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Effect of *Bacillus velezensis* and *Glomus intraradices* on Fruit Quality and Growth Parameters in Strawberry Soilless Growing System

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This study evaluates the effect on the 'Splendor' and 'Primoris' strawberry cultivars of different dates of inoculation with *Glomus intraradices*, an arbuscular mycorrhizal (AM) fungus. Additionally, plants were grown in a soilless growing system with or without *Bacillus velezensis* at the beginning of the experiment. A completely randomized block design (2 biofertilizer treatments × 2 cultivars × 3 inoculation dates) with 2 replications was used. Each replicate consisted of one bag with 10 plants. Fruit weight, fruit quality, growth parameters, and SPAD values in young leaves were monitored from October 2011 to June 2012 in a greenhouse. At the end of the crop cycle, the microbial population of *Bacillus* spp. and the *Glomus intraradices* population were determined from the rhizosphere of the plant. *Bacillus velezensis* and *Glomus intraradices* were established in the strawberry soilless growing system. The effect of arbuscular mycorrhizae on strawberry fruit quality was more important than that on growth parameters. Biofertilizer with arbuscular mycorrhizal fungi had an inhibitory effect on fruit quality, as indicated by low TSS, pH, and TA values. The combined effect of the biofertilizer and inoculation dates of *Glomus intraradices* on growth parameters was more significant in the 'Primoris' cultivar than in 'Splendor'. In both cultivars, an increase in SPAD values was observed from week 12 to week 22 after planting. Depending on the cultivar selected, the date of inoculation may significantly affect plant response to AM fungal colonization in a soilless growing system.

Key Words: firmness, soilless growing system, SPAD, titratable acidity, total soluble solid.

Introduction

The strawberry (*Fragaria* × *ananassa* Duch.) is one of the most commonly consumed berries and an important small fruit crop in Spain and around the world (FAOSTAT, 2014, http://faostat3.fao.org/, December 9, 2014). Low in calories and with high mineral and vitamin content and a high level of antioxidant compounds, the strawberry is a fruit that easily meets basic nutritional requirements. In recent years, concern about the prevention of environmental pollution and food safety has increased. In order to meet criteria on sustainable fruit production, agrotechniques can be improved by application of the best available fertilizers (Pešaković et al., 2013). By introducing arbuscular mycorrhizal (AM) fungi in the substrates, fertilizer and pesticide inputs can be reduced and plants can grow in a more sustainable way (Cordier et al., 2000). Mycorrhizal symbiosis plays a significant role in the nutrition and development of host plants, and mycorrhizae are well known for their ecological role in plant establishment, nutrient uptake, protection against biotic and abiotic stress and in soil aggregation (Smith and Read, 1997), tolerance against pathogens (Matsubara et al., 2009; Vos et al., 2012) and water stress (Borowicz, 2010). Besides contributing to the biological control of root pathogens, they also affect nutrient cycling and/or seedling establishment and soil quality (Barea et al., 1993).

The strawberry is a mycotrophic species that is susceptible to heavy colonization by AM fungi in mineral soil. However, earlier strawberry studies have also shown the negative effect of peat on the AM fungal colonization of strawberry roots. It is unclear whether the

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low AM fungal colonization observed was due to high levels of available P or to the other chemical or biological properties of the peat itself. Calvet et al. (1992) found that certain peat products had a negative effect on the establishment of AM fungal symbiosis, although germination and early mycelial growth were not affected, indicating a biological cause of the inhibition. In contrast to soil, soilless growing systems are poor in terms of microorganisms. Several reports indicate the benefit of AM fungi in substrate culture (Cekic and Yilmaz, 2011; Ikiz, 2003; Schnitzler, 1996; Suzuki et al., 2000).

Research on mycorrhizal strawberry plants in soilless growing systems includes the work of Corkidi et al. (2004), who indicated that some substrates used in soilless growing systems are not suitable for the development of mycorrhizal colonization, such as peat. On the other hand, Cekic and Yilmaz (2011) suggested that, in soilless strawberry growing systems, mycorrhizal plants had more crowns and fruit in the 'Maraline' and 'Camarosa' cultivars when inoculated with *Glomus clarum*. Mycorrhizal plants showed higher biomass accumulation (crowns and shoot) and a more extensive leaf area (Borkowska, 2002).

Strawberry plants establish mutualistic associations with mycorrhizal fungi and so they are now widely used in studies of such symbiotic relationships. Some of the benefits of using AM fungi in strawberry are associated with the influence on plant growth (Vestberg et al., 2004). The increase in yield from inoculation with AM fungi has been studied in different strawberry cultivars (Robertson et al., 1988; Varma and Schuepp, 1994). Koomen et al. (1987) suggested that, in soilless growing systems, strawberry plants inoculated with 4 species of *Glomus* spp. showed stronger growth and more fruit per plant.

The increases in the numbers of flowers and fruit in inoculated strawberry plants have been reported by Robertson et al. (1988) and the increase in the number of stolons has been reported by Niemi and Vestberg (1992). Botham et al. (2009) indicated that mycorrhization with *Entrophospora colombiana* and *G. intraradices* on *Fragaria virginiana* (Wild) resulted in a rise in the number of flowers, and stated that this effect is a result of crop genetics. The increase in fruit quality from inoculation with AM fungi has been studied by Lingua et al. (2013) and Castellanos-Morales et al. (2010), and the increase in fruit production has been studied by Douds et al. (2008).

In order to overcome the adverse effects of chemical fertilizers, the current trend is to use plant growth-promoting rhizobacteria (PGPR), so-called biofertilizers (Pešaković et al., 2013). *Bacillus velezensis* sp. nov. was isolated during a research program to discover novel bacterial strains capable of synthesizing new lipopeptides with surfactant and/or antimicrobial activity. Genomic and phenotypic data demonstrated that *B. velezensis* is a novel species of *Bacillus* (Ruiz-García et al., 2005), now reclassified as *B. amyloliquefaciens* (Wang et al., 2008).

Bacterial plant growth promotion may be achieved by direct (e.g., the production of plant hormones) or indirect (e.g., antagonism and nutrient competition) mechanisms and requires good colonization of the promoting organism on the root surface. Therefore, enrichment of the plant growth-promoting strain in a very early stage of plant development is essential (Waechter-Kristensen et al., 1997). Studies performed in commercial market gardens showed that there were seasonal changes in the quality and quantity of bacteria on the root and in the nutrient solution (Waechter-Kristensen et al., 1997).

Boer et al. (2005) suggested that bacteria and fungi compete for simple plant-derived substrates and have developed antagonistic strategies. Avilés et al. (1996) studied the evolution of fungi during the composting of cork industrial waste to obtain a growth medium and suggested that there is a rising trend in the density of cellulolytic populations.

For the further development of biological control methods in soilless growing systems, it is necessary to understand the ecological and biological characteristics of indigenous microorganisms in such systems. Most bacteria reside on the surface of plant roots (Campbell and Greaves, 1990). In addition, they can utilize a wide range of substances, such as either carbon or nitrogen sources, and grow relatively faster than other microorganisms (Glick, 1995).

PGPR and AM fungi are further components of rhizosphere microflora that can also play a role in plant growth and phytopathogen suppression, mainly due to their synergistic interaction with mychorrhizae (Compant et al., 2005; Jeffries et al., 2003). Synergistic positive interactions between AM fungi and PGPR, such as nitrogen fixers, fluorescent *Pseudomonads* and sporulating bacilli, have been documented by many researchers (Galleguillos et al., 2000; Hameeda et al., 2007), although some neutral effects of AM fungi and PGPR interaction have also been reported (Andrade et al., 1997; Walley and Germida, 1997).

The aim of this research was to study the combined effect of the date of inoculation with AM fungus (*G. intraradices*) and the application of a biofertilizer (*B. velezensis*) on fruit weight, fruit quality, growth parameters and SPAD values in young leaves of two strawberry cultivars produced in a soilless growing system.

Materials and Methods

The experiment was carried out in a greenhouse at the Rábida Campus at Huelva University, Spain (37°12'N latitude, 6°55'W longitude and 24 m above sea level), under natural light and temperature from October 2011 to June 2012. Two short-day strawberry

(*Fragaria* × *ananassa* Duch.) cultivars, 'Splendor' and 'Primoris', were grown in polyethylene bags (100 cm × 18 cm × 16 cm) filled with coconut fiber (Pelemix Spain, S.L., Murcia, Spain). A completely randomized block design (2 biofertilizers × 2 cultivars × 3 inoculation dates) with 2 replications was used. Each replicate consisted of one bag with 10 plants. The sample number per treatment was 20 plants (240 plants in total). The polyethylene bags were placed on support structures 40 cm above the ground and watered by means of a drip irrigation system with four drippers per bag delivering 2 $L \cdot h^{-1}$ per dripper. Coconut fiber growth medium was autoclaved before planting (at the beginning of the crop cycle). The nutrient solution consisted of ($mg \cdot L^{-1}$): N 271, P 702, K 586, Mg 207, S 414, Fe 8, Mn 4, Cu 0.3, Zn 0.8, B 0.7, and Mo 0.3 (Correia et al., 2011). Regarding the physicochemical properties of the growth medium, coconut fiber growth medium showed electrical conductivity of 1.31 $mS \cdot cm^{-1}$ and a pH of 5.92.

The inoculum of *B. velezensis* (Cilus Plus®) was obtained from Ithec Company. Plants inoculated with *B. velezensis* (0.001 g/plant of *B. velezensis* at the beginning of the crop cycle) and plants not inoculated with biofertilizer were used. The microbial population of *Bacillus* spp. was determined at the end of the crop cycle. The density of *Bacillus* spp. was determined by dilution plating on semi-selective media according to Tuitert et al. (1998) with modifications as in Borrero et al. (2005). The *Bacillus* spp. population was determined from the rhizosphere of the plant. In order to collect rhizosphere growth medium samples, plants were carefully dug out with their roots and gently shaken. Growth media that adhered closely to the root system were considered as rhizosphere samples (Dhingra and Sinclair, 1995). Three samples were taken from each polyethylene bag and two bags (replications) were considered for each treatment (inoculation date). Plant growth media (5 to 10 g) were suspended in 250 mL of 0.1% sodium pyrophosphate. The suspension was shaken and tenfold dilution series were prepared with 0.1% water agar. Suspensions were pipetted onto three plates per culture medium and dilution (three replicates). Four dilutions per series were placed on plates. For the isolation of *Bacillus* spp., 100 $\mu g \cdot mL^{-1}$ cycloheximide was substituted for 10 $\mu g \cdot mL^{-1}$ of benomyl (Energía e Industrias Aragonesas, S.A., Madrid, Spain) and 0.3 $\mu L \cdot mL^{-1}$ Previcur (Propamocarb, 72.2%; Schering, Alcácer, Spain).

The commercial inoculum of *G. intraradices* (MYC 4000®) was obtained from Ithec Company. The inoculation dates of *G. intraradices* (5–10 spores/plant of *G. intraradices*) were: T1 (inoculation at the beginning of the crop cycle) and T2 (inoculation 4 weeks after transplantation). The plants not inoculated with *G. intraradices* were used as a control. The *G. intraradices* population was determined at the end of the crop cycle and was measured in the rhizosphere of the plant. Assessment of roots for *G. intraradices* colonization was performed on those plants sampled for root growth analysis. A fraction of the roots was carefully washed, cut into 1 cm segments, cleared in 10% KOH solution and stained with 0.1% trypan blue before estimation of mycorrhizal colonization (Phillips and Hayman, 1970). AM fungal colonization was estimated using a modified line intersect method (Brundett et al., 1996; Giovannetti and Mosse, 1980). These observations were carried out by light microscopy to rate the degree of root infection by AM fungi at the end of the crop cycle.

Ripe fruit from each treatment was harvested throughout the experimental period. Strawberry fruit was graded for size and external color, sorted to eliminate damaged material and transported under refrigeration to the laboratory. Sampling took place between January and May. On each sampling date, the total fruit from each bag and each treatment was gathered for quality assessment and converted into pulp using a mixer.

Total soluble solid (TSS), pH, titratable acidity (TA), ripening index (RI), and firmness in the fruits of the 'Splendor' and 'Primoris' cultivars were measured weekly and evaluated. TSS was determined using an automatic temperature-compensated PR101 digital refractometer (Atago Pallette PR101; Atago Co., Tokyo, Japan). TA (expressed as g of citric acid per 100 g fresh weight) was measured by titrating 10 g of the pulp plus 10 mL of H_2O with 0.1 $mol \cdot L^{-1}$ NaOH up to pH 8.1. RI was calculated by TSS/TA. Firmness was evaluated in a sub-sample of 3–4 fruits from each treatment using a portable penetrometer, and the results are expressed in $g \cdot cm^{-2}$. The growth parameters for 'Splendor' and 'Primoris', the number of leaves per plant, crown diameter (mm), vegetative growth index (VGI = plant height (cm) × plant width (cm) × plant length (cm) × 10^{-4}), number of fruit per plant, and root length (cm), were measured weekly and evaluated. The level of chlorophyll in the youngest expanded leaves was recorded weekly (from week 12 to week 29 after planting) by taking SPAD-502 Chlorophyll Meter (Minolta Camera Co. Ltd., Osaka, Japan) readings, which estimates relative chlorophyll content with the light transmitted through the leaf at 650 nm (photosynthetically active wavelength) and 940 nm. Statistical analyses were performed by analysis of variance (ANOVA) using SPSS software version 19.0 (SPSS; IBM, Chicago, IL, USA). We used Duncan's multiple range test to compare means. The variables analyzed by ANOVA were: fruit weight, TSS, pH, TA, RI, firmness, number of leaves per plant, crown diameter, VGI, number of fruit per plant, and root length. The main effects (AM fungal treatment and biofertilizer) and their interaction were also evaluated.

Results and Discussion

The analysis of the roots showed that the highest value of *G. intraradices* colonization with *B. velezensis* in 'Splendor' cultivar was with T2 treatment (56%) and the highest value of *G. intraradices* colonization with *B. velezensis* in 'Primoris' cultivar was with T1 treatment (30%) (Table 1). At the end of the crop cycle, the *G. intraradices* colonization with *B. velezensis* values were higher than the *G. intraradices* colonization without *B. velezensis* values. Similarly, several studies have demonstrated a synergistic interaction between AM fungi and phosphate-solubilizing bacteria (Barea, 1997; Kim et al., 1998).

It was also shown that structures had developed in all plants inoculated with AM fungi (arbuscles or hyphae) that were typical of mycorrhization. *Bacillus velezensis* and *G. intraradices* were established in the strawberry soilless growing system. Chávez and Ferrera-Cerrato (1990) also reported root colonization of some strawberry cultivars after AM fungal inoculation by endophytes, which varied from 25% to 75%.

Koohakan et al. (2004) suggested that the population of microorganisms was significantly different between soilless culture systems. The coconut fiber system (organic substrate culture) showed the highest amount of fungi, whereas the rockwool system (inorganic substrate culture) contained the highest amount of fluorescent *Pseudomonas*. Aerobic bacteria in roots became equilibrated in all systems. At the end of the crop cycle, *G. intraradices* colonization could have been increased by *B. velezensis*. Koohakan et al. (2004) suggested that fungal populations tended to increase at the end of the crop cycle in soilless culture systems and the population density of microorganisms in soilless culture systems may vary depending on the type of organic substrate.

Results suggested that only the fruit weight was affected in the Treatment × Biofertilizer (T × B) interaction for 'Primoris' cultivar (Table 2). Lütfi and Murat (2009) recorded a significant increase in yield in the 'Selva' strawberry with the use of PGPR (foliar + root application). Günes et al. (2009) reported similar results in their study of the effects of phosphate-solubilizing microorganisms (*Bacillus* FS-3, *Aspergillus* FS9) on strawberry yield.

Fruit weight increment as a result of biofertilizer application might be due to the microorganism's production of plant hormone-like substances (growth regulators). Bull et al. (2005) showed that no differences in market yield were detected between the inoculated and non-inoculated plants of the 'Aromas' or 'Diamante' strawberry cultivar, and that AM fungal colonization failed to produce any significant effect. The 'Primoris' fruit weight was highest in the T1 treatment with *B. velezensis* (17.40 g). Cekic and Yilmaz (2011) reported similar results in g per fruit for the 'Camarosa' and 'Maraline' cultivars inoculated with AM fungi (*Glomus clarum* and *Glomus caledonium*). This could have been due to the cell division increase that occurs in flower development and the early stages of fruit development as a result of the greater vegetative growth. The fruit weight values in 'Splendor' appear to be higher than in 'Primoris', but in the former, the standard deviation (SD) is high.

The chemical parameters analyzed showed the significant effect of AM fungal treatment and biofertilizer (Tables 2 and 3). In 'Splendor', T × B interaction significantly affected all of the chemical parameters, except TSS and firmness. On the other hand, in 'Primoris', a significant interaction effect was observed in TSS and TA. The application of biofertilizer gave the lowest TSS and pH (Table 2). TSS in 'Splendor' fruit ranged from 7.25 $mg \cdot kg^{-1}$ to 7.87 $mg \cdot kg^{-1}$ without *B. velezensis*, and from 6.96 $mg \cdot kg^{-1}$ to 7.35 $mg \cdot kg^{-1}$ with *B. velezensis* (Table 2). TSS in 'Primoris' fruit ranged from 8.76 $mg \cdot kg^{-1}$ to 9.44 $mg \cdot kg^{-1}$ without biofertilizer and from 7.51 $mg \cdot kg^{-1}$ to 8.48 $mg \cdot kg^{-1}$ with fertilizer (Table 2). The TSS values were within the range 4.8–10.9 reported in the literature for ripe strawberries (Karlidag et al., 2009). The TSS content in 'Splendor' fruit was highest in T1 treatment without biofertilizer (7.87 $mg \cdot kg^{-1}$) and lowest in T1 treatment

Table 1. Microbial population of *Bacillus* spp. ($\times 10^6$ CFU·mL^{-1}) of rhizosphere of the plant and *Glomus intraradices* colonization (%) of strawberry roots at the end of the crop cycle in 'Splendor' and 'Primoris' cultivars.

Treatment[z]	Biofertilizer	'Splendor'		'Primoris'	
		Bacillus	Mycorrhiza	*Bacillus*	Mycorrhiza
Control	Without *B. velezensis*	—	—	—	—
	B. velezensis	0.10 b	—	0.15 c	—
T1	Without *B. velezensis*	—	11 c	—	20 ab
	B. velezensis	0.15 b	37 b	1.14 b	30 a
T2	Without *B. velezensis*	—	18 c	—	16 b
	B. velezensis	0.47 a	56 a	2.79 a	21 ab

[z] Inoculation dates: Control (plants without inoculation with *Glomus intraradices*), T1 (inoculation at the beginning of the crop cycle), and T2 (inoculation 4 weeks after transplantation).

Values in each column followed by different letters are significantly different based on Duncan's test at $P < 0.05$.

Table 2. Combined effect of *Bacillus velezensis* and inoculation dates of *Glomus intraradices* on fruit weight and fruit quality (from January to May) in the 'Splendor' and 'Primoris' cultivars.

Treatment[z]	Biofertilizer	Fruit weight (g/fruit)		Total soluble solids (mg·kg^{-1})		pH		Titratable acidity (%)		RI		Firmness (g·cm^{-2})	
		'Splendor'	'Primoris'	'Splendor'	'Primoris'	'Splendor'	'Primoris'	'Splendor'	'Primoris'	'Splendor'	'Primoris'	'Splendor'	'Primoris'
Control	Without *B. velezensis*	19.40±10.71 a	15.66±6.68 bc	7.25±1.96 ab	8.76±2.57 b	3.98±0.18 bc	3.83±0.05 b	1.50±0.27 a	1.36±0.17 a	4.78±1.33 b	5.11±0.63 b	254.27±60.60 a	284.02±64.90 a
	B. velezensis	19.35±10.78 a	14.58±7.34 ab	6.97±1.48 a	7.92±1.64 a	4.01±0.25 c	3.76±0.34 b	1.69±0.12 b	1.65±0.28 b	4.16±0.67 ab	4.40±1.00 a	252.86±59.09 a	277.00±66.97 a
T1	Without *B. velezensis*	19.59±9.63 a	14.96±5.72 ab	7.87±1.45 c	8.80±1.95 b	3.82±0.03 a	3.88±0.08 b	1.66±0.12 ab	1.78±0.20 b	3.97±0.45 a	4.71±0.55 ab	263.28±69.10 a	275.02±57.55 a
	B. velezensis	18.14±10.30 a	17.40±7.17 d	6.96±1.94 a	8.48±1.99 b	3.94±0.11 bc	3.84±0.84 b	1.58±0.36 ab	1.73±0.15 b	4.55±1.34 ab	4.23±0.74 a	267.06±55.23 a	287.13±62.21 a
T2	Without *B. velezensis*	19.17±10.73 a	16.74±6.68 cd	7.62±2.62 bc	9.44±2.72 c	4.00±0.10 a	3.81±0.05 b	1.59±0.26 ab	1.68±0.34 b	4.15±0.78 ab	4.56±0.93 ab	259.71±65.07 a	277.58±71.60 a
	B. velezensis	19.91±11.19 a	13.79±5.88 a	7.35±1.99 abc	7.51±1.80 a	3.87±0.15 ab	3.41±1.20 a	1.64±0.29 ab	1.22±0.46 a	4.15±0.73 ab	4.72±0.35 ab	246.51±62.03 a	277.33±63.12 a
Treatment (T)		NS	NS	NS	NS	**	*	NS	**	NS	NS	NS	NS
Biofertilizer (B)		NS	NS	**	**	NS	*	NS	NS	NS	*	NS	NS
T×B		NS	**	NS	**	**	NS	*	**	*	NS	NS	NS

[z] Inoculation dates: Control (plants without inoculation with *Glomus intraradices*), T1 (inoculation at the beginning of the crop cycle), and T2 (inoculation 4 weeks after transplantation).
Values are mean±SD. Values in each column followed by different letters are significantly different based on Duncan's test at $P<0.05$.
NS non-significant, * significant at $P\leq0.05$, ** significant at $P\leq0.01$.

Table 3. Combined effect of *Bacillus velezensis* and inoculation dates of *Glomus intraradices* on growth parameters (from January to May) in 'Splendor' and 'Primoris' cultivars.

Treatment[z]	Biofertilizer	Leaf (numbers)		Crown diameter (mm)		Vegetative growth index (cm^3)		Flowers (numbers)		Root length (cm)	
		'Splendor'	'Primoris'	'Splendor'	'Primoris'	'Splendor'	'Primoris'	'Splendor'	'Primoris'	'Splendor'	'Primoris'
Control	Without *B. velezensis*	8.45±4.41 a	7.55±4.97 b	18.31±8.08 a	17.27±9.44 ab	9.06±7.63 a	7.76±6.62 abc	2.54±3.05 a	2.81±3.52 a	10.00±1.12 a	18.50±2.12 a
	B. velezensis	8.22±3.38 a	6.02±3.11 a	18.43±7.32 a	14.96±7.82 a	7.20±5.38 a	5.10±6.73 a	2.41±2.63 a	2.22±2.35 a	17.00±1.41 a	19.67±4.16 a
T1	Without *B. velezensis*	8.63±3.55 a	7.69±3.11 b	19.66±9.51 a	20.76±9.07 c	7.42±6.76 a	10.32±7.77 bc	2.50±2.13 a	3.00±2.88 a	16.50±4.95 a	13.38±3.86 a
	B. velezensis	8.13±3.42 a	6.34±2.34 ab	17.09±6.38 a	18.34±7.68 ab	5.74±4.28 a	10.97±9.65 c	2.41±2.06 a	2.28±2.30 a	15.00±2.58 a	17.50±4.51 a
T2	Without *B. velezensis*	7.89±3.42 a	6.80±2.93 ab	17.56±7.38 a	18.74±9.04 bc	6.21±4.46 a	10.19±10.88 bc	2.16±2.28 a	2.47±2.45 a	18.00±1.01 a	18.50±14.85 a
	B. velezensis	8.95±5.64 a	7.05±4.00 ab	18.68±8.80 a	16.74±7.16 ab	8.86±11.73 a	6.20±5.92 ab	2.44±2.85 a	2.50±2.05 a	17.33±3.05 a	20.50±6.36 a
Treatment (T)		NS	NS	NS	**	NS	*	NS	NS	NS	NS
Biofertilizer (B)		NS	*	NS	*	NS	NS	NS	NS	NS	NS
T×B		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

[z] Inoculation dates: Control (plants without inoculation with *Glomus intraradices*), T1 (inoculation at the beginning of the crop cycle), and T2 (inoculation 4 weeks after transplantation).
Values are mean±SD. Values in each column followed by different letters are significantly different based on Duncan's test at $P<0.05$.
NS non-significant, * significant at $P\leq0.05$, ** significant at $P\leq0.01$.

with *B. velezensis* (6.96 mg·kg^{-1}) (Table 2). The TSS content in 'Primoris' was highest in T2 without biofertilizer (9.44 mg·kg^{-1}) and lowest in T2 with *B. velezensis* (7.51 mg·kg^{-1}) (Table 2).

The pH value of 'Splendor' fruit in T2 without *B. velezensis* was 4.00 and it was 3.87 with biofertilizer (Table 2). The value for pH in 'Primoris' fruit in T2 without biofertilizer was 3.81 and it was 3.41 with *B. velezensis* (Table 2). In 'Primoris' with biofertilizer, the pH values tended to be lower in T2 than in T1. Therefore, fruit pH value variation depends on the inoculation date. Cekic and Yilmaz (2011) noticed an increase in pH in 'Camarosa' inoculated with *G. clarum* but a decrease in the plants inoculated with *G. caledonium*, and suggested that pH values also depended on the AM fungi used.

The TA values ranged from 1.50 to 1.69, respectively, for non-inoculated plants with and without *B. velezensis* (Table 2). The inoculation and the application of *B. velezensis* did not affect the 'Splendor' fruit TA but the T × B interaction was significant. These results are in line with those reported by Cekic and Yilmaz (2011), who also found that the date of inoculation did not affect the TA of the 'Maraline' and 'Camarosa' cultivars. 'Primoris' showed significant differences in T2 between plants with and without biofertilizer (Table 2).

Premsekhar and Rajashree (2009) reported higher TA values in mycorrhizal plants of a tomato crop, justified by the strong dependence on plant colonization by fungus and its role in nutrient acquisition. However, Cekic and Yilmaz (2011), reporting on a soilless strawberry growing system, found lower TA values in 'Camarosa' inoculated with *G. caledonium* and in 'Moraline' inoculated with *G. caledonium* or *G. clarum*. In 'Primoris', the application of biofertilizer triggered the lowest TA in T2, 1.22, and 1.36 in control plants without biofertilizer (Table 2). Biofertilizers may enhance strawberry yield without adverse effects on soilless growing system properties and ensure the basic criteria of sustainable fruit production. Given that biofertilizers contain living microorganism cells, they also improve soil composition and the supply of essential nutrients for increasing productivity and quality. Biofertilizers with AM fungi exhibited an inhibitory effect, as indicated by low values of TSS, pH, and TA, which can be associated with changes in the organic matter content of the growth substrate. However, *B. velezensis* application without AM fungi (control plants) affected the chemical properties of the fruit, such as TSS, pH, and TA, a phenomenon that can be influenced by the cultivar. This is also in agreement with the work of Chelpinski et al. (2010), who reported that the application of azotobacter in the 'Chandler' strawberry cultivar had a major impact on some chemical properties of the fruit, such as TSS and total sugar. Likewise, the effect of biofertilizer on chemical properties was higher than the combined effect of AM fungi with *B. velezensis*.

In 'Splendor', the RI was not affected by treatment or biofertilizer application despite the significant interaction. The lowest value was observed in T1 plants without *B. velezensis* (3.97) and the highest was recorded in the control plants (4.78). In 'Primoris', the RI of the fruit of plants without *B. velezensis* was highest in the control plants (5.11) and lowest in T1 with *B. velezensis* (4.71). Comparing the two cultivars, the RI values with and without *B. velezensis* were higher in 'Primoris' than in 'Splendor' (Table 2).

Firmness was not affected by either treatment, and no interaction was observed. In 'Splendor', values varied between 246.51 g·cm^{-2} in T2 with biofertilizer and 267.06 g·cm^{-2} in T1 with biofertilizer. In 'Primoris', the values varied between 277.00 g·cm^{-2} in the control plants with biofertilizer and 287.13 g·cm^{-2} in T1 with biofertilizer (Table 2). Thus, the 'Primoris' fruit was generally firmer than that of 'Splendor'. The differences between 'Splendor' and 'Primoris' in a soilless growing system might have been due to the fact that 'Splendor' was more vigorous than 'Primoris', and started to grow earlier.

The AM fungal colonization of strawberries in a soilless growing system can be affected by *B. velezensis*, the date of inoculation and the cultivar used. Therefore, when inoculation of the 'Splendor' cultivar was performed 4 weeks after transplantation (T2), the AM fungal colonization was more effective than when inoculated at the beginning of the crop cycle (T1). However, in 'Primoris', the AM fungi colonization was more effective when inoculation was performed at the beginning of the crop cycle (T1) than 4 weeks after transplantation (T2).

The response of the plants to AM fungal colonization depended on the cultivar used. The results suggested that only the 'Primoris' growth parameters were affected by the inoculation dates of *G. intraradices* and plants inoculated with *B. velezensis* or not inoculated. The crown diameter value of 'Primoris' in treatment T1 without biofertilizer was 20.76 mm and it was 18.34 mm with biofertilizer (Table 3). The application of biofertilizer produced the lowest crown diameter and VGI in treatment T2 and the control plants, respectively (Table 3). In 'Primoris', crown diameter values suggested that AM fungal colonization was more effective when inoculation was performed at the beginning of the crop cycle (T1) than 4 weeks after transplantation (T2). Mycorrhizal plants had a greater effect on crown diameter than non-mycorrhizal ones (control treatment) (Table 3). Vosatka et al. (1992) found a synergistic effect on strawberry growth following co-inoculation with AM fungi and *P. putida*.

The mycorrhizal plant increased VGI values and crown diameter in all treatments except T2 with biofertilizer (Table 3). Vestberg (1992) reported that micropropagated strawberry inoculation with AM fungi at the

weaning stage increased growth.

The highest values of leaf number (7.69), crown diameter (20.76 mm) and flower number (3.00) were observed in T1 without *B. velezensis* (Table 3). Strawberry plants with high biomass accumulation in their crown and that have been grown in conditions conducive to flower initiation have the greatest potential for fruit production. The effects of AM fungal inoculation in a soilless strawberry growing system include increased fruit production and early flowering and fruiting (Barea et al., 1993).

There was no significant difference in the root length of either cultivar (Table 3). Furthermore, the combined effect of *B. velezensis* and the mycorrhizal plant on root length could vary in different crop systems. In a soilless growing system, the inoculation of biofertilizer and the mycorrhizal plant might not necessarily boost root growth because the root was not under stress. In 'Primoris', the combined effect of *B. velezensis* and the mycorrhizal plant on shoot growth was greater than on root growth. In general, the 'Splendor' cultivar presented higher SPAD values in young leaves than the 'Primoris', with the exception of the final week of measurements (Fig. 1). SPAD values showed different trends during the crop cycle when considering the inoculation treatments only. In both cultivars, there was a change in SPAD over time. An increase was observed between weeks 12 and 22 after planting, and there was a decrease in SPAD from week 22 to week 29 as the crop cycle neared its end (Fig. 1). Similar trends have been reported by Pestana et al. (2011), who suggested that changes in total chlorophyll concentration ($\mu mol \cdot m^{-2}$) in young strawberry plant leaves over time were affected by the foliar application of a grass-clipping extract. Blunden et al. (1997) and Schwab and Raab (2004) reported enhanced chlorophyll concentration in the leaves in the early growth stages during the induction of flower formation. Likewise, Bynum et al. (2007) suggested that enhancing chlorophyll concentration could increase the CO_2 assimilation rate. In 'Splendor', average SPAD values were 53.68 in non-mycorrhizal plants and 53.33 (T1) and 53.37 (T2) in mycorrhizal plants (Fig. 1). It is clear, therefore, that inoculation in 'Splendor' did not enhance SPAD values between weeks 12 and 22 after planting. By contrast, 'Primoris' seems to gain some beneficial effect from late inoculation (T2) with a rise in SPAD, in particular after week 14. In 'Primoris', the average values were 51.92 in non-mycorrhizal plants and 52.78 (T1) and 53.34 (T2) in mycorrhizal ones.

In conclusion, the results suggest that the fruit weight of strawberry could be affected by biofertilizer depending on the cultivar; fruit weight of 'Primoris' was affected by the inoculation dates of *G. intraradices* and whether or not plants were inoculated with *B. velezensis*. It seems that biofertilizer application reduced TSS and pH values in 'Splendor' and 'Primoris', and combination with AM fungi produced an inhibitory effect. The results of fruit weight and TSS for 'Splendor', and fruit weight, TSS, pH, TA, and SPAD for 'Primoris', suggested that the highest values were found in mycorrhizal plants rather than in non-mycorrhizal ones (control). Depending on the cultivar selected, the date of inoculation may significantly affect plant response to AM fungal colonization in a soilless growing system. T2 treatment was more effective in 'Splendor' while T1 had a greater effect on 'Primoris'.

In 'Primoris', the combined effect of biofertilizer and

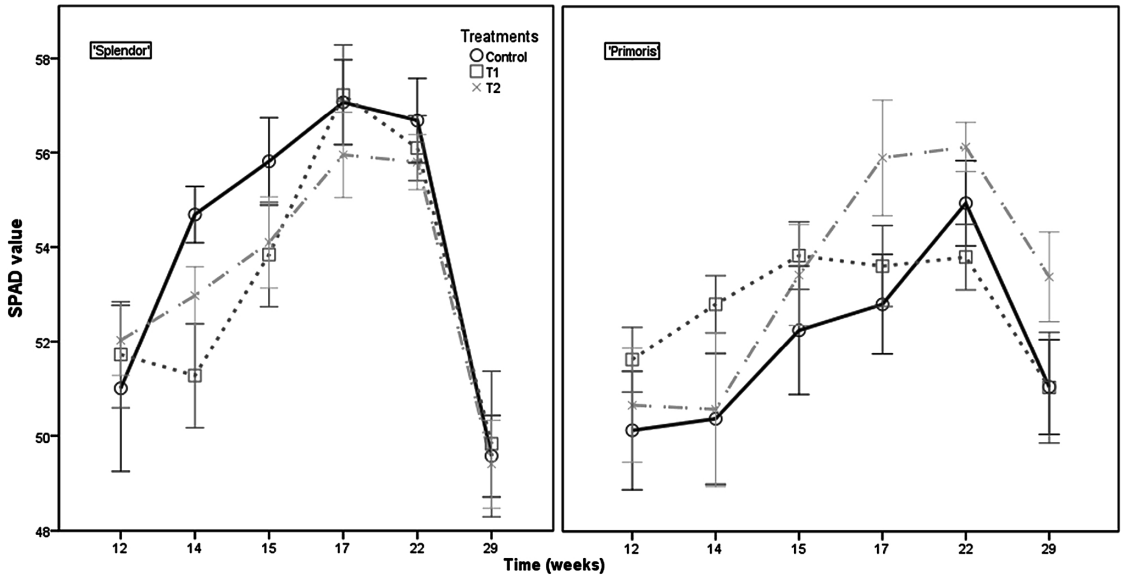

Fig. 1. Effect of strawberry cultivars and inoculation dates of *Glomus intraradices* on SPAD values of young leaves (12 to 29 weeks after planting). Control (plants without inoculation with *G. intraradices*), T1 (inoculation at the beginning of the crop cycle), and T2 (inoculation 4 weeks after transplantation). Values are mean ± SD.

the mycorrhizal plant on shoot growth was greater than on root growth. In a soilless growing system, the effect of AM fungi on strawberry fruit quality was greater than on growth parameters. We believe that further research is needed with different cultivars in order to establish generalizations about the effect of AM fungi and biofertilizer on strawberry fruit weight, fruit quality attributes and growth parameters in a soilless growing system.

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