# Frost Response in Lentil. Part 2 - Detecting early frost damage using proximal sensing

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## Abstract

Frost can have negative impacts on both yield and quality of lentil, and currently there is a gap in methods to provide growers with tools to identify, monitor and assess the degree of damage due to frost. To evaluate the potential for spatial, non-destructive detection of frost damage, a trial was established in lentil (*cv.* Jumbo 2) using active frost treatments over a range of growth stages and intensities. Proximal sensing was used to monitor the crop to determine whether non-destructive methods can detect frost damage, and if post-processing can separate frost effects from natural crop senescence. The results show that in addition to the proximal measurements detecting natural senescence occurring during the period from flowering to pod fill, it also detected a decrease in canopy chlorophyll associated with cold exposure, beyond a threshold cold sum value (5 - 31 °C.hr). SFR\_G from active fluorometer measurements was fit to cold sums, resulting in R<sup>2</sup> values of 0.84 six days following frost application at flowering, and 0.72 eight days following frost at pod filling. NDVI from canopy reflectance measurements was related to cold sums across pooled measurement dates with an R<sup>2</sup> value of 0.81. The reflectance index PRI increased with increasing cold exposure, indicating changes in photosynthetic efficiency with increasing frost damage. These initial results agree with previous research on detection of frost damage in wheat.

# **Key Words**

Remote sensing, frost detection, fluorometer, canopy reflectance

## Introduction

Australian lentil production contributes 419,000 tons grain per annum (ABARES 2017), with an average gross margin of AU\$783 per ha (Rural Solutions SA 2017). Frost can have negative impacts on lentils as with other grains, resulting in reduction of yield and quality. Currently there is a gap in methods to provide growers with tools to identify, monitor and assess the degree of damage due to frost. If methods can be developed, non-destructive measurements (e.g., satellite or UAV imagery) could be used to assess the extent of frost damage at paddock scales. Potentially these methods could be incorporated into breeding programs as a high throughput method to measure susceptibility of breeding lines. In this work we utilised plot trials with active frost treatments at different intensities and development stages. Proximal sensing was used to monitor the crop and test the utility of non-destructive methods for detecting frost damage in lentil, and if analytical methods could be developed to differentiate frost effects from natural crop senescence.

## Methods

## 2017 Trial on Lentil

A trial was established on lentil (*cv.* Jumbo 2) with 14 treatments and 4 replicates. Treatments consisted of 2 controls (protected and ambient), and 3 frost scenarios at each of 4 times: flowering, early pod, flat pod and filling pod. The frost treatments were applied following methods developed for wheat (Nuttall et al. 2018) using dry ice and insulated chambers. Canopy temperatures were monitored using thermistors installed at canopy height, and temperature was logged at five-minute intervals using TGP-4505 external temperature and relative humidity probes. The level of frost exposure was determined by integrating the time over which canopy temperatures were below 0°C, expressed as °C.h (< 0°C). Further details of the trial and results for yield and quality impacts are presented in Delahunty et al. 2019.

## Multispectral fluorescence and reflectance measures

A handheld active light fluorometer (Multiplex 3.6, Force A, Orsay Cedex FRA) with four excitation bands (UV, blue, green, and red) and three detection bands (yellow, red and far red) was used. Measurements were taken every 2 days throughout the trial period (4 Oct to 9 Nov) at canopy level using a 6 cm aperture. Results are presented for the index SFR\_G, an index related to chlorophyll concentration (Ghozlen et al. 2010).

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Spectral reflectance of the canopy was measured on 10 and 17 Oct, using a handheld spectroradiometer (ASD Field Spec FR, Boulder CO USA). Measurements were made 40 cm above ground, for a ground sample diameter of approximately 20 cm. The spectra were normalised to reflectance using a Spectralon<sup>TM</sup> calibration reference. Two reflectance indices were computed for each plot, the Normalized Difference Vegetation Index (NDVI; Rouse et al. 1973) and the Photochemical Response Index (PRI; Gamon et al. 1992).

## **Results and Discussion**

#### Fluorometer results

SFR\_G declined during the measurement period for the control plots (Fig. 1 *a*), indicating there was a need to normalise measurements through time to differentiate the effects of senescence from the frost exposure. SFR\_G measurements were normalised by adding the offset between the mean value of the protected controls for 4 Oct (first reading) and each subsequent measurement date (Fig 1 *b*). The relationships between the normalized values and corresponding cold sums for each plot were evaluated using segmented regression. The regression results are listed in Table 1. The fitted parameters and R<sup>2</sup> values varied by measurement DAFr (days after frost) and growth stage. At flowering, R<sup>2</sup> values from ranged from 0.68 (8 DAFr) to 0.84 (6 DAFr). At pod fill, the R<sup>2</sup> ranged from 0.39 (day following frost) to 0.72 (8 DAFr). SFR\_G was found to decrease more rapidly with cold loads for the flowering treatment than for the early pod fill growth stage (Fig. 2).



Figure 1. Fluorometer measurements were made at 2-day intervals at the canopy scale from flowering through to filled pod. SFR\_G is an indicator of chlorophyll concentration and was seen to decline during the measurement period (*a*). The fluorometer measurements were normalised by subtracting the change (offset) for the mean of the closed control plots (*b*).

#### Canopy reflectance measurements

NDVI and PRI measurements were evaluated by pooling the data across the two measurement dates, combining the flowering and early podding treatments. NDVI and PRI were fitted to cold sums using segmented regression (Fig. 3). NDVI values decreased with increasing cold sum, after a threshold value of 31 °C.hr (Fig. 3 *a*), resulting in an R<sup>2</sup> value of 0.81. PRI was found to increase with increasing cold sums

greater than 22 °C.hr, resulting in an R<sup>2</sup> value of 0.61 (Fig. 3 *b*). The results for NDVI and PRI generally agree with previous work in frost detection in wheat reported by Nuttall *et al.* 2018.

DAFr	Slope 1	Slope 2	Breakpoint x	Breakpoint y	$\mathbf{R}^2$
0		-0.0224	15.7	2.01	0.79
2	-0.0058	-0.0449	22.1	1.88	0.72
4		-0.0429	16.4	2.03	0.79
6		-0.0433	21.5	2.00	0.84
8		-0.0373	21.4	2.02	0.68
0	0.0294	-0.0116	8.2	2.26	0.39
2	0.0366	-0.0135	5.4	2.22	0.54
4	0.0283	-0.0206	12.1	2.25	0.65
6	0.0287	-0.0190	12.4	2.26	0.61
8	0.0215	-0.0219	20.0	2.37	0.72
	DAFr 0 2 4 6 8 0 2 4 6 8	DAFr Slope 1   0 2   2 -0.0058   4 6   8 0   0 0.0294   2 0.0366   4 0.0283   6 0.0287   8 0.0215	DAFr Slope 1 Slope 2   0 -0.0224   2 -0.0058 -0.0449   4 -0.0429   6 -0.0433   8 -0.0373   0 0.0294 -0.0116   2 0.0366 -0.0135   4 0.0283 -0.0206   6 0.0287 -0.0190   8 0.0215 -0.0219	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	$\begin{array}{c c c c c c c c c c c c c c c c c c c $





Figure 2. Fluorometer index SFR\_G declined with increasing cold sum, as shown here for DAFr2 at flowering (open circles) and pod filling (open squares) growth stages.

#### Conclusion

This paper reports on initial work in evaluating non-destructive measures for frost damage detection in lentil. These results show that in addition to natural senescence occurring during the measurement period from flower to pod fill, the proximal measurements also detected a decrease in canopy chlorophyll due to cold load, beyond a threshold cold sum value. This response was consistent across both the indices SFR\_G (from active fluorometer measurements) and NDVI (from canopy reflectance measurements). The reflectance index PRI increased with increasing cold load, indicating changes in photosynthetic efficiency with cumulative frost damage. These initial results agree with previous research on detection of frost damage in wheat. Next steps in the research will include assessment of other fluorescence and reflectance indices, measurements at additional growth stages, and response to frost for other lentil varieties.

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Figure 3. Canopy reflectance was measured using a portable spectrometer following flowering (6 and 13 DAFr) and early podding (3 DAFr). The reflectance spectra were used to derive vegetation indices to evaluate the relationships with cold sum. NDVI (*a*) decreased with cold sum, with the slope increasing at about 32 °C.hr. PRI (*b*) increased with increasing cold sum greater than 23 °C.hr.

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