

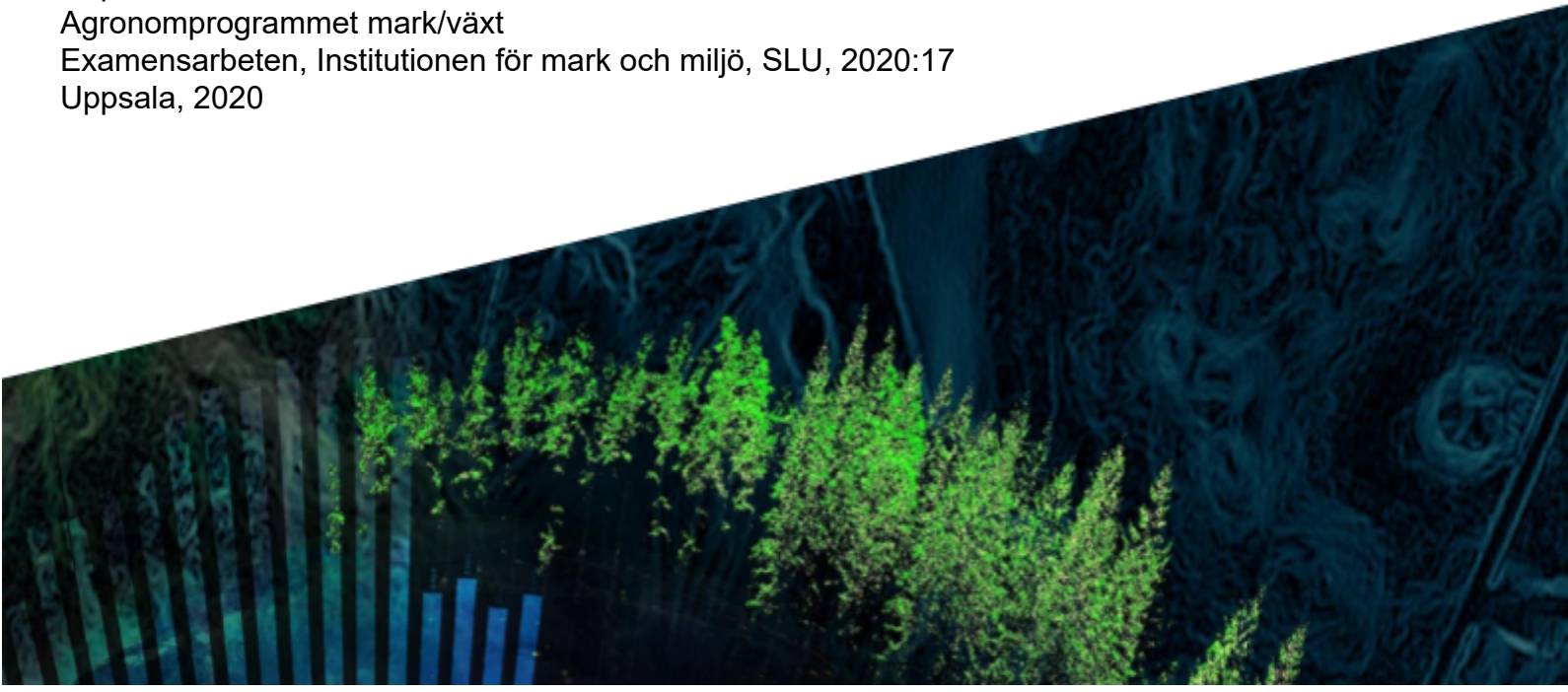


Arbuscular Mycorrhizal Fungi and its agricultural benefits

– how to succeed with AMF inoculation

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Abstract

Arbuscular Mycorrhizal Fungi (AMF) are obligate symbionts to 85% of all land-living plants. They provide P and other nutrients in exchange for carbohydrates produced in the photosynthesis. They have proven to alleviate biotic and abiotic stress and to increase soil structure and biodiversity. They also increase the quality and quantity of crop yield. These perks has sparked an interest in the agricultural field for commercially produced AMF inoculum. However, usage of these products has led to mixed results. The aim of this thesis was to evaluate twelve factors ,sorted into four main areas, and how they impact AMF efficiency from the perspective of yield increase. The areas were “inoculation composition”, “soil management”, “soil conditions and climate” and “crop qualities” (i.e. species and cultivar). A literature review was conducted using 22 articles. The articles were found on the Web of Science when searching for “yield” “field” “mycorrhiza” and handpicked based on themes related to the four areas.

Generally, the factors from the areas “inoculum composition” and “soil conditions and climate” were found to be most influential. Nutrition availability and factors closely related to this (i.e soil pH, fertilization and climate) had the most impact on inoculation efficiency. The effectiveness increased with the N:P ratio under low-moderate nutrient availability. The conclusion was that inoculated AMF has a lot of potential to serve as a way to streamline P uptake and reduce the doses of P fertilizers needed for satisfactory yields.

Keywords: crop yield, field conditions, inoculum, nutrient availability, soil management

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Glossary

Arbuscular Mycorrhizal Fungi (AMF): Fungal obligate symbiont with plant found in the phylum Glomeromycota

Native AMF: AMF species naturally present in the soil

Genus (plural genera): Here, subgroups within Glomeromycota

Inoculum: Here, AMF in the form of spores, mycelia and pieces of infected host root that are introduced to plant roots in order to colonize them

Multi/single species inoculum: Here, using one or more AMF species or isolate for inoculation

Host plant: Here, a temporary plant for mycorrhiza to colonize in order to produce inoculum

Propagule: Here, vegetatively produced spores, mycelia and/or infected host roots, each with the ability to colonize roots

Hyphae: Fungal body that grows underground in filamentous threads

Mycelia: A collection of hyphae that forms a web

Obligate symbiosis: When one or both parts in a mutualistic symbiotic relationship is depending on the other for survival

Biotic stress: Stress caused by living organisms such as insects, bacteria, virus or pathogenic fungi

Abiotic stress: Stress caused by mechanical, chemical and climate related factors. Examples are drought, flooding and pollution.

Abbreviations

AMF	Arbuscular Mycorrhizal Fungi
SOM	Soil Organic Matter

1. Introduction

1.1 The Fungi Kingdom

The fungi kingdom consist of heterotrophic decomposing organisms without the ability to photosynthesize. They can be single-celled, called yeast, or multicellular (Moran 2018). Organisms of this kingdom have cell walls containing chitin, a strong yet flexible material edified by polysaccharides, and multicellular bodies that are made up with filaments called hyphae (Moran 2018). The hyphae are thinner than roots and grow underground, where they absorb nutrients and water (Taylor et al 2009). Mineral nutrient uptake increases the ionic concentration inside the fungal cells and causes water to enter, leading to osmotic pressure. Cross-walls within hyphae, called septa, separate hyphal cells, with pores for nutrients to move through. The nutrients move through hyphae in the cytoplasm, following streams caused by osmosis, making transport of micro- and macro-nutrients possible. Some species lack septa, called coenocytic fungi, which makes nutrient transport easier (Moran 2018). For mycorrhizal fungi, this enables the fungi to effectively transport said nutrients between hyphae and root cells. Fungi put their resources on expanding hyphal length instead of circumference, increasing the total hyphal surface area. Networks of interconnected hyphae are called mycelia (Moran 2018). It is common for fungi to live in association with other organisms, such as pathogenic fungi and mutualistic species like mycorrhiza (Taylor et al 2009).

The fungi kingdom is divided into five phyla; *Ascomycota*, *Basidiomycota*, *Glomeromycota*, *Chytridiomycota* and *Zygomycota* (Tronsmo et al 2020).

1.2 Arbuscular Mycorrhizal Fungi (AMF)

Arbuscular mycorrhizal fungi (AMF) are obligate symbionts to plant roots, exchanging nutrients, especially phosphorus, and water for photosynthetic products. More than 85 % of all land-living plants cooperate with AMF (Tronsmo et al 2020). They are ecologically and agronomically important as they stimulate plant growth and increase production (Menge 1982 and Smith & Read 1997 see Singh & Thomas 2019). The hyphal network helps the plant to reach nutrients and

gain access to insoluble phosphorus and other important minerals such as copper, calcium and zinc (Sing & Giri 2017 see Sing & Thomas 2019). A common trait in mycorrhizal hyphae is the exudation of enzymes, phosphatases, that in reaction with polymeric phosphorus compounds releases phosphate P. P in phosphates is generally non-labile and tightly bound to mineral surfaces, and would be more difficult to access for the plants without mycorrhizal symbiosis. This increases the P-concentration in the pore water. (Danell-Huss & Carlsson 2016). About half of the plant net photosynthesis is consumed below ground, expanding root biomass and feeding mycorrhiza (Danell-Huss & Carlsson 2016)

AMF is found in the phylum *Glomeromycota* and about 280 different species have been identified (Tronsmo et al 2020). The phylum was classified in 2001 based on DNA-analysis, formerly included in *Zygomycota*. They propagate solely asexually, producing large multilayered spores ranging from 40-800 µm in size. The hyphae have no or few septa (i.e. cell walls inside the hyphae separating the nuclei) (Moran 2018). The spores can germinate without host plant roots but are unable to complete their life cycles without them (Parniske 2008).

AMF is characterized by its hyphae penetrating root cell walls where they taper and branch out to tree-like structures (i.e. arbuscules) (Danell-Huss & Carlsson 2016, Moran 2018). The arbuscules never penetrate the cell plasma membrane but form tubes that grow into invaginations in the membrane. Vesicles can be found on the hyphae inside the root cells and are hypothesized to act as food storages for the fungi (Benfey 2018)

The arbuscular structure optimizes the interface between plant and fungi and maximizes the nutrient exchange. (Danell-Huss & Carlsson 2016). There are many genera (singular: genus) in the glomeromycota phylum; *Acaulospora*, *Ambispora*, *Claroideoglomus*, *Diversispora*, *Funneliformis*, *Glomus*, *Paraglomus*, *Rhizophagus*, *Sclerocystis* and *Septoglomus* are some examples. Every genus has a unique spore morphology that serves as an easy way to tell them apart (Souza 2015).

1.3 Agricultural and environmental benefit

AMF acts as an elongation of the roots, reaching several centimetres into the soil from the root surface. The hyphae are thinner than roots and branched. Plants in symbiosis with AMF have ten times the below-ground surface area than plants lacking AMF partners (Danell-Huss & Carlsson 2016). The hyphae are able to reach nutrients inside of micropores inaccessible to roots. The hyphae can grow through air-filled pores and serve as a “transport bridge” between plants and

nutrients in soil water. It is estimated that up to 20% of the carbon fixated by plants through photosynthesis is consumed by AMF (Bago et al 2000 see Parniske 2008) and 1 cm³ of soil can contain up to 100 meters of hyphae (Miller et al 1995 see Parniske 2008)

AMF mycelia stabilize soil texture and form aggregates. Extraradical mycelium provides soil carbon, increasing the amount of organic matter. The hyphae, like roots, creates mechanical pressure on soil aggregates when penetrating the soil substrate. This creates micro aggregates next to the hyphae with unique microclimates for soil microbes. This contributes to soil carbon levels and unique local nutritional properties (Rilling & Mummey 2006, Barbosa et al 2019). Glomalin is a term that has been used to denote a variety of different compounds with hydrophobic and adhesive properties. These are exuded by AMF hyphae and cement soil particles together (Rilling & Mummey 2006).

In vivo different native AMF species affect the plant community composition, favouring certain plant species (Grime et al 1987 and van der Heiden et al 1998 see Rilling & Mummey 2006). The plant communities affect factors such as root biomass and soil fauna, having consequences for local soil aggregation (Rilling & Mummey 2006).

AMF has the potential to reduce plant stress by accumulation and immobilization of heavy metals in polluted soil (Kumar & Saxena 2019).

Several studies have shown that AMF species and isolates from polluted areas have a larger tolerance against heavy metal pollution than other species/isolates. This depends on soil-AMF interactions and AMF-plant interactions. Heavy metal is immobilized by organic acids exuded by the hyphae (Kumar & Saxena 2019). This dissolves the heavy metals which are then translocated from the soil into plant roots and mycorrhizal hyphae (Gonzalez-Chavez et al 2004 and Finlay 2008 see Kumar and Saxena 2019).

AMF fungi impact the plant hormone production in order to maintain a balanced symbiosis development (Gianinazzi-Pearson 1996 see Ravichandran and Muthukumar 2019). During stress such as drought or pollution, the fungi produces growth regulators that help the plant to cope with its environment (Nadeem et al 2014 see Ravichandran and Muthukumar 2019). For example, increased levels of auxins (i.e. growth hormones) has been noted in soybean and maize after inoculation with AMF (Kaldorf and Ludwig-Müller 2000 see Ravichandran and Muthukumar 2019). Plant immune systems are also affected; compounds involved in plant immune systems such as phenolic compounds (Larose et al 2002 see Ravichandran and Muthukumar 2019) and phytohormones involved in pathogen

resistance (Pozo et al 2002 see Ravichandran and Muthukumar 2019) are activated in the symbiosis. It can also be argued that pathogen resistance is indirectly promoted through increased fitness caused by increased nutritional status due to AMF symbiosis.

Because of its many positive effects there is a market in the agricultural field for commercially produced AMF. It is sold in many forms from liquid and gel to powder (BioOrganics LLC 2020). AMF in this form is called *inoculum* and is obtained by extracting spores, mycelia and/or root pieces from plants colonized with AMF. The inoculum can be applied to roots, seed or soil depending on the product (Berruti et al 2016, BioOrganics LLC 2020).

1.5 Aim

Answer the following questions

- Does inoculation with commercial AMF increase yield under field conditions?
- How does inoculum composition, soil management, soil qualities and crop qualities impact AMF effectivity in terms of yield increase?

2. Method

When I started working on this literature review I got recommended two papers; Hoeksema et al (2010) and Berruti et al (2016). I proceeded to search on the Web of Science database for the words “mycorrhiza” “yield” “field”, looking for the most recent literature, therefore limiting the publication years to 2015-2020. I included all results, i.e. experimental studies, reviews, meta-analyses and book chapters, to get a broad range of studies to choose from and ended up with 135 hits (31/5 2020). After sorting out literature about horticulture I had 84 hits. When adding “meta-analysis” in the search field a paper by Zhang et al (2018) could be included. This was particularly useful as it gave me four independent meta-analyses (Hoeksema et al 2010, Pellegrino et al 2015, Zhang et al 2018 and Rúa et al 2016) to compare to each other, adding more gravity to this report compared to working with just individual studies. I proceeded by reading through the abstracts and headings, handpicking articles based on theme, choosing articles discussing facts related to inoculum composition, soil management, soil conditions/climate and crop qualities. Outside of my search result range, I used a couple of articles for basic and additional information (Smith and Read 2008, Moran 2018, Tronsmo et al 2020 to mention a few). The book *Biofertilizers for*

Sustainable Agriculture and Environment by Bhoopander et al (2019) included several articles that came in handy for my thesis.

3. Result

3.1. Inoculum composition

3.1.1 Propagation of inoculum

A problem with finding an effective propagation method is that AMF are obligate symbionts and therefore need a host plant to survive. Working with and growing host plants takes much time and space. Three methods are described in a report by Berruti et al from 2016; propagation with host plants in soil substrate, host plants cultured in hydroponic/aeroponic systems and culturing roots.

Growing host plants in soil is described as the most widespread method for AMF propagation. Soilless culture systems such as aeroponics or hydroponics produce clean spores and suit large-scale production (Ijdo et al 2011 as cited in Berruti et al 2016). The risk of including unwanted soil organisms like pathogenic fungi is reduced in these kinds of systems. Also suited for large scale production is *monoxenic culturing* (i.e. root cultivation). This method consists of culturing carrots infected with “hairy root”, a disease caused by *Agrobacterium rhizogenes*. The bacteria is causing abnormal root growth due to a root-inducing plasmid. Roots containing this plasmid have the ability to grow on Petri dishes with products from photosynthesis replaced by sucrose in the growth medium, thus not needing any above-ground biomass. Using hairy root as hosts enable the cultivation of a large amount of inoculum in a less time- and space consuming way (Bécard & Fortin 1988 see Berruti et al 2016).

Different AMF species prefer different types of propagation. Species from the *glomeraceae* family colonizes roots most effectively when inoculated in the form of fragmented mycelia while spores better suit families such as *gigasporaceae* and *scutellosporaceae* (Brundrett et al 1999 see Berruti et al 2016).

Most studies reviewed by Berruti et al had plants inoculated with one of the three AMF species; *Rhizophagus intraradices*, *Funneliformis mosseae* and *Rhizophagus irregularis*. These species are generalist symbionts that can tolerate long-term storage, colonize a variety of hosts and are found all over the world (Öpik et al

2010 see Berruti et al 2016). They also have the benefit of being easily propagated in large amounts (Berruti et al 2016).

3.1.2. Single- and multispecies inoculum

There are two main types of inoculum compositions; single species and multispecies. Single species means that one AMF species is applied at a time per plant, while multispecies has several species applied. In the literature I found good results from usage of both single species- and multispecies inoculum. Between the two composition types, there was no statistically significant difference in terms of efficiency in the meta-analyses (Zhang et al 2018 and Hoeksema et al 2010). This was the case for field conditions but for lab studies the results were different.

Hoeksema et al came to the conclusion that for soil in a controlled lab environment multispecies inocula was substantially more effective than single species. Furthermore “whole soil inoculum” (i.e multispecies inoculum combined with soil including bacteria and fungi originating from the host plant) was more effective than adding only mycorrhiza.

This result is interesting because it indicates that biodiversity might favour plant response to AMF inoculation. The aspect of biodiversity will be further discussed under the “soil conditions” heading.

Pellegrino et al (2015) never evaluated the importance of inoculum composition but reported in their meta-analysis a 20% overall yield increase in wheat from commercial AMF usage. A majority, 88%, of their sources used single species inoculum.

Results from *table 1* indicate that multispecies inoculum is the most common composition type used in commercial mycorrhiza, as all commercial inoculants contain multiple AMF species, ranging from 2-7 species/ product. This can possibly be explained by the need for many applications. If a company wants to sell AMF inoculum for a variety of culture systems including pots, greenhouses and outdoor farming, multispecies inoculum is likely to give good results for all of these systems, especially for indoors farming.

Because of differences between AMF genera in the way they propagate, the recommended method for using multispecies inoculum is to include all types of propagules (i.e. spores, mycelia, host plant root). This is called *crude inoculum* and it maximizes the chances for the AMF mix to colonize the roots (Berruti et al 2016).

3.1.3. Species and origin

When looking at the different origins of the fungal inoculant Berruti et al found that inoculated native AMF was slightly more efficient than commercial AMF in terms of yield increase. The difference, however, was not statistically significant, with a p-value below 0.20. Studies show that AMF species differ between soils. As discussed in the introduction, soils with high levels of stress and pollution host AMF species adapted to such conditions (Leyval et al 2002 see Berruti et al 2016) (Ravichandran and Muthukumar 2019). Berruti et al therefore suggest that a careful selection of favourable host/niche/fungus combinations could be important for the inoculation outcome.

Different AMF families and genera, has different qualities. The same holds true for isolates within the same species. Based on the fact that AMF are obligate symbionts and share an evolutionary history with plants and soil, there might be an advantage in combining AMF and soil with the same geographic background (Rúa et al 2016). Rúa et al investigated this phenomenon on wild plant species populations in a meta-analysis. Their aim was to find out how inoculation of locally adapted AMF populations influenced plant response in soil compared to non-native AMF. They came to the conclusion that *sympatric* relationships (i.e same geographic origin) between plant, fungi and soil led to a more positive response than *allopatric* relationships (i.e. different geographic origin) between the three. In cases where plant-soil were sympatric to each other and the fungi had a different geographic origin, Rúa et al observed the most biomass increase in response to inoculation. Most importantly, however, there was no advantage for sympatry between fungus-soil with allopatric plants, compared to allopatry between plant, soil and fungus. In agricultural practices the crop is to a large extent annual and cannot be expected to share origin with the soil or local AMF populations. Regardless of geographic origin, fields inoculated with AMF always yielded more biomass than non-inoculated fields (Rúa et al 2016) .

There is much evidence for the efficiency of commercial AMF significance on yield increase in field. Many papers report positive outcomes from usage of commercial fungi (Pellegrino et al 2015, Cely et al., Zhang et al 2018, Hijri 2016). For example, Cely et al (2016) conducted a study with AMF used on soybean and cotton, applying a single species inoculum of *Rhizophagus clarus*. Crude inoculum containing colonized roots, mycelia and spores was used. The cotton and soybean seeds were palletized with the crude inoculum in an organic matrix. *R. clarus* infected the plant roots effectively, proving to be a good competitor with

native AMF. The native AMF activity was high, indicated by the colonization rate in control plants.

To find out which AMF species are agriculturally important I conducted a small study, since that specific information was hard to find in my web of science result range. I googled the words “mycorrhiza product crop” (30/5 2020) and chose the first three commercial producers with information about specific AMF species in their product. Two products from each producer were manually picked based on certification; one for organic farming and one for conventional use, except for one brand with exclusively organic products.

Product	AMF species	Organic certificate	Monospecies/ multispecies
Valent Mycoapply EndoPrime	- <i>Rhizophagus intraradices</i> - <i>Funneliformis mosseae</i> - <i>Rhizophagus aggregatus</i> - <i>Claroideoglossum etunicatum</i>	No	multi
Valent MycoApply Endomaxx Organic	See above	Yes	multi
Atens Aegis Sym Gel	- <i>Rhizophagus irregularis</i> - <i>Funneliformis mosseae</i>	Yes	multi
Atens Team Microgranulo	See above	No	multi
BioOrganics Mycominerals Soil Amendment	- <i>Rhizophagus aggregatus</i> - <i>Claroideoglossum etunicatum</i> - <i>Rhizophagus intraradices</i> - <i>Funneliformis mosseae</i> - <i>Rhizophagus clarum</i> - <i>Septoglossum deserticola</i>	Yes	multi

	- <i>Glomus monosporum</i> - <i>Gigaspora margarita</i> - <i>Paraglomus brasilianum</i>		
BioOrganics Endomycorrhizal Inoculant	See above	Yes	multi

Table 1. AMF species used in six commercial inoculum products from three producers: Valent (ValentBioSciences LLC 2020) , Atens (Agrotechnologias Naturales Atens 2020) and BioOrganics (BioOrganics LLC 2020). Some species names were altered based on new data from *indexfungorum.org* 1/6 2020 (Indexfungorum Partnership 2020)

3.2 Soil Management

3.2.1. Tillage

An option to inoculation is to cultivate and care for the naturally occurring AMF communities in the field. Scientists have hypothesized that agricultural interventions disrupt hyphae and inhibit the positive effects of AMF (Helgason et al., 1998 and Castillo et al., 2006 as cited in Pellegrino et al 2015). Therefore one method to increase colonization and crop yield could be to promote native AMF with reduced or no-tillage soil management. Zhang et al evaluated three forms of interventions to manage AMF; direct application by *inoculation* and manipulation of already present fungi using *rotation* and *no-tillage*. Rotation management consisted of having crops rotated or intercropped with AMF host plants. The no-tillage treatment consisted of trials using no- or reduced tillage in order to create an advantageous environment for AMF, with no disturbance to the mycelial network. Inoculation proved to be the most efficient treatment in terms of yield increase, the other two had no significant effect of AMF impact on grain yield (Zhang et al 2018). Unfortunately in the literature there was no information about AMF inoculation combined with no- or reduced tillage management practices.

Hirji. M. studied 231 field trials where a single-species inoculation using *Rhizophagus irregularis* was applied to potato seed. The trials lasted four years and gave some promising results in terms of AMF effectivity on crop production combined with conventional tillage. The study was done in cooperation with farmers located in northeast America and Europe who inoculated the tubers themselves. All of them used conventional agricultural practices; pesticides, conventional fertilizer and tillage. They exerted a rotation system switching

between potato, wheat and barley. The seed pieces were inoculated with a liquid suspension, containing AMF spores. Yield increased highly significantly, with inoculated fields producing an average yield of + 9.5 % compared to non-inoculated fields (Hijri 2016) .

3.2.2.Fertilization

Fertilization goes hand in hand with nutrient availability. Organic fertilizers comes from manure and commercial fertilizers, such as NPK, are chemically produced. The most important difference between the two is the form in which the nutrients are introduced to the soil. As the name suggests, organic fertilizers are bound to organic compounds and must be mineralized before they become plant available. Commercial fertilizers are already in mineralized form (jordbruksverket 2020). The impact of *nutrient availability* on AMF will be discussed later in the report. Most of the studies reviewed in my literature deal with conventional fertilizers.

Smith and Read found that P-fertilization did not significantly affect plant response to AMF. They found, however, that conventional fertilizer with plant-available P did not promote as much colonization as slower-moving organic P (Ryan et al 1994, 2000 see Smith and Read 2008).

An Australian study was conducted with commercial P-fertilization on AMF inoculated bell pepper. Five treatments of two sets each were fertilized with P-levels ranging between 0 kg P/ha to 135 kg P/ha. One set was inoculated with AMF and the other was not. The yield levels were highest for inoculated plants except for the treatment with the largest fertilization dose of 135 kg P/ha (Olsen et al 1999 see Smith and Read 2008). Zhang et al could not find a significant reduction in AMF impact on grain yield by NPK-fertilization. However, though the difference was not statistically significant, low N- and P-nutrient soils with no fertilization had a slightly higher AMF *effectivity* than other soils (Zhang et al 2018).

Cely et al studied the effectivity of inoculated single-species AMF on soybean and cotton yield compared to the effectivity of chemical fertilizer. The study was conducted using five different treatments: 200 kg NPK/ha, AMF, AMF + 200 kg NPK /ha, AMF + 100 kg NPK/ha and a control without fertilizer or AMF. The authors found that AMF in combination with fertilizer increased both the cotton and the soybean yield. For soybean, the yield was highest in treatments with AMF+200 kg. N and P- uptake increased with 24% compared to using only fertilizer. For cotton, AMF inoculum treatment could compete in terms of yield

with fertilizer-only and AMF+ 100 kg NPK/ha. Naturally occurring soil P availability was reportedly moderate in soils used in the experiment (Cely et al 2016). Similarly, Hijri combined conventional NPK with AMF in potato fields and consequently observed a significant yield increase (Hijri 2015). This effect probably depended on the store of tightly bound P that was inaccessible to non-mycorrhizal plants.

The results indicate that fertilization in itself does not inhibit AMF colonization. On the other hand, as observed by Smith and Read, high P doses might have an inhibiting effect. This can be tied to levels of P-availability rather than fertilization.

Hoeksema et al observed in their meta-analysis that N-fertilization was a highly significant indicator for plant response to AMF. Their results showed that the addition of N correlated with decreasing AMF benefits while P-fertilization was consistently relatively unimportant for the outcome. The authors note that the naturally occurring N and P- levels likely played a part in these results. This was hypothesized to be connected to the N:P ratio, a factor proven to be significant for plant response. The N-fertilized soils naturally contained low N-volumes and relatively high P-levels. This reduced both the nutrient limitation and the N:P ratio, undermining the need for AMF. Tissue in non-inoculated plants showed that the N:P ratio was similar between fertilized and unfertilized soils, leading to P-fertilization seeming relatively unimportant (Hoeksema et al 2010). The mechanisms and influence of the N:P ratio will be further discussed under *nutrient availability*.

3.3 Soil conditions and climate

3.3.1 Climate

There was a limited amount of data for how climate (interpreted as mean annual precipitation and temperature) determined the AMF effectivity in terms of yield. Pellegrino et al's results for this parameter point to the importance of water availability and high enough temperature during early stages of plant development. It is reasonable to hypothesize that climatic factors, as they largely influence soil biochemistry and soil-water conditions, could play an important role in AMF effectivity (Pellegrino et al 2015). Temperature affects evaporation and chemical reactivity with consequences for the ionic concentration and composition in the groundwater. Water transports, dilutes and redistributes the

nutrients. These processes in turn directly and indirectly influence pH, nutrient availability, soil texture and soil structure.

Kilpeläinen et al tested the impact of temperature and humidity on AMF development, based on root colonization of *Populus angustifolia* seedlings. A mild drought was simulated at 14, 20 and 26 °C. Colonization was lowest in 14 °C, both in the dry and adequate watering treatment. Notably, the spore production was considerably lower than in the higher temperatures. Drought led to a colonization reduction of 20% but did not affect root biomass. Plant root growth was mainly inhibited by low temperature (Kilpeläinen et al 2020).

3.3.2 Soil pH

According to Zhang et al (2018), soil pH had a significant impact on the effectiveness of AMF inoculum. On neutral to acidic soils its effect on plant production was higher than in alkaline soils. This can be explained with nutrient availability. The pH value in soil systems plays an important role in soil nutrient availability. At pH<6.0 Fe, Cu and Zn is dissolved and at pH>6.0 N, P, K, S, Ca and Mg is dissolved. Above respectively below pH 6 the nutrients are immobilized and harder for plants to get (Storey A. 2016). As plants generally rely on AMF for proper P supply, AMF is extra important at low P availability. Low pH immobilizes P and makes it less available for plants. Studies indicate that this favours AMF growth (Pellegrino et al 2015, Zhang et al. 2018) as it makes the plant more dependent on AMF (see results for *nutrient availability* below).

3.3.3. Soil texture and structure

The effect of AMF on soil texture and structure-building aggregates has been established in the introduction; AMF contributes to soil aggregation by exuding organic acids and hydrophobic proteins that serves as adhesives for soil particles. This creates microhabitats which increase biodiversity and soil organic matter (Barbosa et al 2019).

Berruti et al evaluated whether or not soil texture and soil organic matter (SOM) had an impact on AMF and its effect on grain yield. There were two levels to the texture parameter: *sandy* referring to soils with 50% sand or higher and *not sandy* for other soils. This factor could not be correlated to AMF and its impact on yield increase.

A well-structured soil with a high amount of organic matter favours root development (Barbosa et al 2019). This would positively affect the allocation of biomass and photosynthesis products below-ground. Fungi would, according to

this reasoning, benefit from the increased production of carbohydrates, provided of course that there is a well-established root-fungus collaboration. Berruti et al evaluated the effect of SOM for low, medium and high proportions of organic matter and found that this factor on its own did not affect the benefit on yield (Berruti et al 2016).

3.3.4 Nutrient availability

Kilpeläinen et al evaluated climate related factors and their impact on AMF hyphal growth (see *climate* above). Low temperature and drought was found to inhibit AMF colonization. Other explanations could have to do with nutrient availability as a direct consequence of the climatic factors. At the lowest temperature of 14°C, levels of soluble S, Ca, and Mg were lowest. P levels remained the same in all three temperatures (14, 20 and 26°C) while N availability was clearly highest in 26°C (Kilpeläinen et al 2020). It seems here that the increased soluble nutrient concentration might have favoured AMF development. Worth noticing is the fact that the P-levels remained unchanged while plant available N increased. This affects the relationship between N and P, increasing the N:P ratio. A positive correlation between high N:P ratios and AMF effectivity has been confirmed in the meta-analysis by Pellegrino et al (2015), who observed that high N-levels combined with low P-levels promote AMF development, based on yield outcome and colonization rate (Pellegrino et al 2015). This is in line with the results from Kilpeläinen et al and Hoeksema et al. I will further discuss the mechanisms behind this phenomenon.

N-limitation negatively affects the plants' photosynthetic ability and with less photosynthesis, there will be fewer carbohydrates for the fungi. That means that general hyphal nutrient uptake will decrease as well. The principle reads: more N in plant tissue leads to a higher P uptake because of increased photosynthesis, and carbohydrate-nutrient exchange between fungi and plant (Hoeksema et al 2010). With an *abundance* of N in the soil however, the response to AMF decreased as results showed a clear negative correlation between N-fertilization and AMF effectivity (Hoeksema et al 2010). This is hypothesized to be explained by plant roots not being dependent on their fungal allies in the search for N, consequently not contributing with C to the hyphae, and by the relationship between available N- and P-levels (Smith and Read 2008, Hoeksema et al 2010) This indicates that there is an upper limit to the correlation between N-availability and positive AMF feedback.

AMF specializes on P-uptake and many plant species depend on AMF for sufficient P-supply. Low P availability correlates with increased benefits of AMF inoculation (Stribley et al 1980 as cited in Hoeksema et al 2010, Smith and Read 2008). This could be explained with the nature of the plant-fungus symbiosis. AMF helps make available P, “making itself useful” for the plant and encouraging investment in the symbiosis. If P is available to the plant without the help from AMF, there’s less need for the plant to supply the fungus with its precious carbohydrates. This principle is generally true for any nutrient limitation (Hoeksema et al 2010), but because of its crucial role as a P-supplier, P-availability has an extra-large impact on AMF development and consequently its contribution to crop yield. This explains the importance of the N:P ratio and serves as a reminder of the complex ecological interplay below-ground. Further, it is possible that in case of low P:N ratio signals are sent to the plant to invest more C in the symbiosis, making it possible for AMF to collect more P.

3.3.5. Soil biodiversity

The process of aggregation creates microhabitats for soil organisms and enhances soil biodiversity (Rilling & Mummey 2006 see Barbosa et al 2019). For Hoeksema et al soil biodiversity in terms of inoculum composition was significant for AMF impact on yield. This conclusion was drawn upon results showing that “whole soil inoculum” (i.e multispecies inoculum with soil including microbes and non-AMF-fungi from the host plant) led to significant AMF benefits (Hoeksema et al 2010). As mentioned, this was the case for lab studies but not for field conditions.

Berruti et al (2015) argue that because of soil microbial activity and its role in mineralization and immobilization, it would be reasonable to assume that this activity impacts AMF.

However in field, Zhang et al found no correlation between AMF effects on plant growth and the addition of a mixed bacterial inoculum. This was also the case for Rúa et al. As whole soil inoculum presumably contains bacteria and other microbes from the same background as the AMF, this “sympatry” if you will, may play a part in the effectiveness compared to the addition of alien microbes. It is, however, unclear whether it is the multiple species aspect or the bacterial/microbial aspect of the whole soil inoculum that determines the AMF effectivity in this case. In lab, since multispecies AMF was important for the outcome but soil sterility and the addition of soil microbes were not, Rúa et al argue that diversity in the form of *other AMF species* may be important rather

than non-mycorrhizal soil organisms (bacteria, other fungi). For Hoeksema et al supplementary microbes had a significant impact only in greenhouse environment but not in field.

Another indication of the relative unimportance of soil biodiversity in terms of yield increase is the fact that soil sterility was not significant for AMF effectivity in any of the meta-analyses (Hoeksema et al 2010, Rúa et al 2016, Zhang et al 2018 , Pellegrino et al 2015).

3.4 Crop qualities

3.4.1 Plant species

The literature covers a wide range of crops; soybean, cotton, wheat, rice, corn, sorghum, barley and potato to mention some. Most species in the literature responded positively to AMF inoculum in terms of yield, growth and nutrition levels. Berruti et al, for example, evaluated plants from 43 different families, with overall good AMF response. Exceptions to the rule were species belonging to Brassicaceae and Amaranthaceae, with no or insignificant AMF response, and the Poaceae family with a reported low response (Nieves et al 2018).

Because the studies were made under different conditions, they are hard to compare fairly to each other without conducting a proper meta-analysis. I will mention crops that stuck out or showed no response to AMF inoculum and compare the different results from the meta-analyses.

Zhang et al (2018) conducted a meta-analysis, analyzing five globally important crops and the effect of mycorrhiza on the grain yield under field conditions; wheat, rice, barley, corn and sorghum. In a majority of field inoculation trials the effect was positive. Overall, inoculation lead to a mean increase of 16% on grain yield for all crops. For each crop the numbers were the following; rice + 17%, wheat +17%, corn +13%, sorghum +37% and barley +0.94%. The authors highlight that in the case of barley and sorghum there was a small amount of data, leading to a high variability in the results.

The meta-analysis about wheat response to AMF inoculation by Pellegrino et al showed that all of the plant traits related to nutrient uptake (i.e. straw and grain yield, P concentration and content, N content in grain) except for N content in straw and N concentration in grain showed statistically significant positive responses to inoculation. Mean increase in grain yield was 20%, and an increase of 20% and 31% in grain P- and N-content, respectively (Pellegrino et al 2015).

The two parameters *grain P-level* and *P-concentration* in biomass above ground both increased by 16%.

This result is comparable in terms of wheat yield to that of Zhang et al (+20% compared to +17%).

Fabaceae with N-fixing bacteria responded less positively to inoculation than other plants (Hoeksema et al 2010). The authors note that they generally used soils with high P-levels. This combined with N-fixing symbionts prevent N- and P-limitation, which in turn can inhibit AMF development (as discussed in *nutrient availability* above).

3.4.2 Cultivar

One factor that made a significant difference on plant response to AMF for Zhang et al was the year of release. Cultivars released after 1950 responded less to the AMF treatment than wild relatives and cultivars released before 1900 (Zhang et al 2018). This could be related to biomass allocation differences produced in the domestication process. Old cultivars or wild relatives, for example, generally develop more underground biomass (root system) than new ones. New cultivars, however, generally have shorter straw and a more shallow root system with thinner roots but more grain biomass (Pellegrino et al 2015, Pérez-Jaramillo et al 2018). As AMF is a root symbiont, altering the root biomass could negatively impact response to inoculation. Another possible explanation is that new cultivars are bred to suit high nutritious conditions where investment in an AMF symbiosis is redundant. The genetic tendency to invest in mycorrhiza could in this case be diminished.

For new cultivars though, a successful and effective AMF colonization could possibly mean that the plants maximize their biomass allocation and that leads to a larger grain yield in relation to required fertilization. Pellegrino et al drew the opposite conclusion to Zhang et al in their meta-analysis on wheat response to AMF. They observed a negative correlation between AMF response and straw biomass. As cultivars of wheat and several annual crops are bred to generate grain harvest at the cost of straw biomass (De Vita et al 2007 see Pellegrino et al 2015), the correlation indicates that plants with shorter straw (new cultivars) respond better to AMF inoculation. The authors hypothesize that in light of this fact, new cultivars are more responsive to AMF than ancestor ones (Pellegrino et al 2015). Nieves et al published a study in 2018 on the impact of domestication of crops and its consequences for AMF response. They compared wild ancestors to modern day crops. The response in terms of biomass increase was equivalent between the

two under low P conditions. For high P conditions, however, while the ancestor plants continued to allocate biomass, the positive effects in domesticated crops decreased more dramatically and the symbiosis became costly or ineffective. Berruti et al raise the thought that plant response to AMF should be taken into consideration in modern plant breeding programs (Berruti et al 2016)

4. Discussion

The aim of this thesis was to answer the two following questions:

- Does inoculation with commercial AMF increase yield under field conditions?
- How does inoculum composition, soil management, soil qualities and crop qualities impact AMF effectivity in terms of yield increase?

The answer to the first question is yes, inoculation does indeed have a significant positive effect on AMF colonization in field. The colonization rate is reflected in crop yield increase, potentially up to 20%, and better nutrient quality (Pellegrino et al 2015, Hijri 2016, Zhang et al 2018).

What motivates AMF usage in agricultural practices, is the potential for an ecologically sustainable complement to fertilization. P in conventional fertilizers is largely recovered from rock-phosphate, a non-renewable resource (Smith and Read 2008, Huey et al 2020) and the global food supply is to a large extent depending on P from rock-phosphate. AMF is specialized through its evolutionary history to extract non-labile P compounds from mineral formations (Samreen and Kausar 2019). Added P that is not absorbed by roots is either transported away via water or accumulated in non-labile form tightly bound to soil particles. With an abundance of P, erosion and heavy rainfall risk to carry it away from the field. Mycorrhiza, as well as increasing uptake, helps dissolve non-labile P, increasing P-mobility (hence plant availability) and counteracts accumulation. The nutritional uptake gets more effective and less fertilizer needs to be used to reach satisfactory yield levels. These are some examples of how AMF symbiosis in agriculture has the potential to reduce the use of rock-phosphate. For the factors with no correlation to yield increase (see *table 2*) there are other benefits worth taking into account when working towards a more ecologically sustainable agriculture, for example long term soil quality and biodiversity as a consequence of structure and texture related improvements.

The idea when starting this literature study was to find out whether it had been proven to be economically sustainable with AMF inoculation. However, most of the literature lacked economic analyses in order to find out if AMF inoculation generated a net profit. Two exceptions were the reports by Hijri 2015 and by Smith and Read 2008. For Hijri, AMF inoculation in potato fields generated a mean yield increase of +3.92 tons/ha, surpassing the threshold for net profit of +0.67 tons/ha with good marginals (Hirji 2016). In the bell pepper trial by Olsen et al a non-inoculated treatment with full fertilization dose was more economically profitable than in its inoculated counterpart. The authors note that if the prices on P-fertilizer would increase, this would change (Olsen et al 1999 see Smith and Read 2008).

The answer to the second question has been evaluated and summarized in *table 2* below. The most important conclusions were that the following factors had a statistically significant correlation to AMF-induced biomass increase in field; AMF source, fertilization, climate, soil pH, nutrient availability, crop type and geographic origin. Other factors such as inoculum composition, AMF species, soil management, texture/structure, soil organic matter, soil biodiversity and cultivar did not. Optimal conditions for AMF impact on yield is a high N:P ratio combined with a general low-moderate nutritional soil status and pH<6.0. The temperature should be at least 14 °C, preferably above 20 °C, and water supply should be adequate. Fertilization had less impact than expected, as studies by Hijri et al, Cely et al and Olsen et al (described by Smith and Read 2008) show that it is possible to achieve a significant yield increase in combination with half and/or a full dose of NPK.

Because of the complex interplay in and the variability between soil ecosystems it is hard to control the exact outcome of AMF inoculation. It remains an exciting challenge for growers and scientists to unlock the potential of AMF and work towards a more sustainable agriculture. Continued research on the mechanisms behind plant AMF response is needed, in order to increase the predictability of inoculation and its impact on yield increase. Observations of wild relatives to crop species and their continued positive net response to AMF under high P conditions may serve as an inspiration for future plant breeding projects.

This thesis goes through the basics of AMF and its effect on crop yield increase. It concludes the current state of knowledge and can be useful for farmers, hobby growers or anyone interested in the workings of AMF.

5. Conclusion

Factor evaluated	Best AMF effectivity	No or negative AMF effectivity
<i>AMF source</i>	Inoculation	Favour already present species (no effect)
<i>Inoculum composition</i>	<ul style="list-style-type: none"> • Multispecies inoculum* • Crude inoculum* 	-
<i>AMF species</i>	-	-
<i>Soil management</i>	-	No-tillage (no effect) Fallow (negative effect)
<i>Fertilization</i>	No fertilization	-
<i>Climate</i>	<ul style="list-style-type: none"> • Adequate water availability • Temperature > 14°C 	-
<i>Soil pH</i>	pH<6.0	-
<i>Soil texture and structure</i>	-	<ul style="list-style-type: none"> • Soil texture (no effect) • Soil structure (no effect)
<i>Soil Organic Matter</i>	-	% SOM (no effect)
<i>Soil nutrient availability</i>	<ul style="list-style-type: none"> • High N:P ratio • P-limitation 	High levels of available P and N (negative effect)
<i>Soil biodiversity</i>	Several species of AMF	Addition of microbes (no effect)
<i>Crop type</i>	Any crop with AMF symbiosis	N-fixing plants in high P-soil inhibit effectivity Brassicaceae and Amaranthaceae (no effect) Poaceae (low effect)
<i>Cultivar</i>	-	-
<i>Geographic origin</i>	Best: sympatry between plant and soil Second best: sympatry between plant/soil/fungus	-

Table 2. *A conclusion of the impact of each factor on AMF effectivity in terms of crop yield increase. Blank spaces indicate that no relevant data was found or that the data was insufficient.*

* *only relevant for lab cultivation*

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