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Seed Composition, Seedling Emergence and Early Seedling Vigour of Red Kidney Bean Seed Produced at Elevated Temperature and Carbon Dioxide

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Abstract

Understanding the influence of growth temperature and carbon dioxide (CO₂) on seed quality in terms of seed composition, subsequent seedling emergence and early seedling vigour is important under present and future climates. The objective of this study was to determine the combined effects of elevated temperature and CO₂ during seed-filling of parent plants on seed composition, subsequent seedling emergence and seedling vigour of red kidney bean (*Phaseolus vulgaris*). Plants of cultivar 'Montcalm', were grown at daytime maximum/nighttime minimum sinusoidal temperature regimes of 28/18 and 34/24 °C at ambient CO₂ (350 μmol mol⁻¹) and at elevated CO₂ (700 μmol mol⁻¹) from emergence to maturity. Seed size and seed composition at maturity and subsequent per cent emergence, early seedling vigour (rate of development) and seedling dry matter production were measured. Elevated CO₂ did not influence seed composition, emergence, or seedling vigour of seeds produced either at 28/18 or 34/24 °C. Seed produced at 34/24 °C had smaller seed size, decreased glucose concentration, but significantly increased concentrations of sucrose and raffinose compared to 28/18 °C. Elevated growth temperatures during seed production decreased the subsequent per cent emergence and seedling vigour of the seeds and seedling dry matter production of seed produced either at ambient or elevated CO₂.

Introduction

Changes in climate, particularly an increasing concentration of atmospheric carbon dioxide (CO₂) and an associated temperature increase (IPCC, 2007) might influence plant growth and reproduction. Growth at elevated CO₂ will increase seed yield in most C₃ crop species due to increased rate of photosynthesis and increased vegetative growth under optimal light, temperature and growth conditions (Kimball 1983, Drake et al. 1997, Kimball et al. 2002). Studies have shown that the negative effects of high temperatures (i.e. 5 °C above ambient, upper end of climate change predictions) on reproductive traits such as

pollen viability, seed-set, seed size and harvest index will nullify the beneficial effects of elevated CO₂ on photosynthesis and growth (Baker et al. 1989, Prasad et al. 2002, 2003, 2006a). Changes in climate may also influence seed composition and subsequent establishment of plant species by influencing seedling emergence and early seedling vigour.

Most of the studies on effects of elevated CO₂ and/or temperature on crop species have given little attention to aspects of seed quality in terms of seed size, seed composition, subsequent seedling emergence and early seedling vigour. Analyses of data from 159 studies over 79 crop and wild species showed that there was a small (4 %) but

significant increase due to elevated CO₂ (500–800 μmol mol⁻¹) on individual seed size or mass (Jablonski et al. 2002). Our previous studies on grain legumes showed no effect of elevated CO₂ on seed size (Baker et al. 1989, Thomas 2001, Prasad et al. 2002, 2003). There has been little information on the effect of elevated CO₂ upon seed composition of legume species with the exception of soybean (*Glycine max* L. Merr.). Thomas et al. (2003) observed no effect of elevated CO₂ (700 μmol mol⁻¹) on seed size, N, P, starch, total oil, fatty acids, and total non-structural carbohydrates in soybean seed when compared to ambient CO₂. Some elevated CO₂ studies reported decreased N concentration in soybean seed (Rogers et al. 1984) while others reported no effect (Allen et al. 1988, Thomas et al. 2003). There were no reported effects of elevated CO₂ on nutrient composition and mineral nutrients in peanut (*Arachis hypogaea* L.) seeds (Wu et al. 1997). There was no effect of parental plant growth at elevated CO₂ on subsequent seedling emergence of soybean (Rogers et al. 1980) and wheat (*Triticum aestivum* L.; Sanhewe et al. 1996).

Growth at elevated (above optimum) temperature decreased seed yield and seed size of dry bean (*Phaseolus vulgaris* L.; Sexton et al. 1994, Sanhewe and Ellis 1996, Prasad et al. 2002, Porch 2006), peanut (Nigam et al. 1998, Prasad et al. 2000, 2003) and soybean (Pan 1996). Several studies have investigated the influence of growth temperature on composition of soybean seed, while no data is available on other grain legumes. There were continuous negative effects of temperature increases from 28/18 through 44/34 °C on N, P, starch, total oil, fatty acids and total non-structural carbohydrates in soybean (Thomas et al. 2003). Oil concentration in soybean seed decreased at temperatures above 28 °C, while protein concentration increased at temperatures above 25 °C (Wolf et al. 1982, Dornbos and Mullen 1992, Gibson and Mullen 1996a, Piper and Boote 1999). Soybean seeds obtained from plants grown at high day (35 °C) and high night (30 °C) temperatures had lower seed germination and seedling vigour (Gibson and Mullen 1996b). Soil moisture conditions during soybean growth could also influence nutritional quality of seed (Al-Tawaha et al. 2007). In cowpea [*Vigna unguiculata* (L.) Walp], establishment of a crop later in the season when environmental conditions were favourable produced better quality seeds measured in terms of germination after controlled deterioration (Sangakkara 1998). However, there was no influence of moisture stress on seed viability and seedling vigour in peanut (Ramamoorthy and Basu 1996).

Studies on combined and interactive effects of elevated growth temperature and CO₂ on seed quality of crops are uncommon and limited to few crop species such as wheat (Sanhewe et al. 1996) and soybean (Thomas 2001,

Thomas et al. 2003). A better understanding of combined effects of super-optimal temperature and elevated CO₂ on seed quality in other crop species is essential and will provide wider impact assessment. We hypothesize that seed composition and quality may change as an acclimation to elevated temperatures and CO₂. The impact of combined effects of elevated temperature and CO₂ on dry bean phenology, growth, reproductive processes and yield were quantified and described elsewhere (Prasad et al. 2002). The objective of this study was to investigate the combined effects of elevated temperature and CO₂ during an entire growing season (including seed-fill) of parent plants on seed composition, subsequent seedling emergence and seedling vigour of red kidney bean (*P. vulgaris*), which is an important food grain legume grown in many parts of the world.

Materials and Methods

Growing conditions during seed formation and maturation

This research was conducted at the controlled environment facility of the University of Florida and United States Department of Agriculture – Agricultural Research Service at Gainesville, Florida, USA. Plants of red kidney bean, cultivar 'Montcalm', were grown at daytime maximum/nighttime minimum temperature regimes of 28/18 and 34/24 °C at ambient CO₂ (350 μmol mol⁻¹) and at elevated CO₂ (700 μmol mol⁻¹) from emergence to maturity in eight sunlit controlled-environment chambers attached to 60-cm deep soil bins. Detailed information on growth chamber growing conditions, uniformity of environmental conditions, and controls of sunlit controlled environment chambers are given in Prasad et al. (2002, 2006a). The temperature was controlled in a sinusoidal function during the day and exponential decay function at night (Parton and Logan 1981). The temperatures were controlled within ± 0.2 °C of the set-points. Dewpoint temperatures in the chambers were controlled so that the relative humidity in each chamber was similar (about minimum of 40–42 % at 1500 h). Daytime CO₂ concentrations in chambers were controlled within 2 μmol mol⁻¹ of set-point. Plants were surface irrigated using sprinklers from sowing (15 August 2000) to 20 days after sowing (DAS); thereafter, subsurface irrigation was provided by automated float valves that controlled the water table at 45 cm below the soil surface. Plants in all treatments were healthy and did not have any biotic or abiotic stress other than the treatment effects. All plants were harvested at maturity when all pods were yellow and seeds were red in colour. Seed from two temperature treatments (28/18 and 34/24 °C) at ambient or elevated

CO₂ were analysed for seed quality (composition, seedling emergence and seedling vigour).

Seed composition

Seed from the final harvest were air dried and two sets of 100-seed samples were taken from each treatment for composition analyses. The seed were ground in a single rotary grinder (Ika Werk, Staufen, Germany) and then milled to fine powder in a blender (Waring, Torrington, CT, USA) to achieve uniform particle size. The ground seed were stored at -20 °C until further analysis. All composition tests were duplicated, utilizing ground seed from these two 100-seed samples. Total N in the seed was estimated by the modified aluminum block digestion procedure of Gallaher *et al.* (1975). Seed samples of 0.25 g were digested for 4 h at 375 °C in a mixture of 1.5 g of 9 : 1 K₂SO₄ : CuSO₄, 6 ml of H₂SO₄ and 2 ml of H₂O₂. The N in the digestate was determined by semi-automated colorimetry (Hambleton 1977). Crude protein in seed was computed by multiplying N concentration by 6.25.

Soluble protein concentration was determined using Bio-Rad[®] (Bio-Rad Laboratories, Inc., Hercules, CA, USA) protein assay, which is based on the Bradford (1976) method. Per cent oil was determined by dissolving seed oil in hexane : methyltertbutyl ether (1 : 1 v/v), centrifuging and collecting the supernatant, then evaporating the solvent with a rotary evaporator. The oil remaining in the tube was then weighed.

Analysis of carbohydrate composition was conducted with the method of LePrince *et al.* (1990), as modified by Sinniah *et al.* (1998) using high pressure liquid chromatography (HPLC). Soluble carbohydrates were extracted by homogenizing 0.5 g of ground seed in 3 ml of ethanol (80 % v/v). The samples were incubated at 80 °C for 15 min in plastic centrifuge tubes in a water bath. The homogenate was then centrifuged for 10 min at 3000 g. The pellet was homogenized two more times with 3 ml of 80 % ethanol, incubated at 80 °C for 15 min and centrifuged each time. The supernatants were removed and used to determine glucose and fructose concentrations, which were analysed on a microplate using the method of Hendrix (1993).

Twenty microlitres of each supernatant was dried and then reconstituted in 50 µl of deionized, distilled water, diluted 1 : 10, and filtered through a 0.45 µm filter. Thereafter, 10 µl of aliquot was taken and subjected to HPLC analysis using a PAD detector (Shimadzu, Kyoto, Japan). The separation of sugars was achieved with a DIONEX-CarboPac PAIO 2741 using 90 mM NaOH as the eluent. Identification and quantification of raffinose, stachyose and sucrose was achieved by comparing the retention times with known standards, using melezitose as an added internal standard (Sigma Chemical Company,

St. Louis, MO, USA), and comparing the eluted peaks with known amounts of standard solutions.

For starch analysis, the pellets from each sample were first air-dried at 40 °C. The dry pellet was then digested with 1 ml 0.2 N KOH in boiling water and, after cooling to room temperature, 0.2 ml of acetic acid and 2 ml of acetate buffer (pH 4.6) containing amyloglucosidase were added. The tubes were incubated at 55 °C for 60 min to complete starch hydrolysis, then 2 ml of water was added and the tubes were mixed and centrifuged. To assay starch, 20 µl of supernatant was added to the wells of standard micro-plate for glucose analysis. Glucose equivalents from standard curves were determined to estimate starch concentration as described by Hendrix (1993).

Seed size, per cent emergence and seedling vigour

Mean seed size (single seed weight) from each treatment was estimated by dividing the total seed weights by the total numbers of seeds. To test subsequent emergence and early seedling vigour, harvested seeds were air dried at 26 °C and stored at 5 °C until sowing. This experiment was conducted on natural field soil, Millhopper fine sand (a loamy, siliceous, hyperthermic Grossarenic Paleudult) in two large polytunnels (27.4 m long, 4.4 m wide and 2.2 m high at the apex). Each polytunnel was further divided into two sections, east and west, for replicated treatments. Thus, there were four replications from two polytunnels. Air temperature was measured with aspirated copper constantan thermocouples at 50 cm above soil surface at 1-s intervals and means were stored at hourly intervals. Similarly, soil temperature at sowing depth (5 cm) was measured at hourly intervals using HOBO StowAway temperatures loggers (Onset computer, Bourne, MA, USA). A complete description of these polytunnels and uniformity of environmental conditions are given by Prasad *et al.* (2006b,c).

Soil in the polytunnels was tilled using a hand operated roto-tiller, then levelled and irrigated before sowing. Seeds randomly selected from the seed lot of each treatment were sown 8 cm apart in three rows, each 1.4 m long and 20 cm apart, on 26 March 2002. A total of 51 seeds were sown for each treatment in each of the four replications (i.e. total of 204 seeds). Irrigation was provided on alternate days by timed automatic micro-sprinklers to maintain adequate moisture for seed germination, emergence and seedling growth. Plots were checked daily during the first 7 days and on alternate days thereafter to determine the number of seedlings emerged (emergence of any green part of the plant) and number of plants reaching vegetative stage V₂ (beginning of separation of second trifoliate leaflets). Subsequent V stages until flowering were recorded. Final emergence was estimated as the ratio of

the number of seedlings emerged to the number of seeds sown and expressed as percentage. Time from sowing to emergence and various V-stages (50 % of plants emerged or reaching corresponding V-stage) were calculated. The rate of seedling emergence was estimated as the inverse of the number of days from sowing to 50 % emergence. Similarly, the rate of development to V₂-stage was estimated as the inverse of the number of days from sowing to V₂-stage. The mean daytime (0700–1800 h)/mean nighttime (1800–0700 h) air temperatures during emergence (0–7 DAS) were 30.5/21.0 °C; and during early growth (8–30 DAS) were 30.7/22.5 °C.

To determine seedling vigour, six seedlings were harvested at 5, 10, 17 and 24 DAS, by manually digging deep to avoid damage and to keep root system intact. At each harvest date, plants were separated into component parts (leaves, stems and roots). Root system was carefully washed by keeping the plant on sieves and gently spraying with water. Plant height (from soil surface to tip of the top emerging leaf) and leaf area (with LI-COR 3000 leaf area meter; LI-COR, Lincoln, NE, USA) were measured. Leaf, stem and root dry weights were recorded after oven drying for 5 days at 65 °C.

Data analysis

Data analyses for all the measured and calculated variables were conducted using ANOVA procedures in SAS (SAS Institute, Cary, NC, USA). The combinations of two CO₂ concentrations (ambient 350 μmol mol⁻¹ and elevated 700 μmol mol⁻¹) and two temperatures (28/18 and 34/24 °C) were regarded as treatments. The data were analysed as a randomized complete block design. There were two replications for seed composition, and four

replications for seedling emergence, seedling vigour and component dry weights.

Results

Seed composition

There were no significant effects of elevated CO₂, temperature and interaction between CO₂ and temperature on total soluble protein concentration, or crude protein concentration (Table 1). Only the interaction between CO₂ and temperature was significant for N concentration, which decreased under elevated CO₂ at growth temperature of 28/18 °C, but not at 34/24 °C. Elevated temperature of 34/24 °C compared to 28/18 °C significantly decreased oil concentration by 23 %. Interaction between CO₂ and temperature showed that elevated CO₂ increased oil concentration by 38 % at growth temperature of 28/18 °C but decreased it by 19 % at 34/24 °C. Elevated temperature decreased concentration of glucose (monosaccharide) by 44 %, while it increased concentrations of sucrose and raffinose (oligosaccharides), by 33 and 116 respectively. There were no effects of elevated growth temperature on concentrations of fructose, starch and total carbohydrates. There were no effects of elevated CO₂ on carbohydrates with the exception of glucose, which was significantly decreased by 27 % at higher CO₂. There were no effects of the interaction of elevated CO₂ and temperature on concentrations of carbohydrates tested.

Seed size, per cent emergence and seedling vigour

Temperature was the only influencing factor on seed size, per cent seedling emergence and early seedling vigour

Table 1 Influence of growth temperature (T, 28/18 and 34/24 °C) at ambient (350 μmol mol⁻¹) and elevated (700 μmol mol⁻¹) CO₂ during seed formation on mature seed composition (nitrogen, protein, oil and various carbohydrates) of red kidney bean when grown in sunlit controlled environment growth chambers

Trait	28/18 °C			34/24 °C			LSD and significance		
	350	700	Mean	350	700	Mean	CO ₂	T	Interaction
Nitrogen (%)	4.62	4.33	4.48	4.40	4.53	4.47	ns	ns	0.20*
Crude protein (%)	28.86	27.09	27.98	27.50	28.31	27.91	ns	ns	ns
Soluble protein (%)	14.63	15.37	15.00	12.68	12.68	12.68	ns	ns	ns
Oil (%)	1.77	2.44	2.11	1.80	1.46	1.63	ns	2.73*	2.73*
Glucose (%)	0.24	0.21	0.23	0.17	0.09	0.13	0.24*	0.24***	ns
Fructose (%)	0.09	0.10	0.10	0.15	0.14	0.15	ns	ns	ns
Sucrose (%)	5.82	5.27	5.55	6.93	7.79	7.36	ns	1.28*	ns
Raffinose (%)	0.40	0.35	0.38	0.74	0.89	0.82	ns	0.27**	ns
Stachyose (%)	3.95	3.96	3.96	4.34	4.83	4.59	ns	ns	ns
Starch (%)	22.14	28.67	25.41	27.58	25.97	26.78	ns	ns	ns
Total carbohydrates (%)	32.65	38.56	35.61	39.91	39.62	39.77	ns	ns	ns

*P < 0.05, **P < 0.01, ***P < 0.001. ns = non-significant P > 0.05.

Table 2 Influence of growth temperature (T, 28/18 and 34/24 °C) at ambient (350 $\mu\text{mol mol}^{-1}$) and elevated (700 $\mu\text{mol mol}^{-1}$) CO₂ during seed formation on seed size, and subsequent per cent seedling emergence, time to emergence and V₂ stage, and rate of emergence and rate to V₂ stage of red kidney bean when grown under field soil conditions under polytunnels

Trait	28/18 °C			34/24 °C			LSD and significance		
	350	700	Mean	350	700	Mean	CO ₂	T	Interaction
Seed size (g seed ⁻¹)	0.53	0.56	0.55	0.37	0.42	0.40	ns	0.02***	ns
Final emergence (%)	95.1	97.0	96.05	87.73	79.87	83.80	ns	9.96**	ns
Time to emergence (days)	5.54	5.63	5.59	6.65	6.63	6.64	ns	0.62***	ns
Rate of emergence (day ⁻¹)	0.180	0.177	0.18	0.150	0.151	0.15	ns	0.02***	ns
Time to V ₂ stage (days)	13.00	13.00	13.00	17.00	18.00	17.50	ns	0.77***	ns
Rate to V ₂ stage (days ⁻¹)	0.078	0.078	0.08	0.059	0.056	0.06	ns	0.003***	ns

P < 0.01, *P < 0.001. ns = non-significant P > 0.05.

(Table 2). There were no significant effects of growth at elevated CO₂ or interaction between CO₂ and temperature on seed size, subsequent total per cent seedling emergence, time to emergence, time to V₂ stage, rates of emergence and rate of development to V₂ stage.

Growth at elevated temperature of 34/24 °C compared to 28/18 °C significantly decreased subsequent total per cent seedling emergence from 96 % to 84 % when averaged across CO₂ treatments (Table 2 and Fig. 1). There was a significant effect of growth temperature on rate of emergence and rate of development to V₂ stage. Seedlings from seed produced at 28/18 °C emerged a day before seedlings from seed produced at 34/24 °C, and the timing of leaf emergence was more uniform, compared to seedlings from seed produced at 34/24 °C. Similarly, developmental time to V₂ stage was longer and rate of development was significantly slower in seeds formed at 34/24 °C compared to 28/18 °C (Table 2). Moreover, seeds produced at 34/24 °C developed unevenly with

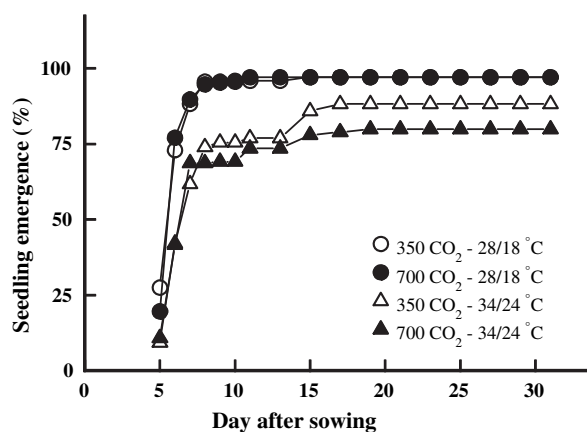


Fig. 1 Seedling emergence (%) of seeds produced at growth temperatures of 28/18 and 34/24 °C under ambient (350 $\mu\text{mol mol}^{-1}$) or elevated (700 $\mu\text{mol mol}^{-1}$) CO₂ at different days after sowing. Each point is mean of four replications.

some individual plants lagging behind the rest of the population. Unifoliate leaves were often crinkled and flowering was slightly delayed in seedlings from seed produced at high temperature.

There were significant effects of growth temperature on subsequent seedling growth and component dry weights at different times after sowing (Figs 2 and 3). Seedlings grown from seed produced at elevated temperature (34/24 °C) were shorter in height (Fig. 2b) with smaller total plant leaf area (Fig. 2c) when compared to those produced at 28/18 °C at 10, 17 and 24 DAS, regardless of CO₂ treatment during seed formation. The per cent decrease due to growth at elevated temperature ranged from 10 % to 20 % in plant height and by 20 % to 40 % in total leaf area. Similar decreases in leaf, stem and total plant dry weight caused by elevated temperature during seed growth were observed across all harvest dates at 5, 10 and 17 DAS (Fig. 3). The percentage decrease in leaf dry weights of seedlings produced from seeds formed at elevated temperature (34/24 °C) when compared to ambient temperature (28/18 °C) were 31 %, 40 % and 33 % at 5, 10 and 17 DAS respectively (Fig. 3). The corresponding decreases at 5, 10 and 17 DAS in stem dry weights were 5 %, 34 % and 37 %, respectively, and total dry weights were 6 %, 25 % and 35 % respectively. There was no effect of parental CO₂ or interaction between CO₂ and temperature on seedling growth or dry matter accumulation across all harvest dates (Figs 2 and 3).

Discussion

Seed quality of dry bean in terms of subsequent seedling emergence and seedling vigour was negatively influenced by growth temperature of the parent plant, but was not influenced by elevated CO₂. Previous studies on soybean (Rogers et al. 1980) and dry bean (Sanhewé et al. 1996) showed no effect of growth at elevated CO₂ on per cent germination and early vigour. In contrast, *Arabidopsis*

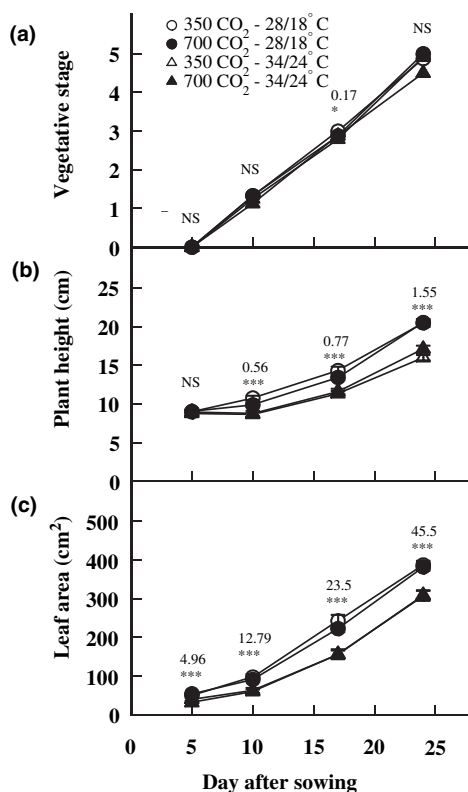


Fig. 2 (a) Vegetative stage, (b) plant height and (c) leaf area of seedlings emerged from seeds produced at growth temperatures of 28/18 and 34/24 °C under ambient (350 $\mu\text{mol mol}^{-1}$) or elevated (700 $\mu\text{mol mol}^{-1}$) CO₂ at different days after sowing. The significance of P-values and LSD of temperature is shown at each harvest time. There were no significant effects of CO₂ or interaction between CO₂ and temperature on any of these traits. Each point is denoted by mean values ($n = 4$) and \pm S.E.

thaliana seed that developed at elevated CO₂ had lower germination and seedling growth (Andalo et al. 1996, 1998). Similarly, total biomass of *Bromus rubens* seedlings produced by elevated CO₂-developed seeds were smaller than those that developed at ambient CO₂ (Huxman et al. 1998); however, *Trifolium repens* seeds harvested from plants developed at elevated CO₂ had higher germination and seedlings with greater mass (Edwards et al. 2001).

Elevated temperature during seed-filling of dry bean seeds significantly decreased subsequent per cent seedling emergence and early seedling vigour (Table 2 and Fig. 1). Growth at elevated temperature decreased the seed size under both ambient and elevated CO₂ conditions (Table 2). In this experiment, there was a positive correlation ($r^2 = 0.64$) between seed size as influenced by growth conditions and subsequent per cent seedling emergence. Similarly, Sanhewe and Ellis (1996) reported that higher temperature during maturation resulted in smaller seed of poor quality.

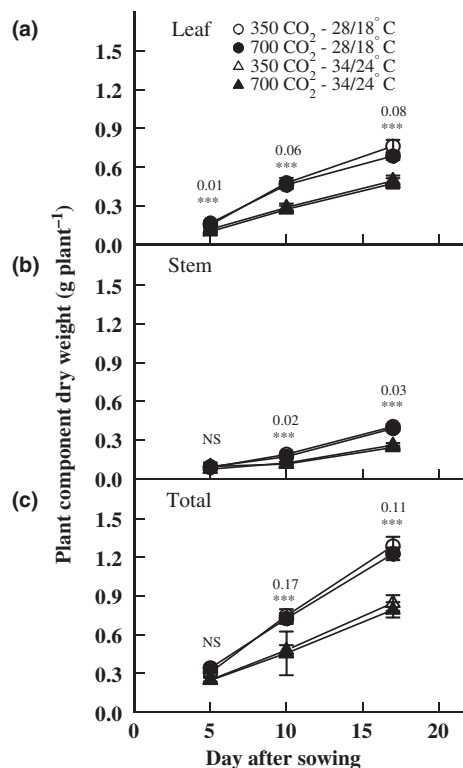


Fig. 3 (a) Leaf (b) stem and (c) total dry weight (leaf, stem, root and cotyledon) of seedlings emerged from seeds produced at growth temperatures of 28/18 and 34/24 °C under ambient (350 $\mu\text{mol mol}^{-1}$) or elevated (700 $\mu\text{mol mol}^{-1}$) CO₂ at different days after sowing. The significance of P-values and LSD of temperature is shown at each harvest time. There were no significant effects of CO₂ or interaction between CO₂ and temperature on any of these traits. Each point is denoted by mean values ($n = 4$) and \pm S.E.

Lower seedling emergence at elevated temperature could be related to carbohydrate concentrations. While elevated growth temperatures of 34/24 °C increased sucrose and raffinose concentrations, they decreased per cent seedling emergence and seedling vigour, regardless of CO₂ (Tables 1 and 2). There were negative relations between sucrose, raffinose, concentrations and final emergence percentage ($r^2 = 0.97$ – 0.98) and total seedling dry weight at 17 DAS ($r^2 = 0.83$ – 0.92). Increases in sucrose and oligosaccharides (such as raffinose) and decreases in monosaccharides (such as glucose and fructose) were reported to be associated with decreases in seed quality (Bernal-Lugo and Leopold 1995). Normal soybean lines and soybean lines with reduced concentration of raffinose were studied by Meis et al. (2003). Seed with low raffinose produced in a subtropical environment, which would include elevated temperatures, had significantly less field emergence (8 %) than the same seed produced in temperate climates (63 %)(Meis et al. 2003). In contrast, some studies showed no relation between raffinose

content and seed quality (Bentsink *et al.* 2000). In another legume, *Medicago truncatula*, stachyose has been shown to positively impact seed longevity (Rosnoblet *et al.* 2007). Raffinose and stachyose have been shown to be osmoprotectants whose levels are often elevated under several abiotic stresses. Thus, concentration changes in these carbohydrates may also be likely indicators of stress (such as high temperature or drought) exposure during seed development and maturation.

Raffinose in beans and bean meal creates digestive problems in both non-ruminant animals and humans, where the intestinal mucosa does not contain the galactosidase enzyme necessary to digest this carbohydrate (Sebastian *et al.* 2000). In contrast, the intestinal microfloras are able to metabolize the raffinose, which results in the production of flatulents (Hawton *et al.* 1996). Therefore, increases in concentrations of raffinose at elevated growth temperature (as observed in this study) would further decrease the nutritional and digestive quality of dry bean seed.

The day/night temperatures of 28/18 °C (cooler treatment) during growth in our study (Prasad *et al.* 2002) and 27/21 °C (cooler treatment) in studies of Sanhew and Ellis (1996) are close to the upper limit of typical temperatures where red kidney type dry bean is currently grown (e.g. higher elevation sites in Central and South America, and high latitudes of Europe). Therefore, any further increase in temperature due to climate change or climate variability in these regions would not only decrease seed yield but also decrease the seed quality due to adverse effects on seed composition and loss of early seedling vigour. To cope with these changes, new production practices such as changes in fertilizer management (increased N and other nutrients) or cultivar selection may be necessary. These issues and interactions of climate change factors and nutrients need further research. In conclusion, our research has shown that elevated temperatures (34/24 °C compared with 28/18 °C) during seed development will decrease seed size, alter seed composition, decrease nutritional value of seeds, and decrease subsequent emergence and seedling vigour. It is important to test if such changes occur over multiple years and also in other plant species, as continuous change may have potential to change crop ecologies and competition.

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References

- Allen, L. H. Jr, J. C. V. Vu, R. R. Valle, K. J. Boote, and J. W. Jones, 1988: Nonstructural carbohydrates and nitrogen of soybean grown under carbon dioxide enrichment. *Crop Sci.* 28, 84–94.
- Al-Tawaha, A. M., P. Seguin, D. L. Smith, and R. B. Bonnell, 2007: Irrigation level affects isoflavone concentrations of early maturing soybean cultivars. *J. Agron. Crop Sci.* 193, 238–246.
- Andalo, C., B. Godelle, M. Lefranc, M. Mousseau, and I. Till-Bottraud, 1996: Elevated CO₂ decreases seed germination in *Arabidopsis thaliana*. *Global Change Biol.* 2, 129–137.
- Andalo, C., C. Raquin, N. Machon, B. Godelle, and M. Mousseau, 1998: Direct and maternal effects of elevated CO₂ on early root growth of germinating *Arabidopsis thaliana* seedlings. *Ann. Bot.* 81, 405–411.
- Baker, J. T., L. H. Allen Jr, K. J. Boote, P. Jones, and J. W. Jones, 1989: Response of soybean to air temperature and carbon dioxide concentration. *Crop Sci.* 29, 98–105.
- Bentsink, L., C. Alonso-Blanco, D. Vreugdenhil, K. Tesnier, S. P. C. Groot, and M. Koornneef, 2000: Genetic analysis of seed-soluble oligosaccharides in relation to seed storability of *Arabidopsis*. *Plant Physiol.* 124, 1595–1604.
- Bernal-Lugo, I., and A. C. Leopold, 1995: Seed stability during storage – raffinose and seed glassy state. *Seed Sci. Res.* 5, 75–80.
- Bradford, M. M., 1976: A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 72, 248–254.
- Dornbos, D. L., and R. E. Mullen, 1992: Soybean seed protein and oil concentration and fatty-acid composition adjustments by drought and temperature. *J. Am. Oil Chem. Soc.* 69, 228–231.
- Drake, B. G., M. A. Gonzales-Meler, and S. P. Long, 1997: More efficient plants: a consequence of rising atmospheric CO₂? *Ann. Rev. Plant Physiol. Plant Mol. Biol.* 48, 609–639.
- Edwards, G. R., P. C. D. Newton, J. C. Tilbrook, and H. Clark, 2001: Seedling performance of pasture species under elevated CO₂. *New Phytol.* 150, 359–369.
- Gallaher, R. N., C. O. Weldon, and J. G. Futral, 1975: An aluminum block digester for plant and soil analysis. *Soil Sci. Soc. Am. Proc.* 39, 803–806.
- Gibson, L. R., and R. E. Mullen, 1996a: Soybean seed composition under high day and night temperatures. *J. Am. Oil Chem. Soc.* 73, 733–737.
- Gibson, L. R., and R. E. Mullen, 1996b: Soybean seed quality reductions by high day and night temperature. *Crop Sci.* 36, 1615–1619.
- Hambleton, L. G., 1977: Semiautomated method for simultaneous determination of phosphorus, calcium and crude protein in animal feeds. *J. Assoc. Official Agric. Chem.* 60, 845–852.
- Hawton, J. D., L. J. Johnston, T. M. Seizer, and G. C. Shurson, 1996: Applications of nutritionally improved corn and

- soybeans for swine. In: Proceedings of the 57th Minnesota Nutrition Conference, p. 304. University of Minnesota, St. Paul, MN.
- Hendrix, D. L., 1993: Rapid extraction and analysis of nonstructural carbohydrates in plant tissue. *Crop Sci.* 33, 1306–1311.
- Huxman, T. E., E. P. Hamerlynck, D. N. Jordan, K. J. Salsman, and S. D. Smith, 1998: The effects of parental CO₂ environment on seed quality and subsequent seedling performance in *Bromus rebens*. *Oecologia* 114, 202–208.
- IPCC, 2007: Intergovernmental Panel on Climate Change Fourth Assessment Report: Climate Change 2007. Synthesis Report. World Meteorological Organization, Geneva.
- Jablonski, L. M., X. Z. Wang, and P. S. Curtis, 2002: Plant reproduction under elevated CO₂ conditions: a meta-analysis of reports on 79 crop and wild species. *New Phytol.* 156, 9–26.
- Kimball, B. A., 1983: Carbon dioxide and agricultural yield: an assemblage and analysis of 430 prior observations. *Agron. J.* 75, 779–788.
- Kimball, B. A., K. Kobayashi, and M. Bindi, 2002: Responses of agricultural crops to free-air CO₂ enrichment. *Adv. Agron.* 77, 293–368.
- Leprince, O., R. Bronchart, and R. Deltour, 1990: Changes in starch and soluble sugars in relation to the acquisition of desiccation tolerance during maturation of *Brassica campestris* seed. *Plant Cell Environ.* 13, 539–546.
- Meis, S. J., W. R. Fehr, and S. R. Schnebly, 2003: Seed source effect on field emergence of soybean lines with reduced phytate and raffinose saccharides. *Crop Sci.* 43, 1336–1339.
- Nigam, S. N., R. C. Nageswara Rao, and J. C. Wynne, 1998: Effect of temperature and photoperiod on vegetative and reproductive growth of groundnut (*Arachis hypogaea* L.). *J. Agron. Crop Sci.* 181, 117–124.
- Pan, D., 1996: Soybean Responses to Elevated Temperature and Doubled CO₂. PhD Dissertation. University of Florida, Gainesville, FL. Dissertation Abstracts International 57, no. 10B, (1996): 5987. Accession No. AAG709292.
- Parton, W. J., and A. Logan, 1981: A model for diurnal variation in soil and air temperature. *Agric. Meteorol.* 23, 205–216.
- Piper, E. L., and K. J. Boote, 1999: Temperature and cultivar effects on soybean seed oil and protein concentrations. *J. Am. Oil Chem. Soc.* 76, 1233–1241.
- Porch, T. G., 2006: Application of stress indices for heat tolerance screening of common bean. *J. Agron. Crop Sci.* 192, 390–394.
- Prasad, P. V. V., P. Q. Craufurd, and R. J. Summerfield, 2000: Effect of high air and soil temperature on dry matter production, pod yield and yield components of groundnut. *Plant Soil* 222, 231–239.
- Prasad, P. V. V., K. J. Boote, L. H. Allen Jr, and J. M. G. Thomas, 2002: Effects of elevated temperature and carbon dioxide on seed-set and yield of kidney bean (*Phaseolus vulgaris* L.). *Global Change Biol.* 8, 710–721.
- Prasad, P. V. V., K. J. Boote, L. H. Allen Jr, and J. M. G. Thomas, 2003: Super-optimal temperatures are detrimental to peanut (*Arachis hypogaea* L.) reproductive processes and yield at both ambient and elevated carbon dioxide. *Global Change Biol.* 9, 1775–1787.
- Prasad, P. V. V., K. J. Boote, and L. H. Allen Jr, 2006a: Adverse high temperature effects on pollen viability seed-set, seed yield and harvest index of grain sorghum [*Sorghum bicolor* (L.) Moench] are more severe at elevated carbon dioxide due to high tissue temperatures. *Agric. For. Meteorol.* 139, 237–251.
- Prasad, P. V. V., K. J. Boote, L. H. Allen Jr, J. E. Sheehy, and J. M. G. Thomas, 2006b: Species, ecotype and cultivar differences in spikelet fertility and harvest index of rice in response to high temperature stress. *Field Crops Res.* 95, 398–411.
- Prasad, P. V. V., K. J. Boote, J. M. G. Thomas, L. H. Allen Jr, and D. W. Gorbet, 2006c: Influence of soil temperature on seedling emergence and early growth of peanut cultivars in field conditions. *J. Agron. Crop Sci.* 192, 168–177.
- Ramamoorthy, K., and R. N. Basu, 1996: Studies on the effect of moisture stress at different growth phases on seed vigor, viability and storability in peanut (*Arachis hypogaea* L.). *J. Agron. Crop Sci.* 177, 33–37.
- Rogers, H. H., G. E. Bingham, J. D. Cure, W. W. Heck, A. S. Heagle, D. W. Israel, J. M. Smith, K. A. Sunaro, and J. F. Thomas, 1980: Field Studies of Plant Responses to Elevated Carbon Dioxide Levels. Response to Vegetation to Carbon Dioxide Series, No. 1. USDE/USDA, Washington, DC.
- Rogers, H. H., J. D. Cure, J. F. Thomas, and J. M. Smith, 1984: Influence of elevated CO₂ on growth of soybean plants. *Crop Sci.* 24, 361–366.
- Rosnoblet, C., C. Aubry, O. Leprince, B. L. Vu, H. Rogniaux, and J. Buitink, 2007: The regulatory gamma subunit SNF4b of the sucrose non-fermenting-related kinase complex is involved in longevity and stachyose accumulation during maturation of *Medicago truncatula* seeds. *Plant J.* 51, 47–59.
- Sangakkara, U. R., 1998: Growth and yields of cowpea [*Vigna unguiculata* (L.) Walp] as influenced by seed characters, soil moisture and season of planting. *J. Agron. Crop Sci.* 180, 137–142.
- Sanhewe, A. J., and R. H. Ellis, 1996: Seed development and maturation in *Phaseolus vulgaris*. II. Ability to germinate and tolerate desiccation. *J. Exp. Bot.* 47, 949–958.
- Sanhewe, A. J., R. H. Ellis, T. D. Hong, T. R. Wheeler, G. R. Batts, P. Hadley, and J. I. L. Morrison, 1996: The effect of temperature and CO₂ on seed quality development in wheat (*Triticum aestivum* L.). *J. Exp. Bot.* 47, 631–637.
- Sebastian, S. A., P. S. Kerr, R. W. Pearlstein, and W. D. Hitz, 2000: Soybean germplasm with novel genes for improved digestibility. In: J. K. Drackely, ed. Soy in Animal Nutrition, pp. 56–74. Federation of Animal Science Society, Savory, IL.
- Sexton, P. J., J. W. White, and K. J. Boote, 1994: Yield-determining processes in relation to cultivar seed size in common bean. *Crop Sci.* 34, 84–91.

- Sinniah, U. R., R. H. Ellis, and P. John, 1998: Irrigation and seed quality development in rapid-cycling Brassica: soluble carbohydrates and heat stable proteins. *Ann. Bot.* 82, 647–655.
- Thomas, J. M. G., 2001: Impact of Elevated Temperature and Carbon Dioxide on Development and Composition of Soybean Seed. PhD Dissertation. University of Florida, Gainesville, FL. Dissertation Abstracts International 62, no. 09B, (2001): 3856. Accession No. AAI3025125.
- Thomas, J. M. G., K. J. Boote, L. H. Allen Jr, M. Gallo-Meagher, and J. M. Davis, 2003: Elevated temperature and carbon dioxide effects of soybean seed composition and transcript abundance. *Crop. Sci.* 43, 1548–1557.
- Wolf, R. B., J. F. Cavins, R. Kleiman, and L. T. Black, 1982: Effect of temperature on soybean seed constituents – oil, protein, moisture, fatty acids, amino acids and sugars. *J. Am. Oil Chem. Soc.* 59, 230–232.
- Wu, W. H., J. Y. Lu, D. G. Mortley, P. A. Loretan, C. K. Bonsi, and W. A. Hill, 1997: Proximate composition, amino acid profile, fatty acid composition, and mineral concentration of peanut seeds hydroponically grown at elevated CO₂ levels. *J. Agric. Food. Chem.* 45, 3863–3866.