ONION (*Allium cepa* 'Calibra')
Host response; mycorrhizal colonization

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The influence of soil phosphorus levels on onion root colonization by arbuscular mycorrhizal fungi from commercial inoculants, 2017.

Symbiotic arbuscular mycorrhizal fungi (AMF) form associations with most plant species. AMF potentially enhance crop performance by helping host plants mine the soil for immobile nutrients, particularly phosphorus (P), and resist some biotic and abiotic stresses. AMF inoculants are proliferating in the marketplace but there is little independent data on their efficacy. This study evaluated the influence of four commercial inoculants on AMF colonization of onion roots, onion growth, and nutrient acquisition as seedlings. Since soil P concentration can influence AMF colonization of roots, the effect of soil P concentration on AMF colonization of onion roots by the four AMF inoculants was tested at a range of soil P concentrations. Soil was collected from a farm in Pasco, WA, that had 25 ppm P (Olsen test), shredded, steam-pasteurized at 65°C for 1 h, then air-dried and sieved to a particle size ≤2 mm. Superphosphate (0-45-0, Wilbur Ellis Co., Mount Vernon, WA) was added to create medium (45 ppm) and high (74 ppm) P treatments. Non-amended soil served as the low P treatment. Liquid, powder, and granular formulations of AGTIV (Premier Tech Agriculture, Rivière du Loup, Quebec) were compared to Mykos Gold Granular (Reforestation Technologies International, Gilroy, CA) and non-inoculated control soil for each soil P level. Each inoculant was added to each soil P treatment at a rate to achieve ~1,500-2,000 AMF spores per kg soil, using a twin shell blender: 20 g Mykos Gold Granular in 2,980 g soil, 30 g AGTIV Granular in 2,970 g soil, 0.354 g AGTIV Powder suspended in 360 ml water in 3,000 g soil, and 30 ml AGTIV Liquid in 2,970 g soil. Each trial was set up as a 3 x 5 factorial, randomized complete block design with five replicates. Each plot was a D40 deepot (25 cm deep × 7 cm diameter, Stuewe & Sons, Inc., Tangent, OR) with 500 g of soil of the appropriate P × AMF treatment. Ten seeds of the cv. Calibra were sown in each deepot. Deepots were watered as needed and fertilized three times weekly from 3 weeks after planting with 0.4-strength, nitrate-type Long Ashton fertilizer (without micronutrients or P). The number of onion seedlings emerged in each pot was counted weekly from one to 8 weeks after planting, and combined into an area under the emergence progress curve (AUEPC). Onion seedlings were harvested 56 days after seeding when leaf length and above-ground dry weight (leaf biomass) of all plants per deepot were measured. Dried leaves were used for foliar nutrient analysis (Soiltest Farm Consultants, Moses Lake, WA). Roots were washed, stained (Verheilig et al. 1998), and examined microscopically (5× to 20× magnification) to quantify AMF colonization in each of 40 root sections per deepot based on a modified gridline intersection method (Giovannetti and Mosse 1980). The trial was repeated with soil from the same field, at final concentrations of 21, 61, and 101 ppm P. Data were evaluated by two-way analyses of variance (ANOVAs). Dunnett's Test or the Games Howell Test (with Bonferroni correction to preserve a family-wise $\alpha = 0.05$) were used post hoc where significant AMF inoculant effects were identified, and Tukey's Test for significant fertilizer effects. Analyses were done in R version 3.1.1. The thermophilic fungus *Peziza* developed after soil steaming in 33% of the plots in Trial 2, impacting onion growth, so data from those plots were treated as missing and imputed using the R package 'mice' (van Buuren and Groothuis-Oudshoorn 2011). The trials were completed in a growth chamber set at 15 ± 1°C with a 12 h photoperiod/day. Trial 1 was planted on 6 Sep and Trial 2 on 29 Sep.

Means ± standard errors by AMF treatment are presented in Table 1. Where ANOVAs identified significant interactions between AMF and soil P treatments, results are separated by soil P treatment. In both trials, onions grown in low P soil amended with AGTIV Granular, AGTIV Powder, and Mykos Gold Granular had significantly more AMF root colonization than those in control soil (0%). Maximum root colonization (30.2%) was observed in low P soil with Mykos Gold Granular in Trial 2. Onions in this soil in Trial 1 had 27.2% root colonization. No other treatment combination in either trial yielded >10% root colonization. For all four inoculants in both trials, AMF root colonization rate decreased as soil P concentration increased, and Mykos Gold Granular yielded significantly greater colonization in low P soil than medium or high P soil. Onions in soil with AGTIV inoculants had lower overall root colonization rates with less differentiation across soil P treatments. AGTIV Liquid resulted in the least colonization. In neither trial was AMF root colonization associated with changes in onion growth (emergence, leaf length, and biomass) or nutrient acquisition. Results by soil P treatment are shown in Tables 2 (Trial 1) and 3 (Trial 2). In both trials, onion leaf length and biomass were greater in soil with medium and high P than in soil with low P. Also, maximum leaf length and biomass were observed in the medium P soil rather than the high P soil, although the differences in leaf length were only significant in Trial 2 (146 mm in high P soil vs. 165 mm in medium P soil, $p < 0.001$). Soil P concentrations covered a larger range in Trial 2, which might explain this discrepancy. For most micro- and macro-nutrients, onion foliar concentration was greater in the low P soil than the medium or high P soils, likely reflecting nutrient dilution in the greater biomass of plants in medium and high P soils. The exception was foliar P, which was significantly greater in concentration with each increase in soil P concentration. Results from this study suggest that, although granular formulations of AGTIV and Mykos Gold resulted in moderate rates of onion root colonization by AMF in the steamed soil used in this study, the increase in AMF root colonization compared to non-inoculated soil did not increase plant growth or nutrient acquisition. The most effective inoculant in this study, Mykos Gold Granular, was 76% less effective at inducing AMF colonization of onion roots in soil with 61 ppm P compared to 21 ppm P; and 64% less effective in soil with 45 ppm P compared to 25 ppm P. The results support prior evidence of higher soil P concentrations inhibiting AMF colonization of onion roots (e.g., Henrichs et al. 2017).

Table 1. Means ± standard errors by AMF treatment, Trials 1 and 2

Plant attribute	Control	AGTIV Granular	AGTIV Liquid	AGTIV Powder	Mykos Gold Granular	Transformation[z]
Trial 1						
AMF root colonization (%)						-
- Low P	0.0 ± 0.0	6.3 ± 1.2 *[y]	1.1 ± 0.2	5.7 ± 1.4 *	27.2 ± 4.8 *	√(x+1)
- Mid P	0.1 ± 0.1	4.0 ± 1.0 *	1.6 ± 0.5	3.2 ± 0.7 *	9.6 ± 1.4 *	√(x+1)
- High P	0.1 ± 0.1	1.8 ± 0.4	1.1 ± 0.3	3.1 ± 0.9 *	8.3 ± 2.1 *	√(x+1)
AUEPC[x]	346 ± 11	329 ± 9	345 ± 11	340 ± 11	358 ± 11	-
Leaf length (mm)	180 ± 7	164 ± 10	164 ± 10	171 ± 8	159 ± 10 *	-
Leaf biomass (g)	0.70 ± 0.06	0.57 ± 0.07	0.70 ± 0.08	0.65 ± 0.07	0.64 ± 0.07	-
Total foliar N (%)	3.17 ± 0.28	3.75 ± 0.36	3.12 ± 0.34	3.06 ± 0.27	3.50 ± 0.30	np
Foliar P (%)	0.38 ± 0.03	0.36 ± 0.04	0.33 ± 0.02	0.37 ± 0.03	0.33 ± 0.03	-
Foliar K (%)	5.02 ± 0.31	5.52 ± 0.47	4.60 ± 0.35	4.79 ± 0.24	4.98 ± 0.35	-
Foliar Ca (%)	0.99 ± 0.04	1.12 ± 0.08	0.99 ± 0.06	0.98 ± 0.04	1.07 ± 0.06	-
Foliar Mg (%)	0.28 ± 0.01	0.30 ± 0.02	0.27 ± 0.01	0.27 ± 0.01	0.30 ± 0.01	-
Foliar Na (%)	0.06 ± 0.01	0.07 ± 0.01	0.06 ± 0.01	0.06 ± 0.00	0.06 ± 0.01	-
Foliar S (%)	0.42 ± 0.03	0.47 ± 0.04	0.39 ± 0.04	0.37 ± 0.03	0.45 ± 0.03	-
Foliar Zn (ppm)	22.8 ± 1.5	27.3 ± 2.8	21.5 ± 2	22.1 ± 1.5	25.9 ± 2.3	-
Foliar Fe (ppm)	483 ± 31	490 ± 31	481 ± 38	550 ± 64	474 ± 51	-
Foliar Mn (ppm)	123 ± 11	148 ± 22	126 ± 19	143 ± 13	147 ± 26	-
Foliar Cu (ppm)	1.29 ± 0.19	1.12 ± 0.22	1.19 ± 0.2	0.87 ± 0.11	0.86 ± 0.13	-
Foliar B (ppm)	16.4 ± 0.5	17.6 ± 1.1	16.2 ± 0.7	15.6 ± 0.5	17.1 ± 0.8	-
Trial 2						
AMF root colonization (%)						-
- Low P	0.0 ± 0.0	6.3 ± 0.6 *	4.7 ± 1.8	5.5 ± 1.6 *	30.2 ± 1.8 *	-
- Mid P	0.0 ± 0.0	4.0 ± 0.9	2.0 ± 0.5	3.5 ± 1.4	7.3 ± 1.6 *	-
- High P	0.0 ± 0.0	2.6 ± 0.9	3.0 ± 1.4	2.7 ± 0.9	5.0 ± 0.8 *	-
AUEPC[x]	417 ± 11	412 ± 11	410 ± 11	417 ± 7	408 ± 11	√
Leaf length (mm)	149 ± 6	141 ± 5 *	142 ± 8	141 ± 7	154 ± 8	-
Leaf biomass (g)	0.56 ± 0.04	0.54 ± 0.04	0.50 ± 0.06	0.53 ± 0.04	0.51 ± 0.05	-
Total foliar N (%)	3.31 ± 0.26	3.30 ± 0.37	3.20 ± 0.32	3.52 ± 0.25	3.83 ± 0.31	√
Foliar P (%)	0.36 ± 0.03	0.34 ± 0.02	0.36 ± 0.02	0.39 ± 0.02	0.34 ± 0.02	-
Foliar K (%)	4.99 ± 0.26	4.69 ± 0.27	4.95 ± 0.26	4.78 ± 0.22	5.16 ± 0.20	-
Foliar Ca (%)	1.26 ± 0.04	1.19 ± 0.04	1.23 ± 0.05	1.32 ± 0.03	1.30 ± 0.03	-
Foliar Mg (%)	0.32 ± 0.01	0.30 ± 0.01	0.31 ± 0.01	0.33 ± 0.01	0.33 ± 0.01	-
Foliar Na (%)	0.06 ± 0.00	0.06 ± 0.00	0.06 ± 0.00	0.06 ± 0.00	0.06 ± 0.00	√
Foliar S (%)	0.45 ± 0.02	0.44 ± 0.03	0.44 ± 0.03	0.42 ± 0.02	0.51 ± 0.02	-
Foliar Zn (ppm)	23.2 ± 1.9	24.9 ± 2.7	24 ± 2.1	23.9 ± 1.9	24.9 ± 2.1	-
Foliar Fe (ppm)	575 ± 56	571 ± 50	591 ± 60	575 ± 53	493 ± 67	-
Foliar Mn (ppm) - Low P	378 ± 25	387 ± 39	374 ± 14	336 ± 24	270 ± 27 *	-
- Mid P	98 ± 8	132 ± 6 *	95 ± 5	146 ± 5 *	66 ± 3 *	-
- High P	107 ± 9	142 ± 24	185 ± 35	97 ± 14	110 ± 12	-
Foliar Cu (ppm)	2.10 ± 0.17	2.19 ± 0.19	1.84 ± 0.18	1.75 ± 0.16	2.21 ± 0.18	-
Foliar B (ppm)	17.2 ± 0.6	16.9 ± 0.6	17.1 ± 0.5	17.7 ± 0.6	17.8 ± 0.6	-

[z] If needed, data were transformed to meet assumptions for parametric analysis: square root (√) or square root of value (x) + one (√(x+1)); - = no transformation; "np" = data analyzed non-parametrically.

[y] * = The treatment differed significantly from the control plot for that variable (Dunnett's test, $0.01 \leq p \leq 0.05$).

[x] AUEPC = Area under emergence progress curve for weekly stand counts for 8 weeks after planting.

Table 2. Means ± standard errors by soil P treatment, Trial 1

Plant attribute	Low soil P (25 ppm)	Medium soil P (45 ppm)	High soil P (74 ppm)	Pr (>F)[z]	Transformation[y]
AMF root colonization (%)					-
- Control	0.0 ± 0.0	0.1 ± 0.1	0.1 ± 0.0		-
- AGTIV Granular	6.3 ± 1.2 a	4.0 ± 1.0 ab	1.8 ± 0.4 b	*	-
- AGTIV Liquid	1.1 ± 0.2	1.6 ± 0.5	1.1 ± 0.3		-
- AGTIV Powder	5.7 ± 1.4	3.2 ± 0.7	3.1 ± 0.9		-
- Mykos Gold Granular	27.2 ± 4.8 a	9.6 ± 1.4 b	8.3 ± 2.1 b	**	-
AUEPC[x]	346 ± 8	337 ± 9	349 ± 8		-
Leaf length (mm)	134 ± 4 b	188 ± 5 a	185 ± 5 a	***	-
Leaf biomass (g)	0.37 ± 0.02 b	0.82 ± 0.04 a	0.81 ± 0.03 a	***	-
Total foliar N (%)	4.55 ± 0.16 a	2.57 ± 0.11 b	2.68 ± 0.13 b	***	np
Total foliar P (%)	0.26 ± 0.01 c	0.36 ± 0.01 b	0.45 ± 0.02 a	***	√
Total foliar K (%)	6.15 ± 0.16 a	4.28 ± 0.17 b	4.34 ± 0.23 b	***	-
Foliar Ca (%)	1.24 ± 0.04 a	0.88 ± 0.02 b	0.94 ± 0.03 b	***	√
Foliar Mg (%)	0.33 ± 0.01 a	0.25 ± 0.01 b	0.27 ± 0.01 b	***	-
Foliar Na (%)	0.08 ± 0.00 a	0.05 ± 0.00 b	0.05 ± 0.00 b	***	√
Foliar S (%)	0.53 ± 0.02 a	0.35 ± 0.02 b	0.37 ± 0.02 b	***	-
Foliar Zn (ppm)	31.8 ± 1.2 a	18.9 ± 0.8 b	19.9 ± 0.9 b	***	-
Foliar Fe (ppm)	600 ± 37 a	454 ± 34 b	423 ± 20 b	***	Log
Foliar Mn (ppm)					
- Control	162 ± 13	114 ± 21	98 ± 7		-
- AGTIV Granular	229 ± 13 a	102 ± 17 b	74 ± 9 b	**	-
- AGTIV Liquid	196 ± 27 a	79 ± 11 b	85 ± 6 b	**	-
- AGTIV Powder	159 ± 7	138 ± 35	131 ± 18		-
- Mykos Gold Granular	250 ± 22 a	108 ± 35 b	67 ± 6 b	**	-
Foliar Cu (ppm)	0.93 ± 0.10	1.25 ± 0.15	1.00 ± 0.15		-
Foliar B (ppm)	18.2 ± 0.6 a	15.1 ± 0.4 b	16.1 ± 0.5 b	**	-

[z] Asterisks indicate variables for which significant differences among treatments were identified in the analysis of variance: * = $0.01 \leq p \leq 0.05$, ** = $0.001 \leq p < 0.01$, *** = $p < 0.001$; values in a row with the same letter are not significantly different according to Tukey's Honestly Significant Difference test at $p < 0.05$.

[y] Where necessary, data were transformed to meet assumptions for parametric analysis: square root (√) or logarithm (Log); - = no transformation was needed; "np" = data were analyzed non-parametrically.

[x] AUEPC = Area under the emergence progress curve based on weekly stand counts for 8 weeks after planting.

Table 3. Means ± standard errors by soil P treatment, Trial 2

Plant attribute	Low soil P (21 ppm)	Medium soil P (61 ppm)	High soil P (101 ppm)	Pr (<F)[z]	Transformation[y]
AMF root colonization (%)					-
- Control	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0		-
- AGTIV Granular	6.3 ± 0.6 a	4.0 ± 0.9 ab	2.6 ± 0.9 b	*	-
- AGTIV Liquid	4.7 ± 1.8	2.0 ± 0.5	3.0 ± 1.4		-
- AGTIV Powder	5.5 ± 1.6	3.5 ± 1.4	2.2 ± 1		-
- Mykos Gold Granular	30.2 ± 1.8 a	7.3 ± 1.6 b	5.0 ± 0.8 b	***	-
AUEPC[x]	418 ± 7	408 ± 10	413 ± 6		-
Leaf length (mm)	126 ± 4 c	165 ± 4 a	146 ± 5 b	***	-
Leaf biomass (g)	0.38 ± 0.02 b	0.63 ± 0.03 a	0.57 ± 0.03 a	***	-
Total foliar N (%)	4.42 ± 0.18 a	3.10 ± 0.22 b	2.78 ± 0.17 b	***	√
Total foliar P (%)	0.26 ± 0.01 c	0.36 ± 0.01 b	0.45 ± 0.01 a	***	Log
Total foliar K (%)	5.78 ± 0.12 a	4.62 ± 0.17 b	4.35 ± 0.15 b	***	-
Foliar Ca (%)	1.29 ± 0.02	1.22 ± 0.03	1.27 ± 0.03		-
Foliar Mg (%)	0.32 ± 0.01	0.31 ± 0.01	0.33 ± 0.01		-
Foliar Na (%)	0.07 ± 0.00 a	0.05 ± 0.00 b	0.05 ± 0.00 b	***	-
Foliar S (%)	0.51 ± 0.02 a	0.44 ± 0.02 b	0.40 ± 0.01 b	***	Log
Foliar Zn (ppm)	33.5 ± 1.3 a	20.5 ± 0.8 b	18.6 ± 0.7 b	***	Log
Foliar Fe (ppm)	759 ± 38 a	419 ± 20 b	505 ± 39 b	***	-
Foliar Mn (ppm)					
- Control	378 ± 25 a	98 ± 8 b	107 ± 4 b	***	-
- AGTIV Granular	387 ± 39 a	132 ± 6 b	142 ± 24 b	***	-
- AGTIV Liquid	374 ± 14 a	95 ± 5 b	185 ± 35 b	***	-
- AGTIV Powder	336 ± 24 a	146 ± 5 b	97 ± 14 b	***	-
- Mykos Gold Granular	270 ± 27 a	66 ± 12 b	110 ± 12 b	***	-
Foliar Cu (ppm)	2.16 ± 0.14	2.03 ± 0.13	1.86 ± 0.14		-
Foliar B (ppm)	19.0 ± 0.4 a	17.0 ± 0.4 b	16.1 ± 0.4 b	***	-

[z] Asterisks indicate variables for which significant differences among treatments were identified in the analysis of variance: * = $0.01 \leq p \leq 0.05$, ** = $0.001 \leq p < 0.01$, *** = $p < 0.001$; values in a row with the same letter are not significantly different according to Tukey's Honestly Significant Difference test at $p < 0.05$.

[y] Where necessary, data were transformed to meet assumptions for parametric analysis: square root (√) or logarithm (Log); - = no transformation.

[x] AUEPC = Area under the emergence progress curve based on weekly stand counts for 8 weeks after planting.