Protein formation and the critical period for yield in lentil

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Abstract
Lentil is a cool season grain legume produced primarily in the Mediterranean and temperate regions of the world where yield is constrained by water and heat stress in critical developmental windows. The effect of stress on yield depends on the timing, intensity and duration of stress; here we focus on timing relative to phenological development. To determine the critical period for grain yield and grain protein, we compared unshaded controls and crops exposed to sequential 10-14 d shading periods using two locally adapted varieties at two sites: Roseworthy, south-eastern Australia, and Valdivia, southern Chile. Yield of unstressed controls varied from 1 t ha^{-1} at Roseworthy to 7 t ha^{-1} at Valdivia, and seed protein from 21% in Valdivia to 27.6% at Roseworthy. Irrespective of growing conditions, the most sensitive period was pod emergence, between 100 and 200 ^{o}Cd after flowering. Grain number and biomass accounted for most of the variation in yield. Grain protein concentration varied according to a bi-linear model, with minor reductions (<10%) before an environment-dependent threshold and linear increase after the threshold, at flowering at Valdivia, and ~ 200 ^{o}Cd after flowering at Roseworthy. Protein yield tracked grain yield but was partially buffered by increased grain protein concentration with late-season stress.

Keywords
Yield; biomass, harvest index; protein; grain number; grain size; stress.

Introduction
Darwin (1859) observed "..very trifling changes, such as a little more or less water at some particular period of growth, will determine whether or not the plant sets a grain". This notion of a critical developmental period has become a core physiological concept, with agronomic practices seeking to reduce the likelihood of stress in the more sensitive crop stages for yield (Flohr et al., 2017; Lake et al., 2021). The primary effect of abiotic stresses is to reduce crop growth rate. Shading reliably replicates the effects of abiotic stress and is used sequentially over the lifecycle of the crop to determine the critical period (Lake et al., 2019). The critical period of determinate cereals spans from about 300 ^{o}Cd before anthesis to 100 ^{o}Cd after anthesis. In indeterminate crops like soybean, pulses and canola, the critical period for yield is displaced towards pod set (Sadras and Dreccer, 2015). This behaviour partially relates to the overlap of vegetative and reproductive growth allowing some compensation for stress at earlier stages (Slafer et al., 2009). The critical developmental window for yield response to stress has not been established in lentil. In addition, the critical developmental windows for deposition of grain components – starch, oil and protein – are well established in crops such as wheat (Slafer et al., 2021), sunflower (Debaeke and Izquierdo, 2021) and soybean (Tamagno et al., 2018), are incipient in other crops such as canola (Kirkegaard et al., 2018) and are largely unknown in lentil. This study aims to determine the critical period of determination of yield and grain protein in lentil in field experiments in two environments spanning actual yield from 1 to 7 t ha^{-1}.

Methods
Two rainfed trials were established on an Andisoil at Valdivia, southern Chile, and on a Calcic Luvisol at Roseworthy, south-eastern Australia. Crops were managed with local practice to control diseases, pests and weeds. The grain was inoculated with Rhizobium and fertilised with 80 kg ha^{-1} mono ammonium phosphate at Roseworthy. Grain was not inoculated with Rhizobium at Valdivia, where crops relied on fertiliser and soil nitrogen mineralisation. In Valdivia, two large-grained, locally adapted cultivars, Calpún and Super Araucana, were sown at 167 seeds m^{-2} on the 3rd of September 2019. In Roseworthy two small-grained locally adapted varieties, PBA HurricaneXT and CIPAL 901, were sown at 120 seeds m^{-2} on the 24th of June 2019. The experiment was a factorial combining (i) two cultivars, (ii) unshaded control and seven sequential 14-d (Valdivia) and six 10-d (Roseworthy) shading periods during the crop cycle, and two shade levels at Valdivia, 50% and 90%. Treatments were laid out in a completely randomised design with three replicates. Fewer and shorter shading treatments sought to accommodate the faster development at Roseworthy compared to Valdivia. Treatments were laid out in a split-plot design with three replicates. Cultivars were allocated to main plot and shading treatments randomised to subplot.

We monitored phenology weekly; flowering date was established when 50% of the plants within the plot had at least one open flower. Phenological stages were expressed on thermal time using a base temperature of 2 ºC. At maturity we sampled shoot samples from the centre rows. Samples were dried for 72 hours at 70 °C or to constant weight. Yield components were separated to determine pod number, grain number, grain size, grains per pod, shoot biomass, and harvest index (HI = grain yield / shoot biomass). Grain samples (≈ 40 g) were dried at 80 ºC, finely ground and analysed for nitrogen concentration. Nitrogen was converted to protein with the factor 6.25.

We used ANOVA to test the effects of experimental sources of variation on yield and its components in each trial separately. We report *p*-value as a continuous quantity, and Shannon information transform [$s = -\log_2(p)$] as a measure of the information against the tested hypothesis (Greenland, 2019). Yield and yield components in shaded treatments were normalised as a fraction of the control, and the trajectory of normalised traits was plotted against the phenology of controls on thermal time scale centred at flowering; polynomials were fitted as in Lake et al. (2019).

Results

Yield and protein in unshaded controls

Yield of controls varied 7-fold, from 110 g m^{-2} at Roseworthy to 789 g m^{-2} for Calpún at Valdivia. Likewise, biomass varied 7-fold. Calpún out-yielded Super Araucana at Valdivia ($p < 0.001$, $s > 9.9$), whereas PBA HurricaneXT and CIPAL0901 returned similar yield at Roseworthy ($p = 0.630$, $s = 0.7$). Crops at Valdivia produced 3-fold more grain which were 2.3-fold larger. Grain protein content averaged 21 at Valdivia and 27.6 % at Roseworthy.

Owing to the difference in experimental design, we analysed the association between yield and component traits separately for each environment. In both environments, yield associated with all four components: grain number, grain size, biomass and harvest index. The association was stronger with biomass (Valdivia: $r = 0.90$, $p < 0.001$, $s > 9.9$; Roseworthy: $r = 0.90$, $p < 0.001$, $s > 9.9$) than with harvest index (Valdivia: $r = 0.63$, $p < 0.001$, $s > 9.9$; Roseworthy: $r = 0.73$, $p < 0.001$, $s > 9.9$), and stronger for grain number (Valdivia: $r = 0.86$, $p < 0.001$, $s > 9.9$; Roseworthy: $r = 0.72$, $p < 0.001$, $s > 9.9$) than for grain size (Valdivia: $r = 0.37$, $p < 0.001$, $s > 9.9$; Roseworthy: $r = 0.47$, $p < 0.001$, $s > 9.9$). Grain protein was negatively correlated with harvest index at both Valdivia ($r = -0.37$, $p < 0.001$, $s > 9.9$) and Roseworthy ($r = -0.59$, $p < 0.001$, $s > 9.9$) and with grain size ($r = -0.63$, $p < 0.001$, $s > 9.9$) and yield ($r = -0.37$, $p < 0.001$, $s > 9.9$) at Roseworthy.

The critical period for yield and components

Fig. 1AB show the response of yield to shading across the crop lifecycle at Valdivia and Roseworthy. A single model fitted across varieties and shading intensity at Valdivia, and across varieties at Roseworthy showed a common stage, 100-200 °Cd after flowering, for maximum yield reduction. Analysis of residuals at Valdivia showed no difference between varieties ($p = 0.103$, $s = 3.3$) and larger reduction in yield with more intense shading ($p = 0.003$, $s = 8.4$) (inset Fig. 1A) with no interaction between varieties and shade intensity ($p = 0.509$, $s = 1.0$). Analysis of residuals showed CIPAL0901 was slightly less sensitive to early shading than PBA HurricaneXT at Roseworthy ($p = 0.049$, $s = 4.4$) (inset Fig. 1B). Grain number fully accounted for the response of yield (Fig. 1CD). Grain size was largely unresponsive to shading at Valdivia (Fig. 1E). At Roseworthy, shading reduced grain size at two stages: immediately before flowering, and late in grain fill (Fig. 1F).

Fig. 2 (A-D) shows the environment-dependent interplay between biomass and harvest index in response to shading. Biomass reduction followed a similar trajectory to yield but was more severe at flowering in Valdivia, and 110 ºCd after flowering at Roseworthy. At Valdivia harvest index began to decline around flowering, at approximately the same stage at which biomass began to increase (Fig. 2C). This decline was gone by the last shade treatment 589 ºCd after flowering. At Roseworthy the decline in harvest index occurred in conjunction with biomass; however, the most significant reduction was at flowering, 110 ºCd earlier than biomass. The last two shading treatments had little effect on harvest index (Fig. 2D). Analysis of residuals for biomass at Valdivia showed no difference between varieties ($p = 0.071$, $s = 3.8$), larger reduction with more intense shading ($p < 0.001$, $s > 9.9$) and no interaction between varieties and shade intensity ($p = 0.849$, $s = 0.2$). Analysis of residuals showed no difference between CIPAL0901 or PBA HurricaneXT at Roseworthy (p, s). Analysis of residuals for harvest index at Valdivia showed no difference between varieties ($p = 0.732$, $s = 0.5$); shade intensity ($p = 0.714$, $s = 0.5$) or interaction ($p = 0.568$, $s = 0.8$).

Analysis of residuals for harvest index showed CIPAL0901 was less sensitive to shade than PBA HurricaneXT at Roseworthy (p = 0.043, s = 4.5).

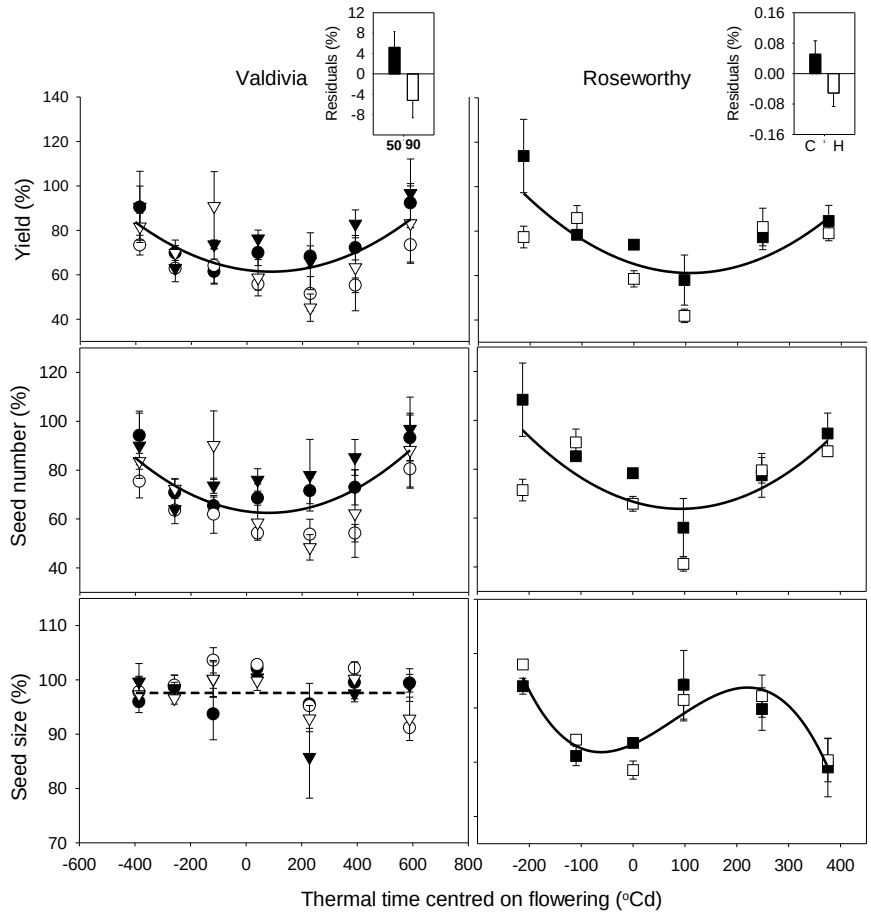

Fig. 1. Effect of timing of shade on normalised (A,B) yield, (C,D) grain number and (E,F) grain size for Valdivia and Roseworthy. Insets show analysis of residuals for shading in Valdivia, and varieties in Roseworthy. For Valdivia, circles are Calpún and triangles are Super Araucana; closed symbols are 50% shade and open symbols are 90%. For Roseworthy closed symbols are CIPAL0901 and open symbols are PBA HurricaneXT. The lines are polynomials except in E where the dashed line is the average across treatments. Error bars are ± S.E. The phenological scale is for the unshaded controls with data presented at the mid- point of the shade period.

Critical period for grain protein and protein yield

Fig. 2 (E-H) shows the effect of timing of shading on grain protein content and protein yield. For the data pooled across varieties and shading intensities at Valdivia and for the data pooled across varieties at Roseworthy, grain protein concentration responded non-linearly to shading. Before a threshold, 40 ± 203.9 °Cd after flowering at Valdivia and 188 ± 71.3 °Cd after flowering at Roseworthy, shading reduced protein concentration by less than 10%. Grain protein concentration increased linearly with shading after these thresholds. Analysis of residuals for grain protein concentration at Valdivia showed Super Araucana was more sensitive than Calpún (p = 0.007, s = 7.2), with no effect of shade intensity (p = 0.223, s = 2.2) or interaction (p = 0.667, s = 0.6). Analysis of residuals for grain protein concentration showed no difference between varieties at Roseworthy (p = 0.571, s = 0.8).

The increases in grain protein content and grain number after flowering were insufficient to overcome the loss in protein yield except in the last 50% shading treatment at Valdivia (Fig. 2 G,H). Analysis of residuals for protein yield at Valdivia showed little difference between varieties (p = 0.068, s = 3.9), a larger effect with more intense shading (p = 0.003, s = 8.4) as well as an interaction (p = 0.048, s = 4.4). Analysis of residuals showed no difference between varieties at Roseworthy (p = 0.577, s = 0.8).

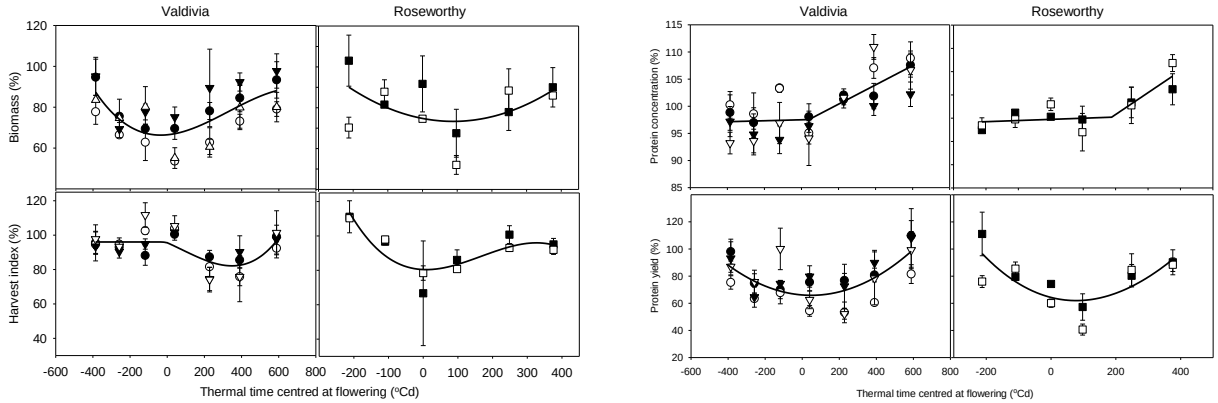

Fig. 2. Effect of shade on normalised biomass (A,B) harvest index (C,D), grain protein concentration (E,F) and protein yield (G,H). For Valdivia, circles are Calpún and triangles are Super Araucana; closed symbols are 50% shade and open symbols are 90%. For Roseworthy closed squares are CIPAL0901 and open squares are PBA HurricaneXT. The lines are polynomials except in G and H which are bi-lineal regressions. Error bars are ± S.E. The phenological scale is for the unshaded controls with data points presented at the mid-point of the shade period.

Conclusion

In two contrasting environments with a 7-fold variation in yield, the most critical period for lentil yield was between 100 and 200 °Cd after flowering, around pod emergence. This reinforces the species-specific nature of the critical period. Grain protein concentration responded bi-linearly to timing of shading, with a slight decrease before an environment-specific threshold close to flowering and a linear increase after this threshold. Site-specific combinations of sowing date and variety phenology are necessary to reduce the likelihood of prevalent stresses to coincide with the critical period. Protein yield tracks grain yield but can be partially buffered by increased grain protein concentration with late-season stress.

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References

Arisnabarreta et al. Field Crop. Res. 107, 196-202.
Darwin, C., 1859. On the origin of species by means of natural selection. J. Murray.
Debaeke and Izquierdo 2021 In Crop Physiology. Case Histories for Major Crops. Academic Press, pp. 483-518.
Estrada-Campuzano et al 2008. Eur. J. Agron. 28, 597-605.
Fischer 1985. Journal of Agricultural Science, UK 105, 447-461.
Flohr et al 2017. Field Crop. Res. 209, 108-119.
Greenland et al 2019. The American Statastician. 73: 106-114.
Lake et al 2021. Eur. J. Agron. 122, 126154.
Lake et al 2019. Field Crop. Res. 241, 107575.
Kirkegaard et al 2018. Field Crops Research. 222: 180-188.
Mahadevan et al 2016. Field Crop. Res. 199, 109-116.
Sadras et al 2015. Crop Past. Sci. 66, 1137-1150.
Savin et al 1991. J. Agr. Sci. 116, 1-7.
Slafer et al 2009. In Crop Physiology: Applications for Genetic Improvement and Agronomy. Academic Press, San Diego, CA, USA, pp. 277–308.
Slafer et al 2021. In Crop Physiology. Case Histories for Major Crops. Academic Press, pp. 99-164.
Tamagno et al 2018. Scientific Reports. 8: 1-11.