Project title:	Pre-colonisation of strawberry runners and tray plants with arbuscular mycorrhizal fungi to manage Verticillium wilt
Project number:	CP 106
Project leader:	Professor Xiangming Xu
	East Malling Research
Report:	Annual report, October 2015
Previous report:	N/A
Key staff:	Benjamin Langendorf
Location of project:	East Malling Research
	New Road, East Malling, Kent, ME19 6BJ
Industry Representative:	Mrs Marion Regan
Date project commenced:	October 2013
Date project completed	September 2016

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AHDB Horticulture,
AHDB
Stoneleigh Park
Kenilworth
Warwickshire
CV8 2TL

Tel – 0247 669 2051

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AUTHENTICATION

We declare that this work was done under our supervision according to the procedures described herein and that the report represents a true and accurate record of the results obtained.

Benjamin Langendorf

PhD student

East Malling Research

Signature .... Date ....28/09/2015.................

Report authorised by:

Professor Xiangming Xu

Programme Leader, Genetics and Crop Improvement

East Malling Research

Signature .... Date ....30/09/2015.................

GROWER SUMMARY

Headline

- The effect of pre-colonisation of plants with arbuscular mycorrhizal fungi (AMF) on Verticillium wilt development depends on both cultivar and AMF species used.

Background and expected deliverables

Strawberry wilt, caused by *Verticillium dahliae* Kleb., can reduce yield by up to 75%. For many years, soil was routinely fumigated with methyl bromide until this was banned by the 1994 Montreal Protocol which became effective in the UK in 2006. Extensive effort has gone into finding alternatives. The incorporation of green manures that release volatile fungitoxic compounds, so-called biofumigation, shows promise as a component of a disease management strategy.

In a recent Defra funded Horticulture LINK project, a group of scientists led by EMR demonstrated that lavender waste can effectively reduce Verticillium wilt severity on strawberry and identified three key terpenoids responsible for the observed suppressive effect. In a follow-on Innovate UK project, EMR is leading a consortium to investigate whether pelletised lavender waste and microencapsulated terpenoids can effectively control strawberry wilt. Results so far, however, indicate limited efficacy of these products. Therefore, other measures in addition to the biofumigation-based approach are needed.

Arbuscular mycorrhizal fungi (AMF) are ubiquitous in terrestrial ecosystems where they are major components of the soil microbial biomass. Mycorrhizal associations are multi-functional, assisting the plants in nutrient acquisition, water uptake, and protecting roots from pathogens. AMF have been shown to increase plant tolerance to *V. dahliae* on several crops, including pepper, strawberry and cotton. However, the beneficial effects offered by AMF can vary considerably.

A recent publication showed that one particular AMF strain significantly reduced strawberry wilt when plants were inoculated at planting. The extent of AMF root colonisation and their beneficial effects to plants are however also dependent on particular AMF strains and strawberry cultivars. Ensuring sufficient colonisation of strawberry planting materials (runners or tray plants) before transplanting may further increase the benefit of AMF-symbiosis through physical exclusion of potential colonisation sites for soil pathogens.

This project aims to find out if AMF pre-colonised planting material leads to reduced incidence or severity of wilt on strawberry.

Summary of the project and main conclusions

To date, we have shown that AMF can colonise *in-vitro* derived plantlets in vermiculite and plants from runner tips in a peat/perlite based substrate. The high moisture conditions during weaning/tipping did not prevent AMF from colonising roots. The effects of the symbiosis on plant growth were variable. AMF can survive in cold stores in colonised roots for several months.

All AMF species tested on the tissue culture derived plant 'EM1996' increased the crown diameter of the plantlets but this increase was only significant with *R. irregularis*. For the runner tip-derived plants, the effects of AMF inoculation on crown diameter varied greatly with specific combinations of AMF and cultivars. There is some evidence of reduced wilt incidence for AMF-colonised plants, which needs to be confirmed in 2015.

Financial benefits

It is too early to speculate the financial benefits.

Action points for growers

This is the second year of the project. Although there are a few interesting results, it is still too early to identify action points for growers as further confirmatory studies are needed over the next twelve months.

SCIENCE SECTION

Introduction

Strawberry wilt, caused by the soil-borne pathogen Verticillium dahliae Kleb., alters water status, plant growth and can reduce berry yield by up to 75 % (Lovelidge, 2004). For many years, soil fumigation with methyl bromide was routinely applied as pre-plant treatment in commercial strawberry production to control verticillium wilt (Martin & Bull, 2002). However, because of its high ozone-depleting potential and toxicity, methyl bromide was made subject to the control arrangements of the 1994 Montreal Protocol (Ristaino & Thomas, 1997). The use of methyl bromide was finally prohibited, within the EU under Regulation 1005/2009, from 18 March 2010 (HSE guidance, 2014). Henceforth, extensive effort has gone into finding economically effective alternatives to manage wilt (Martin, 2003; Goicoechea et al., 2010).

An approach is to exploit arbuscular mycorrhizal fungi (AMF) as a bio-protectant against strawberry wilt. AMF are ubiquitous in agro- and eco-systems, where they are major components of soil microbial biomass (Smith & Read, 2010). At least 80 % of terrestrial plant families form symbioses with AMF (Wang & Qiu, 2006), including strawberry (Daft & Okusanya, 1973). Mycorrhizal associations are multi-functional. They can assist strawberry plants in nutrient acquisition, particularly of phosphate (Holevas, 1966; Dunne & Fitter, 1989), and water up-take (Hernández-Sebastià et al., 1999) and can minimise environmental stresses e.g. drought and salt (Borkowska, 2002; Fan et al., 2011). In addition, AMF was shown to protect strawberry roots from soil-borne pathogens, e.g. Phytophthora fragariae (Norman & Hooker, 2000). The use of AMF increased plant tolerance to V. dahliae on several crops, e.g. tomato, alfalfa, cotton, aubergine (egg-plant), pepper (Bååth & Hayman, 1983; Nursery, 1992; Liu, 1995; Karajeh & Al-Raddad, 1999; Karagiannidis et al., 2002; Garmendia et al., 2004; Porras-Soriano et al., 2006). Two recent studies showed that Funneliformis mosseae (Glomus mosseae), Glomus versiforme and a commercial arbuscular mycorrhizal inoculant containing Glomus spp. significantly reduced strawberry wilt when inoculated at planting (Ma et al., 2004; Tahmatsidou et al., 2006). However, it is well documented that the beneficial effects for plants provided by AMF symbiosis is highly variable regarding factors such as host genotype, AMF species/strains (Marschner & Timonen, 2005) and the growth substrate characteristics (Caron et al., 1985; Caron & Parent, 1987; Duvert et al., 1990; Murphy et al., 2000; Abiala et al., 2013).

Micro-propagation technology is a practice used by strawberry breeders to multiply disease-free plants of new selections before being released to nurseries (Debnath & Teixeira da Silva, 2007; Rowley et al., 2010). In addition to production of commercial bare-rooted runners in the field, modern strawberry nurseries also rely on the production of plug plants in soil-less substrate derived from runner tips (Rowley et al., 2010). Both micro-propagation and tipping methods require either plant propagator or misting systems to keep the atmosphere sufficiently damp for several weeks to ensure plant acclimatisation and rooting. The initial media used for micro-propagation do not contain AMF and hence early colonisation of plant roots by AMF cannot occur. While soil-less substrates (e.g. peat, coir or other compost mixes) may contain AMF propagules, their presence is usually scarce and variable. Therefore, pre-transplant inoculation with AMF inocula may allow post in-vitro plantlets and runner tips to benefit from AMF symbiosis before future transplantation in the field and permit new roots to be colonised during and after plant establishment. The effectiveness of AMF inoculation on post in-vitro strawberry plantlets as well as seedlings has been investigated previously and showed promising results (Kiernan et al., 1984; Chávez & Ferrera-Cerrato, 1990; Niemi & Vestberg, 1992; Vestberg, M., 1992; Vestberg, Mauritz, 1992; Varma & Schüepp, 1994; de Silva et al., 1996) whereas similar studies have not been carried out for the tipping system.

Some empirical evidence suggests that AMF colonisation of root may be limited under damp conditions when using soil-less substrates such as peat and/or vermiculite. If plant root colonisation by AMF is possible in commercially used soil-less substrates under high moisture, we will then investigate whether early colonisation could improve subsequent plant growth and health – particularly tolerance to wilt. The ultimate aim of this work will be to establish a system based on early AMF colonisation to produce vigorous and healthier plants, hence requiring less fertilisers and pesticides.

Objectives

This proposal aims to investigate whether AMF pre-colonised strawberry planting materials would reduce incidence or severity of wilt, focusing on the interaction among strawberry cultivars and AMF strains (species). Transcriptomic and histological studies will be conducted to investigate the likely genetic and physiological bases for the wilt suppressive effects offered by AMF. Finally it will investigate whether wilt can be further reduced when AMF is integrated with lavender waste derived products. Specifically, we have five hypotheses:

- AMF can survive and colonise roots of two different types of strawberry planting materials under high moisture conditions in commercially used soil-less substrates
- AMF species do not differ in their ability to colonise different strawberry cultivars

- Pre-colonisation of strawberries by AMF improves plant growth and development
- Early colonisation by AMF improves plant tolerance to verticillium wilt
- AMF species do not differ in their ability to increase plant tolerance to wilt

During the last twelve months, the research focused on the two aspects: (1) whether AMF in colonised strawberry roots can survive cold storage for several months; and (2) whether inoculating plants with AMF can increase tolerance to wilt. In this report, we used schematic representation to show experimental setups; we hope this will help readers to understand what we are trying to do quickly. For easy reading, we also structured the report based on individual experiments.

Experiment 1: Can AMF in colonised strawberry roots survive a long period in cold storage?

Materials and methods

In this particular experiment, we tested a specific hypothesis regarding the survival of AMF in colonised strawberry roots, namely AMF does not suffer from appreciable amounts of mortality in colonised strawberry roots of tray plants during cold storage for several months at -2 °C.

Misted tip plants of both 'Red Glory' and 'Vibrant' strawberry cvs. colonised with AMF in the autumn 2014 were used for this study. These plants were placed in the cold store in late November 2014, and batches of these plants were potted up at monthly intervals and placed in a growth cabinet. New roots were sampled to estimate the extent of AMF colonisation (Fig. 1). A total of five batches of plants (1 to 5 months) were used; plants were inoculated with each of three AMF species separately during the misting phase.

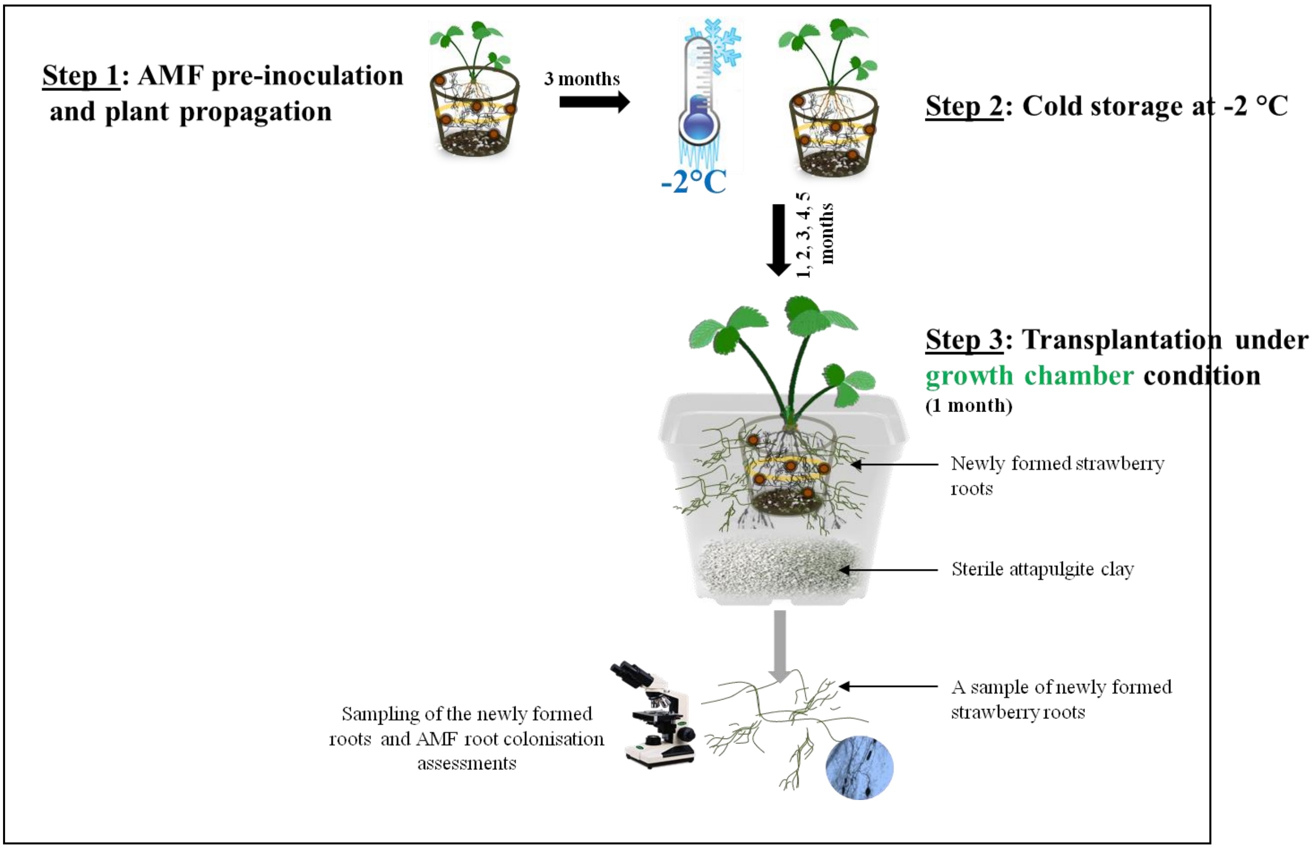

Figure 1: Schematic representation of experimental setup to study the effect of cold storage at -2°C on the survival of AMF in colonised strawberry roots. Plants were potted up at monthly intervals (five batches in total: 1 to 5 months) and placed in a growth cabinet. New roots were examined for the extent of AMF colonisation

Results

After a month of growth in a growth chamber, AMF structures were observed in newly formed roots of both 'Red Glory' and 'Vibrant' strawberry cvs. for all three AMF species tested (Figure 2), irrespective of the length of cold storage (up to 5 months). Thus, we may conclude that AMF can survive inside colonised roots of strawberry tray plants in cold storage at -2 °C for several months.

Currently, we are still assessing the extent of root length colonisation by AMF in order to determine whether the level of root colonisation remained at the same level for five different storage times. It is anticipated that this experiment will be completed and written up by January 2016.

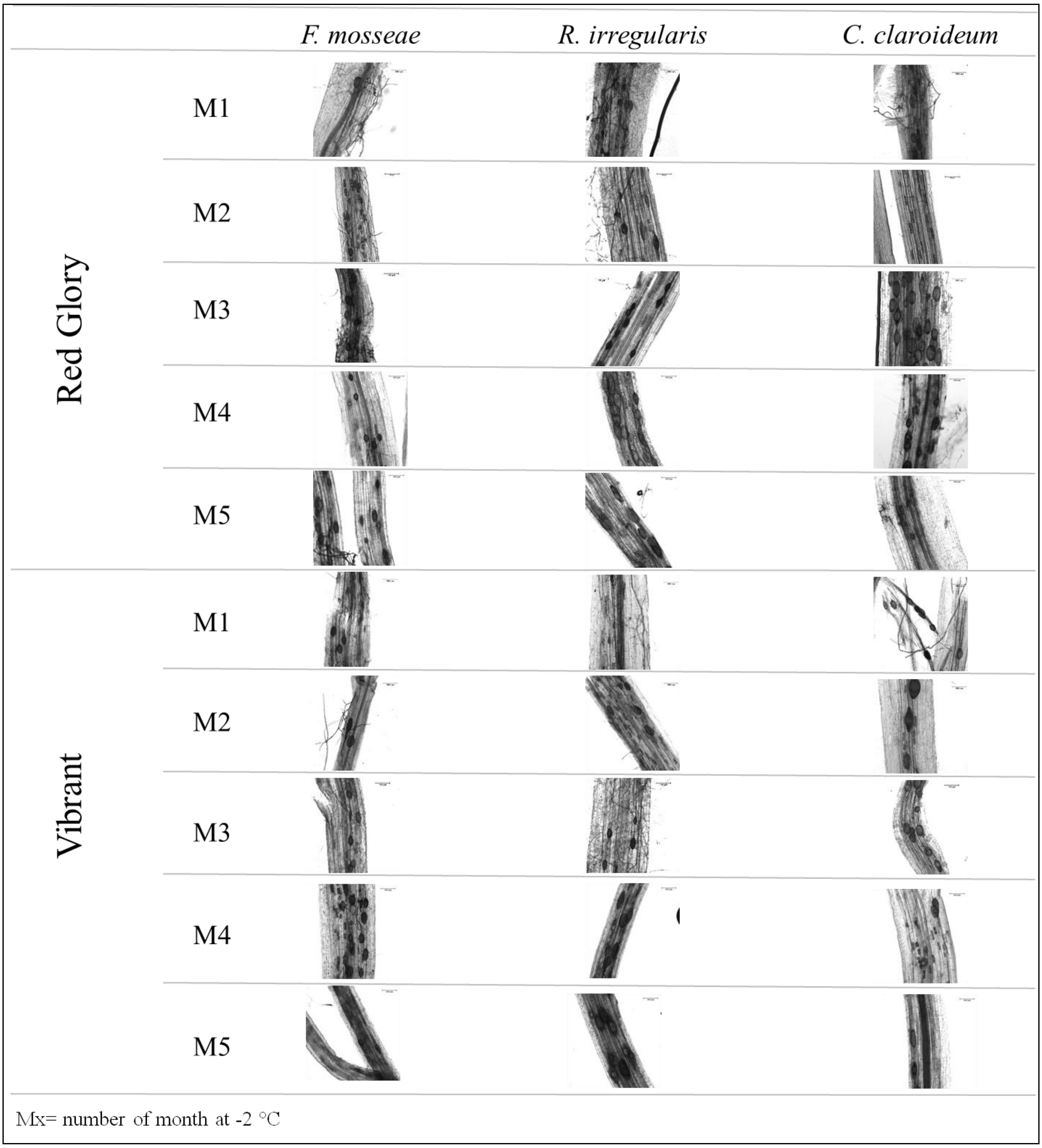

Figure 2: Root colonisation by AMF of plugs of ‘Red Glory’ and ‘Vibrant’ strawberry cvs. after one month of cultivation in autoclaved attapulgite clay in a growth chamber followed by cold storage at -2 °C from one to five months: M1 to M5. Longitudinal squash of roots stained with trypan blue; colonisation by single AMF species: F. mosseae, R. irregularis, or C. claroideum

Experiment 2: Can AMF pre-colonisation increase tolerance to strawberry wilt under field conditions?

Materials and methods

Strawberry tray plants of cvs. 'Red Glory', 'Malling Centenary' and 'Vibrant' strawberry pre-colonised with AMF during the misting phase were stored in a cold store and planted out in June 2016 at EMR. The main objective was to assess whether pre-colonisation by AMF led to increased tolerance to wilt under field conditions. Figure 3 shows the schematic representation of this experiment.

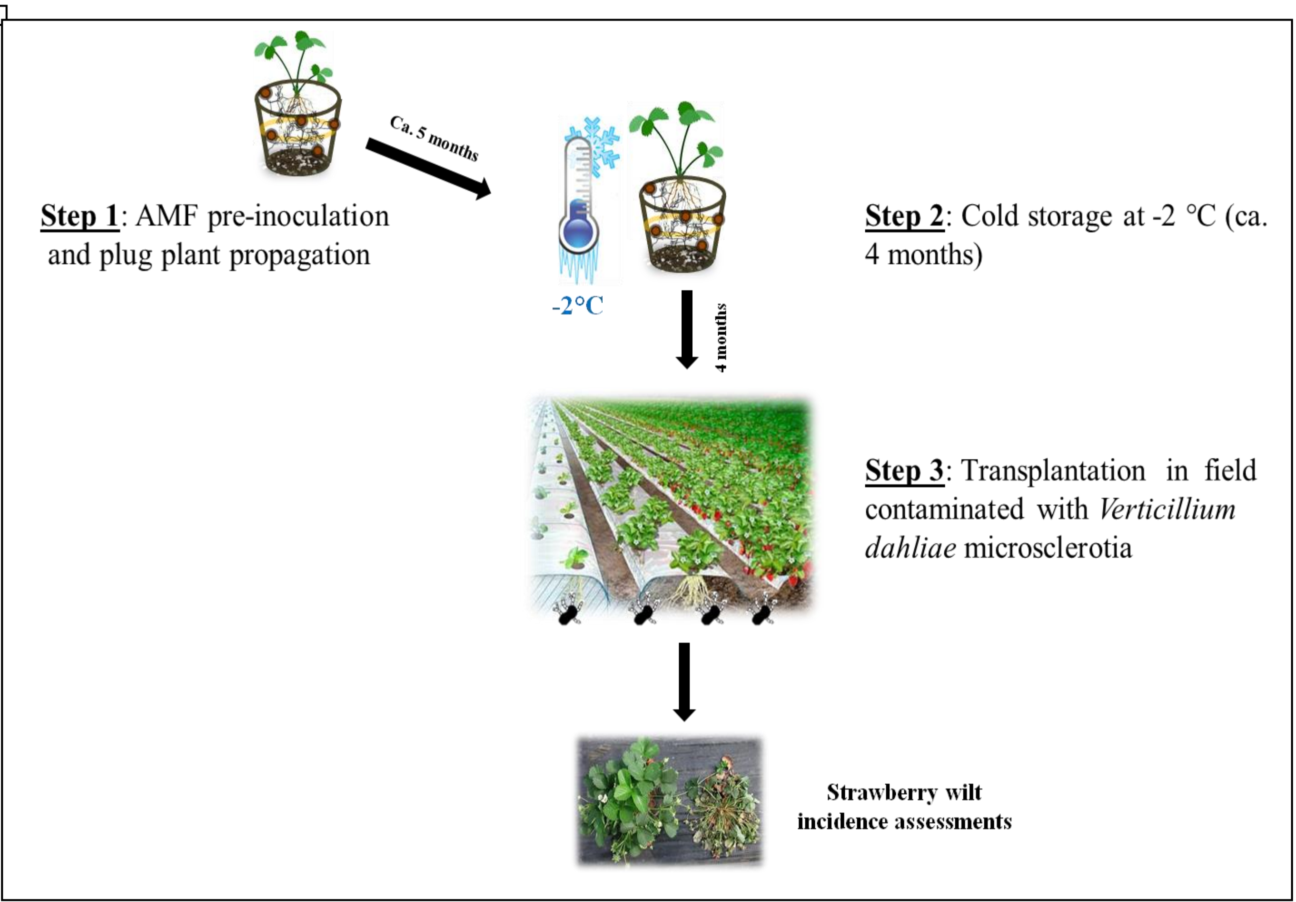

Figure 3: Schematic representation of experimental setups to study the effect of pre-colonisation by AMF on wilt development under field conditions

Results

After 14 weeks of cultivation under field conditions, a very low level of wilt symptoms was observed despite the presence of 1.9 CFU g-1 of soil in the trial site. Wilted plants appeared, (Figure 4). Mycorrhizal inoculation may increase or decrease the number of diseased plants depending on individual AMF species and strawberry cultivars (Figure 5). It should be stressed that we cannot draw any conclusion yet as we need to statistically analyse the data and also conduct further trials.

Figure 4: Verticillium wilt symptoms assessed 14 weeks after strawberry plugs were transplanted in the field

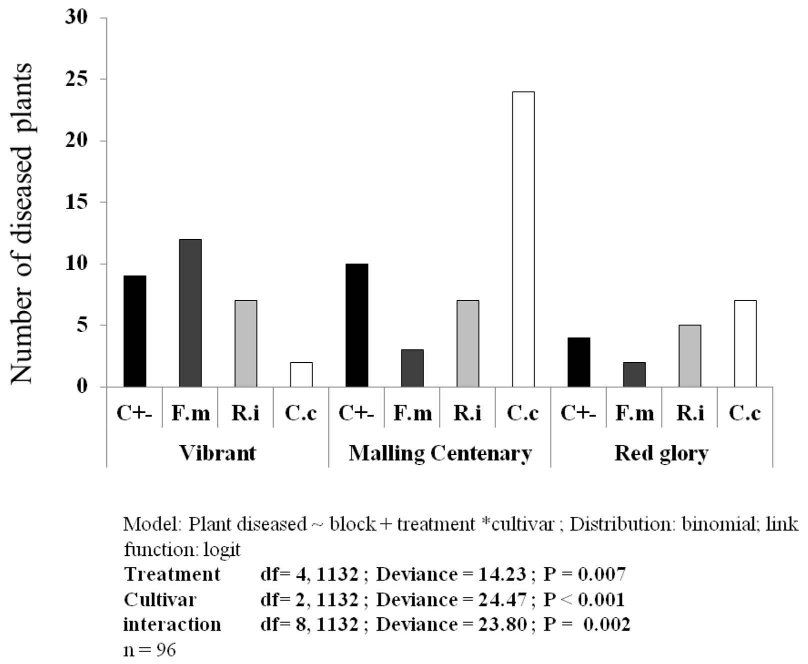

Figure 5: Results of generalised linear models fitting plant diseased without mycorrhiza (C+-) or inoculated with single AMF species (F. mosseae, R. irregularis, C. claroideum) and

three different strawberry cultivars (‘Vibrant’, ‘Malling Centenary’ and ‘Red Glory’). Data are number of diseased plants (n = 96)

Experiment 3: Can autotrophic in-vitro systems be developed to explore the nature of interactions between AMF and pathogens in strawberry plants?

Materials and methods

This experiment was conducted to assess whether we could conduct host-AMF-pathogen interaction studies in an in vitro system. If we could, it will greatly increase research efficiency. Figure 6 shows the key experimental steps in this study. We used two pathogens for this study: wilt pathogen inoculated onto F. vesca (one diploid parent of cultivated strawberry) and P. fragariae inoculated onto cv. ‘Calypso’.

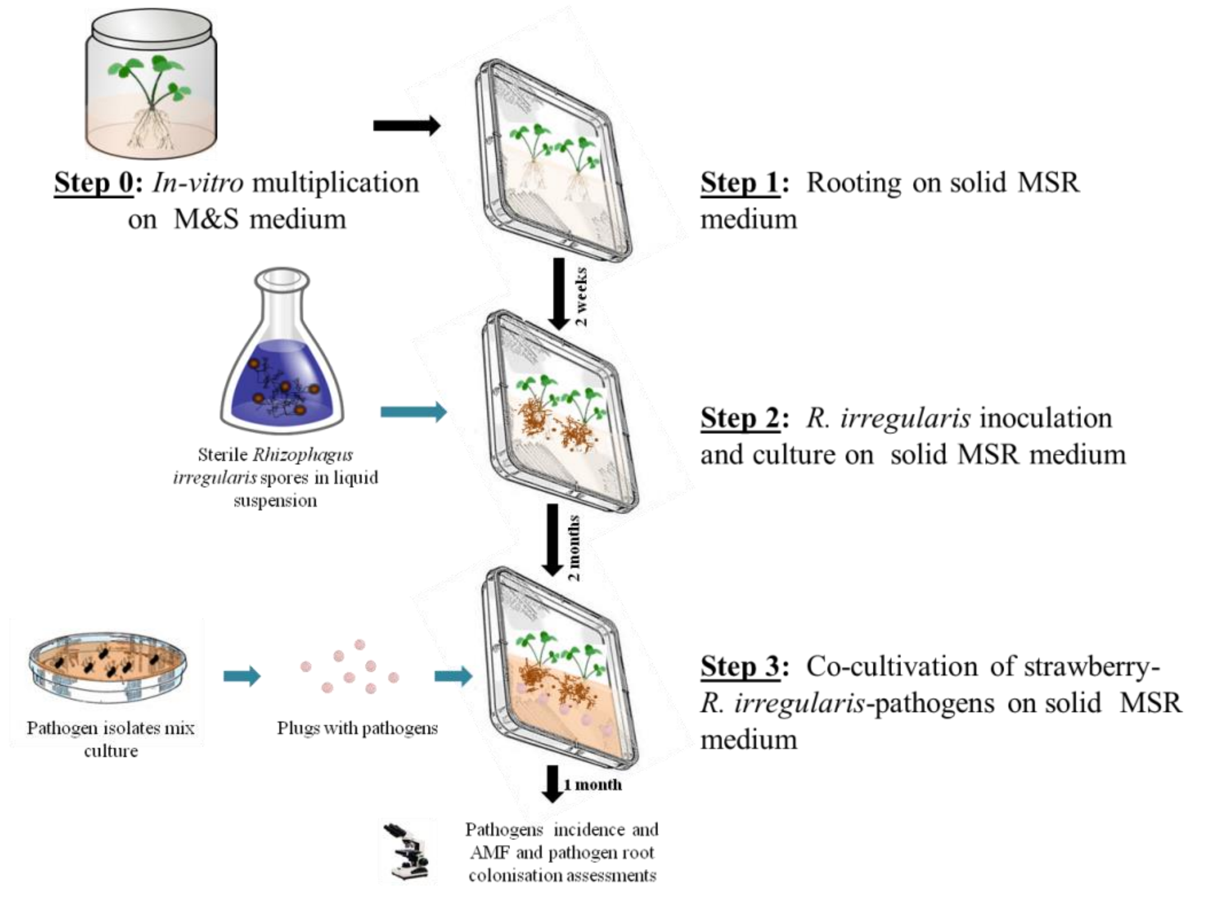

Figure 6: Schematic representation of the experiment to investigate whether we could use an autotrophic in-vitro system to study the interactions between AMF (R. irregularis) and V. dahliae or P. fragariae on micro-propagated strawberry

Results

Both F. vesca and ‘Calypso’ plantlets can produce new roots and establish on solid modified MSR medium (Figure 7). Calypso plantlets were highly infected by P. fragariae (Figure 8). However, the results were not clear for F. vesca inoculated with V. dahliae; F. vesca root straining with trypan blue failed to detect wilt infection.

However, R. irregularis MUCL 43194 could not colonise strawberry roots of F. vesca or F. x ananassa cv. ‘Calypso’ on solid modified MSR medium. Thus we could not use this system to study AMF-pathogen interactions on strawberry.

Figure 7: Fragaria vesca (A) and F. x ananassa cv. ‘Calypso’ (B) after one month of culture on modified MSR medium

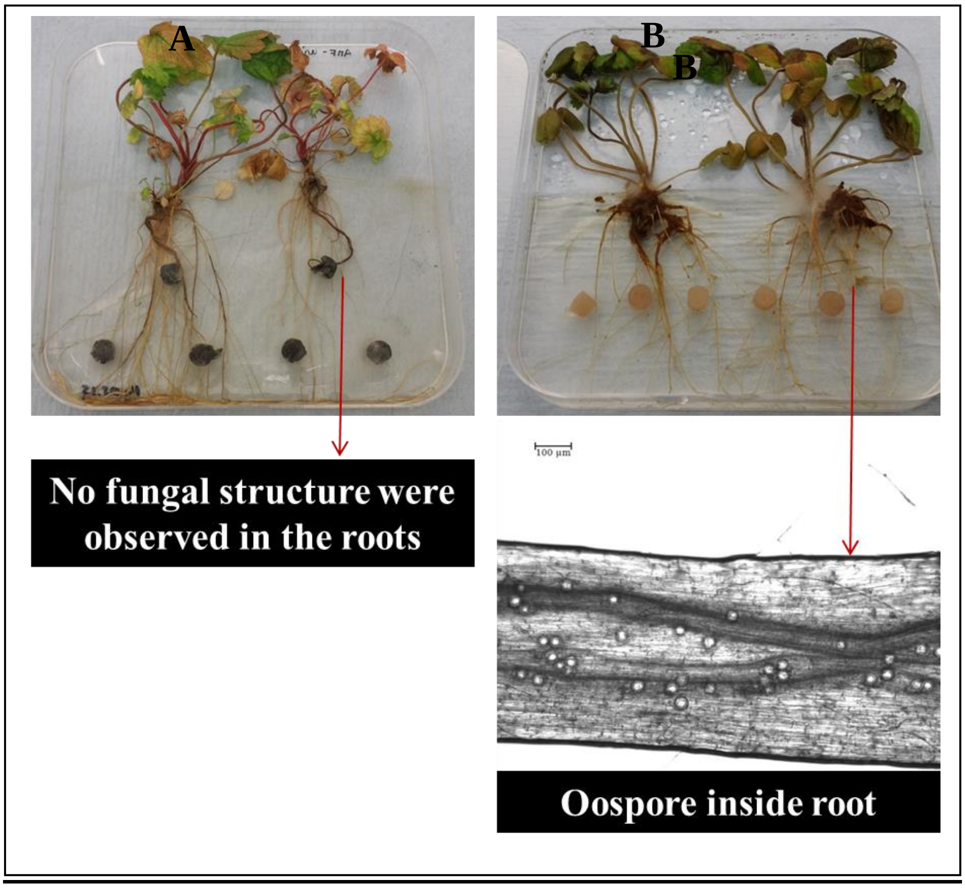

Figure 8: F. vesca plantlets (A) pre-inoculated with R. irregularis and inoculated with V. dahliae (AMF+W+); F. x ananassa cv. 'Calypso' plantlets (B) pre-inoculated with R. irregularis and inoculated with P. fragariae (AMF+P+). The pictures show the appearance of the strawberry plantlets and the presence of intraradical fungal structures one month after inoculation with a pathogen. However, AMF structures were not detected inside plantlets roots for both experiments

Conclusions

We have shown that AMF can colonise in-vitro derived plantlets in vermiculite and runner tip-derived plants in a peat/perlite based substrate. Furthermore, AMF can survive in cold stores in colonised roots for several months.

The effect of pre-colonisation of plants with AMF on wilt development indicated that the effect of wilt varies with cultivars and AMF species. Further experiments are necessary to confirm these preliminary results.

Future work

UK strawberry production systems are rapidly moving towards the table-top system, where strawberry plants are grown in substrate under protection. Strawberry wilt is therefore expected to become less of a problem in UK strawberry production. Table top systems bring several advantages to the strawberry industry but several pathogens, such as P. fragariae (red core), remain an issue. Consequently, we plan to study whether AMF pre-colonisation of strawberry planting material could reduce incidence and severity of strawberry red core within the next six months. These studies may also include PGPR (beneficial bacteria) as well as AMF.

It is not known whether introduced AMF species could persist in substrate or how the introduced AMF species/strain impact rhizosphere microbiota. Therefore, AMF inoculation success and its effect on other microbiota should be investigated using a metagenomic approach.

Finally, AMF have been shown to provide beneficial effects to strawberry growth and yield at EMR. The maintenance of a microbial population may be increasingly fundamental to sustainable food security. Substrates such as coir are usually depleted of beneficial microbes, including AMF, and as such the introduction of beneficial microbes through pre-inoculated strawberry plugs is more likely to generate benefits in such commercial cropping systems. Thus an experiment will be designed to investigate whether or not the use of AMF pre-inoculated strawberry plugs could improve fruit production and berry quality in a substrate growing system.

Knowledge and Technology Transfer

The student attended the international conference on mycorrhizal fungi in August 2015 in USA and presented a poster. The student has produced a summary report of this conference which is available through AHDB Horticulture. The work was also presented at Fruit Focus.

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