Proximal sensors to detect fungal disease in chickpea and faba bean

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Abstract
Pulse legumes are an important part of cropping rotations in the Victorian grains industry and one of the key management decisions relating to their production is the use of fungicides to control disease. Precision agriculture technology has the potential to assist with these decisions; if it were possible to develop sensor calibrations to detect the presence of disease this could potentially enable more targeted use of fungicide inputs and improve productivity. This paper outlines preliminary findings of testing an active light fluorometer and portable spectroradiometer for their ability to quantify disease in both chickpea (*Cicer arietinum*) and faba bean (*Vicia faba*). In general the fluorometer did not indicate strong relationships with ascochyta blight (*Ascochyta rabei*) in chickpea; however some indices offered an encouraging trend dependent on chickpea type (favoured by desi varieties). Spectral reflectance indices were more successful in quantifying disease in faba bean; however these measurements were undertaken at the leaf rather than canopy level. This study has highlighted some indices for further research, but a number of challenges remain both in terms of the research and practical application of this technology to a field environment.

Keywords
Fluorometry, reflectance, precision agriculture, ascochyta blight, chocolate spot.

Introduction
Pulse production in Australia averaged $503 M p.a. from 2003/04 to 2008/09, with diseases costing an estimated $74 M p.a. (Murray and Brennan 2012). Current disease management strategies are based on the use of fungicides, varietal resistance, crop rotation and stubble management. Given the significant potential of precision agriculture (PA) it is warranted to consider if such technologies have utility in disease management. For PA technologies such as unmanned aerial vehicles to be of value to agriculture they must be equipped with sensors providing information that can be turned into actionable insights not otherwise available. If such sensors were available it is foreseeable that growers and advisors could monitor larger areas for disease more efficiently and or map disease progression spatially. Depending on the disease and options available for control this may enable more targeted management at either sub-paddock, farm or district level, leading to better targeting of fungicide use and improved productivity. This paper reports initial research results focussing on identifying the potential utility of both fluorometry and spectral reflectance approaches to identify key diseases in chickpea and faba bean, both important pulses for Victoria's grains industry.

Methods
Trial design and agronomic management
Two field trials were established at Rupanyup in the Victorian Wimmera during 2016 to investigate the effect of variety and fungicide management on disease incidence and crop productivity for chickpea and faba bean. The first trial (sown 13-May) included 22 commercial and 'near release' varieties of chickpea grown with either nil or fortnightly fungicide applications (chlorothalonil 720). The second trial (sown 16-May) included a single variety of faba bean, (*cv.* Farah) treated with a variety of fungicide products and times of application. The chickpea trial was established as a split-plot design with fungicide applications as whole plots and varieties in sub-plots. The faba bean trial was a randomised complete block design. Both trials had four replicates. Chickpea plots were inoculated with ascochyta blight (*Phoma rabiei*, formerly known as *Ascochyta rabiei*) and faba bean plots were inoculated with chocolate spot (*Botrytis spp.*) on 4-August and 17-August, to promote disease infection. Rhizobial inoculation, crop nutrition, weeds and insect pests were managed according to industry best practice.

Monitoring of disease progression visually and using fluorometry in chickpea
Visual assessments of the chickpea trial were undertaken on a total of three dates during the season; 3-August, 26-September and 27-October, with individual plots visually scored on a 1-9 scale for the presence

of ascochyta blight. An active light fluorometer (Multiplex 3.6, Force A, Orsay Cedex FRA) was used to monitor a subset of varieties (Table 1), including both nil and fortnightly fungicide treatments.

Measurements were undertaken on a total of seven dates throughout the season (between 11-August and 4-November) by holding the instrument immediately above the crop canopy at an angle of 45° from horizontal and using a 4 cm aperture plate. The active light fluorometer measures a range of indices relating to plant health (Table 2). A total of 10 measurements were taken per plot on each monitoring day and data was processed according to the manufacturers guidelines before calculating the plot mean. Visual assessment data was clumped into groups of low, medium and high disease and assigned to corresponding fluorometer measurements prior to correlation analysis.

Table 1. Characteristics of varieties monitored using active light fluorescence.

Variety	Chickpea type	Ascochyta blight resistance rating (foliage)*	Ascochyta blight resistance rating (pod)*	Maturity*
Howzat	Desi	S	S	Mid
PBA Maiden	Desi	S	S	Mid
PBA Slasher	Desi	MS	S	Mid
Genesis 090	Kabuli	MS	S	Mid-late
Genesis Kalkee	Kabuli	MS	S	Late
CICA1352	Kabuli	MS[#]	S[#]	Mid

*Couchman and Hollaway (2017).
[#]Provisional ratings.

Monitoring of disease using leaf reflectance in faba bean

Leaf reflectance was measured using a handheld spectroradiometer (ASD Field Spec FR, Boulder CO USA). Cloudy conditions throughout key measurement periods meant that using this passive sensor for canopy level measurements was not possible. Protocols were subsequently changed to connect the spectroradiometer to a leaf clamp with internal illumination. Leaves were collected from the field on a total of five days from 23-August to 10-October. On each sampling day, a representative sample of individual leaves was collected from both nil fungicide and 'complete control' treatments to ensure a spread of disease symptoms. Samples were analysed in a laboratory, with three measurements taken across the upper side of each leaf. Leaves were then individually assessed for presence of disease and assigned a rating on a three point scale from low to high. Samples taken on the final day (10-October) were analysed in further detail by measuring two points per leaf and marking the specific area of measurement, enabling visual assessment of disease symptoms at the sub-leaf level. A range of reflectance indices (Table 2) were calculated prior to correlation analysis.

Table 2. Indices computed from fluorometer and reflectance measurements.

	Index name	Description/ Reference
Fluorometer indices	ANTH_RB, ANTH_RG	Anthocyanin indices, negatively correlated to chlorophyll in the absence of anthocyanin.
	FLAV	Index of compounds and which absorb at 375 nm.
	NBI1, NBI_G, NBI_R	Nitrogen balance indices
	SFR_G, SFR_R	Chlorophyll indices
Spectral indices	Anthocyanin reflectance index 1 (ARI1)	Gitelson et al. (2001)
	Carotenoid reflectance index 2 (CRI2)	Gitelson et al. (2002)
	Normalised difference red-edge index (NDRE)	
	Normalised difference vegetation index (NDVI)	
	Photochemical response index (PRI)	Gamon et al. (1992)
	Plant senescence reflectance index, (PSRI)	Merzlyak et al. (1999)
	Red-edge vegetation stress index (RVSI)	
	Structure insensitive pigment index (SIPI)	Peñuelas et al. (1995)

Results

Seasonal conditions and disease progression

Growing season (April to October) and annual rainfall at Rupanyup was 384 and 512 mm, respectively (both in the top 10% of years, BoM, 2017), including 120 mm during September. As a result, chickpea plots which received no fungicide exhibited increases in ascochyta blight levels during the season (data not shown). Fortnightly fungicide application successfully controlled the disease in approximately 90% of chickpea plots, with the exception of assessments on 26-September when a small percentage of plots exhibited low to moderate infection. Disease levels in the faba bean trial were also high, particularly in the lower canopy.

Prediction of ascochyta blight incidence in chickpea using fluorescence

Fluorescence based indices did not show clear relationships with ascochyta blight severity (typically low correlation). However a number of indices (e.g. ANTH_RB, FLAV, NBI_G) showed trends to suggest an association with ascochyta blight severity (Figure 1). These trends were not necessarily consistent over time with stronger trends shown later in the season. This may have related to phenological stage of the crop and disease progression but also the level of disease i.e. earlier in the season the range of disease symptoms was not as wide. The strength of these trends also changed according to chickpea type with desi varieties showing a stronger trend than kabuli types for a number of indices including FLAV (Figure 2). Desi types typically show distinct visual symptoms in response to stress generally manifesting as reddening of stems and leaves in comparison to kabuli types which tend towards yellowing symptoms.

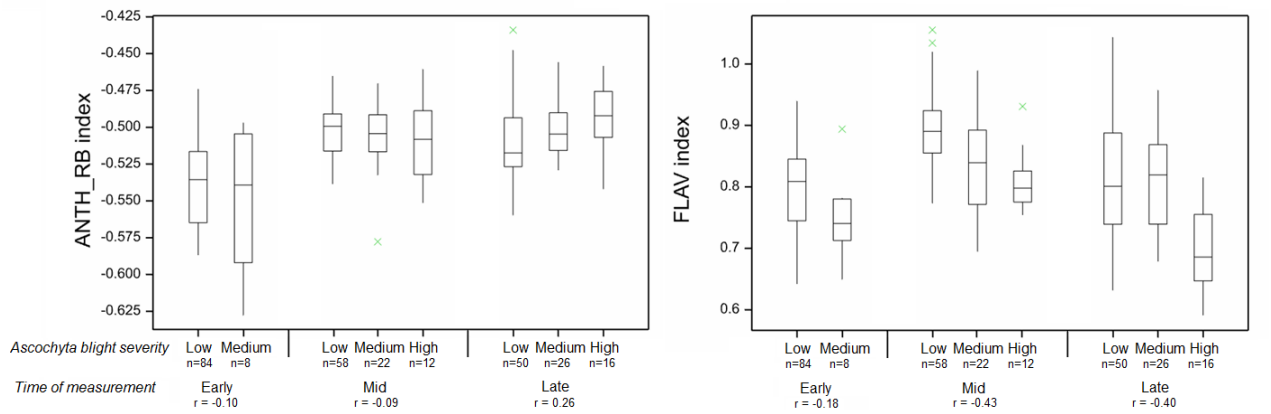

Figure 1. Relationship between ascochyta blight severity in chickpea and the ANTH_RB and FLAV fluorometer indices taken at three points (early, mid and late) in the season between 11-August and 4-November.

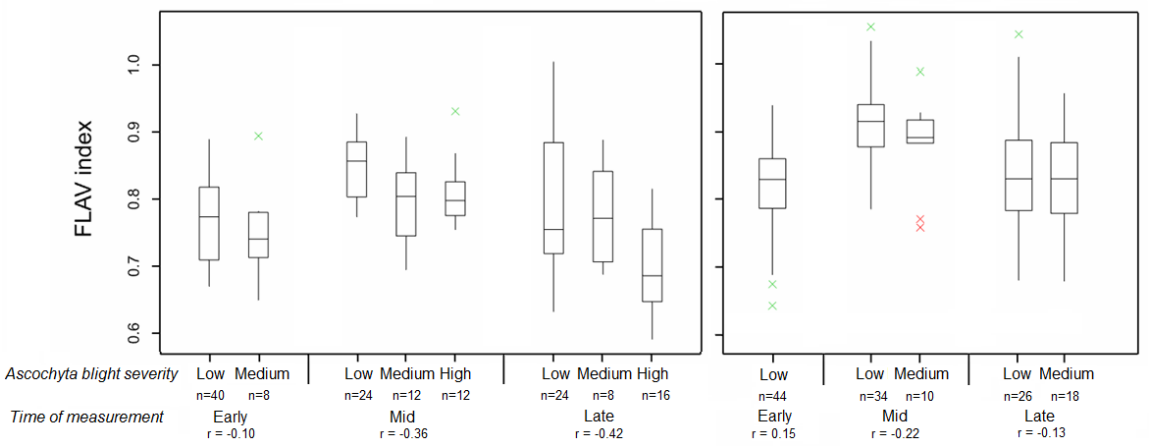

Figure 2. Relationship between ascochyta blight severity in desi (left) and kabuli (right) chickpea varieties and the FLAV fluorometer index taken at three points in the season between 11-August and 4-November.

Prediction of disease symptoms in faba bean using spectral reflectance

A number of spectral indices correlated well with disease severity including the ARI1 (r = 0.77), CRI2 (r = 0.55), PRI (r = 0.65), PSRI (r = 0.64) and SIPI (r = 0.60) indices (Figure 3). Monitoring symptoms at the sub-leaf level on 10-October did not improve the strength of these trends. In some cases the correlation decreased (PSRI and SIPI); while in the case of the RVSI index there was a significant increase (-0.01 to 0.55). How well these indices perform at the canopy level is yet to be tested, particularly in situations where disease symptoms are confined to the lower canopy and they can provide a source of infection for leaves and stems higher in the canopy. In such instances sensors may need to be deployed at varying heights.

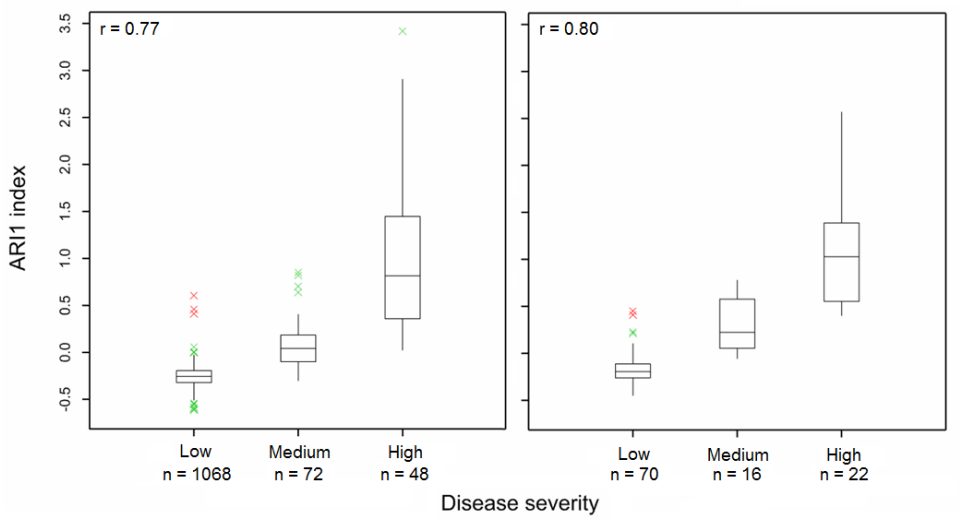

Figure 3. Relationship between disease symptoms in faba bean and the ARI1 spectral index for symptoms measured at the leaf (left) and sub-leaf (right) level.

Conclusion

Weather conditions experienced during 2016 were conducive to disease development in both chickpea and faba bean. Regular monitoring of ascochyta blight in chickpea using an active light fluorometer did not result in any clear indices for reliable detection of disease. However a number of indices (particularly FLAV) indicated a trend towards detection. This depended on chickpea type with a better relationship in desi varieties. Spectral reflectance based indices showed greater promise for detection of disease in faba bean, in particular Anthocyanin Release Index 1. However, these measurements were taken at the leaf level rather than canopy and application at the field level is yet to be tested. For this type of technology to be commercialised a number of challenges will need to be overcome including: research problems such as atmospheric conditions limiting use of passive reflectance sensors and robust analysis techniques for bringing together discrete datasets (e.g. disease rankings) with continuous outputs from sensors. Separating symptoms caused by biological and non-biological agents, dealing with diseases where symptoms are stratified within the canopy and linking sensor outputs to management decisions in a timely manner are all equally challenging. However, PA is a key opportunity to improve productivity in the Australian grains industry and such challenges will need to be overcome to realise this.

Acknowledgements

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