Identification of cultivar resistance against *Leptosphaeria maculans* and *L. biglobosa* in oilseed rape

Oilseed rape (*Brassica napus*) is a popular farmed crop which produces one of the top-quality vegetable oils with a range of beneficial health properties, including high omega-3 content. Sadly, fungal pathogens attack this crop leading to yield losses. Phoma stem canker is a damaging disease of oilseed rape in the UK, causing yield losses worth >£100M p.a., despite the use of fungicides. The disease is caused by two closely related species *Leptosphaeria maculans* and *L. biglobosa*. *L. maculans* is generally considered more damaging than *L. biglobosa*. Therefore, previous research focused on control of *L. maculans*, with little research being done on *L. biglobosa*. Recent research showed that *L. biglobosa* can be as damaging as *L. maculans*, causing both basal stem cankers and upper stem lesions, leading to yield losses. Therefore, there is a need to investigate cultivar resistance against both causal pathogens. Epidemics of phoma stem canker are initiated by ascospores. At the leaf infection stage in the autumn/winter, phoma leaf spots caused by *L. maculans* or *L. biglobosa* can be distinguished by their symptoms. However, it is difficult to distinguish phoma stem canker caused by *L. maculans* or *L. biglobosa* in summer before harvest by visual symptoms; this can only be determined by species-specific PCR. The aim of my project was to identify phoma stem canker causal pathogens (*L. maculans* and *L. biglobosa*) on different winter oilseed rape cultivars from field experiments.

The first part of my project involved sampling plants from the field experiments and assessing the severity of phoma stem canker. Stems of oilseed rape cultivars were sampled from field experiments at two sites. The stems were cut at the stem base (i.e. the crown) and the severity of phoma stem canker was scored on a 0 - 6 scale, based on pathogen-associated necrosis on the stem cross-section (0, no visible symptoms; 6, 100% of the stem cross-section with necrosis). There were differences between the six cultivars in severity of phoma stem canker. For example, severe cankers developed on stems of cultivar Incentive, while there were no or few cankers on cultivar Harper; this makes me understand the importance of using host resistance to control plant diseases. The experience allowed me to learn how to score phoma stem canker symptoms to measure disease severity. In addition, I have learned how to recognise symptoms and assess the severity of other oilseed rape diseases, such as light leaf spot and sclerotinia stem rot.

Etelka in the field sampling oilseed rape plants for assessing phoma stem canker severity

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The second part of my project involved identification of the causal pathogens (*L. maculans* and *L. biglobosa*) of stem canker on different cultivars. After assessment of severity of stem canker, stems with canker symptoms were taken to the lab. The diseased stem tissues were cut off the stem and placed into Eppendorf tubes for freeze-drying. The freeze-dried stem samples were then ground to powder with a pestle and mortar using aseptic techniques to prevent cross-contamination between samples. DNAmite plant kit was used to extract DNA. Extracted sample DNA was checked using a Nanodrop for quality and concentration. The fungal species present in the stem samples were determined using species-specific quantitative PCR using the SYBR green reagent. The amounts of *L. maculans* DNA or *L. biglobosa* DNA present in each stem sample were assessed in relation to the severity of phoma stem canker and the susceptibility of the cultivar. Results showed that there were significant differences between cultivars for *L. maculans* infection; for example, cultivars Harper and Fencer showed good resistance with less *L. maculans* DNA in comparison to cultivar Incentive. For *L. biglobosa,* there were also significant differences between the six cultivars; cultivar Incentive showed good resistance with less *L. biglobosa* DNA in comparison to cultivars Harper and Fencer. This suggests that cultivars with good resistance against *L. maculans* may susceptible to *L. biglobosa.* There is a need to investigate cultivars with resistance against both pathogens.

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