

Inoculation of sweet potatoes with AM fungi produced on-farm increases yield in high P soil

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Abstract

Vegetable farmers who grow seedlings for later outplanting to the field have the opportunity to incorporate arbuscular mycorrhizal [AM] fungus inocula into potting media to produce plants ready to benefit from the symbiosis upon outplanting. Inocula of AM fungi are available commercially or may be grown on-farm. The impact of AM fungus inoculum produced on-farm upon yield of sweet potato (*Ipomoea batatas* L.) was studied in a field experiment over six site-years. Rooted cuttings were inoculated with AM fungi either directly in the planting hole or were grown first in a greenhouse in potting media amended with AM fungus inoculum. Controls received the same compost and vermiculite mixture in which the inoculum was grown. Available P levels in the soil ranged from 242 to 599 kg ha⁻¹. Mean increase in yield of sweet potatoes of the inoculated plants for the experiment was statistically significant at 10.0 ± 1.9 % over uninoculated controls. Further, roots collected at the time of harvest indicated significantly greater colonization by AM fungi of previously inoculated plants than in controls which became colonized by the indigenous population of AM fungi. Utilization of AM fungi produced on-farm reliably increased the yield of sweet potato in high P soils.

Key words: Arbuscular mycorrhizal fungi, on-farm inoculum, *Ipomoea batatas*, sustainable agriculture

Introduction

Arbuscular mycorrhizal [AM] fungi are obligate symbiotic soil fungi that form a mutualistic symbiosis with the majority of crop plants. The extraradical hyphae of the fungi function, in effect, as extensions of the root system for the increased uptake of immobile soil nutrients such as P, Zn and Cu (Neumann and George, 2010). Among other benefits to the plant that are ascribed to the symbiosis are enhanced water relations (Jayne and Quigley, 2014), disease resistance (Maffei *et al.*, 2014; Olawuyi *et al.*, 2014), and salt stress resistance (Vincente-Sánchez *et al.*, 2014). Consideration of these benefits make it easy to understand why many have concluded that optimal utilization of the symbiosis is essential for the sustainability of agriculture (Harrier and Watson, 2003; Jeffries *et al.*, 2003; Fester and Sawers, 2011). However, contrary indications exist for extensive cropping systems in Australia (Ryan and Kirkegaard, 2012).

Vegetable growers have the option of utilizing AM fungus inoculum by growing seedlings in inoculated potting media in the greenhouse growth phase prior to outplanting to the field (Koide *et al.*, 1999). This enables the plant to benefit from the functioning symbiosis immediately upon outplanting, rather than experiencing the delay necessary for the establishment of mycorrhizas by the indigenous AM fungus community in the field soil. This method has been demonstrated to enhance the growth or yield of numerous crops, including kidney bean (Isobe and Tsuboki, 1999), leeks (Sorensen *et al.*, 2008) and strawberries (Douds *et al.*, 2008). Alternatively, inoculum may be added directly to planting holes or furrows in the field in more labor intensive small farms. Here, the inoculum must have sufficient AM fungus propagule numbers to compete with or supplement the

indigenous population to be effective (Sieverding, 1991; Hamel *et al.*, 1997). Nevertheless, this method enhanced the yield of many crops including potatoes (Douds *et al.*, 2007), garlic (Al-Karaki *et al.*, 2002), and maize (Ananthi *et al.*, 2011).

In addition to the status of the indigenous population of AM fungi, crop response to inoculation with AM fungi has been shown to be affected by the availability of P in the soil. The P level above which a response is unlikely differs depending upon crop and soil type, and has been found to range from 50 to 140 mg P kg⁻¹ soil (Thingstrup *et al.*, 1998; Amijee *et al.*, 1989). These levels are well below those common in soils of the northeastern and middle Atlantic states of the US, e.g., 413 mg P kg⁻¹ soil for crop soils of the Delaware coastal plain (Sims *et al.*, 2002), where many decades of fertilizer P and manure application have lead to available P above optimal levels (Fixen, 2006; Bjorkman and Reiners, 2014). Nevertheless, we have demonstrated yield increases upon AM fungus inoculation in that area for a number of crops; including strawberry, potatoes, leeks, and peppers; in soils with available P ranging from 140-330 mg P kg⁻¹ soil (Douds, *et al.*, 2007, 2008, 2012a, 2012b).

Though inocula of AM fungi are available commercially (Ijdo *et al.*, 2011; Faye *et al.*, 2013), they may also be produced on-farm. Methods for the on-farm production of inoculum were originally developed for tropical countries (Sieverding, 1991; Gaur *et al.*, 2000; Maiti *et al.*, 2009; Schlemper and Stürmer, 2014). We have developed a method suitable for temperate climates that can produce inocula containing either introduced AM fungus isolates or those indigenous to the farm (Douds *et al.*, 2010). Work published to date has demonstrated the reliability of this method and the utility of the inoculum in the greenhouse (Douds, 2009; Douds *et al.*, 2013, 2014).

Sweet potato (*I. batatas* L.) is a mycotrophic crop that has been used for AM fungus inoculum production in aeroponic systems (Hung and Sylvia, 1988). Growth and yield of sweet potato were shown to be strongly responsive to P level (Negeve and Roncadori, 1985) making them good candidates for inoculation with AM fungi. Sweet potatoes are an excellent component of the human diet, rich in complex carbohydrates, fiber, vitamin C, and beta-carotene (Center for Science in the Public Interest, 2013). Sweet potato production in the US has increased from 597 x 10³ kg in 1970 to 1082 x 10³ kg in 2010 on approximately 47.3 x 10³ ha (USDA-ERS, 2011). However, approximately 80% of global production is in China (CIP, 2007).

Here, we extend the work noted above for other crops and demonstrate the consistent benefit achieved with the inoculation of sweet potato with AM fungi produced on-farm. Two methods of inoculation, directly to the planting hole in the field and preliminary growth in inoculated potting media, were studied over five growing seasons.

Materials and methods

Experiments were conducted from 2009 through 2014 to study the impact of inoculation with AM fungi upon the yield of sweet potato (*I. batatas* L.) cv. ‘Beauregard’ the first three years and cv. ‘Covington’ thereafter. Mixed species inocula of AM fungi were produced on-farm. Slips were inoculated at outplanting in 2009 and 2010 and in later years by a preliminary two weeks of growth in a greenhouse in an inoculated potting mixture.

Field sites: Experiments were conducted at two farms in southeastern Pennsylvania, USA. Eagle Point Farm (EPF) is a conventional vegetable farm in Kutztown, PA. The second farm, Shenk’s Berry Farm (SBF), is a conventional small fruit and vegetable farm in Lititz, PA. The preceding crops were tomato at EPF and strawberry at SBF. The soil at both farms is a Berks silt loam (loamy-skeletal, mixed, active, mesic typic dystrodepts). Pooled soil samples were collected from each site for soil analyses (Pennsylvania State Agricultural Analytical Services Laboratory, University Park, PA) in all but the first year of the experiment (Table 1). Soil available P greatly exceeds crop needs at both farms, characteristic of the Mid-Atlantic region of the US (Sims *et al.*, 2002). Subsamples of the collected soil were used for most probable number [MPN] bioassays to quantify the populations of AM fungi with which the inoculated AM fungi had to compete (Alexander, 1965). Dilutions ranging from 10⁻¹ to 10⁻⁴, with 3-5 replicates per dilution, were prepared with bahiagrass (*Paspalum*

notatum Flugge) as the host plant. The diluent was an autoclaved mixture of field soil, pool filter sand, vermiculite, and calcined clay (0.75:1.0:1.0:0.75 v/v/v/v). Bioassays were conducted for 4 wks in a controlled environment chamber (Conviron) (day/night 16/8 h, 25/18 °C, and PAR=685 μmol m⁻² sec⁻¹) after which entire root systems were washed free of soil mix. Roots were then stained with trypan blue (Phillips and Hayman, 1970) and scored for presence or absence of AM fungus colonization using a dissecting microscope (20-50X).

Inoculum production, utilization, and outplanting: AM fungus inocula used were produced at each site using the method for on-farm production of AM fungus inoculum described earlier (Douds *et al.*, 2006; 2010). Briefly, seven gallon (26.5 L) plastic bags (“Grow Bags,” Worm’s Way, Bloomington, IN 47404) were two thirds filled with a 1:4 (v/v) mixture of compost and vermiculite. Bahiagrass seedlings, colonized by one of a variety of AM fungi (*Funneliformis mosseae*, *Claroideoglossum claroideum*, and *Glomus* sp. A, isolated from soils of the Farming Systems Trial at the Rodale Institute (Kutztown, PA); *Glomus* sp. B (originating from the Stoneleigh Estate, Villanova PA); and *Rhizophagus intraradices* (DAOM 181602)) were transplanted into the bags after the threat of frost had passed. Some bags prepared in 2012, for the 2013 experiments, propagated an “indigenous mixed” inoculum (Douds *et al.*, 2010). These bags received 100 cm³ of sieved field soil as starter inoculum and nonmycorrhizal bahiagrass seedlings. Bags were weeded and watered as needed throughout the growing season, after which the bahiagrass was winter killed. The bags then overwintered *in situ*. The following spring, the compost and vermiculite mixtures from bags used to propagate 3 or more AM fungi were mixed to yield the inoculum for that year’s experiment (Table 2). MPN bioassays were conducted on the inoculum, as outlined above.

Slips of *I. batatas* cv ‘Beauregard’ were utilized in 2009, 2010, and 2012 and were obtained from Trauger’s Farm Market (Kintnersville, PA 18930). This cultivar was unavailable in 2013, so for that and the next year the replacement cultivar, ‘Covington’, recommended by the supplier, was used. Upon receipt each year, roots of three plants were stained to verify they were not already colonized by AM fungi. Plants were inoculated at outplanting in 2009 and 2010. Thirty cm³ of the compost and vermiculite mixture containing AM fungi was placed directly into the planting hole. Uninoculated controls received the same volume of a freshly-prepared compost and vermiculite mixture (1:4 v/v) not previously used for inoculum production. A different technique was utilized in later years to avoid the labor intensive hand planting and inoculating. Here, plants first were grown for two weeks in 36 cell plastic flats (100 cm³ cell⁻¹) in inoculated potting media (1:3 or 1:4 [v/v] inoculum:potting mix). Controls received the same volume of compost and vermiculite mixture, as above. The high levels of inoculum used were to ensure a) competition with the indigenous population of AM fungi in the field in 2009 and 2010, and b) development of mycorrhizas during the brief residence in the greenhouse in subsequent years (Table 2). Plants grown in the greenhouse received no supplemental fertilization for this two week period. A subsample of three plants from each treatment was withheld from outplanting and used to quantify AM fungus colonization. Percentage root length colonized by AM fungi was measured via the gridline intersect method after

Table 1. Characteristics of soils at sites used for the study of AM fungus inoculation of *I. batatas*¹

Year	Farm ²	Soil pH	Available P (kg ha ⁻¹)	AM fungi ³ (prop. cm ⁻³)
2010	EPF	6.3	242	15.5
2012	EPF	5.6	591	0.1
2013	EPF	5.3	508	0.4
	SBF	7.2	599	0.1
2014	EPF	5.4	502	7.2

¹ Results of one pooled sample from 5 locations within the study plot.

² EPF= Eagle Point Farm, Kutztown, PA; SBF= Shenk’s Berry Farm, Lititz, PA.

³ Results of most probable number bioassay to determine number of propagules of AM fungi cm⁻³ of field soil.

Table 2. Cultivar, inoculation strategy, propagule levels, and experimental design parameters used in the study of AM fungus inoculation of *I. batatas*

Year	Farm ¹	Cultivar ²	Inoculation method	Inoculum ³ (prop. cm ⁻³)	AM fungi ⁴ (prop. plant ⁻¹)	Plants unit ⁻¹	n
2009	EPF	B	Field	72	2160	6	5
2010	EPF	B	Field	400	12000	5	9
2012	EPF	B	GH	132	3300	10	10
2013	EPF	C	GH	155	10000	10	10
	SBF	C	GH	400	3100	3	12
2014	EPF	C	GH	400	8000	10	10

¹ EPF= Eagle Point Farm, Kutztown, PA; SBF= Shenk's Berry Farm, Lititz, PA.

² B= cv 'Beauregard'; C= cv 'Covington'.

³ Results of most probable number bioassay to determine number of propagules of AM fungi cm⁻³ in the compost and vermiculite based inoculum produced on-farm.

⁴ AM fungus propagules per plant, calculated using the inoculum density and volume of inoculum used per plant (30 cm³ for field inoculation, 20 or 25% of the volume of the 100cm³ cell of the flat for the greenhouse method).

clearing and staining (Phillips and Hayman, 1970; Giovannetti and Mosse, 1980).

Outplanting date varied from year to year at Eagle Point Farm and ranged from 25 May in 2010 to 17 June in 2014. Plants were grown on raised beds covered with black plastic at 70 cm spacing with no fertilization. Plants were outplanted somewhat later at Shenk's Berry Farm on 24 July, 2013. Beds were not covered at this site. The experimental design varied slightly from year to year, based upon the available length of beds and needs of the particular farm. Typically, two adjacent beds were utilized, each with alternating runs of 10 inoculated and 10 uninoculated plants arranged to ensure an AM fungus inoculated run in one bed had an uninoculated immediate neighbor in the adjacent bed. Usually there were ten such 10 plant harvesting units per inoculation treatment (Table 2). Beds were weeded as needed, and sprayed twice with "Liquid Fence" (Liquid Fence Co., Mt. Pocono, PA 18344) to inhibit deer browse.

Data collection and analysis: Harvest dates varied slightly from year to year, and occurred from 19 September to 14 October. Sweet potatoes were unearthed by hand using a digging fork. Adhering soil was brushed off and the total yield per sampling unit was weighed in the field. Samples of unearthed roots from each of six to nine paired sampling units of inoculated and control plots were brought back to the lab in 2012 through 2014 and assayed for percentage root length colonized by AM fungi to determine if the inoculation effect persisted to harvest.

Percentage root length colonized data were analyzed via ANOVA after arc sin transformation. Mycorrhizal Yield Response (MYR) was calculated using sweet potato yield data for paired, adjacent inoculated (Myc) and uninoculated (Nonmyc) sampling units according to Hetrick *et al.* (1992):

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

The significance of the mean MYRs over the six site-years of the experiment was determined using a simple 95% confidence interval (CI) calculation. If the range of the CI did not include zero, the MYR was considered significant.

Results

AM fungus colonization of roots at the time of outplanting ranged from 2.1 ± 0.5% of root length (mean ± SEM, n=3) in 2014 to 8.7 ± 2.3% (mean ± SEM, n=3) in 2012, indicating that two weeks of exposure to the inoculum levels used was sufficient to establish

mycorrhizas. No colonization was seen in roots of uninoculated plants after the greenhouse growth period. Further, the roots collected from the field after harvest showed greater percentage root length colonized in previously inoculated plants than for those colonized only by the indigenous population of AM fungi in the field (Table 3).

Yield per plant ranged from a high of 3.8 ± 0.08 (mean ± SEM) kg in 2009 to a low of 1.1 ± 0.1 kg for the short growing period at SBF in 2013 (Fig. 1). Though the MYR was positive each year, and ranged from 19.2% in 2009 to 7.1% in 2014, yields were not significantly different for inoculated vs. uninoculated in any given year (eg. Pr>F = 0.1817 for 2009) (Fig. 1). However, the

Table 3. AM fungus colonization of roots of *I. batatas* at harvest¹

Treatment	2012		2013		2014
	EPF	EPF	SBF	EPF	EPF
Inoculated	33.6 ± 3.1	59.1 ± 3.9	54.0 ± 4.7	25.3 ± 3.2	
Uninoculated	20.7 ± 4.5	39.9 ± 2.7	34.7 ± 4.8	14.3 ± 2.1	
ANOVA					
P>F	0.0326	0.0010	0.0170	0.0131	
MSD	11.68	10.12	15.06	8.28	

¹ Means of 6-9 observations ± SEM. EPF= Eagle Point Farm, Kutztown, PA; SBF= Shenk's Berry Farm, Lititz, PA; MSD= minimum significant difference, Tukey's Method of Multiple Comparisons.

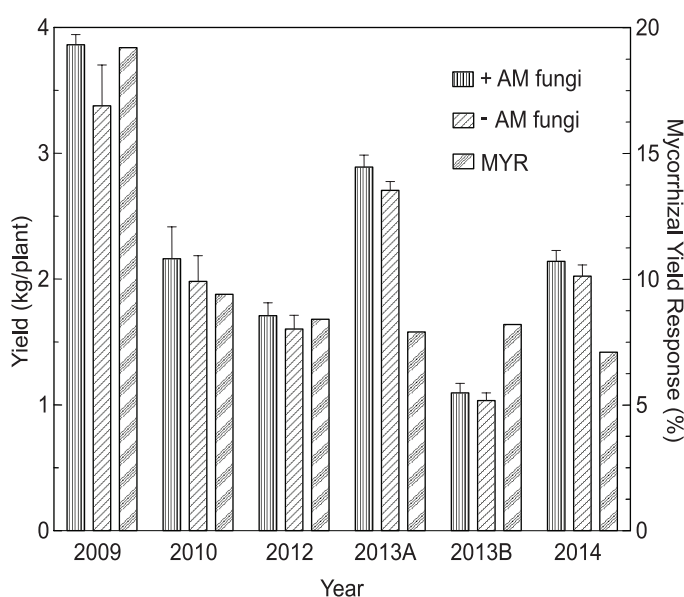


Fig. 1. Yield of *I. batatas* with or without inoculation with AM fungi (left axis) and mycorrhizal yield response. Each bar for the yield is the mean of 5-12 observations ± SEM (see Table 2). Experiments were conducted at Eagle Point Farm except 2013B= Shenk's Berry Farm.

mean MYR, $10.03 \pm 1.86\%$, was significantly different than zero ($95\% \text{ CI: } 5.25\% \leq \bar{y} \leq 14.81\%$), indicating a significant positive response over the long term.

Discussion

Inoculation of sweet potato slips with AM fungi increased yield of tuberous roots by 10% over experimental controls. Relatively few reports exist in the literature in which sweet potatoes with and without AM fungus inoculation were grown to commercial maturity in the field. Greenhouse and growth chamber studies have examined the role of mycorrhizas in plant tolerance of acid soils (Yano and Takaki, 2005), the response to inoculation with a variety of AM fungi (Gai *et al.*, 2006), or the influence of P addition upon the mycorrhizal growth response (Negeve and Roncadori, 1985). These experiments were terminated before significant growth of tubers. Growth of plants in the field to harvest has been conducted in China (Farmer *et al.*, 2007). This experiment found a statistically non-significant trend for AM fungus inoculated sweet potatoes to have greater yields than uninoculated, and also a trend for greater levels of sugar and beta-carotene in the tubers. Yields were much less (approx. $0.6 \text{ kg plant}^{-1}$), than found here. O'Keefe and Sylvia (1993) studied the development of AM fungus colonization of roots and yield of sweet potatoes inoculated in the planting hole with one of two AM fungus species. Inoculation had no significant effect upon shoot growth or total accumulation of P or Zn, but yield data indicated a much earlier production of tubers in the inoculated plants. Twenty weeks after planting, uninoculated plants averaged just $0.2 \text{ kg plant}^{-1}$ while yield from the inoculated plants was significantly greater at 1.7 to $2.3 \text{ kg plant}^{-1}$. Differences narrowed considerably at 27 weeks after planting when yield of controls was not significantly different than that of one of the AM fungus inoculation treatments.

Given that crop growth responses to the AM symbiosis decrease with increasing soil P availability, how can the consistent yield increase noted here with soil available P levels well above optimal be explained? First, AM fungi are known to provide protection from biotic and abiotic stressors, not measured in this experiment, which could have an impact upon yield (Koltai and Kapulnik, 2010). Second, early season P uptake of plants is negatively impacted by the reduction in P solubility by low soil temperatures (Sheppard and Racz, 1984) and the time needed for formation of mycorrhizas by the indigenous community of AM fungi. Early season P nutrition has been shown to be a determinant of yield in many crops (Grant *et al.*, 2001). Enhanced P uptake of the inoculated vs. that of uninoculated plants immediately upon outplanting could have had long-lasting impact upon yield. This idea is supported by the work of O'Keefe and Sylvia (1993) who found early season enhancement of shoot P levels of *I. batatas* cv. 'White Star' by inoculation with the AM fungus *Acaulospora rugosa* even though the soil available P level was 159 mg kg^{-1} in the 30-60 soil depth. There were no differences in shoot P later in the season, yet significant increases in yield of tubers over controls at both 20 and 27 weeks after planting.

On-farm production of inoculum has been demonstrated to be consistently successful (Douds *et al.*, 2014). Further, propagule densities are sufficient for application in the field or as amendments to horticultural potting media. Routine use of AM

fungus inoculum for sweet potato production can confidently be expected to increase storage root yield, even in high P soils.

Acknowledgments

We thank L. Rogers, R. Mitchell, and M. Lohman for assistance with fieldwork.

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