

Effects of elevated carbon dioxide (CO₂) on flowering traits of three horticultural plant species

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Abstract

Carbon dioxide (CO₂) has been increasing homogeneously worldwide since industrial revolution. Current CO₂ concentrations are around 400 ppm, which may increase over this century to 430 ppm (best scenario) to >1000 ppm (worst scenario) (IPCC, 2013). Species-level responses of plant traits to elevated carbon dioxide (eCO₂), especially those that affect interactions with pollinators, could potentially have flow-on effects to both ecological communities and horticultural industries. The impacts of eCO₂ on flowering traits in pepper (*Capsicum annuum* - Giant Bell), tomato (*Lycopersicon lycopersicum*- var. Tomato Roma) and zucchini (*Cucurbita pepo* - Zucchini Blackjack), all economically important horticultural species, were investigated. Twenty plants of each species were grown under two treatments: 1) two glasshouses with ambient CO₂ (400 ppm), and 2) two with eCO₂ (555 ppm). For both treatments, we measured the number of flowers, flower longevity and dry biomass for all species; pollen diameter and number of pollen grains for tomato and zucchini; and the sucrose concentration and nectar production in zucchini. Elevated CO₂ had few significant effects on the measured traits, the main exceptions being in zucchini, which produced more male flowers, fewer pollen grains per plant and fewer female flowers under this treatment. Pepper also produced fewer flowers at eCO₂ while tomato was the least sensitive species. Future studies could be aimed at testing sensitivity of different varieties of these important commercial species to eCO₂.

Keywords: Elevated CO₂; flower longevity; pollen; vegetable crops; tomato (*Lycopersicon lycopersicum*); pepper (*Capsicum annuum*); zucchini (*Cucurbita pepo*).

Abbreviations: eCO₂ Elevated carbon dioxide; carbon dioxide_CO₂

Introduction

Atmospheric carbon dioxide (CO₂) has increased from 280 ppm in pre-industrial times to around 400 ppm, and could reach 1000 ppm by the end of the century unless strong greenhouse gas emission reductions take place (Hare et al., 2011; IPCC, 2013). Plant responses to elevated CO₂ (eCO₂) are species-specific, but in general, crop plants tend to be more sensitive than native flora (Jablonski et al., 2002). Any significant effects of eCO₂ on flowering traits could potentially affect processes from the species level (reproduction and yield) up to the community level (interactions with pollinators, (Potts et al., 2003; Hoover et al., 2012).

Although the effects of eCO₂ and temperature on photosynthesis, plant growth, and fruit and seed yield of crops have been relatively well studied (e.g. Jablonski et al., 2002; Tubiello et al., 2007; Dieleman et al., 2012; Wang et al., 2012), far less is known about the impacts on floral traits, particularly on vegetables (Peñuelas et al., 1995; Osborne et al., 1997; Jablonski et al., 2002; Springer and Ward, 2007), and where they have been investigated, considerable variability has been found, both within and between species (Parmesan and Hanley, 2015). In this study the impacts of eCO₂ on the floral traits of tomato, pepper and zucchini were investigated. All three species are economically important. For example, in 2014 in the USA, the value of production of pepper crops was ca \$619 million, the value of tomato was ca \$1134 million, and the value of zucchini was ca \$192 million (United States Department of Agriculture, 2015). Quantifying

potential effects of altered atmospheric conditions on commercial species such as these will help identify crop varieties that may be better adapted to future conditions and increase our general understanding of future impacts on agroecosystems and food production (Springer and Ward, 2007; DaMatta et al., 2010; Turrall et al., 2011).

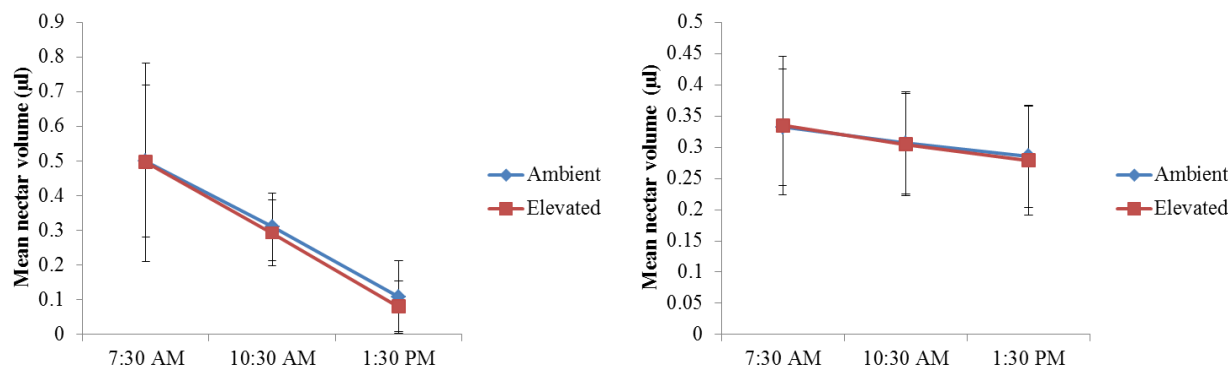
Results and Discussion

Overall, eCO₂ had relatively few significant impacts on floral traits of the three horticultural species investigated. The effects that did occur were individualistic for each species, consistent with the findings of a meta-analysis of 24 crop species in which 63% showed accelerated flowering times, while 29% did not change and 8% had delayed flowering (Springer and Ward, 2007). We found the most significant impacts of CO₂ treatment were flower number in pepper and zucchini, and the number of pollen grains in zucchini (Table 2).

The eCO₂ treatment did not affect flower longevity in any of the species (tomato p=0.70 and pepper p=0.80). There were fewer pepper and zucchini female flowers but more zucchini male flowers at eCO₂ compared to ambient (p=0.038, p=0.047 and p=0.033, respectively; Table 2). The eCO₂ treatment did not affect flowering in tomatoes (p=0.144, Table 2). These results contrast in some respects with several studies that have shown eCO₂ to have a positive impact on some vegetative and floral traits, especially in

Table 1. Details of measurements, abbreviations Z, C, T represent: Zucchini, Pepper and Tomato, respectively.

Measurement	No. plants per glasshouse	No. flowers/fruits/pollen per plant
Nectar secretion rate (Z)	8	4 males and 2-4 females
Nectar sugar concentration (Z)	7	3 (males and females)
Flower lifespan (C, T)	8	5
Sucrose concentration (Z)	7	3
Number of flowers (Z, C, T)	20	All
Pollen grains (Z, T)	8	3
Number of fruits (C, T)	20	All
Fruits biomass (C, T)	7	All
Vegetative biomass (Z, C, T)	7	NA

**Fig 1.** Nectar secretion over the course of the day in zucchini flowers. A. Female flowers, B. Male flowers.

C₃ crops (Osborne et al., 1997; Moya et al., 1998; Lake and Hughes, 1999; Jablonski et al., 2002; Ziska et al., 2005; DaMatta, et al., 2010).

There were no significant differences between CO₂ treatments in the number of grains or pollen size in tomato. In zucchini, there was no significant difference in pollen size between treatments, but there were significantly fewer grains per plant at eCO₂ ($p=0.029$, Table 2). Nectar secretion rates decreased during the course of the day in both male and female zucchini flowers in both CO₂ treatments (Fig. 1) but there were no significant differences between treatments in sucrose concentration in male flowers, nectar volume for females or nectar volume for males ($p=0.15$, $p=0.14$ and $p=0.46$, respectively; Table 2) or secretion rate (Fig. 1). The resource index for male flowers was higher at eCO₂, but for female flowers was not significantly affected by CO₂ treatment (Table 2).

The results of this study support the notion that floral traits of some plant species may be relatively insensitive to variation in abiotic parameters such as atmospheric CO₂, resulting in the presentation of stable floral rewards and in turn ensuring the maintenance of pollinator fidelity (Lanau, 2004). Additionally, presenting a relatively constant amount of floral resources could also benefit outcrossing, where plants are able to attract pollinators by rewarding them but nonetheless not providing excess amounts, thus encouraging pollinators to find other individuals; in other words, the plants keep pollinators “hungry but faithful” (Willmer and Stone, 2004).

Our finding that elevated CO₂ had few significant impacts on flowering traits could have been affected by the length of the experiment. While zucchini tends to be grown as an annual crop, some pepper and tomato plants can live several years and continue to bear fruit. It is possible that longer exposure times to the elevated CO₂ treatment may have resulted in greater differences over time, although many plants acclimate to such treatments (Lambers et al., 2008; Smith and Dukes, 2013). It is also possible that nutrient

constraints and pot size may have limited the differences between treatments in our study (Reich et al., 2006; Reddy et al., 2010; Kirschbaum, 2010).

Although the impact of eCO₂ on pepper and tomato has been previously investigated (Hickleton and Jolliffe, 1978; Micallef et al., 1995; Peñuelas et al., 1995), these studies did not measure the impact on most of the flowering traits of the present study. Our findings are consistent with those of Micallef et al., (1995) and Yelle et al., (1990), in that the number of flowers and fruits of tomato did not change at eCO₂ when the treatment was applied from the seed stage. However, our results contrast with those of Hickleton and Jolliffe (1978), who reported earlier flowering and higher yield in tomato plants that were enriched with CO₂ from the seedling stage. Our finding that pepper produced fewer flowers at eCO₂ contrast with those of Peñuelas et al. (1995) who found that eCO₂ had no effect on flowering in this crop. CO₂ treatment had little impact on the vegetative characteristics of any of the species. In the correlation analyses, the only significant relationships found were between the number of flowers and above ground biomass at ambient CO₂ for both tomato and pepper (Table 3). These results are consistent with several other studies, where changes in these traits tend to be more dependent on factors such as water and nutrient availability. For instance, changes on dry biomass in pepper at eCO₂ increased with high amounts of nitrogen (Peñuelas et al., 1995) and also higher water availability (Peñuelas et al., 1995; Rezende et al., 2008). Similarly, tomato tends to be more sensitive to nitrogen availability than eCO₂ (Van Oosten et al., 1994).

In summary, elevated CO₂ did not significantly affect most of the floral traits measured in the three crop species examined. In those traits that were affected, responses varied among the species, with zucchini the most sensitive to eCO₂, followed by pepper, and tomato the least sensitive. The relative lack of sensitivity of Tomato Roma to eCO₂ could suggest that it is a potential variety for future studies on

Table 2. Summary of statistical analyses of floral traits, pollen, aboveground dry biomass and nectar. Significant interactions between CO₂ treatment and glasshouses are indicated on the table. Letters in brackets: FA: Friedman ANOVA; K-W: Kruskal-Wallis.

			Mean ± s.d.		df	F (or Chi Sqr. for FA)	p
			Ambient	Elevated			
Flowering	Life span (days)	Pepper	4.5±0.54	4.6±0.64	1	0.08	0.804
		Tomato	4.2±0.54	4.3±0.54	1	0.195	0.702
	Number of flowers	Pepper (FA)	1.67±2.82	1.58±2.50	13	23.35	0.038**
		Tomato (FA)	3.72±4.0	3.13±3.47	13	18.38	0.144
		Zucchini females (FA)	0.82±0.85	0.73±0.80	13	22.56	0.047**
		Zucchini males (FA)	2.39±1.70	2.86±1.70	13	23.82	0.033**
Number of fruits	Pepper (K-W)	3.48±1.22	3.28±1.44	1	--	0.992	
	Tomato	17.78±5.11	19.52±4.92	1	0.159	0.728	
Dry biomass	Fruits (g)	Pepper	12.09±4.36	12.36±4.05	1	0.07	0.817
		Tomato	2.95±0.45	2.78±0.32	1	1.547	0.34
	Above ground (g)	Pepper	19.98±6.35	18.41±5.64	1	0.072	0.814
		Tomato (log transformed)	39.22±14.53	42.02±13.91	1	0.014	0.915
		Zucchini	27.92±7.45	27.05±8.53	1	3.687	0.195
Pollen	Number of grains of pollen	Tomato (log transformed)	137.8±51.4	171.7±44.8	1	0.932	0.436
		Zucchini	19.26±6.53	14.30±6.3	1	33.43	0.029**
	Size of grains of pollen	Tomato	17.19±0.52	17.47±0.59	1	1.276	0.376
		Zucchini	130.00±3.51	130.14±3.28	1	0.315	0.631
Nectar of zucchini	Secretion volume (µL)	Male flowers (square root transformed)	0.31±0.28	0.29±0.27	1	0.57	0.456
		Female flowers (FA)	0.54±0.52	0.55±0.54	2	4	0.135
	Sucrose concentration (g/100g)	Male flowers (KW)	40.77±2.96	41.81±4.09	1	--	0.1475
		Female flowers	42.49±3.72	43.47±2.38	1	0.437	0.577
	Resource index	Male flowers	0.74	0.83	--	--	--
	Female flowers	0.44	0.4	--	--	--	

*, **, *** indicate differences at the 0.1, 0.5, or 0.01% level of significance, respectively.

Table 3. Summary of correlations between floral traits and number of fruits, fruits weight and above ground vegetative biomass between treatments.

			R	p
Number of flowers vs. Number of fruits	Tomato	Ambient	-0.019	0.73
		Elevated	0.026	0.46
	Pepper	Ambient	0.011	0.67
		Elevated	0.023	0.59
Number of flowers vs. Fruits weight	Tomato	Ambient	-0.0082	0.35
		Elevated	0.015	0.21
	Pepper	Ambient	0.11	0.67
		Elevated	-0.23	0.18
Number of flowers vs. Above ground vegetation	Tomato	Ambient	0.5	0.0075***
		Elevated	-0.012	0.94
	Pepper	Ambient	0.57	0.044**
		Elevated	0.28	0.23
	Zucchini	Ambient	-0.27	0.57
		Elevated	0.72	0.3

*, **, *** indicate differences at the 0.1, 0.5, or 0.01% level of significance, respectively.

breeding better-adapted crops (Turrall et al., 2011; Wheeler and von Braun, 2013).

This work could be extended in many ways, including investigating the impacts of eCO₂ on a wider range of crop species and varieties; the changes produced in belowground biomass; potential interactions between eCO₂ and temperature; and changes over a longer duration for the perennial species.

Materials and Methods

Experimental design

Twenty replicate plants of each species were grown for 20 weeks in each of four glasshouses at Macquarie University (Sydney, Australia), under two treatments: 1) at ambient CO₂ (400 ± 12 ppm), and 2) at elevated (560 ± 10 ppm), as described in previous studies by Manea and Leishman (2011) and Kelly et al., (2015). All glasshouses were maintained at 24°C day and 16°C night temperatures with a day-length of 12 h and a relative humidity of approximately 60%. Each plant was randomly repositioned within the glasshouse every 25 days. The measurements taken for each species are summarized in Table 1.

Species selection

Tomato (*Lycopersicon lycopersicum* - var. Tomato Roma) and pepper (*Capsicum annuum* - var. Giant Bell) are hermaphroditic plants that can produce fruits without external pollination (Delaplane, K. S. and Mayer, 2000), while zucchini (*Cucurbita pepo* - var. Zucchini Blackjack), is obligately-dependent on insect pollinators to produce fruits (Vidal et al., 2006). All three species have a C₃ photosynthetic pathway.

Sowing and growth

Plants of these three species were grown from seed (Yates^R, Australia) in commercial soil potting mix (DEBCO^R, Australia). In ensure enough replicates, three seeds were sown in each pot (diameter 18 cm, depth 20 cm, 30L volume, commercial potting mix) and after seedlings reached 10-15 cm in height, excess seedlings were removed. Plants were watered by automatic sprinklers four times per day for two

minutes, and received supplementary watering (300 ml) daily once the first fruit appeared. All plants were fertilized weekly with 9g L⁻¹ of ThriveR Complete Plant Food (Australia) at the seedling stage. After flowers appeared, fertilization was increased to 18g L⁻¹ weekly.

Trait measurements

After flowering commenced, the total number of flowers per plant was counted daily for zucchini plants and weekly for tomato and pepper, for a period of 14 weeks. Since zucchini flowers last for only a single day (approximately 6 am until noon; (Nepi et al., 2001)), flower lifespan for this species was not recorded. For tomato and pepper, eight randomly chosen plants per glasshouse were used to estimate the average life span of five flowers per plant.

Hand-pollination was not performed for any of the species, and therefore tomato and pepper fruits were from self-pollination only. Since zucchini plants do not self-pollinate, only flowering was measured for this species. Mature tomato and pepper fruits with uniform red colour were harvested from seven randomly chosen plants per glasshouse and dried at 60°C for ten days. In addition, 17 weeks after sowing, all fruits of all tomato and pepper plants were counted. Seven plants per species per glasshouse were also harvested and dried at 60°C for three days and weighed to estimate aboveground vegetative dry biomass.

Tomato flowers do not produce nectar, and because of the difficulty of manipulating the small flowers of pepper, measurements of nectar volume and sugar concentrations were recorded for zucchini flowers only. Female flowers were far less abundant than male flowers, so fewer female flowers were analyzed for nectar secretion rate (Table 1). Eight plants from each glasshouse were randomly selected for nectar volume and secretion measurements. Four male flowers and 2-4 female flowers were selected per plant. Nectar was collected every three hours from the same flower (7:30 am, 10:30 am, and 1:30 pm) during a single day, using 0.2 µl microcapillary tubes. The content of each capillary was dotted onto filter paper (Whatman's Number 1). The diameter of the nectar dot was measured immediately, and the nectar volume was estimated via correlation using data from Dafni (1992, p. 140). To estimate the overall amount of nectar that would be available for a pollinator, a resource index was

calculated by multiplying the mean number of flowers by the mean volume of nectar per plant.

The sucrose content of nectar has been widely used in studies relating floral traits to foraging behaviour in honeybees (Esch et al., 1993, Pankiw et al., 2001; Pérez and Farina, 2004). We determined the sucrose concentration of nectar in g/100g (International index scale of ICMUSA 1974) from three flowers on each of seven plants per glasshouse, using a refractometer. The nectar was collected from the flowers with a micropipette. All samples were taken at 9:00 am using a micropipette and were analyzed at room temperature in the laboratory.

Three flowers from each of eight randomly selected tomato and zucchini plants per glasshouse were used for the pollen grain counts. During anthesis, tomato flowers presented some variability in colour and exposition of the anthers during the first two days; the anthers were therefore removed after the second day of anthesis. All anthers were removed from the base, placed in 1.5 ml vials, and stored immediately at -20°C for later analysis. Zucchini anthers were removed and stored in the same manner during their single day of life. The procedure to obtain and measure the pollen was adapted from Lake and Hughes (1999) and Georgiady and Lord (2002). At time of analysis, vials were removed from the freezer and 1 ml water added to each. Vials were then shaken using a vortex mixer for one minute to release the pollen. The anthers were removed from the vials and the vials were shaken again for ten seconds to obtain a homogeneous suspension. Two samples of 10 µL were taken from the suspension and the pollen grains were counted using eight squares of a Neubauer chamber under an optical microscope (10X). Photographs of five pollen grains per sample were taken and the diameter of each was measured using the program ImageJ (Rasband) <http://rsb.info.nih.gov/ij/>.

Statistical analysis

Data were analyzed using 2-factor nested ANOVAs, in which glasshouses were considered a random factor nested within CO₂ concentration, which was designated as a fixed factor. For nectar secretion and number of flowers per week, the data were analyzed using repeated measures ANOVA. Data analysis was performed using Statistica 7.0. Some data were log or square root transformed where necessary to improve normality and meet the assumptions of the analyses. When data could not be transformed to meet normality assumptions, non-parametric Kruskal-Wallis or Friedman ANOVA analyses were used. To assess if there was a trade-off between the vegetative traits and sexual traits of the species, correlation analyses were performed with the above ground vegetative weight, the number of fruits and the weight of the fruits compared to the number of flowers and the number of pollen grains.

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References

- DaMatta FM, Grandis A, Arenque BC, Buckeridge MS (2010) Impacts of climate changes on crop physiology and food quality. *Food Res Int.* 43, 1814–1823.
- Delaplane KS, Mayer DF (2000) *Crop pollination by bees* (United Kingdom: CABI Publishing).
- Dieleman WIJ, Vicca S, Dijkstra FA, Hagedorn F, Hovenden MJ, Larsen KS, Morgan JA, Volder A, Beier C, Dukes JS, King J, Leuzinger S, Linder S, Luo Y, Oren R, de Angelis P, Tingey D, Hoosbeek MR, Janssens I (2012) Simple additive effects are rare: a quantitative review of plant biomass and soil process responses to combined manipulations of CO₂ and temperature. *Glob. Change Biol.* 18, 2681–2693.
- Esch H, Goller F, Burns JE (1993) Honeybee waggle dances: the “energy hypothesis” and thermoregulatory behavior of foragers. *J Comp Physiol. B* 163, 621–625.
- Georgiady MS, Lord EM (2002) Evolution of the inbred flower form in the currant tomato, *Lycopersicon pimpinellifolium*. *Int J Plant Sci.* 163, 531–541.
- Hare WL, Cramer W, Schaeffer M, Battaglini A, Jaeger CC (2011) Climate hotspots: key vulnerable regions, climate change and limits to warming. *Reg Environ. Change* 11, S1–S13.
- Hickleton PR, Jolliffe PA (1978) Effects of greenhouse CO₂ enrichment on the yield and photosynthetic physiology of tomato plants. *Can J Plant Sci.* 58, 801–817.
- Hoover SER, Ladley JJ, Shchepetkina AA, Tisch M, Giese SP, Tylianakis JM (2012) Warming, CO₂, and nitrogen deposition interactively affect a plant-pollinator mutualism. *Ecol Lett.* 15, 227–234.
- IPCC (2013) Summary for Policymakers. In *Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*, (Cambridge University Press, Cambridge, United Kingdom and New York), p.
- Jablonski LM, Wang X, Curtis PS (2002) Plant reproduction under elevated CO₂ conditions: a meta-analysis of reports on 79 crop and wild species. *New Phytol.* 156, 9–26.
- Kelly JWG, Duursma RK, Atwell BJ, Tissue DT, Medlyn BE (2015) Drought x CO₂ interactions in trees: a test of the low-intercellular CO₂ concentration (C_i) mechanism. *New Phytol.* 1–13.
- Kirschbaum MUF (2010) Does Enhanced Photosynthesis Enhance Growth? Lessons Learned from CO₂ Enrichment Studies[W]. *Plant Physiol.* 155, 117–124.
- Lake JC, Hughes L (1999) Nectar production and floral characteristics of *Tropaeolum majus* L. grown in ambient and elevated carbon dioxide. *Ann Bot.* 84, 535–541.
- Lambers H, Chapin FS, Pons TL (2008) Effects of the Rising CO₂ Concentration in the Atmosphere. In *Plant Physiological Ecology*, (New York: Springer Science Business Media, LLC), p. 623.
- Lanau K (2004) Adaptive radiation and coevolution — pollination biology case studies. *Org Divers Evol.* 4, 207–224.
- Manea A, Leishman MR (2011) Competitive interactions between native and invasive exotic plant species are altered under elevated carbon dioxide. *Oecologia* 165, 735–744.
- Micallef BJ, Haskins JK, Vanderveer PJ, Roh KS, Shewmaker CK, Sharkey TD (1995) Altered photosynthesis, flowering, and fruiting in transgenic tomato plants that have an increased capacity for sucrose synthesis. *Planta.* 196, 327–334.

- Moya TB, Ziska LH, Namuko OS, Olszyk D (1998) Growth dynamics and genotypic variation in tropical, field-grown paddy rice (*Oryza sativa* L.) in response to increasing carbon dioxide and temperature. *Glob Change Biol.* 4, 645–656.
- Nepi M, Guarnieri M, Pacini E (2001) Nectar secretion, reabsorption, and sugar composition in male and female flowers of *Cucurbita pepo*. *Int J Plant Sci.* 162, 353–358.
- Osborne JL, Awmack CS, Clark SJ, Williams IH, Mills VC (1997) Nectar and flower production in *Vicia faba* L (field bean) at ambient and elevated carbon dioxide. *Apidologie.* 28, 43–55.
- Pankiw T, Waddington KD, Page REJ (2001) Modulation of sucrose response thresholds in honey bees (*Apis mellifera* L.): influence of genotype, feeding, and foraging experience. *J Comp Physiol A.* 187, 293–301.
- Parmesan C, Hanley ME (2015) Plants and climate change: complexities and surprises. *Ann Bot.* 116, 849–864.
- Peñuelas J, Biel C, Estiarte M (1995) Growth, biomass allocation, and phenology responses of pepper to elevated CO₂ concentrations and different water and nitrogen supply. *Photosynthetica.* 31, 91–99.
- Pérez N, Farina WM (2004) Nectar-receiver behavior in relation to the reward rate experienced by foraging honeybees. *Behav Ecol Sociobiol.* 55, 574–582.
- Potts SG, Vulliamy B, Dafni A, Ne'eman G, Willmer P (2003) Linking bees and flowers: How do floral communities structure pollinator communities? *Ecology.* 84, 2628–2642.
- Rasband W, ImageJ (National Institutes of Health, USA).
- Reddy A, Rasineni G, Raghavendra A (2010) The impact of global elevated CO₂ concentration on photosynthesis and plant productivity. *Curr Sci.* 99, 46–57.
- Reddy KR, Prasad PVV, Kakani G (2005) Crop responses to elevated carbon dioxide and interactions with temperature. *J Crop Improv.* 13, 157–191.
- Reich PB, Hobbie SE, Lee T, Ellsworth DS, West JB, Tilman D, Knops JMH, Naem S, Trost J (2006) Nitrogen limitation constrains sustainability of ecosystem response to CO₂. *Nature.* 440, 922–925.
- Rezende FC, Frizzone JA, Botrel TA, Pereira AS (2008) Plantas de pimentão cultivadas em ambiente enriquecido com CO₂. I. Crescimento vegetativo. *Acta Sci Agron.* 24, 1517–1526.
- Smith NA, Dukes JS (2013) Plant respiration and photosynthesis in global-scale models: incorporating acclimation to temperature and CO₂. *Glob Change Biol.* 19, 45–63.
- Springer CJ, Ward JK (2007) Flowering time and elevated atmospheric CO₂. *New Phytol.* 176, 243–255.
- Tubiello FN, Amthor JS, Boote KJ, Donatelli M, Easterling W, Fischer G, Gifford RM, Howden M, Reilly J, Rosenzweig C (2007) Crop response to elevated CO₂ and world food supply A comment on “Food for Thought...” by Long et al. *Eur J Agron.* 26, 215–223.
- Turrall H, Burke J, Faurès JM (2011) Prospects for mitigation. In *Climate Change, Water and Food Security*, (Rome: FAO), p. 200.
- United States Department of Agriculture (2015) *Vegetables 2014 Summary (USA: National Agricultural Statistics Service)*.
- Van Oosten JJ, Wilkins D, Besford RT (1994) Regulation of the expression of photosynthetic nuclear genes by CO₂ is mimicked by regulation by carbohydrates: a mechanism for the acclimation of photosynthesis to high CO₂? *Plant Cell Environ.* 17, 913–923.
- Vidal M, Jong D, Wien HC, Morse RA (2006) Nectar and pollen production in pumpkin (*Cucurbita pepo* L.). *Rev Bras Bot.* 29, 267–273.
- Wang D, Heckathorn SA, Wang X, Philpott SM (2012) A meta-analysis of plant physiological and growth responses to temperature and elevated CO₂. *Oecologia.* 1–13.
- Wheeler T, von Braun J (2013) Climate Change Impacts on Global Food Security. *Science.* 341, 508–513.
- Willmer PG, Stone GN (2004) Behavioral, ecological, and physiological determinants of the activity patterns of bees. *Adv Study Behav.* 34, 347–466.
- Yelle S, Jr Beeson RC, Trudel MJ, Gosselin A (1990) Duration of CO₂ enrichment influences growth, yield, and gas exchange of two tomato species. *J Am Soc Hortic Sci.* 115, 52–57.
- Ziska LH, Reeves JB, Blank B (2005) The impact of recent increases in atmospheric CO₂ on biomass production and vegetative retention of Cheatgrass (*Bromus tectorum*): implications for fire disturbance. *Glob Change Biol.* 11, 1325–1332.