Project title:	Pre-colonisation of strawberry runners and tray plants with arbuscular mycorrhizal fungi to manage Verticillium wilt
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The results and conclusions in this report are based on an investigation conducted over a one-year period. The conditions under which the experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.

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.

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GROWER SUMMARY

Headline

- There is some evidence of reduced wilt development in AMF-colonised plants.

Background

Strawberry wilt, caused by *Verticillium dahliae* Kleb., can reduce yield by up to 75%. For ca. 40 years, strawberry growers routinely fumigated their soils with methyl bromide to reduce levels of soil inhabiting *V. dahliae* before planting. The use of methyl bromide in the UK was banned in 2006. Other soil fumigants have also been used for this purpose, most notably chloropicrin, but its use has also been limited by legislation in recent years. Extensive effort has gone into finding alternative ways of reducing levels of soil inhabiting *V. dahliae*, including the incorporation of green manures that release volatile fungitoxic compounds (so-called biofumigation), which has shown promise as a component of a disease management strategy.

In a recent Defra Horticulture LINK project (HL0177, HDC project SF 77), a group of scientists led by EMR demonstrated that the incorporation of lavender waste in soils prior to planting, can effectively reduce verticillium wilt severity on strawberry. Three key terpenoids were identified as being responsible for the observed suppressive effect. In a follow-on TSB funded project, EMR is leading a consortium to investigate whether pelletised lavender waste and microencapsulated terpenoids can effectively control *V. dahliae* in soils. Results so far, however, indicate limited efficacy of these products. Therefore, other control 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* in 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 trans-planting, may further increase the benefit of AMF-symbiosis through physical exclusion of potential colonisation sites for soil pathogens.

Arbuscular mycorrhizal fungi (AMF) have been shown to confer a number of benefits to their host plant including enhanced pathogen resistance. This project is investigating if pre-colonising strawberry plants with AMF leads to reduced incidence or severity of verticillium wilt.

Summary

To date, we have demonstrated that AMF can colonise *in-vitro* produced plantlets in vermiculite and runner tip produced plants 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. All AMF species tested on the tissue culture produced plant of the cultivar EM1996 increased the crown diameter of the plantlets, but this increase was only significant with *R. irregularis*. For the runner tip produced 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 identify the potential financial benefits from this project.

Action points

- This is only the first year of the project so it is too early to recommend specific action points for growers.

SCIENCE SECTION

Introduction

Strawberry wilt, caused by the soil-borne pathogen *Verticillium dahliae* Kleb., alters water status and plant growth and can reduce berry yield by up to 75 % (Lovelidge, 2004). For ca. 50 years, soil fumigation with methyl bromide was routinely applied as a pre-planting 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). Another effective fumigant, chloropicrin, is to be withdrawn from the UK in the near future. Henceforth, extensive effort has gone into finding economically effective alternatives to manage wilt (Martin, 2003; Goicoechea *et al.*, 2010).

One 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 showed 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 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 provided by AMF symbiosis to plants 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 in soilless substrate of plug plants derived from runner tips (Rowley *et al.*, 2010). Both micro-propagation and tipping methods require suitable equipment 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 does not occur. While soil-less substrates (e.g. peats, composts) 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 tip propagation system.

Some empirical evidence suggests that AMF colonisation of root may be limited under damp conditions when using soil-less substrates such as peats 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 fertiliser and pesticide.

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.

Materials and methods

Three experiments were conducted to study whether AMF can colonise strawberry roots under damp/misting conditions in substrate and whether early colonisation of plants with AMF could lead to reduced wilt development when transferred to soils in pots.

Experiment 1: Pre-colonisation of micro-propagated derived plantlets

In vitro strawberry plantlets:

Sixty micro-propagated uniformly rooted plantlets of a single strawberry accession 'EM1996' were obtained from the strawberry tissue-culture laboratory at EMR, UK. The plantlets were grown for two months on M&S medium (Murashige & Skoog, 1962) supplemented with 0.75 % of agar, 3 % of sucrose, and hormones 1.2 $mL.L^{-1}$ of GA_3 and 8 $mL.L^{-1}$ of IBA to induce rooting. *In-vitro* plants were kept in a growth room (21°C, 50% relative humidity (RH), light:dark 16/8 h, photosynthetic photon fluence rate (PPFR) of 40 $\mu mol.m^{-2}.s^{-1}$).

Growing substrate:

Vermiculite was used as growth substrate during the weaning process of tissue culture plantlets. Vermiculite is a hydrated laminar magnesium-aluminium-iron silicate ((Mg, Fe, Al)3((Al, Si)4O10)(OH)2.4H2O) mineral. It is widely used in horticulture because of its good water holding ability, excellent insulation and nutrient buffering capacity (Indrasumunar & Gresshoff, 2013). Tissue culture derived plantlets were potted on a seed tray with forty 46 cm^3 cells (B&Q 40 Cell Insert 08535B, Kent) filled up with twice-autoclaved (121°C for 20 min, four days between autoclave cycles) exfoliated vermiculite medium (Sinclair Horticulture Ltd, Lincoln; Table 5) mixed with 0.25 $g.L^{-1}$ of once-sterilised bone-meal (121°C, 20 min; Verve-Bonemeal B&Q plc, Kent). The substrate was humidified with non-sterile deionised water (1 L of water for 2 L of vermiculite).

AMF inoculation and the weaning process:

Commercial isolates of F. mosseae, Rhizophagus irregularis and Claroideoglomus claroideum) (PlantWorks Ltd, Sittingbourne) were used to inoculate micro-propagated derived plantlets (Table 2). The three single species inocula (i.e. 4.2 g.plant-1 of calcined

attapulgite clay [Terra-Green®] mixed with host plants root fragments used as inoculum carriers) were placed as a layer ca. 1 cm below the surface of the growing medium.

The roots of tissue culture plantlets were first washed with sterile deionized water to remove any adhering agar and nutrients. Plantlets were then gently potted individually and grown six weeks in a growth chamber (Meridian Refrigeration Ltd, Croydon; day and night 21-22°C, ~ 72% RH, light:dark 16h/ 8 h, photosynthetic photon fluence rate PPFR of 40 $\mu mol.m^{-2}.s^{-1}$). The first two weeks, plants were enclosed inside plastic propagators (52 x 42.5 x 24 cm, Stewart Plastic Ltd, online, Oxon, UK) to allow acclimatisation. During this period, foliage of plantlets was misted once daily with sterile deionised water via a hand-held sprayer. Additionally, a ca. 1 cm layer of water, not in direct contact with the module tray, was left for 3 weeks in the bottom of the propagator to maintain high humidity. Plant roots that grew out of the bottom part of the module tray were systematically cut off to avoid additional water supplies or AMF cross contamination.

According to the standard EMR weaning protocol (K. McLeary pers. comm.), both adjustable vents present on the lid of the propagator were kept closed initially (week 1), and then left open (week 2) before the lid was removed (week 3). Once the lid was removed, each plantlet was watered once a week with 10 mL of sterile deionised water. After three weeks post transplantation, absence of fertilisation resulted in the apparition of plant nutrient deficiency symptoms (Figure s1). From week 4, plants were watered three times a week with 10 mL of half-strength Rorison's nutrient solution without phosphate (Hewitt & Bureaux, 1966) and the nutrient deficiency symptoms were alleviated**.**

Transfer to wilt contaminated field soil:

Six weeks after weaning, experimental plants were re-potted into 1 L plastic pots (11 x 11 x 12 cm, black, Desch Plantpak Ltd, Essex) filled up with non-autoclaved soil collected in a non-fumigated commercial strawberry field at EMR (N 51°17'20.93'', E 00°27'11.52''; soil: Barming series, loamy fine sand, sampled on 17 March 2014, Table 5). The soil was sieved beforehand with a garden riddle (square hole wire mesh ca. 5 mm). During transplanting the vermiculite substrate from each tray cell was completely transferred with the plant. Plug plants were then allowed to grow for ca. 28 weeks under standard greenhouse conditions (> 23°C, ~ 40% RH, natural light:dark cycle, adequate pest control). For 20 weeks pots were watered once daily with tap water and fertilised once a week with 50 mL of full-strength Rorison's nutrient solution without phosphate (Hewitt & Bureaux, 1966). Thereafter, the plants were watered only two or three times a week (including fertilisation). The aim of this change in water supply was to induce a moderate hydric stress to encourage the wilt symptoms to develop quicker.

Experimental design:

This experiment was done once. There were five inoculation treatments (three single AMF species, control C-- and control C+-), each with 12 replicate plants, giving a total of 60 plants. A randomised block (three blocks) design was used. Non-inoculated plants (C--) contained only autoclaved attapulgite clay (Terra-Green® Oil-Dri UK Ltd, Cambridgeshire; two cycles, four days between cycles, 121°C, 20 min), whereas C+- contained an equal amount of the autoclaved inocula (one cycle, 121°C, 20 min). Additionally, control C+- was inoculated with 1 mL of aqueous bacterial filtrate, of the triple species inoculum mix (7.6% suspension, w/v), vortexed for ca. 10 min and filtered first through 45 µm sieve (Laboratory test sieve, Endecotts Ltd, London) and then through filter paper (Range QL100, Fisher Scientific UK Ltd, Loughborough) placed in the bottom of a plastic funnel. This design was preserved when the plants were transferred to individual pots.

Experiment 2: Pre-colonisation of runner tips derived plants

Tipping process:

Runners tips deriving from mother plants of two cultivars: 'Vibrant' (June-bearer, propagated by Hargreaves Plants Ltd, Hillington) and 'Red Glory' (ever-bearer, propagated by Edward Vinson Plants Ltd, Faversham) were used for this trial. Mother plants were grown in coir bags (Botanicoir Ltd, London). Stolons were produced within three months under greenhouse conditions (temperature 20-23°C, ~ 30% RH, light:dark 16/8 h, ample irrigation, appropriate fertilisation regimes and pest control).

Growing substrate:

Potting mix consisting of seven parts of non-autoclaved Irish dark peat (Clover Peat Products Ltd, Dungannon, Ireland) and three parts of non-autoclaved 2.0-5.0 mm perlite (Sinclair Horticulture Ltd, Lincoln) was used as growth substrate during weaning and establishment of runner tips. The growing substrate (Table 5) was limed with 16 $g.L^{-1}$ of non-autoclaved dolomite lime (Omya UK Ltd, Derbyshire, UK) to give a pH of ca. 7 and fertilised with 0.25 $g.L^{-1}$ of sterilised bone-meal (one cycle at 121°C, 20 min).

AMF inoculation and the weaning process:

F. mosseae, R. irregularis and C. claroideum were used as in experiment 1 (Table 2). The three single species inocula (6.4 g.plant-1 of Terra-Green® mixed with host plant root fragments used as inoculum carriers) were placed as a layer ca. 1 cm below the surface of the growing medium before runner transplantation. Fifty uniform runner tips of 'Vibrant' and

'Red Glory' were harvested when root pegs and at least two fully trifoliated expanded leaves were visible. Approximately 1.5 cm of the stolon was left. Runner tips were immediately pinned down in standard plastic module trays (48 cells, 70 cm3 per cell, Desch Plantpak Ltd, Essex). Immediately after transplantation, tips were placed in a growing chamber (> 20°C, no artificial light, ~ 90-100% RH) intermittently water-sprayed with a Macpenny Solarmist VTL misting system (Wright Rain Ltd, Hampshire) for two weeks (misting time of ca. 5 seconds with the frequency depending on light conditions, from six minute intervals on bright days to 20 min intervals on dull days; Wright Rain Ltd pers. comm.). At the end of the weaning period, plants were grown under glasshouse conditions for ca. four extra weeks (> 20°C, light:dark 16/8 h, ~ 40% RH). Plants were then watered daily with tap water**.**

Transfer to wilt contaminated field soil:

After six weeks in module trays, experimental plants were transplanted and grown in 1 L pots filled up with field soil as in trial 1. Plants were allowed to grow for ca. 22 weeks under standard greenhouse conditions (> 23°C, ~ 40% RH, natural light:dark cycle, adequate pest control). For 16 weeks pots were watered daily with tap water and fertilised once a week with 50 mL of full-strength Rorison's nutrient solution without phosphate (Hewitt & Bureaux, 1966). Thereafter, the plants were watered only two or three times a week as in trial 1.

Experimental design:

There were five inoculation treatments (as experiment 1, three AMF species, control C-- and control C+-) with two strawberry cultivars ('Vibrant' and 'Red Glory') each with 10 replicate plants, giving a total of 100 plants. A randomised block (5 blocks) design was used and this design was preserved when the plants were transferred to pots*.*

Experiment 3: Pre-colonisation of runner tips derived plants with different size

Experiment 3 investigated the same questions than experiment 2 but with additional factor of runner quality (Table 1).

Tipping process:

Runners tips deriving from mother plants of four cultivars: 'Elsanta', 'Malling Centenary', 'Vibrant' (June-bearer, propagated by Hargreaves Plants Ltd) and 'Red Glory' (ever-bearer,

propagated by Edward Vinson Plants Ltd) were used for this experiment. Mother plants were grown as in experiment 2.

Growing substrate:

Potting mix was prepared as in experiment 2 (Table 5).

AMF inoculation and the weaning process:

F. mosseae, *R. irregularis*, *C. claroideum*, *Glomus microagregatum* and *Funneliformis geosporum* were tested (Table 2). The five single AMF species were inoculated as in experiment 2. Additionally, control C+- was inoculated with 10 mL of aqueous bacterial filtrate, of the triple species inoculum mix (10% suspension, w/v) prepared as experiment 2. The Macpenny Solarmist VTL misting system (Wright Rain Ltd, Hampshire) was set for two weeks with a misting time of ca. 7 seconds available at frequencies similar as in experiment 2.

Transfer to compost/sand mix:

Eight weeks after weaning experimental plants were re-potted into 250 mL plastic pots (7 x 7 x 8 cm, black, Desch Plantpak Ltd, Essex). Potting mix consisted of one part of non-autoclaved compost (reduced peat mix with added grit from Fargro Ltd, West Sussex) and one part of non-autoclaved sharp sand (Sinclair Horticulture Ltd, Lincoln). Plants were grown in poly-tunnel and watered daily with tap water.

Verticillium dahliae conidial inoculation:

A Verticillium dahliae isolate 12253 provided by the Genetics and Crop Improvement laboratory at EMR was used. The culture was resuscitating from cryostore and cultured at 22°C on Prune Lactose Yeast Agar (PLYA) medium in the dark (Talboys, 1960). After six days, 5 mL of sterile distilled water was pipetted onto the plates. The surface was then rubbed to produce a conidial suspension. Thereafter 0.2 mL of this suspension was pipetted onto new PYLA plates and spread across. Plates were incubated at room temperature and close to a natural solar source for 6 days before harvesting the conidia. A conidial suspension was then prepared with distilled water and filtered through filter paper (Range QL100, Fisher Scientific UK Ltd, Loughborough). The conidial suspension concentration was determined using a haemocytometer.

Twenty days post transplantation onto sandy compost, strawberry plants from all treatment received (20 mL per pot) of a freshly prepared conidial suspension of 4.2×10^5 conidia.mL^{-1} poured on the substrate surface.

Experimental design:

There were seven inoculation treatments (five AMF species, control C-- and control C+-) with four strawberry cultivars ('Elsanta', 'Malling Centenary', 'Red Glory', 'Vibrant') with two different crown sizes (strong and weak; Table 1) each with six replicate plants, giving a total of 336 plants. A randomised stratified block design was used, which was preserved after the plants were transferred to individual pots.

Table 1: Different strawberry runner grades used at the beginning of experiment 3

		Mean crown diameter (mm) ± SE		
Strawberry cultivars	**n**	**Grade 'Strong'**	**Grade 'Weak'**	**P***
Elsanta	21	8.87 ± 0.35	5.20 ± 0.27	P < 0.001
Malling Centenary	21	6.87 ± 0.37	4.42 ± 0.16	P < 0.001
Red Glory	21	5.86 ± 0.24	4.06 ± 0.17	P < 0.001
Vibrant	21	7.79 ± 0.27	4.38 ± 0.13	P < 0.001

*Independent t-test applied for each row (α = 0.05)

Data collection and analysis

*Determination of AMF inoculum infectivity***:**

A most probable number (MPN) bioassay was undertaken to determine the number of infective propagules (*i.e*. infected host root pieces, hyphae and spores) present in each inoculum used for all experiments (Cochran, 1950). For this bioassay *Zea mays* ('F1 Sundance' seeds from Suttons seeds, Devon) was used as trap plant. Plants were watered daily with tap water and roots were harvested after ca. six weeks.

Root samples were cleared in 2% (w/v) potassium hydroxide solution (KOH) for 1 h at 90°C in a water bath. Then roots were rinsed three times with tap water before being submerged for 30 min with 2% (v/v) hydrochloric acid (HCl) at room temperature. Roots were covered with 0.05% (w/v) trypan blue in lactoglycerol (without phenol) for 1 h at 90°C in a water bath (Kormanik & McGraw, 1982). Roots were then placed into a Petri dish with 50% (v/v) glycerol for de-staining and samples were ready for stereomicroscopic observations. Each inoculum was a mixture of infected plant host root fragments, hyphae and spores (estimated inocula Table 2).

Determination of Verticillium wilt inoculum density in field soil:

A field soil sample was initially dried on trays for ca. one week before being sieved through a 2 mm sieve. The detection and estimation of *V. dahliae* in the sieved soil was carried out as Harris *et al.* (1993) on agar selective DOX medium with 2 $g.L^{-1}$ of PGA and 1 $mL.L^{-1}$ of tergitol NPX. The pH was adjustment to 6.4 with KOH prior to adding the agar. Before the

medium was poured, 100 mL of filter sterilized biotin and three antibiotics solution was supplemented to 1 L of DOX medium. This antibiotic and biotin solution was made up into 200 mL of sterile deionised water (DI) water with 0.12 g of streptomycin, 0.12 g of chloramphenicol, 0.12 g of chlortetracycline and 0.012 g of biotin.

The plates were stored in the incubator at 22^{o}C. After four weeks, plates were washed and dried and *Verticillium* wilt colonies were counted using a stereomicroscope. From the counting results the concentration of *V. dahliae* in the soil samples could be calculated. For the field soil used in experiment 1 and 2, inoculum density of *V. dahliae* was estimated to be 22.2 propagules.g^{-1} of field soil.

Table 2: Most probable number (MPN) bioassay results for each AMF specie used in the experiments.

Trials	AMF species	Propagules.mL^{-1}
1,2,3	*F. mosseae*	> 1600
1,2,3	*R. irregularis*	> 1600
1,2,3	*C. claroideum*	> 1600
3	*G. microagregatum*	79
3	*F. geosporum*	360

AMF root length colonisation determination:

Sub-root samples were harvested just prior to transplantation to field soil or sandy compost (Table 3). Root samples of each trial were stained with trypan blue as described above for the MPN test. The percentage of root length colonised (% RLC), as well as frequencies of arbuscules (%) and vesicles (%)vwere recorded at × 250 magnification with a compound microscope equipped with a cross hair eyepiece for a minimum of 100 root intersections per sample (McGonigle *et al.*, 1990). No attempt was made to distinguish between indigenous AMF species potentially introduced with non-autoclaved field soil or compost at the final step of each trial.

The incidence of Verticillium wilt:

In order to assess the protective effect of AMF against wilt, wilt symptoms were scored as follow. The scale was: 0: absence of symptoms, 1: presence of symptoms such as wilted leaf, chlorotic leaf, stunted or dead plant. Time points of disease incidence scoring were summarised in Table 3.

Table 3: Schedule of root sampling for AMF root colonisation and disease incidence assessments.

Stage	Trials	No. of weeks post AMF inoculation (wpi)	
		RLC*	**Disease incidence**
1	1	6 wpi	-
1	2	6 wpi	-
1	3	8 wpi	-
Stage	**Trials**	**No. of weeks post Verticillium wilt inoculation (wpi)**	
		RLC*	**Disease incidence**
2	1	28 wpi	21 wpi, 28 wpi
2	2	20 wpi	17 wpi, 20 wpi
2	3	8 wpi	8 wpi

* Root length colonisation (RLC)

Internal colonisation by Verticillium wilt:

Quantitative real-time PCR (qRT-PCR) methods will be used to quantify pathogen colonisation of plant tissues for experiment 3.

Plant measurements:

Plant growth was measured non-destructively by recording plant height (i.e. the average length of the two longest leaf petioles) for experiment 1 and 2. Plant crown diameter was measured for all experiments. Both measurements were taken using a digital calliper (Designer Habitat Ltd, Manchester). Flowering time was recorded for experiment 1 and 2, but flowers were cut-off for experiment 3 to avoid plant being able to allocate energy for fruiting.

Fruits from experiment 1 and 2 were harvested when ripe. Only the berries of experiment 2 were counted and fresh weighed (yield). At the end of experiment 1 and 2 plant growth was assessed in terms of plant crown diameter and number of runners produced per plant. After the final harvest, shoot, root and runners were destructively harvested, oven-dried at 70°C for ca. 96 h and dry weight was determined. For clarity, the sampling time points of all measurement parameters for each experiment were summarised in Table 4.

Statistical analysis:

All data were analysed using SPSS (IBM SPSS statistics 19). The three experiments were analyses separately. All data sets were tested for normality. Flowering times were analyzed with Kaplan-Meier method using log-Rank test.

General linear model univariate procedure was applied, followed up by a Bonferroni post-hoc test if necessary, to determine significant differences between treatments for the variables: crown diameter, plant height, shoot/root dry weight, berry yield, wilt colonisation and AMF root colonisation.

Root colonisation data were arcsine transformed. Disease incidence data and number of berries produced were analysed for significance using generalised linear model (GLM) with a binomial distribution with logit link function and a Poisson distribution with a log link function respectively. For all statistical analyses the significance level was set at $\alpha = 0.05$.

Table 4: Schedule of plant development parameters assessed during the studies.

		Plant measurements							
Stages	**Trials**	**Plant height**	**Crown diameter**	**Flowering onset**	**Yield**	**No. of fruits**	**No. of runners**	**Shoot dry weight**	**Root dry weight**
1	**1**	(6)	(6)	-	-	-	-	-	-
1	**2**	(6)	(6)	-	-	-	-	-	-
1	**3**	-	(6)	-	-	-	-	-	-
2	**1**	-	[28]	[0~28]	-	-	[28]	[28]	[28]
2	**2**	-	[20]	[0~20]	[0~20]	[0~20]	[28]	[28]	[28]
2	**3**	-	[8]	-	-	-	[8]	[8]	[8]

- Non-recorded

( ) Number of weeks from AMF inoculation

[ ] Number of weeks from wilt inoculation

[0~X] denotes a period from 0 to X weeks

Table 5: Chemical characteristics of the growth substrates used at the start of the experiments, which were carried out by the laboratories of NRM Ltd, on a single measurement of pooled samples

Stages	Trials	Substrates	pH	P	K	Mg	NH_4^+	NO_3^-	Ca^{2+}
				mg/kg					
1	**1**	Vermiculite*	8.09	1.21	36.22	21.93	29.78	<1.21	1.21
1	**2**	Peat/perlite*	6.95	2.11	8.76	40.18	63.14	13.90	25.08
1	**3**	Peat/perlite*	7.24	2.51	6.15	58.38	35.20	39.94	37.71
2	**1+2**	Barming's soil[#]	5.90	31.05	198.63	69.63	0.88	8.11	-
2	**3**	Sand/compost*	6.88	18.4	136.46	42.44	66.93	81.38	82.73

[#] Analyses references: 'Standard soil', code A001 and 'Soil mineral Nitrogen', code A046

* Analysis reference: 'Compost suite 1', code H001

Results

Experiment 1: Micro-propagated derived plants

Six weeks post inoculation the newly formed roots of AMF inoculated treatments were colonised. Different mycorrhizal forms (arbuscules, vesicles, internal hypha, and spores) were observed (Figure 1). The extent of colonisation (RLC), as well the frequency of arbuscules and vesicles were similar for the three AMF species tested (Table 6). The roots of the control C^{--} were AMF free. It is important to note that a single root portion of a non-mycorrhizal control C^{+-} plant contained distinctly stained hypha and arbuscules. This could be due to a remaining AMF spore in the aqueous bacterial filtrate applied to each C^{+-} control plant or a cross-contamination between tray cells during watering.

The proportion of plantlets surviving acclimatisation was 100%. After six weeks, growth parameters of non-mycorrhizal and mycorrhizal plants were variably affected. Figure 2A demonstrated a significant positive effect of *R. irregularis* on the crown diameter compare to the AMF free controls. *F. mosseae* and *C. claroideum* also increased plants crown diameter but the results was not significant compare to the non-mycorrhizal controls. It was important to note that no differences in plant height were observed between non-mycorrhizal controls and AMF inoculated plantlets (Figure 2B).

Figure 3 shows flowering curves obtained for non-mycorrhizal and AMF inoculated plantlet. Log rank test evidenced that there were no differences in the onset of flowering between non-mycorrhizal controls and AMF inoculated plants ($\chi^2 = 4.248$, d.f. = 4, P = 0.373).

Table 6: % colonization of host plant roots in total, in arbuscules and vesicles experiment 1

	RLC (%)	Arbuscules (%)	Vesicles (%)
C^{--}	None	None	None
C^{+-}	0.5 ± 0.5	0.5 ± 0.5	None
F. mosseae	92.5 ± 2.3 a	43.8 ± 3.1 a	21.6 ± 2.7 a
R. irregularis	92.8 ± 1.3 a	46.8 ± 2.5 a	14.0 ± 1.7 a
C. claroideum	93.9 ±1.6 a	39.6 ± 3.9 a	20.3 ± 3.2 a

Non-mycorrhizal controls (C^{--} and C^{+-}) were not included in the analysis. Six weeks post inoculation, no significant difference was found between: the percentage root length colonisation (% RLC) ($F_{2,31} = 0.4$, P = 0.669), arbuscule colonisation, ($F_{2,31} = 1.3$, P = 0.282), vesicle colonisation ($F_{2,31} = 2.5$, P = 0.095) by AMF species. Data are shown as mean ± SE (n = 12). Variables were *arcsine*-root *transformed* to achieve normality.

Appearance of wilt symptoms on leaves were observed 21 weeks post wilt inoculation (Figure 4). The incidence of *Verticillium* wilt in the non-mycorrhizal controls C^{--} and C^{+-} reached 25 and 33.3 % respectively. The incidence of wilt was lower in plants colonised by *F. mosseae* and *C. claroideum* (respectively 8.3 and 16.7 %), but it was similar to the non-mycorrhizal controls with *R. irregularis* (33.3 %). Nevertheless, there was no-significant difference between treatments (GLM; Wald $\chi^2 = 0.21$, d.f. = 2, P = 0.571, Table 7).

Table 7: Effect of AMF colonisation on the incidence degree of Verticillium wilt

	Disease incidence[#]		
Treatments	**Wilt***	**No-wilt**	**Total**
C^{--}	3	9	12
C^{+-}	4	8	12
F. mosseae	1	11	12
R. irregularis	4	8	12
C. claroideum	2	10	12

[#] Recorded 21 weeks after the first contact with soil contaminated with microsclerotia

* Number of wilted plants is not significantly different (P > 0.05) according to GLM with a Binomial distribution; link function: logit.

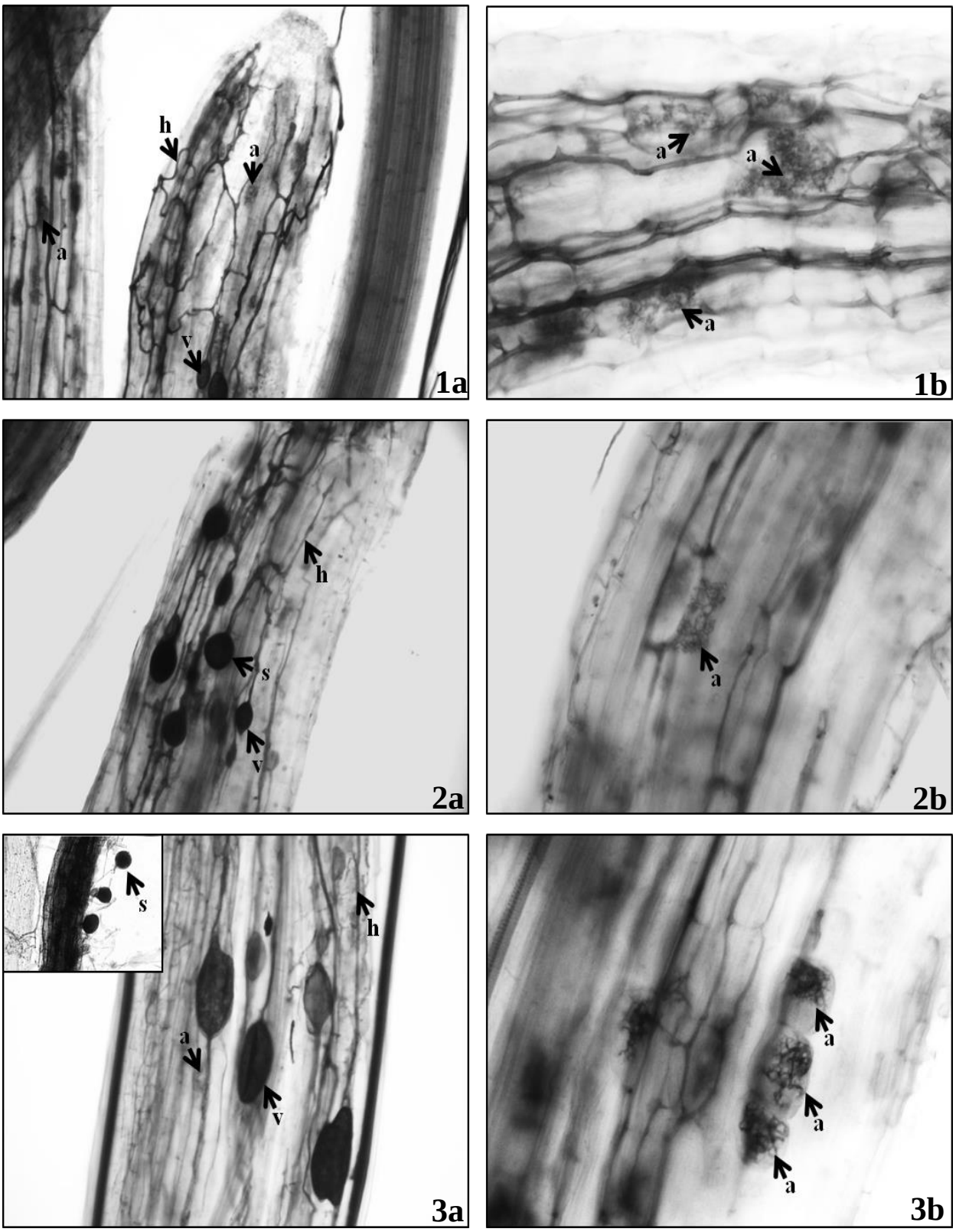

Figure 1: Root infection by AMF of micro-propagated *F. x ananassa* 'EM1996' plantlets after 6 weeks in vermiculite. Longitudinal squash of roots stained with trypan blue (left column x 100, right column x 200). 1a and 1b) colonisation by *F. mosseae*; 2a and 2b) colonisation by R. irregularis; 3a and 3b) colonisation by C. claroideum; a: arbuscule, v: vesicule, h: hypha, s: spore.

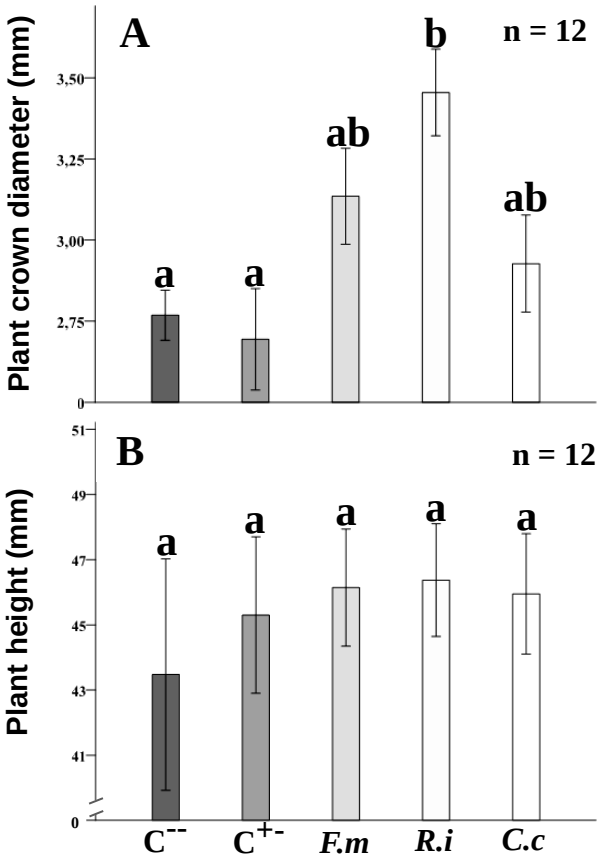

Figure 2: Effect of AMF inoculation on the crown diameter (A) and plant height (B) of *Fragaria* x *ananassa* 'EM1996' from experiment 1, stage 1, measured after 6 weeks post inoculation. Data are shown as mean ± SE (n = 12). Means with the same letter are not significantly different (P > 0.05) according to Bonferroni test. C^{--} contained only autoclaved attapulgite clay, C^{+-} control with bacterial filtrate and autoclaved inoculum mix*, F. mosseae* (*F.m*), *R. irregularis* (*R.i*) and *C. claroideum* (*C.c*).

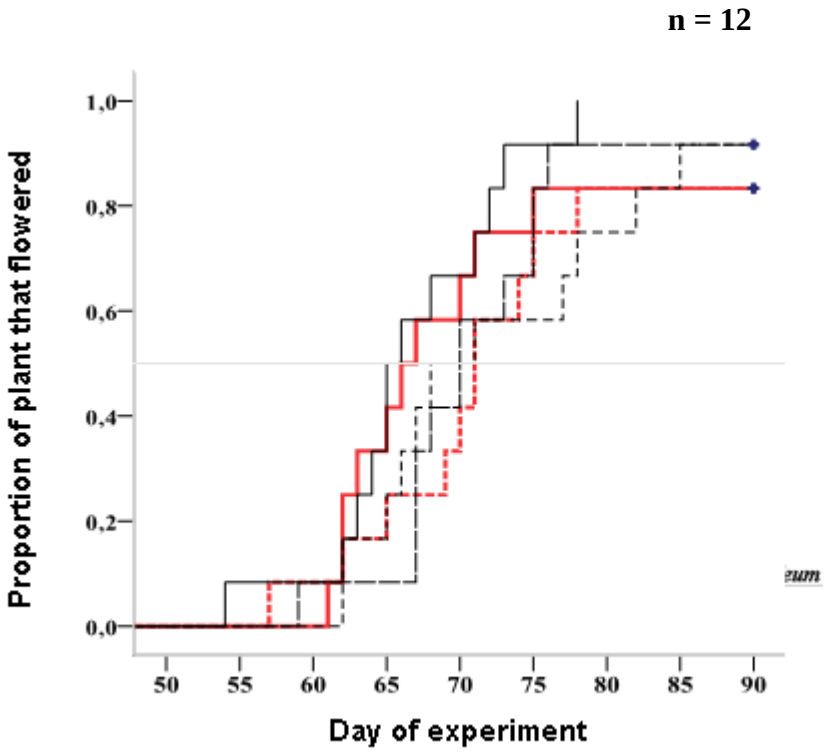

Figure 3: Kaplan-Meier curves showing predicted probability of "flowering" for *Fragaria* x *ananassa* 'EM1996' from experiment 1, stage 2. C^{--} contained only autoclaved attapulgite clay, C^{+-} control with bacterial filtrate and autoclaved inoculum mix, *F. mosseae*, *R. irregularis* and *C. claroideum*.

Figure 4: *Verticillium* wilt symptoms observed 21 weeks post *V. dahliae* inoculation on *F.* x *ananassa* 'EM1996' plants in experiment 1, stage 2: wilted leaves, brown leaves, stunted plants

Experiment 2: Pre-colonisation of runner tips derived plants

Six weeks post inoculation *F. mosseae*, *R. irregularis*, *C. claroideum* had already infected the roots of both strawberry cultivars. Different mycorrhizal forms (arbuscules, vesicles and internal hypha) were distinctly stained (Figure 5). The proportion of runner tip derived plantlets surviving acclimatisation was 100%. Figure 6A demonstrated that the main effect

of AMF treatment on plant crown diameter was not significant ($F_{4,86}$ = 0.65, P = 0.624). There was a significant effect of cultivar on the crown diameter ($F_{1,86}$ = 4.20, P = 0.044). The strawberry cultivar 'Vibrant' presented bigger crowns than 'Red Glory'. There was also a significant interaction between both factors ($F_{4,86}$ = 2.57, P = 0.044). *C. claroideum* significantly increased plant crown diameter for 'Red Glory' but it reduced crown size of 'Vibrant'. Figure 6B confirmed that the main effect of AMF treatment on plant height was not significant ($F_{4,86}$ = 0.56, P = 0.695). 'Vibrant' was significantly higher than 'Red Glory' ($F_{1,86}$ = 7.54, P = 0.007). The interaction of the two factors was close to being significant ($F_{4,86}$ = 2.12, P = 0.086).

Figure 7A and 7B shows flowering curves of controls and AMF inoculated plants obtained from 'Vibrant' and 'Red Glory' respectively. Significance tests evidenced that there were no differences in the onset of flowering between non-mycorrhizal and the three single AMF species tested for 'Vibrant' (log rank test: χ^2 = 8.277, d.f. = 4, P = 0.082) and 'Red Glory' (log rank test: χ^2 = 1.613, d.f. = 4, P = 0.807).

After 17 weeks in contact with field soil containing Verticillium wilt microsclerotia, very few 'Vibrant' plants showed disease symptoms (Figure 8). The incidence of Verticillium wilt in the no-mycorrhizal 'Vibrant' controls C^{--} and C^{+-} reached 10% (Table 8). The incidence of wilt was increased by *F. mosseae* (20 %), but it was not reduced with *R. irregularis* and *C. claroideum* (0%). It is important to note that no wilt symptom was observed for the cultivar 'Red Glory' in all treatments.

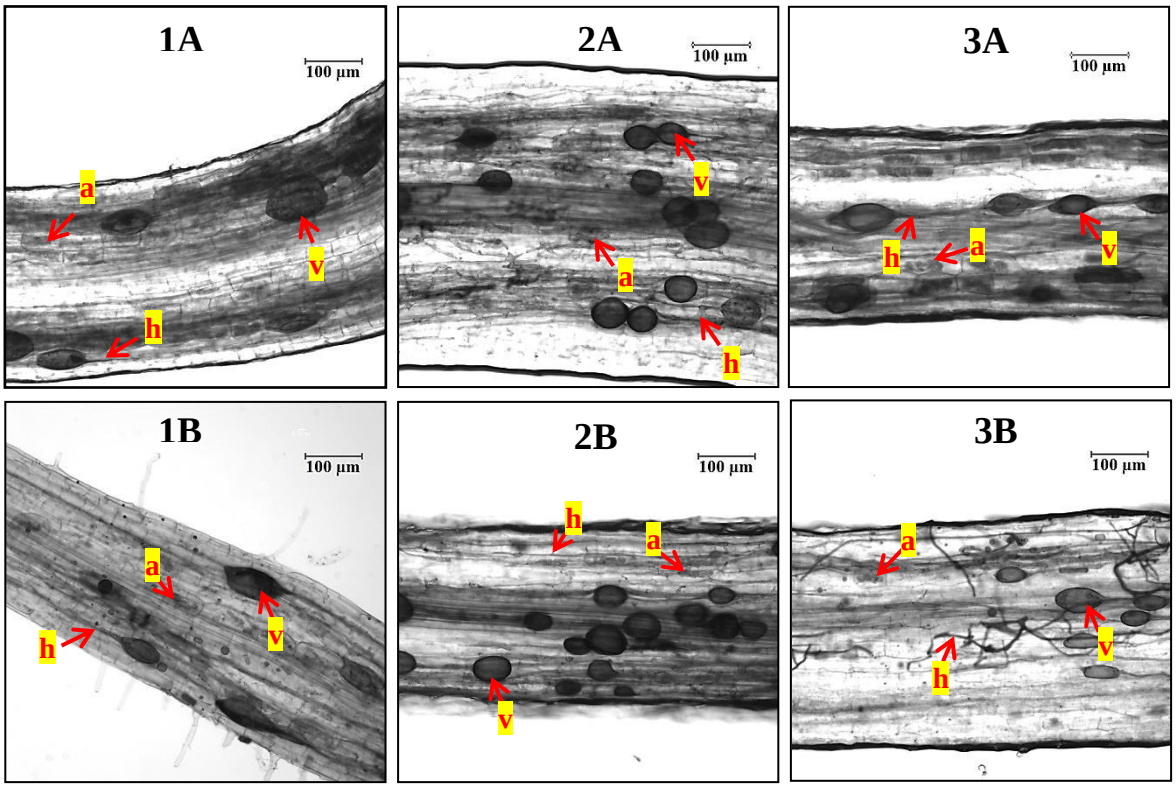

Figure 5: Root infection by AMF of micro-propagated *F. x ananassa* (A) 'Vibrant' and (B) 'Red Glory' after six weeks in peat/perlite, stage 1. Longitudinal squash of roots stained with trypan blue. 1) Colonisation by *F. mosseae*; 2) Colonisation by *R. irregularis*; 3) Colonisation by *C. claroideum*; a: arbuscule, v: vesicule, h: hypha, s: spore

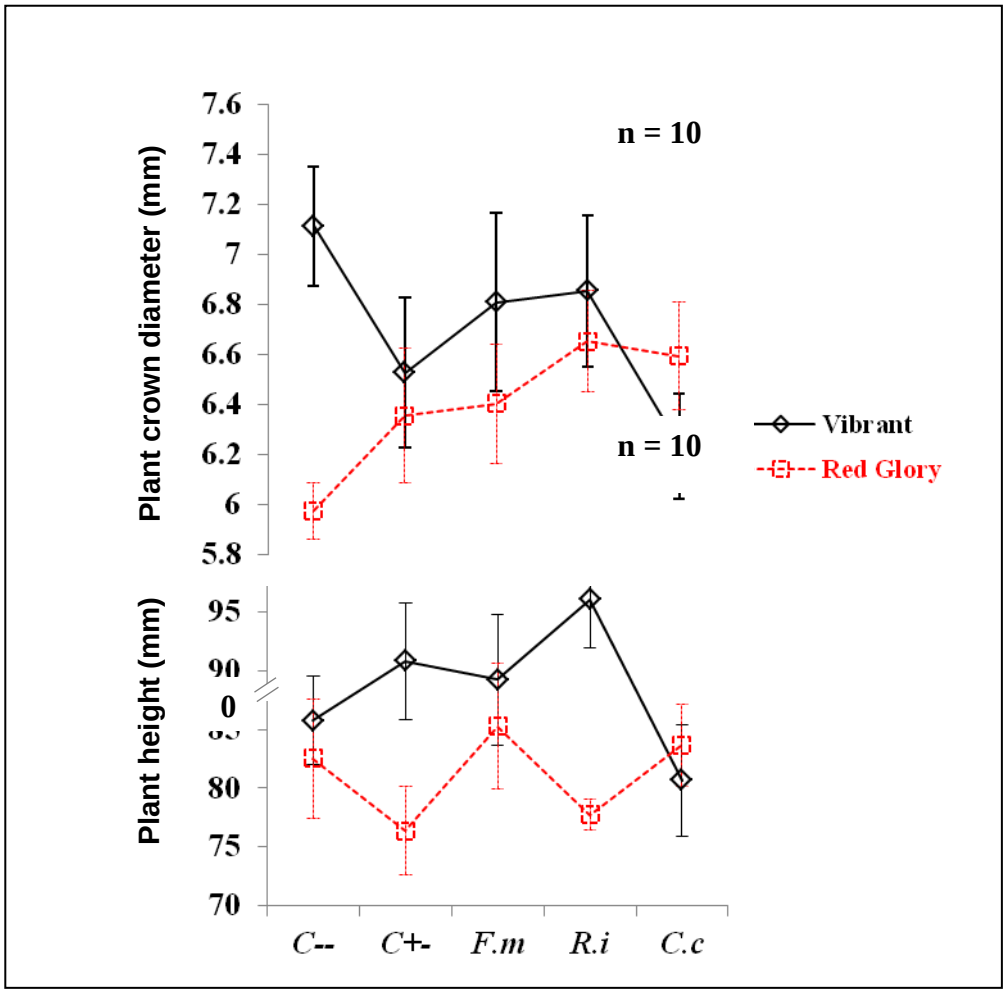

Figure 6: Effect of AMF inoculation on (A) the crown diameter and (B) the plant height of *F.* x *ananassa* 'Vibrant' and 'Red Glory' from experiment 2, stage 1, measured after six weeks post inoculation. Data are shown as mean ± SE (n = 10). Vertical bar represents the standard error of the mean. C^{--} containing only autoclaved attapulgite clay, C^{+-} control with bacterial filtrate and autoclaved inoculum mix, *F. mosseae* (*F.m*), *R. irregularis* (*R.i*) and *C. claroideum* (*C.c*).

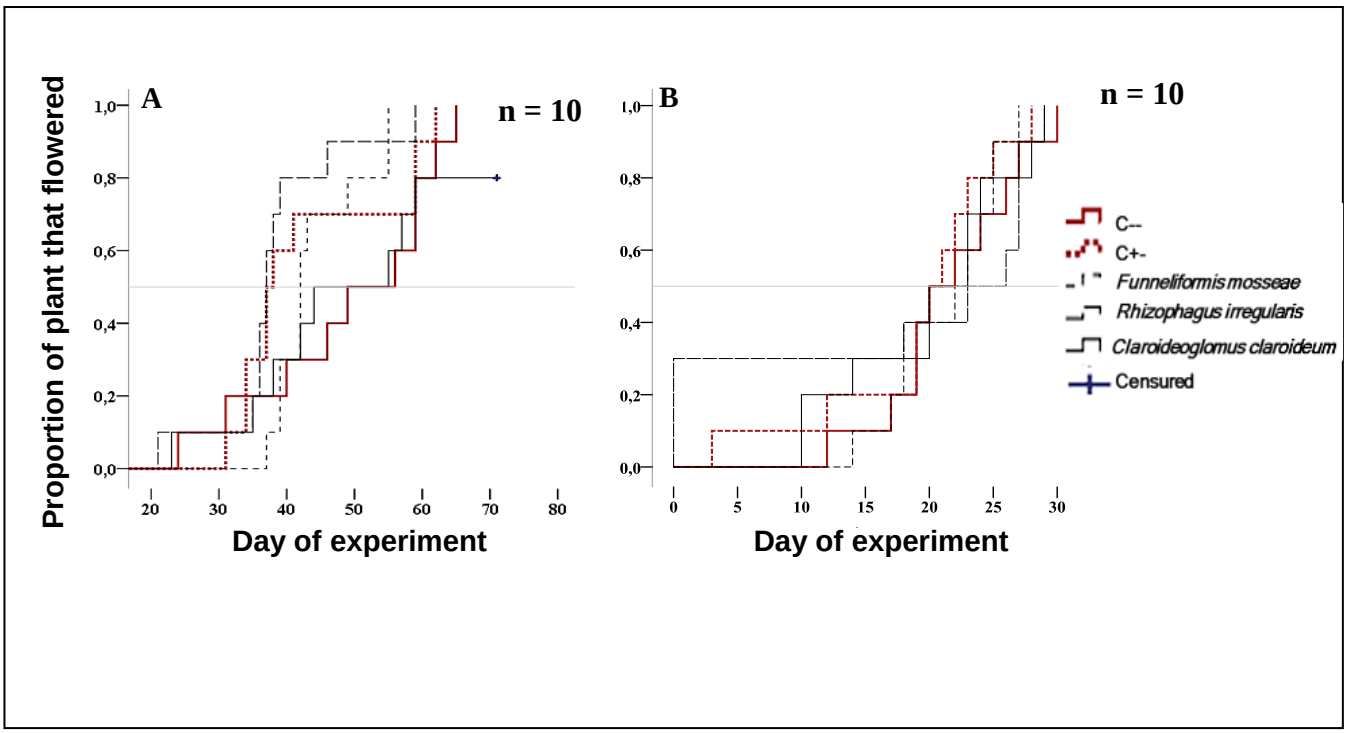

Figure 7: Kaplan-Meier curves showing predicted probability of "flowering" for *F.* x *ananassa* (A) 'Vibrant' and (B) 'Red Glory' from experiment 1, stage 2. C^{--} contained only autoclaved attapulgite clay, C^{+-} control with bacterial filtrate and autoclaved inoculum mix, *F. mosseae*, *R. irregularis* and *C. claroideum*.

Figure 8: *Verticillium* wilt symptoms observed 17 weeks post *V. dahliae* inoculation on 'Vibrant' plants in experiment 1, stage 2: wilted leaves, brown leaves, stunted plants

Experiment 3: Pre-colonisation of runner tips derived plants with different vigour

Table 9 shows the absence of significant differences in crown diameter between non-mycorrhizal and mycorrhizal treatments. There were significant differences in crown size between strawberry cultivars 'Vibrant' > 'Elsanta' > 'Malling Centenary' > 'Red Glory' (Table 9). The runner tips of grade 'Strong' were significantly bigger than the runner tips of grade 'Weak' for each cultivar respectively (Table 1 and 9). No interaction between the previous variables was reported.

Table 8: Disease incidence on *F.* x *ananassa* 'Vibrant' assessed as presence (1)/absence (0) of visible *Verticillium* wilt symptoms

	Disease incidence[#]		
Treatments	**Wilt***	**No-wilt**	**Total**
C^{--}	**1**	9	10
C^{+-}	**1**	9	10
F. mosseae	**2**	8	10
R. irregularis	**0**	10	10
C. claroideum	**0**	10	10

[#] Recorded 17 weeks after the first contact with soil contaminated with microsclerotia

* No statistics applied

Table 9: Results of the general linear model univariate analysis of the crown diameter of four different strawberry cultivars 'Elsanta', 'Malling Centenary', 'Red Glory', 'Vibrant' with two different grade of runners (i.e. 'Strong' and 'Weak') without AMF inoculation (C^{--} and C^{+-}) or inoculated with *F. mosseae*, *R. irregularis*, *C. claroideum*, *G. microagregatum* and *F. geosporum*, measured after 6 weeks post inoculation.

		Mean square [a]
Source	**d.f.**	**Crown**
Block	5	**4.91***
Treatment (T)	5	2.03
Cultivar (C)	3	**54.03****
Runner grade	1	**292.53****
T x C	18	2.35
T x Rg	6	1.64
C x Rg	3	3.04
T x C x Rg	18	2.63
Error	253	2.16

a Mean squares followed by one (*) or two (**) asterisks were statistically significant at $P < 0.05$ or $P < 0.01$ respectively.

Discussion

Results are from the research in Year 1 and need to be confirmed and augmented with further results from larger field trials to be conducted in 2015.

Conclusions

- We have shown that AMF could colonise in-vitro derived plantlets in vermiculite and runner tips derived plants in a peat/perlite based substrate.
- The high moisture conditions maintained during plants' weaning allowed AMF root colonisation.
- The effects of the symbiosis on plant growth were variable. All AMF species tested on the tissue culture derived plant 'EM1996' increased the crown diameter of the plantlets but this size increase was only significant with R. irregularis.
- For the runner tip derived plants, AMF inoculation affected the crown diameter differently with positive, neutral or negative effects being reported.

- In experiment 2, interaction between AMF C. claroideum and the strawberry cultivar ‘Red Glory’ resulted in a significant increase of plant crown size, compared to the non-mycorrhizal control.
- In contrast C. claroideum reduced the crown diameter of ‘Vibrant’. However, this pattern was not reported in experiment 3.
- It was interesting to note that AMF never influenced the onset of flowering.
- Finally, evidence of a reduction of wilt incidence was reported during experiment 1, but it was not statistically significant.
- The low level of disease and the nutrient deficiency symptoms indicated the necessity to adopt a different wilt inoculation technique (e.g. conidial suspension) and substrate (e.g. sandy compost).
- Field trials with a larger sample size are also required to draw robust conclusions concerning the effect of mycorrhizal pre-colonisation against Verticillium wilt.

Knowledge and Technology Transfer

The student attended the AHDB PhD and Fellowship conference held in York in September 2014.

Glossary

None

References

Abiala M, Popoola O, Olawuyi O, Oyelude J, Akanmu A, Killani A, Osonubi O, Odebode A. 2013. Harnessing the potentials of vesicular arbuscular mycorrhizal (VAM) fungi to plant growth–a review. *International Journal of Pure and Applied Sciences and Technology* 14: 61-79.

Atallah Z, Bae J, Jansky S, Rouse D, Stevenson W. 2007. Multiplex real-time quantitative PCR to detect and quantify *Verticillium dahliae* colonization in potato lines that differ in response to *Verticillium* wilt. *Phytopathology* 97: 865-872.

Bååth E, Hayman D. 1983. Plant growth responses to vesicular-arbuscular mycorrhiza. *New Phytologist* 95: 419-426.

Borkowska B. 2002. Growth and photosynthetic activity of micropropagated strawberry plants inoculated with endomycorrhizal fungi (AMF) and growing under drought stress. *Acta physiologiae plantarum* 24: 365-370.

Caron M, Fortin JA, Richard C. 1985. Influence of substrate on the interaction of *Glomus intraradices* and *Fusarium oxysporum* f. sp. *radicis-lycopersici* on tomatoes. *Plant and Soil* 87: 233-239.

Caron M, Parent S 1987. Definition of a peat-lite medium for the use of vesicular-arbuscular mycorrhizae (VAM) in horticulture. *Symposium on Horticultural Substrates and their Analysis* 221: 289-294.

Chávez MG, Ferrera-Cerrato R. 1990. Effect of vesicular-arbuscular mycorrhizae on tissue culture-derived plantlets of strawberry. *HortScience* 25: 903-905.

Daft M, Okusanya B. 1973. Effect of Endogone mycorrhiza on plant growth VI. Influence of infection on the anatomy and reproductive development in four hosts. *New Phytologist* 72: $1333^{-1}339$.

de Silva A, Patterson K, Mitchell J. 1996. Endomycorrhizae and Growth of 'Sweetheart' Strawberry Seedlings. *HortScience* 31: 951-954.

Debnath SC, Teixeira da Silva J. 2007. Strawberry culture in vitro: applications in genetic transformation and biotechnology. *Fruit, Vegetable and Cereal Science and Biotechnology* 1: $1^{-1}2$.

Dunne M, Fitter A. 1989. The phosphorus budget of a field-grown strawberry (*Fragaria* x *ananassa* cv. Hapil) crop: evidence for a mycorrhizal contribution. *Annals of Applied Biology* 114: $185^{-1}93$.

Duvert P, Perrin R, Plenchette C. 1990. Soil receptiveness to VA mycorrhizal association: concept and method. *Plant and soil* 124: 1-6.

Fan L, Dalpé Y, Fang C, Dubé C, Khanizadeh S. 2011. Influence of arbuscular mycorrhizae on biomass and root morphology of selected strawberry cultivars under salt stress. *Botany* 89: 397-403.

FAOSTAT. [WWW document] URL http://faostat.fao.org/site/567/DesktopDefault.aspx?PageID=567#ancor/. [accessed 26 November 2013].

Garmendia I, Goicoechea N, Aguirreolea J. 2004. Effectiveness of three *Glomus* species in protecting pepper (*Capsicum annuum* L.) against *Verticillium* wilt. *Biological Control* 31: 296-305.

Goicoechea N, Garmendia I, Sanchez-Diaz M, Aguirreolea J. 2010. Arbuscular mycorrhizal fungi (AMF) as bioprotector agents against wilt induced by *Verticillium* spp. in pepper. *Spanish Journal of Agricultural Research* 8: 25-42.

Harris DC, Yang JR, Ridout MS. 1993. The detection and estimation of *Verticillium dahliae* in naturally infested soil. *Plant Pathology* 42: 238-250.

Hernández-Sebastià C, Piché Y, Desjardins Y. 1999. Water relations of whole strawberry plantlets in vitro inoculated with *Glomus intraradices* in a tripartite culture system. *Plant science* 143: 81-91.

Hewitt EJ, Bureaux CA. 1966. Sand and water culture methods used in the study of plant nutritionTechnical Communication 22. Farnhan Royal Commonwealth Agricultural Bureau, Bucks, England.

Holevas C. 1966. Effect of vesicular-arbuscular mycorrhiza on uptake of soil phosphorus by strawberry (*Fragaria* sp. var Cambridge Favourite). *Journal of horticultural science & Biotechnology* 41: 57.

HSE. [WWW document] URL http://www.pesticides.gov.uk/guidance/industries/pesticides/topics/pesticide-approvals/enforcement/what-is-the-approval-status-of-methyl-bromide [accessed 26 Augsut 2014].

Indrasumunar A, Gresshoff PM. 2013. Vermiculite's strong buffer capacity renders it unsuitable for studies of acidity on soybean (*Glycine max* L.) nodulation and growth. *BMC research notes* 6: 465.

Karagiannidis N, Bletsos F, Stavropoulos N. 2002. Effect of *Verticillium* wilt (*Verticillium dahliae* Kleb.) and mycorrhiza (*Glomus mosseae*) on root colonization, growth and nutrient uptake in tomato and eggplant seedlings. *Scientia Horticulturae* 94: 145-156.

Karajeh M, Al-Raddad A. 1999. Effect of VA Mycorrhizal Fungus (*Glomus mosseae* Gerd and Trappe) on *Verticillium dahliae* Kleb. of Olive. *Dirasat: Agricultural sciences* 26: 338-341.

Kiernan J, Hendrix J, Stoltz L, Maronek D. 1984. Characterization of strawberry plants produced by tissue culture and infected with specific mycorrhizal fungi. *HortScience* 19.

Kormanik PP, McGraw AC. 1982. Quantification of vesicular-arbuscular mycorrhizae in plant roots. *In Methods and Principles of Mycorrhizal Research* (Ed. by N. C. Schenck), pp. 37-45. The American Phytopathological Society, St Paul, Minnesota.

Liu R-J. 1995. Effect of vesicular-arbuscular mycorrhizal fungi on *Verticillium* wilt of cotton. *Mycorrhiza* 5: 293-297.

Lovelidge B. 2004. Strawberry wilt tests confirm widespread infection. *The Fruit Grower* 2: 18-19.

Ma B-h, Zhen W-c, Cao K-q, Wu Y-h. 2004. A primary study on effect of VAM fungi to strawberry and *Verticillium* wilt. *Journal of Agricultural University of Hebei* 4: 19.

Marschner P, Timonen S. 2005. Interactions between plant species and mycorrhizal colonization on the bacterial community composition in the rhizosphere. *Applied soil ecology* 28: 23-36.

Martin FN. 2003. Development of Alternative Strategies for Management of Soilborne Pathogens Currently Controlled with Methyl Bromide 1. *Annual review of phytopathology* 41: 325-350.

Martin FN, Bull CT. 2002. Biological approaches for control of root pathogens of strawberry. *Phytopathology* 92: 1356-1362.

McGonigle TP, Miller MH, Evans DG, Fairchild GL, Swan JA. 1990. A new method which gives an objective measure of colonization of roots by vesicular-arbuscular mycorrhizal fungi. *New phytologist* 115: 495-501.

Murashige T, Skoog F. 1962. A revised medium for rapid growth and bio-assays with tobacco tissue cultures. *Physiologia plantarum* 15: 473-497.

Murphy JG, Rafferty SM, Cassells AC. 2000. Stimulation of wild strawberry (*Fragaria vesca*) arbuscular mycorrhizas by addition of shellfish waste to the growth substrate: interaction

between mycorrhization, substrate amendment and susceptibility to red core (*Phytophthora fragariae*). *Applied soil ecology* 15: 153-158.

Niemi M, Vestberg M. 1992. Inoculation of commercially grown strawberry with VA mycorrhizal fungi. *Plant and Soil* 144: 133-142.

Norman J, Hooker JE. 2000. Sporulation of *Phytophthora fragariae* shows greater stimulation by exudates of non-mycorrhizal than by mycorrhizal strawberry roots. *Mycological Research* 104: 1069-1073.

Nursery T. 1992. Effects of vesicular-arbuscular mycorrhizal fungi on the development of *Verticillium* and *Fusarium* wilts of alfalfa. *Plant Disease* 76: 239.

Porras-Soriano A, Marcilla-Goldaracena I, Soriano-Martín M, Porras-Piedra A. 2006. Development and resistance to *Verticillium dahliae* of olive plantlets inoculated with mycorrhizal fungi during the nursery period. *The Journal of Agricultural Science* 144: 151-157.

Ristaino JB, Thomas W. 1997. Agriculture, methyl bromide, and the ozone hole: Can we fill the gaps? *Plant Disease* 81: 964-977.

Rowley D, Black B, Drost D. 2010. Strawberry plug plant production. Master thesis, Utah State University, Logan, UT, USA.

Smith SE, Read DJ. 2008. *Mycorrhizal symbiosis*. San Diego, CA, USA: Academic Press.

Tahmatsidou V, O'Sullivan J, Cassells AC, Voyiatzis D, Paroussi G. 2006. Comparison of AMF and PGPR inoculants for the suppression of *Verticillium* wilt of strawberry (*Fragaria* x *ananassa* cv. Selva). *Applied soil ecology* 32: 316-324.

Talboys PW. 1960. A culture-medium aiding the identification of *Verticillium Albo-Atrum* and *V. dahliae*. *Plant pathology* 9: 57-58.

Varma A, Schüepp H. 1994. Infectivity and effectiveness of *Glomus intraradices* on micropropagated plants. *Mycorrhiza* 5: 29-37.

Vestberg M. 1992a. The effect of growth substrate and fertilizer on the growth and vesicular-arbuscular mycorrhizal infection of three hosts. *Agricultural Science in Finland* 1: 95-105

Vestberg M. 1992b. The effect of vesicular-arbuscular mycorrhizal inoculation on the growth and root colonization of ten strawberry cultivars. *Agricultural Science in Finland* 1: 527-535.

Wang B, Qiu Y-L. 2006. Phylogenetic distribution and evolution of mycorrhizas in land plants. *Mycorrhiza* 16: 299-363.

Appendices

Appendix 1 – Images of plant nutrient deficiency symptoms

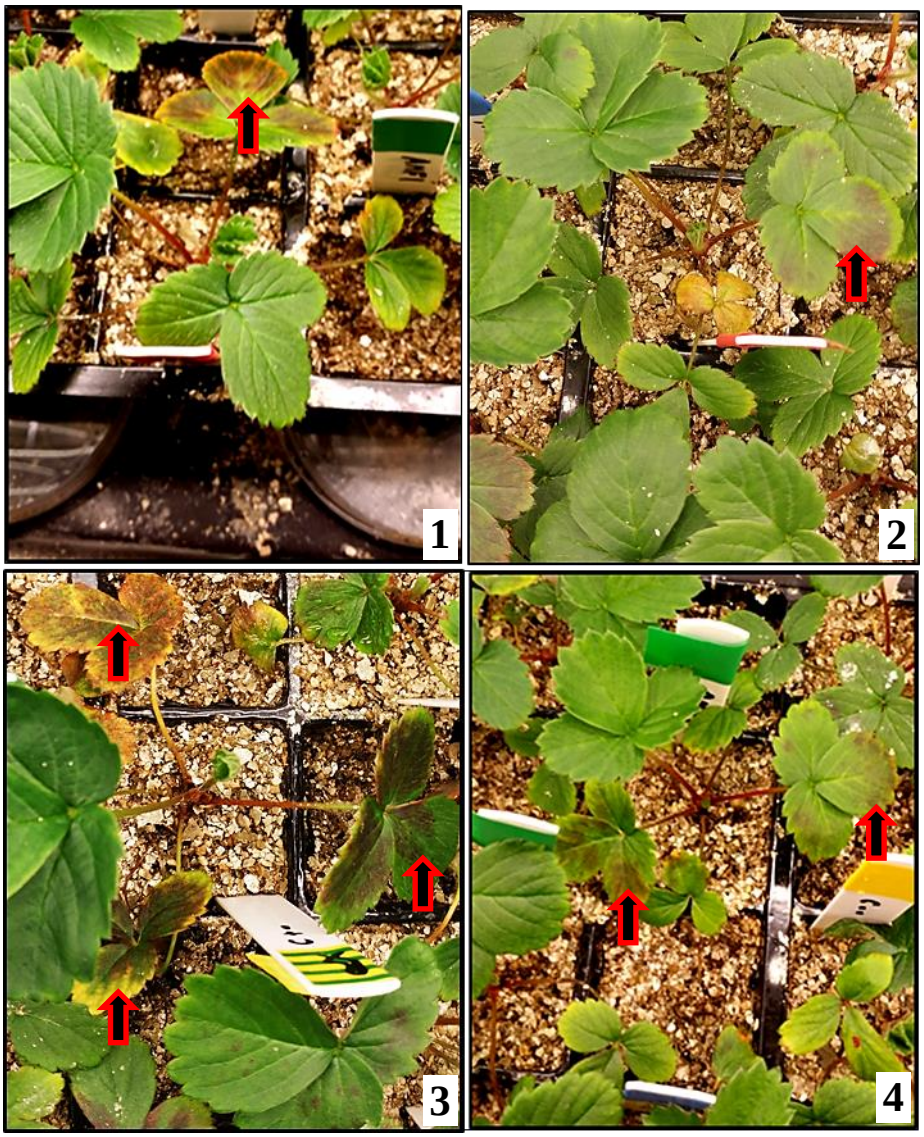

Figure s1: Plant nutrient deficiency symptoms (arrows) observed 3 weeks post transplantation in vermiculite (likely due to nitrogen deficiency, see Maas, 1998*). 1-2) Plants inoculated with *R. irregularis*, 3) Non-mycorrhiza plant (control C^{+-}), 4) Plant inoculated with *F. mosseae*. NB: The control C^{--} and plant inoculated with *C. claroideum* showed the same symptoms (not shown). Symptoms were alleviated following addition of half-strength Rorinson's nutrient solution without phosphate.

*Maas JL, 1998. Compendium of Strawberry Diseases. APS Press, St. Paul, MN.