TITLE: Creating Climate Resilient Organic Systems by Enhancing Arbuscular Mycorrhizal Fungi Associations

Principle Investigator: Dr. Erin Silva, Department of Plant Pathology, University of Wisconsin-Madison (UW-Madison)
Researcher: Michelle Pearson, Department of Plant Pathology, UW-Madison
Research Collaborator: Dr. Jean-Michel Ané, Departments of Bacteriology and Agronomy, UW-Madison

Farm Collaborators and Trial Sites:
Larryville Gardens, Madison, Wisconsin
Olden Produce, Madison, Wisconsin
Winterfell Farm, Madison, Wisconsin
Raleigh Farm, Madison, Wisconsin
Equinox Farm, Madison, Wisconsin
University of Wisconsin West Madison Agricultural Research Station, Madison, Wisconsin

Project Summary

An important aim of organic production is to improve overall soil health. Arbuscular mycorrhizal fungi (AMF) form symbiotic relationships with the roots of host plants in which plants directly receive nutrients. AMF also are reported to indirectly promote plant health through their contribution to soil building. Thriving AMF communities increase the water-holding capacity of soils through the deposition of proteins. This project sought to determine whether the genetic variances between popular cultivars of carrot would promote the growth of different AMF communities over the growing season, thereby altering the quantity of AMF-associated proteins in soils. There were no obvious trends toward increases in AMF-associated soil proteins that correlated to any cultivar of carrot. Most sites had moderate changes in protein quantities from spring to fall, with no clear pattern. One site (Larryville) had consistently increased Fall quantities of AMF-associated proteins compared to Spring quantities for all cultivars for both years (2017 and 2018). We observed more intensive weed management at this site. Storm related challenges impacted some farms during these years, with flooding inflicting considerable carrot losses at multiple sites.

INTRODUCTION

Annual sales of organic produce are maintaining steady growth both in the United States (U.S.) and abroad, with supply continually falling short of consumer demand [1]. Organic carrots have experienced particularly accelerated growth in sales, accounting for 14.35% of total carrot production acreage in 2011, up from 5.8% in 2005 [2]. As a leader in overall organic farm numbers and crop acreage, California is also a leader in organic carrot production, representing 94% of the total organic carrot acreage in the U.S. [3]. Washington State provides the next largest acreage of carrots, with 3.6% of production [3]. Wisconsin is second for certified organic operations and fifth in total organic crop acreage, with organic carrots integrated into the operations of many certified organic diversified vegetable farms, and Wisconsin organic carrot sales exceeding $500,000 in 2014 [3,4].

As global climate change brings rising temperatures and changing precipitation patterns impacting food production worldwide, the need to create greater resiliency in our crop production systems becomes imperative. The aforementioned production figures illustrate that, while organic carrots are a foundational crop within the portfolio of vegetables offered by many fresh market, diversified organic farmers, the vast majority of acreage supplying the significant consumer demand resides within the water-stressed states of California and Washington. Given that carrots require consistent water inputs, and considering the record-breaking annual droughts in these areas, production of organic carrots is at significant risk. In 2015, California's fourth consecutive year of drought resulted in nearly $1 billion in crop losses, with an estimated $93 million in vegetable losses, with water restrictions applied to farmers [5]. Warm temperatures (above 86º F or 30º C) during cultivation (a phenomenon expected to occur more frequently as mean temperatures increase) can limit marketability of carrots due to the development of unpleasant flavors [6]. Thus, carrot can serve as a model crop to assess strategies to mitigate the the impacts of climate change, and a model production system to examine the role of cultivar choice and soil ecological communities in creating greater cropping system resilience.

As organic farmers have observed for decades, healthy soil microbial communities contribute to agriculturally desirable outcomes. Symbiotic microbes offer increased access to nutrients that can be limiting in organic agriculture such as nitrogen (N) and phosphorus (P) [7]. Arbuscular mycorrhizal fungi (AMF) are soil-borne fungi that form structures within host roots called arbuscules, which transfer water and nutrients vital to plant health and performance. Host plants, in return, provide sugars to the AMF. Without the carbon source provided by hosts, AMF would be unable to extend hyphal networks throughout soil. These mycelial networks penetrate pore spaces too fine for plant roots, allowing for the acquisition and transport of plant-available such as P that may otherwise be inaccessible to plants due to slow diffusion through soil [8]. AMF also shuttle water to host roots via transport proteins called aquaporins [9] that are located at the periarbuscular membrane, the interface between the host cell and the arbuscule. Association with AMF contributes to plant health by supporting increased plant biomass and plant disease resistance [10]. Abundant AMF populations also enhance soil moisture retention through deposition of organic materials, which adds to the resiliency of organic systems under drought conditions [8,10].

Organic producers and small-scale gardeners sometimes add commercial mycorrhizal inoculants to their soil with intent to improve vegetable nutrient access and drought tolerance. The actual impact of added inoculants is difficult to assess, partly due to native mycorrhizal community presence. It is unknown whether carrot cultivars demonstrate preference for AMF partners at the species level, and it is unknown whether different AMF species contribute differentially to carrot drought tolerance and yield. Inoculants may be unsuccessful in soils with more competitive AMF communities. Alternatively, host plants may elect to interact with certain AMF species.

While soil microbial communities are diverse and abundant in natural systems, agricultural practices can alter native community composition. Organic systems have the ability to improve soils by stimulating increased species diversification through cultivation of mycorrhizal and rhizobial plant species. Generally, organic management increases AMF diversity and abundance [8,10] and is more sustainable than conventional methods due to its emphasis on land stewardship. Organic systems are enhanced by AMF's contributions to moisture-retention from hyphal turnover of extraradical mycelia. Certain crop rotations are known to play a role in increasing AMF populations; however, rotations that exclude mycorrhizal plants limit the growth of these important soil microbes. Other factors impacting AMF include the use of tillage [11], which is commonplace in organic management. For farms that were previously under conventional management, soils may have low AMF levels due to residual impacts of annual applications of fertilizers, fungicides, nematicides, and herbicides [9,11].

Appropriate cultivar selection is crucial to the success of a crop regardless of farming practice [11]. Vegetable cultivars are bred for a variety of desirable traits including biomass, appearance, flavor profile, texture, sugar content, nutritional density, harvest date, cold tolerance, drought tolerance, disease resistance, competitiveness with weeds, and processability. While overall populations of AMF on organic farms have been broadly described, more detailed studies of specific AMF communities, and their associations with specific crop species and cultivar (as proposed in this work), are lacking. The work we propose is unique in that we seek to determine whether cultivar selection can contribute to an increased AMF community activity in a measurable way. These communities can contribute to the performance and sustainability of organic systems, particularly in the face of water scarcity and climate change.

RESEARCH OBJECTIVES STATEMENT

We sought to determine whether carrot cultivars preferentially recruit AMF communities.
We evaluated if differential recruitment and colonization patterns in organic production systems translated to improved carrot performance in a variety of soil conditions on working organic farms, providing a greater understanding of the impact of cultivar and soil microbial communities on resilience under drought conditions and under organic management more broadly. We also evaluated the role of carrot cultivar on AMF-related proteins, an important factor influencing soil health improvements related to AMF communities. These objectives were developed in partnership with organic farmers, derived from input from organic farmers as to their research and management priorities; improving soil health, biology, and resilience of organic agriculture in the face of climate change emerged as important themes. As such, to expand from these conversations in this study, we worked with six organic farmer members of the Fairshare Community Supported Agriculture (CSA) Coalition in Wisconsin during the 2017 and 2018 field seasons.

MATERIALS AND METHODS

Four cultivars of carrot were planted at each participating farm site in 2017 and 2018 (Table 1). Prior to planting (late May) and at the end of each season (late August), soil samples were collected to be used for the evaluation of AMF-related soil proteins. Three soil samples per cultivar (by site and date) underwent sodium citrate extraction, a process that requires autoclaving to extract proteins often referred to as glomalin-related soil proteins. Soil extracts were quantified using Bradford reaction dye. Root fragments from carrots were evaluated for evidence of AMF colonization. A small sample of carrots were harvested at the end of each season to evaluate and compare growth.

Table 1. Cultivars of carrot planted at farm sites.

Cultivar	Type	Seed Source
Napoli	F1 Hybrid	Johnny's Selected Seeds
Yaya	F1 Hybrid	Johnny's Selected Seeds
Red Cored Chantenay (RCC)	Open Pollenated	Seed Saver's Collective
Scarlet Nantes (Nantes)	Open Pollenated	Seed Saver's Collective

PROJECT RESULTS

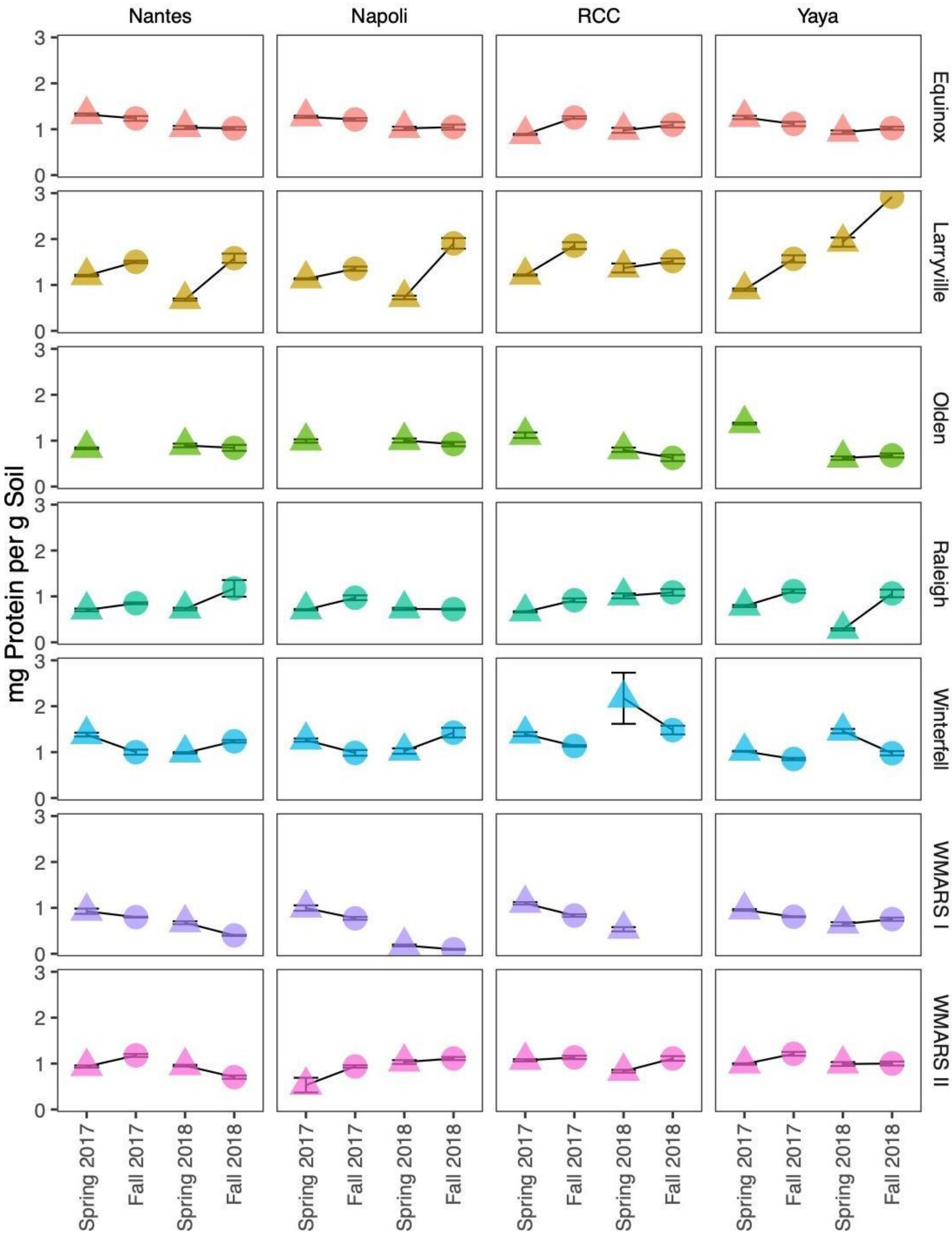
Farm sites did not differ in the quantity of root colonization by AMF, and there was no difference in colonization according to cultivar of carrot (Table 3). Most sites experienced heavy rain during August, which is typically a drier month. This made extracting roots from soils particularly challenging due to the frail nature of the fine roots of carrot. Soil protein fluctuations from start to end of season were not correlated to the cultivar planted, and no consistent pattern emerged among the farm sites (Figure 1).

Table 2. Colonization of roots by arbuscular mycorrhizal fungi at each farm site.

Cultivar	Site name	Year	Mean root colonization percent	Standard deviation
Napoli	Equinox	2017	44.9	2.4
Napoli	Equinox	2018	26.1	9.1
Napoli	Larryville	2017	43.1	2.9
Napoli	Larryville	2018	25.6	7.5
Napoli	Olden	2018	-	-
Napoli	Olden	2017	44.4	4.6
Napoli	Raleigh	2018	18.6	2.5
Napoli	Winterfell	2017	-	-
Napoli	Winterfell	2018	-	-
Napoli	WMARS1	2017	38.6	5.6
Napoli	WMARS1	2018	23.7	2.3
Napoli	WMARS2	2017	42.4	3.8
Red Cored Chantenay	Equinox	2017	21.4	1.1
Red Cored Chantenay	Equinox	2018	10.2	5.0
Red Cored Chantenay	Larryville	2017	37.5	5.6
Red Cored Chantenay	Larryville	2018	20.6	3.4

Red Cored Chantenay	Olden	2017	54.2	5.8
Red Cored Chantenay	Raleigh	2018	15.3	5.5
Red Cored Chantenay	Winterfell	2017	58.0	13.2
Red Cored Chantenay	Winterfell	2018	36.2	10.3
Red Cored Chantenay	WMARS1	2017	27.1	7.0
Red Cored Chantenay	WMARS1	2018	35.9	4.5
Red Cored Chantenay	WMARS2	2017	41.2	4.9
Scarlet Nantes	Equinox	2017	62.3	5.4
Scarlet Nantes	Equinox	2018	26.4	1.6
Scarlet Nantes	Larryville	2017	47.7	9.3
Scarlet Nantes	Larryville	2018	20.7	3.4
Scarlet Nantes	Olden	2017	56.6	2.8
Scarlet Nantes	Raleigh	2018	22.2	5.1
Scarlet Nantes	Winterfell	2017	56.4	10.4
Scarlet Nantes	Winterfell	2018	23.4	4.3
Scarlet Nantes	WMARS1	2017	58.7	7.2
Scarlet Nantes	WMARS1	2018	22.3	5.0
Scarlet Nantes	WMARS2	2017	49.2	1.3
Yaya	Equinox	2017	36.7	19.2
Yaya	Equinox	2018	17.2	0.9
Yaya	Larryville	2017	32.9	5.3
Yaya	Larryville	2018	14.4	1.4
Yaya	Olden	2018	13.0	2.4
Yaya	Olden	2017	64.2	4.2
Yaya	Raleigh	2018	20.5	2.3
Yaya	Winterfell	2017	75.1	20.2
Yaya	Winterfell	2018	28.2	12.3
Yaya	WMARS1	2017	42.7	3.7
Yaya	WMARS1	2018	3.9	3.4
Yaya	WMARS2	2017	50.9	18.8

Figure 1. AMF-related soil protein quantification. Protein was quantified for three samples per cultivar, site, and date. Protein quantities shown are as a proportion of soil using Bradford protein dye. Missing data resulted from crop failure due to flooding.



DISCUSSION AND CONCLUSIONS

Mean root colonization averaged approximately 33% for cultivars ‘Napoli’, ‘Red Cored Chantenay’, and ‘YaYa’, but was significantly greater for one of the open pollinated varieties, ‘Scarlet Nantes’. This aligns with previous research findings for some crops that modern varieties, as compared with older varieties, ancestors, and landraces demonstrate a loss of response to colonization by AMF (14). Over all sites, mean root colonization percent trended lower in 2018 versus 2017. It is interesting to note that 2018 was a year of record rainfall in Wisconsin, and that in the absence of water stress, less colonization may have occurred; other studies have found more colonization in the presence of water stress (15). There were no obvious trends toward increases in AMF-associated soil proteins that correlated to any cultivar of carrot with soil protein trends across cultivars at different farm sites tended to be fairly consistent. Most sites had moderate changes in protein quantities from spring to fall, with no clear pattern. One site (Larryville) had consistently increased Fall quantities of AMF-associated proteins compared to Spring quantities for all cultivars for both years (2017 and 2018). Interestingly, more intensive weed management occurred at this site. Storm related challenges impacted some farms during these years, with flooding inflicting considerable carrot losses at multiple sites.

While the scope of this work is limited, it does highlight two areas that warrant further research and for which we will be pursuing future funding, based on this preliminary data. First, particularly among open-pollinated cultivars, it would be beneficial to screen diverse genotypes for enhanced AMF colonization, to be used either directly be farmers or to be included in breeding programs. Second, as indicated by the Larryville site, this preliminary data shows some interesting interactions in the role of AMF in increasing soil proteins, with their role in enhanced soil aggregation, in highly disturbed environments, which can be characteristic of organic farms with their reliance on tillage and cultivation. It would be valuable to further investigate the role of cultivar selection and AMF inoculation on soil aggregation in heavily tilled/cultivated phases of the organic crop rotation, and in intensive vegetable production.

OUTREACH AND COMMUNICATION

Several outputs were generated from this work, with more in progress. Reports to farmer cooperators were one of the most welcomed outputs, as this also included general soil test information. One of the main outputs has been the reports to our farmer cooperators. Other public-facing communication to farmers included presentations at Organic Vegetable Field days (August 20, 2018 and August 22, 2019). An outreach publication related to the themes of this project was developed by the project team, titled “The Biological Component of Soil Health: Measuring it and Harnessing it”. We will we will continue this momentum by further characterizing changes in soil biology with plant genotypes in organic agriculture with additional funding that is available.

We are grateful to the Organic Farming Research Foundation for the opportunity to pursue this research question. As a follow up to this work, we aim to use sequencing technology to identify the AMF community constituents present in samples obtained during this work. This may be used to correlate species or genus abundance with soil protein quantities to identify whether specific communities contribute to greater protein deposits in agricultural soils.

Photo: Harvesting organic carrot research plots.

Photo: Graduate Student Michelle Keller-Pearson sampling carrots for AMF colonization.

REFERENCES

1. Greene, C. et al. U.S. Market Growth Outpaces Domestic Supply (EIB-55). 3–15 (2009). at http://www.ers.usda.gov/media/452875/eib55b_1_.pdf

2. USDA-ERS: Table 3. Certified organic and total U.S. acreage, selected crops and livestock (1995-2011).

3. US Department of Agriculture, National Agricultural Statistics Service, & USDA's Risk Management Agency. 2014 Organic Survey, Wisconsin. Census of Agriculture (2014).

4. USDA-ERS: Table 4. Certified organic producers, pasture, and cropland. Number of certified operations, by State, 2000-11. Total acreage of certified organic pasture and cropland by State, 1997 and 2000-11.

5. Howitt et al. Economic Analysis of the 2015 Drought For California Agriculture (2015).

6. Seufert, V., Ramankutty, N. & Foley, J. a. Comparing the yields of organic and conventional agriculture. Nature 485, 229–232 (2012).

7. Smith, S. E. Mycorrhizal Symbiosis (Academic Press, Boston, ed. 2, 2008).

8. Verbruggen, E. et al. Positive effects of organic farming on below-ground mutualists : of mycorrhizal fungal comparison in agricultural communities soils. New Phytol. 186, 968–979 (2010).

9. Köhl, L., Lukasiewicz, C. E. & van der Heijden, M. G. a. Establishment and effectiveness of inoculated arbuscular mycorrhizal fungi in agricultural soils. Plant. Cell Environ. n/a–n/a (2015). doi:10.1111/pce.12600

10. Gosling, P., Hodge, a., Goodlass, G. & Bending, G. D. Arbuscular mycorrhizal fungi and organic farming. Agric. Ecosyst. Environ. 113, 17–35 (2006).

11. Baum, C., El-Tohamy, W. & Gruda, N. Increasing the productivity and product quality of vegetable crops using arbuscular mycorrhizal fungi: A review. Sci. Hortic. (Amsterdam). 187, 131–141 (2015).

12. König, S. et al. Taqman real-time PCR assays to assess arbuscular mycorrhizal responses to field manipulation of grassland biodiversity: Effects of soil characteristics, plant species richness, and functional traits. Appl. Environ. Microbiol. 76, 3765–3775 (2010).

13. Phillips, J.M., Hayman, D.S.. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection (1970). Transactions of the British Mycological Society 55 (1): 158e160.

14. Hetrick, B.A.D., Wilson, G.W., Cox, T.S. Mycorrhizal dependence of modern wheat varieties, landraces, and ancestors. Can. J. Bot. 70:2032–2040 (1992).

15. Li, J., Meng, B., Chai, H., Yang, X., Song, W., Li, S., Lu, A., Zhang, T., Sun, W. Arbuscular Mycorrhizal Fungi Alleviate Drought Stress in C_3 (***Leymus chinensis***) and C_4 (***Hemarthria altissima***) Grasses via Altering Antioxidant Enzyme Activities and Photosynthesis. Front Plant Sci.10:499 (2019).