WSDA SPECIALTY CROP BLOCK GRANT PROGRAM
FINAL PERFORMANCE REPORT

Project title: Evaluation of arbuscular mycorrhizal fungi for enhanced growth, phosphorus use efficiency, and management of soilborne pathogens of onion and carrot in the Columbia Basin

Grant Agreement Number: K-1769
Report Submitted By: Lindsey du Toit, Washington State University

Project Summary

1. Provide a background for the initial purpose of your project. Include the specific issue, problem, or need that was addressed by the project.

Onions are an important specialty crop in Washington State. Onion bulb crops were planted on 24,000 acres in this state in 2017, earning a farmgate value of $130 million (USDA National Agricultural Statistics Service). The majority of Washington onion production takes place in the Columbia Basin, where growers typically fumigate the soil the fall prior to planting, and apply soluble fertilizers at medium to high rates of application. Carrot production, for which Washington State has the second-largest fresh market acreage in the nation after California, and the largest processing acreage in the nation, is associated with similar intense production practices. These practices may decrease fertilizer use efficiency because of potential negative impacts on crop-beneficial soil mycoflora such as arbuscular mycorrhizal fungi (AMF). AMF form associations with the roots of many plant species (>80% of plant species) and can help plants access soil nutrients and water. Evidence also exists that AMF help hosts plants limit infection by some pathogens as well as damage by some pests and abiotic stresses such as drought and heat stress.

Published scientific studies which informed this project have demonstrated that onions are responsive to the presence of AMF because of the relatively unbranched root structure with very few root hairs compared to most plant species.[1] It is also known, however, that high application rates of soluble fertilizers, especially phosphorus (P) fertilizers, inhibit the association of most AMF with plant roots.[2, 3]

In recent years, several manufacturers have developed commercial AMF inoculants designed to promote colonization of crop roots with AMF species that have been selected to enhance crop performance. There are liquid, powder, and granular formulations of these inoculants, which can be applied as seed coatings, in-furrow at seeding, or banded or broadcast over the soil and incorporated. By using AMF inoculants, onion growers in the Columbia Basin could potentially increase AMF colonization of onion roots, particularly following soil fumigation, which may improve resistance to some soilborne pathogens and pests, and increase yields and quality of onion bulb crops while also reducing the rates of fertilizer applications. These effects would benefit long-term soil health. The project team members were being informed increasingly by onion growers in the Columbia Basin of the use of AMF inoculants. However, there was very little independent information available regarding the efficacy of these inoculants under typical production practices for onion and carrot crops in the Columbia Basin, including interactions of these AMF products with pesticides commonly used in onion and carrot crops (e.g., seed treatments and soil applications of fungicides with potential efficacy against AMF, and fertilization practices that potentially have adverse effects on AMF colonization of plant roots).

[1] Brewster, J. 2008. Onions and Other Vegetable Alliums. Crop Production Science in Horticulture No. 15. CAB International, Wallingford.
[2] Charron, G., Furlan, V., Bernier-Cardou, M., and Doyon, G. 2001. Response of onion plants to arbuscular mycorrhizae. 1. Effects of inoculation method and phosphorus fertilization on biomass and bulb firmness. Mycorrhiza 11:187-197.
[3] Charron, G., Furlan, V., Bernier-Cardou, M., and Doyon, G. 2001. Response of onion plants to arbuscular mycorrhizae. 2. Effects of nitrogen fertilization on biomass and bulb firmness. Mycorrhiza 11:145-150.

A preliminary (2013-14) study conducted by Dr. Lindsey du Toit's program at Washington State University (WSU), funded by the WSU BIOAg program, demonstrated that the commercial AMF inoculant BioTerra Plus enhanced onion growth and eliminated stunting caused by the soilborne fungus *Rhizoctonia solani* when evaluated in growth chamber trials using pasteurized soil sampled from the Columbia Basin (du Toit et al. 2014a, 2014b, 2014c; Sharma-Poudyal et al. 2014). However, the effects were negated by fungicide seed treatments used widely by onion growers in the Columbia Basin. Furthermore, the formulation of this and other AMF commercial products evaluated were incompatible with most onion growers' equipment, because of the predominance of direct-seeding of onion bulb crops and carrot root crops in this region (>99% of crops are direct-seeded, and <1% are planted using transplants), preventing effective evaluation of these products in onion and carrot grower-cooperator trials.[4]

This WSDA SCBG project No. K-1769 was developed to identify AMF inoculants compatible with onion and carrot growers' equipment as well as their production practices typical for the Columbia Basin, and to test the efficacy of these products in the context of commercial, direct-seeded onion and carrot production in the Columbia Basin. The primary objective was to generate independent data on the efficacy, compatibility, and economic viability of AMF inoculants under growers' field conditions. To provide insight into interpreting results from the field trials, the project also included growth chamber trials in which AMF inoculants were tested using pasteurized soil that had been sampled from the Columbia Basin. The growth chamber trials facilitated greater control than the field trials of the many factors that might interact with AMF inoculants (e.g., temperature, soil moisture, soil fertility, and soil microflora). Both the field and growth chamber trials included evaluation of fertilizer treatments in an attempt to quantify the interaction of fertilizers on AMF and the relative effects of AMF inoculants and soluble fertilizers on onion bulb and carrot root production that are representative of grower practices in the Columbia Basin.

2. Establish the motivation for your project by presenting the importance and timeliness of the project.

Regulations on soil fumigation and nutrient management in agriculture are becoming increasingly strict, putting pressure on farmers to find alternative practices for managing crop health and productivity. A growing understanding of the importance of soil health and quality also is motivating action among agricultural stakeholders within Washington State. Internationally, 2015 was declared the 'Year of Soil' by the United Nations' 68th General Assembly. These factors, together with the increasing promotion of adoption of AMF inoculant use by commercial companies, and published literature on onion responsiveness to AMF, encouraged the exploration of AMF inoculants as a potential tool for reduction of fertilizer rates of application and pesticide applications in onion and carrot production in the Columbia Basin. The primary emphasis was on onion production because of anecdotal evidence that several larger onion production operations in this region had begun using AMF inoculants, partially in an effort to rebuild beneficial soil microflora following soil fumigation prior to planting onion bulb crops.

A further motivator for this project was anecdotal evidence received by the project team from farmers and agronomists in the Columbia Basin who were using AMF inoculants that there was a lack of independent, objective data on the agronomic or economic impacts of these inoculants in the various means by which they were being applied, potentially eroding already-slim profit margins. Growers in the Columbia Basin (and elsewhere) are inundated with promotional materials and products from agricultural product manufacturers, but the independent data needed to assess the manufacturers' claims is scarce or lacking, particularly for direct-seeded onion and carrot crops in the Columbia Basin.

3. If the project built on a previously funded SCBG project, describe how this project complimented and enhanced the previously completed work.

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[4] du Toit, L.J., Paulitz, T., Sharma-Poudyal, D., and Knerr, A.J. 2014. Impact of arbuscular mycorrhizal fungi on P use efficiency and root diseases of onion crops in the Columbia Basin. 2014 Report for the Washington State University Center for Sustaining Agriculture and Natural Resources (CSANR) Biologically Intensive and Organic Agriculture (BIOAg) Project. 10 pp.

This project is not a direct continuation of a previous SCBGP project but was inspired by a recent WSDA SCBGP project No. K-525, also led by Dr. Lindsey du Toit. That project investigated stunting in onion and pea crops planted in the Columbia Basin caused by *Rhizoctonia* spp., soilborne fungal pathogens which can severely limit crop yield and quality in the region. Field trials conducted during that project elucidated the etiology and epidemiology of the pathogens in onion bulb crops grown following cereal cover crops, and highlighted the need for effective management strategies. The study helped develop highly effective recommended management practices, including managing the 'green bridge' from cereal cover crops, and pre-plant, banded and incorporated application of the fungicide azoxystrobin. Additional experiments with AMF inoculants towards the end of that study showed suppression of Rhizoctonia stunting in onion seedlings, suggesting that AMF inoculants could provide an effective and environmentally-sound management tool for Columbia Basin growers seeking to control *Rhizoctonia* in onion crops. Dr. du Toit received an additional year of funding from the BIOAg program of the WSU Center for Sustaining Agriculture & Natural Resources to examine AMF inoculants in further detail for management of *Rhizoctonia* and promotion of onion growth. The results served as the foundation for this proposed SCBGP project.

Project Approach

4. Briefly summarize the activities performed and tasks achieved during the project period. Whenever possible, describe the work accomplished in both quantitative and qualitative terms. Include the significant results, accomplishments, conclusions and recommendations. Describe favorable and unusual developments.

This project included numerous field trials conducted on grower-cooperator farms in the Columbia Basin, and growth chamber trials carried out at the WSU Mount Vernon Northwestern Washington Research & Extension Center (NWREC) in Mount Vernon, WA. In total, eight commercial AMF inoculants were tested along with one mycorrhizal stimulant (Table 1). Interested grower-cooperators were given a list of commercial products and formulations at the start of the project, from which to select the product and formulation they thought would be most compatible with their equipment and interests. After the first field season (2016) and accompanying growth chamber trials, it was evident that the liquid formulations did not result in any AMF colonization of onion roots. Therefore, none of the liquid formulations was used in field trials in the second season (2017).

Table 1. Arbuscular mycorrhizal fungi (AMF) products tested in this WSDA SCBGP study.

Product	Manufacturer
AGTIV Specialty Crops Granular	PremierTech Ag, Quebec, Canada
AGTIV Specialty Crops Liquid	PremierTech Ag, Quebec, Canada
AGTIV Specialty Crops Powder	PremierTech Ag, Quebec, Canada
BioTerra Plus Dry Mix	Plant Health LLC, Corvallis, OR
MycoApply Endo Liquid	Mycorrhizal Applications LLC, Grants Pass, OR
MycoApply Ultrafine Endo Powder	Mycorrhizal Applications LLC, Grants Pass, OR
Myconate AS Mycorrhizal Stimulant[1]	Plant Health Care, Inc., Pittsburgh, PA
Mykos Gold Specialty Crops Granular	RTI-Ag, Gilroy, CA
Mykos Gold Specialty Crops Liquid (also known as "Mykos Gel")	RTI-Ag, Gilroy, CA

[1]Does not contain AMF but contains isoflavonoids that stimulate mycorrhizal fungi in the soil to colonize plant roots.

Field trials

From 2016 to 2018, 18 individual field trials were carried out for this project to test the effects of nine AMF products (Table 1) on crop growth, health, and productivity in three different onion market types (storage [13 trials], dehydration [two trials], and sweet onions [two trials]) and for fresh market carrots (one trial). Six grower-cooperators in the Columbia Basin served as the hosts for these trials. In the majority of the trials, AMF products were distributed from gandy boxes into the seed furrow at planting. The exceptions were the dehydration onion trials and the carrot trial, in which the AMF inoculants were applied to the seed by certified commercial seed conditioning operations, and the treated seed shipped to Drs. du Toit or Waters or the grower-cooperator for planting. The trials were all set up in the growers' crops, and each grower used their standard practices including, in most cases, planting fungicide-coated (pelletized) seed, insecticides, fungicides, and soluble fertilizer applications with a few exceptions in the trial to fit the specific treatments being evaluated.

Field trial evaluations typically included early season stand counts; mid-season sampling for aboveground dry biomass of plants, leaf length, and foliar nutrient concentration; and sampling of the mature crop close to when the grower harvested the surrounding crop, for grading and yield quantification. Disease was also assessed mid-season or on mature crops: severity of pink root was evaluated on the roots of bulbs in all the storage onion trials and the dehydration onion trials, and both pink root severity and white rot incidence were evaluated in the sweet onion trials in Walla Walla. Carrot roots were evaluated for cavity spot and other diseases in the carrot trial. In four of the field trials, onion bulbs were stored for one month following harvest and tested for firmness using a modified penetrometer. Bulbs harvested from the two dehydration onion crops were tested for total dissolved solids (Brix) using a refractometer. To evaluate the direct efficacy of inoculants at inducing root colonization by AMF, root colonization was quantified in nine of the onion field trials. This was not completed for all of the trials because of the extremely time-consuming, tedious microscopic work required to quantify root colonization effectively. For each trial in which this was completed, roots cut off at least five plants sampled from each plot mid-season were then stained according to the method of Verheilig et al.[5] and examined microscopically (10 to 20x magnification). The number of AMF arbuscules and/or vesicles was counted in 40 root sections, using a modified gridline intersection method.[6] At least five plants per plot were evaluated in this manner.

All the field trial results showed no to minimal effects of the AMF inoculants on all measured indicators of crop health, growth, or productivity. Statistically significant (p<0.05) results were limited to the following:

1) In 2016 trials near Paterson, WA (storage onions), Mykos Gold Granular was associated with a reduction in onion stand count relative to the control plots without AMF inoculant (443 vs. 461 plants per 40 ft of bed), and similar results were observed with AGTIV Granular (456 vs. 471 plants per 40 ft of bed), although this did not translate to a difference in bulb yield for either product compared to control plots.

2) In a 2017-18 overwintered sweet onion trial near Walla Walla, plots inoculated with Mykos Gold Granular had significantly greater stand count than the control plots (49 vs. 45 plants per 10 ft of double-row).

3) In a 2017 trial near Othello, WA (storage onions), plots inoculated with MycoApply and AGTIV Granular had significantly reduced yield of medium grade bulbs relative to the control plots (2.5 lb for MycoApply and 2.6 lb for AGTIV Granular vs. 4.4 lb in the control plots), though this was also associated with a trend (not significant statistically) towards greater yield of jumbo grade bulbs in plots with these two AMF inoculants compared to control plots.

A trend of reduced stand counts in AMF-inoculated plots was observed in several trials although this was only significant statistically in two of the trials. This may have been due to inadvertent reduction in seeding rate caused by placing AMF granules alongside seed in the furrow. Future trials could address this issue by using granules of an inert substance as a control treatment. Similar results were reported by Dr. Thaddeus Gourd from Colorado State University at the 2016 National Allium Research Conference in Savannah, GA, for large-scale, grower-cooperator field trials evaluating AMF inoculants in onion crops in the San Juan Valley of Colorado.

In four of the field trials in which root colonization was quantified, there were no significant differences in root colonization levels between inoculated plots and control plots. In one storage onion trial near Othello, WA in 2016, the

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[5]Verheilig H., Coughlan A.P., Wyss U. and Piché Y. 1998. Ink and vinegar, a simple staining technique for arbuscular-mycorrhizal fungi. Applied and Environmental Microbiology 64 (12): 5004-5007.
[6]Giovannette M., and Mosse B. (1980) An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infections in roots. New Phytol 89:489-500.

inoculant MycoApply was associated with 30% AMF colonization of roots of the onion cv. Arcero vs. 18% in the non-inoculated control plots of that cultivar ($p<0.05$). A 2017 field trial in a spring-planted sweet onion crop near Walla Walla revealed an average 70% AMF colonization of onion roots in plots inoculated with Mykos Gold Granular vs. 59% root colonization in the non-inoculated control plots ($p<0.05$). Both MycoApply and Mykos Gold Granular were tested in multiple trials, and yielded non-significant results in all the field trials except the cases noted above.

An important observation in the 18 field trials was that AMF root colonization was observed in all the non-inoculated control plots, despite many of these fields having been fumigated with metam sodium the fall prior to planting. Onion roots generally showed 40-50% AMF colonization in non-inoculated plots, indicating the presence of endemic root colonizing fungi in the soil of these commercial production fields in the Columbia Basin despite the widespread practice in this region of soil fumigation with metam sodium the fall prior to planting. Metam sodium typically is applied either by chemigation through center pivots or injected into the ground with tractor-mounted shanks. Prior to completing the BIOAg proposal that preceded this WSDA SCBG, this result would have been unexpected. However, a survey of organic and conventional onion fields for prevalence of AMF and AMF community diversity, followed by a survey of fumigated and non-fumigated conventional onion fields, revealed no differences in prevalence of AMF root colonization in organic vs. conventional onion crops, and in fumigated vs. non-fumigated conventional onion crops.[7]

For 12 of the 18 field trials, fertilizer treatments were included to examine the potential effects of rates of fertilizer application on AMF inoculants. Onion and carrot growers in the Columbia Basin make multiple fertilizer applications during the crop cycle. Most growers broadcast fertilizer over the field the fall prior to planting, e.g., in the form of composted manures. In addition, almost all onion growers make a pre-planting or at-planting application of 'starter' fertilizer banded over the bed (usually in a 6- to 8-inch-wide band over the bed), which is then incorporated into the soil with a rototiller. Additional side-dress or top-dress fertilizer applications may be made during the season by center-pivot chemigation or other application methods. In the 12 field trials in this study in which two factors were tested (AMF inoculant as one factor and fertilizer treatment as the second factor), a pre-plant, banded fertilizer application was tested with and without AMF inoculant, in addition to no pre-plant, banded fertilizer application with and without AMF inoculant. In two trials in 2016 near Paterson, WA, the grower-cooperator also included a half-rate of the pre-plant, banded fertilizer application, with and without AMF inoculant.

Results showed no evidence of interaction between pre-plant, banded fertilizer application and AMF inoculant effects. Pre-plant, banded fertilizer treatment effects were generally greater than AMF treatment effects. One or more measures of crop growth and productivity showed a statistically significant positive response of onion crops to the pre-plant, banded fertilizer application in five of the 12 trials that included fertilizer treatments (one trial near Paterson, WA in 2016; and two trials near Paterson, WA and two trials near Irrigon, OR in 2017). The lack of a significant pre-plant, banded fertilizer application response in the other seven field trials in which this was tested suggests that the pre-plant, banded fertilizer application may have exceeded the crop demand in these other onion fields. If so, this represents an opportunity for farms to cut back on fertilizer application rates, which will reduce input costs and, potentially, environmental impacts.

Perhaps the most distinct result from the field trials was the negative impact of the pre-plant, banded fertilizer applications on onion root colonization by AMF. Whether in AMF-inoculated or non-inoculated plots, the degree of root colonization by AMF typically was several percentage points less in plots that received a pre-plant, banded fertilizer application compared to plots in the same trial that did not receive this fertilizer application (Fig. 1). As described above, crop performance was more responsive to this pre-plant, banded fertilizer application than to AMF inoculants in these trials, indicating that the potential benefit offered by a pre-plant, banded fertilizer application outweighs the potential benefit of AMF root colonization in the few mildly nutrient-limited situations encountered in this study in growers' fields in the Columbia Basin.

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[7] Knerr, A.J., Wheeler, D., Schlatter, D., Sharma-Poudyal, D., du Toit, L.J., and Paulitz, T.C. 2018. Arbuscular mycorrhizal fungal communities in organic and conventional onion crops in the Columbia Basin of the Pacific Northwest USA. Phytobiomes 2: *in press*. http://dx.doi.org/10/.1094/PBIOMES-05-18-0022-R

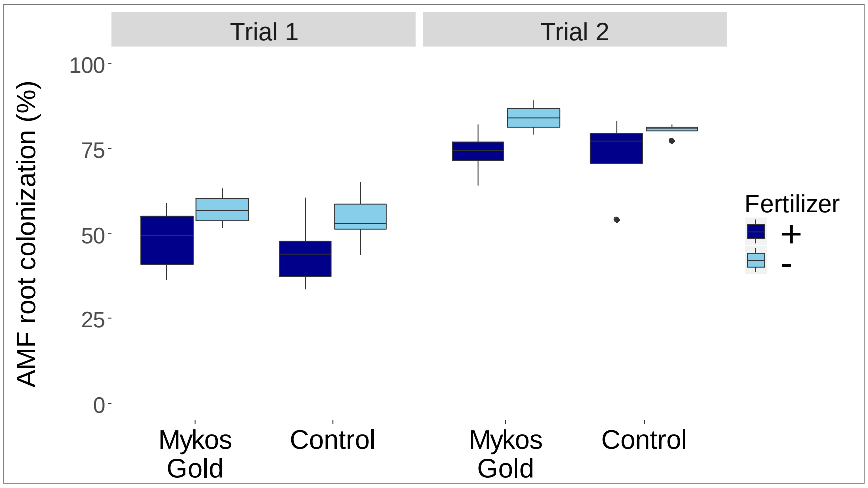

Figure 1. AMF root colonization rates in two field trials from this study in which half the plots received a pre-plant, banded fertilizer application ("+"), and half the plots did not receive this fertilizer application ("-"). Plots inoculated with Mykos Gold Granular were compared to non-inoculated control plots in combination with both fertilizer treatments. Notice that AMF root colonization rates were greater in plots without the pre-plant fertilizer application; and that there was little difference between AMF colonization rates of onion plants in the inoculated (Mykos Gold Granular) plots and the non-inoculated control plots.

Growth chamber trials

Over the duration of this project, eight growth chamber trials were completed to complement the 18 field trials. This included three trials that were each repeated (six trials), plus two individual trials.

To make growth chamber trials as representative as possible of the target growing environment in the Columbia Basin, and based on recommendations of the commercial AMF inoculant manufacturers to get the products in the soil alongside the seed as close to planting as possible, which means early spring for most onion and carrot growers in the Columbia Basin, soil was sampled from onion production fields in the Columbia Basin to set up each growth chamber trial. The soil was pasteurized to eliminate endemic AMF and ensure that any AMF activity detected was a result of the added AMF inoculant(s). Initial soil phosphorus (P) concentration of the soil was approximately 16-18 ppm. The fields sampled were selected for low soil P to facilitate amending soil P to greater concentrations for evaluating the impact of soil fertility on AMF inoculants. In some growth chamber studies, some soil treatments entailed amending the soil with superphosphate to create intermediate (40-60 ppm) and high (75-100 ppm) soil P treatments. Additional fertilizer added to the soil in these growth chamber studies was a nitrate-type Long Ashton suspension without micronutrients or P. One observational unit (replicate) in growth chamber trials was a 7 cm diameter x 24 cm long Conetainer (Deepot) in which 500 g soil was added and seeded with 10 onion seed. Each treatment combination was replicated five times, and each trial was arranged as a randomized complete block design. Most of the growth chamber trials were repeated, for a total of 10 replicates. AMF inoculants were each either blended into the soil or applied as a seed coating, depending on the trial. Seedlings were grown for eight weeks at 15^{o}C to mimic cooler, spring planting conditions. Observations in growth chamber trials typically included weekly seedling emergence counts, and measurement of maximum shoot length (averaged for all seedlings in a conetainer), onion seedling dry weight (total for all seedlings in a conetainer), and foliar onion nutrient concentration 8 weeks after planting. In addition, the degree of root colonization by AMF was evaluated in every growth chamber trial using the root staining and microscopic examination protocol described above in the section "Field Trials".

In the first set of growth chamber trials, Mykos Gold Granular was blended into the soil prior to seeding. This product results in an average AMF root colonization rate of 82% which was significantly greater than 0% colonization observed in the roots of plants growing in non-inoculated control soil. In one of the two repeats of this trial, Mykos Gold Granular was also associated with significantly taller onion plants and greater onion dry weight than that of the plants in the control soil. In both trials, Mykos Gold Granular was associated with elevated concentrations of many foliar nutrients. However, such striking effects on onion growth were not observed in subsequent trials with this product over the duration of this project, despite following near-identical procedures. As a result, new ('fresh') batches of Mykos Gold Granular were requested from RTI-Ag to avoid the potential confounding effect of reduced shelf-life of the product affecting results of the trials. The reason for a reduction in efficacy of Mykos Gold Granular at causing onion root colonization and growth promotion in subsequent trials was not determined conclusively in this study, but highlighted concerns others have noted with variation in efficacy among trials when using commercial AMF inoculants.

While never as extensive as in the first trials, Mykos Gold Granular continued to cause more AMF root colonization than any other AMF inoculants tested in the growth chamber trials. When evaluated in soil with a lower P concentration of 16-18 ppm, Mykos Gold Granular typically resulted in 30-50% root colonization by AMF. The only other product consistently associated with root colonization rates significantly greater than 0% was AGTIV Granular, which was tested in two growth chamber trials. Under the lower soil P conditions that favored AMF root colonization, AGTIV Granular induced an average of 6% root colonization. Liquid formulations of Mykos Gold and AGTIV, however, were ineffective at causing AMF root colonization, whether mixed into soil pre-seeding or applied directly to the seed by commercial seed treaters. Granular and powder formulations of other products mixed into the soil also were ineffective.

Mykos Gold Liquid was tested in a growth chamber trial in 2016 as a seed treatment in the presence and absence of several other treatments typically used in onion seed coatings for bulb crops in the Columbia Basin: Thiram (active ingredient, a.i., = thiram), Apron (mefenoxam), Farmore D300 (mefenoxam + fludioxonil + azoxystrobin), and Farmore I300 (Farmore D300 + the insecticides spinosad + thiamethoxam). Mykos Gold Liquid was also tested using primed onion seed. Many growers had expressed interest in applying AMF inoculants to seed because of the ease of application that would not require modifying their planting equipment if AMF inoculants could be delivered effectively in this manner. For this reason, a number of field trials planted in the first field season (2016) entailed AMF inoculants applied to seed. This growth chamber trial was set up to assess the potential impact on seed-applied AMF inoculants of other seed treatments very commonly used by onion and carrot growers in the Columbia Basin. In this growth chamber trial, weekly seed germination counts were collected as well as onion shoot height, dry biomass, and root colonization by AMF 8 weeks after seeding. Low to no AMF root colonization was observed in any of the treatments with Mykos Gold Liquid (similar to the non-inoculated control soil), even in the absence of any fungicide and insecticides on the seed. A subsequent growth chamber trial comparing Mykos Gold Liquid with Mykos Gold Granular, with both products applied to the soil at equivalent rates of spore amendment, revealed that the liquid formulation failed to result in any root colonization by AMF whereas the granular formulation did result in root colonization. Subsequent communication with staff at RTI-Ag revealed there were issues with the liquid formulation not permitting spores of the AMF to germinate when applied to the soil (proprietary inhibitors added to in the liquid formulation to prevent the spores from germinating during storage of the product apparently prevented the spores from germinating even when the product was added to soil). Given these results and confirmation provided by the manufacturer, no further trials were pursued with Mykos Gold Liquid in this study. Furthermore, results of growth chamber trials funded by the WSU BIOAg grant demonstrated that most of the fungicide seed treatments used by conventional onion growers in the Columbia Basin have an inhibitory effect on AMF, even when the inoculants are applied to the soil.[4] Jin et al. (2013) showed similarly that rhizosphere fungal communities were severely inhibited by seed treatments containing contact and systemic fungicides.[8]

Except for the first growth chamber trial in this study with Mykos Gold Granular, AMF root colonization rates in onion growth chamber studies did not result in any significant improvements in plant growth or foliar nutrient acquisition. In fact, some results indicated a potential negative relationship between AMF root colonization rate and onion growth (significantly shorter shoots and onion dry weight than in the control soil), probably reflecting the cost to the host plant in photosynthate and/or P of the AMF association. Such effects have been observed in other research trials under P-sufficient conditions.[9]

In four growth chamber trials, three levels of soil P concentration were evaluated in a factorial design with AMF treatments to explore the interaction between available soil P concentration and AMF inoculant effects on onion growth. Echoing findings from the field trials, less AMF root colonization was observed as soil P concentration increased.

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[8] Jin, H.Y., Germida, J.J. and Walley, F.L. 2013. Suppressive effects of seed-applied fungicides on arbuscular mycorrhizal fungi differ with fungicide mode of action and AMF species. Applied Soil Ecol. 72:22-30.
[9] Thomson, B.D., Robson, A.D. and Abbott, L.K. 1986. Effects of phosphorus on the formation of mycorrhizas by *Gigaspora calospora* and *Glomus fasciculatum* in relation to root carbohydrates. New Phytol. 103:751-765.

Mykos Gold Granular caused 76% less AMF colonization of onion roots when soil P concentration was at 74 ppm vs. 25 ppm; and 64% less root colonization in soil with 45 vs. 25 ppm P. However, a positive onion plant growth response to increasing soil P was observed from 18 to 40 ppm P, with negligible additional growth response between 40 and greater concentrations.

Conclusions

1. Field and growth chamber studies clearly demonstrated that the two liquid formulations of AMF inoculants evaluated in this study were not effective at causing root colonization by AMF under the conditions of these trials. Personal communication by the project leader with an AMF inoculant company representative confirmed that AMF spore germination was slower in the liquid formulation of AMF products, delaying root colonization. This suggests liquid formulations may not be effective for applying AMF inoculants in direct-seeded crops like onion and carrot, given the importance of rapid colonization of the roots by AMF after germination to promote early growth, potentially outcompete or limit the ability of plant pathogens to colonize the roots, or trigger the host physiological response associated with beneficial effects of AMF to plants.
2. AMF inoculants were not effective at promoting AMF root colonization in soils with medium to high P concentration. Efficacy of AMF inoculants was best at soil P concentrations of 16-20 ppm.
3. AMF colonization rates of less than 80% were not associated with consistent improvements in onion crop health, growth, or productivity. However, the project generated preliminary evidence of performance benefits from colonization rates of 80% or greater.
4. AMF inoculants appear to offer little to no advantage in direct-seeded onion and carrot crops grown under the typical, high-input practices of the Columbia Basin. However, these inoculants may benefit crops exposed to stresses such as soil nutrient scarcity, water deficiency, transplant stress, salt stress, etc. as demonstrated in many published studies. The capacity for Columbia Basin growers to provide almost ideal growing conditions for direct-seeded onion and carrot crops appears to limit the benefits of AMF inoculants in this region.

Recommendations

1. Growers of direct-seeded onion or carrot crops in the Columbia Basin probably will not benefit from the use of AMF inoculants unless planting into soils with significantly lower soil P concentration than 40 ppm (Olsen), and probably should avoid using AMF inoculants unless they anticipate crop stress such as nutrient deficiency, heat stress, salt stress, etc. Conventional fungicide seed treatments (particularly Farmore D300 which has two active ingredients that are inhibitory to AMF) are likely to have adverse effects on the potential benefits of AMF inoculants in direct-seeded onion and carrot crops.
2. Growers of direct-seeded onion or carrot crops probably should avoid liquid formulations of AMF inoculants without independent, direct evidence that the application of such products result in increased root colonization by AMF.
3. Further tests of granular AMF inoculants in direct-seeded crops in field and growth chamber trials are needed to clarify the optimum balance of soil-applied fertilizers and AMF colonization of roots for enhancing crop growth and production. Another avenue of interest for research is testing AMF inoculants in the absence of soluble fertilizers, e.g., in organically-managed fields.
4. Although most onion and carrot growers test their soils to determine appropriate fertilizer application rates, this study provided evidence that some fields have excessive soil P levels needed for optimum crop growth.

5. Describe the significant contributions and roles of project partners.

Dr. Lindsey du Toit was the Principal Investigator and primary contact for grower cooperators and AMF product manufacturers. She coordinated grower surveys, oversaw the evolving research plan and maintained trial data throughout the project. Dr. du Toit supervised the Scientific Assistants based at the WSU Mount Vernon NWREC who worked on field and growth chamber trials, and academic staff who participated in various stages of the project, including almost 8 months of microscopic work required for quantifying AMF root colonization for many of the trials: Dr.

Leonard Kiirika participated on the project from February to mid-April 2016; Mr. Brian Henrichs was involved from May 2016 to April 2017; Ms. Erin Miller assisted from May through October 2017; and Dr. Louisa Winkler assisted with data analyses, report writing, and final collation of the study results for preparation of publications from March through October 2017.

Dr. Tim Waters was co-PI and took primary responsibility for coordinating field trials with grower cooperators. He oversaw Scientific Assistants based at the WSU Benton and Franklin Co. Extension Office. He also worked with grower-cooperators and agronomists on equipment modifications and calibration for applying various AMF inoculants.

Both Dr. du Toit and Dr. Waters contributed to the project outreach to stakeholders by publicizing project results at field days each year and stakeholder meetings such as the Pacific Northwest Vegetable Association Annual Convention & Trade Show in Kennewick each November, and generating publications from the study.

Grower-cooperators who served as hosts for this project in their commercial production fields included River Point Farms (primary contact was Dan Jepsen), Hartley Farms (agronomist Wes Locke), Locati Farms (Michael Locati), Mercer Canyon Farms (Dewey Holliday), and L&L Farms (Kevin Kudsk and Kerrick Bauman). In addition, Klaustermeyer Farms (Jim Klaustermeyer Sr.) was the host for trials in commercial carrot fields. As hosts, each grower-cooperator performed all the field preparation, treatment application, and crop planting and management, providing all the necessary labor and equipment. In some cases, growers also provided additional personnel to help with field trial evaluations.

Skagit Seed Services of La Conner, WA provided custom seed treatments for the trials in this project that entailed AMF seed treatments. Most of the AMF inoculant manufacturers with products tested during the project provided their product free of charge, with the exception of several shipments of AGTIV products. The companies involved were Reforestation Technologies Incorporate-Ag based in Gilroy, CA; AGTIV in Rivière du Loup, Quebec, Canada; Mycorrhizal Applications, Inc. in Grants Pass, OR; Plant Health Care, Inc. in Pittsburgh, PA; and Plant Health LLC in Corvallis, OR.

These stakeholder contributions comprised the cost-sharing for this project.

6. If the overall scope of the project benefitted commodities other than specialty crops, indicate how project staff ensured that funds were used to solely enhance the competitiveness of specialty crops.

This project was focused primarily on onion and secondarily on carrot because of onion and carrot grower interest in AMF, and the lack of independent information on the potential benefits of AMF inoculants in these high-value, high-risk, direct-seeded vegetable crops in the Columbia Basin. Only one trial was carried out in a carrot crop, but the technical difficulties of studying AMF in carrots were substantial, particularly root colonization by AMF. Furthermore, the carrot industry expressed less interest in using AMF products than the onion industry, particularly once the lack of efficacy of the two liquid formulations of AMF inoculants evaluated in this study was demonstrated, together with the adverse effects of fungicide seed treatments on AMF, and the lack of evidence of any benefits of the AMF treatments in the carrot trial at Klaustermeyer Farms. Liquid formulations had been considered of the greatest interest for seed treatments by many of the growers involved in this study.

Goals and Outcomes Achieved

7. Describe the activities that were completed to achieve the performance goals and Expected Measurable Outcomes for the project.

The short-term Expected Measurable Outcome described in the project proposal was:

> "Independent, unbiased assessment of the relative efficacy and potential economic viability of commercial AMF products currently being promoted to onion and carrot growers in the Columbia Basin."

Eighteen field trials and eight growth chamber trials carried out for this project generated independent, unbiased data on the efficacy of nine AMF products. Efficacy was assessed in terms of impacts on root colonization rates and crop performance (health, growth, and productivity). In addition to testing the efficacy of granular or powder product formulations applied in-furrow with seed or applied as seed treatments, the project also implemented replicated, randomized trials to address: (1) the impact of additional nutrients contained in an AMF inoculant product on that product's efficacy; (2) the potential for applying liquid inoculants as onion seed coatings, alone or in combination with commonly used fungicide seed treatments; (3) the impact on product efficacy of applying a liquid AMF formulation as a seed treatment or soil drench; (4) the impact on product efficacy of applying a granular AMF formulation with the seed in the top layer of soil (mimicking banding) or blending into soil (mimicking incorporation); and (5) the interaction of AMF inoculant effects with those of soil nutrient concentrations, particularly P. The field trials were completed over 2 summer field seasons and one overwintered season (latter in Walla Walla), and represented a range of growing conditions and onion market types in the Basin, as well as fresh market carrot production. The dynamic companion growth chamber trials to the field trials allowed research questions to be addressed more thoroughly.

This large body of data is a valuable resource to growers of direct-seeded onion crops considering how the use of AMF inoculants might be integrated into their crop management. It offers direct insight into the economic viability of these products. More broadly, it contributes to scientific understanding of the dynamics of AMF communities in agricultural soils. After sharing results of the growth chamber and field trials using two liquid formulations of AMF inoculants, one larger-scale onion farm in the Columbia Basin that had been using a liquid inoculant informed us they had adjusted their AMF program, resulting in a saving of more than $50,000/year. Another farm stated in their cost-sharing letter for this project that "As growers, we have been bombarded with biological sales consultants claiming their products are the best thing since sliced bread. The work of Dr. du Toit and Waters has been critical in assisting growers to make more informed economic and agronomic decisions" (Josh Gefre, Agronomist at Mercer Ranches).

8. If any Expected Measurable Outcomes were long term, summarize the progress that has been made towards their achievement.

The long-term Expected Measurable Outcome described in the project proposal was:

> "Development of an improved, sustainable production practice for onion and carrot growers, i.e. effective use of AMF inoculants on at least 50% of onion and carrot acres in the Columbia Basin, with a concomitant ~25% reduction in onion and carrot acres subjected to soil fumigation (or ~50% reduced soil fumigation rates) and ~50% reduction in P fertilization rates in onion and carrot crops."

Although initial studies by the project team had indicated that AMF inoculants could have substantial positive impact on crop growth and could become a substitute for at least a part of the soil fumigant and fertilizer applications, subsequent results were disappointing. Results from the project indicated that, under conditions typical of direct-seeded onion and carrot production in the Columbia Basin (relatively high soil P concentration, application of more-than-adequate levels of other plant nutrients in soluble form, and the use of fungicidal seed treatments), there was little to no economic benefit of adopting routine use of AMF inoculants in these cropping systems. Some growers in the Columbia Basin have begun to adopt AMF inoculants (see section 10). Communication of this project's findings to growers is anticipated to save the expense of applying AMF products in circumstances that are not likely to result in any benefit. This project and the preceding project funded by the WSU BIOAg program demonstrated that soil fumigation with metam sodium has a more minor effect on onion root colonization by AMF endemic in fields in the Columbia Basin than medium to high concentrations of soil P (above 20 ppm P).

The trial results build the case for a general reduction in rates of fertilizers applied to onion and carrot crops in the Columbia Basin. This was communicated to growers at the 2018 WSU Onion Cultivar Demonstration and Field Day, and will continue to be communicated through journal and Extension publications, in reports written for individual grower-cooperators, and at grower events. Further research on optimizing fertility practices for onion and carrot production to avoid excessive rates of application is likely to have much greater long-term economic and environmental impact than further studies with AMF inoculants, as surveys of both conventional and organic fields in the Columbia Basin have demonstrated AMF to be prevent in all the fields tested, even those fields that had been subjected to fumigation with metam sodium about once every three to four years.

9. Provide a comparison of the activities and goals established for the project with the actual accomplishments.

Project goals specified in the work plan included 12 field trials in the Columbia Basin. Ultimately, 18 field trials were completed in nine separate onion crops and one carrot crop, substantially exceeding that goal (and not counting an unreplicated AMF inoculant trial set up in an onion seed crop near Soap Lake, WA in 2015-16, which also revealed no benefit to any of the four AMF inoculants applied in that trial). Six grower-cooperators were recruited to host these trials. Although the goal initially was to complete trials in two carrot crops, the difficulty of studying AMF in carrots and subsequent lack of interest from the carrot industry once results with liquid AMF inoculants were shared, led the team not to pursue further carrot trials.

Goals also included a program of growth chamber trials to compare a diversity of commercial AMF inoculants, in Project Years 1 (Oct 2015 - Sep 2016), 2 (Oct 2016 – Sep 2017) and 3 (Oct 2017 – Sep 2018); and the use of growth chamber trial results to direct the design of field studies (the exact number of trials was not specified in the original proposal). Two growth chamber trials were carried out in late 2015, four in 2016, and two in 2017. As detailed above, the field and growth chamber studies were highly beneficial in directing the course of the project. For example, early indications from the field and growth chamber trials that liquid AMF formulations were ineffective, were followed by growth chamber trials designed to establish whether the application method could be adapted to increase efficacy of liquid formulations. When the growth chamber trials demonstrated no efficacy of these liquid products, regardless of application method, liquid formulations were dropped from all the field studies for the 2017 season.

One of the original objectives was to assess the potential benefits of AMF inoculants at suppressing soilborne diseases such as pink root and Fusarium basal rot of onion, and cavity spot of carrot. Although this aspect of the proposal was examined in the numerous field trials, it was not pursued in the growth chamber trials because of the dominating evidence of potential inhibitory effects of soil fertility levels of AMF inoculants. Given the typical grower fertility practices, it quickly became evident that we needed to prioritize examining in detail the impact of typical soil fertility levels encountered in onion and carrot crops in this region on the potential efficacy and benefits of AMF inoculants. The fairly limited expense for achieving optimum (to above optimum) soil fertility levels for carrot and onion production, compared to the risk of yield reduction if fertility levels fall too low and the inability to guarantee that AMF inoculants will compensate for potential losses from low fertility levels, emerged as the main reason AMF inoculants are not likely to be of much benefit under the current onion and carrot production practices in the Columbia Basin.

Outreach goals included preparation of reports for stakeholders and manuscripts for submission to scientific journals. To date, four papers have been published in *Plant Disease Management Reports* (see section 17). A further six reports are in preparation for submission to this same technical journal in December 2018. One longer academic manuscript bringing together the findings and placing the results in the context of published literature will be prepared for submission in 2019. One stakeholder-oriented article has been submitted to the industry publication *Onion World*, and accepted for printing in the January or February 2019 issue.

As planned, initial results from two field trials were presented at the Pacific Northwest Vegetable Association Annual Convention & Trade show in November 2016, and at the National Allium Research Conference / National Onion Association Annual Meeting in Savannah, GA in December 2016. Results were also presented to onion growers at smaller meetings in the winters of 2015 and 2016 in preparation for the subsequent field season and designing grower-cooperator field trials. In August of each of 2017 and 2018, results were shared at the WSU Onion Cultivar Demonstration and Field Day.

10. Clearly convey the achievement of your Expected Measurable Outcomes by describing the baseline data that was gathered and the achievement (or progress toward achievement) of your set targets.

An initial goal of this project was to survey onion and carrot growers in the Columbia Basin to assess current use and understanding of AMF. A survey was disseminated in December 2015 and again in the winter of 2016-17, but only two responses were received. Thus, there was no opportunity to develop a baseline dataset at the beginning of the project.

Instead, an end-of-project survey was used as an opportunity to evaluate the impact of the project and document the industry's evolving relationship with AMF inoculants. This survey was conducted at the 2018 WSU Onion Cultivar and Demonstration Field Day, held in the Columbia Basin near Prosser, WA and received around 70 valid responses. Responses to this survey are summarized in an attachment to this report. They revealed that 29% of crop consultants and seed company representatives had recommended AMF inoculants for onion or other crops, and 43% of growers have used them. Sixty percent of respondents felt they did not have enough evidence to make informed decisions about the use of AMF inoculants, and 80% reported that university research was an important source information to them on this topic. These results underline the importance of publishing findings from Project K-1769 and communicating them to the industry.

Beneficiaries

11. Describe those who have benefited from the completion of your project and its accomplishments.

Growers of direct-seeded onions and carrots in the Columbia Basin are the primary beneficiaries of this research. Crop consultants and agronomists advising such growers on the use of products such as AMF inoculants are also beneficiaries. The project has generated objective research on AMF inoculants, a growing class of commercial products, which will help the onion industry make informed decisions about their use.

12. Clearly state the quantitative data that concerns the beneficiaries of the project and/or that describes the economic impact of the project.

The most effective AMF inoculant tested in this project, Mykos Gold Granular, costs around $5.30/lb. Applied once during the growing season at the recommended rate of 5-10 lb/acre, the cost of using Mykos Gold Granular is approximately $26.50-$53.00/acre, which is more expensive than most fungicide applications. Producers on several of the larger onion farms in the Columbia Basin indicated they had begun using AMF inoculants in onion production on a routine basis, including some liquid formulations, despite not having independent data to verify the benefits that had been promoted by companies selling these products, and not having set up trials on their farms to assess whether the perceived benefits were real from adopting AMF inoculants. After sharing results of the replicated, randomized growth chamber trials and numerous companion field trials on multiple farms, one of the larger onion farms in the Columbia Basin, which was a host for field trials each season, informed us that results of this study reduced their expenses by more than $50,000/year. Other farms have not shared the quantitative nature of economic benefits derived from this study, but the most frequent response from the cooperators and other stakeholders has been respect for the independent data generated under their production conditions and practices in he Columbia Basin that revealed the very limited to no benefits of AMF inoculant use in their cropping systems.

Lessons Learned

13. Offer insights into the lessons learned by project staff as a result of completing this project. Include the positive and negative results and conclusions of the project.

One important lesson learned from carrying out this project was that AMF root colonization readings, done by microscopic examination, are extremely time-consuming. Using the modified gridline-intersection method on 40 root sections per plant, it took 40-60 minutes to read the roots of a single plant. In a typical growth chamber trial with five replicates of a 3 x 5 factorial design, there were 75 plants to examine, equating to around 70 hours of time at the microscope. In a typical field trial, with five plants sampled per plot from a 2 x 2 factorial design with a minimum of three replicates, examining the roots of 60 plants equated to 40-60 hours of work per trial. There were insufficient hours available to perform root colonization readings for every trial, so root readings were not completed for those trials in which the field data (stand counts, disease ratings, and other growth parameters) offered no evidence of AMF inoculant benefits. However, quantifying root colonization by AMF was ultimately the only way to prove that AMF were or were not associated with the impact of the treatments evaluated on the other plant traits measured. The project staff advise future investigators of AMF-related topics to plan trials carefully with an eye to minimizing the required number of root readings; and/or to be prepared for significant labor to quantify root colonization. Although molecular methods of AMF quantification have been developed, the very limited amount of plant tissue that can be used for DNA quantification makes it almost impossible to translate results from a few grams of root tissue for each plot to large-scale field trial results, particularly given the high degree of variability in AMF colonization even among roots on the same plant and among plants in the same plot.

During the growth chamber studies, fruiting bodies of a saprophytic fungus, *Peziza*, developed on the surface of the soil in some of the conetainers. Spores of this fungus must have been present in the field where the soil was collected, as the spores of this fungus are known to be stimulated to germinate after fires, which the soil pasteurization process must have mimicked. The fungi were observed in some conetainers but not others, and where present, dramatically reduced onion growth, negatively impacting the data. In one growth chamber trial, so many plots were affected by this saprophyte that those plots had to be dropped from the statistical analyses.

Although the project goals included adoption of AMF inoculants on a large percentage of the onion and carrot acres planted in the Columbia Basin, with concomitant reduction in reliance on soil fumigation and reduction in soil fertilizer application rates, the results did not justify continued pursuit of this goal. However, as stated in the original proposal, it is just as important to demonstrate objectively which products and application methods do not work in these cropping systems as it is to demonstrate which products/methods of application are effective. Independent data generated by this project and other university research is critical to help protect growers from unscrupulous claims of product manufacturers. While it has been a major disappointment not to find a means of optimizing AMF inoculant use to address the economic and environmental goals/benefits noted in the original proposal, i.e., reducing the reliance on soil fumigation and higher rates of fertilizer application, the growers involved in this project have expressed sincere appreciation for independent research that enables them to make informed decisions regarding AMF inoculants.

14. Describe any unexpected outcomes or results that were an effect of implementing your project.

Prior to conducting field trials with AMF inoculants, the team anticipated finding very low levels of AMF activity in non-inoculated control plots owing to the widespread practice in the Columbia Basin of fumigating fields with metam sodium, a broad-spectrum fungicide and fumigant. However, onion roots generally showed 40-50% rates of colonization in the non-inoculated control plots, which was not increased by any of the AMF inoculant treatments in any of the field trials, and which reflected the presence of endemic AMF in all of these fields. Similar results were observed during field surveys of onion bulb crops conducted by Dr. du Toit's group with Dr. Tim Paulitz, USDA ARS plant pathologist, in 2014-15. The explanation may lie in the fumigation product and method of application: metam sodium most commonly is chemigated by center pivot. The limited depth of movement of the product by chemigation into the soil profile might be a major factor limiting the effects of this product on AMF in the soil profile, as metam sodium has very poor vapor pressure and, hence very poor mobility in soil. Research by Kyle Coleman (NovaSource) with onion and potato growers in the Columbia Basin has shown metam sodium to be limited to the top 4 inches of the soil profile or less when applied by chemigation, whereas shank applications can place the product at precise depths in the soil profile based on the depth of shanking.

15. If activities, goals or Expected Measurable Outcomes were not achieved, identify and share your lessons learned to help others expedite problem-solving.

The project team had hoped to establish a strong baseline for AMF inoculant use among Columbia Basin onion and carrot growers at the start of the project in 2015. A survey was mailed to over 30 growers on two occasions that winter of 2015-16, but only two responses were received. The team considered the probable explanation to be 'survey fatigue' and limited time among growers, because the interest of growers serving as cooperators in this study indicated this lack of response did not reflect a lack of interest in the research. These factors were taken into consideration when designing the end-of-project survey which was completed during the 2018 WSU Onion Cultivar Demonstration and Field Day in August 2018 at Hartley Farms. More than 100 onion growers, crop consultants, seed company representatives, and other stakeholders (including researchers and extension specialists from three universities) were present. The survey consisted of physical ballots which respondents dropped into ballot boxes as they waited in the lunch line. The placement of the survey at the center of this activity made it difficult for attendees to 'dodge' participation, yielding a very high response rate (approx. 80%). The team considered this aspect of the survey to have been very successful. A flaw in the survey design, however, was the use of separate ballot boxes for each question, limiting the ability to track responses of individuals among the questions asked. Future surveys should be designed to provide each respondent with a single sheet on which their responses can be tracked among all the questions and related to their profession. Primary results from the survey were as follows:

1) A large percentage of farmers surveyed (43%) reported having used AMF products. Crop consultants and seed company representatives seem to be more hesitant about AMF products than farmers, with only 29% having recommended these products in the past.
2) The majority of respondents (64% of a total of 69 valid responses) reported that results from this project would influence their use of AMF products, but opinion was split as to whether they were more or less likely to use AMF products (45 and 25%, respectively). We note that results from the project did **not** support the value of AMF products in Columbia Basin onion crops. This survey result may indicate that respondents had not clearly understood the research; perhaps not surprising, since it was presented in the form of a five-minute speech and not all attendees were listening.
3) The majority of respondents (60%) reported not feeling adequately informed about AMF products, demonstrating the need for research results such as those from this project. A number of comments (8, 9, 12) indicated that stakeholders value the opportunity to see research taking place in the field.
4) University research was the single most popular source of information about AMF products, listed as a preferred resource by 80% of respondents. The second most popular source was "colleagues", listed by 41% of respondents.
5) Respondents overwhelmingly (70%) indicated that this research would influence their fertilizer use. Indeed, the research appears to have been more influential for fertilizer use than for AMF product use (64%), perhaps because fertilizer use is more widespread. As with AMF products, however, respondents showed split opinions regarding their inclination to increase (26%) or decrease (34%) their fertilizer use, suggesting that an opportunity to communicate results more clearly to stakeholders is needed.
6) University research was less dominant as a preferred resource for fertilizer decision-making than for AMF product decision-making (58 vs. 80%). Instead, private consultants were the most selected source of fertilizer information. Comments also indicated that stakeholders actively use their own experimentation and farm-specific observations to develop fertilizer programs.

Additional Information

16. Provide the total level of cash or in-kind matching donations utilized for your project. Describe the amounts, sources and ways in which the donations were utilized.

In Project Year 1, four grower-cooperators provided all the equipment, labor/salaries, fertilizers, herbicides, and other maintenance expenses for setting up and maintaining field trials in five onion crops and one carrot crop (based on the size of the trials, each was valued at ~$10,000 in-kind support). 6 trials x an estimated $10,000 = approximately $55,000 in-kind support for grower-cooperator field trials in 2016.

In Project Year 2, four grower-cooperators provided all the equipment, labor/salaries, fertilizers, herbicides, and other maintenance expenses for setting up and maintaining field trials in seven onion crops. Based on the size of the trials, which ranged from 12 single-bed plots of ~50' each to 40 plots of ~250' single-bed plots, the in-kind support was valued at ~$60,000 for grower-cooperator field trials in 2017.

In Project Year 3, one grower-cooperator carried out two overwintered trials with sixteen blocks measuring 8’ by 120’ for a total are of approximately 15,360 square feet. This trial was estimated to have been valued at ~$5,000.

Based on the cost-share letters provided by grower-cooperators, the specific donations/in-kind support from these stakeholders are as follows (total value of $118,500):

- Locati Farms in Walla Walla: $10,000 in estimated support for one trial in 2017 and two overwintered trials in 2017-18 in Walla Walla sweet onion crops.
- L&L Ag Production LLC: $20,000 for onion field trials (2 trials in 2016 and one in 2017) near Connell, WA
- Hartley Farms: $25,000 for onion field trials (2 trials in each of 2016 and 2017) near Paterson, WA
- Klaustermeyer Farms, Inc.: $2,500 for a carrot trial near Basin City, WA
- Mercer Ranches: $25,000 for dehydration onion trials near Paterson, WA
- River Point Farms: $20,000 for 4 onion field trials and other support in 2017 near Irrigon, OR
- Schulthies Farm: $16,000 for onion field trials

This does not include the value of onion seed donated by companies like Bejo Seeds America, AMF inoculants donated by all of the product manufacturers except for some of the AGTIV products used in several growers’ field trials (growers either paid for the product or we paid the cost of the product from this grant), and fungicides donated by chemical companies. These additional donations have an estimated value of ~$5,000.

17. Provide any additional information available (i.e. publications, websites, photographs) that was not applicable to any of the prior sections.

Academic journal publications

Knerr, J.A., Paulitz, T.C. and du Toit, L.J. 2016. Effects of commercial arbuscular mycorrhizal fungi (AMF) products on onion in a growth chamber study, 2015. Plant Disease Management Reports 10: V098.

Henrichs, B.A., Waters, T.D. and du Toit, L.J. 2017. The effect of soil phosphorus levels on colonization of onion roots by arbuscular mycorrhizal fungi, 2016-2017. Plant Disease Management Reports 11: V125

Henrichs, B.A., Derie, M.L, Waters, T.D. and du Toit, L.J. 2017. The effects of arbuscular mycorrhizal fungi inoculants on pink root and onion growth in field trials near Connell, WA, 2016. Plant Disease Management Reports 11: V137.

du Toit, L.J., Derie, M.L., Holmes, B.J., Henrichs, B.A., Winkler, L.R., Waters, T.D., and Darner, J. 2018. The effects of arbuscular mycorrhizal fungal inoculants on pink root and yield in an onion crop near Paterson, WA, 2016. Plant Disease Management Reports 12: V102.

Extension publications

du Toit, L.J., Waters, T.W., Derie, M.L., and Henrichs, B. 2016. The potential role of mycorrhizal inoculants in Columbia Basin onion production. Pp. 8-9 in: 2016 WSU Onion Cultivar Demonstration and Field Day Handout, 25 Aug. 2016, Othello, WA. 16 pp.

du Toit, L., Waters, T., Derie, M., Holmes, B., Darner, J., Henrichs, B., Knerr, J., Paulitz, T., Sharma-Poudyal, D., Miller, E., Morgan, P. and Winkler, L. 2018. *Do AMF inoculants help onion crops?* 2018 WSU Onion Cultivar Demonstration and Field Day packet. https://s3.wp.wsu.edu/uploads/sites/2082/2018/09/2018WSUOnionFieldDayPacket.pdf

Industry publications

Winkler, L.R., du Toit, L.J., and Waters, T.D. 2018. WSDA Specialty Crop Block Grant K1769: Evaluation of arbuscular mycorrhizal fungi for enhanced growth, phosphorus use efficiency, and management of soilborne pathogens of onion and carrot in the Columbia Basin. End-of-project survey report.

Winkler, L.R., Waters, T.D. and du Toit, L.J. 2019. Do onion growers benefit from mycorrhizal inoculants? The effects of mycorrhizal inoculants on onion crops in the Columbia Basin. Onion World, in press (to be printed in January or February 2019).

Additional publications in preparation

du Toit, L.J., Derie, M.L., Holmes, B.J., Miller, C.E., Batson, A., Winkler, L.R., Waters, T.D., and Darner, J. 2019. The effects of arbuscular mycorrhizal fungal inoculants on pink root and yield in an onion crop near Paterson, WA, 2017. Plant Disease Management Reports 13: to be submitted in Dec. 2019.

du Toit, L.J., Derie, M.L., Miller, C.E., Winkler, L.R., Batson, A., Waters, T.D., and Darner, J. 2019. The effects of arbuscular mycorrhizal fungi inoculants and pre-plant, banded fertilizer application on pink root and onion growth in field trials near Irrigon, OR, 2017. Plant Disease Management Reports 13: to be submitted in Dec. 2019.

du Toit, L.J., Derie, M.L., Miller, C.E., Winkler, L.R., Waters, T.D., and Darner, J. 2019. The effects of arbuscular mycorrhizal fungi inoculants on pink root and onion growth in a field trial near Connell, WA, 2017. Plant Disease Management Reports 13: to be submitted in Dec. 2019.

du Toit, L.J., Derie, M.L., Miller, C.E., Winkler, L.R., Waters, T.D., and Darner, J. 2019. The effects of arbuscular mycorrhizal fungi inoculants on pink root and crop growth in dehydrated onion trials near Paterson, WA, 2016. Plant Disease Management Reports 13: to be submitted in Dec. 2019.

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Winkler, L.R., du Toit, L.J., and Waters, T.D. Field-scale evaluation of arbuscular mycorrhizal inoculants in direct-seeded onion bulb crops. HortScience: manuscript in preparation.

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