

Influence of colonization by arbuscular mycorrhizal fungi and a root endophyte on  
selected strawberry cultivars under salt conditions

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I would like to dedicate this thesis to Dame Jane Goodall:  
“My mission is to create a world where we can live in harmony with nature.”

## Abstract

Two factorial greenhouse experiments were performed to determine the effects of four arbuscular mycorrhizal fungi (AMF) species (*Glomus arenarium*, *Funneliformis caledonius*, *F. mosseae*, and *Rhizophagus irregularis*) and a root endophyte (*Piriformospora indica*) on four 'day-neutral' strawberry (*Fragaria × ananassa* Duch.) cultivars ('Albion', 'Charlotte', 'Mara des Bois', and 'Seascape'), and mixed-AMF species (*R. irregularis* + *F. mosseae*) on cv. 'Seascape', under salt conditions (0-200 mM NaCl). In its biomass, 'Seascape' was more tolerant to salinity than the other cultivars. Cultivars responded differently to fungal inoculation as to salinity. *G. arenarium* had a negative effect on plant growth and 'Mara des Bois' responded negatively to inoculation. Among the remaining inoculants and cultivars, fungal-symbiosis was beneficial to growth. *R. irregularis* alleviated the symptoms of salt stress and improved fruit quality to a higher degree than the other AMF species and the root endophyte. Our results support the use of bio-inoculants in salty horticultural areas.

## Résumé

Deux expériences en serre de type factoriel ont été réalisées afin de déterminer les effets de quatre espèces de champignons mycorhiziens arbusculaires (CMA: *Glomus arenarium*, *Funneliformis caledonius*, *F. mosseae* et *Rhizophagus irregularis*) et d'une endophyte racinaire (*Piriformospora indica*) sur quatre cultivars de fraisier à jour neutre (*Fragaria* × *ananassa* Duch., cv 'Albion', 'Charlotte', 'Mara des Bois' et 'Seascape'), ainsi qu'un mélange de deux CMA (*R. irregularis* + *F. mosseae*) sur le cultivar 'Seascape', en conditions salines (0 - 200 mM NaCl). Avec sa biomasse la plus élevée, 'Seascape' était plus tolérante à la salinité que les autres cultivars. Comme pour la salinité, les cultivars ont répondu différemment aux CMA et à l'endophyte racinaire. *Glomus arenarium* a eu un effet négatif sur la croissance des plantes et 'Mara des Bois' a répondu négativement à l'inoculation. Parmi les autres cultivars et inoculants, la symbiose a été bénéfique à la croissance. *R. irregularis* a atténué les symptômes de stress salin et amélioré la qualité des fruits en comparaison aux autres espèces de CMA et à l'endophyte racinaire. Nos résultats supportent l'utilisation de bio-inoculants dans les milieux horticoles salins.

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## Table of Contents

Dedication .....	ii
Abstract .....	iii
Résumé.....	iv
Acknowledgements.....	v
Table of Contents .....	vi
List of Figures .....	viii
List of Tables .....	x
List of Abbreviations .....	xi
Chapter 1: Introduction .....	1
Salinity.....	1
<u>Arbuscular mycorrhizal fungi</u> .....	2
<u>Mycorrhizal fungus and root endophyte inoculants</u> .....	4
<u>Strawberry cultivars</u> .....	7
<u>Objectives, rationale, and hypotheses</u> .....	10
Chapter 2: Materials and Methods.....	11
<u>First experiment</u> .....	11
<u>Propagation of fungal inoculants</u> .....	11
<u>Cultivar selection</u> .....	12
<u>Experimental conditions and plant materials</u> .....	12
<u>Inoculation and salt treatments</u> .....	13
<u>Data collection and measurement</u> .....	13
<u>Mycorrhizal colonization determination</u> .....	14
<u>Statistical analyses</u> .....	14
<u>Second experiment</u> .....	15
<u>Propagation of AMF</u> .....	15
<u>Cultivar selection</u> .....	16
<u>Experimental conditions and plant materials</u> .....	16

__ Inoculation and salt treatments .....	17
__ Data collection and measurement .....	17
__ Root architecture analysis .....	17
__ Fruit quality analysis .....	18
__ Statistical analyses .....	18
Chapter 3: Results .....	19
__ 3.1 First experiment .....	19
__ Mycorrhizal colonization .....	19
__ Shoot and root fresh and dry mass, shoot water content, and root/shoot ratio .....	22
__ Fruit yield .....	25
__ 3.2 Second experiment .....	28
__ Mycorrhizal colonization .....	28
__ Shoot and root fresh mass .....	31
__ Fruit yield .....	38
__ Fruit quality .....	39
__ Root architecture .....	45
__ 3.3 Stolon effect .....	57
Chapter 4: Discussion and Conclusions .....	61
Recommendations and perspectives .....	70
References .....	72

## List of Figures

### First experiment

- Fig. 1.** Root colonization percentages by four AMF species and a root endophyte, *P. indica*, in four strawberry cultivars.
- Fig. 2A-D.** Effect of four AMF species and a root endophyte, *P. indica*, on the fresh mass, dry mass, and water content of shoots in four strawberry cultivars (**A.** 'Albion', **B.** 'Charlotte', **C.** 'Mara des Bois', **D.** 'Seascape').
- Fig. 3.** Effect of salinity on fruit mass in four strawberry cultivars.
- Fig. 4.** Effect of four AMF species and a root endophyte, *P. indica*, on fruit mass in four strawberry cultivars.

### Second experiment

- Fig. 5.** Mean root colonization percentages by three AMF species, plus a mixed-AMF species in 'Seascape'.
- Fig. 6A-C.** Effect of AMF and salinity on fresh mass of plant organs in three strawberry cultivars (**A.** 'Albion', **B.** 'Charlotte', **C.** 'Seascape').
- Fig. 7.** Effect of salinity on fresh mass distribution of plant organs in three strawberry cultivars.
- Fig. 8.** Effect of salinity on the soluble solids content of fruits in three strawberry cultivars.
- Fig. 9.** Effect of salinity on the ratio of soluble solids content to titratable acidity of fruits in three strawberry cultivars.
- Fig. 10.** Effect of salinity on root surface area in three strawberry cultivars.
- Fig. 11.** Effect of salinity on root length in three strawberry cultivars.
- Fig. 12.** Effect of salinity on root volume in three strawberry cultivars.
- Fig. 13.** Effect of salinity on the distribution of root diameter classes in three strawberry cultivars.
- Fig. 14A-C.** Effect of AMF and salinity on root surface area in three strawberry cultivars (**A.** 'Albion', **B.** 'Charlotte', **C.** 'Seascape').

## **Stolon effect**

**Fig. 15.** Effect of stolon treatment on fresh mass of plant organs in three strawberry cultivars.

**Fig. 16.** Effect of stolon treatment on fresh mass distribution of plant organs in three strawberry cultivars.

## List of Tables

### First experiment

- Table 1.** Three-way ANOVA for salinity, AMF or root endophyte inoculation, and cultivar treatments and their interactions on mycorrhizal colonization.
- Table 2.** Three-way ANOVA for salinity, AMF or root endophyte inoculation, and cultivar treatments and their interactions on biomass parameters.

### Second experiment

- Table 3.** Three-way ANOVA for salinity, AMF, and cultivar treatments and their interactions on mycorrhizal colonization.
- Table 4.** Three-way ANOVA for salinity, AMF, and cultivar treatments and their interactions on biomass parameters.
- Table 5.** Three-way ANOVA for salinity, AMF, and cultivar treatments and their interactions on the fruit quality parameters: soluble solids content, titratable acidity, and their ratio.
- Table 6.** Fruit soluble solids content, titratable acidity, and their ratio, of strawberry plants treated with or without AMF and salinity.
- Table 7.** Interactions among AMF and salinity on fruit soluble solids content, titratable acidity, and their ratio, of strawberry cultivars.
- Table 8.** Three-way ANOVA for salinity, AMF, and cultivar treatments and their interactions on root parameters.
- Table 9.** Root parameter measurements of strawberry plants treated with or without AMF and salinity.

### Stolon effect

- Table 10.** Four-way ANOVA for salinity, AMF, cultivar, and stolon treatments and their interactions on biomass parameters.

## List of Abbreviations

AMF	Arbuscular mycorrhizal fungi
ANOVA	Analysis of variance
DAOM	Department of Agriculture (Mycology), Plant Research Institute, Ottawa, Canada
LSD	Least significant difference
PVLG	Polyvinyl alcohol-lactic acid glycerol
P-Ca	Prohexadione-Ca
SRL	Specific root length
SSC	Soluble solids content
TA	Titrateable acidity

## Chapter 1: Introduction

### Salinity

Plants are exposed to various environmental conditions and stressors. Abiotic stressors, such as drought, salinity, extreme temperatures, and metal and chemical toxicity are serious threats to agriculture (Audet and Charest, 2007, 2009; Subramanian and Charest, 2008). These stressors lead to a series of morphological, physiological, and molecular changes that adversely affect plant growth and productivity (Wang *et al.*, 2001). Salinity is considered one of the most limiting factors on plant growth.

Agricultural soils that are salty or subjected to salinity limit crop production and account for more than 70% of all agricultural soils worldwide (Jain *et al.*, 1989). Increased salinization of arable lands is expected to have devastating global effects, resulting in up to 30% land loss by the year 2050 (Wang *et al.*, 2003).

The accumulation of salt in cultivated soils is mainly a result of inappropriate irrigation and climate warming. High levels of salinity, such as >40 mM NaCl or >0.1% soil content (Richards, 1954; Juniper and Abbott, 1993), in soils are mainly due to the soluble salts in irrigation water and fertilizers used in agriculture (Abrol, 1986; Copeman *et al.*, 1996, Al-Karaki, 2000), low precipitation, high temperature, and over-exploitation of water resources (Cantrell and Lindermann, 2001; Al-Karaki, 2006; Mouk and Ishii, 2006).

Most crops grow poorly under saline water and soil. Plant adaptation to hyperosmotic environments is generally associated with reduced growth and ultimately yield loss, making the pursuit of agriculture difficult (Orsini *et al.*, 2012). Salt stress has osmotic, nutritional, and toxic effects that prevent growth in a lot of species (Hasegawa *et al.*, 1986). Reduction in growth response to salinity is usually associated with either ion toxicity or low osmotic potential. Salt stress can affect the plant by disrupting its physiological mechanisms such as decreasing photosynthetic efficiency, gas exchange, membrane disruption, and water status. Symptoms of salt injury generally include loss of turgidity and increased susceptibility to disease, often due to cellular damage.

Strawberry (*Fragaria × ananassa* Duch.) is considered as a plant species particularly susceptible to salt stress (Maas and Hoffman, 1977; Levitt, 1980; Schwarz, 1995; Martínez Barroso and Alvarez, 1997). *Fragaria × ananassa* is by far the most important cultivated species of strawberry. Its root system is rather weak and shallow, with 70% of its root system in the upper 7cm soil layer, where salts tend to accumulate. It prefers light, sub acid soil (pH 5.5-6.5) and suffers from salty soil and water (from both Na<sup>+</sup> and Cl<sup>-</sup>). Reductions in fruit yield due to salinity are first observed between 10-15 mM NaCl, with a decrease in yield of 11-33% for each increasing 10 mM NaCl (FAO, 1998).

### **Arbuscular mycorrhizal fungi**

Arbuscular mycorrhizal fungi (AMF) are obligate symbionts that colonize plant roots and modulate growth in many ways, feeding on the products of photosynthesis from their host

plants. The existence of AMF in salt-laden crops is very common (Juniper and Abbott, 1993). AMF are able to enhance plant growth and production in such salty soils (Al-Karaki *et al.*, 2001; Daei *et al.*, 2009; Benothmane, 2011). Different species of AMF differ in their tolerance to stress.

AMF germination and hyphal growth from spores in the soil was shown to be stimulated by signaling compounds released by roots (Akiyama *et al.*, 2005). The initial hyphal structures called appresoria are the entry points into the rhizodermis and the AMF continue to grow into the root forming intraradical hyphae arbuscules. Arbuscules are surrounded by an invaginated cell membrane, then remaining within the apoplast. The fungus also forms vesicles, swollen structures that act as food storage organs. Hyphae grow out into the soil forming a branched mycelium that functions to give access to a much larger volume of soil than the root itself and take up mineral nutrients. Spores are formed by this extraradical mycelium, completing the life cycle (Smith and Read, 2008).

Plant salt tolerance may involve enhanced nutrient acquisition (eg. P, N, Mg, and Ca), maintenance of the K<sup>+</sup>:Na<sup>+</sup> ratio, biochemical changes (accumulation of proline, betaines, polyamines, carbohydrates, and antioxidants), physiological changes (photosynthetic efficiency, relative permeability, water status, abscissic acid accumulation), molecular changes (eg. expression of genes: PIP, Na<sup>+</sup>/H<sup>+</sup> antiporters, Lsnced, Lslea, and LsP5CS), and ultrastructural changes (Yokoi *et al.*, 2002). The role of AMF in alleviating salt stress is well documented (Evelin *et al.*, 2009; Miransari, 2010). AMF can selectively take up elements such as K and Ca

which act as osmotic equivalents, while they avoid uptake of toxic Na, which can alleviate salt stress in plants.

Strawberry growth response to inoculation depends on cultivar-AMF species combinations (Khanizadeh *et al.*, 1995; Taylor and Harrier, 2001). Additionally, AMF show preference for specific environmental conditions (Davies *et al.*, 2002). Because beneficial combinations would maximize AMF-derived benefits, it may be profitable to identify the AMF inoculants most appropriate for a given cultivar in a given environment.

Biodiversity is related to higher plant productivity; AMF species are functionally different and their impact on a host plant may be complementary (Hart and Klironomos, 2002). A diversity of AMF species may allow AMF populations to better adapt to stress conditions (Koomen *et al.*, 1987). Thus, a multiple-species inoculum could be superior to a single-species inoculum under salt conditions.

## **Mycorrhizal fungus and root endophyte inoculants**

*Glomus* is a genus name of AMF classified in the Glomeraceae family in the Glomeromycota phylum. It comprises ca. 53% of all AMF described to date. *Glomus* species are found in nearly all terrestrial habitats, including arable land, deserts, grasslands, tropical forests, and tundras. *Glomus* earned its name from the spherical appearance of its spores. *Glomus* is the largest genus among the AMF with ca. 85 species described, but is currently defined as non-monophyletic (Kirk *et al.*, 2008). *Glomus* has recently been split into several genera. Many species previously cited as *Glomus* are presently called *Funneliformis*,

*Claroideoglomus* or *Rhizophagus* genera based on morphological parameters and phylogenetic analyses (Schüßler and Walker, 2010; Oehl *et al.*, 2011). Further taxonomic changes are likely as the phylogeny of AMF becomes better understood. Taylor and Harrier (2001) found that *Glomus* species have the highest level of colonization and arbuscule content in strawberry.

*Glomus arenarium* was recovered from maritime sand dunes of Northern Poland (Blaszkowski *et al.*, 2000) and from maritime sand dunes adjacent to Tel Aviv, Israel (Blaszkowski *et al.*, 2001). No data exist of the presence of this fungus in other regions of the world. *G. arenarium* forms spores with a narrow and hyaline subtending hypha. Spores are orange to raw umber, and are born singly in the soil with a single subtending hypha, rarely with two.

*Funneliformis caledonius* (also cited as *Glomus caledonium*) was first characterized by Nicolson and Gerdemann (1968) and again by Gerdemann and Trappe (1974). It has a worldwide distribution and can sporulate abundantly in both poor and rich soils. Its spores are pale yellow to golden yellow and are borne singly in the soil with a single subtending hypha (Blaszkowski, 1989, 1993).

*Funneliformis mosseae* (also cited as *Glomus mosseae*) was first characterized by Gerdemann and Trappe (1974). It is a frequent component of communities of AMF associated with plants of different regions of the world. In Poland it was found to be the third most frequently occurring fungal species, where it markedly preferred cultivated soils (Blaszkowski, 1993). Its spores are pale yellow to golden yellow and borne singly in the soil, and in compact sporocarps. Spores most resemble those of *F. caledonius*. However, the spore wall of *F.*

*mosseae* contains three layers, while that of *F. caledonius* contains a 4-layered wall, only the outermost one being impermanent (Morton, 1996, 2000).

*Rhizophagus irregularis* (also cited as *Glomus irregulare*) was first described by Blaszkowski *et al.* (2008) and found later conspecific with several of the *Rhizophagus intraradices* commonly used as commercial inoculants (Sokolski *et al.*, 2010). Consequently, *R. irregularis* strains, especially the model strain DAOM 197198, are the most intensely studied arbuscular mycorrhizal fungi. To date, > 1200 publications refer to this species, > 130 of which have its name in the title. Phylogenetic analysis by Stockinger *et al.* (2009) concluded that the AMF identified as DAOM 197198 is not *R. intraradices*, but falls into a clade containing the recently described *R. irregularis*. The strain DAOM 197198 was found to be conspecific to *R. irregularis* (Sokolski *et al.*, 2010) based on molecular analyses of protein encoding genes. This species has a worldwide distribution associated with plants colonizing maritime sand dunes of as of crop plants. *R. irregularis* has a frequent irregular-shaped hyaline to pale yellow spore with a spore wall architecture resembling the one of *R. intraradices* and differentiating spore aggregates and intraradical and extraradical spores. The spore wall consists of two semi-permanent, hyaline outer layers and a hyaline to pale yellow, laminate innermost layer (Blaszkowski *et al.*, 2008).

*Piriformospora indica* is a plant growth promoting root endophyte belonging to the order Sebanicales. It is a plant-root-colonizing basidiomycete that was discovered in the Indian Thar desert and described by Verma *et al.* (1998). *P. indica* resembles AMF with respect to physiological characteristics, the mode of invasion of the root, and morphological features,

producing small globoid structures, although these structures are much smaller than AMF vesicles (Varma *et al.*, 1999). Unlike AMF, which require a host plant, *P. indica* can be easily propagated on various complex or minimal substrates. It was shown to provide strong growth-promoting activity with a broad spectrum of plant species (Varma *et al.*, 1999). In experimental trials, it was found to significantly reduce the mortality rate of transplanted micro-propagated plants and increase growth of the plants (Sahay and Varma, 1999). *P. indica* was also reported to induce resistance to fungal diseases in barley (Waller *et al.*, 2005) and wheat (Serfling *et al.*, 2007), and tolerance to salt stress in barley (Waller *et al.*, 2005). Because of its ease of culture and plant growth promotional effect under extreme physical and nutrient stress, it may have potential in plant production systems.

The above *Glomus* species have documented potential in improving plant growth under stress conditions, and have not been investigated on strawberry under salt conditions. Particularly, these species have not been investigated under high salt conditions. In addition, the influence of mixed-AMF species on strawberry under salt conditions has not yet been investigated. *P. indica* has not been tested on strawberry.

## **Strawberry cultivars**

From the Québec strawberry breeding program, four cultivars were selected due to their differences in pedigree, and because they are 'day-neutral'. This allowed flowers and fruiting in the first year, as opposed to 'short-day' strawberry plants, that produce fruits in the second year after going dormant.

'Albion' was produced as a cross between 'Diamante' and numbered selection 'Cal94.16-1' at the University of California in 1997. It produces orange-red conical firm fruit that ripens from the tip towards the top. Its fruit offers a strong sweetness and is valued for its excellent quality and flavour. It produces more runners and larger berries than 'Seascape'. Fruiting season is later than that of 'Seascape' (all summer to the fall) and it is highly tolerant to low temperatures.

'Charlotte' was created in a breeding program by crossing 'Mara de Bois' and numbered selection, 'CAL 19', at the CIREF station, Nébouts à Lanxade, France, in 1995. It was selected for having fruits of good quality, vigour and hardiness, high disease tolerance, and a consistent and distinctive tastefulness. 'Charlotte' is classified as having weaker plant vigour than 'Mara des Bois' but stronger than 'Seascape'. It has larger leaves than both 'Mara des Bois' and 'Seascape'. 'Charlotte' produces medium-sized, conical, medium red skinned fruits.

'Mara des Bois' was cultivated in France. It has small leaves relative to 'Charlotte' and 'Seascape', and small, ovoid fruits. It is known for its high vigour and high taste value but short-shelf-life (Allais and Létang, 2009). It was first classified by the Canadian Food Inspection Agency and abandoned in 1997.

'Seascape' was produced as a cross between 'Selva' and 'Douglas' at the University of California in 1991. It is widely grown due to its high yields. Its fruit is firm, medium to long conic in shape, bright red in colour with an attractive glossy finish and good quality. Fruits are flavourful with an intense, sometimes unpleasant aroma in summer. 'Seascape' produces high yields in August and early September. It is extremely susceptible to powdery mildew, which

causes small, seedy fruit as well as red blotching on foliage. Its leaves are medium in size relative to 'Charlotte' and 'Mara de Bois', and it is considered the most winter hardy of the day-neutral varieties.

## **Objectives, rationale, and hypotheses**

In this research, I examined the effects of AMF and a root endophyte on strawberry plants subjected to salt conditions. My first objective was to determine the level of root colonization by each fungal species under increasing salinity. My second objective was to determine the effects of the inoculants on biomass, root architecture, and fruit quality. My third objective was to determine whether a mixed-AMF species inoculum was more beneficial than single-AMF species to plant productivity under salt stress.

The rationale underlying this study was that under salt conditions, fungal inoculation will benefit strawberry plants in their tolerance to salinity. What is still unknown is how different salinity and inoculation combinations impact growth of the cultivars.

My hypotheses were:

- 1) Fungal inoculant colonization levels decrease with increasing salinity.
- 2) Fungal inoculation improves biomass, root architecture, and fruit quality under salt conditions.

## Chapter 2: Materials and Methods

### First experiment

The first greenhouse experiment was performed to screen the growth response of strawberry cultivar and fungal inoculant combinations under salt conditions. A short communication based on results of the first experiment has been accepted for publication in the *Canadian Journal of Plant Science* (Sinclair *et al.*, 2013).

### Propagation of fungal inoculants

Inoculants of three AMF species, *Glomus arenarium* (DAOM 241280), *Funneliformis caledonius* (DAOM 193528), and *F. mosseae* (DAOM 194475), were obtained from the National Collection of Glomeromycota of Agriculture and Agri-Food Canada (AAFC) in Ottawa, Canada. Each inoculant consisted of colonized substrate and root segments and contained approximately 7 propagules per gram inoculum. Inoculants were kept at 5°C prior to use (Dalpé and Monreal, 2004).

A fourth AMF inoculum, *Rhizophagus irregularis* (developed from the strain DAOM 197198), was purchased commercially as MYKE® Pro Agriculture G from Premier Tech Biotechnologies (Rivière-du-Loup, QC). It contained approximately 15 viable spores per gram inoculum.

A root endophyte, *Piriformospora indica* (DAOM 241284), was propagated at the AAFC. The strain was kindly provided by Ralf Oelmüller, Germany. An agar disc (1 cm in diameter)

seeded with hyphae and spores of *P. indica* was placed in a 9 cm Petri dish containing potato dextrose agar (Difco). Spores propagated in an incubator at 23°C in the dark for five weeks, enough to cover the agar surface. 80 gel plugs (1 cm in diameter) were extracted from the cultures under laminar flow hood, with a copper cork borer that was sterilized over a flame and soaked in 70% methanol to prevent contamination, and used as inoculum.

### **Cultivar selection**

Four 'day-neutral' strawberry (*Fragaria × ananassa* Duch.) cultivars ('Albion', 'Charlotte', 'Mara des Bois', and 'Seascape') were chosen. Plantlets were obtained from Luc Larreault Certified Fruit Plants, Lavaltrie, QC.

### **Experimental conditions and plant materials**

Greenhouse experiments were conducted at L'Acadie Research Sub-Station, AAFC, Saint-Jean-sur-Richelieu, QC. Two-month old plantlets of uniform size were grown in 14.5 cm high × 15 cm diameter pots (one plant per pot) that were filled with hydrated growth medium containing peat moss, perlite and vermiculite (3:1:1, pH 5.5-6.5), Fafard® Agro Mix®, Saint-Bonaventure, QC. Plants were watered thoroughly after planting, and soil was added to bury exposed roots. A factorial block design was used with six blocks (one replicate per block) containing 72 pots per block. The plants were fertilized twice weekly with 100 mL of a N:P:K fertilizer (12:2:14) at a concentration of 5 mL/L (Plant Products®, QC). Plants were watered as

needed. Plants were brushed lightly to spread pollen and stolons were removed throughout the growing season to strengthen the plants.

## **Inoculation and salt treatments**

*G. arenarium*, *F. caledonius*, *F. mosseae*, *R. irregularis*, and *P. indica* were tested against a non-inoculated control. For each cultivar, pots were given inoculum containing approximately 100 propagules. The inoculum was applied at the base of the growing roots of each plant. There were six replicates per treatment.

Forty days after planting, 100 mL of salt solution containing either 0, 50 or 100 mM NaCl (EMD Chemicals Inc, CAS 7647-14-5) was applied twice weekly. Excess solution was allowed to drain. Salt treatments continued over six weeks of growth.

## **Data collection and measurement**

Fruits were harvested upon ripening and separated into sepals and fruit flesh. Only fruit flesh was used for further investigations. Fruits were cut into smaller pieces, frozen in liquid nitrogen, vacuum-sealed, and kept at -80°C. After forty days of salt treatment, all plants were harvested. Total roots were gently extracted from the pots, rinsed in tap water to remove debris, and excess water was removed by blotting with paper towels. Roots and shoots were separated. Fresh mass of shoots and roots was recorded, and dry mass determined after drying in an oven at 50°C for 48 h. Dry roots were stored at room temperature.

## **Mycorrhizal colonization determination**

Roots were stained according to Phillips and Hayman (1970). One gram of each root sample was cut into 1-2 cm pieces, placed into 50 mL vials and covered with 10% KOH, covered loosely and autoclaved for 10 min. at 1 atm, then rinsed with water. Roots were acidified in 1N HCl for 30 min. Root samples were then stained in 0.01% Fuchsin acid in lactoglycerol and autoclaved for 10 min. at 1 atm.

Root colonization percentage was determined using the gridline-intercept method (Giovannetti and Mosse, 1980), in which roots were randomly dispersed in a 9 cm diameter Petri dish with 1 cm grid lines. Scanning along these grid lines with a dissecting microscope to quantify intersections between grid-lines and roots, these intersections were designated as either colonized or not. Vesicles, hyphae, and arbuscules were considered to calculate the AMF colonization percent. Roots were mounted on slides for photography using a drop of polyvinyl alcohol-lactic acid glycerol (PVLG) mounting media (Koske and Tessier, 1983).

## **Statistical analyses**

The data were subjected to a three-way ANOVA using a generalized linear model procedure using R statistical analysis software (version 2.15.0). Means were tested by a least significant difference (LSD) test ( $P < 0.05$ ) when the variance was significant. Orthogonal polynomial contrast was used to study the effect of salinity. Log transformation was used for numerical data before analysis, and for simplicity, the results were presented as original data when the outcomes of the transformed and nontransformed data were the same.

## Second experiment

From the overall results of the first experiment, some changes were brought to the second greenhouse experiment, such that *G. arenarium*, *P. indica*, and cv. 'Mara des Bois' were removed from the study because of their poor growth response. In addition, the other AMF-cultivar combinations were tested under an increased range of salinity, and a mixture of the two AMF species, *R. irregularis* + *F. mosseae*, was tested on cv. 'Seascape' to study its effect relative to its single-species components. A number of changes were made to the methodology of the second experiment. At the time of thesis submission, a full-length manuscript based on results of the second experiment was being prepared for submission to the *Canadian Journal of Plant Science*.

## Propagation of AMF

Two AMF species, *F. caledonius* (DAOM 193528) and *F. mosseae* (DAOM 194475), were propagated in vivo through pot-culture in a greenhouse, Centre for Advanced Research in Environmental Genomics, University of Ottawa. Leek was selected as the host-plant because of its high mycorrhizal potential and its extensive root system. For these two AMF, approximately 250 g of pure AMF strains containing 15 propagules per gram were incorporated into an autoclaved sandy-soil substrate (peat moss and sand) among three pots. Leek seeds were soaked in wet paper towels for one week before planting to accelerate germination. Six seeds were planted per pot. Leek plants were watered almost daily and greenhouse temperatures varied from 22-28°C. Plants were fertilized every three weeks with 15 mL of Long Ashton

nutrient solution (Hewitt, 1966) per pot. After six months, leek plant stems were removed and watering ceased. After two weeks, roots were cut up into 1-2 cm segments and reincorporated into the substrate, which was mixed well. At this time, 1 g of root segments from each pot was stained and percent mycorrhizal colonization calculated for each inoculum (see above, Mycorrhizal colonization determination). The substrate was also examined to determine the number of propagules per gram substrate. *F. caledonius* contained approximately 12 propagules per gram inoculum, and *F. mosseae* contained 9 propagules. Both colonized substrate and roots served as mycorrhizal inoculum for the strawberry cultivar experiment.

### **Cultivar selection**

'Mara des Bois' performed more poorly than the other cultivars, regardless of inoculum or salinity, and was not included in the second experiment. For the second greenhouse experiment, plug plants of the three remaining cultivars were obtained from the same producer.

### **Experimental conditions and plant materials**

Two-month old plug plants of uniform size were used rather than plantlets. The factorial block design contained 52 pots per block. To study the effect of stolon removal on the biomass of strawberry plants inoculated with *Glomus* spp. or not under salt stress, stolons were maintained and counted.

## **Inoculation and salt treatments**

*G. arenarium* and *P. indica* had a depressive effect on growth and the strains were not included in the second experiment. The remaining AMF were tested against a non-inoculated control. A mixed-species inoculant containing 50 spores from each of the two highest-performing AMF species, *R. irregularis* and *F. mosseae*, was tested on cv 'Seascape'.

NaCl solution was applied in the same manner at concentrations of 0, 50, 100, and 200 mM. The 200 mM NaCl level was included to study its effect on strawberry plants grown in the arid areas of North America, where such soil salinity might be found (Dierickx, 2013).

## **Data collection and measurement**

Fresh mass of shoots (including stolons) and roots (no dry mass) was recorded and roots were stored at 5°C.

## **Root architecture analysis**

Roots were rehydrated in water and Tween-20 (0.01%), spread out in a transparent tray, and scanned. Root morphology parameters were determined using WinRHIZO Pro image analysis software (Regent Instruments Inc., Québec, QC). Root length, volume, average diameter, surface area, and number of forks and crossings were automatically analyzed using this software. Following analysis, wet roots were kept at 5°C.

The specific root length (SRL) was calculated as sample root length divided by root fresh mass (Ostonen *et al.*, 2007).

## **Fruit quality analysis**

For the fruit chemical analysis, the juice of five strawberries from the same treatment was extracted using an ACME Supreme Juicerator.

### **Soluble solids**

A refractometer (Sugar/Brix Refractometer, 300010, SPER SCIENTIFIC) was calibrated using a drop of distilled water. A drop of juice was then placed on the refractometer and the soluble solids content (%) reading recorded at 20°C. Three replicates were performed for each sample.

### **Titrateable acidity**

Three trials were done for each sample, consisting of 2 mL of juice diluted in 18 mL of water, then the pH was taken using a pH meter. The solution was titrated with standard NaOH to pH 8.05. The percent acidity was calculated using the following formula:

$$\% \text{ total acid} = \frac{1}{10} \times \frac{\text{equiv. wt. of acid} \times \text{normality of NaOH} \times \text{titer}}{\text{wt. of sample}}$$

Acidity was expressed as g citric acid per 100 mL juice (%).

### **Statistical analyses**

Data were analysed using the same methods as in the first experiment. To study the effect of stolon removal or maintenance on biomass, a four-way ANOVA was used.

## Chapter 3: Results

### 3.1 First experiment

#### Mycorrhizal colonization

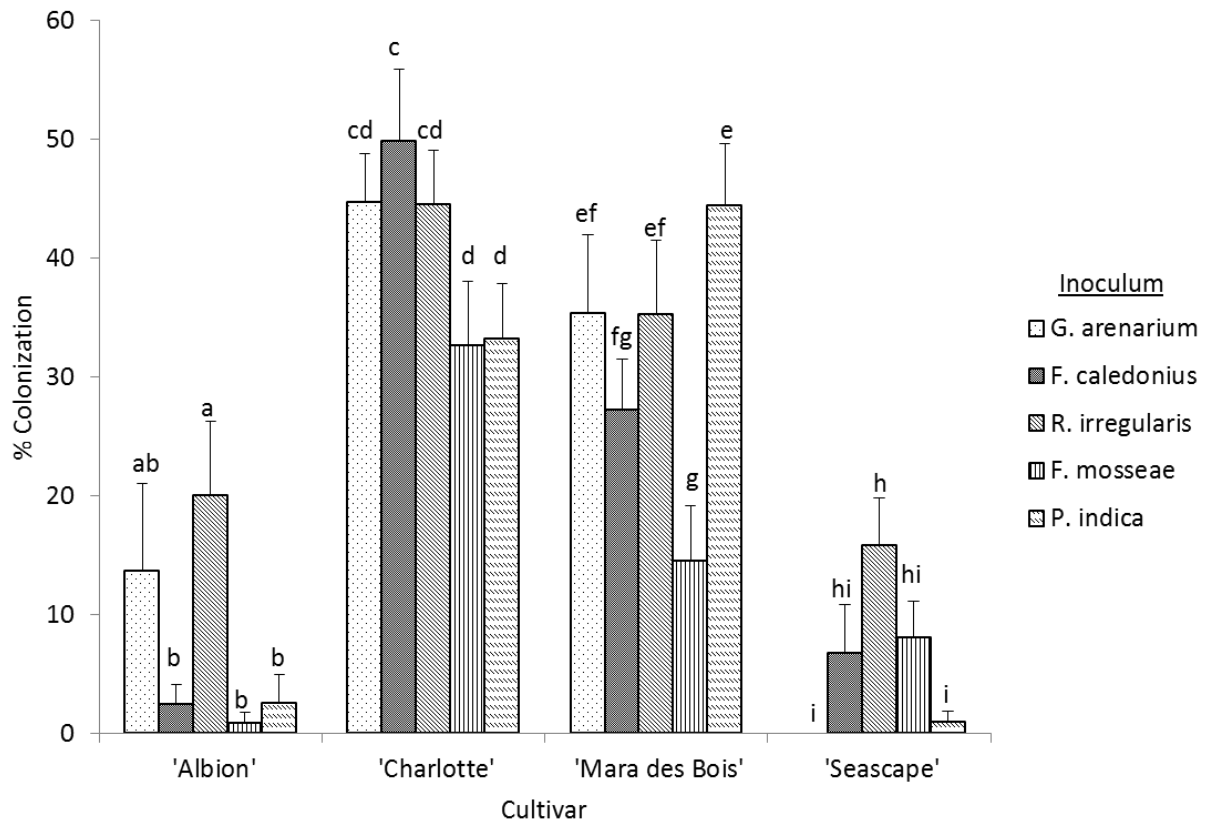
Both cultivar and inoculum ( $P=0.001$  and  $P<0.001$ ) and their interaction ( $P=0.004$ ) were significant on the mycorrhizal colonization percentage (%), without any effect of the salt treatment (Table 1).

Cultivars responded differently to inoculum (Fig. 1). The root colonization level was the highest in 'Charlotte' (41%), followed by 'Mara des Bois' (31%), then the lowest in 'Albion' (8%), and 'Seascape' (6%). 'Charlotte' and 'Mara des Bois' were highly colonized by all mycorrhizal species. The highest colonization was observed in plants inoculated with *F. caledonius* in 'Charlotte' and the root endophyte *P. indica* in 'Mara des Bois'. 'Albion' and 'Seascape' were colonized the most by *R. irregularis*. 'Albion' had trace levels of colonization by *F. caledonius*, *F. mosseae*, and *P. indica*. 'Seascape' had trace levels of colonization by *P. indica*, and no observed colonization by *G. arenarium*.

**Table 1.** Three-way ANOVA for salinity (S), AMF or root endophyte inoculation (I), and cultivar (C) treatments and their interactions on mycorrhizal colonization.

	S	I	C	S × I	S × C	I × C	S × I × C
Mycorrhizal colonization	NS	**	***	NS	NS	**	NS

**Note:** \*, 0.05; \*\*, 0.01; \*\*\*, 0.001; NS, not significant



**Fig. 1.** Mean (n=12) root colonization percentages +SE by four AMF species and a root endophyte, *P. indica*, in four strawberry cultivars. Different letters within each cultivar indicate significant differences at  $P < 0.05$  according to LSD test.

## Shoot and root fresh and dry mass, shoot water content, and root/shoot ratio

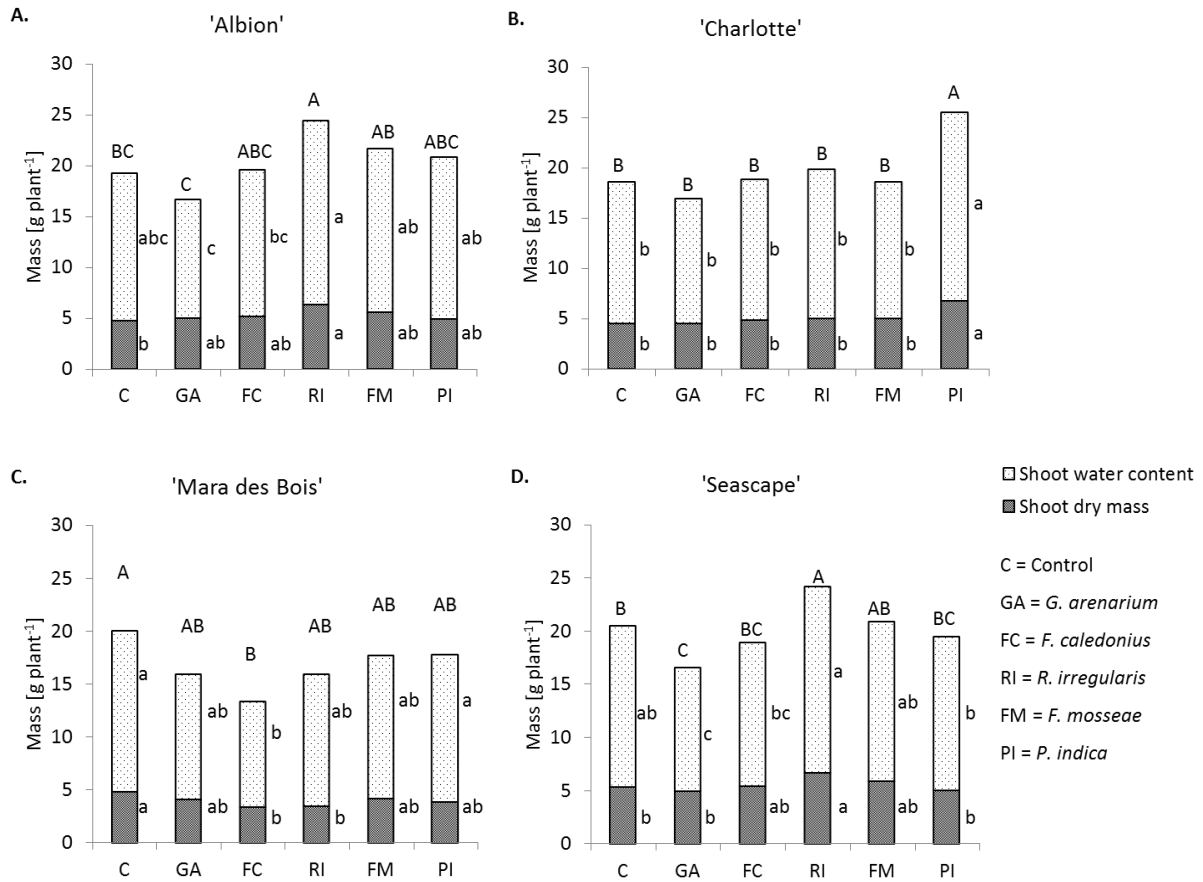
Shoot and root fresh and dry mass varied among cultivars ( $P < 0.001$ , Table 2). Root fresh and root dry mass, and root/shoot (R/S) ratio were significantly reduced by salinity ( $P = 0.001$ ,  $P < 0.001$ ,  $P < 0.001$ ), while shoot fresh and dry mass were unaffected. 'Seascape' had significantly higher shoot water content than the other cultivars. There were no interactions between salinity and cultivar on the fresh or dry mass of shoots or roots; the cultivars responded similarly to salinity.

The inoculum had a significant effect on shoot fresh mass ( $P < 0.001$ ) and shoot water content ( $P < 0.001$ ). The inoculum and cultivar interaction was significant for shoot fresh mass ( $P = 0.037$ ), dry mass ( $P = 0.015$ ), and water content ( $P = 0.011$ ); however, cultivars responded differently to inoculum (Fig. 2A-D). As shown, the growth response of 'Mara des Bois' was negative for all AMF species and root endophyte *P. indica*. The growth response to *G. arenarium* was negative or non-significant for all cultivars.

**Table 2.** Three-way ANOVA for salinity (S), AMF or root endophyte inoculation (I), and cultivar (C) treatments and their interactions on growth parameters.

	S	I	C	S × I	S × C	I × C	S × I × C
Fresh shoot mass	NS	***	***	NS	NS	*	NS
Dry shoot mass	NS	NS	***	NS	NS	*	NS
Shoot water content	NS	***	*	NS	NS	*	NS
Fresh root mass	**	NS	***	NS	NS	NS	NS
Dry root mass	***	NS	***	NS	NS	NS	NS
R/S Ratio	***	NS	***	NS	NS	NS	NS
Fruit mass	NS	**	***	NS	*	*	NS
Fruit size	***	***	***	NS	NS	NS	NS

**Note:** Significant differences: \*, 0.05; \*\*, 0.01; \*\*\*, 0.001; NS, not significant



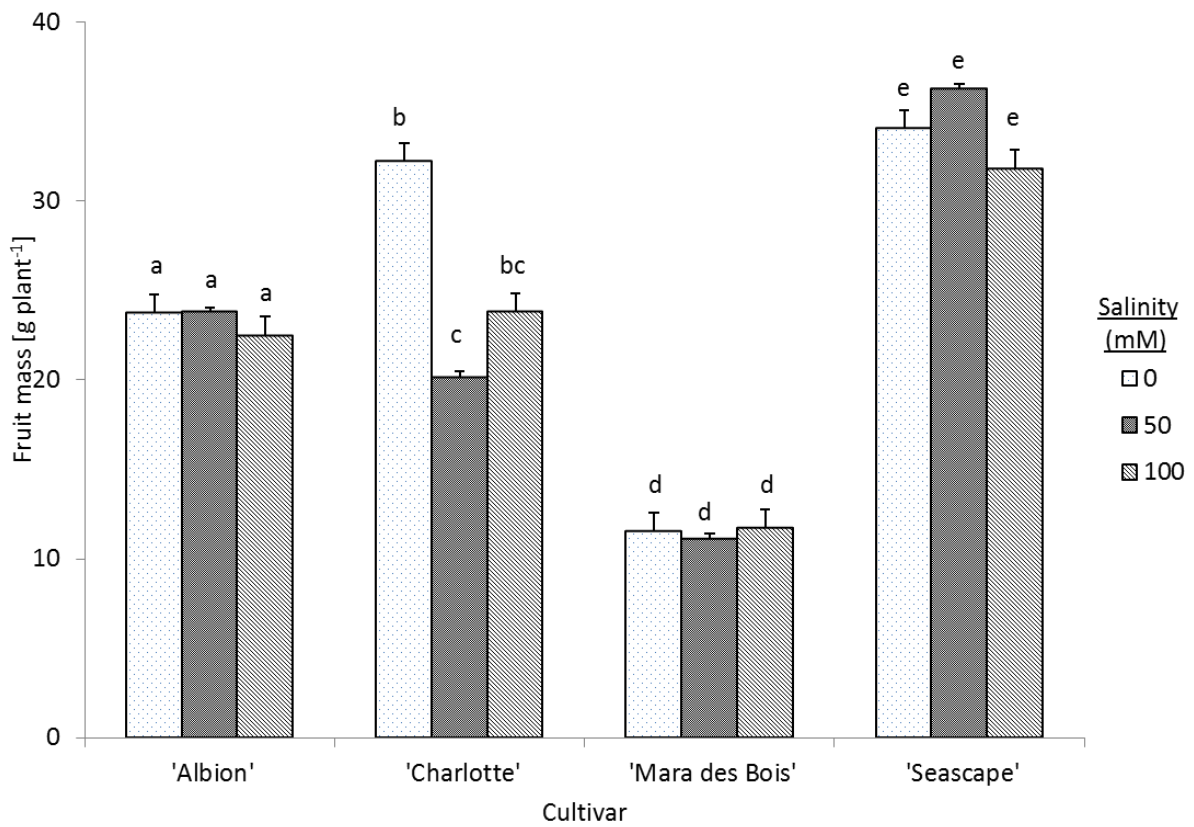
**Fig 2A-D.** Effect of four AMF species and a root endophyte, *P. indica*, on the mean fresh mass, dry mass, and water content of shoots (g plant<sup>-1</sup>, n=12) in four strawberry cultivars (**A.** 'Albion', **B.** 'Charlotte', **C.** 'Mara des Bois', **D.** 'Seascape'). Full bars represent shoot fresh mass. Different letters indicate significant differences for similar parameters at  $P < 0.05$  according to LSD test. Capital letters are used for shoot fresh mass, while lower case letters are used for shoot dry mass and water content.

## Fruit yield

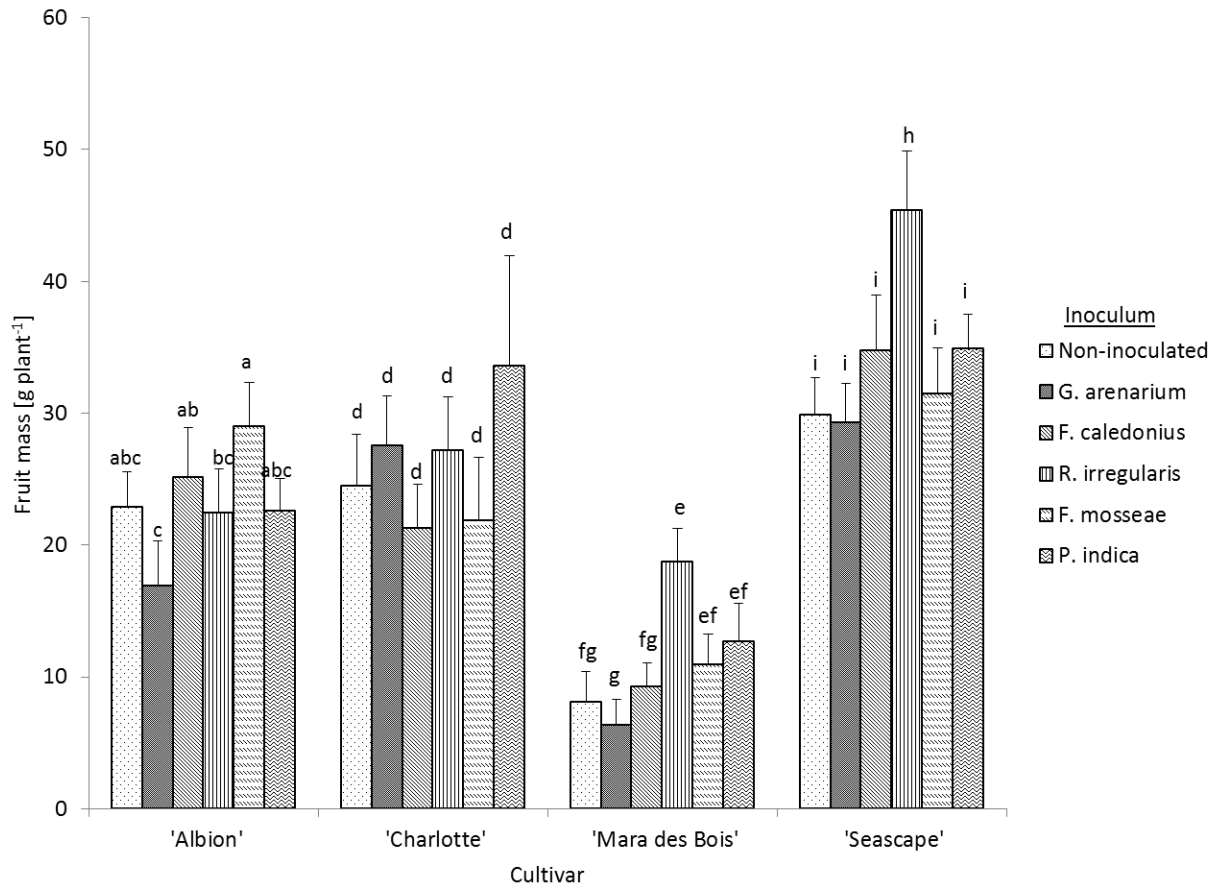
'Albion' produced the largest fruits, followed by 'Seascape', 'Charlotte' and 'Mara des Bois'. Fruit size (g/fruit) decreased with salinity ( $P < 0.001$ ) and inoculum ( $P < 0.001$ ).

Fruit mass (g/plant) varied significantly ( $P < 0.001$ ) among cultivars. 'Seascape' produced the highest fruit mass, followed by 'Charlotte', 'Albion', and 'Mara des Bois'. Fruit mass was significantly affected by the interaction between salinity and cultivar ( $P = 0.021$ ); 'Albion' and 'Seascape' preserved higher fruit mass than the other cultivars under salt conditions (Fig. 3).

Fruit mass was affected by inoculum ( $P = 0.002$ ), but the effect was not always beneficial, as *G. arenarium* reduced fruit yield overall. There was an interaction between inoculum and cultivar ( $P = 0.027$ ). 'Albion' benefitted most from symbiosis with *F. mosseae*, 'Charlotte' from *P. indica*, while 'Mara des Bois' and 'Seascape' benefitted most from *R. irregularis*. 'Charlotte' responded negatively to *F. caledonius* and *F. mosseae* (Fig. 4).



**Fig. 3.** Effect of salinity on mean ( $n=24 +SE$ ) fruit mass ( $g\ plant^{-1}$ ) in four strawberry cultivars. Bars sharing a same letter within each cultivar are not significantly different at  $P<0.05$  according to LSD test.



**Fig. 4.** Effect of four AMF species and a root endophyte, *P. indica*, on mean ( $n=12 +SE$ ) fruit mass ( $g\ plant^{-1}$ ) in four strawberry cultivars. Bars sharing a same letter within each cultivar are not significantly different at  $P<0.05$  according to LSD test.

## 3.2 Second experiment

### Mycorrhizal colonization

In this second experiment, although the colonization levels were much lower, trends were similar as in the first experiment. Colonization was significantly affected by cultivar and AMF ( $P < 0.001$  and  $P = 0.010$ ) and their interaction ( $P = 0.046$ ). Although salinity did not affect colonization overall, there was a significant interaction ( $P = 0.011$ ) between salinity and cultivar (Table 3).

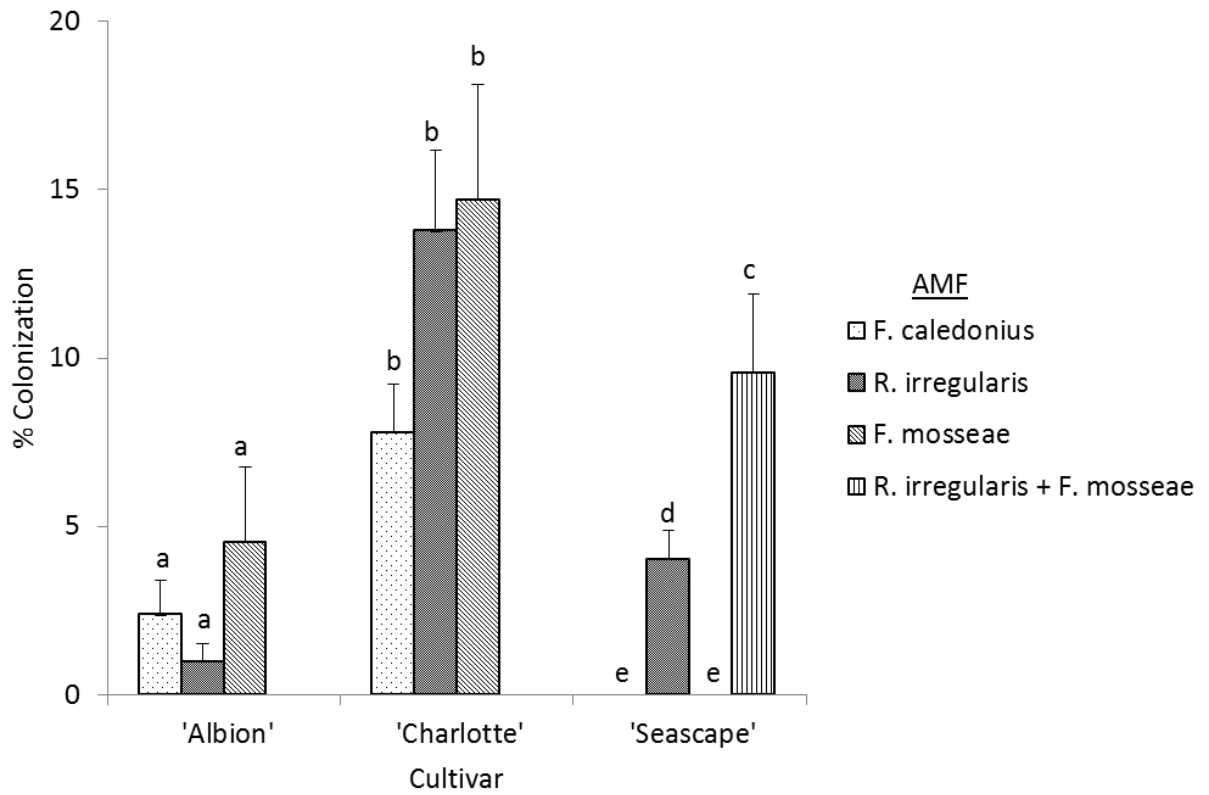
As in the first experiment, cultivars responded differently to inoculum (Fig.5). 'Charlotte' had a significantly ( $P < 0.001$ ) higher level of colonization (12%) than 'Albion' (3%) and 'Seascape' (3%). The highest colonization was observed with *F. mosseae* in 'Albion' and with *R. irregularis* in 'Charlotte'. In 'Seascape' there was no observed colonization by *F. caledonius* or *F. mosseae*. Although there was no observed colonization by *F. mosseae*, the mixed-AMF species, *R. irregularis* + *F. mosseae*, had a significantly ( $P = 0.0097$ ) higher level of colonization than *R. irregularis* alone.

The interaction between salinity and cultivar was shown only in 'Albion', where colonization significantly ( $P = 0.011$ ) decreased sharply at high salinity.

**Table 3.** Three-way ANOVA for salinity (S), AMF, and cultivar (C) treatments and their interactions on mycorrhizal colonization.

	S	AMF	C	S × AMF	S × C	AMF × C	S × AMF × C
Mycorrhizal colonization	NS	**	***	NS	*	*	NS

**Note:** \*, 0.05; \*\*, 0.01; \*\*\*, 0.001; NS, not significant



**Fig. 5.** Mean (n=16 +SE) root colonization percentages by three AMF species, plus a mixed-AMF species in 'Seascape'. Different letters within each cultivar indicate significant differences at  $P < 0.05$  according to LSD test.

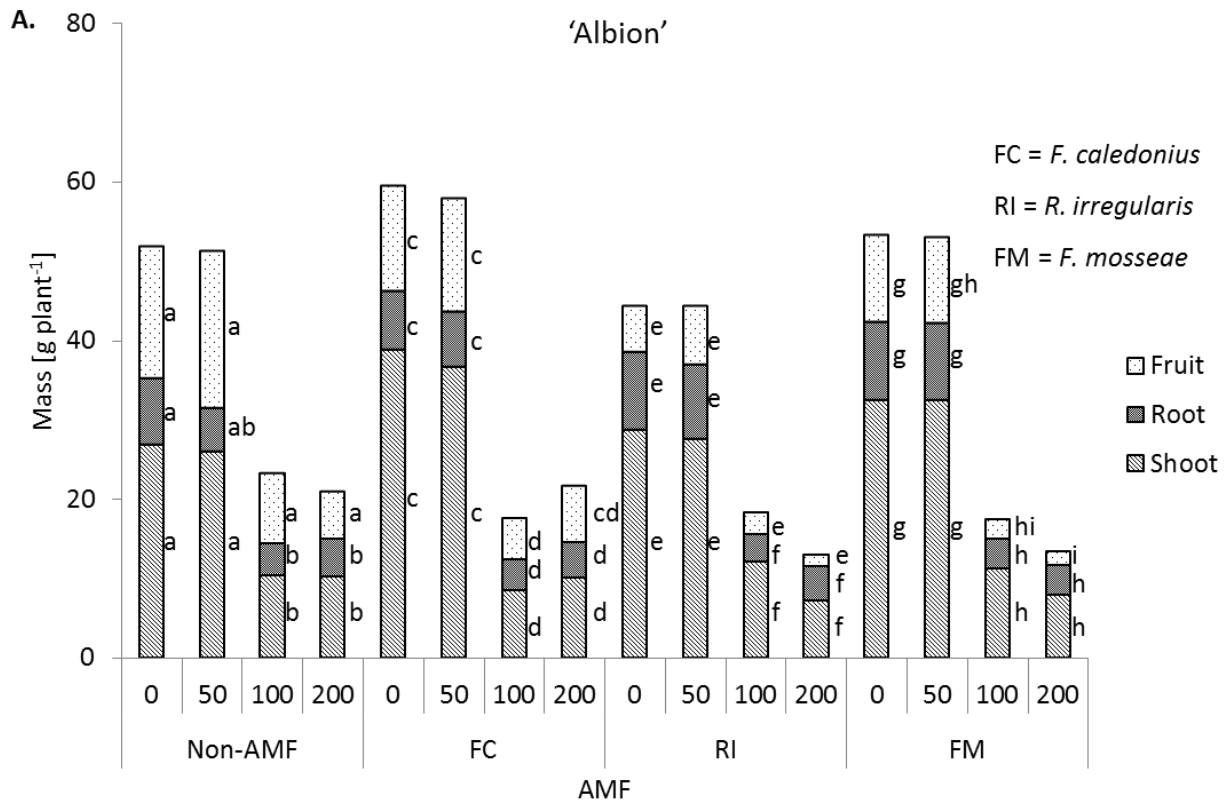
## Shoot and root fresh mass

Fresh mass of shoots, roots, and fruits decreased with increasing salinity ( $P < 0.001$ ), especially between 50 and 100 mM NaCl (Fig. 6A-C). In non-inoculated treatments, shoot fresh mass was more severely affected by salinity than fruit or root fresh mass (Fig. 7). At high salinity, the inhibitory effects of salt on growth were observed in both shoots and roots ( $P < 0.001$ , Table 4).

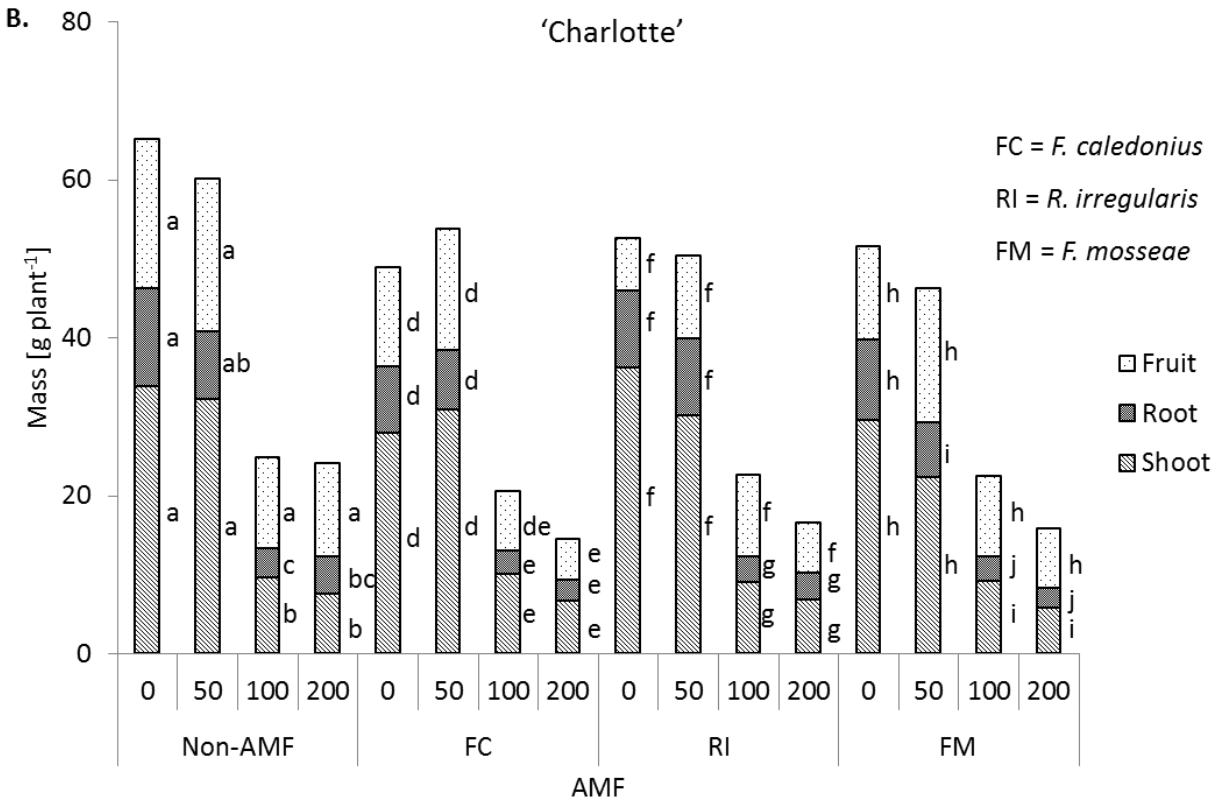
**Table 4.** Three-way ANOVA for salinity (S), AMF and cultivar (C) treatments and their interactions on biomass parameters.

	S	AMF	C	S × AMF	S × C	AMF × C	S × AMF × C
Number of stolons	***	NS	***	NS	NS	NS	NS
Fresh shoot mass	***	NS	NS	NS	NS	NS	NS
Fresh root mass	***	*	***	*	*	NS	NS
Fruit mass	***	***	**	NS	*	NS	NS
Fruit size	**	NS	***	NS	**	NS	NS

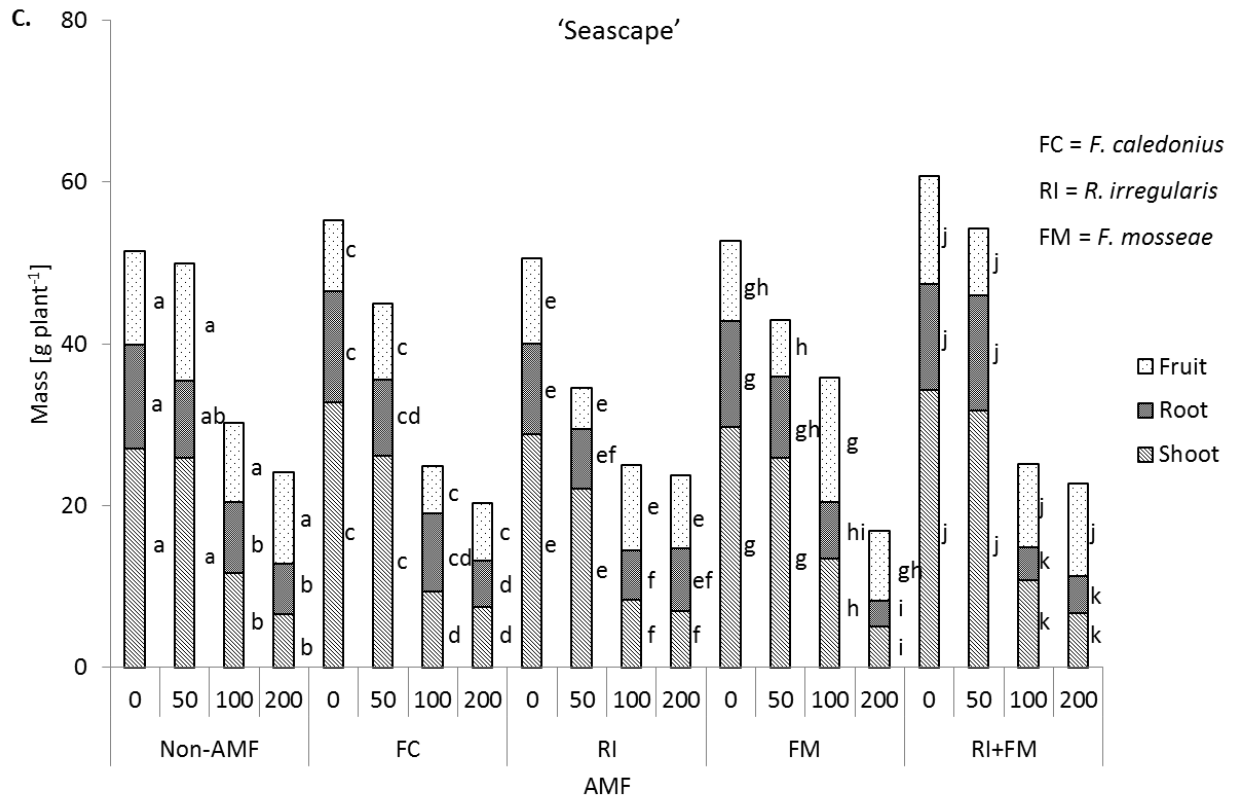
**Note:** \*, 0.05; \*\*, 0.01; \*\*\*, 0.001; NS, not significant



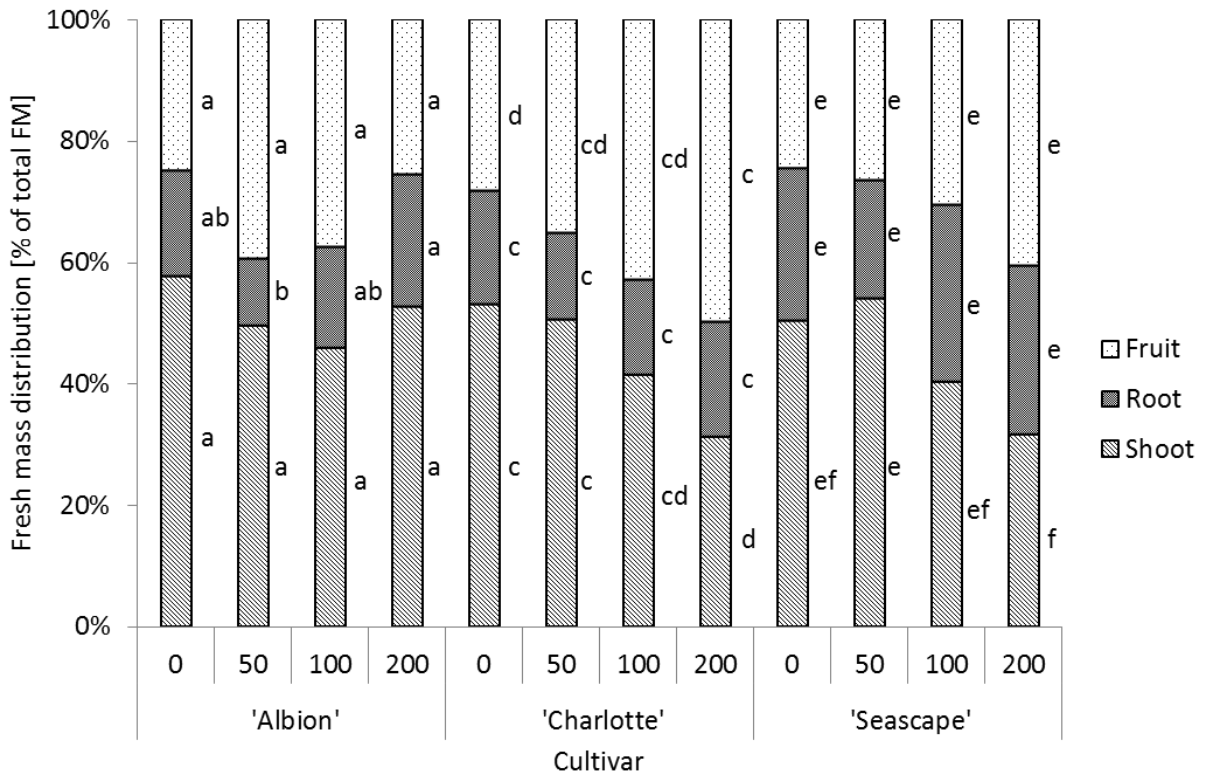
**Fig. 6A.** Effect of salinity (0-200 mM) and AMF on mean fresh mass ( $\text{g plant}^{-1}$ ,  $n=6$ ) of plant organs in strawberry cultivar 'Albion'. Different letters indicate significant differences in each plant organ within each AMF treatment at  $P < 0.05$  according to LSD test.



**Fig. 6B.** Effect of salinity (0-200 mM) and AMF on mean fresh mass (g plant<sup>-1</sup>, n=6) of plant organs in strawberry cultivar 'Charlotte'. Different letters indicate significant differences in each plant organ within each AMF treatment at  $P < 0.05$  according to LSD test.



**Fig. 6C.** Effect of salinity (0-200 mM) and AMF on mean fresh mass (g plant<sup>-1</sup>, n=6) of plant organs in strawberry cultivar ‘Seascape’. Different letters indicate significant differences in each plant organ within each AMF treatment at  $P < 0.05$  according to LSD test.



**Fig. 7.** Effect of salinity (0-200 mM) on mean fresh mass distribution (% of total FM, n=6) of plant organs in three strawberry cultivars. Different letters within each cultivar indicate significant differences in each plant organ at  $P < 0.05$  according to LSD test.

The effect of AMF was significant ( $P=0.046$ ) on root fresh mass, not on shoot (Table 4). There was an interaction ( $P=0.024$ ) between salinity and the cultivar on root fresh mass; 'Seascape' being more salt-tolerant than the two other cultivars. 'Seascape' also responded more positively to inoculation than 'Albion' and 'Charlotte'. Overall, root mass increased with all AMF, however, *R. irregularis* was most efficient at high salinity. In 'Albion', AMF induced a positive growth response in shoot and roots at 0 and 50 mM NaCl, but *R. irregularis* was the only AMF species to increase biomass at high salinity. *F. caledonius* and *F. mosseae* did not improve biomass at high salinity. 'Charlotte' was the least affected by AMF and responded negatively to *F. caledonius* and *F. mosseae*, as in the first experiment, and positively to *R. irregularis*, but only at low salinity.

## **Fruit yield**

'Albion' produced significantly ( $P<0.001$ ) larger fruit than 'Charlotte' and 'Seascape'. Fruit size (g/fruit) tended to decrease with salinity ( $P=0.007$ ), particularly at 100 mM NaCl, and was not significantly affected by AMF.

At low salinity, 'Charlotte' produced the most fruit mass (g/plant), followed by 'Albion', and 'Seascape' the least. Overall, fruit mass decreased with increasing salinity ( $P<0.001$ ) and salinity interacted significantly ( $P=0.015$ ) with cultivar treatment. In its fruit mass, 'Seascape' exhibited exceptional tolerance to salinity in both inoculated and non-inoculated treatments. 'Albion' was less tolerant, and 'Charlotte' the least tolerant, particularly when salinity increased from 50mM to 100mM (Fig. 6A-C).

AMF decreased fruit yield ( $P<0.001$ ), which was inversely related to shoot and root mass; the decrease in fruit mass was greatest in treatments where AMF increased shoot and root mass to the highest degree

## Fruit quality

The soluble solids content (SSC), titratable acidity (TA), and their ratio (SSC/TA) varied significantly ( $P < 0.001$ ) among cultivars. 'Albion' produced fruits with the highest SSC and 'Charlotte' had the highest SSC/TA ratio (Table 6). The SSC and SSC/TA decreased significantly ( $P < 0.001$ ) with increasing salinity (Figs. 8 and 9), while TA was marginally not significantly affected ( $P = 0.076$ ). In SSC/TA, the cultivars responded differently to salinity ( $P = 0.0445$ ); 'Charlotte' was the most tolerant and 'Albion' the least tolerant to salinity. 'Seascape' produced moderately salt-tolerant fruit with lower SSC and SSC/TA than the two other cultivars (Figs. 8 and 9).

The AMF species (Table 5) had a significant effect on SSC ( $P = 0.007$ ), TA ( $P < 0.001$ ), and SSC/TA ( $P < 0.001$ ) and tended to increase SSC and SSC/TA at low salinity (Table 7). In 'Seascape', the effect of the mixed-species AMF was lesser than either of its single-species components. There was a significant interaction between AMF and salinity on SSC ( $P = 0.0341$ ), TA ( $P < 0.001$ ), and SSC/TA ( $P < 0.001$ ). While *G. mosseae* increased SSC/TA to a higher degree than the other AMF species at low salinity, *R. irregularis* improved fruit quality to the highest degree at high salinity (Table 7). In *R. irregularis* inoculated plants, SSC/TA ratios were higher by 5%, 24%, and 25% than in the non-AMF control at the 50, 100, and 200 mM NaCl levels, respectively. 'Charlotte' inoculated with *R. irregularis* produced fruits with the highest SSC, SSC/TA, and had the highest salt tolerance.

**Table 5.** Three-way ANOVA for salinity (S), AMF, and cultivar (C) treatments and their interactions on the fruit quality parameters: soluble solids content (SSC), titratable acidity (TA), and their ratio (SSC/TA).

	S	AMF	C	S × AMF	S × C	AMF × C	S × AMF × C
SSC	***	**	***	*	NS	***	NS
TA	NS	***	***	***	***	***	***
SSC/TA	***	***	***	***	*	NS	NS

**Note:** \*, 0.05; \*\*, 0.01; \*\*\*, 0.001; NS, not significant at  $P=0.05$

**Table 6.** Fruit soluble solids content (SSC), titratable acidity (TA), and the ratio (SSC/TA) of strawberry plants treated with or without AMF and salinity.

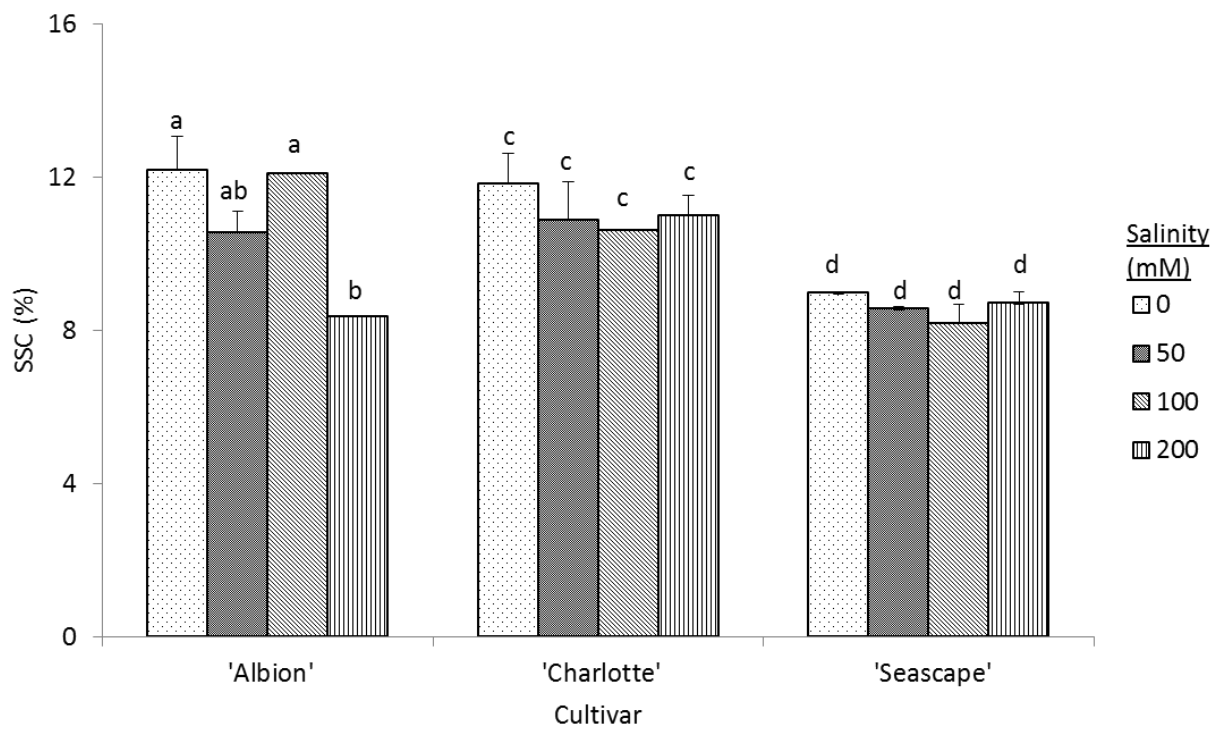
	SSC (%)	TA (%)	SSC/TA
<u>Cultivars</u>			
'Albion'	12.3a	0.93a	11.8b
'Charlotte'	10.9b	0.94a	14.6a
'Seascape'	8.9c	0.89a	9.5c
LSD <sub>0.05</sub>	0.6	0.05	0.82
<u>AMF</u>			
Non-AMF	10.3a	1.03a	11.4a
<i>F. caledonius</i>	10.7a	0.74d	11.7a
<i>R. irregularis</i>	10.5a	0.91c	11.8a
<i>F. mosseae</i>	10.2a	0.96bc	12.7a
<i>R. irregularis</i> + <i>F. mosseae</i>	9.2b	0.98a	9.6b
LSD <sub>0.05</sub>	0.96	0.06	1.42
<u>Salinity levels</u>			
0 mM	11.3a	0.90a	12.8a
50 mM	10.3b	0.92a	11.6ab
100 mM	9.5c	0.94a	10.9b
200 mM	9.4c	0.9a	10.7b
LSD <sub>0.05</sub>	0.8b	0.06	1.2
Orthogonal polynomial contrast	Quadratic***	NS	Quadratic**

**Note:** Different letters within each block indicate significant differences ( $P < 0.05$ ) according to LSD test; Significant differences: \*, 0.05; \*\*, 0.01; \*\*\*, 0.001; NS, not significant at  $P=0.05$

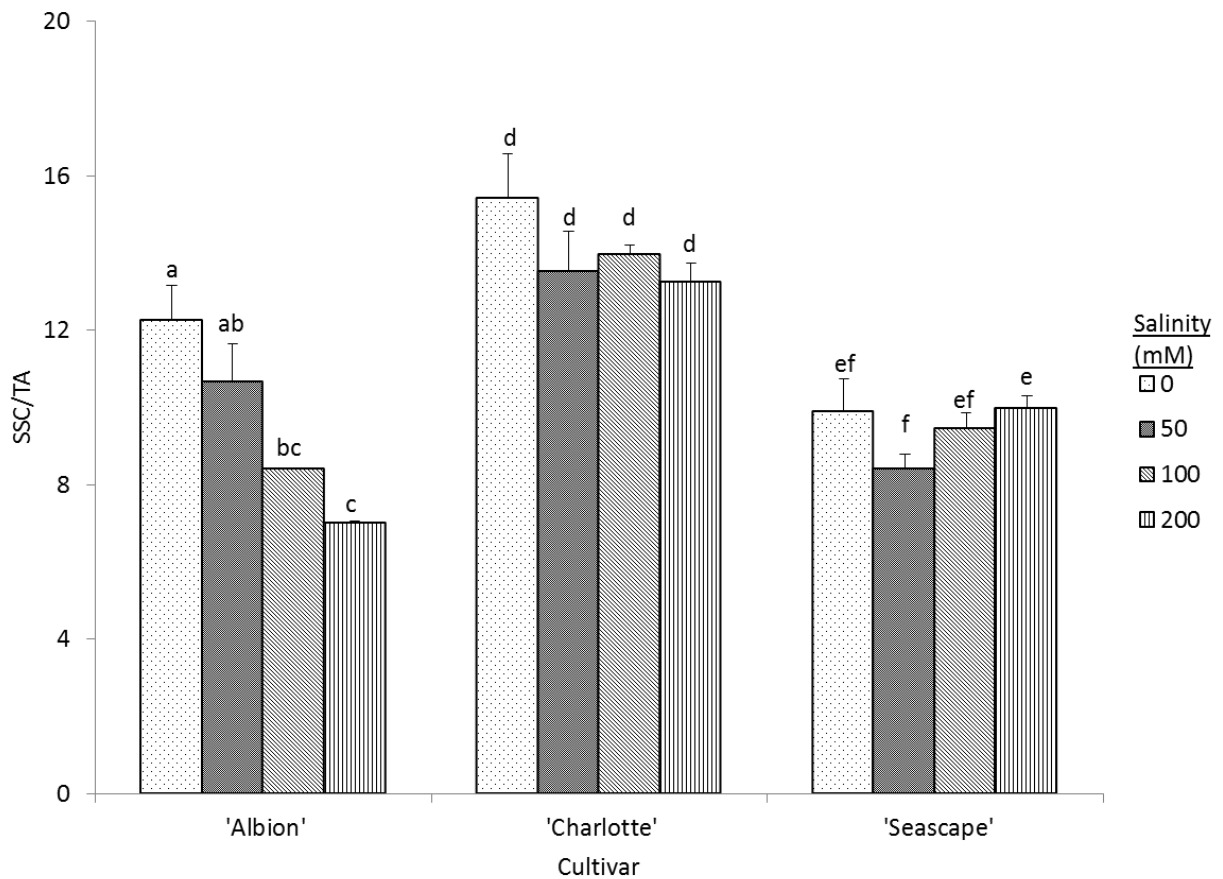
**Table 7.** Interactions among AMF and salinity on fruit soluble solids content (SSC), titratable acidity (TA), and the ratio (SSC/TA) of strawberry cultivars.

	Non-AMF	<i>F. caledonius</i>	<i>R. irregularis</i>	<i>F. mosseae</i>	<i>R. irregularis</i> + <i>F. mosseae</i>
<b>SSC (%)</b>					
<u>Salinity levels</u>					
0 mM	11.3a	11.7a	11.8a	11.3a	9.5a
50 mM	10ab	10.3a	9.9b	11a	9.8a
100 mM	9.8b	10a	9.9b	9.1b	8.1b
200 mM	9.6b	9.9a	10.4ab	8.1b	9.1ab
LSD <sub>0.05</sub>	1.5	1.9	1.7	1.9	1.4
Orthogonal polynomial contrast	NS	NS	L*	Q***	Q*
<b>TA (%)</b>					
<u>Salinity levels</u>					
0 mM	0.9a	0.89a	0.99a	0.89a	0.94a
50 mM	0.94a	0.99a	0.96ab	0.78a	1.13a
100 mM	0.98a	0.98a	0.84b	0.79a	0.99a
200 mM	0.92a	0.93a	0.85b	0.88a	0.96a
LSD <sub>0.05</sub>	0.12	0.12	0.13	0.13	0.24
Orthogonal polynomial contrast	NS	NS	Q**	NS	NS
<b>SSC/TA RATIO</b>					
<u>Salinity levels</u>					
0 mM	12.9a	13.6a	11.9a	13.1a	10.5a
50 mM	10.9ab	10.6b	10.7a	14.4a	8.9a
100 mM	10.4b	10.8b	12.4a	12.1a	8.3a
200 mM	10.7b	10.9b	12.8a	9.3b	9.9a
LSD <sub>0.05</sub>	2.1	2.4	3.2	2.7	3
Orthogonal polynomial contrast	NS	L**	NS	Q**	NS

**Note:** Different letters within each block indicate significant differences between treatments ( $P < 0.05$ ) according to LSD test; \*, 0.05; \*\*, 0.01; \*\*\*, 0.001; NS, not significant



**Fig. 8.** Effect of salinity on the soluble solids content (SSC) of fruits in three strawberry cultivars. Means (n=6) +SE are presented. Bars sharing a same letter within the same cultivar are not significantly different at  $P < 0.05$  according to LSD test.



**Fig. 9.** Effect of salinity on the ratio of soluble solids content to titratable acidity (SSC/TA) of fruits in three strawberry cultivars. Means (n=6) +SE are presented. Bars sharing a same letter within the same cultivar are not significantly different at  $P < 0.05$  according to LSD test.

## Root architecture

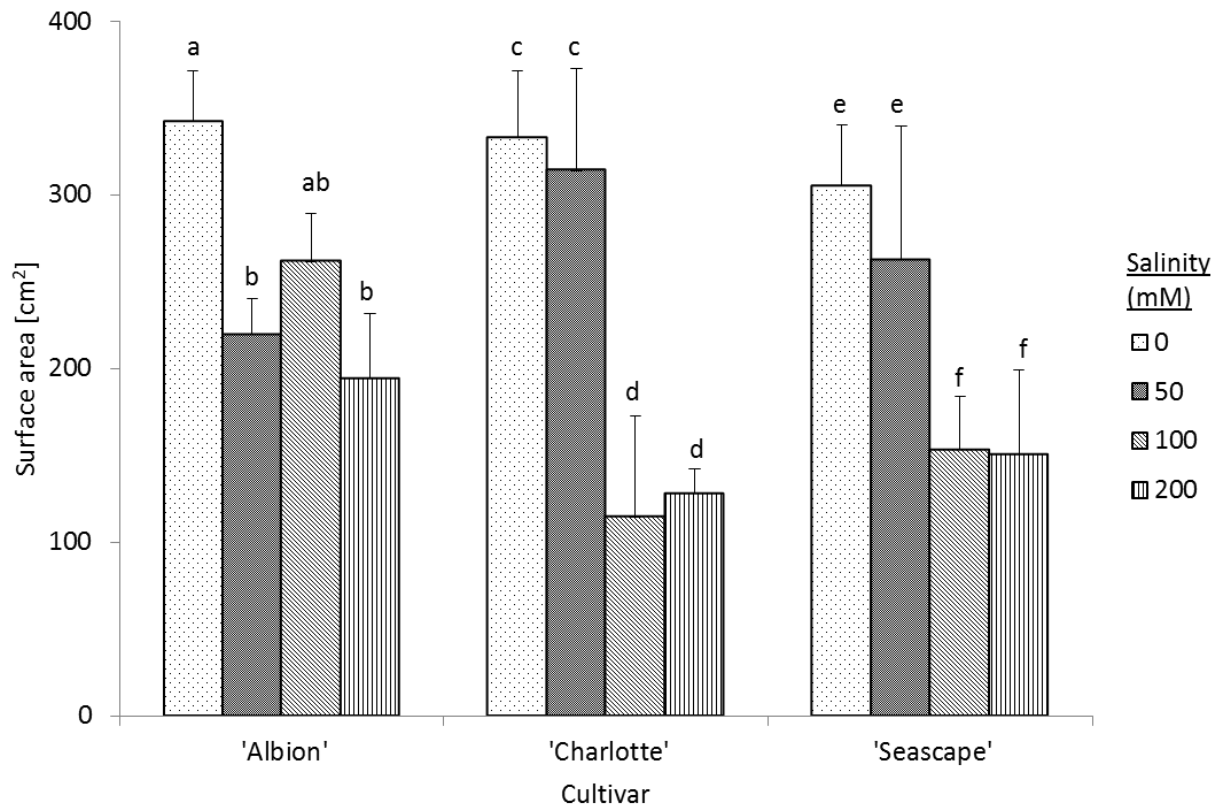
The salt treatment significantly ( $P < 0.001$ ) decreased the root surface area (Fig. 10), length (Fig. 11), volume (Fig. 12), and the number of forks and crossings (Tables 8 and 9). The greatest decrease in all these parameters was observed when salinity increased from 50 to 100 mM NaCl. 'Charlotte' and 'Seascape' were more negatively affected by salinity than 'Albion'. The average root diameter and specific root length (SRL) were not significantly affected by salinity (Table 8).

Without salt or AMF, 'Albion' had the smallest average root diameter and the largest root system (largest surface area, length, volume, and highest number of forks and crossings). 'Charlotte' and 'Seascape' had a relatively large average root diameter, and a smaller root system overall. There was an interaction between salinity and cultivar on root diameter ( $P = 0.039$ ). As salinity increased, the average diameter of roots did not change in 'Albion' or 'Charlotte', but tended to increase in 'Seascape'. There was an interaction between salinity and cultivar on the distribution of root length among the different diameter classes (Fig. 13). 'Albion' had the highest proportion of fine roots ( $\phi \leq 0.5\text{mm}$ ) that remained constant with increasing salinity. 'Charlotte' had a lower proportion of fine roots that did not change with salinity. 'Seascape' had the highest proportion of coarse and medium roots that increased further with salinity, at the expense of fine roots. With the exception of root diameter, there was no significant interaction between the cultivar and salt treatment on the root parameters; the cultivars responded similarly to salinity.

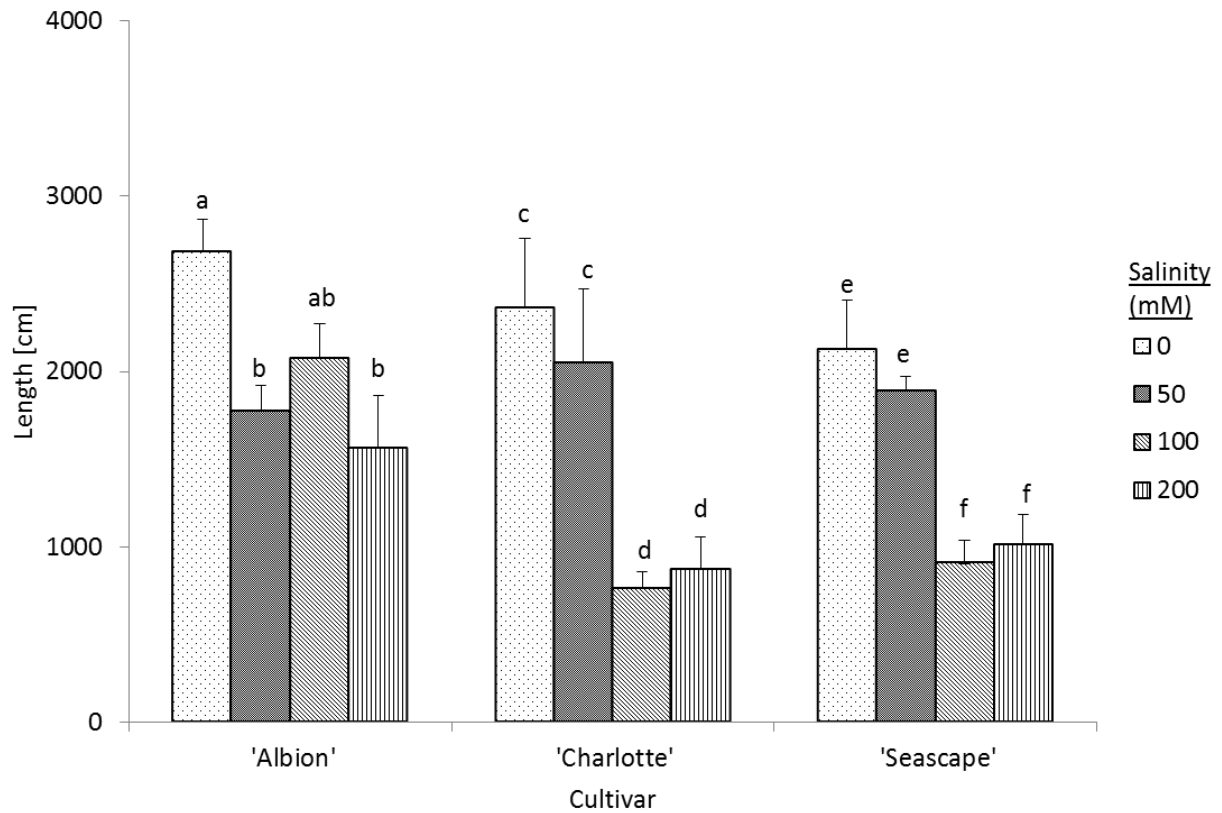
**Table 8.** Three-way ANOVA for salinity (S), AMF, and cultivar (C) treatments and their interactions on root parameters.

	S	AMF	C	S × AMF	S × C	AMF × C	S × AMF × C
Diameter	NS	NS	***	NS	*	NS	NS
Surface area	***	NS	*	NS	NS	NS	NS
Length	***	NS	***	NS	NS	NS	NS
Volume	***	NS	NS	NS	NS	NS	NS
Specific root length	NS	NS	***	NS	NS	NS	NS
Number of forks	***	NS	***	NS	NS	NS	NS
Number of crossings	***	NS	***	NS	NS	NS	NS

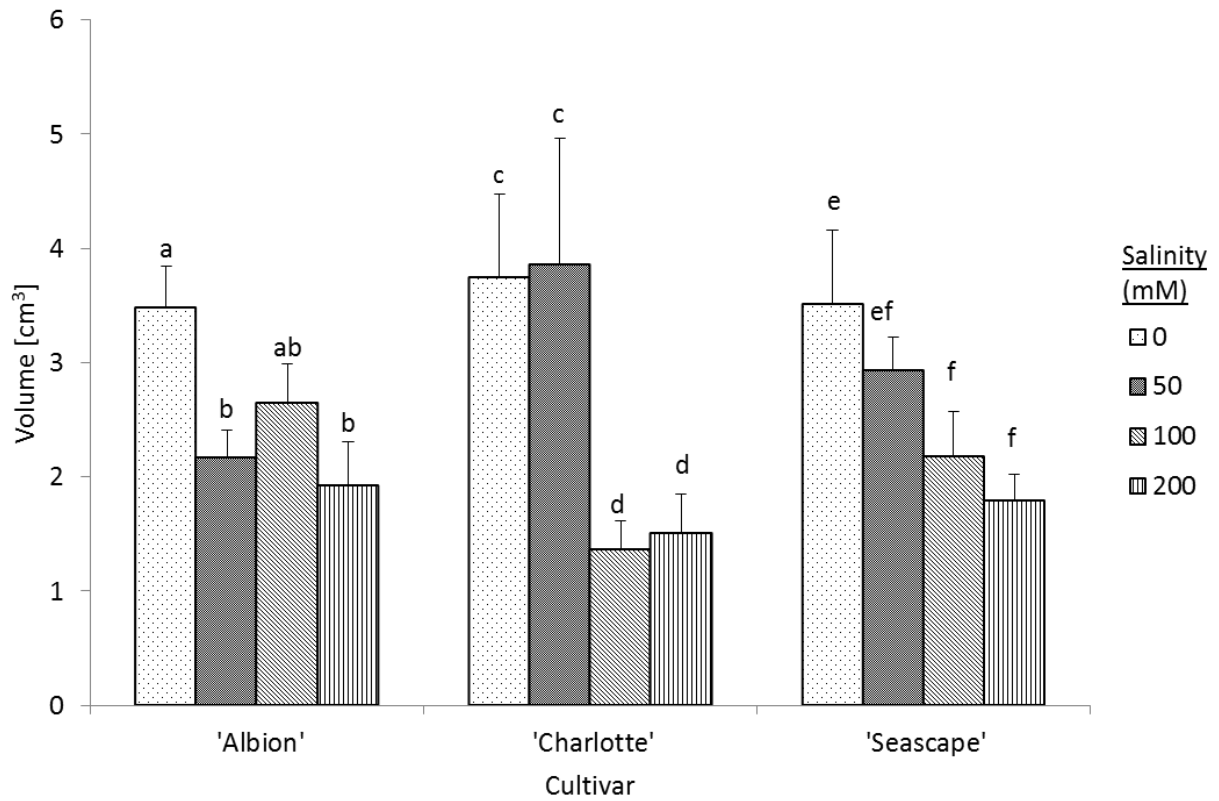
**Note:** \*, 0.05; \*\*, 0.01; \*\*\*, 0.001; NS, not significant



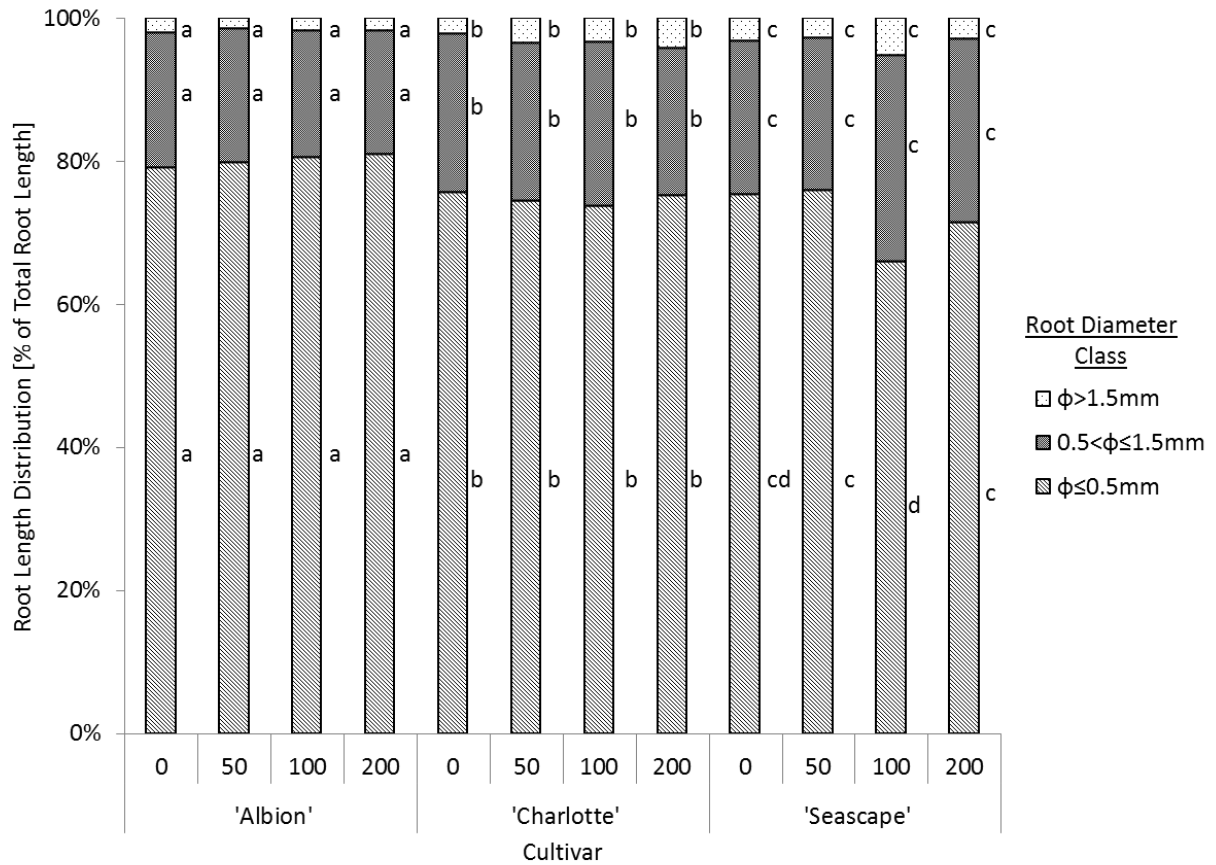
**Fig. 10.** Effect of salinity on the mean ( $n=12$ )  $\pm$ SE root surface area ( $\text{cm}^2$ ) in three strawberry cultivars. Different letters within each cultivar indicate significant differences at  $P<0.05$  according to LSD test.



**Fig. 11.** Effect of salinity on mean (n=4) +SE root length in three strawberry cultivars. Different letters within each cultivar indicate significant differences at  $P < 0.05$  according to LSD test.



**Fig. 12.** Effect of salinity on mean ( $n=4$ ) +SE root volume ( $\text{cm}^3$ ) in three strawberry cultivars. Different letters within each cultivar indicate significant differences at  $P<0.05$  according to LSD test.



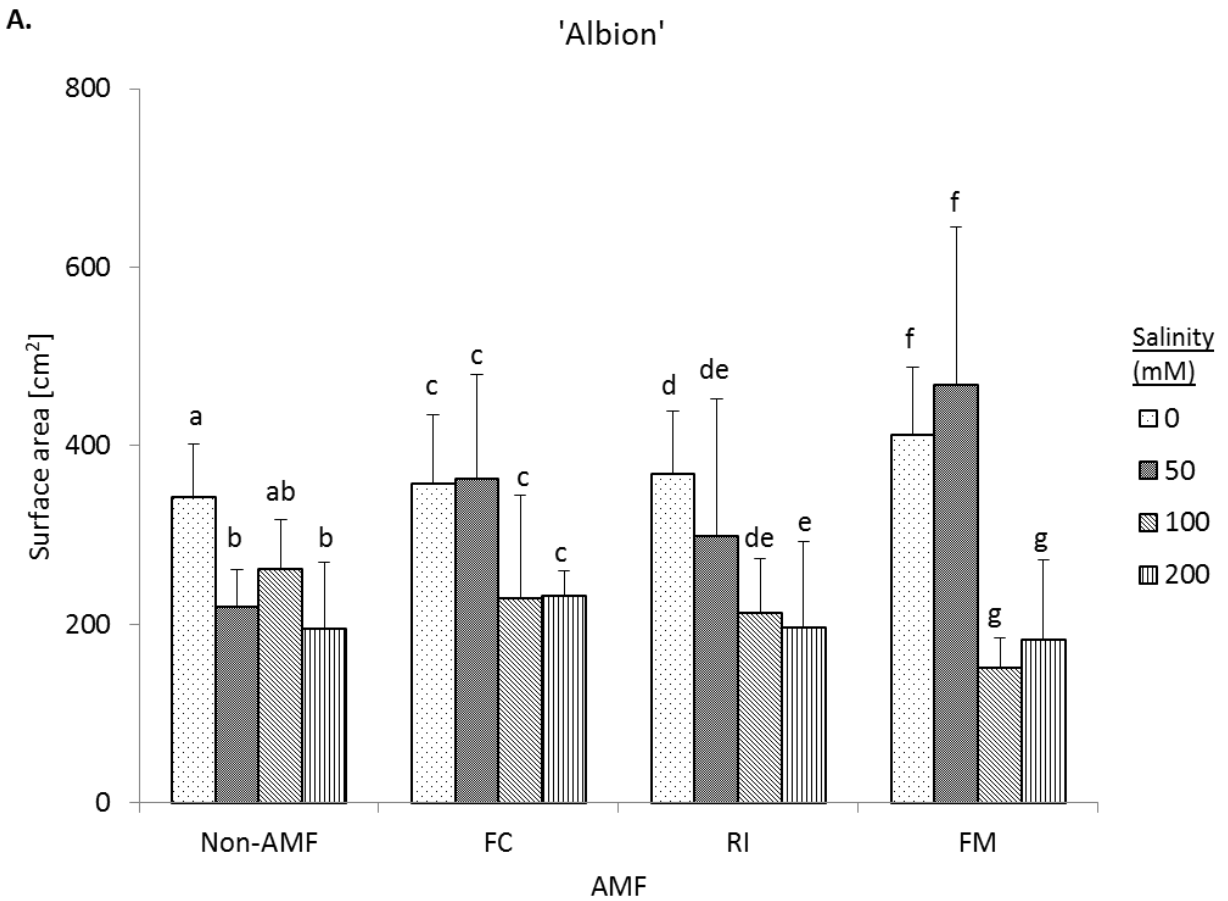
**Fig. 13.** Effect (%; n=4) of salinity (0-200 mM) on the distribution of root diameter classes in three strawberry cultivars. Different letters within each cultivar and root diameter class indicate significant differences at  $P < 0.05$  according to LSD test. SE < 5%.

**Table 9.** Root parameter measurements of strawberry plants treated with or without AMF and salinity.

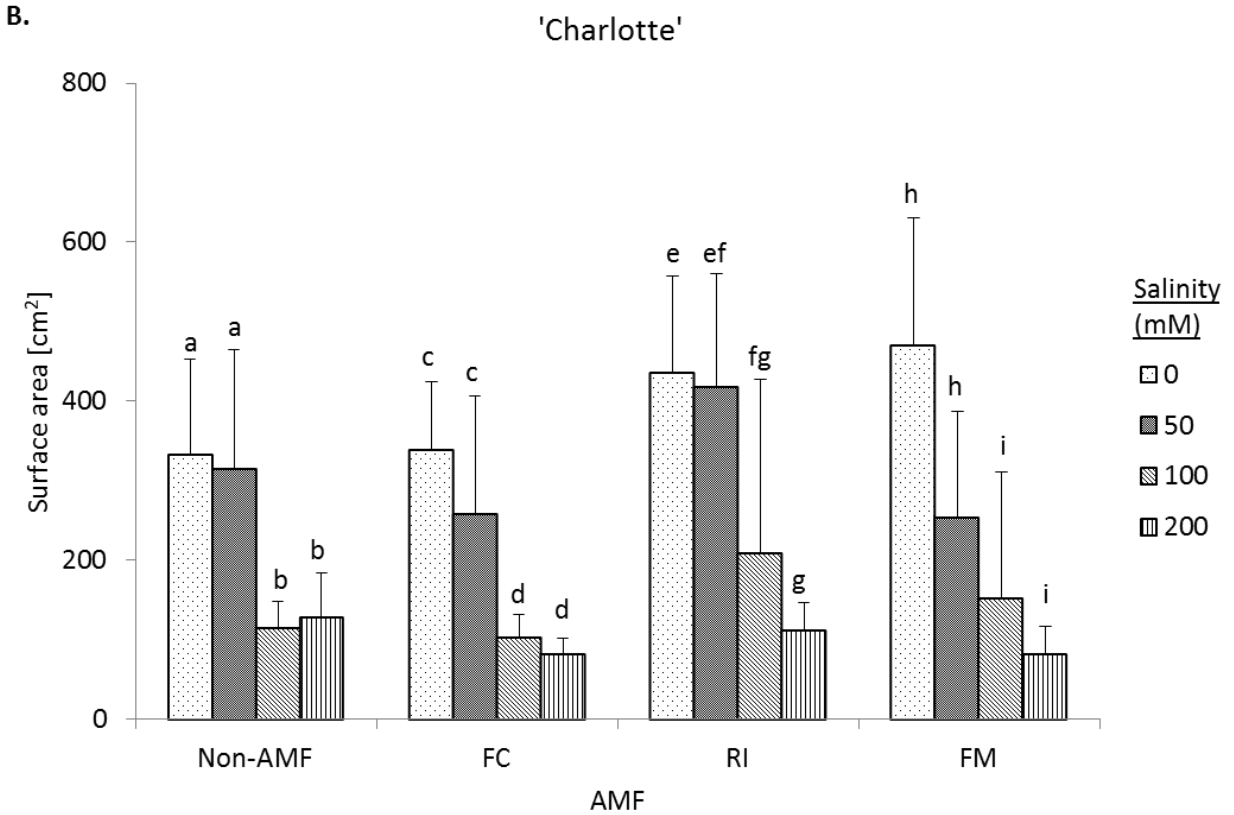
	Average diameter (mm)	Surface area (cm <sup>2</sup> )	Total length (cm)	Volume (cm <sup>3</sup> )	SRL (cm/g)	No. of crossings	No. of forks
<u>Cultivars</u>							
'Albion'	0.42b	280a	2102a	3a	362a	3757a	12160a
'Charlotte'	0.49a	246a	1558b	2.9a	245b	2313b	7798b
'Seascape'	0.48a	237a	1656b	2.9a	209c	2653b	8924b
LSD <sub>0.05</sub>	0.02	48	307	0.6	34	635	1978
<u>AMF</u>							
Non-AMF	0.45b	231a	1673a	2.6b	243ab	2639a	8694a
<i>F. caledonius</i>	0.45ab	242a	1724a	2.8ab	281a	2913a	9468a
<i>R. irregularis</i>	0.46ab	265a	1834a	3.1ab	286a	3010a	10040a
<i>F. mosseae</i>	0.48ab	264a	1771a	3.2ab	274ab	2936a	9810a
<i>R. irregularis</i>	0.48a	289a	1910a	3.5a	223b	3053a	10410a
+							
<i>F. mosseae</i>							
LSD <sub>0.05</sub>	0.03	68	449	0.9	57	945	2928
<u>Salinity levels</u>							
0 mM	0.47a	382a	2601a	4.6a	261a	4622a	14950a
50 mM	0.46a	310b	2149b	3.6b	276a	3525b	11700a
100 mM	0.46a	172c	1224c	2c	285a	1860c	6354c
200 mM	0.47a	150c	1077c	1.7c	246a	1546c	5293c
LSD <sub>0.05</sub>	0.03	41	267	0.6	47	592	1818
Orthogonal polynomial contrast	NS	Q***	Q***	Q***	NS	Q***	Q***

**Note:** Different letters within each block indicate significant differences ( $P < 0.05$ ) according to LSD test; \*, 0.05; \*\*, 0.01; \*\*\*, 0.001; NS, not significant

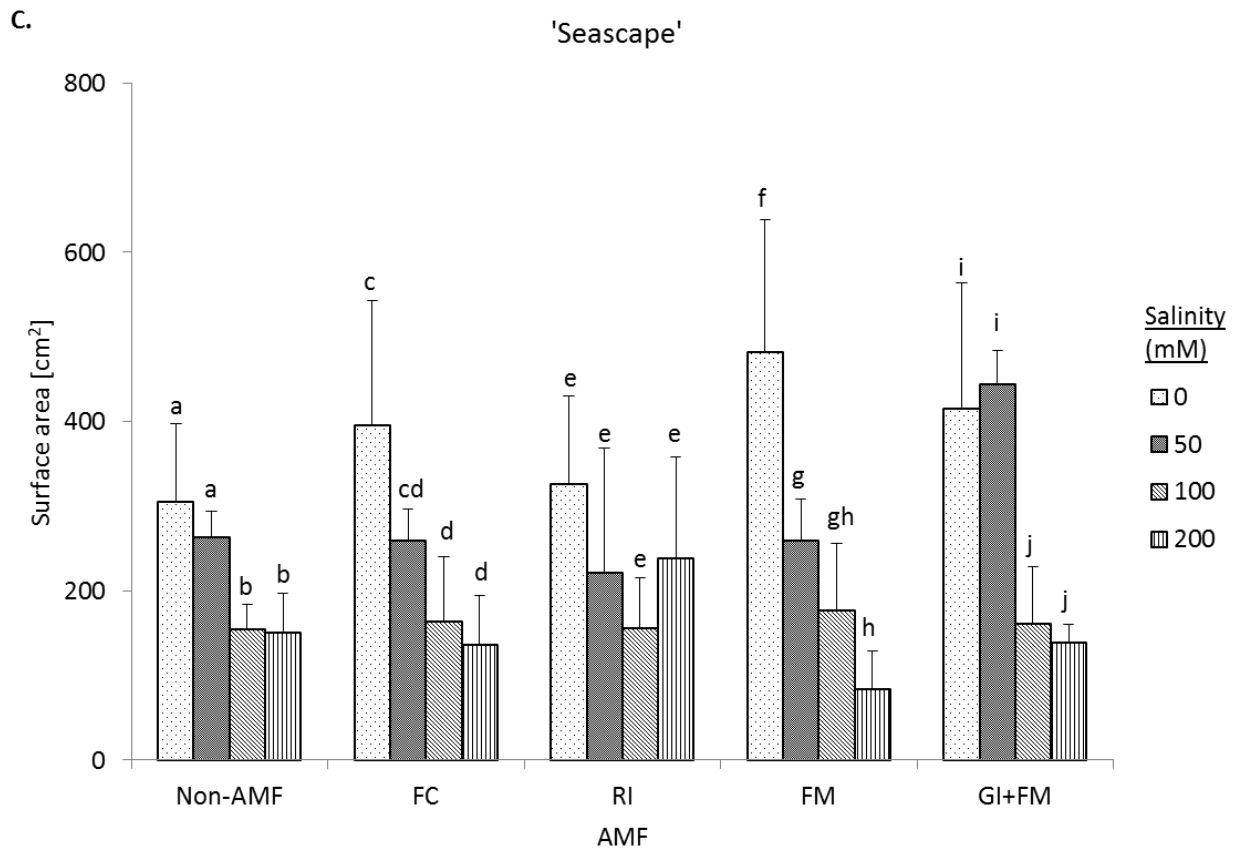
The AMF increased root growth overall (Table 9). Root surface area was increased by AMF (Fig. 14A-C). At low salt level (0-50 mM), *F. mosseae* increased root surface area to a higher degree than the other AMF species. At high salinity (100-200 mM), surface area was increased most by *F. caledonius* in 'Albion' and by *R. irregularis* in 'Charlotte'. At low salinity, plants of 'Seascape' inoculated with the mixed-AMF species of *R. irregularis* and *F. mosseae* produced a significantly larger surface area than with any single AMF species or the non-inoculated control. However, at high salinity, *R. irregularis* improved the surface area to a higher degree than the mixed AMF species. A similar trend was observed for root length and volume.



**Fig. 14A.** Effect of AMF and salinity (mM) on mean (n=4) +SE root surface area in strawberry cultivar 'Albion'. Different letters within each cultivar indicate significant differences at  $P < 0.05$  according to LSD test.



**Fig. 14B.** Effect of AMF and salinity (mM) on mean (n=4) +SE root surface area in strawberry cultivar 'Charlotte'. Different letters within each cultivar indicate significant differences at  $P < 0.05$  according to LSD test.



**Fig. 14C.** Effect of AMF and salinity (mM) on mean (n=4) +SE root surface area in strawberry cultivar 'Seascape'. Different letters within each cultivar indicate significant differences at  $P < 0.05$  according to LSD test.

The AMF increased the root length in all root diameter classes, although the proportion of medium and coarse roots was increased to a higher degree than fine roots. AMF had significant effects on the proportion of medium and fine roots ( $P=0.0068$  and  $P=0.0132$ ), the proportion of medium roots increasing at the expense of fine roots. Similar to the response in surface area to AMF, the mixed-species AMF had the strongest effect on root length distribution at low salinity, but decreased the proportion of coarse roots at 200 mM NaCl, while single species *R. irregularis* increased the proportion of coarse roots at high salinity.

### 3.3 Stolon effect

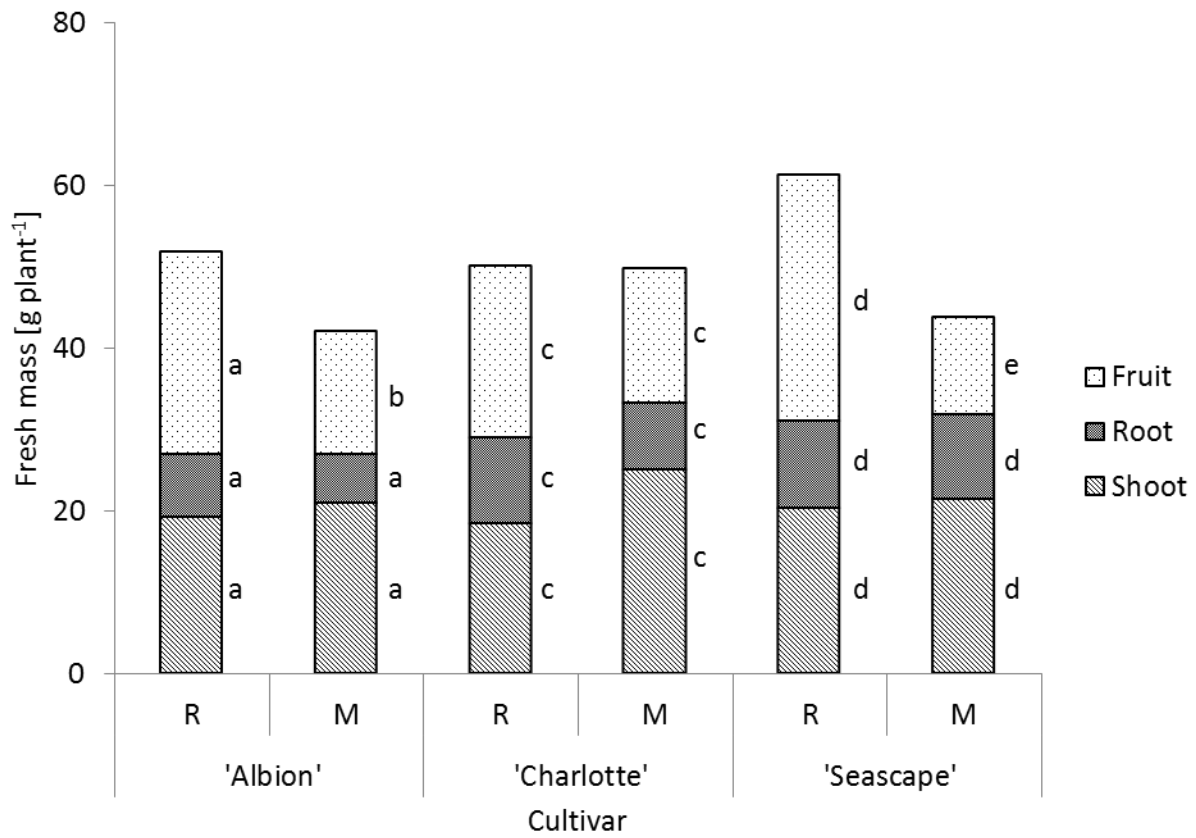
Stolons were removed from plants throughout the growing season in the first experiment and not in the second experiment. Stolon removal was revealed to be highly significant ( $P < 0.001$ ) on fruit, shoot, and root mass (Table 10). The plant mass was greater when stolons were maintained than when removed. Particularly, when stolons were maintained, shoot mass increased while fruit mass decreased (Figs. 15 and 16). Overall, the positive relationship between fresh shoot and fruit mass per plant was 26% greater when stolons were removed. There was an interaction between salinity and stolon removal on shoot ( $P < 0.001$ ) and root ( $P = 0.0016$ ) mass; the effect of salinity was much stronger when stolons were maintained.

The AMF symbiosis was less beneficial when stolons were maintained. When stolons were removed, *R. irregularis* preserved fresh shoot mass by 27% and 26% more efficiently at the 50 mM and 100 mM NaCl levels, respectively, than when stolons were maintained. There was an interaction between AMF and stolon removal on fruit yield ( $P = 0.0012$ ); fruit mass was significantly increased by AMF when stolons were removed and significantly decreased when maintained. Strawberry plants inoculated with *R. irregularis* produced the most fruit mass when stolons were removed, and the least when stolons were maintained. The other *Glomus* species followed the same trend, however to a lesser degree.

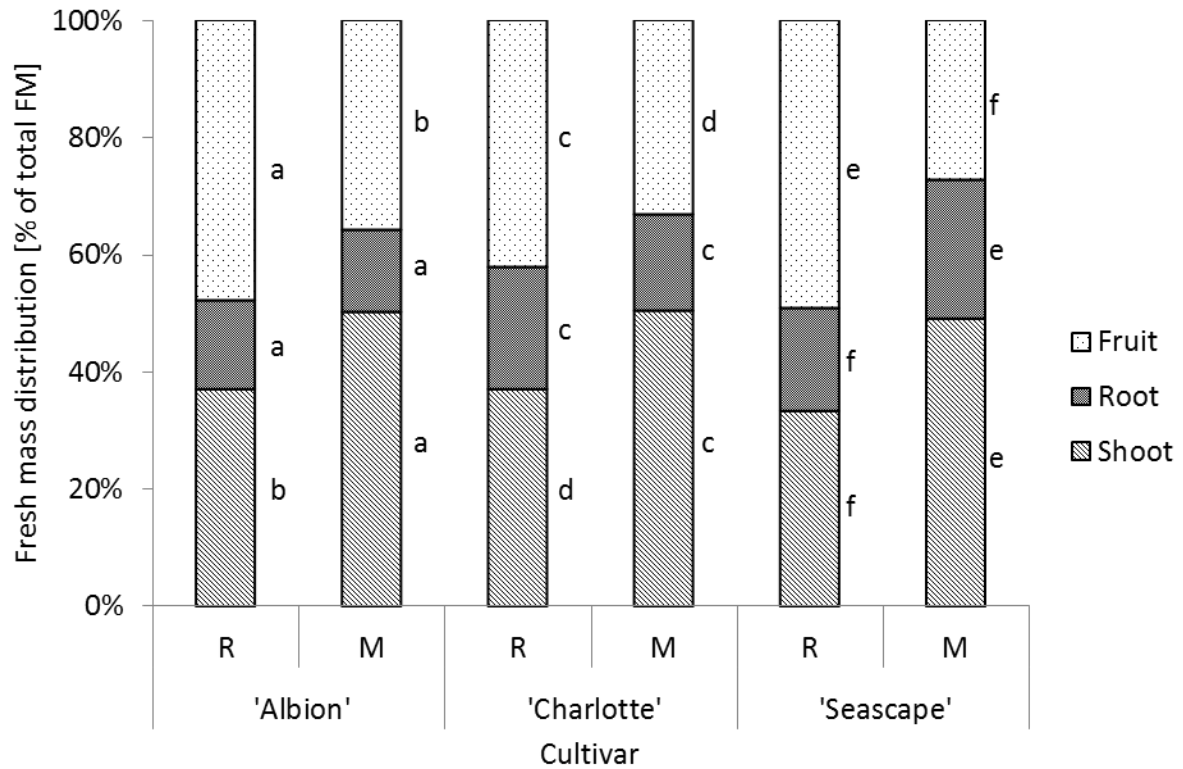
**Table 10.** Four-way ANOVA for salinity (S), AMF, cultivar (C), and stolon (ST) treatments and their interactions on biomass parameters.

	S	AMF	C	ST	S×AMF	S×C	AMF×C	S×ST	AMF×ST	C×ST	S×AMF×C	S×AMF×ST	S×C×ST	AMF×C×ST	S×AMF×C×ST
Fruit mass	*	NS	***	***	NS	NS	*	NS	**	***	NS	NS	**	NS	NS
Fresh root mass	***	NS	***	***	NS	NS	*	**	NS	*	NS	NS	NS	NS	NS
Fresh shoot mass	***	NS	NS	***	NS	NS	NS	***	NS	NS	NS	NS	NS	NS	NS

**Note:** \*, 0.05; \*\*, 0.01; \*\*\*, 0.001; NS, not significant



**Fig. 15.** Effect of stolon removal (R) or maintenance (M) on mean (n=12) fresh mass (g plant<sup>-1</sup>) of plant organs in three strawberry cultivars. Different letters within each cultivar indicate significant differences in each plant organ at  $P < 0.05$  according to LSD test.



**Fig. 16.** Effect of stolon removal (R) or maintenance (M) on mean (n=12) fresh mass distribution (% of total fresh mass) of plant organs in three strawberry cultivars. Different letters within each cultivar indicate significant differences in each plant organ at  $P < 0.05$  according to LSD test.

## Chapter 4: Discussion and Conclusions

Fungal root colonization was not affected by salinity. Thus, we cannot accept the hypothesis that colonization levels decrease with increasing salinity. Overall, fungal inoculation tended to improve biomass, root architecture, and fruit quality under salt conditions. This, we accept the hypothesis that fungal symbiosis benefits strawberry plants in their tolerance to salinity.

The finding that salinity did not affect overall the percent root colonization is inconsistent with others (Yang *et al.*, 2009; Fan *et al.*, 2011a) who reported that AMF colonization rates of strawberry plants significantly decreased with increasing salinity. AMF spore germination was shown to be inhibited by salinity (Hirrel, 1981; Juniper and Abbott, 2006), as well as arbuscular and hyphal growth and development (Pfeiffer and Bloss, 1988; McMillen *et al.*, 1998). Another common response of root systems to salinity is a reduction in growth rate and the appearance of endodermal and exodermal suberization closer to the root apex (Shannon *et al.*, 1994; Reinhardt and Rost, 1995), resulting in a wider root diameter. The root suberization reduces mycorrhizal association, as coarse, suberized roots are responsible for mechanical support and the transport of mineral nutrients between the fine roots and shoot, and are not found to be colonized. Fine, third order roots are associated with most nutrient and water uptake, as well as mycorrhiza formation (Marschner, 1995). The reduction in the proportion of fine roots at high salinity is therefore associated with a lower colonization level.

However, at high salinity, our results indicated an interaction between salinity and cultivar, illustrating that cultivars respond differently to salinity and AMF symbiosis. For example, 'Albion' having a low root colonization at high salinity may be related to the fact that it benefitted from AMF only at low salinity. Audet and Charest (2009) stressed that roots and AMF experience a critical toxicity threshold at which their symbiosis ceases to be beneficial.

In our study, colonization levels varied widely among cultivars. Such variability in colonization by *Glomus* spp. in strawberry had also been shown by Stewart *et al.* (2005). Colonization levels by *R. irregularis* were lower than those reported in literature for *F. intraradices*, its close relative, under similar conditions. Compared with 31% colonization in our study, Fan *et al.* (2011a) reported 57% colonization, 35% in cv. 'Elvira' by Taylor and Harrier (2001), and 41% in cv. 'Avanta' by Varma and Schuepp (1994). Levels of root colonization by *F. mosseae* were also lower than those found in literature. In our study, colonization ranged from 1 to 33%. Under similar conditions, Vestberg (1992) observed colonization by *F. mosseae* in ten strawberry cultivars ranging from 23 to 42%. However, data on mycorrhizal colonization of roots and the distribution of fungal propagules such as spores is often not reliable as it is highly variable and has a non-normal frequency distribution (St. John and Hunt, 1983; Friese and Koske, 1991).

There was no relationship between percent colonization and biomass; the cultivars indicating the lowest percent colonization, 'Albion' and 'Seascape', achieved the highest shoot fresh and dry mass and fruit yield. However, there was a positive relationship between percent colonization and root architecture; 'Charlotte', having the highest percent colonization, the

highest root mass, diameter, surface area, length, volume, and SRL. The other cultivars followed this trend, however, 'Mara des Bois' produced poorly relative to the other cultivars in all measurements, despite its high level of colonization. Similarly, *R. irregularis* achieved the highest level of colonization, and also increased root parameters to the highest degree. This finding is consistent with previous studies that linked the level of mycorrhizal colonization to the level of root system enhancement (Atkinson *et al.*, 1994).

Turhan and Eris (2005) reported that strawberry shoot and root biomass were unaffected by weak salinity. Our results were consistent with this finding; shoot mass, and particularly, root mass, were mostly unaffected at 50 mM NaCl. The growth response to salinity is usually attributed to either ion toxicity or low external osmotic potential (Munns and Termatt, 1986). The resistance of roots to weak salinity is due to the high tolerance of external cortical layers to the presence of ion excess in the circulating water solution in the medium. While the FAO reported decreased fruit yield in strawberry at 10 mM NaCl, in our study, reductions in biomass were not significant until 100 mM.

Responses of cultivated strawberry plants to different AMF and other inoculants have been shown to vary (Khanizadeh *et al.*, 1995; Mark and Cassells, 1996; Murphy *et al.*, 2000). The interactions are dependent on plant-fungus compatibility, as some AMF-plant combinations are more beneficial than others (Klironomos, 2003). In our study, cultivars responded differently to inoculation. In screening the AMF species, it was observed that particular species could have a negative effect on plant growth. *G. arenarium* had a depressive effect on plant growth, and *F. caledonius* and *F. mosseae* particularly on 'Charlotte'. Taylor and

Harrier (2001) reported no positive shoot growth response to *Glomus* species AMF inoculation, and root growth increases for plants colonized by *F. intraradices*. Similarly, in our study, root mass was more strongly affected by AMF than shoot mass, indicating a strong relation between AMF and the root system.

Our results suggest a strong positive link between root mass and salt tolerance, especially in 'Seascape' that produced higher root mass than the other cultivars and exhibited a markedly higher level of salt tolerance. The interaction between AMF and salinity on root mass may also indicate a strong relationship between the root system and salt tolerance such as AMF-inducing root enhancement increased tolerance to salinity (Fan *et al.*, 2011a).

Studies have generally reported beneficial effects of mycorrhizal inoculation on strawberry productivity. Davies *et al.* (2002) stated that the response of a plant to AMF is not only cultivar or isolate specific but depends on the environmental conditions. In our study, *R. irregularis* was shown to increase the root mass at high, but not at low salinity level. Since many AMF have particular preferences for specific environmental conditions, and cultivars respond differently to AMF and environmental conditions, it would be important to screen mycorrhizal-induced plant responses in this way.

The soluble solids content and titratable acidity are the most important indices of fruit quality ubiquitously used in standard quality controls (Fan *et al.*, 2011b). Strawberry flavor is derived from the interactive taste and aromas of many chemical constituents, mainly sugars and volatile compounds. SSC is mainly derived from organic sugars, such as glucose, sucrose

and fructose, which influence the taste, flavor and maturity of strawberries (Kader, 1990). High level sugars and relatively high acid content are required for good flavor.

Galletta *et al.* (1995) reported that in general SSC is in the range of 7-12% in strawberry fruit. Our results were mostly within this range as shown; for cultivars 'Albion' and 'Charlotte' ranging from 10 to 14.5% without salinity but decreasing with the addition of salt.

The ratio of SSC/TA of 8.5-14 is considered an appropriate balance of sweet-tart flavour notes in strawberry for human palatability (Oregon Strawberry Commission, 2006). By this standard, most 'Seascape' fruits under NaCl treatment, regardless of AMF, and non-AMF 'Albion' fruits treated with 100mM or higher NaCl were deemed unacceptable for consumer consumption.

Other studies (Awang *et al.* 1993; Keutgen and Pawelzik 2007, 2008) reported that moderate salinity results in improved strawberry fruit quality, as indicated by higher soluble solids, in some cultivars. This increase being not observed in our study suggests that the tested cultivars should not be grown under moderate salinity levels to optimize fruit quality.

Root-system architecture is an important feature that can be altered by various abiotic and biotic factors (Ostonen *et al.*, 2007). In our study, root system response to salinity varied among cultivars, although the interaction among salinity and cultivar was only significant for diameter. A common response of root systems to salinity is a reduction in growth rate and the appearance of endodermal and exodermal suberization closer to the root apex (Shannon *et al.*, 1994; Reinhardt and Rost, 1995), resulting in roots of a wider diameter. These coarse roots are responsible for mechanical support and the transport of mineral nutrients between fine roots

and the shoot. As shown, salinity increased average root diameter in the tested cultivars. 'Seascape' responded most positively to salinity. It possessed roots of a wider diameter than the other cultivars and salinity increased the proportion of medium and coarse roots. The other cultivars had an increase in coarse root length with salinity, but the response was not as strong as in 'Seascape'. Interestingly, 'Seascape' demonstrated a significantly higher level of salt-tolerance in its biomass than the other cultivars. Results suggest a link between the proportion of coarse roots and salt tolerance.

AMF were shown to improve the rhizosphere and contribute to salt tolerance of crops (Pond *et al.*, 1984; Ruiz-Lozano *et al.*, 1996). Improved salt tolerance following mycorrhizal colonization may be the result of more efficient nutrient uptake, reduced levels of water stress, lower disease resistance, and increased photosynthesis ability (Feng *et al.*, 2000; Augé, 2001; Mohammad *et al.*, 2003; Stewart *et al.*, 2005; Sheng *et al.*, 2008). AMF improved root systems under stress, and root system improvement was related to increased shoot mass. This finding is consistent with results of Marschner (1995), and Fan *et al.* (2011a), who found that the length of suberized roots is increased by mycorrhiza, offering increased mechanical support and mineral nutrient transport between fine roots and the shoot.

SRL is probably the most frequently measured parameter of fine roots, and is indicative of environmental changes. SRL is strongly dependent on fine root classes (Ostonen *et al.*, 2007). These fine, third order roots are associated with most nutrient and water uptake, as well as mycorrhiza formation (Marschner, 1995). SRL has been successfully used as an indicator of nutrient availability in experimental conditions, in which SRL decreased under reduced nutrient availability. In our study, SRL was significantly increased by AMF and not affected by

salinity. By increasing fine root length, AMF increased SRL and may indicate that AMF colonization effectively increased the availability of nutrients to the roots. Similar results were reported by Fan *et al.* (2011a). There was also an interaction between cultivar and AMF on SRL, especially in 'Charlotte' and 'Seascape', however not in 'Albion'. It is of interest to note that inoculated plants of 'Charlotte' and 'Seascape' were more salt tolerant than 'Albion' in their biomass.

Colonization by the mixed-AMF species was higher than either of its single-species components in 'Seascape'. In a similar study, Stewart *et al.* (2005) reported that after six weeks, the colonization level by mixed species of *F. intraradices* + *F. mosseae* + *G. etunicatum* was higher than *F. intraradices* alone in some cultivars, and lower in others. In our study, the high level of colonization by mixed AMF species was related with an improved root system, but only at low salinity level. It also related with higher shoot and fruit mass over single species AMF treatments, but with lower fruit quality. Studies remain inconclusive as to the benefit of mixed-species inoculum over its single-species components on strawberry plant growth. Previous studies have shown that the effect of multiple-AM fungal species is not necessarily additive. Stewart *et al.* (2005) found that the mixed-species inocula were more beneficial to biomass in only some cultivars. Koomen *et al.* (1987) reported that inoculum containing four *Glomus* species was equally or more effective than with only single species in promoting strawberry plant growth under control or stressful conditions.

Stewart *et al.* (2005) reported that *F. intraradices* was more efficient at promoting growth of strawberry plants than a mix of *F. intraradices*, *F. mosseae*, and *G. etunicatum*. In our study, 'Seascape' inoculated with the mixed AMF species *R. irregularis* + *F. mosseae*

increased shoot and root fresh mass at 0 and 50 mM NaCl, but reduced biomass at 100 and 200 mM NaCl, while *R. irregularis* alone increased biomass at high salinity. In 'Seascape', the mix of *R. irregularis* + *F. mosseae* improved fruit quality, but to a lesser degree than either of its single-species constituents. *R. irregularis*-inoculated plants preserved fruit quality under salt stress more efficiently than the other AMF species.

The mixed species inoculum was not different in its effect on root systems from that of the single species inoculum under stress conditions. Similar results were reported by Koomen *et al.* (1987) and Stewart *et al.* (2005). Therefore, the recommendation of multi-species inocula for field inoculation to ensure wider adaptation to different environmental conditions and greater consistency in benefits to the host plant must continue to be screened on a condition- and cultivar-specific basis.

Different cultures and varieties of strawberry plants have very different-sized root systems, depending largely on whether they make stolons freely and express their vigor in number of stolon plants, or whether they make few stolons and express their vigor in making large, individual plants. Within limits, the former can be changed into the latter by restricting stolons, a common practice in strawberry farming (Darrow, 1966). After fruit harvests, stolons, when allowed to proliferate, act primarily as sinks for water, nutrients, and photosynthates, reducing the amount of resources available for fruit production. Our findings that plants were more productive when stolons were removed are consistent with Hrselova *et al.* (1990) who reported that strawberry plants inoculated with *Glomus* spp. produce lesser biomass but more stolons. Overall, when stolons were removed, the effects of AMF were greater, and those of salinity lesser, than when stolons were maintained. Therefore the impact of salinity on

strawberry plants can be lessened, and the benefits of AMF amplified, by the practice of removing or suppressing stolons throughout the growing season.

## Recommendations and perspectives

Soil salinity is an increasing worldwide environment problem leading to huge losses in plant productivity. AMF are significant in alleviating salt stress and have beneficial effects on plant growth and productivity. In our study on strawberry, AMF improved salt tolerance by increasing the proportion of medium ( $0.5 < \phi \leq 1.5\text{mm}$ ) and coarse ( $\phi > 1.5\text{mm}$ ) diameter roots. In the tested cultivars, *R. irregularis* was most efficient at preserving biomass and fruit quality at low and high salinity. 'Seascape' produced the highest fruit mass at low and high salinity, however, 'Charlotte' produced the highest quality fruit, although lower yield. Because cultivars responded differently to fungal inoculants, and the inoculants have particular preferences under specific conditions, we recommend that fungal inoculants continue to be screened on a cultivar- and condition-specific basis.

Because agricultural soils face salinization, indigenous AMF populations might not be the ones that best enhance plant growth. After a while, inoculated plants in the field will face a mixed-AMF species environment. Further studies should examine the effect of such an environment on field strawberry plants. If necessary, cultivation management should include AMF colonization of plants in the greenhouse prior to transplantation in the field to ensure pre-colonization and less competition with natural soil AMF.

Stolon formation has been suppressed by the foliar application of the gibberellin biosynthesis inhibitor prohexadione-Ca (P-Ca) in selected strawberry cultivars (Black, 2004). As it has been shown that stolon suppression increases fruit yield, amplifies the benefits of AMF,

and reduces the effects of salinity, it would be important to investigate the potential of P-Ca in suppressing stolon formation in the tested cultivars.

## References

- Abrol, J.P. (1986) Fuel and forage production from salt affected wasteland in India. *Reclamation and Revegetation Research* **5**: 65–74.
- Allais, T., and Letang, G. (2009) Influence of mist-chilling on postharvest quality of fresh strawberries cv. Mara des Bois and Gariguette. *International Journal of Refrigeration* **32**: 1495-1504.
- Al-Karaki, G.N. (2000) Growth of mycorrhizal tomato and mineral acquisition under salt stress. *Mycorrhiza* **10**: 51–54.
- Al-Karaki, G.N. (2006) Nursery inoculation of tomato with arbuscular mycorrhizal fungi and subsequent performance under irrigation with saline water. *Scientia Horticulturae* **109**: 1–7.
- Al-Karaki, G.N., Hammad, R., and Rusan, M. (2001) Response of two tomato cultivars differing in salt tolerance to inoculation with mycorrhizal fungi under salt stress. *Mycorrhiza* **11**: 41–47.
- Akiyama, K., Matsuzaki, K., and Hayashi, H. (2005) Plant sesquiterpenes induce hyphal growth branching in arbuscular mycorrhizal fungi. *Nature* **435**: 824-827.
- Atkinson, D., Berta, G., and Hooker, J.E. (1994) Impact of mycorrhizal colonization on root architecture, root longevity, and the formation of growth regulators. *Impact of Arbuscular Mycorrhizas on Sustainable Agriculture and Natural Ecosystems*. p. 89-99.

- Audet, P., and Charest, C. (2007) Heavy metal phytoremediation from a meta-analytical perspective. *Environmental Pollution* **147**: 231-237.
- Audet, P., and Charest, C. (2009) Contribution of arbuscular mycorrhizal symbiosis to in vitro root metal uptake: from trace to toxic metal conditions. *Botany* **87**: 913-921.
- Augé, R.M. (2001) Water relations, drought and vesicular-arbuscular mycorrhizal symbiosis. *Mycorrhiza* **11**: 3-42. doi:10.1007/s005720000097.
- Awang, Y.B., Atherton, J.G., and Taylor, A.J. (1993). Salinity effects on strawberry plants grown in rockwood. II. Fruit quality. *Journal of Horticultural Science* **68**: 791-795.
- Benothmane, F. (2011) Mycorrhizal colonization and growth characteristics of salt-stressed *Solanum lycopersicum* L.. MSc Thesis. University of Ottawa, Canada.
- Black, B.L. (2004) Prohexadione-calcium decreases fall runners and advances branch crowns of 'Chandler' strawberry in a cold-climate annual production system. *Journal of the American Society of Horticultural Sciences* **129**: 479-485.
- Blaszkowski, J. (1989) Polish Endogonaceae 1. *Acaulospora bireticulata*, *Entrophosphora infrequens*, *Glomus caledonium*, and *Scutellospora pellucida*. *Karstenia* **29**: 1-10.
- Blaszkowski, J. (1993) Comparative studies of the occurrence of arbuscular fungi and mycorrhizae (Glomales) in cultivated and uncultivated soils of Poland. *Acta Mycol* **28**: 93-140.
- Blaszkowski, J., Czerniawska, B., Wubet, T., Schäfer, T., Buscot, F., and Renker, C. (2008) *Glomus*

- irregulare*, a new arbuscular mycorrhizal fungus in the *Glomeromycota*. *Mycotaxon* **106**: 247-267.
- Blaszkowski, J., Tadych, M., and Madej, T. (2000) *Glomus minutum*, a new species in Glomales (Zygomycetes) from Poland. *Mycotaxon* **76**: 187-195.
- Blaszkowski, J., Tadych, M., and Madej, T. (2001) *Glomus arenarium*, a new species in Glomales (Zygomycetes). *Acta Societas Botanicorum Poloniae* **70**: 97-101.
- Cantrell, I.C., and Linderman, R.G. (2001) Preinoculation of lettuce and onion with VA mycorrhizal fungi reduces deleterious effects of soil salinity. *Plant and Soil* **233**: 269–281.
- Copeman, R.H., Martin, C.A., and Stutz, J.C. (1996) Tomato growth in response to salinity and mycorrhizal fungi from saline or nonsaline soils. *Horticultural Science* **31**: 341–344.
- Daei, G., Ardekani, M., Rejali, F., Teimuri, S., and Miransari M. (2009) Alleviation of salinity stress on wheat yield, yield components, and nutrient uptake using arbuscular mycorrhizal fungi under field conditions. *Journal of Plant Physiology* **166**: 217–225.
- Dalpe and Monreal (2004) Arbuscular mycorrhizal inoculum support sustainable cropping systems. Online. *Crop Management*.
- Darrow, G.M. (1966) *The strawberry; History, Breeding and Physiology*. Holt, Reinhardt, and Winston.
- Davies, F.T. Jr., Olalde-Portugal, V., Aguilera-Gomez, L., Alvarado, M.J., Ferrera-Cerrato, R.C.,

- and Boutton, T.W. (2002) Alleviation of drought stress of Chile ancho pepper (*Capsicum annuum* L. cv. San Luis) with arbuscular mycorrhizae indigenous to Mexico. *Scientia Horticulturae* **92**: 347-359.
- Dierickx, W. (2013) The salinity and alkalinity status of arid and semi-arid lands. In *Land Use, Land Cover and Soil Sciences Vol. V*. Ed. W. Verheye. pp. 163-189.
- Evelin, H., Kapoor, R., and Giri, B. (2009) Arbuscular mycorrhizal fungi in alleviation of salt stress: a review. *Annals of Botany* **104**: 1263-1280.
- FAO (1998) Crop evapotranspiration – Guidelines for computing crop water requirements version 56. <http://www.fao.org/docrep/X0490E/X0490E00.htm>
- Online: 06/22/2013
- Fan, L., Dalpé, Y., Fang, C., Dubé, C., and Khanizadeh, S. (2011a) Influence of arbuscular mycorrhizae on biomass and root morphology of selected strawberry cultivars under salt stress. *Botany* **89**: 1-7.
- Fan, L., Fang, C., Dubé, C., Tremblay, N., and Khanizadeh, S. (2011b) A non-destructive method to predict polyphenol content in strawberry. *Journal of Food, Agriculture, and Environment* **9**: 59-62.
- Feng, G., Li, X., Zhang, F., and Li, S. (2000) Effect of AM fungi on water and nutrient status of corn plants under salt stress. *Chinese Journal of Applied Ecology* **11**: 595-598.
- Friese, C.F., and Koske, R.E. (1991) The spatial dispersion of spores of vesicular-arbuscular

- mycorrhizal fungi in a sand dune: microscale patterns associated with the architecture of American beachgrass. *Mycological Research* **95**: 952-957.
- Galletta, G.J., Maas, J.L., Enns, J., Draper, A.D., and Swartz, H.J. (1995) 'Mohawk' strawberry. *HortScience* **30**: 631-634.
- Gerdemann, J. W., and Trappe, J. M. (1974) The Endogonaceae in the Pacific Northwest. *Mycologia Memoir* **5**: 1-76.
- Giovannetti, M. and Mosse, B. (1980) An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. *New Phytologist* **84**: 489-500.
- Hart, M.M., and Klironomos, J.N. (2002) Diversity of arbuscular mycorrhizal fungi and ecosystem functioning. In.: van der Heijden, M.G.A., and Sanders, I.R. (eds.). *Ecological studies* **157**. Mycorrhizal Ecology. Springer. p. 225-242.
- Hasegawa, P.M., Bressan, R.A., and Handa, A.K. (1986) Cellular mechanisms of salinity tolerance. *HortScience* **21**: 1317-1324.
- Hewitt, E. J. (1966) Sand and water culture methods used in the study of plant nutrition. Commonwealth Agricultural Bureaux. *Technical Communication. No. 22 (2nd ed.) of the Commonwealth Bureau of Horticulture and Plantation Crops*. pp: 431-432.
- Hirrel, M.C. (1981) The effect of sodium and chloride salts on the germination of *Gigaspora margarita*. *Mycologia* **43**: 610-617.
- Hršelova, H., Gryndler, M., and Vancura, V. (1990) Influence of inoculation with VA mycorrhizal

- fungus *Glomus* sp. on growth of strawberries and runner formation. *Agriculture, Ecosystems and Environment* **29**: 193-197.
- Jain, P.K., Paliwal, K., Dixon, R.K., and Gjerstad, D.H. (1989) Improving productivity of multipurpose trees on substandard soil. *Indian Journal of Forestry* **87**: 38-42.
- Juniper, S., and Abbott, L. (1993) Vesicular-arbuscular mycorrhizas and soil salinity. *Mycorrhiza* **4**: 45-57.
- Juniper, S., and Abbott, L. (2006) Soil salinity delays germination and limits growth of hyphae from propagules of arbuscular mycorrhizal fungi. *Mycorrhiza* **16**: 1639-1646.
- Kader, A.A. (1990) Quality and its maintenance to the postharvest physiology of strawberry. *The Strawberry Into the 21<sup>st</sup> Century; Dale, A., Luby, J.J., Eds.; Proceedings of the Third North American Strawberry Conference, Houston, TX.* Timber Press. Portland, OR. p. 145-152.
- Keutgen, A. and Pawelzik, E. (2007) Modifications of strawberry fruit antioxidant pools and fruit quality under NaCl stress. *Journal of Agricultural and Food Chemistry* **55**: 4066-4072.
- Keutgen, A. and Pawelzik, E. (2008) Quality and nutritional value of strawberry fruit under long term stress. *Food Chemistry* **107**: 1413-1420.
- Khanizadeh, S., Hamel, C., Kianmehr, H., Buszard, D., and Smith, D.L. (1995) Effect of three vesicular-arbuscular mycorrhizae species and phosphorus on reproductive and vegetative growth of three strawberry cultivars. *Journal of Plant Nutrition* **18**: 1073-1079.

- Kirk, P.M., Cannon, P.F., Minter, D.W., and Stalpers, J.A. (2008) Dictionary of the Fungi (10<sup>th</sup> ed.). Wallingford, UK: CABI. p. 287.
- Klironomos, J.N. (2003) Variation in plant response to native and exotic arbuscular mycorrhizal fungi. *Ecology* **84**: 2292-2301.
- Koomen, I., Grace, C., and Hayman, D.S. (1987) Effectiveness of single and multiple mycorrhizal inocula on growth of clover and strawberry plants at two soil pHs. *Soil Biology and Biochemistry* **19**: 539-544.
- Koske, R.E., and Tessier, B. (1983) A convenient, permanent slide mounting medium. *Mycological Society of America Newsletter* **34**:59.
- Levitt, J. (1980) Responses of Plants to Environmental Stresses. Volume II, 2<sup>nd</sup> ed. Academic Press, New York.
- Maas, E.V., and Hoffman, G.J. (1977) Crop salt tolerance: current assessment. *Journal of the Irrigation and Drainage Division. ASCE* **103**: 115-134.
- Mark, G.L., and Cassells, A.C. (1996) Genotype dependence in the interaction between *Glomus fistulosum*, *Phytophthora fragariae* and the wild strawberry (*Fragaria vesca*). *Plant and Soil* **185**: 233-239.
- Marschner, H. (1995) Mineral Nutrition of Higher Plants (2nd Ed.) Academic Press.
- Martínez Barroso, M.C., and Alvarez, C.E. (1997) Toxicity symptoms and tolerance of strawberry to salinity in the irrigation water. *Scientia Horticulturae* **71**: 177-188.

- McMillen, B.G., Juniper, S., and Abbott, L.K. (1998) Inhibition of hyphal growth of a vesicular-arbuscular mycorrhizal fungus in soil containing sodium chloride limits the spread of infection from spores. *Soil Biology and Biochemistry* **30**: 1639-1646.
- Miransari, M. (2010) Contribution of arbuscular mycorrhizal symbiosis to plant growth under different types of soil stress. *Plant Biology* **12**: 563-569.
- Mohammad, M.J., Malkawi, H.I., and Shibli, R. (2003) Effects of arbuscular mycorrhizal fungi and phosphorus fertilization on growth and nutrient uptake of Barley grown on soils with different levels of salt. *Journal of Plant Nutrition* **26**: 125-137.
- Morton, J. B. (1996) Redescription of *Glomus caledonium* based on correspondence of spore morphological characters in type specimens and a living reference culture. *Mycorrhiza* **6**: 161-166.
- Morton, J. B. (2000) International Culture Collection of (Vesicular) Arbuscular Mycorrhizal Fungi. West Virginia University: <http://www.invam.caf.wvu.edu/>.
- Mouk, B.O., and Ishii, T. (2006) Effect of arbuscular mycorrhizal fungi on tree growth and nutrient uptake of *Sclerocarya birrea* under water stress, salt stress and flooding. *Journal of the Japanese Society for Horticultural Science* **75**: 26–31.
- Munns, R. and Termaat, A. (1986) Whole-plant responses to salinity. *Australian Journal of Plant Physiology* **13**: 143-160.
- Murphy, J.G., Rafferty, S.M., and Cassells, A.C. (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.
- Nicolson, T.H., and Gerdemann J.W. (1968) Mycorrhizal *endogene* species. *Mycologia* **60**: 313-325.
- Oehl, F., Sieverding, E., Palenzuela, J., Ineichen, K., and Alves da Silva, G. (2011) Advances in *Glomeromycota* taxonomy and classification. *IMA Fungus* **2**: 191-199.
- Oregon Strawberry Commission (2006) Product development guide, 1-4. (<http://www.oregon-strawberries.com>, visited on 03.04.2013).
- Orsini, F., Alnayef, M., Bona, S., Maggio, A., and Gianquinto, G. (2012) Low stomatal density and reduced transpiration facilitate strawberry adaptation to salinity. *Environmental and Experimental Botany* **81**: 1-10.
- Ostonen, I., Püttsepp, Ü., Biel, C., Alberton, O., Bakker, M.R., Lõhmus, K., Majdi, H., Metcalfe, D., Olsthoorn, A.F.M., Pronk, A., Vanguelova, E., Weih, M., and Brunner, I.. (2007) Specific root length as an indicator of environmental change. *Plant Biosystems* **141**: 426-442.
- Pfeiffer, C.M., and Bloss, H.E. (1988) Growth and nutrient of guayale (*Parthenium argentatum*) in a saline soil as influenced by vesicular-arbuscular mycorrhiza and phosphorus fertilization. *New Phytologist* **108**: 315-321.
- Phillips, J.M., and Hayman, D.S. (1970) Improved procedures for clearing roots and staining

- parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *Transactions of the British Mycological Society* **55**: 158-161.
- Pond, E., Menge, J., and Jarrell, W. (1984) Improved growth of tomato in salinized soil by vesicular-arbuscular mycorrhizal fungi collected from saline soils. *Mycorrhiza* **76**: 74-84.
- Reinhardt, D.H., and Rost, T.L. (1995) Salinity accelerates endodermal development and induces an exodermis in cotton seedlings roots. *Environmental and Experimental Botany* **35**: 563-574.
- Richards, L.A. (1954) Diagnosis and improvement of saline and alkali soils. *United States Department of Agriculture, Handbook no. 60*. Washington, DC. p. 4–18.
- Ruiz-Lozano, J.M., Azcón, R., and Gómez, M. (1996) Alleviation of salt stress by arbuscular mycorrhizal *Glomus* species in *Lactuca sativa* plants. *Physiology of Plants* **98**: 767-772.
- Sahay, N.S. and Varma, A. (1999) *Piriformospora indica*: a new biological hardening tool for micropropagated plants. *FEMS Microbiology Letters* **18**: 297-302.
- Schüßler, A., and Walker, C. (2010) The Glomeromycota: A species list with new families and new genera. Glouctester, UK. p. 56.
- Accessed online: June 10, 2013.
- Schwarz, M. (1995) Soilless Culture Management. *Advanced Series in Agricultural Sciences, Vol. 24*.
- Serfling, A., Wirsal, S.G., Lind, V., and Deising, H.B. (2007) Performance of the biocontrol fungus

- Piriformospora indica* on wheat under greenhouse and field conditions. *Phytopathology* **97**: 523-531.
- Shannon, M.C., Grieve, C.M., and Francois, L.E. (1994) Whole-plant response to salinity. "Plant Environment Interactions" (R.E. Wilkinson, Ed.), Dekker, New York. pp. 199-244.
- Sheng, M., Tang, M., Chen, H., Yang, B., Zhang, F., and Yuang, Y. (2008) Influence of arbuscular mycorrhizae on photosynthesis and water status of maize plants under salt stress. *Mycorrhiza* **18**: 287-296.
- Sinclair, G., Charest, C., Dalpé, Y., and Khanizadeh, S. (2013) Influence of arbuscular mycorrhizal fungi and a root endophyte on the biomass and root morphology of selected strawberry cultivars under salt conditions. *Canadian Journal of Plant Science* **93**: 1-3.  
doi:10.4141/CJPS2012-279
- Smith, S.E., and Read, D.J. (2008) Mycorrhizal Symbiosis, Third edition. Academic Press. pp. 523-636.
- Sokolski, S., Dalpé, Y., Séguin, S., Khasa, D., Lévesque, C.A., and Piché, Y. (2010) Conspicuity of DAOM 197198, the model arbuscular mycorrhizal fungus, with *Glomus irregulare*: molecular evidence with three protein-encoding genes. *Botany* **88**: 829-838.
- St. John, T.V., and Hunt, H.W. (1983) Statistical treatment of VAM infection data. *Plant and Soil* **73**: 307-313.
- Stewart, L.I., Hamel, C., Hogue, R., and Moutoglis, P. (2005) Response of strawberry to

- inoculation with arbuscular mycorrhizal fungi under very high soil phosphorus conditions. *Mycorrhiza* **15**: 612-619.
- Stockinger, H., Walker, C., and Schüßler, A. (2009) '*Glomus intraradices* DAOM 197198', a model fungus in arbuscular mycorrhiza research, is not *Glomus intraradices*. *New Phytologist* **183**: 1176-1187.
- Subramanian, K.S., and Charest, C. (2008) Arbuscular mycorrhiza and drought management. In *Arbuscular mycorrhiza in arid and semi-arid ecosystems*. Edited by N.M.M. Arias, S.L.C. Ricalde, R.G. Sánchez, and A.M. Ata. Mundi-Prensa, Mexico. pp. 55-72.
- Taylor, J., and Harrier, L. (2001) A comparison of developmental and mineral nutrition of micropropagated *Fragaria × ananassa* cv. Elvira (Strawberry) when colonised by nine species of arbuscular mycorrhizal fungi. *Applied Soil Ecology* **18**: 205-215.
- Turhan, E., and Eris, A. (2005) Changes of micronutrients, dry weight, and chlorophyll contents in strawberry plants under salt stress conditions. *Communications in Soil Science and Plant Analysis* **36**: 1021-1028.
- Varma, A., and Schüepp, H. (1994) Infectivity and effectiveness of *Glomus intraradices* on micropropagated plants. *Mycorrhiza* **5**: 29-37.
- Varma, A., Verma, S., Sudha, Sahay, N., Bütchorn, B., and Franken, P. (1999) *Piriformospora indica*, a cultivable plant-growth promoting root endophyte. *Applied Environmental Microbiology* **65**: 2741.
- Verma, S., Varma, A., Rexer, K.H., Hassel, A., Kost, G., Sarbhoy, A., Bisen, P., Bütchorn, B., and

- Franken, P. (1998) *Piriformospora indica*, gen. et. Sp. Nov., a new root-colonizing fungus. *Mycologia* **90**:896-903.
- Vestberg, M. (1992) The effect of vesicular-arbuscular mycorrhizal inoculation on the growth and root colonization of ten strawberry cultivars. *Agricultural Science in Finland* **1**: 527-535.
- Waller, F., Achatz, B., Baltruschat, H., Fodor, J., Becker, K., Fischer, M., Heier, T., Hüchelhoven, R., Neumann, C., von Weetstein, D., Franken, P., and Kogel, K. (2005) The endophytic fungus *Piriformospora indica* reprograms barley to salt-stress tolerance, disease resistance, and higher yield. *Proceeding of the National Academy of Sciences* **102**: 13386-13391.
- Wang, W.X., Vinocur, B., Shoseyov, O., and Altman, A. (2001) Biotechnology of plant osmotic stress: physiological and molecular considerations. *Acta Horticulturae* **560**: 285-292.
- Wang, W.X., Vinocur, B., and Altman, A. (2003) Plant responses to drought, salinity and extreme temperatures: towards genetic engineering for stress tolerance. *Planta* **218**: 1-14.
- Yang, R.H., Liu, R.J., Liu, C.L., Wang, Y.Z., Li, P.H., and Yuan, Y.B. (2009) Effects of arbuscular mycorrhizal fungi and salicylic acid on salt tolerance of strawberry (*Fragaria x ananassa* Duch.) plants. *Scientia Agricultura Sinica* **42**: 1590-1594.
- Yokoi, S., Bressan, R. A., and Hasegawa, P.M.. (2002) Salt stress tolerance of plants. *JIRCAS Working Report*. p. 25-33.