Fusarium crown rot detected in-crop using thermal imagery and quantified reduced water use and yield in bread and durum wheat

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Abstract
The cereal disease Fusarium crown rot (FCR), caused by the fungus *Fusarium pseudograminearum,* is a major challenge to winter cereal production in the northern grains region in Australia. A replicated glasshouse experiment, using 1.2 m growth tubes to simulate field conditions, explored water use and associated grain yield in two bread wheat and two durum wheat varieties in the presence and absence of FCR infection. Infection with FCR reduced grain yield on average by 9.5% and water use by 7.5% across all four cultivars. A field study examined the potential of thermal imagery to discriminate between FCR infected and uninfected plots based on canopy temperature. Identification of infected plots at stages before visual symptoms (basal browning) were expressed occurred with temperature increases of approximately 0.9°C compared to non-infected treatments. The mechanistic link between decreased water use with FCR infection and increased canopy temperature extends current understanding of the activity of FCR infection in cereal plants. The management implications of these preliminary findings will be evaluated in further studies.

Keywords
Disease interactions, water optimisation

Introduction

Fusarium crown rot (FCR) caused by *Fusarium pseudograminearum* (*Fp*) is a major fungal disease responsible for yield and quality downgrades of wheat in northern grains region, including New South Wales and cropping regions of Queensland. The disease restricts the plants ability to transfer solutes and water from roots to shoots causing significant productivity loss (Kazan & Gardiner, 2018). FCR has been estimated to cost the Australian wheat industry a total of $88 million in yield and quality losses annually (Murray & Brennan, 2009). The vascular disruption of the plant decreases nitrogen use efficiency (NUE) and is thought to decrease water use efficiency (WUE) (Davis et al., 2009). The impact of the disease is exacerbated in seasons with dry finishes, which are common in northern grains region. Consequently, the plant fails to achieve protein and yield potential, but the impact on plant water use and transpiration remains unknown. It is assumed that vascular disruption caused by the presence of the fungus in the xylem and phloem, as determined in microscopy studies, reduces the supply of water and other resources during periods of peak demand. However, this actual relationship and physiology of FCR infection has not previously been reported.

Detection of FCR in-crop is labour intensive and rarely spatially quantified at a paddock scale. Humpal et al. (2020) successfully identified FCR in pre-symptomatic plants using proximal near infrared sensing and machine learning models, however accuracy decreased significantly when distance between sensor and leaf increased. Further alternatives include molecular techniques to determine the presence of the fungal DNA in plant tissue and while accurate, this is both destructive and costly because of the large number of samples required for spatial analysis. Changes in plant transpiration, leading to a temperature increase within the plant, could provide opportunity for pre-symptomatic detection of infection which could serve as a proxy for selecting genotypes with improved FCR resistance.

The objective of this work was to determine the influence of FCR infection on wheat water use as well as explore the use of thermal imagery for early identification of FCR at a field scale.

Methods

Glasshouse experiment

Soil, tube design and FCR treatments: Polyvinyl chloride (PVC) soil tubes 150 mm diameter x 1200 mm length were used to simulate a field soil profile. The soil used was a Grey Dermosol with a Plant Available Water Capacity of 202 mm/m and starting nitrogen (N) of 36.4 mg N/kg nitrate and 3.8 mg N/kg ammonium. The lower subsoil (bottom 900 mm) was compacted to a bulk density of 1.28 g cm^{-3} and the upper topsoil (top 260 mm) was compacted to a bulk density of 1.1 g cm^{-3}. Two FCR treatments were used, uninoculated and inoculated. The inoculated treatment contained a band of 20 mm of inoculated soil. This was prepared by adding ground FCR infected seed (0.5 - 2 mm fraction) evenly mixed throughout soil at rates of 1 g inoculum / 100 g of soil (Forknall et al., 2019). The uninoculated treatment had 20 mm of clean soil in a similar manner. A further 10 mm of soil was then added to both treatments to minimise colonisation

of the fungus across the soil surface during the experiment. *Plant materials and growing conditions:* Two bread wheat varieties, LPRB Lancer and LPRB Flanker and two durum wheat varieties, DBA Lillaroi and EGA Bellaroi were grown over a six-month period. Seed was treated with Vibrance® and Emerge® at rates of 360 mL/100 kg and 240 mL/100 kg, respectively for standard bunt and smut control and early protection against aphids. Five seeds of each cultivar were sown 4 cm below soil surface and thinned to four plants per tube upon establishment. There were five replicates of each cultivar and treatment. The experiment was conducted in an air-conditioned polyhouse complex at Tamworth Agricultural Institute (TAI), Tamworth New South Wales with a 25˚C day and external ambient night temperature regime. Soil tubes were individually weighed and watered to field capacity each week until flowering. Post flowering, the mild water stressed treatments were managed to 40% of field capacity (-100 kPa matric potential), whilst the non-stressed treatment maintained the original watering regime. At three weeks prior to harvest, soil tubes were not watered for a week to dry the soil column down for core removal and water content was maintained at this level for the remainder of the experiment. Water use per tube was gravimetrically determined for the entirety of the experiment. At GS45 for the bread wheats and GS49 for the durum, soil tubes were treated with the equivalent of 130 kg/ha of potassium nitrate and 120 kg/ha of urea to equate to 49.4 kg/ha of K and 72 kg/ha of N to address deficiency symptoms. *In crop measurements:* Plants were visually scored for the severity of FCR infection on the basis of the extent of browning of tiller bases using a 1-3 scale at GS55 (Forknall et al., 2019). This confirmed all the FCR inoculated treatments physically displayed signs of infection and that no infection occurred in the non-inoculated treatment. Stomatal conductance and leaf surface temperature were also recorded monthly from the onset of jointing to flowering using a LI-6400XT (Biosciences, 2002). Immediately prior to harvest counts were taken of plants, tillers, heads, whiteheads and late maturing heads (small spikelets, less than half the height of the other heads). Main stem heads from each plant were removed followed by their stems, which were cut 5 mm above soil surface. The remainder of the heads and stems were then collected, keeping the white and late maturing heads separate. Both heads and stems where dried at 40°C for 72 h prior to weighing. *Head analysis:* Grain was threshed from the collected heads from the four main stems of plants in each soil tube. Remaining viable heads and whiteheads / late maturing heads were collected separately and threshed to recover grain. All resulting residue was collected and bulked together for plants grown in each soil tube then ground through a 2 mm sieve. Grain weights and counts for main stems, mature heads and white/late maturing heads were taken separately. Main stem sections were plated onto 1/4 strength potato dextrose agar (PDA) + novobiocin medium to confirm infection and percentage vertical colonisation by *Fp*.

Thermal Measurement in Field Experiment

A field trial in Northern NSW (Piallamore, 31°10'16.5"S, 151°03'35.1"E was established with three bread wheat (LRPB Hellfire, LRPB Lancer, Suntop) and three durum wheat varieties (Jandaroi, DBA Lillaroi, DBA Aurora). The trial was inoculated with FCR treatments as well as uninoculated (background) treatments. The experiment was conducted during the 2020 winter growing period. *Equipment and design:* A drone with a radiometric thermal camera (Parrot Anafi Thermal ®) conducted regular fortnightly flights over the field experiment where both thermal and RGB (visual) images were captured. The drone was flown at the same geo-spatial parameters (height, speed, overlap, angle) and under optimum solar radiance (11 am -3 pm) to capture timing of greatest evaporative demand of the crop. Images were stitched together using Pix4D Mapper® and data exported to QGIS 2.8.1® for spatial summary of mean canopy temperature for the trial plots.

The statistical software package R (R Core Team 2019) was used to fit linear models to the datasets, model diagnostics were checked and where necessary, data was transformed to uphold model assumptions. Post hoc multiple comparisons were performed using Tukey's method (package: lsmeans)

Results and discussion

Glasshouse experiment

Plating stem sections for the pot trial confirmed inoculation of the pots produced 100% infection in those treatments. Infection with FCR reduced grain yield in the pot experiment by 9.5% overall (P=0.014) for inoculated treatments when compared with uninoculated treatments (Figure 1). Yield penalties over 40% have been regularly recorded with FCR infection in replicated field experiments (Simpfendorfer et al., 2020). Hence, the magnitude of yield loss from FCR measured in this experiment was not as great as previously observed under field conditions. This may have been a result of the watering regime. While the experimental protocol was designed from laboratory measurements of the moisture release curve, soil packing in the pots resulted in different structure to that tested in the laboratory. This resulted in wetter than anticipated soil

conditions with the two watering regimes. Not all genotypes responded similarly to the water treatments in the glasshouse, with Lillaroi performing better under wetter treatments while Lancer yielded more when exposed to mild moisture stress compared to all other varieties (P=0.001). Compared to the durum wheat varieties, the bread wheat yield was significantly reduced under the non-water stress treatment (Figure 1).

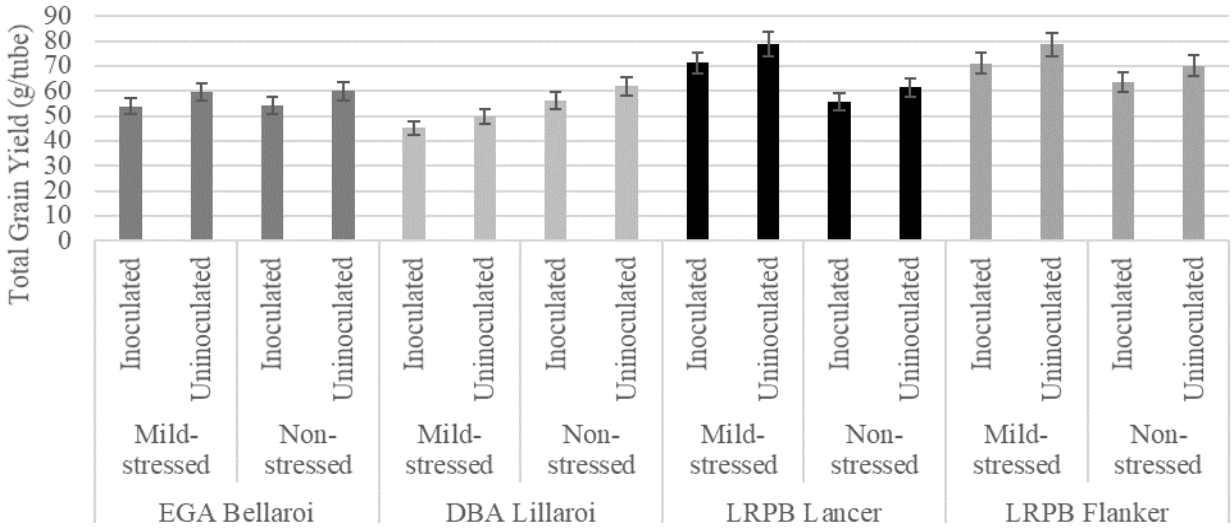

Figure 1. Grain yield of wheat as whole growth tube (four plants) grown with FCR (inoculated and uninoculated) and water (mild-stressed and non-stressed) treatments. Error bars indicate standard error. Conducted at TAI 2020

Water use measurements of the soil tubes demonstrated a range of 22-38 L across all treatments over the duration of the experiment (Figure 2). Infection with FCR decreased water use of plants by 7.5% overall (P=0.047, Figure 2). Measurements of stomatal conductance and leaf temperatures in the glasshouse were variable and highly dependent on the cycling of the climate control, which impacted the strength of the differences, however in some cases mirrored total water use findings. Lillaroi and Flanker demonstrated greater differences between the mild- and non-stressed treatments as compared to Lancer and Bellaroi. Lillaroi and Flanker used significantly less water in the mild-stress treatments compared to the non-stressed treatments.

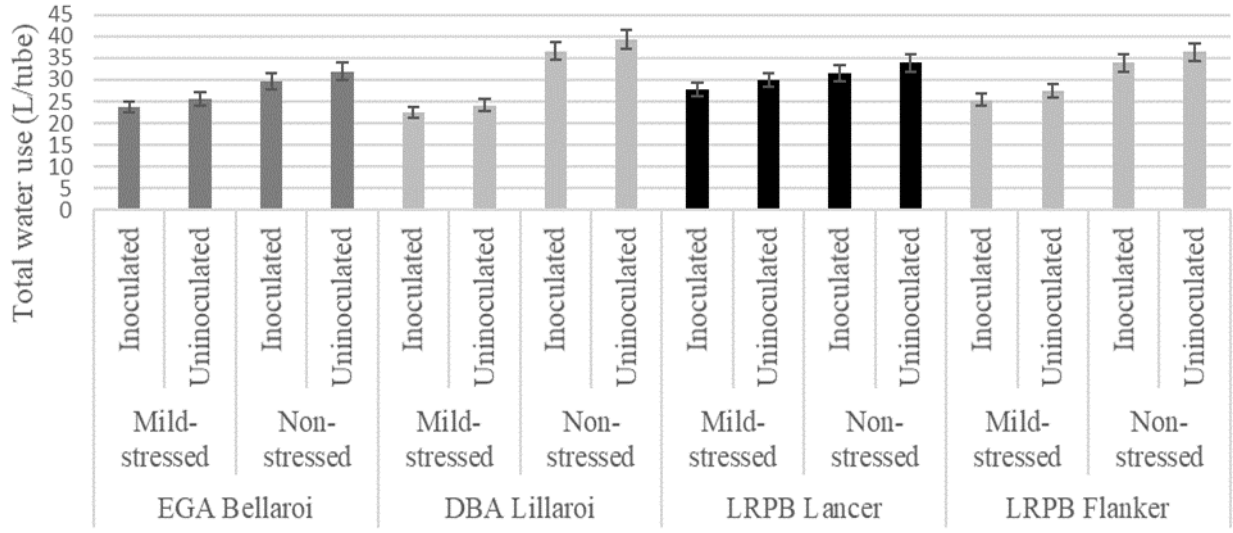

Figure 2. Total water use of wheat plants as the whole growth tube (four plants) grown with FCR (inoculated and uninoculated) and water (mild-stressed and non-stressed) treatments. Error bars indicate standard error. Conducted at TAI 2020

Thermal Measurement in Field Experiment

Thermal imagery was able to detect canopy temperature differences across the field experiment. Ambient temperature determined the timing of drone flights to optimise potential for detection of treatment differences with best results achieved during periods of peak transpirational stress. Initial thermal surveys taken at GS30 indicated up to 4 °C difference in canopy temperature between cultivars thought to be related to varietal canopy structures. There was successful identification of FCR infection when compared to

uninfected plots with an average increase in canopy temperature of 0.9 °C (P= <0.001, Figure 3). The strongest treatment differences were seen as early as GS30 (prior to visual symptoms of basal browning were evident).

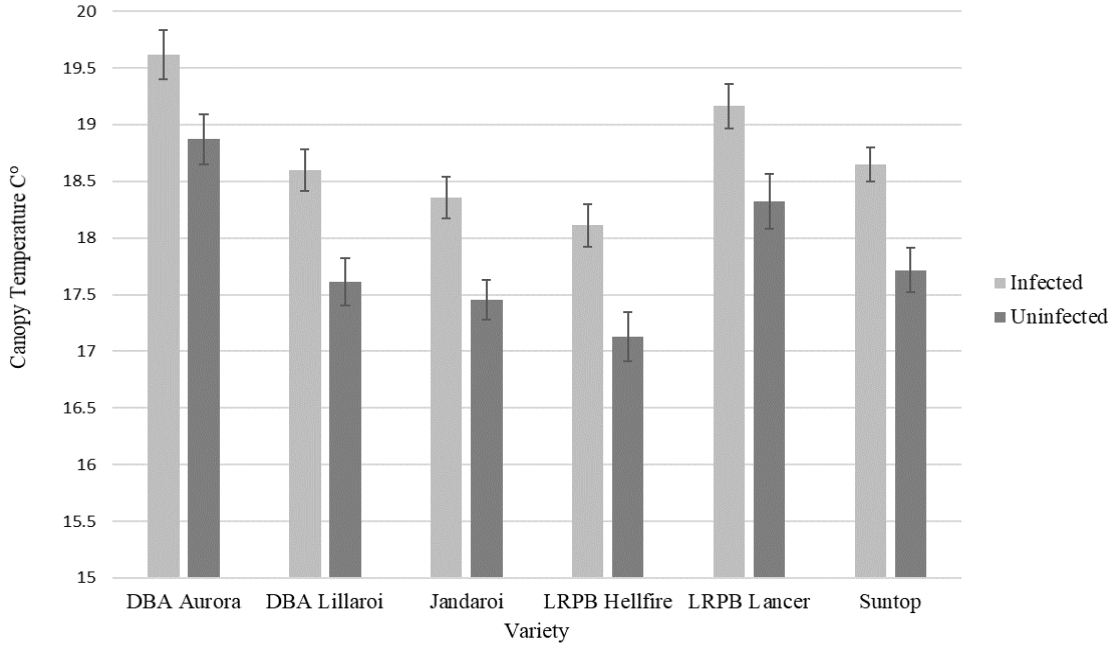

Figure 3. Canopy temperature at GS30 (pre-symptomatic of visual browning) at Piallamore, NSW trial site 2020. Error bars indicate standard error. Temperatures recorded using Parrot Anafi Thermal ® and processed using Pix4D Mapper ® and QGIS 2.8.1 ®.

Conclusion

While this study reports a logical extension of current understanding for how FCR reduces yield, there is limited literature that confirms the reduction of water use by wheat infected with FCR due to a reduction in transpiration caused by FCR infection. The canopy temperature changes suggest reduced water use maybe due to interruption of xylem vessels rather than due to reduced plant size and less surface area for transpiration, though this will also ultimately reduce water use. Infection of plants by FCR was measurable using thermal imagery prior to visual symptoms. This is a novel and unique detection method that may have future applications in disease screening both in the paddock or in glasshouse studies. However, care will need to be taken when considering local environmental conditions to optimise detection.

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