Arbuscular Mycorrhizae Fungi Symbiotic Relationships with *Vitis vinifera* Cultivar Pinot Noir

Capstone Project Paper

Cornell University

Presented to the College of Agriculture and Life Sciences Faculty of the Graduate Program in completion of the Master of Professional Studies in the Field of Integrative Plant Sciences

By

Lloyd Christopher Jones

June 2023

ABSTRACT:

A field trial was conducted to evaluate the symbiotic relationship between Arbuscular Mycorrhizal Fungi (AMF) and *Vitis vinifera* cultivar Pinot noir with commercial inoculation. Pinot noir grapevines were planted and inoculated with MycoApply® Endo-granular in early summer, 2019. MycoApply® Endo is a granular product containing 4 species of fungi (Glomus intraradices, Glomus mossae, Glomus aggregatum, and Glomus etunicatum). The purpose was to evaluate the proposed benefits of the commercially sold fungi and differences between two rootstocks compared to the control. The experiments were conducted in the Finger Lakes AVA at Lansing, NY Cornell University vineyard. The inoculation treatments resulted in greater root length colonization by AMF, but there were no apparent benefits to the vine.

Biography:

My name is Lloyd Christopher Jones. I am 34 years old, and I was born in Brooklyn, New York. I have spent most of my adult life in New York actively learning and working in the amazing field of Horticulture. I am currently a graduate student in the MPS SIPS program as of Fall 2020. My area of focus is within Viticulture and Enology, which has been a very rewarding learning experience to date. I completed my undergraduate studies at SUNY Cobleskill from the years of 2009-2012 earning a bachelor's degree in Horticulture. After graduation I worked in the field of horticulture gaining necessary skills and experience as a Gardener and Technician. Throughout my graduate academic career, I have worked at several wineries and vineyards gaining practical experience and useful knowledge on the east and west coast as a cellar hand and vineyard technician. I recently visited South Australia for several months with the honors of the Dreer Scholarship Award to do viticulture research at the University of Adelaide. My involvement in this research project shares my interests of how biological organisms naturally and sustainably support each other.

Acknowledgements

I would like to acknowledge and praise my advisor Dr. Justine Vanden Heuvel for her insight, support, and guidance throughout my academic career at Cornell University. I only wish that you could have been an earlier influence on my academic career. I have been able to accomplish quite a lot in a short period of time as your advisee.

I would like to thank Steve Lerch for his reputable experience and sharing with me his knowledge of viticulture. It was a pleasure working in the vineyards with you collecting data.

I would like to thank Mariam Patricia Berdeja for taking the time to show me how to quantify and statistically analyze the data in the lab.

I would like to thank Kelly Ivanovski from the Nolen Greenhouses at the NYBG for writing me a letter of recommendation for acceptance into the MPS program at Cornell University.

I would like to thank all my professors for sharing their wisdom in their respected spaces of brilliance. A special thanks to Gregory Peck, Russell Moss and Dwayne Bershaw for all their help with career advice.

Thank you to everyone else that has been a major help in my life!

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Introduction

Soil health is very important in vineyards. To have good soil health, microorganisms need to be present along with adequate levels of organic matter (OM). Grapevines form symbiotic relationships with Arbuscular Mycorrhizae Fungi (AMF). In most if not all soils are a presence of fungal networks. Fungi help decompose organic matter making nutrients more available to plants, they help with water uptake and scavenge nutrients making them available to plants. In the case of AMF, they associate with plant roots to form a mutualistic symbiotic relationship.

AMF form arbuscules and vesicles within the roots of plants forming a symbiotic relationship which becomes mutually beneficial to both plants and fungi. AMF begin their life cycle as a spore. The spore germinates when conditions favor its survival and grows hyphae. The hyphae grow within the soil forming mycelium which is a threadlike root structure. These hyphae are influenced by strigolactones (root exudates) and dependent upon soil phosphorus concentrations to naturally be present. Within the rhizosphere roots exude strigolactones and the hyphae are attracted to the roots. The hyphae penetrate the cortical cells of the roots. Within the cells arbuscules and vesicles form. The arbuscles are formed as an exchange site. They help exchange phosphorus, water, and other nutrients to the plants and in return the plant exchanges carbon to the AMF forming a mutualistic symbiotic relationship. Each organism can benefit each other and proves the benefits of having AMF in the vineyard.

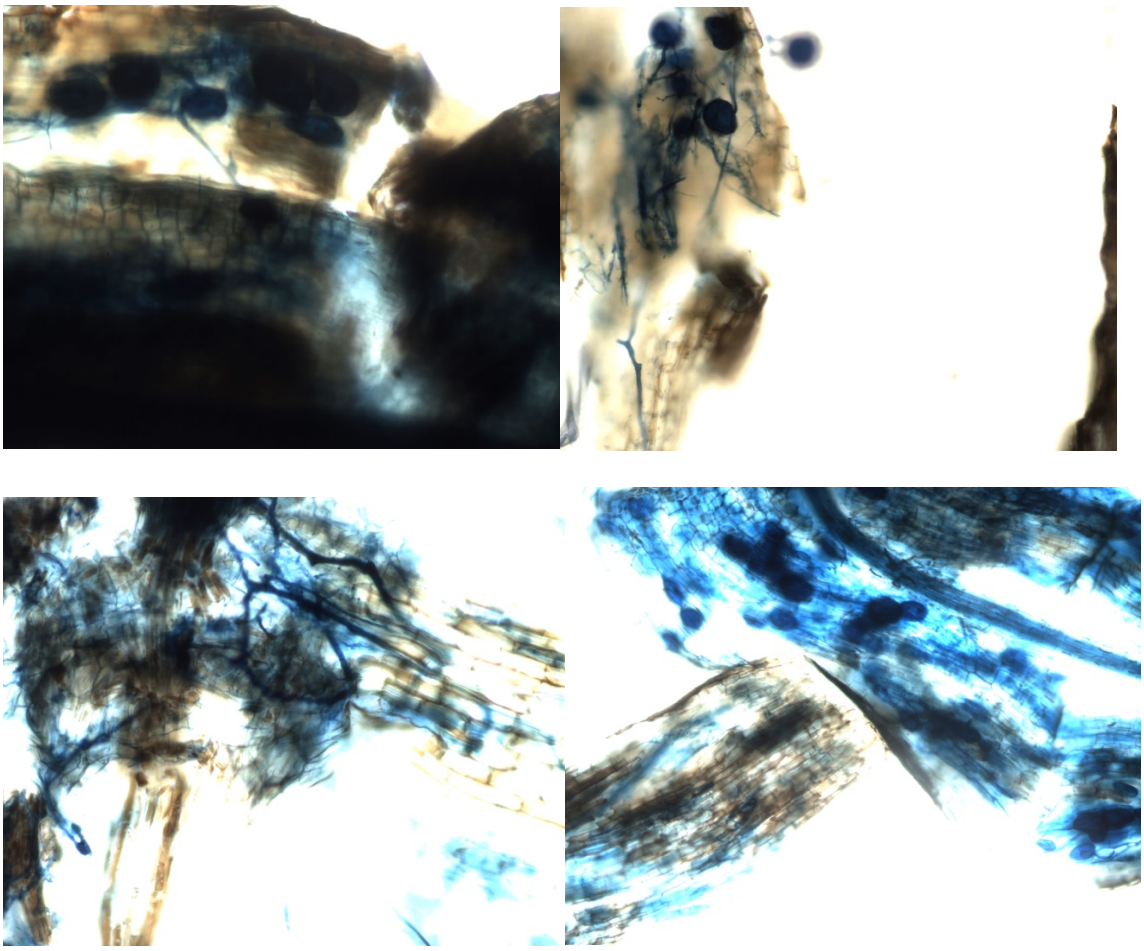

Figure 1: Hyphae and vesicles as a root colonization as evidenced from microscope slides.

Site

The vines were planted at the Cornell experimental vineyard in Lansing, NY, Tompkins County, (Finger Lakes A.V.A.). The site soil composition is made up of an Ovid silt loam and has a 0-6% slope. It is somewhat poorly drained and located relatively close to Cayuga lake. The average precipitation is 43.16 inches annually, average frost dates are in May and mid-October. The typical average growing season is from May to October.

Tompkins County, New York (NY109)

Tompkins County, New York (NY109)

Map Unit Symbol	Map Unit Name	Acres in AOI	Percent of AOI
OaA	Ovid silt loam, 0 to 6 percent slopes	0.3	100.0%
Totals for Area of Interest		**0.3**	**100.0%**

Figure 2: Location and soil map for experimental block. Map courtesy of USDA Soil Web.

Materials and Methods

For this experiment the cultivar Pinot Noir was used to conduct trials of AMF inoculation. Vitis vinifera 'Pinot Noir' scions grafted onto 101-14 and 3309C rootstocks and planted in the experimental vineyard at Lansing, NY in June of 2019. The commercial product MycoApply Endo granular was inoculated to each treated vines root zone. The trellis system used was Vertical Shoot Positioning (VSP). Each third vine of each panel had a minirhizotron tube inserted the year of planting to facilitate root imaging. In May of 2020 there was a replanting of 14 unsuccessfully planted vines from 2019.

Planting Information

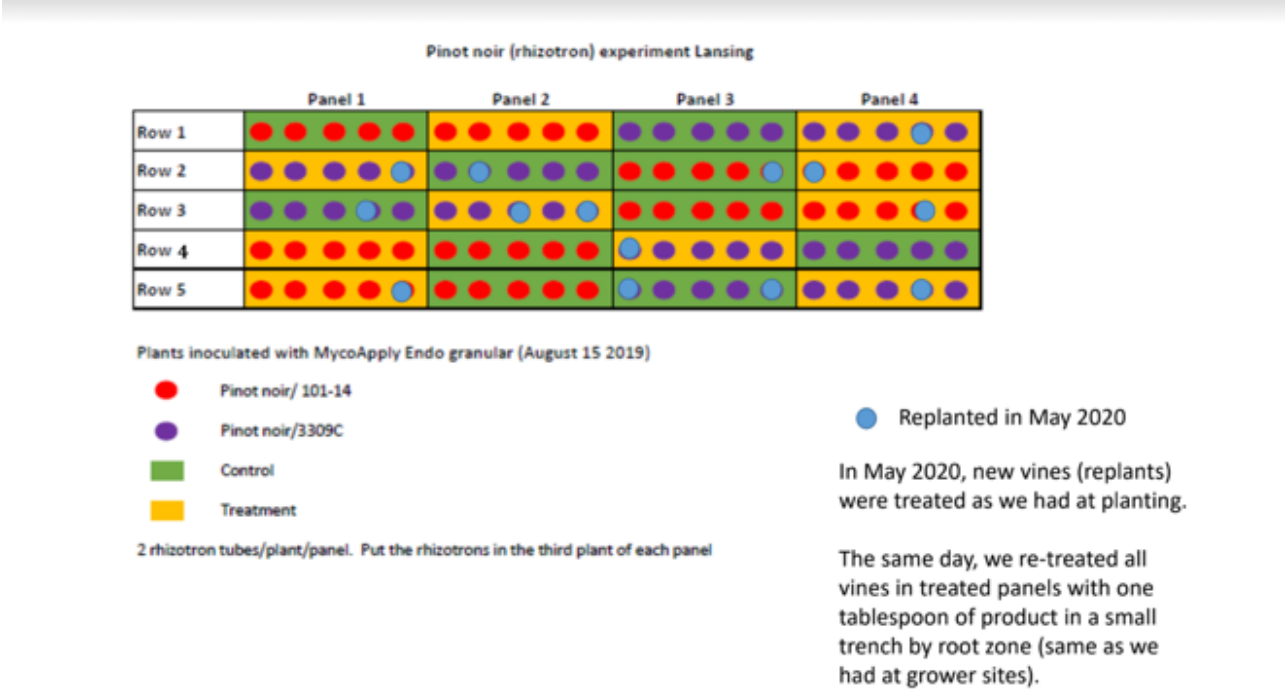

Figure 3: Experimental design of trial. Note the two different rootstocks.

The Pinot Noir vines were planted in 5 rows with 4 panels in a north to south orientation. The plot was divided by rootstock 101-14 and 3309c, where there are 5 reps of inoculated vs. control (non-inoculated) as shown in figure 3.

Veraison Leaf Samples

Leaf samples were taken after veraison and analyzed for nutrient concentration. The graphs below show the total Nitrogen and Carbon for each vine. As the graphs below indicate there was some variability but not significant amount enough to differentiate between control and treatments or rootstock type. The highest total N was 2.47% (PN/3309C control) and the lowest was 1.72% (PN/3309C control). The highest total C was 48.54% (PN/3309C control and the lowest was 41.35% (PN/3309C control). Resulting that the control and rootstock type was dominate on each side of the scale. The average total N was 2.19% and the average total C was 46.66%.

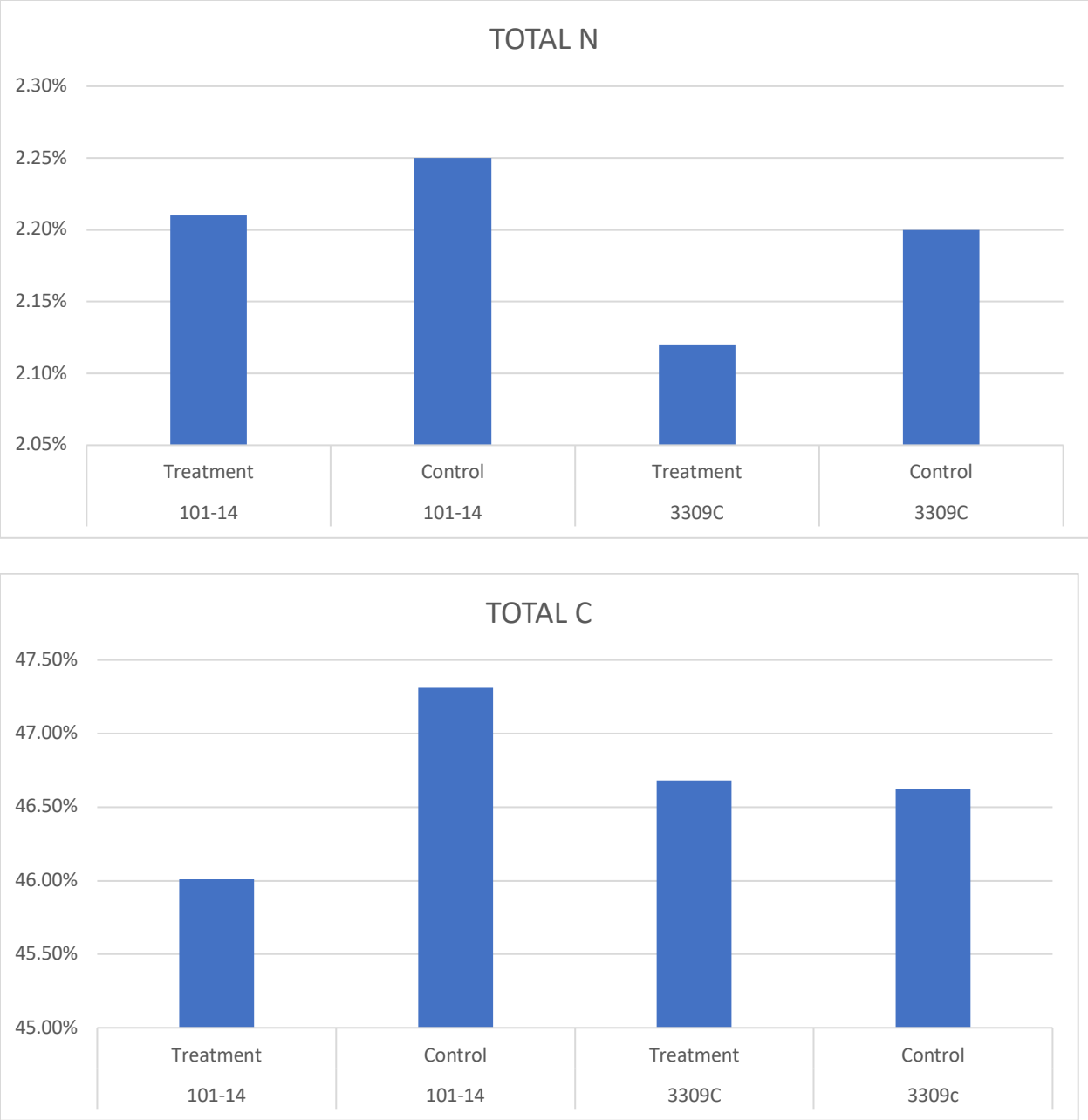

Figure 4: Total nitrogen above and carbon below of leaf blades at veraison.

Statistical Analysis

Core samples were extracted from each vine rhizosphere and sent to the lab. Within the lab the core samples were sifted through a sieve removing the soil and exposing the collected roots. The roots were then placed in a vial and hydrated. Afterwards the individual root samples were delicately placed on a microscope slide and stained to be scrutinized. Next, we identified which slides had AMF present and began quantifying the amounts present. After quantifying the hyphae, vesicles, and arbuscles under a microscope for each root sample. We used the Pvalue of 0.05 to determine significance. It was determined that the Pvalue of 0.00546 was significant between treatment and control but the Pvalue of 0.37787 was not significant between rootstock and treatment, suggesting inoculation resulted in a similar effect of treatment on both rootstocks. Below is the breakdown of each category within the experiment that we analyzed to determine AMF colonization.

Cultivar	Treatment	Row	Panel	Block	Negatives	Vesicles	Arbuscules	Hyphae	Intersection	% Vesicles	% Arbuscules	% hyphae	% RLC Root length colonized
Pinot Noir/101-14	Treatment	5	1	5	16	4	1	26	47	8.5106382978723	2.1276595744681	55.31914893617	65.957446808511
Pinot Noir/101-14	Control	5	2	5	23	4	5	15	47	8.5106382978723	10.63829787234	31.914893617021	51.063829787234
Pinot Noir/3309C	Treatment	5	3	5	32	6	0	3	41	14.634146341463	0	7.3170731707317	21.951219512195
Pinot Noir/3309C	Control	5	4	5	18	5	0	21	44	11.363636363636	0	47.727272727273	59.090909090909
Pinot Noir/101-14	Control	4	1	4	35	7	0	2	44	15.909090909091	0	4.5454545454546	20.454545454546
Pinot Noir/101-14	Treatment	4	2	4	8	13	4	17	42	30.952380952381	9.5238095238095	40.476190476191	80.952380952381
Pinot Noir/3309C	Control	4	3	4	25	4	1	15	45	8.8888888888889	2.2222222222222	33.333333333333	44.444444444444
Pinot Noir/3309C	Treatment	4	4	4	5	19	0	31	55	34.545454545454	0	56.363636363636	90.909090909091
Pinot Noir/3309C	Control	3	1	3	33	10	0	11	54	18.518518518519	0	20.37037037037	38.888888888889
Pinot Noir/3309C	Treatment	3	2	3	11	37	1	36	85	43.529411764706	1.1764705882353	42.352941176471	87.058823529412
Pinot Noir/101-14	Control	3	3	3	21	12	0	19	52	23.076923076923	0	36.538461538462	59.615384615385
Pinot Noir/101-14	Treatment	3	4	3	4	19	1	42	66	28.787878787879	1.5151515151515	63.636363636364	93.939393939394
Pinot Noir/3309C	Treatment	2	1	2	21	7	1	18	47	14.893617021277	2.1276595744681	38.297872340426	55.31914893617
Pinot Noir/3309C	Control	2	2	2	29	5	0	14	48	10.416666666667	0	29.166666666667	39.583333333333
Pinot Noir/101-14	Control	2	3	2	34	4	0	6	44	9.0909090909091	0	13.636363636364	22.727272727273
Pinot Noir/101-14	Treatment	2	4	2	21	17	1	7	46	36.95652173913	2.1739130434783	15.217391304348	54.347826086957
Pinot Noir/101-14	Control	1	1	1	24	19	0	15	58	32.758620689655	0	25.862068965517	58.620689655172
Pinot Noir/101-14	Treatment	1	2	1	16	23	0	16	55	41.818181818182	0	29.090909090909	70.909090909091
Pinot Noir/3309C	Treatment	1	3	1	22	16	0	0	38	42.105263157895	0	0	42.105263157895
Pinot Noir/3309C	Control	1	4	1	37	2	0	2	41	4.8780487804878	0	4.8780487804878	9.7560975609756

Figure 5: Vesicle, Arbuscles, Hyphae, and RLC percentages

Vesicles

Table 1: ANOVA for root length colonization of vesicles in experiment.

Source	Logworth	PValue
Treatment	2.262	0.00546
Cultivar*Treatment	0.423	0.37787
Cultivar	0.232	0.58591

REML Variance Component Estimates

Random Effect	Var Ratio	Var Component	Std Error	95% Lower	95% Upper	Wald p-Value	Pct of Total
Block[Cultivar]	0.4973708	41.127151	46.127421	-49.28093	131.53523	0.3726	33.216
Treatment[Cultivar,Block]		82.68912	41.34456	37.726267	303.4838	<.0001*	66.784
Total		123.81627	46.127421	66.868854	303.07828		100.000

-2 LogLikelihood = 133.55448665

Note: Total is the sum of the positive variance components.

Total including negative estimates = 123.81627

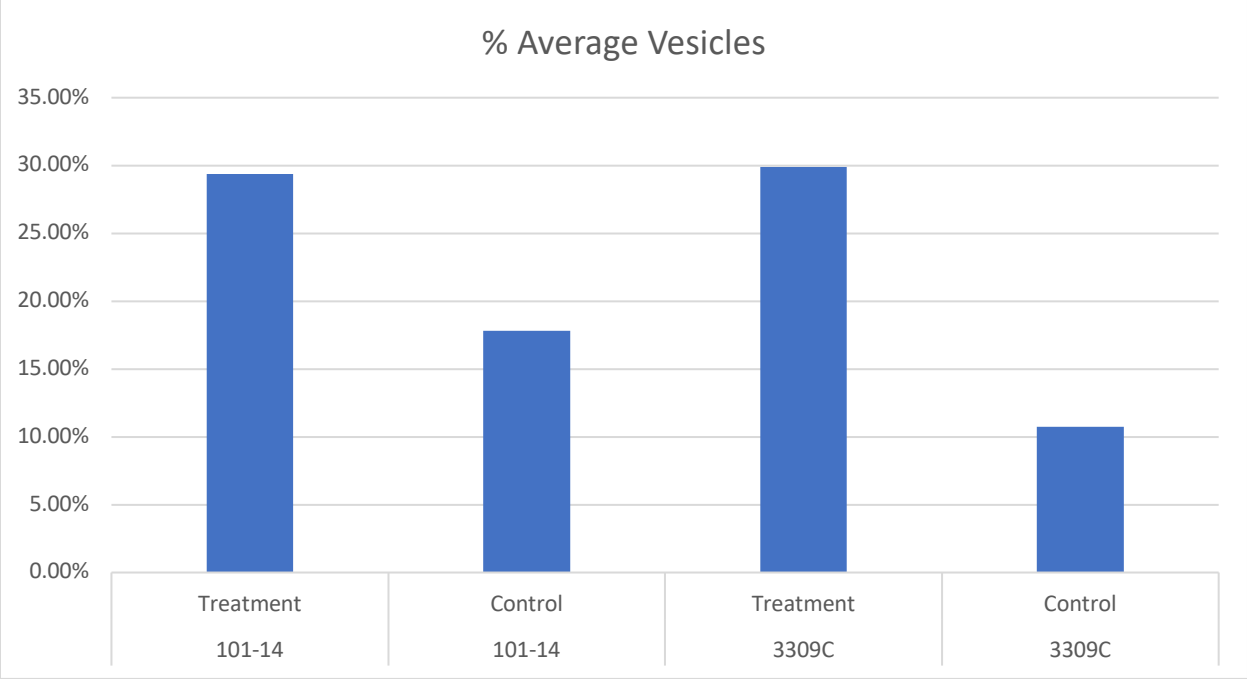

Figure 6: Average. proportion of root length containing vesicles.

Arbuscles

Table 2: ANOVA for root length colonization of arbuscules in experiment.

Source	Logworth	PValue
Cultivar	0.830	0.14791
Treatment	0.151	0.70701
Cultivar*Treatment	0.090	0.81349

REML Variance Component Estimates

Random Effect	Var Ratio	Var Component	Std Error	95% Lower	95% Upper	Wald p-Value	Pct of Total
Block[Cultivar]	-0.13012	-1.434157	3.42745	-8.151836	5.2835212	0.6756	0.000
Treatment[Cultivar,Block]		11.02178	5.5108898	5.0286011	40.451894	<.0001*	100.000
Total		11.02178	5.5108898	5.0286011	40.451894		100.000

-2 LogLikelihood = 93.375492221

Note: Total is the sum of the positive variance components.

Total including negative estimates = 9.5876223

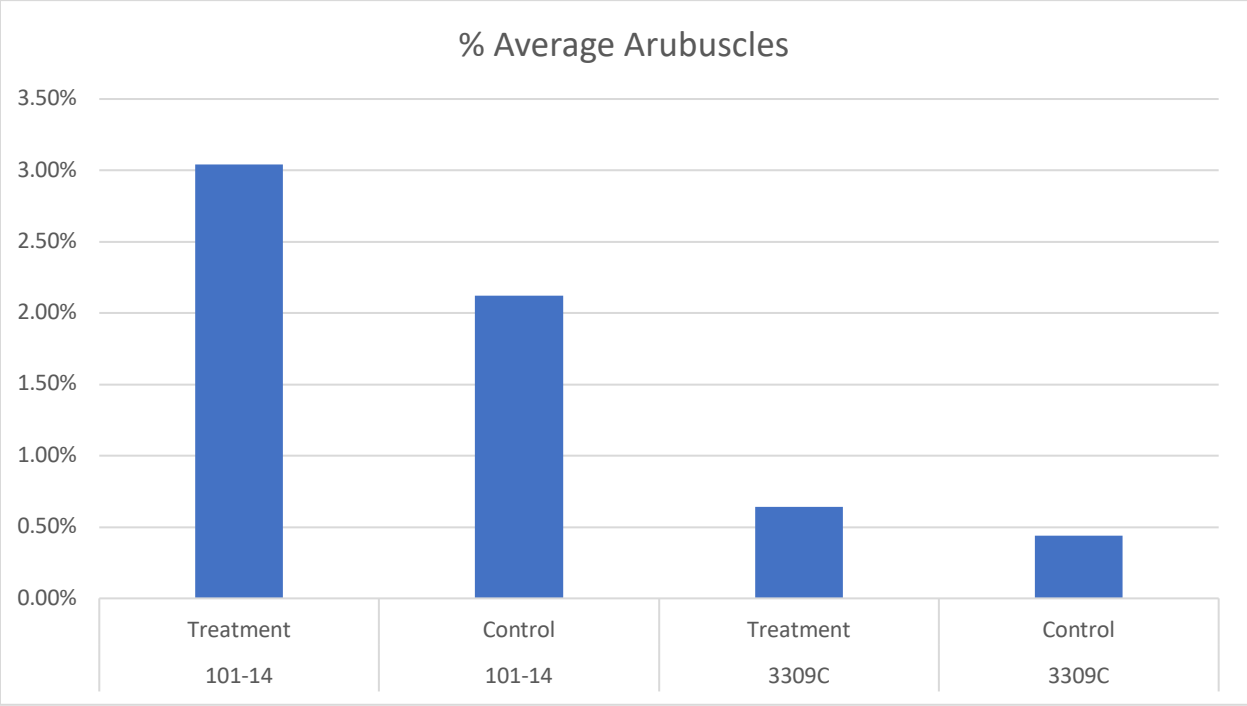

Figure 7: Arbuscles proportion of root length containing arbuscles.

Hyphae

Table 3: ANOVA for root length colonization of hyphae in experiment.

Source	Logworth	PValue
Treatment	0.752	0.17712
Cultivar*Treatment	0.589	0.25785
Cultivar	0.145	0.71560

REML Variance Component Estimates

Random Effect	Var Ratio	Var Component	Std Error	95% Lower	95% Upper	Wald p-Value	Pct of Total
Block[Cultivar]	0.5179381	118.44764	129.67984	-135.7202	372.61546	0.3610	34.121
Treatment[Cultivar,Block]		228.69073	114.34536	104.33836	839.3357	<.0001*	65.879
Total		347.13837	129.67984	187.21072	852.28388		100.000

-2 LogLikelihood = 149.99430148
Note: Total is the sum of the positive variance components.
Total including negative estimates = 347.13837

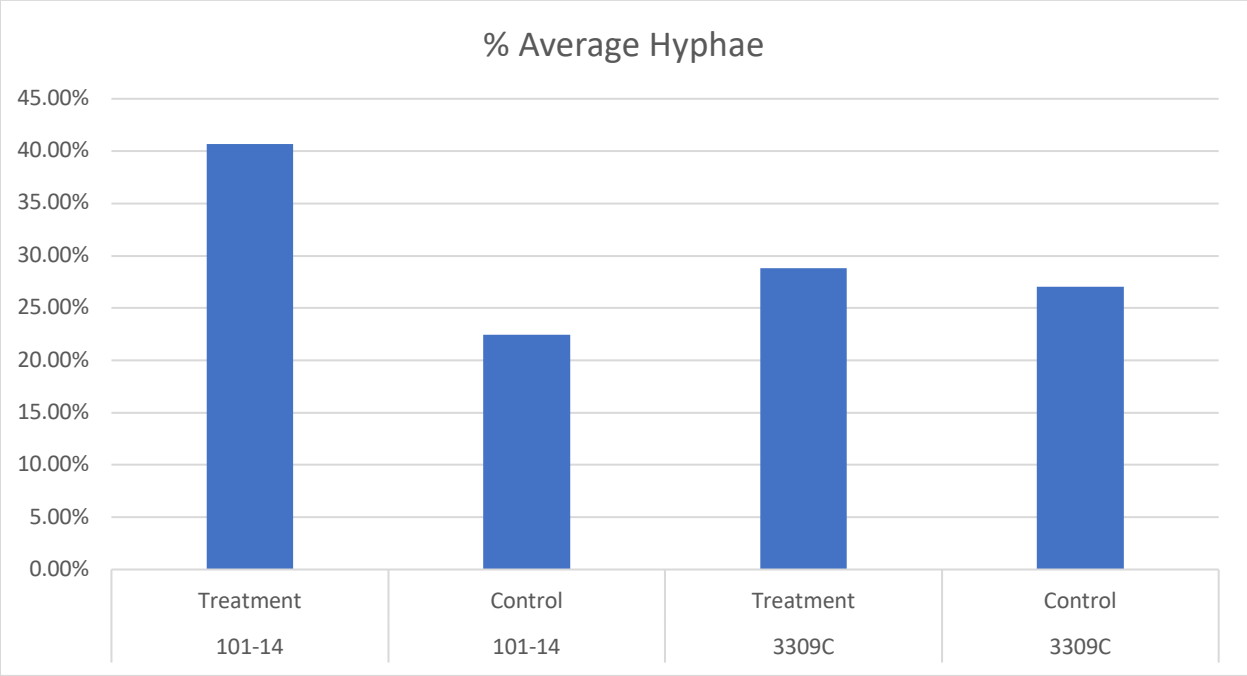

Figure 8: Average proportion of root length colonization of hyphae.

Root Length Colonized

Table 4: ANOVA for total root length colonization in experiment.

Source	Logworth	PValue
Treatment	1.698	0.02005
Cultivar	0.404	0.39487
Cultivar*Treatment	0.217	0.60616

REML Variance Component Estimates

Random Effect	Var Ratio	Var Component	Std Error	95% Lower	95% Upper	Wald p-Value	Pct of Total
Block[Cultivar]	0.117731	47.195739	159.29423	-265.0152	359.40669	0.7670	10.533
Treatment[Cultivar,Block]		400.87766	200.43883	182.89731	1471.2924	<.0001*	89.467
Total		448.07339	159.29423	247.85085	1043.7183		100.000

-2 LogLikelihood = 154.97902298

Note: Total is the sum of the positive variance components.

Total including negative estimates = 448.07339

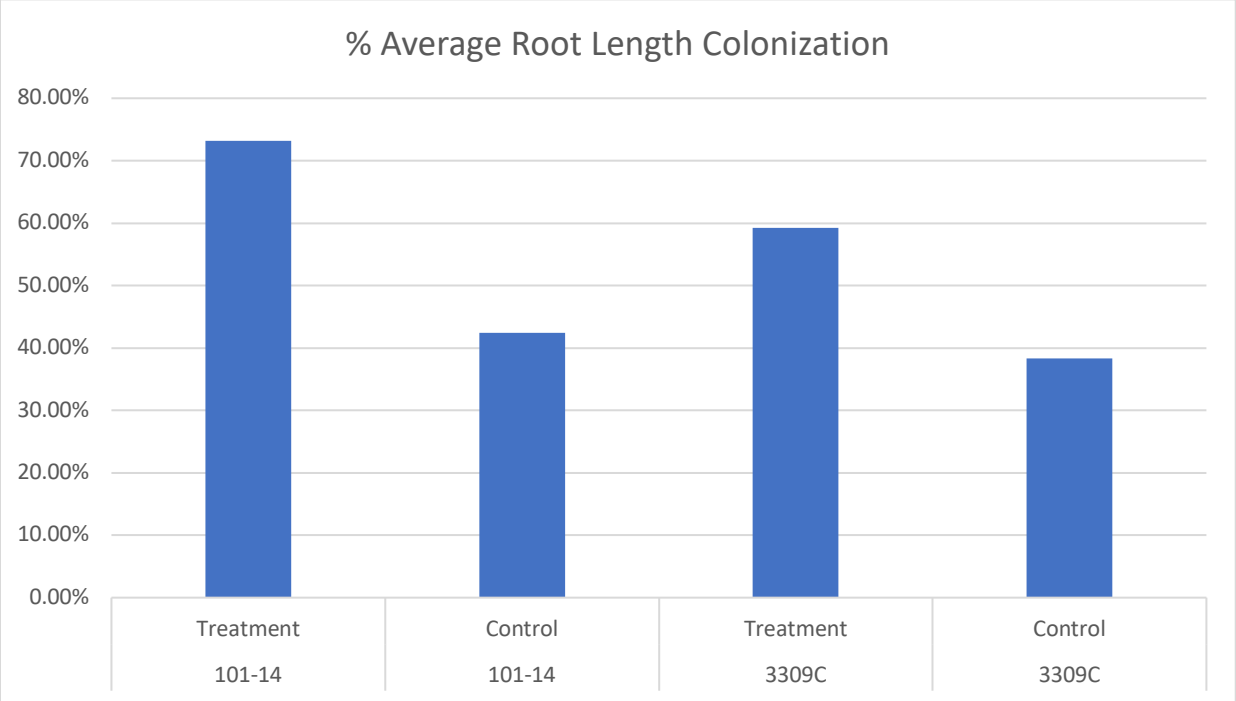

Figure 9: Average proportion of root length colonized.

Microscope Photograph Slides

Live photographs of root samples displaying abundant populations of vesicles and hyphae of the collected root samples. The vesicles and hyphae were highly visible to find and tedious to calculate. The arbuscles were seldom to find and not as easy to distinguish under the microscope. As you can see the AMF populations were present and roots were colonized. Unfortunately, the high-density populations did not correlate with high nutrient uptake or change in growth patterns.

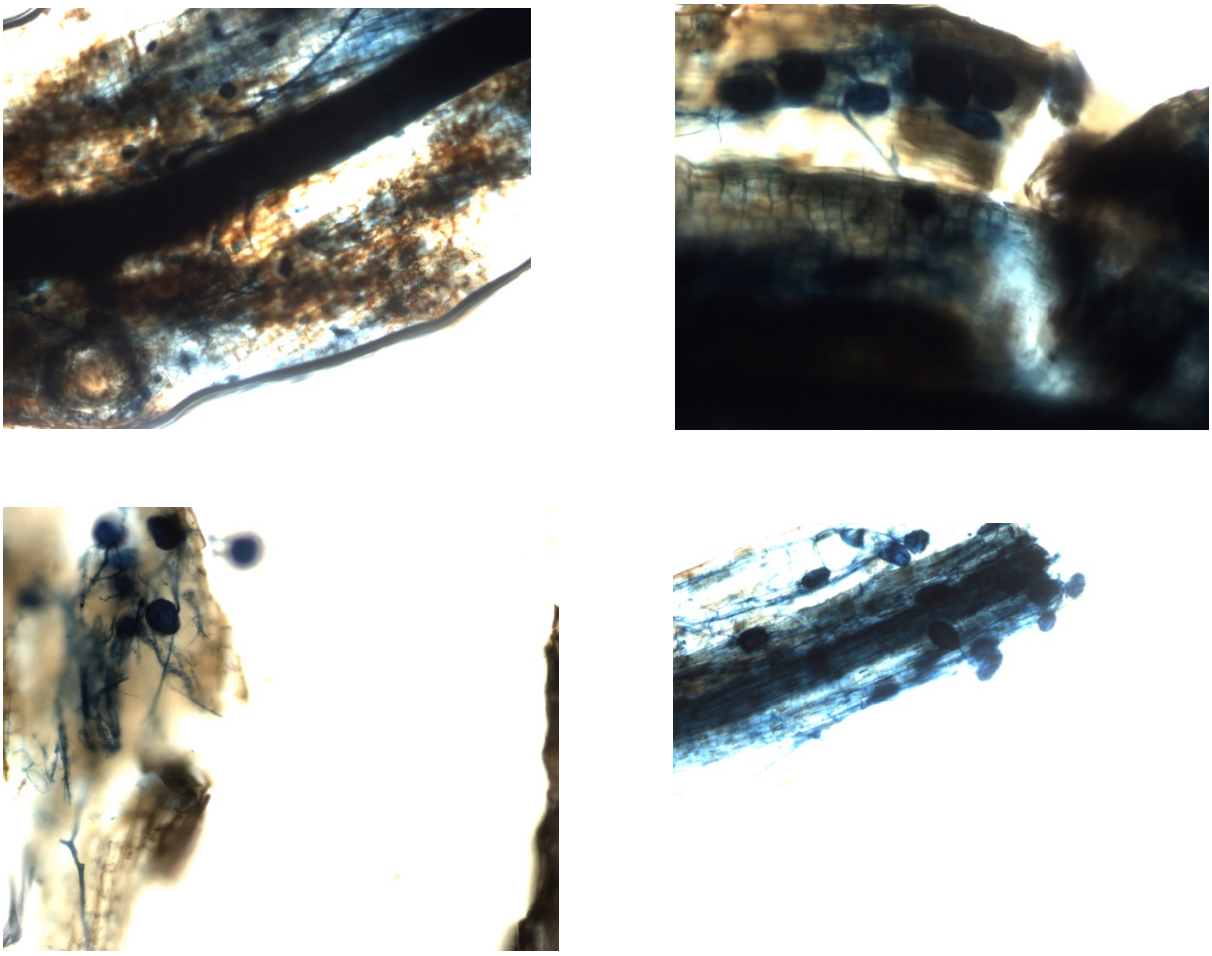

Figure 10: Examples of hyphae and vesicles in stained roots.

Results

In late winter early spring cane and lateral shoot measurements were taken at random from each row and panel.

Cane Measurements

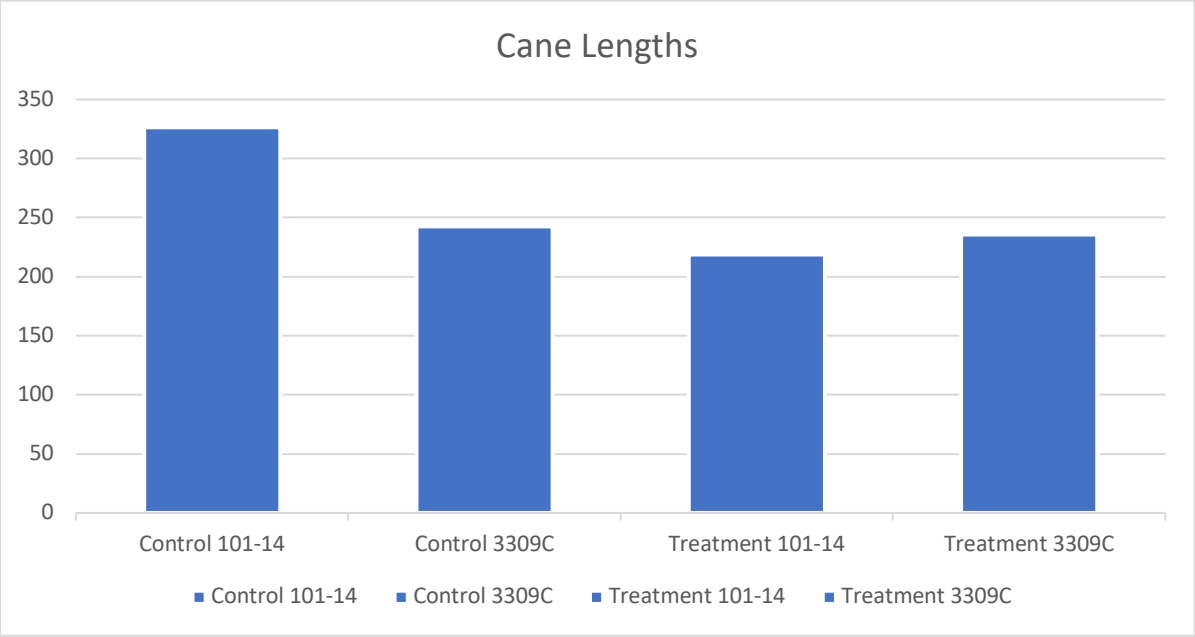

Figure 11: Average cane length (cm) in each of the four treatments.

Pruning Weights

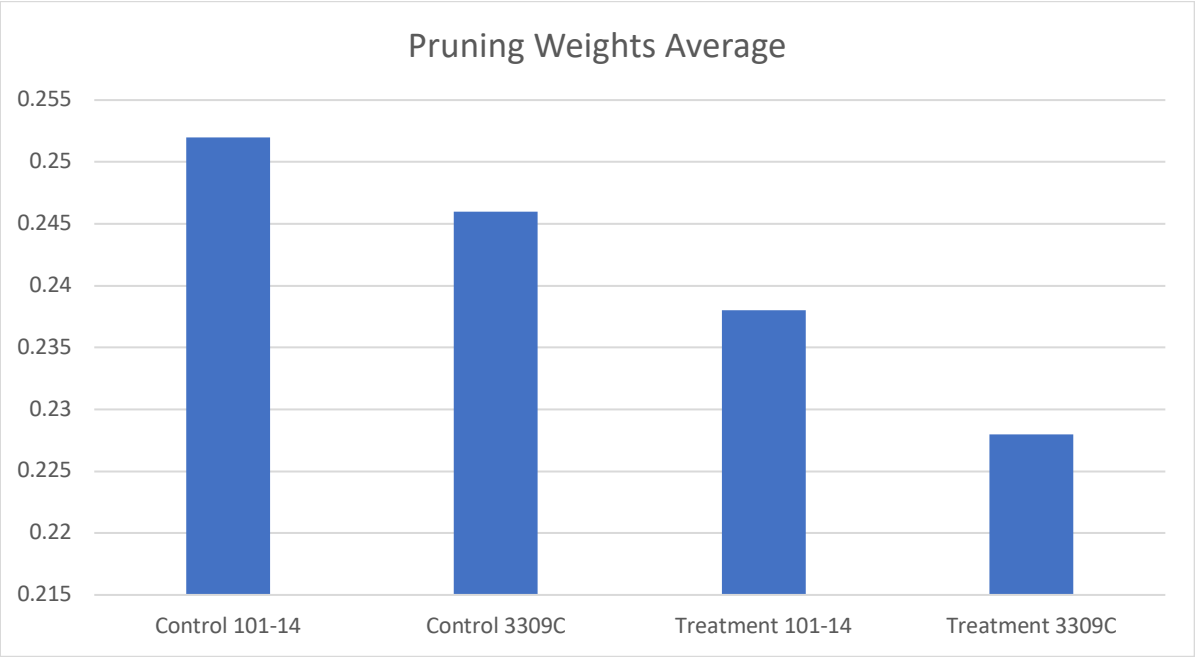

Figure 12: Average pruning weight (kg) in each of the four treatments.

DISCUSSION

For the Pinot noir on rootstocks 3309C and 101-14, the inoculation with AMF may be promising as we determined increased root length colonization as a result. The remaining data collected was somewhat variable. These vines are still young and not quite established, but we were looking for more evidence to show more nutrient uptake benefiting these vines.

Going forward as a suggestion, maybe different applications might bear improved results. Combining biochar and or compost to the mix at various rates might possibly change the outcome. Depending on the year whether it being a wet or dry year the use of irrigation could be influential in the experiments to ensure optimal vine health.

CONCLUSION

In conclusion there was some significant difference between vines treated with AMF, and the control. Further research will be needed to determine the long-term impact of inoculation. The use of other grape varieties and different commercial mycorrhizal inoculants may result in different vine responses as a hypothesis. Soil conditions and climate maybe a pivotal factor for different results, particularly as AMF respond to low phosphorus in the soil. Conducting further experiments is required to reach a conclusion about the impact of inoculating with AMF.

REFERENCES

Schreiner, P. 2007. **Effects of Native and Nonnative Arbuscular Mycorrhizal Fungi on Growth and Nutrient Uptake of 'Pinot Noir' (Vitis vinifera L.) in Two Soils with Contrasting Levels of Phosphorus**, Science Direct, USDA-ARS-Horticultural Crops Research Laboratory, March 1, 2007.

Schreiner, P. 2005. **Mycorrhizas and Mineral Acquisition in Grapevines**, The American Society for Enology and Viticulture, Proceedings of the Soil Environment and Vine Mineral Nutrition Symposium, L.P, USDA-Agricultural Research Service, Horticultural Crops Research Laboratory. https://www.ars.usda.gov/ARSUserFiles/5018/pdf/asev05.pdf

Begum, N., Qin, C., Ahanger, M. A., Raza, S., Khan, M. I., Ashraf, M., Ahmed, N., Zhang, L., 2019. **Role of Arbuscular Mycorrhizal Fungi in Plant Growth Regulation: Implications in Abiotic Stress Tolerance**, Frontiers in Plant Science, September 19, 2019. https://www.frontiersin.org/articles/10.3389/fpls.2019.01068/full

Giese, G., Velasco-Cruz, C., Leonardelli, M., 2020. **Grapevine Phenology: Annual Growth and Development**, Publications Horticulture, College of Agricultural, Consumer and Environmental Sciences, New Mexico University, August 2020. https://pubs.nmsu.edu/_h/H338/

Trouvelot, S., Bonneau, L., Redecker, D., Tuinen, D. van, Adrian, M., Wipf, D., 2015. **Arbuscular Mycorrhiza Symbiosis in Viticulture**, Agronomy for Sustainable Development, September 1, 2015. https://link.springer.com/article/10.1007/s13593-015-0329-7

Heuvel, J. V., Berdeja, M., Ye, Q., 2020. **Enhancing Vine Health with Commercial Arbuscular Inoculants**, Section of Horticulture, School of Integrative Plant Science, Cornell University, 2020. https://grapesandwine.cals.cornell.edu/sites/grapesandwine.cals.cornell.edu/files/shared/2019Research%20Focus2020-4.pdf

Baumgartner, K., 2006. **The Role of Beneficial Mycorrhizal Fungi in Grapevine Nutrition**, USDA-Agricultural Research Service, UC Extension, Davis, March 16, 2006. https://iv.ucdavis.edu/files/24424.pdf

Torres, N., Antolin, M.C., Giocoechea, N. 2018. **Arbuscular Mycorrhizal Symbiosis as a Promising Resource for Improving Berry Quality in Grapevines Under Changing Environments**, Frontiers in Plant Science, June 29, 2018. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6034061/

Thomsen, C., Loverock, L., Kokkoris, V., Holland, T., Bowen, P.A., Hart, M. 2021. **Commercial Arbuscular Mycorrhizal Fungal Inoculant Failed to Establish in a Vineyard Despite Priority Advantage**, Ecology, April 22, 2021.
https://peerj.com/articles/11119/

Rosa, D., Poiatzis, A., Bowen, P., Kokkoris, V., Richards, A., Holland, T., Hart, M. 2020. **Performance and Establishment of a Commercial Mycorrhizal Innoculant in Viticulture**, Sustainable Viticulture and Agriculture, November 10, 2020.
https://www.mdpi.com/2077-0472/10/11/539

Eftekhari, M., Alizadeh, M., Mashayekhi, K., Asghari, H., Kamkar, B. 2010. **Integration of Arbuscular Mycorrhizal Fungi to Grape Vine (Vitis vinifera L.) in Nursery Stage**, Journal os Advanced Laboratory Research in Biology, Volume 1, Issue 2, October 2010.
file:///Users/lloydjones/Downloads/Integration%20of%20arbuscular%20mycorrhizal%20fungi%20to%20grapevine%20_Vitis%20vinifera%20L.pdf

Karoglan, M., Radic, T., Anic, M., Andabaka, Z., Stupic, D., Tomaz, I., Mesic, J., Karazija, T., Petek, M., Lazarevic, B., Polijak, M., Oseracak, M. 2021. **Mycorrhizal Fungi Enhance Yield and Berry Chemical Composition of in Field Grown "Cabernet Sauvignon" Grapevines (Vinifera L.)**, Agricultural Technology, May 19, 2021.