Interspecific interactions between fungal pathogens causing light leaf spot (*Pyrenopeziza brassicae*) and phoma stem canker (*Leptosphaeria maculans* and *L. biglobosa*) *in vitro*.

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Introduction

- Phoma stem canker and light leaf spot caused by *Leptosphaeria maculans* and *Pyrenopeziza brassicae* respectively are the two most economically damaging diseases to UK oilseed rape growers; causing > £150M losses annually.
- These plant pathogens do occur independently; however, they are often observed together on leaves or stem tissues of the same individual plant (Figure 1); their interactions are not clear.
- This work aims to understand the interspecific interactions between these pathogens *in vitro* on different media when inoculated with different extracted secondary metabolites.

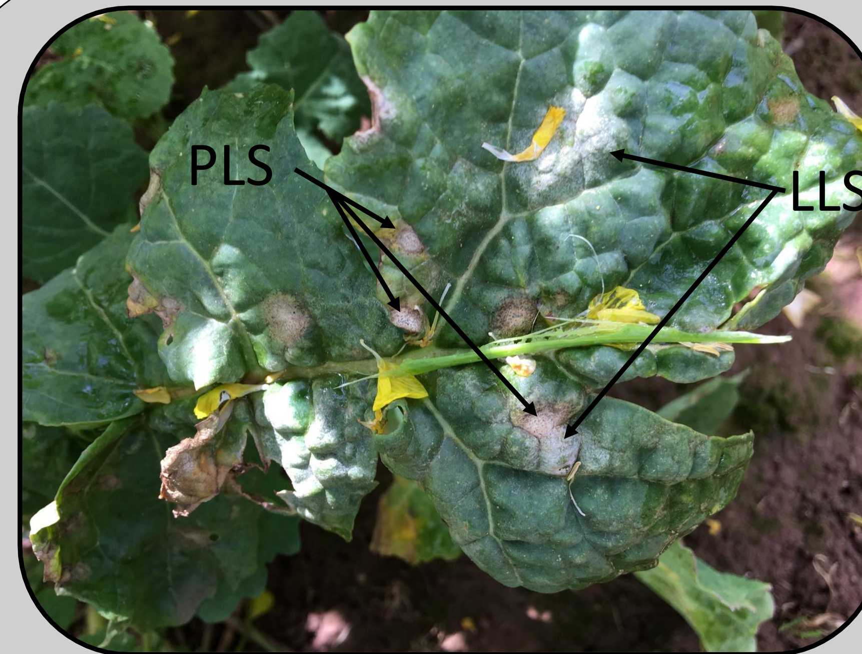

Figure 1. Photograph of light leaf spot (LLS) and phoma leaf spot (PLS) on the same oilseed rape leaf.

Methods

For each treatment (Table 1), a total of 4 plugs (co-cultures 2 plugs of each fungus) were cultured in 75 ml of clarified V8 juice broth in a rotary shaker set at 80RPM and 18°C for 14 days. Each treatment was done in replicate and placed in a randomised position in the rotary shaker.

Table 1. Treatment list for production of secondary metabolites in Liquid culture and for plug bioassay.

Treatment	Description	Replicates	Liquid Culture	Plug Bioassay
1	Media only	3	✓	✓
2	Lm only	3	✓	✓
3	Pb only	3	✓	✓
4	Lb only	3	✓	✓
5	Lm vs Pb	3	✓	✓
6	Lb vs Pb	3	✓	✓
7	Lm vs Lb	3	✓	✓
8	EtOH only	3	×	✓
9	Control	3	×	✓

Using miracloth, the liquid broth was separated away from the fungal colonies and each treatment was pooled into individual Duran bottles. Pour 75 mL of ethyl acetate (EtOH) into each Duran bottle and shake for 45 seconds and allow the two phases to settle for 30 minutes. For each treatment, the top phase was pipetted into two 50ml Falcon tubes and centrifuged at 6000 RPM for 3 minutes. Take 40 mL of the clear supernatant from centrifuged Falcon tubes and pool into two new skirted 50ml Falcon tubes (20ml in each). This was repeated for each treatment. The excess EtOH was evaporated using a sample concentrator. Dried metabolites were re-suspended in 1 mL of EtOH.

Plates were inoculated with a fungal plug onto clarified V8 juice agar (8mm, 8mm and 4 mm diameter fungal plugs of *L. maculans*, *L. biglobosa* and *P. brassicae*, respectively). Pipette 20 µL of extracted secondary metabolites (SM) from each treatment (Table 1) onto a fungal plug in triplicate. Samples were incubated at 18°C. Assessments were made at 2, 7 and 10 days for *L. maculans* and *L. biglobosa,* and weekly for *P. brassicae* by measuring the average colony diameter.

Results

- For Lm, colony growth was significantly reduced when plugs were inoculated with SM from liquid cultures that did not contain Lm, with the exception of ‘Lm vs Lb’ SM (Figure 2).
- SM from ‘Pb only’ caused the greatest reduction in average colony diameter after 7 days (Fig. 2).

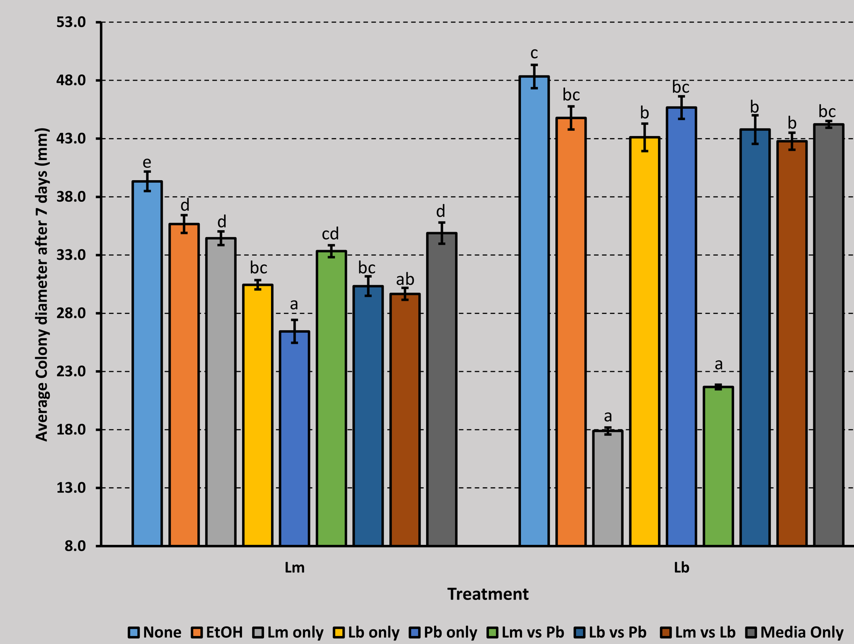

Figure 2. Average colony diameter of *L. maculans* and *L. biglobosa* after 7 days when inoculated with different secondary metabolites. Bars that do not share a letter are significantly different according to Tukey.

- For both Lb and Pb, SM deriving from liquid cultures with ‘Lm only’ or ‘Lm vs Pb’ caused the only significant reduction in colony diameter (Fig. 2,3).
- For Pb, SM from ‘Lb vs Pb’ caused a small yet significant reduction in colony growth compared to EtOH control.
- ‘Lm vs Pb’ SM did not cause a significant reduction to Pb.

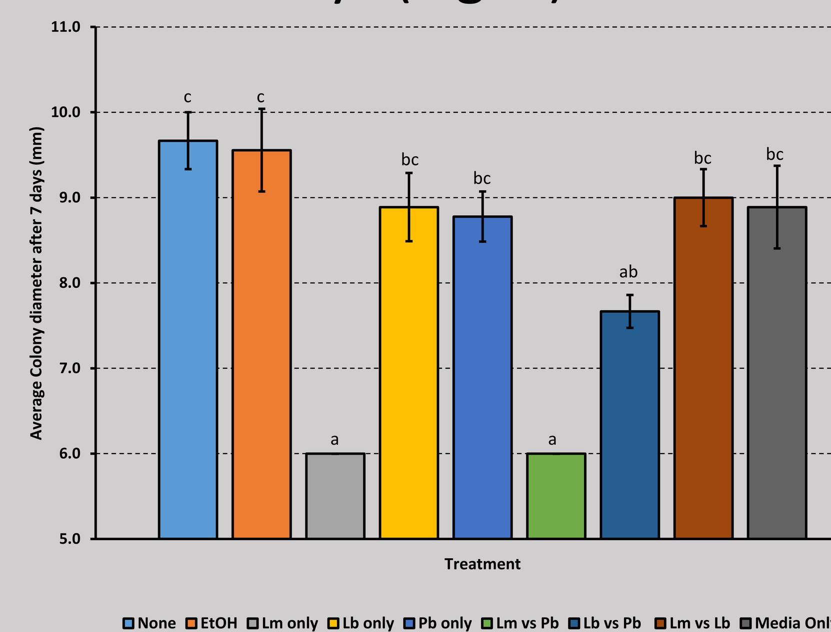

Figure 3. Average colony diameter of *P. brassicae* after 7 days when inoculated with different secondary metabolites. Bars that do not share a letter are significantly different according to Tukey.

Conclusions

- *L. maculans* releases antagonistic secondary metabolite/s that reduce the colony diameter of both *L. biglobosa* and *P. brassicae.*
- *L. biglobosa* can detoxify the antagonistic secondary metabolite/s.
- Secondary metabolites from *L. biglobosa* and *P. brassicae* cause small, but significant reductions in *L. maculans* colony diameter.
- *P. brassicae* release antagonistic metabolites that reduce *L. maculans* colony diameter, however *L. maculans* has a mechanism to detoxify these secondary metabolite/s.

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