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THE EFFECT OF ARBUSCULAR MYCORRHIZAL FUNGI ON VEGETATIVE GROWTH AND NUTRIENT UPTAKE IN THREE APPLE (*MALUSDOMESTICA* BORKH.) CULTIVARS ('RED DELICIOUS', 'GOLDEN DELICIOUS' AND 'STARKINGDELICIOUS')

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ABSTRACT

Mycorrhizal fungi (MF) have a significant impact on plant growth and nutrition, as they transfer inorganic nutrients between plants and fungi. This study has been conducted to investigate the effect of MF on the growth characteristics and nutrient uptake in three apple cultivars. Experiments were performed according to complete block design in a split plot, randomized with four replications. The treatments involved four levels of mycorrhizal fungi (0, 70, 100, and 120 grams per pot) and three cultivars of apples ('Red Delicious', 'Golden Delicious', and 'Starking Delicious'). Some characteristics that measured were included leaf area, plant height and diameter, fresh and dry weight of leaves, chlorophyll a and b content, and phosphorus, nitrogen, zinc, and copper content and uptake. The results show that the use of MF in the apple growth media significantly increased plant height, stem diameter, and leaf area compare to the control. Arbuscular mycorrhizal fungi (AMF) inoculation increased fresh and dry weight of leaves and chlorophyll content of three cultivars in comparison to the non-inoculation treatment. The effect of AMF was not significant on leaf N status and decreased leaf P content in apple seedlings. However, leaf N and P uptake was increased compared to the control. By the application of AMF, Fe and Zn content of leaves decreased. The highest Fe and Zn leaf uptake was found in 'Red Delicious' that were treated with 70 g of AMF fertilizer per pot. According to the results, 70 g of AMF per pot had the biggest effect on vegetative growth and nutrient uptake of apple seedlings.

Keywords: Chlorophyll, Cultivars, Growth characteristics, Nitrogen, Zinc

INTRODUCTION

Apple (*Malus domestica* Borkh.) is one of the most important temperate fruits from the Rosacea family (Anonymous 2008). It is one of the most common fruit consumed regularly around the world and is often produced in the mountainous and cold areas of Iran. About 2.66 million metric tonnes of apples are produced in Iran every year (Anon 2009). Most of these apples are exported to the United Arab Emirates, Turkey, Germany, etc. (Tabatabaeefar and Rajabipour 2005).

Arbuscular mycorrhizal Fungi (AMF) are generally expected to be effective in establishing environmental friendly agriculture. AMF can increase nutrients uptake especially phosphorus (P) and several microelements by plants (Jones et al. 1991), promote growth, increase root length, leaf area, stem diameter (Sharma et al. 2009, 2011b), improve transplant performance, and increase tolerance to abiotic (water, nutrition) stresses (Gohre and Paszkowski 2006; Smith and Read 1997).The beneficial effects of AMF in horticultural crop production, as an effective scavenger of nutrients and as abio-control agent, have been well established. These microorganisms have an ecological importance and widespread associations with most terrestrial ecosystems. They are also benefit in plant establishment. Dual inoculation of *Glomus macrocarpum* and *Pseudomonas striata* significantly increased plant uptake of nutrients like P, Zn, Cu, Mn, and Fe, and plant dry biomass (Sreenivasa and Krishnaraj1992). Only 10–20% of the phosphorus fertilizers applied to soil is absorbed by plants (Holford 1997). Soil microorganisms cannot gain access to the insoluble phytate, while other organic phosphates are easily decomposed by them. Plants with symbiotic AMF apparently utilize the precipitated inorganic phosphate such as Fe–phosphate more efficiently than those with non-mycorrhizal fungi. This is probably due to the spatial utilization of the soil by the fine hyphae of AMF, and not due to the active solubilization of the precipitated phosphate (Smith and Read 2008). Sharma et al. (2012) demonstrated the symbiosis of AM fungal technology and Azotobacterization approach to enhance the growth attributes of apple seedlings under conditions of reduced inorganic nutrient fertilization.

The AM fungal hyphae are extended into the rhizosphere, thereby improving the absorption of water and nutrients such as phosphate and nitrogen (Chalot et al. 2006). When indigenous AMF colonize crops in the field, instead of the direct pathway, the AM pathway plays the

central role in absorption of nutrients. The hyphal network of AMF also can protect soil against erosion. Disturbance of the network by tillage reduces nutrient absorption via the AM pathway (Evans and Miller 1988), depending on the species of the AMF (McGonigle et al. 2003).The production of mycorrhizal plants with the adequate selected fungal symbionts may yield a profit when these young rootstocks are transplanted into the field, where the symbiosis can help the plants tolerate stressful conditions (Calvet et al. 2004). Dutt et al. (2013b) investigated 18 *Prunus* root stock cultivars inoculated with three types of AMF in order to evaluate their affinity to mycorrhizal colonization. Mycorrhizal colonization was low in plants inoculated with *Glomus mosseae* (Nicol. and Gerd.), Gerdemann and Trappe, and *Glomus etunicatum* Becker and Gerdemann. In contrast, *Glomus intraradices* Schenck and Smith proved to be the most infective endophyte, achieving the highest mycorrhizal colonization rate in most of the evaluated rootstocks. The *Prunu sinsititia* L. species was the only botanical group to show a consistently high affinity for mycorrhizal colonization with *G. intraradices*.

Soil inoculation of apple root by *Glomus fasciculatum* and *Azotobacter chroococcum* strains significantly increased all the vegetative growth parameters, microbial consortium of the soil, and leaf nutrient content of N, P, K, and Zn in the seedlings (Sharma et al. 2011a). Meyer et al (2015) stated that Colonization correlated positively with leaf P, Ca and Mg, and with stem circumference, but negatively with leaf N and yield.

AMF-colonized plants are often less likely to be infected by pathogens and show lower disease occurrence than the non-colonized plants (Torres-Barragan et al. 1996). Apple roots are usually colonized by AMF, which may have a positive influence on plant growth and the suppression of diseases. The results of Krishna et al. (2010) suggest that AMF could significantly reduce the injurious effect of *Botryos phaeriaribis* on apple growth. *Glomus versiforme* could be regarded as a biocontrol agent to protect apple trees against apple scar skin viroid infection (Yang et al. 2014). Hosseini and Gharaghani (2015) showed that AMF inoculation generally enhanced the growth parameters of apple rootstocks in calcareous soils.

The aim of the study was to assess the effect of AMF fertilizers on vegetative characteristics and nutrient uptake in three cultivars of apple.

MATERIAL AND METHODS

Experimental site and treatments

The experiment was conducted during 2014–2015 in the Experimental Research Farm of the Department of Horticultural Science, Ardakan University (Iran).Plant materials were obtained from a commercial nursery in Eqlid (Fars, province), Iran. Three-year-old of apple seedlings were grafted on 'Malling' rootstock planted in August 2014 in plastic pots (45 × 25 cm) in one row, 1.2 m apart, in a random block design in four replications. A replication (plot) consisted of three trees; there was a 1mwide isolation strip between the plots. This experiment was consisted of three cultivars of apple ('Golden Delicious', 'Red Delicious', and 'Starking Delicious') and four levels of AMF fertilizers (0, 70, 100, or 120 g/pot). These plants were kept in natural condition of Ardakan city (Yazd province, Iran) by 19° C as the mean temperature in a year and 62 mm annual rainfall.

Soil physicochemical properties

The soil samples that were used for the pot experiment were collected from a commercial apple orchard located in Eqlid region of Fars province.The soil used in the experiment was autoclaved at 121 ˚C for 15 minutes. The texture of soil was sandy-loam, with 68.8% sand, 28.4% silt, and 2.8% clay. It had 0.43% organic carbon by the pH of 7.38 and electrical conductivity of 0.86dS m^{-1}. The initially available N, P, and K content in the soil were 400, 9.5, and 258 $mgkg^{-1}$ respectively. Diethylene triamine penta acetic acid (DTPA) extractable micronutrients, namely, Fe, Cu, Zn, and Mn were 0.6, 0.12, 0.34, and 5.4 mg kg^{-1}respectively.

Inoculation of seedlings with AMF

AMF fertilizer was purchased from MABCO®. It was then inoculated in the rhizosphere zone of seedling spots. In each plastic bag, 0, 70, 100, or 120 g of fertilizer was used. Plants were irrigated with 0.5 liter of water every three, five or seven days (according to the environmental condition). No additional fertilizers were applied. After six months of inoculation, the seedlings were harvested and the relative dependency to AM species was singly assessed by measuring the plant height, stem diameter, leaf area, leaf fresh and dry weight, and total chlorophyll contents of the seedlings. Simultaneously, leaf nutrient status and nutrient uptake by the apple seedlings were also assessed.

Vegetative growth measurements

Plant height and stem diameter of the main shoot were measured using measuring tape and Vernier callipers respectively, at 5 cm above the soil surface. Leaf area was assessed with the help of the leaf-area meter model Win Area-UT-11 and it was expressed in square centimetres

(cm^2). For calculating dry weight, the leaves were rinsed with distilled water and oven-dried at 65 °C for 72 hours. Chlorophyll content of the leaf was estimated according to the process given by Halfacre et al. (1968).

Total N content of leaves was determined using Nitrogen Kjeldahl's method. P content of leaves determined by the phosphovanadomolybdate method (Jackson, 1973). Content was determined by using a flame photometer. Micronutrients viz. Fe, Cu, Zn, and Mn were determined at wavelength of 214–589 nm using atomic absorption spectrophotometer (GBC UV-Visible Spectrometer Cintra 5T model) (Wu *et al.,* 2011). Concentrations of the macronutrients were expressed as percentages of dry weight, while those of micronutrients were expressed as ppm in dry weight. Nutrient uptake measured according to the relation 1 and expressed as mgper pot.

Relation (1): $U(\frac{mg}{pot}) = \frac{C \times W}{100} \times 1000$

U: Amount of element that absorbed by the plant
C: Concentration of element in plant according to the percent
W: Dry weight of plant according to the g pot^{-1}

Data analysis

The experiment was laid out in a random block design. Each treatment was replicated four times. The data thus obtained were subjected to analysis of variance (ANOVA). The least significant differences (LSD) were used to compare the means at 5% level of significance.

RESULTS AND DISCUSSION

Vegetative growth parameters

In general, all the treatments resulted in higher plant height, stem diameter, and leaf area in AMF plots compared to the uninoculated seedlings. The application of AMF to apple seedlings led to significant improvement in growth characteristics compared to the uninoculated control, as shown in Table 1.

Data presented in Table1 indicate that the mycorrhizal colonization of apple seedlings obviously increased plant height compared to the control treatment. Plant height of 'Golden Delicious' seedlings inoculated with 70, 100, and 120 g/pot of MF increased more than that of other seedlings (Table 1).'Starking Delicious' inoculated with 70 g AMF fertilizer per pot also showed increased height (12.33 cm) (Table 1).

Significant increases in stem diameter were detected in seedlings colonized by AMF compared to non-inoculated seedlings (Table 1). Mycorrhizal stem diameter increased in comparison to control seedlings (Table 1). The highest mean of stem diameter (0.19 cm) was observed in 'Red Delicious' treated with 70 g of AMF fertilizer per pot (Table 1).

It is evident from the data given in Table 1 that the leaf number of the seedlings was significantly increased by mycorrhizal treatments compared to uninoculated control.

The maximum significant increase in leaf number of the seedlings was recorded in 70gand 100g of AMF fertilizer per pot in the three cultivars (Table 1).

Different individual treatments also resulted in a significant increase in leaf area of the seedlings. When AMF fertilizer was used, a significant increase (17.65 cm^2) occurred only at the 100g fertilizer level in 'Golden Delicious', and not at lower or higher levels (Table 1).

In general, all treatments resulted in higher plant height, stem diameter, leaf number, and leaf area in AMF plots compared to uninoculated seedlings. These results were consistent with the findings of Hosseini and Gharaghani (2015), Sharma et al. (2011b), Hodge *et al. (*2010) and Kolati *et al. (*2010). Sharma et al. (2011b) found thatAM1 (*G. fasciculatum*) to be the most potent fungal species for promoting all growth parameters of apple seedlings. Sharma et al. (2011a) showed that inoculation of mango saplings with locally isolated *G. fasciculatum* and *A. chroococcum* strain increased the seedlings' height, stem diameter, leaf area, and total root length in plots.

AMF inoculation alleviated leaf fresh and dry weight of three cultivars in comparison to the non-AMF control (Table 1). This improvement was validated by the higher fresh weight of 'Red Delicious' treated by 70 and 120 g of AMF per pot (Table 1).

There was a significant variation in leaf dry weight among the different plant cultivars tested. 'Red Delicious', 'Golden Delicious' and 'Starking Delicious' cultivars at 70, 100, and 120 g of AMF per pot respectively were found to be significantly superior to the others (Table 1).

The AMF inoculation also resulted in higher chlorophyll content of leaves in the seedlings in comparison to uninoculated seedlings (Table 1). The treatment of 'Starking Delicious' and 'Red Delicious' cultivars with 100 and 120 g of AMF fertilizer per pot respectively, significantly increased the chlorophyll a content (Table 1). Also, 'Starking Delicious' treated with 120 g of AMF fertilizer per pot significantly increased the chlorophyll b level (Table 1).The increase in the total chlorophyll content of AMF-treated seedlings was due to the direct consequence of symbiotic association, which led to greater water uptake and nutrients, resulting in higher level of biosynthesis (Dutt et al. 2013a).The differences in AMF colonization frequency could be related to the differences in mycorrhizal dependency among the host plants and to abiotic factors (Yano Melo et al. 1999). Sharma et al. (2011a) found that soil inoculation of apple root by *Gomus fasciculatum* and

Azotobacter chroococcum strains significantly increased all the vegetative growth parameters. The increase in growth parameters of apple seedlings after AMF fertilizer application may be related to elevated nutrient uptake by the seedlings, the release of plant growth regulators such as indole-3-acetic acid and gibberellins in the rhizosphere soils by these microorganisms and the consequent promotion of plant growth (Singh et al. 2010).

Leaf nutrient status

Inoculation of AMF had a non-significant effect on leaf N status of apple seedlings (data not shown), conforming to the results of Dutt et al. (2013b). However, AMF inoculation resulted in the highest increase (3.50%) in the N content of the seedlings compared to the uninoculated control (Table 2). The inoculation of AMF also resulted in higher N uptake of leaves in the treated seedlings in comparison to uninoculated seedlings (Table 2). In all threecultivars, N uptake increased by increasing the AMF levels up to 100g; after that, N uptake decreased (Table 2). The highest N uptake was observed in 'Golden Delicious' and 'Red Delicious' treated with 100 g of AMF fertilizer per pot (119 and 122 mg pot^{-1} respectively) (Table 2). Several studies have also reported that mycorrhizas could significantly increase root nodulation (Duponnois et al. 2002; Duponnois and Plenchette 2003). It has been proposed that the mycorrhizal symbiosis enhanced nodulation and N_2 fixation by enabling P uptake in plants (André et al. 2005) or by progressing root growth, which facilitated rhizobial colonization and infection. Application of AMF also decreased P content of leaves compared to uninoculated control (Table 2), but P absorption of leaves increased in AMF-treated seedlings. The highest P absorption was observed in 'Starking Delicious' treated with 100 g of AMF per pot (Table 2). Inoculation with AMF resulted in 0.45% increase in P content in comparison to uninoculated control. In general, the inoculation of AMF had non-significant effects on leaf K content (data not shown) due to the functional-host specificity and/or compatibility. This is in agreement with the results of Dutt et al. (2013b).Several studies have shown that AMF absorbed more phosphate from soil solution than unaffected and non-colonized roots. The external hyphae of AMF absorb P from the same soil pool as the roots, asit is fixed to increase of the soil volume. This higher P absorption by AMF is because of the excellent efficiency of the uptake from labile forms of soil phosphate and is not attributed to its capacity to mobilize phosphate sources unavailable to non-mycorrhizal roots (Sharma et al. 2002; Dutt et al. 2013a). It is also

documented that AMF and root hairs were fundamentally an alternative mechanism for P uptake (Dutt et al. 2013a). Under sufficient P conditions, mycorrhizal plants could uptake more P than non-mycorrhizal plants. Thus, the metabolites are needed to exudate of mycorrhizal from the root in large quantity to sustain the colonization process. As available P content increases, an increase in the mycorrhizal association may reduce plant growth rate due to carbon cost association. Even at very low levels of P, there might be a transient depression in plant growth associated with the plant for the limited P level (Kahiluoto et al. 2000).

The interaction effects of AMF and apple cultivar were also significant for leaf Fe and Zn content (Table 3). According to Table 3, Fe and Zn content of leaves decreased after applying AMF. Different AMF fertilization rates also resulted in increased leaf Zn uptake up to 508 mg^{-1}per pot. Application of 120 g of AMF fertilizer per pot in 'Red Delicious' resulted in the lowest amount of leaf Fe content (Table 3). The highest Fe and Zn leaf uptake was recorded in 'Red Delicious' treated with 70 g of AMF fertilizer per pot (Table 3). It has been clearly documented that the mycorrhizal effect on plant growth depends on the abundance of infective ectomycorrhizal propagules in soil (Garbaye et al. 1988). Similarly, the inoculation with AMF was most effective and resulted in increasing the leaf Cu uptake compared to uninoculated control. 'Starking Delicious' treated with 70 g of AMF fertilizer per pot showed the highest leaf Cu content and absorption rate (Table 3). This finding was in agreement with that of Smith and Read (2008), who found that AMF improved the nutrient content of the host plants. This also proved that Zn levels above 5.0 mg kg^{-1} soil were in the sufficiency range and as such may not be effective in increasing the mycorrhizal colonization. It has been well-documented that uptake of any element would be enhanced by MF, if it is made available slowly and if its soil diffusion rate is the limiting factor in its uptake. The absorption rate of Zn by the MF is greater than non-mycorrhizal fungi (Sharma et al. 2005).

Leaf micronutrient—namely, Zn, Cu, and Fe—content in leaves was found to have increased. This increase is attributed to maximum root colonization, which in turn increases the surface area for nutrient absorption in the rhizosphere. It is also well-understood that the application of AMF turned the insoluble form of nutrients from the rhizosphere soil to the soluble form and made them easily available, and thereby resulting in increased leaf Zn, Cu, and Fe content (Oliviera et al. 2003).

It has been well-established that Cu and Zn have very low mobility in soil because of their strong adsorption by the soil colloids. Thus, mycorrhizal infection may possibly improve trace metal nutrition by a mechanism similar to that for phosphorus if the metals are absorbed and translocated in the mycelium. Metals must be adsorbed by the mycelium because they are essential for fungal growth. Generally, it has been proved that mycorrhizal plants can absorb higher amounts of slow mobility elements such as P, Zn, and Fe (Yano-melo *et al.,* 1999). The different nutrient absorption capacities through the roots can be result of the differences among the nutrient uptake of rootstocks (Kayan, 2008). Structure of root systems, root cation exchange capacities, rhizosphere pH, and characteristics of root exudates can affect on the nutrient uptake by the plant (Kucukyumuk and Erdal, 2011).

Similar to P, high levels of Zn are toxic to the AMF, although Gildon and Tinker (1981) found Zn-tolerant fungal strains forming mycorrhizas on mine spoil. Zhang et al. (2009) reported that AMF colonization increases the Cu-binding capacity of the root cell wall and reduces uptake across the plasma membrane into the root cells. It has been clearly documented that the mycorrhizal effect on plant growth depends on the abundance of infective ectomycorrhizal propagules in soil (Garbaye et al. 1988).

CONCLUSIONS

This study demonstrates that AM fungal fertilizer can improve productivity parameters with better plant growth, leaf nutrient status, and higher leaf nutrient uptake by the apple seedlings. Among the various treatments, 70 g of mycorrhizal fertilizers per pot had the biggest effect on apple growth.

Table 1: Interaction between cultivars and different levels of AM fungi fertilizer on vegetative growth parameters

Cultivars	AM fungi fertilizer (g per pot)	Increased seedling height (cm)	Increased Stem diameter (cm)	Leaf number	Leaf area (cm^2)	Fresh Weight (g)	Dry Weight (g)	Chlorophyll a (mg/ g fresh weight)	Chlorophyll b (mg/ g fresh weight)
	0	2^{d}	0.05^{f}	48^{b}	5.37^{e}	4.54^{d}	3.35^{cde}	2.21^{abcd}	1.80^{e}
	70	12.33^{a}	0.09^{cd}	112^{a}	6.82^{cde}	4.98^{cd}	3.41^{cde}	2.40^{abcd}	2.20^{bcde}
'StarkingDelicious'	100	5.67^{bc}	0.09^{cd}	108^{a}	6.04^{de}	5.34^{cd}	3.92^{bc}	2.84^{a}	2.76^{ab}
	120	6^{b}	0.13^{b}	72^{ab}	11.30^{bc}	6.01^{bc}	4.68^{a}	2.94^{a}	2.89^{a}
	0	6^{b}	0.07^{e}	70^{ab}	7.23^{cde}	4.82^{cd}	3.65^{cd}	1.78^{d}	1.92^{de}
	70	13.33^{a}	0.09^{cd}	119^{a}	12.55^{b}	5.56^{cd}	3.92^{bc}	2.16^{bcd}	1.90^{de}
'Golden Delicious'	100	14^{a}	0.08^{cde}	85^{ab}	17.65^{a}	7.04^{ab}	5.03^{a}	2.01^{cd}	1.75^{e}
	120	12.38^{a}	0.10^{c}	99^{ab}	12.37^{b}	5.07^{bcd}	3.77^{bcd}	2.41^{abcd}	2.28^{abcde}
	0	3.67^{bc}	0.04^{f}	58^{b}	7.63^{bcde}	4.29^{d}	2.95^{e}	2.45^{abc}	2.04^{cde}
	70	$6b^{c}$	0.19^{a}	118^{a}	10.73^{bcd}	7.72^{a}	5.04^{a}	2.78^{ab}	2.63^{ab}
'Red Delicious'	100	$7b^{c}$	0.14^{b}	121^{a}	8.93^{bcde}	4.30^{d}	4.38^{ab}	2.81^{ab}	2.73^{ab}
	120	5.33^{cd}	0.07^{de}	106^{ab}	8.60^{bcde}	7.56^{a}	3.21^{de}	2.85^{a}	2.51^{abcde}

Means in each column with at least one similar letters are not significantly different at the 5% probability level using Duncan multiple range test

Table 2 Interaction between cultivars and different levels of AM fungi fertilizer on nitrogen and phosphorus absorption of three apple cultivars

Cultivars	AM fungi fertilizer (g per pot)	Concentration of P (%)	P uptake (mg pot^{-1})	Concentration of N (%)	N uptake (mg pot^{-1})
	0	0.45^{a}	11.42^{de}	3.50^{a}	58^{fg}
	70	0.28^{d}	14.67^{b}	3.20^{a}	75^{cde}
'StarkingDelicious'	100	0.35^{c}	10.48^{cd}	3.10^{a}	104^{bc}
	120	0.37^{bc}	17.59^{a}	3.15^{a}	98^{bc}
	0	0.39^{bc}	10.41^{de}	2.87^{a}	69^{fg}
	70	0.26^{d}	12.35^{cd}	3.05^{a}	88^{bc}
'Golden Delicious'	100	0.27^{d}	14.70^{b}	3.32^{a}	119^{a}
	120	0.41^{ab}	12.14^{cd}	3.30^{a}	99^{bc}
	0	0.42^{ab}	9.26^{e}	3.16^{a}	58^{efg}
	70	0.28^{d}	12.70^{cd}	3.50^{a}	122^{a}
'Red Delicious'	100	0.35^{c}	14.90^{b}	3.41^{a}	111^{b}
	120	0.28^{d}	10.25^{de}	3.20^{a}	91^{cde}

Means in each column with at least one similar letters are not significantly different at the 5% probability level using Duncan multiple range test

Table 3: Interaction between cultivars and different levels of AM fungi fertilizer on Fe, Zn & Cu content and absorption of three apple cultivars

Cultivars	AM fungi fertilizer (g pot^{-1})	Cu uptake (mg pot^{-1})	Concentration of Cu (ppm)	Zn uptake (mg pot^{-1})	Concentration of Zn (ppm)	Fe uptake (mg pot^{-1})	Concentration of Fe (ppm)
'StarkingDelicious'	0	62^{bc}	20^{ab}	243^{c}	112^{a}	639^{abc}	126^{def}
	70	90^{a}	22^{a}	441^{a}	77^{bc}	977^{a}	300^{ab}
	100	44^{bc}	14^{bcd}	262^{c}	73^{bc}	688^{abc}	160de
'Golden Delicious'	120	65^{b}	14^{bcd}	243^{c}	51^{c}	761^{abc}	201^{cd}
	0	36^{c}	10^{cd}	276^{bc}	76^{bc}	341^{d}	262^{bc}
	70	53^{bc}	14^{bcd}	286^{bc}	73^{bc}	924^{ab}	172de
	100	45^{bc}	10^{cd}	271^{bc}	54^{bc}	696^{abc}	172de
'Red Delicious'	120	48^{bc}	14^{bcd}	294^{bc}	82^{b}	468^{cd}	68^{f}
	0	41^{bc}	14^{bcd}	191^{c}	116^{a}	347^{ab}	341^{a}
	70	60^{bc}	12^{cd}	508^{a}	78^{bc}	1000^{a}	157de
	100	44^{bc}	10^{cd}	395^{ab}	75^{bc}	788^{ab}	169de
	120	48^{bc}	14^{bcd}	221^{c}	60^{bc}	738^{abc}	107^{ef}

Means in each column with at least one similar letters are not significantly different at the 5% probability level using Duncan multiple range test

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