Influence of colonization by arbuscular mycorrhizal fungi on three strawberry cultivars under salty conditions

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Plant adaptation to hyperosmotic environments is generally associated with reduced growth and ultimately yield loss, making farming difficult. The potential of mycorrhizal symbioses to alleviate salt stress has been documented and benefits to plant revealed to be specific and dependent to both plant cultivars and fungal strains. A factorial greenhouse experiment was performed to determine the effects of three arbuscular mycorrhizal fungi (AMF) species (*Funneliformis caledonius*, *F. mosseae* and *Rhizophagus irregularis*) on three 'day-neutral' strawberry (*Fragaria × ananassa* Duch.) cultivars ('Albion', 'Charlotte' and 'Seascape'), and a mixture of *R. irregularis* and *F. mosseae* on 'Seascape', under four salt conditions (0–200 mM NaCl). The overall results showed that plant biomass decreased with increasing salinity. The cultivars responded differently to both AMF and salinity, and 'Seascape' was more tolerant to salinity than the other cultivars. AMF enhanced plant growth and improved salt tolerance by increasing the proportion of medium ($0.5<\phi\leq1.5$ mm) and coarse ($\phi>1.5$ mm) diameter roots. The mixture of two AMF species increased root and shoot mass to a higher degree than each species alone at low salinity (0–50 mM) but reduced fruit quality. At higher levels (100–200 mM), *R. irregularis* alleviated salt stress and improved fruit quality to a higher degree than the other AMF species. Our results support the use of bio-inoculants in saline horticultural areas. Because cultivars respond differently to fungal inoculants, and inoculants prefer specific environmental conditions, fungal inoculants need to be screened on a cultivar- and condition-specific basis.

Key words: arbuscular mycorrhizal fungi, arid soil, salinity, strawberry, stress

Introduction

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 for plant growth.

More than 70% of all agricultural soils worldwide are saline or affected by salinity problems, which can reduce crop productivity (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 the 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 conditions. Plant adaptation to hyperosmotic environments is generally associated with reduced growth and ultimately yield loss, making farming 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).

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The growth reduction 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 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 a plant species that is considered particularly susceptible to salt stress (Maas and Hoffman 1977, Schwarz 1995, Martínez Barroso and Alvarez 1997).

Arbuscular mycorrhizal fungi (AMF) are obligate symbionts that colonize plant roots and modulate plant growth, while obtaining photosynthetically fixed carbon from their host plants to ensure their own survival. The presence of AMF in salt-laden crops is very common (Juniper and Abbott, 1993). AMF are able to enhance plant growth and production in saline soils (Al-Karaki et al. 2001, Daei et al. 2009, Benothmane 2011). Different species of AMF differ in their tolerance to stress. 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, thereby alleviating salt stress in plants.

The growth response of strawberry to inoculation depends on cultivar-AMF species combinations (Khanizadeh et al. 1995, Taylor and Harrier 2001). Additionally, AMF show a preference for specific environmental conditions (Davies et al. 2002). Because beneficial combinations of these mycorrhizae would maximize the potential benefits for the host plants, it may be profitable to identify the AMF inoculants most suitable for a given cultivar in a given environment.

Increased plant productivity has been linked to higher biodiversity. AMF species are functionally different and they may have complementary effects on a host plant (Hart and Klironomos 2002). The presence of 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 salty conditions.

In this research, the effects of AMF on strawberry plants subjected to salty conditions were examined. The first objective was to determine the level of root colonization by each fungal species under increasing levels of salinity. The second objective was to determine the effects of the inoculants on biomass, root architecture and fruit quality. The third objective was to determine whether an inoculum consisting of a mixture of AMF species was more beneficial than a single AMF species for plant productivity under salt stress.

Materials and methods

Two AMF strains species, *F. caledonius* (DAOM 193528) and *F. mosseae* (DAOM 194475), from the National collection of Glomeromycota, Agriculture and Agri-Food Canada were propagated through pot-culture in a greenhouse at the University of Ottawa's Centre for Advanced Research in Environmental Genomics. Leek was selected as the host plant because of its high mycorrhizal potential and its extensive root system. Leek plants were watered almost daily and greenhouse temperatures varied between 22 and 28 ˚C. Plants were fertilized every three weeks with 15 ml of Long Ashton nutrient solution per pot (Hewitt 1966). 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 was calculated for each inoculum. The substrate containing fungal propagules such as spores, hyphae and colonized roots served as mycorrhizal inoculum for the strawberry experiment.

Three 'day-neutral' strawberry (*Fragaria × ananassa* Duch.) cultivars ('Albion', 'Charlotte' and 'Seascape') were selected for the experiment, and non-AMF plug plants were obtained from Luc Larreault at Certified Fruit Plants in Lavaltrie, Quebec.

The greenhouse experiment was conducted at Agriculture and Agri-Food Canada's L'Acadie Research Sub-Station in Saint-Jean-sur-Richelieu, Quebec. Two-month-old plug plants of uniform size were grown in 14.5-cm-high × 15-cm-diameter pots (one plant per pot) which were filled with Fafard® Agro Mix® (Saint-Bonaventure, QC) hydrated growth medium containing dark peat moss, perlite and vermiculite (3:1:1, pH 5.5–6.5). The Farfard Agro Mix is a sterilized medium and did not contain natural untreated peat. A factorial block design was used with six blocks (one replicate per block) containing 52 pots per block. The plants were fertilized twice weekly with 100 ml of Plant Products® N-P-K fertilizer (12-2-14) at a concentration of 5 ml l^{-1}. They were watered as needed and brushed lightly to spread pollen.

F. caledonius (= Glomus caledonium, DAOM 193528*)*, *F. mosseae (= G. mosseae,* DAOM 194475*)*, and *R. irregularis* (= *G. irregulare*), DAOM 197198 formerly identified as *G. intraradices* (Sokolski et al. 2010) were tested against a non-inoculated control. For each cultivar, pots were given inoculum containing approximately 100 fungal propagules. A mixed-species inoculant containing 50 fungal propagules from two of the AMF species, *R. irregularis* and *F. mosseae*, was tested on 'Seascape'. 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, 100 or 200 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.

Fruits were harvested upon ripening and separated into sepals and fruit flesh. Only fruit flesh was used for further investigation. Fruits were cut into smaller pieces, frozen in liquid nitrogen, vacuum-sealed, and kept at –80 ˚C. After 40 days of salt treatment, all plants were harvested. Total roots were gently extracted from the pots and rinsed in tap water to remove debris, and excess water was removed by blotting with paper towels. The roots and shoots were separated. The fresh mass of the shoots (including stolons) and roots was recorded, and the roots were stored at 5 ˚C.

Fresh roots were hydrated in water and Tween-20 (0.01%), spread out on a transparent tray, and scanned. Root morphology parameters were determined using WinRHIZO Pro image analysis software (Regent Instruments Inc., Quebec City, QC). Root length, volume, average diameter, surface area, and number of forks and crossings were automatically analyzed using this software. Following analysis, the wet roots were kept at 5 ˚C. Due to root damage, 4/6 replicates were used for root analysis.

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

Soluble solids

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

Titratable acidity

Three trials were performed for each sample, consisting of 2 ml of juice diluted in 18 ml of water, then the pH was measured using a pH meter. The solution was titrated with standard NaOH to pH 8.05. 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 of citric acid per 100 ml of juice (%).

Roots were stained according to the method of Phillips and Hayman (1970). Root colonization percentage was determined using the gridline-intercept method (Giovannetti and Mosse 1980. Vesicles, hyphae, and arbuscules were taken into account when the AMF colonization percentage was calculated.

The data were subjected to a three-way ANOVA using a generalized linear model procedure in R statistical analysis software (version 2.15.0). Means were tested using 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 untransformed data were the same.

Results

Colonization was significantly affected by cultivar and AMF (p<0.001 and p=0.010) and their interaction (p=0.046). No colonization was observed for control plants. Although salinity did not affect colonization overall, there was a significant interaction (p=0.011) between salinity and cultivar (Table 1). Cultivars showed different responses to inoculum (Fig.1). 'Charlotte' had a significantly (p<0.001) higher level of colonization (12%) than 'Albion' (3%) and 'Seascape' (3%). The highest level of 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 mixture of two AMF species, *R. irregularis* + *F. mosseae*, showed a significantly (p=0.0097) higher level of colonization than *R. irregularis* alone.

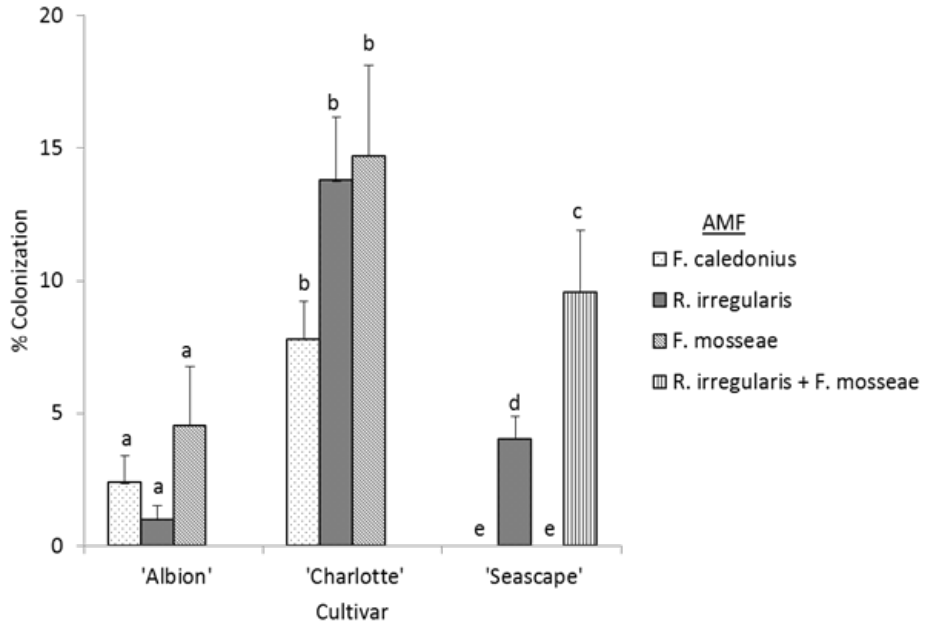

Fig. 1. Mean (n=16) root colonization percentages by three arbuscular mycorrhizal fungi (AMF) species, and a mixed-AMF species, in 'Seascape'. Different letters within each cultivar indicate significant differences at p<0.05 according to Duncan new multiple range test.

Shoot and root fresh mass

Fresh mass of shoots, roots and fruits decreased with increasing salinity (p<0.001), especially at levels between 50 and 100 mM NaCl. In non-inoculated treatments, shoot fresh mass was more severely affected by salinity than fruit or root fresh mass. At high salinity, the inhibitory effects of salt on growth were observed in both shoots and roots. The effect of AMF was significant (p=0.046) on root fresh mass, but not on shoots (Table 1). There was an interaction (p=0.024) between salinity and the cultivar, which affected root fresh mass; 'Seascape' was 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* had the greatest effect at high salinity levels. 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 levels. *F. caledonius* and *F. mosseae* did not improve biomass at high salinity levels. 'Charlotte' was the least affected by AMF and responded negatively to *F. caledonius* and *F. mosseae* and positively to *R. irregularis*, but only at low salinity levels.

Fruit yield

At low salinity, 'Charlotte' produced the greatest fruit mass (g plant^{-1}), followed by 'Albion', and 'Seascape' produced the smallest amount. Overall, fruit mass decreased with increasing salinity (p<0.001) and salinity interacted significantly (p=0.015) with cultivar treatment. In terms of 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 50 mM to 100 mM.

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 were associated with the greatest increase in shoot and root mass.

Table 1. Three-way ANOVA for salinity (S), AMF, and cultivar (C) treatments and their interactions.

	S	AMF	C	S × AMF	S × C	AMF × C	S ×AMF×C
Mycorrhizal colonization	NS	**	***	NS	*	*	NS
Fresh shoot mass	***	NS	NS	NS	NS	NS	NS
Fresh root mass	***	*	***	*	*	NS	NS
Fruit mass	***	***	**	NS	*	NS	NS
Soluble Solids Content (SSC)	***	**	***	*	NS	***	NS
Titratable Acidity (TA)	NS	***	***	***	***	***	***
SSC/TA	***	***	***	***	*	NS	NS
Root diameter	NS	NS	***	NS	*	NS	NS
Root surface area	***	NS	*	NS	NS	NS	NS
Root length	***	NS	***	NS	NS	NS	NS
Root volume	***	NS	NS	NS	NS	NS	NS
Number of forks	***	NS	***	NS	NS	NS	NS
Number of crossings	***	NS	***	NS	NS	NS	NS
Percentage of fine roots (diameter $\phi \leq 0.5$ mm)	NS	*	***	NS	*	NS	NS
Percentage of medium roots ($0.5 < \phi \leq 1.5$ mm)	NS	**	***	NS	*	NS	NS
Percentage of coarse roots ($\phi > 1.5$ mm)	NS	NS	***	NS	**	NS	NS

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

Fruit quality

The soluble solids content (SSC), titratable acidity (TA), and their ratio (SSC/TA) varied significantly (p<0.001) among the cultivars. 'Albion' produced fruits with the highest SSC and 'Charlotte' had the highest SSC/TA ratio (Table 2). The SSC and SSC/TA decreased significantly (p<0.001) with increasing salinity, while TA was marginally 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.

The AMF species (Table 1) 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 3). In 'Seascape', the mixed-species AMF had less of an effect than either of its constituent single species. There was a significant interaction between AMF and salinity which affected SSC (p=0.0341), TA (p<0.001), and SSC/TA (p<0.001). While *F. mosseae* increased SSC/TA to a greater degree than the other AMF species at low salinity, *R. irregularis* had the greatest positive effect on fruit quality at high salinity (Table 3). In *R. irregularis*-inoculated plants, SSC/TA ratios were 5%, 24%, and 25% higher than in the non-AMF control at the 50, 100, and 200 mM NaCl levels, respectively. When inoculated with *R. irregularis*, 'Charlotte' produced fruits with the highest SSC and SSC/TA, and had the highest salt tolerance.

Table 2. Mean fruit soluble solids content (SSC), titratable acidity (TA), and the SSC/TA ratio for strawberry plants treated with or without arbuscular mycorrhizal fungi (AMF) and salinity.

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

Note: Different letters within each block indicate significant differences (p< 0.05) according to Duncan new multiple range test; Significant differences: *, 0.05; **, 0.01; ***, 0.001; NS, not significant at p=0.05

Root architecture

The salt treatment significantly (p<0.001) decreased the root surface area, length and volume, and the number of forks and crossings (Tables 1 and 4). Without salt or AMF, 'Albion' had the smallest average root diameter and the largest root system (largest surface area, length and 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 which affected 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 which affected the distribution of root length among the different diameter classes (Fig. 2). 'Albion' had the highest proportion of fine roots (ϕ≤0.5mm) 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, which showed an additional increase 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 showed similar responses to salinity.

Table 3. Interactions between arbuscular mycorrhizal fungi (AMF) and salinity on fruit soluble solids content (SSC), titratable acidity (TA), and the SSC/TA ratio for strawberry cultivars.

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

Note: Different letters within each block indicate significant differences between treatments (p< 0.05) according to Duncan new multiple range test; *, 0.05; **, 0.01; ***, 0.001; NS, not significant.

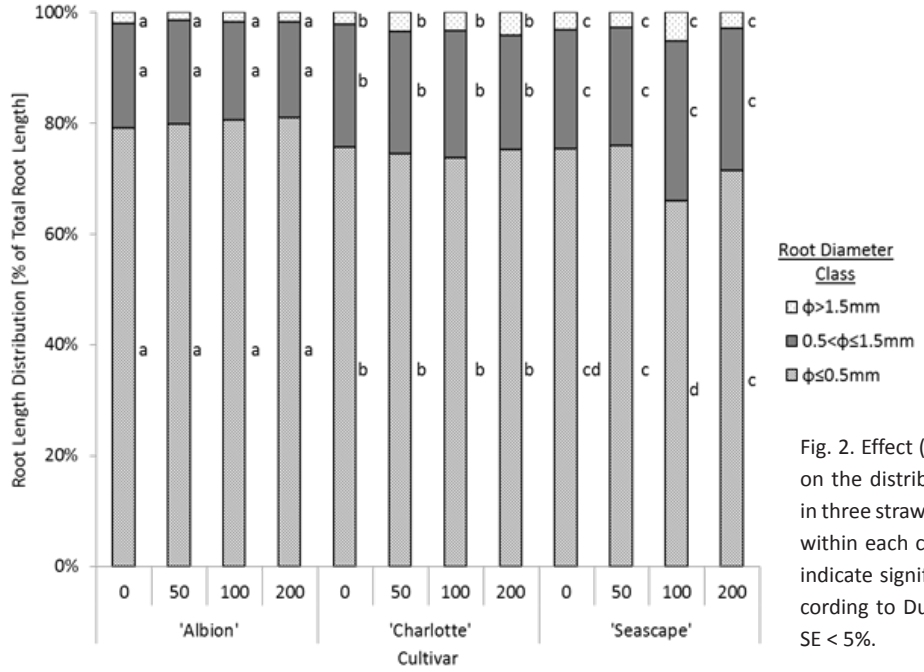

Fig. 2. Effect (%, n=4) of salinity (0–200 mM) on the distribution of root-diameter classes in three strawberry cultivars. Different letters within each cultivar and root-diameter class indicate significant differences at p<0.05 according to Duncan new multiple range test. SE < 5%.

The AMF increased root growth overall (Table 4). Root surface area was increased by AMF. At low salt levels (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, 'Seascape' plants inoculated with the AMF mixture of *R. irregularis* and *F. mosseae* produced a significantly larger surface area relative to 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.

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), with the proportion of medium roots increasing at the expense of fine roots. Similar to the surface area response 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.

Table 4. Mean root parameter measurements of strawberry plants treated with or without arbuscular mycorrhizal fungi (AMF) and salinity.

	Average diameter (mm)	Surface area (cm^2)	Total length (cm)	Volume (cm^3)	Specific root length($cm\ g^{-1}$)	No. of crossings	No. of forks
Cultivars							
'Albion'	0.42^b	280^a	2102^a	3^a	362^a	3757^a	12160^a
'Charlotte'	0.49a	246^a	1558^b	2.9^a	245^b	2313^b	7798^b
'Seascape'	0.48^a	237^a	1656^b	2.9^a	209c	2653^b	8924^b
$LSD_{0.05}$	0.02	48	307	0.6	34	635	1978
AMF							
Non-AMF	0.45^b	231^a	1673^a	2.6^b	243^{ab}	2639^a	8694^a
F. caledonius	0.45^{ab}	242^a	1724^a	2.8^{ab}	281^a	2913^a	9468^a
R. irregularis	0.46^{ab}	265^a	1834^a	3.1^{ab}	286^a	3010^a	10040^a
F. mosseae	0.48^{ab}	264^a	1771^a	3.2^{ab}	274^{ab}	2936^a	9810^a
R. irregularis + F. mosseae	0.48^a	289^a	1910^a	3.5^a	223^b	3053^a	10410^a
$LSD_{0.05}$	0.03	68	449	0.9	57	945	2928
Salinity levels							
0 mM	0.47^a	382^a	2601^a	4.6^a	261^a	4622^a	14950^a
50 mM	0.46^a	310^b	2149^b	3.6^b	276^a	3525^b	11700^a
100 mM	0.46^a	172^c	1224^c	2^c	285^a	1860^c	6354c
200 mM	0.47^a	150^c	1077^c	1.7^c	246^a	1546^c	5293^c
$LSD_{0.05}$	0.03	41	267	0.6	47	592	1818
Orthogonal	NS	Q***	Q***	Q***	NS	Q***	Q***
Polynomial contrast							

Note: Different letters within each block indicate significant differences (p<0.05) according to Duncan new multiple range test; *, 0.05; **, 0.01; ***, 0.001; NS, not significant.

Discussion

Fungal root colonization was not affected by salinity. This is inconsistent with the findings of other studies (Yang et al. 2009, Fan et al. 2011a) which reported that the rates of AMF colonization of strawberry plants decreased significantly with increasing salinity. AMF spore germination was shown to be inhibited by salinity (Hirrel 1981, Juniper and Abbott 2006), as was arbuscular and hyphal growth and development (Pfeiffer and Bloss 1988, McMillen et al. 1998).

Cultivars respond differently to salinity and AMF symbiosis. For example, the low root colonization observed in 'Albion' at high salinity may indicate that it benefitted from AMF only at low salinity. Audet and Charest (2009) pointed out that there is a critical toxicity threshold at which the symbiosis between roots and AMF ceases to be beneficial.

There was no relationship between percent colonization and biomass; the cultivars with the lowest percent colonization, 'Albion' and 'Seascape', had the highest shoot fresh and dry mass and fruit yield. However, there was a positive relationship between percent colonization and root architecture. 'Charlotte' had the highest percent colonization, along with the highest root mass, diameter, surface area, length, volume, and specific root length (SRL). The other cultivars showed a similar trend. *R. irregularis* achieved the highest level of colonization, and also had the greatest positive effect on the root parameters. 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).

We showed that shoot mass, and particularly, root mass, were mostly unaffected at 50 mM NaCl. This is consistent with the findings of Turhan and Eris (2005), who reported that strawberry shoot and root biomass was unaffected by weak salinity. The growth response to salinity is usually attributed to either ion toxicity or low external osmotic potential (Munns and Termaat 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 Food and Agriculture Organization of the United Nations reported decreased fruit yield in strawberry at 10 mM NaCl, in our study, reductions in biomass were not significant until the 100 mM level.

Cultivated strawberry plants vary in their responses to different AMF and other inoculants (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. When screening the AMF species, we observed some species have a negative effect on plant growth. *F. caledonius* and *F. mosseae* had a depressive effect on the growth of 'Charlotte'. Taylor and Harrier (2001) reported no positive shoot growth response to AMF inoculation with *Glomus* species, and root growth increases for plants colonized by *G. intraradices*. Similarly, in our study, root mass was more strongly affected by AMF than was shoot mass, indicating a strong relation between AMF and the root system.

Our study suggests a strong positive link between root mass and salt tolerance, especially in 'Seascape', which produced a 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 which 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 levels. Since many AMF show a preference for specific environmental conditions, and cultivars respond differently to AMF and environmental conditions, it is important to take these factors into account in selecting AMF.

Soluble solids content and titratable acidity are the most important indices of fruit quality which are ubiquitously used in standard quality controls (Fan et al. 2011b). Strawberry flavour is derived from the interactive taste and aromas of many chemical constituents, mainly sugars and volatile compounds, which are found to be increased by AMF (Castellanos-Morales et al. 2010, Lingua et al. 2013). SSC is mainly derived from organic sugars, such as glucose, sucrose and fructose, which influence the taste, flavour and maturity of strawberries (Kader 1990). High levels of sugars and relatively high acid content are required for good flavour.

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

An SSC/TA ratio 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 subjected to NaCl treatment, regardless of AMF, and non-AMF 'Albion' fruits treated with 100 mM NaCl or higher levels were deemed unacceptable for consumer consumption.

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 between 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 wider diameter roots. 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' showed the most positive response to salinity. It had wider diameter roots than the other cultivars, and salinity increased the proportion of medium and coarse roots. The other cultivars showed 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 as evidenced by 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 linked to increased shoot mass. This finding is consistent with the results of Marschner (1995) and Fan et al. (2011a), who found that the length of suberized roots is increased by mycorrhizae, offering increased mechanical support and mineral nutrient transport between fine roots and the shoot.

Specific root lenght 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 of the nutrient and water uptake, as well as with mycorrhizal 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', but not in 'Albion'. It is noteworthy that inoculated plants of 'Charlotte' and 'Seascape' were more salt tolerant than 'Albion' as evidenced by their biomass.

In 'Seascape', colonization by the mixture of two AMF species was higher than either of these species alone. In a similar study, Stewart et al. (2005) reported that after six weeks, the level of colonization by mixed species of *G.intraradices (=R. intraradices)* + G*. mosseae (=F. mosseae*) + *G. etunicatum* was higher than for *G. intraradices* alone in some cultivars, and lower in others. In our study, the relatively high level of colonization by the mixed-AMF species was associated with an improved root system, but only at low salinity levels. It was also associated with higher shoot and fruit mass relative to single-species AMF treatments, but fruit quality was lower. The results of studies carried out to date are inconclusive regarding the benefits of mixed-species inoculum relative to that of constituent single species for strawberry plant growth. Previous studies have shown that the effect of multiple AMF 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 inoculum composed of single species in promoting strawberry plant growth under control conditions or stressful conditions.

Stewart et al. (2005) reported that *G. intraradices* was more efficient at promoting growth of strawberry plants than a mix of *G. intraradices*, *G. mosseae,* and *G. etunicatum*. In our study, 'Seascape' inoculated with the mix of 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* maintained fruit quality under salt stress more efficiently than the other AMF species.

The mixed-species inoculum did not differ in its effect on root systems from the single-species inoculum under stress conditions. Similar results were reported by Koomen et al. (1987) and Stewart et al. (2005). Therefore, we recommend that multi-species inocula be used for field inoculation to ensure wider adaptation to different environmental conditions and more consistent benefits for the host plant, and that AMF continue to be screened on a condition- and cultivar-specific basis.

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