Characterisation of host responses triggered by the fungal pathogens causing phoma stem canker on oilseed rape

Phoma stem canker is a disease of oilseed rape of a world-wide importance that causes significant crop losses in areas such as Europe, Australia and North America. The disease is caused by a complex of two *Leptosphaeria* species; *L. maculans* and *L. biglobosa*. The main aim of this project was to investigate the interactions between *L. biglobosa* and two different oilseed rape cultivars: Ningyou7 and Tapidor. The initial work involved growing seedlings in trays in a growth chamber set at an incubation temperature of 20°C. The cotyledon lobes of both cultivars were inoculated by infiltration at the seedling stage with either 10µl of *L. biglobosa* spore suspension or distilled water as a control.

Following inoculation, cotyledons were harvested at 1 and 2 dpi then snap frozen in liquid nitrogen and stored at -80°C prior to DNA extraction. DNA extraction was done using the DNAmite plant kit and the resulting DNA was quantified using Nanodrop. Quantitative PCR experiments were carried out to determine the amount of pathogen DNA in the tissue sampled. This was done using species-specific primers. The amount of pathogen DNA detected in Ningyou7 was 2-3 times greater than that detected in Tapidor. To enable the visualisation of the pathogen presence in host tissue soon after inoculation, cotyledons of both cultivars harvested at 1, 2, 3 and 4 dpi were incubated for 4 hours in trypan blue for staining the pathogen mycelium. This was followed by individual analysis of the samples under an optical microscope at ×40 magnification. More mycelium was observed at 3 and 4 dpi than at earlier time points in both cultivars. However, the protocol followed did not enable accurate comparisons between samples. Nevertheless, assessments of symptom development on cotyledons 12 days following inoculation showed that lesion size was greater on cotyledons of Ningyou7 than on those of Tapidor.

Vanessa working in the lab

This project showed that *L. biglobosa* colonised both cultivars. However, differences in symptom severity and pathogen abundance between the two cultivars should be investigated further in order to examine mechanisms of host resistance or susceptibility to *L. biglobosa*. Examination of early responses following inoculation with the pathogen will provide more insight about the mechanism of infection. This will help to achieve better management of the pathogen to prevent severe disease epidemics.

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Bursary Report

The vacation bursary has given me the opportunity to work in a laboratory environment, greatly adding to my experience. I enjoyed the challenges and rewards it presented. Through this project, I have enhanced my knowledge of molecular plant pathology and have acquired new laboratory skills including DNA extraction, quantification of nucleic acid, use of gel electrophoresis and qPCR. I would like to thank BSPP for this fantastic opportunity, my supervisor Georgia Mitrousia and the University of Hertfordshire Crop and Environmental Protection Group for their invaluable guidance and support in making the experience such a positive one.

Vanessa Waite

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