *in vitro* and resulted in smaller lesions *in planta*.
- The current guidance for fungicide application for control of phoma stem canker is when there is 10-20% crop incidence of Lm.
- So, if Lm and Lb ascospores are released at the same time, phoma leaf spot lesions may appear later or be smaller, resulting in a later application of fungicide.

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# Fostering Populations Of Arbuscular Mycorrhizal Fungi Through Cover Crop Choices and Soil Management

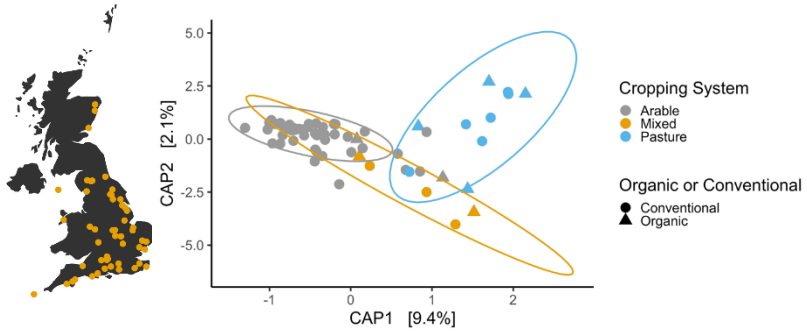
George Crane, Uta Paszkowski, and Lydia Smith

## Motivation

Cover crops are grown for the purpose of 'protecting or improving' soil between periods of regular crop production. Amongst other soil health benefits, cover crops can enhance microbial communities, including arbuscular mycorrhizal (AM) fungi. AM fungi convey a range of benefits, including nutrient uptake, pest and pathogen resistance, and drought tolerance, in exchange for plant derived carbon. Wider research has shown positive impacts of cover crops on AM fungi, both in terms of diversity and abundance. In this project, the diversity of AM fungi in UK agriculture is described, before assessing the impact of various farming practices on AM fungal populations, and how these fungal populations can contribute to soil health, crop growth, and yield.

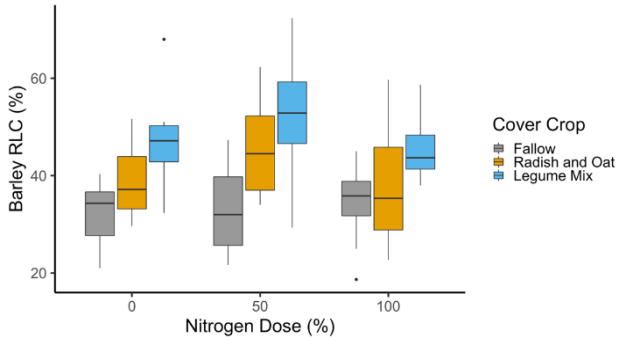
## UK-Wide Analyses

In collaboration with FERA, 148 farm soil samples were sequenced for AM fungi, in order to better understand the diversity and abundance in UK agricultural soils. In total, 87 AM taxa were identified, including three previously unknown to science. Key factors underpinning AM fungal communities were whether a site was organic or conventional, the soil texture, type of cultivation, and whether fungicides had been applied.



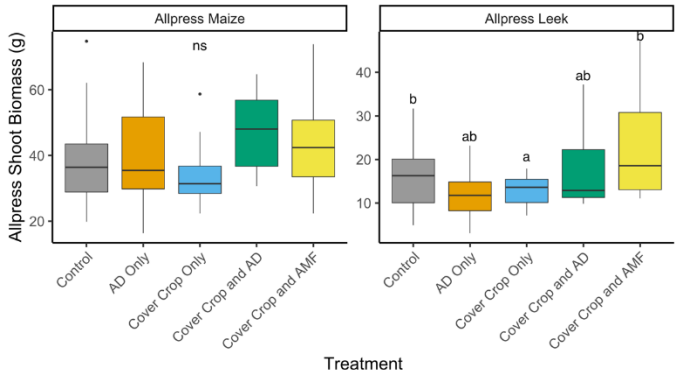

## Replicated Trials

Next, the impact of different cover crop mixtures, AM fungal inoculation, and nitrogen application were investigated in replicated trials. Cover cropping was an effective method of improving the root length colonized (RLC) by AM fungi. Conversely, full rates of N fertilization were shown to negatively impact certain species of AM fungi, including those belonging to the genus *Glomus.* This suggests a role for these taxa in plant N transfer.




## Field Scale

In two other trials, including an Innovative Farmers Field Lab, a commercial AM fungal inoculum had no impact on AM fungal populations, or crop yield in maize, barley, or oat. However, highly mycorrhizal leek had higher RLC and crop biomass following inoculation than some other treatments, but not the control. This suggests some level of species specificity for mycorrhizal induced crop benefit




## Conclusions and Outlook

In this project, we show evidence that AM fungal abundance, but not diversity, can be influenced by cover cropping, and only after multiple iterations of cover crop growth. Leguminous cover crops are most beneficial, at least in terms of increasing cash crop RLC, however, cover crop mixtures including legumes may promote other soil health benefits. Further research on the genetics underpinning plant benefit from AM fungi may result in more effective inocula, or improve crop varieties to maintain yields with lower nutrient additions. Such understanding would contribute to a more sustainable agriculture.

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# Examining Biofumigant Crops for the Management of Pea Foot Rot Complex Pathogens

Lisa M. King*[1], Gary D. Bending[1], Lea Herold[2], John P. Clarkson[1]

*l.king.5@warwick.ac.uk, [1]University of Warwick,
[2]Processors and Growers Research Organisation

## The Pea Foot Rot Complex (PFRC)

In the UK, growth of field pea (*Pisum sativum*) is restricted to eastern England and Scotland, which has led to intensive production and yield declines of up to 40%. This is mostly attributed to a build-up of key fungal and oomycete pathogens involved in the PFRC; *Fusarium solani* f. sp. *pisi* (FSP), *Fusarium oxysporum* (FO), *Didymella pinodella* (DP) and *Aphanomyces euteiches* (AE). Symptoms of PFRC infection include discolouration and destruction of roots (Fig. 1). Current strategies for disease management are limited, especially for the complex as a whole, with research into biofumigant and pure isothiocyanate strategies limited to *in vitro* experiments with AE. Therefore, one of the aims of this project is to identify biofumigant crops that can suppress PFRC pathogens.

**Figure 1.** Symptoms of PFRC infection, including dark root discolouration and stem lesions.

## Materials and Methods

### Growth of Biofumigant Crops and Analysis of Primary Glucosinolate (GSL) Concentrations using High Performance Liquid Chromatography (HPLC)

Biofumigant crop varieties (Table 1) were grown in controlled glasshouse conditions (Fig. 2). Shoot tissue was harvested at mid-flowering, dried and milled to a fine powder. Primary GSLs for each species were identified from the literature. GSLs were released from the milled plant material using hot liquid extraction and the concentration determined using HPLC.

**Figure 2.** Various biofumigant crop varieties being grown in a glasshouse compartment.

## Materials and Methods

**Table 1.** Biofumigant crop varieties and respective primary glucosinolates, sown on 12.04.19.

| Variety | Primary glucosinolate |
|---|---|
| *Brassica juncea* 'Caliente 199' | Sinigrin |
| *Brassica juncea* 'Caliente Rojo' | Sinigrin |
| *Brassica carinata* 'Cappuchino' | Sinigrin |
| *Raphanus sativus* 'Terranova' | Glucoraphanin |
| *Raphanus sativus* 'Contra' | Glucoraphanin |
| *Eruca sativa* 'Nemat' | Glucoerucin |
| *Eruca sativa* 'Trio' | Glucoerucin |
| *Sinapis alba* 'Brisant' | Sinalbin |

### Effect of Biofumigants on Mycelial Growth and Spore Germination of PFRC Pathogens

Experiments were undertaken using a sealed two Petri dish base system (Fig. 3, adapted from Sexton *et al.*, (1999)); One base containing potato dextrose agar, placed on top of a second base containing hydrated biofumigant powders of each variety (water for the control), sealed with parafilm. For mycelial growth experiments, plugs of individual PFRC pathogens were placed onto the agar and percentage inhibition calculated after seven days. For spore germination experiments, approximately 50 spores of individual pathogens were plated onto the agar and exposed to the biofumigant seven days, after which the agar bases were then incubated for a further seven days without the biofumigant. Germination was assessed both after exposure to the biofumigant and after it was removed.

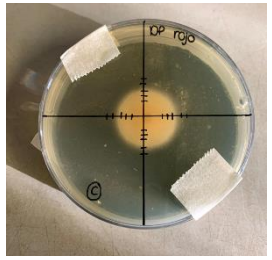


**Figure 3.** Example of Petri dish system used to examine effects of hydrated biofumigants on mycelial growth.

## Results

### Analysis of Primary GSL Concentrations using HPLC

The levels of primary GSLs detected in batch 2 overall were lower than expected for the growth conditions and from previous batches grown in similar conditions (Fig. 4).

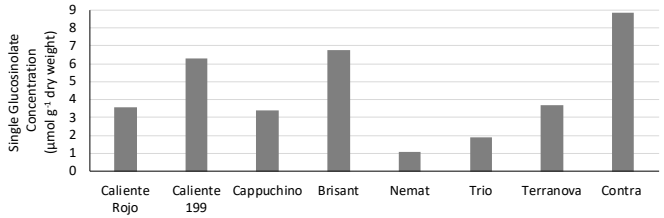


**Figure 4.** Single glucosinolate concentrations for each variety.

### Effect of Biofumigants on Mycelial Growth of PFRC Pathogens

For the three PFRC pathogens tested, the percentage inhibition of growth compared to a control was reduced for all varieties (Fig. 5), particularly for DP, where the varieties *B. juncea* 'Caliente 199', 'Caliente Rojo' and *E. sativa* 'Trio' inhibited mycelial growth by more than 50 %.

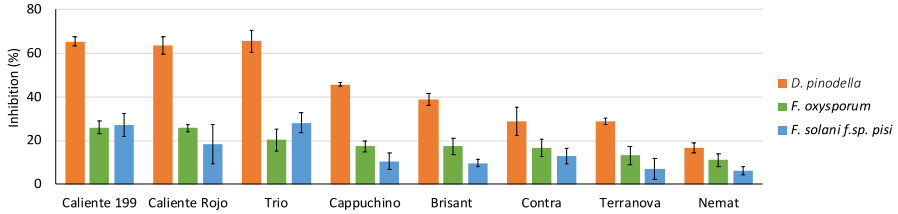


**Figure 5.** Effect of biofumigant treatments on inhibition of PFRC pathogen mycelial growth rates compared to an untreated control.

### Effect of Biofumigants on Spore Germination of PFRC Pathogens

For FO and FSP, spore germination after exposure was reduced for all and two varieties respectively (Fig. 6), with all but *E. sativa* 'Trio' supressing germination by more than 50 % for FSP. However, no variety inhibited germination after removal of the biofumigant by more than 23 % for FO and 31 % for FSP.

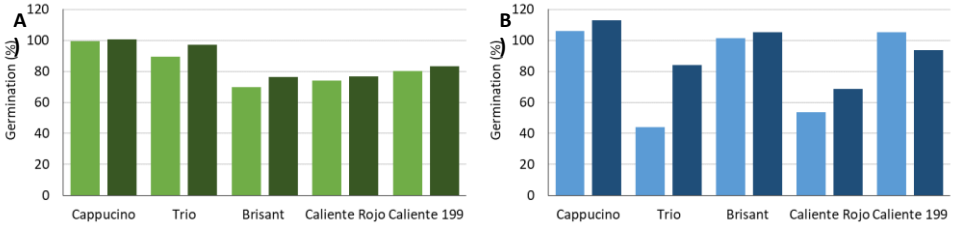


**Figure 6.** Effect of biofumigant treatments on inhibition of A) FO and B) FSP spore germination compared to an untreated control. Lighter bars represent germination after exposure. Darker bars represent germination after non-exposure for a time period.

## Conclusions and Future Research

Results of experiments examining mycelial growth and spore germination, alongside future spore germination experiments with DP, will inform biofumigant variety choice for future glasshouse experiments examining effects of incorporated biofumigants on PFRC disease development. Glasshouse dose response experiments examining effects of different spore concentrations of FO, FSP and DP on disease development in peas have also informed the level of inoculum for these experiments.

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# *Trichoderma* as a biocontrol agent of *Armillaria*

Morgan Millen*[1,2], J. Drakulic[2], M. Cromey[2], A. M. Bailey[1] and G. D. Foster[1]

* Presenting author. [1] School of Biological Sciences, University of Bristol, Bristol, UK. [2] Plant Health, The Royal Horticultural Society, Wisley, Surrey, UK

## 1. Introduction

*Armillaria* or honey fungus effects trees and shrubs causing their decline and death in gardens, forestry, vineyards, stone fruit and nut production. Mycelium can feed on woody residual roots and survive for decades looking for a new host to arrive. This makes replanting susceptible plants in an area which previously had an infection highly risky. Disease is notoriously difficult to cure, and current control methods fall short. Post-infection controls previously relied on environmentally damaging methods, creating the need for new disease management practices.

Figure 1. *Armillaria mellea* mushrooms in Bristol, UK.

*Trichoderma* species are commonly studied as biocontrol agents as they can be endophytic, promote plant growth and antagonize a range of bacterial and fungal pathogens.
I work with a collection of 43 *Trichoderma* isolates (12 distinct species). These isolates have been tested for their ability to antagonize Armillaria isolates through dual culture assays and *in planta* experiments (Rees, 2021). My work looks at how *Trichoderma* is able to control *Armillaria.*

## 2. *In planta* experiments

**In Privet**

- *Trichoderma* isolates (T17/11, T17/15 and T22) were screened in privet plants in 3 soil types: fresh, baked and compost.
- T17/11 (*T. atrobrunneum*) showed potential protection to host plants in baked and fresh soil.
- T22 (*T. harzianum*) exacerbated disease in privet potted in compost.
- Compost groups showed the worse disease symptoms when compared with the other 2 soil types (see figure 2, compost group on the far left side).

Figure 2. Privet experiment carried out at the RHS.

**In Strawberry**

- Ongoing experiment, combinations of *Trichoderma* isolates added per plant to compare to application of isolates individually.
- Deaths measured over time.
- Thus far, T17/11, T17/15 and the combination of these isolates have had the fewest deaths.
- Groups including T17/10 (*T. hamatum*), T17/23 (*T. cerinum*), T17/40 (*T. harzianum*) have not shown biocontrol potential.

Figure 3. Ongoing strawberry experiment testing biocontrol potential of *Trichoderma* (left); dissected strawberry plant from the same experiment showing white *Armillaria* mycelia, where *Trichoderma* was not able to prevent death (right).

## 3. How does *Trichoderma* control *Armillaria*?

**Enzyme Production**

- Plate-based assays carried out for various enzyme groups including cellulase, laccase, amylase and protease (Figure 4).
- The top producers of cellulase were T17/03 (*T. harzianum*), T17/11 (*T. atrobrunneum*) and T17/20 (*T. atrobrunneum*).
- All isolates except T17/10 (T. hamatum) were very high producers of laccase. There was significant variation among the high producers. The highest producers were T17/08 (*T. harzianum*), T17/11 and T22 (*T. harzianum*).
- Successful biocontrol may require several different enzymes. For this reason, high producers in multiple key enzyme groups could be the most promising isolates for use in biocontrol. These assays can be compared to *in planta* results.
- T17/11 was a high producer of all enzyme groups studied here, suggesting that enzyme activity may correlate to biocontrol ability.

**Altering pH**

- 28 *Trichoderma* isolates studied, 3 *Armillaria* isolates studied.
- Media included 0.01% (w/v) Bromocresol purple, which changes from yellow at pH 5.2 to purple at pH 6.8 and above.
- *Trichoderma* and *Armillaria* isolates were tested both together and separately for how they altered pH over time.
- T17/11 and T17/15 (*T. atrobrunneum*) were consistent in making the agar more alkaline, doing so both in the presence of Armillaria and when no Armillaria was present.
- In the presence of *Trichoderma*, *Armillaria* is not able to grow much after the two meet each other. Similarly, *Armillaria* is not able to change the pH of the agar where *Trichoderma* colonizes.

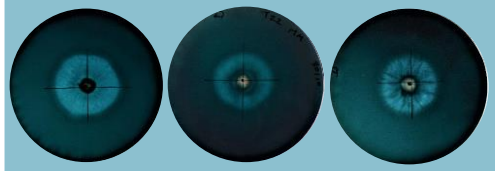

Figure 4. General protease assay. Pale blue marks the protease activity, seen around the inoculation site in center of plate. From left to right: T17/11, T22, T17/34.

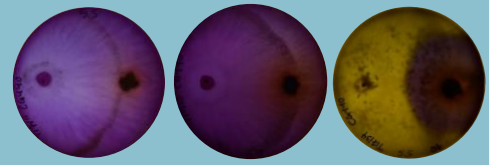

Figure 5. *Trichoderma* and *Armillaria* dual culture pH assay plates. From left to right: T17/11 and CG440, T17/15 and CG440, T17/34 and CG440. *Trichoderma* is on the left of each plate.

## 4. Summary

- *Trichoderma atrobrunneum* isolates show potential as a biocontrol agent of *Armillaria* root rot in privet and strawberry.
- High production of key enzyme groups may indicate an effective biocontrol of *Armillaria.*
- *Trichoderma* significantly reduces the growth and ability of *Armillaria* to alter pH *in vitro*.

## 5. Future studies

- Further investigation into enzyme production, including chitinase.
- Genome sequencing of T17/11. Utilizing this sequence to investigate potential biocontrol related genes.
- *In planta* study to test curative potential of *Trichoderma* in strawberry.
- Further plant studies in privet, testing biocontrol ability of *Trichoderma,* to strengthen previous data.

**Acknowledgements**
This project was funded by the University of Bristol and the Royal Horticultural Society (RHS) as part of a PhD programme.

**About the author:** Morgan is a second year PhD Student based in School of Biological Sciences, University of Bristol.
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