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Effect of fertiliser type and mycorrhizal inoculation on growth and development of sunflower (*Helianthus annuus* L.)

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ABSTRACT

Biological farming practices using bio-inoculants and renewable organic supplements are being increasingly adopted by primary producers although little is known about their benefits compared to conventional fertiliser practices. Two trials were conducted on sunflower (*Helianthus annuus* L., 'Dwarf Sunsation') to compare the influence and interaction of arbuscular mycorrhizal fungi (AMF) inoculation with organic and conventional synthetic fertilisers on plant growth and development. Commercially produced AMF was applied as a spore application with liquid organic fertiliser (Quadshot®) applied at 0 and 20 L ha^{-1} in Trial 1; and 0, 20 L ha^{-1} and 40 L ha^{-1} in Trial 2, or liquid synthetic (inorganic) fertiliser (SF) applied at 0 or 100% concentration (Hoagland's solution regular strength with low P). Results showed limited interaction between AMF and fertiliser type. Sunflower plants inoculated with AMF and synthetic fertiliser had greater plant height and stem diameter in Trial 1 and leaf chlorophyll content at various assessment times in both trials. The presence of mycorrhizal hyphae and arbuscules increased in sunflower plants grown with AMF inoculation and organic fertiliser. There was a strong treatment influence of AMF inoculation on plant height in Trial 2, and number of nodes, flower head diameter, AMF colonisation and AMF structures in both trials. In addition, SF increased the leaf chlorophyll content, number of nodes and flower head diameter in both trials, and flower number in Trial 2. The organic fertiliser had negligible influence on sunflower productivity but improved leaf nutrient status. Standard concentration of SF improved sunflower productivity, and slightly improved leaf nutrient status compared to organic fertiliser. This study has demonstrated that while there were beneficial effects of AMF on plant growth, the use of organic fertiliser at the rates applied in this study did not benefit growth in the short term.

1. Introduction

Most modern farming systems rely on the addition of inorganic fertiliser inputs to maintain or increase crop productivity, yet these fertilisers are considered undesirable in alternative practices such as biological farming and prohibited in certified organic production (Hole et al., 2005; Kirchmann and Bergström, 2001; Norton et al., 2009). As inorganic synthetic fertilisers are a finite resource (Cribb, 2010) and there is growing demand by consumers for sustainably produced crops, research into biological farming approaches to produce high yielding, high quality and nutrient rich products is necessary. However, there is limited research investigating yield and productivity benefits on the growth of annual herbaceous crops using biological farming approaches such as bio-inoculants and renewable organic fertilisers compared to inorganic fertilisers (Treadwell et al., 2007).

The use of bio-inoculants such as arbuscular mycorrhizal fungi (AMF) is of growing interest to primary producers implementing a biological approach to crop production (Barrow, 2012; Wu et al., 2005). Arbuscular mycorrhizal fungi form beneficial associations with more than 80% of recorded species of land plants (Schübler et al., 2001; Wang and Qiu, 2006). Hyphae of AMF extend a considerable distance beyond the host plant roots, acting as carriers of important nutrients especially in weathered and nutrient depleted soil (Brundrett, 1996; Müller et al., 2012).

Multiple studies have demonstrated that AMF promote the absorption of phosphorous (P), nitrogen (N), iron (Fe), zinc (Zn) and copper (Cu) in switchgrass (*Panicum virgatum*), maize (*Zea mays*), cucumber (*Cucumis sativus*) and many other host plants (Clark and Zeto, 2000; Lee and George, 2005; Perner et al., 2006). In modified agricultural systems, research has shown that high levels of inorganic fertilisers (especially N and P) can reduce AMF colonisation of plant roots that would naturally form mycorrhizal associations when lower available nutrients are present (Adesemoye et al., 2008; Egerton-Warburton and Allen, 2000; Egerton-Warburton et al., 2001). Consequently, the inability of plants to form mycorrhizal associations with AMF can lead to a decrease in plant canopy biomass and productivity (Koide, 1985). In

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organic or biodynamic production, AMF colonisation is encouraged through reduced inorganic fertiliser and restricted herbicide and fungicide use. There may be additional benefits to productivity from the use of organic fertilisers and a positive interaction with AMF; however there has been limited research investigating this interaction.

Root colonisation by AMF has a major role in enhancing plant growth through improving plant absorption of non-mobile nutrients, especially P and Zn (Fernandez et al., 2009) and sunflower has been a popular model study species. Chandrashekara et al. (1995) found that AMF inoculation significantly increased spore abundance, percentage of root colonisation, total plant dry biomass, flowering time and maturity, and P content in sunflower tissue at later stages of the experiment. Studies by Koide (1985) showed that low P led to increased AMF colonisation and therefore greater leaf area compared to plants with no colonisation. In high soil P there was less AMF colonisation. While the same outcome could be achieved with inorganic fertilisers, from a sustainability point of view other options are preferable to explore. The same effect was also noticed with high concentration of soil N.

Investigations into the use of organic products based on algae, seaweed residues and humates as renewable organic fertilisers are also increasing (Thirumaran et al., 2009). Kumar and Sahoo (2011) demonstrated that products containing seaweed extracts can improve the physical, chemical and biological characteristics of the soil. Khan et al. (2009) showed that seaweed extracts improve the ability of the soil to retain water, enabling increased nutrient exchange between plant roots and the rhizosphere, which also enhanced the activity of beneficial soil microbes. Organic materials can enhance plant growth indirectly by stimulating the activity of other life forms in the soil, such as bacteria and fungi.

The aim of this study was to investigate the effect of inoculation with mycorrhizal fungi in combination with either a commercial liquid organic fertiliser, Quadshot® (QS) at recommended label rates, or a liquid inorganic (synthetic) fertiliser (SF) on mycorrhizal colonisation and. productivity of an annual crop such as sunflower.

2. Materials and methods

2.1. Experimental design

Two pot trials were conducted under controlled glasshouse conditions at the University of Tasmania in Hobart, Tasmania. Trial 1 commenced in December 2013 and Trial 2 in September 2014, both concluded after 10 weeks. Seeds of sunflower (*Helianthus annuus* L., 'Dwarf Sunsation') were germinated in vermiculite. After two weeks, germinated seedlings were transferred to 160 mm diameter pots (depth 170 mm) containing 2.3 L of a basic potting mix (composted pine bark 80% by volume, coarse sand 20%, lime 3 kg m^{-3} and dolomite 3 kg m^{-3}). The pots were placed on glasshouse benches under natural daylight conditions and temperature of 20 ± 5 °C and watered daily. While light levels were not measured during the two trials, Trial 1 was conducted over the summer and Trial 2 commenced at the beginning of spring, and hence day length was shorter and light intensity lower.

For both trials, treatments were a factorial arrangement of AMF (plus/minus) and fertiliser type (organic/synthetic) (Table 1), established as a randomised complete block design with five replicates per treatment. As Trial 2 was conducted at a different time of year to Trial 1, the same treatments used in Trial 1 were included to determine whether seasonal differences in response would be detected, along with a combination of the two fertiliser types at normal recommended rates to determine whether adding both organic and synthetic fertilisers could further improve plant productivity.

Mycorrhizal fungi were applied using the commercial spore preparation MYCORMAX™ (JH Biotech Australasia Pty Ltd). The MYCORMAX™ preparation, in a clay-based carrier, contained: *Glomus intraradices* (*Rhizophagous irregularis*) 46 propagules cm^{-3} and *Glomus mosseae* 19 propagules cm^{-3}, and ectomycorrhizal fungi: *Laccaria bicolor* 500 propagules cm^{-3}, *Pisolithus tinctorius* 15,300 propagules cm^{-3}, *Scleroderma cepa* 1,760 propagules cm^{-3}, *Scleroderma geastrum* 1,760 propagules cm^{-3} and *Scleroderma citrinum* 1,760 propagules cm^{-3}. According to Brundrett (2009), the ectomycorrhizal fungi *Pisolithus tinctorius*, *Scleroderma cepa*, *Scleroderma geastrum, Scleroderma citrinum* and *Laccaria biocolor* would not have colonised sunflower. The Asteraceae, which includes sunflower, are dominated by AM and ericoid mycorrhizal partnerships.

Table 1
Treatment applications. AMF = arbuscular mycorrhizal fungi; QS = Quadshot® organic fertiliser; SF = synthetic fertiliser (Hoagland's solution).

	Trial 1		Trial 2	
Treatments	Fertiliser rates	AMF	Fertiliser rates	AMF
Control	0	–	0	–
AMF Only	0	+	0	+
QS	20 L ha^{-1}	–	20 L ha^{-1}	–
	20 L ha^{-1}	+	20 L ha^{-1}	+
			40 L ha^{-1}	–
			40 L ha^{-1}	+
SF	100%	–	100%	–
	100%	+	100%	+
QS + SF			20 L ha^{-1} + 100% SF	–
			20 L ha^{-1} + 100% SF	+
			40 L ha^{-1} + 100% SF	–
			40 L ha^{-1} + 100% SF	+

QuadShot® (SLTEC) was applied as the organic fertiliser (QS) and Hoagland's solution – low phosphorous (Foo et al., 2013; Hoagland and Arnon, 1950) applied as the inorganic fertiliser (SF). As the label recommendation for fertigation with Quadshot is 20–60 L ha^{-1}, it was applied to pots at either 20 L ha^{-1} (4 ml per pot, QS-20) or 40 L ha^{-1} (QS-40). The components of the fertiliser solutions are detailed in Table 2.

For the pots receiving AMF treatments, 1.15 g Mycormax was mixed into the surface soil of each pot (equivalent to 500 g m^{-3}) prior to seedling transplant. Fertiliser treatments were commenced a week after transplanting. Fertiliser treatments were applied weekly as a soil drench in 400 ml of water in each pot; the control treatments received 400 ml water.

2.2. Assessments

For both trials, plant height and number of nodes were recorded weekly. Flowering dates were recorded daily from the beginning of flowering until the last plant reached full bloom. The diameter of the primary flower head was measured and the number of axillary flowers counted. At the conclusion of each trial, stem diameter was measured 2 cm above soil level. For both trials, a relative measure of leaf chlorophyll content was estimated weekly on four fully expanded mature leaves per plant from five weeks post transplanting using a SPAD-502 m (Konica Minolta, Osaka, Japan).

At harvest, stems were cut at the soil surface and the fresh weight of stem, leaves and flowers recorded. Below-ground structures were not included as they were used for mycorrhizal assessments. After weighing, plant material was placed in individual paper bags, oven dried at 40 °C for three days and dry weight recorded. Water content and percentage dry matter content (DMC) were calculated using the following formulae:

$$\text{Water content} = \text{Freshweight} - \text{Dryweight}$$

$$\%\ \text{DMC} = \frac{\text{dry weight}}{\text{fresh weight}}\ x\ 100$$

Leaf nutrient status of plants in Trial 2 was analysed by a commercial laboratory (CSBP Soil & Plant Analysis Laboratory, Western Australia).