Wheat response to arbuscular mycorrhizal fungi application in the Western Cape

by

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DECLARATION

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Date: March 2021

Abstract

Wheat (*Triticum aestivum*) is the main cereal crop being planted in the Western Cape of South Africa and is also accounts for over 40% of the world's cereal. Wheat can be grown under a variety of environmental conditions but remains susceptible to adverse temperature and soil conditions. Sustainable methods of crop management such as biofertiliser application, could improve plant adaptability and grain yields in areas affected by droughts and other strenuous environmental conditions. Arbuscular mycorrhizal fungi are one of the most well-known and researched symbiotic organisms in agriculture. The range of benefits AMF provides to a diverse range of crops are indisputable based on both greenhouse and field trials. Despite this, region specific field data is lacking and is necessary to determine if AMF application is viable in a certain area. This study ventured to determine if AMF application in wheat systems would result in colonisation of wheat crops in multiple locations in the Western Cape and the effects of AMF application on plant and yield components. Commercially available AMF products were used in the application process with various strains of mycorrhizal fungi including *Glomus intraradices, Glomus aggregatum, Glomus mosseae* and *Glomus etunicatum*. Several plant and root parameters were measured throughout the growing season including aboveground and belowground biomass as well as grain yield. This study demonstrated that both naturally occurring AMF and applied AMF propagules, were able to colonise wheat plants in the Western Cape. Colonisation was variable across all treatments and sites but were easily visible when root samples were stained and observed under a microscope. Yield increases were observed between AMF treatments and control treatments at the Piketberg (Year 1) and Langgewens (Year 2) trial sites. This observation provides evidence to affirm that AMF application could be beneficial to crop production in the Western Cape. Producers looking to mitigate drought risk and increase grain yields in a sustainable manner would benefit from applying AMF to their soils and ensuring that conservation agriculture practices be followed to maintain the AMF network in the soil.

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1

Introduction

Mycorrhizae is the term used to describe the symbiotic relationship between a mycorrhizal fungus and plant roots. Arbuscular mycorrhizal fungi (AMF; Phylum Glomeromycota) are the most important type of mycorrhizae in agricultural systems (Schüβler et al., 2001). Endomycorrhizal fungi such as AMF, are the most abundant colonisers of agronomical crops such as cereals. Approximately 90% of the plants of the world form symbiotic relationships with mycorrhizal fungi in their natural habitats (Zhu et al., 2010; Ahanger et al., 2014). The fungus colonises the roots of plants and primarily extends the existing root system by growing hyphal networks into the soil. The hyphal roots are much thinner than host plant root hairs, enabling the fungi to reach areas in the soil that plant roots cannot reach.

The plant provides the fungus with carbon (C) in the form of simple carbohydrates and in return the mycorrhizal fungi, together with soil bacteria, increase the uptake of water and soil nutrients such as N, P, K, S, Ca, Fe, Cu, and Zn (Smith and Read, 2008). The effect on biomass and nutrient uptake due to AMF varies widely among plant species and among plant genotypes (Klironomos et al., 2000). Plant species can be classified according to their responsiveness; that is, non-mycotrophic and/or low-, medium-, to high mycotrophic. Mycotrophic plants are reliant on symbiotic fungal relationships to obtain C, water and nutrients from the soil. Cereals, such as wheat (*Triticum aestivum*) are classified as either non-mycotrophic or low/medium mycotrophic according to their genomes (Hetrick et al., 1993). This implies that cereals can obtain C, nutrients and water from the soil without the presence of mycorrhizal fungi; yet, could still benefit from the symbiotic interaction should the roots be colonised. Other benefits of the association with AMF include resistance to biotic and abiotic stresses such as pathogens and drought/salinity, respectively.

The Western Cape region, a winter rainfall area and where this study is based, is the largest wheat producer in South Africa and has widely adopted conservation agriculture (CA) practices. The principles of CA are minimal soil disturbance (no-till), permanent soil cover and crop diversity to allow farmers to manage the soil biota more responsibly. Due to these CA techniques and high crop densities, mycorrhizae can

more easily colonise and spread within wheat crop systems. Using no-tillage systems allows the mycorrhizal fungi hyphae to stay intact in the soil and possibly colonise the following crop in the rotation. The overall yield of the wheat system may also be increased significantly by the addition of mycorrhizal fungi during planting. As such, AMF can be of use to farmers by reducing the need for agrochemicals and fertilisers and increasing soil structure and sustainability. Potential economic and environmental benefits should both be considered by farmers when deciding to inoculate their wheat crops with AMF. This may allow wheat plants to grow better in areas with less rainfall and low soil-nutrient availability such as the Swartland, where many of these study sites are located within the Western Cape.

The aim of this study was to determine if mycorrhizal inoculation affects wheat throughout the life cycle of the plant under dryland conditions in the Western Cape. This aim was fulfilled by achieving two objectives as follows:

1. To evaluate the effect of mycorrhizal inoculation on wheat growth, development, grain yield and quality.
2. To assess whether mycorrhizal inoculum establish in soil in the Western Cape.

CHAPTER 2

Literature review

2.1 Arbuscular Mycorrhizal Fungi

Fungi play an important role in many ecological and microbiological processes, including decomposition of organic matter, increasing soil fertility, cycling of minerals and aiding in plant health and nutrition (Smith and Read, 2008). This beneficial interaction is invaluable in natural environments and even more-so in agricultural practices. It is therefore of the utmost importance to preserve the soil habitat of these fungal communities and to use agricultural methods that facilitate their survival. Arbuscular mycorrhizal fungi (AMF) are fungi that form mutualistic symbiosis with plant hosts and have been termed a “bio-fertiliser” due to their capacity to increase nutrient uptake in plants and potentially improve grain yield in cereal crops such as wheat (Barrow, 2012).

The first AMF interactions with land plants date back nearly 460 million years (Brundrett et al., 2018) and molecular sequence data indicate that the first land plants that were associated with fungi are from the phylum Glomeramycota (Redecker et al., 2000). Currently, AMF are the most common form of mycorrhizal association, found in over 85% of all plant species, including cereal crops. This association is characterised by the fungus colonising roots of plants and forming branched structures, so-called arbuscules that grow intracellularly without penetrating the host cells’ plasmalemma (Finlay, 2008). The host plant provides the fungus with C obtained from photosynthesis in exchange for increased nutrient uptake from the soil and resistance to several abiotic stress factors (Sun et al., 2018).

Mycorrhizal fungi are commonly divided into two main groups termed the endomycorrhizas and ectomycorrhizas. Endomycorrhizal fungi are characterised by the ability of their hyphae to penetrate the cell walls of plant roots and invaginate the cell membrane, while ectomycorrhizal hyphae do not penetrate individual cells within the root (Pegler and Allen, 1993; Harley et. al., 1983). Endomycorrhizal fungi can be further divided into five different sub-groups: Arbuscular endomycorrhizas, Ericoid endomycorrhizas, Arbutoid endomycorrhizas, Monotropoid endomycorrhizas and

Orchidaceous endomycorrhizas (Bagyaraj, 2014). Arbuscular mycorrhizal fungi, being the most common type of mycorrhiza in the soil and infecting most vascular land plants, are the most important type for agricultural uses. Depending on the host-plant species, agricultural crops can benefit from highly diverse AMF communities in the soil albeit with different levels of response to growth and enhanced nutrient uptake (Frew, 2019). Large variations of mycorrhizal species diversity can be present between agricultural sites and even within the same site. This phenomenon cannot only be attributed to host-plant species specificity, but also to edaphic factors such as moisture content, phosphorus (P), nitrogen (N) availability, and soil pH (Burrows, 2002).

The life cycle of AMF begins with the germination of a spore and the formation of extraradical hyphae. Extraradical hyphae are formed outside the roots and grow into the soil to form an extensive network of hyphae also known as a mycelial network (Pumplin and Harrison, 2009). When hyphae come into contact with the surface of plant roots, they form an appressoria, which subsequently penetrate the rhizodermis and spread into the cortical cells of the roots (Bothe et al., 2010). Within the cortex of the root, the fungus forms arbuscules that are extensive, tree-like structures made of intraradical hyphae, which enlarge the surface area for nutrient absorption for both AMF and the plant (Bothe et al., 2010). After each arbuscule is formed, the cortical cells reorganize its shape and envelopes the arbuscules in a symbiosis-specific membrane, called the periarbuscular membrane (Pumplin and Harrison, 2009). This membrane plays a central role in the facilitation of symbiosis by being the primary site of metabolite transfer between host and fungus.

The external hyphae are responsible for the liberation and transport of minerals and water from soil particles and the absorption of nutrient elements such as P, N, zinc (Zn) and copper (Cu) to be used by the host plant (Smith and Read, 2008). It has been demonstrated that spore populations are negatively affected by high P content in the soil (Gaur and Kaushik, 2011). In addition, AMF spore populations fluctuate throughout the year and other than P, may also be influenced by various factors, including soil moisture, soil type, light intensity, nutrient availability, host availability, seasons, and land usage or tillage (Burrows, 2002). However, the spores do remain dormant in the soil until they come into contact with plant roots or until the next crop is planted, and when conditions are optimal. AMF survive in the soil by producing spores that can survive in the soil for several years despite not having host plants to colonise (McGee

et al., 1997). Also, AMF survive and colonises subsequent crops by means of propagules in the form of infected root fragments or extraradical hyphae left in the soil after the host plant was harvested.

It is important to note that studies conducted under field conditions may not yield the same results when compared to studies conducted in a greenhouse environment (Hart, 2018). Greenhouse trials provide the fungus and host plant with ideal soil and environmental conditions to grow, whereas in the field, these conditions are highly variable and often not suitable for AMF infection of plants. Countless greenhouse trials have been conducted to determine the effect of AMF on various factors of plant growth, but only a fraction of those trials have been done under field conditions. It is therefore crucial to conduct field trials that are relevant to farmers and the agricultural industry. By doing these trials we can obtain a clearer indication whether AMF inoculation is a viable agronomic strategy to deal with drought and other abiotic stresses.

2.2 Arbuscular mycorrhizal fungi effect on salinity tolerance

Salinisation of agricultural soils is a widespread problem that severely limits crop production and affects soil health (Villa-Castorena et al., 2003). Many years of intensive agricultural practices such as deep tillage and over-irrigation can cause an increase in soil salinity in certain areas, which has become a problem for cereal farmers (Villa- Castorena et al., 2003). The use of biological methods to address soil imbalances and soil salinity have been thoroughly researched under greenhouse and field conditions in recent years (Al-Karaki and Hammad, 2001; Giri and Mukerji, 2003; Al-Karaki, 2006). Inoculation of AMF being one such method of biological remediation, has been shown to enhance plant tolerance to salinity stress, especially in soils deficient of P by altering the physiology of the plant and increasing water and nutrient uptake (Giri and Mukerji, 2003; Santander et al., 2019). Arbuscular mycorrhizal fungi achieve salinity stress relief by inhibiting the uptake of Na^+ and Cl^- and their transfer to plant shoots in addition to increasing uptake of N, P and Zn. (Giri and Mukerji, 2003; Saqib et al., 2006). Multiple studies have been conducted specifically targeting salinity amelioration of wheat due to AMF inoculation. Studies were conducted under field conditions and share similar results, which strongly indicate that AMF enhanced wheat

plant growth and alleviated osmotic stress under heavy saline conditions (Daei et al., 2009; Mardukhi et al., 2011; Talaat and Shawky, 2014). Certain species such as *Glomus etunicatum* preformed more efficiently in protecting cereal plants from salinity induced stress (Daei et al., 2009).

2.3 Arbuscular mycorrhizal fungi and nutrient uptake

Arbuscular mycorrhizal fungi are key role players of nutrient cycling within the soil. The conversion of immobile, organic nutrient sources such as P, to inorganic nutrient substrates is one of the main benefits of AMF to the plants they colonise. Nutrients such as N, P and Zn are actively converted and absorbed by AMF and transported to the plant in exchange for organic C (Smith and Read, 2008; Al-Karaki and Al-Raddad, 1997; Al-Karaki and Clark 1998; Faber et al., 1990). Arbuscular mycorrhizal fungi are also highly effective in taking up nutrients from nutrient-deficient soils, which can help crops survive and increase yields (Kayama and Yamanaka, 2014).

Nitrogen

The availability of N often limits plant production in agricultural systems. Arbuscular mycorrhizal fungi are able to absorb significant amounts of N from the soil and transport it to the host plant. Depending on soil conditions and the degree of mycorrhizal symbiosis, this method of N uptake may aid plant production significantly (Hodge and Fitter, 2010). While AMF are mostly incapable of decomposition of organic matter for the acquisition of N, they can transport significant amounts of mineral N from organic matter for plant utilization (Hodge, 2014a, b). The delivery of N to the plant is only stimulated when organic C is transferred to the fungus by the plant, indicating C-N trade during symbiosis (Feng et al., 2002).

Phosphorus

Plants require inorganic P to grow and the uptake thereof is significantly reduced under dry soil conditions (Pinkerton and Simpson, 1986). Arbuscular mycorrhizal fungi are responsible for converting organic P in the soil into inorganic P for uptake by plants

especially in ecosystems with limited nutrient availability (Lindahl et al., 2005; Smith and Read, 2008). Enhanced P uptake also directly improves water relations and moisture uptake by fungal hyphae (Ruiz-Lozano et al. 1995). It is important to note that abundant P in the soil, under moisture rich conditions, will inhibit AMF colonisation of crops due to the lack of necessity for symbiosis between plant and fungus (Ryan, Small and Ash, 2000).

Micro-nutrients

Plants require relatively low amounts of micronutrients such as Zn, Cu, Mn, Fe to grow. It has been demonstrated that AMF could be helpful in this regard by enhancing uptake of these elements when subjected to low soil P and adequate water supply (Al-Karaki and Al-Raddad, 1997; Al-Karaki and Clark, 1998; Habte and Manjunath, 1991; Michelsen and Rosendahl, 1990).

2.4 Arbuscular mycorrhizal fungi and disease and pathogen resistance

Due to the non-motile nature of plants, they have inherent coping strategies against pathogens to survive. These include, producing chemicals to attract certain organisms such as pollinators and beneficial root colonisers. Plants can produce a multitude of secondary metabolites with unique and varying structures depending on the specific needs of the plant. Over 100 000 different secondary metabolites have been identified within plants so far, with new ones being discovered regularly (Dixon, 2001). These secondary metabolites also consist of bactericidal and herbicidal compounds that deter infections and prevent diseases (Dixon, 2001). Plant diseases cause serious crop losses annually in agricultural systems and need to be managed responsibly and swiftly to address the problem in a way that is sustainable to the environment. Sustainable approaches to enhancing crop protection by using naturally occurring organisms/substances are necessary for the future of agricultural production. Arbuscular mycorrhizal fungi can serve as biological control agents by preventing soil-borne root diseases in agricultural crops as well as prevent excessive losses due to difficult-to-control root pathogens (Linderman, 1997). Using different species of mycorrhizal fungi or indigenous species adapted to the soil conditions may yield improved results with regards to pathogen resistance as well as the other benefits of AMF inoculation (Zeng, 2006).

Mycorrhizal fungi symbiosis employs several mechanisms to control and prevent pathogenic diseases:

(i) Prevention of pathogen entry by producing polysaccharides and thickening of the cell walls of plant root cells (Dehne 1982).

(ii) Formation of physical barrier by means of sheathing the roots with a mycelial network and surrounding plant root cell walls with hyphae (Ingham, 1991; Maronek, 1981).

(iii) Production of bactericidal and fungicidal compounds that negatively affect pathogenic organisms (Marx, 1972).

(iv) Increasing nutrient uptake by the plant to compensate for the loss of root function due to pathogenic infection (Smith and Reid, 2008).

(v) Directly competing with pathogenic organisms for space and nutrients on plant roots (Reid, 1990).

2.5 Factors affecting arbuscular mycorrhizal fungi colonisation

The symbiotic interaction between AMF and plant roots is one of the tactics that can be used to improve water and nutrient uptake of plants under stress conditions. The degree to which these benefits manifest in practice are affected by various factors such as environmental conditions, host plant species, fungal species diversity and soil conditions. Plants and soil microorganisms alike require substantial amounts of N to grow and multiply. Arbuscular mycorrhizal fungi generally have a higher N concentration per unit of biomass than the plants they colonise. This can lead to competition between host and fungus in N deficient soils, with AMF being superior (Helgason and Fitter, 2009; Hodge and Fitter, 2010).

Several environmental factors determine the efficacy and rate of colonisation of mycorrhizal fungi. Soil acidity as well as soil temperature also affect AMF inoculation and efficiency (Tahat et al., 2010). Factors such as soil type, plant and fungal species determine the overall effect of acidity on symbiosis (Tahat et al., 2010). Some mycorrhizal fungi species grow more effectively in low pH soils, while other species such as *Glomus* spp. adapt better to soils with pH 7 or higher (Abbott and Robson, 1985; Giri and Mukerji, 2003). In addition, AMF species diversity and general resilience of AMF to environmental stresses are factors which improve their survivability, but

each organism has a range of conditions whereby they thrive. Soil temperature may alter the symbiosis between the host plant and mycorrhizal fungi. More inoculum may be produced by changing plant root morphology in addition to plant nutrition and growth due to changes in soil temperature (Hafeel, 2004). Therefore, AMF inoculum rich in diversity that can withstand temperature fluctuations and still maintain symbiotic efficiency would be advantageous.

2.6 Arbuscular mycorrhizal fungi in agriculture

In agriculture, AMF are used as bio-inoculants, but their use as bio-fertilisers in sustainable crop production is gaining traction (Barrow, 2012). Colonised root fragments and extraradical hyphae beneath the soil surface are the main colonisers of future crops. However, agricultural management practices such as tillage, crop rotation and use of chemical inputs affect AMF and propagule survival in the field (Douds et al. 1995). Conventional tillage by means of mould-board ploughs and other intensive tillage implements such as chisel ploughs have been shown to break-up the extraradical hyphal networks beneath the soil, decrease AMF populations, microbial activity, and diversity in the soil (Douds et al. 1995; Evans and Miller 1998). Spores brought to the surface due to tillage practices become exposed to sunlight, which also severely reduce the viability of the spores. In contrast, minimum tillage practices keep hyphal networks intact beneath the soil and allow colonised root fragments and spores to colonise subsequent crops. Yet, there is a paucity of reliable and cost-effective inoculation methods relating to seed treatment and application of the AMF (e.g. in-furrow or and broadcast).

Crop rotation is an essential aspect of conservation agriculture allowing farmers to control weeds and crop diseases more easily and improve soil microbiome diversity. Long fallow periods and usage of canola (*Brassica napus*) or other non-host plants into a crop rotation cycle while applying AMF could result in reduced populations or a complete lack of AMF activity in the soil (Douds et al. 1995; Thompson 1987). Therefore, re-application of AMF is necessary at the start of each new cereal growing season when introducing fallow periods and non-host plants into crop rotation systems.

2.7 Arbuscular mycorrhizal fungi and wheat production in the Western Cape Province (South Africa)

In South Africa, wheat is mainly produced in the Western Cape Province. The region is characterised as having a Mediterranean climate with hot summers and mild, wet winters, but is also prone to experiencing sporadic droughts or dry spells as has been the case the last decade (2010 – 2020). Droughts are detrimental to agricultural systems because they lead to significant yield losses and stunted growth of plants. The average wheat production per hectare in the Western Cape was below the 10 year average (2.68 t ha^{-1}) with 1.8, 2.8 and 1.95 tonnes per hectare in 2017, 2018 and 2019, respectively due to drought conditions according to the annual production reports from Grain South Africa (GrainSA, 2020). In general, shortages in ground water and rainfall result in reduced nutrient uptake by roots and transport of nutrients to the shoots and leaves of the plant due to reduced transpiration and active transport rates. Arbuscular mycorrhizal fungi inoculation has been used on wheat in drought related research for many decades, which has yielded irrefutable evidence that AMF are able to protect plants from severe drought and increase water and nutrient uptake in moisture deficient soils (Al-Karaki, McMichael and Zak, 2003). Wheat plants inoculated with AMF generally have higher grain yield, improved nutrient uptake (especially P), and increased nutrient content in the plant itself when compared to non-inoculated plants. Multiple studies performed under both greenhouse- and field conditions support these findings (Al-Karaki, 1998; Al-Karaki, McMichael and Zak, 2003; Ellis, Larsen and Boosalis, 1985; Saed-Moucheshi, Heidari and Assad 2012).

Agricultural practices in arid or semiarid regions, such as the Western Cape, are characterised by several limitations, most notably the lack of available moisture and soil nutrients, such as P and N. Wheat is mostly grown under rain-fed conditions in a variety of different climatic areas including the semi-arid regions of the Western Cape where drought may occur sporadically during the growing season. Making use of sustainable methods to combat the effect of abiotic stresses on wheat plants would be beneficial to crop production. Bio-fertilisers such as AMF could be a useful tool in sustainable agricultural practices in the Western Cape as an addition to inorganic fertiliser usage. Bio-fertilisers are organic substances that contain naturally occurring microorganisms that improve soil fertility and nutrient uptake of plants to ultimately increase crop production and improve soil health (Sadhana, 2014). Although, localised

research pertaining to the effects of AMF are crucial as results may vary from region to region due to differences environmental and soil conditions, very limited research has been conducted under field conditions in South Africa.

CHAPTER 3

Materials and Methods

3.1 Site description

The Swartland and southern Cape make up the wheat producing area of the Western Cape. The study was conducted at three sites in the Western Cape province of South Africa. Two of these were in the Swartland region with contrasting production potential due to climate differences. The third site was in the southern Cape. The Swartland generally has poorer soil quality than the southern Cape, mostly ascribed to rainfall amount and distribution.

Langgewens Research Farm

Langgewens Research Farm, in the Swartland, is located approximately 18 km north of Malmesbury (33°16'34" S, 18°45'51" E, altitude 179 m). The Swartland region is predominantly a wheat producing area with canola and barley rotations. Average rainfall for this area is around 300 mm per annum. Rainfall during the growing season between April and October (Figures 3.1 and 3.2) was recorded at an average of 232.5 mm and 306 mm for Year 1 and Year 2, respectively. The soils in the area have a sandy loam texture with a high stone content (Table 3.2).

Groenkloof commercial farm (Piketberg)

Groenkloof farm, also located in the Swartland, is approximately 15 km north of Piketberg (32°85'13" S, 18°78'10" E, altitude 155 m). Average rainfall for this area is around 286 mm per annum. Rainfall during the growing season between April and October (Figures 3.1) was recorded at an average of 269 mm for Year 1. The soils in the region range from shallow sandy loam soils to slightly deeper sandy soil (Table 3.1).

Roodebloem Research Farm

Roodebloem Research Farm is located in the southern Cape region in the southern part of the Western Cape Province. The farm is approximately 15 km east of Caledon (34°23'69" S, 19°54'15" E, altitude 1020 m). With a mean annual rainfall of 471 mm, this region has the highest average rainfall of the three trial sites. Rainfall during the growing season between April and October (Figures 3.1 and 3.2) was recorded at an average of 133.6 mm and 570 mm for Year 1 and Year 2, respectively. The soils in this region have a sandy loam texture with a high stone content (Table 3.3).

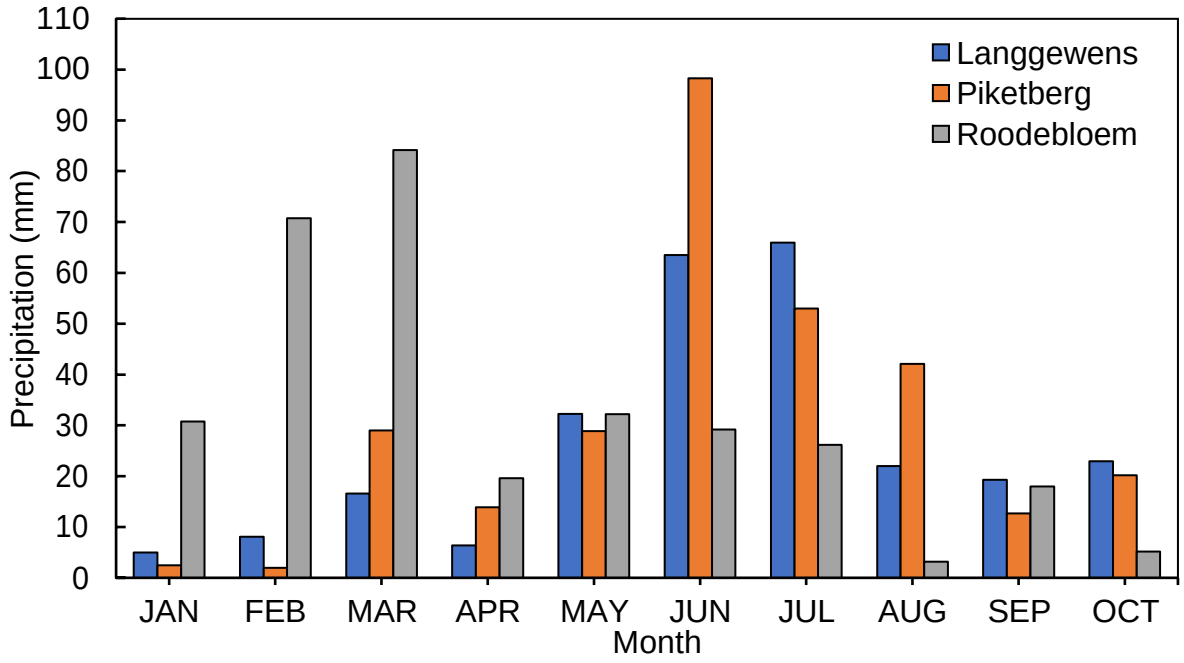

Figure 3.1 Mean monthly rainfall at Langgewens, Piketberg and Roodebloem trial sites during the growing season for 2019 (Year 1).

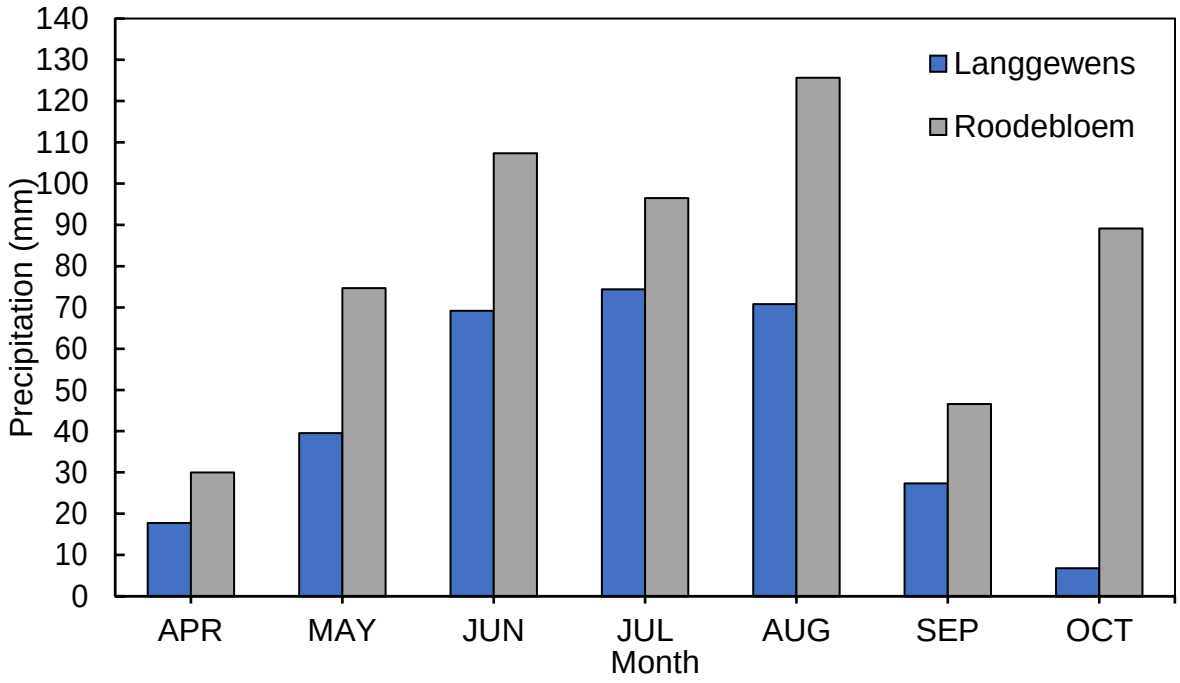

Figure 3.2 Mean monthly rainfall at Langgewens and Roodebloem trial sites during the growing season for 2020 (Year 2).

Table 3.1 Standard soil analysis for the Piketberg trial site in Year 1. Samples were taken at a depth of 30 cm to determine soil quality. Numbers 1 – 5 indicate Treatment plots before treatments were applied to the soil.

Parameter	Piketberg				
	1	2	3	4	5
pH(KCl)	6.1	5.6	5.6	5.2	5.1
Electrical resistance (Ohms)	1190	620	930	530	780
Textural class	Sand	Sand	Sand	Sand	Sandy loam
Exchangeable acidity (cmol(+) kg^{-1})	-	-	-	0.44	0.49
Calcium (mg kg^{-1})	274	256	282	198	222
Magnesium (mg kg^{-1})	54	46.8	63.6	33.6	32.4
Potassium (mg kg^{-1})	123	199	126	157	167
Sodium (mg kg^{-1})	18	40	29	31	25
Phosphorus (mg kg^{-1})	65	53	45	52	44
Total cations (cmol(+) kg^{-1})	2..22	2.36	2.4	2.25	2.41
Copper (mg kg^{-1})	0.47	0.43	0.37	0.33	0.31
Zinc (mg kg^{-1})	0.98	1.16	1.19	1.1	0.75
Manganese (mg kg^{-1})	23.62	40.5	41.23	24.86	35.47
Boron (mg kg^{-1})	0.14	0.13	0.1	0.08	0.1
Carbon %	0.41	0.51	0.46	0.39	0.35
Sulphur (mg kg^{-1})	4.2	12	6.7	6.3	8.2

Table 3.2 Standard soil analysis for the Langgewens trial site in Year 1 and 2. Samples were taken at a depth of 30 cm to determine soil quality. Numbers 1 – 5 indicate Treatment plots before treatments were applied to the soil.

Parameter	Year 1					Year 2				
	1	2	3	4	5	1	2	3	4	5
pH(KCl)	5.5	5.5	6	5.8	5.8	5.5	5.7	5.6	5.6	6
Electrical resistance (Ohms)	290	240	260	470	290	580	680	400	590	630
Textural class	Sandy loam	Sandy loam	Sandy loam	Sandy loam	Sandy loam	Loam	Loam	Loam	Loam	Loam
Exchangeable acidity (cmol(+) kg^{-1})	-	-	-	-	-	-	-	-	-	-
Calcium (mg kg^{-1})	1178	730	1022	1078	1068	4.85	4.07	3.83	3.43	3.86
Magnesium (mg kg^{-1})	180	84	118.8	109.2	104.4	0.91	1.06	0.83	0.78	0.66
Potassium (mg kg^{-1})	182	131	146	215	201	150	161	168	169	150
Sodium (mg kg^{-1})	53	92	69	36	62	33	34	41	31	31
Phosphorus (mg kg^{-1})	99	65	67	81	78	83	81	73	71	76
Total cations (cmol(+) kg^{-1})	8.1	5.1	6.78	7.02	7	6.3	5.7	5.28	4.79	5.05
Copper (mg kg^{-1})	0.37	0.64	0.97	0.63	0.44	-	-	-	-	-
Zinc (mg kg^{-1})	1.08	1.01	0.9	1.43	0.81	1.25	1.46	2.08	0.95	0.99
Manganese (mg kg^{-1})	31.6	59.23	96.35	92.13	27.49	-	-	-	-	-
Boron (mg kg^{-1})	0.19	0.2	0.2	0.22	0.21	-	-	-	-	-
Carbon %	0.78	0.72	0.57	0.68	0.64	0.53	0.63	0.64	0.7	0.73
Sulphur (mg kg^{-1})	16	23	15	11	17	-	-	-	-	-

Table 3.3 Standard soil analysis for the Roodebloem trial site for Year 1 and 2. Samples were taken at a depth of 30 cm to determine soil quality. Numbers 1 – 5 indicate Treatment plots before treatments were applied to the soil.

Parameter	Year 1					Year 2				
	1	2	3	4	5	1	2	3	4	5
pH(KCl)	5.5	5.1	5.6	5.3	4.9	5.3	5.3	5.3	5.4	5.6
Electrical resistance (Ohms)	340	320	190	310	230	410	260	390	360	360
Textural class	Sandy loam	Sandy loam	Sandy loam	Sandy loam	Sandy loam	Loam	Loam	Loam	Loam	Loam
Exchangeable acidity (cmol(+) kg^{-1})	-	0.96	-	0.95	1.16	0.78	0.71	0.72	0.39	-
Calcium (mg kg^{-1})	1072	844	1280	1184	966	5.26	6.28	5.27	5.96	6.06
Magnesium (mg kg^{-1})	147.6	139.2	177.6	133.2	116.4	1.17	1.47	1.32	1.7	1.71
Potassium (mg kg^{-1})	309	241	211	229	185	221	264	267	274	252
Sodium (mg kg^{-1})	67	60	112	53	73	92	103	84	124	117
Phosphorus (mg kg^{-1})	63	59	105	79	62	75	77	83	63	68
Total cations (cmol(+) kg^{-1})	7.68	7.22	8.92	8.8	7.75	8.18	9.58	8.36	9.29	8.93
Copper (mg kg^{-1})	1.26	0.82	0.89	1.15	0.8	-	-	-	-	-
Zinc (mg kg^{-1})	2.51	2.59	3.24	3.09	2.44	2.88	2.57	3.06	3.06	2.72
Manganese (mg kg^{-1})	83.01	63.42	51.32	86.09	70.69	-	-	-	-	-
Boron (mg kg^{-1})	0.38	0.31	0.47	0.39	0.35	-	-	-	-	-
Carbon %	2.18	2.61	2.89	2.32	1.74	1.79	2.05	1.96	1.9	2.02
Sulphur (mg kg^{-1})	7.1	5.6	9.5	7.6	8.5	-	-	-	-	-

3.2 Treatments and experimental design

At each site, a trial was laid out as a randomised block design (RBD) with five treatments replicated in five blocks. The five treatments used for the trial were: i) Endomaxx 5 g ha^{-1}; ii) Endomaxx 10 g ha^{-1}; iii) Endomaxx 20 g ha^{-1}; iv) Industry standard 150 g ha^{-1}; and v) Untreated control (distilled water). The Endomaxx treatments comprised four species of endomycorrhizal fungi spores: *Glomus intraradices, G. aggregatum, G. mosseae*, and *G. etunicatum*. Endomaxx contains a total of 280 propagules per gram of inoculum (70 propagules per gram of each species). The industry standard treatment consists four species of endomycorrhizal spores: *G. intraradices, G. mosseae, G. etinicatum*, and *Scutellospora* spp. The industry standard contains 400 propagules per gram of inoculum and also contains Trichoderma (*Trichoderma harzianum*) at a dosage of $5.2x10^8$ spores per gram of inoculum. Both Endomaxx and the industry standard are mixed with an inert, water soluble powder carriers making application more effective through spreading the spores evenly in the solution.

Each plot in Year 1 (2019) had dimensions of 3 m x 30 m, split lengthwise into halve, to essentially yield two 1.5 m x 30 m plots. Trial plots in Year 2 (2020) were 3 m x 10 m in size and split in half to yield two 1.5 m x 10 m plots. The layout was designed this way to ensure that destructive root and plant sampling could be done on one halve of the plot, without compromising the other half intended for harvesting and yield determination. Each plot was separated from the neighbouring plot with a 2 m buffer zone, which was kept clear of weeds to prevent lateral contamination between treatments. The trial area was surrounded with another 2 m buffer area clear of weeds or other crops, followed by a buffer zone of 1.5 m planted with untreated wheat. New planting sites were used in Year 2 of the study to prevent contamination from the previous year.

3.3 Application methodology

At Langgewens, the treatments were applied to the soil using a hydraulic system fitted to the planter. This would allow the inoculum to be placed directly next to the seed during planting. The inoculum was mixed with nine litres of distilled water and poured into a 25-litre container on the planter. The container was connected to a pump that

split into five tubes with sprayer nozzles which were attached to the seeding tube. The pump was activated whenever seeds were poured into the rotating divider during planting. Langgewens was established using the hydraulic system in Year 1. When planting during Year 2, the knapsack application method was used.

The application method at the Piketberg and Roodebloem farms was adapted to ensure more control over the distribution of the inoculum solution. A knapsack sprayer was used to apply the solution to the soil after planting. The sprayer had a fitting which had five nozzles, one for each of the rows of the planter. Each nozzle was 30 cm apart to coincide with the row width of the planter. Seven litres of water were used with the knapsack solution. This application method proved to be much more effective and ultimately ensured that all the inoculum went into the soil near the seedlings for each treatment.

3.4 Trial Management

Year 1 - 2019

Both Langewens and Roodebloem sites were treated with Sakura (Pyroxasulfone 850 g kg^{-1}) at a dosage rate of 125 g ha^{-1} before planting to eliminate early emerging weeds. The Piketberg site was treated with Roundup (Glyphosate) at a rate of 2 L ha^{-1} before planting. Each of the 25 plots were split in half with 5 rows each. One half was for destructive sampling and the other half for counting and harvest purposes. Each treatment plot was 90 m^2 with an area of 45 m^2 for yield determination.

The planting dates for the Langgewens, Piketberg and Roodebloem trial sites were May 2nd, May 6th and May 10th of 2019, respectively. The wheat variety, SST 0217, were used at all trial sites. The mycorrhizal inoculum was stored in a cool, dry place before weighing out each treatment amount separately for application during planting. The powder inoculum for both Endomaxx and the industry standard were placed into test tubes with screwcaps. The inoculum was mixed with distilled water for application. For the planter system, 9 L of water was used for each treatment to make a solution. For the knapsack application method, 5 L of water was used per treatment (1 L per plot). The untreated control was only distilled water. After each treatment application, the system was thoroughly cleaned with diluted bleach and distilled water to avoid

cross-contamination. Plots at each site were fertilised with NPK fertiliser at a rate of 10 kg N ha^{-1}, 20 kg P ha^{-1}, 10 kg K ha^{-1} in row during planting, with 15 kg N ha^{-1},

30 kg P ha^{-1}, 15 kg K ha^{-1} applied directly after planting. Gypsum was applied at a rate of 16 kg ha^{-1} 14 days after emergence at the Piketberg and Roodebloem sites to increase the sulphur content of the soil. Fertilisation was done according to soil analysis at each site and applied by hand to each plot. Additionally, fertiliser was applied by broadcasting at a rate of 15 kg N ha^{-1}, 30 kg P ha^{-1}, 15 kg K ha^{-1} at each site at 20 DAE and 30 kg N ha^{-1} 60 kg P ha^{-1}, 30 kg K ha^{-1} at 50 DAE.

Pathways were kept clean of weeds by using a non-selective herbicide. Plots were sprayed with Artea (Propiconazole and Cyproconazole) and sulphur to treat White rust (*Puccinia* spp.) at the Langewens site. The Piketberg site was treated with Aurora (Carfentrazone), Glean (Chlorsulfuron) and MCPA for weeds in the planted plots.

Year 2 - 2020

The Piketberg trial site was not used again for Year 2 of the study due to the scaling down of the number of sites. Each treatment plot was 30 m^2 with an area of 15 m^2 for yield determination.

Both Langgewens and Roodebloem trial sites were sprayed with Sakura (Pyroxasulfone 850 g kg^{-1}) at a dosage rate of 125 g ha^{-1} before planting to eliminate early emerging weeds. Each of the 25 plots were split in half with 5 rows each. One half is for destructive sampling and the other half for counting and harvest purposes. The planting dates for Roodebloem and Langgewens trial sites were 5 and 13 May 2020, respectively. The wheat seeds used for all trial sites are of the SST 0217 variety.

The same dosage rates used during Year 1 of the study for the mycorrhizal inoculum were used during Year 2 of the study. The same procedure for inoculum storage and mixing was followed during Year 2 of the study. The total water volume used to make the mixtures was 1.665 L (333 mL per plot). Fertilisation was done at each site according to the common fertiliser usage practices in each area. The Langgewens trial site was applied with LAN (Limestone Ammonium Nitrate) fertiliser at a rate of 15 kg N ha^{-1} in row during planting with 10 kg N ha^{-1} applied as a top-dress, by hand directly after planting. Additionally, 50 kg N ha^{-1} was applied at 20 DAE and

15 kg N ha^{-1} at 50 DAE by hand. The Roodebloem trial site was applied with NPK fertiliser at a rate of 33.8 kg N ha^{-1}, 2.25 kg P ha^{-1}, 5.56 kg K ha^{-1} in row during planting with 9.2 kg N ha^{-1}, 12.75 kg P ha^{-1}, 3.1 kg K ha^{-1} directly after planting as a top-dress application. Additionally, 37 kg N ha^{-1}, 7.4 kg K ha^{-1} was applied at 50 DAE by hand.

The Roodebloem trial site was sprayed with Brush-off (Metsulfuron methyl 600 g kg^{-1}) + MCPA (400 g L^{-1}) for broad leaf weeds, Axial (Pinoxaden 50 g L^{-1}) + Imiboost for grass weed control and Amistar Extra for White rust (*Puccinia* spp.). The Langgewens trial site was sprayed with Logran (Triasulfuron 750 g kg^{-1}) + MCPA for broadleaf weed control, Artea (Propiconazole and Cyproconazole) for White rust (*Puccinia* spp.), and Ceriax (Xemium, F500, Epoxiconazole) for disease control of the wheat.

3.5 Sampling and analysis

3.5.1 Plant parameters

Ten plants per plot were sampled 14 days after emergence (DAE) below the root zone. Spades were used to gently lift the plant out of the soil. The chlorophyll-free coleoptile length was measured to determine the seeding depth. Due to uneven establishment, planting depth was not determined at Roodebloem.

Plant population was determined at 20 DAE by counting ten 1 m rows in the undisturbed half of each plot. Twenty plants were sampled from each plot at 20 and 30 DAE to determine the rooting depth of the plants only during Year 1 of the study. The roots were separated from the shoots, and root length was measured. The same 20 plants used to determine rooting depth were used to determine the dry weight of the roots of each plant at 14, 20 and 30 DAE, respectively. The above- and below ground components were dried for a minimum of 72 hours in an oven at 60˚C to obtain the dry weight. Aboveground biomass determination was done for Roodebloem up to 30 DAE during Year 1 of the study. Root measurements were halted due to hard soils and weed root interference. Root biomass measurements were limited up to 30 DAE for Year 2 due to hardened soils. Rooting depth measurements were omitted for Year 2 for the same reason.

Plant biomass production was determined at 14, 20, 30, 60, 90 and 130 DAE by sampling twenty plants from each treatment plot and separating the aboveground

biomass from the roots of each plant. The plant material was weighed to obtain the fresh weight. The samples were subsequently dried in an oven for a minimum of 72 hours at 60°C and weighed to obtain the dry weight. At 90 DAE, the leaf area index (LAI) of 20 plants from each plot was measured using a LI-COR 3100 leaf area meter. The LAI describes the total potential photosynthetic area of a plant. Higher LAI values may contribute to higher yield potential and biomass of the plant could be (Vina et al., 2011).

Yield components were measured at physiological maturity (130 DAE) to determine potential yield. Five 1 m rows of wheat plants were cut per plot. The total number of spikes were recorded to determine the number of ear-bearing tillers per plant. Twenty spikes were then randomly selected to count the total number of spikelets per spike. Viability of each spikelet was also recorded.

Plots were harvested separately after the plants reached biological maturity with a 10 – 14 % moisture content in the grains. The harvested grains were weighed and used to determine the grain yield. A sub-sample of grains from each plot were fed into a seed counting machine to count and separate 1000 seeds. The 1000 seeds were weighed to determine the 1000 kernel mass of each plot. The 1000 kernel mass was used in conjunction with the spike/spikelet data to determine the potential yield for each plot.

Grain quality parameters were determined by using a Near-infrared spectroscopy machine (Perten Inframatic 9500 – Perten Instruments AB, Huddinge, Sweden) at wavelengths between 570-1100 nm.

3.5.2 Soil measurements

Before planting, soil samples were taken and analysed for sulphur, micro-nutrients and organic carbon (C) content at Langgewens, Piketberg and Roodebloem sites during Year 1 and at Langgewens and Roodebloem during Year 2 of the study. Plant available and unavailable phosphorus, soil C and Zinc were measured at 130 DAE for both Year 1 and Year 2 of the study.

3.6 Mycorrhizal analyses

Root staining was carried out at 90 and 130 DAE on randomly selected root samples taken from sampling five 20 cm rows of plants per plot for Year 1. Root samples were stained and analysed for colonisation at 130 DAE in Year 2. The procedure used to stain the roots is as described by Cao et al. (2013).

Plant roots were cut into 1 – 2 cm segments to ensure complete contact with the reagents and to easily separate the fragments at the end of the procedure. Next, the root samples were cleared with 10% KOH solution in an autoclave for 90 minutes at 95^{o}C and rinsed with water. The samples were then blanched with 10% H_2O_2 solution for 6 – 8 minutes. The roots were rinsed with water whereafter a solution of 7% blue ink-acetic acid was added to the samples and placed in the autoclave for 60 minutes at 90^{o}C. The samples were rinsed again and placed in petri dishes half-filled with water. The root fragments were separated easily and were observed under a stereo microscope. Once potential positive fragments were identified, they were transferred to microscope slides and mounted using glycerol. The root fragment slides were then observed for colonisation under a light microscope.

3.7 Statistical analyses

The data was evaluated by analysis of variance (ANOVA) using mixed models and the restricted maximum likelihood (REML) procedure with the VEPAC package of Statistica (Tibco Inc. 2019). Shapiro- Wilk tests were performed on standardised residuals from the model to verify normality (Shaprio and Wilk, 1965). Bonferroni's test was used at a 5% level to compare means of treatments (Ott, 1998). A probability level of 5% was considered significant for all significance tests.

CHAPTER 4

Results

4.1 Coleoptile length

Planting depth was relatively uniform between treatments at each site during both years of the study (Figure 4.1). In both Year 1 (Y1) and Year 2 (Y2), coleoptile length measurements at 14 DAE, an indication of planting depth, yielded no differences ($p<0.05$) between treatments (Tables 4.1 and 4.2). Due to the high stone content in the soil, the average planting depth of the seeds were below the recommended planting depth of 25 mm for wheat in the Langgewens and Piketberg areas during Y1. Planting depth at Langgewens was close to the recommended depth of 25 mm and Roodebloem had a much deeper planting depth of between 35 mm and 40 mm during Year 2.

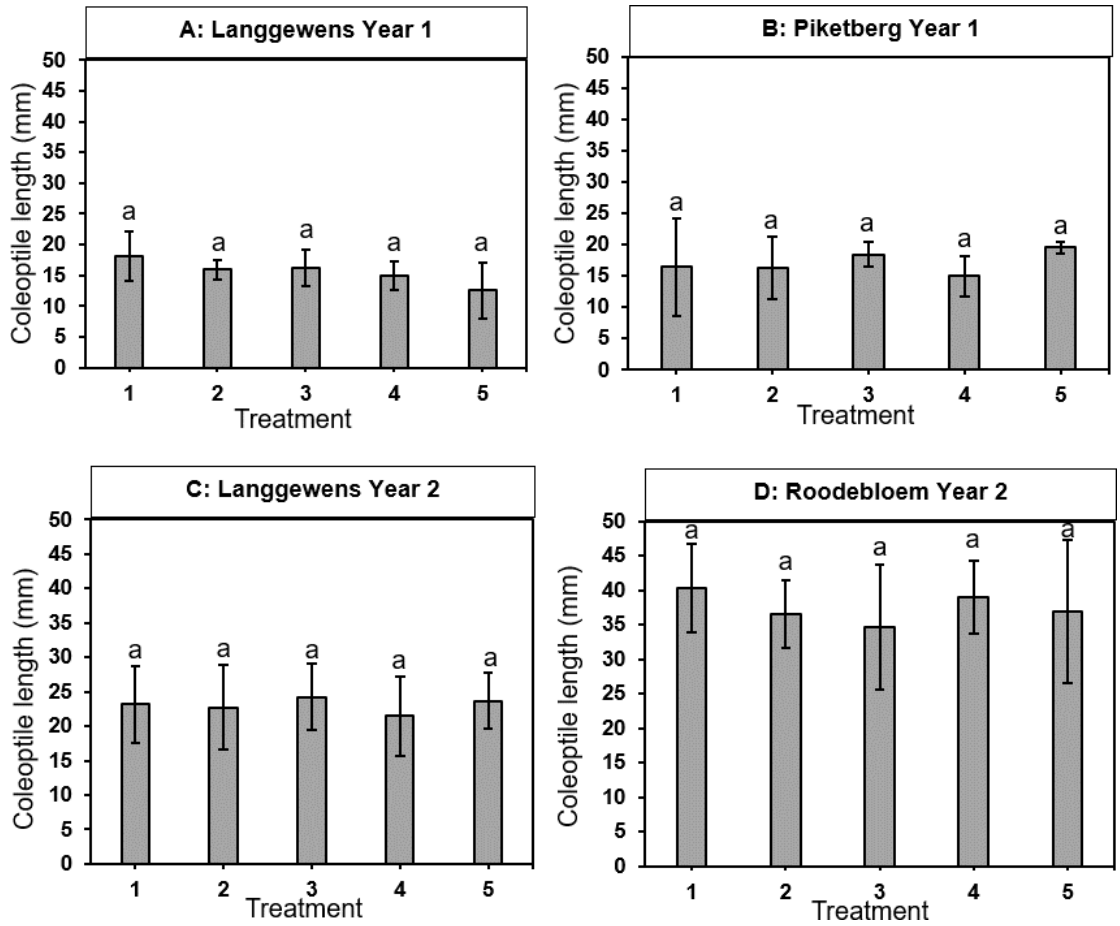

Figure 4.1 Coleoptile length (mm) indicating planting depth at 14 DAE at A) Langgewens (Year 1), B) Piketberg (Year 1), C) Langgewens (Year 2), D) Roodebloem (Year 2). Bars with different letters indicate significant difference at a 5% level. Treatments: 1) Endomaxx 5 g ha^{-1}, 2) Endomaxx 10 g ha^{-1}, 3) Endomaxx 20 g ha^{-1}, 4) Industry standard 150 g ha^{-1}, and 5) Untreated control (distilled water).

Table 4.1. P-values for each of the plant related measurement made throughout the growing season in Year 1 at different localities in the Western Cape. Main effects for treatment and days after establishment (DAE) are shown, and where applicable, the interaction between main factors. Bold-faced values highlight $p < 0.05$.

Parameter	Site	Treatment p-value	DAE p-value	Treatment*DAE p-value
Coleoptile length	Langgewens	0.112		
	Piketberg	0.529		
Aboveground biomass	Langgewens	0.194	**<0.001**	0.116
	Piketberg	0.355	**<0.001**	0.327
	Roodebloem	0.697	**<0.001**	0.453
Plant population	Langgewens	0.943		
	Piketberg	0.264		
	Roodebloem	0.677		
Rooting depth	Langgewens	0.868	**0.012**	0.865
	Piketberg	0.441	**<0.001**	0.728
Root weight	Langgewens	0.508	**0.002**	0.477
	Piketberg	0.714	**0.030**	0.835
Dry matter content	Langgewens	0.805	**<0.001**	0.838
	Piketberg	0.346	**<0.001**	0.893
	Roodebloem	0.330	**<0.001**	0.559
Leaf area index	Langgewens	0.485		
	Piketberg	0.732		
	Roodebloem	0.552		

Table 4.2 P-values for each of the plant related measurement made throughout the growing season in Year 2 at different localities in the Western Cape. Main effects for treatment and days after establishment (DAE) are shown, and where applicable, the interaction between main factors. Bold-faced values highlight $p < 0.05$.

Parameter	Site	Treatment p-value	DAE p-value	Treatment*DAE p-value
Coleoptile length	Langgewens	0.940		
	Roodebloem	0.390		
Aboveground biomass	Langgewens	0.560	**<0.001**	0.960
	Roodebloem	0.030	**<0.001**	0.250
Plant population	Langgewens	0.260		
	Roodebloem	0.760		
Root weight	Langgewens	0.460	**<0.001**	0.100
	Roodebloem	0.230	**<0.001**	0.440
Leaf area index	Langgewens	**<0.001**		
	Roodebloem	0.220		

4.2 Plant population

In both Y1 and Y2, plant populations showed no differences ($p<0.05$) between treatments at any site (Table 4.4, Table 4.5). Moreover, plant populations were uniform between treatments (Figure 4.2). The average plant population was close to, or within the target plant population of 120 to 200 plants m^{-2} at all sites during Y1 and Y2, except for Roodebloem during Y1. During Y1 the plant populations at Langgewens ranged from 165 to 176 plants m^{-2}, Roodebloem from 30 to 40 plants m^{-2} and Piketberg from 92 to 117 plants m^{-2}. During Y2 the plant populations at Langgewens ranged from 118 to 145 plants m^{-2} and Roodebloem from 173 to 193 plants m^{-2}. Severely low plant populations at the Roodebloem site during Year 1 of the study may have been attributed to low soil moisture and low rainfall at the time of planting followed by guinea fowl (*Numida meleagris*) damage after planting.

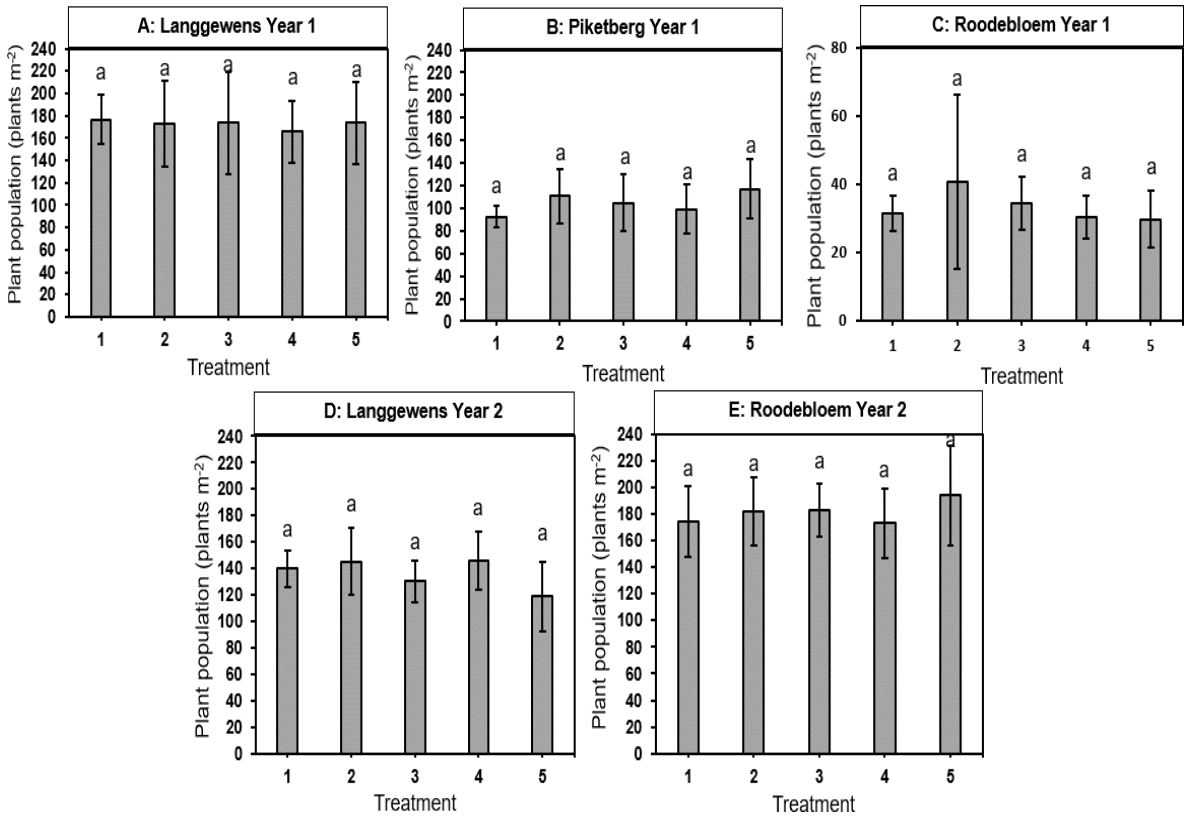

Figure 4.2. Plant population (plants m^{-2}) at 14 DAE at A) Langgewens (Year 1), B) Piketberg (Year 1), C) Roodebloem (Year 1), D) Langgewens (Year 2), E) Roodebloem (Year 2). Bars with different letters indicate significant difference at a 5% level. Treatments: 1) Endomaxx 5 g ha^{-1}, 2) Endomaxx 10 g ha^{-1}, 3) Endomaxx 20 g ha^{-1}, 4) Industry standard 150 g ha^{-1}, and 5) Untreated control (distilled water).

4.3 Root weight

Root weight is a representation of the total belowground biomass of the plants. In Year 1, there were no differences ($p<0.05$) between treatments at Langgewens or Piketberg for root weight over time (Table 4.1, Figure 4.3). However, differences ($p<0.05$) were observed at 20 DAE between Treatment 1 (T1) and Treatments 2 (T2) and 5 at Langgewens. In Year 2, differences ($p<0.05$) were observed at Langgewens (Figure 4.3) between Treatment 4 and Treatment 3 at 30 DAE for root dry weight (Table 4.2), but there were no significant differences at Roodebloem (Figures 4.3.4) for root weight (Table 4.2).

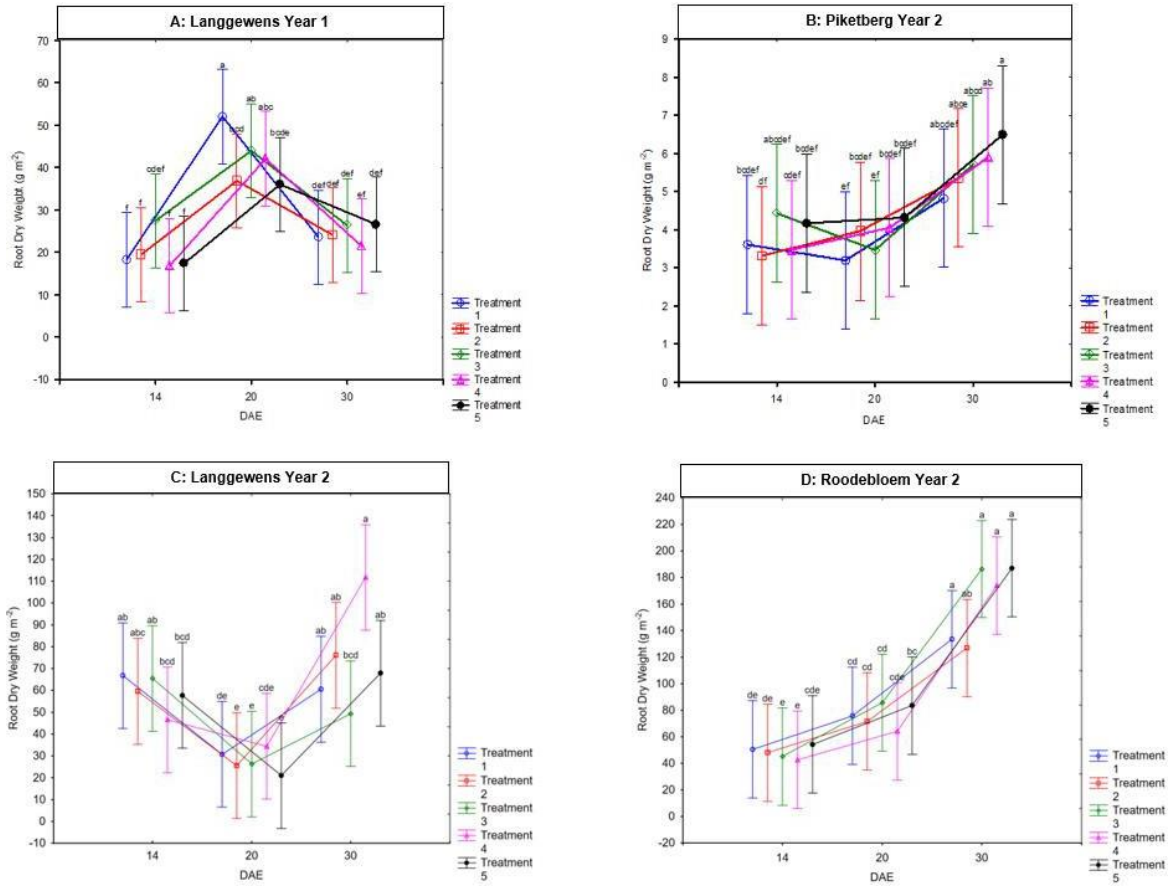

Figure 4.3 Root weight (g m^{-2}) of wheat plants at 14, 20 and 30 DAE at A) Langgewens (Year 1), B) Piketberg (Year 1), C) Langgewens (Year 2), D) Roodebloem (Year 2). Different letters between points in time indicate significant difference at a 5% level. Treatments: 1) Endomaxx 5 g ha^{-1}, 2) Endomaxx 10 g ha^{-1}, 3) Endomaxx 20 g ha^{-1}, 4) Industry standard 150 g ha^{-1}, and 5) Untreated control (distilled water).

4.4 Rooting depth

Rooting depth showed no differences ($p<0.05$) between treatments (Table 4.1) at both Langgewens and Piketberg during Year 1 of the study. (Figures 4.4).

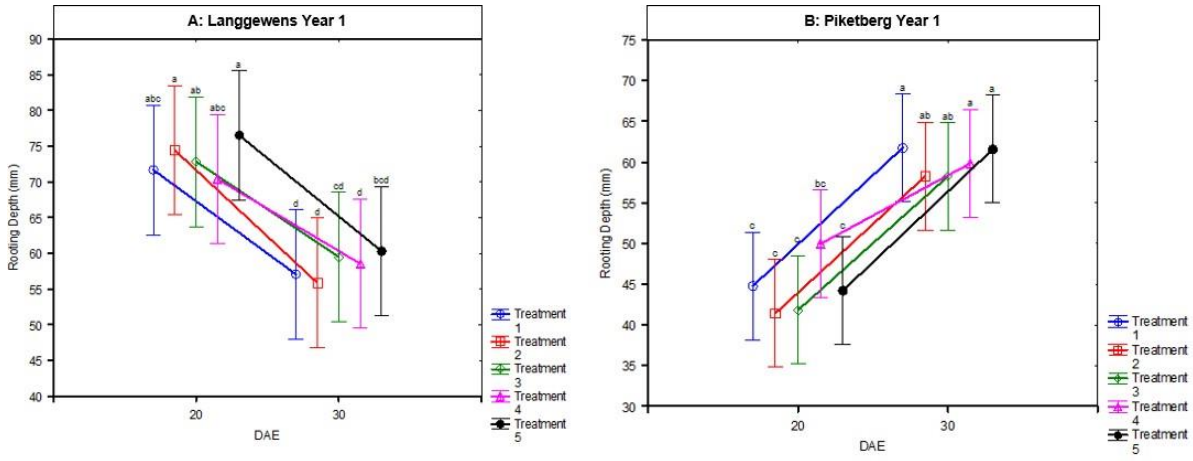

Figure 4.4 Rooting depth (mm) at 20 and 30 DAE at A) Langgewens (Year 1), B) Piketberg (Year 1). No common letters between points in time indicates significant difference at a 5% level. Treatments: 1) Endomaxx 5 g ha^{-1}, 2) Endomaxx 10 g ha^{-1}, 3) Endomaxx 20 g ha^{-1}, 4) Industry standard 150 g ha^{-1}, and 5) Untreated control (distilled water).

4.5 Aboveground biomass production

Wheat plant biomass production was not affected ($p<0.05$) by mycorrhizal inoculation regardless of location (Figure 4.5.1) (Figure 4.5.2) trial sites during Year 1 of the study (Table 4.1). Biomass production remained uniform between treatments at each trial site with slightly more variation from 90 DAE.

In Year 2, the biomass production of wheat plant was affected ($p<0.05$) by mycorrhizal inoculation at Langgewens (Table 4.2). Differences in aboveground biomass at Langgewens (Figure 4.5.3) were observed between Controls and Treatments 1 and 3 at 60 DAE. Differences ($p<0.05$) were also observed between T5 and T1 to T4 at 90 DAE at Langgewens. Similarly, differences ($p<0.05$) were also observed between untreated controls and Treatment 2 and 3 at 60 DAE and 90 DAE, respectively at the Roodebloem trial site (Table 4.2). Plants not supplemented with mycorrhizae exhibited higher biomass production values of 5866 kg ha^{-1} and

16 505 kg ha^{-1} at 60 and 90 DAE, respectively compared to T2 with 3401 kg ha^{-1} and T3 with 10 003 kg ha^{-1} at their respective sampling dates (Figure 4.5.3). The biomass curves followed the standard growth curve as expected with increased biomass between 90 DAE and 120 DAE due to late rains and high soil moisture content.

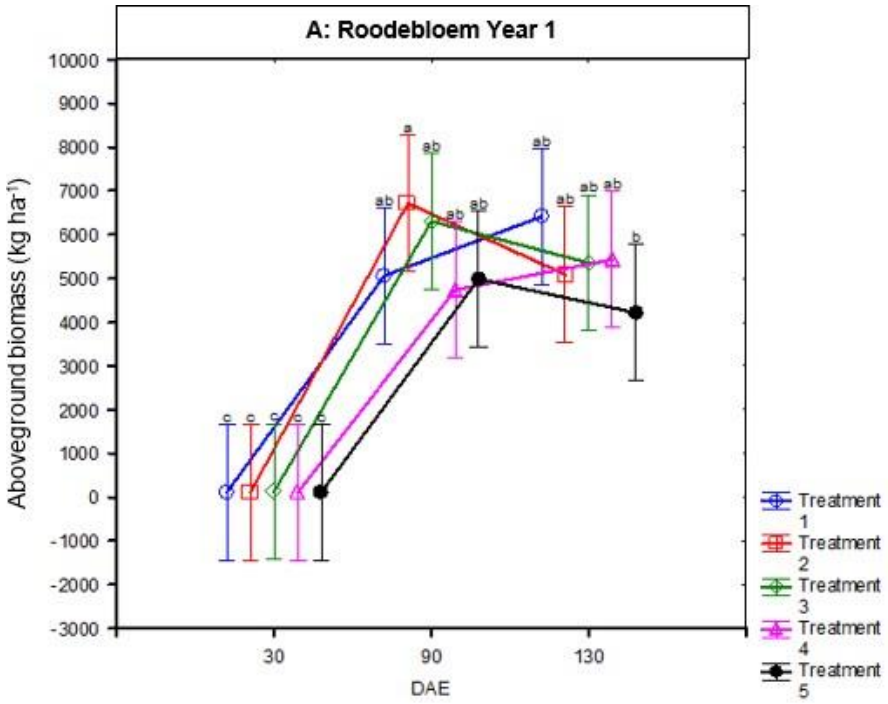

Figure 4.5.1 Aboveground biomass (kg ha^{-1}) at 30, 90 and 130 DAE at A) Roodebloem (Year 1). No common letters between points in time indicates significant difference at a 5% level. Treatments: 1) Endomaxx 5 g ha^{-1}, 2) Endomaxx 10 g ha^{-1}, 3) Endomaxx 20 g ha^{-1}, 4) Industry standard 150 g ha^{-1}, and 5) Untreated control (distilled water).

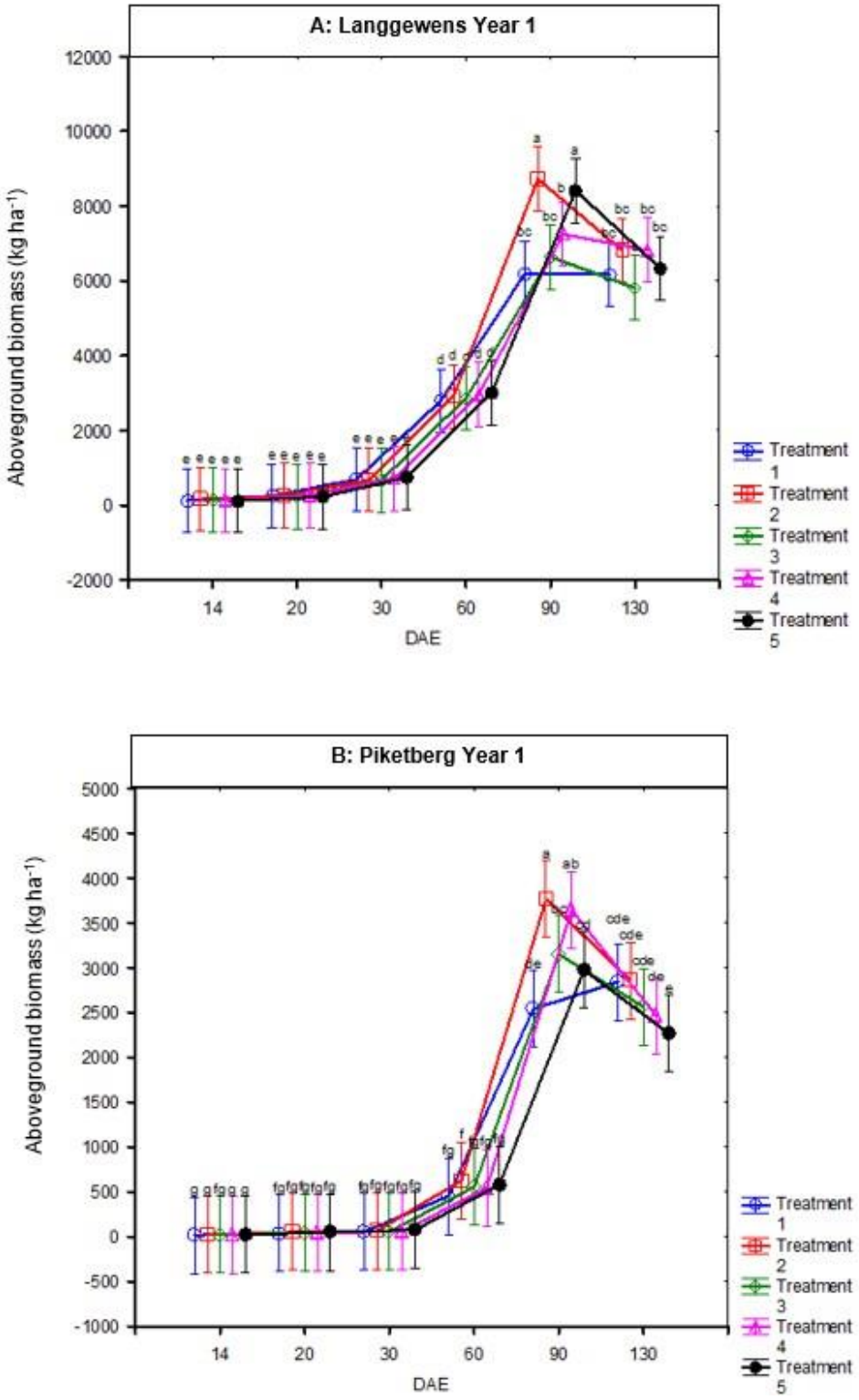

Figure 4.5.2 Aboveground biomass (kg ha^{-1}) at 14, 20, 30, 60, 90 and 130 DAE at A) Langgewens (Year 1), B) Piketberg (Year 1). No common letters between points in time indicates significant difference at a 5% level. Treatments: 1) Endomaxx 5 g ha^{-1}, 2) Endomaxx 10 g ha^{-1}, 3) Endomaxx 20 g ha^{-1}, 4) Industry standard 150 g ha^{-1}, and 5) Untreated control (distilled water).

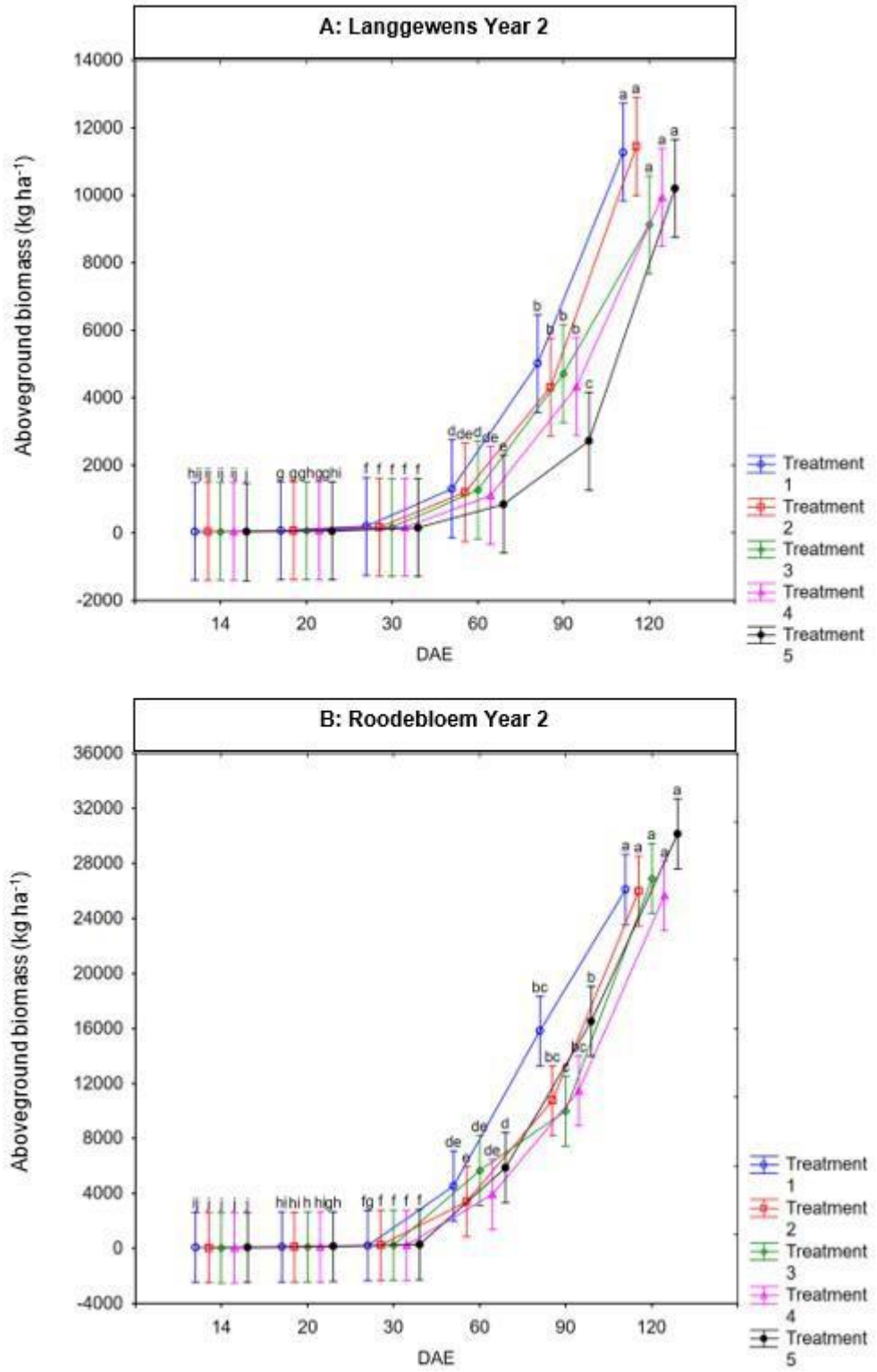

Figure 4.5.3 Aboveground biomass (kg ha^{-1}) at 14, 20, 30, 60, 90 and 120 DAE at A) Langgewens (Year 2), B) Roodebloem (Year 2). No common letters between points in time indicates significant difference at a 5% level. Treatments: 1) Endomaxx 5 g ha^{-1}, 2) Endomaxx 10 g ha^{-1}, 3) Endomaxx 20 g ha^{-1}, 4) Industry standard 150 g ha^{-1}, and 5) Untreated control (distilled water).

4.6 Dry matter content

During Y1, mycorrhizal inoculation had no effect ($p<0.05$) on plant dry matter content at any of (Figure 4.6.1), (Figure 4.6.2) trial sites (Table 4.4).

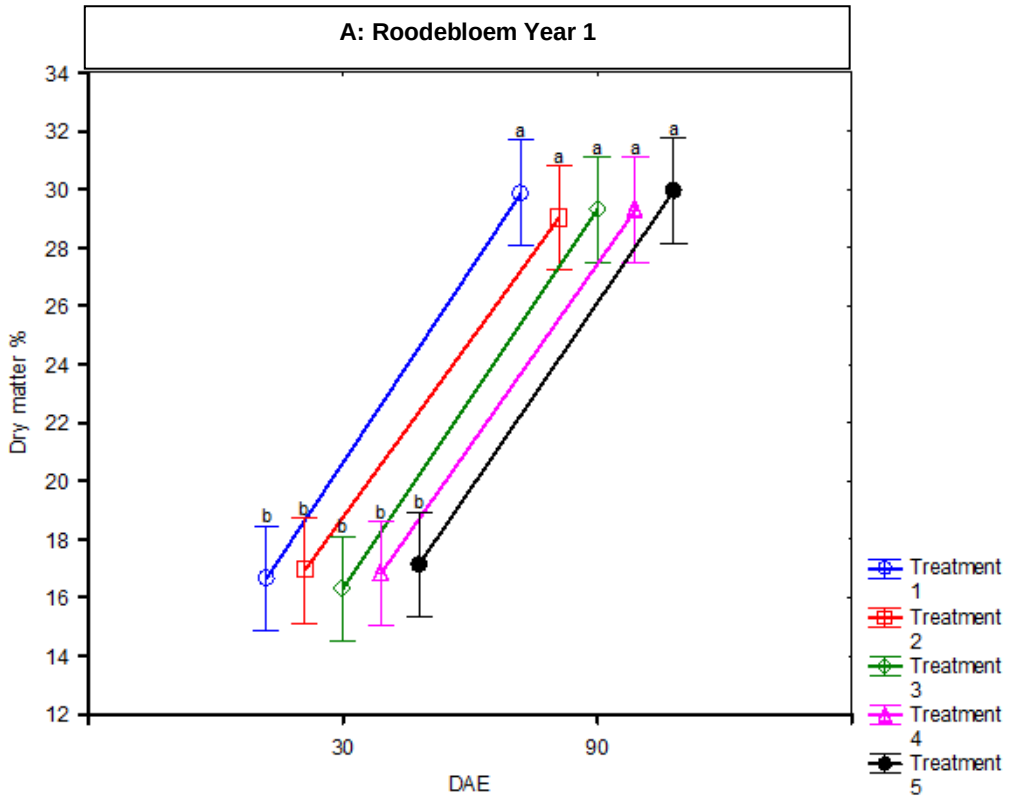

Figure 4.6.1 Dry matter content (%) of wheat plants at 30 and 90 DAE at A) Roodebloem (Year 1). No common letters between points in time indicates significant difference at a 5% level. Treatments: 1) Endomaxx 5 g ha^{-1}, 2) Endomaxx 10 g ha^{-1}, 3) Endomaxx 20 g ha^{-1}, 4) Industry standard 150 g ha^{-1}, and 5) Untreated control (distilled water).

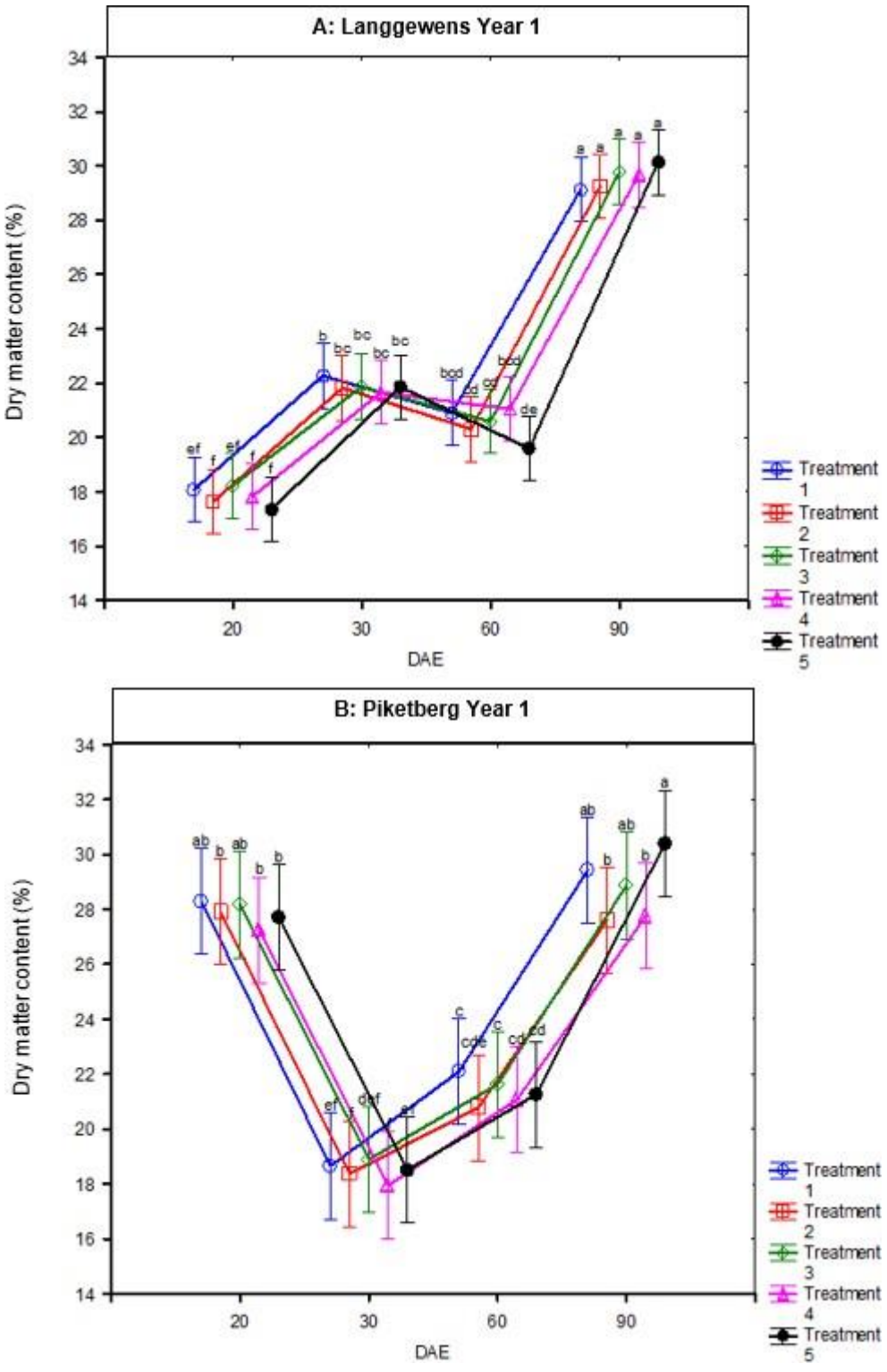

Figure 4.6.2 Dry matter content (%) of wheat plants at 20, 30, 60, 90 DAE at A) Langgewens (Year 1), B) Piketberg (Year 1). No common letters between points in time indicates significant difference at a 5% level. Treatments: 1) Endomaxx 5 g ha^{-1}, 2) Endomaxx 10 g ha^{-1}, 3) Endomaxx 20 g ha^{-1}, 4) Industry standard 150 g ha^{-1}, and 5) Untreated control (distilled water).

4.7 Leaf area index

No differences ($p<0.05$) were observed between treatments for leaf area index at Langgewens, Piketberg and Roodebloem trial sites in both years (Table 4.4 and Table 4.5). Leaf area index remained relatively uniform between treatments (Figure 4.7). Mycorrhizal application did not affect the total leaf area of the plants.

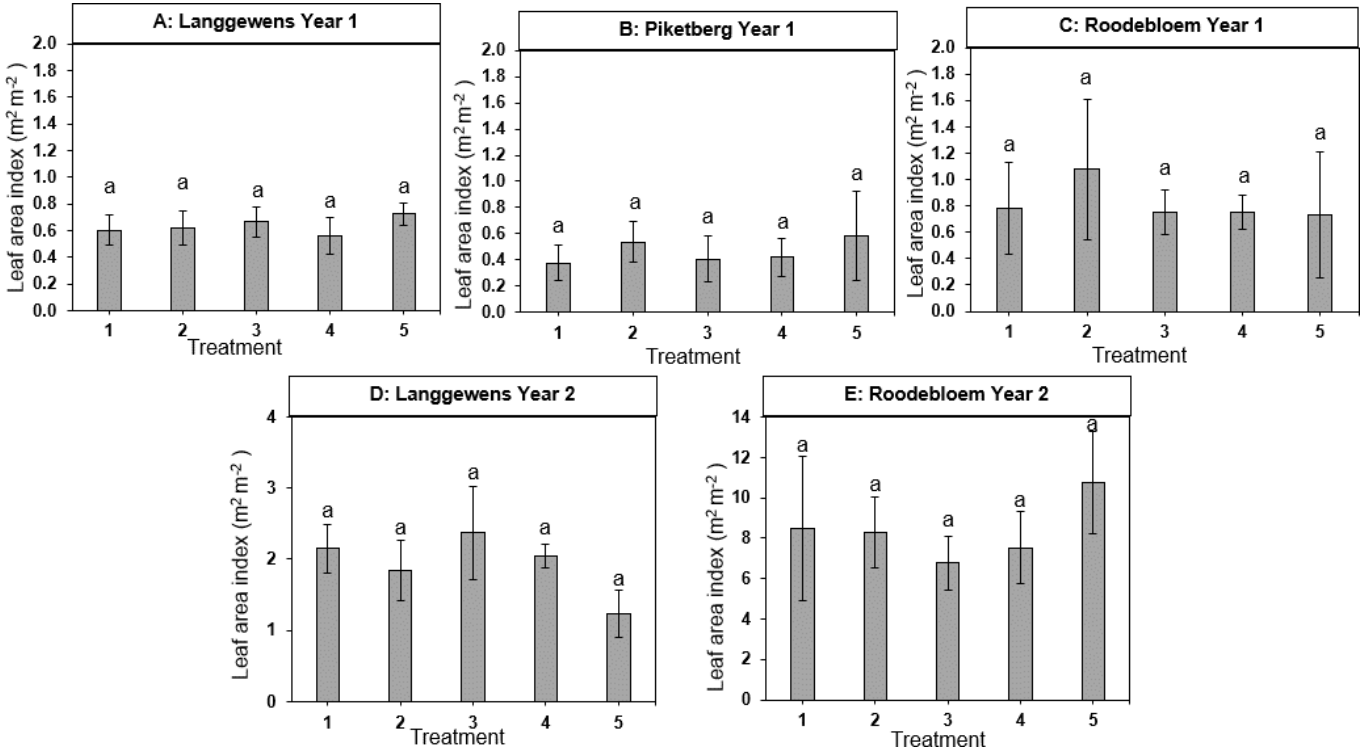

Figure 4.7 Mean leaf area index (m^2 m^{-2}) for each treatment at 90 DAE at A) Langgewens (Year 1), B) Piketberg (Year 1), C) Roodebloem (Year 1), D) Langgewens (Year 2), E) Roodebloem (Year 2). Bars with different letters indicate significant difference at a 5% level. Treatments: 1) Endomaxx 5 g ha^{-1}, 2) Endomaxx 10 g ha^{-1}, 3) Endomaxx 20 g ha^{-1}, 4) Industry standard 150 g ha^{-1}, and 5) Untreated control (distilled water).

4.8 Number of Spikes m^{-2}

The number of spikes did not differ ($p<0.05$) between treatments at each site for both Year 1 and Year 2 (Table 4.3). Mycorrhizal application did not affect the number of spikes of wheat plants at both sites for both Year 1 and Year 2 (Figure 4.8).

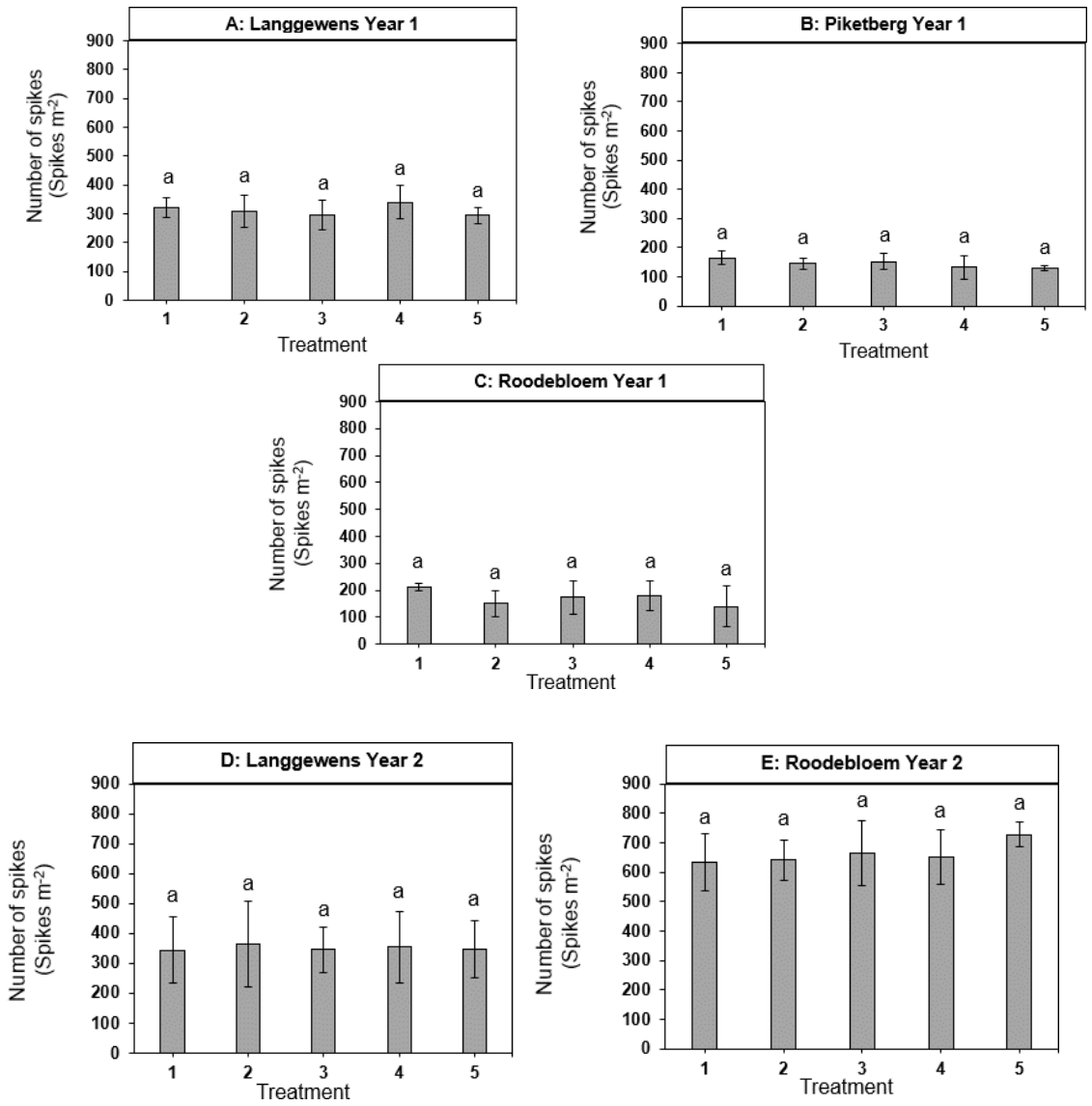

Figure 4.8 Number of spikes (Spikes m^{-2}) (ears) for each treatment at 130 DAE at A) Langgewens (Year 1), B) Piketberg (Year 1), C) Roodebloem (Year 1). D) Langgewens (Year 2), E) Roodebloem (Year 2). Bars with different letters indicate significant difference at a 5% level. Treatments: 1) Endomaxx 5 g ha^{-1}, 2) Endomaxx 10 g ha^{-1}, 3) Endomaxx 20 g ha^{-1}, 4) Industry standard 150 g ha^{-1}, and 5) Untreated control (distilled water).

Table 4.3 P-values for each of the grain quality and yield related measurements made throughout the growing season in Year 1 and Year 2 at different localities in the Western Cape. “Treatment” denotes differences between measurements and treatments only. “Treatments*DAE” denotes differences between measurements and treatments over time. “DAE” denotes differences between measurements and time only.

Parameter	Site	Year 1 Treatment p-Value	Year 2 Treatment p-Value
Spikes	Langgewens	0.440	1.000
	Piketberg	0.184	-
	Roodebloem	0.290	0.410
Spikelets	Langgewens	0.528	0.260
	Piketberg	0.184	-
	Roodebloem	0.245	0.270
Grain Yield	Langgewens	0.813	0.150
	Piketberg	0.038	-
	Roodebloem	0.383	0.380

4.9 Number of Spikelets m^{-2}

The number of spikelets (m^{-2}) (Figure 4.9) did not differ ($p<0.05$) between treatments at each site in Year 1 or at Langgewens during Year 2 (Table 4.3). In contrast, in Year 2, the number of spikelets differed ($p<0.05$) between T1 (2901 spikelets m^{-2}) and T5 (3239 spikelets m^{-2}) at the Roodebloem site (Table 4.6). Mycorrhizal application did not affect the number spikelets at both trial sites during Year 2 due to the control plot having more spikelets than the treatment plots.

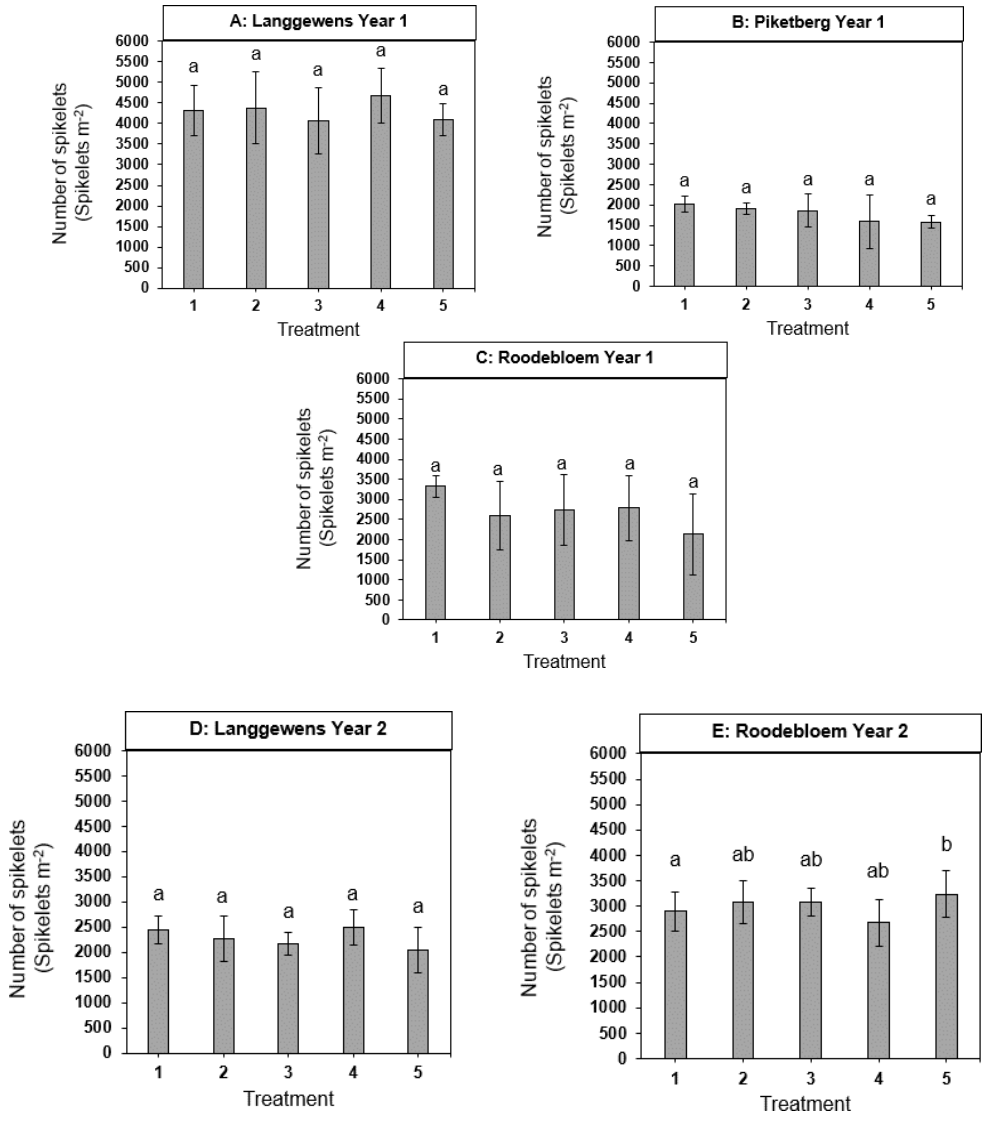

Figure 4.9 Number of spikelets (spikelets m^{-2})for each treatment at 130 DAE at A) Langgewens (Year 1), B) Piketberg (Year 1), C) Roodebloem (Year 1). D) Langgewens (Year 2), E) Roodebloem (Year 2). Bars with different letters indicate significant difference at a 5% level. Treatments: 1) Endomaxx 5 g ha^{-1}, 2) Endomaxx 10 g ha^{-1}, 3) Endomaxx 20 g ha^{-1}, 4) Industry standard 150 g ha^{-1}, and 5) Untreated control (distilled water).

4.10 Grain Yield

Differences ($p<0.05$) for grain yield were observed at the Piketberg (Figure 4.10) trial site during Year 1 and for Langgewens during Year 2 of the study (Table 4.3). Mycorrhizal application resulted in Treatments 1 and 4 having higher ($p<0.05$) grain yield than the untreated control plants at the Langgewens trial site (Figure 4.10) for Year 2. No differences were observed for either Langgewens or Roodebloem (Figure 4.10) sites during Year 1 nor at Roodebloem during Year 2 of the study (Table 4.3). All treatments had no differences ($p>0.05$) in grain yield between treatments due to AMF application at the Roodebloem trial site (Figure 4.10).

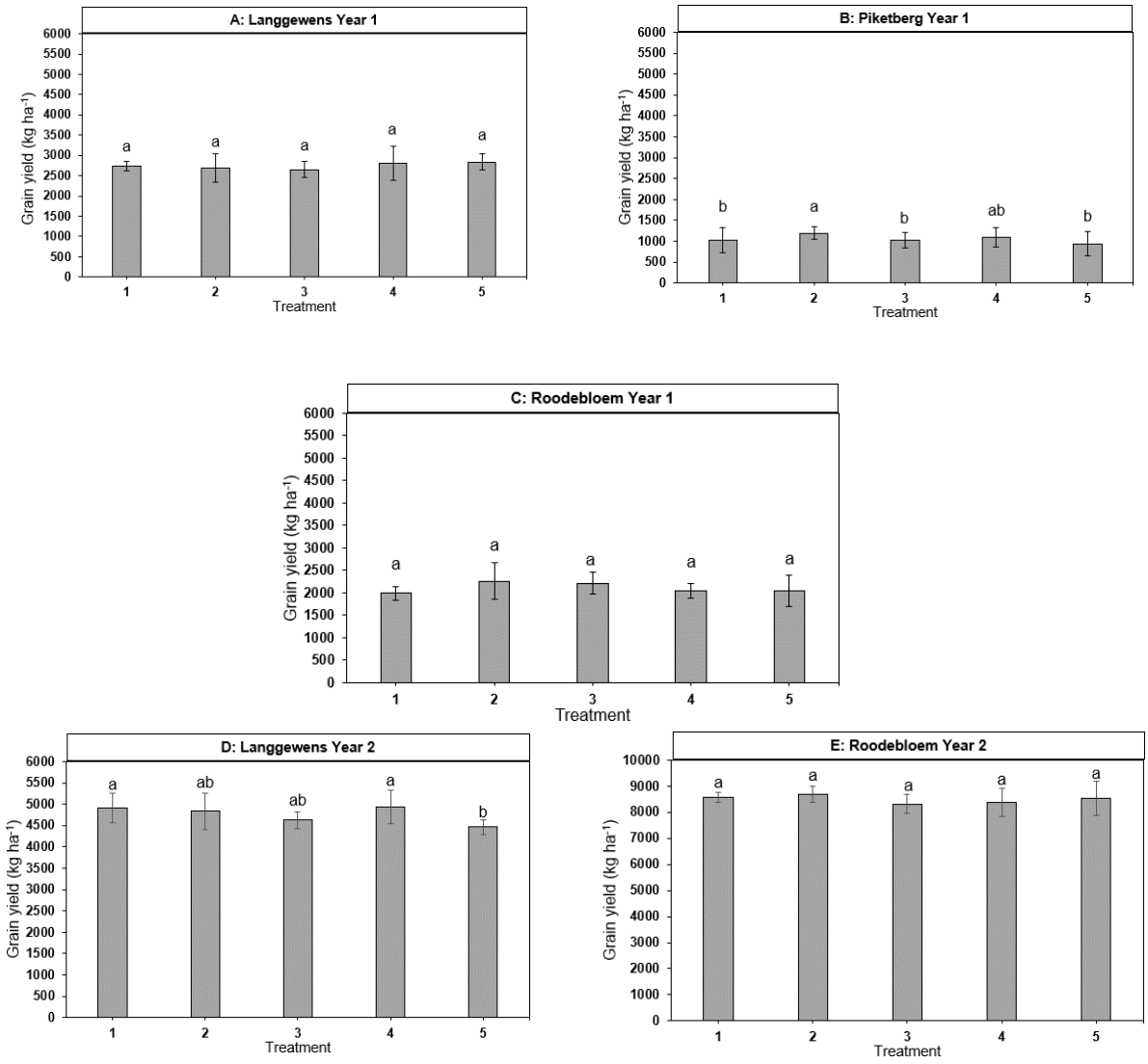

Figure 4.10 Mean grain yields for each treatment at A) Langgewens (Year 1), B) Piketberg (Year 1), C) Roodebloem (Year 1), D) Langgewens (Year 2), E) Roodebloem (Year 2). Bars with different letters indicate significant difference at a 5% level. Treatments: 1) Endomaxx 5 g ha^{-1}, 2) Endomaxx 10 g ha^{-1}, 3) Endomaxx 20 g ha^{-1}, 4) Industry standard 150 g ha^{-1}, and 5) Untreated control (distilled water).

4.11 Grain quality

No differences ($p<0.05$) were observed due to mycorrhizal inoculation regarding the various grain quality parameters (Table 3.2). Langgewens (Table 3.3), Piketberg (Table 3.4) and Roodebloem (Table 3.5) sites had similar values for each grain quality parameter during Year 1 and Year 2.

Table 4.4 Different mean grain quality parameters of each treatment for Langgewens Research farm during Year 1 of the study. No common letters between treatments indicate significant difference at a 5% level. Treatments: 1) Endomaxx 5 g ha^{-1}, 2) Endomaxx 10 g ha^{-1}, 3) Endomaxx 20 g ha^{-1}, 4) Industry standard 150 g ha^{-1}, and 5) Untreated control (distilled water).

Grain Quality Parameter	Treatment	Mean Value	Standard Deviation
Grain Gluten Wet (%)	1	31.18 [a]	0.58
	2	31.16 [a]	0.23
	3	31.24 [a]	0.58
	4	31.24 [a]	0.40
	5	30.96 [a]	0.72
Grain Protein (%)	1	13.54 [a]	0.22
	2	13.52 [a]	0.08
	3	13.56 [a]	0.24
	4	13.54 [a]	0.15
	5	13.48 [a]	0.28
Hectolitre Mass (kg hL^{-1})	1	80.64 [a]	0.75
	2	80.56 [a]	0.97
	3	80.84 [a]	0.97
	4	80.88 [a]	0.59
	5	80.46 [a]	0.49

Table 4.5 Different mean grain quality parameters of each treatment for the Piketberg farm during Year 1 of the study. No common letters between treatments indicate significant difference at a 5% level. Treatments: 1) Endomaxx 5 g ha^{-1}, 2) Endomaxx 10 g ha^{-1}, 3) Endomaxx 20 g ha^{-1}, 4) Industry standard 150 g ha^{-1}, and 5) Untreated control (distilled water).

Grain Quality Parameter	Treatment	Mean Value	Standard Deviation
Grain Gluten Wet (%)	1	35.68 [a]	1.07
	2	36.12 [a]	2.12
	3	35.24 [a]	1.19
	4	36.16 [a]	0.96
	5	35.54 [a]	1.32
Grain Protein (%)	1	15.22 [a]	0.41
	2	15.38 [a]	0.79
	3	15.08 [a]	0.44
	4	15.42 [a]	0.38
	5	15.16 [a]	0.51
Hectolitre Mass (kg hL^{-1})	1	75.02 [a]	1.58
	2	75.66 [a]	1.91
	3	75.90 [a]	1.74
	4	74.84 [a]	0.91
	5	75.80 [a]	1.21

Table 4.6 Different mean grain quality parameters of each treatment for Roodebloem Research farm during Year 1 of the study. No common letters between treatments indicate significant difference at a 5% level. Treatments: 1) Endomaxx 5 g ha^{-1}, 2) Endomaxx 10 g ha^{-1}, 3) Endomaxx 20 g ha^{-1}, 4) Industry standard 150 g ha^{-1}, and 5) Untreated control (distilled water).

Grain Quality Parameter	Treatment	Mean Value	Standard Deviation
Grain Gluten Wet (%)	1	30.70 [a]	2.03
	2	29.64 [a]	1.12
	3	30.48 [a]	1.61
	4	29.94 [a]	1.55
	5	30.08 [a]	1.96
Grain Protein (%)	1	13.34 [a]	0.75
	2	12.96 [a]	0.40
	3	13.26 [a]	0.63
	4	13.06 [a]	0.59
	5	13.12 [a]	0.72
Hectolitre Mass (kg hL^{-1})	1	80.14 [a]	0.43
	2	80.22 [a]	0.55
	3	80.08 [a]	0.49
	4	80.18 [a]	0.24
	5	80.16 [a]	0.50

Table 4.7 Different mean grain quality parameters of each treatment for Langgewens Research farm during Year 2 of the study. No common letters between treatments indicate significant difference at a 5% level. Treatments: 1) Endomaxx 5 g ha^{-1}, 2) Endomaxx 10 g ha^{-1}, 3) Endomaxx 20 g ha^{-1}, 4) Industry standard 150 g ha^{-1}, and 5) Untreated control (distilled water).

Grain Quality Parameter	Treatment	Mean Value	Standard Deviation
Grain Gluten Wet (%)	1	28.12 [a]	0.64
	2	28.20 [a]	0.44
	3	28.42 [a]	1.72
	4	28.14 [a]	0.78
	5	27.98 [a]	0.87
Grain Protein (%)	1	12.36 [a]	0.23
	2	12.36 [a]	0.18
	3	12.50 [a]	0.65
	4	12.34 [a]	0.29
	5	12.30 [a]	0.29
Hectolitre Mass (kg hL^{-1})	1	78.74 [a]	0.74
	2	79.00 [a]	0.57
	3	78.84 [a]	1.57
	4	79.06 [a]	0.73
	5	79.16 [a]	0.65

Table 4.8 Different mean grain quality parameters of each treatment for Roodebloem Research farm during Year 2 of the study. No common letters between treatments indicate significant difference at a 5% level. Treatments: 1) Endomaxx 5 g ha^{-1}, 2) Endomaxx 10 g ha^{-1}, 3) Endomaxx 20 g ha^{-1}, 4) Industry standard 150 g ha^{-1}, and 5) Untreated control (distilled water).

Grain Quality Parameter	Treatment	Mean Value	Standard Deviation
Grain Gluten Wet (%)	1	28.84 [a]	1.76
	2	29.60 [a]	0.50
	3	29.88 [a]	0.81
	4	29.94 [a]	0.54
	5	29.66 [a]	0.66
Grain Protein (%)	1	12.64 [a]	0.66
	2	12.94 [a]	0.18
	3	13.00 [a]	0.31
	4	13.04 [a]	0.21
	5	12.90 [a]	0.25
Hectolitre Mass (kg hL^{-1})	1	81.60 [a]	0.64
	2	81.32 [a]	0.44
	3	81.08 [a]	0.45
	4	81.26 [a]	0.44
	5	81.26 [a]	0.84

4.12 Root colonisation

All hyphae that were observed were intraradical, whereas the extraradical hyphae might have been lost due to the staining process. Root samples were counted as positive if vesicles or hyphae were observed. Colonisation varied throughout all sites for both Year 1 and Year 2 (Figures 4.12.1 to 4.12.5). Light microscope images were taken of infected root fragments to visualise the AMF structures such as vesicles, arbuscules and vesicles (Figure 4.12.6 and Figure 4.12.7).

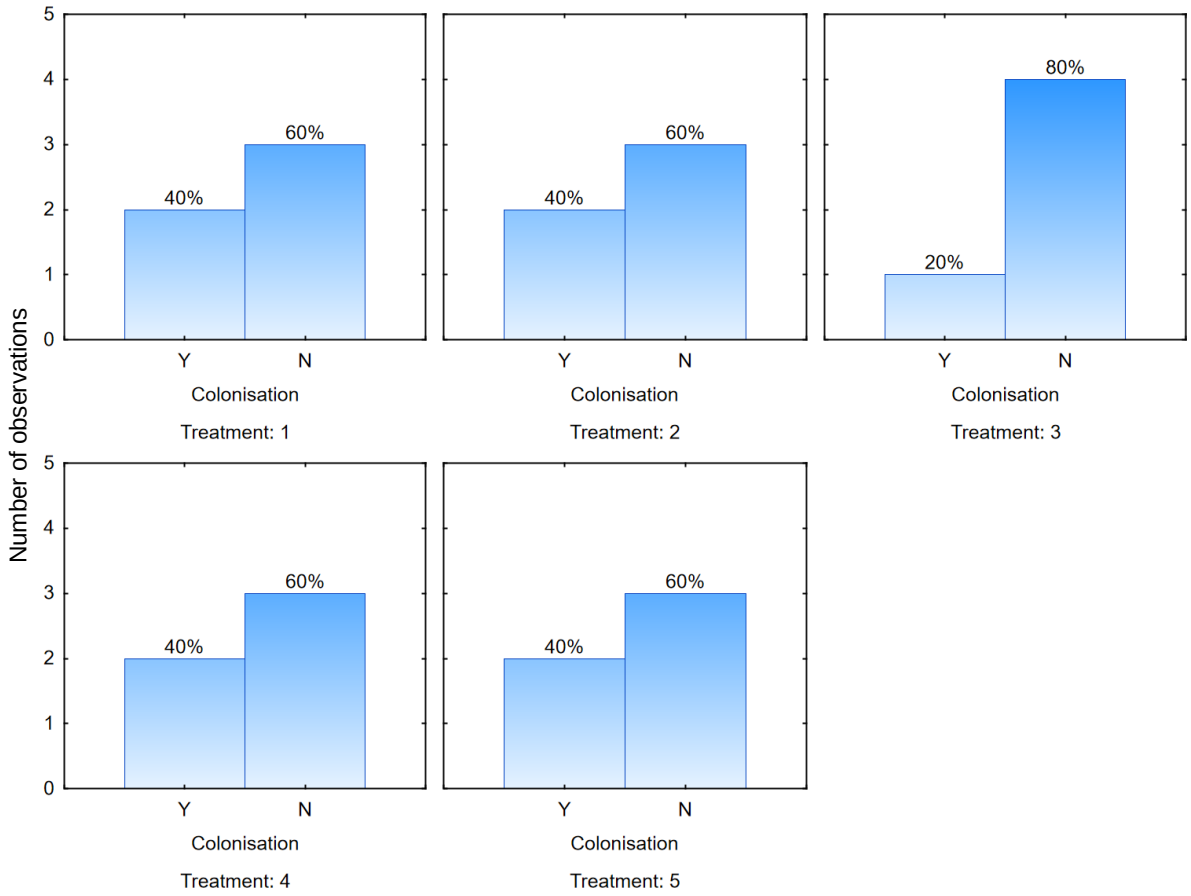

Figure 4.12.1 Colonisation results for each treatment at 130 DAE at Langgewens Research farm during Year 1 of the study. "Y" denotes positive colonisation and "N" denotes an absence of colonisation. Treatments: 1) Endomaxx 5 g ha^{-1}, 2) Endomaxx 10 g ha^{-1}, 3) Endomaxx 20 g ha^{-1}, 4) Industry standard 150 g ha^{-1}, and 5) Untreated control (distilled water).

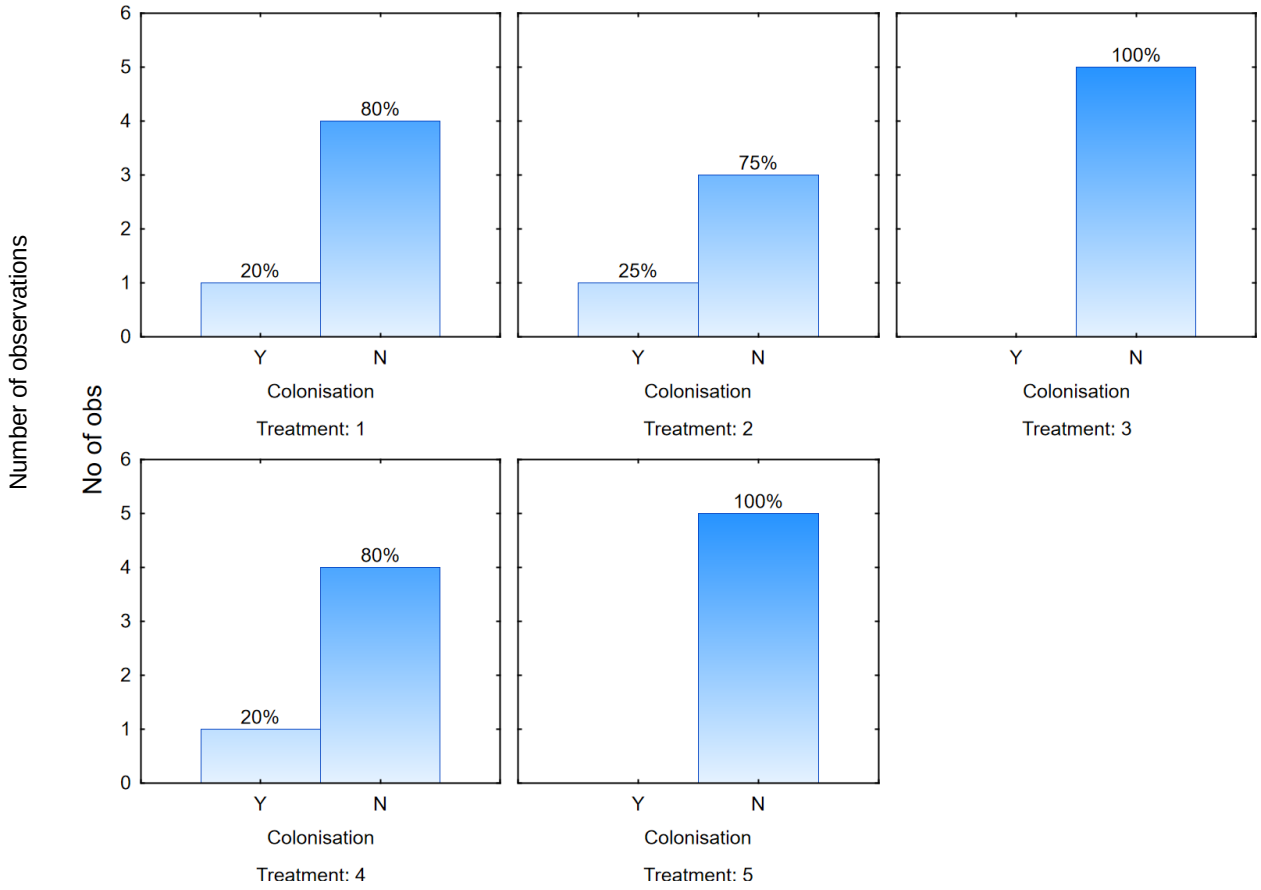

Figure 4.12.2 Colonisation results for each treatment at 130 DAE at the Piketberg farm during Year 1 of the study. "Y" denotes positive colonisation and "N" denotes an absence of colonisation. Treatments: 1) Endomaxx 5 g ha^{-1}, 2) Endomaxx 10 g ha^{-1}, 3) Endomaxx 20 g ha^{-1}, 4) Industry standard 150 g ha^{-1}, and 5) Untreated control (distilled water).

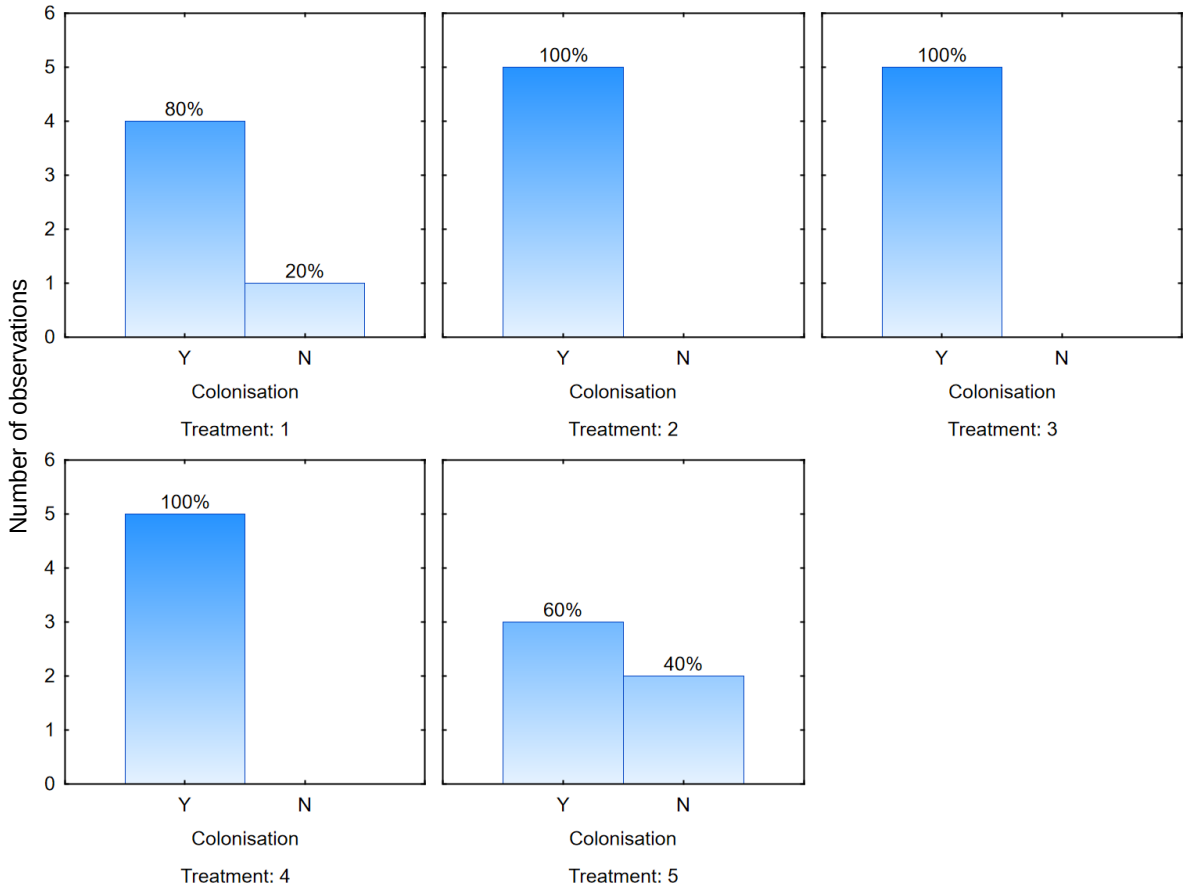

Figure 4.12.3 Colonisation results for each treatment at 130 DAE at Roodebloem Research farm during Year 1 of the study. "Y" denotes positive colonisation and "N" denotes an absence of colonisation. Treatments: 1) Endomaxx 5 g ha^{-1}, 2) Endomaxx 10 g ha^{-1}, 3) Endomaxx 20 g ha^{-1}, 4) Industry standard 150 g ha^{-1}, and 5) Untreated control (distilled water).

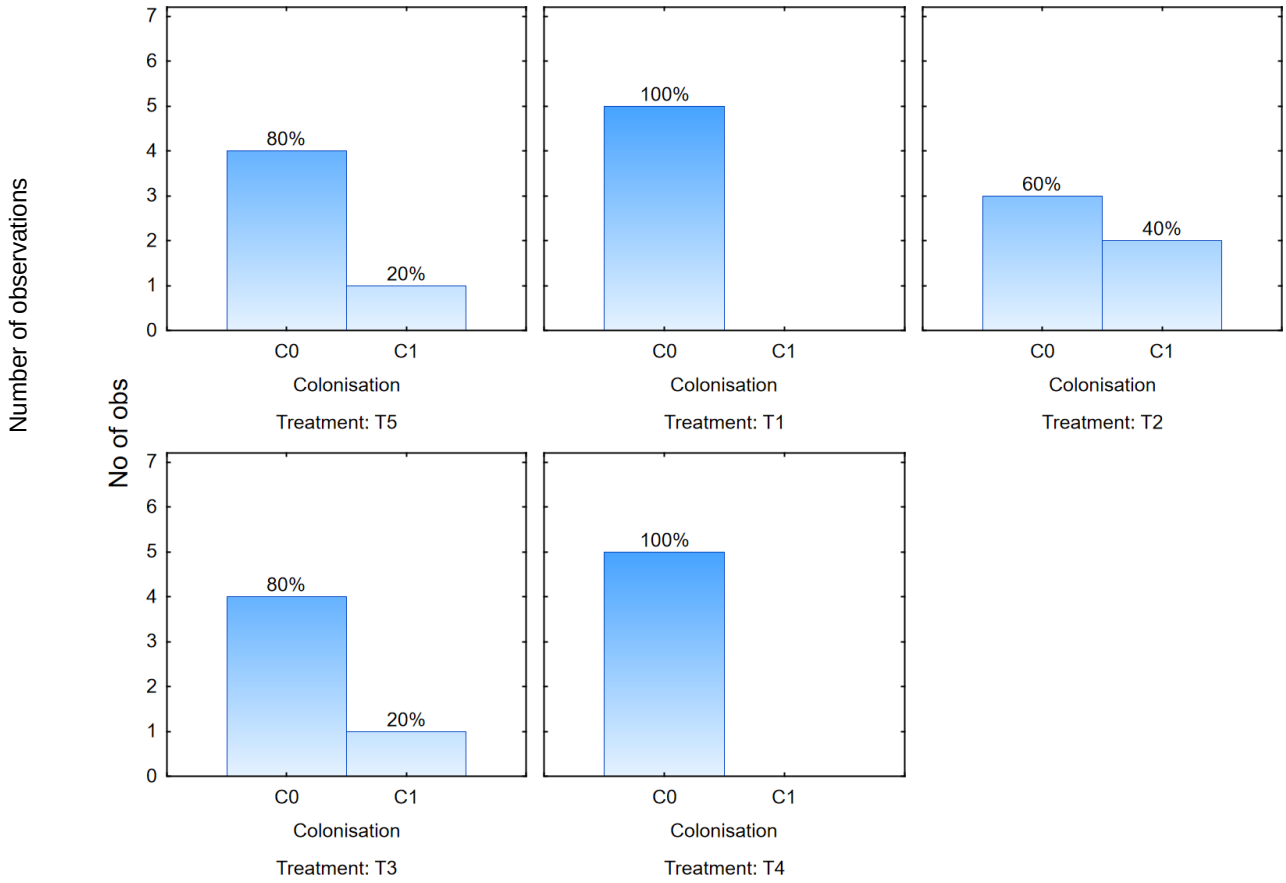

Figure 4.12.4 Colonisation results for each treatment at 130 DAE at Langgewens Research farm during Year 2 of the study. “C1” denotes positive colonisation and “C0” denotes an absence of colonisation. Treatments: 1) Endomaxx 5 g ha^{-1}, 2) Endomaxx 10 g ha^{-1}, 3) Endomaxx 20 g ha^{-1}, 4) Industry standard 150 g ha^{-1}, and 5) Untreated control (distilled water).

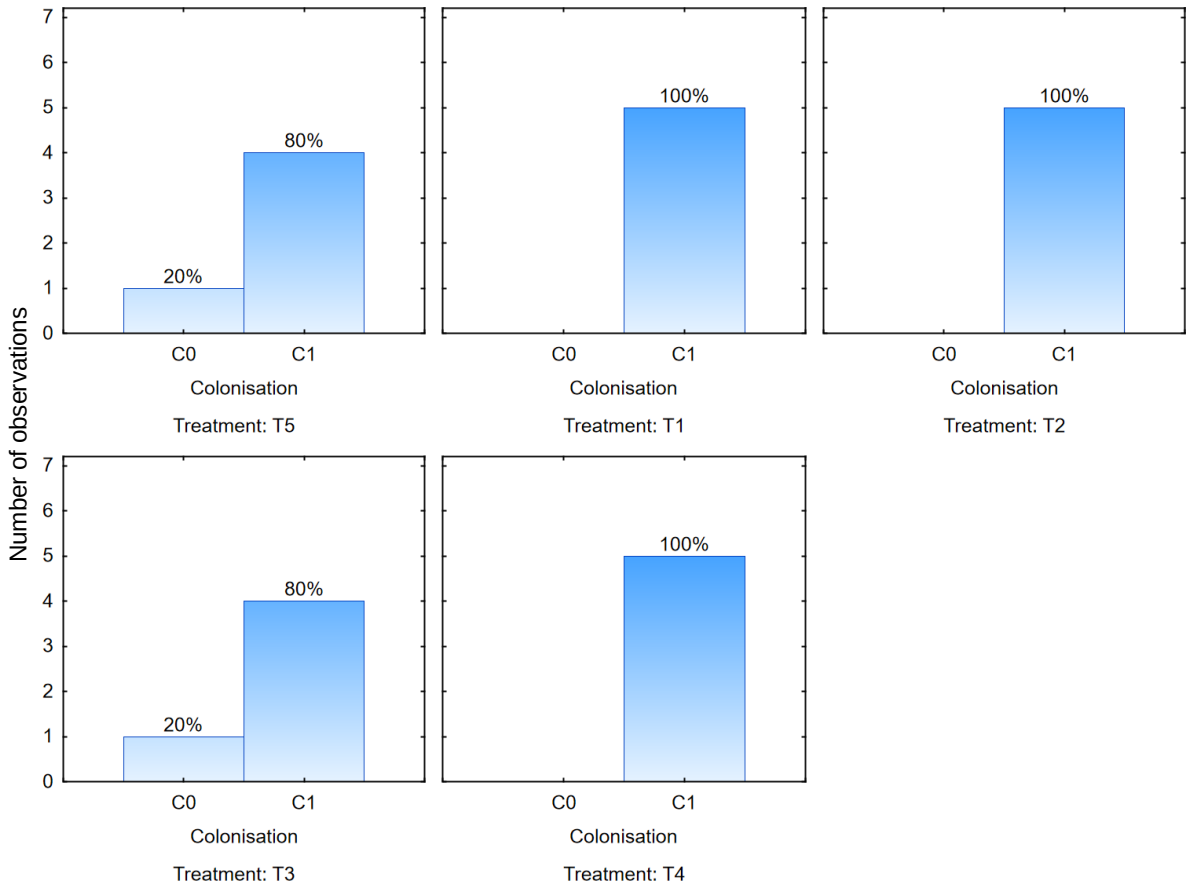

Figure 4.12.5 Colonisation results for each treatment at 130 DAE at Roodebloem Research farm during Year 2 of the study. “C1” denotes positive colonisation and “C0” denotes an absence of colonisation. Treatments: 1) Endomaxx 5 g ha^{-1}, 2) Endomaxx 10 g ha^{-1}, 3) Endomaxx 20 g ha^{-1}, 4) Industry standard 150 g ha^{-1}, and 5) Untreated control (distilled water).

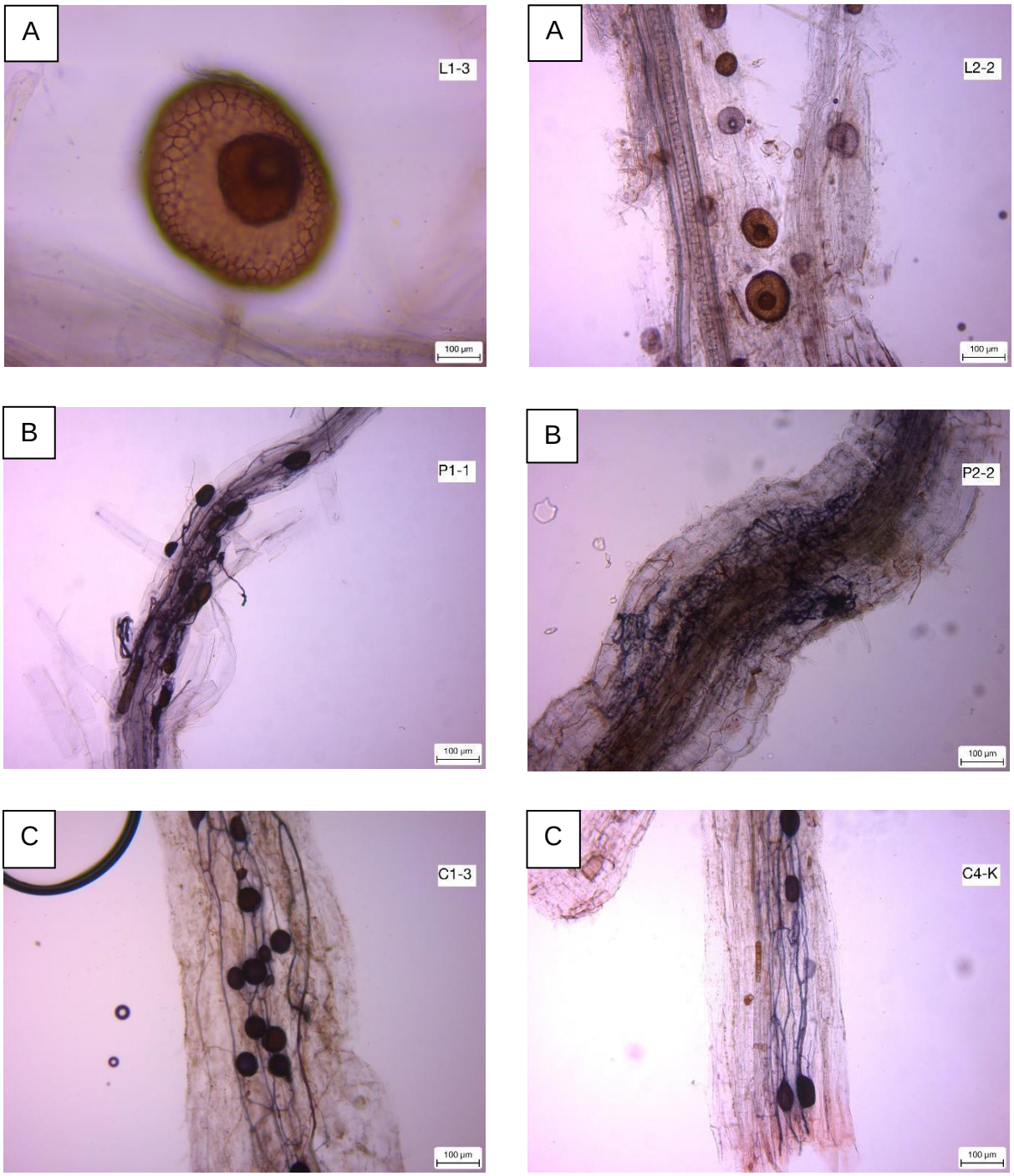

Figure 4.12.6 Light microscope (200x) images of AMF colonised wheat root fragments from A) Langgewens (Year 1), B) Piketberg (Year 1), C) Roodebloem (Year 1). Images were taken from samples taken at 130 DAE.

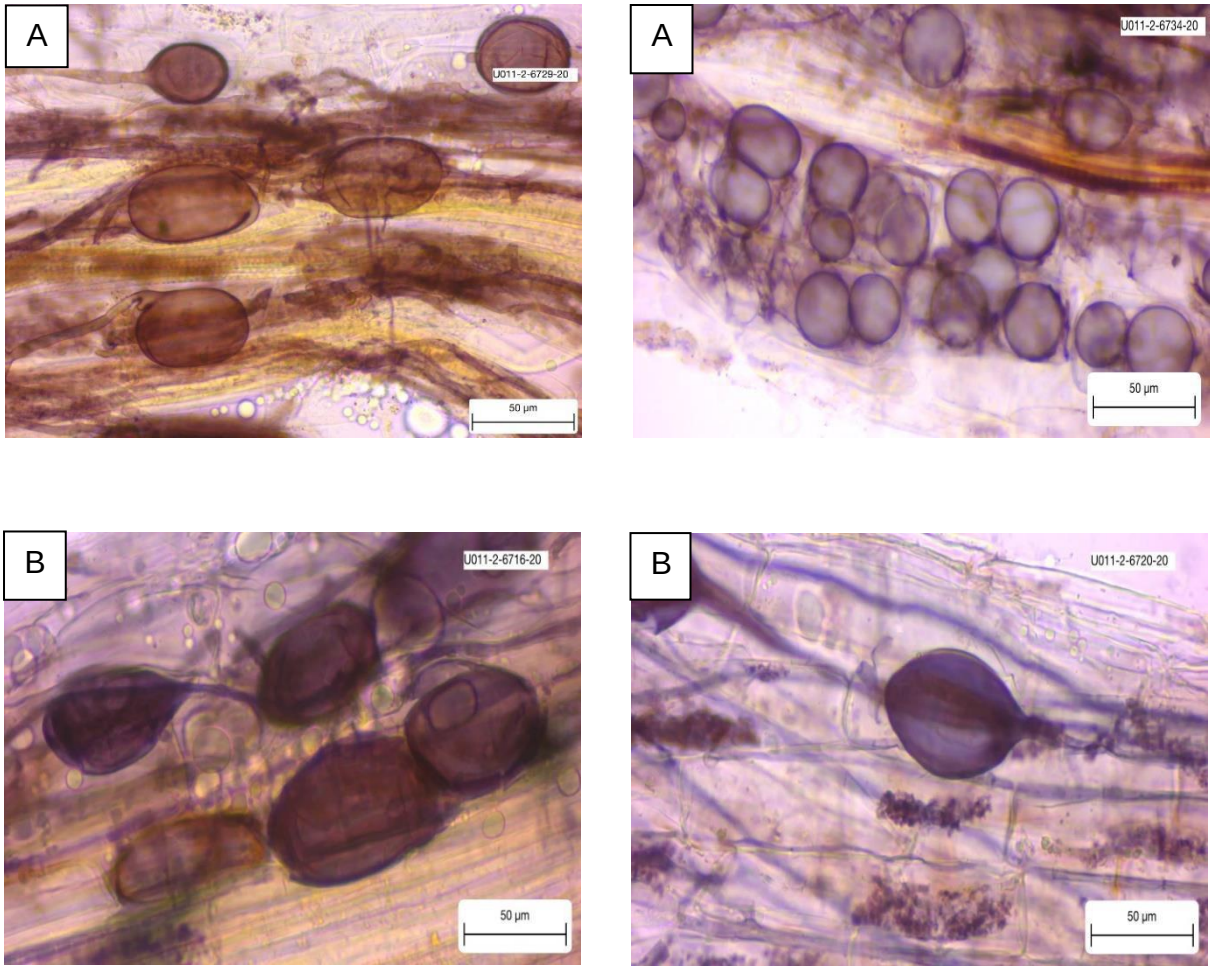

Figure 4.12.7 Light microscope (400x) images of AMF colonised wheat root fragments from A) Langgewens (Year 2), B) Roodebloem (Year 2). Images were taken from samples taken at 120 DAE.

CHAPTER 5

Discussion

This study investigated arbuscular mycorrhizal fungi (AMF) inoculation effects on wheat under dryland conditions. Mycorrhizal fungi are capable of protecting seedlings from harmful bacteria and are able to outcompete certain harmful fungi that might colonise an emerging seedling (Maherali and Klironomos, 2007).

Similar ($p<0.05$) plant populations were observed at each site during Year 1 and Year 2 of the study. Achieving similar plant populations between treatments is favourable. Spatial parameters being relatively constant allows treatments to be compared more reliably with each other. Wheat plant populations in the Western Cape range between 120 and 200 plants m^{-2} with a desired target of 175 to 200 plants m^{-2} under dryland conditions decreasing to 150 and 175 plant m^{-2} in low potential areas (Sensako, 2019). Plant populations (Figure 4.2) were close to, or within the target plant population range of 120 to 200 plants m^{-2} at each site, except for Roodebloem during Year 1. Plant population targets were achieved despite all trial sites having high stone content in the soil, which could have affected planting depth (Figure 4.1) and ultimately the successful emergence of new seedlings. All treatments had relatively equal access to sunlight, water and nutrients. Research has demonstrated that AMF inoculation can increase seedling survivability and resistance to unfavourable environmental conditions and pathogens which may lead to an increase in plant population (Koide and Dickie, 2002). No clear evidence was present to indicate that AMF improved seedling survivability during these trials. Soil and climactic conditions may have been unfavourable for early colonisation of seedling roots after germination. Regardless of colonisation, the trial plots were adequately populated and lead to plots that were well suited for sampling and aboveground biomass and grain yield determination.

Aboveground plant biomass and root biomass are two of the hallmark plant traits affected by mycorrhizal inoculation. Due to the potential increase in nutrient uptake, plants can produce more biomass and root volume compared to plants without inoculation as demonstrated in greenhouse trials on wheat (Al-Karaki and Al-Raddad, 1997). The same results would not be easily achievable in field trials due to several environmental variables that may affect the fungus or the plant especially in a short

period of time such as a year or two. This is because efficacy is entirely reliant on favourable environmental conditions, soil composition, the need for a symbiotic relationship with the host plant and AMF species diversity and fungal community size (Melo et al., 2019). Greenhouse trials allow a large degree of control over these factors to ensure the growth and survival of AMF. In this study, root weight (Figure 4.3) and rooting depth (Figure 4.4) were sampled up to 30 DAE, which might have been too early for AMF to affect the measurements in trials conducted. Root measurements at Langgewens generally decreased over time during Year 1 of the study, which could be due to harder soils and a high stone content in the soil. Root biomass increased over time during Year 2 of the study. This could be attributed to the above average rainfall experienced for the 2020 growing season which loosened the soil and allowed the roots to grow more vigorously. Differences ($p<0.05$) were observed between T4 and T3 at 30 DAE. This could possibly be attributed to very early colonisation of AMF. Plants are often starved of N or P, which may result in reduced above- or belowground biomass in non-intensive systems. Arbuscular mycorrhizal fungi have been shown to increase the uptake of N and P by plants in systems where N and P are in low availability in the soil, often leading to responses in above- and belowground biomass. (Hodge and Storer, 2014; Leigh et al., 2009; Lindahl et al., 2005; Smith and Read, 2008). Common farming practices in the Western Cape include using mineral fertilisers to supplement the soil with these nutrients. This practice leads to an abundance of nutrient availability to the plants, which may have caused a reduction in colonisation and weakened response in AMF activity and a diminished symbiotic relationship with the wheat plants. Usage of organic fertilisers do not suppress AMF activity as severely as inorganic fertilisers and would be recommended to maintain fungal communities and benefit to the plant (Beslemes et al., 2016).

Aboveground biomass (Figures 4.5.1, 4.5.2, 4.5.3) was not affected over time by mycorrhizal inoculation when compared to the control plots, despite mycorrhizal infection being present from 90 DAE to 130 DAE. There was an increase in aboveground biomass throughout the growing season as expected due to the plant undergoing vegetative growth and rapidly gaining biomass. The plants switched to reproductive growth around 90 DAE, which led to a general decrease in biomass during Year 1. Aboveground biomass increased between 90 DAE and 130 DAE during Year 2 of the study mainly due to the high amount of rain late in the growing season.

Consequently, vegetative growth was prolonged before switching to reproductive growth. Differences in aboveground biomass between treatments were observed at both trial sites at 90 DAE. Plants from Treatments 1 – 4 had more ($p<0.05$) biomass than plants from the untreated control at Langgewens (Figure 4.5.3). The aboveground biomass of T3 was higher ($p<0.05$) than the untreated control at Roodebloem (Figure 4.5.3). These observations demonstrate that AMF application can lead to increases in biomass for plants at certain stages of development under dry land conditions. Plants colonised by AMF tend to have better water use efficiency (WUE), which can lead to increases in shoot and leaf biomass (Al-Karaki, 1998). The degree to which the AMF benefit the plants varies during the growing season depending on the needs of the plant at any given time. Research regarding the effects of AMF inoculation on wheat biomass and growth agrees with this observation in both greenhouse and dryland settings (Al-Karaki and Al-Raddad, 1997; Al-Karaki, McMichael and Zak, 2003). A recent meta-analysis study of field trials on wheat (Pellegrino et al., 2015) also demonstrated that wheat biomass and grain yield can be increased by AMF inoculation under field conditions.

Dry matter content indicates the percentage of a plant that consists of dry matter vs. water. The ability of AMF to assist plants with water uptake in times of low soil moisture can be quantified by comparing the wet weight of a sample with the dry weight. The results showed a general increase in dry matter content for all treatments at each site (Figures 4.6.1, 4.6.2) during Year 1 of the study, which was expected because plants continue to lose moisture as they get to the reproductive phase. In addition, LAI measurements (Figures 4.7) were taken at 90 DAE that is approximately the time when colonisation was first detected in root samples. Without observable colonisation of the roots before 90 DAE it seems plausible that the leaf area (LA) of the wheat plants would not have any differences due to AMF inoculation for both Year 1 and Year 2 of the study. The similarities in LA indices between treatments is largely due to the relatively uniform plant population measured at 20 DAE (Figure 4.2). Instances where AMF inoculation affect LA normally result in higher LA due to an in increase in aboveground biomass as seen with barley plants when inoculated with AMF (Beslemes et al., 2016).

Yield components are often the main factors researchers and farmers reference to determine if AMF benefited their wheat system. Mycorrhizal fungi can also affect soil

compaction, soil organic matter content and mineral availability to plants. It is important to consider all factors and potential benefits when determining if AMF had a positive effect on a system. The number of spikes and spikelets m^{-2} (Figures 4.8 and 4.9, respectively) were not affected ($P>0.05$) by mycorrhizal inoculation at any trial site during Year 1 of the study, possibly due to the plants having relatively equal and easy access to nutrients during the early reproductive growth phase. During Year 2, the Langgewens trial site demonstrated no differences in the number of spikes m^{-2} or spikelets m^{-2} between treatments. At Roodebloem, plants demonstrated no differences ($p<0.05$) in the number of spikes m^{-2} between treatments, but Treatment 5 had more ($p<0.05$) spikelets m^{-2} when compared to T1. This could be due to the naturally occurring AMF outperforming the applied AMF, as both treatments had high degrees of colonisation (Figure 4.12.5). Trials on wheat yield components have demonstrated contradictory results on the effect of AMF colonisation on the number of spikes or spikelets (Al-Karaki, McMichael and Zak, 2003). This outcome is often the case with field trials as demonstrated with these results. Mycorrhizal fungi greenhouse trial results are difficult to replicate in the field due to the biological nature of the fungus itself. However, by controlling factors such as date of planting, seed cultivar, application method, inoculum and inoculum concentration as uniform as possible, field trails can be conducted with relative success (Rocha et al., 2019).

Both Langgewens (Figure 4.12.1) and Roodebloem (Figure 4.12.3) control treatment plots had 40% and 60% infection rates, respectively in Year 1. This data could explain why no differences ($p>0.05$) for grain yield were observed between AMF treatments and the control treatment at both these sites. Despite Piketberg having the fewest instances of colonisation (Figure 4.12.2), a difference in grain yield was observed during Year 1 of the study. T2 had an average yield increase of between 69 kg ha^{-1} and 253 kg ha^{-1} when compared to Treatments 3 and 5 (Control), respectively. Treatments 1 and 4 had higher ($p<0.05$) grain yields when compared to T5 at Langgewens during Year 2 of the study. Treatments 1 and 4 had 440 kg ha^{-1} and 467 kg ha^{-1} higher grain yields, respectively than the untreated control (T5) (Figure 4.12.4). These relatively large differences in yield will be financially important to producers. This outcome is in complete agreement with previous studies relating to grain AMF inoculation of wheat and the subsequent quantification of grain yield (Al-Karaki and Hammad, 2001; Al-Karaki, 2006; Pellegrino et al., 2015). Despite having no

differences between yield components, these sites still had higher grain yield in some cases. AMF inoculation demonstrated no ($p>0.05$) effect on grain yield at the Roodebloem trial site during Year 2 of the study (Figure 4.12.5). Sufficient rainfall and abundant nutrient availability in the soil may have resulted in a less efficient symbiosis between plant and fungus with subsequently no noticeable effect on grain yield between treatments (Michelsen and Rosendahl, 1990). Grain quality was not affected by AMF inoculation during both Year 1 and Year 2 of this trial. Grain protein, gluten and hectolitre mass remained uniform between all treatments at each site during Year 1 and Year 2 of the study (Tables 4.4, 4.5, 4.6, 4.7, 4.8). Perez et al. (2016) demonstrated that spring wheat cultivars could exhibit differences in grain protein % due to inoculation with AMF.

Although mycorrhizal colonisation is known to occur at the emergence phase of development, it can also occur later in the development of the wheat plant. In this study, it was seemingly too early for the AMF to infect the seedling roots and make any noticeable difference in emergence at 20 days after emergence. Colonisation was first observed at 90 days after emergence during Year 1 of the study. Indeed, colonisation can be delayed substantially if conditions such as water availability and soil temperature are too unfavourable or if too much P is present in the soil. Spores can lay dormant in soil for years if conditions are not acceptable for the fungi to survive (McGee et al., 1997). Nearly all root samples observed were also colonised with Mucoromycotina fine-root endophytic fungi (Figures 4.12.6 and 4.12.7). These endophytic fungi are associated with most land plants and are often also found in close association with AMF, especially Glomeromycotina fungi (Field et al., 2019; Hoysted et al., 2019). At 130 DAE most treated (and some untreated) plots were colonised. Arbuscules, vesicles and hyphae were easily visible using a stereo microscope (Figures 4.12.6 and 4.12.7). Langgewens (Figure 4.12.1) had the most incidents of colonisation followed by Roodebloem (Figure 4.12.3) and Piketberg (Figure 4.12.2) sites respectively during Year 1 of the study. Warmer soils, lack of regular rainfall and possible over-fertilisation could have attributed to the lack of colonisation in Piketberg compared to the other sites during Year 1 of the study. During Year 2 of the study, Roodebloem (Figure 4.12.5) had exceptionally high rates of colonisation, while Langgewens (Figure 4.12.4) had almost no visible colonisation. It is important to note that the control plots (T5) also had visible colonisation at all locations. This was

expected as no measures were taken to inhibit or control naturally occurring mycorrhizal populations in the soil. No correlation between inoculum concentration and degree of colonisation could be established. Using a higher-than-recommended dosage when planting did not result in a higher degree of colonisation. Establishment of fully functioning and beneficial AMF communities under dryland conditions in the Western Cape might prove challenging considering current fertilisation practices. Minimum tillage greatly improves the conditions needed to establish these communities but much needs to be changed in order for sustainable fungal communities to thrive in the soils of the Western Cape. Gollner et al. (2005) concluded that the minimum amount of time to establish a fully functional AMF community in the soil would take approximately 15 years when converting from conventional tillage to organic farming. Organic farming might not be feasible in the Western Cape, but this finding could be used as a point of reference to determine how long AMF communities might take to establish successfully under conservation agriculture practices.

CHAPTER 6

Conclusions and recommendations

6.1 Synopsis

Conducting field trials in three distinctly different climactic regions allowed for proper investigation into the colonisation and effects of AMF on wheat in the Western Cape. By adhering to common farming practices for each region we were able to determine if AMF inoculation affected wheat development and yield under dryland field conditions. Wheat plants at each site were successfully infected with both applied and naturally occurring AMF.

6.1.1 Objective 1

To evaluate the effect of mycorrhizal inoculation on wheat growth, development, grain yield and quality

Arbuscular mycorrhizal fungi application had no clear effect on root- or plant biomass of wheat in the Western Cape. Grain quality did not differ ($p>0.05$) between treatments either. Grain yield increases were observed between treatments in both Year 1 and Year 2 of the study at the Piketberg and Langgewens, respectively. A yield difference at Piketberg (Year 1) of 69 kg ha^{-1} and 253 kg ha^{-1} when compared to the untreated control was a noticeable increase for farmers looking to increase the yield potential of their crops. During Year 2 of the study, treated plots at Langgewens had between 160 kg ha^{-1} and 467 kg ha^{-1} higher grain yield when compared to the untreated control. These observations provide evidence to the effectiveness of AMF application in the Western Cape and demonstrate that AMF symbiosis with wheat plants in these areas of relatively low rainfall were beneficial.

6.1.2 Objective 2: To assess whether mycorrhizal inoculum establish in soil in the Western Cape

Colonisation results from both Year 1 and Year 2 of the study determined that AMF are indeed capable of colonising wheat plants in the Western Cape, albeit with a lack

of uniformity. Roodebloem consistently had the highest degree of colonisation for both Year 1 and Year 2. This could be due to the higher levels of organic matter in the soil and favourable soil conditions for the AMF. At Roodebloem, no differences ($p>0.05$) between treatments for grain yield or other plant parameters were observed, while Langgewens and Piketberg sites had less colonisation but demonstrated differences in grain yield. Considering these observations, the degree of AMF colonisation and establishment in the soil did not strongly correlate with yield or biomass increases in the Western Cape.

Despite the positive colonisation results, no differences ($p>0.05$) were observed in all the data except for the yield data from Piketberg. Factors such as high soil P content, extreme temperatures, low soil moisture and low organic matter content in the soil might have been contributing factors. Higher or lower concentrations of Endomaxx did not affect the inoculation status of the wheat plants or the other physiological measurements. The industry standard also did not affect ($p>0.05$) the grain yield or other measurements taken throughout the growing season. The industry standard has approximately 120 propagules g^{-1} more than Endomaxx as well as an application concentration of 10 g ha^{-1} (Endomaxx) vs. 150 g ha^{-1} (Industry standard) still did not show up differences between the two products. Greenhouse trials might determine the difference in effect the two products have on wheat development and yield. By controlling environmental factors and removing naturally occurring AMF by fumigation or sterilisation of soil before planting, it would be possible to directly compare the performance of the two products. Western Cape soils vary greatly in quality and nutrient composition and so too will the presence and response of AMF vary in these soils.

6.2 General conclusion

Due to the extensive species diversity associated with AMF and wide range of soil types, it is difficult to determine the immediate effects of AMF inoculation in wheat systems in the Western Cape. The agricultural gain obtained by using AMF can be strongly argued due to the overwhelming evidence of benefits the fungi provide to crops in greenhouse settings. In practice, AMF behaves unpredictably and rarely mirrors the results obtained under greenhouse conditions. Thus, farmers who can afford to experiment and trial mycorrhiza by applying the inoculum at planting should do so for a few years before making the decision whether to continue or stop using the inoculum. Regular colonisation testing, soil sampling and yield analysis should be conducted to determine the extent of mycorrhizal impact on crops and soil health.

However, natural remedies to agricultural problems are on the rise Thus, AMF are not a complete solution to these problems but rather an integral component of sustainable agriculture. Farmers in the Western Cape use different crop rotation combinations, which regularly includes canola. This practice presents a challenge with regards to maintaining high counts of mycorrhizal propagules/spores in the soil. Taking this into account, benefits of using a broadleaf crop, such as canola in rotation with grains cannot be ignored and is regarded as an essential component of conservation agriculture in the Western Cape. Studies such as this thesis attempt to provide a reference point for AMF usage in a certain area. It is still crucial to trial AMF on a farm-to-farm basis to determine if AMF application is effective. By gathering data when AMF is used on commercial farms and trials, we might gain more understanding of AMF usage under field conditions in the Western Cape.

6.3 Recommendations for future research

Future studies should focus on soil composition with relation to how it affects mycorrhizal spores and fungi directly in the Western Cape. Greenhouse trials should be conducted to test for specific soil nutrient abundancies and deficiencies at different moisture levels using both Western Cape soil and growth media to support field data. Understanding what makes AMF spores delay germination by up to three months after planting of wheat in Western Cape soils is the key to improving soil health and applied AMF usability in local agricultural systems. Long-term trials with crop rotations

involving canola and other cereals with wheat in the Western Cape will provide insight into the efficacy of AMF as a biofertiliser over time. However, re-application of AMF is necessary at the start of each new cereal growing season when introducing fallow periods and non-host plants (such as *Brassica* spp.) into crop rotation systems. Bio-fertiliser effectiveness should be assessed over multiple seasons and not judged by its performance after a single season of usage. Another important aspect to consider would be to determine if AMF fits into the crop rotation cycles used and if it would be economical to continually inoculate the soil after each season.

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