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Remote detection of Fusarium crown rot in broadacre bread wheat and durum wheat through use of aerial imagery

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ABSTRACT

Context. The cereal disease Fusarium crown rot (FCR), caused by the fungal pathogen *Fusarium pseudograminearum*, is a worldwide major constraint to winter cereal production, especially in Australia's northern grain region of New South Wales and Queensland. **Aims.** Detection of the disease is labour-intensive and often not spatially quantifiable; hence, the aim of this study was to provide methods for in-crop FCR detection on a broadacre scale. **Methods.** A replicated field experiment across three locations in northern New South Wales explored the use of thermal and multispectral imagery and hyperspectral reflectance data for the spatial detection of FCR in three bread wheat (*Triticum aestivum* L.) and three durum wheat (*T. durum* Desf.) varieties in the presence and absence of inoculation with *F. pseudograminearum*. **Key results.** Canopy temperature was 0.30–0.90°C higher in two-thirds of field sites inoculated with the pathogen during early wheat growth in a slightly wetter than normal season. Some multispectral indices including normalised difference red edge, normalised difference vegetation index, near infrared and red edge also demonstrated the ability to identify inoculated versus uninoculated treatments as early as the first node stage (GS31). **Conclusions.** Although positive identification was achieved with remote detection, environmental conditions (i.e. soil-water availability and ambient temperature) and physiological maturity influenced the accuracy of the technology for detecting FCR infection, particularly in wetter early-season conditions. **Implications.** Early spatial detection of FCR infection on a broadacre scale could allow producers to manage this disease spatially through better agronomic decisions.

Keywords: aerial imagery, Fusarium crown rot, *Fusarium pseudograminearum*, remote disease detection, remote sensing, stubble borne disease, thermal reflectance, wheat.

Introduction

Fusarium crown rot (FCR), caused by *Fusarium pseudograminearum* (*Fp*), is recognised globally as a major limitation to wheat production (Kazan and Gardiner 2018; Petronaitis *et al.* 2021). The disease is responsible for yield reduction and quality downgrades in wheat worldwide, but particularly in the northern grain region of Australia, including New South Wales (NSW) and cropping regions of Queensland, owing to the typically low rainfall at peak water demand (Simpfendorfer *et al.* 2019; Petronaitis *et al.* 2021). The disease restricts the plant's ability to transfer solutes and water from roots to shoots, causing significant productivity loss (Kazan and Gardiner 2018). FCR has recently been estimated to cost the Australian wheat industry approximately AU$404 million in yield and quality losses annually, with $112 million of this loss occurring in the northern grains region (Hollaway *et al.* 2022). Furthermore, spatial detection of FCR in-crop is not possible at present, with the traditional pathological and visual identification of infection being labour-intensive and localised to hand-sampled locations and often requiring specialised equipment (Alahmad *et al.* 2018).

Remote sensing can be useful for measuring the heterogeneity of crop health on a broadacre scale (Franke and Menz 2007). Remote unmanned aerial vehicles (UAVs) have become a useful additional management tool in a range of broadacre agricultural systems, including for disease detection (West *et al.* 2017; Bohnenkamp *et al.* 2019;

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Francesconi *et al.* 2021). Plant spectral reflectance across the electromagnetic spectrum can often change in relation to the metabolic status of the plant (Sankaran and Ehsani 2014). This is most often observed in reduced chlorophyll content following infection with many rust and other leaf-pigment-altering pathogens (He *et al.* 2018). These changes are often observed in the visible spectrum (VIS) as well as red edge and near-infrared (NIR) regions (380–780 nm, 650–780 nm and 800–2500 nm, respectively) (Franke and Menz 2007). NIR using five bands (900–1700 nm) has been demonstrated for non-destructive discrimination between *Fp*-infected and non-infected wheat plants at the seedling stage with an accuracy of 55–100% at 3–11 weeks after infection under glasshouse conditions (Humpal *et al.* 2020*a*). Changes in spectral reflectance are only indicative of a change in homogeneity of crop health, size or biomass and are unable to confirm infection or cause of disease without ground-truthing of locations of interest (Bhandari *et al.* 2018; Nagai *et al.* 2020). These observed wavelengths are commonly incorporated into specific equations and referred to as multi-spectral indices, with common examples including the normalised difference red edge (NDRE), normalised difference vegetation index (NDVI), and the NIR and red edge (Su *et al.* 2018; Boiarskii and Hasegawa 2019).

Thermal imagery can also be used in disease detection, but unlike multispectral reflectance, thermal reflectance can have stronger metabolic links to changes in plant transpiration status, which can be a surrogate indicator for disease (Das *et al.* 2021). Stressed wheat plants often upregulate solute transport and metabolic activity (Cox and Boersma 1967; Li *et al.* 2017). In FCR-affected wheat plants, *Fp* mycelial growth colonises the xylem tissue of the vascular bundles, which results in a restriction of solute transport throughout the plant canopy (Burgess 2014; Knight and Sutherland 2016; Buster *et al.* 2022). It is hypothesised that this restriction increases plant canopy temperature owing to reduced transpirative cooling (Buster *et al.* 2022). Furthermore, it is hypothesised that, as infected wheat plants mature, the effect of *Fp* colonisation of the xylem would increase and exacerbate the increase in canopy temperature by impeding fluid transport and reducing transpiration.

This study investigated the potential of both multispectral and thermal (8–14 μm) reflectance to detect FCR in bread wheat (*Triticum aestivum* L.) and durum wheat (*T. durum* Desf.). Previous work has been conducted to explore the prospects for FCR detection in wheat using NIR sensors (Humpal *et al.* 2020*b*). However, the success of that study was limited to controlled environments with contact NIR sensors, because the accuracy decreased significantly when the sensors were removed from the plant tissue. The work is beneficial in breeding and pre-breeding screening for FCR-tolerant varieties, but not at a field scale (Humpal *et al.* 2020*b*). The benefit of this study is the early and spatially quantifiable detection of FCR infection on a field scale, which could allow growers to manage high risk areas differentially in order to minimise productivity losses and optimise profitability.

Materials and methods

Location and soil characteristics

Field experiments were conducted across three research stations in northwest NSW: Liverpool Plains Research Station (LPRS), Breeza; Australian Cotton Research Institute (ACRI), Narrabri; and Piallamore. The experiments were repeated in sequential years across the 2020 and 2021 winter growing seasons. Details of the site locations, plant available water (PAW) capacity, sowing and rainfall information were reported previously (Buster *et al.* 2022); however, both seasons were wetter and cooler than average for these locations.

Plant materials and growing condition

Three spring bread wheat varieties (LPRB Lancer, LPRB Hellfire and Suntop) and three durum wheat varieties (DBA Lillaroi, DBA Aurora and Jandaroi) were grown in each of the two experimental years across each site. Two water scenarios were created: a natural rainfed treatment, and supplementary irrigation to represent a higher rainfall scenario. Four nitrogen (N) treatments were included in the study for comparison of upfront and split applications of N as urea to support yield potential at both decile 5 and decile 9 rainfall scenarios at each site. This paper reports on the detection of FCR infection, and not specifically on water or N treatments, which have been reported previously (Buster *et al.* 2023).

The pathology of this field trial was described in Buster *et al.* (2022), where visual severity of FCR infection (crown rot index) was assessed post-harvest, demonstrating a significant effect of inoculation treatment at all three sites in the 2020 growing season. Uninoculated treatments had a crown rot index of 12–28%, whereas inoculated treatments ranged from 35% to 66% (Buster *et al.* 2022). FCR severity was not measured in the 2021 season owing to exceedingly wet conditions; however, this environment facilitated growth of many stubble- and soil-borne pathogens including *Fp* and *Alternaria* spp. FCR-induced yield penalties of 6–18% were observed in the inoculated treatment compared with the uninoculated treatment in 2020. However, because of the wet seasonal conditions, FCR yield penalties were not observed at any of the three sites in the 2021 season.

UAV field equipment and measurements

Two UAVs were used. One, providing thermal data, was fitted with a radiometric thermal camera (ANAFI Thermal; Parrot, Paris, France: sensor FLIR Lepton 3.5 microbolometer, sensor resolution 160 × 120, spectral band 8–14 μm, thermal

sensitivity <50 mK (0.050°C)). The other UAV, providing multispectral data, was fitted with a Parrot Sequoia camera and sunshine sensor including four monochrome sensors (pixel size 3.75 μm, focal length 3.98 mm, resolution 1280 × 960; spectral band width: green 530–570 nm, red 640–680 nm, red edge 730–740 nm, NIR 770–810 nm). The two UAVs were used for the duration of the field experiments by conducting flights at predefined wheat growth stages when suitable flying conditions prevailed. Surveys were conducted at a range of crop maturity stages including GS31 (stem elongation, first node present on main stem), GS39 (flag leaf on main stem), GS50 (ear emergence), GS65 (50% of anthers mature), and GS70 (start of grain fill, grain watery ripe) (Zadoks *et al.* 1974). GS31 precedes the onset of the visual crown browning symptoms of FCR, whereas all other growth stages follow the development of visual symptoms, and we expect that yield components would show increasing influence of FCR as growth stages progress. The UAVs were flown at the same geospatial parameters (40 m AGM, 85% overlap, 90° angle and self-calculated speed) and under optimum solar radiance (11:00–15:00) in order to capture timing of greatest evaporative demand within the crops. All flights were controlled via a Pix4D capture application on a tablet device.

Equations for calculated multispectral indices are as follows:

$$\mathrm{NDRE} = (\mathrm{NIR} - \text{red edge})/(\mathrm{NIR} + \text{red edge})$$

$$\mathrm{NDVI} = (\mathrm{NIR} - \mathrm{red})/(\mathrm{NIR} + \mathrm{red})$$

Hyperspectral field equipment and measurements

For the second year of the field experiments (2021), hyperspectral measurements were taken during the growing season. The spectral reflectance measurements were undertaken with an ASD FieldSpec 4 Hi-Res spectrometer (350–2500 nm) (Malvern Panalytical, Malvern, UK) fitted with a Leaf Clip attachment with a sampling resolution of 1 nm. This attachment provided a target area of interest of 2 cm, and because it delivered its own light source from a 4.25 V, 4.5 W halogen lamp (MR6), the measurements were not subject to the influences of differing ambient light conditions caused by intermittent cloud cover. Measurements were obtained at two growth stages for each experimental site GS39 (full flag leaf emergence) and GS69 (grain watery ripe). Two plants were selected at random from the middle row within each plot and were ~2 m from either end of the experimental plot. The newest fully unspooled leaf was selected and placed in the Leaf Clip ~4 cm from the leaf tip, ensuring full coverage of the target area.

Image processing analysis

All images acquired were separated into sensor type and specific flight then stitched together using Pix4D Mapper software (Pix4D, Lausanne, Switzerland). Within Pix4D Mapper, the thermal images were processed under Thermomap and the multispectral images were processed under Ag Multispectral. Visioning theory was applied to compare conjugate points in overlapping images and determine their relative positions and orientations by bundle block adjustment (Bollard-Breen *et al.* 2015). The software produced a radiometric calibrated orthomosaic for thermal and individual orthomosaics of NDRE, NDVI, NIR and red edge from each flight, which was then exported to QGIS 3.2.1 (www.qgis.org, Open Source Geospatial Foundation, Chicago, IL, USA) for spatial summary of mean canopy temperature and previous identified indices for the field experimental plots. Each orthomosaic was processed to remove all non-plant material from the assessed plot following methods outlined in (Parker *et al.* 2020) allowing a true representation of crop reflectance. A vector grid with a spatial coverage of 6 m × 1 m was used to extract the reflectance values from each plot.

Statistical analyses

The statistical software package R (R Foundation for Statistical Computing, Vienna, Austria) was used to fit generalised additive models (GAM package 'mgcv'; Wood 2011) to each survey including a full tensor product smooth to account for underlying spatial variation, and an ANOVA table was extracted from the model. Each model included the survey data as the response variable with inoculation, watering regime, N application, cultivar treatments and replicate (block) fitted as response variables with all interaction terms. A stepwise model simplification function was used to reduce models to the most parsimonious form based on the Akaike information criterion. The inoculation predictor variable was always included in these models. Model diagnostics of the simplest model were assessed to ensure model assumptions were upheld. *Post hoc* multiple comparisons were performed to determine statistical significance, and least significant difference (l.s.d.) was calculated as an approximate measure of the sensitivity of the survey method using the 'agricolae' package (de Mendiburu 2019).

The predictive association between the hyperspectral data and infection category was assessed with partial least square discriminant analysis (PLS-DA). This analysis uses a PLS regression model that transforms a set of correlated dependent variables into a new set of uncorrelated variables and regresses these against dichotomous categorical independent variables (infection category in this case), thereby addressing multicollinearity of variables (Serrano-Cinca and Gutiérrez-Nieto 2013). For the localities of LPRS and Piallamore and the GS39 and GS69 data, PLS-DA was undertaken using the package 'ropls' (Thévenot *et al.* 2015) and function 'opls' in R ver. 4.2.1. Following data exploration, the analysis was undertaken with one predictive axis and one orthogonal axis (to enable basic plotting). Analyses were also undertaken

with automatically generated predictive axes, and the results were compared with the above to ensure that axes selection did not produce different outcomes. *P*-values were generated using 100 permutations as specified in the 'opls' function.

Results

Measurements of canopy temperature made using thermal imagery identified *Fp*-inoculated versus uninoculated treatments at both ACRI and Piallamore in 71% of surveys between GS31 and GS65 in 2020 (Table 1). Detection of FCR was not achieved at LPRS in 2020. The largest differentiation in mean canopy temperature was identified at GS31 at both ACRI and Piallamore (0.67°C and 0.90°C, respectively; Table 1). Typically, statistical significance was observed early in crop growth (before GS50), only once at GS50, and not at GS65. Of the thermal surveys conducted during 2020, 50% measured a significant increase in canopy temperature for inoculated compared with uninoculated treatments ($P < 0.05$; Table 1); although 90% of the thermal surveys measured an increase in canopy temperature, many did not reach statistical significance. Correct identification of *Fp*-inoculated versus uninoculated treatments through an increase in mean canopy temperature was not achieved in the 2021 growing season at any site (Table 2). The l.s.d. values calculated using this imagery indicated that thermal differences of greater than an average of 0.165°C in 2020 could be detected as different (Table 1); however, the l.s.d. values from 2021 were approximately twice as large as those seen in 2020, at 0.35°C (Table 2).

Multispectral imagery distinguished *Fp*-inoculated from uninoculated treatments at both ACRI and Piallamore at GS31 in 2020 (Table 3). Correct identification of inoculation was not achieved at LPRS in 2020 with any of the four indices. At ACRI, the indices NDRE, NDVI and NIR all correctly identified inoculated versus uninoculated treatments, whereas red edge did not ($P = 0.124$). At Piallamore, NDVI and red edge both correctly identified inoculated versus uninoculated treatments, whereas NDRE was unable to distinguish between treatments ($P = 0.19$). NIR was unavailable for Piallamore in 2020 owing to a sensor error. Of the multispectral surveys conducted during 2020, 64% measured a significant decrease in respective indices for inoculated compared with uninoculated treatments ($P < 0.05$; Table 3); although 91% of the multispectral surveys measured a decrease in respective indices, some were of insufficient magnitude to reach statistical significance. No statistical significance was observed with any of the multispectral indices at any site in 2021 (Table 4).

The association between hyperspectral reflectance and FCR infection was determined by PLS-DA. The proportion of the variation in the data explained by the predictive axes is quantified by the R^2X term (Table 5). These measures indicate that the reflectance data were highly consistent and structured. Nonetheless, the R^2Y term, which represents the proportion of variation explained by the infection classification of FCR, was extremely low and ranged between 0.23% and 0.54%. Cross-validation of the model allowed calculation of the predictive power of the model and is represented by Q^2. These values were also very small relative to the R^2Y values, indicating that the model has limited predictive capability (Table 5). When 100 permutations were run, randomly reshuffling infection labels to the model, the proportion of those models that had predictive power equivalent to or greater than the real model (represented by PR^2Y and PQ^2 values) was quite high, indicating that the capacity of the hyperspectral data to predict infection status using PLS-DA was strongly limited under these experimental conditions.

Table 1. Mean canopy temperatures of three bread wheat (LRPB Lancer, Suntop and LRPB Hellfire) and three durum wheat (DBA Lillaroi, Jandaroi and DBA Aurora) varieties at multiple growth stages (GS) when inoculated or uninoculated with *Fusarium pseudograminearum* across three trial sites in 2020: Australian Cotton Research Institute (ACRI), Liverpool Plains Research Station (LPRS) and Piallamore.

Site	GS	*P*-value (infection)	R^2	Mean inoculated temp. (°C)	Mean uninoculated temp (°C)	Difference (°C)	l.s.d. ($P = 0.05$)
ACRI	31	0.03*	0.31	22.53 (2.18)	21.86 (1.95)	0.67	0.40
ACRI	39	0.02*	0.48	12.82 (1.00)	12.56 (0.99)	0.26	0.17
ACRI	50	0.01*	0.54	16.44 (1.28)	16.07 (0.99)	0.37	0.18
ACRI	65	0.56	0.70	23.07 (1.76)	23.17 (1.87)	−0.10	0.23
LPRS	31	0.52	0.79	18.09 (1.05)	18.00 (0.97)	0.09	0.11
LPRS	50	0.88	0.84	27.21 (2.56)	27.18 (1.81)	0.03	0.21
LPRS	65	0.71	0.96	25.68 (1.78)	25.62 (1.76)	0.05	0.09
Piallamore	31	<0.001*	0.92	18.75 (0.99)	17.85 (1.14)	0.90	0.08
Piallamore	39	<0.001*	0.92	14.48 (0.87)	14.17 (0.83)	0.30	0.06
Piallamore	50	0.20	0.87	24.88 (1.36)	24.77 (1.37)	0.10	0.12

P-value represents the significance of infection response from the ANOVA table (* indicates $P < 0.05$). Standard deviation is given in parentheses following the mean temperature. Generalised R^2 represents the model fit.

Table 2. Mean canopy temperatures of three bread wheat (LRPB Lancer, Suntop and LRPB Hellfire) and three durum wheat (DBA Lillaroi, Jandaroi and DBA Aurora) varieties at multiple growth stages (GS) when inoculated or uninoculated with *Fusarium pseudograminearum* across three trial sites in 2021: Australian Cotton Research Institute (ACRI), Liverpool Plains Research Station (LPRS) and Piallamore.

Site	GS	*P*-value (infection)	R^2	Mean inoculated temp. (°C)	Mean uninoculated temp. (°C)	Difference (°C)	l.s.d. ($P = 0.05$)
ACRI	31	0.62	0.40	18.00 (2.09)	17.90 (2.03)	0.10	0.37
ACRI	39	0.32	0.48	24.69 (1.00)	24.91 (0.98)	−0.23	0.62
ACRI	65	0.53	0.65	42.42 (1.76)	42.38 (1.87)	0.04	0.36
LPRS	39	0.77	0.09	32.83 (2.56)	32.92 (2.68)	−0.09	0.58
LPRS	65	0.24	0.24	27.20 (1.48)	26.95 (1.63)	0.25	0.32
Piallamore	39	0.23	0.35	26.53 (2.35)	26.53 (2.54)	−0.01	0.46
Piallamore	50	0.72	0.68	29.74 (1.20)	29.78 (1.30)	−0.04	0.16
Piallamore	65	0.08	0.53	33.61 (1.29)	33.91 (1.55)	−0.29	0.23
Piallamore	70	0.17	0.84	24.83 (0.68)	24.82 (0.73)	0.00	0.07

P-value represents the significance of infection response from the ANOVA table. Standard deviation is given in parentheses following the mean temperature. Generalised R^2 represents the model fit.

Table 3. Mean canopy multispectral reflectance (NDRE, normalised difference red edge; NDVI, normalised difference vegetation index; NIR, near infrared; and red edge) of three bread wheat (LRPB Lancer, Suntop and LRPB Hellfire) and three durum wheat (DBA Lillaroi, Jandaroi and DBA Aurora) varieties at multiple growth stages (GS) when inoculated or uninoculated with *Fusarium pseudograminearum* across three trial sites in 2020: Australian Cotton Research Institute (ACRI), Liverpool Plains Research Station (LPRS) and Piallamore.

Site	Index	GS	*P*-value (infection)	R^2	Mean inoculated reflectance	Mean uninoculated reflectance	Difference	l.s.d. ($P = 0.05$)
ACRI	NDRE	31	0.005*	0.60	0.272 (0.016)	0.282 (0.013)	0.011	0.002
ACRI	NDVI	31	0.000*	0.50	0.893 (0.016)	0.908 (0.010)	0.015	0.002
ACRI	NIR	31	0.040*	0.46	0.367 (0.036)	0.405 (0.040)	0.038	0.007
ACRI	Red edge	31	0.124	0.56	0.212 (0.019)	0.229 (0.021)	0.017	0.004
LPRS	NDRE	39	0.474	0.80	0.245 (0.017)	0.245 (0.017)	0.000	0.002
LPRS	NDVI	39	0.240	0.72	0.888 (0.018)	0.890 (0.015)	0.002	0.002
LPRS	NIR	39	0.625	0.74	0.386 (0.038)	0.387 (0.034)	0.001	0.004
LPRS	Red edge	39	0.526	0.74	0.234 (0.017)	0.234 (0.015)	0.001	0.002
Piallamore	NDRE	31	0.191	0.68	0.127 (0.014)	0.139 (0.015)	0.012	0.002
Piallamore	NDVI	31	0.003*	0.84	0.148 (0.012)	0.167 (0.013)	0.020	0.001
Piallamore	Red edge	31	0.003*	0.84	0.148 (0.012)	0.167 (0.013)	0.020	0.001

P-value represents the significance of infection response from the ANOVA table (* indicates $P < 0.05$). Standard deviation is given in parentheses following the mean reflectance. Generalised R^2 represents the model fit.

Discussion

To the best of our knowledge, this is the first study to demonstrate the potential of remote thermal and multispectral imagery for spatial FCR detection in wheat under field conditions. Previous studies have had varying success under controlled environmental conditions or through the use of proximal (direct contact) sensors (Humpal *et al.* 2020*b*; Xie *et al.* 2021). We observed increased leaf surface temperature, confirming the postulated physiological link between *Fp* colonisation of xylem tissue (Knight and Sutherland 2016) and decreased transpiration and water use (Buster *et al.* 2022). In the 2020 season, thermal imagery was particularly effective at detecting infection early in the season (prior to GS50). Thermal detection identified *Fp*-infected plots before any visual changes (i.e. browning of crown) were evident. This observation is similar to what has been reported with NIR in pot studies (Humpal *et al.* 2020*b*), specifically that identification of FCR infection was possible even 3 weeks post-inoculation (approximately GS22).

Early detection of FCR infection could allow growers to manage their crop to minimise losses from this disease, for example, through decreased or spatially optimised in-crop N application. Buster *et al.* (2023) demonstrated that N applications commensurate with achieving maximal yield and high protein levels increase FCR severity, decrease N use efficiency, or reduce the return on investment for N applications. Another management option could be the

Table 4. Mean canopy multispectral reflectance (NDRE, normalised difference red edge; NDVI, normalised difference vegetation index; NIR, near infrared; and red edge) of three bread wheat (LRPB Lancer, Suntop and LRPB Hellfire) and three durum wheat (DBA Lillaroi, Jandaroi and DBA Aurora) varieties at multiple growth stages (GS) when inoculated or uninoculated with *Fusarium pseudograminearum* across three trial sites in 2021: Australian Cotton Research Institute (ACRI), Liverpool Plains Research Station (LPRS) and Piallamore.

Site	Index	GS	*P*-value (Infection)	R^2	Mean inoculated reflectance	Mean uninoculated reflectance	Difference	l.s.d. ($P = 0.05$)
ACRI	NDRE	39	0.429	0.57	0.301 (0.022)	0.300 (0.021)	−0.001	0.003
ACRI	NDVI	39	0.332	0.63	0.868 (0.023)	0.869 (0.022)	0.000	0.003
ACRI	NIR	39	0.857	0.70	0.388 (0.036)	0.388 (0.035)	0.000	0.005
ACRI	Red edge	39	0.509	0.80	0.209 (0.019)	0.209 (0.018)	0.000	0.002
ACRI	NDRE	65	0.929	0.12	0.080 (0.032)	0.075 (0.037)	−0.005	0.008
ACRI	NDVI	65	0.943	0.40	0.398 (0.124)	0.377 (0.135)	−0.021	0.023
ACRI	NIR	65	0.616	0.30	0.215 (0.034)	0.212 (0.032)	−0.003	0.006
ACRI	Red edge	65	0.387	0.32	0.182 (0.024)	0.182 (0.023)	0.000	0.004
LPRS	NDRE	39	0.323	0.65	0.251 (0.036)	0.249 (0.037)	−0.002	0.005
LPRS	NDVI	39	0.475	0.51	0.865 (0.021)	0.863 (0.024)	−0.003	0.004
LPRS	NIR	39	0.411	0.66	0.410 (0.039)	0.406 (0.040)	−0.004	0.005
LPRS	Red edge	39	0.855	0.73	0.244 (0.019)	0.243 (0.021)	−0.001	0.002
LPRS	NDRE	65	0.172	0.78	0.202 (0.036)	0.203 (0.037)	0.001	0.004
LPRS	NDVI	65	0.391	0.72	0.827 (0.027)	0.827 (0.031)	0.000	0.004
LPRS	NIR	65	0.927	0.70	0.388 (0.023)	0.387 (0.031)	0.000	0.003
LPRS	Red edge	65	0.203	0.634	0.257 (0.016)	0.256 (0.025)	−0.001	0.002
Piallamore	NDRE	39	0.155	0.733	0.272 (0.018)	0.269 (0.019)	−0.003	0.002
Piallamore	NDVI	39	0.279	0.707	0.864 (0.013)	0.862 (0.015)	−0.002	0.002
Piallamore	NIR	39	0.054	0.824	0.349 (0.023)	0.346 (0.024)	−0.003	0.002
Piallamore	Red edge	39	0.214	0.930	0.200 (0.011)	0.199 (0.012)	−0.001	0.001
Piallamore	NDRE	65	0.076	0.920	0.133 (0.027)	0.135 (0.026)	0.002	0.002
Piallamore	NDVI	65	0.221	0.889	0.693 (0.053)	0.699 (0.051)	0.005	0.004
Piallamore	NIR	65	0.102	0.002	0.298 (0.020)	0.299 (0.021)	0.001	0.002
Piallamore	Red edge	65	0.483	0.928	0.228 (0.016)	0.227 (0.016)	−0.001	0.001

P-value represents the significance of infection response from the ANOVA table. Standard deviation is given in parentheses following the mean reflectance. Generalised R^2 represents the model fit.

spatially selective application of fungicide in-crop. Post-emergent fungicide treatments are being investigated for the treatment of FCR in-crop (Zhang *et al.* 2022), and once available, spatial identification of affected areas could allow targeted treatments. Prioritisation of seed treated with a preventative fungicide may also occur where potential FCR risk zones within a field have been identified with the use of imagery from previous seasons. In an irrigated system, supplementary water could be targeted to zones with higher FCR incidence to limit disease expression and resulting yield loss (Buster *et al.* 2022). Spatial mapping of FCR infection within crops could also be used at harvest to manage grain quality by prioritising zones with lower infection levels, which will also have higher quality (i.e. reduced levels of smaller, shrivelled grains) along with increased yield. This could be particularly important in regions prone to weather damage during harvest, such as the northern grains region of Australia, because it would maximise harvest of areas of higher grain quality and yield within fields prior to rain events.

The ability of the remote sensing technologies used in this study to detect FCR infection correctly was appreciably constrained with advancing crop maturity at both ACRI and Piallamore field sites. This is contrary to expectations that, as wheat plants mature, the effect of *Fp* colonisation of the xylem in reducing upward water movement would be increased and thus would be expected to exacerbate the increase in canopy temperature. Three potential explanations for this reduced sensitivity with increasing crop maturity are proposed. First, assuming decreased water use by FCR-affected wheat plants (Buster *et al.* 2022), the *Fp*-inoculated plots may have used less soil water and thus had higher PAW than uninoculated treatments when plants reached maturity. Therefore, the inoculated treatments may have had a lower level of evapotranspirative stress than the

Table 5. Model fit parameters from partial least square discriminant analysis of hyperspectral data collected from three bread wheat (LRPB Lancer, Suntop and LRPB Hellfire) and three durum wheat (DBA Lillaroi, Jandaroi and DBA Aurora) varieties at multiple growth stages (GS) when inoculated or uninoculated with *Fusarium pseudograminearum* across two trial sites in 2021; Liverpool Plains Research Station (LPRS) and Piallamore.

Site	GS	R^2X (cumulative)	R^2Y (cumulative)	Q^2	PR^2Y	PQ^2
Piallamore	39	0.894	0.0054	−0.00065	0.63	0.11
Piallamore	65	0.894	0.0038	−0.0119	0.57	0.76
LPRS	39	0.943	0.0023	−0.00805	0.84	0.64
LPRS	65	0.945	0.0036	−0.00324	0.61	0.30

R^2X is the proportion of variation in the data explained by the predictive axes; R^2Y is the proportion of variation in infection classification explained by the model; Q^2 is a measure of the predictive power of the model; PR^2Y and PQ^2 are the proportion of models with randomised infection status that exceed the predictive value of the real PLS-DA model (and as such approximate *P*-values of R^2Y and Q^2).

uninoculated treatments. This hypothesis aligns with the declining thermal difference observed in the present study between inoculated and uninoculated treatments with advancing growth stage. The differences in canopy temperature were larger in early observations than in later growth stages, and the difference, although not significant, even appeared negative at GS65 at ACRI in 2020. This potential mechanism is also reflected in the observations of Buster *et al.* (2022), where PAW measurements indicated that *Fp*-inoculated treatments had more stored water at early maturity stages but these differences declined at later growth stages. Consequently, the differences in canopy temperature diminished as the crop matured, owing to higher soil-water availability in the *Fp*-inoculated treatments. This hypothesis is supported by the decreased sensitivity and FCR infection detection in the 2021 season where conditions were exceedingly wet (Buster *et al.* 2022) and the different patterns of water usage under inoculated conditions were negated by high levels of water supply.

A second possible explanation for smaller detectable differences later in the season may relate to the physiological manifestation of FCR infection. The specific mechanism of yield loss from FCR infection is not yet fully elucidated but may involve a combination of carbon loss due to fungal growth and reduced photosynthetic efficiency due to xylem blockage and stomatal closure (Knight and Sutherland 2015; Buster *et al.* 2022). In particularly wet seasons, reduction in photosynthetic capacity due to transpirative stress (expressed as increases in leaf temperatures) would be lower because of higher humidity and lower vapour pressure deficits. Under cool, wet conditions, carbon consumption by fungal growth would be expected to continue and represent a physiological cost to the plant causing some depression in yield. This hypothesis may account for the limited success of thermal detection in 2021, where yield loss from FCR infection was not recorded in any of the three field experiments. Furthermore, it is noteworthy that this study was conducted in two seasons that were not conducive to expression of and yield loss from FCR infection, owing to excess in-crop water, especially in 2021 (Liu and Liu 2016; Alahmad *et al.* 2018). However, unlike the 2020 season where significant yield loss was observed with successful detection of FCR infection, the 2021 season recorded no significant yield loss, and subsequently, successful identification of FCR by any remote sensing method was not attained (Buster *et al.* 2022). These explanations have limitations and future research is critical to refine methods further and elucidate which of these potential explanations is correct.

A third (less likely) explanation for the decreased sensitivity observed when plants were more mature may be unquantified limitations of the methods and technology used. The thermal drone used at the time of study was of consumer grade and was chosen to test the suitability of what would be economically viable for growers and consultants. This may have restricted detection sensitivity compared with that of higher grade thermal cameras such as those used by other researchers (Moorhead *et al.* 2019; Sener *et al.* 2019). Therefore, the spectral resolution or the thermal sensitivity may be non-linear, so that as the ambient temperature increased throughout the season, the thermal camera sensitivity diminished. Although this is a common feature of many types of electronic sensors, particularly when approaching the extremes of dynamic range, the sensor used is sensitive between −10°C and 400°C, and the field temperatures ranged between 12°C and 43°C, making this explanation unlikely. In addition, the relatively small l.s.d. values observed for thermal imagery indicate that in many of these surveys, the thermal imagery is quite sensitive to canopy temperature changes, and where no difference was detected, it is likely that transpiration was relatively uniform between inoculated and uninoculated treatments. Furthermore, although only the temperature values for leaf area were assessed in image analysis, it is possible that as the ambient temperatures increased during the season, the darker soil types at the field sites buffered external temperatures and radiated heat through the canopy, diminishing the separation between treatments.

The capacity of multispectral imagery to detect differences between infected and uninfected treatments in the 2020 field trials might suggest the existence of one or many reflectance wavelengths that are influenced by the presence of *Fp*. Identification of a specific wavelength or a group of wavelengths influenced by biochemical changes caused by the presence of *Fp* in the plant would allow the development of highly specific sensors deployable in field management or in breeding programs for screening resistant genotypes. Hyperspectral reflectance was recorded in 2021 in an attempt to identify prospective wavelengths. Substantial interrogation of the spectra indicated that although the data were highly structured and contained substantial information, <1% of the variation was explained by inoculation with *Fp*. This observation

remains consistent with the findings of the other methods presented above where thermal and multispectral imagery were not able to detect differences in the 2021 season. Despite the experimental design successfully establishing differences in FCR infection in 2020, we were not able to confirm this for the 2021 season owing to environmental conditions. From remotely captured data in 2021, we were not able to identify any candidate spectral features that might indicate the presence of *Fp* in the plant; however, the seasonal conditions were not conducive to the expression of the disease.

Some success was achieved in identification through different multispectral indices; however, there is no known FCR-related physiological mechanism for a change in any specific reflectance feature. Unlike thermal imagery, multispectral reflectance does not link to a specific physiological condition, and therefore, it can only generally represent underlying plant constraints. Although the methods described here may have some efficacy in identifying the spatial distribution of FCR, they could also be influenced by other biotic or abiotic constraints to plant function. This does not diminish the value of the methods proposed in this study but rather contextualises the application and interpretation of data obtained in this manner. It is our recommendation that until a clear mechanistic pathway for disease presentation is identified, caution is required when using multispectral imagery for FCR detection in wheat crops.

This is the first study demonstrating the use of remotely sensed data to determine the spatial variability of FCR distribution in broadacre wheat production. Under climatic conditions that allowed some expression of FCR, thermal and multispectral imagery had potential to distinguish between crops inoculated with *Fp* and those without inoculation. The results showed that canopy temperature was 0.30°C to 0.90°C higher at two-thirds of field sites inoculated with *Fp* at early growth stages. Some multispectral indices detected inoculation treatments some of the time (P = 0.01–0.04). Of the methods employed in this study, thermal imagery has more direct links to physiological symptoms of FCR infection than multispectral analysis, and is therefore preferred. Detection of differences using these methods appears to have more potential during early growth stages, with differences becoming less pronounced later in the growing season, perhaps as a result of infection–environment interactions. Hyperspectral reflectance does not appear to have any specificity for detection of FCR in wheat plants and probably more generally reflects plant performance; however, climatic conditions experienced during this study were not conducive to FCR expression, and so further investigation appears warranted. With further research and a clear understanding of the other environmental factors that can influence remotely sensed data, drone imagery could have a role in determining the spatial extent of FCR infection in wheat fields and allow site-specific intervention and disease management. This would have the potential to reduce yield loss and increase profitability of wheat production in the presence of this disease worldwide.

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Data availability. The data that support this study will be shared upon reasonable request to the corresponding author.

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