Impacts of drought on carrots and their symbiotic fungi: from field interactions to gene expression

By

Michelle Lynn Pearson

A dissertation submitted in partial fulfillment

of the requirements for the degree of

Doctor of Philosophy

(Cellular and Molecular Biology)

at the

UNIVERSITY OF WISCONSIN–MADISON

2019

Date of final oral examination: 08/05/2019

The dissertation is approved by the following members of the Final Oral Committee:
Erin Silva, Associate Professor, Plant Pathology
Jean-Michel Ané, Professor, Bacteriology and Agronomy
Nancy Keller, Professor, Bacteriology, and Medical Microbiology and Immunology
Anne Pringle, Professor, Botany and Bacteriology
Richard Lankau, Associate Professor, Plant Pathology

Abstract

In a symbiosis that occurs globally in soils, arbuscular mycorrhizal (AM) fungi engage in mutually beneficial interactions with the roots of plants. Hosts of AM fungi permit and regulate the colonization of their tissues by reorganizing their cellular components to enable the fungus to grow intracellular structures, called arbuscules. These highly branched hyphal projections facilitate exchanges between symbionts, in which the fungus receives lipids and sugars, its only carbon source. Plants benefit from the direct transfer of nutrients as well as from improved tolerance to environmental stress, both of which may be influenced by the genotype of the fungal partner as well as the genotype of the host plant.

Water stress poses challenges to plants and agricultural production. AM symbiosis is known to alter plant water management, partially buffering against the negative impacts of drought. Recent work suggests that AM fungi likely transfer water directly to hosts while also modulating hosts' transpiration (by altering carotenoid and hormone processes) and improving hosts' nutritional status (with direct transfers of nitrogen, phosphorus, and potassium). Although much is known of host responses to AM colonization during water stress, relatively little is known of the stress responses in AM fungi. Recent sequencing and annotation of the AM fungal (*Rhizophagus irregularis*) genome created new opportunities to investigate these organisms.

Carrot (*Daucus carota*) is an excellent plant model for mycorrhizal research. Much of what is known of AM fungal stress biology came out of *in vitro* experiments in which root organ cultures of carrot supported fungal growth. Carrot invests heavily in the production of its taproot, and it permits a high level of AM colonization. The newly available genome of carrot, its status as a highly nutritious and economically important vegetable, and its high level of carotenoid production make it an intriguing plant model for studies involving AM fungi. The work

described below aimed to answer questions about host-fungal interactions during drought with evaluations that range from genotype x genotype experiments to those identifying the changes in gene expression that occur for drought-challenged AM symbionts.

Experimental repetition highlighted the folly of extrapolating trends in AM contributions to plant growth within individual experiments. From multiple iterations of field and greenhouse trials, in which we subjected combinations of cultivars of carrot and isolates of AM fungi to late-season water restriction in organic soils, we observed inter- and intraspecific variability among fungal isolates in their contributions to plant growth, but these impacts differed in each trial. Although we observe what could be called 'functional diversity' among AM isolates, it was not possible to elucidate trends in fungal contributions to carrot yield. This may be in part due to the established mycorrhizal networks of native AM populations (in the field) and due to seasonal light availability and differences among soils (in both the greenhouse and field).

Breeding histories of plants influenced compatibility with AM fungal inoculants. In the field experiments, although no AM isolate provided a consistent impact, carrot genotypes clearly indicated differential response to inoculation. The open-pollinated, heirloom cultivars exhibited a generally positive mycorrhization response (measured in weights of taproots relative to mock-inoculated controls). On the other hand, hybrid cultivars, bred in and for high-input systems, demonstrated neutral to negative mycorrhization response. This suggests that breeding may be an important consideration if we seek to enhance benefits from AM symbiosis.

Gene expression of AM fungi and the carrot hosts contrasted during drought. *R. irregularis* exhibited a high level of gene upregulation, including for symbiotic genes. Carrot gene expression revealed mostly downregulation in response to drought, with reduced expression

of genes involved in the establishment and maintenance of symbiosis. Generally, it seemed that carrot and *R. irregularis* differed in their carbon management strategies during drought stress.

Acknowledgments

I am grateful to my amazing husband, Andrew Pearson, who provided support in every imaginable way throughout my work toward earning my PhD. My graduate experience was peppered with moments of family grief, through which Andrew provided unwavering love, kindness, and compassion. He reliably encouraged me through all moments of self-doubt, always showing his belief in me. Andrew contributed directly to my success by helping me set up and take down experiments when I was short on hands. He also read drafts of manuscripts, despite the fact that he thought it was like reading a foreign language. Most of all, I am thankful to Andrew for being my equal partner, a doting father, and my best friend.

Thanks to my family for believing in and for whole-heartedly supporting my academic pursuits, even when it meant my moving across the country during a period of family loss. My deepest thanks go to my parents, Donna and Larry Wood. Thank you to my sister, Jolene Pollock. I am glad we were able to share so many big moments together, both difficult and wonderful. I also want to acknowledge Corinne and David Pearson, whose interest in and inquiries about my work helped me to articulate and understand my goals more clearly. Thank you to my friends, (Dr.) Kymi Draeger, Tomás Rush, (Dr.) Katie Gold, and (Dr.) Marian Bolton, Monica Snipes, and David Jones, with whom I shared the experience of graduate school. The ability to share knowledge, expertise, advice, and commiseration was priceless.

Thank you to my advisors Drs. Erin Silva and Jean-Michel Ané, who enabled me to pursue my research and professional development interests. I appreciate the time you each dedicated to my growth as an academic. Your support and thoughtful critiques pushed me to become a better scientist, and I am fortunate to have been encouraged to learn new skills under your direction. I also want to thank the members of my thesis committee, Drs. Nancy Keller,

Anne Pringle, and Richard Lankau. Over the past five years, you each took time with me one-on-one to provide advice, feedback, and excellent conversation – thank you.

I would like to give a special thanks to my colleagues from the laboratory, greenhouse, and field. Anthony Bortolazzo and Zachary Keyser, you have been excellent lab mates and great friends. Thank you to Drs. Kevin Cope and Kevin Garcia for their input and feedback early in my graduate progress. Thanks to Dylan Bruce for all the time helping me in the field. Thank you to my former lab mate, and forever friend, Danielle Graham – you helped me to take on and navigate challenging work with your supportive words. Thank you to Bjorn Karlson for your expertise and endless assistance in the Biotron Laboratory.

During my graduate career, I was fortunate to mentor many undergraduate students, and each one contributed to the work presented here. Thank you: Brendan Smith, Kelly Verhaalen, Alyssa Joachim, Holiday (Yang) Liu, Brianna Holubets, Annika Peterson, Kaley Pederson, Ian McIntire, Brent Arnoldussen, Luke Willems, Kahaan Patel, Elisse Pavletich, Alex Johnson, and Tyler Kowalczyk.

I am deeply grateful for the funding and community provided by the Science and Medicine Graduate Research Scholars (known as SciMedGRS). I was also fortunate to also have funding through the National Science Foundation's Graduate Research Fellowship Program. Thanks to all the communities that helped me to learn and grow including my graduate program, Cellular and Molecular Biology; my department, Plant Pathology; and the programs that provide immense professional development in teaching, Delta and WISCIENCE.

Table of Contents

Chapter 1: Introduction

1.1 Drought and Agriculture

The global human population currently exceeds 7 billion people and projections suggest that the population may peak with up to 12 billion people by 2070 (KC & Lutz, 2017). The number of people struggling with undernourishment is on the rise (FAO *et al.*, 2019), and food security is a growing concern. Resource scarcity, including natural limits to water and phosphorus availability, poses challenges to the stability of food production (Mancosu *et al.*, 2015; Chowdhury *et al.*, 2017). In order to feed the growing population, there is an imminent need to shift to more sustainably-minded production practices that conserve natural resources, build soils, and protect biodiversity (OECD, 2012; Van Vuuren *et al.*, 2012).

The changing climate adds to the challenge of feeding a growing population. Climate impacts the ability to grow and distribute food, and severe weather can cause crop failures and food shortages (Mueller *et al.*, 2012; Heino *et al.*, 2018). Human activities fuel climate instability leading to less predictable and, thus, more severe weather events. Drought is among the most devastating disasters for crops and for those who rely solely on their crops for subsistence or income (Morton, 2007). Globally, there is an increase in drought severity and in the amount of land area affected (Howitt *et al.*, 2014; Wang *et al.*, 2014). Recurring droughts pose challenges to crop producers at all scales.

To ensure productive crops in face of scarce resources, agronomic tools must be optimized for sustainability. Organic production is one solution, which relies on recycling of resources on a local level. Instead of imported fossil fuel-intensive synthetic inputs, organic focuses on manure and cover crops (USDA-NOP, 2019). Organic also emphasizes locally-selected varieties adapted to low input, stressful environments.

Another solution to help mitigate the risk of crop failures in scarce resources are augmented soil microbial communities. Since soil microbial communities can buffer against drought stress, farmers may consider adding biological inputs that hold promise of promoting plant health (Hart & Forsythe, 2012; Baum *et al.*, 2015). Fungal inoculants are among the commercially-produced options that farmers add to soils that are sold with promises to increase yields (Pellegrino *et al.*, 2012; Hijri, 2016), enhance water use efficiency (Augé, 2001; Baum *et al.*, 2015), and improve soil quality (Rillig, 2004). There is also potential to exploit the existing variation among cultivars by identifying plant varieties that best stimulate beneficial soil microbial communities. Matching a cultivar or crop to field microbial populations could be a useful strategy in the quest to design more sustainable agronomic solutions.

1.2 Arbuscular Mycorrhizal Fungal Symbiosis

Arbuscular mycorrhizal (AM) fungi form plant symbiotic associations by colonizing the roots of land plants. AM fungi are well known as obligate biotrophs that rely on their hosts to complete their life cycles (Smith and Read, 2008a). Symbiotic plants reap rewards from the interaction through increased access to nutrients and improved stress tolerance conferred by AM fungi. Fossil and genetic evidence date the origins of this symbiosis to about 450 million years ago (Strullu-Derrien et al., 2014; Taylor et al., 1995), with plants following fungi in the colonization of land. Most plant families retained this interaction (Bravo et al., 2016; Delaux et al., 2015), which is characterized by the formation of arbuscules in the cortical cells of host roots (Smith and Read, 2008a).

AM species are members of the Glomeromycota, a ubiquitous group of root-associating fungi. Like other filamentous fungi, AM hyphae explore their environment to scavenge and translocate resources. Unlike many filamentous species, AM fungi produce coenocytic

(aseptate), multinucleate hyphae (Smith and Read, 2008a), which enables the efficient movement of cytoplasmic contents but poses challenges for genetic studies (Riley and Corradi, 2013; Tisserant et al., 2013). Arbuscules are the modified hyphal projections resembling microscopic, intracellular trees, which give rise their name (Nicolson, 1967; Smith and Read, 2008a). These ramified structures facilitate exchanges between AM fungi and their plant hosts.

Prior to the formation of arbuscules, AM fungi and host plants secrete signals into soils that invite symbiotic activity (Harrison, 2005; MacLean et al., 2017). Strigolactones (Besserer et al., 2006), flavonoids, and increased carbon dioxide from plant roots indicate the availability of a receptive host to nearby AM fungi (Bécard et al., 1992). In turn, the plant receives AM fungal signals, known as Myc (mycorrhizal) factors, which comprise short chitooligosaccharides and lipochitooligosaccharides (Genre et al., 2013; Maillet et al., 2011). These communications lead to the joint construction of the symbiotic interface, beginning with the attachment of a fungal hypha to the host root and (Wang et al., 2012), ending with intraradical fungal growth.

Arbuscules are highly branched hyphal structures characteristic of AM symbiosis. Host plants invest in the production of increased plasma membrane to invaginate around the growing arbuscule, forming the ‘periarbuscular membrane’(Gutjahr and Parniske, 2013). It is at this interface that interspecies trade occurs. AM fungi deliver nutrients to hosts that are often limited in soils, such as phosphorus (P), nitrogen (N), and potassium, (Smith and Read, 2008a). Additionally, AM fungi improve plant water status (Augé, 2001), though the mechanisms of this remain in debate. Plants compensate their fungal partners by sharing sugars and lipids (Bravo et al., 2017; Helber et al., 2011; Keymer et al., 2017; Luginbuehl et al., 2017), providing vital carbon sources to the fungus.

AM symbiosis is widespread, with no known plant-microbe specificity or host preference. Despite this, there are species and isolate-level differences among AM fungi that may lead to more or less beneficial plant outcomes (Klironomos, 2003). For example, Mensah *et al.* demonstrated that isolates of AM fungi differ in their propensities to provide P and N to hosts, resulting in growth differences (2015). AM fungi may also contribute to differing plant fitness during water stress. Ruiz-Lozano *et al.* reported that inoculating lettuce plants with different species of AM fungi led to differential plant growth, which enabled the ranking of the least to most beneficial species during drought (1995).

Plants are known to benefit from their AM fungal partners during drought stress. It is well-established that mycorrhizal plants alter water management compared to their non-mycorrhizal (NM) counterparts (Augé, 2001). Knowledge of the mechanisms by which plants benefit from AM fungi during water restriction remains incomplete. Benefits may arise from conditioning of the soils from hyphal turnover as well as from direct molecular impacts of AM fungal colonization (Nichols, 2010; Rillig, 2004; Wright and Upadhyaya, 1998). Recent discoveries of highly expressed fungal aquaporins in the intraradical mycelia of the AM species *Rhizophagus irregularis* and *R. clarus* stoked speculation that water moves directly from fungus to host (Aroca et al., 2009; Kikuchi et al., 2016; Li et al., 2013). Kikuchi *et al.* provides substantial evidence and a plausible model for the flow of water and P that depends on a plant's water potential gradient (2016). Polyphosphates (and likely inorganic P) stream through the cytoplasm of AM hyphae toward host roots, without an energetic cost, then break down to permit transfer of inorganic P to host cells (Kikuchi et al., 2016).

Compared to their hosts, relatively little is known about AM fungi. For example, although there are many reports of inter- and intraspecific functional diversity, it is unclear

whether results of such diversity translate from experiments based in greenhouses to those in the field. Similarly, although we have a clear idea of the degree to which host functioning changes with colonization, very little is known as to the impact of drought stress on AM fungi. Experiments in which root organ cultures of carrots supported the growth of *R. irregularis* led to the first identification of an AM fungal aquaporin (Aroca et al., 2009; Porcel et al., 2007, 2006). Later, this *in vitro* co-culture system provided material that enabled the first assembly of the AM fungal genome (Tisserant et al., 2013), which provided new opportunities to study these cryptic fungi at a molecular level (Chen et al., 2018; Lin et al., 2014). Root organ cultures of carrot provide an excellent model (Bécard and Fortin, 1988), supporting sterile production and propagation of AM fungi. Beyond their use in the laboratory, little is known about the relationship between AM fungi and carrots.

1.3 Carrot as a Plant Model

Carrot (*Daucus catota*) has several attributes that make it an excellent research model. Carrot is a biennial plant that produces a large storage taproot during its first year of growth. It is relatively easy to produce tissue cultures of carrot (Baranski, 2008; Bécard and Fortin, 1988), making it a useful laboratory model. Carrot contains high proportions of beta-carotene (pro-vitamin A), lycopene, and lutein, which make it nutritionally important as well as an excellent model for carotenoid studies (Simon et al., 2008). The genome of carrot was recently published (Iorizzo et al., 2016), paving the way for high-quality transcriptomic studies.

Carrot is a popular vegetable with increasing global production and demand. Globally, carrot production value neared $20 billion USD in 2016 (FAOSTAT, 2019). In the United States (US), sales reached $700 million USD in 2017 (USDA-NASS, 2018a). Organic carrots accounted for 14.35% of total carrot production area in 2011 (USDA-ERS, 2011), and organic

carrot sales reached nearly $90 million USD in 2016 (USDA-NASS, 2018b). Most carrots in the US grow in California, which experiences increasingly severe annual droughts costing nearly $100 million USD in vegetable losses (Howitt et al., 2014). As climate change produces hotter annual conditions and agricultural demand for water continues to grow (Wilson et al., 2017), it is vital that production strategies include drought mitigation.

Greenhouse experiments demonstrate that carrots are sensitive to drought, particularly with limited nutrient inputs (Razzaq et al., 2017). Water stress in field-grown carrots may reduce carrot taproot yield and storability (Sôrensen et al., 1997). Recent studies found that irrigation and varying levels of water stress have greater consequences for storage and processing qualities of carrots than for overall yields (Reid and Gillespie, 2017), and improved drought tolerance is among the breeding goals for carrot (Simon et al., 2008).

Carrot is an excellent model for studies with AM fungi. As a well-known 'mycotrophic' plant (Schreiner and Koide, 1993), carrot is already in use as an *in vitro* model of AM symbiosis, supporting the sterile maintenance of AM cultures. Since carrot permits a high percentage of AM colonization, it is the model of choice for those studying AM genomics. With the recent availability of the carrot and *R. irregularis* genomes, high-quality dual transcriptomics studies are possible. Much of the work investigating the best methods by which to grow carrot in soilless media is complete (Kobayashi et al., 2013), so scaling up to growth chamber and greenhouse studies is the next logical step for carrot and its AM fungal symbionts.

1.4 Research Overview

The drought interactions between plants and arbuscular mycorrhizal fungi are well studied in laboratory and greenhouse settings. Despite the breadth of information arising from these studies, many opportunities to contribute new knowledge remain. Studies of drought in

AM inoculated plants typically occur in sterile media in greenhouses, and these usually include only a single AM species. Molecular mycorrhizal studies focus solely on plants, and as a result, our understanding AM fungal responses to varying conditions is exceptionally limited.

Chapter 2 provides a current review of the state of knowledge of the molecular underpinnings and evolution of AM symbioses. This includes a detailed discussion of the genomics of AM fungi, describing their potential for sexual mating and foreshadowing the discovery of plant-to-fungus lipid transport. Further, an evolutionary overview of the plant mechanisms comprising the 'symbiotic toolkit' are provided.

Chapter 3 reports on multi-year field and greenhouse experiments testing unique combinations of isolates of AM fungi and cultivars of carrot in USDA organic soils, with and without drought pressure. Overall, no single AM fungal inoculant provided a consistent yield benefit to carrot, regardless of water treatment. In the field, heirloom cultivars had more positive inoculation response, relative to mock treatments, compared to hybrid cultivars. This supports the notion that breeding in high-input systems could hinder cultivar responsiveness to beneficial soil microbes. This work also demonstrated the importance of experimental repetition, as trends could not be deciphered from single iterations.

Chapter 4 describes the results of a dual-transcriptomic study of carrot and AM fungi (*R. irregularis* DAOM 197198) during drought. This study provides the first complete comparison of gene expression profiles of droughted and well-watered AM fungi. Several mycorrhizal-specific genes were identified in carrot, symbiosis maintenance genes were downregulated in carrot, and drought had a stimulatory impact on fungal gene expression. These results reveal contrasting responses between the plant and fungus and provide new insights into the drought dynamics of this system.

Chapter 5 offers a discussion and conclusions from the work presented in this document.

1.5 References

Aroca R, Bago A, Sutka M, Paz JA, Cano C, Amodeo G, Ruiz-Lozano JM. **2009**. Expression Analysis of the First Arbuscular Mycorrhizal Fungi Aquaporin Described Reveals Concerted Gene Expression Between Salt-Stressed and Nonstressed Mycelium. *Molecular Plant-Microbe Interactions* **22**: 1169–1178.

Augé RM. **2001**. Water relations, drought and vesicular-arbuscular mycorrhizal symbiosis. *Mycorrhiza* **11**: 3–42.

Baranski R. **2008**. Genetic transformation of carrot (*Daucus carota*) and other apiaceae species. *Trans Plant Journal* **2 (1)**: 18–38.

Baum C, El-Tohamy W, Gruda N. **2015**. Increasing the productivity and product quality of vegetable crops using arbuscular mycorrhizal fungi: A review. *Scientia Horticulturae* **187**: 131–141.

Bécard G, Douds DD, Pfeffer PE. **1992**. Extensive In Vitro Hyphal Growth of Vesicular-Arbuscular Mycorrhizal Fungi in the Presence of CO 2 and Flavonols Extensive In Vitro Hyphal Growth of Vesicular-Arbuscular Mycorrhizal Fungi in the Presence of CO2 and Flavonols. *Applied and Environmental Microbiology* **58**: 821–825.

Bécard G, Fortin a. J. **1988**. Early events of vesicular-arbuscular mycorrhiza formation on Ri T-DNA transformed roots. *New Phytologist* **108**: 211–218.

Besserer A, Puech-Pagès V, Kiefer P, Gomez-Roldan V, Jauneau A, Roy S, Portais J-C, Roux C, Bécard G, Séjalon-Delmas N. **2006**. Strigolactones stimulate arbuscular mycorrhizal fungi by activating mitochondria. *PLoS biology* **4**: e226.

Bravo A, Brands M, Wewer V, Dörmann P, Harrison MJ. **2017**. Arbuscular mycorrhiza-specific enzymes FatM and RAM2 fine-tune lipid biosynthesis to promote development of arbuscular mycorrhiza. *New Phytologist* **214**: 1631–1645.

Bravo A, York T, Pumplin N, Mueller LA, Harrison MJ. **2016**. Genes conserved for arbuscular mycorrhizal symbiosis identified through phylogenomics. *Nature Plants* **2**: 1–6.

Chen ECH, Morin E, Beaudet D, Noel J, Yildirir G, Ndikumana S, Charron P, St-Onge C, Giorgi J, Krüger M, *et al.* 2018. High intraspecific genome diversity in the model arbuscular mycorrhizal symbiont *Rhizophagus irregularis*. *New Phytologist* **220**: 1161–1171.

Chowdhury RB, Moore GA, Weatherley AJ, Arora M. **2017**. Key sustainability challenges for the global phosphorus resource, their implications for global food security, and options for mitigation. *Journal of Cleaner Production* **140**: 945–963.

Delaux P-M, Radhakrishnan G V., Jayaraman D, Cheema J, Malbreil M, Volkening JD, Sekimoto H, Nishiyama T, Melkonian M, Pokorny L, *et al.* 2015. Algal ancestor of land plants was preadapted for symbiosis. *Proceedings of the National Academy of Sciences* **112**: 201515426.

FAOSTAT. **2019**. Crop production database. *http://www.fao.org/faostat/*.

Food and Agriculture Organization of the United Nations (FAO), International Fund for Agricultural Development (IFAD), United Nations Children's Fund (Unicef), World Food Programme (WFP), World Health Organization (WHO). **2019**. *The state of food security and nutrition in the world. Safeguarding against economic slowdwns*.

Genre A, Chabaud M, Balzergue C, Puech-Pages V, Novero M, Rey T, Fournier J, Rochange S, Becard G, Bonfante P, *et al.* 2013. Short-chain chitin oligomers from arbuscular mycorrhizal fungi trigger nuclear Ca2+ spiking in Medicago truncatula roots and their production is enhanced by strigolactone. *New Phytologist* **198**: 179–189.

Gutjahr C, Parniske M. **2013**. Cell and developmental biology of arbuscular mycorrhiza symbiosis. *Annual Review of Cell and Developmental Biology* **29**: 593–617.

Harrison MJ. **2005**. Signaling in the arbuscular mycorrhizal symbiosis. *Annual Review of Microbiology* **59**: 19–42.

Hart MM, Forsythe JA. **2012**. Using arbuscular mycorrhizal fungi to improve the nutrient quality of crops; nutritional benefits in addition to phosphorus. *Scientia Horticulturae* **148**: 206–214.

Heino M, Puma MJ, Ward PJ, Gerten D, Heck V, Siebert S, Kummu M. **2018**. Two-thirds of global cropland area impacted by climate oscillations. *Nature Communications* **9**: 1–10.

Helber N, Wippel K, Sauer N, Schaarschmidt S, Hause B, Requena N. **2011**. A Versatile Monosaccharide Transporter That Operates in the Arbuscular Mycorrhizal Fungus *Glomus* sp Is Crucial for the Symbiotic Relationship with Plants. *The Plant Cell* **23**: 3812–3823.

Hijri M. **2016**. Analysis of a large dataset of mycorrhiza inoculation field trials on potato shows highly significant increases in yield. *Mycorrhiza* **26**: 209–214.

Howitt R, Medellín-azuara J, Macewan D. **2014**. Economic Analysis of the 2014 Drought for California Agriculture. *Center for Watershed Sciences. University of California, Davis, California*: 20p.

Iorizzo M, Ellison S, Senalik D, Zeng P, Satapoomin P, Huang J, Bowman M, Iovene M, Sanseverino W, Cavagnaro P, *et al.* 2016. A high-quality carrot genome assembly provides new insights into carotenoid accumulation and asterid genome evolution. *Nature Genetics* **48**: 657–666.

KC S, Lutz W. **2017**. The human core of the shared socioeconomic pathways: Population scenarios by age, sex and level of education for all countries to 2100. *Global Environmental Change* **42**: 181–192.

Keymer A, Pimprikar P, Wewer V, Huber C, Brands M, Bucerius SL, Delaux P-M, Klingl V, Röpenack-Lahaye E von, Wang TL, *et al.* 2017. Lipid transfer from plants to arbuscular mycorrhiza fungi. *eLife* **6**: 1–33.

Kikuchi Y, Hijikata N, Ohtomo R, Handa Y, Kawaguchi M, Saito K, Masuta C, Ezawa T. **2016**. Aquaporin-mediated long-distance polyphosphate translocation directed towards the host in arbuscular mycorrhizal symbiosis: application of virus-induced gene silencing. *The New Phytologist* **211**: 1202–1208.

Klironomos J. **2003**. Variation in plant response to native and exotic arbuscular mycorrhizal fungi. *Ecology* **84**: 2292–2301.

Kobayashi Y, Mondal F, Ban T, Matsubara H, Adachi F, Asao T. **2013**. Growing carrots hydroponically using perlite substrates. *Scientia Horticulturae* **159**: 113–121.

Li T, Hu Y-J, Hao Z-P, Li H, Wang Y-S, Chen B-D. **2013**. First cloning and characterization of two functional aquaporin genes from an arbuscular mycorrhizal fungus *Glomus intraradices*. *New Phytologist* **197**: 617–630.

Lin K, Limpens E, Zhang Z, Ivanov S, Saunders DGO, Mu D, Pang E, Cao H, Cha H, Lin T, *et al.* 2014. Single nucleus genome sequencing reveals high similarity among nuclei of an endomycorrhizal fungus. *PLoS genetics* **10**: e1004078.

Luginbuehl LH, Menard GN, Kurup S, Erp H Van, Radhakrishnan G V., Breakspear A, Oldroyd GED, Eastmond PJ. **2017**. Fatty acids in arbuscular mycorrhizal fungi are synthesized by the host plant. *Science* **356**: 763–775.

MacLean AM, Bravo A, Harrison MJ. **2017**. Plant Signaling and Metabolic Pathways Enabling Arbuscular Mycorrhizal Symbiosis. *The Plant Cell* **29**: 2319–2335.

Maillet F, Poinsot V, André O, Puech-Pagès V, Haouy A, Gueunier M, Cromer L, Giraudet D, Formey D, Niebel A, *et al.* 2011. Fungal lipochitooligosaccharide symbiotic signals in arbuscular mycorrhiza. *Nature* **469**: 58–63.

Mancosu N, Snyder RL, Kyriakakis G, Spano D. **2015**. Water scarcity and future challenges for food production. *Water* **7**: 975–992.

Mensah JA, Koch AM, Antunes PM, Kiers ET, Hart M, Bücking H. **2015**. High functional diversity within species of arbuscular mycorrhizal fungi is associated with differences in phosphate and nitrogen uptake and fungal phosphate metabolism. *Mycorrhiza* **25**: 533–546.

Morton JF. **2007**. Climate Change and Food Security Special Feature: The impact of climate change on smallholder and subsistence agriculture. *Proceedings of the National Academy of Sciences* **104**: 19680–19685.

Mueller ND, Gerber JS, Johnston M, Ray DK, Ramankutty N, Foley J a. 2012. Closing yield gaps through nutrient and water management. *Nature* **490**: 254–257.

Nichols K a. 2010. Glomalin production and accumulation in soilless pot cultures. *Canadian Journal of Soil Science* **90**: 567–570.

Nicolson TH. **1967**. Vesicular-arbuscular mycorrhiza--a universal plant symbiosis. *Science*

Progress **55**: 561–581.

OECD. **2012**. *OECD Environmental Outlook to 2050 : the Consequences of Inaction.* OECD Pub.

Pellegrino E, Turrini A, Gamper HA, Cafà G, Bonari E, Young JPW, Giovannetti M. **2012**. Establishment, persistence and effectiveness of arbuscular mycorrhizal fungal inoculants in the field revealed using molecular genetic tracing and measurement of yield components. *New Phytologist*: 810–822.

Porcel R, Aroca R, Cano C, Bago A, Ruiz-Lozano JM. **2006**. Identification of a gene from the arbuscular mycorrhizal fungus *Glomus intraradices* encoding for a 14-3-3 protein that is up-regulated by drought stress during the AM symbiosis. *Microbial Ecology* **52**: 575–582.

Porcel R, Aroca R, Cano C, Bago A, Ruiz-Lozano JM. **2007**. A gene from the arbuscular mycorrhizal fungus *Glomus intraradices* encoding a binding protein is up-regulated by drought stress in some mycorrhizal plants. *Environmental and Experimental Botany* **60**: 251–256.

Razzaq M, Akram NA, Ashraf M, Naz H, Al-Qurainy F. **2017**. Interactive effect of drought and nitrogen on growth, some key physiological attributes and oxidative defense system in carrot (*Daucus carota* L.) plants. *Scientia Horticulturae* **225**: 373–379.

Reid JB, Gillespie RN. **2017**. Yield and quality responses of carrots (*Daucus carota* L.) to water deficits. *New Zealand Journal of Crop and Horticultural Science* **45**: 299–312.

Riley R, Corradi N. **2013**. Searching for clues of sexual reproduction in the genomes of arbuscular mycorrhizal fungi. *Fungal Ecology* **6**: 44–49.

Rillig MC. **2004**. Arbuscular mycorrhizae, glomalin, and soil aggregation. *Canadian Journal of Soil Science* **84**: 355–363.

Ruiz-Lozano JM, Azcon R, Gomez M. **1995**. Effects of arbuscular-mycorrhizal *Glomus* species on drought tolerance: physiology and nutritional plant responses. *Appl Environ Microbiol* **61**: 456–460.

Schreiner RP, Koide RT. **1993**. Stimulation of vesicular-arbuscular mycorrhizal fungi by mycotrophic and nonmycotrophic plant root systems. *Applied and environmental microbiology* **59**: 2750–2.

Simon PW, Freeman RE, Vieira J V, Boiteux LS, Briard M, Nothnagel T, Michalik B, Kwon Y-S. **2008**. *Carrot*.

Smith SE, Read D. **2008**. *Mycorrhizal Symbiosis*. Elsevier.

Sôrensen JN, Jôrgensen U, Ku BF. **1997**. Drought Effects on the Marketable and Nutritional Qualit y of Carrots. **379**.

Strullu-Derrien C, Kenrick P, Pressel S, Duckett JG, Rioult JP, Strullu DG. **2014**. Fungal

associations in Horneophyton ligneri from the Rhynie Chert (c. 407 million year old) closely resemble those in extant lower land plants: Novel insights into ancestral plant-fungus symbioses. *New Phytologist* **203**: 964–979.

Taylor TN, Remy W, Hass H, Kerp H. **1995**. Fossil Arbuscular Mycorrhizae from the Early Denovian. *Mycologia* **87**: 560–573.

Tisserant E, Malbreil M, Kuo A, Kohler A, Symeonidi A, Balestrini R, Charron P, Duensing N, Frei dit Frey N, Gianinazzi-Pearson V, *et al.* 2013. Genome of an arbuscular mycorrhizal fungus provides insight into the oldest plant symbiosis. *Proceedings of the National Academy of Sciences of the United States of America* **110**: 20117–22.

USDA-ERS. **2011**. *Table 3. Certified Organic and Total U.S. Acreage, Selected Crops and Livestock*.

USDA-NASS. **2018a**. Carrots - Conventional Production Measured in Sales.

USDA-NASS. **2018b**. Carrots - Organic Sales.

USDA-NOP. **2019**. U.S. Code: Title 7. Part 205—National Organic Program. Subpart G—National List of Allowed and Prohibited Substances §205.600 Evaluation criteria for allowed and prohibited substances, methods, and ingredients.

Van Vuuren D, Kok M, Van der Esch S, Jeuken M, Lucas P, Prins A-G, Alkemade R, Van den Berg M, Hilderink, H. Kram T, Stehfest E, *et al.* 2012. *Roads from Rio+ 20 : pathways to achieve global sustainability goals by 2050 : summary and main findings to the full report*. PBL, Netherlands Environmental Assessment Agency.

Wang E, Schornack S, Marsh JF, Gobbato E, Schwessinger B, Eastmond P, Schultze M, Kamoun S, Oldroyd GED. **2012**. A common signaling process that promotes mycorrhizal and oomycete colonization of plants. *Current biology : CB* **22**: 2242–6.

Wang Q, Wu J, Lei T, He B, Wu Z, Liu M, Mo X, Geng G, Li X, Zhou H, *et al.* 2014. Temporal-spatial characteristics of severe drought events and their impact on agriculture on a global scale. *Quaternary International* **349**: 10–21.

Wilson TS, Sleeter BM, Cameron DR. **2017**. Mediterranean California's water use future under multiple scenarios of developed and agricultural land use change. *PLoS ONE* **12**: 1–22.

Wright SF, Upadhyaya a. 1998. A survey of soils for aggregate stability and glomalin, a glycoprotein produced by hyphae of arbuscular mycorrhizal fungi. *Plant and Soil* **198**: 97–107.

Chapter 2: Biology and evolution of arbuscular mycorrhizal symbiosis in the light of genomics

This chapter was previously published as:

> **Kamel L, Keller-Pearson M, Roux C, Ané JM**. **2017**. Biology and evolution of arbuscular mycorrhizal symbiosis in the light of genomics. *New Phytologist* **213**: 531–536.

Contributions: Jean-Michel Ané and Christophe Roux initiated the planning for this review and contributed heavily to the writing. Laurent Kamel and Michelle Keller-Pearson wrote the majority of the content, and Michelle Keller-Pearson prepared the figure.

2.1 Summary

Arbuscular mycorrhizal (AM) fungi associate with the vast majority of land plants, providing mutual nutritional benefits and protecting hosts against biotic and abiotic stresses. Significant progress was made recently in our understanding of the genomic organization, the obligate requirements, and the sexual nature of these fungi through the release and subsequent mining of genome sequences. Genomic and genetic approaches also improved our understanding of the signal repertoire used by AM fungi and their plant hosts to recognize each other for the initiation and maintenance of this association. Evolutionary and bioinformatic analyses of host and nonhost plant genomes represent novel ways with which to decipher host mechanisms controlling these associations and shed light on the stepwise acquisition of this genetic toolkit during plant evolution. Mining fungal and plant genomes along with evolutionary and genetic approaches will improve understanding of these symbiotic associations and, in the long term, their usefulness in agricultural settings.

2.2 Introduction

Arbuscular mycorrhizal (AM) fungi form symbiotic associations with representatives from most families of land plants, including early-diverging lineages such as liverworts and hornworts (Bonfante and Genre, 2008; Wang et al., 2010). This symbiosis facilitates water and nutrient uptake in host plants, protecting them against various biotic and abiotic stresses. The emergence of AM fungi and land plants strikingly coincided at nearly 450 million yr ago (Taylor et al., 1995) with fossil records dated to 407 million yr ago (Strullu-Derrien et al., 2014) suggesting that associations with fungi probably facilitated land colonization and true root development (Selosse et al., 2015). This ancient association arose in Pangaea and is now present on all continents. Notably, plants can grow in the absence of AM fungi, but AM fungi are obligate symbionts. This feature, called 'obligate biotrophy', is ancient; all extant AM fungi share this feature, and it probably shaped their evolution. Mycorrhizal colonization of plants follows a well-defined sequence of events: germination of fungal spores, hyphal branching in the vicinity of a host root that facilitates recognition, hyphal attachment to root surfaces and hyphopodium differentiation, penetration and spread of the fungus in plant roots guided by a pre-penetration apparatus (Genre *et al.*, 2005), and development of highly ramified arbuscules within reprogrammed root cortical cells that allow efficient exchange of nutrients between symbionts (Gutjahr and Parniske, 2013). These steps occur after a coordinated molecular dialog between AM fungi and host plants. Several host signals, as well as perception mechanisms, are well characterized at the molecular level thanks to studies employing combinations of genetic and biochemical approaches (Oldroyd, 2013). Less is known on the fungal partner. Recent advances in AM fungal genomics provide new opportunities to discover genes, proteins, and regulatory elements involved in a range of key activities either in symbiotic establishment (*i.e.* signaling

and nutrient exchange between AM fungus and the plant host) or in fungal development (*i.e.* reproduction of AM fungi). New evidence adds credence to the highly debated potential of sexual reproduction of AM fungi. Access to AM genomes also enables deeper evaluation of transcriptomic data from plants associated or not with AM fungi such that clearer distinctions can be drawn between species origins for identified sequences.

2.3 Genomics of arbuscular mycorrhizal fungi

The announcement of the genome-sequencing program for the model AM fungus in 2004 posed a real challenge (Martin et al., 2004). First, genomes of AM fungi are among the largest in fungi, ranging from 150 Mb to over 1 Gb depending on the species (Hosny et al., 1998). Second, producing enough tissue to study presents a difficulty since they require co-cultivation with a host plant. *Rhizophagus irregularis* DAOM 197198 was therefore chosen because of its relatively small genome size (153 Mb) (Sedzielewska et al., 2011) and the possibility to propagate it efficiently with *in vitro* root organ cultures. Because their spores are asexual structures filled with hundreds to thousands of nuclei and because the nuclei within a single spore are thought to be extremely diverse, AM fungi were anticipated to be very difficult to sequence from spores (Bécard & Pfeffer, 1993; Kühn *et al.*, 2001). Such heterokaryotic organization would limit shotgun sequencing strategies, as it impairs assembly procedures. Taking advantage of the development of next-generation sequencing technologies, two independent studies published genomic sequences for *R.s irregularis* DAOM 197198 (Lin et al., 2014; Tisserant et al., 2013). The genome was estimated to be 153 Mb with a high frequency of transposable elements (36% of the genome). Thus, 60% and 92% of the deduced genome, respectively, is covered by the two assemblies (Genome assembly length: 91.08 Mb, Tisserant *et al.*, 2013; 141 Mb Lin *et al.*, 2014). Gene expansion was observed in several gene families such as kinases and mating-related genes, whereas, in both assemblies, other genes were strikingly absent, such as glycoside hydrolases that could damage host cell walls. By sequencing individual nuclei, Lin *et al.*, (2014) showed that ribosomal regions are highly variable within each nucleus. Moreover, the rate of single nucleotide polymorphisms (SNPs) found in *R. irregularis* was similar to other fungal genomes. The analysis of genomic sequences from different strains of *R.*

irregularis recently confirmed the low rate of SNPs (Ropars et al., 2016). A similar result was also found from the gene repertoire of another species, *Gigaspora rosea* (Tang *et al.*, 2016). Global analyses of the coding gene repertoires obtained at this time indicate that AM fungi exhibit features of classical genome organization, *i.e.* one genome per strain.

The recent publication of genomic data from strains of *R. irregularis* (Ropars et al., 2016) and gene repertoires from additional AM species (*Rhizophagus clarus* (Sędzielewska Toro and Brachmann, 2016), *Gigaspora margarita* (Salvioli et al., 2015), and *Gigaspora rosea* (Tang et al., 2016) heralds a new era in the investigation of AM fungi. These gene repertoires will enable deeper investigations of the fungal biology through RNAseq analyses, for instance.

These genomic advances drastically changed our views on the (lack of) sexuality of AM fungi. In the absence of an observed sexual cycle at the cellular and morphological levels, AM fungi were thought to be asexual. Recent studies revealed that genes involved in meiosis and sexuality are present in several AM fungi, suggesting a cryptic sexual cycle (Riley et al., 2014; Riley and Corradi, 2013). The comparison of genomic sequences from strains of *R. irregularis* revealed differential allele distributions consistent with the probable existence of homokaryotic and dikaryotic strains (Ropars et al., 2016). This works also revealed the presence of a locus very similar to mating-type (MAT) loci found in heterothallic bipolar Basidiomycetes (Ropars et al., 2016). Interestingly, this locus seems to be the only protein-coding locus shown to differ between dikaryons. Allele occurrence within this putative MAT locus is consistent with the hypothesis that the dikaryotic strains originated from plasmogamy between monokaryotic ones (Ropars et al., 2016). These results strongly suggest that AM fungi have a functional sexual cycle. However, further studies are needed to evaluate the full extent of mating types, to understand the molecular function of this putative MAT locus and, most of all, to show whether

homokaryotic AM parental strains can be crossed in experimental conditions to form a heterokaryotic strain and later homokaryotic progeny (**Figure 1**). A better understanding of the entire life-cycle of AM fungi would facilitate the genetic development of improved fungal strains for agricultural applications.

2.4 Genomic Insights on the Obligate Nature of Arbuscular Mycorrhizal Fungi

Because AM fungi are obligate symbionts, genes involved in hijacking the host metabolism are critical for the stability of these associations. AM fungi obtain carbohydrates derived from hosts' photosynthesis in exchange for nutrients such as phosphorous and nitrogen (Pfeffer et al., 1999; Bago et al., 2003). Until recently, it was thought that AM fungi used this carbon source to synthesize lipids for their vast mycelial networks (Trépanier et al., 2005), but analysis of the *R. irregularis* genome revealed the absence of fatty acid synthase (FAS; Wewer *et al.*, 2014). FASs are also absent in other AM fungal genomes such as *G. margarita* and *G. rosea* (Salviolli *et al.*, 2016; Tang *et al.*, 2016) as well as in five *R. irregularis* strains (Ropars *et al.*, 2016). This observation raises the question of the origin of palmitic acid (C16) in these oleogenic fungi. Lipid droplets released after the collapse of old arbuscules are apparently recaptured by the hyphae (Kobae et al., 2014). Autophagy-like mechanisms could, therefore, facilitate lipid recycling from the host-fungus interface. The lack of FAS in fungi was already reported in the case of a fungal pathogen (*Malassezia globosa*) that acquires its fatty acids from the host via secreted lipases (Xu et al., 2007). Intriguingly, 36 putative secreted lipases are found in *R. irregularis* genome. Mining of host and non-host genomes revealed several non-specific lipid transfer proteins as potentially involved in AM associations (Delaux et al., 2014). These proteins could play a role in the transfer of lipids from host to fungi. Future investigations will probably elucidate how the loss of FAS may be an adaptive strategy of AM fungi to obligate symbiosis (**Figure 1**).

2.5 Interdisciplinary Approaches to Unravel the Host-Symbiont Interactome

Mutual recognition between plants and AM fungi is essential for the initiation and probably the maintenance of symbiotic associations. AM fungi recognize several signals exuded by plant roots into the rhizosphere. In low nutrient soils or drought conditions, plant roots secrete strigolactones (Ruyter-Spira et al., 2013; Lopez-Obando et al., 2015), which activate hyphal growth and branching (Akiyama et al., 2005; Besserer et al., 2006). Other stimulatory compounds have also been described: a higher concentration of carbon dioxide promotes hyphal growth, and flavonoids increase spore germination for several AM fungal species, although they seem dispensable in later steps of the interaction (Bécard et al., 1992; Becard et al., 1995; Larose et al., 2002; Scervino et al., 2007) . Hydroxy fatty acids provoke the branching of hyphae emerging from germinating spores (Nagahashi and Douds, 2011). It was proposed that cutin monomers released from the root surface induce the formation of fungal penetration structures, hyphopodia (Wang et al., 2012). The mechanisms allowing AM fungi to perceive these signals are still unknown, but the availability of genome sequences and comparative genomic approaches will accelerate mechanistic discoveries.

In response to some of these plant signals, AM fungi produce chitin-derived signals collectively referred to as Myc (mycorrhizal) factors. These factors include short chitooligosaccharides (S-COs) and Myc-lipochitooligosaccharides (Myc-LCOs) decorated with various fatty acids and substitutions (Maillet et al., 2011; Genre et al., 2013). Given that decorated LCOs are probably derived from S-COs, it is tempting to speculate that AM fungi may also produce decorated S-COs. Comparisons of plant responses to S-CO and Myc-LCO signals reveal differences between rice (*Oryza sativa*) and legumes as well as among root types and cell types. For example, S-COs and Myc-LCOs induced typical oscillations of the nuclear and

perinuclear calcium concentration called ‘calcium spiking’ in legume atrichoblasts, but only S-COs induced this response in rice atrichoblasts (Sun et al., 2015). Also, this study revealed that a combination of S-COs and Myc-LCOs, present in AM fungal exudates, triggered responses different than those by individual signals. In rice, S-COs trigger calcium spiking in atrichoblasts and only a combination of both S-COs and Myc-LCOs leads to calcium spoking in root hairs (trichoblasts). However, both S-COs and LCOs alone are able to stimulate root development. The observation of a synergy between signals limits the relevance of previous studies investigating host responses to individual signals and indicates that cell responses to a combination of S-COs/Myc-LCOs could differ according to plant species or clade. It also highlights the need to quantify S-CO and Myc-LCO repertoires in the presence of different hosts and possibly at different stages of the symbiotic association.

Since the first description of a small secreted peptidic effector in *R. irregularis* (SP7 - Kloppholz *et al.*, 2011), several studies have analyzed the secretomes of various AM fungi. The number of secreted proteins from *R. irregularis* ranges from 300 to 600 candidates (Tisserant et al., 2013; Lin et al., 2014). The recent publication of secreted protein repertoires for *R. clarus* (Sędzielewska Toro and Brachmann, 2016), *G. margarita* (Salvioli et al., 2015), and *G. rosea* (Tang et al., 2016) revealed that secreted proteins are mostly lineage specific. It is now necessary to investigate the expression of these secreted proteins in various host plants to determine if some of these secreted proteins are host specific and possibly involved in controlling host fitness. It will also be important to determine their expression pattern at different stages of symbiotic interaction (**Figure 1**).

2.6 Evolution of Mycorrhizal Associations Revealed by Comparative Genomics

Host mechanisms allowing the establishment of AM symbioses, sometimes referred to as the 'symbiotic toolkit' (Delaux et al., 2013), were initially discovered in model legumes through forward genetic screens for symbiotic associations, via protein interactions, and reverse genetics (Ané et al., 2004; Kevei et al., 2007; Horváth et al., 2011), and have now also been discovered in other hosts such as tomato (*Solanum lycopersicum*) and rice (Larkan et al., 2013; Gutjahr et al., 2015). Evolutionary studies of these host genes across land plants and in their algal relatives suggests that they were acquired through the classical potentiation-actualization-refinement sequence (Blount et al., 2012; Delaux et al., 2015, 2014; Favre et al., 2014). The functional conservation ofthese genes from legumes to hornworts and even liverworts was demonstrated through rescue assays of the corresponding legume mutants and protein interactions (Wang et al., 2010).

Some proteins of the host 'symbiotic toolkit' are absent in many lineages that have lost symbiotic abilities, such as Brassicaceae, some Caryophyllales, and many gymnosperms (Delaux et al., 2015; Bravo et al., 2016). Several hypotheses have been proposed to explain the loss of apparently beneficial AM associations in these lineages. Clade-specific innovations such as antifungal metabolites, thick cell walls, or extremely fine roots with a limited cortex may have made some plants incompatible with AM fungi. Colonized plants might have had reduced fitness as a result of carbon costs of AM colonization or vulnerabilities inherent in symbiotic signaling pathways. Consistent with this latest hypothesis, the loss of *Reduced Arbuscular Mycorrhization2* (RAM2) in *Medicago truncatula* resulted in the loss of AM associations but conferred resistance to oomycete pathogens (Wang et al., 2012; Gobbato et al., 2015). It will be interesting to determine whether non-mycorrhizal lineages are more resistant to oomycetes,

particularly lineages that lost AM associations fairly recently, such as lupines (Delaux et al., 2014). The repeated loss of the same set of genes in independent lineages prompted several groups to look for more host genes following the same evolutionary pattern and potentially involved in AM associations (Delaux et al., 2014; Favre et al., 2014; Bravo et al., 2016). Functional validation of some of these candidates clearly demonstrated that this evolution-based bioinformatics mining of genomes and transcriptomes is an extremely powerful approach to identify genes involved in specific biological processes and, in particular, in symbiotic associations.

Interestingly, these symbiotic pathways are not lost in all non-host lineages. For instance, Calcium/Calmodulin-Dependent Protein Kinase (CCaMK) and Interacting Protein of DMI3 (IPD3)/CYCLOPS are found in non-host bryophytes (Wang et al., 2010; Favre et al., 2014; Delaux et al., 2015). Even more surprising is the conservation of host mechanisms in Charophyte green algae that are not known to associate with mycorrhizal fungi but are the closest algal relatives to land plants (Delaux et al., 2015; Leliaert et al., 2012). Conservation of these genes in non-mycorrhizal lineages raises questions about other unknown roles that these symbiotic pathways may play and whether unidentified symbiotic interactions occur in these lineages. Interactions between these early diverging lineages and microbes are very much unexplored. In recent years, elegant ecological and physiological studies have revealed endosymbiotic associations between liverworts, hornworts, and lycopods with Mucoromycotina fungi, which are closely related to Glomeromycota (Bidartondo et al., 2011; Favre et al., 2014; Field et al., 2014). Given the strong similarities between these fungal associations, it seems likely that associations with Mucoromycotina fungi may rely on the same host mechanisms as those with Glomeromycota in early diverging lineages.

We believe that significant progress will be made through the development and use of genetic model systems in the early diverging host and non-host lineages (**Figure 1**). Powerful genetic tools are available already in *Physcomitrella patens* (moss) and *Marchantia* (liverwort) (Zimmer *et al.*, 2013; Hiss *et al.*, 2014). Use of these and the Charophyte green alga *Penium margaritaceum* as research models will lead to greater understanding of the host mechanisms that gave rise to efficient AM symbioses (Sørensen et al., 2014).

2.7 Acknowledgements

C.R. and J.M.A. planned and designed the manuscript. L.K., M.K.P, C.R, and J.M.A. wrote the manuscript. This work was supported by USDA-AFRI #2015-67013-22899 to J.M.A and by an NSF GRFP fellowship to M.K.P. L.K. received funding from Agronutrition SA (Labège, France). LRSV is part of the TULIP "Laboratoire d'Excellence" (ANR-10-LABX-41). Authors are grateful for the three anonymous reviewers for constructive comments on the manuscript.

2.9 Figure

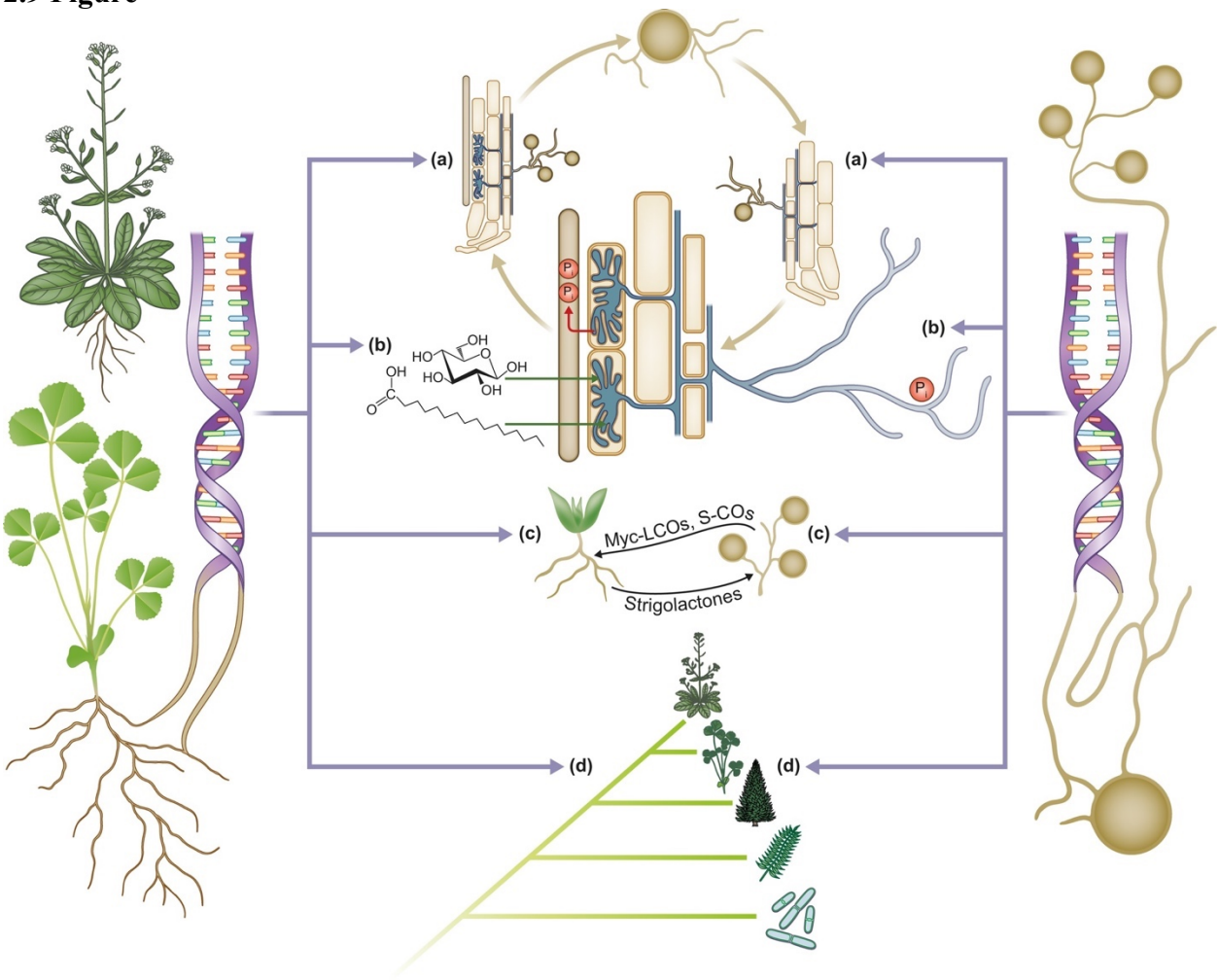


Fig. 1 Genomic approaches shed light on the fungal and host mechanisms controlling arbuscular mycorrhizal (AM) associations. (a) Lifecycle. Recent genomic data have revealed potential homokaryotic and dikaryotic strains of *Rhizophagus irregularis*, as well as a putative mating locus and mating types. At this time, it is unknown whether plasmogamy/karyogamy events occur. (b) Obligate requirements. The absence of essential metabolic pathways and proteins (e.g. fatty acid synthase) in AM fungi has led to new hypotheses being proposed for the origins of fatty acids in these oleogenic fungi. (c) Host–symbiont interactome. In response to plant signals, AM fungi produce lipochitooligosaccharides and short chitooligosaccharides. Our understanding of signal perception in both partners continues to progress. New techniques such as host-induced gene silencing enable the validation of fungal candidate genes. (d) Evolution of AM associations. Acquisition of the 'host symbiotic toolkit' occurred in a stepwise manner starting with nonmycorrhizal Charophyte algae. The correlation between the loss of symbiosis and the loss of essential genes from this toolkit drove innovative genome mining approaches to identify plant genes following the same evolutionary pattern and requirement for AM associations. AM fungal genome comparisons will enable identification of a 'fungal symbiotic toolkit' and its conservation among Glomeromycota.

2.9 References

Akiyama K, Matsuzaki K, Hayashi H. **2005**. Plant sesquiterpenes induce hyphal branching in arbuscular mycorrhizal fungi. *Nature* **435**: 824–7.

Ané J-M, Kiss GB, Riely BK, Penmetsa RV, Oldroyd GED, Ayax C, Lévy J, Debellé F, Baek J-M, Kaló P, *et al.* 2004. Medicago truncatula DMI1 required for bacterial and fungal symbioses in legumes. *Science* **303**: 1364–1367.

Bago B, Pfeffer PE, Abubaker J, Jun J, Allen JW, Brouillette J, Douds DD, Lammers PJ, Shachar-Hill Y. **2003**. Carbon export from arbuscular mycorrhizal roots involves the translocation of carbohydrate as well as lipid. *Plant Physiol* **131**: 1496–1507.

Bécard G, Douds DD, Pfeffer PE. **1992**. Extensive In Vitro Hyphal Growth of Vesicular-Arbuscular Mycorrhizal Fungi in the Presence of CO 2 and Flavonols Extensive In Vitro Hyphal Growth of Vesicular-Arbuscular Mycorrhizal Fungi in the Presence of CO2 and Flavonols. *Applied and Environmental Microbiology* **58**: 821–825.

Bécard G, Pfeffer PE. **1993**. Status of nuclear division in arbuscular mycorrhizal fungi during in vitro development. *Protoplasma* **174**: 62–68.

Becard G, Taylor LP, Douds DD, Pfeffer PE, Doner LW. **1995**. Flavonoids Are Not Necessary Plant Signal Compounds in Arbuscular Mycorrhizal Symbioses. *Molecular Plant-Microbe Interactions* **8**: 252.

Besserer A, Puech-Pagès V, Kiefer P, Gomez-Roldan V, Jauneau A, Roy S, Portais J-C, Roux C, Bécard G, Séjalon-Delmas N. **2006**. Strigolactones stimulate arbuscular mycorrhizal fungi by activating mitochondria. *PLoS biology* **4**: e226.

Bidartondo MI, Read DJ, Trappe JM, Merckx V, Ligrone R, Duckett JG. **2011**. The dawn of symbiosis between plants and fungi. *Biology letters* **7**: 574–577.

Blount ZD, Barrick JE, Davidson CJ, Lenski RE. **2012**. Genomic analysis of a key innovation in an experimental *Escherichia coli* population. *Nature* **489**: 513–518.

Bonfante P, Genre A. **2008**. Plants and arbuscular mycorrhizal fungi: an evolutionary-developmental perspective. *Trends in Plant Science* **13**: 492–498.

Bravo A, York T, Pumplin N, Mueller LA, Harrison MJ. **2016**. Genes conserved for arbuscular mycorrhizal symbiosis identified through phylogenomics. *Nature Plants* **2**: 1–6.

Catoira R, Galera C, de Billy F, Penmetsa R V, Journet EP, Maillet F, Rosenberg C, Cook D, Gough C, Dénarié J. **2000**. Four genes of Medicago truncatula controlling components of a nod factor transduction pathway. *The Plant Cell* **12**: 1647–1666.

Delaux P-M, Radhakrishnan G V., Jayaraman D, Cheema J, Malbreil M, Volkening JD, Sekimoto H, Nishiyama T, Melkonian M, Pokorny L, *et al.* 2015. Algal ancestor of

land plants was preadapted for symbiosis. *Proceedings of the National Academy of Sciences* **112**: 201515426.

Delaux P-M, Séjalon-Delmas N, Bécard G, Ané J-M. **2013**. Evolution of the plant-microbe symbiotic 'toolkit'. *Trends in plant science* **18**: 298–304.

Delaux PM, Varala K, Edger PP, Coruzzi GM, Pires JC, An?? JM. **2014**. Comparative Phylogenomics Uncovers the Impact of Symbiotic Associations on Host Genome Evolution. *PLoS Genetics* **10**.

Favre P, Bapaume L, Bossolini E, Delorenzi M, Falquet L, Reinhardt D. **2014**. A novel bioinformatics pipeline to discover genes related to arbuscular mycorrhizal symbiosis based on their evolutionary conservation pattern among higher plants. *BMC Plant Biology* **14**: 333.

Field KJ, Rimington WR, Bidartondo MI, Allinson KE, Beerling DJ, Cameron DD, Duckett JG, Leake JR, Pressel S. **2014**. First evidence of mutualism between ancient plant lineages (Haplomitriopsida liverworts) and Mucoromycotina fungi and its response to simulated Palaeozoic changes in atmospheric CO2. *The New phytologist* **205**: 743–756.

Genre A, Chabaud M, Balzergue C, Puech-Pages V, Novero M, Rey T, Fournier J, Rochange S, Becard G, Bonfante P, *et al.* 2013. Short-chain chitin oligomers from arbuscular mycorrhizal fungi trigger nuclear Ca2+ spiking in Medicago truncatula roots and their production is enhanced by strigolactone. *New Phytologist* **198**: 179–189.

Gobbato E, Wang E, Higgins G, Bano SA, Schultze M, Oldroyd GED, Gobbato E, Wang E, Higgins G, Bano SA, *et al.* 2015. Aphanomyces euteiches colonization RAM1 and RAM2 function and expression during Arbuscular Mycorrhizal Symbiosis and Aphanomyces euteiches colonization. **2324**: 1–5.

Gutjahr C, Gobbato E, Choi J, Riemann M, Johnston MG, Summers W, Carbonnel S, Mansfield C, Yang S-YS-Y, Nadal M, *et al.* 2015. Rice perception of symbiotic arbuscular mycorrhizal fungi requires the karrikin receptor complex. *Science* **350**: 1521–1524.

Gutjahr C, Parniske M. **2013**. Cell and developmental biology of arbuscular mycorrhiza symbiosis. *Annual Review of Cell and Developmental Biology* **29**: 593–617.

Hiss M, Laule O, Meskauskiene RM, Arif MA, Decker EL, Erxleben A, Frank W, Hanke ST, Lang D, Martin A, *et al.* 2014. Large-scale gene expression profiling data for the model moss Physcomitrella patens aid understanding of developmental progression, culture and stress conditions. *Plant Journal* **79**: 530–539.

Horváth B, Yeun LH, Domonkos Á, Halász G, Gobbato E, Ayaydin F, Miró K, Hirsch S, Sun J, Tadege M, *et al.* 2011. Medicago truncatula IPD3 Is a Member of the Common Symbiotic Signaling Pathway Required for Rhizobial and Mycorrhizal Symbioses. *Molecular Plant-Microbe Interactions* **24**: 1345–1358.

Hosny M, Gianinazzi-Pearson V, Dulieu H. **1998**. Nuclear DNA content of 11 fungal species in Glomales. *Genome* **41**: 422–428.

Kevei Z, Lougnon G, Mergaert P, Horvath G V, Kereszt A, Jayaraman D, Zaman N, Marcel F, Regulski K, Kiss GB, *et al.* 2007. 3-hydroxy-3-methylglutaryl coenzyme A reductase1 interacts with NORK and is crucial for nodulation in Medicago truncatula. *Plant Cell* **19**: 3974–3989.

Kloppholz S, Kuhn H, Requena N. **2011**. A secreted fungal effector of Glomus intraradices promotes symbiotic biotrophy. *Current biology : CB* **21**: 1204–9.

Kobae Y, Gutjahr C, Paszkowski U, Kojima T, Fujiwara T, Hata S. **2014**. Lipid droplets of arbuscular mycorrhizal fungi emerge in concert with arbuscule collapse. *Plant & cell physiology* **55**: 1945–53.

Larkan NJ, Ruzicka DR, Edmonds-Tibbett T, Durkin JMH, Jackson LE, Smith FA, Schachtman DP, Smith SE, Barker SJ. **2013**. The reduced mycorrhizal colonisation (rmc) mutation of tomato disrupts five gene sequences including the CYCLOPS/IPD3 homologue. *Mycorrhiza* **23**: 573–584.

Larose G, Chênevert R, Moutoglis P, Gagné S, Piché Y, Vierheilig H. **2002**. Flavonoid levels in roots of Medicago sativa are modulated by the developmental stage of the symbiosis and the root colonizing arbuscular mycorrhizal fungus. *Journal of Plant Physiology* **159**: 1329–1339.

Leliaert F, Smith DR, Moreau H, Herron MD, Verbruggen H, Delwiche CF, De Clerck O. **2012**. Phylogeny and Molecular Evolution of the Green Algae. *Critical Reviews in Plant Sciences* **31**: 1–46.

Lin K, Limpens E, Zhang Z, Ivanov S, Saunders DGO, Mu D, Pang E, Cao H, Cha H, Lin T, *et al.* 2014. Single nucleus genome sequencing reveals high similarity among nuclei of an endomycorrhizal fungus. *PLoS genetics* **10**: e1004078.

Lopez-Obando M, Ligerot Y, Bonhomme S, Boyer F-D, Rameau C. **2015**. Strigolactone biosynthesis and signaling in plant development. *Development (Cambridge, England)* **142**: 3615–9.

Maillet F, Poinsot V, André O, Puech-Pagès V, Haouy A, Gueunier M, Cromer L, Giraudet D, Formey D, Niebel A, *et al.* 2011. Fungal lipochitooligosaccharide symbiotic signals in arbuscular mycorrhiza. *Nature* **469**: 58–63.

Martin F, Tuskan GA, DiFazio SP, Lammers P, Newcombe G, Podila GK. **2004**. Symbiotic sequencing for the Populus mesocosm. *New Phytol* **161**: 330–335.

Messinese E, Mun JH, Yeun LH, Jayaraman D, Rouge P, Barre A, Lougnon G, Schornack S, Bono JJ, Cook DR, *et al.* 2007. A novel nuclear protein interacts with the symbiotic DMI3 calcium- and calmodulin-dependent protein kinase of Medicago truncatula. *Molecular Plant-Microbe Interactions* **20**: 912–921.

Nagahashi G, Douds DD. **2011**. The effects of hydroxy fatty acids on the hyphal branching of germinated spores of AM fungi. *Fungal Biology* **115**: 351–358.

Oldroyd GED. **2013**. Speak, friend, and enter: signalling systems that promote beneficial symbiotic associations in plants. *Nature reviews. Microbiology* **11**: 252–63.

Pfeffer PE, Douds DD, Bécard G, Shachar-Hill Y. **1999**. Carbon Uptake and the Metabolism and Transport of Lipids in an Arbuscular Mycorrhiza1. *Plant physiology* **120**: 587–598.

Riley R, Charron P, Idnurm A, Farinelli L, Dalpé Y, Martin F, Corradi N. **2014**. Extreme diversification of the mating type-high-mobility group (MATA-HMG) gene family in a plant-associated arbuscular mycorrhizal fungus. *New Phytologist* **201**: 254–268.

Riley R, Corradi N. **2013**. Searching for clues of sexual reproduction in the genomes of arbuscular mycorrhizal fungi. *Fungal Ecology* **6**: 44–49.

Ropars J, Toro KS, Noel J, Pelin A, Charron P, Farinelli L, Marton T, Krüger M, Fuchs J, Brachmann A, *et al.* 2016. Evidence for the sexual origin of heterokaryosis in arbuscular mycorrhizal fungi. *Nature Microbiology*: 16033.

Ruyter-Spira C, Al-Babili S, van der Krol S, Bouwmeester H. **2013**. The biology of strigolactones. *Trends in Plant Science* **18**: 72–83.

Salvioli A, Ghignone S, Novero M, Navazio L, Venice F, Bagnaresi P, Bonfante P. **2015**. Symbiosis with an endobacterium increases the fitness of a mycorrhizal fungus, raising its bioenergetic potential. *The ISME journal* **10**: 1–15.

Scervino JM, Ponce M a, Erra-Bassells R, Bompadre J, Vierheilig H, Ocampo J a, Godeas A. **2007**. The effect of flavones and flavonols on colonization of tomato plants by arbuscular mycorrhizal fungi of the genera Gigaspora and Glomus. *Canadian journal of microbiology* **53**: 702–9.

Sedzielewska KA, Fuchs J, Temsch EM, Baronian K, Watzke R, Kunze G. **2011**. Estimation of the Glomus intraradices nuclear DNA content. *New Phytologist* **192**: 794–797.

Sędzielewska Toro K, Brachmann A. **2016**. The effector candidate repertoire of the arbuscular mycorrhizal fungus Rhizophagus clarus. *BMC Genomics* **17**: 101.

Selosse M-A, Strullu-Derrien C, Martin FM, Kamoun S, Kenrick P. **2015**. Plants, fungi and oomycetes: a 400-million year affair that shapes the biosphere. *New Phytologist* **206**: 501–506.

Sorensen I, Fei Z, Andreas A, Willats WGT, Domozych DS, Rose JKC. **2014**. Stable transformation and reverse genetic analysis of Penium margaritaceum: A platform for studies of charophyte green algae, the immediate ancestors of land plants. *Plant Journal* **77**: 339–351.

Strullu-Derrien C, Kenrick P, Pressel S, Duckett JG, Rioult JP, Strullu DG. **2014**. Fungal

associations in Horneophyton ligneri from the Rhynie Chert (c. 407 million year old) closely resemble those in extant lower land plants: Novel insights into ancestral plant-fungus symbioses. *New Phytologist* **203**: 964–979.

Sun J, Miller JB, Granqvist E, Wiley-Kalil A, Gobbato E, Maillet F, Cottaz S, Samain E, Venkateshwaran M, Fort S, *et al.* 2015. Activation of symbiosis signaling by arbuscular mycorrhizal fungi in legumes and rice. *The Plant cell* **27**: 823–38.

Tang N, San Clemente H, Roy S, Bécard G, Zhao B, Roux C. 2016. A Survey of the Gene Repertoire of Gigaspora rosea Unravels Conserved Features among Glomeromycota for Obligate Biotrophy. *Frontiers in Microbiology* **7**: 1–16.

Taylor TN, Remy W, Hass H, Kerp H. 1995. Fossil Arbuscular Mycorrhizae from the Early Denovian. *Mycologia* **87**: 560–573.

Tisserant E, Malbreil M, Kuo A, Kohler A, Symeonidi A, Balestrini R, Charron P, Duensing N, Frei dit Frey N, Gianinazzi-Pearson V, *et al.* 2013. Genome of an arbuscular mycorrhizal fungus provides insight into the oldest plant symbiosis. *Proceedings of the National Academy of Sciences of the United States of America* **110**: 20117–22.

Trépanier M, Bécard G, Moutoglis P, Willemot C, Gagné S, Avis TJ, Rioux J, Tre M, Be G, Gagne S. 2005. Dependence of Arbuscular-Mycorrhizal Fungi on Their Plant Host for Palmitic Acid Synthesis. *Applied and Environmental Microbiology* **71**: 5341–5347.

Wang E, Schornack S, Marsh JF, Gobbato E, Schwessinger B, Eastmond P, Schultze M, Kamoun S, Oldroyd GED. 2012. A common signaling process that promotes mycorrhizal and oomycete colonization of plants. *Current biology : CB* **22**: 2242–6.

Wang B, Yeun LH, Xue JY, Liu Y, Ané JM, Qiu YL. 2010. Presence of three mycorrhizal genes in the common ancestor of land plants suggests a key role of mycorrhizas in the colonization of land by plants. *New Phytologist* **186**: 514–525.

Wewer V, Brands M, Dörmann P. 2014. Fatty acid synthesis and lipid metabolism in the obligate biotrophic fungus Rhizophagus irregularis during mycorrhization of Lotus japonicus. *The Plant journal* **79**: 398–412.

Xu J, Saunders CW, Hu P, Grant R a, Boekhout T, Kuramae EE, Kronstad JW, Deangelis YM, Reeder NL, Johnstone KR, *et al.* 2007. Dandruff-associated Malassezia genomes reveal convergent and divergent virulence traits shared with plant and human fungal pathogens. *Proceedings of the National Academy of Sciences of the United States of America* **104**: 18730–18735.

Zimmer AD, Lang D, Buchta K, Rombauts S, Nishiyama T, Hasebe M, Van de Peer Y, Rensing SA, Reski R. 2013. Reannotation and extended community resources for the genome of the non-seed plant Physcomitrella patens provide insights into the evolution of plant gene structures and functions. *BMC genomics* **14**: 498.

Chapter 3. Inoculation with arbuscular mycorrhizal fungi has a more significant positive impact on the growth of open-pollinated heirloom varieties of carrots than on hybrid cultivars under organic management conditions

This chapter has been submitted for publication in Agriculture, Ecosystems & Environment.

Michelle Keller-Pearson[a], Yang Liu[b], Annika Peterson[a], Kaley Pederson[a], Luke Willems[b], Jean-Michel Ané[b,c], Erin M. Silva[a,*]

[a]Department of Plant Pathology, University of Wisconsin-Madison

[b]Department of Bacteriology, University of Wisconsin-Madison

[c]Department of Agronomy, University of Wisconsin-Madison

*Corresponding Author: Erin M. Silva

Contributions:

Michelle Keller-Pearson, Jean-Michel Ané, and Erin Silva designed the research. Michelle Keller-Pearson, Yang Liu, Annika Peterson, Kaley Pederson, and Luke Willems performed the experiments. Michelle Keller-Pearson analyzed the data and wrote the paper with input from Yang Liu, Annika Peterson, Kaley Pederson, Luke Willems, Jean-Michel Ané, and Erin Silva.

3.1 Abstract

To meet the high demand for organic produce, farmers must select crop cultivars that perform well under the low-input conditions of organic production systems. Most cultivars grown on organic farms are genotypes selected through conventional breeding programs, which may impact responsiveness to microbial symbionts. The use of biological inputs such as mycorrhizal inoculants offers the promise of improving yield, quality, and stress-responsiveness of crops, but evidence of efficacy in the field remains elusive. Moreover, interspecific and intraspecific variability may impact the ability of mycorrhizal inoculants to provide benefits. This work evaluated four cultivars (two heirlooms and two hybrids) of carrots and their propensities to benefit from inoculation with isolates of arbuscular mycorrhizal fungi in organic field conditions with and without late-season water restriction. Inoculants included geographically-distinct isolates from four species (*Funneliformis mosseae*, *Rhizophagus clarus, Rhizophagus intraradices,* and *Septoglomus deserticola*). Heirloom cultivars demonstrated a higher propensity to benefit compared to hybrid cultivars from inoculation with arbuscular mycorrhizal fungi. We observed benefits and costs with respect to yield associated with inoculation within experiments, but these changed across site-year, regardless of water conditions. Breeding histories of plant genotypes likely contribute to their mycorrhizal responsiveness.

3.2 Introduction

Organic produce sales maintain steady growth both in the United States (U.S.) and abroad, with supply continually falling short of consumer demand (Greene et al., 2009). To bring more hectares of land under certified organic management and to meet the need for more organic products, strategies must be developed to overcome the production challenges faced by organic farmers, ensuring that organic production is both productive and resilient.

Like their conventional counterparts, organic farmers struggle with production issues, but management strategies and available tools limit organic farmers' ability to mitigate these challenges. Organic regulations prohibit the use of synthetic inputs like herbicides and fertilizers (USDA-NOP, 2019), so farmers rely on more systems-based solutions, including crop variety selection. For example, two of the most challenging aspects of organic production are weed and fertility issues (Clark et al., 1999; Jerkins and Ory, 2016; Poudel et al., 2002; Tsvetkov et al., 2018). To overcome these, organic farmers select high-yielding crop cultivars that exhibit high nutrient use efficiency and weed competitiveness (Jerkins and Ory, 2016; Lammerts Van Bueren et al., 2011; Tsvetkov et al., 2018). To increase the effectiveness of crop variety selection as a management tool, organic farmers have strongly expressed their need for crops adapted explicitly to organic management systems (Lyon et al., 2015).

Most modern cultivars available to farmers, even those available as organically produced seed, originate from plant breeding under exclusively conventional production practices. Thus, the traits that confer high yield potential in these cultivars may be explicitly associated with high-input, synthetic approaches of conventional management (Lammerts van Bueren et al., 2011; Murphy et al., 2007; Singh et al., 2011). For example, many elite modern cultivars are bred under conventional management, with synthetic nitrogen (N) and phosphorus (P) fertilizers

added to ensure minimal impacts from crop deficiencies, and resultant plant genotypes may not be adapted to the limited mineral N and P pools typical of organic management (Dawson et al., 2008). Conversely, the historical development of land races from wild relatives through the production of monocultures without fertilization may have indirectly selected for increased mycorrhizal dependence in these early cultivated genotypes (Hetrick et al., 1992).

One factor that could improve crop performance under the more nutrient-limited conditions in organic environments is enhanced symbiotic relationships with soil microbes, including arbuscular mycorrhizal fungi (AMF). AMF form symbiotic relationships with host plants, facilitating the uptake of phosphorous, nitrogen, and other trace minerals for most land plants. Mycorrhizal symbiosis also improves water stress responsiveness (Augé, 2001; Smith and Read, 2008a), which may derive from the direct transfer of water via fungal aquaporins (Kikuchi et al., 2016; Li et al., 2013), improved water-holding capacity of soils resulting from proteins deposited by extraradical mycelium of AMF (Rillig, 2004; Wilson et al., 2009; Zhu and Miller, 2003), or improved physiological response due to a "priming" of plant defenses (Gosling et al., 2006; Ruiz-Sánchez et al., 2010). In a recent review, Ryan and Graham (2018) argue that management of AMF is unnecessary, but as Rillig et al. (2019) point out, their focus was on studies of cereal crops grown in high-input, conventional systems. In systems where readily-available nutrients might be limited, including low-input and organic management, AMF may be particularly beneficial to increase resilience and crop performance (Gosling et al., 2006). Although most data showing AMF benefits are rooted in greenhouse studies, some studies suggest that field inoculations may increase yields of cassava, potato, and wheat (Ceballos et al., 2013; Hijri, 2016; Mäder et al., 2011; Rodriguez and Sanders, 2015).

Though plants that host AMF are known to reap benefits from their symbiosis, the degree

to which they benefit may differ among crop genotypes. Genotypic variability arising from selection within breeding programs can alter phytohormone production, leading to notable differences in symbiosis-derived benefits among various crop genotypes (Singh et al., 2012; Venturi and Keel, 2016). Mustafa *et al.* (2016) showed that wheat cultivars, differing in susceptibility to wheat powdery mildew, exhibit differing levels of disease protection from mycorrhizal inoculation, and the protective effects may be tied to the choice of mycorrhizal inoculant. Numerous studies conducted over a wide range of essential crop species indicate that plant genotypes vary in their responsiveness to AMF (Burleigh et al., 2002; de Novais et al., 2014; Smith and Read, 2008b; Turrini et al., 2016). Further, a significant body of literature documents a loss in plant response to AMF from older genotypes to more modern cultivars (Lehmann et al., 2012; Sawers et al., 2008; Schmidt et al., 2016). For example, modern varieties of wheat, compared with older varieties, ancestors, and landraces demonstrate a loss of response to colonization by AMF (Hetrick et al., 1992). The authors hypothesize that the ability to host mycorrhizae declined in wheat cultivars bred after the 1950s due to increasing levels of soil fertility in breeding programs, making such symbiosis unnecessary and potentially detrimental to plant growth. However, this response is not consistent across all crop types; Chu et al. (2013), analyzing the mycorrhizal responsiveness of maize genotypes across a range of release dates showed that mycorrhizal associations were not negatively impacted by selection and that AMF may promote the growth of modern varieties in high-P soils.

Complicating the evaluation of mycorrhizal responsiveness of plant genotypes is the range of impacts that different AMF genotypes may solicit from their host plant. In a meta-analysis, Van Geel et al. (2016) found no single arbuscular mycorrhizal fungus strain elicited a "one-size-fits-all" consistent positive response across all host plants. Investigations of the effects

of mycorrhization on citrus production tested five mycorrhizal species (*Funneliformis mosseae* (UK), *F. mosseae* (USA), *Rhizophagus clarus*, *F. caledonius* and *Claroideoglomus. etunicatum*), resulting in significant differences between AMF species in growth, nutrient uptake, and percentage of mycorrhizal infection of the citrus plants, with plants inoculated with *R. clarus* growing taller than other mycorrhizal species (Ortas et al., 2006). Similar results were found in a study focused on another perennial crop, persimmon, with one of five AMF species tested (*C. etunicatum*) associated with the highest total plant dry weight, and another (*F. caledonius*) resulting in a higher concentration of leaf chlorophyll (İncesu et al., 2015). In a study of green peppers, an annual crop, three successive experiments found differing impacts on vegetative biomass and uptake of P and Zinc for plants inoculated with *F. mosseae*, *R. intraradices*, *C. etunicatum*, *R. clarus*, or *F. caledonius* (Ortas et al., 2011). Avio *et al.* found intraspecific variability between isolates of *F. mosseae* and *R. intraradices* in their ability to grow extraradical mycelial networks and in their impacts on alfalfa shoot phosphorus content (2006). Similarly, Mensah *et al.* reported a high level of functional diversity for intraspecific isolates of AMF grown with alfalfa and designated some isolates as high, medium, or low performers based on biomass and nutrient contents in colonized plants (2015). Understanding the interactions between non-native, exogenously-applied AMF versus native AMF species and plant genotype is of particularly timely importance to the agricultural community, within which the use of biological soil amendments is increasing (Lehman et al., 2015).

Plant breeding for growth and resilience under organic management may increase the ability of plants to interact with and benefit from soil symbionts. Our research seeks to understand the role of crop genotype (both as specific genotypes and as categorized by open-pollinated heirloom varieties and modern cultivars bred under high-input conditions) in

determining the response to AMF colonization under organic management, using carrot as a model crop. While related studies held similar aims, much of this research was conducted on field crops under conventional management using synthetic inputs. In addition to the evaluation of crop genotype, we also sought to understand the impact of inoculating with geographically distinct isolates of four closely-related AMF species under organic management with the absence of synthetic inputs. While most studies that compare inoculants from various AMF species and isolates occur under growth chamber and greenhouse conditions (Table S1), this study took place in agricultural fields with multi-year replication. Our research investigates these questions with a high-value vegetable crop under organic management conditions, a unique production environment as compared to conventional management due to inherent differences in nutrient inputs; resource homogeneity; fungicide, pesticide and herbicide application; tillage practices; and crop rotation strategies.

We hypothesized that under organic management conditions, modern hybrid cultivars, bred under high-input conditions, will exhibit less benefit (as measured by plant above and below-ground biomass) from AMF inoculation as compared to the older, open-pollinated heirloom cultivars, which arise from breeding conditions that likely favored symbiosis with AMF. Additionally, we hypothesized that specific AMF isolates might confer different magnitudes of advantages, with some isolates contributing to the growth of carrots more than others during water restriction.

3.3 Materials and Methods

3.3.1 Field Experiments

Field experiments were conducted at the West Madison Agricultural Research Station (WMARS, University of Wisconsin-Madison, Madison, WI, USA) on certified organic land during 2016 and 2017 (for cropping history see **Table S2)**. The land had been certified organic for over a decade, with crop rotations of diverse cereal grains, vegetables, soybeans, and cover crops. Soil samples were taken to a depth of 20 cm from two points within each tunnel, with a total of 8 samples, prior to planting each year. Soils were silt loam (13% sand, 67% silt, and 20% clay) with low potassium and phosphorus levels documented through soil tests (UW Soil and Plant Analysis Lab, **Table S3**). Before planting, winter rye (*Secale cereale*) cover crop was incorporated by discing, with planting following incorporation by at least three weeks to allow for cover crop decomposition. Each year included two separate experiments planted two weeks apart (**Table S4**).

Rain-excluding tunnels, covered with 6-millimeter greenhouse plastic, permitted control of water inputs. Tunnels measured 3 m wide, 2 m tall, and 40 m long. During 2016, tunnel orientation was East-West, and in 2017 tunnel orientation was North-South, to best account for the contours and slopes of the field. We installed plastic to a minimum depth of 20 cm along the tunnels' edges to limit movement of rainwater into tunnels. Each experiment comprised two tunnels; the well-watered tunnel received adequate water input during the whole growing season (**Table S4**). Water-restriction tunnels received no water inputs during the six weeks preceding harvest, whereas well-watered tunnels received water via drip irrigation up to three times per week. Drip irrigation lines ran parallel to carrot rows. Onset® HOBO data loggers tracked weather conditions within each tunnel.

This study evaluated two open-pollinated heirloom carrot cultivars ('Red Cored Chantenay' and 'Scarlet Nantes', Seed Savers Exchange, USA) and two hybrid cultivars ('Nelson' and 'Napoli', Johnny's Selected Seeds, USA), varieties commonly used by organic farmers in the upper Midwestern US. Organically-produced seeds were used for all experiments. To compare the performance among 4 closely-related species of AMF and their geographically-distinct representatives, we selected isolates from California and North Carolina to serve as inoculants (*Funneliformis mosseae* CA127, *Funneliformis mosseae* NC302C, *Rhizophagus clarus* CA401, *Rhizophagus clarus* NC112A, *Rhizophagus intraradices* CA502, *Rhizophagus intraradices* NC200, *Septoglomus deserticola* CA113, *Septoglomus deserticola* NC302A). The International Vesicular-Arbuscular Mycorrhizal Culture Collection (West Virginia University, USA) provided the isolates used to produce inoculants. Whole inoculants for each isolate comprised pasteurized organic soil, autoclaved coarse sand, roots of *Sorghum bicolor* (cv. 'Sugar Drip', Seed Savers Exchange, USA), and mycorrhizal propagules produced in open pot culture. Mock inoculant, produced without mycorrhizal propagules, served as a control. Whole inoculants are similar to commercially-available inoculants, and these often comprise multiple species.

Within each tunnel, combinations of carrot cultivar and a single mycorrhizal inoculant (36 combinations in total) were planted in a completely randomized design for the experiment (Expt) 1 and a randomized complete block design in Expt 2-4, with three replicate blocks per treatment housed in each tunnel (**Figure 1a**) and a total for 4 site-years. Each block comprised three rows, spaced approximately 75 cm apart and centered within tunnels, in which cultivar-inoculant combinations were divided (**Figure 1b**). Individual carrot-inoculant combinations were planted in 60 cm sections of rows, separated by 30 cm sections of non-inoculated guard carrots

(**Figure 1c**). Each treatment section had a randomly assigned number. To ensure root-inoculant contact and to prevent inoculant spread, we dug 15 cm-deep trenches, evenly spread 650 g inoculant, re-covered with soil, and sowed carrot seeds in a single row. Carrots were thinned to 2.5 cm spacing, with 24 – 30 carrots remaining in each cultivar-inoculant combination per replicate.

3.3.2 Field Sampling/Harvest

Carrots were harvested manually using a broad fork approximately 110 days after planting (**Table S4**). In water-restricted tunnels, soils showed clears signs of droughtiness, and the resulting soil hardness increased the difficulty of harvest digging compared to well-watered tunnels. We randomly selected 10 marketable carrots from each treatment section for data collection unless fewer than 10 marketable carrots were present, in which case we selected as many as possible. In year 1, we aimed to harvest 15 marketable carrots for mock treatments, and in year two we added a second mock treatment section per block and per cultivar, harvesting 10 from each. For each carrot harvested in all experiments, we measured the fresh weight and length of the taproot and shoot. To confirm the effectiveness of the water restriction treatment, we weighed taproots from Expt 3 and Expt 4 after drying at 60˚C for 6 weeks and calculated taproot water content for each carrot. Prior to harvesting taproots, we collected fine roots from up-turned carrots and their surrounding soils from each cultivar-isolate pair and stored them in 60% ethanol for mycorrhizal evaluation. Fine roots were more time-consuming to collect than their well-watered counterparts, and target volume of 8 ml of fine roots were collected for each replicate of each cultivar-isolate pair. A random subsampling of fine roots were cleared and stained with Sheaffer ink according to established methods and microscopically evaluated for colonization (Brundrett et al., 1996).

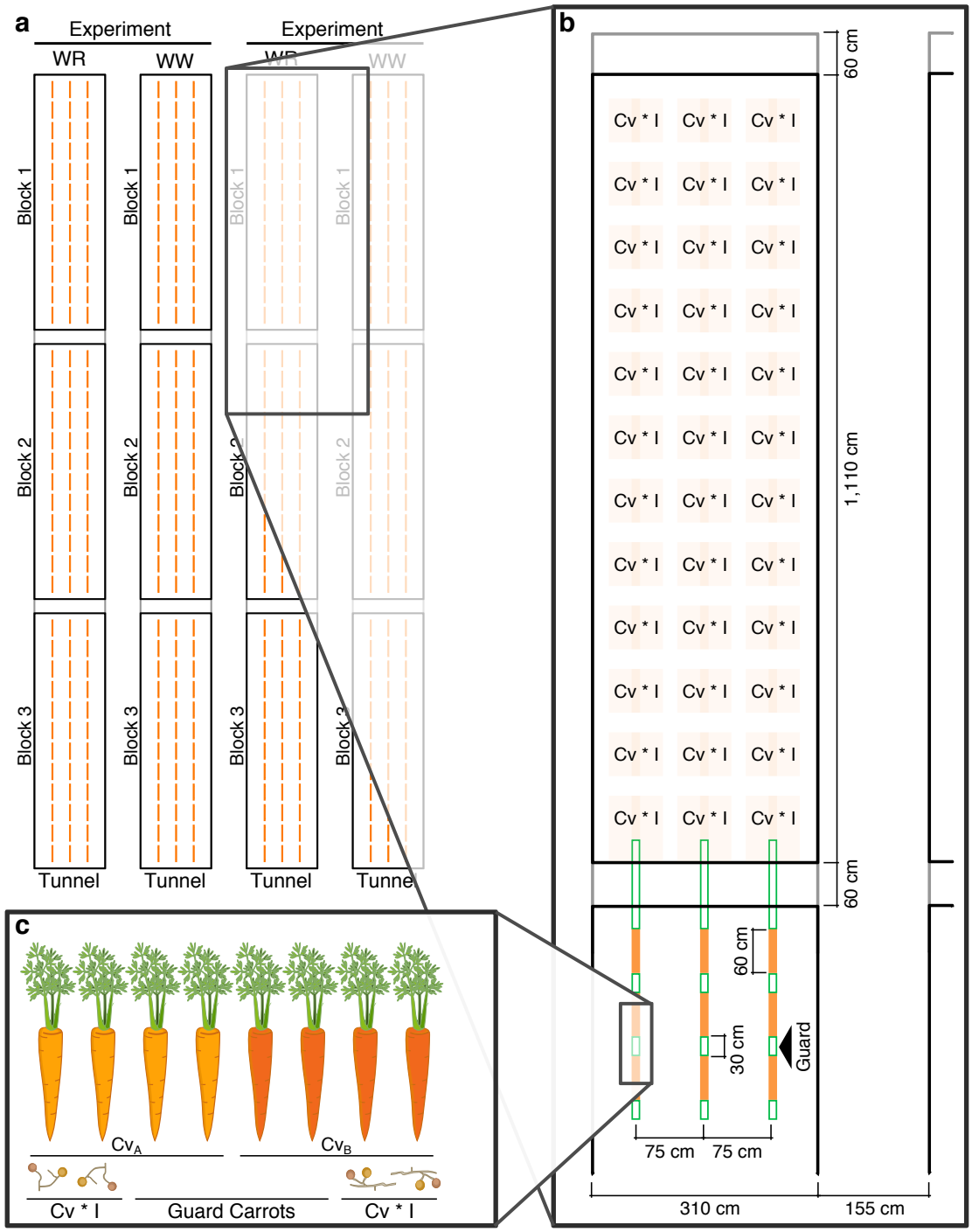


Figure 1. Experimental layout of inoculated carrots in the field. Within each year, 4 rain-excluding tunnels were planted with two, well-watered (WW) and water restricted (WR) comprising a single 'Experiment' (a). Experiments were planted two weeks apart (Table S2). Each tunnel was divided into three replicate blocks in which 36 unique pairings of cultivars and isolates (Cv * I) were randomly assigned to one of 3 rows (b), spaced as indicated. Non-inoculated 'guard' carrots spanned the spaces between cultivar-isolate pairings, and these were

planted to the midpoint with cultivars of carrot from each adjacent treatment (c). Guard carrots also spanned blocks and were planted at tunnel ends.

3.3.3 Statistical analysis

To compare the change in fresh taproots relative to mock treatments, we took the difference from each carrot taproot weight from the average of the mock treatment weight in each block. A linear mixed effects model was used to determine whether carrot cultivars responded differently to mycorrhizal inoculation in the field, with tunnel, cultivar, isolate, and cultivar-isolate interaction as fixed effects and cultivar-isolate location as nested random effects, adjusting for variability from the experimental design. We used analysis of variance (ANOVA) with Type III Sums of Squares using Satterthwaite's method to calculate degrees of freedom, followed by Tukey's HSD post hoc test. Comparisons between the growth of carrots within experiments and water treatments were made for each cultivar using one-way ANOVA followed by Dunnett's method to determine whether inoculants differed from mock treatments. Differences between combined inoculated and mock treatments were evaluated using Student's T-test. We conducted statistical analyses in R version 3.4.3 (R Core Team, 2019) and used the following packages: *argicolae* (Mendiburu, 2019)*; lattice* (Sarkar, 2018)*; lmerTest* (Kuznetsova, A., Brockhoff, P. B., & Christensen, 2014)*; multcomp* (Hothorn et al., 2019)*; ggplot2* (Wickham et al., 2019)*; dplyr* (Wickham et al., 2018). Due to the significant differences between experiments, results are analyzed and presented by individual experiments.

3.4 Results

3.4.1 Cultivars differ in their responses to inoculation in the field

Across experiments, we observed substantial differences between carrot genotypes, with heirloom cultivars more likely to show a positive response to inoculation in the field (Two-Way ANOVA, $F_{3,706.63} = 13.4667$, p-value < 0.001). Increases to inoculated carrots' fresh taproot weights relative to mock-treatments (inoculated sample minus mean of mock treatment within each block) occurred more often in heirloom varieties than in hybrids (**Figure 2; Figure S1**). While significant differences were not observed between the genotypes within heirloom versus hybrid classes, certain genotypes exhibited more frequent significant responses. Within the heirloom class, 'Scarlet Nantes' tended to demonstrate a significant positive response to inoculation more often. Within the hybrid class, 'Nelson' tended to demonstrate a significant negative response to inoculation more often.

Overall, inoculated heirloom genotypes tended to grow heavier, longer carrots, regardless of water treatment (**Table 1 , Table S5**). One exception to this trend occurred in Expt 4, during which inoculated Red Cored Chantenay carrot taproot weights were significantly greater under water-restricted (WR; 98.9 g inoculated vs 81.5 g mock, p-value = 0.0260) conditions but significantly less than mock carrots under well-watered (WW; 103.8 inoculated vs 118.0 mock, p-value = 0.0598) conditions. Conversely, inoculated hybrid varieties rarely increased taproot weight or length when compared to mock treatments, and there was no trend related to water condition. Inoculated hybrids did not respond consistently to water conditions. In the 2017 WR conditions, Expt 3 and 4 showed opposite effects for inoculated Napoli taproots (Expt 3: 114.5 g inoculated vs 127.2 g mock, p-value = 0.0875; Expt 4: 107.8 g inoculated vs 95.1 g mock, p-value = 0.0389) and no effect for Nelson carrots.

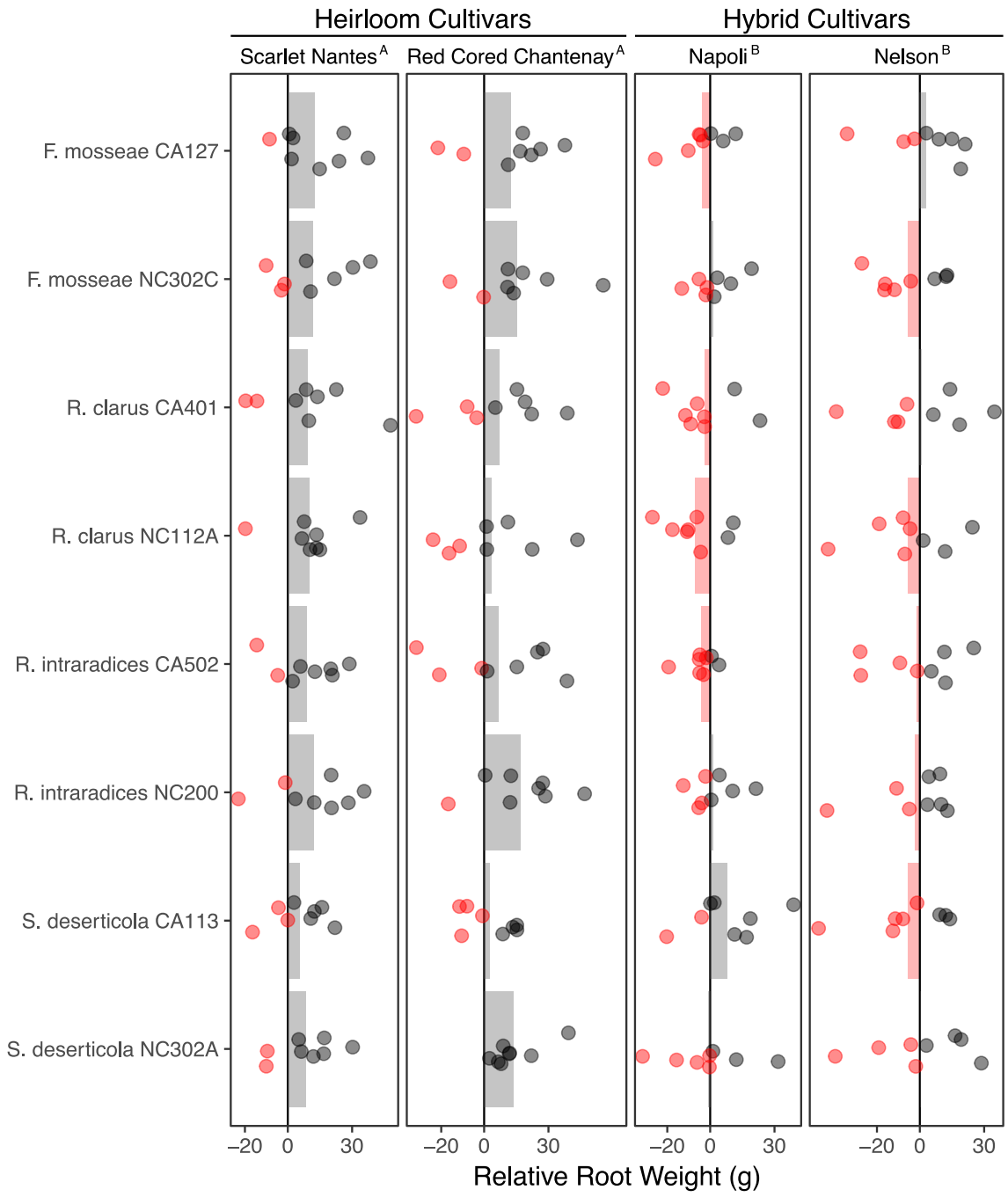


Figure 2. Comparison of inoculated carrot taproot weights relative to the means of mock treatments for each experimental condition. Dots represent the mean taproot weight gain or loss of inoculated cultivars relative to mock (inoculated sample minus mean of mock treatment within each block) for each water treatment within each experiment. Bars represent collective mean relative taproot weight gain or loss for all experiments combined. Relative taproot weights were significantly higher for heirloom cultivars than for hybrid cultivars (p-value < 0.001, $n \geq 24$ per dot, different superscripts following cultivar names indicate differences between groups). No differences were detected for relative taproot weights between Scarlet Nantes and Red Cored Chantenay or between Napoli and Nelson cultivars.

Table 1. Performance of heirloom and hybrid carrot genotypes with and without AMF inoculation as compared to mock treatments in each experiment. Mean fresh taproot weights (Root Wt), lengths (Root Length) and shoot weights (Shoot Wt) are given for each inoculation category and water regime (well-watered, WW, and water-restricted, WR). Differences were determined by Students t-test.

			Heirloom Cultivars						Hybrid Cultivars					
			Red Cored Chantenay			Scarlet Nantes			Napoli			Nelson		
Expt No.	Water Regime	Treatment	Root Wt (g)	Root Length (cm)	Shoot Wt (g)	Root Wt (g)	Root Length (cm)	Shoot Wt (g)	Root Wt (g)	Root Length (cm)	Shoot Wt (g)	Root Wt (g)	Root Length (cm)	Shoot Wt (g)
1	WW	Inoculated	128.0	13.3	54.4	90.4	16.3	31.0	114.5	17.6	22.9	94.5	16.5	13.4
1	WW	Mock	105.0	12.3	40.2	66.6	15.2	26.5	113.0	18.1	19.6	132.1	17.0	14.4
		p-value	*0.0071*	*0.0199*	*0.0003*	*<0.0001*	*0.0367*	*ns*	*ns*	*ns*	*ns*	*0.0002*	*0.0848*	*ns*
1	WR	Inoculated	112.8	12.8	58.0	76.9	16.0	26.2	95.1	17.0	18.3	78.6	15.8	12.7
1	WR	Mock	96.5	12.3	47.4	85.6	15.6	35.8	96.5	15.6	20.2	73.3	14.9	11.4
		p-value	*0.0437*	*ns*	*0.0746*	*ns*	*ns*	*0.0126*	*ns*	*0.0056*	*ns*	*ns*	*0.0675*	*ns*
2	WW	Inoculated	83.5	11.1	35.7	70.4	14.2	22.5	90.2	15.8	14.0	82.2	15.1	12.1
2	WW	Mock	56.4	9.6	25.2	57.5	13.2	21.1	86.4	14.7	15.8	80.7	14.9	12.5
		p-value	*0.0045*	*0.0059*	*0.0435*	*0.0395*	*0.0713*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*
2	WR	Inoculated	82.9	11.3	37.2	65.5	13.8	21.8	81.8	15.3	13.2	76.2	14.6	10.7
2	WR	Mock	83.4	11.8	34.7	49.4	12.9	18.3	81.7	15.6	11.9	88.2	15.8	12.0
		p-value	*ns*	*ns*	*ns*	*0.0003*	*0.0492*	*0.0928*	*ns*	*ns*	*ns*	*0.0581*	*0.0201*	*ns*
3	WW	Inoculated	105.6	12.9	43.7	80.4	16.3	24.5	92.4	16.9	17.7	90.6	16.5	14.2
3	WW	Mock	111.2	13.2	47.4	60.8	15.2	20.6	96.1	18.0	17.4	81.4	17.0	12.9
		p-value	*ns*	*ns*	*ns*	*<0.0001*	*0.0071*	*0.0505*	*ns*	*0.0107*	*ns*	*0.0512*	*ns*	*ns*
3	WR	Inoculated	110.9	13.5	43.8	80.5	16.5	25.0	114.5	18.4	20.5	96.2	17.7	15.4
3	WR	Mock	95.4	13.4	36.1	70.0	16.6	20.3	127.2	19.8	22.5	95.2	17.7	15.1
		p-value	*0.0111*	*ns*	*0.0267*	*0.0698*	*ns*	*0.0131*	*0.0875*	*0.0052*	*ns*	*ns*	*ns*	*ns*
4	WW	Inoculated	103.8	12.3	35.9	92.1	15.8	26.1	138.3	18.9	22.9	108.4	17.2	14.5
4	WW	Mock	118.0	13.3	43.8	81.7	15.3	20.4	146.0	19.0	23.8	92.2	16.9	11.2
		p-value	*0.0598*	*0.0090*	*0.0348*	*0.0676*	*ns*	*0.0011*	*ns*	*ns*	*ns*	*0.0005*	*ns*	*<0.0001*
4	WR	Inoculated	98.9	12.6	37.5	72.5	15.3	19.3	107.8	18.0	17.4	93.9	16.7	12.2
4	WR	Mock	81.5	11.6	30.4	79.7	15.4	21.1	95.1	16.9	15.7	94.4	16.7	13.4
		p-value	*0.0260*	*0.0031*	*0.0270*	*ns*	*ns*	*ns*	*0.0389*	*0.0063*	*ns*	*ns*	*ns*	*ns*

Shoot weights followed similar trends as the root weights with heirloom varieties demonstrating more instances of significantly increased shoot weights with inoculation than hybrid varieties. As with taproot weights and lengths, impacts of inoculation varied with cases of significantly positive response to inoculation (heirloom varieties in the WR treatment of Expt 4) and significantly negative response to inoculation (heirloom cultivars in WW treatment in Expt 4). The shoots, with one exception of hybrid varieties, did not increase or decrease significantly with inoculation, even when taproots differed.

Although harvest data revealed no differences between fresh weights by water condition, late-season water restriction significantly reduced the percent of taproot water content in all cultivars except Nelson (**Figure S2**), confirming the effectiveness of our water restriction treatment.

3.4.2 Effects of inoculation by carrot cultivar and isolate across water treatments

Inoculation outcomes with specific AMF isolates were inconsistent across field experiments (**Table 2**). While instances of significant root weight increase and decrease arose with single inoculations within experiments, no consistent trends emerged by water treatments or across experiments. For example, under the WW condition in Expt 2, Red Cored Chantenay and Scarlet Nantes carrots grew significantly heavier taproots with two different isolates of *R. clarus* as compared to mock (95.14 g inoculated with CA401 vs. 56.36 g mock [p-value < 0.05] and 95.15 g inoculated with NC112A vs. 57.46 g mock [p-value < 0.01], respectively), but results such as these did not repeat. In another instance, *R. intraradices* CA502-inoculated Nelson carrot taproot weight increased under WR conditions in Expt 4 but decreased in Expt 3 (105.91 g inoculated vs 94.45 mock [p-value < 0.05] and 67.72 g inoculated vs 95.24 g mock [p-value <

0.01], respectively). Due to inconsistent responses such as these, inoculants could not be ranked with confidence nor could we assign optimal AMF isolate and carrot genotype pairings.

Root staining confirmed mycorrhizal colonization for all roots, including those that received mock inoculants, which suggest that native AMF contributed to colonization (**Figure 3**). There were no substantial differences between isolates in percent colonization.

3.4.3 Weather

Environmental conditions during the production season varied between 2016 and 2017. Before the implementation of WR in the tunnels, daily temperatures and relative humidity were lower in 2016 as compared to 2017 (**Figure S3**). However, these trends reversed after WR was imposed, with the temperature and relative humidity in the tunnels higher in 2017 as compared to 2016. Photosynthetically active radiation was consistently higher in the tunnels in 2017 versus 2016.

Table 2. Performance of specific heirloom and hybrid carrot genotypes inoculated with AMF isolates under well-watered and water-restricted conditions as compared to mock treatments. Differences between carrot growth with inoculation treatments within experimental water conditions were compared by one-way ANOVA followed by Dunnett's test; significant differences from mock treatments are indicated (* p-value < 0.05; ** p-value < 0.01; *** p-value < 0.001).

			Heirloom						Hybrid					
			Red Cored Chantenay			*Scarlet Nantes*			*Napoli*			*Nelson*		
Expt No	Water Regime	Isolate	Root Wt (g)	Root Length (cm)	Shoot Wt (g)	Root Wt (g)	Root Length (cm)	Shoot Wt (g)	Root Wt (g)	Root Length (cm)	Shoot Wt (g)	Root Wt (g)	Root Length (cm)	Shoot Wt (g)
1	WW	Mock	104.97	12.33	40.2	66.59	15.21	26.51	113.0	18.14	19.63	132.15	17.01	14.44
		F. mosseae CA127	142.64	14.01	61.25*	103.97**	17.47**	32.65	109.68	17.95	18.56	98.23**	16.76	13.27
		F. mosseae NC302C	122.98	13.02	52.21	63.56	14.72	19.96	114.89	16.97	26.55	105.12**	17.41	14.59
		R. clarus CA401	127.17	13.61	52.58	114.51***	17.77**	45.89***	124.4	19.07	20.71	93.19***	16.56	12.41
		R. clarus NC112A	106.22	12.04	45.19	81.57	16.12	33.02	95.4	16.4	22.65	89.36***	16.2	12.39
		R. intraradices CA502	129.84	13.5	50.84	87.36	15.96	31.48	113.63	17.7	21.27	104.33*	16.8	15.54
		R. intraradices NC200	130.41	12.73	65.72**	86.8	16.88	25.05	113.51	18.1	20.53	88.85***	16.05	13.84
		S. deserticola CA113	120.27	13.76	49.56	88.58	15.25	29.44	129.98	16.88	33.29*	84.93***	15.71	11.44
		S. deserticola NC302A	144.3*	13.71	58.12	96.84*	15.84	30.25	114.29	17.67	19.6	92.71***	16.17	13.57
		p-value	*0.0714*	*ns*	*0.0205*	*<0.0001*	*0.0001*	*<0.0001*	*ns*	*ns*	*0.0891*	*<0.0001*	*ns*	*ns*
1	WR	Mock	96.54	12.29	47.39	85.6	15.61	35.81	96.5	15.57	20.18	73.28	14.89	11.42
		F. mosseae CA127	113.38	12.87	48.39	87.35	17.71*	28.06	96.82	17.57*	18.77	82.21	16.07	14.52
		F. mosseae NC302C	107.65	12.58	63.69	75.51	15.56	28.18	94.48	17.69*	17.03	69.06	15.4	11.12
		R. clarus CA401	111.87	13.21	58.86	71.21	15.94	23.11*	87.44	16.03	17.71	79.58	16.0	13.37
		R. clarus NC112A	118.98	12.12	66.94	65.83	15.52	23.55*	90.26	16.95	17.53	65.5	15.15	11.2
		R. intraradices CA502	123.99	12.82	67.72	87.86	16.6	27.32	91.66	16.41	18.12	72.09	15.12	13.55
		R. intraradices NC200	108.66	12.73	49.12**	62.54	14.18	25.46	106.99	17.26	18.73	82.69	16.02	12.39
		S. deserticola CA113	109.94	13.41	49.88	88.48	17.02	27.91	96.66	17.5*	19.79	85.36	16.35	11.92
		S. deserticola NC302A	108.26	12.27	60.06	76.06	15.63	25.58	96.15	16.44	18.4	92.56*	16.63*	13.35
		p-value	*ns*	*ns*	*0.1170*	*ns*	*0.0007*	*0.06*	*ns*	*0.0279*	*ns*	*0.0077*	*0.0521*	*ns*
2	WW	Mock	56.36	9.64	25.18	57.46	13.22	21.14	86.41	14.71	15.79	80.72	14.87	12.55
		F. mosseae CA127	78.35	11.49*	33.16	83.59*	15.0	27.9	98.34	15.93	17.94	99.8	15.87	14.66
		F. mosseae NC302C	85.76	11.32*	39.5	79.21	14.94	29.15	105.72	16.91*	14.17	93.42	15.68	12.67
		R. clarus CA401	95.14*	12.04**	41.93	66.04	13.85	17.42	74.88	14.69	13.45	70.56	14.67	10.54
		R. clarus NC112A	67.45	10.13	25.98	91.15**	14.69	29.79	97.2	16.27	13.76	76.19	14.78	10.72
		R. intraradices CA502	95.0*	11.38*	33.96	52.77	13.29	20.46	83.29	14.98	13.79	92.7	15.83	15.36
		R. intraradices NC200	103.22**	11.73**	50.41	56.25	12.84	17.34	73.86	13.99	12.51	84.27	15.46	11.67
		S. deserticola CA113	65.04	9.99	29.97	69.85	15.38	19.9	88.35	16.37	13.37	68.16	14.2	9.42
		S. deserticola NC302A	78.37	10.8	31.1	69.39	14.19	20.17	98.61	16.72	12.98	76.53	14.26	11.96
		p-value	*0.0060*	*0.0004*	*0.0046*	*0.0024*	*ns*	*0.0138*	*ns*	*0.0056*	*ns*	*0.0095*	*ns*	*0.0118*
2	WR	Mock	83.38	11.83	34.73	49.38	12.88	18.27	81.7	15.62	11.86	88.2	15.78	11.98
		F. mosseae CA127	94.62	11.64	44.09	64.23	13.36	20.65	77.1	14.49	13.19	85.71	15.3	11.54
		F. mosseae NC302C	94.29	11.92	36.8	57.97	13.39	16.26	85.01	14.85	13.57	76.38	14.46	11.28
		R. clarus CA401	75.47	10.66	28.53	63.2	14.14	16.55	104.94	15.97	15.9	76.37	14.51	11.44
		R. clarus NC112A	84.51	11.7	38.08	57.03	13.21	18.52	71.45	15.81	11.43	69.25	13.95*	9.79
		R. intraradices CA502	62.47	10.69	26.98	78.08*	15.08*	24.23	76.61	15.22	13.81	78.91	14.52	11.26
		R. intraradices NC200	83.88	10.97	45.15	77.62*	14.78	29.73*	85.99	15.98	13.37	77.34	14.91	10.0
		S. deserticola CA113	72.89	10.99	35.0	60.09	13.27	24.46	77.67	15.27	13.01	76.74	14.74	9.49
		S. deserticola NC302A	95.31	11.74	43.02	66.14	13.35	23.75	75.56	14.64	11.15	69.04	14.3	11.01

Table 2. continued

			Heirloom						Hybrid					
			Red Cored Chantenay			*Scarlet Nantes*			*Napoli*			*Nelson*		
Expt No	Water Regime	Isolate	Root Wt (g)	Root Length (cm)	Shoot Wt (g)	Root Wt (g)	Root Length (cm)	Shoot Wt (g)	Root Wt (g)	Root Length (cm)	Shoot Wt (g)	Root Wt (g)	Root Length (cm)	Shoot Wt (g)
		p-value	*ns*	*ns*	*ns*	*0.0139*	*0.0255*	*0.0020*	*ns*	*ns*	*ns*	*ns*	*0.1085*	*ns*
3	WW	Mock	111.25	13.17	47.45	60.79	15.17	20.65	96.12	17.96	17.4	81.39	16.95	12.94
		F. mosseae CA127	101.76	12.64	42.98	84.66*	16.85	28.62	70.54*	14.85***	13.1	96.36	17.53	15.91
		F. mosseae NC302C	111.01	13.58	39.06	91.13***	15.97	28.02	94.66	18.08	18.08	88.34	16.31	12.51
		R. clarus CA401	116.5	12.46	46.4	83.41*	16.6	28.48	93.54	16.92	18.16	95.46	17.41	13.73
		R. clarus NC112A	94.9	12.87	38.39	67.39	15.78	23.64	91.69	16.99	15.56	82.97	15.6	12.57
		R. intraradices CA502	79.66*	11.79*	34.95	80.81	16.44	23.08	94.31	16.63	15.33	86.74	15.06*	14.76
		R. intraradices NC200	123.73	13.48	54.48	81.18	16.95	20.01	90.75	17.22	17.69	91.3	16.48	14.32
		S. deserticola CA113	103.25	13.32	43.09	76.71	15.13	18.5	107.51	17.73	26.04***	73.51	15.58	12.45
		S. deserticola NC302A	113.72	13.29	49.96	77.8	16.28	25.24	95.86	17.06	17.7	110.09**	18.41	17.79
		p-value	*0.0300*	*0.0384*	*ns*	*0.0032*	*ns*	*ns*	*0.0440*	*0.0014*	*0.0001*	*0.0061*	*<0.0001*	*ns*
3	WR	Mock	95.39	13.37	36.15	70.03	16.58	20.33	127.15	19.79	22.53	95.24	17.69	15.07
		F. mosseae CA127	113.41	13.27	42.61	70.75	16.84	21.55	122.04	18.98	24.04	98.25	17.65	14.37
		F. mosseae NC302C	150.93***	13.46	59.52***	80.64	16.76	27.96	121.94	18.33	21.18	79.1	17.94	11.62
		R. clarus CA401	91.94	13.79	33.99	79.84	16.52	23.92	121.09	18.39	21.03	89.26	17.28	16.05
		R. clarus NC112A	83.98	13.06	31.96	83.42	16.57	28.53	100.21	18.21	16.41	119.75*	18.32	20.23*
		R. intraradices CA502	110.66	13.59	52.07	82.68	16.22	22.18	107.77	17.4*	17.25	67.72**	15.91*	8.68***
		R. intraradices NC200	123.96	14.09	44.73	105.65***	17.12	30.17	124.99	19.33	24.36	99.45	17.52	15.81
		S. deserticola CA113	110.63	13.82	43.01	65.67	15.95	19.72*	106.85	17.46*	18.22	104.47	18.6	17.77
		S. deserticola NC302A	101.97	12.88	42.13	75.13	15.76	25.99	111.47	18.78	21.89	111.7	17.99	18.72
		p-value	*<0.0001*	*ns*	*0.0009*	*0.0046*	*ns*	*0.0297*	*ns*	*0.0658*	*ns*	*<0.0001*	*0.0238*	*<0.0001*
4	WW	Mock	118.01	13.26	43.83	81.73	15.31	20.45	145.99	19.04	23.75	92.2	16.86	11.25
		F. mosseae CA127	96.45	12.23	33.13	84.18	15.37	24.41	152.06	19.1	23.69	113.35	17.73	15.42*
		F. mosseae NC302C	102.09	12.04	32.52	120.25***	16.76	37.54***	132.75	18.86	22.29	104.47	16.96	14.76
		R. clarus CA401	86.31*	11.45**	31.12	85.49	15.35	23.08	123.87	18.36	19.73	127.0	17.99	17.26***
		R. clarus NC112A	94.19	12.3	28.57*	95.03	16.01	26.01	135.2	18.95	21.13	104.01	16.87	12.87
		R. intraradices CA502	116.85	12.82	43.96	87.64	15.75	26.41	141.07	18.64	23.53	117.33	17.69	14.64
		R. intraradices NC200	101.3	12.54	35.7	94.05	15.71	25.42	142.09	18.74	24.01	105.05	16.77	14.45
		S. deserticola CA113	106.52	12.53	35.79	81.72	15.49	22.38	164.73	19.99	29.69	106.24	17.24	14.92
		S. deserticola NC302A	126.91	12.37	46.53	88.03	15.63	23.32	114.46	18.57	18.7	90.31	16.53	11.46
		p-value	*0.0240*	*0.1129*	*0.0033*	*0.0121*	*ns*	*0.0011*	*ns*	*ns*	*ns*	*0.0006*	*ns*	*0.0012*
4	WR	Mock	81.54	11.61	30.41	79.66	15.45	21.12	95.11	16.89	15.66	94.45	16.7	13.38
		F. mosseae CA127	107.88	13.63**	37.95	71.13	15.18	16.13	84.89	16.45	11.07	86.93	16.66	10.34
		F. mosseae NC302C	95.31	11.98	39.99	78.12	15.62	22.06	104.75	18.04	17.17	77.96	15.71	10.77
		R. clarus CA401	100.67	11.72	34.47	60.01	14.84	16.62	92.42	16.76	15.43	113.01***	17.01	15.33
		R. clarus NC112A	125.07***	12.89	49.11**	90.01	15.92	25.44	103.43	17.8	15.97	87.47	16.35	11.82
		R. intraradices CA502	82.97	12.8	33.4	65.18	14.31	18.04	99.23	17.74	16.48	105.91*	17.36	13.41
		R. intraradices NC200	108.88	13.18	43.25	83.2	15.47	18.03	116.46	18.3	19.68	89.56	17.19	10.34
		S. deserticola CA113	80.74	12.54	27.11	63.21	15.48	19.67	134.02**	19.53***	23.41**	93.16	16.4	11.45
		S. deserticola NC302A	89.46	12.43	34.55	69.63	15.41	18.35	126.82*	19.41***	19.89	97.48	16.82	13.73
		p-value	*0.0003*	*0.0316*	*0.0041*	*0.0200*	*ns*	*ns*	*0.0002*	*<0.0001*	*0.0004*	*0.0212*	*ns*	*ns*

** p-value < 0.05; ** p-value < 0.01; *** p-value < 0.001*

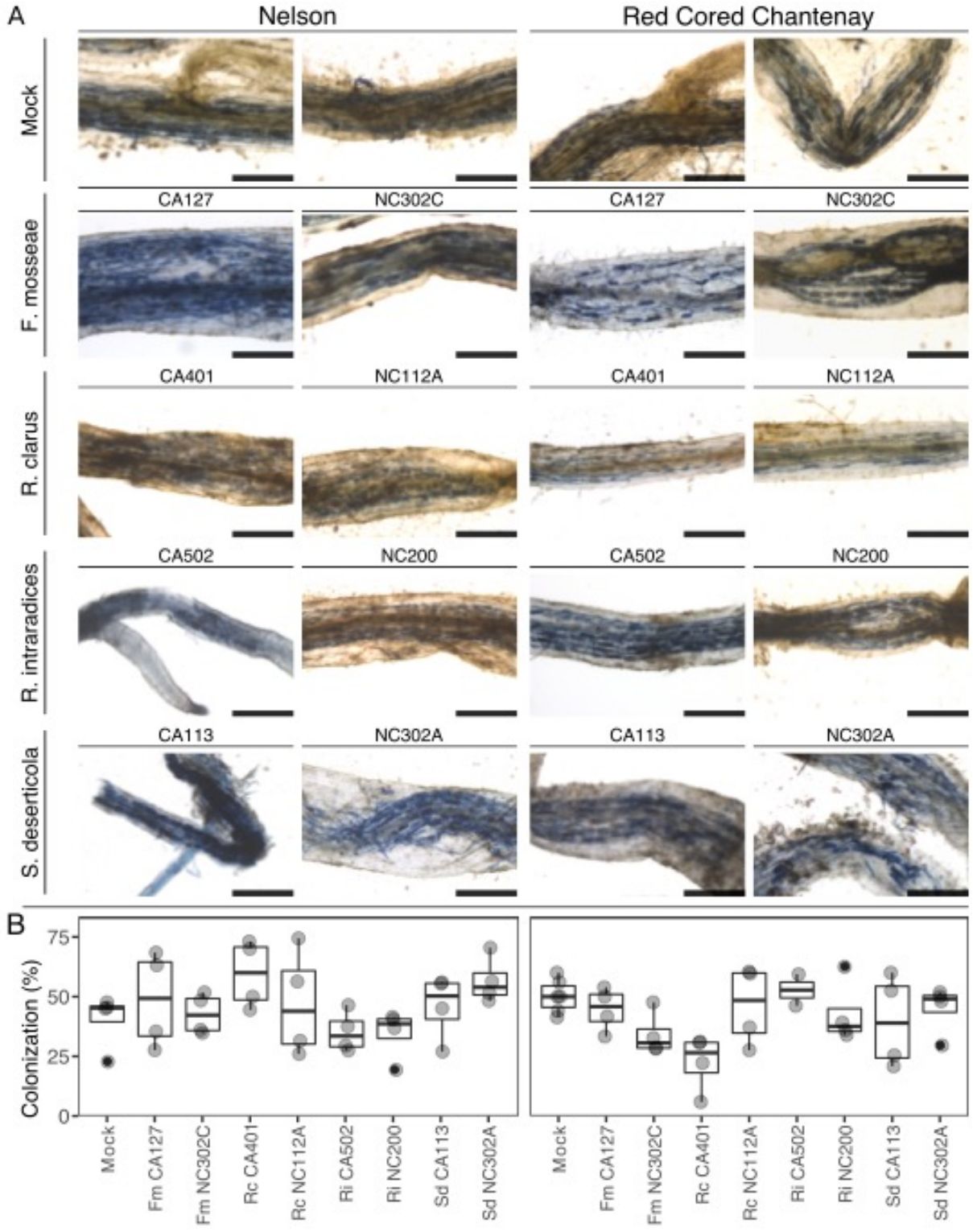


Figure 3. Comparison of colonization for each isolate in two cultivars. (A) Stained roots show mycorrhizal structures in blue (scale bar = 200 µm). (B) Percent root colonization range is shown in boxplots with replicate colonization counts shown as dots for each inoculant for Nelson carrots (left) and Red Cored Chantenay (right).

3.5 Discussion

Open-pollinated heirloom varieties showed consistent responses to mycorrhizal inoculation compared to modern hybrid cultivars under organic management in both WW and WR conditions as measured by root and shoot weight. The results of our study align with similar studies performed on other crops (greenhouse-grown sorghum (Raju et al., 1990; Symanczik et al., 2018); greenhouse-grown wheat (Hetrick et al., 1995, 1993); greenhouse-grown corn (Khalil et al., 1994), as well as a greenhouse-based multi-species analysis (Martín-Robles et al., 2018). Because these studies took place in greenhouses and relied on sterilized soil mixtures, there was an absence of background AMF communities, thus the similarities to our results are even more intriguing. Overall, data across these studies indicate that modern breeding programs produced genotypes less adapted to overcome the more resource-limited conditions of low-input and organic agriculture.

While modern cultivars may not be optimized to benefit from potential soil symbionts fully, further understanding of the interactions of plant hosts and microbial partners could allow breeders to enhance these symbiotic relationships rather than inadvertently lose their benefits through selective breeding (Milla et al., 2015). In many breeding programs, advancement of genotypes through the breeding program is typically based on easily measured traits (e.g., yield), which ignores underlying mechanisms contributing to productivity. Breeding for modern traits of interest in high-input, high productivity environments – including a focus on above-ground growth, such as yield and dwarf growth habit – may have altered plant hormone expression, which in turn may change the regulation of resource trafficking between AMF and associated plant hosts and root system architecture, both of which may affect the potential for AMF colonization (Burton et al., 2013; Gaudin et al., 2014; Hedden, 2003; Martín-Robles et al., 2018;

Schmidt et al., 2016). In addition to differences in the degree of AMF association between older and more modern cultivars, the trends in our study also align with other published studies that demonstrate that host response to inoculation can vary with cultivar (Buysens et al., 2016; Douds et al., 2016; Pellegrino et al., 2015) and fungal genotype (de Novais et al., 2014; Mensah et al., 2015; Pringle and Bever, 2008).

Our study found inconsistent results with respect to inoculation outcomes with specific AMF isolates and carrot cultivar across water regimes. These results are not surprising, given the outcomes of other published studies which have observed effects ranging from positive to negative with respect to plant performance upon inoculation with AMF under drought conditions (Neuman et al., 2009; Al-Karaki et al. 2004; Ashfar et al., 2014; Sendek et al., 2019). As observed by Sendek et al. (2019), the implementation of these experiments under field conditions with the presence of native soil biota may confound experimental results as compared to those conducted in controlled-environment conditions, particularly with the complexity of the other genotypic effects on plant drought response.

Our data also highlights the challenges of evaluating the impact of application of AMF on a commercial scale on farms. In recent years, companies emerged promoting AMF inoculants for use by organic farmers (Park et al., 2010). Although inoculants may benefit crops, incondistent positive yield response has been observed. While plant cultivar selection may be a key factor in the degree of response to application of these inoculants, competition with resident AMF across diverse soil environments also may be impacting results. In commercial vegetable production, with the exceptions of a few crops such as carrots and onions, transplanting of starter plants is the preferred method for establishing vegetables in organic fields. Plants sown and inoculated in greenhouse-conditions may benefit from their mycorrhizal inoculants even after establishment in

the field (Pringle and Bever, 2008; Tawaraya et al., 2012). This method may prove more beneficial than field applications of mycorrhizal inoculants since the applied inoculants lack competition with resident soil AMF to colonize and establish an initial network in greenhouse settings. To assess the prospective utility of mycorrhizae in greenhouse grown vegetables destined for transplant to fields, future studies should compare field and transplant applications of inocula.

Isolate selection poses another challenge in gauging the potential on-farm impacts of mycorrhizal inoculants. While intraspecific and interspecific variation in plant response to mycorrhizal colonization is well-documented in controlled greenhouse experiments (Marulanda et al., 2003; Mensah et al., 2015; Ruiz-Lozano et al., 1995; Symanczik et al., 2018), studies such as these usually lack replication and result in the reporting of "high performing" species and isolates. While contributing to a deeper understanding of the mechanisms underlying host plant responses, this approach to identifying the AMF "high performers" leaves the impression that some isolates will always be high performers. The experiments presented here highlight that changes to the specific field and annual environmental conditions, differences in plant genetic background, and different production variables such as planting dates contribute to varying outcomes across experiments. Across the published literature, positive inoculation effects vary with specific fungal isolates (Abbott et al., 1983; Abbott and Robson, 1982), soil fertility (Tawaraya et al., 2012), inoculation timing (Mummey et al., 2009), site disturbance level (Antunes et al., 2009) and plant-fungal co-adaptation (Koyama et al., 2017; Pringle and Bever, 2008; Rúa et al., 2016). Further complicating the interpretation of results is the fact that many older studies do not identify the AMF species used, either with their number/name or with the name of the collection from which they originated. Thus, multi-year, multi-site experiments

evaluating multiple crop genotypes and identified AMF inoculants are critical to understanding the range of responses to be anticipated with the application of any AMF inoculant and to establish predictable commercial inoculation outcomes.

In addition to the uncertainty of plant performance as it relates to applications of exotic AMF, other concerns exist to relying on the use of AMF to compensate for lower propensity to form AMF associations. While data is still lacking, concerns exist about the longer-term impacts of applying exotic AMF to a new land (Hart et al., 2018). Although studies show limited establishment and persistence of introduced AMF (Antunes et al., 2009), the concern remains that these new species may become invasive or negatively impact native species of plants and native microorganisms (Loján et al., 2017; Pellegrino et al., 2012). Additionally, the strategy of using exotic AMF inputs mirrors the input-intensive strategy of conventional agriculture, which deviates from the systems-based approach required by organic regulation. Our data, in tandem with similar data from studies on other crops, indicates that cultivar choice may provide an alternative means to promote beneficial microbial communities, rather than introducing a single microbe or a blend of microbial inoculants (Van Geel et al., 2016).

Selective breeding within organic systems, specifically incorporating AMF association and response, could provide organic farmers with cultivars possessing traits that confer resilience across a range of production stresses. Our results indicate that it is imperative for organic crop breeders to include plant-microbial partnerships as an additional focus for breeding programs to deliver efficient genotypes for sustainable agricultural systems. Beneficial soil fungi present an excellent opportunity to make global agriculture more efficient, more sustainable, and more productive (Ellouze et al., 2014; Rillig et al., 2019; Rodriguez and Sanders, 2015). Therefore, it is critical that crop genotypes be assessed for symbiotic potential, that crop genomes are mapped

to uncover the traits associated with mycorrhizal partnership, and that these traits are linked to productivity and food nutrition.

3.6 Acknowledgments

This material is based upon work supported by the National Science Foundation Graduate Research Fellowship Program under Grant No. [DGE1256259, 2015-2017; DGE1747503, 2017-2018]. Any opinions, findings, and conclusions or recommendations expressed in this material are those of the author(s) and do not necessarily reflect the views of the National Science Foundation. This work was also supported by the Advanced Opportunity Fellowship through SciMed Graduate Research Scholars at University of Wisconsin --Madison and USDA-NIFA-AFRI Award # 016-51300-25721. We thank Dr. Richard Lankau for his valuable comments and suggestions to improve our manuscript. Thank you to Maria Kamenetsky for her in depth consultation of statistical analyses.

3.7 Supplementary Figure and Tables

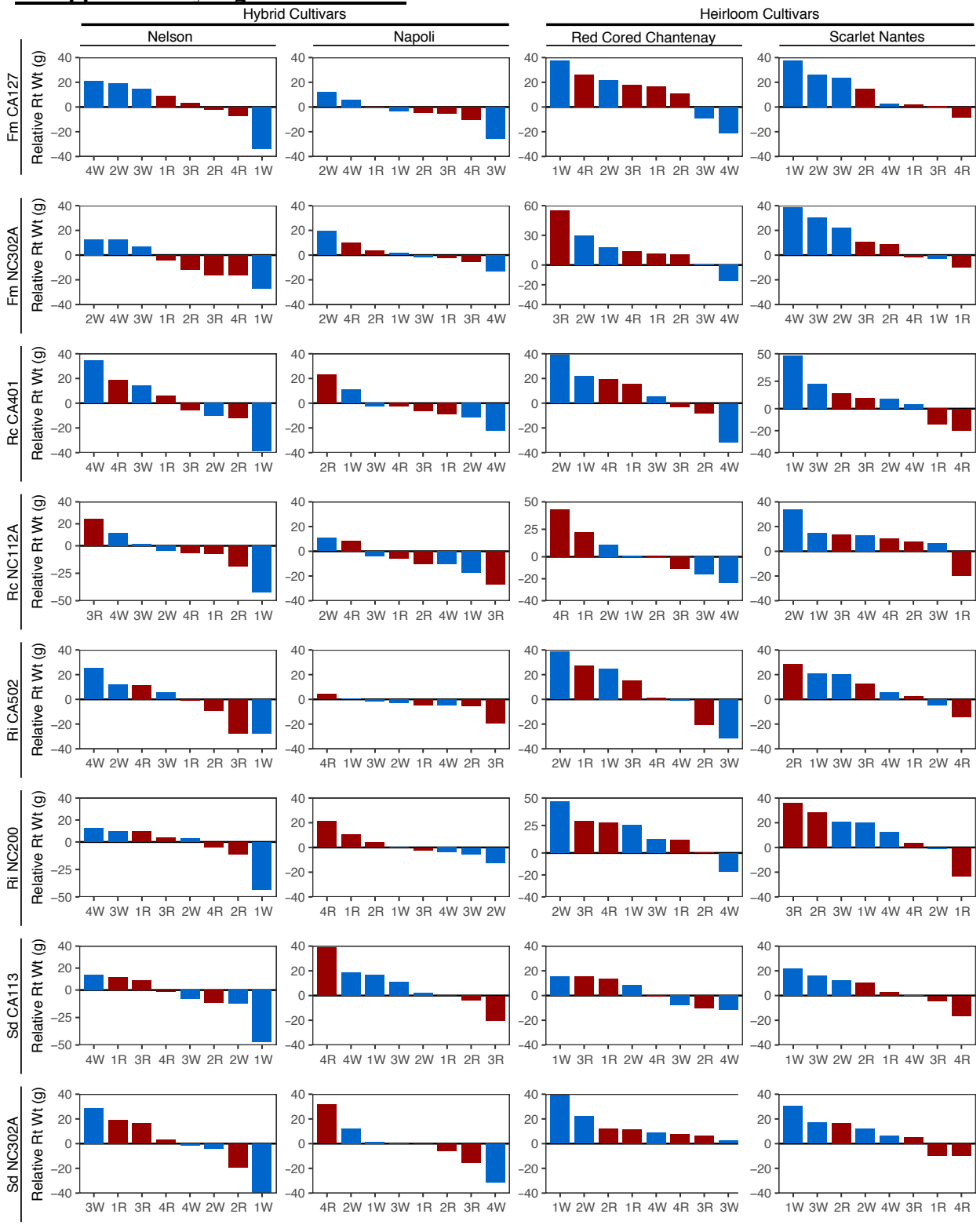


Figure S1. Ranked comparisons of inoculated carrot taproot weights relative to the means of mock treatments for each experimental condition. Bars represent the mean taproot weight gain or loss of inoculated cultivars relative to mock (inoculated sample minus mean of mock

treatment within each block) for each water treatment within each experiment. Each tunnel is indicated with experimental number and water condition, well-watered (W) or water-restricted (R). Relative taproot weights were significantly higher for heirloom cultivars than for hybrid cultivars (p-value < 0.001, n ≥ 24 per dot, different superscripts following cultivar names indicate differences between groups). No differences were detected for relative taproot weights between Scarlet Nantes and Red Cored Chantenay or between Napoli and Nelson cultivars.

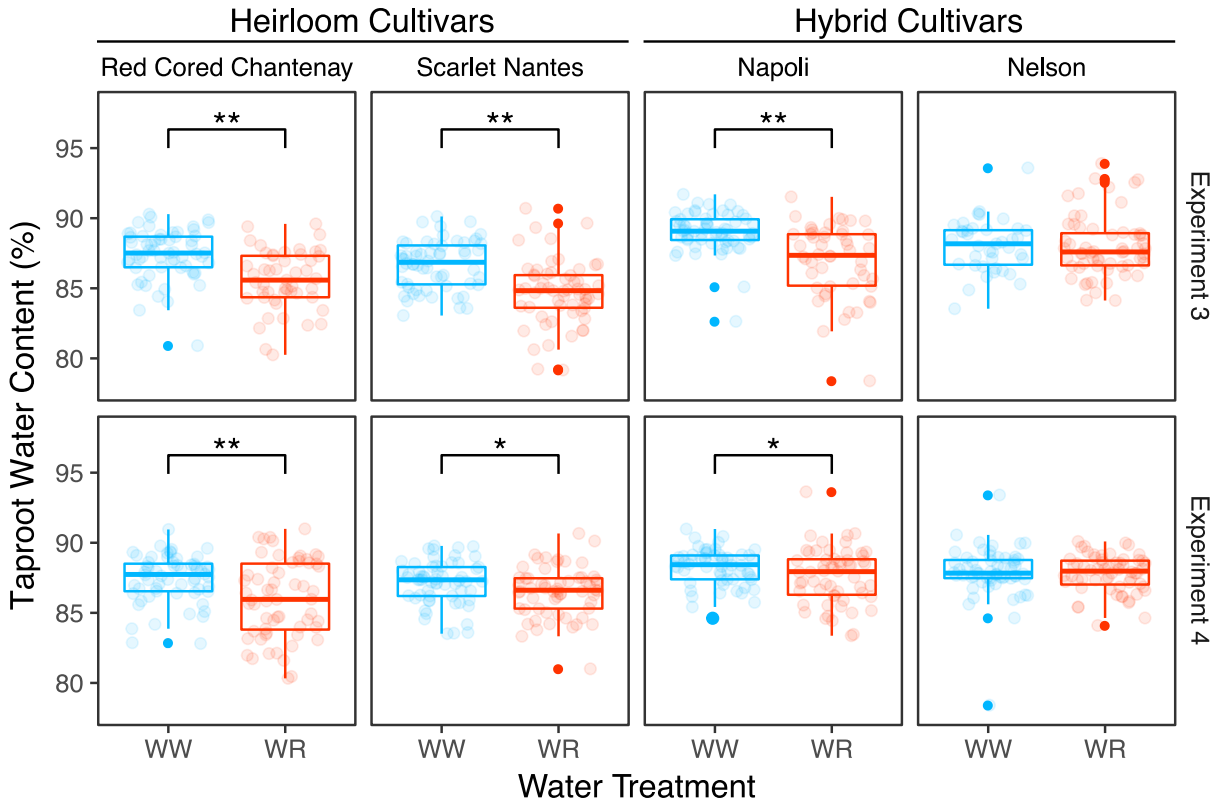


Figure S2. Distributions of taproot water content from field experiments 3 and 4. Well-watered controls (WW, orange) and late-season water restriction (WR, red) treatment were compared for each cultivar; n ≤ 40, paired T-test, *p-value < 0.05, **p-value<0.001

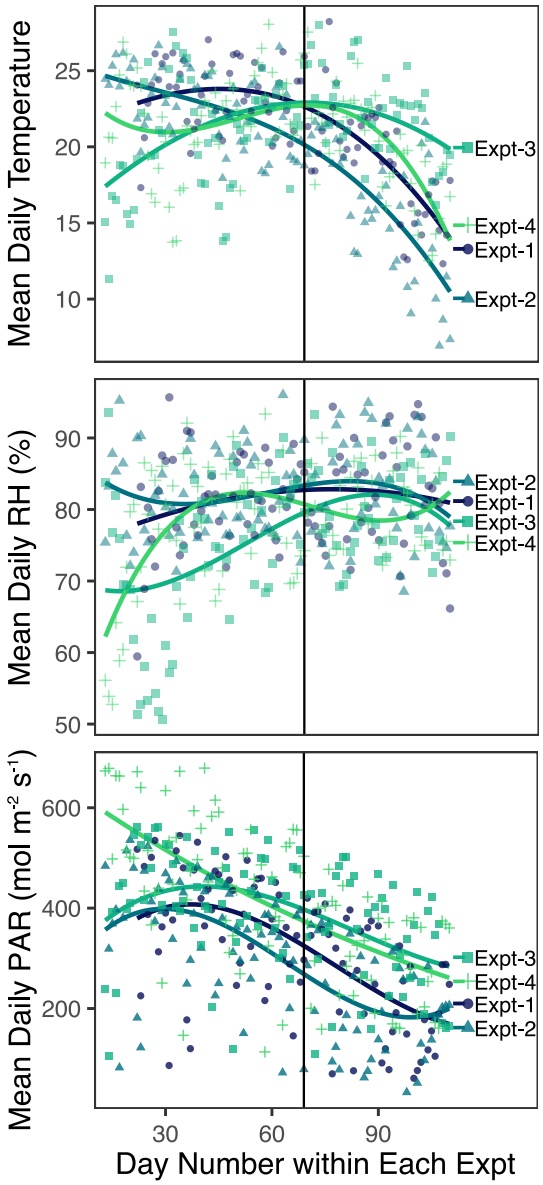


Figure S3. Weather conditions varied during each experiment. Mean daily temperatures (A), relative humidity (RH, B), and photosynthetically active radiation (PAR, C) are represented with different shapes and colors. Trend lines for each experiment illustrate the patterns during each trial. The legends rank each experiment and metric from high to low for the water restriction period.

Table S1. Comparison of experimental designs employed by cited literature.

Author(s)	Trial Type	Plant Species	Inoculant Type	Species	Inoculant Description	Measurements
Abbott *et al.,* 1983	**Field**	Clover (*Trifolium subterraneum* cv. Seaton Park)	Whole	Single	*Glomus fasciculatum* (Thaxter sensu Gerd.) Gerd., and Trappe, *Glomus monosporum* Gerd. and Trappe (AMF isolated from soils in Western Australia sharing similar pH to soils in this trial)	Colonization %; shoot biomass
Antunes *et al.,* 2009	Greenhouse	Maize (*Zea mays* L hybrid 192)	Whole	Single	*Glomus intraradices* (Myke® Pro SG2, Premier Tech Biotechnologies, Riviére-du-Loup, Quebec, Canada)	Colonization %; shoot phosphorus content; structure of AMF community
Avio *et al.,* 2006	Expt. 1: Growth chamber	*Medicago sativa* cv. Messe	Spores (germinated)	Single	*Glomus mosseae* (Nicol. & Gerd.) Gerdemann & Trappe, isolates IMA1 from UK and isolate AZ225C from US *Glomus intraradices* Schenk & Smith	Extent of extradical mycelial networks; spore germinations and hyphal growth; root and shoot biomass
	Expt 2: Greenhouse		Whole		IMA5 from Italy and isolate IMA6 from France	% Colonization; N and P content of leaves and roots; root and shoot biomass
Buysens *et al.,* 2016	**Field**	Potato	Spores	Mixed with non-AMF	*R. irregularis* MUCL 41833 and *T. harzianum MUCL 29707* (non-AMF, fungus), both from the Glomeromycota *in vitro* collection, grown on maize for mass production, then extracted spores were entrapped in alginate beads	Potato tuber weight
Ceballos *et al.,* 2013	**Field**	Cassava	Whole	Single	*R. irregularis*, Glomygel®	Plant tissue weights, root diameter, and colonization %
Chu *et al.,* 2013	Greenhouse	Corn	Spores	Single	*R. irregularis*	Colonization %

Table S1. continued

Author(s)	Trial Type	Plant Species	Inoculant Type	Species	Inoculant Description	Measurements
de Novais *et al.,* 2014	Greenhouse/ other	Corn	Spores	Single	*Aculospora colombiana, A. morrawiae, A. scrobiulata, A, spinosa, Clarodigeoglomus etunicatum, Glomus formosanum, Rhisophagus clarus, R. manihotis, Gigaspora albida, G. candida, G. margarita, G. rosea, Racocetra gregaria, Scultellospora calospora, S. heterogama,* and *S. pellucida* grown on *Urochloa decumbens* (Stapf) R.D.Webster (syn. *Brachiaria decumbens)*	P leaf content, stem diameter, shoot dry mass, root colonization, number of spores in 25 mL substrate, morphological and sequence analysis
Douds *et al.,* 2016	Greenhouse	Corn	Spores	Mixed	*R. irregularis*, *C. clairoidem*, *F. mossae*, *Glomus* sp (white), *Glomus* sp. grown outside (over-wintered) in pot cultures on *Paspalum notatum* (Flugge)	Heights, weights, colonization %
Hetrick *et al.,* 1992	Greenhouse	Wheat	Spores	Mixed	Various *Glomus spp.*	Weights and colonization %
Hetrick *et al.,* 1993	Greenhouse	Wheat	Spores	Mixed	Various *Glomus spp.*	Weights and colonization %
Hetrick *et al.,* 1995	Greenhouse	Wheat	Spores	Mixed	Various *Glomus spp.*	Growth increase % and colonization %
Hijri, 2016	**Field**	Potato	spores	single	*Rhizophagus irregularis* (Myke® Pro SG2, Premier Tech Biotechnologies, Riviére-du-Loup, Quebec, Canada)	Yield, presence of AMF colonization
İncesu *et al.,* 2015	Greenhouse	Persimmon	Spores	Mixed	Various *Glomus spp.*	Colonization %, height, chlorophyll concentrations
Khalil *et al.,* 1994	Greenhouse	Soybean, corn	Spores	Mixed	*Glomus intraradices* (Nutri-Link, Salt Lake City, UT, USA) and *Gigaspora margarita* (International Culture Collection of Arbuscular and VA Mycorrhizal Fungi, Morgantown, WV)	Colonization %, weights, leaf area, phosphatase activity

Table S1. continued

Author(s)	Trial Type	Plant Species	Inoculant Type	Species	Inoculant Description	Measurements
Koyama *et al.,* 2017	Greenhouse	Sudangrass and leek	Spores	Single	*Rhizophagus intradices* isolated from the Ontario Forest Research Institution Arboretum and *R. clarus* MN414B (International Culture Collection of Arbuscular and VA Mycorrhizal Fungi, Morgantown, WV)	Root and shoot weights
Loján *et al.,* 2017	**Field**	Potato	Spores	Single	*Rhizophagus irregularis* 1. Mycorise® ASP; liquid (Premier Tech Biotechnologies) 2. Myke® Pro P-801; powder (Premier Tech Biotechnologies) 3. Myke ® Pro GR; granular (Premier Tech Biotechnologies) 4. Symplanta ®; powder (SYMPLANTA)	Plant emergence, plant height, dry weight of shoots, leaves, roots and tubers, Total N P and K,
Martín-Robles *et al.,* 2018	Greenhouse	27 herbaceous crops	Spores	Single	*Rhizophagus irregularis* (Blaszk., Wubet, Renker & Buscot) C. Walker & A. Sch€ußler strain EEZ 58	AM colonization, above ground biomass, leaf P concentration
Marulanda *et al.,* 2003	Greenhouse	Lettuce- Lactuca sativa	Whole	Single	*Glomus coronatum, G. intraradices, G. claroideum, G. mossae, G. constrictum,* and *G. geosporum*	
Mensah *et al.,* 2015	Greenhouse	Medicago sativa L (alfalfa)	Whole	Single	Isolates from 31 diverse AM fungi, includng within the following species: *Rhizophagus irregulare, R. intraradices, Funneliformis mosseae, Croideoglomus claroideum, Gigaspora margarita, Acaulospora scrobiculata, A. morrowiae, A. colomiana, Paraglomus occultum,* and *Ambispora leptoticha*	Root and shoot biomass, P and N content in roots and shoots

Table S1. continued

Author(s)	Trial Type	Plant Species	Inoculant Type	Species	Inoculant Description	Measurements
Mummey *et al.,* 2009	Greenhouse	Ox-eye Daisy (*Leucanthemum vulgare*) Lam.	Whole	Mixed	*Gigaspora margarita* NC121A, *Gigaspora gigantea* NC150A, *Glomus claroideum* NC106A, *and Glomus deserticola* NC302A (International Culture Collection of Arbuscular and VA Mycorrhizal Fungi, Morgantown, WV) cultured on *Sorghum vulgare* var.	Nutrient contents, percent AMF by gridline intersect, PCR & ran gel, plant shoot biomass, leaf number and length,
Mustafa *et al.,* 2016	Greenhouse	Wheat (*Triticum aestivum* L.) cv. 'Orvantis' and 'Lord'	Whole	Single	1. *Rhizophagus irregularis* DAOM 197198, propagated on *Trifolium repens* L. (UCEIV, France) 2. *Funneliformis mosseae* FR140 (MycoAgro Ltd., France) 3. *Glomus* sp. mix, Solrize® (SZE; Agrauxine Ltd., France)	Mycorrhizal percent, plant height, leaf and stem counts, root and shoot dry weight, and number of powdery mildew colonies for susceptible ('Orvantis') and resistant ('Lord') cultivars of wheat
Ortas *et al.,* 2006	Greenhouse	Citrus	Spores	Single	*Glomus mosseae* (1) from UK *G. mosseae* (2) from Germany *G. clarium* from Nitri-Link, USA *G. caladonium* from UK *G. etunicatum* from Nitri-Link, USA	Fresh weight of root shoot and whole plant, dry weight root and shoot, root length, % mycorrizal colonization
Ortas *et al.,* 2011	Greenhouse	Green Pepper	Whole	Single and Mixed	Glomus etunicatum, G. clarum, G. intraradacis, G. caledonium, G. mossea, cocktail mixture of 5 AMF species in equal parts	Dry weight of root and shoot, phosphorus and zinc concentration,
Pellegrino *et al.,* 2012	**Field**	Corn	Spores	Single	F. mosseae (AZ225C and IMA1)	PCR, Root colonization levels measured by Gridline intersect method, plant heights, dry weights of leaf, stem and root, above ground dry matter, shoot N to P concentrations

Table S1. continued

Author(s)	Trial Type	Plant Species	Inoculant Type	Species	Inoculant Description	Measurements
Pringle and Bever, 2008	**Field, transplant from growth chamber**	*Allium vineale* L., *Anthoxanthum odoratum* L., *Cerastium glomeratum* Thuillier, *Plantago lanceolata* L., *Rumex acetosella* L., and *Veronica arvensis* L.	Whole	Single	AM fungi from grassland trap-culture: *Acaulospora morrowiae*, *Archaeospora trappei*, *Gigaspora gigantea*, and *Scutellospora pellucida, and Glomus* 'white' were cultured from single spores in pot culture on *Sorghum vulgare* Persoon.	Depended on the plant species, included: root and shoot biomass, seed counts, and fungal sporulation
Raju *et al.,* 1990	Greenhouse	Sorghum (SC6 and SC97)	Whole	Single	*Glomus fasciculatum* cultured on sudangrass	Percent root colonized, N concentrations, total root length, dry root weight, dry shoot weight
Ruiz-Lozano *et al.,* 1995	Growth Chamber	Rice (Oryza sativa, cv INCA LP-5)	Whole	Single	*Glomus etunicatum* (Becker et Gerd), *Glomus fasciculatum* (Thax. sensu Gerd.) Gerd. et Trappe, *Glomus mosseae* (Nicol. et Gerd.) Gerd. et Trappe, *Glomus deserticola* (Trappe, Bloss et Menge), *Glomus caledonius* (Nicol. et Gerd.) Trappe et Gerd., *Glomus intraradices* (Schenck et Smith), and *Glomus occultum* (Walker); all from Zaidín Experimental Station	% Colonization; proline, H2O2, and oxidative damage to lipids in leaves; photosynthetic response; and root and shoot biomass
Ruiz-Sánchez *et al.,* 2010	Growth Chamber	*Lactuca sativa* L. cv. Romana	Whole	Single	*Glomus intraradices* (Schenck and Smith), isolate EEZ 01	% Colonizatioe; proline, N, P, K, Ca, and Mg content of leaves; photosynthetic response; and root and shoot biomass

Table S1. continued

Author(s)	Trial Type	Plant Species	Inoculant Type	Species	Inoculant Description	Measurements
Singh *et al.,* 2012	Greenhouse	Cultivars of Durum Wheat (*Triticum tugidum* var. *durum* Desf.): 'Commander', 'DT710', 'Strongfield', 'Mongibello', and 'AC Morse'	Spores	Single	*Glomus irregularis* DAOM 197198	AM Root colonization level(%),durum wheat grain and straw production,grain and straw P concentrations, and straw K concentration
Symanczik *et al.,* 2018	Greenhouse	*Sorghum bicolor* (L.) Moench, cv. Plant-5	Spores	Single	*Rhizophagus arabicus* from sand plain in Oman and *R. irregularis* BEG-75 from field in Wädenswil, Switzerland, grown in pot culture on *Plantago lancelata, Trifolium pratense,* and *Lolium perenne*	Transpiration rates, plant growth, and nutrient uptake (indication of drought stress)
Tawaraya *et al.,* 2012	**Field, transplanted from greenhouse**	Welsh Onion	Whole	Single	*Glomus* R-10 (Idemitsu Kosan Co. Ltd., Tokyo, Japan)	AM fungus colonization, shoot P concentration, shoot dry weight, shoot length, and leaf sheath diameter

"Whole" inoculants refer to inoculants produced by open pot culture, which comprise roots, extraradical mycelia and spores, and substrate media.
"Spores" refer to sterile spores harvested from sterile *in vitro* culture.

Table S2. Cropping histories for field sites.

Expt	Year	Previous Years	Crop History	Notes
1 & 2	2016	2015	Winter rye	Harvested for grain / straw
		2014	Soybeans	
		2013	Oats	
		2012	Alfalfa	
3 & 4	2017	2016	Sweet corn	Sustaine 8-2-4 for fertility*
		2015	Sorghum sudangrass	Cover crop
		2014	Sweet corn	Sustaine 8-2-4 for fertility*
		2013	Sorghum sudangrass	Cover crop

*Organic slow-release fertilizer

Table S3. Soil analysis for field sites.

Year	Experiment	pH	P	K	OM	NO3-N	NH4-N
			ppm	ppm	%	ppm	ppm
2016	1 and 2	6.9 ± 0.1	25 ± 6.4	87 ± 13.9	3.4 ± 0.2	32.2 ± 9.5	6.4 ± 1.6
2017	3 and 4	6.6 ± 0.3	22 ± 4.0	106 ± 21.4	3.1 ± 0.1	29.0 ± 4.1	5.8 ± 0.4

Table S4. Dates for critical production-related events for Experiments 1-4 conducted on the organically certified land at the West Madison Agricultural Research Station, 2016 and 2017.

Experiment	Year	Planting Dates	Water Restriction Start*	Harvest Dates	Tunnel Orientation
1	2016	21 June - 25 June	22 August	3 October - 14 October	East - West
2	2016	5 July - 8 July	8 September	20 October - 27 October	East - West
3	2017	9 May - 15 May	18 July	28 August - 1 September	North - South
4	2017	23 May - 26 May	3 August	11 September - 17 October	North - South

*Water restriction only applies to water-restriction tunnel; the control tunnel received normal irrigation.

3.8 Additional Data

3.8.1 Greenhouse Experiments and Sampling

We conducted 4 experiments with carrots carrots in a greenhouse in the Biotron Laboratory (University of Wisconsin-Madison). Supplementary lighting turned on automatically, as needed, such that daytime hours began at 0700 and ended at 2000. Daytime and nighttime temperatures were 25˚C and 18˚C, respectively. Substrates comprised sieved and pasteurized organic soil from fields at WMARS and perlite. All carrots grew in Deepots with 6.9 cm diameters and 35.6 cm lengths.

We used the same organic carrot cultivars and inoculants (described in section 3.5, above) for a total of four experiments (**Table I**). We filled pots halfway with substrate, added approximately 30 ml of whole inoculant, filled the pots to their tops, then sowed 5-10 seeds per pot. Plants received reverse osmosis water via automatic drip emitters for 3 minutes per day at 0900 (**Figure I**). Each pot received a single water emitter during plant establishment. Well-watered treatment pots received a second emitter during the 6 weeks preceding harvest. We arranged pots in two randomized complete blocks, each with three replicates.

At 110 days post-sowing, we harvested the carrots, measuring the fresh weight and length of each shoot and taproot. We recorded shoot dry weights for all carrots. We collected, stored, and evaluated fine carrot roots as described above. Taproots from Experiment 1 were diced into pieces <1 cm, which were lyophilized, then ground using 6 mm beads and a paint shaker. Following an overnight extraction in petroleum ether (50 mg ml^{-1}), samples were prepared and analyzed with high performance liquid chromatography (Bowman et al., 2014).

3.8.2 Mycorrhizal outcomes vary across greenhouse experiments

All mycorrhizal inoculants produced colonized carrot roots in greenhouse experiments, and roots of mock-inoculated carrots showed no signs of colonization. Inoculated carrots tended to have increased taproot fresh weights compared to mock treatments in low-water conditions, but these increases were not consistent across experiments nor were they always significant (**Tables II-V**). *S. deserticola* NC302A and *R. intraradices* NC200 contributed to taproot fresh weight increases for most experiments, regardless of water treatment (**Tables II-V**).

Increases to taproot fresh weights did not differ by cultivar. As opposed to the field experiment (**Figure II**), heirloom cultivars allocated more biomass into shoot production than hybrids (**Figure III**). During the first greenhouse experiment, shoot fresh weights and lengths were somewhat reduced for all inoculated carrots compared to controls (**Tables II-V)**, but this was not the case in subsequent experiments. Differences between greenhouse experiments likely arose from differences in bulk density of soil and seasonal factors.

Beta-carotene levels increased for ‘Nelson’ and ‘Scarlet Nantes’ inoculated with *Rhizophagus clarus* CA401 compared to mock treatment (**Figure IV;** two-way ANOVA followed by Dunnett’s contrast, p-value < 0.05, n=3). No other isolate contributed to a change in levels of the carotenoid in taproots.

Colonization patterns differed between isolates within the AMF species. The *Septoglomus deserticola* NC302A isolate invested more heavily in arbuscule production than CA113 (**Figure V**).

Table I. Details of greenhouse experimental designs and timelines.

Planting Dates	Water Restriction Start	Harvest Dates	Cultivars	Inoculants	Soil:Perlite Ratio
Experiment 1, 36 cultivar-inoculant combinations, n = 6					
1 August 2016	7 October	17 - 20 November 2016	Nelson	*F. mosseae* CA127	1:1
			Napoli	*F. mosseae* NC302C	
			Red Cored Chantenay	*R. clarus* CA401	
			Scarlet Nantes	*R. clarus* NC112A	
				R. intraradices CA502	
				R. intraradices NC200	
				S. deserticola CA113	
				S. deserticola NC302A	
				Mock	
Experiment 2, 36 cultivar-inoculant combinations, n = 6					
10 February 2016	23 March	10 - 11 May 2016	Nelson	*F. mosseae* CA127	1:2
			Napoli	*F. mosseae* NC302C	
			Red Cored Chantenay	*R. clarus* CA401	
			Scarlet Nantes	*R. clarus* NC112A	
				R. intraradices CA502	
				R. intraradices NC200	
				S. deserticola CA113	
				S. deserticola NC302A	
				Mock	
Experiment 3, 6 cultivar-inoculant combinations, n = 6					
20 June 2017	29 August	10 October 2017	Napoli	*S. deserticola* CA113	1:1.5
			Red Cored Chantenay	*S. deserticola* NC302A	
				Mock	
Experiment 4, 6 cultivar-inoculant combinations, n = 6					
2 November 2017	27 December	7 February 2018	Napoli	*R. intraradices* CA502	
			Red Cored Chantenay	*R. intraradices* NC200	
				Mock	1:1.5

Figure I. Experimental plants growing in the greenhouse. During taproot maturation, well-watered carrots received water from two drip emitters (arrows), and water-restricted carrots received water from one emitter (A). Carrots grew in a greenhouse (B).

Table II. Mean weights of taproots, lengths of taproots, and shoot lengths of inoculated 'Napoli' carrots grown in a greenhouse with inoculation.

Exp. No	Water Regime	AMF Isolate	N	Mean Root Weight	Mean Root Length	Mean Shoot Length
1	HighH2O	Mock	6	23.42 ± 4.35	10.02 ± 3.05	27.47 ± 4.30
		Funneliformis mosseae CA130	6	24.23 ± 4.90	12.18 ± 1.94	23.03 ± 3.92
		Funneliformis mosseae NC302C	6	23.59 ± 4.30	10.55 ± 2.02	24.88 ± 4.09
		Rhizophagus clarus CA404	6	23.60 ± 4.19	12.33 ± 3.44	19.30 ± 1.91
		Rhizophagus clarus NC112A	6	24.05 ± 3.41	11.18 ± 1.30	23.32 ± 1.83
		Rhizophagus intraradices CA505	6	21.72 ± 4.67	10.12 ± 4.06	23.03 ± 1.50
		Rhizophagus intraradices NC203	6	22.27 ± 6.02	10.28 ± 2.57	19.83 ± 2.98
		Septoglomus deserticola CA116	6	21.45 ± 6.01	10.77 ± 2.61	23.42 ± 3.73
		Septoglomus deserticola NC305	6	26.53 ± 6.57	11.67 ± 3.10	23.12 ± 1.74
	LowH2O	Mock	6	16.90 ± 7.84	8.25 ± 2.67	27.73 ± 3.13
		Funneliformis mosseae CA130	6	20.81 ± 5.58	9.98 ± 2.37	24.67 ± 3.91
		Funneliformis mosseae NC302C	6	21.43 ± 3.28	10.92 ± 1.98	20.25 ± 2.37
		Rhizophagus clarus CA404	6	20.01 ± 2.90	10.42 ± 1.49	22.63 ± 2.49
		Rhizophagus clarus NC112A	6	22.38 ± 4.75	11.27 ± 1.11	24.48 ± 4.17
		Rhizophagus intraradices CA505	6	19.60 ± 3.67	10.00 ± 2.01	23.15 ± 3.17
		Rhizophagus intraradices NC203	6	22.68 ± 6.10	11.35 ± 1.72	21.30 ± 4.04
		Septoglomus deserticola CA116	6	16.10 ± 5.16	10.63 ± 1.48	18.97 ± 1.80
		Septoglomus deserticola NC305	6	25.66 ± 5.74	12.03 ± 2.71	19.37 ± 2.10
2	HighH2O	Mock	6	15.08 ± 2.00	8.65 ± 2.60	22.02 ± 2.67
		Funneliformis mosseae CA130	6	13.79 ± 2.39	9.12 ± 1.81	22.97 ± 1.82
		Funneliformis mosseae NC302C	6	14.01 ± 2.44	8.92 ± 1.12	22.78 ± 3.87
		Rhizophagus clarus CA404	5	14.39 ± 1.18	10.10 ± 2.46	23.18 ± 5.25
		Rhizophagus clarus NC112A	6	14.21 ± 3.98	9.50 ± 2.33	23.10 ± 4.02
		Rhizophagus intraradices CA505	6	11.71 ± 2.11	7.55 ± 2.36	20.00 ± 6.02
		Rhizophagus intraradices NC203	5	17.38 ± 2.80	10.86 ± 1.11	22.78 ± 2.62
		Septoglomus deserticola CA116	6	15.12 ± 4.62	9.30 ± 3.46	23.50 ± 2.22
		Septoglomus deserticola NC305	6	18.21 ± 4.28	10.32 ± 1.42	24.53 ± 2.03
	LowH2O	Mock	6	13.90 ± 3.31	9.22 ± 1.23	22.98 ± 3.59
		Funneliformis mosseae CA130	6	14.95 ± 2.95	10.62 ± 2.33	19.82 ± 2.79
		Funneliformis mosseae NC302C	6	17.14 ± 5.69	10.40 ± 2.32	21.67 ± 1.07
		Rhizophagus clarus CA404	6	13.86 ± 4.02	10.52 ± 1.42	20.02 ± 2.48
		Rhizophagus clarus NC112A	6	16.08 ± 3.92	10.97 ± 1.42	23.30 ± 2.44
		Rhizophagus intraradices CA505	6	13.23 ± 4.37	9.38 ± 2.48	21.53 ± 2.56
		Rhizophagus intraradices NC203	6	17.85 ± 2.80	9.82 ± 2.28	22.20 ± 1.44
		Septoglomus deserticola CA116	5	14.14 ± 2.83	9.64 ± 1.49	25.08 ± 1.16
		Septoglomus deserticola NC305	6	16.30 ± 2.51	9.48 ± 2.09	23.57 ± 1.89
3	HighH2O	Mock	6	27.26 ± 5.09	13.53 ± 2.69	22.48 ± 2.48
		Septoglomus deserticola CA116	6	20.13 ± 9.42	10.88 ± 1.93	22.88 ± 3.30
		Septoglomus deserticola NC305	6	23.58 ± 8.45	12.68 ± 2.79	21.68 ± 2.65
	LowH2O	Mock	6	14.64 ± 5.60	12.10 ± 4.33	22.17 ± 1.09
		Septoglomus deserticola CA116	6	11.30 ± 3.70	11.62 ± 3.72	20.90 ± 1.56
		Septoglomus deserticola NC305	6	17.80 ± 4.02	11.50 ± 1.67	22.10 ± 3.71
4	HighH2O	Mock	6	23.49 ± 5.85	11.13 ± 2.37	31.38 ± 5.53
		Rhizophagus intraradices CA505	6	25.19 ± 3.43	10.63 ± 1.75	29.23 ± 4.65
		Rhizophagus intraradices NC203	6	25.94 ± 5.53	12.70 ± 1.87	29.15 ± 3.51
	LowH2O	Mock	6	19.15 ± 3.77	12.02 ± 1.65	35.88 ± 3.29
		Rhizophagus intraradices CA505	6	23.09 ± 3.55	10.95 ± 1.70	31.77 ± 3.80
		Rhizophagus intraradices NC203	6	22.01 ± 9.43	12.05 ± 2.44	32.30 ± 5.18

Table III. Mean weights of taproots, lengths of taproots, and shoot lengths of inoculated 'Nelson' carrots grown in a greenhouse with inoculation.

Exp. No	Water Regime	AMF Isolate	N	Mean Root Weight	Mean Root Length	Mean Shoot Length
1	HighH2O	Mock	6	24.69 ± 3.07	9.62 ± 1.99	29.02 ± 2.80
		Funneliformis mosseae CA130	6	24.56 ± 2.57	10.92 ± 0.57	23.83 ± 3.53
		Funneliformis mosseae NC302C	6	25.41 ± 5.15	10.15 ± 0.75	25.87 ± 2.32
		Rhizophagus clarus CA404	6	20.19 ± 4.47	9.67 ± 2.77	22.35 ± 2.87
		Rhizophagus clarus NC112A	6	21.50 ± 5.12	10.20 ± 2.21	25.88 ± 1.52
		Rhizophagus intraradices CA505	6	21.61 ± 3.73	10.82 ± 1.93	22.73 ± 2.39
		Rhizophagus intraradices NC203	6	27.84 ± 6.31	12.62 ± 0.57	22.85 ± 3.99
		Septoglomus deserticola CA116	6	25.53 ± 4.25	12.40 ± 0.99	23.05 ± 2.82
		Septoglomus deserticola NC305	6	26.85 ± 3.03	12.50 ± 0.26	24.48 ± 1.53
	LowH2O	Mock	6	18.39 ± 5.79	10.58 ± 1.02	30.30 ± 3.73
		Funneliformis mosseae CA130	5	19.61 ± 5.24	9.94 ± 1.11	25.50 ± 3.23
		Funneliformis mosseae NC302C	6	17.74 ± 2.53	10.28 ± 1.03	22.92 ± 0.70
		Rhizophagus clarus CA404	6	22.66 ± 3.89	11.00 ± 1.11	22.23 ± 2.54
		Rhizophagus clarus NC112A	6	16.77 ± 4.08	9.03 ± 0.91	25.27 ± 3.58
		Rhizophagus intraradices CA505	6	20.41 ± 5.77	9.48 ± 1.41	22.47 ± 2.58
		Rhizophagus intraradices NC203	6	20.94 ± 2.90	11.42 ± 0.75	21.25 ± 3.47
		Septoglomus deserticola CA116	6	14.84 ± 6.97	9.28 ± 1.88	22.33 ± 1.90
		Septoglomus deserticola NC305	6	20.68 ± 8.74	10.85 ± 2.02	21.73 ± 3.51
2	HighH2O	Mock	5	13.34 ± 5.00	7.95 ± 3.05	24.72 ± 1.50
		Funneliformis mosseae CA130	6	18.12 ± 4.88	10.88 ± 1.43	22.48 ± 1.73
		Funneliformis mosseae NC302C	6	15.33 ± 4.56	8.77 ± 1.79	21.42 ± 3.05
		Rhizophagus clarus CA404	6	15.05 ± 7.87	9.52 ± 1.39	24.32 ± 4.48
		Rhizophagus clarus NC112A	6	16.49 ± 4.72	8.47 ± 2.95	21.97 ± 1.99
		Rhizophagus intraradices CA505	4	16.28 ± 3.35	9.80 ± 1.90	21.85 ± 1.18
		Rhizophagus intraradices NC203	6	16.39 ± 7.10	8.46 ± 1.49	23.93 ± 2.85
		Septoglomus deserticola CA116	6	15.40 ± 6.78	8.98 ± 3.18	25.08 ± 2.79
		Septoglomus deserticola NC305	6	16.28 ± 3.25	8.53 ± 2.63	24.83 ± 1.93
	LowH2O	Mock	5	14.48 ± 0.96	8.82 ± 0.68	27.02 ± 2.28
		Funneliformis mosseae CA130	6	16.32 ± 4.26	9.47 ± 2.49	24.00 ± 2.00
		Funneliformis mosseae NC302C	5	16.71 ± 3.62	9.14 ± 2.32	20.54 ± 2.03
		Rhizophagus clarus CA404	6	12.37 ± 6.11	8.32 ± 2.41	23.13 ± 3.78
		Rhizophagus clarus NC112A	6	17.66 ± 3.64	9.87 ± 2.08	24.45 ± 3.12
		Rhizophagus intraradices CA505	5	16.74 ± 4.83	8.98 ± 2.45	20.48 ± 0.61
		Rhizophagus intraradices NC203	4	11.73 ± 4.44	9.93 ± 0.90	17.90 ± 0.80
		Septoglomus deserticola CA116	6	16.15 ± 3.34	9.53 ± 2.13	23.55 ± 2.49
		Septoglomus deserticola NC305	6	16.65 ± 1.69	9.07 ± 2.19	25.72 ± 2.68

Table IV. Mean weights of taproots, lengths of taproots, and shoot lengths of inoculated 'Scarlet Nantes' carrots grown in a greenhouse with inoculation.

Exp. No	Water Regime	AMF Isolate	N	Mean Root Weight	Mean Root Length	Mean Shoot Length
1	Well Watered	Mock	6	18.07 ± 4.61	8.13 ± 1.79	33.65 ± 5.29
		Funneliformis mosseae CA127	6	16.15 ± 5.06	7.95 ± 3.43	27.97 ± 3.92
		Funneliformis mosseae NC302C	6	20.90 ± 4.46	9.75 ± 2.59	24.63 ± 2.22
		Rhizophagus clarus CA401	6	14.02 ± 5.60	7.98 ± 2.66	23.40 ± 2.51
		Rhizophagus clarus NC112A	6	17.47 ± 5.31	9.40 ± 3.38	29.00 ± 4.40
		Rhizophagus intraradices CA502	6	15.88 ± 4.72	9.15 ± 1.53	21.45 ± 3.86
		Rhizophagus intraradices NC200	6	16.74 ± 5.03	10.53 ± 1.90	23.45 ± 3.89
		Septoglomus deserticola CA113	6	20.83 ± 3.69	10.30 ± 0.93	23.90 ± 3.40
		Septoglomus deserticola NC302	6	18.84 ± 4.73	9.60 ± 2.32	26.02 ± 3.63
	Water Restricted	Mock	6	16.36 ± 6.70	11.08 ± 1.75	27.90 ± 6.18
		Funneliformis mosseae CA128	6	15.13 ± 3.80	10.82 ± 1.39	26.08 ± 4.72
		Funneliformis mosseae NC302C	6	15.71 ± 4.96	9.85 ± 1.74	21.98 ± 2.84
		Rhizophagus clarus CA402	6	14.43 ± 3.95	9.15 ± 2.73	23.98 ± 2.03
		Rhizophagus clarus NC112A	6	15.07 ± 3.68	9.23 ± 2.34	27.93 ± 3.36
		Rhizophagus intraradices CA503	6	14.52 ± 4.19	9.77 ± 1.93	23.33 ± 3.03
		Rhizophagus intraradices NC201	6	14.35 ± 5.11	10.27 ± 2.90	23.42 ± 1.80
		Septoglomus deserticola CA114	6	16.10 ± 2.66	9.13 ± 1.69	22.75 ± 2.14
		Septoglomus deserticola NC303	6	17.98 ± 2.60	10.27 ± 1.74	23.88 ± 4.10
2	Well Watered	Mock	6	13.55 ± 4.01	8.60 ± 2.51	23.73 ± 1.40
		Funneliformis mosseae CA129	6	10.47 ± 4.31	9.08 ± 1.50	24.78 ± 3.54
		Funneliformis mosseae NC302C	6	9.85 ± 3.85	8.35 ± 1.61	24.33 ± 1.72
		Rhizophagus clarus CA403	6	8.69 ± 1.27	8.16 ± 1.79	24.98 ± 0.72
		Rhizophagus clarus NC112A	6	11.71 ± 3.81	8.38 ± 2.48	25.68 ± 2.99
		Rhizophagus intraradices CA504	6	11.19 ± 2.53	8.48 ± 2.55	22.30 ± 3.53
		Rhizophagus intraradices NC202	6	7.92 ± 1.67	6.94 ± 1.22	23.85 ± 4.28
		Septoglomus deserticola CA115	6	12.20 ± 5.38	10.15 ± 3.21	24.00 ± 4.40
		Septoglomus deserticola NC304	5	14.84 ± 3.57	9.28 ± 2.31	23.24 ± 2.76
	Water Restricted	Mock	6	8.63 ± 1.67	8.18 ± 1.90	25.70 ± 4.30
		Funneliformis mosseae CA130	6	8.39 ± 2.04	8.02 ± 2.15	23.88 ± 3.40
		Funneliformis mosseae NC302C	6	9.27 ± 3.07	7.02 ± 2.29	20.95 ± 3.07
		Rhizophagus clarus CA404	6	10.16 ± 2.48	7.85 ± 1.95	25.13 ± 2.62
		Rhizophagus clarus NC112A	6	12.25 ± 2.08	8.82 ± 1.71	25.33 ± 5.71
		Rhizophagus intraradices CA505	6	11.76 ± 2.56	7.72 ± 1.56	24.97 ± 3.88
		Rhizophagus intraradices NC203	6	9.39 ± 1.79	8.14 ± 1.46	23.44 ± 5.03
		Septoglomus deserticola CA116	6	9.39 ± 3.75	8.80 ± 3.56	23.70 ± 5.11
		Septoglomus deserticola NC305	6	11.86 ± 3.37	9.63 ± 2.16	27.12 ± 2.31

Table V. Mean weights of taproots, lengths of taproots, and shoot lengths of inoculated 'Red Cored Chantenay' carrots grown in a greenhouse with inoculation.

Exp. No	Water Regime	AMF Isolate	N	Mean Root Weight	Mean Root Length	Mean Shoot Length
1	HighH2O	Mock	6	18.50 ± 4.05	5.95 ± 1.35	31.87 ± 4.37
		Funneliformis mosseae CA130	6	19.69 ± 4.52	7.88 ± 2.53	25.87 ± 2.86
		Funneliformis mosseae NC302C	6	27.70 ± 8.52	9.30 ± 2.37	24.10 ± 2.02
		Rhizophagus clarus CA404	6	21.49 ± 2.72	9.13 ± 1.79	23.68 ± 1.86
		Rhizophagus clarus NC112A	6	18.85 ± 1.46	7.13 ± 1.16	26.38 ± 3.97
		Rhizophagus intraradices CA505	6	16.56 ± 3.71	6.70 ± 1.54	25.65 ± 4.34
		Rhizophagus intraradices NC203	6	25.48 ± 5.79	8.70 ± 1.37	25.23 ± 2.28
		Septoglomus deserticola CA116	6	20.04 ± 6.74	7.73 ± 2.73	25.72 ± 3.80
		Septoglomus deserticola NC305	6	19.87 ± 4.10	7.12 ± 1.52	25.07 ± 3.75
	LowH2O	Mock	6	15.97 ± 7.10	7.75 ± 1.52	29.67 ± 3.95
		Funneliformis mosseae CA130	6	17.02 ± 5.03	7.12 ± 1.27	27.62 ± 2.66
		Funneliformis mosseae NC302C	6	20.37 ± 3.23	7.95 ± 1.32	25.33 ± 3.02
		Rhizophagus clarus CA404	6	20.44 ± 4.77	8.22 ± 1.82	25.55 ± 4.66
		Rhizophagus clarus NC112A	6	18.63 ± 4.13	7.58 ± 1.55	27.40 ± 4.55
		Rhizophagus intraradices CA505	6	17.76 ± 5.90	7.53 ± 0.63	24.15 ± 5.35
		Rhizophagus intraradices NC203	6	18.47 ± 3.93	7.62 ± 2.08	22.83 ± 2.63
		Septoglomus deserticola CA116	6	16.11 ± 3.91	7.22 ± 0.64	23.62 ± 2.82
		Septoglomus deserticola NC305	6	17.04 ± 6.26	7.43 ± 2.36	27.10 ± 3.21
2	HighH2O	Mock	6	11.99 ± 3.46	7.00 ± 1.14	25.37 ± 2.98
		Funneliformis mosseae CA130	5	15.36 ± 2.54	7.00 ± 1.08	24.82 ± 4.91
		Funneliformis mosseae NC302C	6	13.14 ± 3.11	6.64 ± 0.83	25.87 ± 3.77
		Rhizophagus clarus CA404	6	11.72 ± 2.44	6.46 ± 0.46	24.43 ± 3.77
		Rhizophagus clarus NC112A	6	16.08 ± 7.18	7.04 ± 1.11	24.70 ± 4.82
		Rhizophagus intraradices CA505	6	11.08 ± 1.86	6.98 ± 1.13	24.75 ± 3.64
		Rhizophagus intraradices NC203	6	10.95 ± 3.81	6.43 ± 1.78	22.00 ± 3.49
		Septoglomus deserticola CA116	5	14.96 ± 3.47	8.56 ± 2.27	26.28 ± 1.20
		Septoglomus deserticola NC305	6	13.95 ± 3.65	7.47 ± 2.02	24.45 ± 3.96
	LowH2O	Mock	6	12.09 ± 0.91	7.02 ± 1.07	26.00 ± 2.65
		Funneliformis mosseae CA130	6	12.59 ± 4.20	8.17 ± 1.45	22.07 ± 2.45
		Funneliformis mosseae NC302C	5	13.29 ± 3.93	9.25 ± 3.46	24.58 ± 1.72
		Rhizophagus clarus CA404	6	11.65 ± 3.87	6.42 ± 1.22	25.32 ± 4.53
		Rhizophagus clarus NC112A	6	14.60 ± 2.86	7.87 ± 1.18	25.87 ± 2.69
		Rhizophagus intraradices CA505	6	12.36 ± 4.53	7.18 ± 1.40	26.10 ± 3.06
		Rhizophagus intraradices NC203	6	9.89 ± 1.99	6.02 ± 1.04	24.30 ± 2.59
		Septoglomus deserticola CA116	6	13.18 ± 3.54	7.48 ± 2.29	25.42 ± 2.65
		Septoglomus deserticola NC305	6	13.55 ± 3.10	6.14 ± 2.36	29.60 ± 4.18
3	HighH2O	Mock	6	26.53 ± 4.87	10.40 ± 3.73	27.22 ± 1.97
		Septoglomus deserticola CA116	6	22.42 ± 3.24	8.75 ± 1.57	26.03 ± 3.96
		Septoglomus deserticola NC305	6	24.12 ± 5.70	12.47 ± 4.66	27.92 ± 4.73
	LowH2O	Mock	5	12.71 ± 4.14	10.86 ± 4.01	25.04 ± 4.45
		Septoglomus deserticola CA116	6	13.95 ± 6.00	8.55 ± 1.78	22.98 ± 3.14
		Septoglomus deserticola NC305	6	15.19 ± 2.65	7.87 ± 2.74	25.83 ± 3.75
4	HighH2O	Mock	6	18.27 ± 6.80	7.87 ± 0.91	36.82 ± 3.55
		Rhizophagus intraradices CA505	6	23.39 ± 8.49	9.60 ± 1.94	32.72 ± 4.01
		Rhizophagus intraradices NC203	6	22.69 ± 10.22	9.53 ± 1.41	35.53 ± 7.45
	LowH2O	Mock	6	12.93 ± 4.50	8.10 ± 1.52	35.08 ± 2.49
		Rhizophagus intraradices CA505	6	17.10 ± 2.28	9.10 ± 1.79	32.25 ± 3.07
		Rhizophagus intraradices NC203	6	17.09 ± 4.45	7.62 ± 1.36	35.85 ± 3.59

Table VI. Mean weights of taproots, lengths of taproots, and shoot lengths of inoculated 'DH1' carrots grown in a greenhouse with inoculation.

Exp. No	Water Regime	AMF Isolate	N	Mean Root Weight	Mean Root Length	Mean Shoot Length
3	HighH2O	Mock	6	7.91 ± 1.99	8.78 ± 2.21	19.33 ± 3.40
		Septoglomus deserticola CA116	6	9.70 ± 3.24	8.88 ± 2.00	22.05 ± 2.08
		Septoglomus deserticola NC305	6	13.77 ± 5.76	13.77 ± 4.26	23.55 ± 2.51
	LowH2O	Mock	6	6.12 ± 1.61	9.15 ± 2.44	22.50 ± 1.40
		Septoglomus deserticola CA116	6	6.26 ± 1.47	8.33 ± 3.25	20.32 ± 2.22
		Septoglomus deserticola NC305	6	7.06 ± 3.44	9.55 ± 2.21	18.93 ± 5.11

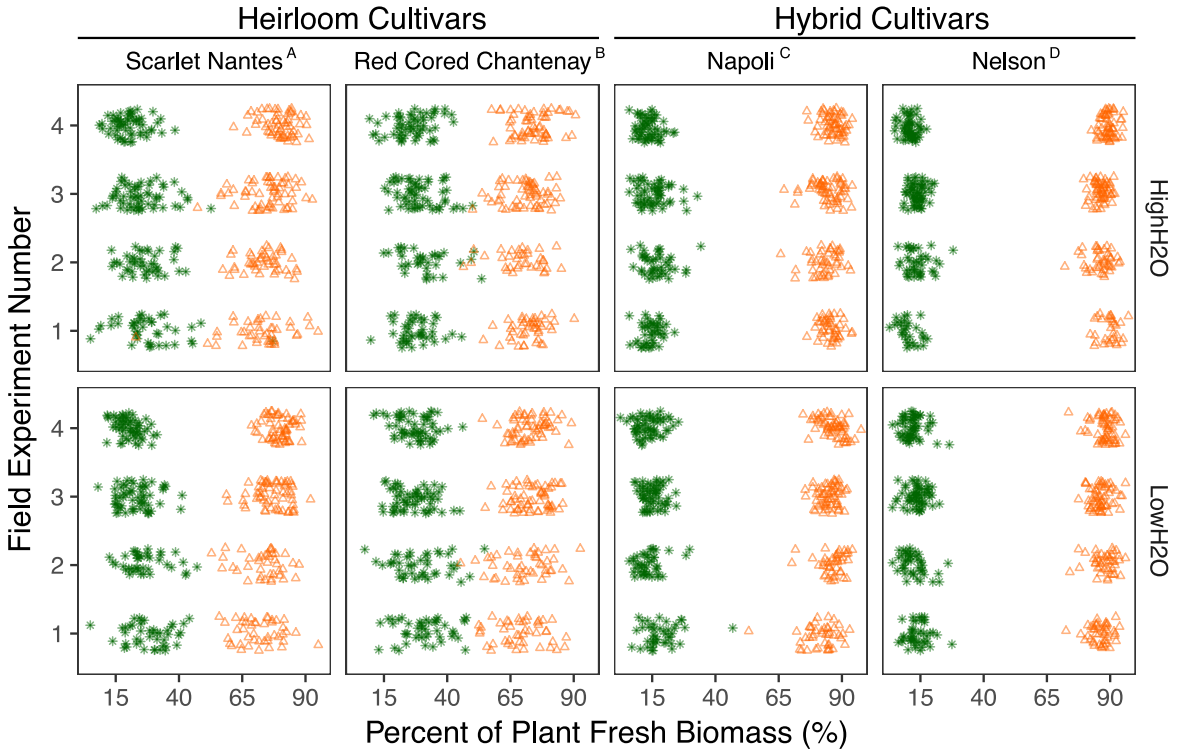


Figure II. Percent of fresh biomass allocation to shoots and taproots for mock-treated cultivars across field experiments and water regimes. Green stars and orange triangles represent the percent of total fresh biomass each plant allocated to shoots and taproots, respectively. Cultivars significantly differed in their biomass allocation to taproots (Two-way, type III ANOVA followed by Tukey's post hoc test, p-value < 0.001).

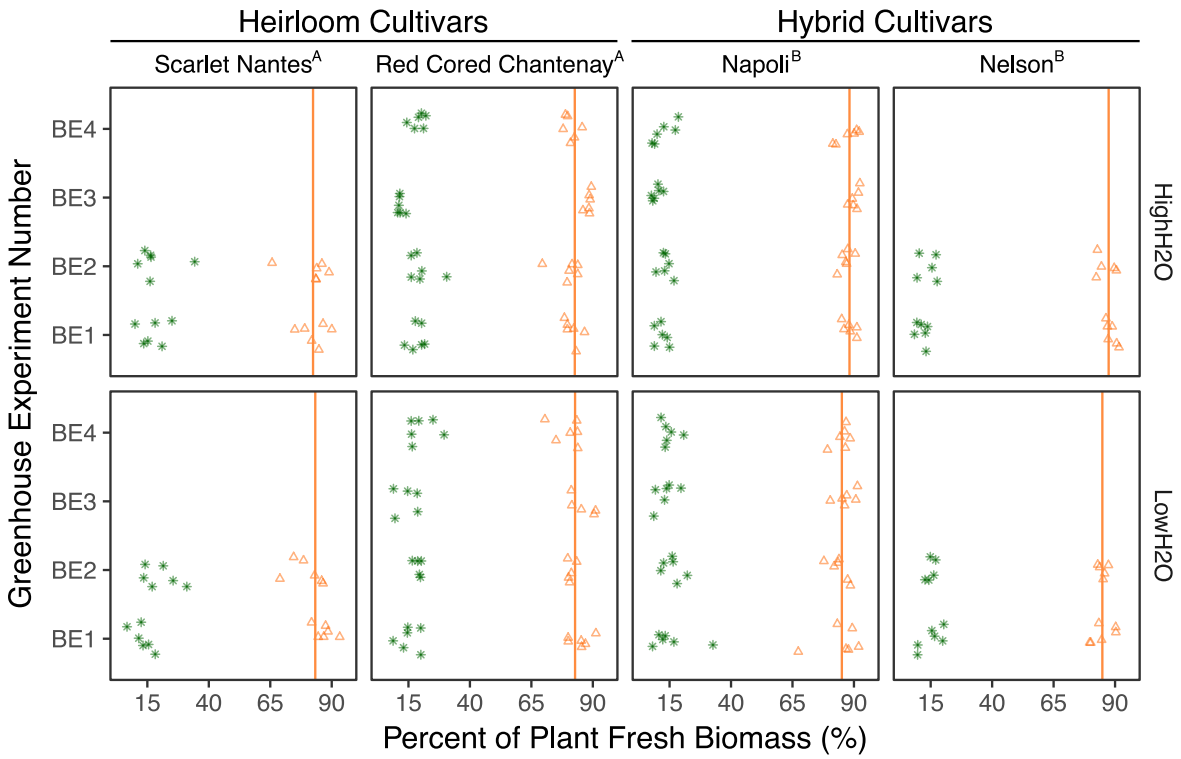


Figure III. Percent of fresh biomass allocation to shoots and taproots for mock-treated cultivars across greenhouse experiments and water regimes. Green stars and orange triangles represent the percent of total fresh biomass each plant allocated to shoots and taproots, respectively. Cultivars significantly differed in their biomass allocation to taproots (Two-way, type III ANOVA followed by Tukey's post hoc test, p-value < 0.001).

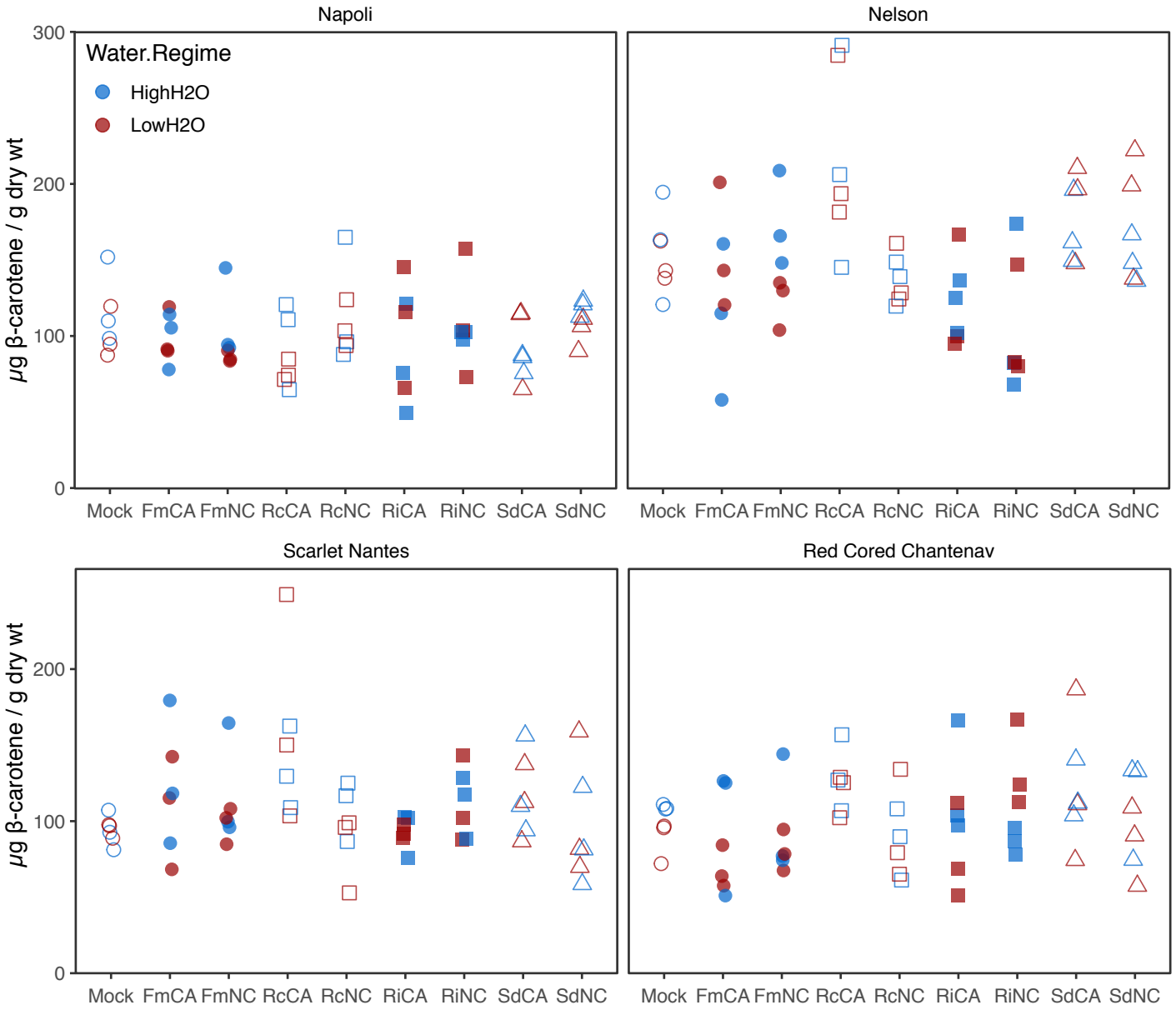


Figure IV. (Legend on next page).

Figure III. Beta carotene content of carrot taproots grown with or without inoculation by arbuscular mycorrhizal fungi in a greenhouse experiment. Mock inoculalted carrots are indicated with 'Mock'; *Funneliformis mosseae* CA127, 'FmCA'; *Funneliformis mosseae* NC302C, 'FmNC'; *Rhizophagus clarus* CA401, 'RcCA'; *Rhizophagus clarus* NC112A, 'RcNC'; *Rhizophagus intraradices* CA502, 'RiCA'; *Rhizophagus intraradices* NC200, 'RiNC'; *Septoglomus deserticola* CA113, 'SdCA'; *Septoglomus deserticola* NC302A, 'SdNC'.

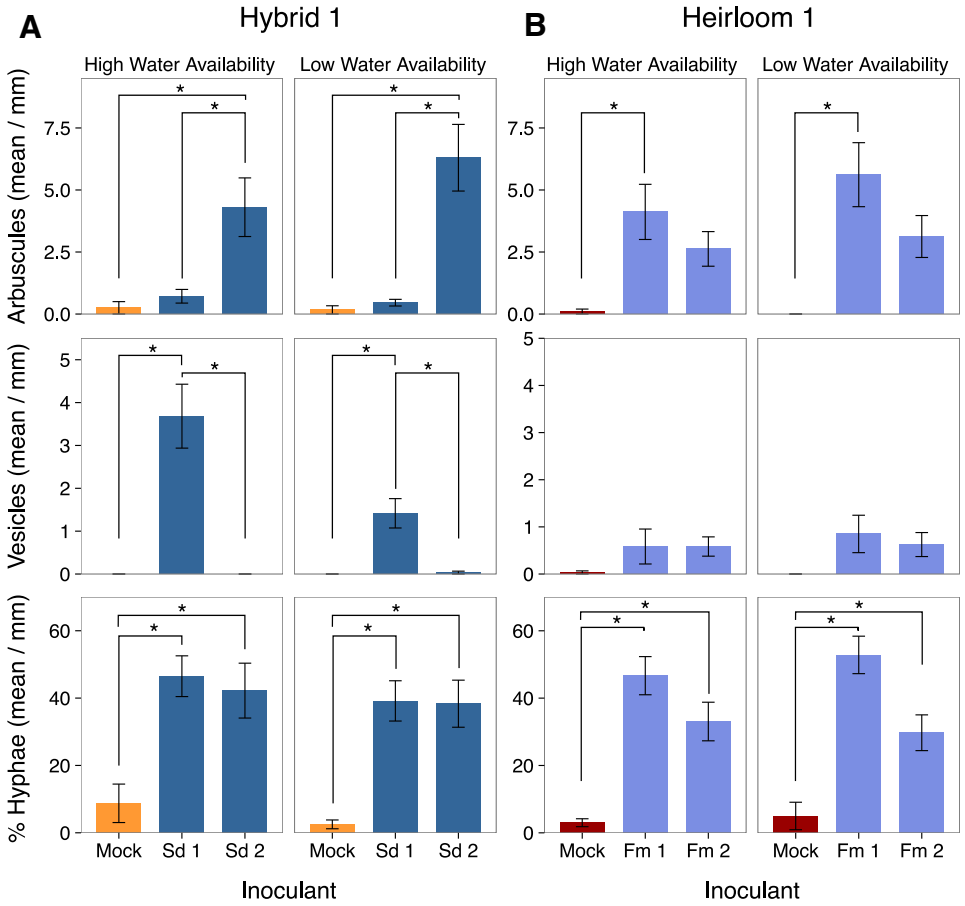


Figure V (Left). Mycorrhizal structures in Nelson and Napoli cultivars of carrot. Arbuscules, vesicles, and hyphae were quantified as a function of root length from a sampling of roots from 6 experimental plants with 6 root lengths randomly selected on a microscopic grid 'Nelson' (Hybrid 1) colonization with *Septoglomus deserticola* (Sd) isolates (panel A) and 'Scarlet Nantes' (Heirloom 1) grown with *Funneliformis mosseae* (Fm) (panel B) revealed differing fungal investments to symbiotic structures. Asterisks (*) indicate significant differences between fungal structures observed between isolates or between mycorrhizal and non-mycorrhizal (Mock) roots (* p-value ≤ 0.05, One-way ANOVA followed by Tukey HSD test, n = 36).

3.9 References

Abbott, L.K., Robson, A.D., 1982. The role of vesicular arbuscular mycorrhizal fungi in agriculture and the selection of fungi for inoculation. Aust. J. Agric. Res. 33, 389–408. https://doi.org/10.1071/AR9820389

Abbott, L.K., Robson, A.D., Hall, I.R., 1983. Introduction of vesicular arbuscular mycorrhizal fungi into agricultural soils. Aust. J. Agric. Res. 34, 741–749.

Afshar, R.K., Jovini, M.A, Chaichi, M.R., Hashemi, M., 2014. Grain sorghum response to arbuscular mycorrhiza and phosphorus fertilizer under deficit irrigation. Agron. J., 106, 1212–1218 (2014)doi:10.2134/agronj13.0589

Al-Karaki, G., McMichael, B., Zak, J., 2004. Field response of wheat to arbuscular mycorrhizal fungi and drought stress . Mycorrhiza, 14, 263-269.

Antunes, P.M., Koch, A.M., Dunfield, K.E., Hart, M.M., Downing, A., Rillig, M.C., Klironomos, J.N., 2009. Influence of commercial inoculation with *Glomus intraradices* on the structure and functioning of an AM fungal community from an agricultural site. Plant Soil 317, 257–266. https://doi.org/10.1007/s11104-008-9806-y

Augé, R.M., 2001. Water relations, drought and vesicular-arbuscular mycorrhizal symbiosis. Mycorrhiza 11, 3–42. https://doi.org/10.1007/s005720100097

Avio, L., Pellegrino, E., Bonari, E., Giovannetti, M., 2006. Functional diversity of arbuscular mycorrhizal fungal isolates in relation to extraradical mycelial networks. New Phytol. 172, 347–357. https://doi.org/10.1111/j.1469-8137.2006.01839.x

Brundrett, M., Bougher, N., Dell, B., Grove, T., Malajczuk, N., 1996. Working with mycorrhizas in forestry and agriculture. https://doi.org/10.1046/j.1469-8137.1997.00703-7.x

Burleigh, S.H., Cavagnaro, T., Jakobsen, I., 2002. Functional diversity of arbuscular mycorrhizas extends to the expression of plant genes involved in P nutrition. J. Exp. Bot. 53, 1593–1601. https://doi.org/10.1093/jxb/erf013

Burton, A.L., Brown, K.M., Lynch, J.P., 2013. Phenotypic Diversity of Root Anatomical and Architectural Traits in *Zea* Species. Crop Sci. 55, 1042–1055. https://doi.org/10.2135/cropsci2012.07.0440

Buysens, C., César, V., Ferrais, F., Dupré, H., Boulois, D., Declerck, S., 2016. Inoculation of *Medicago sativa* cover crop with *Rhizophagus irregularis* and *Trichoderma harzianum* increases the yield of subsequently-grown potato under low nutrient conditions. Appl. Soil Ecol. 105, 137–143. https://doi.org/10.1016/j.apsoil.2016.04.011

Ceballos, I., Ruiz, M., Fernándes, C., Penã, R., Rodríguez, A., Sanders, I., 2013. The *In Vitro* Mass-Produced Model Mycorrhizal Fungus, *Rhizophagus irregularis*, Significantly Increases Yields of the Globally Important Food Security Crop Cassava. PLoS One 8. https://doi.org/10.1371/journal.pone.0070633

Chu, Q., Wang, X., Yang, Y., Chen, F., Zhang, F., Feng, G., 2013. Mycorrhizal responsiveness of maize (*Zea mays* L.) genotypes as related to releasing date and available P content in soil 23, 497–505. https://doi.org/10.1007/s00572-013-0492-0

Clark, M.S., Horwath, W.R., Shennan, C., Scow, K.M., Lantni, W.T., Ferris, H., 1999. Nitrogen, weeds and water as yield-limiting factors in conventional, low-input, and organic tomato systems. Agric. Ecosyst. Environ. 73, 257–270. https://doi.org/10.1016/S0167-8809(99)00057-2

Dawson, J.C., Murphy, K.M., Jones, S.S., 2008. Decentralized selection and participatory approaches in plant breeding for low-input systems. Euphytica 160, 143–154. https://doi.org/10.1007/s10681-007-9533-0

de Novais, C.B., Borges, W.L., Jesus, E. da C., Júnior, O.J.S., Siqueira, J.O., 2014. Inter- and intraspecific functional variability of tropical arbuscular mycorrhizal fungi isolates colonizing corn plants. Appl. Soil Ecol. 76, 78–86. https://doi.org/https://doi.org/10.1016/j.apsoil.2013.12.010

Douds, D.D., Wilson, D.O., Seidel, R., Ziegler-Ulsh, C., 2016. A method to minimize the time needed for formation of mycorrhizas in sweet corn seedlings for outplanting using AM fungus inoculum produced on-farm. Sci. Hortic. (Amsterdam). 203, 62–68. https://doi.org/10.1016/j.scienta.2016.03.015

Ellouze, W., Taheri, A.E., Bainard, L.D., Yang, C., Bazghaleh, N., Navarro-borrell, A., Hanson, K., Hamel, C., 2014. Soil Fungal Resources in Annual Cropping Systems and Their Potential for Management. Biomed Res. Int. Vol. 2014. https://doi.org/10.1155/2014/531824

Gaudin, A.M., McClymont, S.A., Soliman, S.S.M., Raizada, M.N., 2014. The effect of altered dosage of a mutant allele of Teosinte branched 1 (tb1-ref) on the root system of modern maize. BMC Genet. 15, 1–14. https://doi.org/10.1186/1471-2156-15-23

Gosling, P., Hodge, a., Goodlass, G., Bending, G.D., 2006. Arbuscular mycorrhizal fungi and organic farming. Agric. Ecosyst. Environ. 113, 17–35. https://doi.org/10.1016/j.agee.2005.09.009

Greene, C., Dimitri, C., Lin, B.-H., McBride, W., Oberholtzer, L., Smith, T., 2009. U.S. Market Growth Outpaces Domestic Supply (EIB-55) 3–15.

Hart, M.M., Antunes, P.M., Chaudhary, V.B., Abbott, L.K., 2018. Fungal inoculants in the field: Is the reward greater than the risk? Funct. Ecol. 126–135. https://doi.org/10.1111/1365-2435.12976

Hedden, P., 2003. The genes of the Green Revolution. Trends Genet. 19, 5–9. https://doi.org/10.1016/S0168-9525(02)00009-4

Hetrick, B.A.D., Wilson, G.W.T., Cox, T.S., 1993. Mycorrhizal dependence of modern wheat cultivars and ancestors: a synthesis. Can. J. Bot. 71, 512–518. https://doi.org/10.1139/b93-056

Hetrick, B.A.D., Wilson, G.W.T., Cox, T.S., 1992. Mycorrhizal dependence of modern wheat varieties, landraces, and ancestors. Can. J. Bot. 70, 2032–2040. https://doi.org/10.1139/b92-253

Hetrick, B.A.D., Wilson, G.W.T., Gill, B.S., Cox, T.S., 1995. Chromosome location of mycorrhizal responsive genes in wheat. Can. J. Bot. 73, 891–897. https://doi.org/10.1139/b95-097

Hijri, M., 2016. Analysis of a large dataset of mycorrhiza inoculation field trials on potato shows highly significant increases in yield. Mycorrhiza 26, 209–214. https://doi.org/10.1007/s00572-015-0661-4

Hothorn, Bretz, F., Westfall, P., Heiberger, R.M., Schuetzenmeister, A., Scheibe, S., 2019. multcomp: Simultaneous Inference in General Parametric Models.

İncesu, M., Yeşİloğlu, T., ÇİmEn, B., Yilmaz, B., Akpinar, Ç., Ortaş, İ., 2015. Effects on growth of persimmon (*Diospyros virginiana*) rootstock of arbuscular mycorrhizal fungi species. Turkish J. Agric. For. 39, 117–122. https://doi.org/10.3906/tar-1405-134

Jerkins, D., Ory, J., 2016. National Organic Research Agenda: Outcomes and Recommendations from the 2015 National Organic Farmer Survey and Listening Sessions.

Khalil, S., Loynachan, T., Tabatabai, M.A., 1994. Mycorrhizal Dependency and Nutrient Uptake by Improved and Unimproved Corn and Soybean Cultivars. Agron. J. 86, 949–958. https://doi.org/10.2134/agronj1994.00021962008600060005x

Kikuchi, Y., Hijikata, N., Ohtomo, R., Handa, Y., Kawaguchi, M., Saito, K., Masuta, C., Ezawa, T., 2016. Aquaporin-mediated long-distance polyphosphate translocation directed towards the host in arbuscular mycorrhizal symbiosis: application of virus-induced gene silencing. New Phytol. 211, 1202–1208. https://doi.org/10.1111/nph.14016

Koyama, A., Pietrangelo, O., Sanderson, L., Antunes, P.M., 2017. An empirical investigation of the possibility of adaptability of arbuscular mycorrhizal fungi to new hosts. Mycorrhiza 27, 553–563. https://doi.org/10.1007/s00572-017-0776-x

Kuznetsova, A., Brockhoff, P. B., & Christensen, R.H.B., 2014. lmerTest: Tests in Linear Mixed Effects Models.

Lammerts van Bueren, E.T., Jones, S.S., Tamm, L., Murphy, K.M., Myers, J.R., Leifert, C., Messmer, M.M., 2011. The need to breed crop varieties suitable for organic farming, using wheat, tomato and broccoli as examples: A review. NJAS - Wageningen J. Life Sci. 58, 193–205. https://doi.org/https://doi.org/10.1016/j.njas.2010.04.001

Lammerts Van Bueren, E.T., Jones, S.S., Tamm, L., Murphy, K.M., Myers, J.R., Leifert, C., Messmer, M.M., 2011. The need to breed crop varieties suitable for organic farming, using wheat, tomato and broccoli as examples: A review. NJAS - Wageningen J. Life Sci. 58, 193–205. https://doi.org/10.1016/j.njas.2010.04.001

Lehman, R.M., Cambardella, C.A., Stott, D.E., Acosta-Martinez, V., Manter, D.K., Buyer, J.S., Maul, J.E., Smith, J.L., Collins, H.P., Halvorson, J.J., Kremer, R.J., Lundgren, J.G., Ducey, T.F., Jin, V.L., Karlen, D.L., 2015. Understanding and enhancing soil biological health: The solution for reversing soil degradation. Sustainability 7, 988-1027. https://doi.org/10.3390/su7010988

Lehmann, A., Barto, E.K., Powell, J.R., Rillig, M.C., 2012. Mycorrhizal responsiveness trends in annual crop plants and their wild relatives—a meta-analysis on studies from 1981 to 2010. Plant Soil 355, 231–250. https://doi.org/10.1007/s11104-011-1095-1

Li, T., Hu, Y.-J., Hao, Z.-P., Li, H., Wang, Y.-S., Chen, B.-D., 2013. First cloning and characterization of two functional aquaporin genes from an arbuscular mycorrhizal fungus *Glomus intraradices*. New Phytol. 197, 617–630. https://doi.org/10.1111/nph.12011

Loján, P., Senés-Guerrero, C., Suárez, J.P., Kromann, P., Schüßler, A., Declerck, S., 2017. Potato field-inoculation in Ecuador with *Rhizophagus irregularis*: no impact on growth performance and associated arbuscular mycorrhizal fungal communities. Symbiosis 73, 45–56. https://doi.org/10.1007/s13199-016-0471-2

Lyon, A., Silva, E., Zystro, J., Bell, M., 2015. Seed and Plant Breeding for Wisconsin's Organic Vegetable Sector: Understanding Farmers' Needs. Agroecol. Sustain. Food Syst. 39, 601–624. https://doi.org/10.1080/21683565.2015.1017786

Mäder, P., Kaiser, F., Adholeya, A., Singh, R., Uppal, H.S., Sharma, A.K., Srivastava, R., Sahai, V., Aragno, M., Wiemken, A., Johri, B.N., Fried, P.M., 2011. Inoculation of root microorganisms for sustainable wheat-rice and wheat-black gram rotations in India. Soil Biol. Biochem. 43, 609–619. https://doi.org/10.1016/j.soilbio.2010.11.031

Martín-Robles, N., Lehmann, A., Seco, E., Aroca, R., Rillig, M.C., Milla, R., 2018. Impacts of domestication on the arbuscular mycorrhizal symbiosis of 27 crop species. New Phytol. 218, 322–334. https://doi.org/10.1111/nph.14962

Martín-Robles, N., Morente-López, J., Freschet, G.T., Poorter, H., Roumet, C., Milla, R., 2019. Root traits of herbaceous crops: Pre-adaptation to cultivation or evolution under domestication? Funct. Ecol. 33, 273–285. https://doi.org/10.1111/1365-2435.13231

Marulanda, A., Azcón, R., Ruiz-Lozano, J.M., 2003. Contribution of six arbuscular mycorrhizal fungal isolates to water uptake by *Lactuca sativa* plants under drought stress. Physiol. Plant. 119, 526–533. https://doi.org/10.1046/j.1399-3054.2003.00196.x

Mendiburu, F. de, 2019. Agricolae: Statistical Procedures for Agricultural Research.

Mensah, J.A., Koch, A.M., Antunes, P.M., Kiers, E.T., Hart, M., Bücking, H., 2015. High functional diversity within species of arbuscular mycorrhizal fungi is associated with differences in phosphate and nitrogen uptake and fungal phosphate metabolism. Mycorrhiza 25, 533–546. https://doi.org/10.1007/s00572-015-0631-x

Milla, R., Osborne, C.P., Turcotte, M.M., Violle, C., 2015. Plant domestication through an ecological lens. Trends Ecol. Evol. 30, 463–469. https://doi.org/10.1016/j.tree.2015.06.006

Mummey, D.L., Antunes, P.M., Rillig, M.C., 2009. Arbuscular mycorrhizal fungi pre-inoculant identity determines community composition in roots. Soil Biol. Biochem. 41, 1173–1179. https://doi.org/10.1016/j.soilbio.2009.02.027

Murphy, K.M., Campbell, K.G., Lyon, S.R., Jones, S.S., 2007. Evidence of varietal adaptation to organic farming systems. F. Crop. Res. 102, 172–177. https://doi.org/https://doi.org/10.1016/j.fcr.2007.03.011

Mustafa, G., Randoux, B., Tisserant, B., Fontaine, J., Magnin-Robert, M., Lounès-Hadj Sahraoui, A., Reignault, P., 2016. Phosphorus supply, arbuscular mycorrhizal fungal species, and plant genotype impact on the protective efficacy of mycorrhizal inoculation against wheat powdery mildew. Mycorrhiza 26, 685–697. https://doi.org/10.1007/s00572-016-0698-z

Neumann, E., Schmid, B., Römheld, V., George, E., 2009, Extraradical development and contribution to plant performance of an arbuscular mycorrhizal symbiosis exposed to complete or partial rootzone drying. Mycorrhiza 20, 13.

Ortas, I., Ortakçi, D., Kaya, Z., 2006. Various mycorrhizal fungi propagated on different hosts have different effect on citrus growth and nutrient uptake. Commun. Soil Sci. Plant Anal. 33, 259–272. https://doi.org/10.1081/CSS-120002392

Ortas, I., Sari, N., Akpinar, Ç., Yetisir, H., 2011. Screening mycorrhiza species for plant growth, P and Zn uptake in pepper seedling grown under greenhouse conditions. Sci. Hortic. (Amsterdam). 128, 92–98. https://doi.org/10.1016/j.scienta.2010.12.014

Park, S., Cao, C., McSpadden Gardener, B.B., 2010. Inoculants and soil amendments for organic growers [WWW Document]. Ohioline Fact Sheet SAG-17-10. URL http://ohioline.osu.edu/sag-fact/pdf/0017.pdf (accessed 3.1.19).

Pellegrino, E., Öpik, M., Bonari, E., Ercoli, L., 2015. Responses of wheat to arbuscular mycorrhizal fungi: A meta-analysis of field studies from 1975 to 2013. Soil Biol. Biochem. 84, 210–217. https://doi.org/10.1016/j.soilbio.2015.02.020

Pellegrino, E., Turrini, A., Gamper, H.A., Cafà, G., Bonari, E., Young, J.P.W., Giovannetti, M., 2012. Establishment, persistence and effectiveness of arbuscular mycorrhizal fungal inoculants in the field revealed using molecular genetic tracing and measurement of yield components. New Phytol. 810–822. https://doi.org/10.1111/j.1469-8137.2012.04090.x

Poudel, D.D., Horwath, W.R., Lanini, W.T., Temple, S.R., Van Bruggen, A.H.C., 2002. Comparison of soil N availability and leaching potential, crop yields and weeds in organic, low-input and conventional farming systems in northern California. Agric. Ecosyst. Environ. 90, 125–137. https://doi.org/10.1016/S0167-8809(01)00196-7

Pringle, A., Bever, J.D., 2008. Analogous effects of arbuscular mycorrhizal fungi in the laboratory and a North Carolina field. New Phytol. 180, 162–175.

R Core Team, 2019. R: A language and environment for statistical computing.

Raju, P.S., Clark, R.B., Ellis, J.R., Maranville, J.W., 1990. Mineral uptake and growth of sorghum colonized with VA mycorrhiza at varied soil phosphorus levels. J. Plant Nutr. 13, 843–859. https://doi.org/10.1080/01904169009364121

Rillig, M.C., 2004. Arbuscular mycorrhizae, glomalin, and soil aggregation. Can. J. Soil Sci. 84, 355–363. https://doi.org/10.4141/S04-003

Rillig, M.C., Aguilar-Trigueros, C.A., Camenzind, T., Cavagnaro, T.R., Degrune, F., Hohmann, P., Lammel, D.R., Mansour, I., Roy, J., van der Heijden, M.G.A., Yang, G., 2019. Why farmers should manage the arbuscular mycorrhizal symbiosis. New Phytol. 222, 1171–1175. https://doi.org/10.1111/nph.15602

Rodriguez, A., Sanders, I.R., 2015. The role of community and population ecology in applying mycorrhizal fungi for improved food security. ISME J. 9, 1053–1061. https://doi.org/10.1038/ismej.2014.207

Rúa, M.A., Antoninka, A., Antunes, P.M., Chaudhary, V.B., Gehring, C., Lamit, L.J., Piculell, B.J., Bever, J.D., Zabinski, C., Meadow, J.F., Lajeunesse, M.J., Milligan, B.G., Karst, J., Hoeksema, J.D., 2016. Home-field advantage? Evidence of local adaptation among plants, soil, and arbuscular mycorrhizal fungi through meta-analysis. BMC Evol. Biol. 16, 1–15. https://doi.org/10.1186/s12862-016-0698-9

Ruiz-Lozano, J.M., Azcon, R., Gomez, M., 1995. Effects of arbuscular-mycorrhizal *Glomus* species on drought tolerance: physiology and nutritional plant responses. Appl Env. Microbiol 61, 456–460.

Ruiz-Sánchez, M., Aroca, R., Muñoz, Y., Polón, R., Ruiz-Lozano, J.M., 2010. The arbuscular mycorrhizal symbiosis enhances the photosynthetic efficiency and the antioxidative response of rice plants subjected to drought stress. J. Plant Physiol. 167, 862–869. https://doi.org/10.1016/j.jplph.2010.01.018

Ryan, M.H., Graham, J.H., 2018. Little evidence that farmers should consider abundance or diversity of arbuscular mycorrhizal fungi when managing crops. New Phytol. 220, 1092–1107. https://doi.org/10.1111/nph.15308

Sarkar, D., 2018. lattice: Trellis Graphics for R Author.

Sawers, R.J.H., Gutjahr, C., Paszkowski, U., 2008. Cereal mycorrhiza: an ancient symbiosis in modern agriculture. Trends Plant Sci. 13, 93–97. https://doi.org/10.1016/j.tplants.2007.11.006

Schmidt, J.E., Bowles, T.M., Gaudin, A.C.M., 2016. Using Ancient Traits to Convert Soil Health into Crop Yield: Impact of Selection on Maize Root and Rhizosphere Function. Front. Plant Sci. https://doi.org/10.3389/fpls.2016.00373

Sendek, A., Karakoç, C., Wagg. C., Domínguez-Begines, J., do Couto, G.M., van der Heijden M.G.A.,Naz, A.A., Lochner, A., Chatzinotas, A., Klotz, S., Gómez-Aparicio, L., Eisenhauer, N., 2019. Drought modulates interactions between arbuscular mycorrhizal fungal diversity and barley genotype diversity. Sci Rep, 4; 9650. doi: 10.1038/s41598-019-45702-1.

Singh, A.K., Hamel, C., Depauw, R.M., Knox, R.E., 2012. Genetic variability in arbuscular mycorrhizal fungi compatibility supports the selection of durum wheat genotypes for enhancing soil ecological services and cropping systems in Canada. Can. J. Microbiol. 58, 293–302. https://doi.org/10.1139/W11-140

Singh, S.P., Terán, H., Lema, M., Hayes, R., 2011. Selection for Dry Bean Yield On-Station Versus On-Farm Conventional and Organic Production Systems. Crop Sci. 51, 631–630. https://doi.org/10.2135/cropsci2010.02.0063

Smith, S.E., Read, D., 2008a. Mycorrhizal Symbiosis, Mycorrhizal Symbiosis. Elsevier. https://doi.org/10.1016/B978-012370526-6.50006-4

Smith, S.E., Read, D., 2008b. Mycorrhizal Symbiosis, Mycorrhizal Symbiosis. https://doi.org/10.1016/B978-012370526-6.50019-2

Symanczik, S., Lehmann, M.F., Wiemken, A., Boller, T., Courty, P., 2018. Effects of two contrasted arbuscular mycorrhizal fungal isolates on nutrient uptake by *Sorghum bicolor* under drought. Mycorrhiza 28, 779–785. https://doi.org/10.1007/s00572-018-0853-9

Tawaraya, K., Hirose, R., Wagatsuma, T., 2012. Inoculation of arbuscular mycorrhizal fungi can substantially reduce phosphate fertilizer application to *Allium fistulosum* L. and achieve marketable yield under field condition. Biol. Fertil. Soils 48, 839–843. https://doi.org/10.1007/s00374-012-0669-2

Tsvetkov, I., Atanassov, A., Vlahova, M., Carlier, L., Christov, N., Lefort, F., Rusanov, K., Badjakov, I., Dincheva, I., Tchamitchian, M., Rakleova, G., Georgieva, L., Tamm, L., Iantcheva, A., Herforth-Rahmé, J., Paplomatas, E., Atanassov, I., 2018. Plant organic farming research–current status and opportunities for future development. Biotechnol. Biotechnol. Equip. 32, 241–260. https://doi.org/10.1080/13102818.2018.1427509

Turrini, A., Sbrana, C., Avio, L., Njeru, E.M., Bocci, G., Bàrberi, P., Giovannetti, M., 2016. Changes in the composition of native root arbuscular mycorrhizal fungal communities during a short-term cover crop-maize succession. Biol. Fertil. Soils 52, 643–653. https://doi.org/10.1007/s00374-016-1106-8

USDA-NOP, 2019. U.S. Code: Title 7. Part 205—National Organic Program. Subpart G—National List of Allowed and Prohibited Substances §205.600 Evaluation criteria for allowed and prohibited substances, methods, and ingredients.

Van Geel, M., De Beenhouwer, M., Lievens, B., Honnay, O., 2016. Crop-specific and single-species mycorrhizal inoculation is the best approach to improve crop growth in controlled environments. Agron. Sustain. Dev. 36. https://doi.org/10.1007/s13593-016-0373-y

Venturi, V., Keel, C., 2016. Signaling in the Rhizosphere. Trends Plant Sci. 21, 187–198. https://doi.org/10.1016/j.tplants.2016.01.005

Wickham, H., Chang, W., Henry, L., Pedersen, T.L., Takahashi, K., Wilke, C., Woo, K., Yutani, H., 2019. ggplot2: Create Elegant Data Visualisations Using the Grammar of Graphics Description.

Wickham, H., François, R., Henry, L., Müller, K., 2018. dplyr: A Grammar of Data Manipulation.

Wilson, G.W.T., Rice, C.W., Rillig, M.C., Springer, A., Hartnett, D.C., 2009. Soil aggregation and carbon sequestration are tightly correlated with the abundance of arbuscular mycorrhizal fungi: results from long-term field experiments. Ecol. Lett. 12, 452–461. https://doi.org/10.1111/j.1461-0248.2009.01303.x

Zhu, Y., Miller, R.M., 2003. Carbon cycling by arbuscular mycorrhizal fungi in soil – plant systems. Ecol. Lett. 8, 407–409. https://doi.org/10.1016/S1360-1385(03)00192-4

Chapter 4. Arbuscular mycorrhizal fungi and carrot demonstrate contrasting responses to drought stress as revealed through a dual transcriptomic approach

Michelle Keller-Pearson[a], Luke Willems[b], Brendan Smith[b], Anthony Bortolazzo[b], Annika Peterson[a], Erin M. Silva[a], Jean-Michel Ané[b,c,*]

[a]Department of Plant Pathology, University of Wisconsin-Madison

[b]Department of Bacteriology, University of Wisconsin-Madison

[c]Department of Agronomy, University of Wisconsin-Madison

Contributions: Michelle Keller-Pearson designed the research with input from Erin Silva and Jean-Michel Ané. Michelle Keller-Pearson, Luke Willems, Brendan Smith, Anthony Bortolazzo, and Annika Peterson conducted the experiments. Michelle Keller-Pearson analyzed the data and wrote the article with input from all co-authors.

4.1 Summary

- Arbuscular mycorrhizal fungi are known for offering host plants improved drought tolerance, and although some mechanisms for this were explored in plants, we know very little about the fungal response to drought. In this study, we evaluated the drought responses of host and symbiont, using carrot as a plant model.
- To test the impacts of drought on host and fungus, we inoculated carrots (*Daucus carota*) with spores of *Rhizophagus irregularis* DAOM 197198. Carrots grew in a greenhouse, and at the beginning of taproot development, some experienced a 10-day water restriction. We evaluated plant growth and physiological responses, colonization level, and plant and fungal gene expression.
- Drought caused diminished photosynthetic activity, reduced plant growth, lower root colonization, and differential gene expression in both plants and fungi. In the fungus, 768 genes were upregulated, and 58 were downregulated. Overall, the expression of genes related to symbiosis and nutrient exchange were downregulated in carrot and upregulated in the fungus.
- We observed a contrast in the regulation of gene expression between carrot and its fungal partner during drought, with carrot reducing its apparent investment in the symbiosis and the fungus increasing its symbiotic efforts.

4.1 Introduction

Arbuscular mycorrhizal (AM) fungi are known to improve plant performance during drought stress, likely through a combination of direct water transfer, priming of stress response systems, and soil conditioning (Wright & Upadhyaya, 1998; Augé, 2001; Li *et al.*, 2013). Symbiotic interactions between plants and AM fungi rely on a coordinated system of molecular communications and nutrient exchanges (Javot *et al.*, 2007; Fellbaum *et al.*, 2014; Kamel *et al.*, 2017). Signaling between the plant and fungus initiates a series of cellular remodeling events leading to fungal colonization of host plants (Harrison, 2005; MacLean *et al.*, 2017), which is characterized by the growth of intraradical hyphae, arbuscules, and vesicles. The highly branched arbuscules grow into cortical cells of host roots and facilitate exchanges between the symbionts.

Gene expression studies provided key information on symbiotic functioning of AM fungi during conditions of abiotic stress (Porcel *et al.*, 2006; Ocón *et al.*, 2007; Aroca *et al.*, 2009; Jia *et al.*, 2017). Studies such as these enabled the discovery and characterization of the first AM fungal aquaporin in *Rhizophagus irregularis*, *AQP1* (Aroca *et al.*, 2009), which differentially expressed during cold and drought stress in host roots but did not function when heterologously expressed. Later, two more aquaporins (AQPF1 and AQPF2) were found, and these were shown to be functional during heterologous expression in yeast (Li *et al.*, 2013). Other studies of osmotic stress on AM fungi relied on *in vitro* co-culture of AM fungi and root organ cultures of carrot (*Daucus carota;* Bécard and Fortin, 1988), with stress imposed by the addition of osmoticum such as polyethylene glycol (Porcel *et al.*, 2007; Aroca *et al.*, 2009).

Despite the vast quantity of research on AM associations, AM fungi are poorly understood compared to their hosts. As the most ancient and widespread root-endosymbiosis

(Humphreys *et al.*, 2010; Delaux *et al.*, 2013), AM fungal associations are well studied with respect to the impacts on plant growth, physiology, and molecular dynamics (Smith & Read, 2008). Because AM fungi lack cell wall degrading enzymes, they rely on their host to permit their presence through host-mediated cellular remodeling (Bucher *et al.*, 2014). Lacking in the ability to colonize like a pathogen, AM fungi wield a suite of tools that stimulate host cooperation. For example, AM fungi produce secreted peptides (SPs) that can act as effector molecules, stimulating host plants to promote colonization by dampening defense responses (Kloppholz *et al.*, 2011).

The recent sequencing of the *R.irregularis* genome provided a bounty of insights into this cryptic fungal symbiont (Tisserant *et al.*, 2013; Lin *et al.*, 2014; Chen *et al.*, 2018). It is well-established that AM fungi depend on plant-produced carbon. Recent work documented the transfer of monosaccharides and lipids from plant to fungus and characterized some of the key molecular entities facilitating such exchanges. On the fungal side, there is one known functional monosaccharide transporter, *Ri*MST2 (Helber *et al.*, 2011). On the plant side, a suite of genes facilitate the movement of lipids to fungal symbionts (Bravo *et al.*, 2016; Keymer *et al.*, 2017; Luginbuehl *et al.*, 2017). The availability of the *R. irregularis* genome also made possible the discovery of meiosis and mating-related genes, casting doubt on the presumed asexual nature of AM fungi (Corradi & Brachmann, 2017).

The use of carrot in the research of AM fungi and symbiosis dates to the 1980s with root organ cultures of carrot providing an *in vitro* model. Beyond its use in the laboratory, there is little mention of carrot and mycorrhizae in the literature. Carrot is commonly thought to be ‘mycotrophic’ or somewhat reliant on root symbionts (Schreiner & Koide, 1993). This could partially be due to the low phosphorus use efficiency of carrot (Dechassa *et al.*, 2003); so it may

benefit immensely from the efficiency of phosphate scavenging and transfer offered by AM fungi (Kikuchi *et al.*, 2016; Walder *et al.*, 2016). Carrot permits a high level of AM colonization, making it an excellent model for transcriptomic studies of AM fungi, since ample fungal RNA can be extracted.

The impacts of drought on carrot production are not well quantified, but increasingly severe droughts result in losses of nearly 100 million USD each year for vegetable crops alone (Howitt *et al.*, 2014). Carrot is an economically valuable crop with sales reaching $700 million USD annually in the US alone (USDA-NASS, 2018). The nutritional value of carrot is well known with its high proportions of the carotenoids beta-carotene and lutein (Baranski, 2008). Carotenoids have photoprotective properties in plants and protect them from a variety of stresses (Young, 1991) and they play a role in symbiotic interactions (Fester *et al.*, 2002, 2005; Baslam *et al.*, 2013). The recent availability of the carrot genome enables more sophisticated study of mycorrhizal interactions using carrot as a model plant (Iorizzo *et al.*, 2016).

AM fungi are known to offer plants protection from drought. There is immense interest in understanding the dynamics of drought in mycorrhizal plants, and they are reviewed extensively by others (Augé, 2001; Santander *et al.*, 2017; Begum *et al.*, 2019). Only few studies report on drought dynamics in AM fungi (Porcel *et al.*, 2006; Aroca *et al.*, 2009; Li *et al.*, 2013). Recently, transcriptomic studies provided insights into the gene expression of plants during stress events (Zhang *et al.*, 2014; Xue *et al.*, 2016; Greenham *et al.*, 2017; Jia *et al.*, 2017). In a study of drought in common bean, Recchia *et al.* (2018) identified transcriptional stress responses for AM and non-mycorrhizal (NM) plants. Fungal responses were not assessed, probably due to their use of a mixed-species, whole inoculant (Recchia *et al.* 2018). The present study is the first report of transcriptional responses in both host and AM fungal symbiont following a progressive, 10-day

long drought. We hypothesized that drought stress would increase the reliance of carrot on its AM fungal symbiont and that *R. irregularis* would exhibit both stress-related and symbiosis-related differential gene expression.

4.3 Methods

Experimental Setup and Design

Seeds of *Daucus carota* cv. 'Napoli' (Johnny's Selected Seeds, USA) were rinsed in DI water and placed inside a Petri dish containing a water-saturated cotton ball. After four days, 5 germinated seedlings were transferred to prepared Deepots with (6.35 cm diameter and 35.56 cm length; Greenhouse Megastore, Danville, IL, USA), containing washed and autoclaved calcined clay substrate (Garcia *et al.*, 2017). Each pot was then watered with 100 ml of Enshi solution, which is formulated to provide carrot with optimal nutrition (Kobayashi *et al.*, 2013).

Pots were designated as either AM or NM. AM pots were inoculated with 400 spores each of *Rhizaphagus irregularis* DAOM 197198 (Mycorise® ASP, lot #10487174, Premier Tech, Quebec, Canada). Pots were arranged in a randomized complete block with 20 plants per treatment. Each pot was then covered with a clear plastic cup to create moist chambers and were watered every third day to maintain moisture until the first true leaves emerged. Once all pots contained carrots with true leaves, carrots were thinned leaving one plant per pot. Water delivery via drip irrigation occurred each morning delivering 28 ml (± 1.8 ml) per plant over a 2-minute interval, maintaining a substrate water holding capacity (WHC) of 33 – 36%. The concentration and frequency of Enshi nutrient solution application increased over time to promote optimal carrot growth (**Table S1**), based on our preliminary studies.

Plants grew in a dedicated greenhouse room with climate control at the Biotron Laboratory (University of Wisconsin-Madison, Madison, WI). Supplemental lighting provided a consistent 13-hour photoperiod (25˚C) and 9-hour nighttime period (18˚C). Following 7 weeks of establishment, plant sizes were assessed by measuring shoot heights and counting leaves. Plants within the AM and NM groups were ranked by size and equitably assigned to either the well-

watered (W) or drought (D) treatment group. Abnormal carrots were omitted from the study at this point leaving 10 plants in the AM+D treatment, 9 in AM+W, and 8 each in NM+D. and NM+W. The remaining plants were re-randomized and drip irrigation equipment was removed. At day 50, we initiated water restriction for plants in the drought treatment. For the 10-day water treatment period, plants received W or D treatments targeting WHC of 33 – 36% or at 22 – 24%, respectively (**Table S2**).

Plants were periodically evaluated for photosynthetic assimilation, stomatal conductance, and transpiration using a Licor LI-6400 portable photosynthesis system (LI-COR Biosciences, Lincoln, NE, USA). Photosynthetic measurements were collected during midday, prior to watering. Leaf areas were quantified using Fiji (Schindelin *et al.*, 2012), which enabled calculation of photosynthetic measurements (**Figure 1a**). Plant heights were recorded prior to harvest on day 11. To prevent plant response to the harvest procedure, carrots were minimally handled, and flash frozen within 90 seconds of removal from their pots without rinsing. Plant extraction from pots occurred in a single motion, enabling them to slide out intact. Carrots remained whole and were gently shaken to remove substrate and placed on a prepared piece of foil and weighed. Each sample was flash frozen in liquid nitrogen and stored at -80˚C. The substrate was weighed and placed in a drying oven, which allowed for a final evaluation of water content.

Sample Selection Criteria

To ensure that the individual plants used for RNA sequencing were comparable within their respective treatment categories, we refined treatment groups based on colonization of root samples and post-harvest water content of the substrate. Plants that received inoculation but

showed no colonization were omitted from analyses (2/9 in W and 3/10 in D). The mean water contents of substrates at harvest were 23.0% and 27.0% for the D and W treatments, respectively

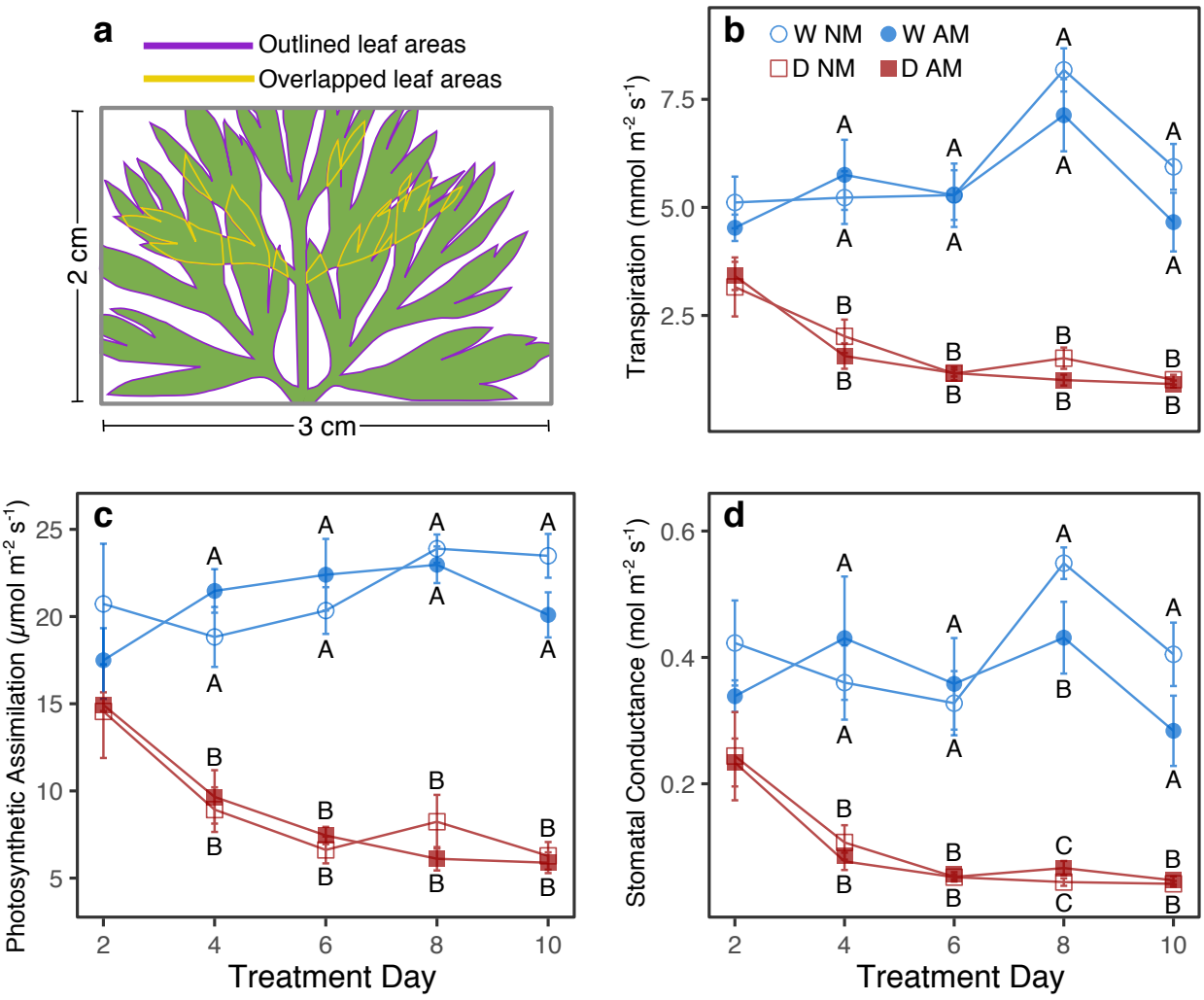


Figure 1. Physiological measurements of carrots during five timepoints within the drought treatment period. Photosynthetic values were adjusted to reflect individual leaf areas with accounting for total leaf area, including overlapping leaflets and excluding negative space (a). Mycorrhizal (AM) and non-mycorrhizal (NM) plants demonstrated consistently lower (b) transpiration, (c) photosynthetic assimilation, (d) stomatal conductance over time when grown in drought (D) conditions as compared to well-watered controls (W). Differing uppercase letters designate differences between treatments at each timepoint (ANOVA followed by Tukey's HSD post-hoc test; p-value < 0.01).

(**Figure S1**). Carrots whose substrates' water content exceeding 1.5% above or below the mean in each group were removed from further analyses. Colonization of fine roots, cleared with 10%

KOH and stained with 5% Schaeffer ink in 5% acetic acid, was quantified microscopically using the gridline intersect method (Brundrett et al., 1996).

RNA Extraction and Sequencing

Fine roots were separated from the bulk sample and ground under liquid nitrogen. RNA was extracted with the PureLink RNA Mini Kit, and DNA contaminants removed with treatment by a Turbo DNA-free kit (Life Technologies, Carlsbad, CA, USA). Gel electrophoresis confirmed that only RNA remained in each sample. RNA integrity and quantity were tested by Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). Libraries of the 16 samples (4 biological replicates within each condition) were prepared for and sequenced on Illumina NovaSeq 6000, generating more than 20M paired-end reads per biological replicate (Novogene LTD Co., Sacramento, CA, USA).

Transcriptomic Analysis

Read quality was evaluated using FastQC *v*0.11.5 (Andrews, 2016). Read quality was good and adapter sequences were not overrepresented, so further trimming was unnecessary. Transcriptome data for *Daucus carota v*2 and for *Rhizophagus irregularis* 197198 *v*2 were downloaded from the Joint Genome Intitute's Phytosome 12 and Mycocosm databases, respectively (Iorizzo *et al.*, 2016; Chen *et al.*, 2018). Reads were pseudo-aligned to an index containing these merged transcriptomes with *kallisto v*0.4.3 (Bray *et al.*, 2016) and subsequently analyzed in R 3.5.2 (R Core Team, 2019), using the s*leuth* package (Pimentel *et al.*, 2017). We evaluated pairwise comparisons of each treatment category (*i.e.* W-NM vs W-AM; D-NM vs D-AM; W-NM vs D-NM; and W-AM vs D-AM) to identify which conditions led to expression differences. Sequences with a false discovery rate (FDR; q-value) < 0.05, a p-value < 0.001, and

an effect size (beta-value) > |1| were considered differentially expressed. Gene ontology analysis of carrot and fungal genes were carried out using AgriGOv2 (Tian *et al.*, 2017).

Carotenoid, Nutrient, and Abscisic Acid Analysis

Taproots and shoots of carrot were cut into pieces and lyophilized, then ground into a fine powder. Leaf tissues of each sequenced plant were analyzed for nutrient content by the Soils & Forage Analysis Laboratory (University of Wisconsin). Due to small sample size, total nitrogen reflects a composite sample comprising equal proportions of material within each treatment group. Taproot tissues were extracted overnight in petroleum ether (50 mg ml^{-1}), then prepared and analyzed with high performance liquid chromatography (Bowman *et al.*, 2014). Abscisic acid (ABA) was extracted from 100 mg of leaf tissues based on established methods (Walker-Simmons, 1987) and quantified with the Phytodetek® ABA Test Kit (Agdia Inc., Elkhart, IN, USA).

Statistics

Two-way ANOVA with Tukey post hoc tests for nutrients and carotenoids were tested in R (3.5.2) using the *agricolae* package *v*1.3-1 (Mendiburu, 2019). Quality control and pseudo-alignment were performed with tools on Cyverse.org (Goff *et al.*, 2011).

4.4 Results

Physiological, growth, and mycorrhizal responses of carrot

Carrots in the drought treatment exhibited reduced photosynthetic assimilation, stomatal conductance, and transpiration, compared to well-watered controls (**Figure 1b-d**). Analysis of leaf ABA content revealed elevated levels in the drought groups (**Figure 2**). Droughted carrots grew shorter shoots and weighed less than well-watered controls (**Figure 3a & 3b**). Colonization percentages ranged 9.9 – 30.9% and 26.6 – 65.1% for drought and control groups, respectively (**Figure 3c**). Mycorrhizal status did not substantially alter physiology, growth, or leaf ABA content during the 10-day drought period. Non-mycorrhizal carrots showed no signs of colonization.

Overall transcriptional regulation contrasted in carrot and fungus

RNA sequencing data showed that the primary driver of differential expression was water status, with mycorrhizal status being the secondary driver of difference for carrot and for the fungus (**Figure 4a & 4b**). Volcano plots show differential expression of transcripts for both carrot (orange dots; **Figure 4c**) and fungus (violet dots; **Figure d**). Overall, most of the differentially expressed gene transcripts (DEGs) of carrot exhibited downregulation (**Figure 4c**) compared to well-watered controls. In contrast, fungal DEGs tended toward upregulation (**Figure 4d**).

To identify differences in carrot gene expression between treatment groups, we conducted pair-wise comparisons within each treatment level (W vs. D in each mycorrhizal category, and AM vs NM in each W and D). Of the four comparison models, most DEGs occurred in the well-watered versus drought (W vs D) comparisons, with 1,124 DEGs shared

between the AM and NM plants (**Figure 4e**). For W vs D, there were 953 and 536 uniquely regulated DEGs for AM and NM plants, respectively (**Figure 4e, A & D**).

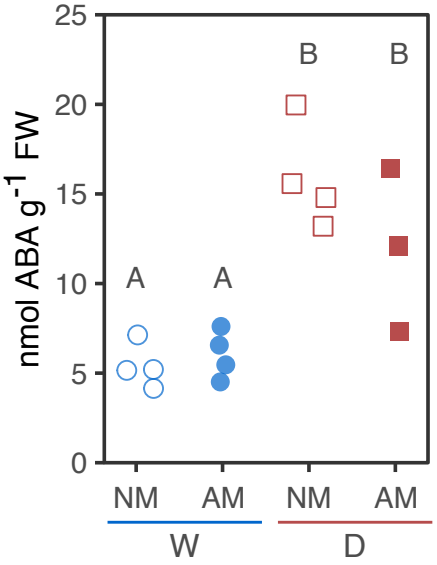


Figure 2. Abscisic acid content in leaves of carrot. Concentrations of abscisic acid (ABA) were elevated under drought (D) conditions for both non-mycorrhizal (NM) and mycorrhizal (AM) carrots compared to well-watered (W) controls. Uppercase letters indicated differences between groups (type III ANOVA, followed by Tukey's HSD post-hoc test, p-value < 0.05).

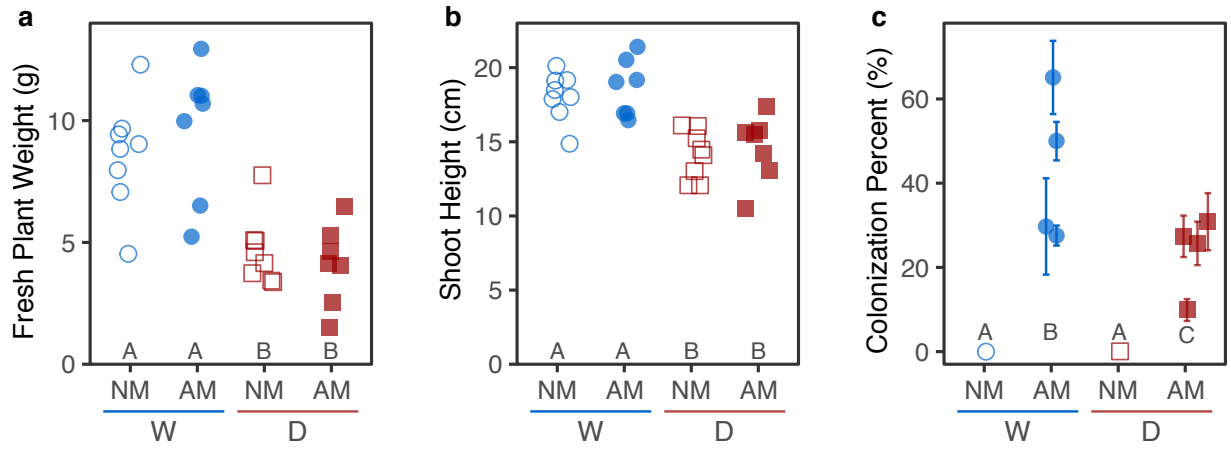


Figure 3. Heights, weights, and colonization of carrots differed by water regime. Whole plant fresh weights and shoot heights differed between drought (D) and well-watered (W) conditions. Non-mycorrhizal (NM) roots contained no fungal structures. Gridline colonization percent was reduced for arbuscular mycorrhizal (AM) roots in the drought group. Differences are designated with uppercase letters inside plot areas (ANOVA followed by Tukey's HSD post-hoc test; p-value < 0.01).

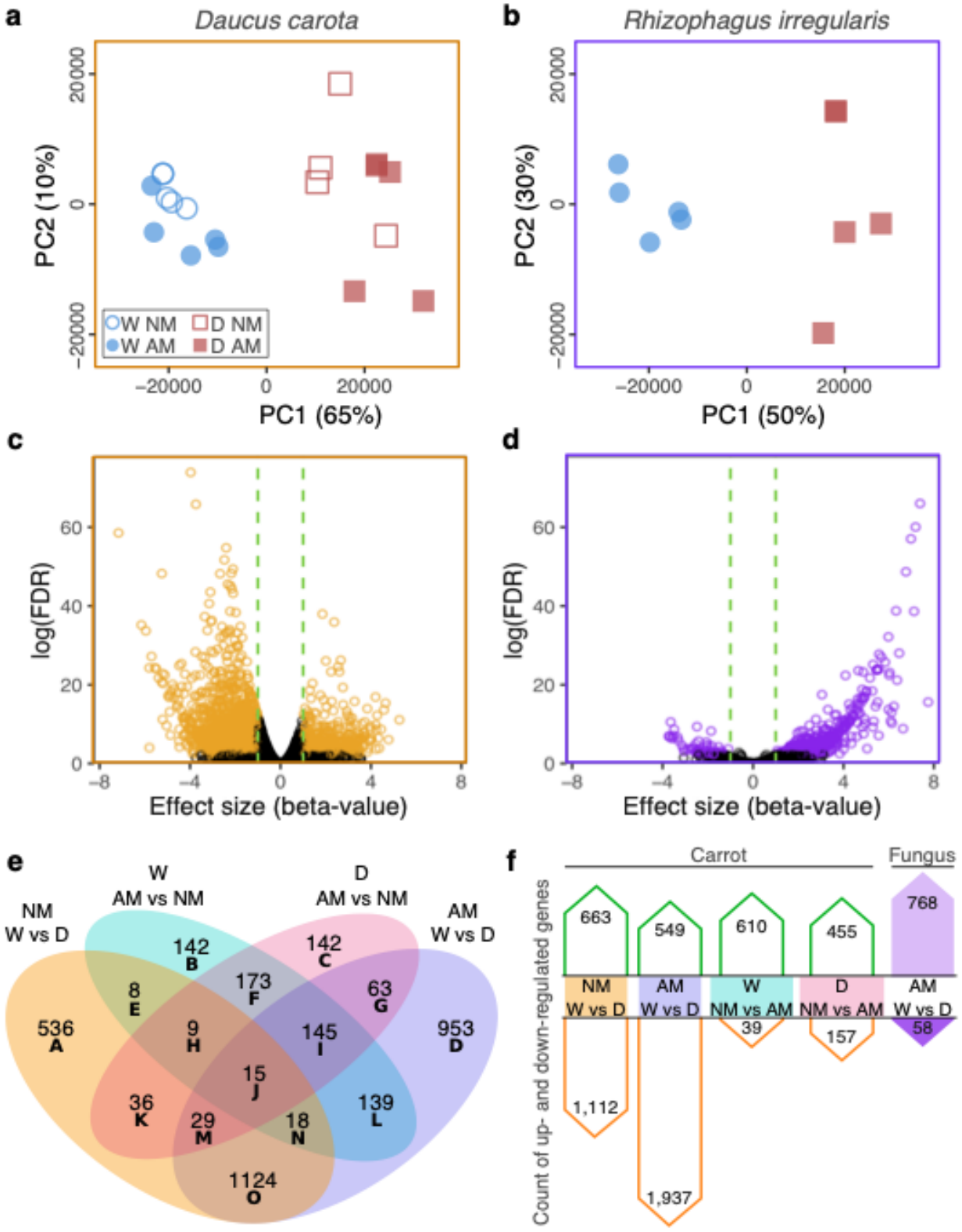


Figure 4. (see next page for legend)

Figure 4 (Previous page). **The effects of water stress outweigh the impact of inoculation treatment on gene expression of plant and fungus**. Principle Component Analyses (PCA) reveals that drought explained most of the variance for gene expression of carrot (a), as evaluated by comparison of all treatment groups. Similarly, drought drove the majority of variation for fungal gene expression (b). Based on the of the normalized transcripts per million, each principle component includes the percent variation explained. Differentially expressed genes (DEGs) of carrot (c) and fungus (d) were defined as those with a false discovery rate (FDR, q-value) below 0.05, p-value below 0.001, and effect size (beta value) greater than |1|. Orange dots represent DEGs of carrot, violet dots represent fungal DEGs, and black dots are non-DEGs; the green dashed line indicates the effect size cutoff. A four-way Venn diagram (e) shows the pair-wise comparisons of differential gene expression of carrot for each treatment level: non-mycorrhizal (NM); arbuscular mycorrhizal (AM); well-watered (W); and drought (D). Each area of the Venn diagram indicates the number of differentially expressed genes unique to the given comparison or of overlapping comparison. Uppercase letters identify each area of the Venn diagram, and corresponding transcripts are listed in Supplementary Table 5. The count of upregulated and downregulated genes differed among comparison (f) for the carrot and fungus, with mostly downregulation in carrot and upregulation in the fungus.

Carrots and *R. irregularis* contrasted in their regulation of gene expression. For *R. irregularis,* 93% of the 826 DEGs were upregulated during drought, whereas 78% of the 2,486 DEGs in AM carrot were downregulated during drought (**Figure 4f**). Similarly, 63% of the 1,112 DEGs in NM carrot were downregulated during drought. Compared to NM carrots, AM colonization led to more upregulation of genes in both control and drought conditions, 94% of 649 DEGs and 74% of 455 DEGs, respectively. There were no *R. irregularis* DEGs in the NM carrot groups.

Expression of symbiosis-stimulating genes of arbuscular mycorrhizal fungi

To assess whether the expression of genes involved in nutrient transport changes in *R. irregularis*, we evaluated the expression of transcripts annotated as potassium, phosphate, ammonium, and nitrate transporters. Additionally, we evaluated the expression of known aquaporins (*GiAQP1*, *RiAQPF1*, and *RiAQPF2*) and a sugar importer (*RiMST2*), but only one aquaporin was differentially expressed *RiAQPF2* (**Figure 5**). Phosphate transporters and several predicted transporters of potassium showed upregulated expression during drought (**Figure 5**).

One phosphate transporter gene, *RiPT2,* was likely upregulated during drought, but fell slightly beyond our strict cutoff (q-value < 0.05, p-value = 0.0013, and b-value > |1|).

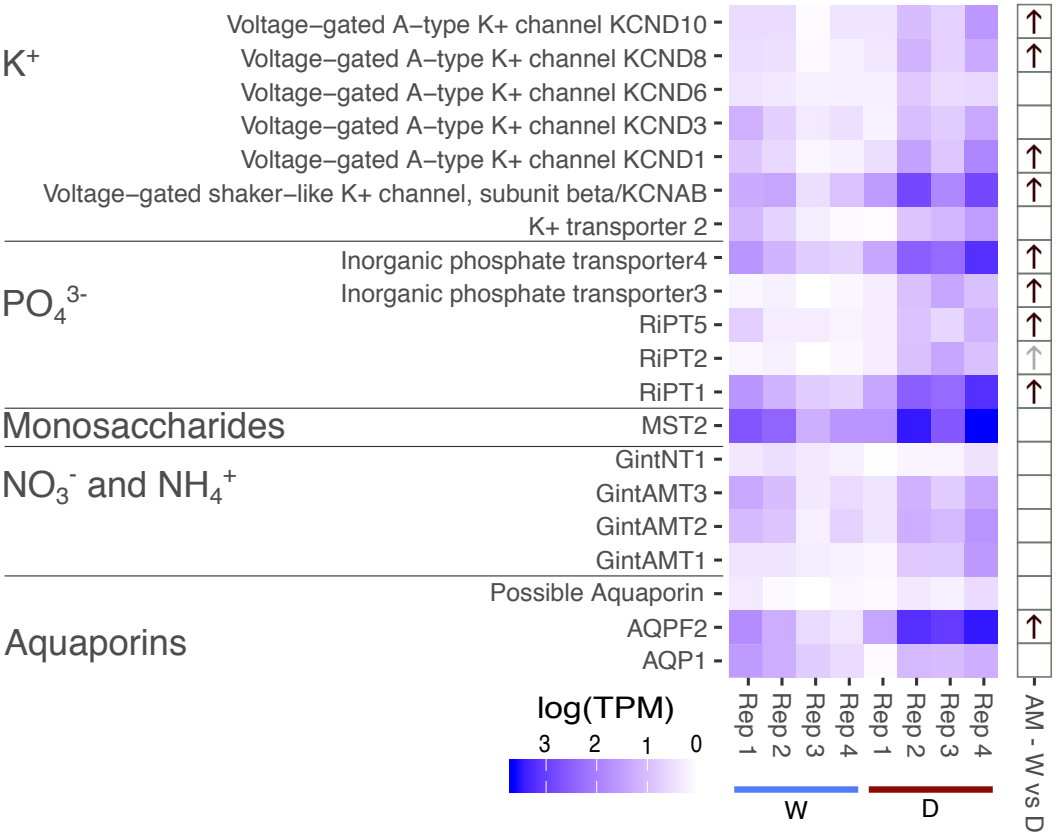


Figure 5. Heatmap of expression of genes associated with symbiosis in *Rhizophagus irregularis* DAOM 197198. Color represents the natural logarithm of transcripts per million (TPM) with an offset of 1 for each gene of interest and each biological replicate (Rep). Gene expression increased for several genes involved in symbiotic transport during drought (D) compared to well-watered (W) controls. All transcripts of genes with a false discovery rate (q-value) < 0.05 are shown. Black arrows denote upregulated genes. Gray arrows highlight genes that are likely upregulated but fall beyond the cutoff (q-value < 0.05, p-value < 0.001, and beta-value > |1|).

Since few genes of AM fungi are characterized, we looked at genes discussed in recent works, including those encoding for SPs, trehalose production, mating type, and meiosis genes. Transcripts associated with SP7 were highly upregulated as well as two other putative SPs (**Table S3**). Of the 4 genes likely involved the production of the osmoprotectant trehalose (Ocón *et al.*, 2007; Iturriaga *et al.*, 2009), 3 were upregulated during drought (**Table S3**). Lastly, there were 3 upregulated genes with putative roles linked to meiosis (Halary *et al.*, 2011), and 6

upregulated genes that may have involvement in reproduction or pheromone signaling (**Table S3**) (Halary *et al.*, 2013).

Negative regulation of symbiosis-associated genes in droughted carrot

Several genes known to play roles in the establishment and regulation of mycorrhizal symbiosis were differentially regulated. Among these, 145 shared regulation differences driven by water condition and mycorrhizal status (**Figure 4e**). As expected, all symbiosis-specific genes were upregulated exclusively in the AM condition (**Figure 6**). Drought caused downregulation of several of these genes including *FatM*, *RAM2*, and *STR*, all three of which are implicated in lipid transfer from the host to the fungus (Bravo *et al.*, 2017). Other genes associated with detection of fungal symbiotic signal, such as *DMI2* and *IPD3* (MacLean *et al.*, 2017), were downregulated during drought regardless of AM status.

To determine whether gene expression in other plant species exhibited similar downregulation of symbiotic genes, we evaluated root gene expression data from comparable drought studies, which excluded AM fungal inoculation. These included a 10-day drought experiment on *Medicago truncatula* and an 8-day drought experiment on *Populus simonii* (Zhang et al., 2014 and Jia et al., 2017, respectively). In *M. truncatula*, *DMI2*, *IPD3*, *LYK3*, *NSP2*, and *NFP* were downregulated during drought (**Table S4**). In *P. simonii*, homologs of *DMI2* and *IPD3* were downregulated (**Table S4**).

Expression of genes related to water, photoprotection, and nutrition in carrot

Aquaporin-related transcripts in carrot roots generally exhibited downregulation during drought (**Figure 7**). We identified three AM-specific aquaporin transcripts (DCAR_005175, DCAR_027966, and DCAR_008738), and only one downregulated with drought

(DCAR_008738; **Figure 6**). A single aquaporin transcript upregulated during drought (DCAR_023234), regardless of mycorrhizal status.

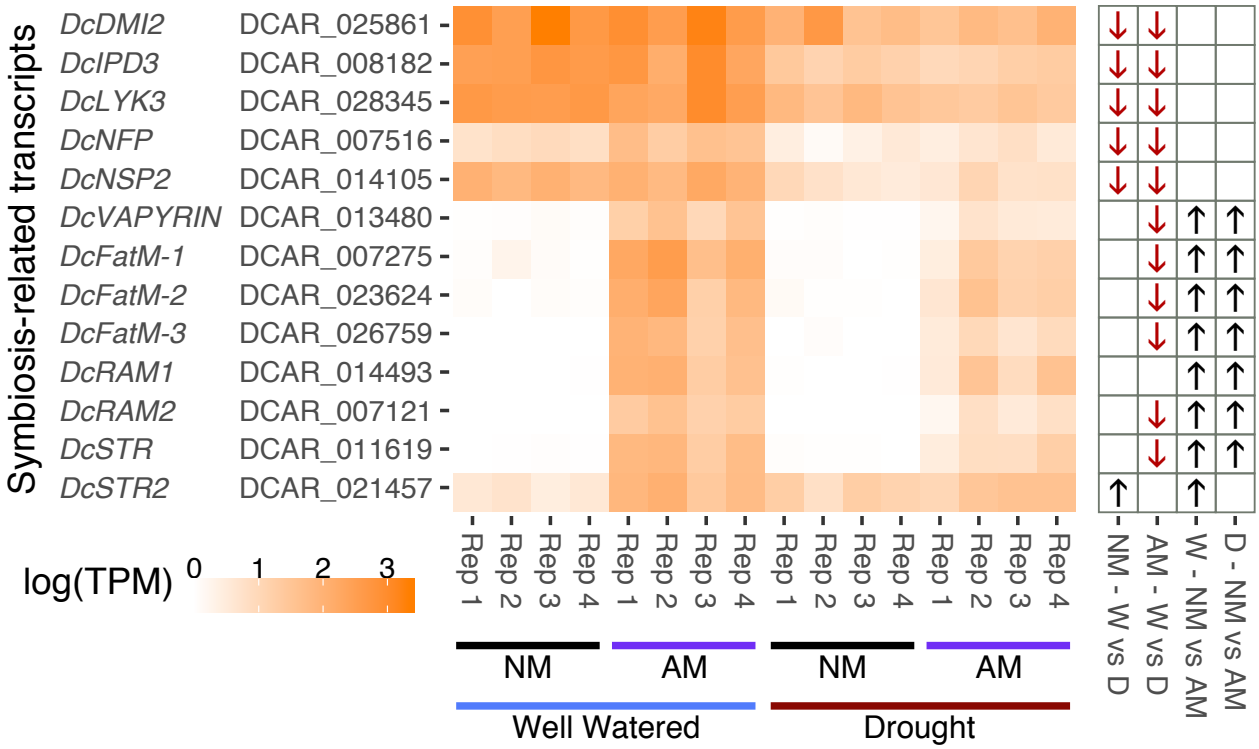


Figure 6. Symbiosis-associated gene expression levels in carrot roots during drought with and without mycorrhizal colonization. Color represents the natural logarithm of transcripts per million (TPM) with an offset of 1 for each gene of interest and each biological replicate (Rep). Carrot had several genes that expressed differentially between drought (D) and well-watered (W) controls as well as between mycorrhizal (AM) and non-mycorrhizal (NM). Up and down arrows indicate whether a transcript was upregulated or downregulated within each comparison model, respectively. Transcripts shown to be differentially expressed transcripts are those with false discovery rate < 0.05, p-value < 0.001, and beta-value > |1|.

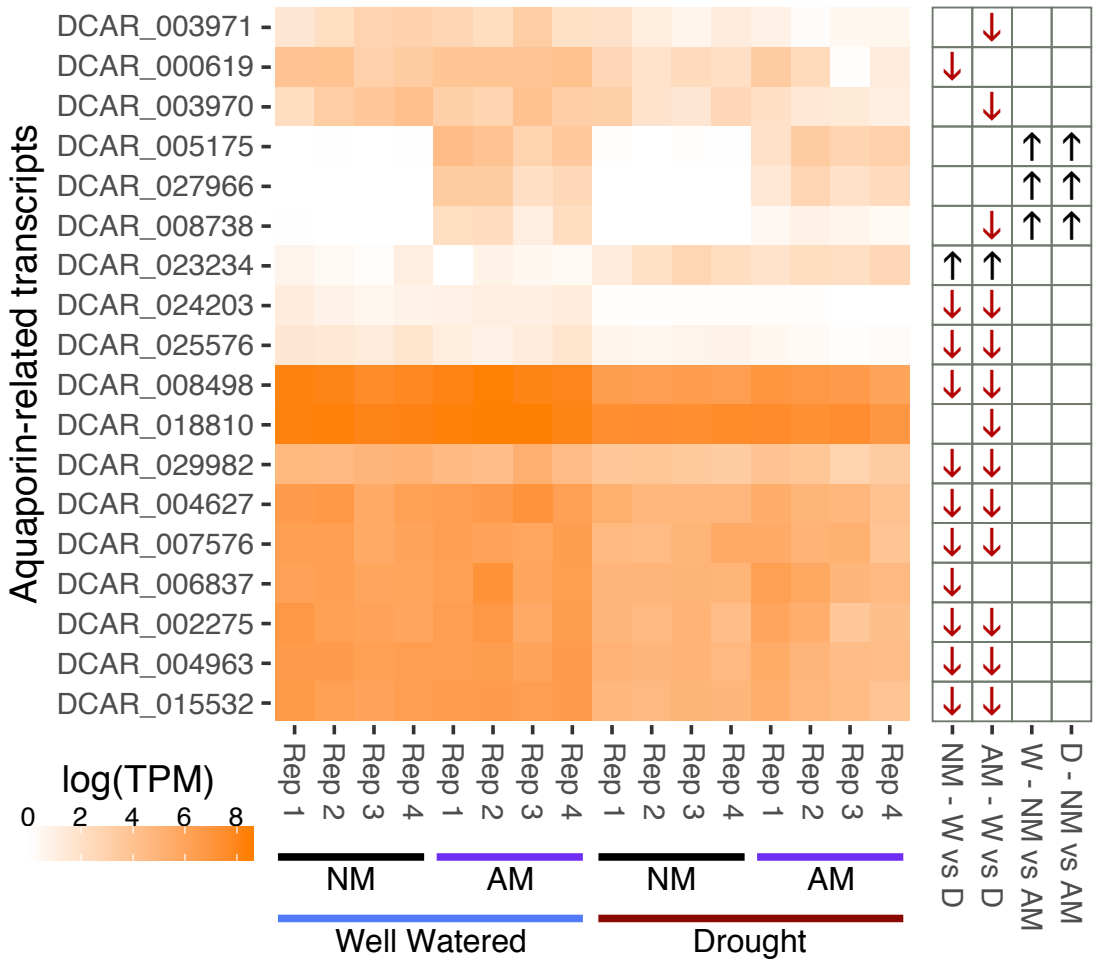


Figure 7. Aquaporin-associated gene expression levels in carrot roots during drought with and without mycorrhizal colonization. Color represents the natural logarithm of transcripts per million (TPM) with an offset of 1 for each gene of interest within each replicate (Rep). Carrot had several genes that expressed differentially between drought (D) and well-watered (W) controls as well as between mycorrhizal (AM) and non-mycorrhizal (NM). Up and down arrows indicate whether a transcript was upregulated or downregulated within each comparison model, respectively (false discovery rate < 0.05, p-value > 0.001, and beta value > |1|).

Several genes involved with carotenoid synthesis had differential regulation, and carotenoid concentrations in taproots differed by water regime. Overall, genes associated with enzymes that catalyze syntheses of carotenoids downstream of phytoene were downregulated during drought (**Figure 8a & b**). In contrast, drought upregulated genes associated with the synthesis and catabolism of ABA (**Figure 8a & b**). Two phytoene synthase genes (PSYs) were upregulated with drought (DCAR_023043 and DCAR_010057) and a phytoene desaturase (PDS,

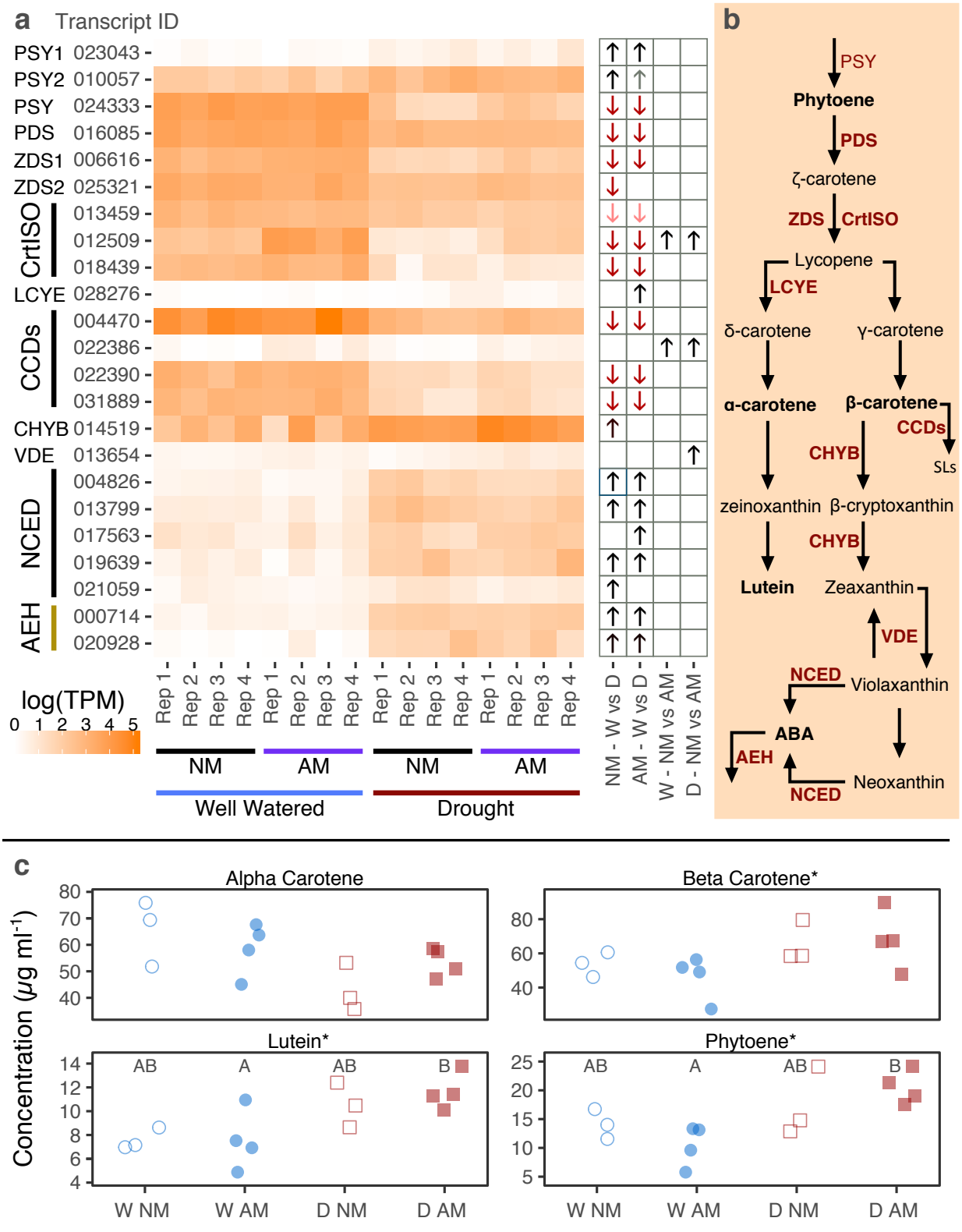


Figure 8. Carotenoid-associated gene expression levels in carrot roots during drought with and without mycorrhizal colonization and carotenoid concentrations in taproots of carrots. In the heatmap (a), color represents the natural logarithm of transcripts per million (TPM) with

an offset of 1 for each gene of interest within each replicate (Rep). Carrot had several genes that expressed differentially between drought (D) and well-watered (W) controls as well as between mycorrhizal (AM) and non-mycorrhizal (NM). Up and down arrows indicate whether a transcript was upregulated or downregulated within each comparison model, respectively (false discovery rate < 0.05, p-value > 0.001, and beta value > |1|). Carotenoid biosynthesis pathway (b) adapted from Rodriguez-Concepcion *et al.* (2013). Concentrations of carotenoids that differed by water treatment are indicated with an asterisk (*, c). Uppercase letters denote differences between treatment groups (two-way ANOVA followed by Tukey's post-hoc test, p-value < 0.05). Carotenoid pathway-related acronyms: phytoene synthase, PSY; phytoene desaturase, PDS; zeta-carotene desaturase, ZDS; carotenoid isomerase, CrtISO; lycopene epsilon-cyclase, LCYE; carotenoid cleavage dioxygenase, CCD; carotenoid epsilon-hydroxylase, CHYB; violaxanthin deepoxidase, VDE; 9-*cis*-epoxycarotenoid dioxygenase, NCED; abscisic acid 8'-hydroxylase 4-like, AEH; abscisic acid, ABA.

DCAR_016085) was downregulated (**Figure 8a**). Given this, we expected to find elevated phytoene in carrot taproots (**Figure 8b**). Beta-carotene, lutein, and phytoene were each present in elevated levels in droughted compared to well-watered carrots (p-value < 0.05; **Figure 8c**). Upregulation of genes associated with NSEDs and AEHs were upregulated (**Figure 8a**), and given their roles in production and breakdown of ABA, this aligns with the observed increase of leaf ABA (**Figure 2**). Genes encoding carotenoid cleavage dioxygenases (CCDs) were mostly downregulated during drought, except one (DCAR_022386), which was upregulated in AM roots.

Since several CCDs have known symbiotic functions in *M. truncatula* and other species, we used NCBI's BLAST to identify homologous proteins and found that DCAR_022386 has the greatest amino acid sequence similarity to *Mt*CCD1 (Floss *et al.*, 2008), which has a role in the production of mycorradicin in mycorrhizal roots. Another transcript with homology to CCD1 was not specifically regulated in the AM condition (DCAR_022390). We further identified DCAR_031889 and DCAR_004470 to encode homologs CCD7 and CCD8, respectively.

Transcripts associated with nutrient transport in roots tended to downregulate during drought, with a couple notable exceptions. We found AM-specific ammonium and nitrate

transporters, all of which had sensitivity to drought (**Figure 9a**). We identified 3 AM-specific phosphate transporters, 2 of which maintained constant expression levels during drought (DCAR_008909 and DCAR_002320). Drought reduced total leaf nitrogen content, but since this measurement was of pooled sample material, we were unable to compare statistically between groups (**Figure 9b**). Reduction in leaf phosphate occurred during drought (two-way ANOVA, $F_{1,13}$= 11.01, p-value = 0.0055), and there was a non-significant increase in leaf phosphate for AM carrots during drought compared to NM carrots (**Figure 9c**). There were no obvious changes to the expression of potassium transporters or to leaf accumulation of potassium (**Figure 9d**).

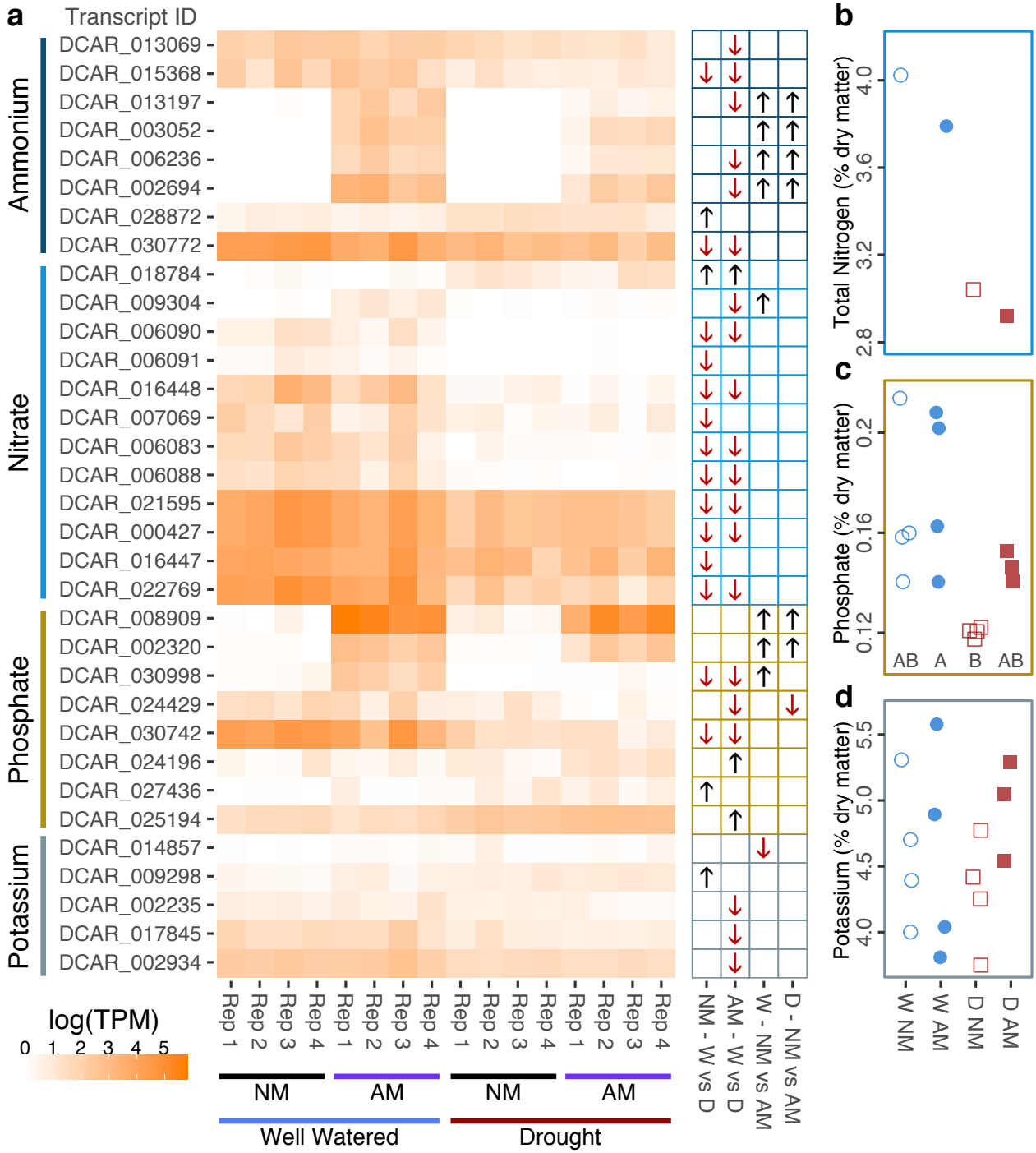


Figure 9. Nutrient transport-associated gene expression levels in carrot roots during drought with and without mycorrhizal colonization and macronutrient content in carrot leaves. Color represents the natural logarithm of transcripts per million (TPM) with an offset of 1 for each gene of interest within each replicate (Rep). Several genes that expressed differentially between drought (D) and well-watered (W) controls as well as between mycorrhizal (AM) and on-mycorrhizal (NM). Up and down arrows indicate whether a transcript was upregulated or downregulated within each comparison model, respectively (false discovery rate < 0.05, p-value > 0.001, and beta value > |1|). Drought negatively impacted the levels of total nitrogen (b) and phosphate (c) in leaves of carrot but did not substantially impact levels of potassium levels (d).

Uppercase letters denote differences between treatment groups (two-way ANOVA followed by Tukey's post hoc test, p-value < 0.01)

4.5 Discussion

Droughted plants displayed negative photosynthetic responses to drought treatment. Similar to Recchia *et al.*'s report in common bean, mycorrhizal status did not substantially impact photosynthetic assimilation, transpiration, or stomatal conductance (2018). Others reported improved photosynthetic efficiency and stomatal conductance arising from mycorrhizal status in lettuce and tomato under long-term drought conditions (Ruiz-Lozano *et al.*, 2016). Additionally, there are several reports of increased photosynthetic response to salinity stress in mycorrhizal monocots (reviewed in Santander *et al.*, 2017).

Because our drought period was 10-days, sufficient growing time elapsed for us to observe differences between plant weights and shoot heights. Although there was no increase in growth for mycorrhizal carrots during drought, there also was no obvious cost of maintaining the symbiosis. The decreased colonization during drought was unexpected; previous reported increased colonization during drought for strawberry, lettuce, and tomato (Boyer *et al.*, 2015; Ruiz-Lozano *et al.*, 2016).

The contrast in the expression of carrot and fungal genes was striking. Given the ubiquity of the notion that AM fungi improve the drought performance of plants, we expected to observe an upregulation of genes associated with establishment and maintenance of symbiosis. Instead, we found a clear downregulation of these genes in carrot during drought, while genes associated with symbiotic exchange in the fungus were upregulated. This upregulation is even more striking as it occurs despite a decrease in colonization levels in the drought treatment. Although the carrot symbiotic gene expression levels decreased during drought and corresponded to a decrease in

colonization, the fungal symbiotic gene expression increased. Taken together, this may indicate a contrast in carbon management strategies for the host and symbiont, wherein the host conserves carbon by reducing colonization levels, and the fungus ramps up its uptake and delivery of P and K to entice the plant to continue its association. On the other hand, the drought-induced composition of plant exudates may have acted as a stimulant, increasing the symbiotic activity of the fungus (de Vries *et al.*, 2019).

Aquaporins are expected to have altered expression levels during drought as a mechanism to modify water use in roots. Of the 18 aquaporin-related transcripts having differential expression patterns, only one was upregulated and three had symbiosis specific regulation. Interestingly, and in opposition to the majority of carrot aquaporins, two of the AM-specific aquaporins in carrot were not regulated by drought. Our results differ from the finding by Recchia *et al.* (2018) in which aquaporin expression in common bean roots were largely upregulated, and one transcript was under exclusive regulation in the AM condition. This difference could arise due to the differing choices in plant models (carrot versus common bean), from the duration of drought (this study had a 10-day progressive drought, whereas Recchia *et al.* withheld water for 4 days, yielding a shorter and more severe drought), from the choice of inoculant (we used a single species, spore based inoculant compared to Recchia *et al.*'s whole inoculant comprising 3 species), or from a combination of these factors.

The expression pattern of carotenoid-associated genes aligned with the expectation that genes involved with ABA synthesis and breakdown would upregulate during drought. Interestingly, CCDs were largely downregulated during drought, except one with symbiosis-specific regulation. The AM regulated transcript DCAR_022386 produces a protein homologous to *Mt*CCD1, which acts as part of a two-step mechanism to produce mycorradicin (Floss *et al.*,

2008). CCD7 and CCD8 are associated with the production of strigolactones, which act as signaling molecules in the rhizospere (Besserer *et al.*, 2006; Ruyter-Spira *et al.*, 2013; Lopez-Obando *et al.*, 2015; Stauder *et al.*, 2018). The carrot transcripts we identified as CCD7 and CCD8 were both downregulated during drought, with no clear influence from mycorrhizal status. Conversely, Ruiz-Lozano *et al.* (2016) found upregulation of CCD7 in tomato in response to mycorrhization, which increased with drought severity.

We observed a stark contrast between the regulation patterns of the plant and fungus under drought conditions, with the host downregulating most nutrient transporters and the fungus upregulating nutrient transporters. In a study of nitrogen starvation in poplar, Calabrese *et al.* (2017) also recognized upregulation of genes associated with fungal nutrient transfer. On the plant side, nitrogen starvation led to generally reduced expression of genes of phosphate transporters (Calabrese *et al.*, 2017). In our study we observed clear reductions in expression of genes associated with nitrogen transport, but there was less difference for those associated with phosphate transport. The lack of drought regulation of most of the symbiosis-specific genes for aquaporins and phosphate transporters in carrot, along with the downregulation of their non-specific counterparts during drought, partially supports Kikuchi *et al.*'s (2016) model in which water and inorganic phosphate travel from AM fungi along the host plant's transpiration gradient. Given the downregulation of many of the other aquaporin genes, we hypothesize that as part of an energy conservation strategy during drought, the plant favors the constant stream of water likely transferred by its fungal partner, which may be why the symbiosis-specific aquaporins are not downregulated during drought.

Throughout this discussion, we highlighted differences between our results in carrot and previous results reported in other species. Currently, the primary laboratory model for

mycorrhizal studies is *Medicago truncatula*, a consequence of its ease of use and dual role as a model of rhizobial-legume symbiosis. Other plants commonly used in mycorrhizal studies include poplar, tomato, potato, and maize. Carrot is an interesting model since it is a biennial plant that invests heavily in its taproot. It would be interesting to evaluate the effects from different durations of drought on various plant models with differing carbon investment strategies as well as how drought impacts resources provided to and by AM fungi. Future research should also evaluate the transcriptomes of different AM fungal species to determine their drought responses and to elucidate which features correspond to favorable host outcomes. If differences occur, tests of host preferences during drought would be an interesting follow up study.

In conclusion, the work presented here reveals that drought stress adds a new layer of complexity to the relationship between AM fungi and their plant hosts. *R. irregularis* increased symbiotic gene expression during drought. Progressive drought appeared to diminish carrot's investment in its AM fungal symbiont, although it maintained a reduced affiliation and likely benefited from the resources it continually provided.

4.6 Acknowledgements

This material is based upon work supported by the National Science Foundation Graduate Research Fellowship Program under Grant No. [DGE1256259, 2015-2017; DGE1747503, 2017-2018]. Any opinions, findings, and conclusions or recommendations expressed in this material are those of the author(s) and do not necessarily reflect the views of the National Science Foundation. This work was also supported by the Advanced Opportunity Fellowship through SciMed Graduate Research Scholars at University of Wisconsin --Madison and USDA-NIFA-AFRI Award # 016-51300-25721. We thank Jason Williams for his technical assistance with CyVerse, which was made possible through CyVerse's Extended Collaborative Support program. We appreciate Dr. Kate McCulloh for the use of photosynthesis equipment and thank Dr. Kimberly O'Keefe for her assistance with it. We are grateful to Erin Lalor, Dr. Douglas Senalik, and Dr. Philipp Simon for their help with carotenoid quantification. We also thank Dr. Thomas B. Irving for his valuable comments and suggestions to improve our manuscript.

4.7 Supplemental Figures and Tables

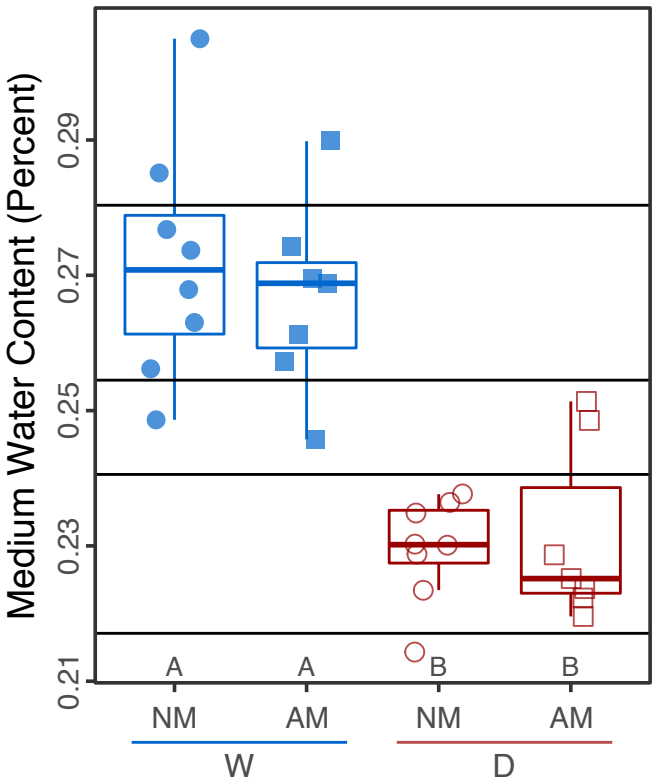


Figure S1. Water content of calcined clay substrate at harvest. Substrate water content differed between well-watered and drought carrots but not between mycorrhizal (AM) and non-mycorrhizal plants (NM). Uppercase letters denote differences between treatment groups (ANOVA followed by Tukey's HSD post-hoc test; p-value < 0.01). Individual plants whose substrates fell beyond one standard deviation of the mean (represented with horizontal black lines) of each water treatment were excluded from sequencing analyses.

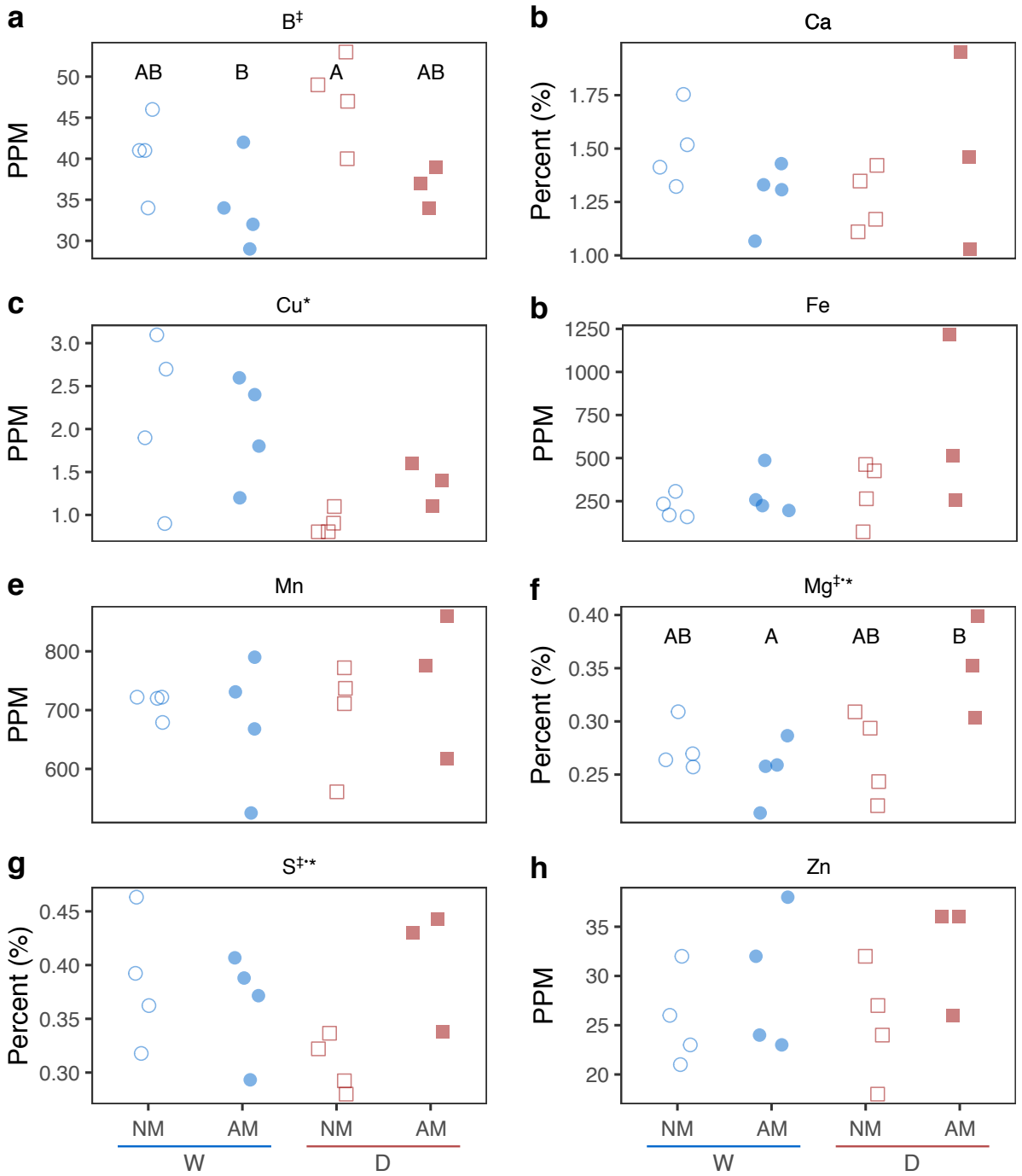


Figure S2. Content of micronutrients in leaf tissues. Micronutrients were quantified for leaf tissues to evaluate differences between non-mycorrhizal (NM) and mycorrhizal (AM) plants and between plants that were droughted (D) versus well-watered (W) controls. Differences between treatments are marked with ‡ (difference due to mycorrhizal status) or * (difference due to water status for each of the following nutrients: (a) boron (B); (b) calcium (Ca); (c) copper (Cu); (d) iron (Fe); (e) manganese (Mn); (f) magnesium (Mg); (g) sulfur (S); (h) zinc (Zn). Differences between individual groups are indicated with differing uppercase letters (two-way ANOVA with Tukey's post hoc test, p-value < 0.05).

Table S1. Nutrient additions throughout plant growth.

Day	Date	Plant Maintenance Activities
0	4 June	Carrot seedlings and water added to Petri dish and placed in the dark for germination
6 - 23	10 June - 27 June	Plants were covered with plastic cups, limiting water loss during establishment
6, 9, 14, & 22	10, 13, 20, & 26 June	Plants watered by hand
25 - 47	29 June - 21 July	Plants watered by drip lines (approximately 28 ml [± 1.8 ml] per pot per day)
18	22 June	Plants received 25 ml 1/4X Enshi
30	4 July	Plants received 25 ml 1/2X Enshi
39	13 July	Plants received 25 ml 1/2X Enshi
48	20 July	Plants received 25 ml 1X Enshi
49	22 July	All plant received 100 ml water, saturating medium and causing 10 - 15 ml water to drain from each pot
50	23 July	No plants received water
51	24 July	Water restriction began for drought treatment plants
60	3 Aug	Plants harvested

Table S2. Inputs of water and nutrients per pot during drought period.

Day	Date	Inputs per pot in well-watered condition	Inputs per pot in drought condition
1	24 July 2018	+ 25 ml water	
2	25 July 2018	+ 25 ml water	+ 25 ml water
3	26 July 2018	+ 25 ml water	
4	27 July 2018	+ 25 ml 1X Enshi Solution	+ 25 ml 1X Enshi Solution
5	28 July 2018	+ 30 ml water	
6	29 July 2018	+ 30 ml water	+ 20 ml water
7	30 July 2018	+ 25 ml water + 10 ml 1/4X Enshi Solution	+ 10 ml 1/4X Enshi Solution
8	31 July 2018	+ 20 ml water	
9	01 August 2018	+ 30 ml water	
10	02 August 2018	+ 30 ml water	
Harvest	03 August 2018		

Table S3. Genes of interest in *Rhizophagus irregularis.*

Genes of interest	Defined Homolog of	Transcript ID	pval	qval	beta
Secreted Peptides	SP7	1742072	2.4E-24	6.7E-22	6.37
	SP7	1659119	6.5E-27	2.5E-24	4.66
		1457703	4.5E-06	6.9E-05	2.36
		1522661	9.0E-07	1.7E-05	2.38
		1788504	0.1290	0.2539	0.75
		1765105	0.2767	0.4313	0.75
Trehalose synthesis		1582882	3.3E-04	0.0027	1.78
		1603975	0.0029	0.0158	1.53
		1625132	0.0016	0.0097	1.34
		1481264	0.0257	0.0809	1.40
Meiosis-related		1458443	0.0013	0.0082	1.56
		1497092	0.0012	0.0080	1.15
		1741430	0.0044	0.0217	1.44
Phermone-sensing		323424	0.0118	0.0459	1.11
		1715024	0.0033	0.0173	1.41
		1562368	2.4E-07	5.1E-06	2.73
		1540399	0.0009	0.0060	1.83
		1656039	2.2E-07	4.8E-06	2.64
		1562368	2.2E-07	4.8E-06	2.64

Table S4. Symbiosis-associated genes in other drought studies

Identifying information	Symbiotic gene of interest	Drought period	Drought regulation in roots
Medicago truncatula, Zhang *et al.* 2014		10 days	
Medicago Probset ID			
Mtr.51192.1.S1_at	DMI2/NORK		Down
Mtr.42174.1.S1_at	IPD3/CYCLOPS		Down
Mtr.142.1.S1_s_at	Lyk3		Down
Mtr.15789.1.S1_at	NFP		Down
Mtr.44789.1.S1_at	NSP2		Down
Mtr.51186.1.S1_at	DMI1		No change
Mtr.44225.1.S1_at	DMI3/CCaMK		No change
Populus simonii, Jia *et al.* 2017		8 days	
Poplar Gene ID			
Potri.007G004700	DMI2 / NORK		Down
Potri.001G130800	IPD3 / CYCLOPS		Down

4.8 References

Andrews S. **2016**. FastQC: A quality control tool for high throughput sequence data.

Aroca R, Bago A, Sutka M, Paz JA, Cano C, Amodeo G, Ruiz-Lozano JM. **2009**. Expression Analysis of the First Arbuscular Mycorrhizal Fungi Aquaporin Described Reveals Concerted Gene Expression Between Salt-Stressed and Nonstressed Mycelium. *Molecular Plant-Microbe Interactions* **22**: 1169–1178.

Augé RM. **2001**. Water relations, drought and vesicular-arbuscular mycorrhizal symbiosis. *Mycorrhiza* **11**: 3–42.

Baranski R. **2008**. Genetic transformation of carrot (*Daucus carota*) and other apiaceae species. *Trans Plant Journal* **2 (1)**: 18–38.

Baslam M, Esteban R, García-Plazaola JI, Goicoechea N. **2013**. Effectiveness of arbuscular mycorrhizal fungi (AMF) for inducing the accumulation of major carotenoids, chlorophylls and tocopherol in green and red leaf lettuces. *Applied Microbiology and Biotechnology* **97**: 3119–3128.

Bécard G, Fortin a. J. **1988**. Early events of vesicular-arbuscular mycorrhiza formation on Ri T-DNA transformed roots. *New Phytologist* **108**: 211–218.

Begum N, Qin C, Ahanger MA, Raza S, Khan MI, Ahmed N, Ashraf M, Zhang L. **2019**. Role of Arbuscular Mycorrhizal Fungi in Plant Growth Regulation: Implications in abiotic stress tolerance. *Frontiers in Plant Science* **10**: 1068.

Besserer A, Puech-Pagès V, Kiefer P, Gomez-Roldan V, Jauneau A, Roy S, Portais J-C, Roux C, Bécard G, Séjalon-Delmas N. **2006**. Strigolactones stimulate arbuscular mycorrhizal fungi by activating mitochondria. *PLoS biology* **4**: e226.

Bowman MJ, Willis DK, Simon PW. **2014**. Transcript Abundance of Phytoene Synthase 1 and Phytoene Synthase 2 Is Associated with Natural Variation of Storage Root Carotenoid Pigmentation in Carrot. *Journal of the American Society for Horticultural Science* **139**: 63–68.

Boyer LR, Brain P, Xu X-M, Jeffries P. **2015**. Inoculation of drought-stressed strawberry with a mixed inoculum of two arbuscular mycorrhizal fungi: effects on population dynamics of fungal species in roots and consequential plant tolerance to water deficiency. *Mycorrhiza* **25**: 215–227.

Bravo A, Brands M, Wewer V, Dörmann P, Harrison MJ. **2017**. Arbuscular mycorrhiza-specific enzymes FatM and RAM2 fine-tune lipid biosynthesis to promote development of arbuscular mycorrhiza. *New Phytologist* **214**: 1631–1645.

Bravo A, York T, Pumplin N, Mueller LA, Harrison MJ. **2016**. Genes conserved for arbuscular mycorrhizal symbiosis identified through phylogenomics. *Nature Plants* **2**: 1–6.

Bray NL, Pimentel H, Melsted P, Pachter L. **2016**. Near-optimal probabilistic RNA-seq quantification. *Nature Biotechnology* **34**: 4–8.

Brundrett M, Bougher N, Dell B, Grove T, Malajczuk N. **1996**. *Working with mycorrhizas in forestry and agriculture*.

Bucher M, Hause B, Krajinski F, Küster H. **2014**. Through the doors of perception to function in arbuscular mycorrhizal symbioses. *New Phytologist* **204**: 833–840.

Calabrese S, Kohler A, Niehl A, Veneault-fourrey C, Boller T, Courty P. **2017**. Transcriptome analysis of the *Populus trichocarpa – Rhizophagus irregularis* Mycorrhizal Symbiosis : Regulation of Plant and Fungal Transportomes under Nitrogen Starvation. **58**: 1003–1017.

Chen ECH, Morin E, Beaudet D, Noel J, Yildirir G, Ndikumana S, Charron P, St-Onge C, Giorgi J, Krüger M, *et al.* 2018. High intraspecific genome diversity in the model arbuscular mycorrhizal symbiont *Rhizophagus irregularis*. *New Phytologist* **220**: 1161–1171.

Corradi N, Brachmann A. **2017**. Fungal Mating in the Most Widespread Plant Symbionts? *Trends in Plant Science* **22**: 175–183.

Dechassa N, Schenk MK, Claassen N, Steingrobe B. **2003**. Phosphorus Efficiency of Cabbage (*Brassica oleraceae* L. var. capitata), Carrot (*Daucus carota* L.), and Potato (*Solanum tuberosum* L.). *Plant and Soil* **250**: 215–224.

Delaux P-M, Séjalon-Delmas N, Bécard G, Ané J-M. **2013**. Evolution of the plant-microbe symbiotic 'toolkit'. *Trends in plant science* **18**: 298–304.

Fellbaum CR, Mensah JA, Cloos AJ, Strahan GE, Pfeffer PE, Kiers ET, Bücking H. **2014**. Fungal nutrient allocation in common mycorrhizal networks is regulated by the carbon source strength of individual host plants. *New Phytologist* **203**: 646–656.

Fester T, Schmidt D, Lohse S, Walter M, Giuliano G, Bramley P, Fraser P, Hause B, Strack D. **2002**. Stimulation of carotenoid metabolism in arbuscular mycorrhizal roots. *Planta* **216**: 148–154.

Fester T, Wray V, Nimtz M, Strack D. **2005**. Is stimulation of carotenoid biosynthesis in arbuscular mycorrhizal roots a general phenomenon? *Phytochemistry* **66**: 1781–1786.

Floss DS, Schliemann W, Schmidt J, Strack D, Walter MH. **2008**. RNA Interference-Mediated Repression of MtCCD1 in Mycorrhizal Roots of *Medicago truncatula* Causes Accumulation of C27 Apocarotenoids, Shedding Light on the Functional Role of CCD1. *Plant Physiology* **148**: 1267–1282.

Garcia K, Chasman D, Roy S, Ané J-M. **2017**. Physiological Responses and Gene Co-Expression Network of Mycorrhizal Roots under K^+ Deprivation. *Plant Physiology* **173**: 1811–1823.

Goff SA, Vaughn M, McKay S, Lyons E, Stapleton AE, Gessler D, Matasci N, Wang L, Hanlon M, Lenards A, *et al.* 2011. The iPlant Collaborative: Cyberinfrastructure for Plant Biology. *Frontiers in Plant Science* **2**: 1–16.

Greenham K, Guadagno C, Gehan M, Mockler T, Weinig C, Ewers B, Mcclung C. **2017**. Temporal network analysis identifies early physiological and transcriptomic indicators of mild drought in *Brassica rapa*. *elife* **6**: 1–26.

Halary S, Daubois L, Terrat Y, Ellenberger S, Wöstemeyer J, Hijri M. **2013**. Mating type gene homologues and putative sex pheromone-sensing pathway in arbuscular mycorrhizal fungi, a presumably asexual plant root symbiont. *PLoS ONE* **8**: 1–12.

Halary S, Malik SB, Lildhar L, Slamovits CH, Hijri M, Corradi N. **2011**. Conserved meiotic machinery in *Glomus* spp., a putatively ancient asexual fungal lineage. *Genome Biology and Evolution* **3**: 950–958.

Harrison MJ. **2005**. Signaling in the arbuscular mycorrhizal symbiosis. *Annual Review of Microbiology* **59**: 19–42.

Helber N, Wippel K, Sauer N, Schaarschmidt S, Hause B, Requena N. **2011**. A Versatile Monosaccharide Transporter That Operates in the Arbuscular Mycorrhizal Fungus *Glomus* sp Is Crucial for the Symbiotic Relationship with Plants. *The Plant Cell* **23**: 3812–3823.

Howitt R, Medellín-azuara J, Macewan D. **2014**. Economic Analysis of the 2014 Drought for California Agriculture. *Center for Watershed Sciences. University of California, Davis, California*: 20p.

Humphreys CP, Franks PJ, Rees M, Bidartondo MI, Leake JR, Beerling DJ. **2010**. Mutualistic mycorrhiza-like symbiosis in the most ancient group of land plants. *Nature communications* **1**: 103.

Iorizzo M, Ellison S, Senalik D, Zeng P, Satapoomin P, Huang J, Bowman M, Iovene M, Sanseverino W, Cavagnaro P, *et al.* 2016. A high-quality carrot genome assembly provides new insights into carotenoid accumulation and asterid genome evolution. *Nature Genetics* **48**: 657–666.

Iturriaga G, Suárez R, Nova-Franco B. **2009**. Trehalose metabolism: From osmoprotection to signaling. *International Journal of Molecular Sciences* **10**: 3793–3810.

Javot H, Penmetsa RV, Terzaghi N, Cook DR, Harrison MJ. **2007**. A *Medicago truncatula* phosphate transporter indispensable for the arbuscular mycorrhizal symbiosis. *Proceedings of the National Academy of Sciences* **104**: 1720–1725.

Jia J, Zhou J, Shi W, Cao X, Luo J, Polle A, Luo Z. **2017**. Comparative transcriptomic analysis reveals the roles of responsive genes in poplars exposed to high temperature and drought. *Scientific Reports* **7**: 1–17.

Kamel L, Keller-Pearson M, Roux C, Ané JM. **2017**. Biology and evolution of arbuscular mycorrhizal symbiosis in the light of genomics. *New Phytologist* **213**: 531–536.

Keymer A, Pimprikar P, Wewer V, Huber C, Brands M, Bucerius SL, Delaux P-M, Klingl V, Röpenack-Lahaye E von, Wang TL, *et al.* 2017. Lipid transfer from plants to arbuscular

mycorrhiza fungi. *eLife* **6**: 1–33.

Kikuchi Y, Hijikata N, Ohtomo R, Handa Y, Kawaguchi M, Saito K, Masuta C, Ezawa T. **2016**. Aquaporin-mediated long-distance polyphosphate translocation directed towards the host in arbuscular mycorrhizal symbiosis: application of virus-induced gene silencing. *The New Phytologist* **211**: 1202–1208.

Kloppholz S, Kuhn H, Requena N. **2011**. A secreted fungal effector of *glomus intraradices* promotes symbiotic biotrophy. *Current Biology* **21**: 1204–1209.

Kobayashi Y, Mondal F, Ban T, Matsubara H, Adachi F, Asao T. **2013**. Growing carrots hydroponically using perlite substrates. *Scientia Horticulturae* **159**: 113–121.

Li T, Hu Y-J, Hao Z-P, Li H, Wang Y-S, Chen B-D. **2013**. First cloning and characterization of two functional aquaporin genes from an arbuscular mycorrhizal fungus *Glomus intraradices*. *New Phytologist* **197**: 617–630.

Lin K, Limpens E, Zhang Z, Ivanov S, Saunders DGO, Mu D, Pang E, Cao H, Cha H, Lin T, *et al.* 2014. Single nucleus genome sequencing reveals high similarity among nuclei of an endomycorrhizal fungus. *PLoS genetics* **10**: e1004078.

Lopez-Obando M, Ligerot Y, Bonhomme S, Boyer F-D, Rameau C. **2015**. Strigolactone biosynthesis and signaling in plant development. *Development (Cambridge, England)* **142**: 3615–9.

Luginbuehl LH, Menard GN, Kurup S, Erp H Van, Radhakrishnan G V., Breakspear A, Oldroyd GED, Eastmond PJ. **2017**. Fatty acids in arbuscular mycorrhizal fungi are synthesized by the host plant. *Science* **356**: 763–775.

MacLean AM, Bravo A, Harrison MJ. **2017**. Plant Signaling and Metabolic Pathways Enabling Arbuscular Mycorrhizal Symbiosis. *The Plant Cell* **29**: 2319–2335.

Mendiburu F de. **2019**. Agricolae: Statistical Procedures for Agricultural Research.

Ocón A, Hampp R, Requena N. **2007**. Trehalose turnover during abiotic stress in arbuscular mycorrhizal fungi. *New Phytologist* **174**: 879–891.

Pimentel H, Bray NL, Puente S, Melsted P, Pachter L. **2017**. Differential analysis of RNA-seq incorporating quantification uncertainty. *Nature Methods* **14**: 687–692.

Porcel R, Aroca R, Cano C, Bago A, Ruiz-Lozano JM. **2006**. Identification of a gene from the arbuscular mycorrhizal fungus *Glomus intraradices* encoding for a 14-3-3 protein that is up-regulated by drought stress during the AM symbiosis. *Microbial Ecology* **52**: 575–582.

Porcel R, Aroca R, Cano C, Bago A, Ruiz-Lozano JM. **2007**. A gene from the arbuscular mycorrhizal fungus *Glomus intraradices* encoding a binding protein is up-regulated by drought stress in some mycorrhizal plants. *Environmental and Experimental Botany* **60**: 251–256.

R Core Team. **2019**. R: A language and environment for statistical computing.

Recchia GH, Konzen ER, Cassieri F, Caldas DGG, Tsai SM. **2018**. Arbuscular Mycorrhizal Symbiosis Leads to Differential Regulation of Drought-Responsive Genes in Tissue-Specific Root Cells of Common Bean. **9**: 1–24.

Rodriguez-Concepcion M, Stange C. **2013**. Biosynthesis of carotenoids in carrot: An underground story comes to light. *Archives of Biochemistry and Biophysics* **539**: 110–116.

Ruiz-Lozano JM, Aroca R, Zamarreño ÁM, Molina S, Andreo-Jiménez B, Porcel R, García-Mina JM, Ruyter-Spira C, López-Ráez JA. **2016**. Arbuscular mycorrhizal symbiosis induces strigolactone biosynthesis under drought and improves drought tolerance in lettuce and tomato. *Plant Cell and Environment* **39**: 441–452.

Ruyter-Spira C, Al-Babili S, van der Krol S, Bouwmeester H. **2013**. The biology of strigolactones. *Trends in Plant Science* **18**: 72–83.

Santander C, Aroca R, Ruiz-Lozano JM, Olave J, Cartes P, Borie F, Cornejo P. **2017**. Arbuscular mycorrhiza effects on plant performance under osmotic stress. *Mycorrhiza* **27**: 639–657.

Schindelin J, Arganda-Carreras I, Frise E, Kaynig V, Longair M, Pietzsch T, Preibisch S, Rueden C, Saalfeld S, Schmid B, *et al.* 2012. Fiji: An open-source platform for biological-image analysis. *Nature Methods* **9**: 676–682.

Schreiner RP, Koide RT. **1993**. Stimulation of vesicular-arbuscular mycorrhizal fungi by mycotrophic and nonmycotrophic plant root systems. *Applied and environmental microbiology* **59**: 2750–2.

Smith SE, Read D. **2008**. *Mycorrhizal Symbiosis*. Elsevier.

Stauder R, Welsch R, Camagna M, Kohlen W, Balcke GU, Tissier A, Walter MH. **2018**. Strigolactone Levels in Dicot Roots Are Determined by an Ancestral Symbiosis-Regulated Clade of the PHYTOENE SYNTHASE Gene Family. *Frontiers in Plant Science* **9**: 1–18.

Tian T, Liu Y, Yan H, You Q, Yi X, Du Z, Xu W, Su Z. **2017**. agriGO v2 .0: a GO analysis toolkit for the agricultural community, 2017 update. **45**: 122–129.

Tisserant E, Malbreil M, Kuo A, Kohler A, Symeonidi A, Balestrini R, Charron P, Duensing N, Frei dit Frey N, Gianinazzi-Pearson V, *et al.* 2013. Genome of an arbuscular mycorrhizal fungus provides insight into the oldest plant symbiosis. *Proceedings of the National Academy of Sciences of the United States of America* **110**: 20117–22.

USDA-NASS. **2018**. Carrots - Conventional Production Measured in Sales.

de Vries FT, Williams A, Stringer F, Willcocks R, McEwing R, Langridge H, Straathof AL. **2019**. Changes in root exudate induced respiration reveal a novel mechanism through which drought affects ecosystem carbon cycling. *New Phytologist*.

Walder F, Boller T, Wiemken A, Courty P, Walder F, Boller T, Wiemken A. **2016**. Regulation of plants' phosphate uptake in common mycorrhizal networks: Role of intraradical fungal phosphate transporters. *Plant Signaling & Behavior* **11**: 1–4.

Walker-Simmons M. **1987**. ABA Levels and Sensitivity in Developing Wheat Embryos of Sprouting Resistant and Susceptible Cultivars. *Plant physiology* **84**: 61–6.

Wright SF, Upadhyaya a. 1998. A survey of soils for aggregate stability and glomalin, a glycoprotein produced by hyphae of arbuscular mycorrhizal fungi. *Plant and Soil* **198**: 97–107.

Xue L, Frost CJ, Tsai C, Harding SA. **2016**. Drought response transcriptomes are altered in poplar with reduced tonoplast sucrose transporter expression. *Nature Publishing Group*: 1–14.

Young AJ. **1991**. The photoprotective role of carotenoids in higher plants. *Physiologia Plantarum* **83**: 702–708.

Zhang J, Carvalho MHC De, Torres-jerez I, Kang Y, Allen SN, Huhman D V, Tang Y, Murray J, Sumner LW, Udvardi MK. **2014**. Global reprogramming of transcription and metabolism in *Medicago truncatula* during progressive drought and after rewatering. *Plant, Cell & Environment* **37**: 2553–2576.

Chapter 5. Discussion and future directions

The work presented here adds to the body of knowledge of symbiosis between arbuscular mycorrhizal (AM) fungi and plants. Data generated through the field and greenhouse experiments presented in Chapter 3 provides highlights the need for repetition within and across years for research involving AM inoculants. Despite our efforts to create parallel experiments in the greenhouse and the field, inherent differences between these systems led to inconsistent outcomes (*e.g.* heirloom and hybrid cultivars of carrot differed in their responsive to AM inoculation in the field, but there was no such pattern in the greenhouse). Within each experiment, in both the field and greenhouse, individual inoculants appeared to be over- or under-performing relative to the others. Again, no clear pattern could be established, since a high performing isolate in one experiment could be the low performing isolate in the next. This serves as a reminder of the importance of replication, which is especially challenging when conditions cannot be controlled (*e.g.* weather).

The main finding reported in Chapter 3, that heirloom cultivars respond more substantially to inoculation in the field than do their hybrid counterparts, supports the idea that modern breeding practices may indirectly select against compatibility with AM fungi and other beneficial soil microorganisms. Others suggested this previously (Milla et al., 2015), and some also tested this notion in other systems (Hetrick et al., 1995, 1993; Khalil et al., 1994; Raju et al., 2008; Symanczik et al., 2018).

Of course, it must be noted that the response to mycorrhizal inoculations likely were influenced by the native soil microbial populations, the fact that the introduced AM fungi did not

have established mycorrhizal networks, and there was no ability to compare the quantity of colonization levels attributed to inoculants versus native species.

The report in Chapter 4 provides detailed analyses of the gene expression profiles of carrot (*Daucus carota* cv 'Napoli') and its AM fungal symbiont (*Rhizophagus irregularis* DAOM 197198). Most of the differentially expressed genes of *R. irregularis* were upregulated following the 10-day drought period. Among the genes of interest were *RiAQPF2*, an aquaporin gene that has previously been shown to be upregulated during water stress in intraradical mycelia (Li et al., 2013). Several known phosphate (P) transporters as well as potassium (K) were highly upregulated. Additionally, the genes of several secreted peptides were upregulated, including one with a known effector-like role in dampening host defense responses. Taken together, this suggests that the fungal response to the drought stress of its host was to invest heavily in its symbiotic relationship.

In contrast to the fungal response to drought, carrot downregulated genes associated with the establishment and maintenance of AM symbiosis. Carotenoid-associated genes were also differentially expressed. Overall, there was downregulation of the genes leading to carotenoid synthesis and modification, and there was upregulation of genes associated with those involved in production and breakdown of abscisic acid (ABA). This is consistent with the increase in ABA content that we expected and observed due to the drought conditions. Interestingly, the carotenoid cleavage dioxygenases (CCD7 and CCD8) involved in the synthesis of strigolactones were also downregulated, suggesting that strigolactone signaling may be diminished, which may contribute to decreased AM colonization. Lastly, given that AM colonization of roots was reduced in the drought condition, it seemed that carrot deprioritized its fungal symbionts, possibly in a bid to conserve carbon.

There were also symbiosis-specific genes whose regulation was not hampered, such as those encoding aquaporins and phosphate transporters, whose regulation did not change. The constant regulation of symbiosis-specific plant and fungal aquaporins and phosphate transporters provides support for Kikuchi *et al.*'s model, which states that the flow of water and polyphosphates from AM mycelia toward plant roots occurs passively (Kikuchi et al., 2016). Taken together, it seems that carrot's response to drought enabled it to conserve resources and energy by diminishing its investment in its AM symbiosis, from which it appeared to still benefit. A recent study showed that droughted plants alter their root exudate compositions, causing the stimulation of soil microbial activity (de Vries et al., 2019). Increased AM fungal activity in plant roots during drought may also be fulfilling a 'lifeboat' scenario, in which the fungus increases its symbiotic activity not only to increase its own carbon reserves, but also to remain inside the host tissues. AM fungi grow faster from propagules stored in plant tissues than from isolated spores, so it is reasonable to expect that continued residence in host roots would permit increased fitness following the stress, in addition to benefiting to the fungus by way of protection from external factors.

Comparative transcriptomics of AM fungi could provide the next breakthrough discoveries of their key symbiotic and asymbiotic functions. For example, a data mining strategy compiling the expression profiles of *R. irregularis* under drought-stress, nitrogen-stress (Jia et al., 2017), and grown with varying lipid-availability (Kameoka et al., 2019) could provide leads to the mechanisms by which AM fungi import lipids from their host. We observed an uptick in regulation of some MAT and pheromone-sensing genes that are potentially involved in sexual mating in AM fungi. Recently, a species of diatoms, previously assumed to be asexual (*Thalassiosira pseudonana*), were found to undergo a conspicuous sexual reproduction under

ammonium stress (Moore et al., 2017). If sexual reproduction does occur in AM fungi, it may be stimulated by stress conditions, and if so, this could be the key to diagnosing such reproduction.

In Chapter 4 we suggested investigating drought-responsiveness of various mycorrhizal plants at differing durations of drought. Studies such as these could eventually integrate multi-species inoculants, modeling more realistic growing conditions and testing for synergies and antagonisms between and among AM fungal species. There are varying numbers of symbiosis-specific genes (*i.e.* those under exclusive regulation during AM colonization) among plant species, including those encoding aquaporins and nutrient transporters. If there is also intraspecific variability in the number of symbiosis-specific genes, this may offer a means by which plant breeders could select for microbial interactions. For example, if a genotype of carrot has 5 symbiosis-specific aquaporins that exhibit positive drought regulation, then it may be a better candidate to recruit and benefit from AM fungi in a field setting. We do not yet know whether symbiosis-specific genes have regulation that differs by AM fungal identity, and this should also be explored.

References

Hetrick BAD, Wilson GWT, Cox TS. **1993**. Mycorrhizal dependence of modern wheat cultivars and ancestors: a synthesis. *Canadian Journal of Botany* **71**: 512–518.

Hetrick BAD, Wilson GWT, Gill BS, Cox TS. **1995**. Chromosome location of mycorrhizal responsive genes in wheat. *Canadian Journal of Botany* **73**: 891–897.

Jia J, Zhou J, Shi W, Cao X, Luo J, Polle A, Luo Z. **2017**. Comparative transcriptomic analysis reveals the roles of responsive genes in poplars exposed to high temperature and drought. *Scientific Reports* **7**: 1–17.

Kameoka H, Tsutsui I, Saito K, Kikuchi Y, Handa Y, Ezawa T, Hayashi H, Kawaguchi M, Akiyama K. **2019**. Stimulation of asymbiotic sporulation in arbuscular mycorrhizal fungi by fatty acids. *Nature Microbiology*.

Khalil S, Loynachan T, Tabatabai MA. **1994**. Mycorrhizal Dependency and Nutrient Uptake by Improved and Unimproved Corn and Soybean Cultivars. *Agronomy Journal* **86**: 949–958.

Kikuchi Y, Hijikata N, Ohtomo R, Handa Y, Kawaguchi M, Saito K, Masuta C, Ezawa T. **2016**. Aquaporin-mediated long-distance polyphosphate translocation directed towards the host in arbuscular mycorrhizal symbiosis: application of virus-induced gene silencing. *The New phytologist* **211**: 1202–1208.

Li T, Hu Y-J, Hao Z-P, Li H, Wang Y-S, Chen B-D. **2013**. First cloning and characterization of two functional aquaporin genes from an arbuscular mycorrhizal fungus *Glomus intraradices*. *New Phytologist* **197**: 617–630.

Milla R, Osborne CP, Turcotte MM, Violle C. **2015**. Plant domestication through an ecological lens. *Trends in Ecology & Evolution* **30**: 463–469.

Moore ER, Bullington BS, Weisberg AJ, Jiang Y, Chang J, Halsey KH. **2017**. Morphological and transcriptomic evidence for ammonium induction of sexual reproduction in <i>Thalassiosira pseudonana</> and other centric diatoms. *PLoS ONE* **12**.

Raju PS, Clark RB, Ellis JR, Maranville JW. **2008**. Mineral uptake and growth of sorghum colonized with VA mycorrhiza at varied soil phosphorus levels. *Journal of Plant Nutrition* **13**: 843–859.

Symanczik S, Lehmann MF, Wiemken A, Boller T, Courty P. **2018**. Effects of two contrasted arbuscular mycorrhizal fungal isolates on nutrient uptake by *Sorghum bicolor* under drought. *Mycorrhiza* **28**: 779–785.

de Vries FT, Williams A, Stringer F, Willcocks R, McEwing R, Langridge H, Straathof AL. **2019**. Changes in root exudate induced respiration reveal a novel mechanism through which drought affects ecosystem carbon cycling. *New Phytologist*.

Appendix. Improving Central Dogma Understanding Using Web-based Practice Tools Teaching-as-Research Project and Internship

The present project occurred through a teaching-as-research internship with the Delta Program in Research, Teaching, and Learning (University of Wisconsin-Madison). This internship provides graduate students and post-doctoral researchers an opportunity to address a teaching challenge, usually through design and implementation of an experimental intervention aimed to promote student learning and classroom equity. Internship cohorts plan, conduct, and evaluate student outcomes through individual teaching as research projects. The cohort meets weekly during the semester in which projects occur, enabling interns to discuss project design, progress, and data management. Interns partner with teaching faculty to identify a topic of interest, arrange interventions and assessments, and to reflect on project results.

Motivation

For my internship, I chose to work with schools comprising a diverse population of introductory biology students. I met with faculty from three teaching-focused institutions to identify potential challenges for students and learned that each pinpointed the same challenge: *students struggle to understand or reconcile misconceptions of how genes are expressed* (transcription and translation). Each course comprised non-biology majors, so it is unlikely that students would have their misconceptions addressed in future biology courses. For this project, I partnered with faculty at the University of Wisconsin-Whitewater (UW-W), Madison College-Truax (MC-T), and Madison College-Downtown (MC-D). Each institution comprises highly diversified student populations, making them ideal for this project where we aim to improve student learning outcomes for a broad set of students. In this work, we ask: *Does interaction with online tools that provide instruction, practice, and instant-feedback to students*

help them to better develop their conceptual understanding of biology's central dogma? A basic understanding of these concepts is increasingly important given recent advances in molecular technologies that have commercial applications and potential impacts on the public.

Background

Undergraduates, especially those in introductory biology courses, tend to misunderstand the concept of the central dogma (generalized as DNA -> RNA -> Protein). The central dogma of biology, generally and briefly, states that DNAs serve as templates for nascent RNAs (specifically messenger RNAs {mRNAs}), that serve as templates for amino acid assembly (leading to protein synthesis). Wright *et al.* found that students held two persistent misconceptions: (1) RNA is a transformed version of DNA, and (2) mRNA exists independent of transcription (2014). Students' confusion about central dogma may be compounded by poor prior scaffolding and lack of definitions in biological modeling (*e.g.* What do arrows represent in the standard model?). Most students fail to connect the concept of genetic information and information flow with central dogma[1], and despite correct use of jargon, students likely have misconceptions regarding the meaning of technical language such as "transcribe" and "translate."

Reinagel and Speth found that repeated model-building activities with peer-review, **self-assessment, and instructor feedback increased students' ability to correctly describe and depict the gene-to-phenotype process**, though a quarter of learners remained confused regarding protein outputs and relationships to phenotypes[2]. The use of optional online tools such as virtual biology laboratories seems to generally be well-received by students since they perceive these resources as a benefit to their success in introductory biology courses, and surveys of students who accessed these resources report a high degree of self-efficacy.[3] Student success in introductory courses is often measured by high performance on multiple choice exams, which

can lead instructors to believe students understand concepts. On assessments requiring written responses, challenges arise in that students may use correct terminology, but their wording may be too vague to enable a grader to determine whether students understand concepts. Because of these challenges, Newman *et al.* developed and vetted a Central Dogma Concept Inventory (CDCI), assessment tool which relies on carefully crafted questions and multiple select answer options, making it easier for instructors to identify misconceptions.[4]

For the present project, I developed web-based tools that provided opportunities to practice using terminology and making predictions using DNA, RNA, or amino acid sequences. Tools provided penalty-free practice with instant feedback tailored to the answers students provided, with the intent that feedback could help correct misconceptions. **This work evaluated whether optional, non-graded, online tools that offer students practice and instant feedback promoted student learning of central dogma concepts.** As part of this, the online tools focused on improving learner self-efficacy and concept clarity for the most confusing concepts, as identified by recent research.[4] This work also tested whether students could connect conceptual understandings to their own lives or experiences.

Research Question

Do optional online study materials that provide instant-feedback to learners affect students' demonstration of their understanding of the central dogma, as measured by CDCI assessment?

Methods

To evaluate whether students' conceptual understanding of the central dogma improved with access to the study materials, I partnered with teaching faculty at three nearby institutions: MC-T, n = 32; MC-D, n = 32; and UW-W, n = 70. These introductory biology courses for non-majors contained comparable curricula. All students took in-class pre- and post-quizzes comprising selected questions from the CDCI assessment (not included here to honor my agreement with the authors who produced it).[4] Quiz questions are multiple-select, so to have correct answers for most questions students must select all the correct options rather than a single choice. To evaluate whether students could identify real-life applications or relevance of the central dogma, I provided an open-ended question at the end of each quiz. For this, students encountered a prompt with a brief explanatory statement asking them to state of how the central dogma related to them. Students took the pre-quiz, then had access to online tools during unit instruction. Prior to course summative assessments, students took the post-quiz (**Figure I**), for which they received participation points.

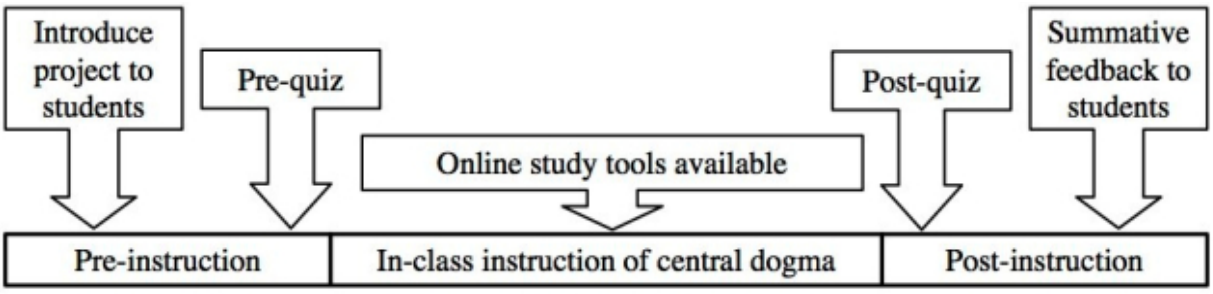


Figure I. Timeline of project activities and assessments.

Student interaction with online tools was voluntary but encouraged by instructors via in-class and email reminders. Tools were accessed through learning management systems (LMS, Blackboard and Desire 2 Learn). Students self-reported use of online tools on the post-quiz and on a survey linked to their institution's LMS. The survey recorded students' self-reported time

spent on each activity and allowed students to rate how helpful they believed the tools were to their studying. The design of the online tools intended to help students by (1) providing basic instruction regarding the overview of central dogma concepts presented in self-guided slides that slowly introduced details, included embedded videos, and solicited students to answer increasingly challenging Bloom's-type questions,[5] (2) offering practice with terminology used to describe mechanisms and molecules involved in transcription and translation (**Figure II**), and (3) giving prompts to stimulate thinking about what sequences (amino acids and nucleotides) give rise to other molecules with known sequences and vice-versa. Student assessments (pre- and post-quizzes) and surveys were paired and then de-identified prior to scoring and data analysis.

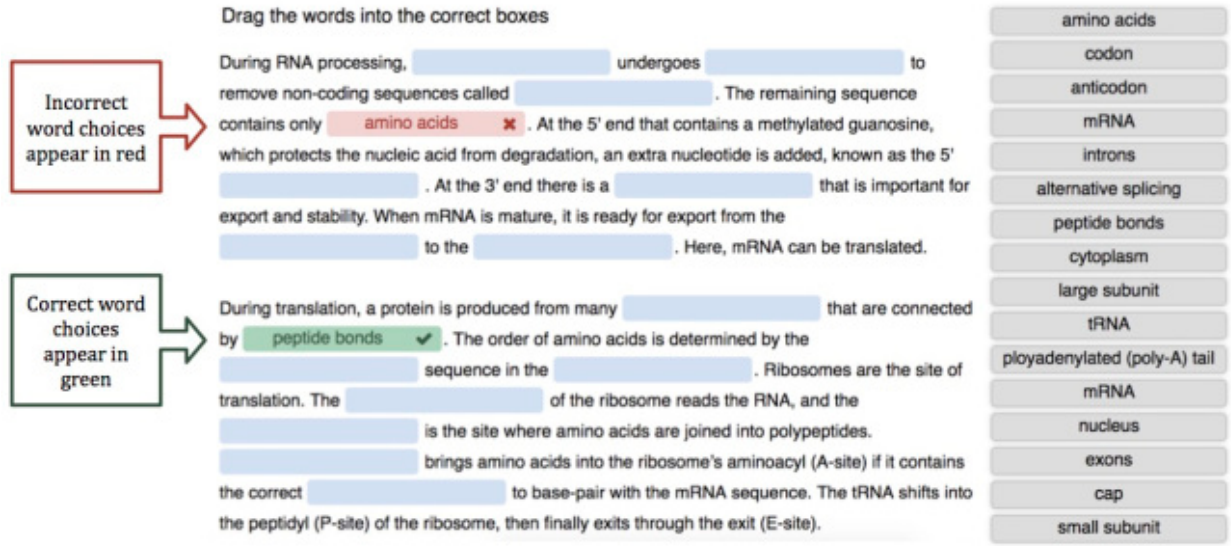


Figure II. Vocabulary practice tool that permits students to drag terms from the list on the right into the blue blanks provided within the paragraph. Correct responses turn green with a checkmark. Incorrect responses turn red and an "X" appears enabling students to recycle the term to the main list.

Results and Discussion

Generally, student responses on post-quizzes demonstrated increased understanding (as measured by quiz scores) compared to pre-quiz assessments (**Table**). Student survey responses regarding whether students believed the tools were beneficial provided mixed responses, and no correlation detectable between self-reported time spent, helpfulness perceived (data not shown), and student understanding. At both Madison College locations, too many students opted to use the online tools to permit robust statistical comparison between the performance of students who did and did not access them (**Table**). The opposite was true of UW-Whitewater. The number of students reporting that they used the online tools differed drastically by institution and may be linked to prompting by instructors.

Table. Student participation and scores for concept inventory quizzes by participating institution.

	Academic Institution		
Item	MC-D	MC-T	UW-W
Student participants of all assessments (n)	20	20	58
Proportion of participants who used online tool (%)	90	90	6
Pre-quiz score (% correct out of 100%)	43	48	37
Post-quiz score (% correct out of 100%)	57	62	49
Change in scores over time (%)	14	14	12

Student performance varied between institutions. Generally, students at the community colleges performed better on the post-quiz (**Figure III**), and these students also had the greatest learning gains, measured in changes in scores over time (**Table**). Combining questions into categories revealed that students from all three institutions struggled with questions about nucleic

acids (**Figure IV**). Performance on advanced metacognitive questions, which required students to incorporate all aspects of transcription and translation into a single concept, was poor at University of Wisconsin-Whitewater (UW-W) whereas both Madison College-Downtown (MC-D) and Madison College-Truax (MC-T) clearly improved with this. During the redesign and iteration of this project with the Madison College faculty partners, analyses of these data are on hold.

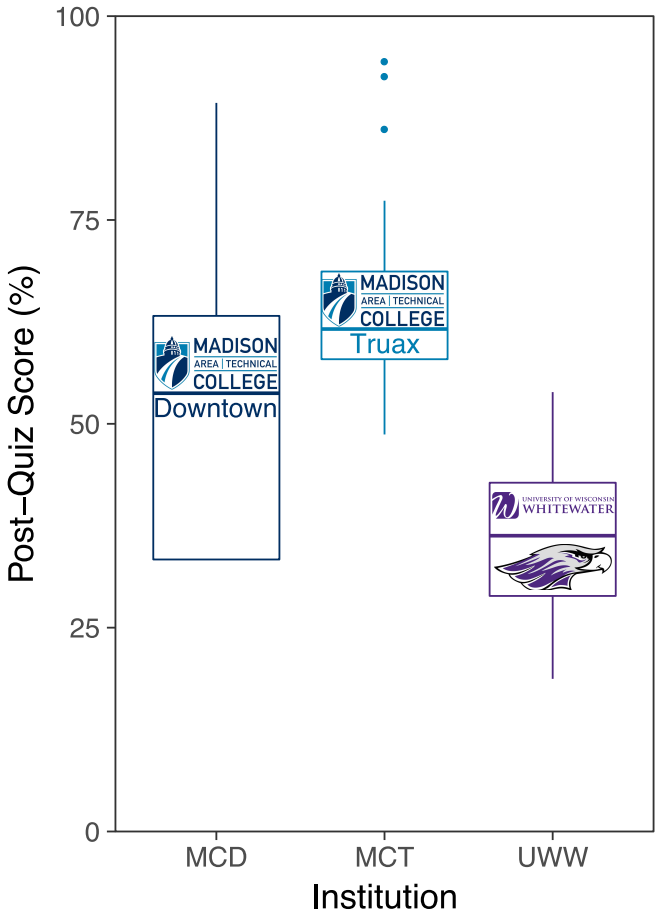
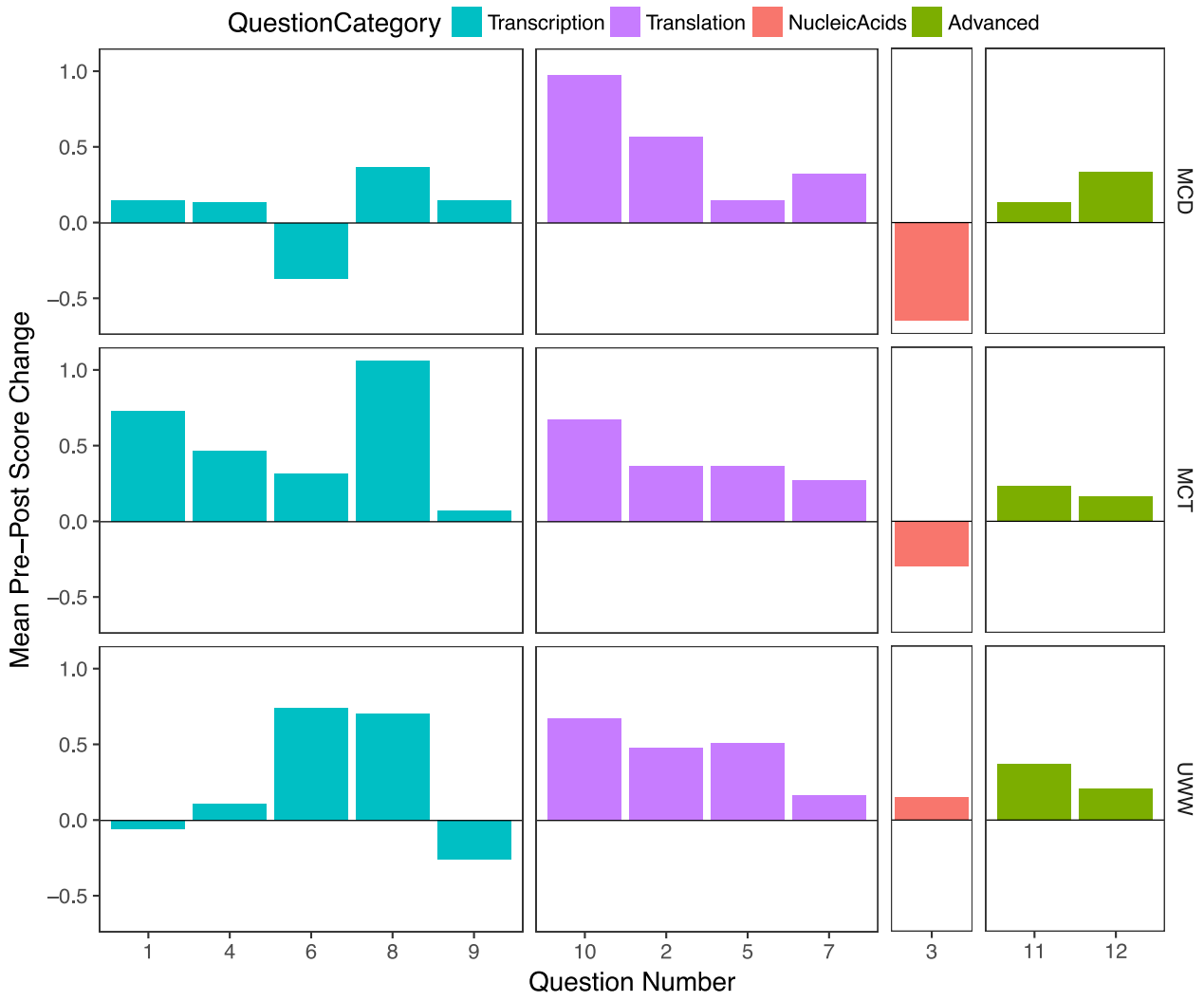


Figure III (right). Post-quiz scores by institution. Inroductory biology students from three institutions participated, Madison College-Downtown (MCD), Madison College-Truax (MCT), and the University of Wisconsin-Whitewater (UWW).

Figure IV. Student final scores relative to pre-quiz scores for each quiz question, presented by category. Responses to questions were scored giving full points for completely correct responses, partial points for responses that were correct but did not select all possible correct answer choices, and negative points for marking incorrect answer choices on a multiple select Central Dogma concept inventory. Inroductory biology students from three institutions participated, Madison College-Downtown (MCD), Madison College-Truax (MCT), and the University of Wisconsin-Whitewater (UWW).



Reflection

This internship was a valuable experience, providing me with insights into qualitative and quantitative data collection and management for teaching-as-research. It did not occur to me that faculty attitude would be an important factor with this project, but this emerged as the main determinant of whether students engaged with extra learning materials (*i.e.* students voluntarily engaged with supplementary materials when nudged by instructors, despite knowing there was no direct credit for this).

The authors of the CDCI assessment designed their questions for use in introductory biology classes populated by students majoring in the sciences. Although I adapted this assessment by selecting only some of its questions, using the provided course materials as a guide, a couple questions covered content presented in online tools but not in class. Despite inclusion in the in-class presentation slides and textbooks, classroom observations revealed that two of the faculty partners did not discuss the directionality of nucleic acids. By including this in the online supplement, students did not connect nucleic acid directionality to the mechanisms discussed in class or in the extra materials. Students in these classes failed to demonstrate improved understanding when asked about nucleic acids, according to the results from the CDCI assessment, and I suspect the lack of conversation around directionality as one contributor.

References

1. Wright, L. K., Fisk, J. N. & Newman, D. L. DNA -> RNA: What Do Students Think the Arrow Means? *Cell Biol. Educ.* **13,** 338–348 (2014).
2. Reinagel, A. & Speth, E. B. Beyond the central dogma: Model-based learning of how genes determine phenotypes. *CBE Life Sci. Educ.* **15,** 1–13 (2016).
3. Swan, A. E. & O'Donnell, A. M. The contribution of a virtual biology laboratory to college students' learning. *Innov. Educ. Teach. Int.* **46,** 405–419 (2009).
4. Newman, D. L., Snyder, C. W., Fisk, J. N. & Wright, L. K. Development of the central dogma concept inventory (CDCI) assessment tool. *CBE Life Sci. Educ.* **15,**1–14 (2016).
5. Adams, N. E. Bloom's taxonomy of cognitive learning objectives. *J. Med. Libr. Assoc.* **103,** 152–153(2015).