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 CO\textsubscript{2} concentrations. A mycorrhiza-defective tomato (\textit{Solanum lycopersicum} L.) mutant with reduced mycorrhizal colonisation (\textit{rmc}) and its mycorrhizal wild-type progenitor (76R MYC \textsuperscript{+}) were grown under ambient and elevated atmospheric CO\textsubscript{2} concentrations (eCO\textsubscript{2}) 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 \textsuperscript{+} plants generally had higher concentrations of P, N and Zn than their rmc counterparts. Consistent with earlier studies, mycorrhizal colonisation was not affected by eCO\textsubscript{2}. Growth of the two genotypes was very similar under ambient CO\textsubscript{2} conditions. Under eCO\textsubscript{2} 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 CO\textsubscript{2}, mycorrhiza mutant, mycorrhizas, \textit{Solanum lycopersicum}.

Introduction

While biota play a central role in soil nutrient cycling and have important impacts on above and belowground processes (Warde 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 CO\textsubscript{2} concentrations (eCO\textsubscript{2}). eCO\textsubscript{2} affects photosynthetic rates, thereby influencing C supply to AMF (Loveless 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 eCO\textsubscript{2} because of changing plant C acquisition costs (Hooijkaas and Bruna 2000; Sterner and Elser 2002; Johnson et al. 2005). eCO\textsubscript{2} 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 eCO\textsubscript{2}; however, P uptake in mycorrhizal plants was higher than in non-mycorrhizal plants when growing under eCO\textsubscript{2} (Jongen et al. 1996; Syvertsen and Graham 1999). Responses of AM to eCO\textsubscript{2} 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 eCO\textsubscript{2} 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 eCO\textsubscript{2} (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 (Marchner and Dell 1994; Smith and Read 1997; Cavagnaro et al. 2006), and under eCO\textsubscript{2}, AMF improved N nutrition of plants after a period of selection (Gammer et al. 2005). Uptake of micronutrients via the mycorrhizal pathway may be especially important under eCO\textsubscript{2} 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 eCO\textsubscript{2}, probably indirectly as a result of eCO\textsubscript{2} effects on plants (Gavito et al. 2003). Although some studies show an increase in intraradical percent colonisation of roots by AMF with eCO\textsubscript{2} (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 eCO\textsubscript{2}, were taken into account, these differences in colonisation were insignificant (Staddon and Fitter 1998; Fitter et al. 2000). At a finer scale of resolution, eCO\textsubscript{2} can lead to an increase in the abundance of arbuscules in roots (Killing 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 fungicide treatments used to establish such controls. Most studies of eCO2 effects on plant growth, plant nutrition, with a few exceptions (e.g. Gavito et al. 2002), used sterilised soils inoculated 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 (rmw) 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. 2006), 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 interaction affecting colonisation of rmw 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 functions without the complicating effects of size-asymmetry between mycorrhizal and non-mycorrhizal plants.

Here, a growth chamber-based experiment compared the rmw 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 nutrition, and mycorrhizal colonisation occurred using a genotypic approach for controlling mycorrhiza symbioses 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 two 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, Molllic Hapludoll), collected from a tomato field on an organically managed farm, Jim and Deborah Duer 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 (rmw) 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 969 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 ms 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 were 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...

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 v. root P and Zn contents (µg plant−1) between Harvastes 3 and 4 (as only nutrient data were available for these harvests), were calculated as in Eqs 1, (following Cavagnaro et al. 2003):

\[ \text{specific P uptake rate} = \frac{\text{mean P content (72 days)}}{\text{mean RDW (72 days)}} \] (1)

Results

Mycorrhizal colonisation

Mycorrhizal colonisation of 76R MYC+ roots ranged from ∼6–80% over the course of the experiment (Fig. 1a, b), with arbuscules, intercellular hyphae, hyphal coils and arbusculate coils clearly visible in the root cortex. Conversely, mycorrhizal colonisation of rmc roots at all harvests was low (≤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 Harvastes 3, 4 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. 2a, 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 Harvastes 2 and 3 in the eCO2 chamber.

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. 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 (± 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 Harvastes. For comparisons between chambers (t-tests) see text (n = 4).
Mycorrhizal effects on tomato under eCO2

Plant nutrition

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), yet there was a large SE 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 arbuscule 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 (Essiennstat et al. 1993; Johnson et al. 1997).

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 4, root %N was significantly higher in 76R MYC+ (2.0 ± 0.1%) than rmc (1.6 ± 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 ± 0.1%) than rmc (1.7 ± 0.2%) plants. There were no other differences in shoot or root N concentrations.

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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 arbuscule 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 (Essiennstat et al. 1993; Johnson et al. 1997).
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 eCO2 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 eCO2 conditions due to differences in the developmental stages of the plants or 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 eCO2, 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 eCO2 at Harvest 4 (see above). Johnson et al. (2005) reported that with some plant/AMF combinations (e.g. Lespedeza spp./Glomus spp.), eCO2 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 eCO2 (Johnson et al. 2005).

Carbon assimilation and allocation within plants is impacted by both eCO2 and AM (Johnson et al. 1997; Staddon and Fitter 1998). AM represent an important tradeoff in the context of eCO2. Allocation of C to root production is typically higher under eCO2 (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 demand, e.g. during sporulation, under eCO2. 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 eCO2, 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 eCO2 at Harvest 4 (see above). Johnson et al. (2005) reported that with some plant/AMF combinations (e.g. Lespedeza spp./Glomus spp.), eCO2 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 eCO2 (Johnson et al. 2005).

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<table>
<thead>
<tr>
<th>Uptake rate</th>
<th>76R MYC+</th>
<th>rmc</th>
<th>76R MYC+</th>
<th>rmc</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (s.e.)</td>
<td>Mean (s.e.)</td>
<td>Mean (s.e.)</td>
<td>Mean (s.e.)</td>
</tr>
<tr>
<td>Specific P</td>
<td>5090.7 a (333.9)</td>
<td>923.2 b (504.4)</td>
<td>8773.3 j (676.2)</td>
<td>4518.5 k (456.8)</td>
</tr>
<tr>
<td>Specific Zn</td>
<td>17.1 (5.5)</td>
<td>8.4 (1.5)</td>
<td>35.0 x (4.8)</td>
<td>3.1 y (2.4)</td>
</tr>
</tbody>
</table>

Means (± 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).

Table 2. Mean shoot and root P and Zn concentrations (µg g−1) of 76R MYC+ and rmc genotypes of tomato at Harvests 1–4, grown under ambient, or elevated atmospheric CO2 concentrations (eCO2)

<table>
<thead>
<tr>
<th>Concentration</th>
<th>76R MYC+</th>
<th>rmc</th>
<th>76R MYC+</th>
<th>rmc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Harvest 3</td>
<td>Mean (s.e.)</td>
<td>Mean (s.e.)</td>
<td>Mean (s.e.)</td>
<td>Mean (s.e.)</td>
</tr>
<tr>
<td>Shoot P</td>
<td>2245.1 a (118.2)</td>
<td>1234.3 b (33.7)</td>
<td>3675.6 a (170.0)</td>
<td>1302.2 b (98.0)</td>
</tr>
<tr>
<td>Root P</td>
<td>2648.4 a (256.2)</td>
<td>1499.5 b (37.2)</td>
<td>3095.7 a (177.9)</td>
<td>1978.0 b (218.7)</td>
</tr>
<tr>
<td>Shoot Zn</td>
<td>40.3 (4.4)</td>
<td>34.1 (4.0)</td>
<td>56.9 a (5.9)</td>
<td>34.8 b (3.8)</td>
</tr>
<tr>
<td>Root Zn</td>
<td>69.8 (6.2)</td>
<td>49.0 (7.0)</td>
<td>99.9 a (8.4)</td>
<td>45.9 b (1.2)</td>
</tr>
</tbody>
</table>

Means (± 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).
first harvest, the RDM 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 arbuscule 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 was not reported at the same soil (Cavagnaro et al. 2003), this may not necessarily accurately reflect AMF biomass and/or activity. Furthermore, since AM morphology, i.e. formation of arbuscules, hyphal coils, arbuscule 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 (~4–5 cm 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 changes 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 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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