In-field differentiation of frost and heat stress in wheat

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Abstract
Frost and acute high temperature are both extreme temperature events that have a significant impact on crops grown within Mediterranean-type climates. During the late spring period which coincides with dryland crops flowering, frost and high temperature can occur within days of each other, and often confound interpretation of which causes a yield penalty. As a means of assessing the effect of the two stresses on wheat and defining the main and interactive effects of heat and frost, a factorial trial was established in a wheat crop at Horsham, Victoria. Insulated chambers were utilised to apply artificial frost during the evenings at two intensities. Following one day of recovery, daytime high temperature treatments at two intensities were applied, using heat chambers, to plots with and without previous frost treatments. The results of the trial show a significant (P <0.001), linear relationship between cumulative frost (C.hr (<0 ºC)), yield and components. No significant relationship was found for heat or frost by heat interaction on yield, most likely due to a lack of intensity for the heat treatments applied. This work provided a backdrop to collect non-destructive, remote sensing data, as a potential tool for rapid assessments of crop damage to extreme temperature events.

Introduction
Extreme weather events (heat waves and frost) constitute a significant challenge to grain growers and are predicted to increase in the future climate (Barlow et al., 2015). For field crops grown in Australia's grain belt, economic losses from frost have been estimated to be as high as $360 million per year (March et al., 2015). Similarly, acute high temperature (HT) events (>32ºC), that occur during anthesis and grain fill cause significant reduction in yield potential and economic losses of between 17-35% (Alexander et al., 2010; Stone and Nicolas, 1995; Talukder et al., 2010, Nuttall et al 2018a). Extreme weather events can operate independently, or alternatively frost occurring in late spring can be coupled with acute HT stress within a relatively short period of time. While crop responses to HT stress and frost damage differ at the genetic, molecular and physiological level, wheat responses during the flowering and reproductive phase to either stress are similar through a step change, non-reversible effect on yield potential (e.g. leaf senescence, death of reproductive organs). Although the main effects of acute HT (Asseng et al., 2011 Nuttall et al., 2018a) and frost (Barlow et al., 2015, Nuttall et al 2018b) on wheat has been quantified, the interactive effect and relative sensitivity of these constraints to a crop is less well understood. Differentiating frost and acute HT would support on-farm, in-season decision making (e.g., cutting for hay or harvesting for grain) to maximise returns, inform crop models on the interactive effect of these factors and enable characterisation of crop reflectance signatures using remote sensing . This paper seeks to quantify the response of wheat to frost, HT, and interaction and support the future validation of these effects using rapid, non-destructive assessments of damage.

Methods
A field trial with factorial combinations of applied frost and HT applied to wheat at anthesis, was established at Horsham, Victoria. Treatments included factorial combinations of two intensities of frost, and two intensities of HT and was compared with nil frost and HT treatments. Specialised mobile chambers constructed from RHS rectangle frames (600mm W x 600mm D x 1200mm H) that were clad with Foilboard ® were used to apply frost to treatments as described in Nuttall et al. 2018*b*, which reduced the temperature from ambient to below 0 ºC for one or two consecutive nights (F1, F2), respectively. Similarly, mobile heat chambers constructed from a RHS frame (1200mm W x 800mm D x 500mm H), clad with Sun Tuff Greca Laserlite, were used to simulate acute HT (>32 ºC) during day time hours for one (H1) or two days (H2), at approximately 30 hours post frost treatment. Nil

treatments (F0, H0) were protected from natural 'extreme' temperature events (Figure 1) using protection boxes for frost. Canopy temperature was measured at 5-minute intervals throughout the treatment period in all plots using TinyTag® TGP-4505. The intensity of applied frost was determined by integrating the time over which the temperature at canopy level was below 0°C, expressed as C.hr (< 0°C) for both applied and natural frost events, similar to the methodology reported in Nuttall et al 2018*b*. Similarly, heat stress was determined based on degree hours above the 32°C threshold. The cumulative sum of C.hr (< 0°C) and C.hr (>32°C) were defined as cold loads and heat loads, respectively. Wheat was harvested on 3/12/2018, as quadrate cuts (0.3 m^2). Yield components measured and data analysis using ANOVA and regression analysis using Genstat (Payne et al., 2011).

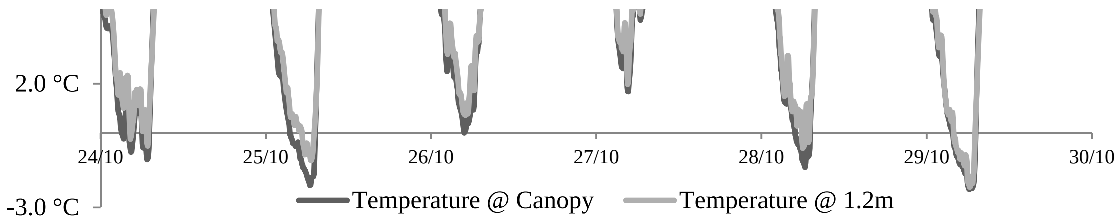

Figure 1. Site weather station temperature recordings showing all natural frost events during anthesis and early grain fill. Temperatures were recorded at canopy height and 1.2m.

Results and Discussion

Frost

Frost chambers effectively imposed cold temperature (frost) treatments to wheat over single/double night combination (23/10 and 24/10) to generate the two intensities F1 and F2, which coincided with crops at 50% anthesis. Average temperature during the F1 and F2 frost treatments were -2.6 and -3.9°C and this compared with ambient temperatures during these nights of 4.9 and 5.2 °C, respectively. The corresponding average cold loads for F1 and F2 were 11.5 and 55.5°C (Table 1).

High temperature

High temperature was applied, at either one or two days post the frost treatments (26/10 & 27/10). The heat chambers raised air temperature at canopy level to above the critical temperature of 32 °C, when average ambient temperature during application 21 and 22 °C, respectively. High temperatures, greater than 40 °C were recorded at canopy level (Table 1). Average heat loads were 20.1 for H1 and 23.8 °C.hr (>32 °C) for H2. The magnitude of heat loads, however, were likely not to have been sufficient to induce a clear response of crop to acute HT, where loads were lower than that imposed in previous HT studies (Nuttall et al., 2018a).

Table 1. Treatment average canopy air temperature during frost (F) and acute high temperature (H) applications. For the F and H treatments, values (0 to 2) represent intensities. Values in parentheses are the standard error of means for treatments. Underlined values represent ambient temperatures at application.

	Applied load		Average temperature		Min/max temperature	
Temperature Treatment	Cold C.hr (<0 °C)	Heat C.hr (>32 °C)	Cold <0 °C	Heat >32 °C	Cold °C	Hot °C
F0H0			<u>4.8 (0.3)</u>	<u>26.5 (1.4)</u>	-2.8 (0.1)	35.0 (2.0)
F0H1		24.4 (n=1)	<u>5.2 (0.5)</u>	34.6 (1.2)	-1.3 (0.1)	37.1 (3.5)
F0H2		17.7 (n=1)	<u>4.8 (0.1)</u>	33.3 (0.4)	-1.6 (0.1)	38.9 (1.4)
F1H0	11.8 (2.5)		-2.7 (0.4)	<u>22.8 (0.2)</u>	-5.0 (0.8)	32.7 (0.9)
F1H1	11.1 (3.2)	18.0 (4.4)	-2.6 (0.3)	34.9 (0.2)	-4.5 (1.1)	39.0 (1.2)
F1H2	11.5 (2.5)	26.9 (4.8)	-2.9 (0.3)	33.7 (0.4)	-5.3 (0.7)	37.2 (1.2)
F2H0	60.1 (10.0)		-4.0 (0.4)	<u>23.6 (1.1)</u>	-7.2 (0.9)	33.7 (0.6)
F2H1	53.0 (5.8)	20.1 (3.4)	-3.9 (0.3)	34.4 (0.4)	-6.4 (0.7)	40.3 (3.5)
F2H2	53.4 (4.6)	26.9 (6.9)	-3.7 (0.2)	34.3 (0.3)	-6.5 (0.4)	39.1 (0.9)

Wheat response

The F1 frost treatment (pooled for HT) caused a 21% reduction in yield compared with the control. In comparison, the F2 caused a 77% reduction in yield, where the reduction was linked with significant reduction in grain-set where frost treatments were applied. There was no significant effect of frost treatment on kernel size (Table 2). Overall, these results indicate a cumulative response to F1 and F2 frost treatments and that is consistent with results seen in Nuttall et al. 2018*b*. This yield reduction corresponded to a step reduction in grain number, however, there was no effect of frost treatment on kernel size.

The high temperature did not cause a significant reduction in either yield or grain number. Additionally, compensatory kernel size increases with reduction in grain number were not significant. Significant reductions in yield because of heat stress have been described in a number of studies, where temperatures exceeding the threshold were applied for 6 and 12 days, causing grain yield decreases of 15 and 24%, respectively (Nuttall et al., 2018*a*). Nuttall et al., 2018*a* describes heat loads between 20 and 276 C.hr (>32 ^{o}C) for yield reductions, indicating that longer exposure to acute HT may be required to observe a main effect.

Where combinations of frost and HT were applied, there was no significant interactive effects of these factors. Again, the low intensity of HT applied may be limiting expression of a potential interaction.

Table 2. Analysis of variance results of frost, heat and combination and nil treatments with yield and yield components.

	Heat treatments								
Frost treatments	H0	H1	H2	H0	H1	H2	H0	H1	H2
	Grain number (grains per m^2)			*Kernel size (mg/1000)*			*Grain yield (kg/ha)*		
F0	7951	8000	9512	48.7	37.9	38.3	3076	3023	3659
F1	6491	6946	6779	38.4	37.7	37.9	2487	2639	2539
F2	1643	2410	1955	38.6	36.4	38.6	627	888	751
F lsd (P<0.001)	625			NS			249.2		
H and H x F	NS			NS			NS		

For the significant main effect of cold load on wheat yield, a strong negative linear relationship between grain number and cold load (R^2=0.84) (Figure 2 a) and yield and cold load (R^2=0.84) (Figure2 b) was observed. This equated to a 1.2 and 1.7% reduction in grain number and yield per degree Celsius below zero. A cumulative response to cold load is consistent with previous studies, where grain number has been observed to decrease by 2.2% per C.hr (<0 ^{o}C) (Nuttall et al., 2018b).

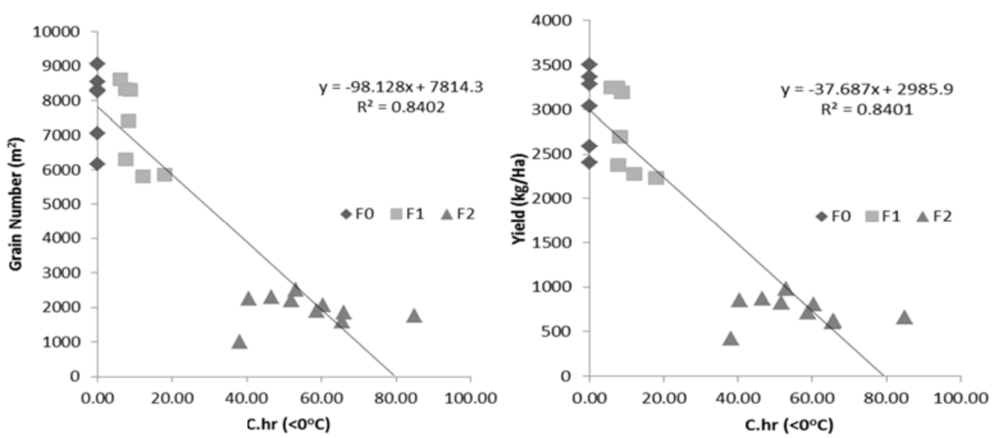

Figure 2. Grain number (a) and yield (b) response of wheat (*cv*. Beckham) to cold load as a simulated frost (F) event applied at anthesis. F0, F1 and F2 are varying intensity treatments.

Conclusion

A strong linear response was observed between frost and yield and grain number(R^2=0.84, 0.84) . For HT treatments, significance was not observed due to the conservative heat loads. This study provides response data of artificially applied extreme temperature events to field wheat. Specially designed chambers provided an effective application method of frost treatments to wheat in a field context, however applied HT appear insufficient to promote a discernible crop response. This work provides the basis for evaluating non-destructive, remote-sensing tools in detecting frost and HT damage in field wheat in future studies. The next step will be to establish the link between cold and heat loads with proximal sensing. Upcoming trials will be designed with greater heat loads, to further understand the frost by HT interactions as well as the effect pf other abiotic stresses on field wheat such as nitrogen and water availability.

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