

Mycorrhizal effects on growth and nutrition of tomato under elevated atmospheric carbon dioxide

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Abstract. Arbuscular mycorrhizas are predicted to be important in defining plant responses to elevated atmospheric CO₂ concentrations. A mycorrhiza-defective tomato (*Solanum lycopersicum* L.) mutant with reduced mycorrhizal colonisation (*rmc*) and its mycorrhizal wild-type progenitor (76R MYC+) were grown under ambient and elevated atmospheric CO₂ concentrations (eCO₂) in a controlled environment chamber-based pot study. Plant growth, nutrient contents and mycorrhizal colonisation were measured four times over a 72-day period. The 76R MYC+ plants generally had higher concentrations of P, N and Zn than their *rmc* counterparts. Consistent with earlier studies, mycorrhizal colonisation was not affected by eCO₂. Growth of the two genotypes was very similar under ambient CO₂ conditions. Under eCO₂ the mycorrhizal plants initially had higher biomass, but after 72 days, biomass was lower than for *rmc* plants, suggesting that in this pot study the costs of maintaining carbon inputs to the fungal symbiont outweighed the benefits with time.

Additional keywords: climate change, elevated CO₂, mycorrhiza mutant, mycorrhizas, *Solanum lycopersicum*.

Introduction

While biota play a central role in soil nutrient cycling and have important impacts on above and belowground processes (Wardle *et al.* 2004), relatively little is known about how soils and their biota will respond to climate change (Staddon *et al.* 2004). Arbuscular mycorrhizal fungi (AMF) are predicted to be important in defining plant responses to elevated atmospheric CO₂ concentrations (eCO₂). eCO₂ affects photosynthetic rates, thereby influencing C supply to AMF (Lovelock *et al.* 1997; Jifon *et al.* 2002). In turn, AMF may help alleviate the increased plant nutrient limitation associated with increased photosynthetic rates (Treseder and Allen 2000). The costs and benefits to plants of forming AM are a function of the balance between the C cost of the fungi, and the benefits such as nutrient supply to the plant (Fitter 1991; Johnson *et al.* 1997). An increase in the relative benefits of formation of AM is anticipated under eCO₂ because of changing plant C acquisition costs (Hoeksema and Bruna 2000; Sterner and Elser 2002; Johnson *et al.* 2005). eCO₂ may also mitigate plant growth depressions that are generated when C costs of AMF outweigh their nutrient uptake benefits (Jifon *et al.* 2002).

Phosphorus concentrations of plants have been shown to be lower under eCO₂; however, P uptake in mycorrhizal plants was higher than in non-mycorrhizal plants when growing under eCO₂ (Jongen *et al.* 1996; Syvertsen and Graham 1999). Responses of AM to eCO₂ have also been shown to differ temporally; for example, while no effect on P inflows in mycorrhizal plants was found early in their growth, a negative effect was found later in their growth (Staddon *et al.* 1999). In one study

eCO₂ led to an increase in the production of AMF hyphae in soil, but direct hyphal uptake of P was unaffected (Sanders *et al.* 1998). Direct hyphal uptake of P by a mixture of AMF species did not increase under eCO₂ (Gavito *et al.* 2002), or in another study where a range of AMF inoculum types were compared (Gavito *et al.* 2003). AMF play an important role in the uptake and transfer of P, N, Zn and other nutrients to plants (Marschner and Dell 1994; Smith and Read 1997; Cavagnaro *et al.* 2006), and under eCO₂, AMF improved N nutrition of plants after a period of selection (Gamper *et al.* 2005). Uptake of micronutrients via the mycorrhizal pathway may be especially important under eCO₂ because of higher nutrient demand associated with higher photosynthetic rates, although this remains to be widely assessed.

Both the intra- and extraradical phases of AMF growth are altered by eCO₂, probably indirectly as a result of eCO₂ effects on plants (Gavito *et al.* 2003). Although some studies show an increase in intraradical percent colonisation of roots by AMF with eCO₂ (reviewed by Treseder 2004), others do not (reviewed by Staddon and Fitter 1998; Staddon *et al.* 2004). However, when the confounding effects of increased root biomass and C allocation to roots, arising from generally greater plant biomass under eCO₂, were taken into account, these differences in colonisation were insignificant (Staddon and Fitter 1998; Fitter *et al.* 2000). At a finer scale of resolution, eCO₂ can lead to an increase in the abundance of arbuscules in roots (Rillig and Allen 1998), although not always (Klironomos *et al.* 1998).

One of the challenges in studying AM is the establishment of non-mycorrhizal controls with the wider soil biota intact. This

is largely due to non-specific effects of fumigation/fungicide treatments used to establish such controls. Most studies of eCO₂ effects on AM have, with a few exceptions (e.g. Gavito *et al.* 2002), used sterilised soils reinoculated with a single AMF species. In order to avoid indirect effects of establishing non-mycorrhizal controls on the wider soil biota, we used a mycorrhiza defective tomato mutant (*rmc*) and its mycorrhizal wild-type progenitor (76R MYC+), that have been used successfully in studies of AM effects on plant growth (Cavagnaro *et al.* 2004, 2006; Poulsen *et al.* 2005), nutrition (Poulsen *et al.* 2005; Cavagnaro *et al.* 2006) and competition (Cavagnaro *et al.* 2004), and soil ecology/plant–microbe interactions (Marschner and Timonen 2005; Cavagnaro *et al.* 2006, in press; Gao *et al.* 2006). The growth of the two genotypes has been found to be very similar under a range of circumstances (Cavagnaro *et al.* 2004, 2006; Poulsen *et al.* 2005), including non-mycorrhizal conditions, suggesting that the mutation affecting colonisation of *rmc* by AMF has no pleiotropic effects on other plant processes (Cavagnaro *et al.* 2004). Furthermore, in a field study on an organic tomato farm soil (Cavagnaro *et al.* 2006), the growth of the two genotypes was very similar but there were large differences in plant nutrient concentrations. Thus, this experimental system allows for comparisons of AM functioning without the complicating effects of size asymmetry between mycorrhizal and non-mycorrhizal plants.

Here, a growth chamber-based experiment compared the *rmc* and 76R MYC+ tomato genotypes under ambient and eCO₂ conditions. It has been suggested that studies of AM should use plant and AMF combinations that co-occur in the field (Johnson *et al.* 2005). Therefore, we used soil collected from a long-term (20 years) organically managed tomato farm, that has previously been shown to have high AMF inoculum potential (L.E. Jackson, unpubl. data), in which the tomato genotypes used here both grow well, and the indigenous AMF enhance the nutrition of the mycorrhizal genotype (Cavagnaro *et al.* 2006). Specifically the aims of this study were:

- (1) to confirm if previously reported effects of eCO₂ on the growth, plant nutrient concentrations, and mycorrhizal colonisation occurred using a genotypic approach for controlling mycorrhizal symbiosis that allows for comparisons of mycorrhizal and non-mycorrhizal plants with the wider soil biota in tact; and
- (2) test the hypothesis that the growth and allocation patterns of 76R MYC+ and *rmc* genotypes of tomato are not altered by atmospheric CO₂ concentration.

Materials and methods

Soil and plant material

Plastic, free draining pots were filled with 1 kg (dry weight basis) of a 20:80 (w/w) soil:sand mixture. The soil was a Zamora loam (a fine-silty, mixed thermic, Mollic Haploxeralfs), collected from a tomato field on an organically managed farm, Jim and Deborah Durst Farming, in Esparto, Yolo County, California. Soil properties are presented in Cavagnaro *et al.* (2006). The sand was twice autoclaved and oven dried.

Two pre-germinated seeds of either the mycorrhiza defective tomato mutant (*rmc*) or its mycorrhizal wild-type progenitor *Solanum lycopersicum* L. cv. 76R (see Barker *et al.* 1998) (76R MYC+) were planted in each pot. Seeds were pre-germinated at 25°C in the dark, following surface sterilisation by immersion in an aerated 3% sodium hypochlorite solution for ten minutes, rinsing with double deionised (DDI) water to remove any trace of sodium hypochlorite, and immersion in aerated DDI water for 20 min. Seeds germinated within ~5 days. Seedlings were thinned to one per pot 7 days following planting.

Growth conditions

Plants were grown in two controlled environment chambers at the University of California Davis Controlled Environment Facility. The atmospheric CO₂ concentration in the first chamber was set at 970 ppm (elevated chamber), the projected atmospheric CO₂ concentration in the year 2100 under the Intergovernmental Panel on Climate Change A1Fi ‘business as usual’ scenario (IPCC 2001). The second chamber was not amended with CO₂, giving an ambient CO₂ concentration (D. Lewis, pers. comm.). Otherwise both chambers were maintained under the same conditions; day/night length was 16/8 h, light intensity was 960 μmol m⁻² s⁻¹, day/night temperatures were 25/20°C and relative humidity 80%. In order to ensure that the chambers were well matched, biomass of tomato plants grown under the same atmospheric CO₂ concentration in the two chambers were compared and found to not differ significantly ($P = 0.74$, data not shown).

Plants were watered every second day with DDI water for the first 25 days following planting. For the remainder of the experiment plants were watered every second day with a 1/10 strength modified Long Ashton solution minus P (Cavagnaro *et al.* 2001b), except once a week when the nutrient solution was amended with 2.6 mM K₂HPO₄. Watering solutions were added until solution drained out the bottom of the pots.

Harvesting

There were four destructive harvests 22, 37, 57 and 72 days after planting. Plants were carefully washed free from the soil with water. The roots and shoots were separated and fresh weights determined. A weighed sub-sample of root material was kept for staining and assessment of AM colonisation (see below). The remainder of the roots and the whole shoots were dried in an oven at 60°C for 48 h and dry weights determined. Following dry weight determination, plant material was ground. Root and shoot material from harvests on days 57 and 72 harvests were analysed for nutrient contents; there was insufficient plant material for full nutrient analysis at the earlier harvests. Shoot and root B, Ca, Fe, K, Mn, Na, P, S and Zn contents were determined on plant material that was microwave-digested with nitric acid (Sah and Miller 1992) and analysed by ICP-AES (Thermo Jarrell Ash Corp., Franklin, MA). Shoot and root total C and N contents were determined by dry combustion on a Carlo Erba NA 1500 (Fisons Instruments, Beverly, MA). Data are expressed as tissue nutrient concentrations (μg g⁻¹). Roots were cleared with KOH and stained with Trypan blue using a modification of the method by Phillips and Hayman (1970), omitting phenol from all reagents. Colonisation of roots was determined using the gridline intersect

method by Giovannetti and Mosse (1980) as in Cavagnaro *et al.* (2006).

Calculations and statistical analysis

Plant and soils data were analysed using the SAS statistical software (version 8.02, SAS Institute, Cary, NC). Data from each chamber were analysed separately using GLM; that is, with *Genotype* as the sole factor in the analysis. Since the two chambers represent separate populations of plants, comparisons between them using GLM (or ANOVA) would violate the assumptions underlying these methods of analysis (Zar 1999). However, in such experiments it is important that such comparisons be made. Thus, to overcome this issue the data were analysed in a second way that allowed for valid comparisons between different populations. That is, comparisons between plants from different *Chambers* (different populations) were made using *t*-tests. Specifically, the following comparisons were made with *t*-tests: *rmc* ambient *v.* *rmc* elevated, 76R MYC+ ambient *v.* 76R MYC+ elevated, elevated *v.* ambient pooled over genotype, and *rmc v.* 76R MYC+ pooled over chamber. Comparisons of genotypes within chambers (using GLM) are presented first, followed by the comparisons made using *t*-tests. There were four replicates for each treatment at each harvest time. Specific P and Zn uptake rates (SPU and SZnU, respectively), based on total shoot + root P and Zn contents ($\mu\text{g plant}^{-1}$) between Harvests 3 and 4 (as only nutrient data were available for these harvests), were calculated as in Eqn 1, (following Cavagnaro *et al.* 2003):

$$\text{specific P uptake rate} = \frac{\text{P content (72 days)} - \text{mean P content (57 days)}}{0.5[\text{RDW (72 days)} + \text{mean RDW (57 days)}]} \quad (1)$$

Results

Mycorrhizal colonisation

Mycorrhizal colonisation of 76R MYC+ roots ranged from ~6–80% over the course of the experiment (Fig. 1*a, b*); with arbuscules, intercellular hyphae, hyphal coils and arbusculate coils observed in the root cortex. Conversely, mycorrhizal colonisation of *rmc* roots at all harvests was low ($\leq 2\%$), and was restricted to the root surface and epidermis. When chambers were analysed separately (using GLM), the percentage of the root length colonised by AMF of 76R MYC+ plants was significantly higher than that of the *rmc* plants at all harvests in the ambient CO₂ chamber, and at Harvests 2, 3 and 4 in the eCO₂ chamber. Colonisation of 76R MYC+ plants did not differ between chambers (*t*-tests $P > 0.05$). The same was true of *rmc* plants.

Plant biomass

The shoot dry weights (SDW) and root dry weights (RDW) of the two genotypes (Fig. 2*a, b*) were not significantly different ($P > 0.05$) in the ambient chamber across all harvests. However, in the eCO₂ chamber at Harvest 1, the SDW and RDW of the 76R MYC+ plants were greater than those of *rmc*. Conversely, in the same chamber at Harvest 4, the SDW of the *rmc* plants was greater than that of 76R MYC+. There were no significant differences ($P > 0.05$) between genotypes at Harvests 2 and 3 in the eCO₂ chamber.

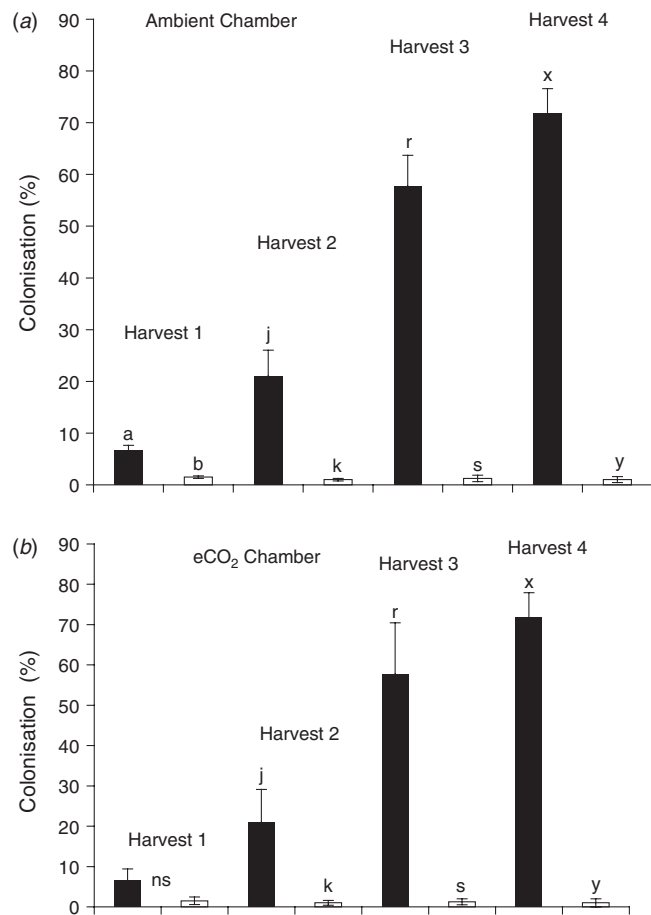


Fig. 1. Mean percentage of root length colonised by AMF of 76R MYC+ (solid bar) and *rmc* (open bar) genotypes of tomato grown under (a) ambient or (b) elevated (eCO₂) atmospheric CO₂ concentrations at Harvests 1–4. Means (\pm s.e.) followed by the same letter are not significantly different at the $P < 0.05$ level (GLM). Note: valid statistical comparisons cannot be made between eCO₂ and ambient chambers or Harvests. For comparisons between chambers (*t*-tests) see text ($n = 4$).

Comparisons between chambers (*t*-tests) revealed that at Harvest 1 the RDW of 76R MYC+ plants grown in the eCO₂ chamber was significantly higher (*t*-test $P = 0.04$) than when grown in the ambient chamber; a similar trend (*t*-test $P = 0.07$) was observed in SDW also. When pooled over chambers, the SDW of the 76R MYC+ plants was significantly greater (*t*-test $P = 0.03$) than that of the *rmc* plants at Harvest 1. At Harvest 2 the RDW of *rmc* plants grown in the ambient chamber was greater than that of *rmc* grown in the eCO₂ chamber (*t*-test $P = 0.05$). Both the SDW (*t*-test $P = 0.03$) and RDW (*t*-test $P = 0.04$) of plants grown in the ambient chamber (pooled over genotype) were significantly higher than those grown in the eCO₂ chamber. At Harvest 3, there were no significant differences within (GLM) or between chambers or genotypes (*t*-tests). At Harvest 4 the SDW of the plants in the eCO₂ chamber were larger than those in the ambient chamber for both 76R MYC+ (*t*-test $P = 0.006$) and *rmc* (*t*-test $P = 0.003$).

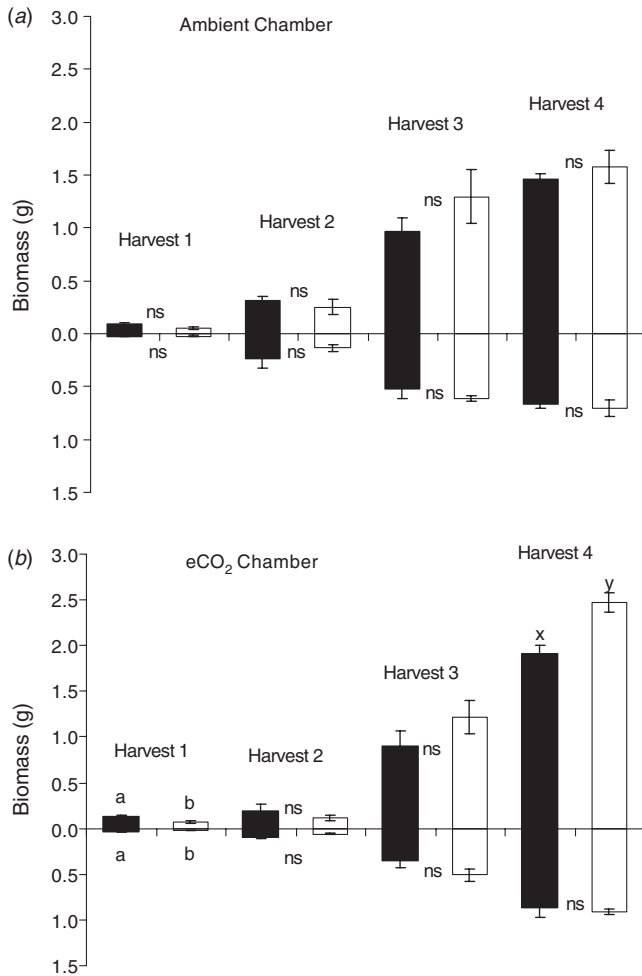


Fig. 2. Mean shoot (above *X*-axis) and root (below *X*-axis) dry weights of 76R MYC+ (solid bar) and *rmc* (open bar) genotypes of tomato at Harvests 1–4, grown under (a) ambient, or (b) elevated (eCO₂) atmospheric CO₂ concentrations. Means (\pm s.e.) followed by the same letter are not significantly different at the $P < 0.05$ level (GLM). Note: valid statistical comparisons cannot be made between Harvests, shoots and roots, or eCO₂ and ambient chambers. For comparisons between chambers (*t*-tests) see text ($n = 4$).

Plant nutrition

At Harvest 3 the shoot and root P concentrations of 76R MYC+ plants were higher than those of *rmc* plants in both chambers (Table 1). When pooled over chamber, the 76R MYC+ plants had a higher shoot P concentration (*t*-test $P < 0.0001$) and root P concentration (*t*-test $P = 0.001$) than *rmc*. Similarly, the shoot P concentration of *rmc* plants was higher (*t*-test $P = 0.002$) in the eCO₂ than ambient chamber. There were no significant differences (reflected in the relatively large S.E.) in shoot or root Zn concentrations at Harvest 3 (Table 1).

At Harvest 4, 76R MYC+ roots and shoots had higher P and Zn concentrations than *rmc* plants in both chambers (Table 1). *rmc* shoots had a higher P concentration in the eCO₂ chamber than the ambient chamber (*t*-test $P = 0.003$). Shoot and root Zn and P concentrations were significantly higher in 76R MYC+ plants than *rmc* when pooled over chamber (*t*-test $P \leq 0.0002$).

At Harvest 4, root %N was significantly higher in 76R MYC+ ($2.0 \pm 0.1\%$) than *rmc* ($1.6 \pm 0.1\%$) plants in the eCO₂ chamber ($P = 0.03$) (data not shown). A marginally significant effect ($P = 0.054$) was observed in the ambient chamber with root %N being higher in 76R MYC+ ($2.2 \pm 0.1\%$) than *rmc* ($1.7 \pm 0.2\%$) plants. There were no other differences in shoot or root N concentrations.

Specific P uptake (SPU) and specific Zn uptake (SZnU) rates of 76R MYC+ plants were significantly higher than those of the *rmc* plants (Table 2). SPU was higher in the eCO₂ chamber than the ambient chamber in both genotypes (*t*-tests 76R MYC+: $P = 0.003$; *rmc* $P = 0.002$). Similar differences in SZnU between chambers were not detected ($P > 0.05$), yet there was a large SE that may have obscured differences.

Discussion

Plant growth and nutrition

Growth of the *rmc* and 76R MYC+ genotypes under ambient CO₂ conditions was very similar, as in earlier experiments in this (Cavagnaro *et al.* 2006) and a range of other soils (Cavagnaro *et al.* 2004; Poulsen *et al.* 2005). Similarly, higher concentrations of P and Zn in the 76R MYC+ plants were also found (Cavagnaro *et al.* 2006; T.R. Cavagnaro, L.E. Jackson, unpubl. data). Matched growth represents a situation where the benefits to the plant of forming an association with AMF are equivalent to the cost of construction and maintenance (Johnson *et al.* 1997). Growth depressions occur when the benefits to the plant of forming AM, such as increased nutrient supply are outweighed by the cost, and positive growth responses occur where benefits outweigh the cost. Both positive and negative growth responses were seen under eCO₂ in this study, supporting the hypothesis that atmospheric CO₂ concentration can alter the cost:benefit ratio of forming AM (Johnson *et al.* 1997; Hoeksema and Bruna 2000; Sterner and Elser 2002; Johnson *et al.* 2005), and suggesting that the costs and maintenance of fungi by the plant can negatively impact plant growth over time.

At Harvest 1, there was a positive mycorrhizal growth response under eCO₂, as in earlier studies with other plant species (Gavito *et al.* 2002; Staddon *et al.* 2004), despite low levels of mycorrhizal colonisation. Plant C acquisition costs may have decreased, making more C available for supply to the AMF, thereby increasing the relative benefits of nutrient uptake by AMF (Hoeksema and Bruna 2000; Sterner and Elser 2002; Johnson *et al.* 2005). Further, the percentage of the root length colonised by AMF was not different between the two genotypes at this harvest. However, colonisation of *rmc* roots was restricted to the epidermis, whereas colonisation of the 76R MYC+ roots extended to the formation of arbuscules, hyphal coils and arbusculate coils in the root cortex. This is consistent with earlier studies that show that mycorrhizal colonisation is not always related to AM functioning (McGonigle 1988; Smith *et al.* 2004; Johnson *et al.* 2005).

By Harvest 4, the biomass of the 76R MYC+ was significantly lower than that of the *rmc* plants in the eCO₂ treatment. Thus, the cost:benefit ratio of forming AM, in terms of biomass, at Harvest 4 was no longer in favour of the plant as at Harvest 1, or neutral as at Harvests 2 and 3. Resource limitation is a key factor in cost:benefit analysis of effects of AMF on plant fitness (Eissenstat *et al.* 1993; Johnson *et al.* 1997).

Table 1. Mean shoot and root P and Zn concentrations ($\mu\text{g g}^{-1}$) of 76R MYC+ and *rmc* genotypes of tomato at Harvests 1–4, grown under ambient, or elevated atmospheric CO_2 concentrations (eCO_2)

Means (\pm s.e.) followed by the same superscript letter are not significantly different at the $P < 0.05$ level (GLM). Note: valid statistical comparisons can only be made between genotypes within the same tissue type (roots or shoots), chamber and harvest time. For comparisons between chambers (*t*-tests) see text ($n = 4$)

Concentration ($\mu\text{g g}^{-1}$)	Ambient							
	Harvest 3				Harvest 4			
	76R MYC+		<i>rmc</i>		76R MYC+		<i>rmc</i>	
	Mean	(s.e.)	Mean	(s.e.)	Mean	(s.e.)	Mean	(s.e.)
Shoot P	2245.1 ^a	(118.2)	1234.5 ^b	(33.7)	3075.6 ^j	(170.0)	1302.2 ^k	(98.0)
Root P	2640.4 ^t	(256.2)	1949.5 ^s	(37.2)	3039.7 ^x	(177.9)	1978.8 ^y	(218.7)
Shoot Zn	40.3	(4.4)	34.1	(4.0)	56.9 ^a	(5.9)	34.8 ^b	(3.8)
Root Zn	69.8	(6.2)	49.0	(7.0)	99.9 ^x	(8.4)	45.9 ^y	(1.2)
	eCO_2							
Shoot P	2095.8 ^a	(150.7)	1558.7 ^b	(54.1)	3147.6 ^j	(201.4)	1870.5 ^k	(69.7)
Root P	2764.0 ^r	(279.6)	1994.4 ^s	(21.6)	2591.1 ^x	(224.8)	1627.6 ^y	(130.0)
Shoot Zn	36.9	(1.4)	44.1	(8.8)	53.0 ^a	(4.8)	31.6 ^b	(2.5)
Root Zn	57.7	(6.3)	82.7	(31.0)	83.7 ^x	(14.8)	46.0 ^y	(3.8)

Table 2. Specific P uptake (SPU) and specific Zn uptake (SZnU), rates of 76R MYC+ and *rmc* genotypes of tomato grown under ambient or elevated atmospheric CO_2 concentrations (eCO_2), calculated using total plant P or Zn content between Harvests 3 and 4

Means (\pm s.e.) followed by the same superscript letter are not significantly different at the $P < 0.05$ level (GLM). Note: valid statistical comparisons can only be made between genotypes within the same chamber. For comparisons between chambers (*t*-tests) see text ($n = 4$)

Uptake rate	Ambient				eCO_2			
	76R MYC+		<i>rmc</i>		76R MYC+		<i>rmc</i>	
	Mean	(s.e.)	Mean	(s.e.)	Mean	(s.e.)	Mean	(s.e.)
Specific P	5090.7 ^a	(333.9)	923.2 ^b	(504.4)	8773.3 ^j	(676.2)	4518.5 ^k	(456.8)
Specific Zn	17.1 ^r	(5.5)	0.4 ^s	(1.5)	35.0 ^x	(12.4)	3.1 ^y	(2.4)

This is in contrast to the ambient chamber where there were no inter-genotypic differences at Harvest 4 (see above). Furthermore, biomass of both genotypes was greater in the eCO_2 chamber. Thus, although both genotypes were impacted by an increase in atmospheric CO_2 concentration (eCO_2 v. ambient chambers), the increase in growth was relatively larger in the *rmc* plants. The 76R MYC+ plants had significantly higher P and Zn concentrations than the *rmc* plants at Harvest 4, and the specific P and Zn uptake rates of the mycorrhizal plants between Harvests 3 and 4 were higher than those of the *rmc* plants. AM colonisation of roots may be especially important under eCO_2 conditions given the potential for C fertilisation of plants. Interestingly, %N was higher in 76R MYC+ plants than *rmc* under eCO_2 , but only marginally so under ambient CO_2 conditions. Manganese was the only nutrient that was significantly ($P = 0.002$) lower in the 76R MYC+ than *rmc* plants at Harvest 4 and 76R MYC+ plants grown under eCO_2 concentration were higher (*t*-test $P = 0.0004$) than those in the ambient chamber (data not shown). This lower Mn concentration in 76R MYC+ than *rmc* plants is consistent with our earlier studies with no difference in growth between the genotypes (Cavagnaro *et al.* 2006; T.R. Cavagnaro, L.E. Jackson, unpubl. data). Thus, nutrient deficiency, e.g. Mn, is not a likely explanation for the relatively lower biomass of the 76R MYC+ than *rmc* plants in the eCO_2 chamber at this harvest.

The significantly lower biomass of the 76R MYC+ plants than *rmc* in the eCO_2 chamber, but not ambient chamber, at Harvest 4 could be explained by a period of increased AMF C

demand, e.g. during sporulation, under eCO_2 . That is, the C cost of maintaining the association was greater than the benefit of enhanced P and Zn nutrition (Johnson *et al.* 1997). That the biomass of both genotypes in the ambient chamber was similar at this harvest, where plant photosynthetic capacity and hence C assimilation were presumably lower than under eCO_2 , may be due to differences in the developmental stages of the plants or AMF in the two treatments (Staddon and Fitter 1998; Staddon *et al.* 1999). This is in part supported by the higher plant biomass of both genotypes under eCO_2 at Harvest 4 (see above). Johnson *et al.* (2005) reported that with some plant/AMF combinations (e.g. *Lespedeza sp./Glomus spp.*), eCO_2 resulted in a decrease in beneficial effects of AM on plants. They concluded that this may be related to differences in C sink-strength among AMF taxa, which in turn may influence their responses to changes in host plant physiology that accompany eCO_2 (Johnson *et al.* 2005).

Carbon assimilation and allocation within plants is impacted by both eCO_2 and AMF (Johnson *et al.* 1997; Staddon and Fitter 1998). AM represent an important tradeoff in the context of eCO_2 . Allocation of C to root production is typically higher under eCO_2 (e.g. Rogers *et al.* 1995); conversely mycorrhizal colonisation can lead to a decrease in root production (Smith and Read 1997). AM colonisation can also alter plant C allocation via effects on root exudation and root respiration in some (Langley *et al.* 2005), but not all circumstances (T. R. Cavagnaro, L. E. Jackson, A. Langley and G. W. Koch, unpubl. data). At the

first harvest, the RDW of the 76R MYC+ plants was greater under eCO₂, consistent with earlier studies; however, the same was not true of *rmc*. Calculation of root : shoot ratios did not yield any additional insight (data not shown). It is estimated that up to 20% of a plant's photoassimilates are supplied to AMF (Jakobsen and Rosendahl 1990). This C may have otherwise been allocated to root production. When colonised by AMF, a plant's nutrient demands can be met via the mycorrhizal pathway (Smith *et al.* 2004), resulting in a plastic response to produce relatively lower root biomass. Conversely, in non-mycorrhizal plants, more C is allocated to root biomass, because plant nutrient demand is relatively higher in the absence of the mycorrhizal pathway.

Mycorrhizal colonisation

Mycorrhizal (percent) colonisation of roots was unaffected by eCO₂, whether root length was taken into account (analysis of co-variance, data not shown) or not, as in other studies (Staddon and Fitter 1998; Fitter *et al.* 2000; Staddon *et al.* 2004). 76R MYC+ roots contained arbuscules, hyphal coils and arbusculate coils, all of which may be involved in nutrient transfer between the plant and fungi (Burleigh *et al.* 2002; Cavagnaro *et al.* 2003). Although an increase in the percentage of the root length containing arbuscules in response to eCO₂ but not total percent colonisation has been reported (e.g. Rillig and Allen 1998), this is not always the case (e.g. Klironomos *et al.* 1998). This may be reflection of the fact that percent colonisation may not necessarily accurately reflect AMF biomass and/or activity. Furthermore, since AM morphology, i.e. formation of arbuscules, hyphal coils, arbusculate coils, of tomato is in part determined by AMF identity (Cavagnaro *et al.* 2001a), this may present a significant challenge in identifying effects of eCO₂ on AM colonisation at the structural level where multiple species of AMF are present (as was the case here). There is also the need to consider other measures of AMF such as the area of symbiotic interface, and the activity of external hyphal networks, both of which are technically challenging. By the end of the experiment, colonisation of the 76R MYC+ roots (but not *rmc*) was approximately three times higher than in our earlier work in the same soil (Cavagnaro *et al.* 2006). This may be due to the restricted rooting volume in this study, reflected in the higher root length density (~4–7 cm/g dry soil at Harvest 4, data not shown) than in the earlier field study (Cavagnaro *et al.* 2006).

Conclusions

The data presented here demonstrate that AMF can play a defining role in determining plant responses to eCO₂. The balance between cost(s) and benefit(s) to the plant of forming AM changed over time. An important consideration in this experiment is the limited rooting volume of the soil which may have led to nutrient limitation. Although effects of eCO₂ on the growth of these two genotypes in the field has not been assessed, their relative growth under ambient CO₂ conditions was very similar as when grown in the same field from which the soil used in this experiment was collected (Cavagnaro *et al.* 2006). This suggests that eCO₂ in the field, with a larger rooting zone, might indeed produce similar tradeoffs as the pot study. The data here highlight the importance of atmospheric CO₂ concentrations on plant growth and nutrition; eCO₂ effects on micronutrient as well

as macronutrient concentrations and uptake should be a priority. Tradeoffs associated with forming AM, especially effects on C allocation and metabolism clearly need to be assessed under a wider range of plant and AMF combinations and environmental conditions.

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