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Mycorrhizal inoculation affects the phytochemical content in strawberry fruits

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ABSTRACT. The aim of this research was to evaluate the effect of the inoculation date of arbuscular mycorrhizal fungi on the fruit quality and the content of phytochemicals in a strawberry soilless growing system. The experiment was performed in Huelva (Spain) and was conducted in a greenhouse on the La Rábida Campus of Huelva University under natural light and temperature from October 2013 to June 2014. Three short-day strawberry cultivars ('Splendor', 'Sabrina' and 'Fortuna') were grown in polyethylene bags filled with coconut fibres. Randomized block design, with 3 repetitions and factorial arrangement (3 cultivars x 3 treatments), was established. Each replicate consisted of one bag with 12 plants supporting structures at 40 cm height. The treatments were: T1 = mycorrhizal inoculation in the transplantation; T2 = mycorrhizal inoculation 30 days after transplantation (DAT); and T0 = control treatment, without inoculation. Arbuscular mycorrhizal fungi inoculation significantly affected the contents of anthocyanin and phenolics. When the inoculation is performed in the transplantation, the fruits showed a high content of anthocyanin and total phenolics. The mycorrhizal inoculation influences decreasing the acidity in fruit throughout the growing season and increase firmness only during the early stage of production.

Keywords: *Fragaria* x *ananassa* Duch., anthocyanin, phenolics, ascorbic acid.

Inoculação micorrízica afeta o conteúdo de fitoquímicos em frutos de morango

RESUMO. O objetivo do trabalho foi avaliar o efeito da época de inoculação dos fungos micorrízicos arbusculares na qualidade dos frutos e no teor de fitoquímicos em sistema de cultivo sem solo de morangueiro. O experimento foi realizado em Huelva (Espanha) e foi conduzido em casa de vegetação no Campus La Rábida da Universidade de Huelva, sob luz e temperatura natural, de outubro 2013 a junho de 2014. Três cultivares de morangueiro de dias curtos (Splendor, Sabrina e Fortuna) foram cultivadas em sacos de polietileno preenchido com fibras de coco. Delineamento em blocos casualizados, com 3 repetições e arranjo fatorial, foi estabelecido. Cada repetição consistiu de um saco com 12 plantas apoiadas em estruturas de 40 cm de altura. Os tratamentos foram: T1 = micorrização no transplante; T2 = micorrização 30 dias após o transplante (DAT); e T0 = testemunha, sem inoculação. A inoculação de micorrízas afeta significativamente o conteúdo de antocianina e compostos fenólicos. Quando a inoculação é realizada no transplante, os frutos apresentaram elevado teor de antocianina e compostos fenólicos total. A inoculação micorrízica influencia diminuindo a acidez das frutas durante todo o ciclo de produção e aumenta a firmeza apenas durante a produção precoce.

Palavras-chave: *Fragaria* x *ananassa* Duch., antocianinas, fenólicos, ácido ascórbico.

Introduction

Several soil beneficial micro-organisms, such as the arbuscular mycorrhizal fungi (AMFs), may provide improvement to plant growth, development and resistance to various abiotic agents (López-Ráez, Flors, García, & Pozo, 2010; Folli-Pereira, Meira-Haddad, Bazzolli, & Kasuya, 2012). Moreover, mycorrhizal fungi may influence the secondary metabolism by increasing, for example, the concentration of phenolics and anthocyanin in plants (Baslam & Goicoechea, 2012; Baslam, Garmendia, & Goicoechea, 2013; Chen et al., 2013).

Currently, select research groups around the world are interested in the secondary metabolites present in plants. This interest results from these compounds being related to the natural defence of the plant, producing a more stress-resistant plant, and from the health benefits these compounds

display in preventing chronic and degenerative diseases including cancer and heart diseases (Nadtochiy & Redman, 2011; Lee et al., 2013).

Strawberries (*Fragaria x ananassa* Duch.) are consumed and commercialized worldwide. Spain is the European leader in strawberry production and is the second largest producer globally after the United States (Statistical of Strawberry Production in World Faostat [Faostat], 2013). Statistical of Strawberry Production in World Faostat (Faostat, 2012) estimates that approximately 7,600 hectares of strawberries are grown in Spain, producing a yearly production of approximately 289,900 tons or 10% of the global production. Moreover, Spain is responsible for providing almost all fresh strawberry fruits to the European Community during the first months of the year. The production of Spanish strawberries is primarily intended for the consumption of fresh fruit, since only 10% of exports are in the form and frozen fruit (López-Aranda, 2008).

The quality and consumption of strawberries are determined by parameters such as size, firmness, contents of soluble sugar and acid concentration. The content of sugar is an important nutritional characteristic because it determines the caloric value of the fruit. Additionally, when combined with organic acids, the sugar content improves the flavour. Firmness is an important characteristic of the texture, whereas organic acids influence the flavour, pH and colour of the fruit by directly intervening in pigment (such as anthocyanin) formation. Ascorbic acid (AsA) is the most important organic acid in strawberry fruits (Ornelas-Paz et al., 2013). Furthermore, strawberry fruits are an important source of phytochemicals. In particular, the phenolics composition of the plant appears to strongly influence the quality, contributing to sensorial and organoleptic characteristics and to the nutritional value of the fruit (Tulipani et al., 2008; Aaby, Mazur, Nes, & Skrede, 2012; Van De Velde, Tarola, Güemes, & Pirovani, 2013). Anthocyanins are responsible for the red colour of the fruit and are quantitatively the most important phenolic compound. Anthocyanins may vary in concentration from 200 to 600 mg kg^{-1} (Fazeelat, Afzal, Asif, Zamir, & Saleem, 2007). Lower concentrations have been reported by Silva, Escribano-Bailón, Alonso, Rivas-Gonzalo, and Santos-Buelga (2007) and Tulipani et al. (2008). However, qualitative variations in the anthocyanin profile occur among cultivars or even inside the identical cultivar, depending on the genetics, ripeness degree, post-harvest processing and storage of the plants and weather factors on the plants (Tulipani et al., 2008).

The application of mycorrhizal inoculum in the conventional strawberry cultivation increases the diversity of these fungi in the rhizosphere, raising the opportunities for symbiosis and bringing benefits to the crop. However, mycorrhizal fungi application in soilless systems is not completely clear. Most studies in soilless systems evaluated the growth and acclimatization of micropropagated strawberry plants (Chávez & Ferrera-Cerrato, 1990; Vestberg, 1992; Cassells, Mark, & Periappuram, 1996; Alarcón, Ferrera-Cerrato, González-Chávez, & Villegas-Monter, 2001; Taylor & Harrier, 2001). Other studies refer to the behavior of inoculated plants in relation to different nitrogen levels (Castellanos-Morales, Villegas-Moreno, Vierheilig, & Cárdenas-Navarro, 2012), phosphorus levels (Cekic & Yilmaz, 2011), influence on vegetative parameters (Martinez, Weiland, & Palencia, 2013), and his performance as root bioprotectors (Murphy, Rafferty, & Cassells, 2000; Norman & Hooker, 2000). Current studies on the increase of anthocyanins and influence of inoculum in fruit quality (Castellanos-Morales et al., 2010; Lingua et al., 2013; Palencia, Martinez, & Weiland, 2013) has been conducted. Research on mycorrhizal strawberry plants in soilless growing systems includes Corkidi, Rowland, Johnson, and Allen (2002), who indicated that select substrates used in soilless growing systems are not suitable for the development of mycorrhizal colonisation (such as peat). Previous research has shown that the salinity of the substrate may reduce mycorrhizal colonisation by inhibiting the germination of spores, growth of hyphae in the soil and hyphal spread in roots after the initial infection occurs (Mcmillen, Juniper, & Abbott, 1998) and by reducing the number or arbuscules. However, other studies have shown that AMFs are able to promote plant growth and salinity tolerance. AMFs promote salinity tolerance by employing various mechanisms such as the enhancement of nutrient acquisition (Al-Karaki & Al-Raddad, 1997), production of plant growth hormones, improvement of rhizospheric and soil conditions (Lindermann, 1994) and alteration of the host physiological and biochemical properties (Smith & Read, 1997). Navarro, Elia, Conversa, Campi, and Mastrorilli (2012) suggested that salt tolerance in carnations increased when roots are colonised by *G. intraradices*. However, Cekic and Yilmaz (2011) suggested that in soilless strawberry growing systems, mycorrhizal plants displayed more crowns and fruits in the 'Maraline' and 'Camarosa' cultivars when inoculated with *Glomus clarum*.

Mycorrhizal plants showed a higher biomass accumulation (crowns and shoot) and a more extensive leaf area (Borkowska, 2002).

As far as our knowledge reaches, no study to date has identified the best time to inoculate and whether it may influence the phytochemical characteristics and content of phytochemicals in the strawberry fruits.

The aim of this research was to evaluate the effect of the inoculation date of AMFs on the fruit quality and the content of phytochemicals in strawberry soilless growing systems.

Material and methods

The experiment was performed in Huelva (Spain) and was conducted in a greenhouse on the Rábida Campus of Huelva University (37° 12' N latitude, 6° 55' W longitude and 24 m above sea level) under natural light and temperature from October 2013 to June 2014. The average temperature and relative humidity during the experiment were 23ºC and 65%, respectively. We used fresh plants from high altitude nurseries (Niharra, Ávila, Spain) that were autumn-planted.

From 2013, with more than 69% of cultivated areas, the strawberry cultivars prevalent in the province of Huelva (Spain), reason why these cultivars were chosen, were Fortuna, from crossing 'Winter Dawn' × 'FL 99-35' and registered by University of Florida, Sabrina from crossing Sel. Nº 90-020 X 97-19 and registered by Planasa® and Splendor from crossing Camarosa × 'PS-1269' and registered by PlantSciences-BerryGenetics, all of them of short days (Finn & Clark, 2008; Consejería de Agricultura, 2013; European Food Safety Authority [EFSA], 2014). Three short-day strawberry cultivars, (Splendor, Sabrina and Fortuna) were grown in polyethylene bags (100 x 18 x 30 cm) filled with coconut fibres (Pelemix Spain, S.L., Murcia-Spain). Each bag contained 12 strawberry plants (8.3 strawberry plants m^{-2}). Polyethylene bags were placed on supporting structures 40 cm high and were watered with a drip irrigation system that had four drippers per pot and delivered 2 L h^{-1} per dripper. The coconut fibre was washed and autoclaved at 121ºC for 1 hour and two cycles, with a 24 hours interval after each cycle, at the beginning of the crop cycle.

A completely randomised block design (3 cultivars x 3 treatments) with 3 replicates was established. Each replicate consisted of one bag with 12 plants. The treatments were as follows: T1 = mycorrhizal inoculation in the transplantation; T2 = mycorrhizal inoculation 30 days after transplantation (DAT); and T0 = control treatment, without inoculation. Inoculation was performed during transplantation and at 30 DAT. The treatments were designed in relation to the minimum and maximum time set for the commercial product. The commercial product (Mycogrowth®) employing a mycorrhizal inoculant based on *Glomus iranicum var tenuihypharum* (1.2×10^4 NMP in 100 mL substrate) was used. The inoculation was accomplished using a syringe, in which the product was diluted in water to a concentration of 3 kg ha^{-1} (0.09 g bag^{-1}). In total, 10 mL was added to the rhizosphere of the plant.

The nutrient solution for all treatments was the following: 1 of NH_4NO_3; 1.5 of KNO_3; 2.3 of $Ca(NO_3)_2$; 0.8 of $Mg(NO_3)_2$ and 1 mEq L^{-1} of KH_2PO_4. The content of phosphorous (KH_2PO_4) was reduced to 50% during the late production phase until the end of the crop cycle (from March to May, 2014).

The mycorrhizal colonisation rate was determined during the early, late and at the end of the crop cycle. One plant from each treatment was removed from the polyethylene bag and used for the mycorrhizal colonisation rate determinations during the three periods mentioned. At the end of the crop cycle, three plants from each treatment and the cultivar were used for the mycorrhizal dependence analysis.

The plant mycorrhizal colonisation rate was determined using root colouring with Trypan blue according to Phillips and Hayman (1970). Later, the percentage of mycorrhizal colonisation was evaluated according to Giovannetti and Mosse (1980). Thirty root segments from each treatment and cultivar were observed through a microscope. The counting was evaluated whether colonisation was noted in the sight field ('Yes') or not ('No'). The percentage of colonisation was obtained through a rule of three.

The mycorrhizal dependence (MD in %) of the plants was calculated according to Gendermann (1975): Mycorrhizal dependence (%) = (total dry weight of mycorrhizal plant – total dry weight of non-mycorrhizal plant) ⋆ 100/ total dry weight of mycorrhizal plant. Therefore, the plants were weighed before and after drying at 65ºC until a constant weight was achieved.

Fruits from each treatment and cultivar were harvested throughout the experimental period when the fruits reached the commercial standard, i.e., between 75 and 100% of the red colour in the epidermis. Strawberry fruits were graded for size and external colour, sorted to eliminate damaged material and transported under refrigeration to the laboratory. The fruits harvested between the months

of January and March (from week 13 to week 19 after planting) were called early production, and the fruits harvested during the months from April to May (from week 20 to week 30 after planting) were called late production (both in g per bag). The total yield was determined by adding the fruit originating from the early production to the fruits originating from the late production. On each sampling date, all fruits from each pot and from each treatment were gathered for quality assessment and converted into pulp using a mixer.

After harvesting, five homogeneous fruits from each treatment and the cultivar were separated to determine the fruit quality and fresh weight. To evaluate the total phenolic compounds and anthocyanins, five other fruits were sampled per cultivar and treatment, and 3 replicates for each sample were analysed.

The fruit quality was evaluated in three cultivars by means of fruit size (mm), total soluble solid (TSS), pH, titratable acidity (TA), TSS/TA ratio, firmness, AsA, total phenolic compounds and anthocyanins. The fruit quality, total phenolic compounds and anthocyanins were measured weekly during the crop cycle.

Fruit size was determined by the maximum diameter of the equatorial section. The TSS was determined using an automatic temperature-compensated PR101 digital refractometer (Atago Pallette PR101). TA (expressed as g of citric acid 100 g^{-1} fresh weight) was measured in each treatment by titrating 10 g of pulp plus 10 mL of H_2O with 0.1 mol L^{-1} NaOH up to a pH of 8.1. The firmness was evaluated in a sub-sample of 3 - 4 fruits from each treatment using a portable penetrometer, and the results were expressed in g cm^{-2}. The AsA was measured weekly during the late crop cycle and was quantified with the Merck Co reflectometer set (Merck RQflex) according to the manufacturer's protocol for red fruit juice (Ascorbic Acid in Red Coloured Fruit Juices, Merck). The results were expressed as mg AsA 100 g^{-1} of fruit fresh weight.

For the analysis of total anthocyanins, the extracts were prepared according to the methodology described by Revilla, Ryan, and Martín-Ortega (1998), in which 30 g of fruits were extracted through sonication for 60 min at room temperature with 30 mL of a hydroethanolic solution 70°GL (°Gay Lussac) at pH 1.0. The samples for determining the total phenolic compounds were extracted using an identical method as for the anthocyanin analysis; therefore, the solvent was 70% methanol. After the extractions, the samples were filtered and stored in a freezer for future analyses.

The analysis of the phenolic content was performed using the Folin-Ciocalteu method as described by Singleton, Orthofer, and Lamuela-Raventos (1999). Sample readings were taken using a spectrophotometer Shanghai Spectrum, model SP – 2000 UV-VIS. The readings were measured in triplicate, and the results were described in milligrams of gallic acid per 100 g of fruits (mg gallic acid 100 g^{-1} of fruit fresh weight).

The content of total monomeric anthocyanin was determined according to the methodology described by Giusti and Wrolstad (2001) and Lee, Durst, and Wrolstad (2005). The absorbance readings were measured in triplicate on a spectrophotometer Shanghai Spectrum model SP – 2000 UV-VIS at two wavelengths (520 and 700 nm). The results were described in milligrams of cyanidine 3-glucoside for each 100 g of fruit fresh weight.

The results of the evaluations were assessed by an analysis of variance. When a significant difference between the averages was noted, the averages were compared using Tukey's test at 5% error probability. To improve the distribution of the residual standard error, the percentage data for the mycorrhizal colonisation were transformed into arcsin $\sqrt{x/100}$.

Results and discussion

In February 2014, the evaluation of the root colonisation regarding the time of early production (when the roots of strawberry plants were analysed microscopically) was performed to verify the presence of fungi. In early production, roots without typical fungal structures of mycorrhizae (spores, hyphae and arbuscules) were observed in the control treatment, showing that the plants received from the seedling nursery do not have natural mycorrhiza. In T1 and T2 treatments, the incidence of fungus in the roots was up to 10%, and the fungal structures noted were spores. In the second evaluation, which coincided with the late crop cycle and, consequently, in the period with a greater growth stress, the colonisation average rate was 50% of roots in treatments in which the fungus was inoculated, indicating a higher dependence on fungi (Figure 1a). At the end of the crop cycle, the rate of root colonisation remained constant, and the fungi were still absent from the control plants. Despite not showing differences in the frequency of colonisation between the treatments, the Fortuna cultivar presented the greatest colonisation rate in both the T1 and T2 treatment. Additionally, Fortuna was the only cultivar that presented mycorrhizal dependence (MD ≥ 10%) (Figure 1b).

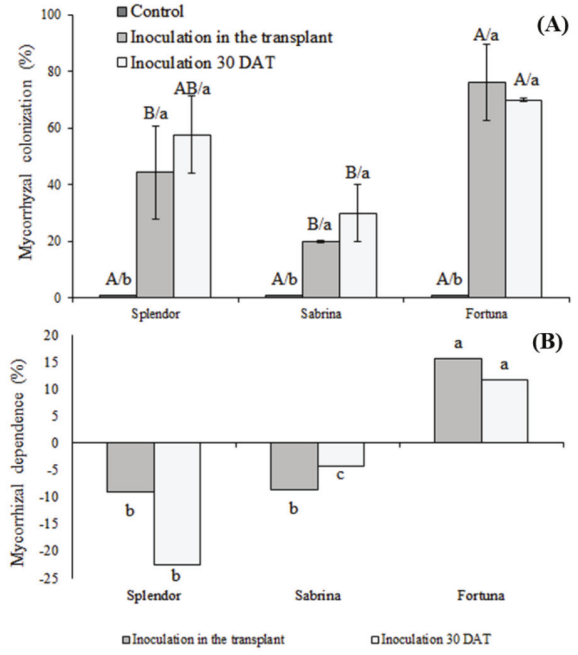

Figure 1. Mycorrhizal colonization on strawberry in soilless system at the end of the production cycle. (A) Percentage of colonization. Means with common lowercase letter do not differ by Tukey test ($p \leq 0.05$) among test treatments (T1 and T2). (B) Mycorrhizal dependence of strawberry in soilless culture. Means with common uppercase letter do not differ by Tukey test ($p \leq 0.05$) test between varieties.

Flavonoids are the main product excreted from plant roots during a phosphorous deficiency (Nagahashi & Douds, 2000), and the flavonoids act as stimulant in the germination of fungal spores (Nadal & Paszkowski, 2013). In our work, the levels of root colonisation were nearly tripled in the late crop cycle (≥ 30% frequency) when compared to the early crop cycle (10%). This increase likely occurred because the stress condition caused by the reduced phosphorous may have provoked the plant to release a higher amount of flavonoids, stimulating the inoculated fungus in the substrate. However, mycorrhizal inoculation fungi can be performed in substrates with a higher fertility (Gianinazzi, Trouvelot, & Gianinazzi-Pearson, 1990). To display the benefits of mycorrhizal fungi, aspects related to the content of organic matter and phosphorous (which can influence the feasibility and effectiveness of mycorrhizal fungi treatment) must be considered.

In this present work, the fungal structures of hyphae and spores were mostly found in the roots; no arbuscular structures were noted. Alarcón, Ferrera-Cerrato, González-Chávez, and Villegas-Monter (2001) observed a high rate of mycorrhizal colonisation in the matrix of strawberry plants; therefore, the incidence rate of arbuscules was low, and the presence of intra-root spores was approximately 50.6%. Previous studies reported that the effectiveness of the mycorrhizal fungi is not always correlated with the fungi infection capacity. For instance, Smith and Gianinazzi-Pearson (1988) noted that the establishment of the arbuscular interface in the cortical cells of the host was the primary form of bidirectional exchange of nutrients between both symbionts. However, the exchange of nutrients is not solely performed by arbuscules, evidence has shown that the intercellular hyphae contribute significantly to the release of nutrients from the cells of the host (Bago et al., 2003). The use of inoculants with mycorrhizal fungi may have been one of the problems of the low colonization and its influence on the parameters evaluated in this study. Using these inoculants, according to Garland, Schroeder-Moreno, Fernandez, and Creamer (2011), has as main drawback the presence of only one or a few species of the fungus that may or may not adapt to the environmental conditions that will be used.

Even when the effect of mycorrhizal inoculation in the total and late production phases does not occur, the effect is more significant when the plant is in a more stressed period (i.e., during the late crop cycle, coinciding with high temperatures). These fluctuations of temperatures throughout the crop cycle allow us to differentiate between two different periods, an early harvest date characterized by low temperatures and high relative humidity, between January and March, and a warmer late harvest date with high temperatures and low relative humidity between April and May (Ariza, Cid-Atencia, Soria, Medina, & Miranda, 2009).

AMFs are known to improve the plant growth, productivity and tolerance to several abiotic agents. In strawberry plants, these influences have been reported in several studies (Douds, Nagahashi, & Shenk, 2008; Matsubara, Ishigaki, & Koshikawa, 2009; Borowicz, 2010; Vos, Van Den Broucke, Lombi, De Waele, & Elsen, 2012). Therefore, only a few works evaluate the quality of the fruit (Castellanos-Morales et al., 2010; Lingua et al., 2013). Few studies have been conducted to determine the most effective time for strawberry mycorrhizal inoculation. Using *Glomus intraradices* Palencia, Martinez, and Weiland (2013) rose two inoculation dates (at the time of transplantation and four weeks) to study its effect on the production of the 'Primoris' cultivar, but found no significance. However, similar studies performed in *Pelargonium*

horturom by Csima, Hernádi, and Posta (2012) conclude that a pre-inoculation is more practical as lower expenses of inoculum is required in addition to result in taller plants with a higher nutrients absorption capacity. But there are not studies to determine the most effective time for strawberry mycorrhizal inoculation in relation to quality.

From Table 1 and 2, no interaction was noted between the inoculation periods and the variables used ($p \geq 0.05$) in this soilless growing system; only isolated factors of significance were displayed.

In the T2 treatment, the fruits were firmer only during the early production (Table 1). However, no significant differences were noted between the control and 30 DAT treatments. Fruits produced in the early stage differ between cultivars in diameter and firmness, considering the average of plants with and without inoculum. Fortuna had a larger fruit diameter. Sabrina was firmer in both production stages.

In relation to the TA (Table 2), the effect of the mycorrhizal fungi inoculation was observed in the total average, a notable difference between treatments. The T2 treatment displayed the largest titratable acidity in the fruits, despite not being different than the control treatment (without inoculation). During the early production, TA values in T1 and T2 treatments were significantly lower than the control treatment. Therefore, the mycorrhizal fungi inoculation decreases TA values in the early production.

Among the cultivars used in this study, Fortuna presents higher values of pH (Table 1) in the late production phase and in the general average (total). Until the twenty-second week of production, Fortuna fruits displayed a larger diameter (Table 1). From the twenty-third week until the end of the cycle, however, this difference disappears. The Sabrina cultivar showed a greater firmness during the productive period (Table 1). The Sabrina cultivar does not differ from the Fortuna cultivar when the general average was evaluated. Sabrina cultivar fruits had lower acidity and higher content of vitamin C when they were harvested in the late production cycle. Confirming that the cv. Sabrina is sweeter than Splendor and Fortuna, a fact noted by strawberry growers in the province of Huelva, in relation to the ratio SST/ATT no difference was found (Table 2).

During the productive cycle, the content of sugar and AsA in the Sabrina cultivar was higher than the Fortuna cultivar. However, no significant differences were noted between the Sabrina and Splendor cultivars (Table 3).

Table 1. Diameter, firmness, pH in fruits of three cultivars of strawberry grown in coconut fiber and inoculated with mycorrhizal fungi in two moments.

Cultivars	Diameter (mm)			Firmness (g cm^{-2})			pH		
	Early	Late	Total	Early	Late	Total	Early	Late	Total
Splendor	22.47 ± 3.43b	27.61 ± 1.62a	26.68 ± 1.82a	296.16 ± 10.75b	220.88 ± 22.76b	235.37 ± 24.31b	3.00 ± 0.03a	2.99 ± 0.88ab	2.99 ± 0.06ab
Sabrina	22.91 ± 4.72b	40.72 ± 3.35a	35.35 ± 2.88a	309.71 ± 12.67a	265.02 ± 22.70a	276.86 ± 13.00a	3.00 ± 0.08a	2.91 ± 0.22b	2.95 ± 0.06b
Fortuna	27.25 ± 2.63a	30.15 ± 3.44a	28.67 ± 2.00a	282.73 ± 11.97b	232.08 ± 21.04b	258. 28 ± 14.85a	3.04 ± 0.06a	3.10 ± 0.10a	3.07 ± 0.06a
Treatments									
Control	22.74 ± 3.80a	42.7 ± 3.86a	36.24 ± 2.56a	293.12 ± 11.25ab	237.95 ± 31.73	254.99 ± 26.92a	3.04 ± 0.04a	2.92 ± 0.20a	2.96 ± 0.15a
Inoc. in the transplant	27.93 ± 4.32a	27.41 ± 1.41a	27.85 ± 1.29a	280.4 ± 15.47b	241.48 ± 38.10a	252.36 ± 32.15a	3.05 ± 0.02a	3.04 ± 0.18a	3.04 ± 0.1a
Inoc 30 DAT	24.32 ± 3.51a	28.35 ± 2.99a	26.62 ± 2.2a	304.73 ± 14.90a	238.54 ± 13.92a	263.17 ± 11.08a	2.97 ± 0.07a	3.04 ± 0.08a	3.01 ± 0.06a
Mean	25.61 ± 4.07	27.88 ± 2.32	30.23 ± 1.49	296.94 ± 18.88	240.01 ± 27.87	256.84 ± 24.51	3.02 ± 0.07	3.04 ± 0.13	3.00 ± 0.11
Cultivars (cv)	*	ns	ns	*	*	*	ns	**	*
Treatments (Trat)	ns	ns	ns	*	ns	ns	ns	ns	ns
Trat x cv	ns	ns	ns	ns	ns	ns	ns	ns	ns

Means ± SD followed by the same letter in the column do not differ by Tukey test. *Significant at $p \leq (0.05)$. nsnot significant.

Table 2. Titratable acidity (TA), total soluble solids (TSS), ratio TSS/TA in fruits of three cultivars of strawberry grown in coconut fiber and inoculated with mycorrhizal fungi in two moments.

Cultivars	TA (% citric acid)			TSS (°Brix)			Ratio TSS/TA		
	Early	Late	Total	Early	Late	Total	Early	Late	Total
Splendor	0.77 ± 0.14a	0.69 ± 0.05a	0.71 ± 0.05a	6.80 ± 0.61ab	5.75 ± 0.45b	5.96 ± 0.6b	8.99 ± 1.92a	8.48 ± 0.75a	8.59 ± 0.97a
Sabrina	0.84 ± 0.21a	0.59 ± 0.09b	0.66 ± 0.09a	7.36 ± 1.47a	6.42 ± 0.39a	6.75 ± 0.6a	9.27 ± 2.34a	16.23 ± 0.88a	13.61 ± 9.53a
Fortuna	0.75 ± 0.06a	0.64 ± 0.05ab	0.70 ± 0.05a	5.67 ± 0.57b	5.31 ± 0.35c	5.51 ± 0.32b	8.11 ± 1.15a	9.03 ± 0.72a	8.46 ± 0.84a
Mean	0.78 ± 014	0.64 ± 0.07	0.69 ± 0.06	6.52 ± 1.18	5.82 ± 0.60	6.07 ± 0.72	8.72 ± 1.80	11.25 ± 9.53	10.22 ± 5.86
Treatments									
Control	0.90 ± 0.17a	0.65 ± 0.03a	0.73 ± 0.05a	6.91 ± 1.51a	5.66 ± 0.72a	6.16 ± 0.9a	8.14 ± 2.07a	9.03 ± 0.86a	8.79 ± 1.03a
Inoc in the transplant	0.69 ± 0.08b	0.63 ± 0.11a	0.66 ± 0.08b	5.76 ± 0.97a	5.86 ± 0.69a	5.86 ± 0.76a	8.71 ± 2.20a	14.64 ± 1.64a	12.24 ± 10.09a
Inoc 30 DAT	0.73 ± 0.07b	0.65 ± 0.07a	0.69 ± 0.05ab	6.59 ± 0.81a	5.95 ± 0.34a	6.21 ± 0.48a	9.24 ± 1.30a	10.07 ± 1.65a	9.64 ± 1.31a
Mean	0.72 ± 0.07	0.64 ± 0.09	0.69 ± 0.06	6.29 ± 0.93	5.91 ± 0.53	6.07 ± 0.72	9.05 ± 1.61	12.36 ± 1.61	10.22 ± 5.87
Cultivars (cv)	ns	*	ns	*	*	*	ns	ns	ns
Treatments (Trat)	*	ns	*	ns	ns	ns	ns	ns	ns
Trat x cv	*	*	*	*	*	*	*	*	*

Means ± SD followed by the same letter in the column do not differ by Tukey test. *Significant at $p \leq (0.05)$. nsnot significant.

Table 3. Ascorbic acid in fruits of three cultivars of strawberry grown in coconut fiber and inoculated with mycorrhizal fungi in two moments.

Cultivars	Ascorbic acid (mg 100 g^{-1} Fruit fresh weight)
Splendor	58.92 ± 8.83a
Sabrina	65.60 ± 5.16a
Fortuna	47.35 ± 4.37b
Mean	56.44 ± 11.32
Tretatments	
Control	55.99 ± 10.59a
Inoc. In the transplant	59.29 ± 11.75a
Inoc. 30 DAT	56.59 ± 7.61a
Mean	57.29 ± 9.85
Cultivars (cv)	*
Treatments (Trat)	ns
Trat x cv	ns

Means ± SD followed by the same letter in the column do not differ by Tukey test. *Significant at $p \leq (0.05)$. nsnot significant.

The physical and sensorial quality of strawberries is linked to characteristics such as size, firmness, colour, pH, sugar/acids ratio, flavour and scent. In our study, the greatest differences are found among strawberry cultivars. The total soluble solids, an important feature for the acceptance of the product by consumers, presented variation between the cultivars, as previously noted in several studies (Cecatto et al., 2013).

The Sabrina cultivar developed sweeter and firmer fruits with less acidity when cultivated in coconut fibre. The cultivar Sabrina developed a greater content of sugar (ºBrix); however, this level remains below the ideal level for consumption *in natura* (i.e., ºBrix exceeding 7%) (Mitchell, Mitcham, Thompson, & Welch, 1996; Namesny, 1999).

According to Consejería de Agricultura (2013), the strawberry cultivars predominant in Huelva until 2012 were 'Candonga' and Camarosa. Therefore, an improvement in the exchange of cultivars was noted, increasing the interest for early cultivars such as Fortuna, Sabrina and Splendor. However, no scientific information regarding the quality of these cultivars is available because the initial testing has just begun.

In the T2 treatment, the influence of the mycorrhizal fungi on the basic aspects of quality was observed only for the firmness and titratable acidity at the beginning of the productive period (the twenty-second week of production). Castellanos-Morales et al. (2010) verified that the mycorrhizal fungi inoculation in the 'Aromas' cultivar cultivated in coconut fibre and perlite (1:3 v v^{-1}) did not affect the diameter, titratable acidity and ºBrix of the fruit, corroborating with the results obtained in our work (Table 1 and 2).

Strawberry fruits are an important source of bioactive compounds because of their high levels of AsA and secondary plant metabolites, substances that present beneficial properties for health (Tulipani et al., 2008; Pineli et al., 2011; Van De Velde et al., 2013; Akhatou & Fernández-Recamales, 2014). In our study, the presence of mycorrhizal fungi did not affect the contents of AsA; the only difference was among the cultivars. The contents of the AsA found in this study (44.2 to 67.6 mg 100 g^{-1} fruit fresh weight) were similar to the contents in other studies of strawberry cultivars. Flores-Cantillano, Ávila, Peralba, Mara, and Toralles (2012) found levels of 55.56 fruit fresh weight and 53.50 mg 100 g^{-1} fruit fresh weight of AsA for 'Camino Real' and Camarosa cultivars, respectively. Mazur et al. (2014) obtained 49 mg 100 g^{-1} fruit fresh weight, 43 fruit fresh weight and 44 mg 100 g^{-1} fruit fresh weight of AsA for the 'Blink', 'Polka' and 'Senga' cultivars, respectively. Bona et al. (2015) suggested that the inoculation with a mixture of AMFs and strains of plant growth-promoting bacteria increased the flower production, fruit production, fruit size, and concentrations of sugars, ascorbic acid and folic acid when compared with the fruits from plants that were not inoculated.

This is the first study that shows that mycorrhizal inoculation time on strawberry plants grown out of the soil affects the physical and chemical characteristics and especially anthocyanin and phenolic compounds in fruits. Mycorrhizal fungi inoculation significantly affected the contents of anthocyanin and phenolics. The fruits showed a greater content of anthocyanin (514 mg 100 g^{-1} fruit fresh weight) and phenolics (235.6 mg 100 g^{-1} fruit fresh weight) when the plant was inoculated during transplantation. Treatment T1 displayed the greatest content of anthocyanin in the Fortuna cultivar (514.04 mg 100 g^{-1} fruit fresh weight) (Figure 2a). The Sabrina cultivar presented the lowest contents in all treatments. In the T1 treatment, the content of anthocyanin in the Splendor cultivar was reduced by 23%, and in the T2 treatment, the contents of anthocyanin was reduced by 53%. The inverse was observed for the content of phenolics (Figure 2b). The Fortuna cultivar showed the lowest quantities of phenolics in all treatments, and the Sabrina cultivar showed the highest quantities. However, no significant differences were noted among cultivars. In the T1 treatment, the content of phenolics was 235.56 mg 100 g^{-1} fruit fresh weight. When the Splendor cultivar was inoculated with mycorrhizae, the content of anthocyanins did not increase. However, the phenolics content increased. The phenolics content in the Sabrina and Splendor cultivars were similar when mycorrhizal was inoculated in the transplant.

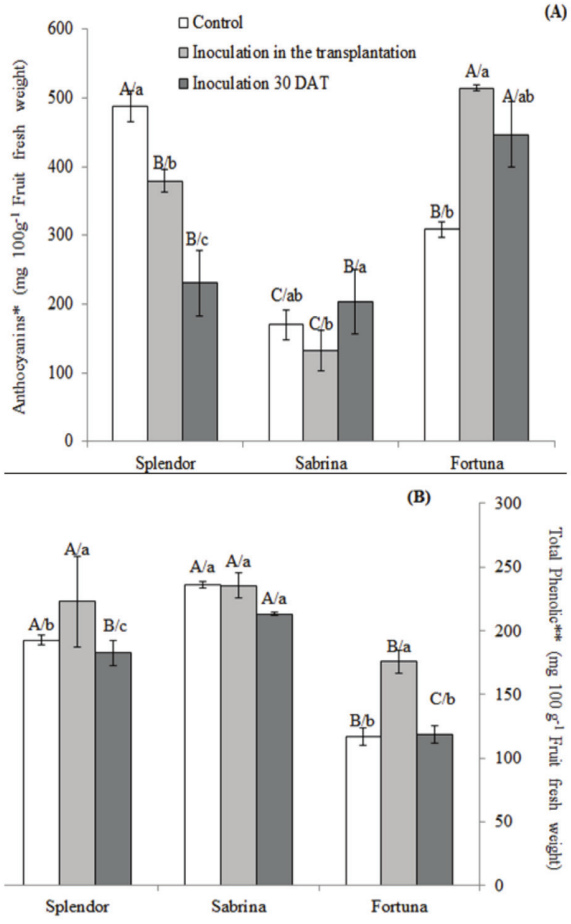

Figure 2. Content of anthocyanins and total phenolics in fruits of three varieties of strawberry plants grown in coconut fiber and inoculated with mycorrhizal fungi at different times. (A) Contents of anthocyanins. *Expressed as cyanidine-3-glucoside. (B) Content of total phenolics. **Expressed as gallic acid. Mean ± standard deviation with common lowercase letter do not differ by Tukey test ($p \leq 0.05$) among test treatments. Means with common uppercase letter do not differ by Tukey test ($p \leq 0.05$) among test varieties ($n = 3$).

The benefits related to the production of phenolic compounds in strawberry fruits inoculated with AMFs were also observed. Castellanos-Morales et al. (2010) showed that the symbiosis between the strawberry plants and mycorrhizal fungi caused an increase in cyaniding 3-glucoside and other phenolic compounds. Lingua et al. (2013) reported concentrations of 350 μg g^{-1} fruit fresh weight of pelargonidine-3-glucoside and 4.5 μg g^{-1} fruit fresh weight of cianidine-3-glucoside when growing the Selva cultivar with the mycorrhizal fungi *Glomus*. Improved quality aspects for the fruits, mainly bioactive compounds, were also obtained in other crops after mycorrhizae inoculation. Baslam, Garmendia, and Goicoechea (2011) observed that the content of total soluble sugars in the leaves increased in mycorrhizal lettuce after inoculation; additionally, the contents of phenolic compounds and anthocyanin also increased, mainly in the internal leaves. In a study of inoculated tomato plants under greenhouse conditions by Giovannetti et al. (2012), it was observed that the symbiosis positively affect the nutritional value of fruit, increasing the levels of lycopene.

This study suggests additional research is required that identify the exact date of mycorrhizal inoculation and the ideal conditions to perform the symbiosis in a soilless growing system. The main objective of employing mycorrhizal fungi in soilless growing systems is to minimise the use of water, pesticides and fertilisers, developing an alternative that produces economic and ecological advantages. In future studies, the action of lowering the content of phosphorous should be performed in the beginning of the experiment when the plants are transplanted, increasing the stress in the plants to verify whether the establishment of the symbiosis occurs earlier.

Conclusion

Inoculations with mycorrhizal fungi significantly affect the concentrations of anthocyanin and phenolic compounds, increasing the levels of these compounds when the inoculation is performed during transplantation.

The mycorrhizal inoculation influences decreasing the acidity in fruit throughout the growing season and increase firmness only during the early stage of production.

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