Mungbean responses to elevated day- and night-time temperatures

Alwyn Williams[1], Kylie Wenham[2], Millicent Smith[1,2], RCN Rachaputi[2] and Marisa Collins[3]

[1] School of Agriculture and Food Sciences, The University of Queensland, Gatton, QLD 4343, Email: alwyn.williams@uq.edu.au [2] Queensland Alliance of Agriculture and Food Innovation, The University of Queensland, Gatton, QLD 4343
[3] School of Life Sciences, La Trobe University, Melbourne, VIC 3086

Abstract
Little is known about the relative importance of elevated day- vs. night-time temperatures for mungbean growth and development. We investigated mungbean responses to elevated day- vs. night-time temperatures. Mungbean plants were grown under ambient glasshouse conditions until flowering and then transferred to one of four temperature-controlled glasshouses without water limitation until maturity: 1) Ambient Day—Ambient Night [ADAN: 30/20 °C]; 2) Ambient Day—High Night [ADHN: 30/29 °C]; 3) High Day—Ambient Night [HDAN: 40/20 °C]; 4) High Day—High Night [HDHN: 40/29 °C]. Mature pod number declined under HDHN, while pod length, grains/pod and grain biomass declined under both HDAN and HDHN. Vapour pressure deficit (VPD) was highest under HDAN and HDHN and correlated negatively with pod length and grains/pod. The results demonstrate substantial impacts on mungbean productivity from elevated day-time temperatures and combined elevated day- and night-time temperatures but with limited independent effects of elevated night-time temperatures.

Keywords
Climate change; heat stress; vapour pressure deficit, photosynthesis

Introduction
The frequency of high temperature days (>35 °C) in eastern Australia is forecast to increase by 60–80 % by 2030 (Hennessy et al., 2010). Increasing temperatures have been demonstrated to cause higher frequency of heat stress in crops, particularly during critical reproductive phases, driving yield declines globally (Zhao et al., 2017). Much societal and research attention has focused on rising day-time temperatures or average daily temperatures with the impacts of elevated nigh-time temperatures largely overlooked. However, night-time temperatures have risen 1.4 times faster than day-time temperatures over the last century (Sadok and Jagadish, 2020). Rising night-time temperatures have been shown to have negative impacts on several major crops including cotton and wheat (García et al., 2015; Loka and Oosterhuis, 2010). Given that mungbean flowering and pollination occurs nocturnally, this represents an important knowledge gap.

High day-time temperatures can damage crop functional components through protein denaturation and oxidative damage. Such damage undermines yield-critical parameters by causing chlorophyll degradation and low pollen viability, among others. These negative impacts on crop growth and development result in yield loss. High night-time temperatures increase crop respiration, leading to reduced crop carbon (C)-use efficiency and loss of photoassimilated-C needed for grain-fill. Little is known about the relative importance of day- vs. night-time temperatures on mungbean growth and development. For example, recent research demonstrated that mungbean is sensitive to elevated temperatures (>40/25 °C day/night) (Kaur et al., 2015) but did not isolate the independent effects of elevated day- vs. night-time temperatures. Furthermore, elevated temperatures can exert stress on plants through its impact on vapour pressure deficit (VPD). Vapour pressure deficit is a measure of environmental evaporative demand and is a curvilinear function of air temperature. High VPD results in stomatal closure, which inhibits photosynthesis (Grossiord et al., 2020).

We grew two varieties of mungbean under controlled glasshouse conditions to investigate the independent and combined effects of elevated day- and night-time temperatures. Plants were initially grown under ambient temperature conditions (30/20 °C day/night) then moved to their respective temperature treatments upon flowering. We assessed a range of crop productivity and crop physiological parameters with the aim of better understanding the mechanisms by which mungbean responds to elevated temperatures.

Methods

The experiment was conducted in controlled-environment glasshouses at The University of Queensland Gatton campus from November 2019 to February 2020. The experiment had two factors: temperature regime and mungbean variety. A total of four temperature regimes were implemented:

1) Ambient Day / Ambient Night (30/20 °C)
2) Ambient Day / High Night (30/29 °C)
3) High Day / Ambient Night (40/20 °C)
4) High Day / High Night (40/29 °C)

Within each temperature regime, two mungbean varieties were grown: Jade-AU and Celera II-AU. To establish the experiment, three seeds of a single variety were planted into a pot filled with potting mix; these were later thinned to give one plant per pot. A total of 12 pots (240 mm dia.) per variety per temperature regime were planted (96 pots in total). Each pot represented an experimental unit, giving twelve replicates. Pots were arranged in a randomised complete block design within an ambient 30/20 °C (day/night) glasshouse and watered daily. The position of each pot and replicate was re-randomised once per week to minimise within-glasshouse environment effects.

When 50 % of the plants were flowering (late December 2019), pots were evenly distributed across the temperature regimes. Twelve pots of each variety (24 pots in total) were placed into each temperature regime and kept there until at least 90 % of plants had reached physiological maturity. Pots were well-watered daily to avoid water stress. This ensured that plant responses to the temperature regimes were due to temperature-related effects and not confounded by drought stress.

At pod initiation, leaf-to-air vapour pressure deficit (VPD$_L$) for each plant was measured once using a LI-COR 6800 infra-red gas analyzer (LI-COR, Lincoln, NE, USA). A fully expanded, non-shaded leaf was chosen for measurement. Conditions in the chamber were set to photosynthetically active radiation (PAR) of 1500 µmol/m^2/s; CO_2 mole fraction of reference air in the measuring chamber was set at 400 µmol/mol. Leaf water content was determined by weighing at harvest, drying in the oven at 80 °C and reweighing. Once plants reached 90 % physiological maturity, plants were harvested by cutting plants at soil level. The following crop productivity parameters were quantified: the number of black pods, pod length, grains per pod, and grain biomass. Crop productivity parameters were all quantified on a per pot (i.e., per plant) basis.

The crop productivity responses of the two mungbean varieties to the temperature regimes were analysed by two-way factorial analysis of variance (ANOVA) followed by Tukey means separation. Relationships between the crop productivity parameters and VPD$_L$ were explored to indicate the mechanistic basis underpinning the observed results.

Results

Both mungbean varieties exhibited the same directional responses to the elevated temperature regimes; however, Celera II-AU showed greater sensitivity (Table 1). Both varieties showed no clear response in vegetative biomass to elevated temperatures. Similarly, for both varieties, the number of black (mature) pods produced per plant was stable across three out of four of the treatments, only showing a decline in response to combined elevated day- and night-time temperatures (Table 1).

However, the extent of decline in the number of black pods produced under combined elevated day- and night-time temperatures was highly significant, with Jade-AU showing a two-thirds reduction and Celera II-AU producing zero pods (Table 1).

In terms of pod length, grains per pod, and grain biomass, both varieties were unaffected by elevated night-time temperatures but showed significant declines in response to elevated day-time temperatures (Table 1). For both varieties, pod length declined by ~25 % due to elevated day-time temperatures, while grains per pod, and grain biomass both declined by up to 50 %. Under combined elevated day- and night-time temperatures, Jade-AU grain biomass declined further, showing ~85 % reduction compared with ambient temperatures (Table 1). For Celera II-AU, pod length, grains per pod, and grain biomass could not be assessed under combined elevated day- and night-time temperatures due to the absence of any black pods.

Table 1. Mungbean variety responses to elevated temperature treatments (± 1 standard error). Superscript letters within columns indicate significant differences.

Variety	Temperature regime	Vegetative biomass (g/pot)	Number of black pods (#/pot)	Pod length (mm)	Grain number/pod	Grain biomass (g/pot)
Celera II-AU	ADAN	9.3 ± 1.0^a	13 ± 2^a	68 ± 2^b	10.7 ± 0.3^b	4.2 ± 0.7^b
	ADHN	9.8 ± 1.0^a	15 ± 2^a	69 ± 2^b	11.6 ± 0.1^b	4.3 ± 0.6^b
	HDAN	10.7 ± 0.5^a	14 ± 1^a	53 ± 2^a	6.3 ± 0.6^a	2.3 ± 0.1^{ab}
	HDHN	12.0 ± 0.7^a	0 ± 0^c	NA	NA	NA
Jade-AU	ADAN	8.9 ± 0.7^a	12 ± 1^a	94 ± 2^c	10.7 ± 0.3^b	7.7 ± 0.9^c
	ADHN	13.3 ± 0.8^b	15 ± 1^a	93 ± 2^c	11.1 ± 0.3^b	8.1 ± 0.7^c
	HDAN	11.6 ± 0.5^a	13 ± 1^a	69 ± 2^b	6.3 ± 0.4^a	3.7 ± 0.3^b
	HDHN	11.7 ± 0.7^a	5 ± 1^b	65 ± 3^b	5.4 ± 0.8^a	1.3 ± 0.2^a
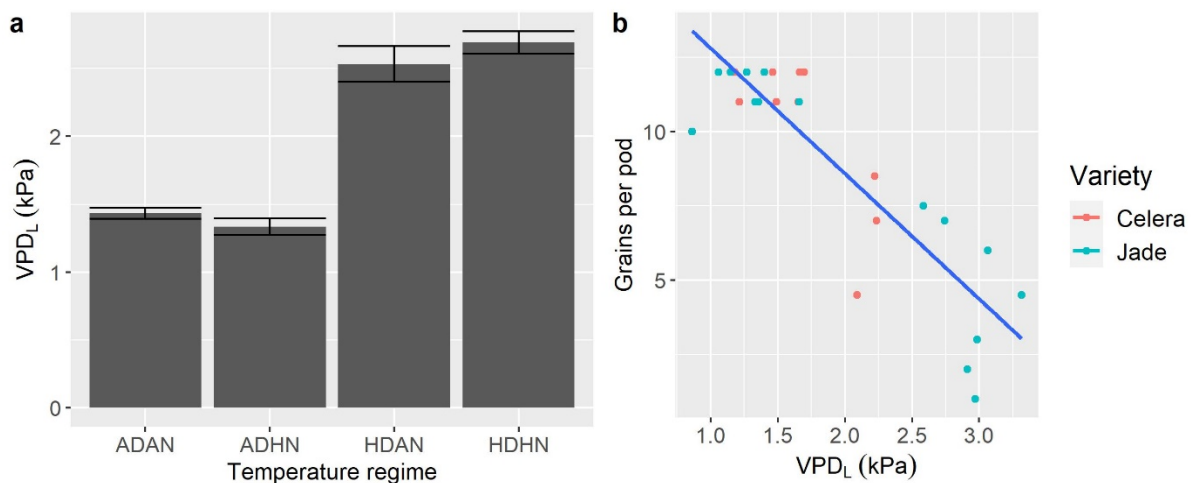
Fig. 1. Mungbean physiological responses measured during late flowering/early pod fill: a) Leaf-to-air vapour pressure deficit (VPDL) under the different temperature regimes. Bars show ± 1 standard error; b) Association between the number of grains per pod and VPDL ($y = -4.2x + 17.0$; $r^2 = 0.75$). Note there are fewer points for Celera II-AU due to the absence of pods in the HDHN regime.

Leaf-to-air VPD differed significantly by temperature regime, with the elevated day and combined elevated day- and night-time temperature regimes having the highest VPDL (Fig. 1a). Moreover, VPDL showed a strong negative correlation with the number of black pods, pod length, and grain biomass; the strongest negative correlation was found between VPDL and grains per pod (Fig. 1b). Leaf water content did not differ across the treatments.

Discussion and Conclusions
The results demonstrate the clear responses of mungbean to elevated day- and night-time

temperatures. When applied independently, mungbean showed significant response to elevated day-time temperatures, showing substantial declines in pod length, grains per pod and grain biomass compared with elevated night-time temperatures. However, when elevated day- and night-time temperatures were applied in combination, the effects on mungbean productivity were severe, with dramatic declines in the production of black pods and a further reduction in grain biomass. These results align well with recent research on elevated temperature effects on mungbean (Kaur et al., 2015).

The temperature regimes that included elevated day-time temperatures (HDAN and HDHN) caused significant increases in VPD_L, which showed strong negative correlation with black pod production, pod length, grain biomass, and grains per pod. This indicates the importance of evaporative demand alongside the direct effect of elevated temperatures for mungbean yield determination. Interestingly, leaf water content showed no relationship with the temperature regimes, VPD_L, or with the crop productivity responses. This indicates that the observed results were not driven by water stress. The absence of any detectable plant water stress suggests mungbean is able to counter elevated VPD, but this comes at high metabolic cost that reduces grain set and fill (Grossiord et al., 2020).

The results demonstrate the potential for significant negative effects on mungbean yield with climate change. Additionally, the data suggest that heat stress can drive broader impacts on mungbean productivity beyond issues of pollen viability. Further research is required to isolate the independent effects of elevated temperatures vs. elevated VPD.

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