Using chlorophyll fluorescence and real-time PCR to detect *Zymoseptoria tritici* in wheat to inform decisions for disease management

Septoria tritici blotch (STB) is an important foliar disease of wheat in the UK caused by the fungus *Zymoseptoria tritici*. STB is a polycyclic disease that occurs early in the growing season, with lesions appearing on the infected leaves on which pycnidia develops as the disease progresses. Symptoms often occur 14-28 days after infection when the pathogen has established in the plant tissue and fungicide applications may be limited in their efficacy by this stage. Therefore, early detection methods as well as optimised preventative fungicide applications are essential to control the disease.

Chlorophyll Fluorescence (CF) is a rapid, non-destructive detection method for abiotic and biotic stress in plants. It is based on the principle that when absorbed light from the photosynthetic apparatus cannot be used to drive photosynthesis, the energy is dissipated as heat, or re-emitted as fluorescence radiation. Among the various CF methods used in crop science, the OJIP fluorescent transient method provides information on the structure and function of photosystem II (PSII) and the energy flux through its reaction centres (RC). There are three core fluxes during the energy cascade from PSII light absorption to electron transport; ABS which refers to the photon flux absorption by the antenna pigments per RC, TR_o which is the trapped energy flux per RC to be converted to redox energy by a reduction of the electron acceptor Q_A to Q_A^- and ET_o which is the electron transport flux per RC by re-oxidising Q_A^- to Q_A and transporting electrons to the CO_2 fixation chain. These OJIP parameters are correlated with photosynthetic efficiency (F_v'/F_m') and have been used to detect changes in the physiological status of the plant when a pathogen causes infection. Thus, this study aimed to investigate the use of CF to detect responses to fungicide treatments against STB in two different wheat genotypes and validate them using real-time PCR.

Winter wheat varieties Cougar and Dickens were grown at Sutton Bonington farm, University of Nottingham. All plots were naturally infected with STB. Five combinations of fungicides including demethylation inhibitors (DMIs), succinate dehydrogenase inhibitors (SDHIs) and multi-site fungicides applied at key growth stages (GS) were tested in comparison to untreated plots. CF measurements were taken at GS 31 on the youngest, fully-expanded leaf and at GS 39, 61 and 75 on the flag leaf. At GS 61 and 75, SPAD measurements were also taken on the flag leaf in order to determine chlorophyll content. Furthermore, visual disease assessment of randomly selected tillers from each plot was undertaken in order to record the percentage leaf area infected with the pathogen at GS 31, 61 and 75. Only the flag leaf at GS 61 was used for DNA extraction and quantification of fungal DNA using real-time PCR.

Dimitra working in the field trial

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Differences in resistance between the varieties were shown early in the season (GS 31) whereas the effect of the fungicide treatments on disease severity was most apparent at GS 61 and 75. Dickens was found to be more susceptible than Cougar and was characterised by higher ET_o/RC and lower PSII efficiency (F_v'/F_m') irrespective of fungicide treatment. Treatments that included an SDHI significantly reduced disease severity and increased chlorophyll content compared to DMI based treatments and multi-site compounds. In addition, the two varieties responded differently to fungicide treatments, for example disease severity was lower in Cougar when protected early in the season whilst Dickens benefited from treatments applied once the flag leaf emerged. Effective fungicide applications of DMI, SDHI and multi-side compounds reduced ABS/RC, TR_o/RC and ET_o/RC and increased F_v'/F_m' compared to the untreated control. In agreement with the observed differences in disease severity and CF, more pathogen DNA was quantified in asymptomatic flag leaves of Dickens than in Cougar, whereas the treatment that included the mixture of DMI, SDHI and multi-side compounds resulted in the lowest fungal DNA present.

Overall, this project demonstrated the potential of CF to detect changes in the photosynthetic activity of the crops due to biotic stress caused by STB, as well as an insight on the efficacy of SDHIs against STB.

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