The role of mycorrhizas in plant nutrition: field and mutant based approaches.

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Abstract
The majority of plants, including most crops, form arbuscular mycorrhizas (AM). These associations play an important role in the growth and nutrition of plants. Here we present results from our ongoing research on the role of AM in sustainable production systems. Firstly, we present results of a survey of the formation of AM by field grown tomato plants from across the south-eastern Australian processing tomato industry. This survey revealed low levels of colonisation, which could be explained by various farm management and edaphic factors. Secondly, to explore the role of AM in these farming systems, we grew a mycorrhiza defective tomato mutant and its mycorrhizal wild type progenitor in in-tact cores containing tomato farm soils. This novel genotypic approach to controlling for AM colonization allows us to study the formation and functioning of AM in field soils without the need to fumigate soils to establish non-mycorrhizal controls. Using this approach we found an important role for AM in the nutrition of tomato plants. Given the role of AM in plant nutrition, and the low levels of colonization in the survey, our ongoing research aims to identify better ways to manage AM in the field.

Key Words
Mycorrhizas, tomato, plant mutant, plant nutrition.

Introduction
Arbuscular mycorrhizas (AM) are associations formed between the roots of most terrestrial plant species and a group of specialized soil fungi (Smith and Read 2008). The formation of AM has a significant impact upon plant growth and nutrition, with bi-directional exchange of inorganic nutrients (P, Zn and others) and carbon between the plants and fungi (Cavagnaro 2008; Marschner and Dell 1994). It has been demonstrated that the plants can receive up to 100% of their P via the mycorrhizal pathway (Smith *et al.* 2004), and 4-20% of a plants C can be transferred to the fungi (Cavagnaro *et al.* 2008; Jakobsen and Rosendahl 1990). This transfer of resources between the plants and fungi have profound effects on plant growth, nutrition and ecology, and have been the focus of considerable interest (Smith and Read 2008). However, if we are to capitalize on the potential benefits to agriculture of forming AM, it is essential that the formation and functioning of these associations be studied in a field setting. Here we present results of field and glasshouse based studies of AM in tomato framing systems.

AM have an important role to play in increasing the sustainability of agricultural systems (Cardoso and Kuyper 2006). However, few studies have directly assessed the role of AM under field conditions. This can in part be attributed to difficulties associated with the establishment of non-mycorhrizal controls in field soils. For example, fumigation of soils can eliminate other members of the soil biota (many of which are involved in nutrient cycling) as well as affect crop growth and nutrition through other mechanisms (Cavagnaro *et al.* 2006; Ryan and Angus 2003). We have overcome this issue using a novel genotypic approach to establishing non-mycorrhizal controls (Cavagnaro and Jackson 2007; Cavagnaro *et al.* 2007; Cavagnaro *et al.* 2006; Cavagnaro *et al.* 2008; Cavagnaro *et al.* 2004). By growing a mycorrhiza defective tomato mutant (named *rmc*) (Barker *et al.* 1998) and its mycorrhizal wild-type progenitor in field soils, it has been possible to explore mycorrhizal functioning, in particular nutrient interception, without the need to fumigate soils, thereby leaving the wider soil biota intact.

The overarching goal of this research is to understand the formation and functioning of AM in the field. This information will be essential in developing farming systems that promote AM and their potential benefits. Here we present results from selected studies which explore different aspects of this goal.

1. Formation of AM in the field:
To assess the formation and functioning of AM in the field, we undertook a survey of AM formed by

tomatoes in southeastern Australia. This survey sought to determine the levels of AM colonization on processing tomato farms, and identify factors impacting levels of colonization.

2. Nutrient uptake and interception by AM:
AM play an important role in plant nutrient uptake. This is important with respect to both plant nutrition and minimizing the loss of nutrients from soils via leaching. Using our genotypic approach for controlling AM colonization, we have studied nutrient uptake by AM in the field, and in field collected soils. Here we present results of selected studies to illustrate the role of AM in nutrient uptake by tomato plants.

Methods
1. Formation of AM in the field
Soils and roots were collected from 17 tomato farms from across northern Victoria, Australia. Samples were collected as fruits were maturing, in January-February, 2009. Briefly, soils were collected from immediately between tomato plants growing in beds. Roots were washed from the soil with reverse osmosis water, cleared with KOH and stained with ink and vinegar using a modification of the method of Vierheilig *et al.* (1998). Colonization of roots was determined using a modification of the methods of McGonigle *et al.* (1990). Soils were also analysed for a wide range of key physicochemical properties.

2. Nutrient uptake and interception by AM
To assess the functioning of AM we have grown the mycorrhiza defective tomato mutant (*rmc* hereafter), and its wild type progenitor *Solanum lycopersicum* L. cv. 76R (see Barker *et al.* 1998) (76R, hereafter) in a range of field soils. In this study, soil was collected from tomato fields using in-tact cores (0-15 cm depth). The cores were returned to the laboratory and seeds of either the *rmc* or 76R genotypes of tomato planted in them. The plants were grown in a glasshouse and harvested after 2 months. Mycorrhizal colonization of roots was assessed (as above) and plant growth and nutrient contents and concentrations (via ICP-AES) were measured (Cavagnaro *et al.* 2006). Data from one site are presented in this paper.

Results and Discussion
1. Formation of AM in the field
In our detailed survey of mycorrhizal colonization of roots from across the processing tomato sector of southeastern Australia, we found that mycorrhizal colonization of roots was generally low. Indeed, ca. 40% of root samples contained no colonization at all. This indicates that the potential benefits of forming AM (i.e. nutritional and biomass) are not being realized in these tomato fields. Furthermore, where roots were colonized, the mean level of colonization across all sites was very low (ca. 5% of root length). While these levels are low, they are in line with a previous study focusing on field grown tomatoes in a southern Florida research station (Rasmann *et al.* 2009), but not in organically managed tomato farms in California (Cavagnaro *et al.* 2006). Analysis of farm management records revealed that AM colonization was not present predominantly in fields where soils had been fumigated at the start of the growing season. Moreover, there was a ca. five fold reduction in colonization of roots where plants were grown in soils that had been fumigated. While not necessarily unexpected, this highlights the importance of farm management on AM. If we are to capitalize on the benefits for forming AM, the impact of soil fumigation needs to be carefully considered. Tomatoes are grown in nurseries prior to translating in the field. Our ongoing research is investigating the potential to pre-inoculate seedlings with AM fungi in the nursery phase of production to improve levels of AM colonization in the field.

In addition to the influence of soil fumigation on AM we also explored the relationship between AM colonization (where it was present) and a wide range of edaphic factors. While colonisation was correlated with soil N nutrition, it was not related to soil P nutrition, as in previous studies (Smith and Read 2008). Together these data highlight the need to consider AM in the management of agricultural soils. To this end, the relationship between the formation of AM, soil physicochemical properties and other edaphic factors in this field survey is currently being assessed in detail.

2. Nutrient uptake and interception by AM
When grown in in-tact cores containing tomato farm soil, the mycorrhiza defective tomato mutant (*rmc*) had essentially no colonisation (less than 2%). Conversely, the mycorrhizal tomato genotype was well colonized for tomato in field collected soils (>15%). This differential colonisation of the two genotypes is consistent with our earlier studies(Barker *et al.* 1998; Cavagnaro *et al.* 2007; Cavagnaro *et al.* 2006; Cavagnaro *et al.*

2008; Cavagnaro *et al.* 2004), and thus, the two genotypes provided mycorrhizal and non-mycorrhizal plants for comparing the functioning of AM in a field soil.

While the two genotypes were differentially colonised, there was little difference in the total biomass of the genotypes. Specifically, growth of the tomato plants was marginally higher (P=0.07) in the mycorrhizal (3.2+/-0.3 g dry weight) than the non mycorrhizal plants (2.2+/-0.4 g dry weight). This finding is in agreement with earlier studies using a range of field soils in both the field and glasshouse (Cavagnaro *et al.* 2006; Cavagnaro *et al.* 2008; Cavagnaro *et al.* 2004; Poulsen *et al.* 2005). This "matched growth" of the genotypes represents a situation where the cost (C for the fungus) and benefit (nutrients to plant, see below) of forming the AM are equivalent (Johnson *et al.* 1997). It is important to note, however, that in most studies biomass is quantified before the plants have reached maturity. Thus, the lack of a vegetative growth response does not necessarily preclude a mycorrhizal "benefit" at a later stage of development. This is an important focus of our ongoing research.

In this study there was no difference in the P concentration of the genotypes (data not shown), in contrast to our earlier studies (Cavagnaro *et al.* 2006; Cavagnaro *et al.* 2008). There was, however, a significant difference in whole plant Zn content. Specifically, the whole plant Zn content (roots+ shoots) of the non-mycorrhizal genotype (77+/-15 µg/plant) was significantly (P<0.001) lower than that of than in the mycorrhizal plants (243+/-18 µg/plant). This considerable increase in Zn content of the mycorrhizal plants highlights the role of AM in plant nutrition generally, and the interesting role that AM play in plant Zn nutrition (Cavagnaro 2008).

Conclusion

The findings reported here, and in our past and ongoing research, indicate that AM have an important role to play in the growth and nutrition of tomatoes. The results of our sector wide field survey indicate that these potential benefits may not be being fully realized. This presents an important challenge and opportunity to capatalize on the benefits that forming AM. Improvements in the formation of AM in the field must, however, be predicated upon a sound understanding of the impacts of AM on plant growth, nutrition and physiology. Our research seeks to address this knowledge gap by using a novel genotypic approach to address a long standing challenge in the study of AM in "real world" soils.

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