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Nutrient interactions and arbuscular mycorrhizas: a meta-analysis of a mycorrhiza-defective mutant and wild-type tomato genotype pair.

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Abstract

Background and aims

Arbuscular mycorrhizas (AM) enhance plant uptake of a range of mineral nutrients from the soil. Interactions between nutrients in the soil and plant, are complex, and can be affected by AM. Using a mycorrhiza-defective mutant tomato genotype (*rmc*) and its wild-type (76R), provides a novel method to study AM functioning.

Methods

We present a meta-analysis comparing tissue nutrient concentration (P, Zn, K, Ca, Cu, Mg, Mn, S, B, Na, Fe), biomass and mycorrhizal colonisation data between the 76R and *rmc* genotypes, across a number of studies that have used this pair of tomato genotypes. Particular attention is paid to interactions between soil P or soil Zn, with tissue nutrients.

Results

For most nutrients, the difference in concentration between genotypes was significantly affected either by soil P, soil Zn, or both. When soil P was deficient, AM were particularly beneficial in terms of uptake of not only P, but other nutrients as well.

Conclusions

Colonisation by AMF significantly affects the uptake of many soil macro- and micro-nutrients. Furthermore, the soil P and Zn status also influences the difference in nutrient concentrations between mycorrhizal and non-mycorrhizal plants. The interactions identified by this meta-analysis provide a basis for future research in this area.

Introduction

Arbuscular mycorrhizas (AM) are associations formed between the majority (80%) of terrestrial plant species, and a specialised group of soil fungi now classified as Glomeromycota (Smith and Read 2008). The formation of AM can benefit plants through enhanced acquisition of nutrients, particularly phosphorus (P), nitrogen (N), copper (Cu) and zinc (Zn) (Smith and Read 2008; Marschner and Dell 1994; Clark and Zeto 2000; Bolan 1991; Lambert and Weidensaul 1991; Watts-Williams and Cavagnaro 2012). In addition, plant uptake of other soil-derived mineral elements such as iron (Fe), potassium (K), calcium (Ca) and magnesium (Mg), has also been reported (Marschner and Dell 1994; Marschner 2012), although responses can be variable (Clark and Zeto 2000; Marschner 2012). Nevertheless, it is for their capacity to increase plant nutrient acquisition that AM are increasingly recognised as having an important role to play in sustainable agricultural production systems (Gianinazzi et al. 2010; Cardoso and Kuyper 2006; Burns et al. 2012). While much is know about the role of AM in improving plant nutrient acquisition, most studies of AM have focused on only one nutrient at a time, although there are some exceptions (Li et al. 1991; Lambert et al. 1979; Kothari et al. 1991a).

Acquisition of nutrients is strongly influenced by the multifarious and complex interactions among nutrients both in the soil and *in planta* (Fageria 2001; Epstein and Bloom 2005). Soil P fertilisation can also impact upon plant uptake of Z, Fe, Cu, Mn, and other nutrients (Lambert and Weidensaul 1991). One of the most frequently studied nutrient interactions is that between P and Zn, specifically, the occurrence of "P-induced Zn deficiency" (Robson and Pitman 1983; Warnock 1970). This interaction is predominant when the soil is high in plant-available P (naturally or through fertilisation) and low in plant-available Zn, and can lead to decreased concentrations of Zn in plant tissues (Broadley et al. 2012). There are many factors that contribute to the complex interactions between P and Zn, such as soil chemical factors (especially soil pH), production of phytosiderophores, and expression of P and Zn transporter genes in plants (see Alloway 2008; Loneragan et al. 1979; Loneragan and Webb 1993; Broadley et al. 2012 and references therein for details). While the effect of soil P fertilisation upon the uptake of other nutrients has also been reported, these interactions and the effect of AM on them are much less understood (Liu et al. 2000; Lambert et al. 1979).

Much in the same way that soil P fertilisation can affect plant Zn nutrition, soil Zn fertilisation can affect the uptake and translocation of other nutrients. For example, Zn fertilisation can increase translocation of Mn to the shoots, and can even induce Mn-toxicity symptoms in plants (Foy et al. 1978). Conversely, soil Zn fertilisation can reduce the uptake of Fe and Cu in rice (Cayton et al. 1985). Taken together, it is clear that further investigation into the effect of Zn fertilisation (including toxic levels) upon tissue nutrient concentration, will be important.

Few studies have considered the effect of AM upon interactions between nutrients, and vice versa. However, it is likely that if the supply of one nutrient affects the formation of AM, this will in turn have an impact on uptake of other nutrients by AM. For example, the formation of AM is affected by both soil P and soil Zn fertilisation. In the case of P there is an inverse relationship between soil P fertilisation and root length colonised by AM (Marschner 2012). In contrast, for Zn, the relationship between soil Zn fertilisation and AM colonisation is not as clear, with positive (Lee and George 2005; Zhu et al. 2001), neutral (Diaz et al. 1996; Ortas et al. 2002) and negative (Shen et al. 2006; Gildon and Tinker 1983a; Chen et al. 2004) responses reported. Furthermore, if the formation of AM increases the capacity of plants to acquire one nutrient, there may be consequences for the acquisition, translocation and internal cycling of other nutrients; this however, has received little attention.

One of the challenges of studying AM is that of establishing non-mycorrhizal controls that avoid non-target effects upon soil nutrient availability. Using a genotypic approach to control for mycorrhizal fungal colonisation, that is, comparing a mycorrhiza-defective mutant plant genotype to its mycorrhizal wild-type counterpart, reduces confounding effects upon the experiment (Rillig et al. 2008), including nutrient availability and cycling. The mycorrhizal 76R and reduced-mycorrhizal *rmc* tomato genotypes (Barker et al. 1998) have been used in numerous studies of plant nutrition, and to explore nutrient interactions, including those between P and Zn (Watts-Williams and Cavagnaro 2012; Watts-Williams et al. 2013; Cavagnaro et al. 2010), but also N and P (Cavagnaro et al. 2006). Furthermore, while some of these studies also present data on other nutrients, interactions between these nutrients are not considered in detail. These data, however, provide an opportunity to explore the impact of AM on plant nutrient interactions. Therefore, results of a meta-analysis are presented here, in which we aimed to answer two main questions:

1. Do tissue nutrient concentrations, biomass, and mycorrhizal colonisation differ significantly between the two genotypes?
2. Does soil P and Zn fertilisation affect the acquisition of P, Zn and other nutrients, by the two genotypes?

Methods

Literature search and data collection

We identified all publications using the *rmc* and 76R tomato genotypes by searching Web of Science (Thomson Reuters) using the search term "76R and rmc", and also sourcing all papers that cite Barker et al. (1998), in May 2013. Once we had determined that a study grew both genotypes, we further screened papers for those that met our inclusion criteria, as follows. To warrant inclusion in the meta-analysis, all studies must: (i) have grown the genotypes separately from each other (ie. not in the same pot), and (ii) report a measure of variance (either standard error or standard deviation). We also screened publications for data on biomass and tissue nutrient concentrations, although not all studies presented data beyond that of mycorrhizal colonisation. We identified 22 papers comprising 97 trials (different treatments within a study), for inclusion in the meta-analysis (see Table 1). We extracted information on mycorrhizal colonisation, biomass, and shoot and root nutrient concentrations (where available), in both genotypes. Each response variable was reduced to a subset of data, as not all studies reported all response variables. We also harvested data on variance, sample size (n), and six moderator variables, where available (see below). When raw data were not available from the lead author or from Tables in the papers, the freeware program DataThief III (ver. 1.6) was used to extract data from Figures.

We were only able to directly retrieve measures of variance in the form of standard deviation (s.d.) from the 11 studies where raw data were available. Where only standard error (s.e.) was reported, standard deviation was calculated as follows:

Eqn. 1: $s.d. = s.e. * \sqrt{n}$

In the handful of papers where no measure of variance was reported, standard deviation was estimated as 10 % of the mean (Rose et al. 2014).

Statistics

All analyses were conducted using the "metafor" package (Viechtbauer 2010) with the R statistical program (R Development Core Team, 2005). Effect sizes were calculated as standardised mean difference (Cohen's *d*, referred to as SMD hereafter), using the "escalc" function in metafor, following Eqn. 2.

Eqn. 2: $$d = \frac{m_2 - m_1}{s_{pooled}}$$

Influential case diagnostics were investigated by constructing plots for each response variable with the "influence" function in metafor (Viechtbauer 2010). From these plots, trials that exerted considerable influence upon the fit of the model were identified and removed.

To quantify heterogeneity (inconsistency among studies), we calculated I^2 statistics for each response variable dataset (Table S1) (Higgins and Thompson 2002; Higgins et al. 2003). Low, moderate, and high heterogeneity are classed as 25, 50 and 75 %, respectively (Higgins et al. 2003). Many of the response variables had medium or high heterogeneity (>50 %, Table S1), thus, we incorporated moderator variables into the model in order to help explain some of the heterogeneity, as follows.

Moderator (explanatory) variables

(i) Trial had two levels: *glasshouse* and *field*. Separates trials where plants were grown in a climate-controlled glasshouse in pots, from those grown outdoors, with unrestricted rooting volume. This moderator variable was not tested for root biomass, as all studies reporting this response variable were glasshouse trials.

(ii) Plant age, a continuous variable: in days, at time of harvest.

(iii) Soil P had two levels: *deficient* or *non-deficient*. We chose to include measures of soil P from only those studies that had quantified soil P by the most commonly used method in the studies included in our analysis (Colwell plant available P), for consistency. Deficient soil P is defined as less than 10 mg P kg soil^{-1}, while non-deficient soil P is defined as anything above 10 mg P kg soil^{-1} (based on Peverill et al. 1999).

153 *(iv) Soil Zn* had three levels: *deficient*, *non-deficient*, *high*. We used measures of soil DTPA-extractable
154 Zn from studies reported in the studies included in this analysis. Plant Zn stress can occur as a result of
155 either there being too little Zn (ie. deficient) or too much Zn (ie. toxic) in the soil, so there were three
156 levels for this moderator variable. Deficient soil Zn was classified as < 0.5 mg Zn kg soil^{-1}, non-
157 deficient soil Zn was classified as 0.6 – 10 mg Zn kg soil^{-1}, and high soil Zn was classified as > 10 mg
158 Zn kg soil^{-1} (based on Reuter and Robinson 1997; Watts-Williams and Cavagnaro 2012).
159 *(v) Soil pH* had three levels: *acidic*, *neutral* and *alkaline.* Categories followed the USDA Natural
160 Resources Conservation Service's Soil Survey Manual's (http://www.nrcs.usda.gov) criteria for pH as
161 follows; acidic < 6.5, neutral = 6.6 – 7.3, alkaline > 7.4.
162 *(vi) Inoculation* had two levels: *un-inoculated*, where the soil comprised native AMF communities
163 only, and *inoculated*, where soil had been sterilised, and then provided with inoculum of a known AMF
164 species (for both genotypes), in order to specifically study that species of AMF. This variable was only
165 tested for colonisation and biomass analyses, as all studies that reported tissue nutrient concentrations
166 were un-inoculated trials.
167 *(vii) Colonisation phenotype*, with three levels: *pen*$^{-}$, *coi* and *myc*$^{+}$(based on Gao et al. 2001), was
168 applied to a subset of *mycorrhizal colonisation* data comprising plants that were *inoculated*, and a
169 separate analysis was conducted on this data set. Most species of AMF studied display the *pen*$^{-}$
170 phenotype (i.e. all colonisation of the roots is restricted) with *rmc*. However, a few AMF species
171 display the *coi* phenotype, which indicate that they can penetrate the root epidermis, but cannot
172 colonise the root cortex (Gao et al. 2001; Manjarrez et al. 2008). One species of AMF (*Glomus*
173 *intraradices* WFVAM23) displays the *myc*$^{+}$ phenotype with roots of *rmc*; that is, complete and
174 functional, yet relatively slow, internal colonisation of roots (Gao et al. 2001; Manjarrez et al. 2008;
175 Poulsen et al. 2005).
176
177 Publication bias was investigated by constructing and viewing funnel plots for each response variable
178 (Egger et al. 1997). Fourteen response variable datasets demonstrated significant (P <0.05) funnel plot
179 asymmetry (Table S1). However, interpretation of funnel plot asymmetry should be approached with
180 caution, as it is largely dependent on the method used to construct the plot (Tang and Liu 2000). In
181 addition, plot asymmetry is not a reliable indicator of publication bias, and could instead be due to
182 chance, data irregularities, or true heterogeneity (Nakagawa and Santos 2012). Heterogeneity can be

partially accounted for by including moderator variables in the model, as we have done in this meta-analysis. Regardless, the trim and fill method was applied to the datasets with significant funnel plot asymmetry (see Table S1 for results).

We conducted a separate mixed-effects multivariate model for each response variable, respectively. Majority of the studies included in the analyses contained multiple trials, which violates the assumption of the independence of studies. However, none of the treatments from individual trials shared a control, which somewhat deals with the violation. In addition to this, "Study" was included as a random factor in every model, which meant all trials within the same study (publication) were allocated the same random effect, while different studies were still considered independent, and allocated different random effects (Thompson and Higgins 2002). Initially, we ran a model for each response variable without the inclusion of moderator variables, before a full model containing all relevant moderator variables, and "Study" as a random effect, was run for each response variable separately. From the output of this full model, moderators with a significant *p*-value ($P < 0.05$) were identified. Two reduced models for the soil P and soil Zn moderator variables were then run, to identify any significant differences in response variable estimated SMD in different soil P (deficient and non-deficient) and soil Zn categories (deficient, non-deficient and high).

Results

Mycorrhizal colonisation

Overall, mycorrhizal colonisation in the 76R genotype was significantly higher than in the *rmc* genotype ($I^2 = 86.22$, $n = 83$, $P < 0.0001$, Figure 1). The mean values corresponding to this result were 5.6 and 39.2 % root length colonised in *rmc* and 76R, respectively.

When we considered just the studies that had inoculated the soil with a specific AMF species, colonisation phenotype and plant age had a significant effect on mycorrhizal colonisation SMD. At each of the three levels of colonisation phenotype (pen^-, *coi* and myc^+), colonisation was significantly higher in 76R than *rmc* ($P < 0.0001$ for all colonisation phenotypes). Specifically, mean values for mycorrhizal colonisation for the *rmc* and 76R genotypes in the pen^- category were; 2.0 and 28.5 % ($P =$

212 0.0001), for the *coi* category; 8.0 and 41.6 % (P <0.0001), and for the *myc*$^+$ category; 30.2 and 72.0 %
213 (P <0.0001) root length colonised, respectively.
214
215 *Biomass*
216 Root dry weight (RDW, I^2 = 55.19) was not, while shoot dry weight (SDW, I^2 = 4.89) was (n = 44, P =
217 0.0298, Figure 1), overall significantly different between genotypes, with 76R plants' SDW
218 significantly larger than *rmc*.
219
220 *Plant nutrition*
221 Phosphorus: Shoot P concentration (I^2 = 84.96) was significantly higher in the 76R genotype than the
222 *rmc* genotype, overall (n = 41, P = 0.0019). Unsurprisingly, soil P had a significant influence upon both
223 root and shoot P concentration SMD (Table S1). Soil pH also had a significant influence on shoot P
224 SMD. Shoot P was significantly higher in the 76R genotype at both deficient (n = 7, P <0.0001, Figure
225 2) and non-deficient (n = 31, P = 0.02) soil P. Root P (I^2 = 89.11) was significantly higher in the 76R
226 genotype only at deficient soil P (n = 6, P<0.0001). Shoot P was significantly higher in the 76R
227 genotype at deficient (n = 9, P = 0.0191) and non-deficient soil Zn (n = 11, P = 0.001), but not high
228 soil Zn.
229
230 Zinc: There were significant effects of soil Zn upon shoot Zn concentration SMD, but no significant
231 effects of moderators on root Zn SMD (Table S1). Root Zn concentration (I^2 = 59.03) was significantly
232 higher in the *rmc* genotype at high soil Zn (n = 19, P = 0.041, Figure 3).
233
234 Calcium: There was a significant effect of soil P on shoot Ca and root Ca concentration SMD (Table
235 S1). Specifically, at non-deficient soil P, shoot Ca (I^2 = 59.03, n = 22, P = 0.0161, Figure 2) and root
236 Ca (I^2 = 59.03, n = 23, P = 0.0223) concentrations were higher in *rmc* than 76R.
237
238 Copper: Shoot Cu concentration (I^2 = 60.91) was significantly higher overall in 76R than *rmc* (n = 26,
239 P = 0.0107). Shoot Cu concentration SMD was significantly influenced by pH, while root Cu SMD
240 was significantly affected by soil P and soil Zn (Table S1). Shoot Cu concentration was significantly
241 higher in the 76R genotype at both deficient (n = 8, P = 0.0147) and non-deficient (n = 19, P = 0.0114)

soil P, while root Cu concentration (I^2 = 86.59) was significantly higher in the 76R genotype at deficient soil P only (n = 7, P = 0.0013). Similarly, shoot Cu concentration was significantly higher in the 76R genotype at deficient (n = 6, P = 0.0155, Figure 3) and high (n = 21, P = 0.0114) soil Zn, and root Cu only at deficient soil Zn (n = 7, P = 0.011).

Potassium: Shoot K concentration (I^2 = 27.17) was significantly higher in the *rmc* genotype at deficient soil Zn (n = 6, P = 0.0479), while root K concentration was significantly higher in the 76R genotype at high soil Zn (I^2 = 59.13, n = 18, P = 0.033). Soil P had a significant influence upon shoot K SMD (Table S1).

Magnesium: Soil P had a significant influence on shoot Mg concentration SMD (Table S1), and shoot Mg (I^2 = 66.59) was significantly higher in the *rmc* genotype at deficient soil P only (n = 7, P = 0.0074, Figure 2).

Manganese: The *rmc* genotype had significantly higher shoot Mn concentration (I^2 = 45.62) than the 76R genotype, overall (n = 29, P = 0.0126). There was a significant effect of soil P and soil Zn upon root Mn concentration SMD (Table S1). At deficient soil P, root Mn (I^2 = 69.91) was significantly higher in the 76R genotype than *rmc* (n = 7, P <0.0001). Conversely, at non-deficient soil P, shoot Mn was significantly higher in the *rmc* genotype than 76R (n = 22, P = 0.0045). When soil Zn was considered, shoot Mn was significantly higher in the *rmc* genotype at deficient soil Zn (n = 7, P = 0.0387, Figure 3).

Boron: Soil Zn had a significant impact upon root B concentration SMD (Table S1). At deficient soil Zn, root B concentration (I^2 = 44.7) was significantly higher in the *rmc* genotype than the 76R genotype (n = 3, P <0.0001).

Iron: Soil P significantly affected root Fe concentration SMD (Table S1), and at deficient soil P, root Fe concentration (I^2 = 26.6) was significantly higher in 76R plants, than *rmc* (n = 7, P = 0.0233, Figure 2).

Sodium: Root Na concentration was significantly higher in the *rmc* genotype than 76R, in general (n = 24, P <0.0001). None of the moderators included in this analysis had significant influence on the root Na concentration SMD. Root Na (I^2 = 0) was significantly higher in the *rmc* genotype at both deficient (n = 6, P = 0.0008) and non-deficient (n = 18, P <0.0001) soil P. Root Na was also significantly higher in *rmc* at deficient (n = 6, P <0.0001, Figure 3) and high (n = 17, P <0.0001) soil Zn.

Sulphur: Shoot S concentration (I^2 = 79.65) was overall significantly higher in the 76R genotype (n = 34, P = 0.0276). Soil P had significant influence on both root and shoot S concentration SMD (Table S1), and at deficient soil P, both root S (I^2 = 62.24, n = 6, P = 0.0208, Figure 2) and shoot S (n = 8, P = 0.0015) concentrations were higher in the 76R genotype than the *rmc*.

Discussion

General patterns

The results of the meta-analysis confirmed that colonisation of the reduced-mycorrhizal genotype *rmc* was significantly lower than that of the mycorrhizal 76R genotype, across many studies. Specifically, 76R was colonised by AMF to a greater extent than *rmc*, both overall and within all of the levels of the moderator variables. Furthermore, colonisation phenotype significantly affected mycorrhizal colonisation SMD (in inoculated plants only), which can be attributed to the differing levels of internal colonisation found in *rmc* plants, depending on colonisation phenotype (discussed above).

Growth of the two genotypes did not differ dramatically, although shoot biomass of the mycorrhizal 76R genotype was overall significantly larger than that of the non-mycorrhizal genotype. In other tomato genotypes, positive mycorrhizal growth responses have been reported (Subramanian et al. 2006; Al-Karaki et al. 2001; Plenchette et al. 1983). There were insufficient data to compare the genotypes in terms of harvestable yields (see Cavagnaro et al. 2012; Cavagnaro et al. 2006, for available data), and future investigation into fruit yield in these genotypes will be of interest. However, studies using other genotypes of tomato have demonstrated a significant positive effect of AM upon fresh fruit yield (Al-Karaki and Hammad 2001; Abdel Latef and Chaoxing 2011; Al-Karaki 2006; Subramanian et al. 2006).

Across all studies, concentrations of P, S, and Cu were significantly higher in the mycorrhizal genotype than the non-mycorrhizal genotype. For P and Cu, this pattern been demonstrated in other genotypes of tomato (Al-Karaki and Hammad 2001; Abdel Latef and Chaoxing 2011; Al-Karaki 2006; Bryla and Koide 1998; Subramanian et al. 2006), and other plant species (Rhodes and Gerdemann 1978a; Li et al. 1991). However, the reverse was true for root Na and shoot Mn concentrations, which were significantly higher in the non-mycorrhizal genotype. While the higher concentrations of nutrients in the mycorrhizal genotype are not unusual, the elevated concentration of Na in the roots of the non-mycorrhizal genotype do not have a clear explanation, but may relate to the salinity status of the soils used in the included studies (Juniper and Abbott 1993; Giri and Mukerji 2004). Elevated concentrations of Mn in non-mycorrhizal plants compared to mycorrhizal have, however, been observed before, and may simply be due to reduced Mn uptake by AM (Marschner 2012). Lower Mn concentrations in AM plant tissue may also be due to an increase in Mn-oxidising bacteria, or a decrease in Mn-reducing bacteria and exchangeable Mn (Mn^{2+}) found in the rhizosphere of mycorrhizal plants (Arines et al. 1989; Kothari et al. 1991b). There were no other significant differences between the genotypes observed where the moderator variables were not included in the model.

Influence of soil P on AM and tissue nutrient interactions

In the meta-analysis, soil P category (deficient or non-deficient) had a significant influence on tissue concentration SMD of all of the nutrients (except Zn, Na and B), in shoots and/or roots. The greatest (often significant) differences between the 76R and *rmc* genotypes were found when soil P was deficient. For example, tissue P, Cu, Mn, Fe and S concentrations were significantly higher in the 76R genotype at deficient soil P. In contrast, the *rmc* genotype had significantly higher concentrations of Mg (shoots) and Na (roots), where soil P was deficient. It is widely accepted that AM are particularly beneficial in terms of P uptake when P is low, or unavailable in the soil (Smith and Read 2008), and this benefit at low P appears to extend to other macro-nutrients, as well as some micro-nutrients. However, at higher soil P concentrations, mycorrhizal colonisation is often lower, so the potential for AM to take up these other nutrients may be reduced. Due to a limited amount of information on the availability of soil nutrients aside from P and Zn in the studies included in the meta-analysis, we could not explore the efficiency of AM to take up other nutrients when they were deficient in the soil.

Shoot P concentration was higher in the 76R genotype, where soil P was not deficient. This supports the hypothesis that AM plants continue to accumulate 'luxury' P when it is not limiting in the soil (Smith and Read 2008). Interestingly, shoot and root Ca, shoot Mn, and root Na concentrations were significantly higher in the *rmc* genotype, where soil P was not deficient. There is no clear explanation for these results, but they may relate to differences between genotypes in root/shoot partitioning of nutrients, discussed further below.

Influence of soil Zn on AM and tissue nutrient interactions

Soil Zn category had a significant impact upon the SMD of shoot or root concentrations of Zn, Cu, Mn and B. When explored further, we found that root Zn concentrations were significantly higher in the *rmc* genotype, at high soil Zn only. This result may be indicative of the "protective effect" of AM, whereby mycorrhizal plants take up less Zn than non-mycorrhizal plants on a Zn-contaminated soil (Chen et al. 2003; Watts-Williams et al. 2013; Christie et al. 2004). The mechanisms that underpin this "protective effect" of AM remain unknown, but the 76R and *rmc* system may provide a good system for further investigation into them (Watts-Williams et al. 2013).

The enhanced uptake of Cu by AM occurred at both deficient and high soil Zn. Similar to Zn, uptake of soil Cu is generally enhanced by AM (Gildon and Tinker 1983a; Lambert et al. 1979), and has been demonstrated in studies that have used other tomato genotypes (Al-Karaki 2006; Al-Karaki and Hammad 2001), and other plant species (Liu et al. 2000; Li et al. 1991; Lambert and Weidensaul 1991). The results of the meta-analysis suggest that soil Zn stress (be it deficiency or toxicity) had no effect on the ability of AM to enhance Cu uptake. However, if the soil had been contaminated with Cu instead of, or in conjunction with Zn, we may have seen evidence of a "protective effect" for Cu uptake (Hildebrandt et al. 2007; Meier et al. 2011; Gildon and Tinker 1983a, b); this however, is speculative, and warrants further investigation.

In the deficient soil Zn category, root B concentration was extremely high in the *rmc* genotype compared to the 76R genotype. However, at toxic Zn, root B was not different between the genotypes. Previously, interactions between Zn and B on plant growth and nutrition have been investigated, and B

accumulation in plant tissue has been shown to be enhanced by soil Zn deficiency in many crop species, including maize, barley and oilseed rape (Graham et al. 1987; Grewal et al. 1998; Hosseini et al. 2007). Also, it appears from this meta-analysis that the 76R genotype can, to some extent, resist B-accumulation when Zn is deficient, compared to the *rmc* genotype. The ability of AM to reduce B-toxicity in wheat has been observed previously (Sonmez et al. 2009); however, the effect of AM on the Zn-B interaction has not, to our knowledge, been investigated.

Patterns of nutrient allocation above- and below-ground

For some nutrients, the difference in concentration between the two genotypes displayed very different patterns above- and below-ground. For example, Mn concentration in the shoots was generally higher in the *rmc* genotype than the 76R genotype; however, the opposite was seen in the roots. Similarly, shoot Na was generally the same between the two genotypes, while root Na was significantly higher in the *rmc* genotype. These particular results may be influenced more by differences in resource allocation of nutrients in mycorrhizal and non-mycorrhizal plants, rather than differences in uptake between the two. That is, the two genotypes may have a similar overall concentration of Mn, but the 76R plants allocated more Mn to the roots than the *rmc* plants, or the Mn may be bound in fungal structures. Such differences in allocation of nutrients between genotypes has been demonstrated previously for Zn (Watts-Williams et al. 2013), and other plant resources (Miller et al. 2014). This highlights the need to consider whole plant responses and patterns of nutrient allocation in studies of plant nutrition.

Conclusions

The intention of this meta-analysis was to synthesise data arising from studies using the *rmc* and 76R tomato genotypes. The results confirm that the *rmc* genotype can be used as an effective non-mycorrhizal control. Also, that plant biomass is essentially matched between the two genotypes, under a wide range of conditions. In this meta-analysis, emphasis was placed on interactions between soil nutrients, plant tissue nutrients, and the formation of AM. The results suggest that AM and the soil nutrients examined here (P and Zn), influence plant nutrition beyond commonly reported response variables (plant tissue P and Zn concentrations), and should be considered in the future. Taken together, the results of this meta-analysis indicate that changes in soil P and Zn concentration not only

affect uptake of these nutrients, but other nutrients too. Most often, it is when soil P and Zn are deficient, that mycorrhizal plants have an advantage over non-mycorrhizal plants, not just in terms of improved growth or P and Zn nutrition, but also in the uptake of a range of other nutrients.

While some studies using the *rmc* and 76R genotypes have focused on N, most focused on P and Zn. With increasing recognition of the importance of AM in the uptake of N (Veresoglou et al. 2012), this is an important area to continue research in. In particular, studies that use a mycorrhizal and non-mycorrhizal genotype to study N uptake, and interactions between N and other nutrients, will be of particular interest. It has been reported that the formation of AM can reduce N loss via leaching (Asghari and Cavagnaro 2011, 2012), and further studies of this nature will be useful. Thus far, much of the work on N has been done using leguminous mycorrhizal mutant plant species, and it will be important to follow up this work using a non-legume mycorrhiza-defective mutant.

Further research that directly compares plant nutrient uptake via the direct and mycorrhizal pathways could utilise mycorrhiza-defective mutant and wild-type pairs (as in Poulsen et al. 2005). Particularly, in conjunction with the use of stable or radioactive isotopes of the mineral element of interest (Merrild et al. 2013). For example, direct evidence of delivery of P, Zn, N, Ca, and S to plants by arbuscular mycorrhizal fungi (AMF) has been demonstrated using isotope tracer techniques (Rhodes and Gerdemann 1978a, 1975, 1978b; Smith et al. 2003; Burkert and Robson 1994; Cooper and Tinker 1978; Jansa et al. 2003; Johansen et al. 1993). However, many of the above studies (except for P) did not explicitly quantify the amount of the nutrient that was delivered to the plant by AM (Marschner and Dell 1994).

Taken together, this meta-analysis highlights the usefulness of mycorrhiza-defective mutant and wild-type pairs in the study of plant nutrition and nutrient interactions. It also begins to explore interactions between nutrients that have thus far received little attention. Based on the findings of this meta-analysis, there is evidence that AM affect these interactions. It is hoped that this analysis will stimulate more work in this area.

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Table S1. I^2 statistic, Egger's regression test for funnel plot asymmetry (p <0.05 indicates asymmetry), p-value for estimated SMD before and after trim and fill method (p <0.05 indicates significant estimated SMD), for each response variable. Bold values are significant p-values from Egger's regression test, and p-values that changed to non-significant following the trim and fill method (see in text for details and interpretation).

Response variable	I^2 (%)	Egger's regression p-value	p-value before trim and fill	p-value after trim and fill	Influence of soil P moderator variable	Influence of soil Zn moderator variable
Myc. colonisation	86.22	**<0.0001**	<0.0001	<0.0001	ns	ns
Shoot dry weight	4.89	ns			ns	ns
Root dry weight	55.19	ns			ns	ns
Shoot P	84.96	**<0.0001**	<0.0001	0.0016	<.0001	ns
Root P	89.11	**0.0004**	0.0001	**0.071**	<.0001	ns
Shoot Zn	72.18	ns			ns	0.007
Root Zn	59.03	**0.033**	ns	ns	ns	ns
Shoot Cu	60.91	**<0.0001**	<0.0001	<0.0001	ns	ns
Root Cu	86.59	**0.0006**	0.006	**0.1436**	<.0001	0.0002
Shoot Mn	45.62	**<0.0001**	<0.0001	0.0035	ns	ns
Root Mn	69.91	ns			<.0001	0.0003
Shoot Mg	66.59	ns			0.0097	ns
Root Mg	72.03	**<0.0001**	0.0003	0.0003	ns	ns
Shoot Fe	16.32	ns			ns	ns
Root Fe	26.6	**0.027**	ns	ns	0.01	ns
Shoot Ca	0	ns			0.019	ns
Root Ca	43.41	**0.001**	0.04	**0.8825**	0.004	ns
Shoot Na	47.38	**0.0069**	0.02	0.04	ns	ns
Root Na	0	**<0.0001**	<0.0001	<0.0001	ns	ns
Shoot B	33.3	ns			ns	ns
Root B	44.7	ns			ns	<.0001
Shoot S	79.65	ns			<.0001	ns
Root S	62.24	**0.035**	ns	ns	<.0001	ns
Shoot K	27.17	**0.0002**	0.01	**0.4584**	0.048	ns
Root K	59.13	ns			ns	ns

Table 1. List of papers used in the meta-analysis, number of trials within each study, and response variables extracted.

	Trials within study	Mycorrhizal colonisation	Shoot biomass	Root biomass	Shoot P conc.	Root P conc.	Shoot Zn conc.	Root Zn conc.	Soil P conc.	Soil Zn conc.	ICP-AES data
Barker et al. (1998)	2	*									
Cavagnaro et al. (2001)	6	*									
Gao et al. (2001)	5	*							*		
Cavagnaro et al. (2004)	1	*							*		
Marschner and Timonen (2005)	4	*	*	*					*		
Poulsen et al. (2005)	6	*							*		
Cavagnaro et al. (2006)	4	*	*		*		*		*	*	*
Cavagnaro et al. (2007)	1	*	*	*	*	*	*	*	*	*	
Cavagnaro et al. (2008)	2	*	*	*	*		*		*	*	
Manjarrez et al. (2008)	19	*							*		
Manjarrez et al. (2009)	3	*									
Hallett et al. (2009)	1	*	*								
Cavagnaro et al. (2010)	6	*	*	*	*	*	*	*	*	*	
Manjarrez et al. (2010)	2	*	*	*	*				*		
Cavagnaro and Martin (2011)	4	*	*	*	*	*	*	*	*	*	*
Schwarz et al. (2011)	1	*	*								
Cavagnaro et al. (2012)	2	*	*		*		*		*	*	*
Asghari and Cavagnaro (2012)	1	*	*	*							
Watts-Williams and Cavagnaro (2012)	10	*	*	*	*	*	*	*	*	*	*
Ruzicka et al. (2012)	1	*									
Watts-Williams et al. (2013)	10	*	*	*	*	*	*	*	*	*	*
Watts-Williams et al. (2014)	6	*	*	*	*	*	*	*	*	*	*

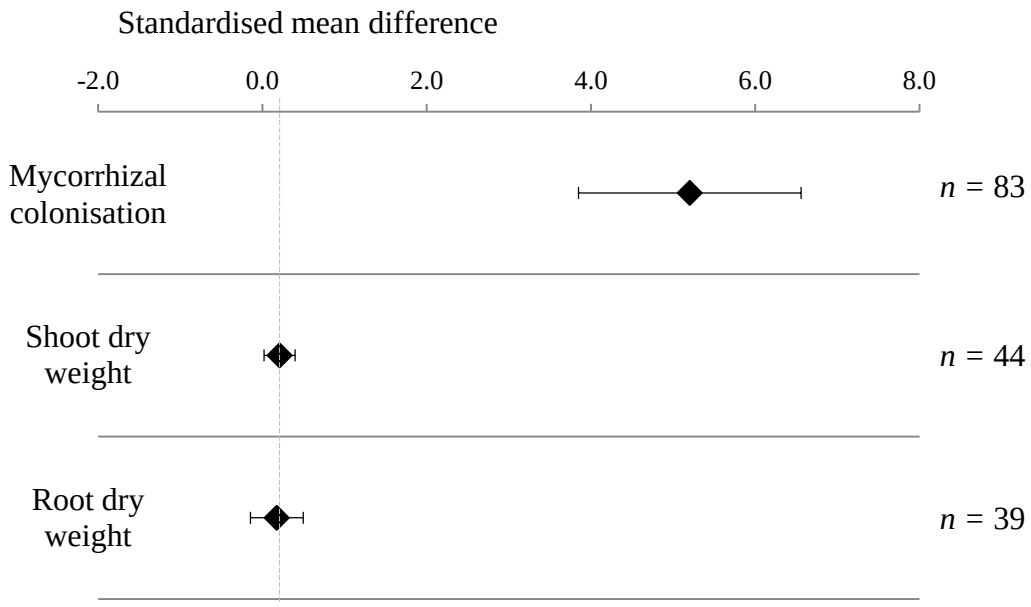

Figure 1. Forest plot of mean ± 95% CI SMD values for overall mycorrhizal colonisation, SDW and RDW. SMD values >0 indicate 76R genotype was significantly higher than *rmc*, while SMD values <0 indicate *rmc* genotype was significantly higher than 76R. Error bars overlapping 0 indicate the two genotypes were not significantly different. The number of trials included for each point is given.

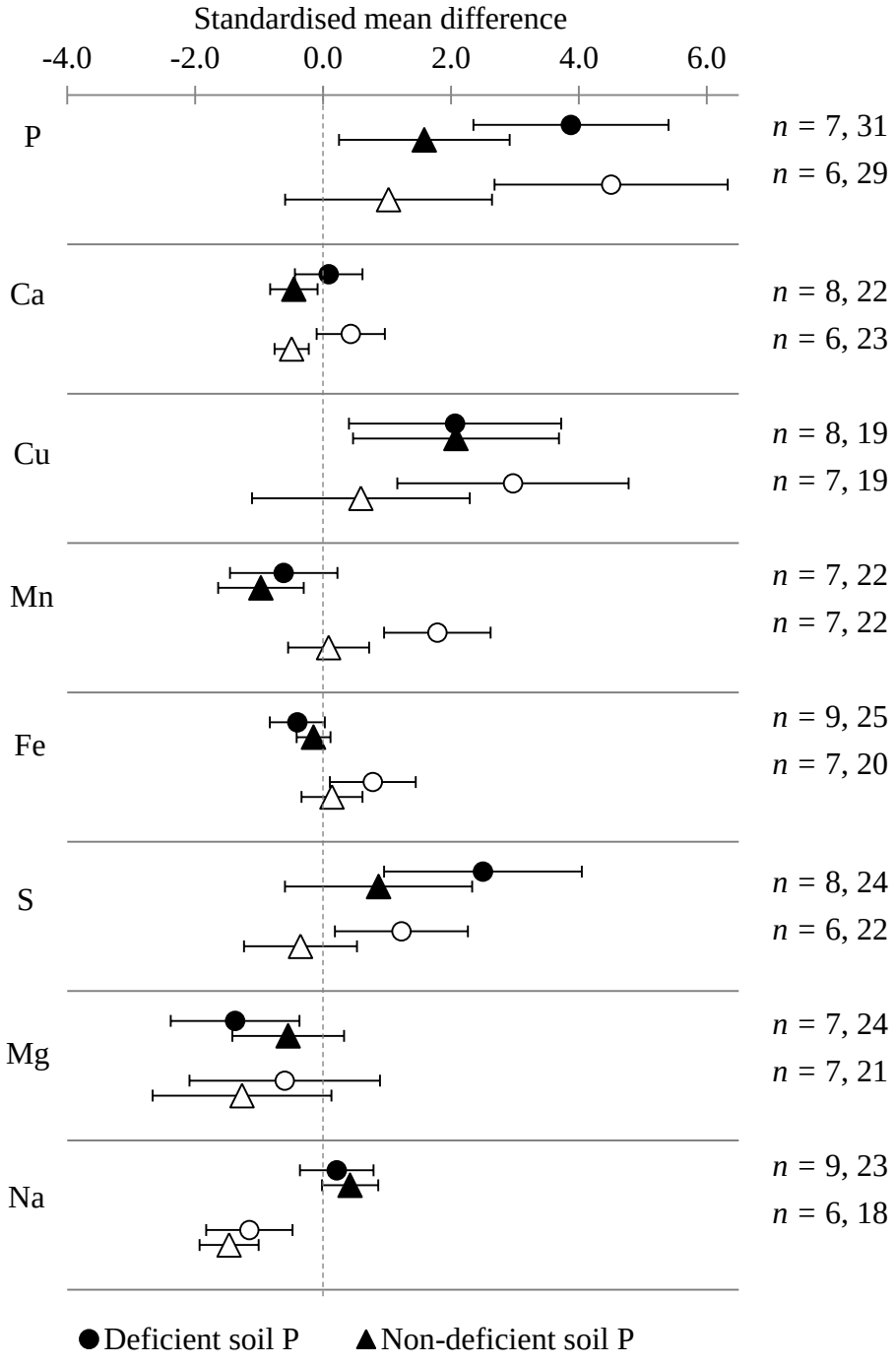

Figure 2. Forest plot of mean ± 95% CI SMD values for various tissue nutrient concentrations (mg kg^{-1} dry weight) in the shoots (black symbols) and roots (white symbols), at deficient soil P (circles) and non-deficient soil P (triangles). SMD values >0 indicate 76R genotype was significantly higher than *rmc*, while SMD values <0 indicate *rmc* genotype was significantly higher than 76R. Error bars overlapping 0 indicate the two genotypes were not significantly different. Error bars overlapping within the same nutrient and tissue type (root or shoot) indicate that SMD was not significantly different between deficient and non-deficient soil P. The number of trials included is given by *n*, where the first and second numbers refer to Deficient soil P and Non-deficient soil P categories, respectively.

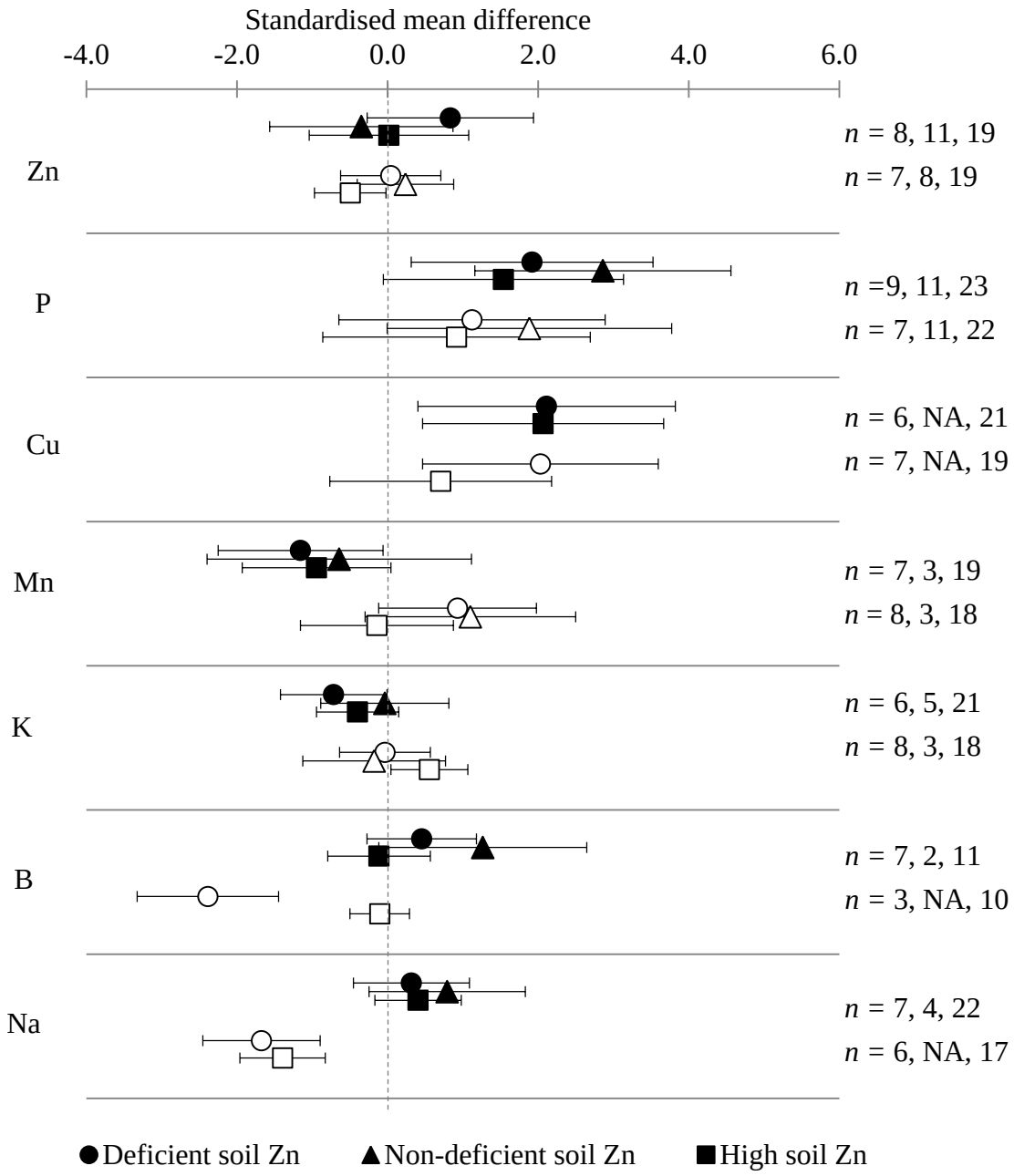

Figure 3. Forest plot of mean ± 95% CI SMD values for various tissue nutrient concentrations in the shoots (black symbols) and roots (white symbols), at deficient soil Zn (circles), non-deficient soil Zn (triangles) and high soil Zn (squares). SMD values >0 indicate 76R genotype was significantly higher than *rmc*, while SMD values <0 indicate *rmc* genotype was significantly higher than 76R. Error bars overlapping 0 indicate the two genotypes were not significantly different. Error bars overlapping within the same nutrient and tissue type (root or shoot) indicate that SMD was not significantly different between soil Zn categories. The number of trials included for each point is given by *n*, where the first, second, and third numbers refer to Deficient soil Zn, Non-deficient soil Zn, and High soil Zn categories, respectively.

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