Leptosphaeria diseases of oilseed rape and swede: Identification and epidemiology

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by

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Leptosphaeria maculans and *L. biglobosa* cause stem canker and dry rot disease on brassica crops. Although the disease has been reported to be present in New Zealand brassica cropping areas a comprehensive assessment of the causal agent and epidemiology has not been carried out. The aim of this study was to determine the causal agent(s) and to investigate the epidemiology under New Zealand conditions. Sampling of the disease tissue characteristic to stem canker/dry rot collected from different regions revealed that both *Leptosphaeria maculans* and *L. biglobosa* are present. *Leptosphaeria maculans* was the predominant species accounting for 97% (n=127) of the colonies recovered with the remaining 3% (n=4) identified as *L. biglobosa*. Initial species identification based on colony morphology was confirmed using molecular methods (species-specific PCR and sequencing). *Leptosphaeria maculans* was recovered from both symptomatic oilseed rape (OSR) and swede plants. Determination of the mating type ratio from 39 representative isolates of *L. maculans* showed that the population deviated from the expected 1:1 mating type ratio, being 5:1 ratio (*MAT1-1:MAT1-2*), with differences between regions. The previously sequenced avirulence alleles *Avr1*, *Avr6*, and *Avr4-7* were present in the *L. maculans* with *Avr6* being the most common and amplified from all 39 isolates. The most common allele structure was a single *Avr6* (n=20), followed by multiple avirulence alleles *Avr1*, *6* (n=3), *Avr1*, *Avr6*, *4-7* (n=12), and *Avr4-7*, *6* (n=4).

Pathogenicity tests using conidial suspensions of different *L. maculans* isolates showed that this species is pathogenic on both OSR and swede, with no correlation between pathogenicity and crop origin observed. Leaf lesions which developed on the *L. maculans* inoculated seedlings were characteristic of the reported symptoms being pale grey lesions with abundant pycnidia. *Leptosphaeria maculans* progressed systemically from the leaf lesions into the petiole and the adjoining stem to cause stem lesions. Stem lesions were first observed 42 day-post inoculation (dpi) on OSR and swede in the greenhouse experiments. The systemic progression was verified by isolation of the pathogen from the symptomless petiole and stem of inoculated plants, and also from the observation of fungal hyphae in the same tissue under fluorescent microscopy. At 65 dpi, some of the inoculated OSR and swede plants were killed. Swede was more susceptible than OSR, regardless of the tolerance ranking of cultivars. Mating type did not influence disease development in either OSR or swede. Although *L. biglobosa* was also pathogenic on OSR and swede, developing characteristic leaf lesions, stem/tuber lesions did not develop on inoculated plants after 65 dpi.

Stem cankers developed both from systemic infection following leaf inoculation and also from direct conidial inoculation of the stem at the six-leaf stage for both swede and OSR. Symptom

expression resulting from direct infection was more severe on swede compared to OSR. Mature leaves of seven month old swede plants were not susceptible to either ascospore or conidial infection and dry rot symptoms did not develop on the bulbs indicating no systemic progression. Direct inoculation of mature swede bulbs with either ascospores or conidia resulted in the development of dry rot symptoms. Symptoms developed four weeks earlier with ascospore inoculation compared with conidia.

Spore trapping conducted in 2012-2013 using a 7-day Burkard and Rotorod spore sampler trapped ascospores of *L. maculans/L. biglobosa* in the field. Ascospores of both species were confirmed by nested-PCR using species-specific primers of the DNA extracted from the melinex tape, and showed that ascospores from both species were released concurrently. In field experiments, the effects of different stubble management practices on disease development in OSR were studied in two years. In 2012, there was no significant difference (P=0.844) in the disease incidence between stubble treatments (in direct drill, slashing and disking plots) and was probably due to cross-contamination by ascospores between plots. In 2013, the disease incidence in the direct drill treatment (72.7%) was significantly higher than in the ploughing (39.3%) and disking treated plots (36.0%). Results showed that the development of stem canker in the field resulted from systemic progression, with the timing of leaf lesion development coinciding with the timing of ascospore release. Systemic progression was verified by isolation of *L. maculans* from the symptomless petiole and stem of the plants which developed leaf lesions.

The effect of the combination of burial and treatment of the OSR stubble with 5% urea on the development of pseudothecia was studied. The results showed that burial stimulated stubble degradation, with urea having no significant effect (P=0.234) on stubble weight. The combination of urea and burial reduced pseudothecial development with no fruiting bodies recovered from the stubble after 17 weeks. Both bacterial and fungal diversity assessed using denaturing gradient gel electrophoresis (DGGE) differed across assessment times. The burial treatment affected the carbon utilisation profile, analysed using the MicroResp^{TM} system, of the soil microbial community associated with the stubble at the April (8 weeks) assessment, but urea application had not effect.

Overall findings from this study showed that both ascospores and conidia of *L. maculans* were able to cause stem canker/dry rot disease. The disease was initiated by ascospores in the field with the high risk period of ascospore release between May-August. The disease can be reduced by targeting and eliminating the overwintering inoculum and hence primary spore production. From this study, a combination of 5% urea application and burial of the stubble after harvest was indicated as a method to reduce inoculum carry over.

Keywords: *Leptosphaeria maculans*, *L. biglobosa*, pseudothecia, ascospores, conidia, urea, burial, 7-day Burkard spore sampler, Rotorod, disease progression, management practices, PCR-DGGE, MicroResp.