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Effects of arbuscular mycorrhizae on tomato yield, nutrient uptake, water relations, and soil carbon dynamics under deficit irrigation in field conditions

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Abstract

Plant strategies to cope with future droughts may be enhanced by associations between roots and soil microorganisms, including arbuscular mycorrhizal (AM) fungi. But how AM fungi affect crop growth and yield, together with plant physiology and soil carbon (C) dynamics, under water stress in actual field conditions is not well understood. The well-characterized mycorrhizal tomato (*Solanum lycopersicum* L.) genotype 76R (referred to as MYC+) and the mutant nonmycorrhizal tomato genotype *rmc* were grown in an organic farm with a deficit irrigation regime and control regime that replaced evapotranspiration. AM increased marketable tomato yields by ~25% in both irrigation regimes but did not affect shoot biomass. In both irrigation regimes, MYC+ plants had higher plant nitrogen (N) and phosphorus (P) concentrations (e.g. 5 and 24% higher N and P concentrations in leaves at fruit set, respectively), 8% higher stomatal conductance (g_s), 7% higher photosynthetic rates (P_n), and greater fruit set. Stem water potential and leaf relative water content were similar in both genotypes within each irrigation regime. Three-fold higher rates of root exudation in detopped MYC+ plants suggest greater capacity for water uptake through osmotic driven flow, especially in the deficit irrigation regime in which root exudation in *rmc* was nearly absent. Soil with MYC+ plants also had slightly higher soil extractable organic C and microbial biomass C at anthesis but no changes in soil CO_2 emissions, although the latter were 23% lower under deficit irrigation. This study provides novel, field-based evidence for how indigenous AM fungi increase crop yield and crop water use efficiency during a season-long deficit irrigation and thus play an important role in coping with increasingly limited water availability in the future.

Keywords: arbuscular mycorrhizal fungi, *Solanum lycopersicum* (tomato), water relations, water stress, soil ecology, root hydraulics

1. Introduction

Increases in the intensity and frequency of droughts predicted with climate change (Trenberth et al., 2014) will affect crop production (Hatfield et al., 2011), even in irrigated cropping systems as freshwater supplies become increasingly limited (Elliott et al., 2014). Plant strategies to cope with drought, such as avoiding water stress by stomatal regulation (Chaves et al., 2003), can be enhanced by associations between roots and soil microorganisms (Bardgett and van der Putten, 2014; Mohan et al., 2014), including arbuscular mycorrhizal (AM) fungi (Augé, 2001).

AM fungi affect a suite of interrelated plant processes, especially nutrient uptake and water relations, that could affect growth under drought (Augé, 2001; Smith and Read, 2008). AM plants often have higher stomatal conductance (g_s) at lower soil moisture (Augé et al., 2015) and sometimes regulate stomatal closure differently (Duan et al., 1996; Lazcano et al., 2014) in ways that may optimize responsiveness to variable soil moisture conditions. Higher g_s in AM plants has been attributed to differences in plant size between AM and non-AM plants or higher leaf phosphorus (P) concentrations, which can affect g_s (Augé et al., 2015). Since P diffusion is severely limited in dry soil (Suriyagoda et al., 2014), AM contributions to plant P may be especially important when soil moisture is low (Neumann and George, 2004). But differences in g_s also occur when AM and non-AM plants have similar size and P levels (Augé et al., 2015). AM fungi can change root hydraulic properties (Aroca et al., 2008; Bárzana et al., 2012; Sánchez-Blanco et al., 2004) that increase water supply to shoots, which may be another mechanism by which they affect g_s. AM plants can also have higher net photosynthetic rates (P_n) under both well-watered and water-stressed conditions (Augé, 2001; Birhane et al., 2012; Huang et al., 2011), which may be related to higher leaf N and/or higher C sink strength of the AM association (Kaschuk et al., 2009).

But how AM fungi affect crop growth and yield under water stress in actual field conditions, and the underlying physiological mechanisms, are not well-known, since most studies have occurred in controlled environments (Augé et al., 2015; Jayne and Quigley, 2014; Worchel et al., 2013), which differ substantially from field environments (Passioura, 2006; Suzuki et al., 2014). For instance, since the much larger volume of soil available to field roots allows them to access more water and nutrients compared to the restricted space in pots, the effect of AM fungi on water relations and nutrient uptake may not be as great as in controlled environments during reduced water availability. Conversely, greater light intensity in the field may allow plants to produce more photosynthate and direct it to AM fungi and thereby increase benefits relative to costs (Johnson et al., 1997). Field studies are thus essential to provide a more complete understanding of AM vs. non-AM plant physiological, biogeochemical, and agronomic processes during an entire crop life cycle in response to long dry spells that occur with reduced rainfall or deficit irrigation (Suriyagoda et al., 2014).

Whole root system measurements are difficult in field studies and belowground processes like soil C dynamics are challenging to measure directly, thus necessitating the use of indicators. Root sap exudation may be a useful indicator of osmotic driven flow and root system size or capacity to access soil water (Pickard, 2003). Indicators of soil C cycling, such as soil CO_2 efflux, which results from respiration of roots and soil microorganisms, and labile soil C pools have been shown to increase in the presence of AM fungi (Cavagnaro et al., 2008; Peng et al., 1993) and may reflect higher belowground C allocation in AM plants, although it is not clear how they might change under water stress.

A major issue in field research on AM effects is achieving non-mycorrhizal controls. Typical tactics to create non-mycorrhizal controls in the field, such as fumigation (Sylvia et al., 1993) or use of soils severely depleted in AM spores (Douds et al., 2011; Subramanian et al., 2006) alter non-target belowground communities and their ecological functions. A well-characterized (Watts-Williams and Cavagnaro, 2014) tomato (*Solanum lycopersicum* L.) mutant with reduced mycorrhizal colonization, named *rmc* (Barker et al., 1998) and its nearly isogenic (Larkan et al., 2013) mycorrhizal wildtype progenitor (cv. 76R, referred to as MYC+) have similar growth and nutrient uptake when not inoculated with AM fungi (Cavagnaro et al., 2004; Facelli et al., 2010), thus serving as a model system for isolating the effects of AM fungi without other interventions (Watts-Williams and Cavagnaro, 2015). Under field conditions on organic farms, AM colonization of MYC+ roots is typically 10–25% and elicits pronounced changes in leaf P, N, and Zn uptake (Cavagnaro et al., 2006), and on expression of root genes for P and N metabolism (Ruzicka et al., 2011).

The main hypothesis of this field study was that the AM symbiosis would increase crop yield under a deficit irrigation, and thus result in higher agronomic water use efficiency (yield per unit of water applied). There were three specific hypotheses regarding plant physiological and belowground effects: 1) Uptake of N and P would be higher in AM plants, especially P in the deficit irrigation regime; 2) Rates of P_n and g_s would be higher and more responsive to soil moisture availability in AM plants; and 3) Indicators of whole root system characteristics (root sap exudation rates) and soil C cycling (soil CO_2 efflux and labile C pools) would be higher in AM plants compared to non-AM plants, but reduced under deficit irrigation. To test these hypotheses, the mycorrhizal tomato MYC+ and the mutant non-mycorrhizal tomato genotype *rmc* were grown in an organic farm in the Sacramento Valley of California, with deficit and well-watered irrigation regimes.

2. Material and Methods

2.1 Field site, experimental design, and water regimes

The experiment was conducted in a field under certified organic management at the University of California Davis Student Farm in Davis, California, USA (38°32'29.49"N, 121°46'0.94"W) during the 2014 growing season. During the winter fallow prior to the experiment, weeds (2.4 ± 0.6 Mg ha^{-1} just before spring tillage), were mainly henbit (*Lamium amplexicuale*) and groundsel (*Senecio vulgaris*), both of which are AM hosts (Ishii et al., 1998). Preparation of the 0.1 ha field (18.3 m × 55 m) included disking and bed formation (1.52 m wide from furrow to furrow) followed by incorporation of 40 kg N ha^{-1} as feather meal (12-0-0) on 15 April 2014.

The soil series was mapped as a Reiff very fine sandy loam, a fine-silty, mixed, nonacid, thermic Typic Xerorthents (Soil Survey Staff, Natural Resources Conservation Service, 2011). Available P (Olsen) was 12.1 µg P g^{-1} and would be considered low for conventional tomato production in California (Table 1). From 21 April to 7 August 2014 (transplanting and harvest, respectively), mean temperatures were 30.9 °C (maximum) and 13.2 °C (minimum), with a maximum of 40.6 °C and a minimum of 5.3 °C (California Department of Water Resources 2014). The only precipitation event >1 mm was on 25 April (8.4 mm).

The split plot, randomized complete block design had two blocks. Irrigation regime was the main plot with two levels (control and 50% deficit, see below) and genotype was the sub-plot, also with two levels (MYC+ and *rmc*, see below), replicated three times within each main plot. Thus, there were six experimental units for each irrigation regime and genotype combination. To minimize effects of adjacent irrigation treatments, one buffer bed on each side of an experimental bed was planted but not sampled (3 beds total per main plot). Plots contained 20 plants at 30 cm spacing and each plot was separated by a 1 m buffer space with no plants.

Transplants of MYC+ and *rmc* were grown from surface sterilized seed provided to Westside Transplant, LLC (Winters, CA). After 8 wk under certified organic management, seedlings were transplanted on the bed center by hand on 21-22 April 2014, followed by 1.9 cm of water applied via a single surface drip line in the center of each bed. Subsequently, subsurface irrigation consisted of two drip lines (buried 10 cm deep, each 23 cm from the center of each bed) pressurized from both ends to minimize time lags during irrigation events.

Irrigation scheduling in the control treatment used guidelines for California tomato production under drip irrigation (Hartz et al., 1994; Johnstone et al., 2005). Daily reference evapotranspiration (derived from a weather station ~1 km from the experimental site) and canopy cover was used to calculate crop evapotranspiration. Canopy cover was measured 16, 28, 42, 58, 74, and 98 d after planting (DAP) using an infrared digital camera (ADCLite; Tetracam Inc., Chatsworth, CA, USA; Fig. 1c; Barrios-Masias *et al.*, 2013). The deficit irrigation treatment began 29 DAP (Fig. 1a) and was achieved by providing 50% of the water as the control at each irrigation event. The total water applied from transplanting until harvest was 32.7 cm (control) and 18.7 cm (deficit irrigation), i.e. a 43% decrease.

2.2 Aboveground biomass and nutrients

Aboveground biomass was measured near tomato anthesis (52 DAP), fruit set (72 DAP), and harvest when most fruit (>75%) were ripe (107 DAP) (Fig. 1a). These times correspond to the BBCH growth stages of “flowering”, “development of fruit”, and “ripening of fruit” for tomato. At anthesis and fruit set one and two plants in each plot, respectively, were cut at the base and separated into leaves, stems, and fruit and then dried at 60 °C for 7 d. Leaves and stems were weighed and then analyzed for total C and N by combustion on a ECS 4010 CHNSO analyzer

(Costech Analytical Technologies Inc., Valencia, CA, USA) and for P by nitric acid/hydrogen peroxide digestion followed by colorimetric analysis of the digest using the molybdate-blue method (Murphy and Riley, 1962). At harvest five adjacent plants from each plot were cut at the base and red fruit (i.e. of harvestable quality) was separated from green and decayed fruit (i.e. unharvestable), using criteria similar to that for commercially harvested tomatoes (Bowles et al., 2015). Biomass of fruits and shoots were weighed in the field (fresh weight) and then subsamples were dried at 60 °C and analyzed for total C, total N, and $\delta^{13}C$ on a PDZ Europa ANCA-GSL elemental analyser interfaced to a PDZ Europa 20–20 isotope ratio mass spectrometer (Sercon Ltd, Cheshire, UK) at the UC Davis Stable Isotope Facility. Nutrients in red fruit at harvest, including P, potassium (K), sulfur (S), boron (B), calcium (Ca), magnesium (Mg), zinc (Zn), manganese (Mn), iron (Fe), and copper (Cu), were determined at the UC Davis Analytical Laboratory by nitric acid/hydrogen peroxide microwave digestion and Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES). Total soluble solids (TSS) of ripe fruit were measured using a refractometer.

2.3 Leaf gas exchange and water status

Leaf gas exchange measurements were taken on mature, fully expanded leaflets from the top of the canopy with a field portable open flow infrared gas analyzer (model 6400, LI-COR Inc., Lincoln, NE, USA). Measurements were taken between 10:15 and 12:30 h with a 6-cm^2 leaf-chamber, with the CO_2 reference set at 400 µmol mol^{-1} and with a light intensity of 2000 µmol m^{-2} s^{-1} using a light-emitting diode source. During both the anthesis and fruit set samplings, plots were sampled over five consecutive days (10 days total). Data from 48 and 50 DAP were not used due to high wind and air temperature. Three leaflets per plot were collected on one day in each sampling period for analysis of relative water content (RWC), total C, total N, $\delta^{13}C$, specific leaf area (SLA), and specific leaf area nitrogen (SLAN). One leaflet had been used for gas exchange measurements and was analyzed separately for photosynthetic N use efficiency (PNUE), calculated as P_n divided by total N concentration. SLA was calculated as the hydrated area divided by the dry mass. Leaf RWC was calculated according to:

$$RWC\ (\%) = \left(\frac{FW - DW}{TW - DW}\right) \times 100$$

where FW is leaf fresh weight; DW is leaf dry weight after 48 h at 60 °C, and TW is leaf turgid weight after submergence of the petiole in water overnight at 4 °C.

Stem water potential (Ψ_{stem}) was measured at mid-morning on one day each during the anthesis and fruit set samplings. Shaded mature leaflets were covered for at least 15 min in plastic bags wrapped in aluminum foil to prevent leaf transpiration, excised, and measured with a Scholander-style pressure chamber (#3005; Soil Moisture Equipment Corp., Goleta, CA, USA) (Choné et al., 2001).

2.4 Root exudation and osmolality

For root exudation rates, exuded sap was collected from one detopped plant per plot when Ψ_{stem} was measured. Immediately after cutting plants for aboveground biomass at anthesis and fruit set (see above), the stump was rinsed with ddH_2O and blotted with an absorbent tissue. PVC tubing was fitted over the stump and sap was collected four times (~30 min intervals) in pre-weighed vials for up to 2 h after ensuring there was no leakage. Collected sap was immediately frozen on dry ice and then weighed in the lab. The osmolality of the exuded sap (excluding the first collection to avoid contamination from cut cells) was determined using a vapor pressure osmometer (VAPRO 5600; Wescor, Logan, Utah, USA). The osmotic potential of the exuded sap was expressed in MPa, where 40.75 mOsmol kg^{-1} corresponds to 0.1 MPa (Fricke et al., 2014).

2.5 Colonization of roots and soil sampling

For determination of AM fungal colonization, roots were collected at 85 DAP 10 cm from the plant row from a 6 cm dia. × 10 cm deep core. After wet sieving of soil, roots were stained with trypan blue (Cavagnaro et al., 2006) and colonization was determined using the gridline intersect method (Giovannetti and Mosse, 1980).

Soil CO_2 fluxes were measured during the same 5-d runs as for leaf gas exchange using a LI-COR 8100 soil respiration system (LI-COR, Lincoln, NE, USA). Measurements were made between 1000 and 1200 h from a PVC collar, 20 cm in dia. × 10 cm deep, inserted between two plants 15 cm from plant row. Volumetric water content (VWC) was determined at the same time using a time domain reflectance (TDR) probe (EC-5; Decagon Devices, Inc, Pullman, Washington, USA) installed at 10 cm depth.

Soil was sampled just prior to starting deficit irrigation (21 DAP), and at anthesis (49 DAP), fruit set (70 DAP), and harvest (108 DAP) samplings at four depths (0–15, 15–30, 30–60, and 60–100 cm; two 6.3 cm dia. cores composited per plot, 15 cm from plant row). Gravimetric water content (GWC) was measured on all samples by drying a subsample at 105 °C for 48 h. Microbial biomass carbon (MBC) and 0.5 M K_2SO_4-extractable organic C (EOC) were measured at all but the harvest sampling in surface soil (0–15 cm) by chloroform fumigation-extraction followed by UV-persulfate oxidation (Wu et al., 1990). No correction factors were used for MBC. EOC was quantified in non-fumigated samples.

2.6 Statistical analysis

Mixed model analysis of variance (ANOVA) was performed using the proc mixed procedure in SAS v.9.4 (Cary, NC). Genotype and irrigation were treated as fixed effects while block and block×irrigation were considered random effects to account for the split plot experimental design. For leaf gas exchange data (i.e. g_s, P_n, and WUE_i), date was considered a repeated measure. Degrees of freedom were adjusted as described by Kenward and Roger (1997). Transformations were used as needed to meet assumptions of homoscedasticity and normality.

Principal components analysis (PCA) of fruit elemental concentrations and quantities was performed using the *vegan* package in R (Oksanen et al., 2012). PCA was selected because these data were normally distributed and the relationships were linear.

3. Results

3.1 AM colonization, canopy cover, and soil moisture

The mutant tomato genotype *rmc* had 6-fold lower root colonization by AM fungi than its wildtype progenitor, MYC+ (2.1 vs. 12.3%, respectively, $F_{geno,1,17}=33.7$, $p < 0.0001$). Colonization was not affected by deficit irrigation.

Canopy cover reached a maximum of 60±2.7% and 71±3.8% in the deficit and control irrigation regimes 98 DAP, respectively (Fig. 1). Canopy cover was similar across all treatments prior to the beginning of deficit irrigation 29 DAP and then was significantly higher in the control irrigation regime 42, 58, 74, and 98 DAP (Fig. 1). There were no significant differences in canopy cover between the genotypes.

Gravimetric water content was similar at all depths prior to the onset of deficit irrigation (Fig. 1). Later changes in GWC were most pronounced at 0–15 cm depth, which was significantly lower in the deficit irrigation regime at the anthesis, fruit set, and harvest samplings. There were no differences in GWC in plots with MYC+ vs. *rmc* at any sampling time.

3.2 Aboveground biomass

At anthesis and fruit set samplings, aboveground dry biomass (leaves, stems, and fruit) was similar for MYC+ and *rmc* and in both irrigation treatments (Fig. 2; Table S1), except for stem biomass at the fruit set sampling, which was 12% higher in MYC+ compared to *rmc*.

At harvest, MYC+ had 25% higher red fruit dry biomass than *rmc* but similar shoot biomass within each irrigation regime (Fig. 2; Table S1). Red fruit fresh biomass (i.e. yield) was 28% and 24% higher for MYC+ than *rmc* under control and deficit irrigation, respectively (Table 2). Total fresh fruit biomass was also 19% higher in MYC+, since green and decayed fruit fresh biomass was similar in both genotypes. In both tomato genotypes, red and green fruit fresh biomass were 11% ($p<0.1$) and 30% ($p\leq0.05$) lower under deficit irrigation, respectively. The fresh biomass of individual red fruit was 67±1.2 g and did not vary among water regime or genotype.

Irrespective of genotype, total aboveground dry biomass (fruit and shoots) at harvest was 12% lower under deficit irrigation treatment, mostly due to lower shoot biomass (Fig. 2; Table S1). Thus, the main effect of AM fungi on plant biomass was in fruit rather than shoots and did not depend on water regime.

3.3 Plant N and P concentrations and contents

At anthesis, concentration of N in tomato leaves was similar across genotypes and water regimes. But at fruit set, concentration of N in leaves was 5% higher in MYC+ than *rmc* (3.14 vs. 3.00%, respectively) considering both water regimes together (Fig. 3; Table S2). Leaf N content was similar across genotypes and water regimes at anthesis and fruit set (Table S2). At anthesis, stem

N concentration and content were 13% and 19% higher, respectively, in MYC+ than *rmc*, but were similar at fruit set (Fig. 3; Table S2). At harvest, N concentration of red fruit was 8% higher in *rmc* than MYC+ (Table S3). But the N content of red fruit was 19% higher in MYC+ than *rmc*, resulting from higher red fruit biomass in MYC+ (Fig. 4; Table S3). There was a trend toward higher total aboveground N content in MYC+ than *rmc* at harvest (Fig. 4; Table S3). Considering both genotypes together, the deficit irrigation reduced N concentration in leaves at fruit set by 5% and reduced total aboveground N content at harvest by 12%.

For the terminal leaflet on the most recently-expanded leaf at anthesis, N concentration was slightly (5%) higher in MYC+ than *rmc* (4.9 vs. 4.7%), which resulted in 5% lower SLAN in MYC+ than *rmc* (i.e. more N per unit leaf area), since SLA was similar in both genotypes (Table 2). Leaflet N concentration was not affected by the irrigation regime at either sampling, but SLA and SLAN were 5% and 3% lower, respectively, under deficit irrigation at anthesis.

Phosphorus concentration and content in plants with AM fungi generally increased, especially later in the growing season, but these effects were more pronounced under the control than the deficit irrigation regime. At anthesis, concentration and content of P in leaves were similar in MYC+ and *rmc*. But at fruit set, P concentration in the leaves was 24% higher in MYC+ than *rmc* in the water control (0.19 vs. 0.15%, respectively) but with only slight differences between genotypes under deficit irrigation (Fig. 3; Table S2). This corresponded to a lower leaf N:P ratio for MYC+ in the water control at fruit set (Fig. 3; Table S1) indicating relatively more plant P uptake than N uptake in these plants. Stem P concentration was higher in MYC+ vs. *rmc* at both anthesis and fruit set (14% and 11%, respectively). Reduced P in leaves of plants under deficit irrigation regime was apparent at anthesis with leaves having 18% lower P concentration, 25%

lower P content, and a 16% higher N:P ratio considering both genotypes together (Fig. 3; Table S2). Stem P concentration and content were not affected by the water treatments.

Whereas red fruit P concentration at harvest was similar in MYC+ and *rmc* (Table S3), P content of all red fruit was 28% higher in AM plants (Fig. 4; Table S3), again resulting from higher red fruit biomass. Total aboveground P content at harvest was 25% higher in MYC+ than *rmc* and was 17% lower under deficit irrigation when considering both genotypes together, but there was a trend toward a stronger genotype effect under the control than the deficit irrigation regime. The N:P ratio in shoots was 17% lower for MYC+ than *rmc* at harvest considering both water regimes together. The N:P ratios in fruit and total biomass were also lower in MYC+, but mainly in the water control (Fig. 4; Table S3).

3.4 Fruit macro- and micronutrients and fruit quality

Considering each nutrient individually, concentrations of K, Mg, Mn, and Cu were significantly lower in red fruit of MYC+ than *rmc* (9, 11, 14, and 12% lower, respectively; Table S4). On the basis of total content in red fruit per plant, all nutrients except Ca, Mn, Zn, and Fe were significantly higher in MYC+ than *rmc* (Table S4). There were no effects of irrigation regime on concentration or content of these nutrients in red fruit at harvest. The PCA of macro- and micro-nutrients in red fruit at harvest showed that nutrient concentrations were strongly correlated with one another and most tended to be lower in MYC+ vs. *rmc* (Fig. 5). Macro- and micro-nutrient content, however, were higher in MYC+ than *rmc* (Fig. 5), reflecting higher red fruit biomass in AM plants. The irrigation regimes did not significantly affect nutrient concentrations or contents in red fruit (Table S4).

Total soluble solids in red fruit were similar in MYC+ and *rmc* but were 6% higher in the deficit irrigation considering both genotypes together (Table S4). Fruit pH had a mean of 4.48 and was similar in both genotypes and irrigation regimes.

3.5 Plant water status

Stem water potential (Ψ_{stem}) at mid-morning was similar in both genotypes and irrigation regimes at anthesis, with a mean of -0.09 MPa (Table 2). But at fruit set, Ψ_{stem} reached -0.35 MPa in the deficit irrigation regime, 29% lower than the control (-0.26 MPa), indicating slightly higher water stress under deficit irrigation 43 days after the deficit began (72 DAP). A trend towards less water stress in MYC+ at fruit set was indicated by less negative Ψ_{stem} than *rmc* but only in the control irrigation (-0.22 vs. -0.30 MPa, respectively; $F_{water\times geno,1,19}$=3.9, *p*=0.06). Leaflet RWC was similar in MYC+ and *rmc* and in both irrigation regimes at the anthesis and fruit set samplings with a mean of 82% (Table 2).

3.6 Leaf gas exchange and water use efficiency

Considering all measurement dates together, P_n and g_s were 7 and 8% higher, respectively, in MYC+ than *rmc* (P_n: $F_{geno,1,18}$=37.0, $p < 0.0001$; g_s: $F_{geno,1,18}$=9.6, p =0.006) but were not affected by the irrigation regimes. Mean P_n was 29.4 and 27.4 µmol m^{-2} s^{-1} and mean g_s was 0.81 and 0.74 mol m^{-2} s^{-1} in MYC+ and *rmc*, respectively. Since P_n and g_s both increased in MYC+, there was no difference in intrinsic water use efficiency (WUE_i, i.e. the amount of CO_2 fixed per unit of H_2O lost) between MYC+ and *rmc*, but WUE_i was 12% higher in the deficit irrigation regime compared to the control at fruit set (Table 3), considering both genotypes together. There was also no difference in leaflet $\delta^{13}C$ for MYC+ vs. *rmc* at either sampling time (Table 2).

Contrasting patterns of P_n and g_s occurred in MYC+ vs. *rmc* during the multi-day runs, and this appears to be related to soil moisture availability and air temperature (Fig. 6). During the anthesis sampling, P_n and g_s increased sharply for MYC+, but not *rmc*, in the deficit irrigation regime at 51 DAP (Fig. 6a and 6b). Water had been applied shortly before gas exchange measurements that day as indicated by an increase in surface soil VWC (Fig. 6d), after several days of hot, windy weather. At fruit set, soil moisture was more consistent (Fig. 5d), but P_n declined by 23% in *rmc* vs. only 10% in MYC+ between 69 and 70 DAP (i.e., 25.4 vs. 20.7 μmol m^{-2} s^{-1} in MYC+ vs. *rmc*). The maximum temperature on 70 DAP was 40.1 °C vs. 36.6 and 32.4 °C on 69 and 71 DAP, respectively Fig6c). At fruit set, similar leaflet N concentrations (see above) but higher P_n contributed to 16% higher PNUE (i.e. P_n N^{-1}) in MYC+ vs. *rmc* under deficit irrigation, but not in the control (Table 2).

3.7 Root exudation rates and osmolality

Root exudation rates from detopped plants were similar in MYC+ and *rmc* at anthesis but were 3-fold lower in *rmc* than MYC+ at fruit set (Fig. 7a), when plants showed more water stress (see Ψ_{stem} above), considering both irrigation regimes together. At fruit set, *rmc* plants in the deficit irrigation treatment exuded virtually no sap. Root exudation rates were approximately 2-fold lower in the deficit irrigation treatment compared to the control at anthesis and fruit set considering both genotypes together. The osmotic potential of exuded sap was 36% higher under deficit irrigation at anthesis but similar in both genotypes (Fig. 7b), whereas at fruit set it was nearly 2-fold higher in *rmc* than MYC+, but unaffected by deficit irrigation.

3.8 Soil C dynamics

Early in the season before plants were present, mean soil EOC and MBC were 43.9 and 89.9 µg C g^{-1}, respectively (data not shown). At anthesis, there was a trend toward slightly higher MBC and EOC in soil with MYC+ plants compared to *rmc* plants (MBC: 98.7 vs. 91.1 µC g^{-1} soil; and EOC: 43.4 vs. 38.8 µC g^{-1} soil in MYC+ and *rmc*, respectively) but at fruit set there were no differences between genotypes (Table 4). Midday soil CO_2 emissions were similar in both genotypes but 23% lower under deficit irrigation.

4. Discussion

This study provides field-based evidence that AM fungi can increase crop yield and crop water use efficiency during season-long deficit irrigation, along with higher plant N and P uptake, higher P_n and g_s, higher soil labile C pools, and possible changes in water uptake capacity. Association with AM fungi increased tomato dry red fruit biomass and fresh red fruit biomass (i.e. yield) by ~25% under field conditions in both the control and deficit irrigation regimes but without other substantial changes in aboveground biomass. Greater fruit set likely occurred in MYC+ plants. Higher rates of root sap exudation in MYC+ plants may reflect higher root osmotic hydraulic conductance, a pathway for water uptake that may play an important role under dry conditions (Barrios-Masias et al., 2015). Surprisingly, the substantial reduction in irrigation (43% less water applied) was not severe enough to impact plant water status, based on little change in Ψ_{stem} and leaf RWC, suggesting that roots could extract substantial water from deep in the soil profile or that plants regulate daily leaf gas exchange to maximize C gain before high vapor pressure deficits begin. These findings suggest that AM affect a suite of interrelated plant drought responses that together enabled plants to produce higher yields.

4.1 AM colonization, plant biomass and nutrient uptake

The substantially lower root colonization of the tomato genotype *rmc* by AM fungi compared to its wildtype progenitor MYC+ provided an effective non-AM control under field conditions. The ratio of root colonization between MYC+ and *rmc* (6-fold higher in MYC+) was similar to that of previous field experiments (Cavagnaro *et al.*, 2006; 2011) and a recent meta-analysis of studies with these genotypes (Watts-Williams and Cavagnaro, 2014), but the rate of colonization was lower (12% in this study vs. 20-25% in previous studies), though still within the range typically found on field tomato roots (Cavagnaro and Martin, 2010; Ruzicka et al., 2011). Winter tillage and bare fallow in the experimental field were reflective of typical agricultural practices in the study region but may have limited the colonization potential of the soil (Lekberg and Koide, 2005). Intense drying and rewetting cycling in surface soil where roots were sampled (0–10 cm) may have also limited AM colonization. Identical growth and physiology (e.g. P uptake) of MYC+ and near-isogenic *rmc* when grown without AM fungi present (Facelli et al., 2010) mean that the large genotypic differences shown here can be attributed to association with AM fungi, and possibly also to changes in rhizosphere microbial communities induced by AM fungi, such as hyphal-associated bacteria or plant-growth promoting bacteria (Scheublin et al., 2010). Previous work showing similar microbial communities in the soil around MYC+ and *rmc* roots (via PLFA profiles) suggest that these changes may be relatively minor (Cavagnaro et al., 2006), but there is still a possibility that there are micro-scale fungal-bacterial interactions that affect nutrient availability and uptake by the plant.

Greater fruit biomass in MYC+ plants and few differences in shoot biomass compared with *rmc* through the season point to a specific effect of AM fungi on fruit rather than a general effect on plant size. Since the size of individual fruits was similar in both genotypes and water regimes, higher fruit biomass must have been a result of an increase in fruit number in MYC+ plants. AM fungi can affect plant reproductive growth (Bryla and Koide, 1990; Poulton et al., 2002), including increasing the total number of flowers in tomato (Subramanian et al., 2006), as well as the number of flowers per truss, and the proportion of flowers setting fruit (Conversa et al., 2013). High temperatures (>32 °C daytime) impair pollen and anther development in tomato at anthesis and reduce fruit set (Peet et al., 1998). Such temperatures were exceeded in this study, as typically occurs in the Mediterranean-type climates where tomatoes are widely grown. Higher g_s in MYC+ plants could suggest higher transpiration rates, since canopy size was similar (Fig. 1), and thus cooler canopies (Fischer et al., 1998).

The higher P concentration of leaves and total plant P content in AM plants is typical for MYC+ plants grown in P-deficient soil (Watts-Williams and Cavagnaro, 2014), as in this study with 12.1 µg P g^{-1} soil. But shoot P concentrations would still be considered low for tomatoes in this region (Hartz et al., 1998). Similarly, N concentrations in shoots were close to the critical N concentration (i.e. the minimum N concentration needed for maximal plant growth) for Roma-type tomatoes (Tei et al., 2002; 3.35% and 2.80% measured aboveground N concentration at anthesis and fruit set, respectively, vs. 3.49% and 2.77% critical N concentration at anthesis and fruit set). But even the slight increases in plant N and P concentrations observed in AM plants may have affected growth, especially fruit production (Tei et al., 2002), and physiology (e.g. root hydraulics and leaf gas exchange, see below; Clarkson *et al.*, 2000; Cramer *et al.*, 2009).

The enhanced capacity to forage for P by AM fungi was expected to be more beneficial in drier soil (Neumann and George, 2004; Smith et al., 2009), since fungal hyphae can access smaller water-filled pores than roots (Nadian et al., 1998), but mycorrizae increased P uptake more in the control than the deficit irrigation treatment. AM contributions to plant P uptake, however, can be substantial even when differences in total plant P are small or absent compared to a non-AM control plant (Li et al., 2006), i.e. the AM contribution to P uptake can be “hidden” when direct root uptake of P decreases but AM transfer of P to roots increases (Smith and Smith, 2011).

Increases in N and P uptake in MYC+ plants likely contributed to the large increase in fruit biomass since fruit are a major nutrient sink (e.g. 43% and 65% of total aboveground N and P uptake at harvest, respectively). For instance, the higher N and P content of MYC+ stems earlier in the growing season could be translocated to the greater fruit load of these plants. The lower concentrations of some nutrients (e.g. N, K, Mg, Mn, and Cu) in fruits of MYC+ vs. *rmc* suggests that fruit elemental stoichiometry is flexible and that these nutrients were not limiting fruit production.

4.2 Water relations, photosynthesis, and soil C dynamics

The magnitude of the increase in g_s in MYC+ plants, compared to *rmc*, was similar under both control and deficit irrigation and is consistent with results from a meta-analysis of experiments, conducted mainly in controlled settings, at similar levels of root colonization and when AM and non-AM plants are similarly sized (Augé et al., 2015). AM fungi may have contributed directly or indirectly to a higher g_s in MYC+ plants at a ψ_{stem} similar to *rmc* plants. Differences in g_s in AM vs. non-AM control plants have been attributed to differences in plant size, leaf P nutrition, as well as C dynamics (see below) of host leaves (Augé et al., 2015). Similar aboveground biomass in MYC+ and *rmc* at anthesis and fruit set, and similar canopy cover over the whole growing season (Fig. 1), rules out canopy size asymmetry as a driver of differences in water relations.

The 3-fold higher root exudation rates in MYC+ plants than *rmc* at fruit set also highlights the possibility for AM effects on root hydraulic properties, which has been observed in greenhouse studies (Aroca et al., 2007; Bárzana et al., 2012) but not yet in the field. Is it possible that higher root exudation rates indicate higher osmotic root hydraulic conductance in MYC+? Relative differences in root hydraulic conductance between MYC+ and *rmc* would depend mainly on the root hydraulic conductivity, the osmotic potential gradient between soil solution and the xylem sap, and the size of the root system. The osmotic potential of the soil solution was likely similar in MYC+ and *rmc* plots, since GWC was similar. Greenhouse studies have shown that MYC+ and *rmc* have similar root biomass (Watts-Williams and Cavagnaro, 2014), although this may change under field conditions. Higher osmotic driven flow may be especially important during periods of water stress when plants rely less on hydrostatic forces (i.e. lower g_s) for water uptake (Aroca et al., 2012; Barrios-Masias et al., 2015).

Higher P_n in MYC+ plants allowed assimilation of enough C to support additional fruit biomass and the C cost of the AM fungi while maintaining a similar canopy size to *rmc*. Building and maintaining a larger canopy may not be advantageous when soil moisture is low due to higher water loss through transpiration. Enhanced P_n in MYC+ plants may result from higher g_s, increased N and P nutrition, and/or higher C sink stimulation. Higher stomatal conductance would increase CO_2 diffusion to sites of carboxylation and support higher P_n. Higher leaflet N concentration and lower SLAN (i.e. more N per unit leaf area), as found in MYC+ plants at the anthesis sampling, may indicate more photosynthetic machinery and a higher capacity for C fixation (Evans, 1989). At fruit set, higher PNUE in MYC+ plants under water stress may be related to differences in N partitioning in the leaf (Barrios-Masias et al., 2013) or evidence of C sink stimulation of photosynthesis (Kaschuk et al., 2009).

Both above- and belowground C sink strengths of MYC+ plants were likely higher than *rmc* since MYC+ plants had more fruit and AM fungal C demand can reach 5–20% of photosynthate

(Jakobsen and Rosendahl, 1990). Just a slight shift in plant belowground C allocation could account for higher MBC in soil associated with MYC+ plants vs. *rmc* because the difference was small (1.4 g MBC m^{-2}), e.g. representing just ~0.6% of aboveground biomass C at anthesis (230 g C m^{-2}). Higher belowground plant C allocation may also have stimulated slightly greater organic matter turnover (Cheng, 2009), thus accounting for higher EOC. Variation in plant allocation of C to AMF during development (Mortimer et al., 2005) may explain why these effects were only apparent at anthesis; root allocation decreases after anthesis in field-grown tomatoes (Jackson and Bloom, 1990). The lack of differences in soil CO_2 emissions between MYC+ and *rmc* shows that total soil respiration was not affected by the AM associations, though the relative contributions of roots, soil heterotrophs, and AMF may have changed (Cavagnaro et al., 2008). Reductions in soil CO_2 emissions under deficit irrigation could reflect lower respiration of soil microbes, since microbial activity decreases with lower soil moisture (Manzoni et al., 2012).

Not only did AM plants have higher mean P_n and g_s, they also appeared to optimize responses to environmental conditions in ways that would maximize growth. The large increase in P_n and g_s in MYC+ but not *rmc* plants following irrigation after several days of hot, dry weather (51 DAP), agrees with studies in controlled environments that show AM plants to respond more quickly than non-AM plants to changes in soil moisture (Duan et al., 1996; Lazcano et al., 2014). This response occurred even in a field environment when changes to soil moisture would inevitably occur more gradually than the rapid rewetting of a pot. Future work could also examine whether AM fungi also affect how plants regulate diurnal patterns of leaf gas exchange, for instance by maximizing C gain through increased stomatal conductance early in the day when vapor pressure deficit is lower, followed by a reduction in g_s in the afternoon (Richards, 2000).

This could help explain the higher g_s we observed prior to late afternoon, when daily air temperature peaks, and that despite a similar canopy size in MYC+ plants, soil water use was similar in MYC+ and *rmc* plants.

Since P_n and g_s increased in parallel in MYC+ plants there was no increase in WUE_i compared to *rmc*, as also reflected in similar leaflet $\delta^{13}C$ in the two genotypes. But since red fruit biomass was higher in MYC+ than *rmc* but with the same amount of water applied, the crop water use efficiency (i.e. yield cm^{-1} water applied) of MYC+ plants was ~30% higher: 2.46 and 3.72 Mg ha^{-1} cm^{-1} in MYC+ vs. 1.85 and 2.94 Mg ha^{-1} cm^{-1} in *rmc* under control vs. deficit irrigation regimes, respectively. Increasing yield per unit of water used will be increasingly important as climate change affects water availability in both rainfed and irrigated agricultural systems.

4.3 Conclusions

The AM symbiosis increased ecosystem provisioning (i.e. yield) and regulating services, which was associated with higher nutrient uptake, higher g_s and P_n at similar water availability, and potentially greater root water uptake capacity. This shows that AM fungi play an important role in plant responses to deficit irrigation in actual agroecosystem conditions. Strategies that boost AM fungal populations like minimizing soil disturbance and fallow periods in agriculture may in turn increase the services provided by mycorrhizal associations in a changing climate.

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