Effect of Inoculation with Arbuscular Mycorrhizal Fungi on Enhancing Root Growing Systems and Improved Nutrient Uptake Efficiency in Tomato

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Abstract

Tomato is a valuable crop around the world. Rising agricultural input costs and increased demand increases the need to investigate more efficient growing systems and improve nutrient uptake efficiency. This paper documents a project which investigated ways of improving nutrient uptake and efficiency of tomato plants in protected growing environments with the use of arbuscular mycorrhizal fungi (AMF). Micro-Tom tomato cultivar were grown in two different inorganic manufactured substrates, Rockwool and Fytocell, and an organic peat compost in a controlled environment glasshouse. Substrates were also treated with arbuscular mycorrhizal fungi (AMF). Indices of plant growth and development regarding the roots, leaves and fruits in tomato plants were measured to determine the effect of the treatments compared to the control plots. Macro- and micro-nutrient levels in the leaves and fruit were analysed post-harvest. Substrate type had a significant impact on the nutrient uptake in both fruit and leaves. Tomato grown in Fytocell produced fruit that assimilated the most nutrients. Application of AMF also had a significant impact on the nutrient uptake by fruit and leaves. Rockwool treated with AMF had a significantly higher biomass, Na and Mo levels. Further pressures on global food security are the rising world population and loss of traditional agricultural land. Therefore, it is vital to ensure that crops such as tomatoes are grown optimally. Applying this knowledge and approach to growing tomato crops could lead to more competent applications which in turn increase nutrient use efficiency and crop production.

Abbreviations

AMF	Arbuscular mycorrhizal fungi
ANOVA	Analysis of variance
C	Carbon
C&N	Carbon and Nitrogen
DEFRA	Department for Environment Food & Rural Affairs in the United Kingdom
DNA	Deoxyribonucleic acid
EC	Electrical conductivity
GM	Growing media
HPS	High pressure sodium light
IC	Ion Chromatography
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
K	Potassium
LAM	Leaf Area Measurement
LRs	Lateral roots
N	Nitrogen
P	Phosphate
pH	Potential of Hydrogen
qPCR	Quantitative polymerase chain reaction
RLC	Root length colonisation assessment
RNA	Ribonucleic acid
SPAD	Soil-Plant Analysis Development
TDS	Technical data sheet
UF	Urea formaldehyde

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1. Introduction

It is increasingly important for all citizens, national governments, and policy makers to consider global food security and sustainable agriculture. The concern about how future global demand for food will be met is driven by population growth and the associated pressures on land use, fertiliser and water availability, and climate change (Fitter, 2012; FOA, 2018; UNFCCC, 1997).

Understanding and optimising the response of plant roots to nutrient conditions and uptake is of central importance in enhancing nutrient use efficiency. Root hairs are important organs for the uptake of nutrients and water from the rhizosphere and serve as sites of interaction with soil micro-organisms. The lateral roots (LRs) play a key role in the root system architecture and its response to local environmental constraints, which make them an essential plant adaptation. Much is known about root growth in the soil. In contrast, much less is known about root development in manufactured substrates (e.g. Fytocell and Rockwool). These manufactured substrates are being used extensively in protected crop growing systems and thus it is important to understand the significant differences in the relationships between soil, substrates and other systems on the root architecture and nutrient uptake. According to DEFRA farming statistics, in 2019 there was 2,825 ha of greenhouse and protected crops under production in the United Kingdom (DEFRA, 2019). Grunert *et al.* (2016) suggests that approximately 95% of all vegetables produced in greenhouses in Europe, the United States and Canada use substrate growing systems. Blok *et al.* (2021) recorded that 0.9 Mm^3y^{-1} of Rockwool was used in 2017.

Traditionally crop production was increased by applying large amounts of organic and inorganic fertilisers to adjust for assumed deficiencies in the soil chemistry. Evidence now shows that by improving the rhizosphere and the plant root structures, plants can make better use of existing nutrients thus decreasing the need for large amounts of fertiliser. This is beneficial because these large amounts of fertilisers, especially nitrogen and phosphorus, leach through or are washed off the soil, polluting groundwater, rivers and coastal

areas. In addition, over-application wastes fertiliser and drives up costs to growers (Sainju *et al*., 1999; Bindraban *et al.,* 2015; Good and Beatty, 2011).

Growing food locally for the end user reduces the carbon footprint and transport miles. The ultimate in local crop production involves growing in cities, but the development of vertical and urban farming on a highly efficient and compact scale is only possible with the aid of new green technologies like improving nutrient use efficiency with AMF and manufactured substrates. These technologies have been studied individually in the past, but they need to be combined for maximum benefit (Despommier, 2011).

Despommier (2011) describes a "cradle to cradle" waste-free economy where food is grown in our urban centres with farms arranged vertically in buildings without soil. These buildings will become the "functional equivalent of a natural ecosystem" and allow nature to convert old rural farms back to the original ecosystems. We already grow crops in glasshouses and new cutting-edge technologies allow us to produce food in vertical farms all year round. One acre of vertical farm is equivalent to as many as ten to twenty traditional soil-based acres, subject to the crop type.

Sustainable agriculture strives to achieve the most efficient ratio between the applied nutrients and the amount absorbed by the plants, thereby limiting the economic waste and negative impacts on the environment (Heins and Yelanich, 2013).

1.1 Tomato

Tomatoes are grown and eaten in many different ways and are an important source of essential nutrients in humans (Labate *et al*., 2018). Tomato is a significant crop grown intensively under protected conditions in greenhouses around the world. It is one of the most widely consumed fruits, providing up to 60% of the adult recommended daily allowance of vitamin C and up to 85% in children. Approximately 20% of the vitamin A recommended daily allowance can be found in 100ml of tomato juice. Consumption can reduce the risk of many diseases, including colon, rectal, and stomach cancer. A significant reduction in the risk of prostate cancer has also been observed

when the primary form of carotenoid, the antioxidant lycopene, is consumed by adult males (Kucuk, 2001).

The tomato industry produces commercial crops sold by weight. Increasing fruit biomass is an important measure in determining whether the industry will adopt new growing methods. According to the World Processing Tomato Council (WPTC), 37.38 million metric tonnes of tomato were produced in 2019 globally generating 64 billion USD in trade (Branthôme, 2020).

Tomatoes are preferably grown in substrates systems to give full control over the nutrients, oxygen, and water, and reduce the effects of soil-borne pathogens. These substrates can be organic or inorganic. The organic substrates traditionally used, like peat, are being phased out due sustainability and environmental issues and replaced with inorganic manufactured substrates. Other methods include soil grown, aeroponics, and hydroponics.

Growth habits are either determinate, indeterminate, or semi-determinate. Determinate types are bushy with a limited number of trusses, depending on plant and climatic conditions. They have between one to three leaves or internodes between inflorescence, no lateral shoots and are suitable where space is limited but fruit is required. Indeterminate types are tall standard or vines with three leaves or internodes between inflorescences. The sympodial segment produces three buds, and the terminal bud forms a flower. One of the axillary buds becomes a lateral shoot and produces the next three buds continuously repeating along the stem. Semi-determinate terminates stem growth after the ninth inflorescence (Schwarz, 2014).

Tomato is widely used as a model crop for a variety of research purposes and Micro-Tom determinate tomato cultivar plants were used for this trial. They were bred as an ornamental plant by crossing Florida Basket and Ohio 4013-3 cultivars. It displays a dwarf phenotype, and the ripened fruits are small and red. This small size, rapid growth, and easy transformation provides a convenient model system for research on the regulation of berry fruit development (Meissner *et al.*, 1997; Eyal and Levy, 2002). Due to its pedigree, it has been suggested that the phenotype of Micro-Tom is the result of two major recessive mutations: *dwarf* (*d*) and *miniature* (*mnt*) (Meissner *et al.*,

1997). Lima *et al*. (2004) reported that allelism tests support the theory that Micro-Tom carries a mutation in gene *D.* The determinate phenotype of Micro-Tom suggests that it has also a mutation in the self-pruning (*SP*) gene (Pnueli *et al.*, 1998).

1.2 Arbuscular Mycorrhizal Fungi

A key part of efficient crop growth systems is the presence and functioning of arbuscular mycorrhizal fungi (AMF). Farmers globally are becoming more concerned about infertile soils that have low nutrient availability, reduced biological diversity, and increased pathogen populations. Through ensuring that soil contains a developed and diverse population of AMF (and other soil micro-organisms), soil fertility can be maintained, and the over-use of fertilisers and pesticides avoided. Therefore, AMF play an important role in maintaining soil fertility, enhancing nutrient use efficiency, and the achievement of sustainable agriculture (Helgason *et al*., 1998; Jeffries and Barea, 2012). AMF are becoming increasingly fundamental to sustainable food security (Ceballos *et al*., 2013).

When studying plant-soil interactions it is important to consider AMF as they account for a large and important part of the microbial biomass in terrestrial ecosystems (Rosendahl, 2008). In particular, AMF have an essential role in the structure and biodiversity of the soil microflora (Sanders *et al*., 1996) and in sustaining plant communities. The increased diversity of AMF positively affects the productivity of plants (van der Heijden *et al*., 1998; van der Heijden, 2002) as well as plant biodiversity.

According to Young (2008), AMF are "fiendishly difficult to study" for several reasons. As AMF are obligate biotrophs, it is difficult to study the functional dynamics of the fungi in both natural and laboratory-based investigations (Tisserant *et al*., 1998). Using *in-vitro* methods, the culture of AMF has been developed for a limited number of species, in complex media and in the presence of genetically transformed carrot root (Cranenbrouck *et al*., 2005).

However, there is limited variation in the morphological characteristics of the fungal spores which creates difficulties in identification and morphotyping requiring a vast knowledge of the morphological characteristics for a wide range of different species (Clapp *et al*., 2001; Tisserant *et al*., 1998). Despite fungal studies requiring a consensus on taxonomy and species recognition (Rosendahl, 2008), the precise identification of AMF is still a subject of fierce debate. Generally, traditional taxonomy was based purely on morphological characteristics of spores (Walker *et al*., 2007). However, over the past few years, the use of phylogeny based on the ribosomal RNA genes has become a much more significant tool in identifying and classifying AMF (Young, 2012).

Currently, there are approximately 230 described species of AMF (Kruger *et al*., 2012) but this number is continually increasing and is likely to have been underestimated in the past. AMF are one of the most widely distributed fungal groups on Earth. They are located in most ecosystems (Jansa *et al*., 2002) and can colonise the rhizosphere and form mutualistic symbioses with more than 80% of vascular plant families. Arbuscules are thought to be sites of nutrient exchange between the fungus and plant roots (Smith and Read, 1997). Plant health and growth are improved when mineral nutrients (mainly phosphorus and nitrogen) as well as water, are extracted from the soil via the extensive hyphal network and transferred to the plant. This process improves plant performance according to numerous studies (Smith *et al*., 2010; Fitter *et al*., 2011; Ruzicka *et al*., 2012; Ngwene *et al*., 2013). In return, organic carbon compounds are transferred from the plant to the AMF. The AMF buffer their host plants against adverse environmental conditions, especially drought (Smith *et al*., 2010), and protect plants from pathogens (Borowicz, 2001; Ismail and Hijri, 2012; Ren *et al*., 2013). The external mycelium of AMF improves soil structure by formation of soil aggregates (Rilling, 2004; van der Heijden *et al*., 2006).

Originally, AMF were placed in the order Glomales and the division Zygomycota but now they form the division Glomeromycota (Schussler *et al*., 2001). Subsequent genome sequencing information has led to the proposal that they may be closer to the Mucoromycotina within the paraphyletic Zygomycota (Lin *et al*., 2014; Tisserant *et al*., 2013). The Zygomycota share a common ancestry with the Ascomycotan and the Basidiomycotan clades. However, currently the taxonomy remains unchanged.

The new genera, *Archaeopora* and *Paraglomus*, were introduced to the Glomeromycota by Morton and Redecker (2001) to remove some of the polyphylogenic anomalies within the *Glomus* genus. However, the taxonomy of AMF was questioned even further when Schwarzotte *et al*. (2001) confirmed that *Glomus* (the largest generic group) was polyphyletic through the use of nearly full-length SSU rRNA gene sequences. They suggested *Glomus* formed two major clades, described as *Glomus* group A and *Glomus* group B.

Recently, other authors have notably revised the taxonomy of this group. Oehl *et al*. (2011, cited in Robinson Boyer, 2014) summarised these findings, using rRNA sequence data and morphological characteristics, to suggest a reorganisation of the Glomeromycota with the addition of new genera. This work was only accepted in part by Redecker *et al*. (2013) who proposed another classification of the Glomeromycota. The most recent classification of Glomeromycota was based on a consensus of regions spanning ribosomal RNA genes, 18S (SSU), ITS1-5.8S-ITS2 (ITS), and/or 28S (LSU). The phylogenetic reconstruction underlying this classification is currently accepted as the taxonomic structure which is used by the majority of research groups as well as the curators of the two culture collections of the International Bank of Glomeromycota (IBG) and the Glomeromycota *in vitro* collection (GINCO) (Robinson Boyer, 2014).

There is considerable genetic diversity within morphologically recognisable species in the Glomeromycota, shown by molecular analyses. The most recent analyses cluster sequence data into 'species groups' or 'phylogenetic clusters'. Additionally, there is a significant number of AMF isolates that have sequences registered in sequence databases which do not always have morphotypes and *vice versa* with *in vitro* isolates held in culture collections (Öpik *et al*., 2010).

It has been demonstrated by many researchers that the below-ground AMF community affects the structural diversity and productivity of plant communities (Grime *et al*., 1987; Van der Heijden *et al*., 1998). Van der Heijden *et al*. (1998) reported that AMF are needed to maintain a basic level

of plant biodiversity and those treatments, which included different AMF taxa, create significantly varied plant communities.

Due to the ability of AMF species to colonise a range of host plants, a lack of absolute specificity has been assumed (Sanders, 2003). However, the different species of AMF may alter their effects on plant growth. Several studies have found that there is some host specificity within AMF. Vandenkoornhuyse *et al*. (2003) studied AMF diversity in eighty-nine root samples from three co-existing grass species in Scotland and found that, using T-RFLP profiling, host preferences were apparent. In DNA profiles from legumes and non-legumes, Scheublin *et al*. (2004) found fourteen sequence groups revealing that different plants hosted different communities. Furthermore, the legume root nodules hosted different communities to the parent roots of the plant. Using qPCR and traditional microscopy, Alkan *et al*. (2006) showed that both *Glomus intraradices* and *Glomus mosseae* demonstrated host preference. Comparing three different methods (molecular probes, bait plants and trap plants) Sykorova *et al*. (2007) found that the AMF which colonise greenhouse trap plants are not necessarily reflective of the AMF colonising bait plants placed directly into field soils.

It is commonly accepted that AMF from different genera or species can simultaneously occupy a single root fragment (Van Tuinen *et al*., 1998; Reddy *et al*., 2005; Alkan *et al*., 2006). It is unclear if this co-occupancy is competitive, synergistic, or antagonistic (Alkan *et al*., 2006; Krak *et al*, 2012).

Several research groups (Koide, 2000; Reddy *et al*., 2005; Alkan *et al*., 2006) have described more than one AMF species colonising a root synergistically, with functional complementarity. In contrast, some data showed that a single effective AMF species provides maximum benefit when colonising the host plant. Koide (2000) suggested that certain AMF might be more beneficial to some plant hosts than others and he proposes that the co-colonisation of two or more AMF species may enable a wider spectrum of benefits. When studying *Glomus mosseae*, *Glomus claroideum,* and *Glomus intraradices*, Jansa *et al*. (2008) demonstrated evidence for functional complementarity in a single host root system. Wagg *et al*. (2011) found a positive AM fungal richness-plant productivity relationship overall, but they discovered a range of AMF interactions ranging from facilitation to antagonism. This gave rise to

positive and negative plant effects that were dependent on abiotic conditions and host plants.

According to Robinson Boyer (2014), ecosystem processes can be influenced by AMF via many different mechanisms. The symbiotic associations that are formed are multi-functional and display complementarity, which help plants in nutrient and water uptake, pathogen protection and mediating carbon transfer. AMF play an important part in low-input sustainable agricultural systems, especially when intensive farming and forestry have been shown to reduce AMF diversity and compromise AMF function. AMF is essential in sustainable farming and forestry. The below-ground biological system needs the same care and attention as the above-ground systems. By identifying the species that are introduced into the rhizosphere and monitoring their growth in the roots of the plants, bio-inoculants can be developed for farming and forestry (Robinson Boyer, 2014).

The addition of AMF to a protected crop substrate is a relatively new approach to growing. Previously, substrates were kept as sterile as possible. The colonisation of fungi and bacteria in the root zone is not usually monitored and only becomes apparent when plants show visible symptoms. In this study, the addition of AMF in tomatoes has been investigated to understand the effects of mycorrhizal colonization on tomato plants when used as a 'crop improver'.

1.3 Substrates

Smith (1996) described a substrate in horticulture as the material into which the plant roots grow. This is not very helpful as there are many different growing media, so he divided this classification into three groups. The first is called "soils", the second "organic media" and the third is "inert substrate" or just "substrates". To further complicate matters, some materials fall into two groups, e.g. pumice, which can be a soil, is not chemically inert and not manufactured.

The advantage of using non-soil substrates is that it gives control over the oxygen, moisture and nutrient contents, temperature and pathogens in the root zone (Smith, 1996). Rockwool or Stonewool is made by melting basaltic or similar diabasic rock with coke and spinning the molten material into fibres. Rockwool was developed as a substrate in the beginning of the 1970s as a rooting material. It allowed free watering and drainage and could be controlled to give the optimum air: water ratio in the root zone (Smith, 1996). The main form of Rockwool used by growers is slabs with different orientations of fibres to suit different growing conditions and moisture requirements. The slabs are usually 5% fibres and 95% pores. When watered to full capacity and left to drain, the moisture content is about 65% with 30% air content. The crop can use up to 90% of the available water.

Smith (1996) lists the following advantages of growing in Rockwool:

- Large pore capacity for water and air
- Good ratio between water and air
- Chemically inert
- Structurally stable
- Consistent quality
- No pathogens
- Can be sterilised with steam for reuse

Additionally, he highlights the low volume of the slabs as limiting the nutrient and moisture buffer available to plants.

Fytocell is an organic synthetic hydrophilic foam substrate manufactured by Aqua Resins Technologies BV, Druten, The Netherlands. The aminoplast foam is made by spraying urea formaldehyde resin through a catalyst. This produces a white open cell foam which has unique properties making it an ideal substrate for soil–free culture.

Fytocell has the following properties:

- Biodegradable
- Optimum capillarity
- Provides a perfect water:air ratio of 60:40 for plant roots even when over watered

- Water/air ratio does not change with the depth of substrate
- Homogenous open structure
- Light weight
- Inert and sterile due to residual formaldehyde

1.4 Nutrients

Protected crops require high input costs to maintain good production. By finding new and efficient ways of growing tomato, fertiliser inputs can be reduced with the associated savings. High tech, greenhouse farming uses a variety of techniques to allow the full control of the growing environment. This includes controlling the various concentrations of macro- and micro-nutrients introduced into the fertigation systems. The tomato industry uses soluble plant nutrients suspended in irrigation water to 'fertigate' plants grown in substrates. Fertigation is the technique of combining irrigation water and fertiliser before it is delivered directly to the root zone of the plants. The actual source of this fertiliser has to be chosen very carefully to ensure compatibility with the specific substrate as well as irrigation water characteristics. The loss of nutrients such as P, K or NH_4^+, or of micro-nutrients, through adsorption and/or precipitation processes may lead to reduced nutrient concentrations in the rhizosphere, thus making nutrient deficiency a common occurrence even when the nutrient concentration in the irrigation water is adequate. Furthermore, the quality of the irrigation water and the chosen fertiliser should be free from any potentially toxic constituents (Silber, 2008). If modified rhizospheres can result in more efficient uptake of nutrients without affecting quality and yield, their concentration in the fertigation recipe can be reduced with the resultant savings of input costs.

The mineral content of tomato fruit depends on the amount of nutrients taken up from the substrate. Growth systems should ensure that sufficient nutrients are available for successful production at all stages of growth as well as optimised nutritional content of the fruit crop. Essential elements like potassium, calcium, and magnesium determine fruit colour, shape,

hollowness, acidity, and flavour along with uniformity of ripening. Sodium is not essential but can limit potassium and calcium uptake (Labate *et al.,* 2018).

Inadequate concentrations of available nutrients can negatively affect quality and yield of the fruit. Excess concentrations of nutrients can also have a negative impact on the plants. High concentrations of nitrogen will promote biomass production at the expense of fruit yield. High biomass also makes harvesting the fruit more difficult. Additionally, high concentrations of manganese, boron, and chloride can be toxic to plants (Sainju *et al*., 1999).

Tomatoes require at least twelve nutrients which are named the "essential elements", for normal growth and reproduction. These are nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sulfur (S), boron (B), iron (Fe), manganese (Mn), copper (Cu), zinc (Zn), and molybdenum (Mo). The function of each of these nutrients and their concentrations in different parts of tomatoes are shown in Tables 1.1. and 1.2. These nutrients are essential for tomatoes to grow and bear fruit. For example, N is the essential component of many compounds that are responsible for biochemical changes in tomato growth including amino acids, proteins and enzymes. Some of the nutrients are needed in such large quantities that they are named macro-nutrients; N, P, K, Ca, Mg, and S. Those needed in smaller quantities; B, Fe, Mn, Cu, Zn, and Mo, are named micro-nutrients (Sainju *et al*., 1999). The yield and fruit quality depend to a large extent on the plant taking up sufficient nutrients to utilise in the growing processes.

Nitrate (NO_3-) ions provide nitrogen for the construction of vital molecules like amino acids. These anions dissolve in water and are translocated in the plant to where they were required. Nitrogen (N) is a constituent of protein and amino acids. It plays a vital role in the growth and reproduction of plants as well as production of enough foliage to protect the fruit from exposure to the hot sun (Gould, 1983). However, it is the most limiting nutrient for tomato growth as tomatoes remove large amounts of N from the substrate. When there is not enough available N it can result in stunted spindly growth and yellowing of the leaves at the base of the tomato plant (Needham, 1973).

High concentrations of N in the substrate can encourage excessive vegetative growth, which can delay the setting and maturity of tomato fruits thereby reducing tomato production (Winsor *et al*., 1967; Kaniszewki *et al*., 1990). It may make younger leaves smaller, darker, and often puckered or curled (Needham, 1973). Root tips could turn brown then die and, in severe cases, most of the root system may be killed. The N that is applied from fertilizers is readily converted into NO_3- for plant uptake. In addition, Maynard *et al*., (1966) and Miliev, (1966) observed that the type of N fertilizer used can affect tomato production because NH_4-N can be toxic unlike NO_3-N.

Phosphorus (P) is important in supporting the early establishment of the tomato plants as it helps to initiate root growth. P is a component of nucleic acid. It helps to produce large amounts of blossom during early tomato growth, as well as the early setting of fruits and seeds (Zobel, 1966), leading to an increase in the number of tomatoes produced, amount of soluble solids, and acidity content (Adb-Alla *et al*., 1996b). In addition, Su, (1974) found that P improves the colour of the skin and pulp, taste, hardiness, and vitamin C content.

Tomatoes take up relatively small amounts of P when compared to N and K so its concentration is small (Tables 1.1., 1.2.) and less needs to be added to fertilizers. In addition, it is relatively immobile. Von Uexkull (1979) observed that water soluble P fertilizers, such as nitro-phosphate or triple super phosphate, provide rapid availability.

Table 1.1. Recommended levels of nutrients for tomatoes (Sainju *et al*., 1999)

	Substrate (mg kg $^{-1}$)		**Plant (mg kg $^{-1}$)**	
Nutrients	**Desirable**	**Toxic**	**Desirable**	**Toxic**
P	60-70		4000	
K	600-700		60000	
Mg	350-700		5000	
Ca	1000		12500	
N	50-100		30000-50000	
B	1.5-2.5	3	40-60	100
Mn	5-20	80	30	1000
pH (no unit)	6.5-7.5			
Salt conductivity (mmho cm $^{-1}$)	80-100			

Table 1.2. Target nutrient concentrations in tomato leaves and their functions (Sainju *et al*., 1999)

Nutrient	**Content (mg kg $^{-1}$)**	**Function**
N	48000	Constituent of proteins and amino acids
P	5000	Constituent of nucleic acids
K	55000	Activates enzymes (e.g. pyruvate kinase); regulates pH of tomato fruit
Mg	5000	Constituent of chlorophyll
Ca	25000	Component of plant cell wall. Affects the permeability of cells
S	16000	Constituent of proteins and amino acids (e.g. methionine)
B	35	Regulates the level of growth substances
Fe	90	Constituent of enzymes (e.g. peroxidase, catalase)
Mn	350	Activates enzymes (e.g. malic)
Cu	15	Constituent of oxidizing enzymes (e.g. phenolase)
Zn	80	Constituent of enzymes (Carbonic anhydrase)
Mo	0.5	Involved in the utilization of NO_3-N (nitrate reductase)

Like N, potassium (K) is absorbed in large amounts by tomatoes demonstrated by the higher concentration of K than the other nutrients (Tables 1.1. and 1.2.). K helps in the vigorous growth of tomato and increases the number of tomatoes per plant by stimulating the early flowering and setting of the fruits (Varis, 1985).

K deficiency results in brown marginal scorching with interveinal chlorosis and the yellowing of tomato leaves (Needham, 1973) and shortened internodes (von Uexkull, 1979). The older leaves of the tomatoes show these symptoms

first then they spread throughout the plant as it matures. Furthermore, fruits ripen unevenly, and tomatoes may have a lower lycopene content, according to Gould (1983). These deficiencies can appear rapidly in tomatoes grown in substrates that are low in K content.

Excess K has hardly any direct effect on tomatoes, but it can reduce the availability of Mg in soil. Needham (1973) suggested that a ratio of 2:1 K to Mg should be maintained in order to reduce Mg deficiency whilst applying K. K, like N, is soluble in water so it can be leached out of substrates. It is important to prevent the leaching of K to reduce fertilization costs.

Calcium (Ca) is another macronutrient needed by tomato in large quantities because of its higher concentration in the plant components (Tables 1.1 and 1.2). Ca in the form of $CaCO_3$ raises the pH of irrigation water extracted from the mains water supply. Excessive $CaCO_3$ should be avoided as it can result in the deficiency of micronutrients, such as Mn and Fe (Sainju *et al*., 1999). High concentrations of free $CaCO_3$ can decrease P availability in the substrates (Abdel-Samad *et al*., 1996a). A Ca deficiency within tomato fruits caused by the lack of movement and distribution of Ca can cause 'blossom-end rot'. In these cases, the leaves may contain an abundance of Ca, but the fruits may not. The affected fruits start to rot at the bottom, and it then spreads up the plant. Wiersum (1966) discovered that the disorder occurred below the Ca concentration of 800mg per kg in the fruits.

Magnesium (Mg) is a component of pectin, organic acids, chlorophyll and coenzymes. In the greenhouse production of tomatoes, Mg deficiency is common, and production is significantly increased by applying Mg fertilizer.

Sulfate (SO_4^{2-}) is the soluble form of sulfur (S) and is a vital element of plant proteins, hormones, and amino acid. S deficiency is rare because it is usually applied along with the N, P and K fertilizers. Additionally, tomatoes absorb S as SO_2 from the atmosphere. However, if tomatoes are exposed to greater than 0.5 mg kg^{-1} SO_2, the mid and lower leaves can get water-spots which become white, dry, and papery as well as the fruits having sunken white spots.

Boron (B) plays an important role in the insemination and reproductive growth of tomatoes. B influences the production of tomato flowers and fruits. The deficiency of B is one of the most widely reported nutritional disorders in commercial tomato production especially in manufactured substrates.

Copper (Cu) is another important element in plants. Cu deficiency can be observed in tomatoes that are grown in greenhouse substrates low in Cu content. The symptoms appear as curled leaves that have a tubular appearance, downwardly curled petioles, and necrotic spotting near the veins of leaves. Additionally, Murphy *et al*. (1980) observed that this deficiency can occur when excess concentrations of fertilizer containing P are applied which decreases Cu availability.

Iron (Fe) is a constituent of many enzymes in the nutritional metabolism of tomatoes (Table 1.2.). Iron deficiency is most likely in high pH substrates and fertigation. Furthermore, excess concentrations of P in soils can decrease the solubility of Fe and its translocation, thereby increasing Fe deficiency (Sainju *et al*., 1999).

Like iron, manganese (Mn) deficiency is induced by high substrate pH. Mn deficiency is most common in tomatoes grown in organic and peat substrates. Beneficial microorganisms reduce organically bound Mn and Mn^{3+} into Mn^{2+} at high temperatures (Winsor, 1973). Compacted and wet substrates are more likely to show Mn toxicity (Sainju *et al*., 1999).

Molybdenum (Mo) is needed for N metabolism in tomatoes. The deficiency of Mo can occur in acid substrates and peat compost (Sainju *et al*., 1999).

Zinc (Zn) is a constituent of the enzyme carbonic anhydrase which is essential for the metabolism of nutrients in tomatoes. Zn deficiency is common in soilless medium or water cultures low in Zn content. Additionally, high P concentrations reduce the availability of Zn (Abdel-Samad *et al*., 1996a).

Sodium (Na) is not essential but can limit K and Ca uptake. Humans often do not consume enough essential minerals, but their daily intake of minerals like Na can be excessive (Labate *et al*., 2018). Studies revealed that the consumption of Na ranked the highest of 13 elements across 20 diverse human diets, followed by K, Ca, and Mg (Mir-Margues *et al*., 2016)

Chloride (Cl^-) is an anion which forms when the element chlorine gains an electron. This anion can bond with the cation Na^+ to form NaCl, sodium chloride. NaCl is toxic to plants in high concentrations.

Cl^- in higher plants is responsible for osmotic and stomatal regulation, evolution of oxygen in photosynthesis, disease resistance, and tolerance. It can also limit yield and growth when available in concentrations above the range of 2 to 20g kg^{-1} depending on the species. Globally, growers are more concerned about toxic concentrations of Cl^- in plants than deficiencies and therefore tend to avoid fertilisers containing metallic salts. This could lead to deficiencies and associated reductions in yields. Many researchers have recorded increases in yield after applying Cl^- containing fertilisers and Chen *et al.* (2010) stress the importance of more research in this area.

Chloride is not an essential element and tomatoes do not suffer from the deficiency of Cl, however, large concentrations of Cl can damage the growth of tomatoes. High concentrations of Cl and increased concentrations of NO_3 can increase vegetative growth at the cost of fruit reproduction (Sainju *et al*., 1999).

1.6 Objectives

The aims of this study were to identify the effect of growing media in terms of nutrient use efficiency and to understand the effect of AMF on nutrient uptake in different growing systems for tomato. To achieve this my objectives were as follows:

- Evaluate growing systems for tomato crops by modifying the rhizosphere.
- Supply all required nutrients through fertigation.

- Investigate the associations between plant roots and AMF, then determine whether AMF can increase plant growth and nutrient uptake.
- Measure efficiency of nutrient-uptake by analysing N, P, K, macro- and micro-nutrient acquisition of tomato leaf and fruit between different growing media using IC and ICP-MS analysis.

2. Materials and methods

2.1 Outline methodology

Micro-Tom tomato plants were grown in individual one litre plastic plant pots placed in plastic trays under controlled conditions in a greenhouse on Sutton Bonington Campus, University of Nottingham for three months until the fruit were ready to harvest. Physiological measurements were taken throughout the growing phase. Harvested fruit and whole plants were examined, data analysed and reported in Chapter 3.

2.2 The trial site

A 50 m^2 glass house on University of Nottingham, Sutton Bonington Campus was used to conduct the trials and the site is located at 52.833°N, 1.251°W. The glass house used was a traditional vented glass house with concrete floor and the weather history is attached in the appendix.
Services available were:

Mains water (Chemical analysis in Table 2.1.)
Automatic ventilation
Supplementary lighting by 600 watt high pressure sodium (HPS) lights
Heating

Mesh plant benches were used which allowed good air circulation around the trays of tomato plants.

The trial design allowed for any variation in glasshouse conditions between the benches by rotating the tomato plant positions on the benches every week.

An 18-hour photoperiod was maintained with the aid of HPS lights. The temperature was controlled by heating and venting to maintain a daytime range of 22 to 28^{o}C and night-time range of 15 to 18^{o}C.

Table 2.1. Analysis of mains tap water supply to glass house.

Analysis	Results
Calcium (mg L^{-1})	58
Magnesium (mg L^{-1})	15
Manganese (mg L^{-1})	<0.01
Boron (mg L^{-1})	0.06
Copper (mg L^{-1})	0.13
Molybdenum (mg L^{-1})	<0.01
Iron (mg L^{-1})	0.02
Zinc (mg L^{-1})	0.05
Sulphur (mg L^{-1})	38
Phosphorus (mg L^{-1})	0.620
Potassium (mg L^{-1})	4
pH	7.2
Nitrate N (mg L^{-1})	2.61
E.C. (mmhos cm^{-1})	0.83
Ammonia N (mg L^{-1})	0.01
Sodium (mg L^{-1})	34
Chloride (mg L^{-1})	53
Bicarbonate (mg L^{-1})	122

2.3 Trial design

Micro-Tom (*Solanum lycopersicum* cv. Micro-Tom) tomato variety was used for this trial as they have a short rotation of 90 days and are free standing. This allowed them to be moved around to eliminate variations between plants and to facilitate artificial blocking in the trial. It also made more logistical sense for measurements and analysis in the laboratory.

Heirloom Micro-Tom seeds from previous trials were germinated in seed plug trays. The seedlings were propagated in compost before being transferred to one-litre plastic pots filled with three different substrates.

There were seven plants per treatment and the one-litre pots were placed in plastic trays in their treatment groups. Plastic trays were used to contain the water draining from each pot: this reduced the watering frequency and prevented AMF spores from the treated plant pots spreading to the untreated pots. Watering was carried out manually as required by the Glasshouse Technician with added soluble fertiliser.

For each mycorrhizal treatment, 10 grams of Plantworks Rootgrow Mycorrhiza inoculum was placed into a planting hole. The seedling plug was placed into the planting hole and the substrate firmed around the root ball.

Three substrates were chosen based on current tomato industry use. Nutrient Film Technique (NFT) has been omitted due to the problems establishing mycorrhiza in these systems. NFT uses a shallow stream of re-circulating nutrients flowing over the plant roots in a channel. Compost is the traditional growing medium and Rockwool has been a popular substrate in protected tomato crops. Fytocell is a synthetic organic substrate which has unique growing properties and is emerging as a protected crop substrate.

There were a total of six treatments as follows:

1. F(1-7) Fytocell
2. FM(1-7) Fytocell with mycorrhiza
3. R(1-7) Rockwool
4. RM(1-7) Rockwool with mycorrhiza
5. C(1-7) Compost
6. CM(1-7) Compost with mycorrhiza

A total of 42 plants were used. Measurements were recorded against these labels.

2.4 Experimental design and statistical analysis

The trays with the potted plants were placed on benches in the glasshouse. The mycorrhizal treated plants were put on a separate bench from the untreated pots to reduce possible cross contamination of mycorrhizal spores, which can be transferred through water. Airborne spore transmission is more likely in windy environments and the probability of this happening in a closed glasshouse is low.

It was suggested that, by keeping the mycorrhizal plants separate from the non- mycorrhizal ones, created 'blocking' with each pot on the bench representing a subplot. To prevent any potential environmental conditions contributing to blocking, the trays were rotated weekly on the benches. Normally, the pots would be placed randomly in the test area to avoid any bias created by treating the plants and their treatment groups separately.

Two-way analysis of variance (ANOVA) was carried out for each data set using Genstat (19th Edition, VSNI) to determine if the two null hypotheses should be accepted or rejected, namely:

- Substrate type has no effect on nutrient uptake efficiency in tomato.
- Addition of AMF to growth substrates does not improve nutrient uptake efficiency in tomato.

Where a two-way ANOVA showed no significant interaction between the substrate and treatment with AMF, the analysis was re-run with a one-way ANOVA for all the plots. This allowed the pooling of the non-significant data with an increase in the degrees of freedom (df) of the error term, thus improving the statistical power of the ANOVA (Labate *et al.,* 2018). If a one-way ANOVA showed results that were significant with $p \leq 0.05$, a Tukey HSD post-hoc test was carried out and the results recorded to indicate where the significant differences in the means were.

Qualitative data (e.g. mycorrhizal root colonisation) were analysed visually.

The following physiological data were recorded during the trial from the time of planting to the time of harvest and analysed statistically:

- Height of the plant
- Spread of canopy
- Number of flowers
- Number of fruits
- SPAD (Soil-Plant Analyses Development)

At the end of the growing period, the following data were collected.

- Leaf area measurement
- Plant tissue nutrient analysis
- Biomass of leaves and shoots
- Root mapping
- Mycorrhizal mapping with Root Length Colonisation Assessment
- Fruit yield, biomass and ripening
- Brix (°Bx) measurements for sugar content of fruit
- Lycopene measurements using spectrometry of the fruit skins
- Fruit chemical analysis

2.5 Fertigation

HortiPonic 7-6-33+MgO+6SO_4+TE soluble fertiliser was used for this trial. It is supplied by Hortifeeds, UK and is formulated for hydroponic tomatoes grown in Rockwool and other inert substrates such as Fytocell. HortiPonic was used at a rate of 0.75g L^{-1} of tap water. Table 2.2. shows the nutrient analysis of the fertiliser and these values were added to the tap water giving a final fertigation solution analysis as shown in Table 2.3. Table 2.3. also shows the total amount of nutrients available to the plants.

HortiPonic is formulated for hydroponic tomatoes grown in Rockwool and other inert substrates such as Fytocell. The same plant feed was used for the compost substrate even though the compost had some added nutrients. The compost was not analysed, and no adjustment was made to the fertigation to allow for these additional nutrients.

Table 2.2. Hortiponic Standard Nutrient Analysis for 7-6-33+MgO+6SO_4+TE
Refer to Appendix for data sheet.

Nutrients	**% by weight**	**mg L^{-1}**
Nitrate - NO_3	7.000	52.00
Ammonium - NH_4	0.000	0.00
Urea - $(NH_2)_2CO$	0.000	0.00
Phosphate -P_2O_5	6.000	45.00
Potassium - K_2O	33.600	252.00
Magnesium - MgO	6.000	45.00
Calcium - CaO	0.000	0.00
Iron - Fe EDTA	0.153	1.15
Manganese - Mn	0.100	0.75
Boron - B	0.030	0.23
Zinc - Zn	0.027	0.20
Copper - Cu	0.020	0.15
Molybdenum - Mo	0.012	0.09
E.C. mS cm^{-1}, local water E.C. should be added		0.88

The soluble fertiliser has a ratio of macro nutrients 7:6:33 N:P:K (nitrogen : phosphate : potassium). Magnesium oxide and sulfate, along with trace elements (TE) were added to create a balanced plant feed suitable for tomatoes. The trace elements included were chelated iron (Fe), manganese (Mn), boron (B), zinc (Zn), copper (Cu), and molybdenum (Mo). The EC of the fertigation water as measured with a Bluelabs EC pen was 1.7mS cm^{-1}. This was kept less than the industry reference of 2mS cm^{-1} mentioned by Li (2001) to accommodate the added nutrition of the compost used in this trial. The fertigation water was found to be pH 7 as measured with a Bluelabs pen in the freshly prepared solution.

N:P:K is the ratio of these elements in a particular fertiliser given as a percentage of the total volume. The elements are not available in their pure form but are mobile in a soluble ionic form. Nitrogen ions are bound in nitrate, phosphorus binds with oxygen to form phosphates and potassium bonds with oxygen to form potassium oxide.

The premixed soluble fertiliser was dissolved with tap water in a tank at a ratio of 75g L^{-1} to create a stock solution. The stock solution was then put into an auto diluter set at 100:1. A watering can was filled from the diluter and fertigation water was hand applied to the tomato plants every second day to a level of no more than 1cm from the bottom of the trays. No fertigation water was allowed to leach and drip onto the floor of the glass house.

The fertigation water was analysed, and the results shown in Table 2.3. The nutrient spectrum can be compared to the analysis of the plant tissue in Tables 3.5., 3.6., 3.7., and 3.8., thus allowing direct comparison of macro- and micro-nutrients that have been taken up by the plant.

Table 2.3. Summary of results of fertigation nutrient analysis

Macro-Nutrients					
P	K	Ca	Mg		
(mg kg^{-1})	(mg kg^{-1})	(mg kg^{-1})	(mg kg^{-1})		
2946.50	19684.75	1879.13	972.88		
Micro-Nutrients					
B	Cu	Fe	Mn	Mo	Zn
(mg kg^{-1})	(mg kg^{-1})	(mg kg^{-1})	(mg kg^{-1})	(mg kg^{-1})	(mg kg^{-1})
35.42	16.87	100.58	107.52	10.94	23.24

No fertigation runoff was collected for analysis in this trial. In commercial greenhouses, fertigation runoff is collected after it has passed through the substrate and analysed in a laboratory. The nutrient spectrum should be similar in the fertigation solution and the runoff, although the runoff is expected to have lower concentrations of nutrients due to absorption by roots. If there is a large residual balance or a deficit of a particular nutrient, this can be adjusted in the fertigation and rechecked.

However, foliar analysis was used to observe how the nutrients were utilised by plants and to indicate the health of the crop. Again, adjustments to nutrient solution composition can be made and runoff and foliar analysis checked. Commercial growers carry out these analyses every two weeks and use the data to help identify interactions or antagonisms among plant nutrients.

2.6 Sampling

Plant physiology, photosynthesis, and root growth parameters were quantified using visual inspections, physical measurements, SPAD, GFS-3000, and CHNS Analyzer. Destructive measurements of plant biomass were made at the end of the trial and expressed on a wet and dry weight basis. Destructive 'Brix' measurements of the sugar concentrations in the fruit were made post-harvest with refractometer.

Non-destructive physiological measurements were taken every week. These were:

- Height of the plant
- Spread of canopy
- Number of flowers
- Number of fruits
- Photo record of each tray
- SPAD (Soil-Plant Analyses Development)

Once all non-destructive tests have been carried out, the leaves and fruit were harvested and prepared for chemical analysis.

2.6.1 Height of the plant

The height of each plant was measured using a tape measure, from the top of the substrate to the top of the tallest branch including the leaf and recorded in millimetres. As the branches grew, they bowed down under their own weight and the weight of the fruit. These branches were not lifted or 'corrected' to show increased growth but measured *in situ*. After the tenth week the overall height of individual plants started to decrease.

The plants all had branches growing from the main stem; the stem height was measured separately to the overall plant height as the latter would be affected by leaf and fruit weight.

2.6.2 Spread of canopy

Spread was measured by measuring across the top of the plant on the two longest axes (90°apart) in millimetres. No adjustment for uneven formation of the crown was made. This measurement was used to compare increases in plant growth. The spread increased as the branches and leaves grew, but also as the branches bowed under the weight of the fruit. No adjustment was made for this.

2.6.3 Number of flowers

Anthesis began after four weeks whereafter flowers were counted weekly. A flower was regarded as a fully formed bud to the point at which petals dropped off. Once the petals dropped off, the ovary was counted as a fruit.

2.6.4 Number of fruits

Post-harvest fruit was divided into two stages. Ripe fruit which was red in colour and green fruit. Fruits were counted once they had formed and continued to ripen. Only ripe fruit were processed for nutrient analysis. However, the total number of green and red fruit was recorded to compare if the treatments had any effect on yield and biomass.

2.6.5 Photographic record of each tray

A photograph was taken of each tray to show the development of the plants during the trial and to record any abnormalities such as symptoms of disease. At the start of anthesis, an infra-red photograph was taken of each tray to detect variations in the leaf temperature. This would show if plants were stressed and transpiring unevenly, an indication of disease in the leaves.

Figure 2.1. Mature tomato plants bearing ripe fruit showing black plastic plant pots in green plastic trays.

2.6.6 SPAD (Soil-Plant Analyses Development)

SPAD measurements of each plant were taken during a four-week period once anthesis had started using a Minolta SPAD-502 leaf chlorophyll meter. The SPAD units record the penetration of red light through the leaves and provide an indication of the amount of chlorophyll in the leaves (Markwell *et al.,* 1995). This in turn could be correlated to the amount of nitrogen in the leaf cells. Nitrogen is an important element in chlorophyll. Chlorophyll pigment molecules in chloroplasts capture light energy and are used to generate high-energy electrons used in the reduction process of photosynthesis (Berg *et al.,* 2002).

At the end of the growing period, the following destructive tests were conducted:

- Leaf area measurement
- Plant tissue nutrient analysis

- Biomass of leaves and shoots
- Root mapping
- Mycorrhizal mapping with Root Length Colonisation Assessment
- Fruit yield, biomass and ripening
- Brix measurements for sugar content of fruit
- Lycopene measurements using spectrometry of the fruit skins
- Fruit chemical analysis

2.6.7 Leaf area measurement (LAM)

LAM is an important measure of plant growth and was measured using a LI-3100C (LI-COR, USA) benchtop leaf area meter. The leaves of each plant were cut off at the stem and placed flat on the guide rollers which fed them through the optical sensor. A total leaf area per plant was recorded in cm^2. The leaves were saved for plant tissue nutrient analysis.

2.6.8 Plant tissue nutrient analysis

After measuring the surface area of the leaves, they were placed in oven drying bags and weighed to give a wet weight. The stems were bagged separately and weighed. Both the leaves and stems were left for 48 hours in an oven at 80°C to dry and then weighed.

Only the ripe fruit were placed in oven drying bags and weighed to give a wet weight. Bags of fruit were left for 48 hours at 80°C to dry. The bags of dry fruit were weighed to give their respective dry weights. Fruit were considered ripe when they had no visible green tissue on the outer skins.

Dry leaves and fruit were prepared for carbon and nitrogen (C&N), ion chromatography (IC) and inductively coupled plasma mass spectrometry (1-MS) analysis, as follows:

Each sample of dried plant material was individually placed in a mortar with a small amount of liquid nitrogen to freeze them and make them brittle. A pestle was used to reduce the leaves to a fine powder. After the liquid nitrogen had evaporated, the dry leaf powder was transferred to a labelled storage bottle for processing later.

For C and N analysis, 20 mg of plant material powder was placed in foil capsules for processing in a Vario Micro Cube (Elementar UK Ltd). The capsules were dropped into a tube where, in the presence of external oxygen, flash combustion occurs at 1800 °C. The gaseous combustion products N_2, NO_x, H_2O, SO_2, O_2 and CO_2 were carried by the helium (He) through a column filled with copper oxide (CuO), then to a Cu-column where nitrogen oxides were reduced to elementary nitrogen, and O_2 to CuO. The remaining gases passed through a Temperature Programmed Desorption (TPD) column which separates N_2 from the other gases. The other gases were released separately with a programmed temperature raise in the column. They flow along a Thermal Conductivity Detector (TCD) which produces an electrical signal proportional to the concentration of nitrogen, carbon, hydrogen and sulfur.

ICP-MS analysis was carried out using an iCAP (Thermo Scientific) and IC analysis using a Dionex™ (Thermo Scientific). 200mg of plant material powder was prepared for microwave digestion. The digestate was diluted and placed in tubes for analysis.

ICP-MS is a method that totally decomposes samples into their primary elements and converts them into ions. It uses argon gas to carry nebulised samples into a high temperature (6,000 – 8,000K) plasma which is produced by a high frequency electrical induction coil. All elements are ionised and carried into a mass spectrometer which identifies and quantifies elements and isotopes.

The IC analysis measures the anions in the digestate to show the total amount of each element irrespective of its chemical form. Elements do not exist freely in the plant tissue but form compounds.

IC separates ions based upon their interactions with resin (stationary phase) and the eluent (mobile phase). There is an anion column, which attracts anions, and a cation column, which attracts cations. Columns can only measure conductivity of the specific type of ion that it attracts. Ions will move through the columns of the ion chromatograph at different rates depending on their affinity for the specific resin. They will separate from each other based upon differences in ion charge and size. Ions with a weaker affinity for the resin will move through the column faster and be eluted first, while ions with a stronger affinity for the column will move slowly through the column.

When the ions leave the column, they are measured by an electrical conductivity detector which produces a chromatogram of conductivity vs. time. The height and area of each ion peak are proportional to the relative ion concentration in the injected solution.

2.6.9 Mycorrhizal mapping with root length colonisation assessment

Mycorrhizal mapping was done by sampling the roots of each plant. In order to see the fungal material on and inside the root cells, it needs to be stained. The protocol described on the International Culture Collection of Vesicular Arbuscular Mycorrhizal Fungi (INVAM) website (www.invam.wvu.edu) was used. About 0.5g of hand selected root was placed inside a histocassette (Simport, Canada). The roots were prepared by cleaning them with 2 % (w/v) KOH at 90°C for one hour and then rinsed three times in water. The histocassettes were then incubated at 20°C in 2 % (v/v) HCl for 30 min. After this they were put into a mixture of Trypan Blue at 0.05 % (w/v) in lactoglycerol (lactic acid, glycerol, water in equal parts at 90°C for one hour. Excess Trypan Blue was removed by rinsing the roots in the histocassettes with 50 % (v/v) glycerol (Phillips and Hayman, 1970).

Stained roots were placed on microscope slides in four rows running length ways. Each slide was labelled with the treatment number and examined under a microscope. When hyphae, arbuscules or spores were found, the numbers were noted and recorded.

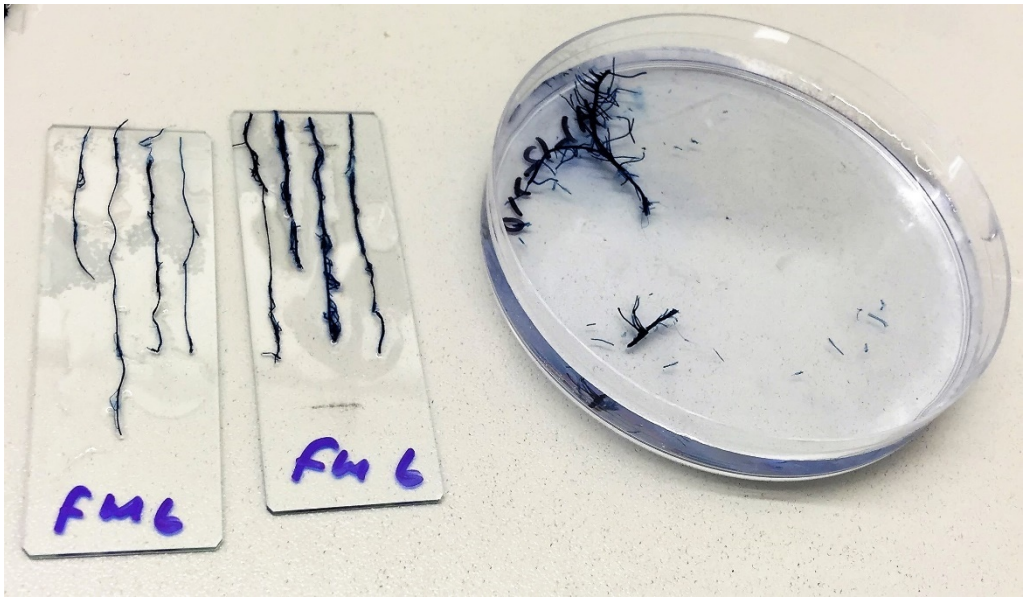


Figure 2.2. Stained roots mounted on microscope slides

2.6.10 Measurements of fruit yield, biomass and ripening

Fruit was separated into two categories, ripe and green. Fruit were considered ripe if they had no green colour visible on the fruit skins. Wet fruit weight was measured and compared to determine if the treatments significantly improved yield.

Dry weight was used to calculate the conversion of nutrients into plant matter. Each group (ripe or green) was counted and weighed before placing them in an oven drying bag. Fruit was placed in the drying oven for 48 hours at 80°C to dry. The dry fruit was weighed, and the biomass recorded.

2.6.11 Brix as a measure of sugar content

Degrees of Brix (°Bx) is a cheap, easy and reliable measure of the quality of fruit and vegetables which can be carried out in the field with a refractometer. It is an indication of total dissolved solids and is used as a measure of sugar in tomatoes. A drop of juice from the tomato was placed on the refractometer, readings taken and recorded.

One degree Brix (°Bx) is equivalent to 1 gram of sucrose in 100 grams of solution and represents the strength of the solution as percentage by weight. Brix is measured in ripe fruit by extracting the fresh juice immediately after harvesting and placing a small quantity on a refractometer. The refractometer is held up to a light source and a reading taken of the degree to which light is refracted through the solution (Bumgarner and Kleinhenz, 2012).

2.6.12 Lycopene

Lycopene is an important nutritional compound in tomato and can be estimated with non-destructive spectrometry (Hyman, 2004). The antioxidant lycopene gives tomato its red pigment and is associated with health benefits such as reduction of the incidence of some cancers. A rapid in-field, non-destructive technique has been developed to measure the lycopene content of tomato using a chromo meter. Linear regressions of the correlation values, L*, a*, b* are used and the correlating lycopene content calculated (Darrigues *et al.,* 2008). Kaur *et al.* (2006) suggested that previous studies show the Hunter (a^* x b^*) values measures the colour of the tomato skin and is a good estimate of the lycopene content. The Hunter correlation values, L*, a*, b* are based on the Opponent-Colour Theory.

A hand-held Minolta CR400 Chroma meter was used to take colour measurements of the ripe fruit skins and the output readings were recorded

for analysis later. L* measures the lightness of the skin colour, a* measures the colour range from green to red. The correlation value b*, measures the colour range from blue to yellow (Darrigues *et al.,* 2008).

2.7 Substrates

Three different growing media used in this trial based on the industry standard are compost, Rockwool, and Fytocell.

2.7.1 Compost

For this trial, Everris Levington Professional Growing Media was used. The major constituent is *Sphagnum* moss peat with an electrical conductivity of 280-380 μSm^{-1}. Dolomite [$CaMg(CO_3)_2$] 5-10%, calcium nitrate [$Ca(NO_3)_2$] 1-5% and ammonium sulfate [$(NH_4)_2SO_4$] 1-5% are added to the peat base to provide a source of nutrients for the plants and to adjust the pH. The pH measured in water is 5.3 to 6.0. Macro-nutrients are added at the rate of 204 mgL^{-1} N, 104 mgL^{-1} P, 339 mgL^{-1} K (Technical Information MSDS in Appendix).

Ammonium, as a source of N, is freely and immediate available to the plants. This was in contrast to the other substrates in this trial, which were inert and offered the plants no additional nutrients.

2.7.2 Rockwool

Grodan Vital from Grodan, The Netherlands, was cut into cylindrical shapes to fit one litre plastic plant pots. The pots were then flushed with tap water (Refer to the MSDS in Appendix).

2.7.3 Fytocell

Fytocell flakes from Aqua Resin Technologies B.V., The Netherlands, was soaked for 24 hours in tap water, drained and flushed before being placed in one litre plastic plant pots (Refer to the MSDS in Appendix).

2.8 Mycorrhiza

AMF inoculum was supplied by Plantworks Limited, Sittingbourne, Kent, UK. The inoculum was manufactured on site and consisted of four pure cultures of the following species: *Funneliformis mosseae* BEG 25, *F. geoporus* BEG 11, *Rhizophagus intraradices* BEG 72, *Glomus microaggregatum* BEG 56.

2.9 Data Recording

All data were prepared and checked for normality and integrity in Excel before being transferred to Genstat (19th Edition, VSNI) for full statistical analysis using ANOVA.

2.10 Pathogens

Glasshouse environments are favourable environments for not only growing plants but also pathogens. While every effort was made to prevent pathogens entering the glasshouse, it is not possible to stop them all. Unfortunately, there were three outbreaks of *Botrytis cinerea* during the course of the trial. These were treated immediately with Amistar, a broad-spectrum fungicide with the active ingredient Azoxystrobin. The Technical Datasheet (TD) of Amistar from Syngenta was studied to see if it would have any effect on the mycorrhizal fungi used in the trial. Diedhiou, Oerke and Dehne, (2004) recorded that strobilurin fungicides like Azoxystrobin had no

effect on arbuscular mycorrhiza when applied at the recommended dosages and therefore it was assumed to be safe to apply to all the plants. The Amistar was applied as a drench over the plants from a watering can at a rate of 1litre/ha. Refer to the appendix for the Safety Data Sheet.

Whitefly, *Trialeurodes vaporarium*, was detected and controlled biologically with Encarline f from Syngenta (United Kingdom), according to the manufacturer's instructions. Encraline f cards with female hymenopterous whitefly parasites, *Encarsia formosa,* were used. Hoddle *et al.,* (1998) records successful control of Whitefly in greenhouse crops when *E. Formosa* attack the Whitefly larvae at their 3rd and 4th stages. The cards were placed between the tomato plants and between 60 to 100 female wasps hatch.

3. Results

The findings in Tables 3.1. to 3.10. show there are significant differences across the spectrum of nutrients analysed in the tomato leaves and fruit.

Table 3.1. Summary of results of physiological measurements. Means of physiological variables are shown with standard errors in brackets. F ratios are derived from one-way analysis of variance (ANOVA) (with degrees of freedom indicated in brackets), p is the associated probability that the means are equal between treatments.

Treatment	Plant height Wk 10 (mm)	Canopy spread (mm)	Number of Flowers (-)	Number of Fruit (-)	Number of Flowers + Fruit (-)	SPAD (unit) Week 8	Leaf area (cm^2)
C	152 (11)	230 (6)	15 (1)	25 (2)	40 (3)	45 (2)	487 (77)
CM	175 (10)	236 (18)	19 (1)	27 (1)	46 (2)	48 (1)	441 (54)
F	157 (7)	253 (5)	18 (3)	26 (3)	45 (3)	47 (1)	581 (68)
FM	140 (7)	258 (10)	20 (3)	21 (2)	41 (2)	49 (1)	636 (76)
R	142 (6)	249 (11)	20 (4)	22 (2)	43 (6)	49 (1)	493 (60)
RM	161 (9)	268 (4)	15 (1)	26 (2)	41 (2)	47 (2)	671 (80)
$F_{5,36}$	2.12	1.97	0.75	1.64	0.83	1.5	4.26
p	0.086	0.106	0.591	0.175	0.54	0.228	0.004

3.1 Height of the plant

The overall height of each plant was measured weekly in millimetres for fifteen weeks. The stem height was also measured to give a better reflection of overall height after the branches started to bow due to their own weight and the weight of the fruit in week eleven.

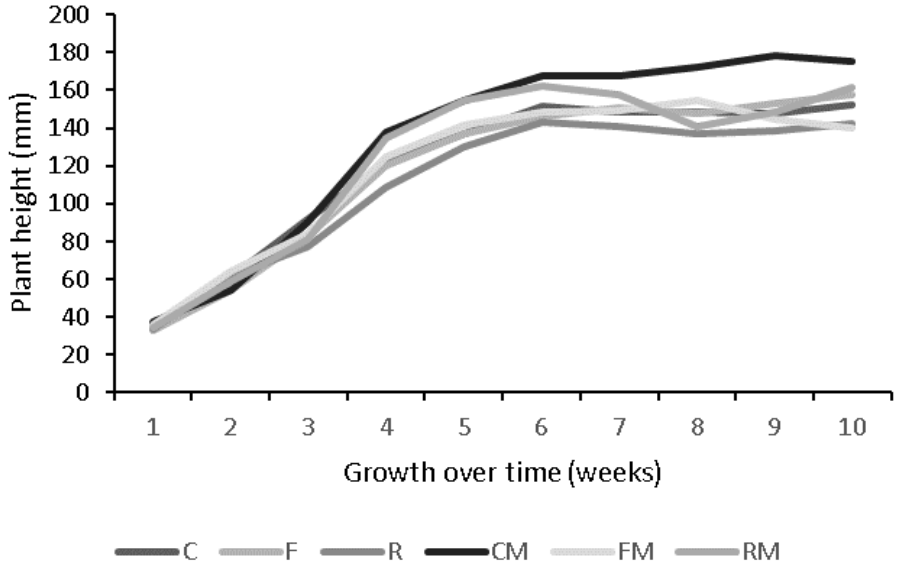


Figure 3.1. Mean tomato plant height (mm) over time (weeks), grown in 3 different growing media, with and without arbuscular mycorrhizal fungi (AMF). C = compost, F = Fytocell, R = Rockwool. M = addition of AMF.

ANOVA of the data at week 10 showed no significant difference in plant height between plots ($F_{5,36}$=2.12, p=0.086, Table 3.1).

3.2 Spread of canopy

Canopy spread allows the plant to present the widest surface area and optimise the available light for photosynthesis. Longer branches also allows more space for more leaves and fruit. There was no significant difference in canopy spread between treatments ($F_{5,36}$=1.97, p=0.106, Table 3.1.), although Rockwool treated with AMF showed the greatest spread with a crown diameter of 268.2mm.

3.3 Number of flowers

The maximum number of flowers set per species of plant is determined genetically but can reduce under physiological stress (Samach and Lotan 2007). Flowers need to be pollinated to produce fruit, so there is a

correlation with flower-set and the number of fruits that develop. Micro-Tom is self-pollinating so no external factors would limit the number of flowers fertilised. There was no significant difference between treatments in the number of flowers ($F_{5,36}$=0.75, p=0.591, Table 3.1.), although Rockwool treated with AMF showed a 33% reduction in flowers from 20 to 15.

3.4 Number of fruits

The total number of fruits per plant were counted at the end of the trial and no distinction was made between green and ripe fruit. There was no significant difference in the number of fruits per plant between plots ($F_{5,36}$=1.64, p=0.175, Table 3.1.). Plants grown in compost treated with AMF had the highest number of fruits with 27.

3.5 Number of flowers and fruit: total potential yield

At the end of the trial, all plants were destroyed for analysis. The flowers were not allowed to continue to develop into fruit and this may have skewed the final yield results. The number of flowers and fruit were added together to give a total potential yield quantity for each treatment. Plants grown in compost treated with AMF had the highest potential yield with 46 fruits and flowers combined. There was no significant difference in the sum of flowers and fruit between treatments ($F_{5,36}$=0.83, p=0.54, Table 3.1.).

3.6 SPAD (Soil-Plant Analyses Development)

SPAD was used as a simple non-destructive test to measure chlorophyll content in plant leaves and can be carried out on live plants in the field. There is a good correlation between chlorophyll content and nitrogen. Jiang *et al.,* (2017) analysed the chlorophyll content of leaves along with their SPAD

values and found a good correlation between the two as can be seen in Figure 3.2 and 3.3.

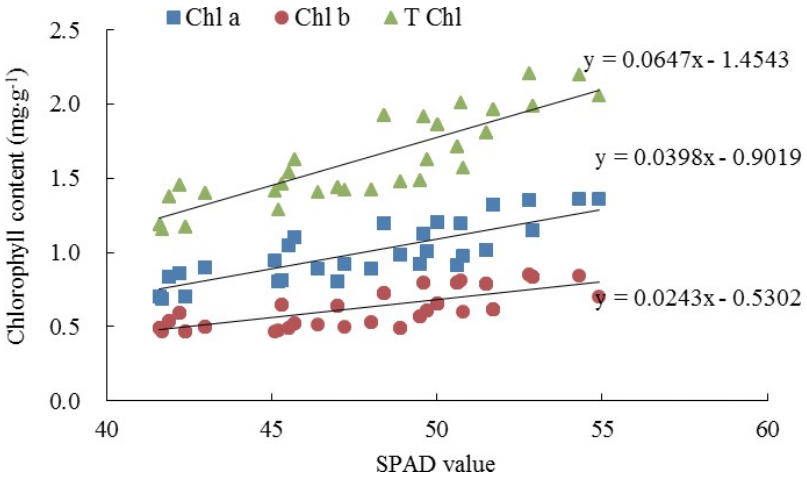


Figure 3.2. Correlation of Soil Plant Analyses Development (SPAD) value and chlorophyll content (mg g^{-1}) in leaves at vegetative growth stage. Chl a = chlorophyll a, Chl b = chlorophyll b, T Chl = Total chlorophyll (Jiang *et al.,* 2017).

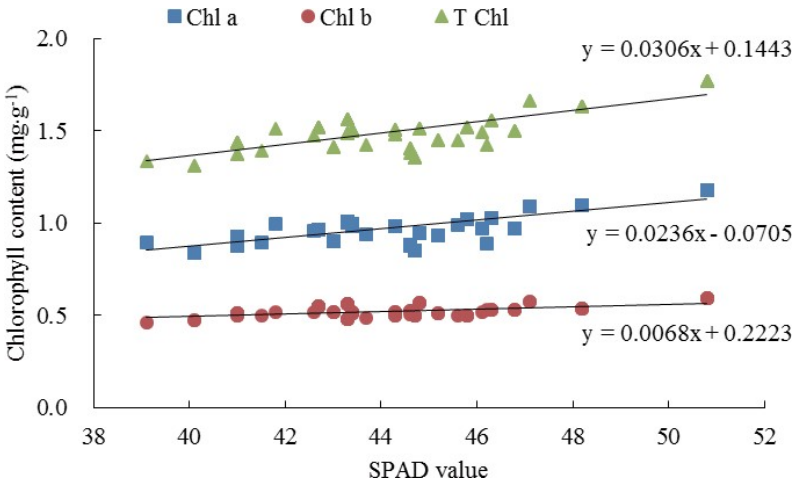


Figure 3.3. Correlation of Soil Plant Analyses Development (SPAD) value and chlorophyll content (mg g^{-1}) in leaves at reproductive growth stage. Chl a = chlorophyll a, Chl b = chlorophyll b, T Chl = Total chlorophyll (Jiang *et al.,* 2017).

Healthy plants should have the optimum N in their leaves in order to maximise photosynthesis. See Table 1.1. for the recommended nutrient levels.

SPAD readings were taken at weeks 4, 6 and 8 and ANOVA analysis performed. There was no significant difference between plots and the results of week 4 were recorded in Table 3.1. as $F_{5,36}$=1.56, p=0.228.

The tomato plants were all grown under optimum horticultural conditions and had similar SPAD readings ranging from 45 to 48.8 units. The application of AMF improved SPAD units in compost and Fytocell, but Rockwool showed a non-significant decrease in SPAD units from 48.8 to 46.5 units. This must be studied in correlation with the IC analysis for nitrate and the CN analysis to check for consistencies.

A repeated measures ANOVA was carried out for week 4,6 and 8 and with a significant increase of nitrogen in the leaves over time but not between treatments. $F_{2,78}$=17.80, p<0.001.

3.7 Leaf area measurement

The chloroplasts in the leaves are the engine of plants converting CO_2 to sugars. The bigger the leaves, the more surface area for photosynthesis. However, excess leaf biomass can come at the expense of fruit production (Sainju, 1999).

The larger leaf areas give the plants more surface area to transpire and store nutrients. However, larger leaves also shade the lower areas of the plant and reduce light penetration. The leaves nearest the fruit produce the compounds needed for fruit development. There was a significant difference in leaf surface area between treatments ($F_{5,36}$=4.26, p=0.004, Table 3.1.). Tukey HSD post-hoc test indicated that RM had significantly larger leaf area than the other treatments (mean=551; p=0.004). Rockwool treated with AMF saw the biggest increase from 493m^2 to 671cm^2 equating to 36% (Table 3.1.).

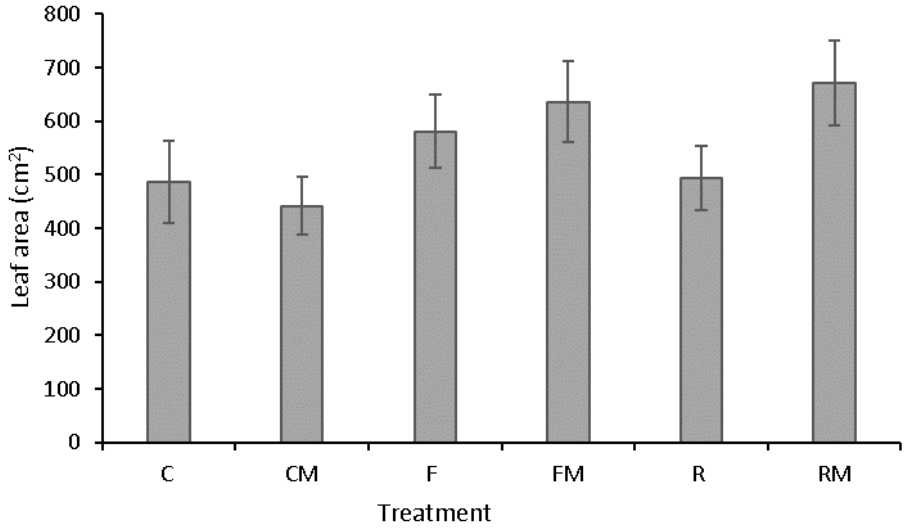


Figure 3.4. Mean ± SE of tomato plant leaf surface area (cm^2) post-harvest, from 3 different growing media, with and without arbuscular mycorrhizal fungi (AMF). C = compost, F = Fytocell, R = Rockwool. M = addition of AMF. A significant difference was found between R and RM.

3.8 Biomass of leaves and shoots

Plant development is usually increased by the colonisation of AMF in the roots. However, there is debate about the relationship between the degree of mycorrhizal colonisation and benefits to the plant in terms of enhanced growth. The extent of this effect is variable due to many factors, including P-availability and AMF genera (Hoeksema *et al*., 2010). Treseder's (2013) meta-analysis showed that, generally, increased AMF colonisation leads to an increased plant biomass and P content. In contrast, there are several reports of negative growth responses in plants to AMF inoculation (Smith *et al*., 2010).

Table 3.2. lists the results of a one-way ANOVA. Rockwool treated with AMF showed a significant increase in leaf biomass over the other treatments and control. Biomass was 4.33g (0.66) $F_{5.36}$ 2.73, p=0.034. This was an increase of 24% over compost.

Table 3.2. Summary of results of post-harvest measurements. Means of post-harvest variables are shown with standard errors in brackets. F ratios are derived from one-way ANOVA (with degrees of freedom indicated in brackets), p is the associated probability that the means are equal between treatments.

Treatment	Biomass of leaves and shoots (g)	Fruit Yield Dry Weight (g)	Fruit Yield Wet Weight (g)	BRIX (°Bx)	Lycopene (unit)
C	3.49 (0.24)	5.53 (0.4)	70.07 (4.2)	5.02 (0.15)	926 (66)
CM	3.62 (0.18)	6.17 (0.3)	78.61 (2.6)	4.83 (0.08)	1,050 (56)
F	4.29 (0.09)	6.27 (0.5)	74.45 (3.2)	5.33 (0.09)	936 (64)
FM	4.20 (0.15)	5.69 (0.3)	75.78 (2.6)	5.17 (0.16)	822 (37)
R	3.87 (0.31)	5.69 (0.6)	72.78 (6)	5.33 (0.23)	872 (60)
RM	4.33 (0.25)	6.84 (0.3)	81.53 (6)	5.20 (0.17)	714 (37)
$F_{5,36}$	2.73	1.39	0.87	1.35	3.72
p	0.034	0.251	0.514	0.264	0.008

3.9 Root mapping

Root mapping was difficult because the roots in the Rockwool substrate intertwined with the fibres, making it impossible to remove roots intact and wash off the substrate. It was decided that no post-harvest root mapping and analysis will be done in this study.

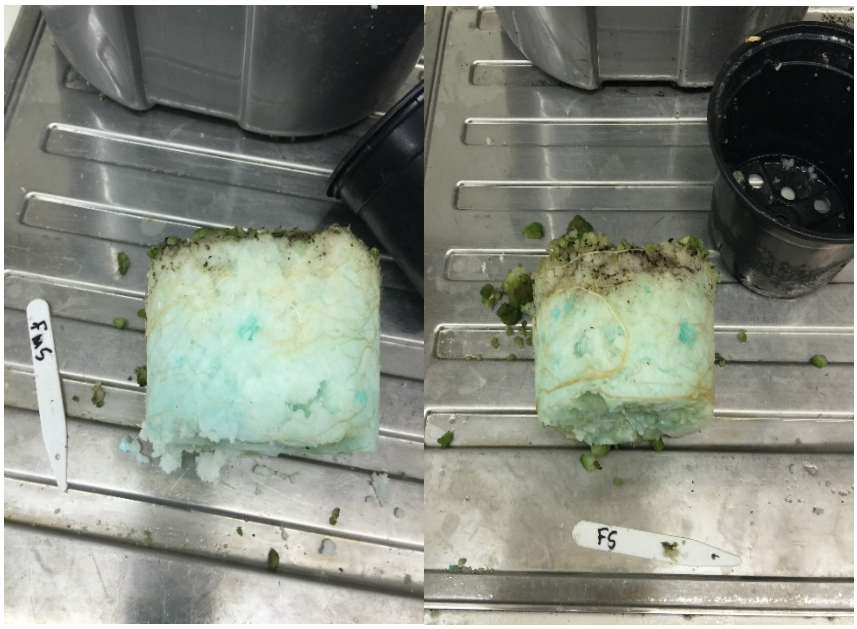
Instead, the root balls were removed from the plastic plant pots and cut in half for visual inspection and analysis. Photographs were taken, as shown in Figures 3.5., 3.6., and 3.7.

The plastic plant pots were placed directly on the bottom of the plastic trays to contain the mycorrhizal spores in the substrates treated with AMF and prevent the spores leaching onto the glasshouse floor and spreading to the untreated plots. For consistency, the untreated pots were also placed in trays. This prevented the pots from draining freely and kept the substrates saturated. Rockwool in Figure 3.7. appeared to be the most saturated and the roots grew down through the holes in the bottom of the plant pots in search of aerobic conditions. Compost in Figure 3.6. on the other hand, had an even distribution of finer fibrous roots throughout the rootzone. Fytocell, Figure 3.5. is known to maintain a near perfect water to air ratio of 60:40 and does not become over saturated. The Fytocell substrate had thicker roots compared to the compost substrate and an evenly distributed fibrous root system throughout the rootzone.

The roots in the substrates treated with AMF appeared to be stronger and thicker than those within the untreated pots. The AMF spores are not visible to the naked eye, so the roots were studied with the aid of a microscope to see if any AMF colonisation took place as described in section 3.11 below.

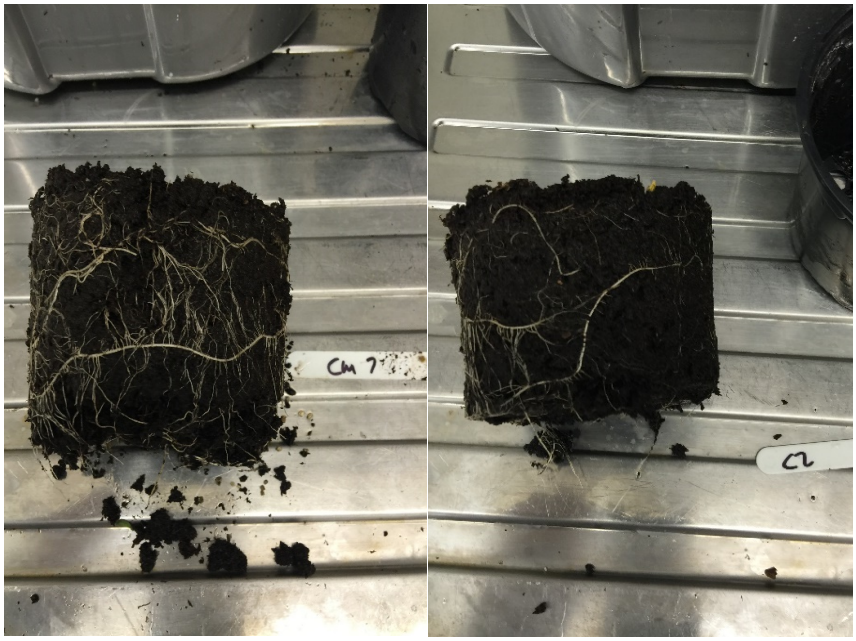
It would appear from visual inspections that the substrates displayed a notable variation between their root development. A large root system presents the maximum possible surface area for nutrient absorption but is not necessarily the most efficient root system. In this trial, the plants were fertigated with a balanced nutrient regime and the fertigation placed on the top of the root ball. Therefore, there was no reason for roots to extend beyond the immediate area of fertiliser and water application to search for nutrients, unless they did so for other reasons, for example to search for aerobic conditions.

The AMF treatment appeared to have improved root growth in the three substrates. By comparing the photographs of the untreated substrates labelled C, F, M with the treated substrates labelled CM, FM, RM a qualitative assessment could be made.



(a) (b)

Figure 3.5. Tomato roots in Fytocell. (a) FM5 Fytocell treated with AMF (b) F5 Fytocell untreated



(a) (b)

Figure 3.6. Tomato roots in Compost. (a) CM7 Compost treated with AMF (b) C2 Compost untreated

(a) (b)

Figure 3.7. Tomato roots in Rockwool. (a) RM7 Rockwool treated with AMF (b) R4 Rockwool untreated

3.10 Mycorrhizal mapping with Root Length Colonisation (RLC) Assessment

Plant roots were harvested and treated for RLC assessment as described in section 2.6.12. Slides were prepared and studied under a microscope at 200× magnification. When AMF were observed, a score was recorded against the plot label.

There was no evidence of AMF observed in the non-treated plots and some observations of AMF colonisation in the plots treated with RootGrow (Plantworks Ltd) inoculum as recorded below:

CM 1 observation

FM 1 observation.

RM 3 observation

The photographs of the observations below show limited colonisation.

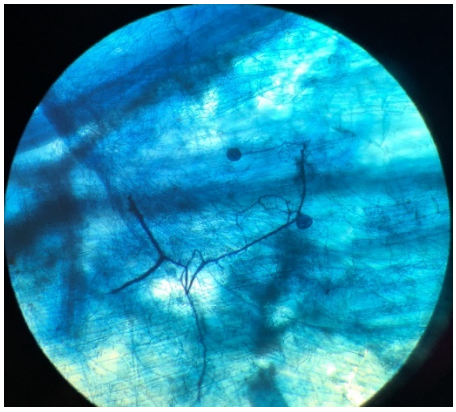


Figure 3.8. Microscopic image of mycorrhizal extraradical hyphae with a couple of spores.

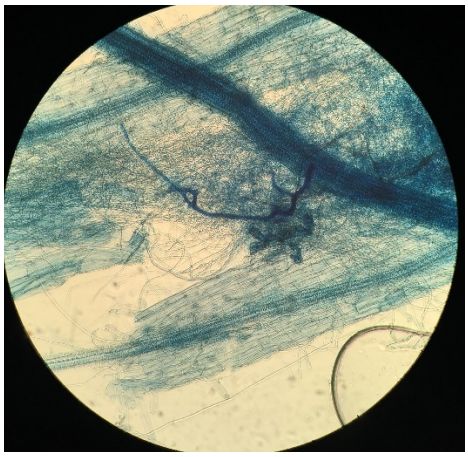


Figure 3.9. Microscopic image of mycorrhizal hyphae in root tissue.

Physiological data and plant tissue analysis did indicate some significant differences between AMF treatments and the control pots but the interactions of AMF and their effect on plant growth was difficult to interpret (see Table 3.2.). The statistical analysis tables throughout this chapter illustrate the differences.

3.11 Fruit yield, biomass and ripening

The fruit yield wet weight (g) per unit of production area in farming (ha), depends on the variety. Micro-Tom is a dwarf variety and produces small fruit. The yield of ripe tomato in each treatment plot was analysed using a

one-way ANOVA to compare the yield per treatment. There was no significant increase in yield ($F_{5,36}$=0.87, p=0.514, Table 3.2.) but all plots showed an increase when treated with AMF. AMF had the least effect on Fytocell, and the most effect on Rockwool. RM increased by 12% with the highest wet weight of 81.53 g.

Biomass was significantly greater in RM at 4.33g (0.25) ($F_{5,36}$=2.73, p=0.034, Table 3.2.) as indicated by Tukey HSD post-hoc test.
The water content is calculated by subtracting the dry weight from the wet weight and expressed as a percentage of the wet weight. All fruit in this trial had a water content of 92%.

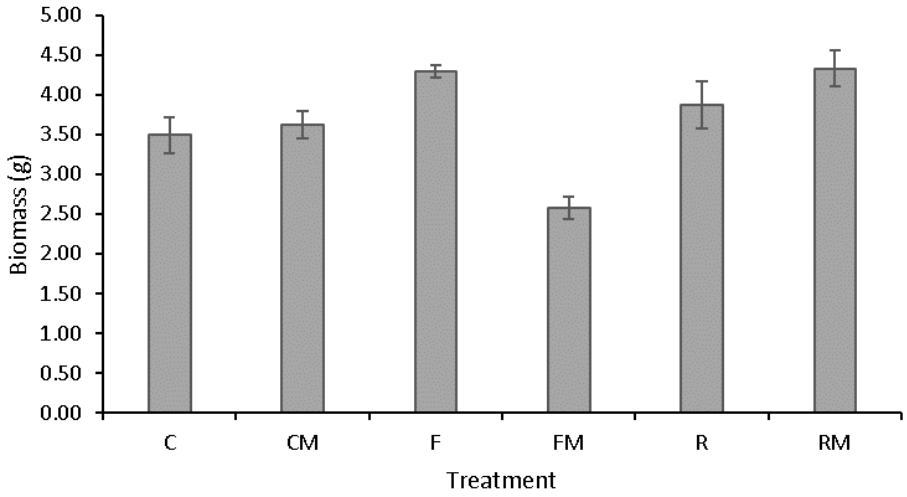


Figure 3.10. Mean ± SE of tomato fruit biomass (g) post-harvest from 3 different growing media, with and without arbuscular mycorrhizal fungi (AMF). C = compost, F = Fytocell, R = Rockwool. M = addition of AMF. A significant increase was found in Rockwool with addition of AMF.

3.12 Brix measurements of fruit

Statistical analysis showed that there was no significant difference between treatments ($F_{5,36}$=1.35, p=0.264, Table 3.2.). The type of substrate and the AMF treatment had little effect on the °Bx of the tomato fruit. The °Bx in all substrates decreased slightly when treated with AMF.

The mean ^{o}Bx of 5.15 for all treatments correlates to 5.15 g of sucrose per 100 g of tomato fruit.

3.13 Lycopene measurements using spectrometry of the fruit skins

One-way ANOVA indicated that Hunter (a^{*} x b^{*}) values varied significantly between treatments ($F_{5,36}$=3.72, p=0.008). Tukey HSD post-hoc test indicated that the Hunter (a^{*} x b^{*}) value increased significantly in CM but Fytocell and Rockwool showed a decrease after treatment (Refer to Table 3.2.).

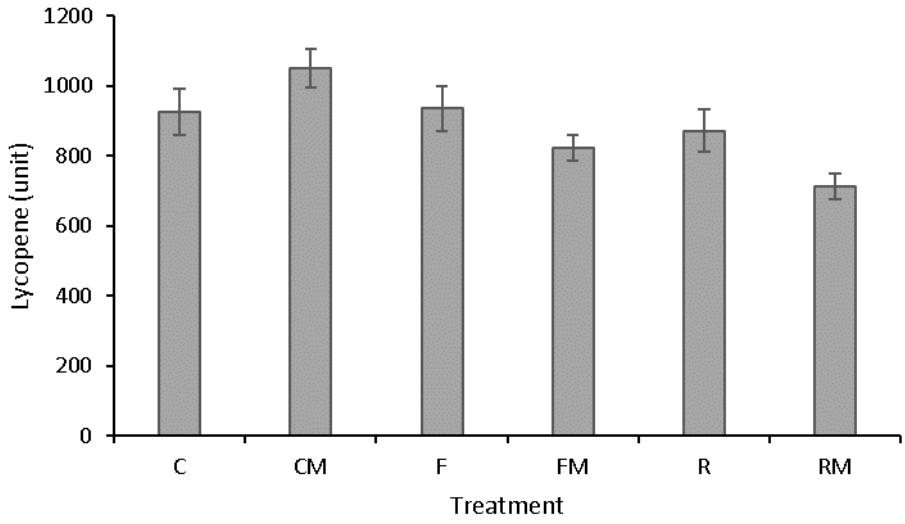


Figure 3.11. Mean ± SE of Lycopene content of ripe tomato fruit using Hunter (a^{*} x b^{*}) colour index. Tomatoes grown in 3 different growing media, with and without arbuscular mycorrhizal fungi (AMF). C = compost, F = Fytocell, R = Rockwool. M = addition of AMF. A significant increase was found between C and CM.

3.14 Plant tissue nutrient analysis

ICP-MS, IC and CN Elementar were used to analyse the concentrations of the elements in both the leaves and the ripe tomato fruit. Statistical analysis was carried out for macro nutrients listed in Table 3.3. for leaves and Table 3.4. for fruit. Statistical analyses for micro-nutrients are listed in Tables 3.5. and 3.6 for leaves and fruit respectively. Macro and micro refer to the quantity of nutrients required by plants and not the size of the elements.

Table 3.3. Summary of results of leaf macro-nutrient analysis. Means of leaf macro-nutrient variables are shown with standard errors in brackets. F ratios are derived from one-way ANOVA (with degrees of freedom indicated in brackets), p is the associated probability that the means are equal between treatments**.**

Treatment	NO_3^- (mg kg^{-1})	PO_4^{3-} (mg kg^{-1})	K (mg kg^{-1})	Ca (mg kg^{-1})	Mg (mg kg^{-1})	SO_4^{2-} (mg kg^{-1})
C	174.3 (23.9)	128.9 (8.6)	70,668 (9,967)	26,739 (3,581)	10,171 (395)	376.6 (2.1)
CM	140.7 (4.9)	130.1 (5.5)	66,165 (2,242)	26,300 (911)	9,887 (459)	484.5 (10.7)
F	158.3 (9.3)	153.1 (9.9)	75,806 (6,997)	28,229 (3,076)	3,971 (268)	407.5 (23.6)
FM	152.4 (16.2)	154.5 (3.2)	73,434 (1,902)	31,228 (1,008)	4,044 (196)	409.1 (13.5)
R	177.9 (4.8)	139.8 (3.0)	80,278 (1,626)	19,858 (1,004)	4,362 (258)	351.3 (15.2)
RM	188.6 (3.7)	150.7 (2.4)	78,825 (2,432)	23,948 (1,541)	4,430 (270)	391.1 (10.6)
$F_{5,36}$	0.81	1.51	6.38	8.15	88.58	4.37
p	0.564	0.257	<0.001	<0.001	<0.001	0.017

Table 3.4. Summary of results of fruit macro-nutrient analysis. Means of leaf macro-nutrient variables are shown with standard errors in brackets. F ratios are derived from one-way ANOVA (with degrees of freedom indicated in brackets), p is the associated probability that the means are equal between treatments.

Treatment	PO_4^{3-} (mg kg^{-1})	K (mg kg^{-1})	Ca (mg kg^{-1})	Mg (mg kg^{-1})	SO_4^{2-} (mg kg^{-1})
C	58.3 (5.6)	45,013 (1,750)	1,314 (54)	2,214 (59)	27.3 (2.4)
CM	56.2 (1.4)	46,466 (1,053)	1,422 (43)	2,274 (43)	28.7 (1.9)
F	54.8 (1.9)	49,622 (6,332)	1,939 (232)	2,022 (279)	26.0 (0.7)
FM	61.5 (4.4)	42,701 (911)	1,714 (59)	1,751 (55)	26.4 (2.5)
R	49.1 (3.6)	42,565 (1,271)	1,035 (44)	1,840 (57)	20.1 (1.6)
RM	65.1 (3.3)	45,279 (1,035)	1,032 (64)	1,960 (21)	28.9 (1.7)
$F_{5,36}$	0.98	0.86	11.70	2.80	1.28
p	0.469	0.515	<0.001	0.031	0.334

Table 3.3. shows there was no significant improvement of NO_{3-} across plots with $F_{5,36}$=0.98, p=0.564. Leaf NO_{3-} in compost and Fytocell treatments decreased compared to Rockwool when treated with AMF, while leaf nitrate in Rockwool increased by 6% when treated with AMF.

PO_4^{3-} uptake was not significantly improved in either leaves or fruit. Rockwool treated with AMF had 16% more phosphate than compost treated with AMF in the tomato leaves (Table 3.3.). Table 3.4. lists RM as having the highest concentrations of phosphate at 65.1 mg kg^{-1} in fruit and a 33% increase from 49.1 mg kg^{-1}.

Leaf K concentrations varied significantly between treatments, $F_{5,36}$=6.38, p<0.01 and concentrations also decreased in all substrates when treated with AMF. CM had the lowest concentration at 66,165 mg kg^{-1}. Tukey HSD post-

hoc test indicated R had the highest significant concentration at 80,278 mg kg $^{-1}$ (Table 3.3).

The K concentrations in the fruit were not significantly different ($F_{5,36}$=0.86, p=0.515). Rockwool showed an increase of 6% after treatment with AMF from 42,565 mg kg $^{-1}$ to 45,279 mg kg $^{-1}$. The untreated Fytocell had the highest concentrations of K at 49,622 mg kg $^{-1}$ but decreased to 45,279 mg kg $^{-1}$ when treated with AMF (Table 3.4.).

The change in the Ca concentration in the leaves was significant across treatments. Concentrations also rose when the manufactured substrates were treated with AMF. Tukey HSD post-hoc test showed FM had the highest significant concentrations of Ca at 31,228 mg kg $^{-1}$ with R the lowest concentration of 19,858 mg kg $^{-1}$. C showed a small decrease when treated with AMF (Table 3.3.).

Ca analysed in the fruit showed a significant change in the concentrations across the plots but not when substrates were treated with AMF. Tukey HSD post-hoc test indicated F had the highest significant concentrations but showed a reduction from 1939 mg kg $^{-1}$ to 1714mg kg $^{-1}$ when treated with AMF. R and RM had the lowest concentrations, showing no real difference between treated and untreated plants (Table 3.4.).

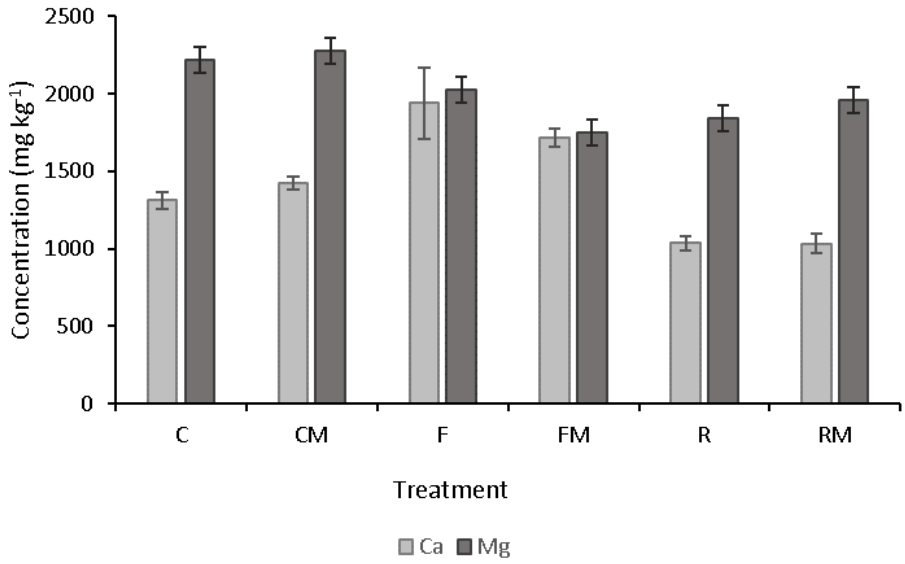


Figure 3.12. Mean ± SE concentrations (mg kg $^{-1}$) of calcium (Ca) and magnesium (Mg) in tomato fruit post-harvest, from 3 different growing media, with and without arbuscular mycorrhizal fungi (AMF). C = compost, F = Fytocell, R = Rockwool. M = addition of AMF.

AMF did not change the concentrations of Mg in the leaves significantly when added to the substrates. However, there was a significant difference between Compost and the manufactured substrates Fytocell and Rockwool, $F_{5,36}$=88.58, p<0.001. Compost had more than twice the concentrations of Mg when compared to R and F (Table 3.3.).

In addition, fruit Mg concentrations were significantly varied across the plots $F_{5,36}$=2.80, p=0.031. Tukey HSD post-hoc test indicated CM had the highest concentration at 2,274 mg kg $^{-1}$ compared to the manufactured substrates, with Fytocell showing a decrease from 2022 mg kg $^{-1}$ to 1751 mg kg $^{-1}$ when treated with AMF (Table 3.4.).

Table 3.3. shows significant variations of SO_4 between treatments with $F_{5,36}$=4.37, p=0.017. Tukey HSD post-hoc test indicated FM had the highest concentrations at 409.1 mg kg $^{-1}$ SO_4^{2-} in the leaves. Compost on the other hand had the largest increase of SO_4^{2-} concentrations from 376.6 mg kg $^{-1}$ to 484.5 mg kg $^{-1}$ in the leaves, 29% with the addition of AMF.

SO_4^{2-} in fruit did not show a significant increase between substrates or treatment with AMF. C showed a higher level (28.7 mg kg^{-1}) of SO_4^{2-} than F (26.4 mg kg^{-1}) but neither responded to AMF treatment. Rockwool had the biggest increase after treatment with AMF from 20.1 mg kg^{-1} to 28.9 mg kg^{-1}, 44% and the highest level compared to the other substrates (Table 3.4.).

Table 3.5. Summary of results of leaf micro-nutrient analysis. Means of leaf micro-nutrient variables are shown with standard errors in brackets. F ratios are derived from one-way ANOVA (with degrees of freedom indicated in brackets), p is the associated probability that the means are equal between treatments.

Treatment	B (mg kg^{-1})	Cu (mg kg^{-1})	Fe (mg kg^{-1})	Mn (mg kg^{-1})	Mo (mg kg^{-1})	Zn (mg kg^{-1})
C	56.70 (9.62)	7.64 (1.16)	162.8 (26.03)	240.5 (34.74)	3.22 (1.49)	47.3 (5.62)
CM	50.70 (3.00)	7.17 (0.51)	128.9 (5.72)	209.2 (5.72)	2.3 (0.34)	41.9 (2.22)
F	86 (9.57)	13.12 (1.57)	134.5 (11.60)	282.2 (11.60)	13.44 (1.70)	53.3 (4.60)
FM	79.8 (3.57)	14.39 (0.43)	131.2 (7.56)	223.2 (7.56)	14.48 (1.07)	59.3 (4.99)
R	90.7 (1.53)	11.59 (0.56)	138.1 (6.56)	309.9 (6.56)	12.93 (0.66)	55.7 (6.24)
RM	98.9 (3.36)	15.3 (1.01)	135 (8.10)	282.1 (8.10)	16 (0.95)	72.1 (5.72)
$F_{5,36}$	34.94	23.93	1.61	7.01	52.91	4.89
p	<0.001	<0.001	0.183	<0.001	<0.001	0.002

Table 3.6. Summary of results of fruit micro-nutrient analysis. Means of fruit micro-nutrient variables are shown with standard errors in brackets. F ratios are derived from one-way ANOVA (with degrees of freedom indicated in brackets), p is the associated probability that the means are equal between treatments.

Treatment	B (mg kg^{-1})	Cu (mg kg^{-1})	Fe (mg kg^{-1})	Mn (mg kg^{-1})	Mo (mg kg^{-1})	Zn (mg kg^{-1})
C	15.69 (0.49)	6.66 (0.20)	46.30 (4.09)	19.75 (0.93)	0.65 (0.05)	20.42 (0.53)
CM	17.09 (0.50)	6.65 (0.23)	38.00 (1.48)	18.89 (0.31)	0.64 (0.03)	19.17 (0.70)
F	18.89 (2.18)	7.79 (0.88)	47.40 (7.29)	26.08 (4.15)	2.88 (0.53)	19.79 (2.24)
FM	16.62 (0.74)	7.09 (2.68)	34.50 (4.91)	19.85 (1.92)	2.54 (0.16)	19.87 (1.36)
R	17.45 (2.04)	6.61 (0.19)	31.90 (2.00)	22.88 (0.67)	2.57 (0.11)	17.54 (0.45)
RM	16.96 (0.55)	7.87 (0.23)	37.00 (2.69)	23.60 (1.03)	2.82 (0.05)	22.3 (0.97)
$F_{5,36}$	0.65	1.93	2.21	2.01	30.43	1.66
p	0.661	0.113	0.074	0.101	<0.001	0.169

B concentrations in tomato plant leaves were significantly different over the treatments. $F_{5,36}$=34.94, p<0.001. Compost and Fytocell showed a decrease after the treatment with AMF but Tukey HSD post-hoc test indicated B in Rockwool increased significantly. The plants grown in Rockwool had the highest level of B and improved 9 % when treated with AMF from 90.7 mg kg^{-1} to 98.9 mg kg^{-1}. RM had 95% more B than CM (Table 3.5.). This is more than two and a half times higher than the recommended level in Table 1.2. but less than the toxic level of 100 mg kg^{-1} (Table 1.1.) and could account for the reduction in tomato flowers and fruit as seen in Table 3.1.

In Table 3.6, the B concentrations in fruit increased in compost when treated with AMF but decreased in Fytocell and Rockwool. $F_{5,36}$=0.65, p=0.661. There was no significant change in concentrations across treatments.

Cu in leaves showed a significant variation in uptake over the treatments ($F_{5,36}$=23.93, p<0.001). Tukey HSD post-hoc test indicated Fytocell and Rockwool had over double the levels of compost. Cu level in compost decreased when AMF was added but increased in both Fytocell and

Rockwool. Rockwool had an increase of 32% in the plants treated with AMF, from 11.59 mg kg^{-1} to 15.30 mg kg^{-1}. The target level given by Sainju *et al.* (1999) is 15 mg kg^{-1} (Table 1.2). RM was also the highest level overall (Table 3.5). The changes in concentrations of Cu in fruit were not significant. $F_{5,36}$=1.93, p=0.113. The concentrations were similar between treatments.

Table 3.5 shows Fe in leaves has no significant variation across treatments and was one of the few elements where treatment with AMF resulted in all substrates decreasing their plant concentrations of Fe. Compost saw the biggest drop from 162.8 mg kg^{-1} to 128.9 mg kg^{-1}. Fytocell and Rockwool remained at similar concentrations and

Changes in Fe levels in fruit were not significant $F_{5,36}$=2.21, p=0.074. Overall compost and Fytocell saw a decrease when treated with AMF while Rockwool increased from 31.9 mg kg^{-1} to 37.0 mg kg^{-1}. Fytocell fruit had the highest level of Fe at 47.4 mg kg^{-1} (Table 3.6.).

There were significant differences across plots in Mn concentrations in leaves and all decreased when treated with AMF. $F_{5,36}$=7.01, p<0.001. Fytocell showed the biggest decrease of 26% from 282.0 mg kg^{-1} to 223.2 mg kg^{-1}. Tukey HSD post-hoc test indicated R had the highest concentration at 309.9 mg/kg and CM the lowest at 209.2 mg kg^{-1} (Table 3.5). This is consistent with the findings made by Winsor (1973). Mn showed no significant differences across plots with $F_{5,36}$=2.01, p=0.101 in the fruits. Compost and Fytocell decreased when treated with AMF while Rockwool increased (Table 3.6).

Mo in leaves showed significant differences in concentrations across plots with concentrations in compost decreasing. Tukey HSD post-hoc test indicated Fytocell and Rockwool increasing significantly. This micro-nutrient showed the biggest variations between compost and the other two substrates. Fytocell and Rockwool had on average more than five times the Mo concentration of compost. Rockwool increased 24% when treated with AMF ($F_{5,36}$=52.91, p<0.001, Table 3.5).

Mo in the fruits had significant changes in concentrations across plots $F_{5,36}$=30.43, p<0.001. Compost and Fytocell saw decreased concentrations of Mo when treated with AMF. Rockwool increased from 2.536 mg kg^{-1} to 2.816

mg kg $^{-1}$. Tukey HSD post-hoc test indicated both the manufactured substrates significantly improved Mo concentrations by more than four times that of compost (Table 3.6.).

Referring to Table 3.5., Zn concentrations in leaves showed significant variations with $F_{5,36}$=4.89, p=0.002. Compost showed a decrease when treated with AMF but Tukey HSD post-hoc test indicated Fytocell and Rockwool showed significant increases. Rockwool responded the best overall with a 29% increase from 55.7 mg kg $^{-1}$ to 72.1 mg kg $^{-1}$. In Table 3.6, Zn concentrations in the fruit were $F_{5,36}$=1.66, p=0.169 with no significant variation. Concentrations of Zn in compost decreased when treated with AMF but increased in Fytocell and Rockwool. The biggest gain was when Rockwool was treated with AMF with a 27% increase of Zn from 17.54 mg kg $^{-1}$ to 22.30 mg kg $^{-1}$.

Table 3.7. Summary of results of leaf carbon and nitrogen analysis. Means of leaf carbon and nitrogen variables are shown with standard errors in brackets. F ratios are derived from one-way ANOVA (with degrees of freedom indicated in brackets), p is the associated probability that the means are equal between treatments**.**

Treatment	C (%)	N (%)	C:N Ratio
C	33.74 (0.30)	4.32 (0.03)	7.81
CM	34.21 (0.40)	4.03 (0.01)	8.49
F	34.02 (0.31)	4.64 (0.17)	7.33
FM	33.48 (0.40)	4.56 (0.11)	7.34
R	36.62 (0.11)	4.61 (0.04)	7.94
RM	37.16 (0.35)	4.82 (0.05)	7.71
$F_{5,12}$	10.1	4.26	
p	<0.001	0.018	

C and N are the building blocks of plants and are measured as percentages of tissue mass and are often expressed as a C:N ratio, which an important metric in phytology. Table 3.7 shows leaf C varied significantly between treatments ($F_{5,12}$=10.10, p<0.01). Variation in C appears to relate to the type of substrate used and not to inoculation with AMF. Rockwool treated with AMF showed the highest percentage of carbon in the leaves of 37.16 %.

Leaf N also varied significantly between treatments ($F_{5,12}$=4.26, p=0.018). Tukey HSD post-hoc test indicated R treated with AMF saw the highest concentration of N in leaves of 4.82%, consistent with the highest leaf carbon content. Compost treated with AMF showed a decrease in leaf N content.

Table 3.8. Summary of results of fruit carbon and nitrogen analysis. Means of fruit carbon and nitrogen variables are shown with standard errors in brackets. F ratios are derived from one-way ANOVA (with degrees of freedom indicated in brackets), p is the associated probability that the means are equal between treatments.

Treatment	C (%)	N (%)	C:N Ratio
C	44.81 (0.76)	3.12 (0.04)	14.36
CM	46.02 (0.41)	3.24 (0.11)	14.20
F	46.00 (0.22)	2.89 (0.07)	15.92
FM	45.93 (1.91)	2.97 (0.16)	15.46
R	50.36 (0.70)	3.51 (0.06)	14.35
RM	45.64 (0.87)	3.29 (0.03)	13.87
$F_{5,12}$	1.74	2.7	
p	0.2	0.073	

Fruit C % was non-significant and fairly consistent across treatments with $F_{5,12}$=1.74, p=0.2. (Table 3.8.)

Fruit N % variation was not significant $F_{5,12}$=2.70, p=0.073 with the untreated Rockwool showing the highest percentage of N at 3.51%.

Na concentrations in the leaves varied significantly with $F_{5,36}$=11.6, p<0.05 (Table 3.9.). Tukey HSD post-hoc test indicated R had the highest concentrations and increased 24% after treatment with AMF from 5,114 mg kg^{-1} to 6,337 mg kg^{-1}.

Na concentrations in fruit also varied significantly with $F_{5,36}$=5.6, p<0.05 (Table 3.10.). Tukey HSD post-hoc test indicated R had the highest concentration of Na in the fruit and increased from 1,217 mg kg^{-1} to 1,564 mg kg^{-1} when treated with AMF.

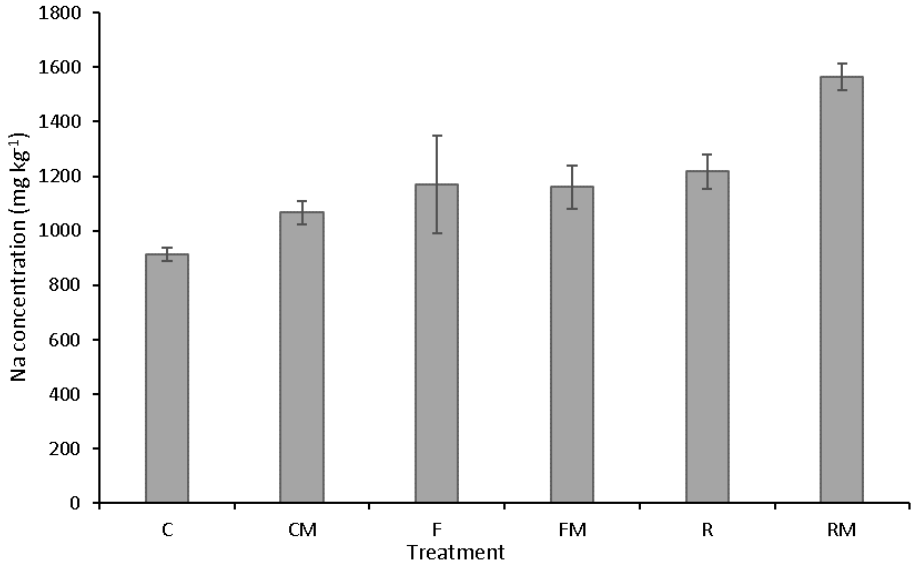


Figure 3.13. Mean ± SE of sodium (Na) concentrations (mg kg^{-1}) in tomato fruit post-harvest from3 different growing media, with and without arbuscular mycorrhizal fungi (AMF). C = compost, F = Fytocell, R = Rockwool. M = addition of AMF. A significant increase was found in Rockwool treated with AMF.

Table 3.9. Summary of results of leaves sodium and chloride analysis. Means of leaves sodium and chloride variables are shown with standard errors in brackets. F ratios are derived from one-way ANOVA (with degrees of freedom indicated in brackets), p is the associated probability that the means are equal between treatments.

Treatment	**Na (mg kg $^{-1}$)**	**Treatment**	**Cl^- (mg kg $^{-1}$)**
C	3846.41 (37.01)	**C**	70.63 (7.72)
CM	4718.142 (57.95)	**CM**	60.19 (1.78)
F	4141.95 (158.44)	**F**	99.55 (2.79)
FM	4575.35 (89.20)	**FM**	137.56 (8.02)
R	5113.50 (52.96)	**R**	87.06 (1.84)
RM	6336.86 (86.15)	**RM**	126.63 (4.77)
$F_{5,36}$	11.6	**$F_{5,17}$**	3.24
p	<0.05	**p**	0.044

Table 3.10. Summary of results of fruit sodium and chloride analysis. Means of fruit sodium and chloride variables are shown with standard errors in brackets. F ratios are derived from one-way ANOVA (with degrees of freedom indicated in brackets), p is the associated probability that the means are equal between treatments.

Treatment	Na (mg kg $^{-1}$)	Treatment	Cl^- (mg kg $^{-1}$)
C	912.63 (25.24)	**C**	119.29 (6.15)
CM	1065.99 (42.73)	**CM**	134.50 (6.55)
F	1169.83 (179.58)	**F**	138.14 (5.39)
FM	1160.67 (79.47)	**FM**	149.69 (6.15)
R	1217.36 (62.62)	**R**	121.77 (11.65)
RM	1563.99 (47.61)	**RM**	163.00 (4.01)
$F_{5,36}$	5.6	$F_{5,17}$	2.38
p	<0.05	**p**	0.101

Table 3.9. shows Cl^{-1} concentrations of leaves increased significantly with AMR inoculation in the Fytocell and Rockwool substrates as indicated by Tukey HSD post-hoc test. The biggest increase was in Fytocell while compost saw a reduction in leaf Cl^- content.

Fytocell increased from a mean of 99.6 mg kg $^{-1}$ to 137.6 mg kg $^{-1}$ and Rockwool increased Cl^- in leaves from 97.1 mg kg $^{-1}$ to 126.6 mg kg $^{-1}$ while compost decreased Cl^- in leaves from 70.6 mg kg $^{-1}$ to 60.2 mg kg $^{-1}$.

Table 3.10. shows Cl^- concentrations in the fruit across substrates did not increase significantly. Rockwool showed the greatest increase of Cl^- concentrations when treated with AMF from 121.8 mg kg $^{-1}$ to 163.0 mg kg $^{-1}$.

4. Discussion

Consumers are becoming more aware of the importance of nutrition and dieticians are encouraging production of healthy crops with a balance of the important nutrients our bodies require. Currently there is no legal requirement to analyse the nutrients in crops but perhaps in the future this information will be required.

One of the aims of the trial was to determine whether AMF improves nutrient uptake in tomato. The value to the grower is to be able to sell the highest yield per ha of farmed area with the lowest inputs. If the application of AMF to substrates can increase nutrient uptake, fertiliser input can be decreased proportionally. With the growing awareness of food with low nutritional value in the supply chain, AMF can also be used to increase the level of nutrients and essential elements in tomato.

As chemical analysis techniques improve, we are able to achieve a greater understanding of what concentrations of nutrients a plant requires to grow in the most efficient and productive way. Making the required nutrients available for the plant roots to absorb and process in the growth and photosynthetic phases will produce the maximum and most nutritious crop yield.

Chemical analyses of both leaves and fruit were carried out together, under the same processes, and the data analysed and listed in Tables 3.4. and 3.6. for fruit and in Tables 3.3. and 3.5. for leaf chemical analysis. Leaf chemical analysis can indicate if nutrients have increased in the tomato plant leaves and correlations can be studied between this and nutrient concentrations in fruit. Fruit nutrient content was the most important aspect of this research. However, similar trials should also be carried out to determine if AMF can increase nutrients in leafy vegetables where fruit are not harvested and therefore irrelevant to this type of study.

Plant tissue analysis is also used as an indicator of crop health based on nutrient status. It is combined with integrated nutritional management programs to help increase volume and quality of yield.

The findings in Tables 3.1. to 3.10. show there are significant difference across the spectrum of nutrients analysed in the tomato leaves and fruit. The variations are greater in the leaves than in the fruit, suggesting that the tomato plant is able to regulate the final utilisation of nutrients. This process is called homeostasis and creates a stable equilibrium between interdependent elements, especially as maintained by physiological processes. The vegetative parts of the plant appear to act as a reservoir for nutrients and the tomato plant can access these stores of nutrients to develop the fruit in the desired way.

Significant changes in the concentrations of Ca across the substrates were observed, but not after AMF treatment. F had the highest concentrations but showed a reduction from 1939 mg kg^{-1} to 1714 mg kg^{-1} when treated with AMF. R and RM had the lowest concentrations, showing no real difference between treated and untreated plants (Table 3.4.).

CM had a significantly higher concentration of Mg at 2,274 mg kg^{-1} compared to the manufactured substrates, with F showing a decrease from 2022 mg kg^{-1} to 1751 mg kg^{-1} when treated with AMF (Table 3.4.).

Both the synthetic substrates significantly improved Mo concentrations by more than four times that of compost (Table 3.6.). C and F saw decreased concentrations of Mo when treated with AMF. R increased from 2.536 mg kg^{-1} to 2.816 mg kg^{-1}.

Na concentrations in fruit also varied significantly and a Tukey HSD post-hoc test indicated R had the highest concentration of Na in the fruit and increased from 1,217 mg kg^{-1} to 1,564 mg kg^{-1} when treated with AMF.
Rockwool treated with AMF saw the most significant levels of nutrients in the fruit.

The fertiliser added to the compost by the manufacturer may have given compost an unfair advantage in this trial. Post planting, all pots received the same irrigation and nutrition. The physiological and chemical analysis of the plants revealed that the tomato plants grown in compost with added nutrients did not have a significant advantage and the nutrients were used efficiently.

According to Liebig's Law of Minimum, plant growth is affected by the nutrient that is least available to plants. The abundance of some nutrients would not necessarily give one substrate an unfair advantage (van der Ploeg, 1999). Van der Ploeg (1999) lists the three parts of the Law of Minimum as follows:

1. "By the deficiency or absence of one necessary constituent, all others being present, the soil is rendered barren for all those crops to the life of which that one constituent is indispensable.
2. With equal supplies of the atmospheric conditions for the growth of plants, the yields are directly proportional to the mineral nutrients supplied in the manure.
3. In a soil rich in mineral nutrients, the yield of a field cannot be increased by adding more of the same substances."

It is important to keep all the necessary macro- and micro-nutrients available to plants and to prevent "nutrient lockout". This occurs when certain nutrients become unavailable to plants due to soil chemistry. pH needs to be within the range of 6.0 to 7.5 (Karadjov, 2012).

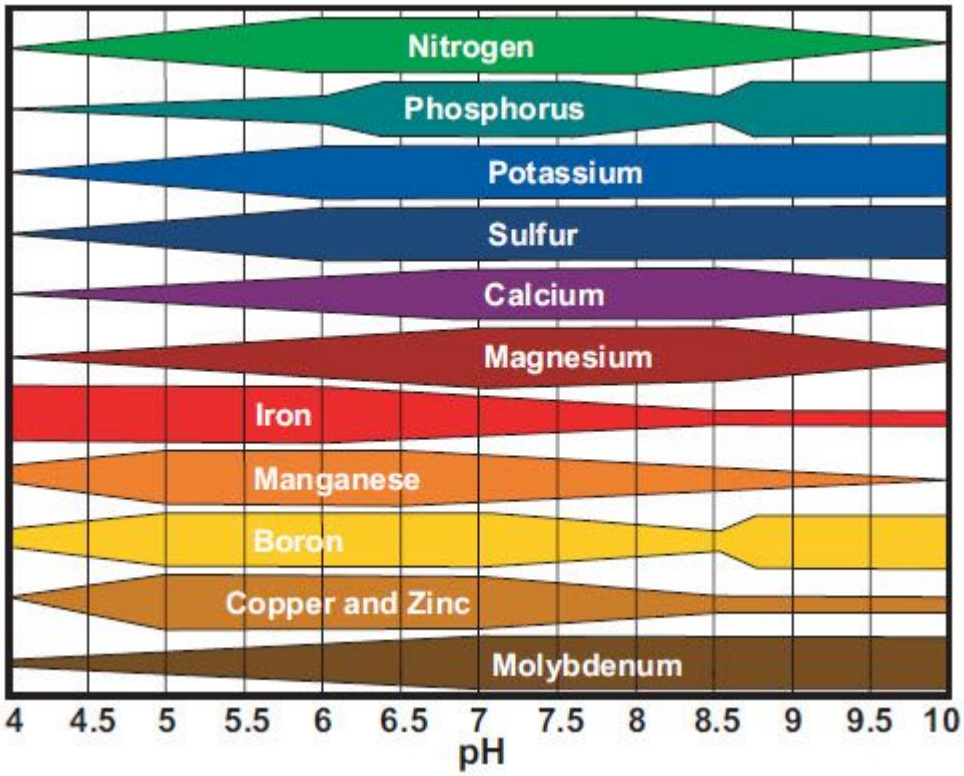


Figure 4.1. Nutrient availability versus pH ranges illustrating the critical points resulting in nutrient lockout (Karadjov, 2012).

Li *et al.* (2001) uses a reference EC of 2 dS m^{-1} in a trial to study the effect of EC and transpiration on production of greenhouse tomato with the commercial tomato production EC range of 2-5 dS m^{-1}. They note that saleable fresh yield reduces by 5.1% for every dS m^{-1} increase above 2 dS m^{-1}. This illustrates the importance of keeping the concentration of nutrients at the optimum level for plants. Whilst in this trial the pH and EC of the substrate and fertigation runoff was not measured, the applied fertigation had a pH of 7 and EC of 1.7 dS m^{-1}.

It was noted that there was no statistically significant difference in fruit yield dry weight (g) between treatments (Table 3.2.). Li *et al*. (2001) mentioned that increased substrate electrical conductivity (EC) resulted in an increased nutrient uptake and therefore dry weight in tomato. In this trial all treatments received the same nutrient feed at the same pH and EC and therefore had no effect on dry biomass. RM had the highest dry weight at 6.84 g and therefore the highest overall mineral content.

Rockwool and Fytocell are inert and therefore do not contribute to the pH or EC of the rhizosphere. However, compost is by nature acidic and the added nutrients during production could have increased the EC, negatively affecting the ability of the tomato plants to absorb nutrients. Table 4.1. shows tomato plants grown in compost treated with AMF had the highest concentration of macro-nutrients in their fruit and therefore increased EC did not negatively affect nutrient uptake.

Barker and Ready (1994) give an example of how excessive ammonium in tomato substrates reduce calcium uptake whilst not affecting potassium and magnesium concentrations. Both genotype and environment affect the level of essential elements in the fruit. Equally high concentrations of potassium uptake can suppress magnesium and calcium absorption (Adams, 1986). Ammonium concentrations were not recorded in this trial and whilst the organic matter in the compost substrate could contribute to higher concentrations of ammonium, Ca uptake was not reduced in the leaves or fruit of these plants. The higher concentrations of Na in the tomato grown in Rockwool for both treatments (Table 3.10.) could account for the lower concentrations of K and Ca, consistent with claims by Labate *et al*. (2018). K and Ca concentrations were higher in Fytocell, refer to Table 3.3. and 3.4.

Sainju *et al*. (1999) and Sweeney *et al*. (1987), observed that tomatoes are unable to recover all of the applied N in fertiliser. In this trial a low N fertiliser in the form of Hortiponic 7-6-33+MgO+SO_4+TE was applied to all plants, and there were no visual signs or SPAD reading of N deficiencies. N in all fruit (Table 3.8.) was within the ranges described by (Sainju *et al*., 1999) in Table 1.1.

The trial plant pots were placed in waterproof plastic trays to prevent the irrigation water leaching onto the floor and spreading the AMF spores around the glasshouse. These spores could potentially infect the control plants. The fertigation water was applied with a watering can and the tomato plants were drenched every second day. The runoff pooled in the bottom of the tray and the plant pots stood directly in this water. This meant that some of the substrate was waterlogged and the only path for air to enter the rootzone

was via the top of the pot. Future trials are needed to establish if the waterlogging had a negative effect on the colonisation of the AMF in the tomato plant roots. These trials should allow the plant pot to drain freely.

The roots grew between the sides of the Rockwool substrate and the plastic pot, down though the holes and into the trays. This was possibly to avoid the waterlogged centres of the substrate which can reduce the effectiveness of the AMF. It is understood that AMF do not survive in waterlogged substrates and require aerobic conditions to survive (Wang *et al.,* 2011). The relationships between AMF and plants in water-stressed environments were also the subject of a detailed literature review by Auge (2001), who found that drought only affected levels of root colonisation in about half of studies examined. In these reports the level of root colonisation increased during drought rather than decreased. The level of AMF sporulation seemed to be reduced by extreme conditions, either chronically dry or permanently water-logged soils. Many studies measuring the physiological response of shoots to soil moisture found that soils inoculated with AMF needed to be drier in order to trigger a comparable response in growth to non-colonized soils (Augé, 2001). This is consistent with the low colonisation observed in the RLC assessment in section 3.10.

Other biotic factors in the rhizosphere could interact with AMF to affect the results in this trial and further research is needed to fully understand the rhizosphere. The literature should be studied to find correlations with the results observed in this trial.

Table 4.1. Matrix with data ranked from 1 to 6, lowest to highest

Treatment Matrix						
Ranked lowest to highest	Treatment					
Metric	**C**	**CM**	**F**	**FM**	**R**	**RM**
Physiological						
Plant height (mm)	4	6	5	3	2	1
Canopy spread (mm)	1	2	4	5	3	6
Number of Flowers	1	4	3	5	6	2
Number of Fruits	3	6	4	1	2	5
Number of Flowers + Fruits	1	6	5	2	4	3
SPAD (unit)	1	4	2	5	6	3
Leaf area (cm^2)	2	1	4	5	3	6
Biomass of leaves and shoots (g)	1	2	6	4	3	5
Fruit Yield Dry Weight (g)	1	4	5	2	3	6
Fruit Yield Wet Weight (g)	1	5	3	4	2	6
BRIX (oBx)	2	1	4	3	6	4
Lycopene (unit)	4	6	5	2	3	1
Subtotal	22	47	50	41	43	48
Leaf Macro-nutrients						
Nitrate (mg kg^{-1})	4	1	3	2	5	6
Phosphate (mg kg^{-1})	1	2	5	6	3	4
Potassium (mg kg^{-1})	2	1	4	3	6	5
Calcium (mg kg^{-1})	4	3	5	6	1	2
Magnesium (mg kg^{-1})	6	5	1	2	3	4
Sulfate (mg kg^{-1})	2	6	4	5	1	3
Subtotal	19	18	22	24	19	24
Fruit Macro-nutrients						
Phosphate (mg kg^{-1})	4	3	2	5	1	6
Potassium (mg kg^{-1})	3	5	6	2	1	4
Calcium (mg kg^{-1})	3	4	6	5	2	1
Magnesium (mg kg^{-1})	5	6	4	1	2	3
Sulfate (mg kg^{-1})	4	5	2	3	1	6
Subtotal	19	23	20	16	7	20
Leaf Micro-nutrients						
Boron (mg kg^{-1})	2	1	4	3	5	6
Copper (mg kg^{-1})	2	1	4	5	3	6
Iron (mg kg^{-1})	6	1	3	2	5	4
Manganese (mg kg^{-1})	3	1	5	2	6	4
Molybdenum (mg kg^{-1})	2	1	4	5	3	6
Zinc (mg kg^{-1})	2	1	3	5	4	6
Subtotal	17	6	23	22	26	32

Table 4.1 cont: Matrix with data ranked from 1 to 6, lowest to highest

Fruit Micro-nutrients						
Boron (mg kg $^{-1}$)	1	4	6	2	5	3
Copper (mg kg $^{-1}$)	5	4	6	2	1	3
Iron (mg kg $^{-1}$)	5	4	6	2	1	3
Manganese (mg kg $^{-1}$)	2	1	6	3	4	5
Molybdenum (mg kg $^{-1}$)	2	1	6	3	4	5
Zinc (mg kg $^{-1}$)	5	2	3	4	1	6
Subtotal	20	16	33	16	16	25
Leaf						
Sodium (mg kg $^{-1}$)	1	4	2	3	5	6
Chloride (mg kg $^{-1}$)	2	1	4	6	3	5
Subtotal	3	5	6	9	8	11
Fruit						
Sodium (mg kg $^{-1}$)	1	2	4	3	5	6
Chloride (mg kg $^{-1}$)	1	3	4	5	2	6
Subtotal	2	5	8	8	7	12
Leaf						
Carbon %	2	4	3	1	5	6
Nitrogen %	2	4	3	1	5	6
C:N Ratio	6	4	5	1	2	3
Subtotal	4	8	6	2	10	12
Fruit						
Carbon %	1	5	4	3	6	2
Nitrogen %	3	4	1	2	6	5
C:N Ratio	1	5	4	3	6	2
Subtotal	4	9	5	5	12	7
Total Score	110	137	173	143	148	191

Each metric recorded was ranked from lowest to highest with one being the lowest and six being the highest. The scores were added up for each treatment and totalled for each section.

The results of this study show significant improvement in uptake for several nutrients across treatments; Tables 3.1 to 3.10, Figure 4.2 and 4.3. The summary matrix in Table 4.1 demonstrated that Rockwool treated with AMF has the highest overall score.

The fruit from tomato grown in Fytocell without the addition of AMF had the highest overall concentrations of nutrients. The nutrient concentrations are ranked highest to lowest as follows, with total fruit nutrient score in brackets:

1. Fytocell (53)
2. Rockwool with AMF (45)
3. Compost with AMF/ Compost (39)
4. Fytocell with AMF (32)
5. Rockwool (23)

Rockwool treated with AMF had the highest yield of 81.53 g, 16 % higher than compost.

There was no correlation between changes in nutrient levels in the leaves and fruit for the different treatments.

Traditionally, the macro-nutrient which is mostly associated with AMF and plant symbiosis is phosphate. The findings did not show a significant increase in the uptake of phosphate in either the leaves or the fruit when substrates were treated with AMF. The reason for this could be that phosphate was abundant in the rootzone and was freely available for the uptake by plant.

Tomato fruit are marketed and sold to the consumer based on appearances. The perception is that colour is an indicator of fruit quality and according to a survey carried out by Oltman *et al.* (2014), the traditional red colour was the most familiar and preferred. This trial measured the colour of the tomato fruit skin to determine the lycopene content. The colour red has a strong correlation with lycopene content and the human eye has a genetic bias to select food with the highest nutritional value. Tomato with the most intense red colour will therefore have the highest lycopene content. Unfortunately, lycopene is not the only important nutrient in tomato and consumers can be deceived with fruit grown to look red but low in vital elements and flavour.

In this trial tomato grown in compost and treated with AMF had significantly higher levels of lycopene at 1,050 units in their fruit while the plants grown in Rockwool treated with AMF produced the highest fruit yield with the lowest concentration of lycopene at 714 units. Refer to Table 3.2 and Figure 3.11.

There is a good correlation between soluble plant sugars and the refraction index of sampled fluid (Bumgarner and Kleinhenz, 2012). Brix can also be used as an indirect indication of overall crop health. The soluble solid content can be proportional to the ripeness of the fruit and helps growers determine the best time to harvest a crop (Bumgarner and Kleinhenz, 2012). No significant differences in Brix were detected.

5. Conclusion

This trial has shown that is it possible to influence the nutrient content of biomass using horticultural practices like substrates and inoculation with AMF. AMF inoculated tomato plants grown in Rockwool had significantly greater levels of Mo and Na than non-AMF plants. The fruit from tomato grown in Fytocell without the addition of AMF had the highest overall concentrations of nutrients and the highest levels of Ca. AMF inoculated tomato plants grown in compost had significantly greater levels of Lycopene.

Substrates with very nutritious rhizospheres do not appear to maximise the symbiotic effect of AMF. Future trials should be carried out with nutrient deficient gradients in the substrates to study this further.

Intensive horticulture requires the greatest crop yield for the lowest input costs to maximise profits. There are added pressures to achieve this in the most sustainable way possible and which is environmentally sensitive. This can only be achieved by continued research and updating horticultural practices to the latest proven technologies.

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Appendices

- Amistar MSDS
- Hortifeeds data sheet for standard soluble fertiliser
- Levingtons Compost data sheet
- Grodan Rockwool data sheet
- Fytocell data sheet
- Local weather history Sutton Bonington

AMISTAR

Version 15- This version replaces all previous versions.
Revision Date 31.07.2013

SECTION 1: IDENTIFICATION OF THE SUBSTANCE/MIXTURE AND OF THE COMPANY/UNDERTAKING

1.1 Product identifier

Product name : **AMISTAR**

Design code : A12705B

1.2 Relevant identified uses of the substance or mixture and uses advised against

Use : Fungicide

1.3 Details of the supplier of the safety data sheet

Company Syngenta UK Limited
CPC4, Capital Park
Fulbourn, Cambridge
CB21 5XE

Telephone : (01223) 883400

Telefax : (01223) 882195

Website : www.syngenta.co.uk

1.4 Emergency telephone number

: +44 (0) 1484 538444

SECTION 2: HAZARDS IDENTIFICATION

2.1 Classification of the substance or mixture

Classification according to Regulation (EU) 1272/2008

Acute aquatic toxicity	Category 1	H400
Chronic aquatic toxicity	Category 1	H410

For the full text of the H-Statements mentioned in this Section, see Section 16.

HortiPonic Standard Nutrient Analysis

7-6-33+6MgO+6SO_4+TE		Nutrient applied at fertigation strength below in mg/litre				
Nutrients	**% by weight**	**0.25g/L**	**0.5g/L**	**0.75g/L**	**1.0g/L**	**1.5g/L**
Nitrate - NO_3	7.0	17	35	52	70	105
Ammonium - NH_4	0	0	0	0	0	0
Urea – $(NH_2)_2CO$	0	0	0	0	0	0
Phosphate -P_2O_5	6	15	30	45	60	90
Potassium - K_2O	33.6	84	168	252	336	504
Magnesium - MgO	6.0	15	30	45	60	90
Calcium - CaO	0	0	0	0	0	0
Iron – Fe EDTA	0.1533	0.39	0.77	1.15	1.53	2.3
Manganese - Mn	0.100	0.25	0.50	0.75	1.0	1.5
Boron - B	0.030	0.08	0.15	0.23	0.3	0.45
Zinc - Zn	0.027	0.07	0.14	0.20	0.27	0.40
Copper - Cu	0.020	0.05	0.1	0.15	0.2	0.30
Molybdenum - Mo	0.012	0.03	0.06	0.09	0.12	0.18
E.C. mS/cm, local water E.C. should be added		0.30	0.59	0.88	1.17	1.76

Hortifeeds Park Farm, Kettlethorpe, Lincoln LN1 2LD.
Tel: **01522 704404** Fax: **01522 704748** Email: **sales@hortifeeds.co.uk**
www.hortifeeds.co.uk

Safety Data Sheet

Revision Date: 19-Jul-2016 **Version:** 1

Section 1: IDENTIFICATION OF THE SUBSTANCE/MIXTURE AND OF THE COMPANY/UNDERTAKING

1.1. Product identifier

Product Name: Levington Professional Growing Media
Product Code 019275A
Synonyms: Humax Multi-Purpose Compost
Humax Potting, Basket & Tub Compost No.2
Humax Original Compost
Humax Decorative Bark
Humax Grow Bag
Humax Ericaceous Compost
Humax John Innes Seed No.1
Humax John Innes Potting no.2
Humax John Innes Potting No.3

1.2. Relevant identified uses of the substance or mixture and uses advised against

Recommended Use: Growing media.
Uses Advised Against: None.

1.3. Details of the supplier of the safety data sheet

Manufacturer
Everris International BV
Nijverheidsweg 1-5; 6422 PD Heerlen (NL); Tel: +31 (0) 45-5609100; Fax: +31 (0) 45-5609190

For further information, please contact
INFO-MSDS@EVERRIS.COM

1.4. Emergency telephone number
IN CASE OF AN EMERGENCY CALL: +44 1235 239 670 (24h)

Section 2: HAZARDS IDENTIFICATION

2.1. Classification of the substance or mixture
Mixture

Regulation (EC) No 1272/2008

This mixture is classified as not hazardous according to regulation (EC) 1272/2008 [EU-GHS]

2.2. Label elements
This mixture is classified as not hazardous according to regulation (EC) 1272/2008 [EU-GHS]
Signal Word:
None

EUH210 - Safety data sheet available on request

Section 3: COMPOSITION/INFORMATION ON INGREDIENTS

3.1 Substances

To present knowledge of the supplier, this product does not contain any hazardous ingredients in accordance to EU regulations or National regulations

Grodan Vital

Product data sheet

The Grodan Vital stone wool slab for vegetable crops is based on NG2.0 technology. The improved slab structure and physical properties guarantee rapid resaturation within a wide steering range. Water and nutrients are distributed homogeneously throughout the slab reducing mutual differences in substrate values between the slabs to zero. Even with a basic irrigation strategy, growers can easily steer the crops to encourage uniform development. Grodan Vital combines simplicity with uniform crop development, reliable technical results, as well as being ideal for Precision Growing.

Grodan Vital

Grodan Vital is highly versatile and perfect for growing tomatoes, sweet peppers, cucumbers and eggplants.

55-75%

Control range

Product characteristics
Tomatoes benefit from the even water distribution and good refreshment properties, while sweet peppers show rapid initial growth and early fruit set.

Block-slab combination
Grodan Vital, together with the Plantop propagation block, ensures a stable plant balance for the entire growing season.

NG2.0 Technology
NG2.0 is the successor of the Next Generation Technology and adds new benefits to the existing advantages. Water distribution is even more uniform and the crop utilises the total substrate volume better. Continual new growth of roots in both the block and the slab results in a healthier and more vigorous crop throughout the whole growing season. These benefits translate into higher yields, improved fruit quality and reduce the sensitivity of the crop to diseases. The technology is available in plugs, blocks and slabs.

Product functionality	
WC control range (practical conditions)	55-75%
Resaturation after 1 day from 30%	68%
Resaturation after 1 day with a weak at 30%	62%
WC uniformity within one slab	★★☆☆☆
Irrigation efficiency	★★☆☆☆

Water distribution over the height

Product specifications

- Single-layer slab
- Vertical fiber structure
- Inert, hydrophilic fibers

Key benefits
- Generative crop response
- Fast and stable EC correction
- Rapid, weather-dependent crop response

Grodan
Industrieweg 15
6065 JG Roermond, NL
Tel: +31 (0)475 35 35 35
www.grodan.nl

The substrates from Grodan are the only stone wool growing media awarded an EU Ecolabel

Material Safety Data Sheet

Date: 06-02-2012
Revision: nr.II
Replaces all previous publications

Product name: FYTOCELL® SYNTHETIC HARDFOAM "dry foam" or older than one year

1. Identification of the Producer and the Marketing Company
   a. Production Plant: Resins Agro BV
      Nijverheidsweg 17a
      6651 KS Druten, the Netherlands
   b. Marketing Company: Resins Agro BV
      Ambachtsweg 6
      6657 CK Boven Leeuwen
      The Netherlands
      T:+31(0)487593778 // F:+31(0)487 594836
   c. Distributor:

2. Composition.
   a. Chemical Name: Urea-Formaldehyde Hardfoam
   b. Synonyms: Aminoplast Hardfoam
   c. Chemical Family: Aminoplasts - Duroplasts

3. Hazards Information
   R: not applicable
   S: 51

4. First Aid Measures.
   No particular first aid measures are needed

5. Fire Fighting Measures.
   a. Flammability: The hardfoam is non flammable, does nQt ignite nor burn
   b. Suitable extinguishing Media: not applicable
   c. Unsuitable Extinguishing Media not applicable
   d. Special Hazards: not applicable
   e. Protective Equipment: see 8

6. Accidental Release Measures.
   a. Personal Precautions: Keep away from non involved people
   b. Environmental precautions No particular precautions needed
      The hard foam is water-based and biodegradable
   c. Methods for cleaning: Brush up / dig up etc

7. Handling and Storage.
   a. Handling: Operators must be trained to work with foams. Operators must avoid breathing fine particles of product (dust).
   b. Storage: Keep storage container/area ventilated. Protect from direct sun light. Store in ventilated area.

8. Exposure Protection and Personal Protection.
   a. Exposure Protection: Always work in well ventilated area
   b. Personal Protection: Wear a simple mouth cap for breath protection

SUTTON BONINGTON WEATHER BY MONTH // WEATHER AVERAGES

	January	February	March	April	May	June	July	August	September	October	November	December
Avg. Temperature (°C)	3	3.8	6.3	8.8	12.2	15.3	17.1	16.6	14	10.3	6.3	4.2
Min. Temperature (°C)	0.3	0.9	2.8	4.8	7.8	10.8	12.6	12.1	9.8	6.7	3.3	1.5
Max. Temperature (°C)	5.8	6.7	9.9	12.8	16.6	19.9	21.7	21.1	18.2	14	9.3	7
Avg. Temperature (°F)	37.4	38.8	43.3	47.8	54.0	59.5	62.8	61.9	57.2	50.5	43.3	39.6
Min. Temperature (°F)	32.5	33.6	37.0	40.6	46.0	51.4	54.7	53.8	49.6	44.1	37.9	34.7
Max. Temperature (°F)	42.4	44.1	49.8	55.0	61.9	67.8	71.1	70.0	64.8	57.2	48.7	44.6
Precipitation / Rainfall (mm)	55	44	47	47	52	54	53	59	55	51	56	58

The difference in precipitation between the driest month and the wettest month is 15 mm. The variation in annual temperature is around 14.1 °C.

https://en.climate-data.org/europe/united-kingdom/england/sutton-bonington-187399/