**Title: Utilization of inoculum of AM fungi produced on-farm increases the yield of
Solanum lycopersicum: a summary of 7 years of field trials on a conventional vegetable
farm with high soil phosphorus**

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Abstract

Adding arbuscular mycorrhizal [AM] fungus inoculum to potting media enables vegetable farmers to better take advantage of the AM symbiosis. On-farm production of AM fungus inoculum is a viable alternative to commercially-available inocula. We conducted a seven year experiment at a conventional vegetable farm in southeastern Pennsylvania with high soil available P typical of the Mid-Atlantic region of the USA (210 mg kg^{-1} soil). Seedlings of three to seven cultivars of *Solanum lycopersicum* L were inoculated in the greenhouse phase of production with a mixed species inoculum of AM fungi produced on-farm. Performance of the inoculation treatment was evaluated based on growth response in the greenhouse and fruit production in the field compared to uninoculated controls. Colonization levels were typically only 1-10% of root length at the time of outplanting. The mean mycorrhizal growth dependency based upon shoot growth at the time of outplanting was significant at $12.5 \pm 3.55\%$ ($\alpha = 0.05$). In addition, the mean mycorrhizal yield response over the seven year experiment was significant at $6.02 \pm 1.92\%$ ($\alpha = 0.05$). Routine use of AM fungus inoculum produced on-farm provided a modest but significant increase in yield of tomato fruit with minimal change in farm management even in a high P soil.

Keywords: sustainable agriculture, tomato, biofertilizer, mycorrhizal yield response

1. Introduction

Arbuscular mycorrhizal [AM] fungi are naturally-occurring soil fungi that form a mutualistic symbiosis with the majority of crop plants. AM fungi are obligate symbionts that benefit through the receipt of fixed carbon from their host plants. The benefits to the plant ascribed to the symbiosis are enhanced: uptake of immobile soil nutrients, disease resistance, and water relations (Smith and Read, 2008). In addition, AM fungi are believed to play an important role in the stabilization of soil aggregates (Miller and Jastrow, 2000; Wright and Anderson, 2000). These benefits make increased utilization of the AM symbiosis particularly attractive to organic farmers, and the role of AM fungi in the general sustainability of agriculture is widely promoted (eg. Rouphael et al., 2015).

The primary benefit plants receive from colonization of their roots by AM fungi is enhanced uptake of immobile soil nutrients, notably P. The zone of P depletion around a nonmycorrhizal root extends to approximately the length of the root hairs, 1-2 mm. The extraradical hyphae of the fungi extend 8 cm or more away from the root's surface, exploring a much larger volume of soil for P, and transporting it back to the root cortex (Rhodes and Gerdemann, 1975). Even though most agricultural soils of the Mid-Atlantic region of the US are very high in soil test P due to decades of fertilizer P and manure addition and subsequent fixation in soils (Russell, 1973; Fixen, 2006), the median available P (Bray P-1 extraction) in North America is $31 \mu\text{g kg}^{-1}$, with 42% of soils testing below $25 \mu\text{g kg}^{-1}$, a moderately critical level (Fixen, 2006). Availability of P to plants is low in the cold soils of spring when crops are planted, even where soil test P levels are high (Björkman and Reiners, 2014), indicating the necessity of application of starter P fertilizers (Sheppard and Racz, 1985). Though inexpensive sources of P fertilizer may be exhausted by 2050 (Vance et al., 2003), calculations of future P

fertilizer needs should take into account crop use of residual soil P (Sattari et al., 2012).

Availability of P fertilizers is expected to be a particular problem in developing countries and tropical/subtropical areas, such that ‘sustainable management of P in agriculture requires that plant biologists discover mechanisms that enhance P acquisition’ by plants (Vance et al., 2003). Though the role of AM fungi in P uptake has been known for decades, intentional use of the AM symbiosis by farmers has not met expectations (Fester and Sawers, 2011).

Farmers have two options for enhancing their utilization of the AM symbiosis (Bagayaraj, 1992). Adoption of farm management practices that enhance the functioning of the AM fungus community indigenous to the soil is the primary option available to row crop farmers (Koltai et al., 2010; Smith and Smith, 2011; Ortas, 2012). Vegetable farmers and other growers who produce seedlings for later outplanting to the field can inoculate plants with effective isolates of AM fungi (Ortas, 2012). Inocula mixed into horticultural potting media produce colonized seedlings ready to take advantage of the symbiosis immediately upon outplanting, rather than experience a lag before colonization by the AM fungi indigenous to the field (Waterer and Coltman, 1989; Koide et al., 1999; Koltai et al., 2010).

Inocula containing one or more AM fungi are available commercially and are produced in a variety of ways (Ijdo et al., 2011; Faye et al., 2013). Commercially-available inocula all require processing for the isolation of AM fungus propagules from the growth medium, formulation, and packaging for transport and sale. These add to the cost of the inoculum, plus the producer loses control over the storage conditions, a major determinant of future viability, once the product leaves the facility (Santana et al., 2014). Though commercially-available inocula have improved over the years so that they now are typically mixtures of AM fungus species to take advantage of functional diversity, comparisons of these inocula to those produced in-house by researchers

consistently have found many to be ineffective (Corkidi et al., 2004; Tarbell and Koske, 2007; Wiseman et al., 2009; Yidliz et al., 2010). Recent research also indicates that inocula produced in vitro with root organ cultures, one method whereby commercial inocula are produced, are not as effective in colonizing plants or stimulating plant growth as inocula produced in pot culture with whole plants (Calvet et al., 2013).

These and other reasons precipitated the development of methods for the on-farm production of inoculum of AM fungi. Techniques were first developed for the tropics (Sieverding, 1991; Gaur, 1997; Gaur et al., 2000; Maiti et al., 2009; Schlemper and Stürmer, 2014), utilize a variety of substrates ranging from beds of field soil to agrowastes, and produce indigenous or introduced isolates of AM fungi. Another method for the on-farm production of AM fungus inoculum suitable for temperate climates has produced an average of 300 propagules cm^{-3} over 40 site years (Douds et al., 2014). On-farm production of inoculum of indigenous AM fungi was encouraged as an alternative to the non-native isolates found in commercially available inocula (Pelligrino et al., 2011).

Tomato (*Solanum lycopersicum* L.) production worldwide is second among vegetables to potato at 163.4×10^6 tonnes annually (FAOSTAT, 2015). US production ranks third at 12.5×10^6 tonnes. China is the leading producer at 50×10^6 tonnes. Tomatoes are a good source of vitamins A and C, carotenoids, phenolics, and α -tocopherol (Beecher, 1998; Abushita et al., 1997). Colonization of roots by AM fungi increased the levels of vitamin C (Ordookhani et al., 2013; Nedorost and Pokluda, 2012), lycopene, and anti-oestrogenic activity of fruits (Giovannetti et al., 2012) vs. uninoculated controls.

Here we present results of seven years of field studies conducted with the objective to transfer the technology for the on-farm production and utilization of AM fungus inoculum to a

conventional vegetable farm. A mixed species inoculum of AM fungi was grown on-farm each year in compost and vermiculite mixtures and utilized to inoculate three to seven cultivars of *S. lycopersicum* seedlings during the greenhouse phase of their production. Field performance of the inoculation treatment was monitored with harvests throughout the production period according to the routine practice of the farm.

2. Materials and methods

This long term study was conducted at the same time (2005-2011) and place (Eagle Point Farm) as a previously-published work on *Capsicum annuum* (Douds et al., 2012b). Therefore, many of the details, procedures, and presentations of the data reported here will be similar to those used in the previous report.

2.1 The Site

Eagle Point Farm is a conventional vegetable farm of 6.1 ha located in Kutztown PA. The soil type is a Berks silt loam (loamy-skeletal, mixed, active, mesic typic dystrochrepts) and typical of the middle Atlantic region of the US in that the level of available P in the soil tests as high (Table 1) (Sims et al., 2002). A variety of vegetables are grown for sale at the local produce auction and at an owner-operated farm market. Crops are grown in rotation. Tomatoes typically followed Cucurbitaceae crops. Two plantings of tomato occur each year, the second planting was studied in 2008-2011 only.

Table 1. Soil analyses of field plots utilized at the Eagle Point Farm for *Solanum lycopersicum* production.^a

Characteristic	YEAR		
	2005	2006	2010
pH	6.0	6.4	5.3
Organic matter (%)	4.1	3.2	ND ^b
Available P (mg kg ⁻¹)	257	114	258
Available K (mg kg ⁻¹)	69	137	176

^aAnalyses were conducted by the Pennsylvania State University Agricultural Analytical Services Laboratory, University Park, PA 16802. Avg. of 2 pooled samples in 2005 and 2010, one in 2006.

^bNot determined.

2.2 Production of AM fungus inoculum on-farm

Inoculum of AM fungi was produced on-farm as described earlier (Douds et al., 2006). Briefly, leaf and yard clippings compost was obtained from the Lehigh Valley Municipal Composting Facility (Allentown, PA), and mixed 1:4 [v/v] with medium texture vermiculite to fill 28 L black plastic bags (7 gallon “Grow Bags” (Worm’s Way, Bloomington, IN 47404)) approximately two thirds full. Three-month-old bahiagrass (*Paspalum notatum* Flugge) seedlings, precolonized with AM fungi (see below), were transplanted into the compost and

vermiculite mixture, five seedlings per bag, one AM fungus isolate per bag. The seedlings were inoculated with one of the following AM fungi originally isolated from the Rodale Institute Experimental Farm, Kutztown, PA: *Funneliformis mosseae* (Nicol. & Gerd) Gerdemann & Trappe, *Claroideoglossum claroideum* Schenck & Smith, *Funneliformis geosporum* (Nicol. Gerd.) Walker, and *Claroideoglossum etunicatum* Becker & Gerdemann (Franke-Snyder et al., 2001) in addition to *Rhizophagus intraradices* Schenck and Smith (DAOM 181602).

Bags were watered and weeded as needed throughout the growing season. The bahiagrass was winter-killed, and the AM fungi in the bags overwintered outdoors. Samples of compost and vermiculite mixture were collected from each bag after plant senescence yet before freezing of the media. A pooled sample was utilized for a Most Probable Number bioassay to estimate the number of propagules of AM fungi per cm³ of mixture as described earlier (Alexander, 1965; Douds et al., 2012b). Briefly, 10⁻¹ through 10⁻⁵ dilutions of the inoculum were prepared using a sterilized soil, sand, vermiculite, and calcined clay mixture. Three replicate 65 cm³ conical plastic pots (RLC-4 Pine Cell, Stuewe and Sons, Corvallis, OR, USA) were prepared per dilution, each receiving a *P. notatum* seedling. Plants were grown for four weeks, after which entire root systems were recovered, cleared with KOH, stained with trypan blue (Phillips and Hayman, 1970) and scored as simply colonized or not for use in the statistical tables (Alexander, 1965).

2.3 Inoculation and greenhouse culture of S. lycopersicum

Inoculum was mixed into peat based horticultural potting media (Farfard Canadian Growing Mix 2; Conrad Farfard Inc., Agawam, MA 01001) and used to fill 48 cell flats, 80-100 cm³ per cell. The ratio of inoculum: potting media varied from 1:2 [v/v] in 2005 and 2006 to 1:9

thereafter (Table 2). The high inoculum:potting mix ratio early in the experiment was to supply more propagules to try to ensure colonization at the high P fertility regime in the early years (see below). The target AM fungus propagule density was ≥ 500 per plant. No sterilized inoculum was added to control flats because the goal was to compare the standard practice of the farm to the utilization of the inoculum. Also, the contribution of the mostly nutritionally-spent compost component of the inoculum to the final volume of the potting media in the flats was only 2-7% (20% of the volume of the inoculum, which then was 10-33% of the final potting mixture). This was considered to not add significantly to the nutrients available to the seedling. Seedlings of *S. lycopersicum* were hand transplanted into the flats and grown in a greenhouse on-site for 4.5 to 5 weeks prior to outplanting. Date of transplanting into the flats varied slightly each year, and occurred from April 16-20 for the first planting and June 7 for the second planting. Date of outplanting occurred May 19- 31 for the first planting and June 29-30 for the second planting. Three to seven cultivars of *S. lycopersicum* were utilized each year (Table 3). Cultivars utilized in a given year varied due to availability of seed and recommendations received at local growers' meetings.

Greenhouse nutrient regimes were not the same throughout the experiment. Particular attention must be paid to available P level in nutrient solutions to achieve AM fungus colonization of *S. lycopersicum* (Douds, 2009) because high P inhibits colonization of roots. The fertilization solution was changed four times over the course of the experiment, from 18-16-18 soluble fertilizer (Plantex Soluble Fertilizer; Plant Products Co. Ltd., 314 Orenda Rd., Brampton, ONT L6T 1G1) in 2005 and 2006, to 21-5-20 [NPK] (Peters, 6656 Grant Way, Allentown PA 18106) in 2007, 20-3-10 (Jack's Professional Water Soluble Fertilizers, J.R. Peters, Inc., 6656 Grant Way, Allentown PA 18106) in 2008 and 2009, and finally 21-2-18 (Jack's Professional

Table 2. Density of AM fungus propagules in the original inoculum produced on-farm, after mixture into the potting media, and total propagules per *Solanum lycopersicum* seedling.

Year	Inoculum ^a (propagules cm ⁻³)	In potting media ^b	Per Plant ^c (total)
2004	21.5	7.2	576
2005	46.5	15.5	1442
2006	1200.0	120.0	10800
2007	120.0	12.0	1200
2008	46.5	4.7	423
2009	46.5	4.7	423
2010	75.0	7.5	675

^a Density of propagules of AM fungi as determined by an MPN bioassay in the year of production.

^b Density of propagules after amendment of the potting mixture = $\frac{\text{units of inoculum}}{(\text{units of inoculum} + \text{units of potting media})} \times \text{propagules cm}^{-3} \text{ inoculum}$. Ratio of inoculum : potting media varied from 1:2 in 2005 and 2006 to 1:9 thereafter; 80-100 cm³ volume per cell in the compartmented flat. These data are for year n+1, i.e. inoculum produced in 2004 was utilized the spring of 2005.

^c Total propagules of AM fungi to which the seedling was exposed in the cell of the compartmented flat = $\text{propagules cm}^{-3} \text{ potting media} \times \text{cm}^3 \text{ cell}^{-1}$.

Table 3. Cultivars of *Solanum lycopersicum* used in the experiment.

Cultivar	2005	2006	2007	2008	2009	2010	2011	Source ^a
Applause						(X)	(X)	3
BHN 961						X	X(X)	2
Celebrity						(X)		1
Celebrity Supreme							(X)	1
Daybreak	X	X	X		(X)			3
Empire	X	X		X	X(X)	X(X)		4
Florida	X							2
JTO 99197		X	X	X(X)	X(X)	X	X	3
Monica					X			3
Mountain Fresh			X	X(X)	X(X)	X		1
Mountain Fresh Plus						(X)	X	1
Red Sun			X					5

X= early planting, (X) = late planting.

^aSeed source codes: 1= Harris Seeds (355 Paul Rd., Rochester, NY 14624), 2= Siegers Seed Co. (13031 Reflections Drive, Holland, MI 49424), 3= Johnny's Selected Seeds (955 Benton Ave, Winslow ME 04901), 4= Holmes Seed Co. (2125 46th St Northwest, Canton, OH 44709); 5= Tomato Growers Supply (P.O. Box 60015, Fort Meyers, FL 33906).

Water Soluble Fertilizer) in 2010 and 2011. Concentrated fertilizers were mixed with water to provide a final target N concentration of 100 ppm. The farmer watered all seedlings daily, or as needed, with the nutrient solution with the exception of one application per week of water only. This farm also produces a variety of horticultural crops as well as other vegetable seedlings for outplanting which were not part of this experiment. A practical constraint on this experiment, which to a large extent gave it real-world validity, is that all plants in the production greenhouse

received the same nutrient solution. Therefore, the inoculated flats received a fertilization regime amenable to satisfactory growth of all plants. The only allowance given for this experiment was the yearly evaluation and consideration of modification of P level. Interestingly, given this change, no noticeable deterioration in growth of other plants was found with the decreasing P level (S. Ganser, personal communication).

2.4 Outplanting and experimental design

Seedlings were outplanted in raised beds with drip irrigation covered with black plastic mulch (width= 1 m). Bed preparation was done with a roto-vator. Nutrient addition to field plots included 17-5-16 granular fertilizer (Timac Agro USA Inc., 153 Angstadt Ln., Reading PA 19007) broadcast to apply 80-100 kg N ha⁻¹. There was one row per bed, 68 cm between plants. Bed length varied each year due to field plot location within the farm, but was typically 60 m. Seedlings were outplanted in alternating runs of 8-12 seedlings per inoculation treatment, with 6-10 such 8-12 plant units per inoculation X cultivar treatment combination. Plants of a cultivar were kept together, with typically two beds per cultivar. Care was taken so that the two adjacent beds had alternating sequences of inoculated and uninoculated plants to ensure that a mycorrhizal sampling unit in one bed had an adjacent nonmycorrhizal unit in the paired bed. Field data on yield was collected on one 4-plant sampling unit within each run of 8-12 plants, yielding 6-10 replicate units per inoculation X cultivar treatment combination. Sampling zones were marked with colored ribbons prior to the maturation of the first fruit.

2.5 Sampling, data collection, and analysis

Soil samples were collected to a depth of 20 cm on the date of planting. Each sample analyzed was a pooled sample from five locations within the selected production field. Samples were air dried, passed through a 2 mm sieve, and sent to the Pennsylvania State University Agricultural Analytical Services Laboratory for routine soil fertility analysis in 2005, 2006, and 2010 (Table 1). Samples collected in the same manner in 2006, 2007, and 2010 were utilized to quantify propagules of indigenous AM fungi via MPN bioassays, as described above for the inoculum.

Random samples of three to six seedlings per inoculation X cultivar treatment combination were withheld from outplanting and returned to the laboratory for characterization of growth and AM fungus colonization. Shoot dry weight and P concentrations were determined as described previously (Douds, 2009). Percentage root length colonized by AM fungi was determined microscopically via the gridline intersect method (Giovannetti and Mosse, 1980) after clearing and staining with trypan blue solution modified to omit phenol (Phillips and Hayman, 1970).

Harvests were conducted once or twice each week (depending on rate of fruit production) and began with the maturation of the first fruit, which occurred between July 27 and August 2 for the first planting and August 26 and September 10 for the second planting. Harvesting continued for the first planting until the plants were spent (varying from August 29 through October 10) and until the first killing frost (ranging from October 13 in 2009 to October 21 in 2007 and 2010) for the second planting. Fruit were weighed in the field *en masse* for each 4 plant sampling unit, and returned to the farmer for sale.

Data were analyzed via analysis of variance. Mycorrhizal Yield Response (MYR) was calculated using total fruit yield for paired, adjacent inoculated vs. uninoculated 4 plant sampling units following Hetrick et al. (1992):

$$\text{MYR} = 100. (\text{Myc} - \text{Nonmyc}) / \text{Nonmyc}.$$

Mycorrhizal Growth Dependence (MGD) for the seedlings at the end of the greenhouse growth period was calculated using shoot dry weights following Plenchette et al. (1983):

$$\text{MGD} = 100. (\text{Myc} - \text{Nonmyc}) / \text{Myc}.$$

These measures were differentiated according to the discussion in Smith and Smith (2011). The significance of the mean MYR or MGD for a given cultivar over the experiment, the mean across cultivars for a given year, or the mean for the entire experiment was determined using a simple 95% confidence interval (CI) calculation: if the range of the CI did not include zero, the average MYR or MGD over time was considered to be significant. The relationship between MGD (independent variable) and MYR (dependent variable) and between AM fungus colonization of roots at outplanting (independent variable) and MYR (dependent variable) were studied through linear regression using Graph Pad Prism (GraphPad Software Inc., San Diego, CA) for cultivars with three or more years of data for each parameter.

3. Results

3.1 *AM fungi in inoculum and field soil*

Inoculum of AM fungi was successfully produced on-farm each year of the study (Table 2). Final propagule density in the potting media hit the target of approximately 500 propagules per cell of the compartmented flat in four years, but overshot particularly in 2007 due to the extremely high number of propagules in the inoculum. The background of indigenous AM fungi into which these seedlings were outplanted was found to be 0.15, 3.8, and 40 propagules cm⁻³ soil for 2006, 2007, and 2010, respectively.

3.2 Status of plants at outplanting

Mean AM fungus colonization of roots of seedlings over all cultivars at the time of outplanting ranged from a low of 0.6% in 2007 to a high of 10.5% of root length in 2009. No colonization was seen in uninoculated roots. The biological significance of the low colonization level seen in 2007, however, is evidenced by the significant effect of inoculation upon shoot growth for cultivar JTO 99197 (2.133 g vs. 1.514 g, $n=4$; $\text{Pr}>F=0.0210$). Only in 2005 was there an overall significant positive effect of inoculation (across all cultivars) upon shoot dry weight ($\text{Pr}>F=0.0481$, data not shown). In all other years there was no significant difference among shoot weights for inoculated vs. uninoculated seedlings at the time of outplanting, as shown for the representative years of 2008 and 2011 (Tables 4 and 5). The progressive lowering of the P concentration in the nutrient application while the plants were in the greenhouse did not appear to have the desired effect of enhancing colonization of roots: levels of colonization of inoculated plants at the end of their greenhouse growth phase were similar through the years of the experiment. Inoculation had no statistically significant effect upon shoot P concentration whenever it was measured (eg. Table 4).

MGD varied with cultivar, and by year for most cultivars (Table 6). Of the cultivars with three or more observations, only Empire and BHN 961 exhibited significantly positive average MGD ($13.6\% \pm 6.1\%$, mean $\pm$ SEM; 95% CI of $1.8\% \leq \mu \leq 25.5\%$ and $25.1\% \pm 11.7\%$, mean $\pm$ SEM; 95% CI of $2.3\% \leq \mu \leq 48.0\%$, respectively). The 95% CI for mean MGD for other cultivars for which there were three or more observations spanned ranges that included zero. However, the mean MGD over all observations was statistically significant ($12.5\% \pm 3.5\%$, $n=25$, 95% CI of $5.7\% \leq \mu \leq 19.3\%$).

Table 4. Status of *Solanum lycopersicum* seedlings at time of outplanting, 22 May, 2008. Plants were inoculated or not with AM fungi produced on-farm in a compost and vermiculite mixture. (n=5 $\pm$ SEM)

Cultivar	Inoculation	Height (cm)	Shoot dry (g)	Shoot P (% dry wt)	Colonization (% root length)
JTO 99197	-	16.9 $\pm$ 1.9	0.794 $\pm$ 0.122		0.0 $\pm$ 0.0
	+	19.0 $\pm$ 1.0	0.917 $\pm$ 0.104		5.0 $\pm$ 1.4
<i>Pr>F</i>		0.3757	0.4664		0.0065
Empire	-	24.3 $\pm$ 0.5	0.806 $\pm$ 0.050	0.300 $\pm$ 0.028	0.0 $\pm$ 0.0
	+	25.0 $\pm$ 1.2	1.059 $\pm$ 0.144	0.246 $\pm$ 0.028	8.1 $\pm$ 2.3
<i>Pr>F</i>		0.6023	0.1358	0.2028	0.0070
Mountain Fresh	-	23.4 $\pm$ 1.0	0.810 $\pm$ 0.090		0.0 $\pm$ 0.0
	+	25.4 $\pm$ 0.6	0.861 $\pm$ 0.077		3.8 $\pm$ 0.5
<i>Pr>F</i>		0.1229	0.6788		<0.0001
Full Model				Pr>F	
<i>Cultivar</i>		<0.0001	<0.6099		0.1508
<i>Inoculation</i>		0.1003	0.1023		<0.0001
<i>C x I</i>		0.8013	0.6132		0.1508

Table 5. Status of *Solanum lycopersicum* seedlings at time of outplanting, 31 May, 2011. Plants were inoculated or not with AM fungi produced on-farm in a compost and vermiculite mixture. (n=3 $\pm$ SEM)

Cultivar	Inoculation	Height (cm)	Shoot dry (g)	Colonization (% root length)
Mountain Fresh	-	52.8 $\pm$ 2.4	2.139 $\pm$ 0.327	0.0 $\pm$ 0.0
	+	59.5 $\pm$ 1.4	1.895 $\pm$ 0.478	5.8 $\pm$ 1.3
		<i>0.0750</i>	<i>0.5160</i>	<i>0.0113</i>
BHN 961	-	46.0 $\pm$ 1.8	1.395 $\pm$ 0.104	0.0 $\pm$ 0.0
	+	57.8 $\pm$ 1.3	2.203 $\pm$ 0.043	7.9 $\pm$ 1.3
		<i>0.0055</i>	<i>0.0530</i>	<i>0.0032</i>
JTO 99197	-	47.0 $\pm$ 0.4	2.225 $\pm$ 0.091	0.0 $\pm$ 0.0
	+	62.4 $\pm$ 1.3	2.614 $\pm$ 0.198	5.4 $\pm$ 2.3
		<i>0.0003</i>	<i>0.1301</i>	<i>0.0780</i>
Full Model		Pr>F		
<i>Cultivar</i>		0.0011	0.0132	<0.0001
<i>Inoculation</i>		<0.0001	0.2453	<0.0001
<i>C x I</i>		0.0006	0.4095	<0.0001

3.3 Response of yield to inoculation with AM fungi

The response of yield to inoculation with AM fungi varied both with cultivar and year. Average yield, when calculated across all cultivars, appeared to vary little between inoculated vs. uninoculated treatments (Figure 1). Yields averaged from a high of nearly 10 kg per plant in 2010 to a low of 4 kg per plant in 2008, a particularly wet growing season. Yearly response to AM fungus inoculation can be demonstrated for cvs. Empire, JTO 99197, and Mountain Fresh, for which there is the most data (Figure 2). Yields for the late planting, for a given cultivar, were consistently less than those of the early planting. MYR varied by cultivar, and by year within cultivar (Table 7). There were five individual cultivar by year combinations in which the MYR was statistically significant ($\alpha=0.05$): Daybreak in 2005 (-8.8%) and 2009 (17.8%), Empire in 2008 (29.2 %), Florida in 2005 (12.7%), and JTO 99197 in 2007 (17.0%). Empire was the only cultivar that exhibited a significantly positive average MYR ($9.7\% \pm 4.0\%$; 95% CI of $1.9\% \leq \mu \leq 17.4\%$) (Table 7, Figure 2A). The CI for the mean MYR of all other cultivars for which there were three or more observations included zero. Mountain Fresh was the only cv. with three or more years of data with a mean MYR that was negative ($-0.46\% \pm 2.59$) (Table 7, Figure 2C). The mean MYRs for 2007 and 2009 were positive and statistically significant (10.8 ± 2.5 , 95% $5.9\% \leq \mu \leq 15.7$ and 9.4 ± 3.3 , 95% CI of $2.9\% \leq \mu \leq 15.8\%$, respectively). The overall MYR for the entire experiment was significant at $6.02\% \pm 1.92\%$ (mean $\pm$ SEM, $n=37$; 95% CI of $2.25\% \leq \mu \leq 9.79\%$).

Figure 1. Average fruit yield for *Solanum lycopersicum*, summed across all cultivars, throughout the study, first planting only. Means of 3-4 cultivars each year, $\pm$ SEM. Plants were inoculated, or not, with AM fungi during the greenhouse growth phase prior to outplanting.

Figure 2. Average yearly yield of fruit for *Solanum lycopersicum* cv Empire (A), JTO 99197 (B), and Mountain Fresh (C). Each point is the mean total harvest per plant of 6-8 sampling units $\pm$ SEM. Plants were inoculated, or not, with AM fungi during the greenhouse growth phase prior to outplanting. Year followed by a “L”= the second (Late) planting.

Table 6. Mycorrhizal Growth Dependence of *Solanum lycopersicum* cultivars inoculated during the greenhouse production phase with mixed species AM fungus inoculum produced on-farm.^a

Cultivar	2005	2006	2007	2008	2009	2010	2011
Daybreak	20.6	X	-18.0				
Empire	6.2	X		23.9	-4.6	13.5	
						(29.2)	
Florida	X						
JTO 99197		X	29.0	13.4	4.1	-20.8	14.9
Mountain Fresh			25.4	5.9	-0.2	35.3	
						(-13.8)	
Mountain Fresh Plus							-12.8
							(23.1)
Monica					17.7		
BHN 961						36.9	36.7
							(1.8)
Applause						(30.3)	(Y)
Celebrity/Supreme						(14.4)	(Y)

^aMycorrhizal Growth Dependence= 100. (Myc-Nonmyc)/Myc. Each value is calculated from the mean shoot dry weight for inoculated and uninoculated plants for each cultivar at the time of outplanting, each year. Means of 3-6 observations. Numbers in parentheses are for the second planting. X= Not enough plants left after outplanting to analyze. Y= Colonization not found in all sampled plants.

The potential relationship between MGD during the seedling growth phase (independent variable) and the MYR of plants in the field (dependent variable) was explored through linear regression for four cultivars which appeared three or more years (Figure 3). Only JTO 99197 had a significant positive relationship between MGD and MYR ($\alpha = 0.0305$, Table 8). No significant relation between level of AM fungus colonization at outplanting and MYR was found for these cultivars (data not shown). The slopes were negative for all cultivars except Empire, but no slopes were significantly different than zero (e.g. $P = 0.191$ for Empire and 0.7861 for BHN 961).

3.4 Economic analysis

An economic return on the use of AM fungus inoculum in this experiment can be estimated. The inoculum was produced at a cost of \$27.30 (US) per bag (\$4.64 for 14.4 L of vermiculite, \$1.26 for 3.6 L of compost (eg. “Black Gold Garden Compost,” Worm’s Way), \$1.00 for the bag (Worm’s Way), and \$0.40 for the five 65 cm³ conical plastic pots (RLC-4 Pine Cell, Stuewe and Sons) for production of the *P. notatum* nurse host seedlings)), including a generous labor charge of \$20.00 (10 min week⁻¹ for 12 weeks, at \$10.00 hr⁻¹). The average bag of inoculum had a propagule density of 59.3 cm⁻³ (Table 2, excluding the outlier year 2006), translating to 1.07×10^6 propagules bag⁻¹. Each seedling received an average of 790 propagules (Table 2, again excluding year 2006), so each bag inoculated 1354 plants. Therefore, the cost of the on-farm inoculum per plant was \$0.02. This assumes propagation of the indigenous AM fungus community. The average yield for the uninoculated plants, across all years and cultivars, was 6.55 kg plant⁻¹. The 6.02% MYR therefore translates to an increase in yield of 0.393 kg

plant⁻¹. The mid-season price for fresh market tomatoes was \$2.00 lb⁻¹ (\$4.41 kg⁻¹) resulting in an increased income of \$1.75 plant⁻¹. The plant and row spacing yielded approximately 8030 plants ha⁻¹, translating to an overall yield increase of 3156 kg ha⁻¹ and a gross return of \$14053, a cost of \$161, and a net of \$13892 ha⁻¹.

Figure 3. Relationship between Mycorrhizal Growth Dependence (MGD), as measured after the greenhouse growth phase, and Mycorrhizal Yield Response (MYR) of plants outplanted to the field. Shown are data for cultivars of *Solanum lycopersicum* for which there are three or more years of data. See Table 8 for statistics.

Table 7. Mycorrhizal Yield Response (MYR) of tomatoes, calculated via paired plots.^a

Cultivar	2005	2006	2007	2008	2009	2010	2011
Daybreak	-8.77*	6.88	12.48		(17.78)*		
Empire	2.20	2.59		29.20*	15.46	-0.03	
					(13.71)	(4.47)	
Florida	12.71*						
JTO 99197		-0.74	16.98*	3.07	1.62	-3.13	7.16
				(49.29)	(2.87)		
Red Sun			8.15				
Mountain Fresh/Plus			5.62	4.51	-4.79	0.80	1.84
				(-16.23)	(5.86)	(-1.28)	
Monica					22.34		
BHN 961						6.59	-6.13
							(6.74)
Celebrity/Supreme						(5.97)	(4.04)
Applause						7.83	(-14.80)

^a MYR= ((yield MYC- yield NONMYC)/ yield NONMYC)*100%. Late plantings in parentheses. Each number is the mean of MYR calculations for 6-10 paired, adjacent inoculated and uninoculated sampling units. *= significant (α =0.05). Overall mean MYR= 6.02 ± 1.92% (n=37).

Table 8. Linear regression of Mycorrhizal Growth Response (independent) vs. Mycorrhizal Yield Response (dependent variable) for *Solanum lycopersicum* cultivars for which there are three or more data points. See Figure 3.

Cultivar	n ^a	r ²	slope	Significance ^b
BHN 961	3	0.2545	-0.1844	0.6634
Empire	6	0.0116	0.0963	0.8634
JTO 99197	5	0.8332	0.3591	0.0305
Mountain Fresh	5	0.1492	0.0735	0.4495

^aNumber of years for which there is MGD data for the cultivar (Table 6).

^bPr>F in test of significance of slope. Pr <0.05 indicates the slope is significantly different than zero.

4. Discussion

The impact of inoculation with AM fungi upon growth and yield of *S. lycopersicum* has been studied in the greenhouse and field and under a variety of abiotic stresses. Both greenhouse (Daft and Okusanya, 1973; Sylvia et al., 2001; Latef et al., 2011; Ortas et al., 2013) and field studies (Clough, 1981; Mohandas, 1987; Conversa et al., 2013) have shown increased growth or yield due to inoculation with AM fungi. Growth responses were greater at low P availability/addition (Daft and Nicolson, 1966; Bryla and Koide, 1998; Schroeder and Janos, 2004; Watts-Williams and Cavagnaro, 2014). Additional physiological and phenological

responses of tomato attributed to AM fungus colonization are earlier flowering (Ortas et al., 2013), prolonged fruiting (Regvar et al., 2003), retention of lower leaves (Daft and Nicolson, 1969), increased vascular tissue (Daft and Okusanyo, 1973), and increased drought tolerance (Subramanian et al. 2006) and salt stress resistance (Al-Karaki, 2006; Hajiboland et al., 2010) relative to nonmycorrhizal plants.

Strong evidence exists, however, for tomato being non-responsive to AM fungus colonization. In addition to studies in which no response was found (e.g. Smith et al., 2004), response to mycorrhization has been studied using mutant tomato lines, resistant to colonization by AM fungi. Comparison of wild type vs. rmc ('reduced mycorrhizal colonization') mutant tomato in an organic production field showed no significant differences in vegetative growth or yield between the two populations (Cavagnaro et al., 2006). In addition, the response to AM fungus inoculation has been demonstrated to be cultivar dependent (Bryla and Koide, 1998) as also was found in this study.

The mean MYR for this seven year study was approximately 6%. This compares to an average MYR of 8.5% for a two year study by Conversa et al (2013) with processing tomato (*S. lycopersicum* cv. Ercole) in a calcareous soil with available P of only 18 to 35 mg kg⁻¹. This potential benefit of the use of inexpensively-produced AM fungus inoculum can be put into better perspective by comparison to the mean yearly yield increase over time for maize, a crop that has received intensive effort in genetic engineering. The average yield of maize in the US from 1986 through 2010 was 82.8 x 10³ kg ha⁻¹ with an increase per year of 1053.4 kg ha⁻¹ yr⁻¹ (FAOSTAT, Heinemann et al., 2014). This translates to a mean yearly increase of 1.27%.

Many have concluded that optimal utilization of the AM symbiosis is essential for the sustainability of agriculture (Bethlenfalvay and Schüepp, 1994; Harrier and Watson, 2003;

Jeffries et al., 2003). Situations in which they may not have a role include extensive cropping systems in Australia (Ryan and Kirkegaard, 2012) and in high P soils (Ryan and Graham, 2002). Generally, plant growth response to mycorrhizas is inversely proportional to the level of available P in the soil (Koide, 1991). However, this broad statement may apply better for situations lacking other stressors for which mycorrhizas provide a measure of resistance. For example, more sophisticated experiments studying the enhanced resistance of mycorrhizal vs. nonmycorrhizal plants to water stress seek to compare plants of the same P and/or growth status. These studies have found that AM fungus colonization of roots imparts to the host drought resistance independent, or in the absence of, a growth or nutritional effect (Augé, 2000). Further, proposed mechanisms for AM fungus enhancement of resistance to plant pathogens include mechanisms independent of nutritional effects (Dalpé, 2005). Therefore, farmers of high P soils, such as in this study with *S. lycopersicum*, should not necessarily assume use of AM fungus inoculum to be futile, as demonstrated here and with other crops in our work with the on-farm production and utilization of AM fungus inoculum (Douds et al., 2007; 2012a; 2012b). One strong advantage of the on-farm inoculum production method is it is inexpensive enough to utilize routinely, year after year, positioning the farmer to combat stresses, biotic or abiotic outlined above, with the symbiosis.

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References

- Abushita AA, Hebshi EA, Daood HG, Biacs PA. 1997. Determination of antioxidant vitamins in tomatoes. *Food Chem* 60: 207-212.
- Al-Karaki G. 2006. Nursery inoculation of tomato with arbuscular mycorrhizal fungi and subsequent performance under irrigation with saline water. *Sci Hort* 109: 1-7.
- Alexander M. 1965. *Methods of Soil Analysis, part 2 Chemical and Microbiological Properties*. C.A. Black, D.D. Evans, L.E. Ensminger, J.L. White, and F.E. Clark (eds). Madison (WI): American Society of Agronomy. Chapter 100, Most-probable-number method for microbial populations. p. 1467-1472.
- Augé, RM. 2000. Stomatal behavior of arbuscular mycorrhizal plants. In: Kapulnik Y, Douds DD, editors. *Arbuscular Mycorrhizas: Physiology and Function*. Dordrecht: Kluwer Academic Publishers. p. 201-238.
- Bagyaraj DJ. 1992. Vesicular-arbuscular mycorrhiza: application in agriculture. *Met Microbiol*. 24: 359-373.
- Bethlenfalvay GJ, Schüepp H. 1994. Arbuscular mycorrhizas and agricultural sustainability. In: Gianinazzi S and Schüepp H, editors. *Impact of Arbuscular Mycorrhizas on Sustainable Agriculture and Natural Ecosystems*. Basel: Birkhäuser Verlag; p. 117-131.
- Beecher GR. 1998. Nutrient content of tomatoes and tomato products. *Proc Soc Exp Biol Med* 218: 98-100.
- Björkman T, Reiners S. 2014. Application of bicarbonate to high-phosphorus soils to increase plant-available phosphorus. *Soil Sci Soc Amer J*. 78: 319-324.

- 573 Bryla DR, Koide RT. 1998. Mycorrhizal response of two tomato genotypes relates to their
574 ability to acquire and utilize phosphorus. *Ann Bot* 82: 849-857.
- 575 Calvet C, Camprubi A, Pérez-Hernández A. 2013. Plant growth stimulation and root
576 colonization potential of in vivo versus in vitro arbuscular mycorrhizal inocula. *HortSci*. 48:
577 897-901.
- 578 Cavagnaro TR, Jackson LE, Six J, Ferris H, Goyal S, Asami D, Scow KM. 2006. Arbuscular
579 mycorrhizas, microbial communities, nutrient availability, and soil aggregation in organic
580 tomato production. *Plant Soil* 282: 209-225.
- 581 Clough KS. 1981. Field inoculation of tomatoes and broad beans with *Glomus mosseae* and
582 *Glomus epigaeus*. Poster at the: Fifth North American Conf. on Mycorrhizas. Quebec,
583 Canada. P 53.
- 584 Conversa G, Lazzizera C, Bonasia A, Elia A. 2013. Yield and phosphorus uptake of a
585 processing tomato crop grown at different phosphorus levels in a calcareous soil as affected
586 by mycorrhizal inoculation under field conditions. *Biol Fertil Soils* 49: 691-703.
- 587 Corkidi L, Allen EB, Merhaut D, Allen MF, Downer J, Bohn J, Evans M. 2004. Assessing the
588 infectivity of commercial mycorrhizal inoculants in plant nursery conditions. *J Environ Hort*.
589 22: 149-154.
- 590 Daft MJ, Nicolson TH. 1966. Effect of *Endogone* mycorrhiza on plant growth. *New Phytol* 65:
591 343-350.
- 592 Daft MJ, Nicolson TH. 1969. Effect of *Endogone* mycorrhiza on plant growth. III. Influence of
593 inoculum concentration on growth and infection in tomato. *New Phytol* 68: 953-961.

- 594 Daft MJ, Okusanya BO. 1973. Effect of *Endogone* mycorrhiza on plant growth. VI. Influence
 595 of infection on the anatomy and reproductive development in four hosts. New Phytol 72:
 596 1333-1339.
- 597 Dalpé Y. 2005. Mycorrhizae: a potential tool for plant protection but not a panacea.
 598 Phytoprotect. 86: 53-59.
- 599 Douds DD. 2009. Utilization of inoculum produced on-farm for production of AM fungus
 600 colonized pepper and tomato seedlings under conventional management. Biol Agr Hort. 26:
 601 353-364.
- 602 Douds DD, Nagahashi G, Reider C, Hepperly PR. 2007. Inoculation with arbuscular mycorrhizal
 603 fungi increases the yield of potatoes in a high P soil. Biol Agr Hort. 25: 67-78.
- 604 Douds DD, Nagahashi G, Shenk JE. 2012a. Frequent cultivation prior to planting to prevent
 605 weed competition results in an opportunity for the use of arbuscular mycorrhizal fungus
 606 inoculum. Sust Agr Food Sys. 27: 251-255.
- 607 Douds DD, Lee J, Rogers L, Lohman ME, Pinzon N, Ganser S. 2012b. Utilization of inoculum
 608 of AM fungi produced on-farm for the production of *Capsicum annuum*: a summary of seven
 609 years of field trials on a conventional vegetable farm. Biol Agr Hort. 28: 129-145.
- 610 Douds DD, Chaiyasen A, Vasquez LR, Wertheim FS. 2014. On-farm production of arbuscular
 611 mycorrhizal fungus inoculum in compost and vermiculite mixtures: results of on-farm
 612 demonstrations and impact of compost microbiological quality. J Nat Assoc County Agr
 613 Agents. 7(2) Dec 2014. <http://www.nacaa.com/journal/index.php?jid=44>
- 614 FAOSTAT 2015. (<http://faostat3.fao.org/browse/Q/QC/E>).
- 615 Faye A, Dalpé Y, Ndung'u-Magiroi K, Jefwa J, Ndoeye I, Diouf M, Lesueur D. 2013. Evaluation
 616 of commercial arbuscular mycorrhizal inoculants. Can J Plant Sci. 93: 1201-1208.

- 617 Fester T, Sawers R. 2011. Progress and challenges in agricultural applications of arbuscular
618 mycorrhizal fungi. *Crit Rev in Plant Sci.* 30: 459-470.
- 619 Fixen PE. 2006. Soil test levels in North America. *Better Crops* 90: 4-7.
- 620 Franke-Snyder M, Douds DD, Galvez L, Phillips JG, Wagoner P, Drinkwater LE, Morton JB.
621 2001. Diversity of communities of arbuscular mycorrhizal (AM) fungi present in
622 conventional versus low-input agricultural sites in eastern Pennsylvania, USA. *Appl Soil*
623 *Ecol.* 16: 35-48.
- 624 Gaur A. 1997. Inoculum production technology development of vesicular-arbuscular
625 mycorrhizae. Ph.D. Thesis, Dept of Botany, Univ of Delhi, Delhi-100 007 India.
- 626 Gaur A, Adholeya A, Mukerji. KG. 2000. On-farm production of VAM inoculum and vegetable
627 crops in marginal soil amended with organic matter. *Trop Agr.* 77: 21-26.
- 628 Giovannetti M, Mosse B. 1980. An evaluation of techniques for measuring vesicular-arbuscular
629 infection in roots. *New Phytol.* 84: 489-500.
- 630 Giovannetti M, Avio L, Barale R, Ceccarelli N, Cristofani R, Iezzi A, Mignoli F, Picciarelli P,
631 Reali D, Sbrana C, Scarpato R. 2012. Nutraceutical value and safety of tomato fruits
632 produced by mycorrhizal plants. *Brit J Nutrition* 107: 242-251.
- 633 Hajiboland R, Aliasgharzadeh N, Laiegh SF, Poschenreider C. 2010. Colonization with
634 arbuscular mycorrhizal fungi improves salinity tolerance of tomato (*Solanum lycopersicum*
635 L.) plants. *Plant Soil* 331: 313-327.
- 636 Harrier LA, Watson CA. 2003. The role of arbuscular mycorrhizal fungi in sustainable cropping
637 systems. *Adv in Agron.* 79: 185-225.
- 638 Heinemann JA, Massaro M, Coray DS, Agapito-Tenfen SZ, Wen JD. 2014. Sustainability and
639 innovation in staple crop production in the US Midwest. *Internat J Agr Sustain.* 12: 71-88.

- 640 Hetrick BAD, Wilson GWT, Cox TS. 1992. Mycorrhizal dependence of modern wheat
641 varieties, landraces and ancestors. *Can J Bot.* 70: 2032-2040.
- 642 Ildo M, Cranenbrouck M, Declerck S. 2011. Methods for large-scale production of AM fungi:
643 past, present, and future. *Mycorrhiza.* 21: 1-16.
- 644 Jeffries P, Gianinazzi S, Perotto S, Turnau K, Barea JM. 2003. The contribution of arbuscular
645 mycorrhizal fungi in sustainable maintenance of plant health and soil fertility. *Biol Fertil*
646 *Soils* 37: 1-16.
- 647 Koide, RT. 1991. Nutrient supply, nutrient demand, and plant response to mycorrhizal infection.
648 *New Phytol* 117: 365-386.
- 649 Koide RT, Landherr LL, Besmer YL, Detweiler JM, Holcomb EJ. 1999 Strategies for
650 mycorrhizal inoculation of six annual bedding plant species. *Hortsci.* 34: 1217-1220.
- 651 Koltai H, Gadkar V, Kapulnik Y. 2010. Biochemical and practical views of arbuscular
652 mycorrhizal fungus-host association in horticultural crops. *Hort Rev.* 36: 257-288.
- 653 Latef AAHA, Chaoxing H. 2011. Effect of arbuscular mycorrhizal fungi on growth, mineral
654 nutrition, antioxidant enzymes activity, and fruit yield of tomato growth under salinity stress.
655 *Sci Hort* 127: 228-233.
- 656 Maiti D, Barnwal MK, Singh RK, Variar M. 2009. A new protocol for on-farm production of
657 arbuscular mycorrhizal mass inoculum of rainfed upland rice. *Indian Phytopath.* 62: 31-36.
- 658 Miller RM, Jastrow JD. 2000. *Arbuscular Mycorrhizas: Physiology and Function.* Kluwer
659 Academic Publishers, Dordrecht (The Netherlands): Y. Kapulnik and D.D. Douds, eds.
660 Chapter 1, Mycorrhizal fungi influence soil structure; p 3-18.

- 661 Mohandas S. 1987. Field response of tomato (*Lycopersicon esculentum* Mill 'Pusa Ruby') to
 662 inoculation a VA mycorrhizal fungus *Glomus fasciculatum* and with *Azotobacter vinelandii*.
 663 Plant Soil 98: 295-297.
- 664 Nedorost L, Pokluda R. 2012. Effects of arbuscular mycorrhizal fungi on tomato yield and
 665 nutrient uptake under different fertilization levels. Acta Univ Agric Silvic Mendel Brun
 666 60(8): 181-186.
- 667 Ordookhani K, Moezi A, Khavazi K, Rejali F. 2013. Effect of plant growth promoting
 668 rhizobacteria and mycorrhiza on tomato fruit quality. Acta Hort 989: 91-96.
- 669 Ortas I. 2012. The effect of mycorrhizal fungal inoculation on plant yield, nutrient uptake, and
 670 inoculation effectiveness under long-term field conditions. Field Crops Res. 125: 35-48.
- 671 Ortas I, Sari N, Akpinar C, Yetisir H. 2013. Selection of arbuscular mycorrhizal fungi species
 672 for tomato seedling growth, mycorrhizal dependency and nutrient uptake. Europ J Hort Sci
 673 78: 209-218
- 674 Pellegrino E, Bedini S, Avio L, Bonori E, Giovannetti M. 2011. Field inoculation effectiveness
 675 of native and exotic arbuscular mycorrhizal fungi in a Mediterranean agricultural soil. Soil
 676 Biol Biochem. 43: 367-376.
- 677 Phillips JM, Hayman DS. 1970. Improved procedures for clearing roots and staining parasitic
 678 and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Trans Br Mycol
 679 Soc. 55: 158-160.
- 680 Plenchette C, Fortin JA, Furlan V. 1983. Growth responses of several plant species to
 681 mycorrhizae in a soil of moderate P-fertility. II. Soil fumigation induced stunting of plants
 682 corrected by reinoculation of the wild endomycorrhiza flora. Plant Soil. 70: 211-217.

- 683 Regvar M, Vogel-Mikus K, Severkar T. 2003. Effect of AMF inoculum from field isolates on
684 the yield of green pepper, parsley, carrot, and tomato. *Folia Geobot.* 38: 223-234.
- 685 Rhodes LH, Gerdemann JW. 1975. Phosphate uptake zones of mycorrhizal and non-
686 mycorrhizal onions. *New Phytol.* 75: 555-561.
- 687 Rouphael Y, Franken P, Schneider C, Schwarz D, Giovannetti M, Agnolucci M, De Pascale S,
688 Bonini P, Colla G. 2015. Arbuscular mycorrhizal fungi act as biostimulants in horticultural
689 crops. *Sci Hort* 196: 91-108.
- 690 Russell DW. 1973. Soil conditions and plant growth. Longman Group Ltd. NY, USA.
- 691 Ryan MH, Graham JH. 2002. Is there a role for arbuscular mycorrhizal fungi in production
692 agriculture? *Plant Soil.* 244: 263-271.
- 693 Ryan MH, Kirkegaard JA. 2012. The agronomic relevance of arbuscular mycorrhizas in the
694 fertility of Australian extensive cropping systems. *Agric Ecosys Environ* 163: 37-53.
- 695 Santana, AS de, Cavalcante UMT, de Sa Barreto Sampaio EV, Maia LC. 2014. Production,
696 storage and costs of inoculum of arbuscular mycorrhizal fungi (AMF). *Braz J Bot* 37: 159-
697 165.
- 698 Sattari S, Bouwman AF, Giller K, van Ittersum MK. 2012. Residual soil phosphorus as the
699 missing piece in the global phosphorus crisis puzzle. *PNAS* 109: 6348-6353.
- 700 Schelmpier TR, Sturmer SL. 2014. On farm production of arbuscular mycorrhizal fungi inoculum
701 using lignocellulosic agrowastes. *Mycorrhiza.* 24: 571–580.
- 702 Schroeder MS, Janos DP. 2004. Phosphorus and intraspecific density alter plant responses to
703 arbuscular mycorrhizas. *Plant Soil* 264: 335-348.
- 704 Sheppard SC, Racz GJ. 1985. Shoot and root response of wheat to band and broadcast
705 phosphorus at varying soil temperatures. *Can. J. Soil Sci.* 65: 79-88.

- 706 Sieverding, E. 1991. Vesicular-arbuscular mycorrhiza management in tropical agrosystems.
 707 Eschborn: Duetsche Gesellschaft für Technische Zusammenarbeit (GTZ) GnbH.
- 708 Sims JT, Maguire RO, Leytem AB, Gartley KL, Pautler MC. 2002. Evaluation of Mehlich 3 as
 709 an agri-environmental soil phosphorus test for the mid-Atlantic United States of America.
 710 Soil Sci Soc Amer J 66: 2016-2032.
- 711 Smith FA, Smith SE. 2011. What is the significance of the arbuscular mycorrhizal colonization
 712 of many economically important crop plants? Plant Soil. 348: 63-79.
- 713 Smith SE, Read DJ. 2008. Mycorrhizal Symbiosis. 3rd Ed. Amsterdam: Academic Press.
- 714 Smith SE, Smith FA, Jakobsen I. 2004. Functional diversity in arbuscular mycorrhizal
 715 symbiosis: the contribution of the mycorrhizal P uptake pathway is not correlated with
 716 mycorrhizal responses in growth or total P uptake. New Phytol 162: 511-524.
- 717 Subramanian KS, Santhanakrishnan P, Balasubramanian P. 2006. Responses of field grown
 718 tomato plants to arbuscular mycorrhizal fungal colonization under varying intensities of
 719 drought stress. Sci Hort 107: 245-253.
- 720 Sylvia DM, Alagely AK, Chellemi DO, Demchenko LW. 2001. Arbuscular mycorrhizal fungi
 721 influence tomato competition with bahiagrass. Biol Fertil Soils 34: 448-452.
- 722 Tarbell TJ, Koske RE. 2007. Evaluation of commercial arbuscular mycorrhizal inocula in a
 723 sand/peat medium. Mycorrhiza 18: 51-56.
- 724 Vance CP, Uhde-Stone C, Allan DL. 2003. Phosphorus acquisition and use: critical adaptations
 725 by plants for securing a nonrenewable resource. New Phytol. 157: 423-447.
- 726 Waterer DR, Coltman RR. 1989. Response of mycorrhizal bell peppers to inoculation timing,
 727 phosphorus, and water stress. HortSci. 24: 688-690.

- 728 Watts-Williams SJ, Cavagnaro TR. 2014. Nutrient interactions and arbuscular mycorrhizas; a
729 meta-analysis of a mycorrhiza-defective mutant and wild-type tomato genotype pair. *Plant*
730 *Soil* 384: 79-92.
- 731 Wiseman PE, Colvin KH, Wells CE. 2009. Performance of mycorrhizal products marketed for
732 woody landscape plants. *J Environ Hort.* 27: 41-50.
- 733 Wright SF, Anderson RL. 2000. Aggregate stability and glomalin in alternative crop rotations
734 for the central Great Plains. *Biol Fertil Soils.* 31: 249-253.
- 735 Yildiz A. 2010. A native *Glomus* sp from fields in Aydin Province and effects of native and
736 commercial mycorrhizal fungi inoculants on the growth of some vegetables. *Turk J Biol.*
737 34: 447-452.
- 738
- 739