

**The effect of formulation and application method of arbuscular mycorrhizal fungi inoculants on onion seedlings in a growth chamber study, 2016.**

Arbuscular mycorrhizal fungi (AMF) inoculants are of interest for the potential to enhance crop performance, manage soilborne diseases, and reduce reliance on fertilizer and fungicide inputs. Commercial AMF inoculants are available in several formulations and may be applied in various ways, but little independent data exist on the relative effectiveness of formulation × application method combinations. This study assessed four such combinations for the effects on onion root colonization by AMF and onion seedling growth (shoot height, dry weight, and foliar nutrient concentration) in a growth chamber setting, using the AMF product Mykos Gold (RTI-Ag, Gilroy, CA). The growth chamber was set at  $15 \pm 1^\circ\text{C}$  with a 12 h photoperiod/day. A randomized complete block experimental design was used with five replicate blocks. One plot consisted of one D40 Deepot (25 cm deep × 7 cm diameter, Stuewe & Sons, Inc., Tangent, OR) with 500 g of soil in which 10 onion seeds of the cv. Calibra were planted. Treatments were: 1) No AMF product applied to the seed or soil (control treatment); 2) Mykos Gold Liquid applied as a seed treatment; 3) Mykos Gold Liquid drenched over the soil to mimic an in-furrow application; 4) Mykos Gold Granular banded over the soil to mimic an in-furrow application; and 5) Mykos Gold Granular blended into soil to mimic a banded, incorporated application prior to planting. Soil was sampled from a certified organic field in the Columbia Basin, for which tests indicated a relatively low soil phosphorus (P) concentration (18 ppm, Olsen P test). The soil was steam-pasteurized twice for 1 h each time at 68-70°C, with a 24 h interval between pasteurizations. The soil was then air-dried and sieved to a particle size  $\leq 2$  mm. For the control treatment, there was no AMF amendment to the soil or onion seed. For the Mykos Gold Liquid seed treatment, Mykos Gold Liquid was applied to seed by Skagit Seed Services (La Conner, WA) and the treated seed planted directly into pasteurized soil. For the Mykos Gold Liquid drench treatment, 50 ml of water was mixed with Mykos Gold Liquid and the suspension drenched over the soil surface in the Deepot at a rate of 2,400 propagules per plot. For the Mykos Gold Granular banded treatment, 5 g of Mykos Gold Granular (80 propagules) was spread over the top of the pasteurized soil in each replicate plot. For the Mykos Gold Granular incorporated treatment, 30 g of Mykos Gold Granular (80 ppg) was blended with 2,970 g of pasteurized soil using a Gustafson blender, with 500 g subsamples placed in each replicate Deepot. Prior to adding soil to each Deepot, the drain holes at the lower end were sealed with crushed paper towels. After placing 500 g soil from the appropriate treatment into each Deepot, water (150 ml/Deepot) was added in 50 ml increments. Ten onion seeds were placed on the soil surface and covered with one tablespoon of dry soil that was not amended with an AMF product. Deepots were watered as needed and fertilized weekly from four 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 eight weeks after planting, and the counts combined into an area under the emergence progress curve. Onion seedlings were harvested 56 days after planting when leaf length and above-ground dry weight of all plants per plot were measured. Dried leaves were used for foliar nutrient analysis (Mukang Labs, Pasco, WA). Roots were washed, stained according to Verheilig et al. (1998), and examined microscopically (10× to 20× magnification) to quantify AMF colonization in each of 40 root sections per plot based on a modified gridline intersection method (Giovannette and Mosse 1980). Results were analyzed in R version 3.3.1 (R Foundation for Statistical Computing, Vienna, Austria) by fitting a one-way analysis of variance (ANOVA) for each of 15 plant growth indicators measured. Model residuals were examined visually and using the Shapiro-Wilk test to check suitability of the modelling approach; where necessary, data were transformed. Where significant ( $p < 0.05$ ) treatment effects were identified in the ANOVA, Dunnett's test was used to compare each AMF treatment to the control treatment.

Significant AMF treatment effects were detected for AMF root colonization, foliar nitrogen (N) concentration, and foliar calcium (Ca) concentration. AMF colonization was not observed for any of the roots examined from the control treatment, as expected for pasteurized soil. Interestingly, AMF root colonization was not observed for either of the Mykos Gold Liquid treatments (seed or drench). In contrast, both granular AMF treatments resulted in root colonization by AMF, with colonization averaging 34% for onion plants in soil with the Mykos Gold Granular banded treatment, and 39% for plants in soil with the Mykos Gold Granular incorporated treatment. Dunnett's tests indicated these values were significantly different from those of the control treatment (both  $p < 0.001$ ). Foliar N concentration was significantly greater for plants grown in soil with the Mykos Gold Granular banded treatment than the control treatment (4.95 vs. 3.90%,  $p = 0.008$ ), and trended towards a greater concentration for plants in soil with the Mykos Gold Granular incorporated treatment (4.29%). Foliar Ca concentration was significantly greater in the foliage of onion plants grown in soil with the Mykos Gold Granular incorporated treatment than in control soil (0.80 vs. 0.72%,  $p = 0.016$ ). Perhaps the most important indicators of seedling growth and development – onion height and biomass – were not affected by any AMF treatment. The results indicated that Mykos Gold Liquid was not effective at inducing colonization of onion roots by AMF, regardless of whether the product was applied to seed or soil. Mykos Gold

Granular caused an average 34% root colonization by AMF when banded onto soil, and 39% when mixed into soil. However, AMF root colonization was not associated with increases in seedling growth (height and dry biomass).

Seedling attribute <sup>z</sup>	Control	Mykos Gold Liquid seed treatment	Mykos Gold Liquid banded	Mykos Gold Granular banded	Mykos Gold Granular incorporated	Pr (>F) <sup>y</sup>
AMF root colonization (%)	0 ± 0	0 ± 0	0 ± 0	34 ± 3*	39 ± 8*	0.000
Area under the emergence progress curve	435 ± 11	416 ± 19	439 ± 16	407 ± 25	415 ± 23	0.679
Dry weight of all plants/plot (g)	0.55 ± 0.06	0.53 ± 0.04	0.55 ± 0.04	0.43 ± 0.04	0.50 ± 0.04	0.245
Plant height (cm)	13.9 ± 0.7	13.8 ± 0.4	13.5 ± 0.4	11.9 ± 0.5	14.2 ± 0.5	0.067
Foliar nitrogen (%)	3.90 ± 0.22	4.00 ± 0.23	3.81 ± 0.17	4.95 ± 0.37*	4.29 ± 0.20	0.008
Foliar phosphorus (%)	0.16 ± 0.02	0.16 ± 0.01	0.16 ± 0.01	0.17 ± 0.01	0.17 ± 0.01	0.797
Foliar potassium (%)	3.26 ± 0.14	3.45 ± 0.10	3.46 ± 0.05	3.41 ± 0.14	3.33 ± 0.13	0.702
Foliar sulfur (%) <sup>x</sup>	0.31 ± 0.02	0.32 ± 0.01	0.31 ± 0.01	0.33 ± 0.02	0.35 ± 0.02	0.218
Foliar boron (ppm)	9.4 ± 0.8	12.3 ± 1.7	11.9 ± 1.3	10.9 ± 0.5	10.0 ± 0.5	0.154
Foliar zinc (ppm)	13.7 ± 1.4	13.0 ± 0.7	12.9 ± 0.4	14.3 ± 0.6	13.7 ± 0.7	0.498
Foliar manganese (ppm)	189.1 ± 13.7	200.7 ± 16.5	211.7 ± 14.8	204.3 ± 21.8	149.9 ± 7.4	0.090
Foliar iron (ppm)	331 ± 37	320 ± 34	330 ± 20	316 ± 24	323 ± 36	0.927
Foliar copper (ppm)	13.2 ± 1.7	13.3 ± 1.1	12.3 ± 0.6	12.9 ± 0.7	13.2 ± 0.7	0.857
Foliar calcium (%)	0.72 ± 0.02	0.78 ± 0.02	0.74 ± 0.01	0.80 ± 0.02*	0.75 ± 0.02	0.011
Foliar magnesium (%)	0.22 ± 0.02	0.22 ± 0.00	0.21 ± 0.00	0.23 ± 0.01	0.21 ± 0.00	0.696

<sup>z</sup> Values are means ± standard errors based on five replicates per treatment.

<sup>y</sup> Probability associated with the treatment term in the analysis of variance fitted for each variable. Treatment means differing significantly ( $p < 0.05$ ) from that of the control treatment according to Dunnett's test are highlighted with an asterisk.

<sup>x</sup> Data were square-root-transformed to meet assumptions of parametric analysis.