Sveriges lantbruksuniversitet
Swedish University of Agricultural Sciences

Faculty of Landscape Architecture, Horticulture and Crop Production Science

The effect of Arbuscular mycorrhizal fungi and biostimulating algae extract on establishment, growth and development of *Vitis vinifera*

– A field study conducted in Sweden

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– En fältstudie genomförd i Sverige

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Degree Project • 30 hec
Hortonomprogrammet /Horticultural Science
Alnarp 2020

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Credits: 30 hec
Project level: A2E, master's thesis
Course Title: Independent Project in Biology, A2E
Course Code: EX0856
Subject: Biology

Programme: Hortonomprogrammet /Horticultural Science

Place of Publication: Alnarp
Year of Publication: 2020
Cover Art: Hanna Silwer
Online Publication: http://stud.epsilon.slu.se

Keywords: Arbuscular mycorrhiza, Biostimulator, Crop physiology, Mineral fertilizers, Precision farming, *Vitis vinifera*

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Acknowledgements

This masters thesis would not have been possible if not Kullabergs vineyard was as genoruos with both lending of land, plant material and time. I would like to dedicate a big thank you to all involved in the vineyard.

I would also like to elevate the importance of good supervisors. Lotta Nordmark and Malin Hultberg made a huge difference in both field trial implementation and writing process. Thank you for all the support, well targeted critique, time and motivation.

Additional acknowledgement is due to Siri Caspersen, who aided me in the lab and supervised my analysis of the root samples. She was generous enough to aid me, even though she was not an assigned supervisor. Jan-Eric Englund, who helped me with the large amount of collected data and statistical analysis of the field trial, diserve all cred possible.

Gratitude is also adressed to Partnerskap Alnarp and The Royal Swedish Academy of Agriculture and Forestry. They supported the thesis field trial financially as well as enabled me to travel and take part of both viticulture research and precision farming around the world.

Hanna Silwer

Alnarp, January 2020

Abstract

About 23% of total global net anthropogenic emissions of greenhouse gases (GHG) come from agricultural and forestry related activities. One of the largest contributors of GHG is the usage of nitrogen (N) and phosphorous (P) fertilizers, causing eutrophication and contributing to global warming. Agriculture needs to become more productive, but with a reduced environmental impact. The solution might be usage of plant biostimulators.

Biostimulators are fairly new within the agricultural sector and are used in order to improve plant growth. They can neither be classified as a fertilizer, nor plant protection. Biostimulators increase availability and uptake of macro- and micronutrients and the most famous plant biostimulator is Arbuscular mycorrhiza (AMF).

AMF and a biostimulating algae extract were inoculated on bare root *Vitis vinifera* plants. During a period of 8 weeks they were phenologically assessed with a BBCH-scale, soil samples were taken and roots extracted and analyzed in microscope.

The result showed no increased growth of vine shoot, when inoculated with biostimulator. An increase in vegetative growth of axillary shoot was observed for plants inoculated with AMF in comparison to a control, suggesting increased uptake of N and P.

Factors possibly affecting the result might have been abiotic factors, amount of inoculum applied prior to planting, damage to the vine apex or levels of P in the soil. Future studies would have to investigate whether other concentrations of inoculum would generate a different result as well as if symbiosis is viable over time and not only for one season.

Keywords: Arbuscular mycorrhiza, Biostimulator, Crop physiology, Mineral fertilizers, Precision farming, *Vitis vinifera*

Sammanfattning

Ungefär 23% av totala växthusgaser, från mänsklig aktivitet, kommer från jordbruks- och skogsindustrin. En av de största bidragande faktorerna till växthusgasutsläpp är användandet av kväve- (N) och fosfor- (P) mineralgödningsmedel, vilka bidrar till övergödning och global uppvärmning. Jordbrukssektorn behöver bli mer produktiv för att kunna upprätthålla jordens befolkning, men till en reducerad inverkan på miljön. En del av lösningen kan vara att använda växtbiostimulatorer.

Växtbiostimulatorer är relativt nya inom jordbrukssektorn och används i syfte att öka grödors tillväxt. De kan varken klassificeras som gödnings- eller växtskyddsmedel. Biostimulatorerna ökar tillgänglighet samt upptag av mikro- och makronäringsämnen, den mest använda växtbiostimulatorn är Arbuskulär mykorrhiza (AMF).

AMF och biostimulerande algextrakt inokulerades på *Vitis vinifera* barrotsplantor. Under en period på 8 veckor utvärderades plantornas tillväxt enligt en BBCH-skala, jordprov togs på försöksfältet samt rötter extraherades och analyserade i mikroskop.

Resultatet visade ingen signifikant skillnad i vegetativ tillväxt på vinplantans skott för plantor inokulerade med biostimulator. En ökning i vegetativ tillväxt observerades för sidoskotten på plantor inokulerade med AMF i jämförelse med en kontroll, vilket kan tyda på ökat upptag av N och P.

Faktorer som potentiellt kan ha påverkat resultatet var abiotiska faktorer, mängd inokulum av biostimulatorerna som tillsattes på rötterna innan plantering, mekaniska skador på plantans skott samt mängd P i jorden. Framtida studier skulle behöva undersöka huruvida andra koncentrationer av inokulum skulle generera ett annorlunda resultat samt om symbios är livskraftig över tid och inte under endast en säsong.

Nyckelord: Arbuskulär mykorrhiza, Biostimulator, Mineralgödningsmedel, Precisionsodling, *Vitis vinifera*, Växtfysiologi

Popular scientific summary

Did you know that when you purchase a bottle of wine, you have contributed to global warming? You might even be considered a bad guy, since eutrophication kills several aquatic species every year.

Applications of fertilizers, which occur in viticultural practices prior to the winemaking, is a major issue for the sustainability of vine cultivation. In order to supply the weekend celebrators with this much desirable and cultural beverage, more vines has to be cultivated in order to supply the wine industry with sufficient yield. But how could the vine produce more, to a lower environmental cost?

Usage of plant biostimulators has showed reduced need of fertilizer application. How could that be possible? Easy, they aid the crop in nutrient uptake! Arbuscular mycorrhiza (AMF) is the most famous and known plant biostimulator, but algae and algae extracts seem to climb in status within the agricultural sector.

In this trial, vines were inoculated with AMF and algae extracts. The result showed that there can in fact be an increased vegetative growth of the crop, however only if inoculated with AMF. The other treatments need further development and research.

So what does this mean? If vines are inoculated with AMF, the vegetative growth is increased and thus there is a more efficient uptake of P from the soil. This uptake would reduce risk of nutrient runoff, reduce the need of fertilizer application and reduce greenhouse gas emissions since agricultural machines would be used to a lower extent.

What can be concluded from this study is that with the aid of a little fungus, you could celebrate the weekend with a clear conscience. With a bit of help from AMF, you will no longer contribute as much to global warming and you will be cleared of any criminal charges. Cheers!

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Abbreviations

Abscisic acid – ABA

Ammonium – NH_4^+

Apatite – $Ca_5(PO_4)_3OH$

Arbuscular Mycorrhizal Fungi – AMF

Carbon dioxide – CO_2

Dihydrogen phosphate – $H_2PO_4^-$

Growing Degree Days – GDD

Hydrogen phosphate – HPO_4^{-2}

Inorganic phosphate – P_i

Intergovernmental Panel on Climate Change – IPCC

Nitrate – NO_3^-

Nitrous oxide – NO_x

Vitis vinifera L – *V. vinifera*

1 Introduction

The Intergovernmental Panel on Climate Change (IPCC) frequently publishes reports on how climate change affects the earth. They also identify which sectors are responsible as well as suffering from climate change. In April 2019, they published a report regarding climate change, desertification, land degradation, sustainable land management, food security, and greenhouse gas fluxes in terrestrial ecosystems. According to IPCC (2019), 25-33% of land's potential net primary production is used by human in order to produce food, feed, timber and energy. The net primary production is defined by IPCC (2019) as "The amount of carbon accumulated through photosynthesis minus the amount lost by plant respiration over a specified time period that would prevail in the absence of land use".

Since the early 1960s there has been a fast growth of the global population and there has also been a change in consumption of food and natural resources per capita (IPCC, 2019). The growth of population has been supported by agricultural production systems and the productivity has increased remarkably. Nevertheless, this has caused an increased greenhouse gas emission as well as eutrophication, which has deprived earth of many natural ecosystems and reduced global biodiversity

IPCC (2019) finds that the global warming has caused food security related issues. Many lower latitude regions suffer from reduced yield, due to warming and more frequent extreme weathers. In comparison, many higher latitude regions have benefited on these events, resulting in increased yield of some crop varieties and enabled new ones to establish during the recent decades.

During the period of 2007-2016, agriculture and forestry related activities accounted for about 23% of total net anthropogenic emissions of greenhouse gases (IPCC, 2019). The net anthropogenic emission of greenhouse gases is made up from release of CO_2, methane and nitrous oxide emissions (NO_x). With predicted global population growth and land utilization for food and natural resources production, agriculture and forestry are in a near future estimated to make up for 21-37% of total net anthropogenic greenhouse gas emissions. In order to reduce the environmental impact that current agricultural practices has, there is a need to develop new and innovative cultivation techniques.

Within the area of agricultural production systems, there are many aspects contributing to emissions of greenhouse gases. Application of chemical fertilizers can cause, among other, N_2O, NO and NO_2 emissions from the soil into the atmosphere (Akiyama et al, 2000; Savci, 2012). The global usage of nitrogen (N) fertilizers has increased significantly since 1950s, in order to meet the growing needs of food and high standard of quality. According to FAOSTAT (2019a) the global N fertilizer used in agricultural and horticultural production systems has increased from 83.4 million tons in 2002, to an amount of 109.1 million tons in 2017. Even though there is a natural reason to why N fertilizers have increased in agricultural and horticultural production systems, it comes to a cost. Further on the effects of mineral fertilizers will be elevated in section 1.1.

In order to reduce mineral fertilizer applications, new innovative solutions and techniques has to be developed as well as applied within the agricultural production systems. Ongoing research shows that arbuscular mycorrhiza fungi (AMF) and other plant biostimulators (microorganisms or substances derived from them) aid crops in uptake and facilitation of soil nutrients such as N, phosphorus (P) and sulfur (S) (Schüβler et al., 2001; Calvo et al., 2014; Popescu, 2016; Varma et al., 2017). Through an established symbiosis between AMF or other biostimulators and the plant, mineral fertilizer application could be reduced and thus the contribution of greenhouse gases and eutrophication lowered.

Biostimulators are fairly new within the agricultural sector and are, according to Swedish Board of Agriculture (2019), used in order to improve plant growth. However since June 2019 they can neither be classified as fertilizer, nor plant protection. Regulation (EU) 2019/1009 of the European Parliament and of the council of 5 June 2019 states the following (2019, 34-35);

> "Plant biostimulant" means a product stimulating plant nutrition processes independently of the product's nutrient content with the sole aim of improving one or more of the following characteristics of the plant or the plant rhizosphere:
>
> (a) nutrient use efficiency;
>
> (b) tolerance to abiotic stress;
>
> (c) quality traits;
>
> (d) availability of confined nutrients in soil or rhizosphere.

Further information about plant biostimulators will be accounted for in section 2.4-2.5.

Research on the effect and usage of AMF on the other hand is much more advanced and well developed. According to Schüβler et al. (2001), it is the most prevalent plant symbiosis known to mankind and is a highly evolved mutualistic relationship. The AMF is so common that it is found in at least 80% of all vascular plant families on earth. Within the literature study, a deep dive into the AMF world will be provided, see section 2.5.

If plants were to be inoculated with these AMF and other plant biostimulators, what would happen? And within which part of the Swedish agricultural sector can they be applied? In this thesis the area of interest landed in viticulture. *Vitis vinifera* L is a highly sensitive crop regarding response to environmental condition and fluctuations, as well as viticultural practices at farm-level management (Keller, 2015).

In current global viticulture, most devastating for the vines is the increase of average temperature in spring and autumn, which will alter the vegetation period and thus generate extended growing seasons. This will alter phenological development stages such as earlier budburst, bloom, veraison and harvest. The global temperature rise will probably cause a shift of vineyard sites to more northern latitudes within the next 50 years. With climate change and the global mean temperature rise, cold climate viticulture has become possible in southern Sweden and vineyards are establishing fast. In order for Swedish viticulture to become more sustainable, the innovative usage of plant growth promoters should be thoroughly tested and investigated at vineyard establishment. This thesis will further investigate how Swedish viticulture, through inoculation of plant biostimulators at vineyard establishment, can become more climate resilient and adapted to a world in need of innovative growing methods.

1.1 Agricultural production systems

Agriculture needs to become more productive and be able to feed more people. The solution was, for a long time in the 1900s, to apply more fertilizers in order to increase yield. According to Savci (2012) the global increase of N fertilizer use has boosted yield, but simultaneously caused severe environmental issues. Water, air and soil are polluted due to extensive usage of N and P fertilizers. Some areas around the world are even toxic, due to increased soil salinity, and not productive anymore.

Water can be polluted by the N and P fertilizers through drainage, leaching and water flow through the soil (Savci, 2012). About 22% of all cultivated areas in Europe exceed the recommended level of nitrate concentration in their drinking water.

Besides from health issues, excessive usage of chemical fertilizer cause eutrophication. Ongley (1996) defines eutrophication as the enrichment of plant nutrients in surface water, which occurs when water goes from oligotrophic (nutrient poor) to hypertrophic (nutrient rich). This change affects the growth of organic matter, such as algae and changes the ecosystem dynamics. In 1990 it was calculated that agriculture was responsible to 55-65% of the total riverine flux of nitrogen to the North Sea and the Baltic Sea. Ryding (1986) observed the lake Oren in Sweden over the period of 1973-1981, where it was found that due to agricultural activities, transparency of the lake declined from 6.2 m to 2.6 m due to algal blooming. Still in present day, eutrophication is said to be the biggest environmental threat of the Baltic Sea (BalticSea2020, 2019). Perennial species of kelp, eel grasses and bladder wrack have been compromised due to excessive growth of plankton and fine fibered algae. The decomposing plankton sink to the sea bottom, which is an oxygen consuming process and cause bottom living organisms to die due to oxygen deprivation. Eutrophication will be hard to reverse, but the effects will be lowered once N and P flow to surface waters are limited.

The effect of N and P fertilizers on the soil are many, however according to Savci (2012), they are not as obvious due to the soil buffering capacity. However, after long term usage of N and P fertilizers there are alterations on soil microbial activity and population (Akiyama et al., 2000; Wang et al., 2018), soil structure, soil pH and other chemical soil properties. Soil microorganisms are important in the nutrient flow cycles as well as decomposition of organic matter. Wang et al. (2018) specified that the microorganism interactions and species composition of the soil are sensitive to changes in microorganism population sizes. This can cause a trophic cascade in the soil ecosystem, resulting in leakage of nutrients and lower plant vigor due to reduced symbiosis. The direct effect on soil microorganisms at nitrogen fertilizer addition is due to a higher osmotic potential and ion toxicity as well as alteration of available soil N. An addition of N to the soil could kill microorganisms sensitive to high osmotic potential, as well as alleviate N limitation to nitrifying and denitrifying microorganisms. Nitrifying and denitrifying organisms use inorganic N as an electron acceptor or utilize it as energy resource. With N addition, N_2 fixating microbes will experience a higher energy cost and thus could decline in population size due to more available N. The end result and inevitable consequences? An incessant need of N and P fertilizer, due to the reduced soil self-sufficiency capacity (through mineralization and fixation).

By using excessive N fertilizers, the soil will undergo an acidification process. This process will lead to leaching of essential base cations, such as Mg^{2+}, Ca^{2+} and Na^{+}, simultaneously as Al^{3+} becomes mobilized and cause toxicity in the soil (Chen et al., 2015; Tian & Niu, 2015).

Besides from environmental issues and consequences of an excessive N and P fertilizer usage, there is an economic trade-off for the growers. Pannell (2017) investigated the economic perspectives of N and P fertilizer usage when it comes to environmental issues, production and yield as well as risks for the grower. The balance between national policies, to ensure lowered risks of eutrophication and water pollution, and farm-level management to boost yield as well as economic turnover is fragile. Education has been identified to be a key point in order to reduce chemical fertilizer usage. Once farm-level management has insight of how much N and P will be fixated and utilized by the crops and soil microorganisms, the environment can be spared simultaneously as the grower saves money.

With understanding and insight of the above-mentioned issues, how can N and P fertilizer usage be reduced and yield still maintained high and satisfactory?

1.2 Swedish climate & climate change

The Swedish climate cannot be generalized due to the length of the country. According to Sandvik et al. (2019) about 15% of Sweden is geographically situated within the Arctic Circle. Sweden is also considered to have fairly favorable climate, in comparison to as Russia and Greenland, which are partly located on the same latitude and have far colder climate. What gives Sweden its favorable climate is influenced by Atlantic low-pressure winds (which bring warm winds from the North Atlantic Current) and continental high pressures to the east.

Due to the length of Sweden, there is a great regional difference in winter climate (Sandvik et al., 2019). The northern parts of Sweden have an average temperature of -12°C, but it can be as low as -30°C or -40°C. In contrast to the winters of the north, southern winters are far more unpredictable and snowfall is irregular. In Scania, which is the most southern part of Sweden, average winter temperatures range from 0°C to -5°C, but just as up north there can be colder periods.

The summer temperature is much more stable all over the country. The differences in mean temperature in summer are 15°C in northern Sweden and 17°C in southern Sweden. What

differs is when meteorological seasons come, such as spring, which in the south occurs in February and as late as May-June in the north.

These fairly warm winter temperatures and early spring has made viticulture possible in southern Sweden. But climate change is faster than ever and the way Sweden is now, climate wise, might never occur again. Persson (2015) made a report for SMHI, collecting climate data from Sweden over the years 1860-2014. The mean year temperature in Scania, of the period of 1961-1990, was 7.2°C and in 1991-2010 the mean yearly temperature was 8.0°C

Swedish climate change has resulted in an alteration of the vegetation period (Persson, 2015). The vegetation period is defined as the part of the year when the daily temperature exceeds a certain threshold/limit, which can vary depending on the plant species. Most common is between 3°C-5°C, which in Sweden means a vegetation period of halfway into the spring and into mid-autumn. The vegetation period of northern Sweden has increased with about two weeks over the past 40 years. However, in southern Sweden, the vegetation period has not increased as dramatically as in the north. The increase of southern Sweden's vegetation period in concentrated to the most recent decade, where the biggest change of the vegetation period is that it starts earlier than it did in the mid-1900s. This is due to the increased spring temperatures. In 2000-2010 the vegetation period of southern Sweden started around 9th of April, whereas it started around the 23rd of April in 1980-1990. This change of vegetative period would become beneficial for Swedish viticulture

The change of Swedish rainfall is of particular interest for the rise of Swedish viticulture. During the period of 1920-1980 the average annual rainfall was 600 mm, but after 1980 this amount of rainfall seems small in comparison. The average annual rainfall has increased significantly in Sweden and rainfall during the summer period has increased the most. The ideal annual amount of rainfall for *V. vinifera* is 500-850 mm, however the average annual rainfall in 1991-2010 at the geographical area of the field trial (the peninsula of Kullaberg, Scania) was 825-1200 mm. Much of this evaporates and ends as runoff; however the annual precipitation is more than sufficient in order to support a viable viticulture. Worth mentioning, even though it will not be further investigated in this thesis, is that an increased rainfall and over all moist could become problematic due to elevated risks of fungal infections (Moyo, 2017).

Persson (2015) stated that the precipitation will probably continue to increase, however the periods of drought and extreme temperatures in Sweden will also become more frequent. Rainfall patterns will change into becoming more extreme and less frequent over the season,

meaning that the groundwater reservoirs and the soil capacity to hold water is crucial for the future of Swedish viticulture. But as is seems today, with the future predictions of Persson (2015), Swedish climate will with time support a vigorous viticulture.

1.3 Aim & research questions

1.3.1 Aim

The aim of this thesis is to investigate weather innovative growing techniques conducted during vineyard establishment could increase sustainability of farm-level management. Thorough and innovative planning and configuration prior to vineyard establishment is crucial to ensure a sustainable production system, especially since vineyards are perennial cropping systems. The aim is further stated as to investigate if inoculation of biostimulating microorganisms at vineyard establishment could invigorate and sustain a viable plant/microorganism symbiosis. In order to address the aim of this thesis, the following research questions have been stated.

1.3.2 Research questions

- Is symbiosis between AMF and *V. vinifera* viable in newly established Swedish vineyards?
- Can inoculation with AMF, prior to planting, on bare root *V. vinifera,* increase vine growth in newly established Swedish vineyards?
- Can inoculation with plant biostimulating algae extract, prior to planting, on bare root *V. vinifera*, increase vine growth in newly established Swedish vineyards?
- Is a combination of inoculation with both AMF and plant biostimulating algae extract, prior to planting, on bare root *V. vinifera* more efficient in increase of vine growth in newly established Swedish vineyards?

1.4 Limitations

In this thesis, only vineyard management will be analyzed in order to target sustainability issues and possible solutions. Viticulture and farm-management ends at harvest, which means no further research will be conducted regarding wine making or other parts within the spectra of oenology.

The field trial was based on three treatments and a control in order to investigate the possibilities of plant biostimulators increasing crop establishment and viability, which in the long run could reduce the environmental impact of viticulture. No further treatments will be investigated in

this thesis. The main focus of this thesis will concern AMF, since usage of plant biostimulating alga is fairly new and thus not enough information regarding its effects could be derived for this thesis. However, it was found to be an interesting treatment to try simultaneously and in combination with the AMF, due to its possible future potentials in increasing vineyard management sustainability.

Due to the size of the field trial (160 vines), only three vines within each plot were measured according to the BBCH-scale during the growth season of 2019. This made up a total of 48 vines being observed, through randomized selection. Field observations and phenological assessment ended earlier than expected. Thus there is only 8 weeks of observations. The reason was that the vines were no longer accessible due to a protecting tube, which ensured a good microclimate and protected against unwanted animals. See appendices 1 for illustration of field trial design.

2 Literature study

2.1 Grapevine – botany, history, phenology & physiology

2.1.1 Botany & history

Grapevine belongs to the higher/vascular plants, meaning they are a part of the subkingdom Tracheobionta. They form flowers, which once fertilized swell and form the fruit (grape berry), further *V. vinifera* are a part of the phylum angiosperms (with modern terminology also called magnoliophytes).

As most plants, grapevines are dicots and are further divided into the order Rhamnales. However taxonomists have recently separated *Vitiaceae* from this order and placed them in the order Vitales (Keller, 2015).

The genus *Vitis* consists only of perennial vines or shrubs, which all are characterised by their tendril-bearing shoots. There are about 60-70 species within the Vitis genus, however further in this thesis there will only be focus on the Eurasian species (*Vitis vinifera* L.).

Keller (2015) stated that Vitaceae family is as a collective referred to as grapevines and this family comprise about 1000 species, which are allocated into 17 genera. One species within this family has now become one of the most produced fruit crops and is cultivated in about 90 countries in purpose of wine, table grape and juice production.

Vitis genus consists of two sub-genera, which are *Euvitis* (the true grapes) and *Muscadinia* (Winkler et al., 1974). The most commonly known species is *Vitis vinifera*, which is native to south of the Caucasus Mountains and Caspian Sea.

Both historically and in modern days, the *V. vinifera* grapes have been utilized in different ways (Winkler et al., 1974). In more modern days, commercial classes of grapes have been divided in four major groups and one minor. The major groups are table grapes, raisin grapes, wine grapes and sweet juice grapes and the minor group is caning grapes. Wine grapes are the only ones considered for this thesis.

Wine grapes are the commercially biggest major group and most of the vineyards in Europe, North Africa, South Africa and South America are dedicated to wine grape production (Winkler et al., 1974). Wine grapes are further divided into two subgroups, table wine and dessert wine. Table wine contains 14% or less alcohol and is said to be made from less sweet varieties, resulting in a "dry" wine. In comparison, dessert wine contains 17-20% alcohol and is made from grape varieties with considerably higher sugar content and lower levels of acid.

2.1.2 Morphology roots

The vine consists of vegetative organs (roots, trunk, shoot, leaves and tendrils) as well as reproductive organs (flowers/berries) (Keller, 2015). All above ground parts are referred to as the vines canopy. The majority of the biomass is made up by the trunk and roots (50-75%); and the proportion of trunk and root biomass increases with age. The root morphology will be thoroughly explained below, since it is of high value for the understanding of root/AMF symbiosis.

The root system of *V. vinifera* provides physical support for the vine as well as enables water and nutrient flow from the soil. Within the roots there is a carbohydrate and nutrition storage, which enables initiation of vegetative growth in the spring (Rasmussen et al., 2013). Plant hormones, such as ABA and cytokinins are also sourced from the roots. Root growth initiation from the cambium is promoted by auxin and can be suppressed by cytokinin and strigolactone. *V. vinifera* has a primary root as well as many adventitious roots, which branch off into secondary and tertiary lateral roots in order to spread into water and nutrient favourable areas within the soil.

Roots are mostly concentrated around the top 0.5-1 m of the soil, whereas some roots can grow into a depth of 30 m if no obstacles are encountered (Lehnart et al., 2008).

When considering the root morphology, the root tip (apex) is of particular interest of this thesis. The root apex is covered by a root cap, which has a starch rich columella (Keller, 2015). The purpose of this root cap is to protect the root meristem from the surrounding as well as facilitate further penetration through the soil. As the root facilitation through the soil advance, the root cap is continuously shed and restored from the inside. This results in that mucigel/mucilage, a gel containing polysaccharide, from the root cap is secreted over the maturing root (Bais et al., 2006). The mucigel attracts and ensures nutritional value for microorganism and could potentially aid the establishment of symbiotic AMF. The AMF forms a fungal mycelium and in exchange for the polysaccharides they support the nutrient and water uptake.

Active cell division occurs in the apical meristem (which is located behind the root cap) and is stimulated by the hormone auxin. Auxin is synthesised and derived from unfolding leaves at shoot tips, through cell-to-cell transport from the vascular cambium and xylem parenchyma (Aloni, 2013). The cell elongation as well as differentiation is promoted by gibberellins (which is produced through stimulation of auxin at site by the expanding cells (Ross et al., 2011). The hormone cytokinin is demanded in high concentrations in order to enable the activity of the meristem and is produced in the root tips, however contradictory enough the cell elongation requires the opposite (Wang & Li, 2008). Cytokinin is referred to as the "cell-division hormone", but in the root tip the effect is the opposite. By counteracting the effect auxin has on cell division, the cytokinin restrains the rate of cell differentiation, which finally determines the length of the root meristem.

Cells produced within the meristem form the endodermis, which is divided into cortex (responsible for soil nutrient uptake and storage of starch as well as other nutrients) and stele (responsible for nutrient transport) (Keller, 2015). Further, the stele is differentiated into the pericycle, primary phloem, and primary xylem (creating the vascular cylinder) and the cortex develops into the exodermis and epidermis.

Lateral root growth is initiated by nearby shoot derived auxin, brassinosteroids and small amounts of gaseous ethylene (which is produced and released as xylem differentiates). Auxin cause the ethylene production, which in turn block further auxin movement and cause a local accumulation of auxin in root pericyle cells and this induce lateral root growth (Keller, 2015). Simultaneously, cytokinin movement from the root tip inhibit lateral root formation, ensuring that lateral root growth is not occurring near the root tip. This to ensure that lateral root growth does not interfere with the continued growth of the apex.

Behind the growth region of the root tip follows an area called the absorption zone, which is an area important for water and nutrient uptake. This area is covered by root hairs, which is formed by epidermal cells and are as thin as 10-15 µm in diameter (Gilroy & Jones, 2000). This root hair zone can make up more than 60% of the total root surface area, which increase the area of contact between root and soil. Muday et al. (2012) found that the elongation of root hair is stimulated by auxin and ethylene. As root growth proceeds, new root hairs for behind the elongation zone simultaneously as the old root hairs are torn away. This results in an advance of the absorption zone along the root tip.

The vascular tissue/vascular system consist of the phloem, vascular cambium and xylem (Stafford, 1988). These three different tissues are separated through compact parenchyma cells, which has thin primary walls and contain a lot of nutritional starch and proteins. The phloem and xylem are arranged parallel and become interconnected with parenchyma tissues, which ensure a possible transfer of nutrients between the phloem and xylem.

2.1.3 Phenology & growth cycle

The growth cycle of *V. vinifera* is divided into a vegetative and a reproductive stage. The reproductive stage is of no further interest of this thesis, since the years following vineyard establishment focus on the vegetative stage and growth of the vine.

The growth of *V. vinifera* is driven by the seasons and their changes (Keller, 2015). Day length and temperature are of biggest importance, which is alternated with the winter dormancy. Bleeding of the xylem sap, derived from wound areas of which pruning was done, is what marks active growth in spring. This is when the vine transits from dormant to active growth in the spring, which seems to occur when soil temperatures are about 7°C. Dormant buds are rehydrated and in combination with increasing temperatures, budbreak and shoot growth is initiated.

Sap bleeding occurs due to an increased root pressure (Keller, 2015). This root pressure is possible due to a remobilization of protein and starch derived nutrient reserves as well as flow of amino acids into the xylem tracheary elements. These remobilizing nutrient reserves cause an increased osmotic pressure and drive an osmotic water uptake. This is what cause hydration of the dormant buds and allow them so swell.

The cell division and auxin production of buds starts about 2-3 weeks before budbreak (Aloni, 2001), causing a decline in ABA concentration, which reduce the growth inhibition of the bud.

The hormone ABA acts as an inhibitor of vegetative growth, which keeps the vine dormant in winter.

Together with the bleeding sap and rehydration of the buds, cytokinin is transported to the cambium cells of the vine as a result of the root pressure. According to Cookson et al. (2013), it is not known if root tip production of cytokinin is possible in early spring; however it is found in the bleeding root sap. This suggests a possible interaction with microorganisms that can, in symbiosis with plants, produce plant hormones (see section 2.4-2.5).

Bud break is increased with increasing temperatures, but above 30°C it declines again. It is the temperature of the bud itself, rather than the surrounding temperature, that is of highest importance for a rapid bud break (Keller & Tarara, 2010).

What follows bud break is shoot growth (Keller, 2015), which starts at apical buds and an apical dominance is established. Apical dominance inhibits outgrowth of lateral buds, which enables the most distal to break early and grow vigorously. The shoots close towards the vine base are suppressed and thus do not grow at all or very weekly. If apical dominance is not as strong and too many buds break, the lower shoots can be broken off. Just as well can the apical dominance be broken, if to persistent, by removal of shoot apex.

Apical dominance result in a sugar supply concentrated to the dominant shoot (Mason et al., 2014). This reduced sugar supply to other buds or shoots deprive them of the possibility to initiate cell division. Cell division is also suppressed by an auxin flow from leaf primordia as well as from young leaves around the shoot apex (Müller & Leyser, 2011). The auxin flow stimulates internode elongation. However, according to Berleth et al. (2007) if shoot growth is too rapid and flow of auxin becomes too increased, the effect of apical dominance can be lowered, allowing other buds to break.

The shoot growth is rapid, with leaves developing every few days. The growth pattern of the shoot is generally upwards, which is due to the effect of negative gravitropic response (the opposite of gravitropism) (Petrásek & Friml, 2009). The gravitropic response is possible due to the sedimentation of starch in some specialised amyloplasts in the endodermis. The sedimentation results in an increased level of auxin and lower extracellular pH and cause the lower side to elongate more than the upper.

As new leaves develop on the elongating shoot, the vine becomes more photosynthetically competent (Turgeon, 2010). The vine is much dependent on the rapid growth and development

of photosynthetically active leaves. Before carbohydrates can be synthetized and transported from the leaves, the shoot growth stage of development literally cause the vine to loose dry weight during the primal growth weeks of spring (Keller, 2015) The loss of dry weight stops once the vine has about 5-6 leaves, which are sufficient to generate new energy and support a gain of biomass.

What affect the rate of leaf unfolding and internode extension are day length as well as temperature, where long days and high temperatures are the most favourable (Alleweldt, 1957).

Following the shoot growth stage, there is a flowering stage and a grape setting stage (which is the reproductive stage and will not be further investigated) (Keller, 2015).

Once fruit set is done, the shoot growth is resumed. As grapes develop and ripen, the shoot will form a periderm and going from green in colour to yellow/red/brown. This process is called shoot maturation and starts at the base of the shoot, working its way to the shoot tip (Keller, 2015). In areas of high rainfall and good nutrient supply (fertile soils), this process can be delayed and thus possess a threat to the ripening of the grapes and ultimately affect grape and wine quality.

The decrease of day length and temperature cause cell division to stop in the apical meristem, prompt buds and cambium (Garris et al., 2009), which prevents lateral shoot emergence and initiate dormancy of the vine. Shoot and trunk dormancy is induced after first occasion of frost in winter and some of the phloem might even not become dormant at all. The phloem can be injured at cold winter temperatures and is especially vulnerable at freezing in, an otherwise warm, autumn (Keller, 2015)

Shoot growth cycle is completed at leaf senescence, which is when nutrients from the leaf are relocated into permanent parts of the vine. According to Niklas (2006), 50-80% of leaf N, 50% of leaf S and 20% of leaf Fe is remobilized before leaf senescence. Abscission follows leaf senescence, which is when the vine sheds its leaves. The leaf senescence is triggered by day length and according to Keller (2015), a reduction of temperature has no effects on this initiation.

After a walkthrough of the phenology of *V. vinifera*, some temperatures of interest could be elevated. As mentioned, many growth stages are affected by temperatures. Growth of *V. vinifera* accelerates rapidly at temperatures up to the optimum, which is 25-30°C (Keller, 2015). However, this varies greatly among varieties and what each plant is adapted to. The warm days

(25-30°C) will promote accumulation of CO_2 and limitation of CO_2 respiratory loss is limited at somewhat lower night temperatures (15-20°C).

Thomas (2013) assume a linear increase of growth as mean temperatures rise, making it possible to calculate so called growing degree days (GDD). How it is calculated is further explained by Keller (2015). However, thanks to GDD, it has been standardized, that *V.vinifera* has a 7 month "standard growing season", which for the Northern hemisphere is April-October (Amerine & Winkler, 1944; Keller, 2015).

The root morphology has already been covered and thus root phenology will be briefly covered within this section. The roots of *V. vinifera* are never dormant (van der Schoot & Rinne, 2011); they only become suppressed in development and activity by cold temperatures (below 6°C).

Just as shoots, the root has a form of apical dominance, which as covered by section 2.1.2, meaning that lateral roots are inhibited to form by the growth of the root tip (Aloni, 2013). This apical dominance allows the root to search for nutrient and water in deeper parts of the soil profile. However, if nutrients such as nitrate and phosphate are not encountered, root tip synthesis of cytokinin can seize, which will allow formation of lateral roots in more shallow soil profiles.

Root growth peaks at the time of bloom and early fruit growth, since assimilation of photosynthetic compounds are highest at that time (Comas et al., 2010). It is estimated that about 30-60% of the vines net photosynthetic products can be stored in the roots and in unfavorable soil this percentage can be even higher. The fine roots (< 1mm in diameter) are replaced continuously due to their short life. These roots are the ones most important for water and nutrient uptake and if they survive, they can become larger structural roots.

As roots form, they start out white in color, turning brown after about 5 weeks and black at the age of 8-11 weeks. Browning of the root is due to a reduced metabolic activity and death of tissue, meaning it stops absorbing water and nutrients and becomes the source of new lateral roots (Comas et al., 2010). As the root dies, it becomes black and can, after degradation, serve as "new" nutrients for the vine.

2.1.4 Water & nutrient uptake

The vine consists of about 70-95% of water; even the trunk and roots can have a water content of about 60% and those are considered to be woody parts of the vine (Keller, 2015). The purpose of the water is to act as solvent of ions and organic molecules within the cells. Water diffuses

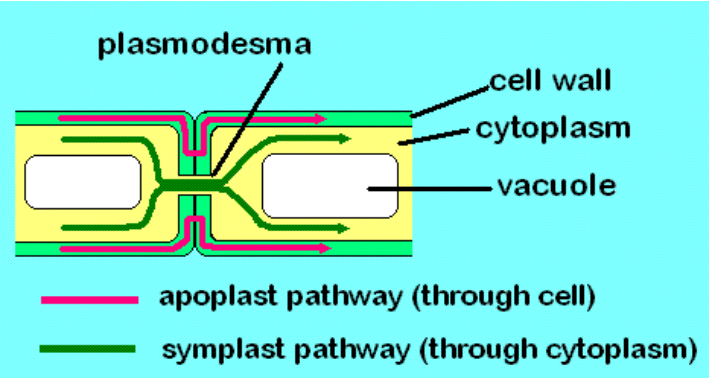


Figure 1: Illustration of the symplastic and apoplastic pathways. Source: Wikimedia Commons, 2009. Public domain.

freely into the cells and through aquaporins. Aquaporin pathway is a form of active transport, since it demands energy and the pores can be closed. This is a way for the cells to control water flow of the cell. Ions on the other hand cannot flow as easily as water through the cell membrane. This makes the membranes selectively permeable/semipermeable. There are gates, with transport proteins and ion channels that facilitate the passage through the membrane.

Osmosis is a water movement of high importance for the vine (Kramer & Myers, 2013). Osmosis is a movement of water, due to a concentration gradient of dissolved ions and small organic molecules between the interior and exterior of a cell. This concentration tends to be higher within the cell, causing a passive water movement through the aquaporin into the cell. Thus, water molecules from the exterior of the cell are pulled and cause a tension, called osmotic potential. The osmotic pressure will build up as water flows into the cell, to restore hydraulic equilibrium. The major osmotic solutes of the vine cells are sucrose, organic acids and inorganic ions. These solutes have, other than a nutritional function, an osmotic function as for instance to neutralize ion charges.

Transpiration is the water evaporation of plants (Keller, 2015). The transpiration of *V. vinifera* occurs across the cuticle (5-10%) and through the stomata pores (Sperry et al., 2002). Stomata react easily on environmental changes, such as open at sunrise (which is light induced) and close when it sets in order to reduce water loss and produce photosynthetic components. Low leaf water pressure also causes the stomata to close, which is a response in order to protect the xylem conduits from cavitation. Speirs et al. (2013) found that dry soils promote ABA synthesis, which stimulate stomata to close in order to reduce water loss.

Evaporation is necessary due to the need of gas exchange and thus it is important to have sufficient water availability in the soil (or irrigation) (Keller, 2015). The created tension in the

xylem, due to evaporating water, will result in a water extraction from the soil, which simultaneously will attract nutrients that can be transported from the roots through the xylem conduits.

The availability of soil nutrients varies a lot, depending on soil type amongst other factors (Bleby et al., 2010). Ions are dissolved in soil water and are thus transported to the different plant organs through the roots. The vast majority of plant nutrients are found in more shallow depths of the soil, since the humus rate and biological activity is higher there. For instance, P and K are taken up from topsoil layers, whereas nutrients that leach deeper into the soil profile (as nitrate) are derived from longer roots.

Water and soluble nutrients enter the root initially through the epidermis and cortex through the apoplastic (extracellular pathway) and symplastic (intracellular pathway) routes (Keller, 2015), see illustration in figure 1. The symplastic pathway ensures water transport from cell to cell through plasmodesmata (Lough & Lucas, 2006). The apoplastic pathway ensures water transport in the intercellular spaces. Only small molecules, such as ions, amino acids and sucrose can be facilitated with the water through the cell walls. Due to the lack of membranes within the apoplast, the water flow is dominant within this pathway.

Transport of ions can be with or against the electrochemical potential gradient. If transport occurs against the electrochemical potential gradient, pumps are necessary and thus it is considered active transport and need energy (ATP) (Grossman & Takahashi, 2001). The active transport is essential for *V. vinifera*, since it allows the crop to concentrate and store nutrients in the roots (Keller, 2015). This storage is what generates the energy of the vine in spring, before it starts to photosynthesise.

Many anions are taken up actively against the electrochemical potential gradient and cations passively through ion channels (Keller, 2015). Carriers are generally needed for macronutrients and the balance between anions and cations are rarely in balance, even though it is important in order to maintain a neutral charge of the plant and its roots.

After passage through the endodermis, the water and nutrients are further transported to the xylem (Dechorgnat et al., 2011). This creates a mutual exchange between parenchyma cells and xylem conduits, which enable *V. vinifera* to modify sap composition (aminoacids, hormones, organic acids and nutrient availability) for the shoots, which can vary depending on developmental stages or abiotic stress factors. In the leaves, photosynthetic compounds are

synthetized and transported back to the roots and other organs (source/sink relocation) (Keller, 2015).

2.2 General viticulture

In the following subheadings there will be a brief walkthrough in the art of viticulture, since the crop used for the field trial was *Vitis vinifera*. This will grant further understanding for the underlying reasons of the conducted field trial.

2.2.1 Soil & soil properties

All above ground parts of the vine are affected by the fundamental soil properties and well-being of the root system. The soil *physiological properties* consider everything that has to do with the volume of soil that the roots can utilise and expand into. Soil structure is the primary controller of this volume and is made from the arrangement of primary particles and air-filled space between them. The soil structure ensures biological and chemical reactions and soil aeriation, as well as water and nutrient holding capacity. According to Grainger and Tattersall (2016) there are many types of soil that are suitable for vine growing. Vines are even considered to be well established and vigorous in vastly inhospitable soils and in unlikely sites. Good drainage is crucial to a good vineyard soil structure. In a viticulture perspective, the ideal soil is the one with natural drainage. Deeper water reservoirs are thought to have higher mineral content

The following soils are found suitable for viticulture: limestone, chalk, clay, marl, granite, gravel, greywacke, sand, schist, slate basalt and volcanic soils. Limestone is especially desired in cooler wine regions, due to that the roots are forced to burrow deep into the soil fissures to excess water and nutrients (Keller, 2015).

Suitable soil pH range between 5-8.5 and affect soil nutrient availability (Keller, 2015). Low pH result in less availability of some macronutrients (P and K), whereas higher pH result in less availability of micronutrient (iron and zinc). Depending of the origin of the *Vitis* species, it can be more or less tolerant to low pH soils, *V. labrusca* is for instance more tolerant than *V.vinifera*. Soil pH is altered through addition of lime in acidic soils. Regardless of soil properties, soil samples should be performed in order to map out the soil characteristics and make a tailored fertilizer plan for the specific field and crop.

2.2.2 Climate requirements of *V. vinifera*

In order to address the climate requirements of *V. vinifera*, the Köppen climate classification system will be used (subdivision of five major types of terrestrial climates) The five climate types are represented with the letters A-E and are all but B defined by temperature criteria, whereas B has the controlling factor of the vegetation dryness rather than coldness (Arnfield, 2019).

A- Moist tropical climates, all months have an average temperature of 18°C or more.
B- Dry (dry arid and dry-semi-arid) climates.
C- Moist and mid latitude climates with mild winters, further divided into humid subtropical, maritime and Mediterranean.
D- Moist and mid latitude climate, however with cold winters.
E- Polar climate, extreme cold summer and winter.

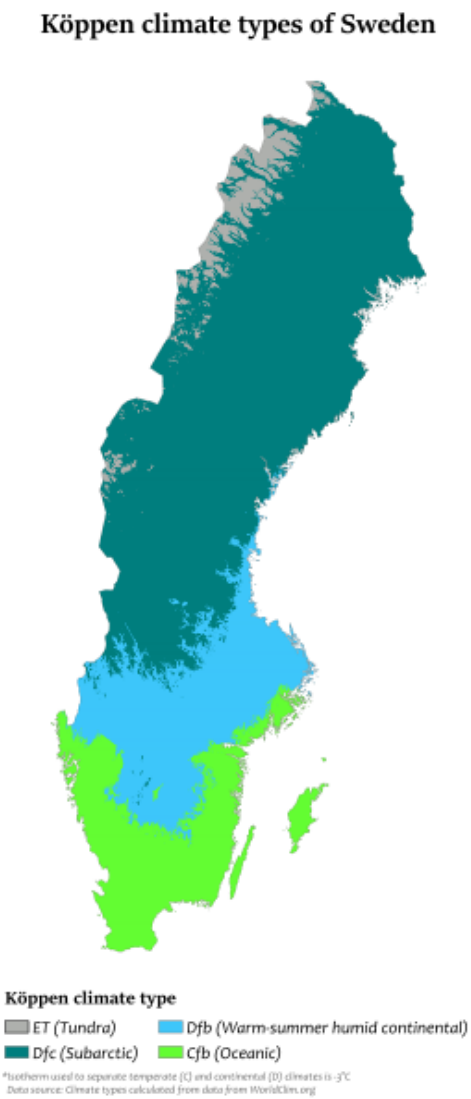


Figure 2: Illustration of the Swedish Köppen climate type. Source: Wikipedia Commons, 2016. Creator Adam Peterson. CC BY-SA 4.0

The climates most suitable for viticulture are, according to Grainger and Tattersall (2016), B (semi-dry) and C. Sweden is considered to be part of the Köppen climatic type D, however local areas such as in Scania are considered to be climatic type C (maritime), meaning it is suitable for viticulture (Climate-data, 2019). Figure 2 illustrates the Köppen climate classifications of Sweden. In comparison, Winkler et al states that in the 1970s "The most northerly vineyards of the world are in Germany." (1974, 44). This suggests that in the last 50 years, both breeding for abiotic stress tolerance and climate change has excelled rapidly and affected global viticulture.

The most important climatic requirements of *V.vinifera* are sunshine, warmth during summer and cold during winter as well as sufficient rainfall. General assumptions are, according to Grainger and Tattersall (2016):

- Sunshine, minimum 1400 hours per year and ideally 7-8 hours/day in the Northern hemisphere during April-October. Too much sun and heat result in produced wines with too high levels of alcohol and not enough acid. In comparison, not enough sunshine result in grapes not ripening in time and they contain too much acid and, when fermented, not enough alcohol as well as unpleasant flavors. However, the ripening of the grapes is not only dependent on the light itself, but rather the heat it generates.
- Warmth, growth stops at temperatures below 10°C and in order to flower the threshold temperature is 15°C. Depending on the variety and surrounding climate, the time between flowering and harvest ranges from 80-150 days and during this period the average temperature has to be at least 18-20°C in order to ripen. In comparison, average temperatures above 23°C can cause unpleasant flavors in the wine and damage both vegetative part and grapes during the growth season.
- Cold winter is needed to ensure rest for the vine. If rest is not induced by cold winter climate, the vine could yield twice a year, resulting in a shortened life for the crop. The cold temperatures are also needed in order to kill fungal pathogens or kill pests that host on *V. vinifera*. Generally, a dormant period induced by cold winter that lasts five months is ideal.
- Rainfall, 500-850 mm is ideal but can differ depending on climatic conditions or fluctuations as well as soil type. European vineyards are seldom in need of irrigation.

There are also climatic drawbacks for *V.vinifera*, where abiotic factors reduce growth or inhibit development of the grape. Grainger and Tattersall (2016) list these following factors as the biggest climatic enemies of *V.vinifera*.

- Frost, if severe enough it can cause freezing of the sap and split the roots. Besides from damaging the roots, vegetative parts are especially sensitive in early spring. Frost during budding time can damage new shoots and buds, resulting in a reduced yield. Circulation of air and sprinkler systems can protect buds at days with risk of freezing, since the water contains heat that will be transferred to the bud as the water surrounds the bud.
- Hail cause severe physiological damages to both vegetative parts of the vine as well as the grapes, causing reduced yield and increase susceptibility to diseases. Hail can, depending on the location of the vineyard be problematic both during spring and before harvest in the autumn.

- Strong winds can damage all vegetative parts of the vine, breaking both trunk and shoots. Flowering can also be compromised and thus the yield reduced. This is especially problematic in valleys and in areas close to the sea.
- Excessive heat and drought cause burning of leaves and cracking of berries, resulting in poor vitality and quality. At temperatures above 40°C, the vine can even die.

2.2.3 *Vitis vinifera*, var. Solaris

V. vinifera Solaris (from now on referred to as Solaris) is a fairly new variety, cultivated in Denmark, southern Sweden, England, and northern European countries such as Germany and Poland (Ambrosi et al., 2011). The variety originates from Germany and is a hybrid. The parent plants were initially crossed in 1975, but it was not until 2001 that it became a protected variety. The crossing was made between prime parent 1, Merzling (Seyve-villard 5276 x (Riesling x Pinot gris), mother), and prime parent 2, Geisenheim 6493 (Zarya severa x Muskat Ottonel, father) leading to pedigree as given by breeder, Norbert Becker, Merzling X Geisenheim 6493. Solaris was then given the official variety number VIVIC 20340, which is a number in The Vitis International Variety Catalogue (VIVIC).

According to Ambrosi et al. (2011), Solaris is a disease tolerant variety that ensures good yield, despite the Northern European cold climate. Teissedre (2018) stated that interspecific hybridization of *V. vinifera* became popular in the 19th century, which initially was in purpose to develop pest and disease resistant varieties. Resistance is a phytopathological concept, which refers to the plants, in this case Solaris, capacity to defend itself from pathogens such as fungal or bacterial. However today, further mentioned by Ambrosi et al. (2011), the breeding and hybridization is also to improve tolerance against various abiotic stresses. The need of both stress tolerant varieties and pest/pathogen resistance within grapevine production has led to the development of such varieties as Solaris.

Weinmann (2018) describes Solaris to be earlier in stages such as to undergo budding and flowering, which is about one week earlier than most varieties. Grape closing or grape development is also said to be earlier than standard varieties, but this occurs about two weeks prior to the standard varieties. Even softening of the grape occurs earlier than standard varieties, which according to Weinmann (2018) is about 10 days. It ripens very early, which in North European countries are favourable due to the reduced growing season. The early ripening varieties are generally said to be tolerant against fungal infections. Solaris is considered to be resistant to the most occurring fungal diseases and is even PIWI International recommended

(Pedneault & Provost, 2016). PIWI stands for Pilzwiderstandsfähige (German), which translates into “disease resistant (Pedneault & Provost, 2016). Initially, these disease resistant varieties were the result of an interspecific cross-breeding between the true grapevines, which are the *V. vinifera*, and their relatives from North America and Asia, which could be for instance *V. ripria, V. rupestris* or *V. amurensis*. The North American and Asian *Vitis* ssp had much higher resistance towards fungal diseases and abiotic stress than *V. vinifera*.

Pedneault and Provost (2016) listed Solaris as resistant towards powdery mildew. They also graded Solaris as moderately susceptible to both *Botrytis cinerea* and downy mildew.

2.2.4 Establishment of a vineyard, site selection & soil preparation

A well planned and executed vineyard establishment is crucial for long term productivity and health of the crops. According to Creasy and Creasy (2018), the site of the vineyard or field is probably the most important aspect of establishment.

Creasy and Creasy (2018) says that there is no such thing as “the perfect site” for a vineyard. It all depends on the variety. However the decision regarding placement of a vineyard or field site should consider aspects of importance for the desired cultivar. Such include heat accumulation, winter minimum temperatures, availability of water and nutrients as well as soil characteristics and type. It is also important to see the whole landscape picture, since slopes can affect the site characteristics (air flow, drainage, frost incidence, sun exposure and temperature).

Prior to planting, soil sample on various depths should be performed, in order to see nutritional status, pH, amount of organic matter and trance for potential pathogens. Again, Creasy and Creasy (2018) states that there is no “perfect soil” for vines, as long as the nutritional demands are reached vines will cope at various sites and soil types. The rootstocks and varieties are what make site choice harder, since these can be more specific in needed soil characteristics.

Sloping land is of interest in cold climate viticulture (Creasy & Creasy, 2018). This is due to gained benefits of water drainage and air exposure, as well as an increased interception of solar radiation. This will also heat up the air and soil more efficiently and promote wine growth throughout the season. Important to keep in mind though is that it can be more difficult for machines to operate.

New technologies, regarding usage of digital tools, artificial intelligence and drones, has enables remote sensing (photographs) of vegetation and site properties. These are, nevertheless,

not to be trusted completely but they are a good tool in aiding vineyard managers in location selection (Filippini Alba et al., 2017).

Once the site is chosen, soil preparation is to be done. It is recommended to clear the field and sow a cover crop the season prior to planting of the vines (Creasy & Creasy, 2018). Cover crops prevent pests to build up and allow the soil to rest prior to planting. It is also important to look at the crop rotation scheme or at what was at the site before planting, since three could be soil borne diseases or nutrient utilization differences. Rows are most often oriented north to south (in the northern hemisphere), which allow even ripening and sun exposure.

The soil is treated as follows: potential usage of herbicides in order to reduce weed pressure at the site (*V.vinifera* is sensitive to many herbicides and thus mechanical methods are recommended in vineyards), usage of a ripper in order to break up compact soil layers and particles (allowing the vine root to penetrate faster and easier) (Creasy & Creasy, 2018). Deep tillage is applied in order to turn the soil over and incorporate organic matter followed by usage of a harrow, to create a loose and even soil. In some cases, chemical fertilizers are also applied prior to planting or usage of manure is applied and incorporated when tilling. If soil is wet, with risk of waterlogging, and of high clay content drainage should be applied to the field.

At this stage, the vines can be planted. The spacing is important and different measures are used around the world, creating more or less dense vineyards. It all depends on the farm level management and machinery. Inter and intra row distance will decide the productivity or vine number, which can range between 9000 vines/ha to 1100 vines/ha (or even less). In Sweden, the recommended amount of vines is 2500-3000/ha (Nordmark, 2017). Planting depth is 35-40 cm, depending on root stock variety. Common is to get waxed cutting delivered to the vineyard, which are incubated in order to allow callus forming at the graft union and will grow fast once emerged into the soil (Creasy & Creasy, 2018).

Planting is done either by hand or machine (Creasy & Creasy, 2018). The common ways are with a mechanical furrow planter, auger (not recommended in high clay content soil, can cause stunted root growth) or waterjet and after soil emergence they are secured around the cutting base, by putting pressure to the soil in order to ensure good contact with the soil. Cover crops should be sowed between the rows, in order to reduce pests, weeds, erosion and loss of nutrients as well as increasing biodiversity, humus and soil nutrients. In order to support the vines in the future, there also has to be posts, intermediate posts and wires installed.

2.2.5 Nutritional requirements

Just as any other plant, *V.vinifera* requires both macro- and micronutrients. Important macronutrients are listed in table 1.

Table 1: Table 1 illustrates the most important macro- and micro nutrients of *V. vinifera*. Both nutrients and brief information regarding effect and importance is listed. Source: Proffitt, T. & Campbell-Clause, J. 2012. *Managing grapevine nutrition and vineyard soil health*. Available: www.winewa.asn.au

Nutrient	***Function***
Nitrogen (N)	N is required for most of the vines metabolic functions and is a major component for different synthetized compounds. Affect shoot growth in spring as well as inhibit premature leaf fall. Other properties of N is that this nutrient can influence inflorescence initiation, fruit set and growth
Potassium (K)	K is of importance for the vacuoles and for the cell protein synthesis. Stomata is also affected by P. Shoot growth is partly regulated by K as well as berry size and fruit Development throughout ripening.
Calcium (Ca)	Ca is needed for cell membrane and cell wall structure and function. Ca mediates many enzymatic processes. This nutrient can also increase risk of physiological disorders (bunch stem necrosis).
Magnesium (Mg)	Mg is necessary for synthetizing chlorophyll molecules and for several important metabolic processes as well as fruit formation and ripening
Phosphorus (P)	Important component for cell membranes and cell and for carbon dioxide fixation. P is necessary for sugar metabolism, genetic material, energy storage and energy utilization. It mediates shoot growth in spring, initiate inflorescence and fruit set.
Sulphur (S)	Important component of amino acids and other metabolic compounds, proteins, enzymes chlorophyll molecules and vitamins.
Boron (B)	B is an important nutrient for the formation of plant hormones, translocation of sugar molecules. Influences reproductive processes and affects cane maturation and fruit set.
Iron (Fe)	Chlorophyll synthesis is dependent on Fe. Fe is also of high importance for other photosynthetic processes and respiration. Inhibits premature leaf fall.
Manganese (Mn)	Important for the synthesis of chlorophyll.
Zinc (Zn)	Mediates cell metabolism, needed for chloroplast development and synthesis of various plant hormones. Fruit set and intermodal elongation is affected by Zn.
Copper (Cu)	Needed for chlorophyll synthesis and is required as a compound of oxidative enzymes. Helps with lignin formation and can affect cane maturation.
Molybdenum (Mo)	Mediates conversion of nitrates for protein synthesis. Influences fruit set.

The nutrients further investigated are N and P.

The acquired form of N, taken up by *V.vinifera* as well as other plants, is inorganic nitrate NO_3^- which is challenging for the vine since most N is bound in organic matter and cannot be taken up directly by the roots (Keller, 2015). The N uptake is small in early spring, since the vine seems to only utilize its reserves and then uptake increases progressively through the different phases of bloom, fruit set, and berry growth (Keller et al., 1995). It seems like N uptake is dependent on water availability in the soil. As drought becomes prevalent and soil moisture is reduced, the uptake is reduced. However, if water is not scarce, nitrogen uptake maximum occurs at the warmest period of the growing season (Keller, 2005). The N uptake continues to increase until harvest, when it drops. Vines tend to have a minimum of endogenous N reserves around flowering, which means that if N is not available in the soil after flowering, the vine could suffer from N deficiency as grapes develop (Holzapfel & Treeby, 2007).

Lateral root growth is inhibited if soil N levels are too high (Zerihun & Treeby, 2002). The inhibition is due to ethylene, which is increased when high NO_3^- levels are encountered by NO_3^- transporters in the root (Wang et al., 2012). If soil N availability is low, the lateral root growth is instead stimulated by auxin in order to find N resources located further away (Gifford et al., 2008).

In N sufficient soils, chlorophyll degradation is delayed and more photosynthetic assimilates can be produced and stored for the vine (Keller, 2015). The N uptake and high N levels also stimulate an increased production of cytokinin, which is a senescence inhibitor. If N levels are low, the opposite can be observed. The vines will then show signs of chlorosis, a reduced yield and growth as well as early leaf abscission. An enhanced level of cytokinin also delays lignification (Kiba et al., 2011; Krouk et al., 2011; Schreiner et al., 2013), which can make the vine more vulnerable to pests and physiological disorders. As mentioned in section 2.1, cytokinin stimulates division in the shoot apical meristem and inhibits cell division in the root apical meristem, which means that an increased cytokinin level in the root zone can inhibit N uptake in order to maintain a balanced N level within the vine (Kiba et al., 2011).

For rubisco formation, N is especially important and the N supply strongly correlates with the vines photosynthetic capacity. If N levels are too low, translocation of sugars decline and starch accumulates in the leaves (Schreiner et al., 2013).

If N availability is low or too high at flowering, the cluster formation will be compromised and fruit set lowered (Keller, 2015). Berries also seem to be enlarged if N levels are too low, which might be due to a reduced competition with shoot meristem growth, leaving more photosynthates to the grapes.

If too much N is applied to the soil, especially young vines suffer. High NO_3^- concentrations could decrease stomatal conductance, due to a decrease of the osmotic potential and less water uptake (Keller et al, 1995). The consequence is the opposite of what the fertilizer applier would expect, since this cause a growth reduction instead of the expected growth increase.

The N status of the soil also seems to affect the availability of other nutrients in the soil. Levels of K^+, Ca^{2+} and Mg^{2+} seem to increase with higher N availability, but anions such as $H_2PO_4^-$ seem to decrease (Keller et al., 1995). The uptake of K^+, Ca^{2+} and Mg^{2+} is not as high as N. If too much N fertilizer, during a longer period of time, are applied to the soil, there is a risk of nutrient leaching and deprivation of essential vine nutrients. In some occasions, too high N levels also suppress P uptake. This could be due to that the higher N uptake induces a reduction in root carbohydrate status, which in turn could limit the carbohydrate availability for arbuscular mycorrhiza fungi (AMF) (Hilbert et al., 2003).

The anion phosphate ($H_2PO_4^-$) is often referred to as P_i (inorganic phosphate) and is important for the formation of ATP as well as many nucleic acids (for DNA & RNA), phospholipids (cell membranes), gibberellins and carotenoids. The P is also important for photophosphorylation, which is essential for photosynthesis. According to Shen et al. (2011), about 90% of total cell P is located within the vacuole. The P uptake pattern is the same of N, which is mentioned above.

Soil content of P is most often sufficient but scarce in availability, as P is highly unstable and immobile, which has to do with its affinity to Ca^{2+}, Mg^{2+} and Al^{3+}. Insoluble complexes with P and above-mentioned cations are frequently formed (Shen et al., 2011). In alkaline soils, P is dominating in the shape of HPO_4^{2-} and in more acid soils the predominant form of P is $H_2PO_4^-$

The most usual form of P taken up by the roots is $H_2PO_4^-$ (more available in shallow soil depths), which is absorbed in the root tips and root hairs (Shen et al., 2011), however the uptake in these roots could deplete the rhizosphere fast of available P. Once this occurs, root growth is increased in order to form lateral roots and to form new root hairs. This is due to an accumulation of auxin and ethylene as well as a depletion of cytokinin and gibberellin in the

root zone. As mentioned in section 2.1, this will result in a denser and shallower root system due to a shift in root morphology (Chiou & Lin, 2011), which ultimately enables to roots to explore more shallow areas of the soil where $H_2PO_4^-$ levels are higher.

Some species of *V. vinifera* can also exude organic acids, which will lower the pH in the rhizosphere in more alkaline soils. The acids also act as anion exchangers, which could make bound P available (Plaxton & Tran, 2011). Availability of K^+ and Mn^{2+} is also said to increase through this mechanism. Besides from exudes of acids from the roots, vines also get an increased uptake of P by the help of AMF. The lower P availability and uptake, the greater the colonization of the roots there seems to be. Strigolactone sesquiterpenes are exuded by the roots, which promotes the AMF to establish contact with the roots (Bais et al., 2006).

Deficiency of P can easily be identified as an early symptom is lowered leaf number and a significantly reduced leaf size, as well as compromised shoot apical meristem growth (Bais et al., 2006). This is due to an increased supply of carbon assimilates to the roots, in order to them to expand and find new sources of P. Thus, the ratio of root:shoot is highly affected by soil P availability. It is also seen that P deficient vines store sugar and starch in source leaves (Hermans et al., 2006), which could be connected to a reduced need of shoot and leaf growth.

2.3 Soil nutrient cycles & availability

2.3.1 Nitrogen

According to Haynes (1986), 98% of the earths N is bound in rocks and minerals, together with iron, titanium and other metals. But it is also found in the chemical form NH_4^+, which is found in primary silicate minerals. The vast majority of earths N is not recycled, due to that it is bound in the rocks and minerals. Instead, the percentage of N in the atmosphere, which is as low as 1.9% of total N, has become the largest contributor of N to all living organisms.

In the ocean, 50% of N content is in the form of organic N, and on land about 73% of all N is organic (Haynes, 1986). The organic material has to be degraded by microorganisms, into mineral forms such as NH_4^+ and NO_3^-. Plant available forms of N are NH_4^+ and NO_3^-, of which only as low as 1% can be found free as soil stored mineral. Thus only 1% of the global terrestrial N is available to the plant. Major additions of N to the soil come from deposition and activity of microorganisms, meaning both fixation of N_2 from the atmosphere and degradation of organic material. Besides from the natural in and out flux of nitrogen, humans are also adding

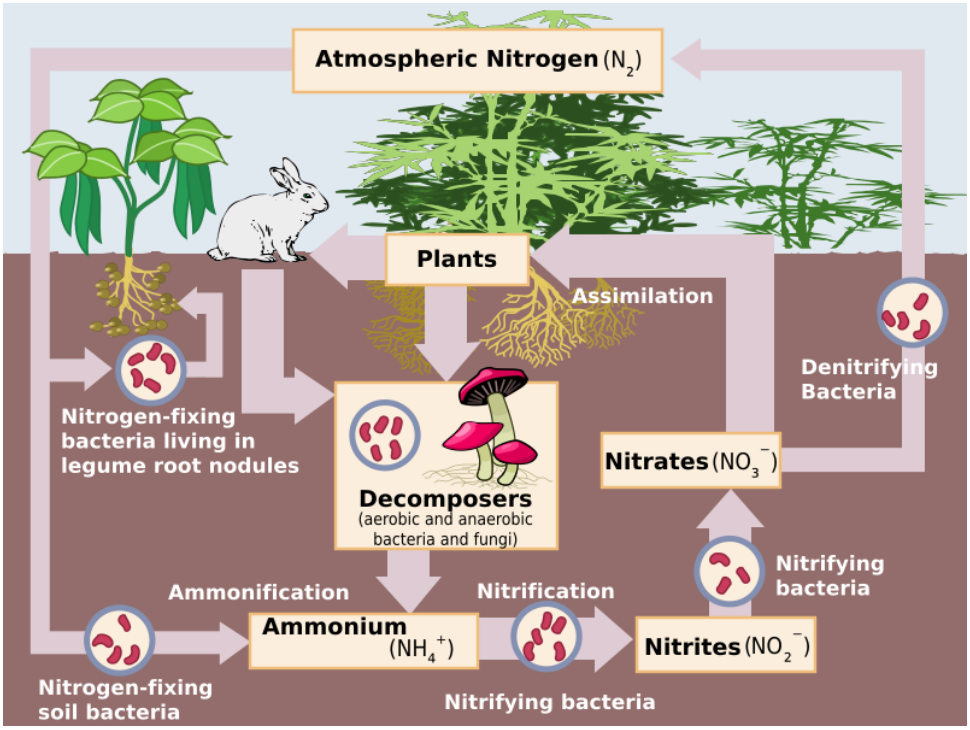


Figure 3: Illustration of the nitrogen cycle, showing how nitrogen is fixated and incorporated in organic matter. Source: Wikipedia Commons, 2009. Creator: Johann Dréo. CC BY-SA 3.0

nitrogen to the ecosystems through industrial activities, usage of machines and addition of fertilizers.

The major losses of N are related to leaching (primarily of NO_3^-), soil erosion, runoff, leaching of ammonia and other N containing gases as well as through agricultural practices and removal of organic matter from the production systems.

A simplified illustration of N cycle is provided in figure 3. Within the N cycle, there is an internal cycle mediated by the soil microorganisms (Haynes, 1986). They decompose nitrogenous organic residues, which result in a release of NH_4^+ (mineralization). Oxidization of NH_4^+ can give the mineral form of N as NO_3^-.

The mineral forms of N are of importance for both the soil microorganisms, which immobilize them, and the plants. The NH_4^+ is often bound to soil colloids or fixed in clay minerals, whereas NO_3^- is highly mobile and not bound to any particles in particular.

Haynes (1986) states that here is a big difference between the natural ecosystem cycle of N and the cycle within an agricultural production system. In natural ecosystems, as one in a steady state, the N inputs by precipitation and biological N_2-fixation, balances the outputs (denitrification processes, volatilization and losses by water flow). In agricultural systems on

the other hand, a higher N input becomes necessary due to the removal of organic material at harvest. In natural ecosystems, a few kilos of N loss are assumed each year. But in agricultural systems, a loss of 100-200 kg N ha^{-1} yr^{-1} is estimated due to runoff and N incorporated in the harvested material.

In the agricultural production systems, it is easily miscalculated how much N has to be applied (Haynes, 1986). Many producers are also afraid that they won't apply enough N, resulting in an increase of N flux to the atmosphere and surface waters, causing an imbalance in the N cycle. Gaseous losses of NO_x are concerned as major air pollutants, since they generate phytochemical smog and degrade ozone. Other contributors to NO_x are the agricultural machines, which utilize fossil fuels.

NO_x flow from the soil seems to be a necessary evil. Many essential microorganisms seem to be directly linked to NO_x flow through chemodenitrification (Haynes, 1986), which is a result of several reactions with NO_3^-. Autotrophic nitrogen fixating bacteria also seem to increase soil loss of NO_x.

Within the soil nitrogen cycle, the key processes are mineralization and immobilization (Haynes, 1986). Mineralization is when inorganic N (such as NH_4^+) is released through a process called catabolism. The last step of mineralization is known as ammonification, which is when simple organic nitrogenous substances are metabolized. What the result of catabolism can be seen as is the energy released for anabolic activity. The anabolic activity further requires uptake and use of mineral N derived from decomposer microorganisms, e.g. immobilization. Thus, it becomes clear that mineralization and immobilization goes hand in hand. Immobilization of N can be said to be responsible for the fate of a large amount of the soil N mineralization.

2.3.2 Phosphorous

Liu and Chen (2014) states that P is one of the most essential building blocks on earth. It cannot be replaced and sustains all food production. The biochemical cycle of P on earth is not in balance at the moment, meaning that much of the available P is translocated into surface water. Additionally, the human activity has resulted in a quadrupling of the P mobilization. The result is eutrophication, a major environmental concern.

The P cycle is more complex than the N cycle. The P cycle has three natural cycles within the cycle. N can be industrially processed in a much greater extension than P, thus being more

available for farmers to distribute. What also differentiates P from N is that the P cycle does not have any significant gaseous compounds. A simplified illustration of the P cycle is provided in figure 4.

The vast majority off all P on earth is derived from weathering of calcium phosphate minerals, especially apatite ($Ca_5(PO_4)_3OH$). The P cycle is not as investigated as the N cycle, resulting in lack of knowledge of biochemical processes of P_i.

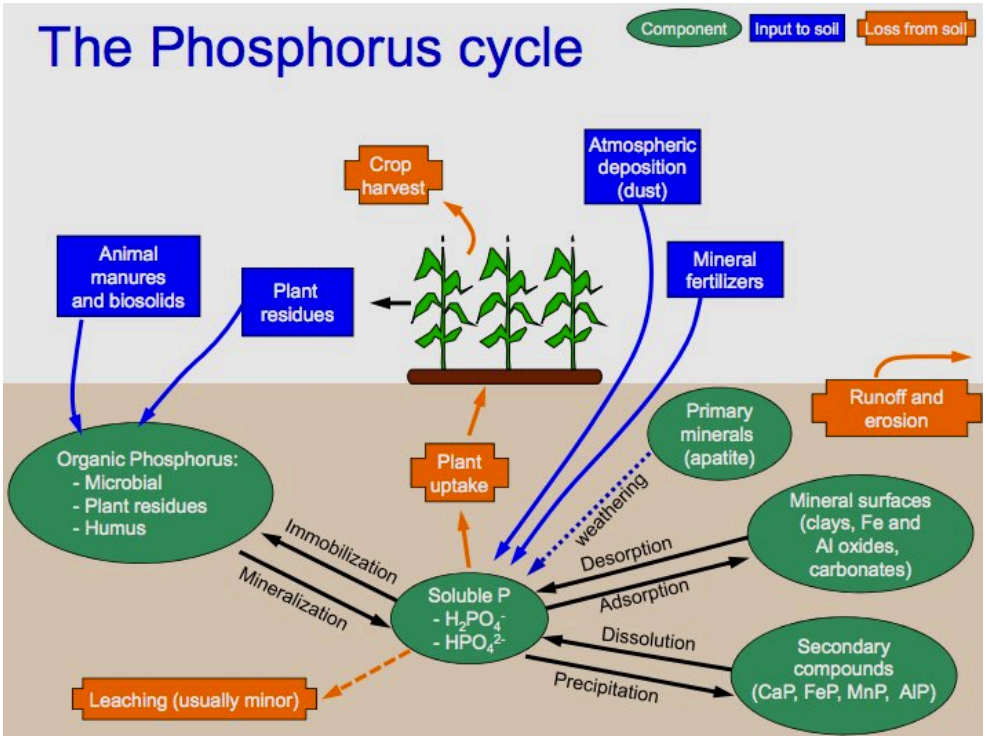


Figure 4: Illustration of the phosphorous cycle, showing how phosphorous is fixated, mineralized and incorporated in organic matter. Source: Wikipedia Commons, 2014. CC BY 3.0

Organic P has two cycles within the P cycle, one for land living circulation and one for water circulation. Almost all living biomass accumulates P from the organic cycles. The P that is incorporated in organic cycles are extracted from phosphate rocks and distributed as fertilizers. In 2017, the global usage of phosphate fertilizers alone was about 45.5 million tons (FAOSTAT, 2019b). This fertilization is necessary in modern agricultural production systems, since the natural weathering and depositions cannot make up for the loss at harvest. What can be done is to restore some P levels by leaving plant residues in situ (Liu & Chen, 2014). However, the application of P fertilizers tends to slow down the natural P weathering in the soil, since the concentration equilibrium is disturbed.

Within the soil, P distribution and availability is controlled by both chemical and physical processes (Liu & Chen, 2014). Only about 15-20% of applied P fertilizer is taken up and accumulated in the plant, meaning that the rest and natural occurring P become insoluble.

The major factor of P mineral solubility is pH. Valsami-Jones (2004) states that increased pH can give an increased solubility of sediment bound P, which is due to an increased charge of Fe and Al hydrous oxides. This increased charge increases the competition between hydroxide and phosphate anions.

Out of the total soil P, organic phosphates are said to make up 30-65% (depending on soil type). Acid soils are said to accumulate more total organic P than alkaline soils tend to do. The reason behind this is thought to be because of the above-mentioned reaction P has with Al and Fe, which occurs during acid conditions. The salts of metal complexes release P through hydrolysis, but slow. Acids and phosphatase enzymes produced by microorganisms can aid in making P available for the plant, by acting as catalyzer (Liu & Chen, 2014).

2.4 Plant biostimulators

As mentioned in the introduction, plant biostimulators are fairly new on the market and as recent as in 2019 the regulations changed regarding the classification of biostimulators (2019/1009/EU). Plant biostimulators are said to consist of diverse substances and/or microorganisms that will enhance plant growth. The vast majority of plant biostimulators are used in European agriculture and production systems. According to Calvo (2013), the global market for biostimulators was in 2014 predicted to reach a financial value of 2.241 million US dollars. In 2012, there was reportedly 6.2 million hectares that was cultivated with biostimulators in Europe alone (European Biostimulants Industry Council, 2013).

The biostimulators are to be applied to the plant or the rhizosphere, which supposedly stimulate natural processes regarding uptake and efficiency of nutrients as well as enhance tolerance to abiotic stress. Some biostimulators has, prior to 2019, been considered as pesticides. However, biostimulators have no direct effect on pests, rather they induce resistance against pests through increased growth and stress tolerance (European Biostimulants Industry Council, 2013), segregating biostimulators from biological control agents. Other biostimulators were considered to fall under the legislation of fertilizers. However, the European Biostimulants Industry Council describes biostimulators as products with some nutritional value, but not purposed as direct fertilization. Biostimulant Coalition (2013) also stated that biostimulators are not plant nutrients and thus they do not offer a nutritional guarantee for the plant.

Depending on what kind of plant biostimulators it is, the effect differs. Some of the effects biostimulators have are reported as reducing nutrient losses from the soil, soil structure improvers, solubilization of nutrients, plant response enhancers, increasing nutrient supply, increasing root growth and biomass through stimuli, compliments to mineral fertilizers, facilitation and assimilation of nutrients through the plant and asymbiotic nitrogen fixation (Vessey, 2003; Canbolat 2006; Adesemoye et al., 2008; Biostimulant Coalition, 2013).

Microbial inoculants are by far the most used biostimulators in agriculture and has increased dramatically during the past 20 years (Hayat et al., 2010). This is due to the fast climate change and the research and development within agriculture and production systems has started to develop solutions to sustainability issues of agricultural production systems in modern days. The most used microorganisms are mainly free-living bacteria, fungi and AMF (Dodd & Ruiz-Lozano, 2012). The effect of AMF will be further investigated in 2.5.

Other plant biostimulators are seaweed extracts, which besides from AMF was investigated as a growth promoter for *V. vinifera* in this thesis. There is a long tradition, even as far as several millennia, to use seaweed as direct additive or compost in order to increase soil fertility and crop yield (Craigie, 2011). Today, it seems like seaweed extracts are more common than the seaweed itself and liquid extracts have been produced since the 1950s. The main effects of the seaweed extracts are said to act as chelators (boost soil mineral nutrients utilization and improve soil aeration (which also stimulates root growth). The improvement of soil aeration is of especial interest in heavy clay soils.

2.4.1 Algae & seaweed as plant biostimulators

In section 2.4, the main effect of plant biostimulating algae was mentioned as acting as a chelator. Besides from acting as chelators, seaweed extracts also enhance plant establishment and growth. It can, in later developmental stages, improve flower set and fruit production, which will give a higher productivity and quality of the crop (Craigie, 2011). The vast majority of seaweed extracts, purposed as plant biostimulators are made from brown seaweed (*Ascophyllum nodosum*, *Laminaria*, *Fucus*, *Turbinaria* and *Sargassum* spp) (Sharma et al., 2012). Some seaweed extracts are given in liquid form, some as dried product and can be used as they are or incorporated in micronutrients or fertilizers. The extracts themselves are needed in very low dosage, as low as 1:1000 or even lower, strengthening the claim that biostimulators cannot be accounted for as plant nutrients themselves (Khan et al., 2009).

So, what does seaweed contain that makes them such, supposedly, good plant biostimulators? They contain a wide range of organic and mineral components, such as complex polysaccharides (laminarin and alginates for instance) that cannot be found in terrestrial plants, as well as plant hormones (Sharma et al., 2012). The content of auxin of seaweed can range from 3-47ng/g and concentrations of minerals such as Fe, I, K, Mg and S vary significantly.

The specific effect seaweed extracts have on plant nutrient uptake, growth and yield are said to be many. Foliar application of seaweed extract has shown enhanced root development of many plant species, including *V. vinifera* (Mugnai et al., 2008). Enhanced root growth has also been detected on *Picea abies* in Norway, showing the efficiency of biostimulators in cold climate (Slávik, 2005). The root development is due to plant hormones (auxin and cytokinin) in the seaweed extract, causing both increase of total root volume, root length and lateral root formation (Khan et al., 2011).

Mineral nutrient uptake was, according to Mancuso et al. (2006), stimulated in *V. vinifera*. Accumulation of macro- and micronutrients also increased. The AMF hyphal growth was also improved by the application of brown algae extract, which suggests that biostimulating algae/seaweed extracts stimulate microbial activity and diversity at the rhizosphere.

Above ground growth, such as shoot growth, has also been reported (Craigie, 2011) as a beneficial effect of seaweed extracts. The leaf of *V. vinifera* has also showed to gain an increase of chlorophyll after applications of algae extracts (Mancuso et al., 2006).

The effects of algae/seaweed derived biostimulators, regarding resistance to abiotic stress, are associated to drought, salinity and temperature (both cold and warm extremes) (Craigie, 2011). The mechanism on how seaweed biostimulators enhance stress tolerance is not understood yet, however betaines (bioactive molecules) and cytokinins might play a role. What is known is that the extracts from seaweed/algae increase endogenous concentrations of stress-related molecules (cytokinins, antioxidants and proline) (Fan et al., 2013).

Particularly interesting, for cold climate viticulture, is that biostimulators seem to increase tolerance against abiotic stress related to cold temperatures. The tolerance is associated with protection of membrane integrity, reduced expression of chlorophyllase genes, increase in genetic response at several tolerance genes and increase of soluble sugars in the cytosol as well as unsaturated fatty acids (Rayirath et al., 2009; Nair et al., 2012).

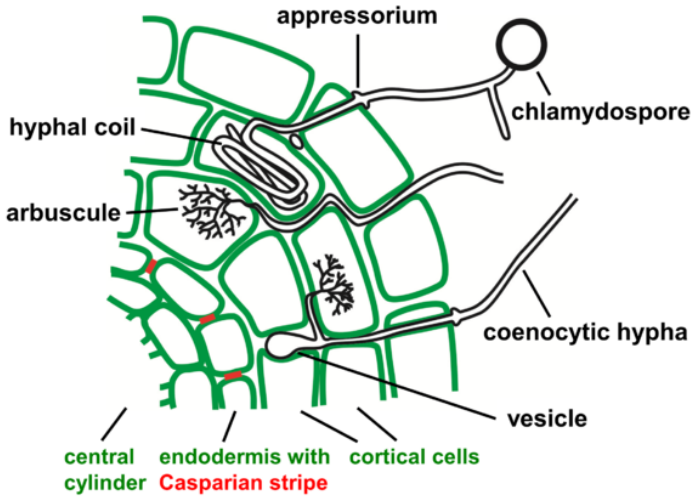


Figure 5: Illustration of the different structures of AMF and the AMF orientation in plant cell structures. Structures are such as arbuscules, hyphae and vesicle. Source: Wikipedia Commons, 2015. Creator: M. Piepenbring. CC BY-SA 3.0

2.5 Arbuscular mycorrhiza

Out of all mycorrhizal fungi, the arbuscular mycorrhiza fungi (AMF) are the most common. Besides from AMF, there are ecto, ericoid, orchid and mycoheterotrophic mycorrhizas (Smith & Read, 2008). However, no other mycorrhiza than AMF will be further considered in this thesis.

Glomeromycota is the common phylum of AMF (Schüßler et al., 2001). It is estimated that for as long as there were plants, there was AMF and that their role was crucial in plants colonization of land (Smith & Read, 2008). However, it was not until the more recent decades of the 19th century that they were discovered and described. The name arbuscular comes from a description of the organism's structure and characteristics, since arbuscules are formed by AMF within the root cortical cells. Besides from arbuscules, there are other fungal structures within and between root cells and together with the root and the characteristic extraradical mycelium in the soil these build up the components of an AMF. The arbuscules are considered to be the main structures of nutrient exchange. In figure 5, the different structures of AMF are illustrated as well as the orientation of the AMF in plant cell structures.

Spores of AMF are considered to be large, since they can become as large as 500µm in diameter (Lemoine et al., 1995). The spores are high in content of lipids, carbohydrates and are supported by a thick wall containing chitin. The number of nuclei is very much depending on the species, since it can range from 800-35 000 (Hosny et al., 1998).

Spore germination is what initiate root colonization of the AMF. The hyphal growth initiates nuclear division, using stored carbohydrates and lipids, resulting in a production of mycelia (Bianciotto et al., 1995). Mycelia is produced in limited amounts and further hyphal branching is stimulated by signal molecules from the plant root (Akiyama et al., 2005). As symbiosis between AMF and host establishes, mycelial growth continues within the root as well as in the soil and give rise to the formation of new spores on the hyphae.

There has been plenty and still ongoing research regarding the ability of AMF to form a relationship with more than one plant species and vice versa (Smith & Read, 2008). Some AMF seem to be host specific, however due to the amount of AMF (150 or more species) and the fact that about 80-90% of all terrestrial plants are hosts to AMF, the AMF cannot afford to be host specific. Observations *in situ*, *in vivo* and *in vitro* show that one single root of a plant can host many different AMF. Other experiments also show that isolated AMF from one plant host can colonize almost any other species that support AMF symbiosis.

2.5.1 Anatomy of Arbuscular mycorrhiza and root colonization

The AMF colonization can arise from three main inoculums within the soil, which are spores, infected root fragments and hyphae. These three are referred to as propagules (Smith and Read, 2008). Spores are dispersed through water, wind or animals and growth as well as dispersal of AMF is inevitably dependent on mycelial growth or by dispersal of plant host.

One site can contain spores of 20-50 species, which are at different stages of dormancy and germination (Fitter, 2005). The fact that germination of different spores is slow and uneven enables the soil to become a reservoir of inoculum that can persist for a long time, ensuring that colonization of the roots can happen several years ahead. Additionally, the mycelial and hyphal network created by different AMF creates big colonization feasibilities and is of high importance in perennial cropping systems.

Germination generates a small amount of presymbiotic mycelium, which enables the spores to undergo growth without the presence of host root (Bago et al., 1999). Once germination is initiated, the root exudates start to stimulate mycelial growth and branching. However, some AMF species have evolved and do not have to colonize the plant immediately after germination. Plant derived flavonoids seem to promote spore germination significantly (Buee et al., 2000; Akiyama et al., 2005).

According to Giovannetti et al. (2004), the mycelium is built up by a network of hyphae. The main hypha contains cytoplasm and nuclei and from this hypha, lateral and finely branched

hyphae systems are developed and separated, linking plants in the soil. The hyphal network enables a rapid colonization of plant seedlings and helps them establish by efficient nutrient mobilization. The hyphae spread from plant root to plant root through a bridge analogous system, creating multiple colonizing hyphae and appressoria (Smith and Read, 2008) which cause secondary colonization.

As mentioned above, colonization is initiated by hyphae from one of the tree inoculums. The main hyphae start to grow towards the root. As the main hyphae approach the root it starts to branch and create a fan-shaped complex of lateral hyphal branches (Smith and Read, 2008). From this fan-shaped lateral hyphal complex, the colonization is initiated.

As the fan-shaped lateral hyphal complex comes in contact with the root, the hypha undergoes adhesion to the root surface. About 2-3 days later, appressoria is formed and start to swell (Giovannetti & Sbrana, 1998) and roots are penetrated. As hyphae penetrate the cell wall, they exude pectinases and hydrolytic enzymes as well as exercise a pressure. Penetration of epidermis and endodermis is done by morphological changes of the hyphae, making them pointier.

After appressorium formation and penetration of epidermis and endodermis, the hyphae spread into middle and inner cortex of the root (Cox & Sanders, 1974). From there it grows longitudinally within the intercellular spaces. The development of fungal hyphae spread across the cortex, which are associated to closely located entry points on the cell epidermis. The entry points on the epidermis are called “infection units”, which grow longitudinally in the root cortex and give rise to intercellular arbuscules.

The intercellular network of hyphae makes up the communication pathway of an AMF infection unit, acting as an agent for translocation of nutrient between the host plant as well as the extraradical mycelium (Smith & Read, 2008).

The AMF form vesicles, which can be located in either inter- or intracellular parts of the cortex (Abbott, 1982). Both number of arbuscules and vesicles are reduced at high levels of P or low irradiance. The vesicles have high lipid content and have several nuclei, suggesting that they are a storage organ for the AMF and are important propagules within parts of the root.

It is hard to estimate how much of the root biomass is made up by AMF, since external parts of the fungus could be very widely spread. Hepper (1977) estimated that the fungal biomass, internal and external hyphae, other organs and spores make up for 8-20% of the root dry weight.

The external hyphae act as the inoculum for colonization for both colonized roots as well as neighboring plants (Smith & Read, 2008). It is thought that the external branching is due to the constant need of new carbon (C) sources for the AMF. On the external mycelium, spores can be formed and enable further spreading of the AMF. The production of spores demands high levels of organic C, forcing the plant to transfer C to the rhizosphere and support the growth of the symbiont. The external hyphae cannot grow unless arbuscules are formed, meaning that after colonization and infection of the host, the AMF has to produce arbuscules before the external hyphae can expand and grow.

The organic matter of the soil is in near contact with external AMF hyphae, making them reach in contact with nutrient mineralization (Hepper & Warner, 1983). Depending on the level of mineralized P, the AMF growth can be reduced, or the hyphae length can be increased. This can be due to a response of the AMF, moving away from the high P level areas, but resulting in a broader infection area of the fungi. Other than the effect of P level in the soil, little is known about the soils effect on external hyphae development (Drew et al., 2003). Changes in pH, soil compaction and root presence have shown to reduce external hyphae growth as well as hyphae biomass (Smith & Read, 2008). Overall colonization of the root seems to also be affected by the density of inoculum and temperature. High propagule density speeds up the colonization and soils prone to erosion seem to reduce propagule density of the soil. Colonization rate is the highest in soil temperatures between 10-30°C.

As colonization proceeds, both root and AMF structure develops. Root growth is described in section 2.1.2. As the root grows and develops, the AMF initiate both primary and secondary infection units (Smith & Read, 2008). The primary and secondary infection units spread towards the cortex and colonize it as the root grows. The colonization rate is therefore limited by both root growth and rate of infection unit formation.

The effect AMF colonization has on root growth is investigated quite extensively. It is suggested that there is some changes in apical root growth pattern, but not to what extent. Berta et al. (1991) found root growth to be slowed down after colonization of some AMF species. Simultaneously the rate of lateral root growth is increased, which is a response to the reduced activity of the root apex of the primary roots. New types of lateral roots can also be produced as a response to stimuli from AMF (Paszkowski & Boller, 2002). These roots are very effective in P uptake and together with external mycelium these roots could sustain viable plant growth even in low P available soil.

2.5.2 Plant & fungal symbiosis

The symbiosis between plants and AMF is considered to be mutualistic, since they both obtain nutrients from each other (Smith & Read, 2008). Plant species can be more or less dependent on the activities of the AMF; however most plants can be viable without the AMF. The AMF on the other hand are much more dependent on photosynthetic assimilates from the plant to be viable. The plant gains a lot from the symbiosis with AMF, more resistance to pests and pathogens as well as becomes more drought resistant. The AMF gains from the symbiosis since they cannot complete a life cycle without the host plant.

The AMF nutrient uptake can be derived from two sources, the soil and other hosts (Smith & Read, 2008). The pathways depend on three processes; 1) mycelial uptake from the soil, 2) translocation of nutrients from external hyphae to fungal structures that are located within the host and 3) transfer from the AMF to the plant cells. The contact surface of the plant with the soil becomes more exploited as the extraradical hyphae and mycelium spreads of the AMF, meaning that nutrient are absorbed from the soil beyond the inherent capacity of the plant. N, Zn and P seem to be the most important nutrients that the AMF facilitates into the plant. The most obvious measurement of a successful mutualistic symbiosis is an increased plant growth and larger AMF colonized root area. But if soil P is increased, some AMF colonized hosts might have a reduced growth in comparison to non-colonized plants, since the plant self-sufficiency of P declines as symbiosis and colonization rate increases.

As the plant gains in above ground growth, the symbiont is rewarded with organic C, derived from photosynthates (Smith & Read, 2008). Hexoses and minerals are transferred from plant to fungus both intercellular in root cortex at sites of hyphal growth and intracellular, where arbuscules are. The transport of organic C through plant membranes must be facilitated as any other transport within the plant, see section 2.1.4. Electrochemical gradient and balance of cytoplasmic pH must be maintained, meaning that the easiest facilitation of hexoses from plant to fungus is done through passive facilitated diffusion.

But how much C does the AMF use? Some scientists have measured a usage of as much as 20% of total assimilated CO_2 (Jakobsen & Rosendahl, 1990), others as little as 1% of net photosynthesis (Heinemeyer et al., 2006). The differing findings could be due to different AMF species has different C need, the AMF utilize different amounts of C depending on where in their life cycle they are or it could be environmental circumstances. The question does arise; does the plant suffer from the AMF usage of C? It seems that generally it does not, since the

symbiont increases shoot growth and thus increases the ability to fixate more C. Smith and Read (2008) states that the plant photosynthetic rate generally is higher for AMF colonized plants. It could, however, be that the plant supplies the rhizosphere with less organic C when soil P availability is abundant, affecting AMF symbiont population. This due to that if there is enough P, the plant cannot support the AMF and thus the cost of maintaining AMF could potentially be too high.

So, what can be concluded of the plant fungus symbiosis? The AMF are dependent on organic C, which they obtain from the host plant (Smith & Read, 2008). Everything between 1-20% of the photosynthetically assimilated C is supplied to the AMF. The AMF uses the organic C to grow and reproduce. As a reward, the AMF act as a nutrient uptake facilitator. The AMF increase the roots contact surface with the soil and thus more nutrients, such as N and P, can be taken up. These nutrients allow the crop to increase vegetative growth and perhaps also increase yield.

2.5.3 Arbuscular mycorrhiza & *V.vinifera*; soil and research

The soil has a significant effect on colonization of AMF on the root as well as their activity in the rhizosphere. Alguacil et al. (2016) found pH as well as levels of Mn and Zn to be the soil characteristics of highest importance for AMF community activity and colonization rate. Besides from soil type, biochemical characteristics and composition, agricultural activities and natural disturbance also affect the soil AMF communities and colonization rates.

Alguacil et al. (2016) states that in previous studies, limited amounts of data of the effects soil characteristics has on AMF communities is available. But all studies, at the same time, point out that there is a significant effect of the soil properties on the AMF communities. They also state that there is no complete soil characterization study reported for AMF. There is also evidence found that AMF has an effect on the soil structure itself, as they improve soil structure and aggregation. Furthermore, Bainard et al. (2015) concluded that soil pH seems to be the only variable that is a key factor for the AMF community.

Ouzounidou (2015) found that alkaline soils (pH 8.2), in comparison to acid soils (pH 5.8) and neutral soils (pH 7.1), had a higher rate of AMF colonization on host roots. Sandy soils also generated more colonization, which correlated with low P levels within the sandy soils. In a similar field study, Wang et al. (1993) found that in acid soils (pH <5.5) few or no colonies of AMF were detected. As early as 1979, Graw also investigated the effect of AMF on P uptake

and reproduction. Some species seemed to be able to sustain a P uptake at pH 5.6 but at a slower rate and at pH levels below 5.6, no viable AMF colonies were found.

It is found that usage of fungicides, as targeted for pest fungi, also suppress AMF colonies and viability (Jin et al., 2013). The tradeoff between using plant protection and gaining from ecosystem services of the AMF is hard to balance. It seems like knowledge of farmers about the consequences of fungicide usage on the AMF is low. The effect of the fungicide is that the AMF population is lowered and thus competition with other microorganisms in the rhizosphere becomes prevalent. The result might be higher risk of infection of pathogenic microorganisms via the roots as well as above ground due to a reduced resistance rate.

Alizadeh et al. (2010) states that there are few species as well as low population density of AMF in vineyard soils. Thus it seems like inoculation of AMF prior to planting and establishment of new vineyards or fields is necessary. But they also emphasize that it is not to a cost, since if the AMF are not at the site, they are considered to be foreign species and thus competes with other species of the "natural" microclimate.

Schreiner (2007) inoculated Pinot noir grapevine cuttings with AMF and investigated the vines development in two different soils. What Schreiner found was that in one of the soils, the vines uptake of P increased with an extreme 833% increase and that the growth of the vine was significantly increased (274%). But in the other soil type, there was no significant difference in growth at all.

In trials conducted by Tomislav et al. (2012), there are evidences that neighboring weeds of the vine affect colonization and AMF community structure. Development of AMF intra- and extraradical mycelium and spores was affected due to competition from other colonizers. Herbaceous weeds also promoted other populations or species of AMF to colonize the roots of the vine. The outcome of the study was that intercropping and weed management is of high importance in order to sustain a diverse AMF community as well as minimize risk of competition with other microorganism from the weeds, which potentially could suppress the AMF colonies of the *V. vinifera.*

Khalil (2013) found that *Vitis* became more resistant towards salinity when inoculated with AMF. As the plants were irrigated with different concentrations of NaCl, the ones inoculated with AMF showed significant increase of several plant growth parameters, in comparison to

the ones not inoculated with AMF. Growth parameters of interest were, amongst other, shoot length, leaf area and root biomass.

There could also be a difference in the effectiveness of the symbiosis between native and other selected AMF and the host plant (Camprubi et al., 2008). It seems as in some cases, native AMF symbiosis can be more effective in the beginning of colonization, but after given time the selected AMF species that are introduced to the site can be as efficient in establishing a viable symbiosis with the host plant. Caglar and Bayram (2006) tested different species of AMF inoculum on grapevine cuttings and measured the level of P in leaves and other organs. They found that not all species can increase uptake of P, meaning that not all AMF species are suitable to pair with *V. vinifera* in order to establish a good symbiosis.

Research conducted by Schreiner (2003) showed that the AMF symbiosis and vine growth is affected by soil moist and that in dry soils, colonization is slower on the variety of their trial.

Trials have also been conducted on older plant material. Usha et al. (2005) inoculated 18-year-old vines with different AMF strains and the result showed increased vegetative growth of the vines. Nutrient uptake was higher of the plants inoculated with AMF and yield was also higher. A conclusion of Usha et al. was that the used strains of AMF can be used as a supplement to the fertilizing strategy, in order to reduce chemical fertilizer usage and thus improve farm-level management. AMF was considered to be a good tool in order to increase the sustainability of viticulture.

3 Material and method

3.1 Field trial

Soil preparation of the field was done in the autumn 2018 and early spring 2019, which was previously owned by a conventional farmer. The soil was ploughed down to 60 cm depth and horse manure (unknown amount) was spread and mixed into the soil in autumn 2018. Soil improvement was also conducted in May 2019, when the soil was turned over, rocks removed and a disc harrow was used. Incorporated in the soil was also Amfert P20 (granulated P-fertilizer, unknown dosage), K+SKaliSop (Kaliumsul42, K-fertilizer, unknown dosage), Magnum 12 Granul (granulated dolomitic lime with incorporated Mg-fertilizer, unknown dosage) and additional lime (unknown dosage).

Plant material was imported from Germany. Plant material grafted vine (*Vitis vinifera* L, variety Solaris), clone FR 360, rootstock SO_4 ((Selection Oppenheim de Teleki No. 4) - *V berlandieri* x *V. riparia*). Full description of plant material in figure 7, language German. The vine roots were cut back to about 10 cm and then the plants started to be emerged in the soil by the German company Florian Hoffmans. GPS and a Fendt tractor together with a customized vine planting machine was used in order to plant the vines (see figure 6).

Field trial site is located at Kullabergs vineyard, in the southern Swedish region Scania. Coordinates to the vineyard are: Latitude: 56.257045 | Longitude: 12.539901. The vineyard consists of 14 hectares of vine plantation. In 2017-2018 Kullabergs vineyard planted eight hectares of *V. vinifera*, consisting of the varieties Souvignier Gris, Binova, Solaris, Muscaris and Donauriesling. In 2019 an additional four hectares were planted, in which the field trial was conducted. In this new field, the following varieties were planted: Pinot Nova, Solaris and Cabernet Noir. The vast majority of their rootstocks are of the variety SO_4.

Figure 6: Illustration of field trial setup and vine planting machine. Field trial was carried out through 3 separate analysis processes. 1) Phenology, study of vegetative growth. 2) Soil sample, to analyze nutrient status of the soil and soil pH. 3) Root samples, to investigate if colonization of AMF was successful. Source: Hanna Silwer

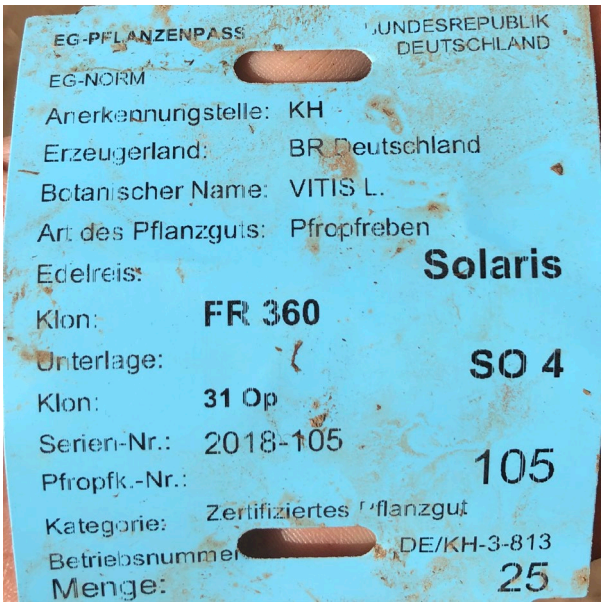


Figure 7: Plant material used in field trial. *Vitis vinifera* L, variety Solaris. Source: Hanna Silwer

The field trial consisted of four blocks, with four treatments in which there were ten vines in each plot. See appendices 1 for complete field trial setup. The four treatments were AMF, biostimulating algae extract, mix between AMF and biostimulating algae extract and a control. Roots were cut prior to inoculation with the treatments and planting was conducted at 13th of May.

AMF treatment was done by inoculation with *Rhizophagus irregularis* (syn: *Glomus irregulare*) SYMPLANTA-001 research grade supplied in 250 g water-insoluble fine, humid (50%) diatomite powder. Precisely 3g of the powder was weighed and put into a plastic bag, in which the roots were submerged and covered with the powder. They were then set aside, with plastic bags on, in a bucket prior to planting.

Biostimulating algae treatment was done by inoculation with PhytoGreen®-Algae extract, containing cold-pressed seaweed juice (*Ascophyllum nodosum*), 2% fulvic and humic acids and 1% iron. The roots were dipped in a 1% solution (10 ml PhytoGreen® roots in 1 liter of water) and set aside in the solution in a bucket prior to planting.

The mix treatment was an inoculation of biostimulating algae (1% solution), followed by submergence in a plastic bag with the AMF powder (3g). The plants were set aside, with plastic bags on, in a bucket prior to planting.

Control plants were only cut by the roots and then put in a bucket prior to planting.

Bare root plants were then given to the machinists, marking the first and last vine of the treatment with a pole. They were planted with precision methods and GPS, with an intra row distance of 0.9m and an inter row distance of 3m. The vines were marked with tags.

Three out of the ten vines in each plot were selected through randomization for observations. See appendices 1 for vine selection. One shoot per vine was also tagged and selected for assessment. A BBCH-scale for grapevines was used to phenologically assess vine growth and development throughout the period of May-August, see appendices 2. Phenology and plant development were assessed once a week, on Tuesdays, and the phenology assessment was revised and based on the research of Coombe (1995). A final measurement was done regarding shoot length in 24th of September. The growth and establishment rate were measured by leaf number counting, according to the BBCH-scale grading criteria. Leaf number was counted on both primary shoot, which was tagged, as well as one axillary shoot of the vine (also tagged). See figure 8a and 8b.

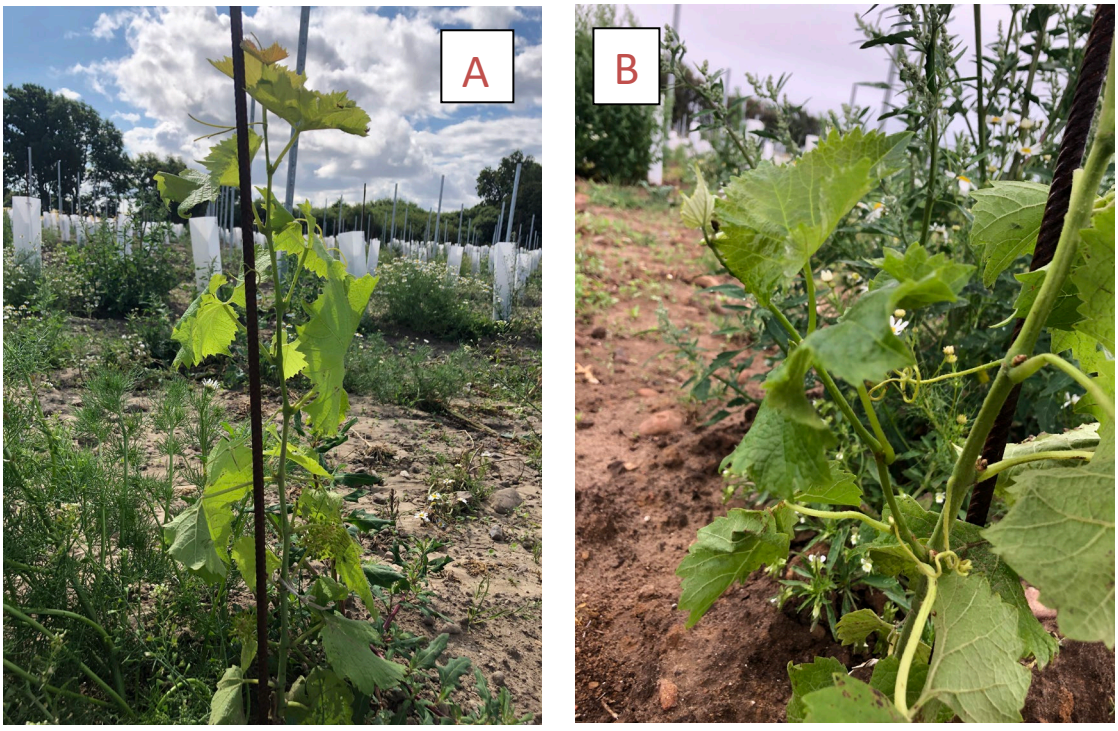


Figure 8A & 8B: Illustration of primary shoot (8A) and axillary shoot (8B), which were measured according to a revised BBCH-scale. Source: Hanna Silwer

Soil samples were taken with two different soil augers 26th of June. First sample of each block was taken with an auger that sampled from 0-20cm of the soil profile. Multiple samples from each control plot was extracted in order to fill a soil sample box from Eurofins, making up a

total of 4 samples, one for each plot. The second soil auger sampled from 20-60cm of the soil profile, of each block. Multiple samples from each control plot was extracted in order to fill a soil sample box from Eurofins, making up a total of 4 samples, one for each plot. There was a total of 8 soil samples. Soils samples were not analyzed *in situ* but sent away for analysis at Eurofins (see appendices 3). Analysis method was by inductively coupled plasma optical emission spectrometry (ICP-OES) (ISO 11885:2007).

Root samples were extracted with an Eijkelkamp Bi-partite root auger set on 3rd of October. The soil profile of 0-15cm was removed and then two samples were taken on the depths 15-30cm and 30-45cm. Root samples were only extracted on control and AMF plots. There was a total of 32 root samples.

3.2 Laboratory analysis

Root samples were stored in a refrigerator 10°C for 20 days. They were then soaked individually in room temperature water for 30 minutes each. All soil was removed from the samples and roots were strained in a 0.5 mm strainer. The roots from each sample were stored in bottles overnight in a refrigerator 10°C. Precisely 15 ml of 2,5% KOH was added to the root samples and they were all heated for 1 min 2 sec or 1 min 23 sec, depending on batch (see appendices 4). After heating they were left for 24 hours in room temperature.

After 24h the roots were washed in deionized water and placed back in their storage container. The container was filled up with 1% HCl, until all root sample was covered and left in the acid over the weekend in room temperature.

Root samples were dyed with blue ink for 24h and then examined in light microscope (Carl Zeiss Germany). For documentation of mycorrhizal colonization an eyepiece camera (VWR International, LLC) connected to WaveImage Image Analysis software was used.

3.3 Literature study

Literature study was conducted both with books, E-books and published articles. Search engines used were, amongst many, SLU Library service Primo, Google scholar, Pubmed and NCIB.

Words searched for included such as cold climate viticulture, arbuscular mycorrhiza, plant biostimulators, viticulture, vine phenology, *Vitis vinifera*, Solaris, nitrogen fertilizer, mineral fertilizer and grapevine physiology.

3.4 Statistical analysis

Statistical analysis was conducted on collected data of BBCH phenotyping and occurrence of AMF on root samples. Used programs were Excel and Minitab, further explained in 4.1.

Statistical analysis methods used were general linear model (ANOVA), Dunnett Multiple Comparisons with a Control, Two-Sample T-Test and Mixed Effects Model. Due to root sample size, no statistical analysis was conducted on root samples. Raw data presented in appendices 5.

4 Results

4.1 Phenological assessment, vine BBCH-scale

During a period of 8 weeks, four blocks were assessed. Vine growth was assessed through a modified vine BBCH scale (appendices 2), where total shoot leaves and axillary shoot leaf number (N_O) was counted and graded according to the BBCH scale. Raw data can be found in appendices 5. Data was processed in Excel and Minitab18. Each observation of the different blocks was performed three times (three out of ten replicates were studied), of which a mean (N_O leaf and N_O axillary shoot leaf) was calculated for each block and treatment.

Provided in table 2 is a schematic compilation of obtained statistical result.

Table 2: Obtained mean leaf number (N_O) at final assessment with BBCH-scale as well as mean grading number according to BBCH-scale development criteria. Data from week 8.

	Shoot leaf		**Axillary leaf**	
Treatment	*BBCH*	*N_O*	*BBCH*	*N_O*
AMF	18.92	19.33 ± 5.16	33.67	2.67± 0,94
Biostimulating algae extract	18.92	16.50 ± 5.67	32.25	1.25 ± 0.92
Combination	19.0	18.42 ± 3.88	32.42	1.42 ± 0.32
Control	18.75	12.42 ± 3.26	31.75	0.75 ± 0.88

In Minitab18, an ANOVA test (general linear model) was performed to see if N_O leaf had a significant difference amongst the treatments, see figure 9. A 95% confidence level was used with responses, N_O leaf and factors block and treatment.

General Linear Model: N_O Leaf versus Block; Treatment

Factor Information

Factor	Type	Levels	Values
Block	Fixed	4	1; 2; 3; 4
Treatment	Fixed	4	Biostimulating algae; Combination; Control; Mycorrhiza

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Block	3	44,11	14,70	0,63	0,612
Treatment	3	113,06	37,69	1,62	0,252
Error	9	209,06	23,23		
Total	15	366,22			

SD
4,81958

Figure 9: Statistical analysis, Anova test (general linear model) of treatment effect on vegetative growth measured in N_O leaf on vine shoot.

What figure 9 illustrates is that P>0.05 for both block (P=0.612) and treatments (P=0.252). This means that there is no significant difference of vegetative growth, measured in N_O leaf on vine shoot, for neither blocks nor treatments. Standard deviation (SD) demonstrates the variation/dispersion of the N_O leaves on shoot. SD=4.81958 is, in this case, high and indicate that the N_O leaves on shoot is spread over a wider range.

In order to investigate the effect of the treatments on the N_O axillary shoot leaf of the vines, another ANOVA test (general linear model) was performed, see figure 10. A 95% confidence level was used with responses, N_O axillary shoot Leaf and factors block and treatment.

General Linear Model: N_O axillary shoot Leaf versus Block; Treatment

Factor Information

Factor	Type	Levels	Values
Block	Fixed	4	1; 2; 3; 4
Treatment	Fixed	4	Biostimulating algae; Combination; Control; Mycorrhiza

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Block	3	1,021	0,3403	0,45	0,723
Treatment	3	7,965	2,6551	3,52	0,062
Error	9	6,785	0,7539		
Total	15	15,771			

SD
0,868250

Figure 10: Statistical analysis, Anova test (general linear model) of treatment effect on vegetative growth measured in N_O axillary shoot leaf on vine shoot.

Similar to figure 9, P>0.05 for both block (P=0.723) and treatment (P=0.062). This meaning that there is no significant difference in vegetative growth, measured in N_O axillary shoot leaf, of the treatments in comparison to the control or between the different blocks. Standard deviation (SD) demonstrates the variation/dispersion of the N_O axillary shoot leaves. SD=0.868250 is, in this case, low and indicate that the N_O axillary shoot leaf is close to the mean.

Due to the high SD in N_O leaf (SD=4.81958), the low SD in N_O axillary shoot leaves (SD=0.868250) as well as the P value of treatment effect on N_O axillary shoot Leaf (P=0.062), further statistical analysis was found necessary. Investigation of the data was conducted in order to see if any of the treatments alone would give a significant difference, in comparison to the control. In order to do that, comparisons with a control (Dunnett test) was performed for N_O axillary shoot leaf, see figure 11.

Comparisons for N_O axillary shoot Leaf

Dunnett Multiple Comparisons with a Control: Treatment

Grouping Information Using the Dunnett Method and 95% Confidence

Treatment	N	Mean	Grouping
Control (Control)	4	0,75000	A
Mycorrhiza	4	2,66667	
Combination	4	1,41667	A
Biostimulating algae	4	1,25000	A

Means not labeled with the letter A are significantly different from the control level mean.

Figure 11: Statistical analysis, Comparison with a control (Dunnett test) for N_O axillary shoot Leaf. Comparison of the treatments Mycorrhiza, Combination and Biostimulating algae against the control.

In Dunnett Multiple Comparisons with a control, confidence level 95%, all means but one is labeled with the letter A. Mycorrhiza mean is not labeled with A, meaning that AMF treatment is significantly different from the control. In order to investigate this significant difference, a Two sample T test was performed, see figure 12.

Two-Sample T-Test and CI: N_O axillary shoot leaf mycorrhiza; N_O axillary shoot leaf control

Method

μ_1: mean of : N_O axillary shoot leaf mycorrhiza

μ_2: mean of N_O axillary shoot leaf control

Difference: $\mu_1 - \mu_2$

Equal variances are assumed for this analysis.

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
N_O axillary shoot leaf mycorrhiza	4	2,667	0,943	0,47
N_O axillary shoot leaf control	4	0,750	0,877	0,44

Test

Null hypothesis	$H_0: \mu_1 - \mu_2 = 0$
Alternative hypothesis	$H_1: \mu_1 - \mu_2 \neq 0$

T-Value	DF	P-Value
2,98	6	0,025

Figure 12: Statistical analysis, Two sample T test, for N_O axillary shoot Leaf. Analyzed treatment, mycorrhiza (AMF), in comparison to the control.

In figure 12, the obtained P<0.05 indicates that there is a significant difference in vegetative growth, measured in N_O axillary shoot leaf, (P=0.025). The null hypothesis (H_0: $\mu_1 - \mu_2 = 0$) can be rejected and thus it is proven that there is a significant difference vegetative growth, measured in N_O axillary shoot leaf, of the AMF treatment and control. Alternative hypothesis, H_1 is accepted.

Due to the significant result obtained in figure 11 and 12 (P<0.05) it became necessary to investigate the statistical block effect. The result in figure 9 and 10 clearly states that there is no significant difference between the blocks and treatments (P>0.05), meaning there is some statistical disturbance. In order to assess the block effect on the result, a Mixed Effect Model on N_O axillary shoot leaf versus block was performed with the blocks as a random factor. See figure 13.

Mixed Effects Model: N_O axillary shoot Leaf versus Block; Mycorrhiza; Biostimulating algae

Factor Information

Factor	Type	Levels	Values
Block	Random	4	1; 2; 3; 4
Mycorrhiza	Fixed	2	0; 1
Biostimulating algae	Fixed	2	0; 1

Variance Components

Source	Var	% of Total	SE Var	Z-Value	P-Value
Block	0,000000	0,00%	*	*	*
Error	0,650463	100,00%	0,265550	2,449490	0,007
Total	0,650463				

Tests of Fixed Effects

Term	DF Num	DF Den	F-Value	P-Value
Mycorrhiza	1,00	12,00	6,67	0,024
Biostimulating algae	1,00	12,00	0,86	0,371
Mycorrhiza*Biostimulating algae	1,00	12,00	4,71	0,051

Figure 13: Statistical analysis, Mixed Effect Model, for N_O axillary shoot Leaf. Analyzed treatments are mycorrhiza (AMF) and biostimulating algae and the combination (Mycorrhiza*Biostimulating algae). The blocks are removed as a randomized factor, making the field trial setup a completely randomized trial instead of blocks.

As seen in figure 13, the block effect is estimated as 0 and thus the block effect is removed by the Kenward-Roger model. The figure also provides P-values different to those in figure 10 (P=0.062 for treatments) and 12 (P=0.025 for mycorrhiza treatment), when the blocks were considered. This suggest that the block effect on the trial had a significant effect on the result. P<0.05 (P=0.024) for mycorrhiza in figure 13 further strengthens the significant difference of AMF in comparison to control. In figure 13, the P-value (P=0.051, P>0.05) for the combination treatment is almost significant, which demanded further research regarding the synergistic effect between AMF and the biostimulating algae extract.

If figure 14, the effect of biostimulating algae and AMF on vegetative growth, measured in N_O axillary shoot leaf is illustrated in an interaction plot, which explains the synergistic effect of biostimulating algae and AMF. What figure 14 illustrates is that the effect of AMF treatment is reduced at presence of biostimulating algae, see red line. But the effect of the biostimulating algae is strengthened at presence of AMF, see blue line. The graph is based on the results obtained in week 8.

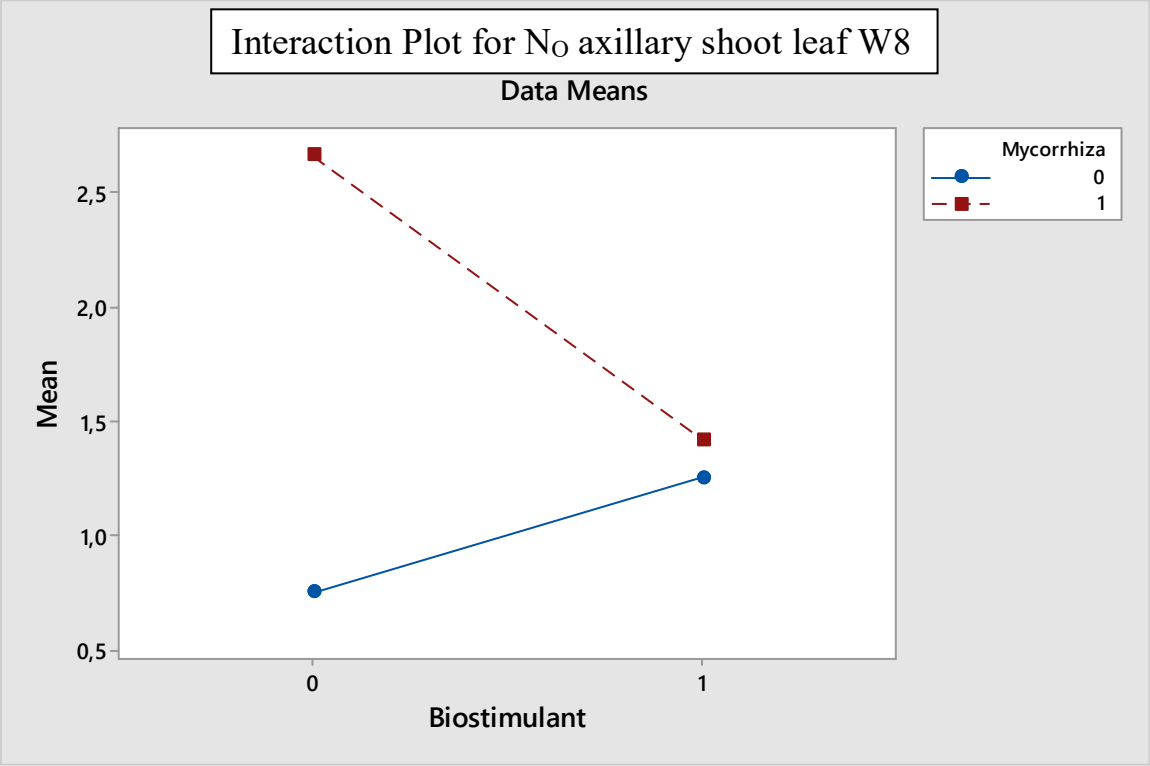


Figure 14: Interaction plot, illustrating the synergistic effect of biostimulating algae (biostimulant) and mycorrhiza (AMF). Red line illustrates the effect on vegetative growth, measured in N_O axillary shoot leaf, of AMF treatment. Blue line illustrates the effect on vegetative growth, measured in N_O axillary shoot leaf, of biostimulating algae treatment. The interaction plot shows that the biostimulating algae treatment is better in combination of AMF, but the AMF treatment is less efficient in combination with biostimulating algae in week 8.

Regardless of the result, the following scatterplots visualize the development of the plants over time. Figure 15 illustrate that the BBCH-scale is not an as good measurement of vegetative growth, in purpose of statistical analysis. Figure 15, x-axis is numbered after the weeks of observation and the y-axis has the BBCH-scale development values. In comparison, figure 16 and 17 also have their x-axis numbered after the weeks of observation, but the y-axis is graded after N_O leaves on shoot and axillary shoot. What can be observed in figure 16 and 17 is that the growth of the control is not as fast as the combination, AMF and biostimulating algae treatment. However, the statistical evidence in figure 9, 10, 11 (besides from mycorrhiza treatment) and 13 show that the development is not significantly faster or greater for any treatment but the AMF treatment.

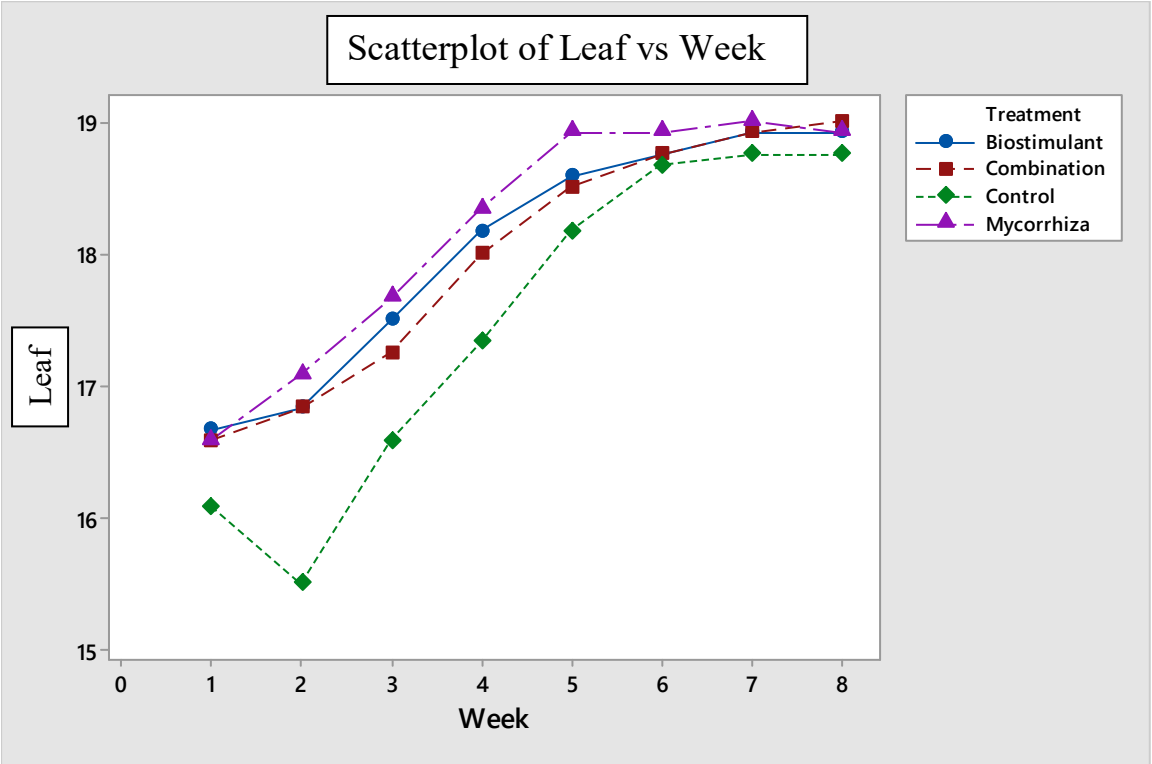


Figure 15: Scatterplot of Leaf vs Week, BBCH-scale phenotypical assessment. Values on y-axis represents given values of the BBCH-scale, values on x-axis represents week of observation.

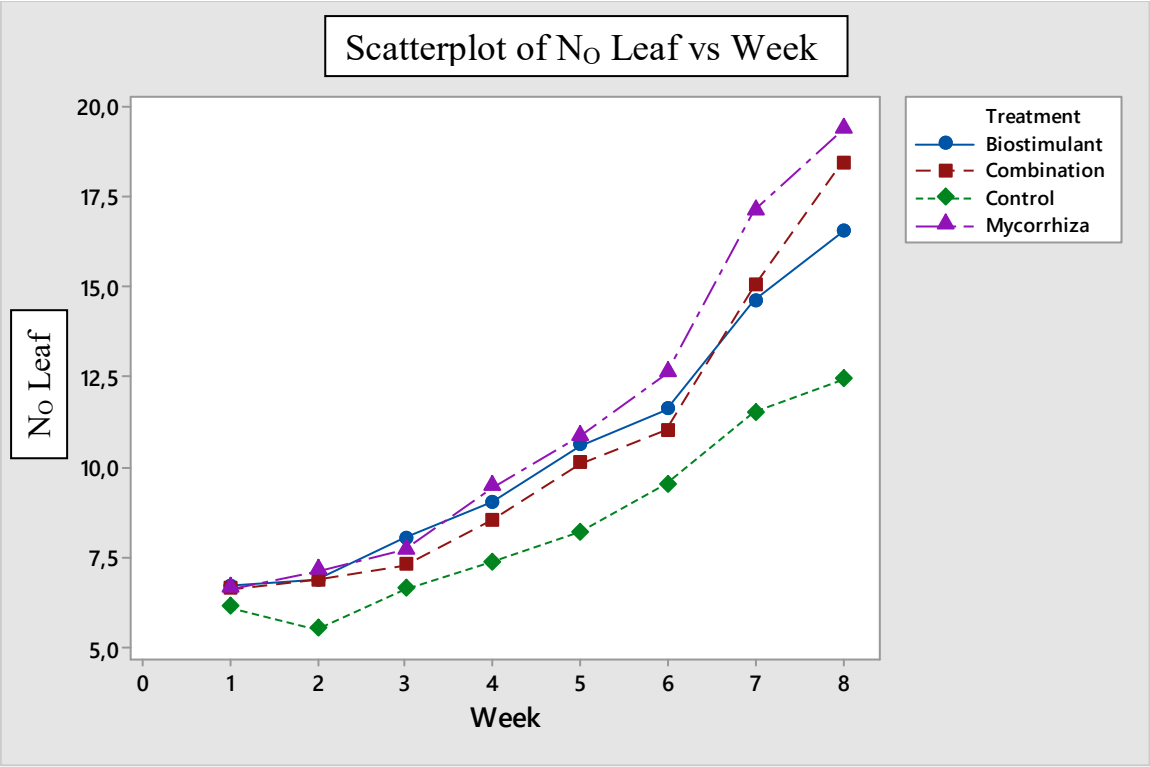


Figure 16: Scatterplot of N_O Leaf vs Week. Values on y-axis represents number of leaves on shoot, values on x-axis represents week of observation.

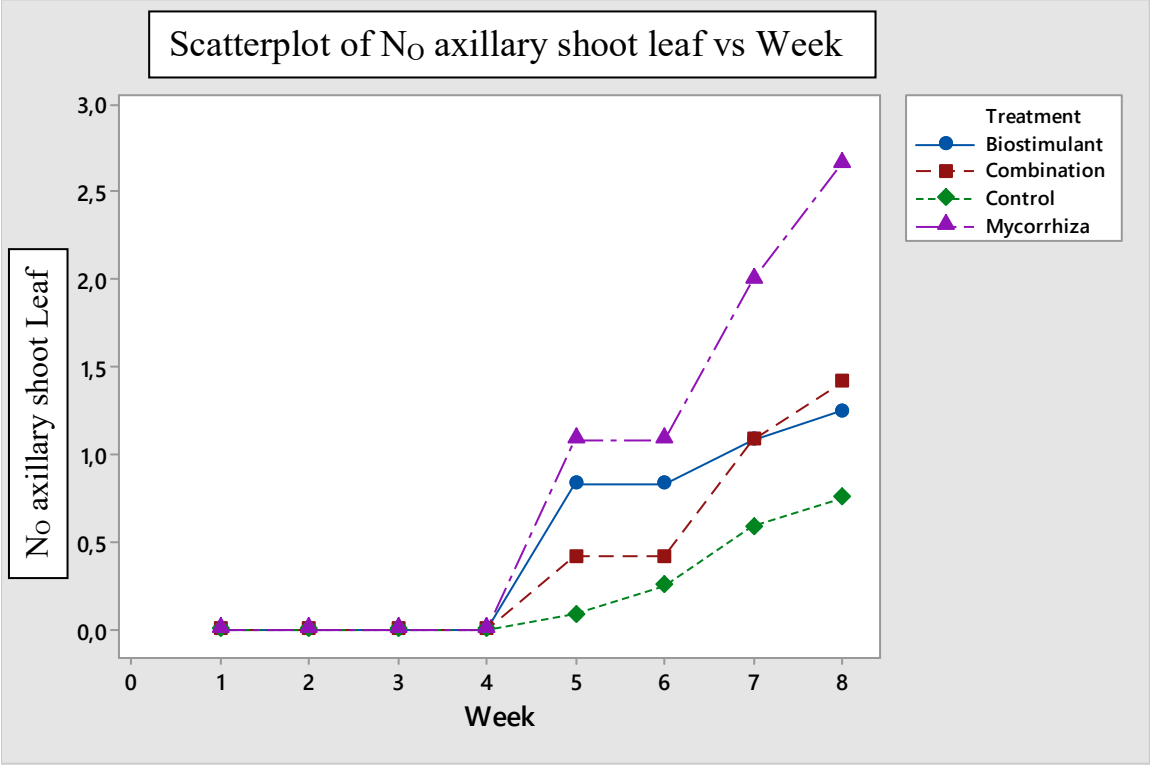


Figure 17: Scatterplot of N_O axillary shoot Leaf vs Week. Values on y-axis represents number of leaves on axillary shoot, values on x-axis represents week of observation

4.2 Soil sample

Full soil sample data and analysis report can be found in appendices 3, where the ones named Hanna are of relevance for this thesis. The soil samples were processed at Eurofins, Kristianstad Sweden. From table 3, the most important data and information has been compiled.

Table 3: Compiled information of relevance for soil samples from field trial. In table 3, the following information can be found for each sample: pH, P-Al, Pal class, Humus content (%), Soil class (Swedish classification system), Clay content and Sand (%).

Depth (cm)	Block	pH	P-Al mg/100g	P-Al class	Humus content %	Soil class	Clay content %	Sand %
0-20	1	6,8	14	IVB	2,1	nmh l Mo	13	59
20-60	1	6,6	13	IVB	2,1	nmh l Mo	12	60
0-20	2	6,7	17	V	2,2	nmh l Mo	12	61
20-60	2	6,5	8,3	IVA	1,4	mf l Mo	13	59
0-20	3	6,8	36	V	2,5	nmh l Sa	11	65
20-60	3	6,4	34	V	2,2	nmh l Sa	10	66
0-20	4	7,1	17	V	1,5	mf sa LL	15	60
20-60	4	7,0	12	IVA	1,5	mf sa LL	15	58

Table 3 shows that there is a slight difference in soil pH over all blocks. P-Al value stands for the plant available P, measured in mg P/100 dry soil, where class I is low amount of plant available P and class V is high plant availability of P. Class III is the class which describes "normal" P status for most crops and if P-Al class is below III, more P has to be added (P shortage), whereas less P has to be added in P-Al classes above III (P abundance). For all samples, P-Al value is above class III, meaning that plant available P is high.

The soil classification system gives information regarding the size of the soil particle sizes and texture, based on the ratio between clay, sand and silt. Humus content (%) represents the organic matter in the soil and the percentage of humus is between 2-6% in a mineral soil.

All 8 soil samples are categorized as one of four representing soil classes (nmh l Mo, mf l Mo, nmh l Sa and mf Sa LL), further described below.

Samples categorized as nmh l Mo all contain some humus (2-3%), are considered to be loamy soils (5-15% clay) and fall in the spectra of the soil type fine sand.

Samples categorized as mf l Mo are poor in humus content (0-2%), considered to be loamy soil (5-15% clay) and fall in the spectra of the soil type fine sand.

Samples categorized as nmh l Sa contain some humus (2-3%), considered to be loamy soil (5-15% clay) and fall in the spectra of the soil type sand.

Samples categorized as mf sa LL are poor in humus content (0-2%), and fall in the spectra of the soil type sandy loam (15-25% clay).

4.3 Root sample

Root sample analysis was conducted in laboratory and microscope. Due to the low amount of roots recovered from sampling in field, no statistical analysis or calculation of colonization rate or colonized area was possible. Each root sample was analyzed twice and it was concluded if there was any colonization of the sample or not. Obtained result can be found in table 4 and 5. Table 4 contain samples from AMF treatment, table 5 contain samples from control. Further information can be found in appendices 4.

Table 4: Results of microscopic analysis on roots derived from AMF plot samples. Illustrated is sample dept, Number of colonized roots (N_O) and percentage of colonized roots.

Treatment	Depth (cm)	N_O colonized vines	%
AMF	15-30	10/11	90.9
AMF	30-45	7/11	63.6
Total	15-45	17/22	77.3

Table 5: Results of microscopic analysis on roots derived from control plot samples. Illustrated is sample dept, Number of colonized roots (N_O) and percentage of colonized roots.

Treatment	Depth (cm)	N_O colonized vines	%
Control	15-30	7/11	63.6
Control	30-45	2/11	18.2
Total	15-45	9/22	40.1

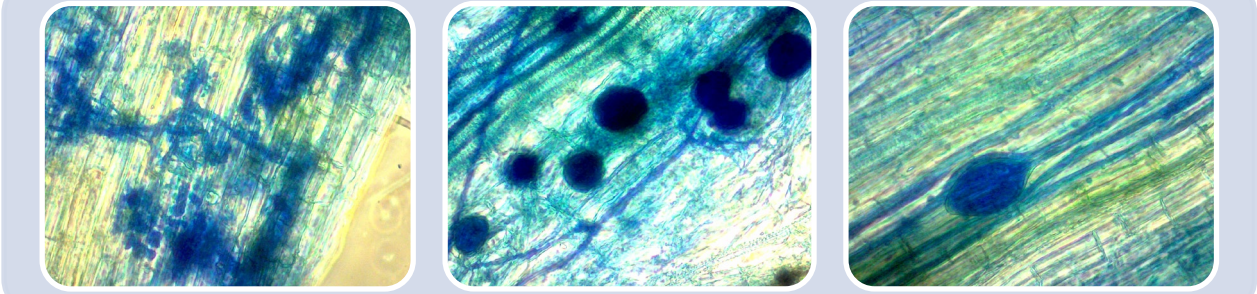
Two samples from AMF treatment plots could not be included in the study, due to that the roots were not thin enough and thus no cell structures or AMF could be visible in the microscope. Corresponding sample numbers for control are also excluded from the result in order to compare the AMF and control.

In table 4, 17 of 22 root samples were found colonized, resulting in that in total 77.3% of the AMF treated roots became successfully colonized. The shallow soil profile samples (15-30cm), 10 of 11 samples were colonized (90.9%). Whereas in deep soil profile samples (30-45cm), 7 of 11 samples were colonized (63.6) Some samples contain other structures from unknown fungal or bacterial species. Other found structures were hyphae and evidence of cells being colonized by other microorganisms.

In table 5, 9 of 22 samples were found colonized, resulting in that in total 40,1% of the control roots were colonized by some AMF species. The shallow soil profile samples (15-30cm), 7 of 11 samples were colonized (63.6%). Whereas in deep soil profile samples (15-30cm), 2 of 11 samples were colonized (18.2%). Some samples contain other structures from unknown fungal or bacterial species. Other found structures were hyphae and evidence of cells being colonized by other microorganisms.

Figures 18 and 19 below are pictures taken with WaveImage Image Analysis software.

In figure 5, the structures of AMF are illustrated. Comparing to this figure, the following structures of AMF can be found in figure 18 and 19:

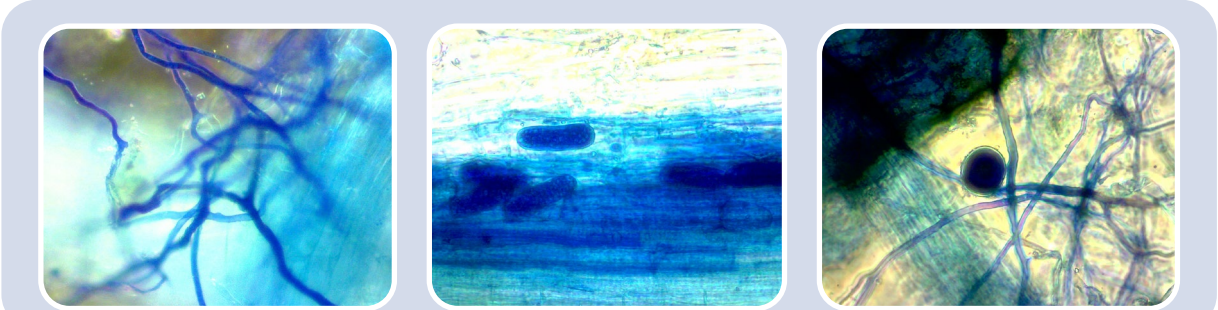


Arbuscules found in root sample of AMF treatment.

Multiple vesicles and hyphae found in root sample of AMF treatment.

Single vesicle and multiple strains of hyphae found in root sample of Control.

Figure 18: Different structures found of the AMF in root samples of AMF treated and control vines. Illustrated is arbuscules, hyphae and vesicles. Source: Hanna Silwer



Hyphae of unknown fungal species, found in root sample of Control.

Structures of unknown microorganism species, found in sample of Control.

Vesicle/chlamyd -ospore and hyphae of AMF, found outside of cellular structures in sample of AMF treatment.

Figure 19: Different structures of the AMF found in AMF treated and control vines. Illustrated is vesicles, hyphae outside of the root cell structures, structures and evidence of infection/colonization of other microorganisms and hyphae of unknown fungal species. Source: Hanna Silwer

5 Discussion

The field trial consisted of three independent analyses, a phenological study, soil samples and root samples in order to investigate if inoculation with biostimulating algae at vineyard establishment would increase growth of *V. vinifera*. The obtained result and circumstances of the trial will now be discussed, in order to identify reasons behind the result and suggestions for further studies in the field.

5.1 Discussion of result, field study BBCH-scale phenological assessment

There was no clearly formulated hypothesis of this thesis; however there were a number of research questions to answer of which statistical analysis was necessary. Figures 9, 10, 11, 12 and 13 are based on the raw data found in appendices 5, where it becomes clear that the AMF treatment is effective, but only for vegetative growth of the shoot axillary shoot (measured in number of leaves), rather than the shoot itself.

At first, as seen in figure 9, there seems to be no significant difference neither between the blocks (P=0.612) nor the treatments (P=0.252) at the 95% confidence level. The fact that there is no significant difference between the blocks suggest that there is some homogeneity of the field or the abiotic factors affecting the plants. However the large standard deviation (SD=4.81958) suggested that there is some difference between the plants, since ≈ 5 leaves differing from the mean number of leaves is a high figure. The reasons behind the potential factors affecting the growth of the vine will be further discussed in section 5.1.2.

If P-value had not been so close to 0.05 (P=0.062) as seen in figure 10, suggesting that there was a trend in increase of axillary shoot vegetative growth, further statistical analysis would probably not have been conducted. However, the trend triggered further analysis and thus it was found that the AMF treatment actually increased vegetative growth of the axillary shoot, but still not for the vine shoot. Thus it was concluded that no further statistical analysis was necessary for assessment of shoot growth.

Dunnett´s test (comparisons with a control) was the primary test showing that there was a significant difference in axillary shoot vegetative growth, see figure 11. Further investigation of the AMF treatment generated the need of the Two sample T-test, where the AMF treatment turned out significant different in comparison to the control, than in the initial statistical analysis where all treatments were considered. The standard deviation in figure 10 (SD=0.868250) and

figure 12 (S_{AMF}=0.943, $SD_{Control}$=0.877) are fairly alike, however the P value differ significantly more. The primary P value obtained in figure 10 (P=0.062) suggests a trend, but not a significant difference between the AMF treatment and control. Whereas in figure 12, P=0.025, which suggests the opposite of the result obtained in figure 10. According to figure 12, there is a significant difference in vegetative growth when comparing the AMF treatment to a control.

The reason behind these contradictory results should be due to the design of the field trial in combination with choice of statistical analysis method. The field trial setup was arranged in blocks, which in case of this field trial was not beneficial. The block effect showed significant impact on the result, as seen in figure 13. Once the field trial was treated as a completely randomized trial, the result showed that there is a significant difference between the control and the AMF on axillary shoot vegetative growth. Mixed Effect Model removed the blocks as a random factor. Once again there were contradicting P values from previous statistical analyses, as seen in figures 9, 10 and 12. What is most interesting from figure 13 is that there is a P-value almost significant for the combination treatment (AMF and biostimulating algae), however P=0.51 is not significant.

The scatter plots in figure 15-17 suggest that the number of leaves on shoot and axillary shoot are more suitable for statistical analysis than the BBCH-scale. The BBCH-scale might be good for field observations and comparisons of developmental stages but not suitable for this study.

The effect the treatments have on each other, biostimulating algae and AMF is of particular interest. The synergistic effect is illustrated in figure 14, where it is a trend that the biostimulating algae treatment gains by co inoculation with AMF. Simultaneously, the effect of the AMF treatment goes down when co inoculated with biostimulating algae. With support the literature study, there are many aspects to discuss over how this outcome might be.

5.1.1 Potential reasons behind synergistic effect of AMF treatment & biostimulating algae

Mancuso et al. (2006) found that the AMF hyphal growth was improved by the application of brown algae extract. Further, Mancuso et al. (2006) suggest that biostimulating algae extracts would stimulate the microbial activity and diversity in the rhizosphere. Contradictory enough, the opposite effect is showed in figure 14. The effect of the biostimulating algae, on the AMF, seems to be non-beneficial, since vegetative growth of the combination treatment is not significantly different from the control (figure 9-11 and 13).

Despite the visible synergistic effect of the AMF treatment and the biostimulating algae, figure 13 suggests that there is a trend, since P=0,051 and a significant result would be obtained at P<0.05. Stated by Sharma et al. (2012) and Khan et al. (2011) is that the biostimulating algae produce hormones such as auxin and cytokinin. These hormones stimulate both root growth as well as above ground foliage development. It might be that the biostimulating algae extract did not contain enough of these hormones, or too much. If levels of biostimulator derived cytokinin are too high, auxin stimulated root growth (initiated from the cambium) could be supressed and thus nutrient and water uptake be less efficient (Keller, 2015). If for instance level of biostimulating auxin is too high, root hair formation might be higher since Muday et al. (2012) stated that the elongation of root hair is stimulated by auxin. If root hair and fine roots are formed at a higher rate, the N and P uptake might be better or sufficient for the plant, meaning it would not gain from a symbiosis with AMF.

The result obtained in figure 14 could also be interpreted as the biostimulating algae had no effect on the crop and that the only effect on growth came from the AMF. This hypothesis is further strengthened by figure 13, where P=0.371 for biostimulating algae only and for combined treatment, P=0.051. Despite the good effects biostimulating algae are said to have, regardless if alone or in co inoculation with AMF, the biostimulating algae could be concluded to not be efficient for *V. vinifera* in this trial. The reasons could be that the synergistic effects the treatments had on each other was non beneficial for the plant, the amount of biostimulating algae inoculation was too high or too low or abiotic factors further discussed in section 5.3.2.

5.1.2 Potential reasons behind AMF treatment not giving significant result on shoot vegetative growth

As figure 9 illustrates, there was no significant difference in vegetative growth of the shoot, measured in number of leaves. Why might this be, since there was a difference in vegetative growth of the axillary shoot of those plants inoculated with AMF? An initial though is connected to the shoot apical dominance and auxin concentrations.

Apical dominance is established as apical buds break (Keller, 2015), meaning that axillary shoot growth and lower shoot growth is supressed and inhibited. It might be that the apical dominance was weak, due to a rapid growth that would lower the shoot auxin flow, as stated by Berleth et al. (2007). The inoculation of AMF could have caused a more rapid growth of the vines, in comparison to the control, which there are signs of in the scatterplots (figures 15-17).

Keller (2015) also stated that the apical dominance could be broken if apex is removed, which occurred multiple times for several plant shoots, see figure 20 below. The shoot apex could for instance get stuck in the tube that was placed around the plants for protection from biotic factors as well as creating a beneficial microclimate. The plant belonging to the picture below is from block 2, vine 49, which is inoculated with AMF.

Figure 20: Picture of vine 49, block 2. Inoculated with AMF. Apex visibly separated from vine, tube illustrated for microclimate and protection from biotic factors. Source: Hanna Silwer.

Apical dominance could also be reduced if more than one bud bursts and allows developing at the same site of the stem, as in figure 21. The vine illustrated in figure 21 is from block 1, vine 17, and inoculated with biostimulating algae extract. This means it is not representative for the claim of whether AMF increase vegetative growth of the shoot or not, but is rather illustrative regarding the effect a reduced apical dominance might have on the obtained result.

Figure 21: Picture of vine 17, block 1, with two shoots on the same site on stem. Inoculated with biostimulating algae. Source: Hanna Silwer

Smith and Read (2008) stated that root:shoot ratio was to be larger for AMF inoculated vines, which was not the case of this field study. The AMF utilize 1-20% of assimilated CO_2, which generally does not affect the plant shoot growth (Jakobsen & Rosendahl, 1990; Heinemeyer et al., 2006). In this case, it might be the opposite, since the shoot growth was not significantly larger for the AMF inoculated vines than for the control. It might have been that the vine could not supply the AMF with enough C, making the vine not able to support the symbiont. Thus, it could be hypothesised that newly established vines vegetative shoot growth initially is inhibited by the AMF symbiosis, however gains in vegetative growth of other structures of the crop. Or it could be hypothesised that newly established vines cannot sustain a viable symbiosis with AMF, due to low levels of exuded C.

One final theory of why shoot growth was not significantly larger of AMF inoculated plants in comparison to the control, but that the vegetative growth of the axillary shoots was, could be connected to the colonization capacity of the AMF. Mycelial growth of the AMF is stimulated by root exudates as well as plant derived flavonoids (Buee et al., 2000; Akiyama et al., 2005). It could be hypothesised that juvenile vines at newly established vineyards might not exude

enough stimulating molecules to initiate enough mycelial branching and thus colonization of the root might not be as large as to make an actual growth difference.

5.2 Potential soil factors affecting the obtained result of field study

Soil sample result, found in both table 3 and appendices 3, showed that all soil samples had high levels of P, since they are all in P-Al classes above P-Al class III. The reason could be that there is either high P content in the soil due to the soil type, or that there had been P added to the field prior to planting.

According to Bleby et al. (2010) P availability and uptake is at its highest in topsoil layers Samples in shallow soil profiles (0-20cm) found in table 3 and appendices 3 should contain more P (or have a higher P-Al class), which is the case in all samples. The reason that there was no increased vegetative growth of vine shoot could be that there was too much available P, meaning that a colonization of a wider range did not happen, even though some AMF structures were found in many samples. If root samples are further mentioned, it became clear that colonization was less extensive for both AMF treatment and control vines in deeper soil profiles (20-60cm).

It might be of importance to discuss the impact of the soil pH on the obtained result. The major factor of P mineral solubility is pH (Valsami-Jones, 2004) and an increased pH would give higher solubility of sediment bound P. The soil samples, as seen in table 3 and appendices 3, range in pH between 6.4-7.1, which is slightly acid to neutral. This means that the solubility of sediment bound P would be reduced as in comparison to a soil with higher pH. However, since P-Al class states that there is enough P, there is no need for further solubility of sediment bound P.

Additionally, the soil pH effect on colonization rate and AMF community vigor was covered by Ouzounidou (2015). There it was found that AMF communities are not viable in pH 5.6 and that the community is reduced significantly at pH 5.8. Even in neutral soils, AMF community is less abundant than in alkaline soils (pH 8.2). The soil samples in table 3 and appendices 3 show that one reason that vegetative growth was no significantly increased for vine shoot could be due to that all sample pH is either neutral or slightly acidic. Graw (1979) also found that P uptake by AMF is reduced in acidic soil, meaning that if AMF colonization was persistent, as seen in many samples found in table 4 and 5, the P uptake ability of the AMF might have been reduced.

Since there are high levels of P, it would reduce colonization rate and thus reduce AMF community in the rhizosphere (Abbott, 1982). However, as stated by Smith and Read (2008), AMF hyphae can also move away from high P level areas, resulting in a broader infection area of the fungi. It is hard to conclude if the high P values in the soil reduced colonization rate and thus did not give and increased vegetative growth of the shoot, or if it enabled the AMF to spread to other areas and vines. The spreading of the AMF, due to high P levels, might have increased colonization rate of some vines, resulting in vegetative growth increase as seen in the number of axillary shoot leaves.

Worth discussing is also the impact of N on field trial result. No data was obtained regarding N status of the soil, meaning it can only be speculated regarding potential impacts of N on the result. Since no vegetative growth increase was visible of the shoot in AMF inoculated vines, it could be assumed that N levels of the soil were high. Lateral root growth is inhibited if soil N levels are too high (Zerihun & Treeby, 2002; Gifford et al., 2008), meaning that less roots in shallow soil profiles would be colonized by AMF, thus reducing the ability to increase shoot growth via AMF symbiosis. Higher N level uptake would also limit root carbohydrates, giving less exudes for AMF and thus potentially reducing colonization rate (Hilbert et al., 2003).

5.3 Other potential factors affecting the obtained result of field study

There are several factors other than nutrient status of the soil and plant material that could have affected the result, both biotic and abiotic. These will be further discussed below.

5.3.1 Inoculation of AMF & biostimulating algae extract

As all field experiments, there are human factors that could affect the outcome of the treatments and result. It is worth discussing the effect wrong amount of inoculant could have had on the obtained result and what might be different if the amounts were altered.

Why was there not any increase in vegetative growth of the shoot? Could it have been that there was not enough *Rhizophagus irregularis* (syn: *Glomus irregulare*) SYMPLANTA-001? It might be that 3g/bare root vine is too little inoculum and that the powder did not stick enough to the plant, meaning it was too much left in the plastic bag the plants were kept in. It might also be that once emerged to the soil, the suspended powder was dispersed in the soil in a too large area around the roots, making it hard for the AMF spores to germinate due to lack of exudes from the roots. It would be interesting to try several different amounts of the AMF powder, in order to see at which point there would be an increase of vegetative growth.

The biostimulating algae was suspended in liquid form, meaning it probably was not taken up by the vine roots during field trial preparation or enough of the liquid stuck to the roots. Desirable would be to allow the vine to absorb some of the liquid or apply it more directly to the rhizosphere after planting, in order to ensure that larger volumes of the biostimulating algae would be beneficial and available for the vine. It would also be interesting to try different concentrations of the biostimulating algae as well as a more frequent application to the crop in order to see if that would generate a different response to the treatment, regarding vegetative growth and vine establishment in the vineyard.

5.3.2 Extraction of roots for root sample analysis & potential factors affecting AMF colonization

The number of colonies found in microscope analysis could not be used to calculate any colonization rate, only to state if colonization or infection had occurred at all. What was found was that it seemed like colonization occurred more frequently in topsoil layers, both for control and inoculated plants.

The result showed that for AMF, see table 4, a total of 77.3% samples were colonized, 90.9% in shallow soil profile and 63.6% in deeper soil profile. However it is not known if the colonizer is the inoculant species, but it could be assumed since the high concentration of spores in contact with the roots would give *Rhizophagus irregularis* (syn: *Glomus irregulare*) a competitive advantage over other AMF or microbial species. Some samples found in table 4 contained other structures from unknown fungal or bacterial species. Other found structures were hyphae, with absence of vesicles and arbuscules, as well as evidence of cells being colonized by other

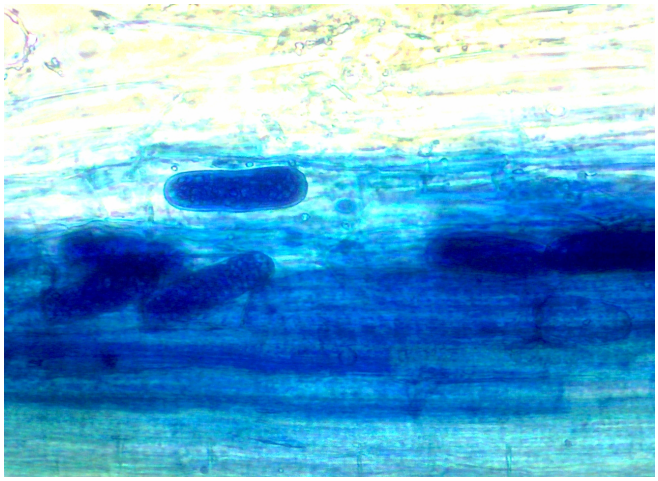


Figure 22: Illustrative photo of root sample from control vine root. Root sample is collected from deeper soil profile (30-45cm) and is colonized by unknown microorganism species. Source: Hanna Silwer

microorganisms. In figure 22 there are other microorganisms that have colonized the root, but it could not be concluded of which species they belong.

In comparison to the control, the AMF inoculated plants had much more colonized roots. But still, as seen in table 5, a total of 40.1% vine roots were colonized by AMF and as much as 63.6% of the root samples from topsoil layers. However, since the control plats were not inoculated and not in especially near location in relation to the AMF inoculated vines, it could be assumed that these vines are either infected/colonized by other AMF species, native to the field.

There are several factors that have to be taken under consideration when discussing the outcome of the root samples. Why are so many control plants colonized and how come that the AMF inoculated vines are not colonized to a greater extent? Some reasons are discussed above in section 5.1 (discussion of result, field study BBCH-scale phenological assessment) and its subheadings. But some other effects are not discussed, which are related to the complexity of the micro flora and fauna of the field.

There could be a case of sporadic colonization, meaning that the availability of inoculum in combination with unfavorable environmental conditions as well as competition with other microorganisms might cause uneven spore germination, causing spatial colonization.

Fitter (2005) stated that spore density of different AMF species in one site can be very dense. The spores can also be in different developmental stages, meaning that for both inoculated and control vines there could have been a large suspension of spores that were ready to germinate and colonize the roots. It is not strange that there is as high colonization rate of the control vines as the result showed. The mycelial network created in the soil would also increase the chances of infection for the control, meaning it could have been an already established and viable mycelial and hyphal network within the trial site.

It would also be interesting to analyze the effect of the weeds within the field on AMF colonization. Tomislav et al. (2012) found that the neighboring weeds of *V. vinifera* affected both colonization and AMF community structure. As seen in figure 23, a change of the field weeds over the trial is illustrated. Initially, there were no weeds, nor any intercropping. However, weeds started to emerge and successfully covered the field trial, to then be removed mechanically to some extent. Flower mix was later sowed between the rows. It would be further interesting to see if the weeds disturbed the colonization, since weed hosted microorganisms

competed with vine colonizing AMF, or if herbaceous weeds would also promote other populations or species of AMF to colonize the roots of the vine, as found by Tomislav et al. (2012).

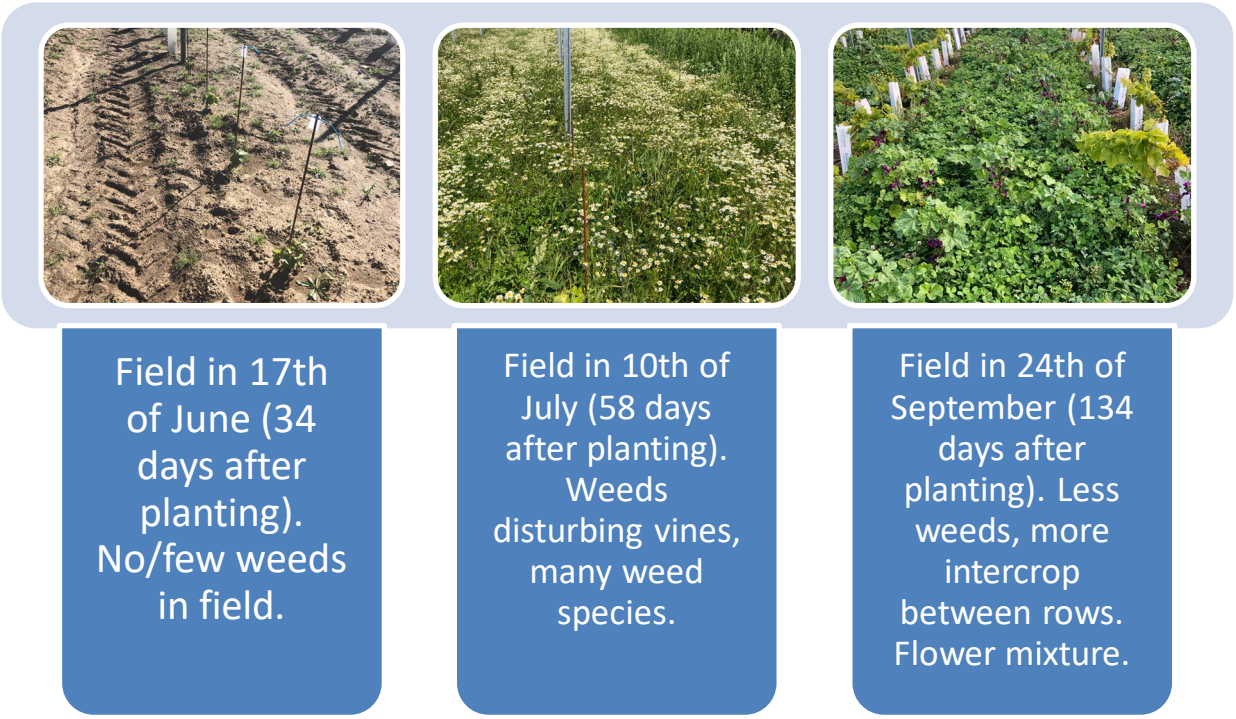


Figure 23: Three illustrative photos of the weeds in the field over time in field study. Source: Hanna Silwer

Figure 24: Photo of weed compeeting with vine in trial (block 1, vine 4). Source: Hanna Silwer

The weeds could also over all possess a competitive threat for the vine over nutrients etcetera. For instance, one vine in the trial (block 1, vine 40, control), could have suffered from competition by the weed in figure 24 (*Chenopodium album*).

A major abiotic factor affecting the trial might have been the heavy rainfall and hail during the field trial period. The rain caused erosion, leaving vine roots and stems exposed. Erosion could cause reduced propagule density and thus leading to a non-significant result in vegetative growth of the shoot. See figure 25 for vine affected by erosion.

A final reflection regarding why the obtained size of the samples were so small would be that the distance samples were taken from, in relation to the vine, was too big. It is stated by and Read (2008) that the ratio of roots to shoot is decreased once symbiosis with AMF is established, meaning that 20cm away from the vine could potentially be a too far distance. Samples should have been obtained closer to the vine in order to get a more significant and analyzable result.

Figure 25: Photo of vine with exposed stem and potentially near surface roots due to soil erosion. Source: Hanna Silwer

6 Conclusion

For the conclusion, I would like to answer my research questions and state if the aim of this thesis was fulfilled.

Research questions stated were as follows:

- *Is symbiosis between AMF and V. vinifera viable in newly established Swedish vineyards?*

Answer: **Yes**, symbiosis between AMF and *V. vinifera* **is** viable in newly established Swedish vineyards. The results given indicate that AMF, both inoculated and native species can cause infection and colonization of *V. vinifera* in present study.

- *Can inoculation with AMF, prior to planting, on bare root V. vinifera, increase vine growth in newly established Swedish vineyards?*

Answer: **Yes** and **No**. Inoculation with AMF, prior to planting, on bare root *V. vinifera*, can partly increase vine growth in newly established Swedish vineyards. Vegetative growth of shoot is not increased in present study; however there is a significant difference in growth of the vine axillary shoot of AMF inoculated vines in comparison to a control. There are many reasons discussed of why this result was obtained and it cannot be concluded which factors are most significant on affecting the obtained result. A mix between human factors, soil chemical and structural properties as well as abiotic factors could have had a significant impact.

- *Can inoculation with plant biostimulating algae extract, prior to planting, on bare root V. vinifera, increase vine growth in newly established Swedish vineyards?*

Answer: **No**, inoculation with plant biostimulating algae extract, prior to planting, on bare root *V. vinifera*, cannot increase vine growth in newly established Swedish vineyards (in present study). Results might be different if other biostimulators are tested, if other concentrations of the used plant biostimulating algae extract is applied or at higher frequency to rhizosphere.

- *Is a combination of inoculation with both AMF and plant biostimulating algae extract, prior to planting, on bare root V. vinifera more efficient in increase of vine growth in newly established Swedish vineyards?*

Answer: **No**. a combination of inoculation with both AMF and plant biostimulating algae extract, prior to planting, on bare root *V. vinifera* is not more efficient in increase of vine growth in newly established Swedish vineyards (in present study). There is a trend suggesting that a co-inoculation would increase vine growth, however the synergistic effects the treatments have on each other suggest that the AMF is the only treatment that would cause a significant increase in growth.

So in order to conclude the answers of the research questions: there is a viable symbiosis between the AMF and vines of the trial. The only treatment that partly worked, since vegetative growth was only observed for one of two measured parameters, is the AMF treatment.

It can be concluded that the aim of this thesis is fulfilled. The AMF does increase uptake and availability of soil macro- and micronutrients, which was measured through an increased vegetative growth of the vine axillary shoot. This could lower the need of N and especially P mineral fertilizers. The nutrient uptake does increase vegetative growth, in some extent, and vine establishment. Farm-level management would potentially become more sustainable if the AMF inoculation was common practice at vineyard establishment in Sweden (and other countries).

Potential future aspects to take in consideration would be to investigate the range of AMF species that colonized the root in order to see if it is the actual inoculant that has colonized the root. It could be that the native AMF species are more beneficial and thus they might be used instead or should be promoted in order to increase the profits of the symbiosis. An analysis of plant material should also be conducted, in order to measure if P uptake was increased or if the increased growth was due to other factors. Further studies has to be conducted in order to see if AMF symbiont is viable over winter and if symbiosis is persistent over a longer period of time and not only for one season.

Further studies are necessary in order to investigate if the plant biostimulating algae extract is more efficient in higher concentrations or if it has to be applied more frequently in order to promote vegetative growth.

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Appendices

1. Field trial setup & vine selection

Planning of trial prior to trial start:

- Four blocks needed for the trial, three treatments and a control in each block. All four blocks contain same three treatments and control.
- Each block contains four plots, one for each treatment and one for control. Each plot contains 10 plants with assigned treatment.
- Block 1 placed in vine row 2 (one treatment/control, total 10 plants) and 4 (three treatments/control, total 30 plants).
- Block 2, placed in vine row 3 (three treatments + control, total 40 plants)
- Block 3, placed in vine row 3 (three treatments + control, total 40 plants)
- Block 4, placed in vine row 4 (three treatments + control, total 40 plants)
- Total amount of plants in trial: 160

Complete description of field trial setup:

- Treatments for trial: biostimulant, AMF, combination of biostimulant and AMF and a control.
- Bunches of 10 vines selected randomly from vine shipping container. Roots cut and treated with inoculum.
- Vines planted in field, one plot of treatments at a time. First and last vine of treatment marked. Accordingly to number in the trial, all vines were assigned block numbers and corresponding plot numbers (1-160) with plastic labels.
- Randomization with excel was conducted, where 3 of 10 vines were selected in each plot for observation. Total number of plants selected for observation: 48

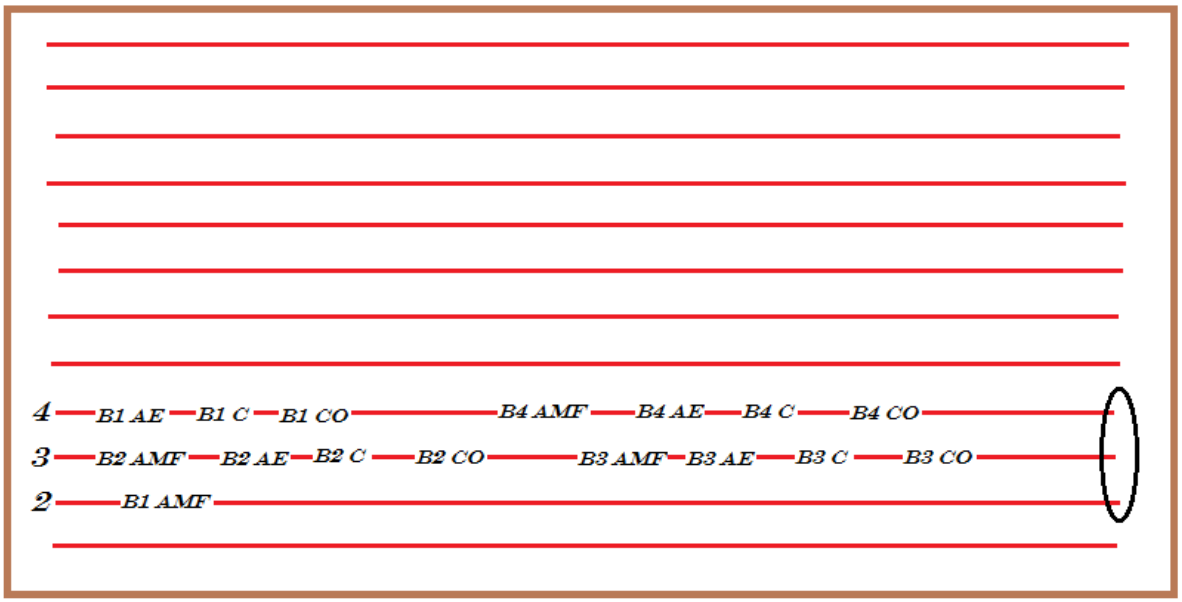

Figure 26: Illustration of field trial setup. Blocks arranged in marked rows. Row 2, 3 and 4 were selected for field trial. Block 1(B1) is situated in row 1 &4. Block 2 (B2) & 3 (B3) are situated in row 3. Block 1 (B1) & 4 (B4) are arranged in row 4. Treatments are AMF, biostimulating algae extract (AE), combined treatment (CO) and control (C). Field illustration is not according to correct scale; however field is rectangular as illustrated in figure and placement of plots is according to field trial setup. Source: Hanna Silwer

2. Grapevine BBCH-scale, revised

Table 6: Revised BBCH-scale for *V. vinifera*. Based on BBCH-scale by Coombe (1995).

Development	Code	Description
Bud	0	Winter bud. Leaf bud pointy or round, light or dark brown in color, Bud scales more or less closed, variety dependent
	1	Buds start to swell; bud starts to expand under bud scales.
	3	End of bud swell, round buds, not green
	5	Wooly bud
	7	Budburst, leaf tip starting to become visible
	8	Budburst, leaf tip clearly visible
Leaf	11	First leaf developed and separated from shoot tip
	12	2nd leaf developed
	13	3rd leaf developed
	14	4th leaf developed
	15	5th leaf developed
	16	6th leaf developed
	17	7th leaf developed
	18	8th leaf developed
	19	9th leaf developed
Sucker	31	Beginning of shoot growth, axils of developed shoots visible
	32	First leaf on sucker developed and separated from shoot
	33	2nd leaf developed
	34	3rd leaf developed
	35	4th leaf developed
	36	5th leaf developed
	37	6th leaf developed
	38	7th leaf developed
	39	8th leaf developed
Flower bud	53	Flower buds fully visible
	55	Flower buds starts to swell, compactly placed flowers
	57	Flower buds fully developed, flowers start to separate
Flower	60	First flower caps loosening
	61	Beginning to flower; 10% caps off
	62	20% caps off
	63	Early flowering; 30% caps off
	64	40% caps off
	65	Full bloom; 50% caps off
	66	60% caps off
	67	70% caps off
	68	80% caps off
	69	Cap fall complete
Berries	71	Berries start to develop & swell, some caps left.

	73	Berries pepper corn size
	75	Berries pea size
	77	Beginning of bunch closure, berries touching
	79	Berries close bunch
Maturing	81	Beginning of ripening; berries start to shift toward variety color
	83	Berries have variety color
	85	Berries soften and touch and close bunch
	89	Berries harvest-ripe (Brix, TA etc.)
Ageing	91	Post-harvest; cane maturation complete
	92	Leaf start to change color
	93	Leaf start to fall
	95	50% leaf fall
	97	End of leaf fall
	99	Dormancy

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Analysrapport

AR-19-LT-010545-01

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Uppdragsnummer:	EUSEKR-00042706	**Uppdragskommentar**	Ref.nr 643LNK	**Proverna ankom**	2019-07-02
Kundnummer	LT3000529			**Utskriftsdatum:**	2019-08-01
Provtyp	Jordprov (M)				

Journalnr	Märkning	pH	pH-mål	Kalk (CaO) pH ton/ha	Kalk (CaO) pH 6,3 ton/ha	Kalk (CaO) pH 6,8 ton/ha	P-AL mg/100 g luftTorrt prov	P-AL klass	K-AL mg/100 g luftTorrt prov	K-AL klass	Mg-AL mg/100 g luftTorrt prov	K/Mg-kvot	Ca-AL mg/100 g luftTorrt prov	Mullhalt % Ts	Jordart	Fe-AL mg/100 g luftTorrt prov	Al-AL mg/100 g luftTorrt prov	Lerhalt %	Sand o Grov % Ts
528-2019																			
07030286	Freja 1	7.6	6.12	0.0	0.0	0.0	50	V	33	V	21	1.6	420	5.4	mmh mo	27	12	15	52
07030287	Freja 2	7.3	6.35	0.0	0.0	0.0	24	V	30	IV	19	1.6	200	1.3	mf sa LL	41	12	18	54
07030288	Freja 3	7.7	6.20	0.0	0.0	0.0	30	V	32	IV	20	1.6	330	4.0	mmh sa LL	26	11	16	54
07030289	Freja 4	7.5	6.35	0.0	0.0	0.0	15	IVB	30	IV	24	1.3	210	2.0	nmh mo	31	12	21	51
07030290	Freja 5	7.7	6.19	0.0	0.0	0.0	34	V	24	IV	16	1.5	300	3.7	mmh l Mo	29	11	14	56
07030291	Freja 6	7.7	6.21	0.0	0.0	0.0	28	V	27	IV	20	1.4	330	3.7	mmh sa LL	24	11	16	52
07030292	Freja 7	7.7	6.20	0.0	0.0	0.0	40	V	32	IV	17	1.9	310	3.6	mmh l Mo	32	12	14	55
07030293	Freja 8	7.7	6.18	0.0	0.0	0.0	35	V	28	IV	18	1.6	330	4.4	mmh sa LL	22	11	16	56
07030294	Freja 9	7.3	6.13	0.0	0.0	0.0	17	V	51	V	13	3.9	310	4.8	mmh l Sa	33	23	13	68
07030295	Freja 10	7.2	6.10	0.0	0.0	0.0	17	V	58	V	15	3.9	280	5.4	mmh l Sa	36	25	13	68
07030296	Freja 11	6.7	6.27	0.0	0.0	0.28	5.2	III	18	IV	6.6	2.7	170	1.9	mf l Sa	27	23	13	71
07030297	Freja 12	7.3	6.14	0.0	0.0	0.0	17	V	52	V	13	4.0	340	4.5	mmh l Sa	33	24	13	70
07030298	Hanna 1	6.8	6.26	0.0	0.0	0.0	14	IVB	34	V	8.5	4.0	130	2.1	nmh l Mo	16	19	13	59
07030299	Hanna 2	6.6	6.25	0.0	0.0	0.56	13	IVB	38	V	12	3.2	120	2.1	nmh l Mo	17	19	12	60
07030300	Hanna 3	6.7	6.24	0.0	0.0	0.28	17	V	41	V	9.4	4.4	140	2.2	nmh l Mo	16	19	12	61
07030301	Hanna 4	6.5	6.29	0.0	0.0	0.78	8.3	IVA	19	IV	8.3	2.3	110	1.4	mf l Mo	19	16	13	59
07030302	Hanna 5	6.8	6.22	0.0	0.0	0.0	36	V	39	V	9.3	4.2	140	2.5	nmh l Sa	27	40	11	65
07030303	Hanna 6	6.4	6.22	0.0	0.0	1.0	34	V	28	IV	8.3	3.4	120	2.2	nmh l Sa	33	43	10	66
07030304	Hanna 7	7.1	6.31	0.0	0.0	0.0	17	V	19	IV	13	1.5	180	1.5	mf sa LL	20	18	15	60
07030305	Hanna 8	7.0	6.31	0.0	0.0	0.0	12	IVA	16	III	9.8	1.6	150	1.5	mf sa LL	21	18	15	58

3. Soil sample

Uppdragsnummer:	EUSEKR-00042706	**Uppdragskommentar**	Ref.nr 643LNK	**Proverna ankom**	2019-07-02
Kundnummer	LT3000529			**Utskriftsdatum:**	2019-08-01
Provtyp	Jordprov (M)				

	pH	pH-mål	Kalk (CaO) pH ton/ha	Kalk (CaO) pH 6,3 ton/ha	Kalk (CaO) pH 6,8 ton/ha	P-AL mg/100 g lufttorr prov	P-AL klass	K-AL mg/100 g lufttorr prov	K-AL klass	Mg-AL mg/100 g lufttorr prov	K/Mg-kvot	Ca-AL mg/100 g lufttorr prov	Mullhalt % Ts	Jordart	Fe-AL mg/100 g lufttorr prov	Al-AL mg/100 g lufttorr prov	Lerhalt %	Sand o Grov % Ts
07030306 Freja 13	7.3	6.14	0.0	0.0	0.0	19	V	60	V	13	4.6	240	4.2	mmh l Sa	32	23	12	73
07030307 Freja 14	7.1	6.12	0.0	0.0	0.0	17	V	52	V	12	4.3	210	4.7	mmh l Sa	39	26	12	70
07030308 Freja 15	6.6	6.30	0.0	0.0	0.57	5.3	III	12	III	5.5	2.2	170	1.6	mf l Sa	28	22	14	69
07030309 Freja 16	7.1	6.12	0.0	0.0	0.0	16	IVB	51	V	13	3.9	240	4.8	mmh l Sa	41	25	12	70

Uppdragsnummer:	EUSEKR-00042706	**Uppdragskommentar**	Ref.nr 643LNK	**Proverna ankom**	2019-07-02
Kundnummer	LT3000529			**Utskriftsdatum:**	2019-08-01
Provtyp	Jordprov (M)				

	Metod	Mätosäkerhet
Al-AL	SS 028310 + T1	
Ca-AL	DIN EN ISO 11885:2009-09	± 32.05%
Fe-AL	SS 028310 + T1	
Jordart	Nilsson, Lantbrukshögskolan, Rapport 47, 1976	
K-AL	DIN EN ISO 11885:2009-09	± 23.38%
Lerhalt	SS ISO 11277mod	
Mg-AL	DIN EN ISO 11885:2009-09	± 30.58%
Mullhalt	KLK 1965 nr 1	
P-AL	DIN EN ISO 11885:2009-09	± 35.13%
pH	ISO 10390: 2005-12	± 5.22%
Sand o Grovmo	SS ISO 11277mod	

Ulla-Britt Nilsson

Rapportansvarig
Denna rapport är elektroniskt signerad

Laboratoriet/laboratoriema är ackrediterade av respektive lands ackrediteringsorgan. Ej ackrediterade analyser är markerade med *

Förklaringar

*Ej ackrediterad analys

Mäto: Mätosäkerhet

Mätosäkerheten, om inget annat anges, redovisas som utvidgad mätosäkerhet med täckningsfaktor 2. Undantag relaterat till analyser utförda utanför Sverige kan förekomma. Ytterligare upplysningar kan lämnas på begäran. Upplysning om mätosäkerhet och detektionsnivåer för mikrobiologiska analyser lämnas på begäran.

4. Laboratory analysis

Table 7: First batch of samples, heating with 2,5% KOH. Time heating in microwave: 1 min 23 sec

AMF treatment	Control
1	13
2	14
3	15
4	16
5	19
6	20
7	21
8	22

Table 8: Second batch of samples, heating with 2,5% KOH. Time heating in microwave: 1 min 2 sec

AMF treatment	Control
9	17
10	18
11	23
12	24

Table 9: Result of microscopic analysis on roots of AMF treatment. Table content illustrates sample number, if colonization is visible under microscope and other comments of importance. Samples are numbered 1-12 followed with -1 or -2, depending on if it was analyze 1 or 2 of the same sample.

Sample number	Depth (cm)	Colonization	Other comments
1-1	15-30	No	Trace of other fungi, visible hyphae of unknown species
1-2	30-45	Yes	
2-1	15-30	No	Signs of colonization, no current infection. Other fungi visible
2-2	30-45	No	
3-1	15-30	Yes	
3-2	30-45	Yes	
4-1	15-30	Yes	
4-2	30-45	Yes	
5-1	15-30	Yes	
5-2	30-45	Yes	
6-1	15-30	No	
6-2	30-45	Yes	
7-1	15-30	Yes	
7-2	30-45	Yes	
8-1	15-30	No	
8-2	30-45	/	Roots too dark, no result. Excluded from result
9-1	15-30	Yes	
9-2	30-45	/	Roots too dark, no result. Excluded from result
10-1	15-30	Yes	
10-2	30-45	Yes	
11-1	15-30	Yes	Contain hyphae and structures of other fungi
11-2	30-45	Yes	
12-1	15-30	Yes	
12-2	30-45	Yes	

Table 10: Result of microscopic analysis on roots of control. Table content illustrates sample number, if colonization is visible under microscope and other comments of importance. Samples are numbered 13-24 followed with -1 or -2, depending on if it was analyze 1 or 2 of the same sample.

Sample number	Depth (cm)	Colonization	Other comments
13-1	15-30	Yes	
13-2	30-45	Yes	
14-1	15-30	No	Trace of other fungi, visible hyphae of unknown species
14-2	30-45	No	
15-1	15-30	No	
15-2	30-45	Yes	
16-1	15-30	Yes	Contain hyphae and structures of other fungi
16-2	30-45	No	
17-1	15-30	Yes	
17-2	30-45	Yes	Contain hyphae and structures of other fungi
18-1	15-30	No	
18-2	30-45	No	
19-1	15-30	No	
19-2	30-45	No	
20-1	15-30	No	
20-2	30-45	Yes	Excluded from result
21-1	15-30	No	
21-2	30-45	No	Excluded from result
22-1	15-30	No	
22-2	30-45	No	
23-1	15-30	Yes	Contain hyphae and structures of other fungi
23-2	30-45	Yes	Contain hyphae and structures of other fungi
24-1	15-30	No	
24-2	30-45	No	

5. Raw data, BBCH-scale phenotyping

Table 10: Raw data from final screening, week 8. Following information is listed: block number, treatment, Leaf BBCH-scale number, number of leaves (N_O Leaf), Sucker BBCH-scale number and number of leaves on axillary shoot (N_O axillary shoot Leaf).

Block	Treatment	Leaf	N_O Leaf	Axillary shoot	N_O axillary shoot Leaf
1	Mycorrhiza	19,0000	20,0000	33,6667	2,6667
2	Mycorrhiza	18,6667	13,6667	34,3333	3,3333
3	Mycorrhiza	19,0000	26,0000	34,3333	3,3333
4	Mycorrhiza	19,0000	17,6667	32,3333	1,3333
1	Biostimulant	18,6667	11,0000	31,3333	0,3333
2	Biostimulant	19,0000	16,3333	32,6667	1,6667
3	Biostimulant	19,0000	24,3333	33,3333	2,3333
4	Biostimulant	19,0000	14,3333	31,6667	0,6667
1	Combination	19,0000	24,0000	32,6667	1,6667
2	Combination	19,0000	18,0000	32,3333	1,3333
3	Combination	19,0000	15,3333	32,0000	1,0000
4	Combination	19,0000	16,3333	32,6667	1,6667
1	Control	19,0000	16,0000	33,0000	2,0000
2	Control	19,0000	14,3333	31,3333	0,3333
3	Control	18,0000	9,3333	31,0000	0,0000
4	Control	19,0000	10,0000	31,6667	0,6667