Size-controlling interstocks affect growth vigour by downregulating photosynthesis in eight-year-old ‘Red Fuji’ apple trees

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Summary

To understand how interstocks reduce vigour and positively influence yield efficiency, we investigated ‘Red Fuji’ apple (*Malus domestica*) scions that were grafted onto five different interstocks. Morphological and physiological parameters, such as growth vigour, gas exchange, leaf area index, soil-plant analysis development value, 100 leaf weight, dry biomass, yield efficiency, and fruit quality, were examined during their eighth growing season in 2018. Compared to semi-dwarf apple trees, dwarf apple trees had lower growth vigour, photosynthetic capacity, root-shoot ratio, and yield per tree, but had a greater short/medium shoot ratio, higher water use efficiency, yield efficiency, and better fruit quality, especially scions grafted onto the interstock Mark. The downregulated photosynthetic capacity induced by dwarfing interstocks could be primarily ascribed to a reduced SPAD value (chlorophyll content or leaf nitrogen). Throughout the experiment, a strong positive linear correlation was observed between the photosynthetic rate and total dry biomass. The distribution of dry biomass in the aboveground and belowground organs further indicates that the differing growth vigour and biomass among the five interstocks were induced by the variation of the accumulation of photoassimilates and translocation. This may have led to greater carbohydrate availability in scions.

Keywords

interstock, gas exchange, root-shoot ratio, yield, fruit quality

Significance of this study

What is already known on this subject?

- The apple dwarfing phenotype is observed with the application of interstock, yet there is limited influence on the root system, which potentially determines their abiotic stress resistance.

What are the new findings?

- We find that the application of interstocks with greater dwarfing effect causes a decrease in photosynthesis and root-shoot ratio but yield better fruit quality in eight-year-old ‘Red Fuji’. The greater ratio of short and medium shoots compared to long shoots is obtained mainly by the reduced long shoot number.

What is the expected impact on horticulture?

- The results provide references for the underlying apple tree vegetative and reproductive responses to interstocks of differential dwarfing ability by examining morphological and photosynthetic parameters; the findings will also facilitate the utilization of suitable interstocks for future dense apple cultivation systems.

Introduction

Apple (*Malus domestica*), a deciduous fruit tree with an old and deep-rooted history of cultivation worldwide, has a production of over 41.39 million tons in China, which accounts for the half of the worldwide production of apples (FAO, 2017). In the past thirty years, size-controlling rootstocks have been widely used to control vegetative growth and promote earlier fruit-bearing, particularly since the development of the modern high-density planting regime (Costes et al., 2001). Size-controlling rootstocks are economically important for high-density apple tree planting, and trees with size-controlling rootstocks may produce higher single fruit weight, increased production per hectare

(Tworkoski and Miller, 2007), minimised pruning requirements, be more adaptable to partial mechanisation, and provide easier access for management (Robinson et al., 2013).

Size-controlling rootstocks can be utilized in two ways, namely, as dwarfing self-rooted rootstocks and dwarfing interstocks (a third genotype grafted between a rootstock and a scion). Dwarfing self-rooted rootstocks are used to induce apple scion dwarfing while they are poorly anchored due to their shallow roots and are more sensitive to water stress and nutrient deficiency (Di Vaio et al., 2009). Dwarfing interstock is another method used to not only induce apple scion dwarfing, but also to increase tolerance to cold, provide better anchor for windy conditions, and improve resistance to disease (Di Vaio et al., 2009). At present, this method is widely used in New Zealand (Seleznova et al., 2008), Turkey (Karlidağ et al., 2014), and China (Zhou et al., 2015).

Researchers have found that dwarfing interstock grafted onto vigorous rootstock engenders a reduction of the natural tree development, accelerating fruit cropping, minimizing the demand of pruning, and facilitating economic and intensive management of the orchard (Di Vaio et al., 2009). Di Vaio et al.

(2009) reported that utilization of M.9 and M.27 interstocks respectively determined a 50% and 80% reduction of canopy growth while increasing the average single fruit weight and fruit production shown in apple trees when compared with the control apple trees. Other fruit trees have shown similar results. For example, Watson et al. (2012) found that the largest dwarfing rootstocks produced the highest number of floral buds in pear. In both peach (Rufato et al., 2006) and apple (Karlidağ et al., 2014) studies showed that the impact of plant growth induced by several interstocks was associated with the height of the graft on the stem. Although the cause of the reduction of scion vigour induced by interstocks has been previously reported (Seleznova et al., 2008; Karlidağ et al., 2014), little is known about the dry biomass and fruit quality in fruiting apple trees induced by interstocks.

Biomass accumulation and fruit production are potentially impacted by photosynthesis, thus it is also essential to understand how these interstocks can directly affect the photosynthetic apparatus and its function (Liu et al., 2012; Greer, 2015). In a previous study, Fallahi et al. (2001) found that 'BC-2 Fuji' apple scion grafted onto the semi-dwarfing M.7 EMLA rootstock had lower net photosynthesis and transpiration rates than the same apple scion grafted onto the dwarfing M.9 rootstock, indicating that a greater dwarfing effect induced a higher photosynthetic rate. However, container-grown 'Golden Delicious' apple trees grafted onto various rootstocks showed that photosynthesis and dark respiration were not influenced by rootstocks (Ferree and Barden, 1971). On the other hand, Baugher et al. (1994) highlighted the adverse effects of size-controlling rootstocks on photosynthetic CO₂ assimilation in apple scion leaves, which coincides with the results from other studies (Gonçalves et al., 2005; Sotiropoulos, 2008). The high or low values of the net CO₂ assimilation rate may reflect differences in palisade thickness, chlorophyll content, leaf nitrogen content, specific leaf weight, and hydraulic conductance (Vemmos et al., 2013; Dayer et al., 2017; Greer, 2018) among the size-controlling rootstocks.

Few studies have been conducted on the photosynthetic properties of the leaves of apple trees that utilize an interstock during the fruit growth period. It is also not clear whether the dwarfing effect induced by interstocks could result in the same photosynthetic assimilation as the dwarfing rootstocks. Moreover, the influence of interstocks on the root-to-shoot ratio is unknown. Given these unknowns related to interstocks, the aims of the present experiment were to: (1) evaluate the performance of scion growth vigour, leaf gas exchange, yield, and fruit quality using five different types of interstocks; and (2) determine fruiting characteristics related to photosynthesis and the relationship between net CO₂ assimilation and dry biomass accumulation. We hypothesize that the utilization of dwarfing interstock would reduce growth vigour and photosynthetic assimilation, but improve yield efficiency and fruit quality. Our findings might help predict the vigour and fruit yield of apple trees using various interstocks.

Materials and methods

Plant materials and growth conditions

This study was conducted on eight-year-old 'Red Fuji' apple (*Malus domestica*) trees in the orchard of the China Agricultural University, Beijing, China, located at latitude 40°08'49", longitude 116°10'06", and altitude of 44 m a.s.l. This study began in 2010, when 25 cm long segments of

Mark, GM.310, SH.6, SH.38, and 77-34 interstocks were grafted onto Baleng rootstocks (additional rootstock information in Table S1) at a height of 10 cm and 'Red Fuji' scions were grafted onto the top of the interstocks.

Apple trees were planted in 4-m rows with a space of 2 m between trees. Rootstock-interstock-scion combinations were randomly distributed in four blocks in the orchard for a randomized complete block design experiment and designed with four replicates. The orchard soil was characterized as deep clay loam with the following characteristics: pH 7.65, 100.85 mg kg⁻¹ available nitrogen, 23.29 mg kg⁻¹ available phosphorus, 208.77 mg kg⁻¹ available potassium, and 10.30 g kg⁻¹ organic matter. Standard apple tree management practices, including control for insect pests and diseases, were followed.

Tree growth parameters

The following plant growth parameters were collected at designated times throughout the growing season. Plant height, trunk girth, canopy width, main branch number, main branch diameter, shoot number, length of different types of annual shoots (i.e., short shoot: less than 5 cm long; medium shoot: greater than 5 cm and less than 20 cm long; long shoot: more than 20 cm long), and shoot diameter were measured at the end of October 2018. Trunk girth was recorded with a flexible ruler 15 cm above the upper graft union and then converted into trunk cross-section area (TCSA), calculated as $TCSA = c^2/4\pi$, where c is trunk girth. The diameter of the main branch and shoots was measured using a digital caliper and was converted into branch cross-section area (BCSA) and shoot cross-section area (SCSA) to calculate the accumulation of branch cross-section area ($\Sigma BCSA$) and shoot cross-section area ($\Sigma SCSA$).

Bud break and flowering typically occur from April 12th to April 23rd in the Beijing area. Flower clusters were recorded daily, starting when the first tree flowered (15th April, 2018) until the end of flowering (25th April, 2018), for all rootstock-interstock-scion combinations. The number of fruit was counted on 28th July 2018, and fruit set per 100 flower clusters were calculated.

LAI, SPAD, 100 leaf weight, and gas exchange

Soil-plant analysis development (SPAD) values were measured with a portable chlorophyll meter (SPAD 502; Minolta Co. Ltd., Osaka, Japan), and the leaf area index (LAI) was monitored with a LAI-2200 plant canopy analyser (LI-COR Biosciences, Inc., Logan, NE, USA). A total of 100 leaves were randomly collected from each apple tree and weighed fresh using an electronic balance.

Leaf gas exchange, including net CO₂ assimilation rate (A_n), stomatal conductance (g_s), intracellular CO₂ concentration (C_i), and transpiration (E), were monitored with a portable gas exchange system (LI-6400XT interfaced with a 6400-02 B 6 cm² leaf chamber with an LED light source, LI-COR Biosciences, Lincoln, NE, USA). The water use efficiency (WUE) was calculated based on an equation defined as A_n/E (Tworkoski et al., 2016). Measurements were conducted on fully expanded leaves from the middle region of the canopy between 9:00 and 11:00 h on sunny days. The conditions of the leaf chamber throughout the photosynthesis measurements were controlled as follows: a flow rate of 500 $\mu\text{mol s}^{-1}$, a PPFD of 1,000 $\mu\text{mol (photon) m}^{-2} \text{s}^{-1}$, a block temperature of $24 \pm 1^\circ\text{C}$, and a leaf vapour pressure deficit (VPDL) of $1.5 \pm 0.1 \text{ kPa}$. The mean atmospheric CO₂ concentration was 400 $\mu\text{mol (CO}_2\text{) mol (air)}^{-1}$ (C_a), the mean air temperature was 26°C, and the relative humidity was 65%.

Yield and fruit quality

All fruit per apple tree was hand-picked at commercial maturity (on approximately 20th October 2018) and assessed for yield (total fruit weight per tree), yield efficiency, and fruit quality. The number of fruit per tree was counted and all the fruit harvested from one tree were weighed using an electronic balance to obtain yield. Yield efficiency was calculated as fruit yield (kg tree⁻¹) per TCSA (cm²) (Kviklys et al., 2016). To determine the fruit quality, 12 apples sampled from each type of combination treatment were used to determine the length/diameter ratio, firmness, soluble solids content (SSC), titratable acidity (TA), and sugar/acid ratio (SSC/TA). Fruit length and diameter were recorded using a digital caliper. The length/diameter ratio was estimated by dividing the fruit length by fruit diameter. Firmness was assessed using a TA.XT Plus Texture Analyser (Stable Micro Systems, Texture Technologies Corporation, Godalming, Surrey, UK) equipped with a load cell of 5 kg, a pre-test speed 1.00 mm s⁻¹, a test velocity of 1.00 mm s⁻¹, and a post-test speed of 1.00 mm s⁻¹. The puncture test was performed with a P/2 probe (cylinder with a 2-mm diameter and a flat end). For the whole apple with the skin, the texture was measured on two diametrically opposite sides (coloured and non-coloured) and applied as the maximum force required to achieve an estimated distance of 8 mm. SSC was assessed with a portable digital refractometer (PAL-1; Atago Co., Ltd., Tokyo, Japan) and TA was determined with a portable digital fruit acidity meter (GMK-835F, G-WON, Korea). SSC/TA was estimated by dividing the SSC by TA.

Aboveground and belowground biomass

Destructive aboveground and belowground biomass harvest was conducted at the end of the experiment on 20th November 2018. Apple trees were removed from the soil intact and roots were thoroughly cleaned. The aboveground and belowground parts of each tree were separated and then the fresh weight was recorded. Biomass was determined by drying the fresh material in a forced air oven at 98°C until a constant weight was reached (Berdeni et al., 2018). The measurements were carried out in quadruplicate.

Data analysis

All statistical analyses were conducted using SPSS 20.0 statistical software (IBM software, New York, USA). A one-

way analysis of variance (ANOVA) was used to test the interstock effect on tree growth, photosynthetic parameters, dry biomass, fruit yield, and quality parameters. Variation of means among the interstocks was considered significant when $P \leq 0.05$, as determined by Duncan's multiple range test. The coefficient of determination (R^2) between the leaf-fruit ratio and the number of flowers was calculated using linear regression analysis. The relationship between A_n and total dry biomass was examined using Pearson's correlation coefficient. OriginPro 9.0 was used to draw 3D scatter plots (OriginLab Corporation, Northampton, MA, USA). The ECharts (<https://echarts.baidu.com/examples/editor.html?c=bar3d-punch-card&gl=1>) package was used to achieve a display of 3D bars of the number of fruit produced by different tree heights.

Results

Growing season climate

During the experimental period from April to October 2018, the mean daily air temperature ranged between 0°C and 31.5°C (Figure 1), with an average temperature of 15.8°C at the start of the growing season (April and May), an average temperature of 27.5°C during the summer (June, July, and August), and an average temperature of 14°C at the end of the growing season (September and October). The mean monthly precipitation ranged between 22 mm and 178 mm throughout the whole growing season (Figure 1). The maximum average precipitation was 178 mm in July and August, while the minimum average precipitation was 22 mm in April and October.

Tree growth

'Red Fuji' grafted onto Mark as the interstock was the shortest tree and had the lowest trunk girth, TCSA, and Σ BCSA (Table 1), followed by GM.310, SH.6, SH.38, and 77-34 interstock. A 34.9% and 50.0% reduction in plant height and TCSA was observed in 'Red Fuji' grafted onto Mark (227.8 cm and 65.9 cm², respectively) in comparison with the interstock 77-34 (349.8 cm and 131.9 cm², respectively). There was a significantly higher value of the number of main branches per meter observed in 'Red Fuji' grafted onto Mark, GM.310, and SH.6 as the interstocks than that of the 77-34 interstock. The canopy width and Σ SCSA were significantly

FIGURE 1. Seasonal changes in daily temperature and mean monthly precipitation recorded at the research orchard of the China Agricultural University, Beijing, throughout the experiment (from April to October 2018).

TABLE 1. Plant height, trunk girth, canopy width, number of main branches per metre, trunk cross-section area (TCSA), the accumulation of branch cross-section area (Σ BCSA), and shoot cross-section area (Σ SCSA) of different interstocks with the 'Red Fuji' cultivar measured in the eighth year (2018) after grafting.

Interstock	Plant height (cm)	Trunk girth (cm)	Canopy width (cm)	Main branch per metre (No. m ⁻¹)	TCSA (cm ²)	Σ BCSA (cm ²)	Σ SCSA (cm ²)
Mark	227.8 d	28.6 c	203.5 d	3.29 a	65.9 c	35.9 d	48.8 c
GM.310	284.5 c	34.4 b	262.3 ab	3.34 a	94.6 bc	101.5 c	91.9 a
SH.6	313.0 b	37.0 ab	250.5 b	3.19 ab	110.0 ab	114.5 b	80.9 b
SH.38	328.0 b	38.5 ab	231.9 c	2.97 bc	118.3 ab	125.1 a	90.6 a
77-34	349.8 a	40.6 a	273.1 a	2.93 c	131.9 a	134.1 a	96.9 a

All measurements were performed in four replicates. Different lowercase letters after the mean values $\pm$ SE within the same column indicate a significant difference at $P < 0.05$ (by Duncan's multiple range test).

TABLE 2. Net CO₂ assimilation rate (A_n), stomatal conductance (g_s), intracellular CO₂ concentration (C_i), transpiration (E), water use efficiency (WUE), leaf vapour pressure deficit (VPDL), and leaf temperature (T_{leaf}) of different interstocks with the 'Red Fuji' cultivar measured in the eighth year (2018) after grafting.

Interstock	A_n ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	g_s ($\text{mol m}^{-2} \text{s}^{-1}$)	C_i ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	E ($\text{mmol m}^{-2} \text{s}^{-1}$)	WUE ($\mu\text{mol mmol}^{-1}$)	VPDL (kPa)	T_{leaf} ($^{\circ}\text{C}$)
Mark	14.29 b	0.159 c	222.1	2.46 c	5.82 a	1.60	25.3
GM.310	14.89 ab	0.174 bc	227.9	2.62 bc	5.69 a	1.57	25.1
SH.6	15.27 ab	0.187 ab	233.0	2.78 ab	5.52 a	1.56	25.2
SH.38	15.78 a	0.193 ab	230.7	2.87 ab	5.58 a	1.55	25.2
77-34	15.90 a	0.200 a	234.2	3.06 a	5.25 b	1.61	25.6

All measurements were performed in 12 replicates. Different lowercase letters after the mean values $\pm$ SE within the same column indicate a significant difference at $P < 0.05$ (by Duncan's multiple range test).

($P < 0.05$) greater in 77-34 and GM.310 interstocks, with an average difference of 31.5% and 93.4%, respectively, than that of the Mark interstock.

LAI, SPAD, 100 leaf weight, and gas exchange

According to the 3D scatter plots (Figure 2) of the SPAD value, the weight of 100 leaves and the LAI of 'Red Fuji' with different interstocks were measured in 2018. The high or low values of each of these parameters were consistent with the degree of dwarfing of each interstock.

FIGURE 2. The 3D scatter plot of soil-plant analysis development (SPAD), 100 leaf weight, and leaf area index (LAI) on different interstocks with the 'Red Fuji' cultivar. Different colours represent different interstocks, and the size of the spheres represents the photosynthetic rate (range from 14.29 to 15.90 $\mu\text{mol m}^{-2} \text{s}^{-1}$) of corresponding interstocks.

The average A_n of the Mark interstock (14.29 $\mu\text{mol m}^{-2} \text{s}^{-1}$) was 4.0%, 6.4%, 9.4%, and 10.1%, lower than that of the GM.310 (14.89 $\mu\text{mol m}^{-2} \text{s}^{-1}$), SH.6 (15.27 $\mu\text{mol m}^{-2} \text{s}^{-1}$), SH.38 (15.78 $\mu\text{mol m}^{-2} \text{s}^{-1}$), and 77-34 (15.90 $\mu\text{mol m}^{-2} \text{s}^{-1}$) interstocks, respectively (Table 2). Similarly, the g_s of the Mark interstock was the lowest followed by the GM.310, SH.6, SH.38, and 77-34 interstocks. The highest value of g_s was observed in leaves of 'Red Fuji' grafted onto the 77-34 interstock (0.200 $\text{mol m}^{-2} \text{s}^{-1}$), which was 25.8% higher than that of the Mark interstock (0.159 $\text{mol m}^{-2} \text{s}^{-1}$). Compared to the 77-34 interstock, E was significantly lower in leaves of 'Red Fuji' grafted onto the Mark and GM.310 interstocks. Furthermore, although the Mark interstock had the lowest C_i , it was not significantly lower than that of the other interstocks. The opposite was observed for WUE; the Mark interstock had a significantly larger WUE than that of the 77-34 interstocks.

Dry biomass

The Mark interstock had a significantly higher number of short shoots ($P < 0.05$) than the 77-34 interstock, but the latter had almost two times more long shoots than the former (Figure 3A). In each rootstock-interstock-scion combination, the number of long shoots was much higher than that of short or medium shoots, especially for the SH.38 and 77-34 interstocks. The GM.310 interstock had the greatest number and dry weight of medium shoots compared with the other interstocks (Figure 3B). The SH.38 and 77-34 interstocks had a significantly greater dry weight of long shoots than the dry weight of short and medium shoots, which corresponded to the number of long shoots in these two interstocks.

For the belowground and aboveground dry weight, the 77-34 and SH.38 interstocks yielded the highest value followed by the SH.6, GM.310, and Mark interstocks (Figure 3C); this was consistent with the vigour of each interstock. 'Red Fuji' grafted onto the Mark interstock had significantly

($P < 0.05$) lower aboveground and belowground dry biomass than the other interstocks. Additionally, compared to the Mark interstock, the GM.310, SH.6, SH.38, and 77-34 interstocks had a significantly ($P < 0.05$) higher root-shoot ratio (Figure 3C). This resulted from a greater decrease in belowground dry weight than aboveground dry weight in the root-shoot ratio equation, defined as underground dry weight/aboveground dry weight.

FIGURE 3. Shoot number (A), shoot dry weight (B), and total dry biomass (C) were measured on different interstocks with the 'Red Fuji' cultivar at the end of the experiment (20th November 2018). A and B, short shoot: less than 5 cm long; medium shoot: greater than 5 cm and less than 20 cm long; long shoot: more than 20 cm long. (C) Root-shoot ratio is inset with different interstocks with the 'Red Fuji' cultivar. All measurements were performed in four replicates. Columns correspond to the means values, and vertical bars represent the SE. Different letters above the individual bar and box indicate a significant difference at $P < 0.05$ (Duncan's multiple range test) for the five different interstocks.

Yield, yield efficiency, and fruit quality

The relationship between the number of flowers as a function of the leaf-fruit ratio showed a nearly linear decrease with the increase of the leaf-fruit ratio (Figure 4A) among the different interstocks, except for the Mark interstock. However, 'Red Fuji' grafted onto the Mark interstock had the lowest leaf-fruit ratio and lowest number of flowers. There was a highly significantly positive effect ($P < 0.001$) of short and medium shoots per annual shoots on the fruit set/100 flower

FIGURE 4. (A) A negative correlation between leaf-fruit ratio and flower number ($n = 4$) of four different interstocks (except Mark) with the 'Red Fuji' cultivar. The function of y , R^2 , and P -value were calculated using the total data. SE bars are shown for both leaf-fruit ratio and flower number. (B) A positive correlation between short and medium shoots per annual shoots and fruit set/100 flower clusters ($n = 4$) of five different interstocks with the 'Red Fuji' cultivar. The function of y , R^2 , and P -value were calculated using the total data. SE bars are shown for both short and medium shoots per annual shoots and fruit set/100 flower clusters. Short and medium shoot: less than 20 cm long. (C) A positive correlation between net CO₂ assimilation (A_n) and total dry biomass ($n = 4$) of five different interstocks with the 'Red Fuji' cultivar. The function of y , R^2 , and P -value were calculated from the average data of each rootstock-interstock-scion combination. SE bars are shown for A_n and dry biomass.

TABLE 3. Yield, yield efficiency, length/diameter ratio, firmness, soluble solids content (SSC), titratable acidity (TA), and sugar/acid ratio (SSC/TA) of different interstocks with the 'Red Fuji' cultivar measured in the eighth year (2018) after grafting.

Interstock	Yield (kg tree ⁻¹)	Yield efficiency (kg cm ⁻²)	Length/diameter ratio	Firmness (kg cm ⁻²)	SSC (%)	TA (% malic acid)	SSC/TA
Mark	20.0 b	0.32 a	0.89 a	8.84 b	18.47 a	0.44 a	43.08
GM.310	28.3 a	0.31 a	0.79 c	8.90 b	17.72 abc	0.39 ab	45.45
SH.6	27.9 a	0.26 ab	0.79 c	8.35 b	18.08 ab	0.41 ab	45.02
SH.38	27.5 a	0.24 b	0.84 b	9.64 a	16.99 c	0.37 b	46.62
77-34	27.8 a	0.21 b	0.81 bc	8.46 b	17.32 bc	0.36 b	48.48

Yield and yield efficiency measurements were performed in four replicates, and other measurements were performed in 12 replicates. Different lowercase letters after the mean values $\pm$ SE within the same column indicate a significant difference at $P < 0.05$ (by Duncan's multiple range test).

clusters in all apple plants (Figure 4B), which indicated that the short and medium shoots per annual shoots effect on the fruit set could be related to the number of short and medium shoots. Furthermore, a strongly positive linear correlation was observed for A_n vs. total dry biomass, with an R^2 of 0.925 and $P < 0.01$ (Figure 4C).

'Red Fuji' grafted onto the Mark interstock (20.0 kg tree⁻¹) had the lowest average yield (Table 3). Specifically, the Mark interstock had a 27.3%, 28.1%, 28.3%, and 29.3% decrease of yield compared with the SH.38, 77-34, SH.6, and GM.310 interstocks, respectively. However, yield efficiency was significantly higher in the Mark and GM.310 interstocks than that of SH.38 and 77-34 interstocks. Although Mark had lower vigour and lower yield per individual tree, its dwarfing traits and higher fruit quality more than made up for those negatives. For instance, Mark had the highest length/diameter ratio of fruit, SSC, and TA compared to SH.38 and 77-34 interstocks. However, there was no significant difference in SSC/TA in all of the rootstock-interstock-scion combinations. Fruit firmness was significantly higher ($P < 0.05$) in the SH.38 interstock, with an 11.6% increase compared with the aver-

age firmness of the other interstocks.

Additionally, the number of fruit per tree and the height of fruit on the tree were measured in each rootstock-interstock-scion combination. The majority of fruit was located between 100 cm and 180 cm (see Figure 5) above the ground in the Mark interstock. Nearly 90% of the apples from the SH.6 and GM.310 interstocks were located between 100 cm and 240 cm. Over 70% of apples from the SH.38 and 77-34 interstocks were located at a tree height between 140 cm and 220 cm.

Discussion

Dwarfing interstocks reduced vigour and positively influenced fruit quality

Apple dwarfing interstocks or rootstocks have been chosen for high-density cultivated orchards and can induce scion vigour, yield efficiency, and fruit quality (Di Vaio et al., 2009; Zhao et al., 2016). In the present study, the utilized interstocks showed a dwarfing effect, shifting the architecture of the canopy to more short/medium length branches and few-

FIGURE 5. The 3D bars showing the fruit number of different tree heights on different interstocks with the 'Red Fuji' cultivar. Different colours represent different fruit numbers (range from 0 to 30). For better presentation, GM.310 is shown in the last position.

er long shoots. The Mark and GM.310 interstocks had fewer long shoots but had higher short or medium length branches than other interstocks, indicating more flowers and earlier productivity. The short and medium length shoots are more floriferous than long shoots, which is likely because short and medium length shoots had pre-formed floral primordia instead of growing longer. In contrast, long shoots are vegetative growth in the current season, so are not floriferous. This was consistent with the positive correlation between short and medium shoots per annual shoots and fruit set/100 flower clusters. Similarly, Seleznyova et al. (2008) suggested that dwarfing rootstocks and interstocks could decrease the number of extension shoots but promote the formation of floral shoots. It is possible that dwarfing rootstocks or interstocks induced earlier fruit because dwarfed apple plants promoted both flowering and early shoot termination due to the role of the flowering genes *MdFT* or *MdBFT*, which also accelerated the transition from juvenility to maturity of apple scions (Foster et al., 2013). Alternatively, in our previous study (unpublished), we found that dwarfing apple trees reserved more starch in both shoots and roots, accelerating the productivity of apple trees.

In this study, the interstock Mark reduced the canopy volume and increased the aspects of the fruit quality, such as length/diameter ratio, SSC, and TA, compared with SH.38 and 77-34 interstocks. Interestingly, the single fruit weight tends to be larger on dwarf apple trees than that on vigorous apple trees (Atkinson and Else, 2001). Vercammen et al. (2007) found that fruit size (the 70–85 mm size class) and colouring of 'Jonagold' were improved by 'Granny Smith' or 'Delcorf' interstocks compared with P22 or 'knipbomen' interstocks. However, some researchers reported that mean fruit weight, independent of rootstocks, was inversely related to crop load (Samuolienė et al., 2016; Iwanami et al., 2018). Skrzyński (2007) reported that the firmness of apple flesh from 'Jonagold' scions grafted onto M.26 and P60 was higher than 'Jonagold' grafted onto M.9 and P22, which was in contrast to another study that showed that dwarf trees had higher flesh firmness (Autio, 1994). The rootstock or interstock can affect apple fruit quality indirectly via altering the mineral concentration of leaves and fruit (Fallahi et al., 2013; Reig et al., 2018). This is logical as the rootstocks or interstocks uptake nutrients from the soil and distribute them to leaves and/or fruit (Fazio et al., 2015). Good fruit quality induced by these rootstocks or interstocks may be explained by the limitation or declined translocation of photoassimilates from the scion leaves to roots, which could significantly contribute to the greater availability of photoassimilates partitioning in the scion; this leads to enhanced carbon transport to the fruit (Martínez-Cuenca et al., 2016).

Dwarfing interstocks reduced leaf photosynthesis and improved WUE

The photosynthetic characteristics measured in leaves of 'Red Fuji' suggest that the regulation of the scion leaf photosynthetic rate is primarily dependent on the interstock and the dwarfing effect induced by the interstocks. Dwarf plants had a significantly lower photosynthetic rate than semi-dwarfing apple trees, which is in agreement with other reports in apple (Sotiropoulos, 2008; Zhao et al., 2016), pear (Corelli-Grappadelli et al., 2001), and sweet cheery (Gonçalves et al., 2005). However, there is a lack of consensus regarding the correlation between dwarfing rootstocks or interstocks and reduced photosynthesis of apple leaves. For example, Fallahi et al. (2001) indicated that the dwarfing

rootstock and interstock improve the photosynthetic rate of leaves of the scion in apple. Furthermore, An et al. (2017) reported that the photosynthetic rate of 'Red Fuji' apple trees was not influenced by grafting onto various rootstocks or interstocks.

In addition to the parameters that have been proposed to cause the reduced photosynthetic rate observed in dwarfing plants, such as hydraulic resistance (Martínez-Alcántara et al., 2013), leaf thickness, and palisade parenchyma (Vemmos et al., 2013), the results from this study suggest that reduced chlorophyll content or leaf nitrogen reflected by measured SPAD value might partially explain the decline of photosynthesis. These observations underline the potential importance of SPAD in photosynthetic assimilation because a lower SPAD value is generally related to the degradation or reduced synthesis of chlorophyll (Croft et al., 2014). It is inferred that the lower chlorophyll content might cause a greater decrease of A_n in dwarf plants than in vigorous apple plants.

When compared to 77-34 interstock, higher WUE (Table 2) was seen in the dwarfing interstock Mark, resulting from a greater decline in E than A_n in the WUE equation, defined as A_n/E . Similarly, via a whole canopy gas exchange investigation, Glenn (2010) demonstrated that A_n and E tended to reduce in dwarf 'Empire' apple plants, which resulted in an increase in WUE. This evidence indicated that a lower E apparently benefitted the WUE in dwarfing interstocks since it partially buffered against the reduction in the supply of photosynthate.

Photosynthetic assimilation was positively correlated to root-shoot ratio and yield

It is inferred that the reduced SPAD or leaf nitrogen induced by dwarfing interstocks has an essential effect on the lower photosynthetic assimilation, but how significant was this result for biomass and yield? The 77-34 and SH.38 interstocks had the greatest belowground and aboveground dry biomass (Figure 3C), which was consistent with their vigour. Coupled with a strongly positive linear correlation observed between A_n and total dry biomass, we concluded that the main reason for the differing growth vigour and root-shoot ratio among the interstocks were variations in photosynthetic assimilation, partitioning of assimilates, and dry matter accumulation. This is in accordance with a study conducted by Foster et al. (2017), which showed that the vigour and development of scions are induced by imbalanced carbon allocation. Whether other factors, such as reduced hormone production, mineral uptake, or water uptake, induce declined root system effects is poorly understood. Nevertheless, the root-shoot ratio does act as a contributor for the determination of interstocks and/or rootstock effects on fruit crop and scion vigour (Webster, 1995). Additionally, biomass accumulation in plants is tightly linked to fruit yield and utilisation of metabolites, which occurs throughout the diurnal cycle, especially of non-structural carbohydrates (De Souza et al., 2018).

Rootstocks are well-known to alter scion development by modifying stem weight and yield (Cookson et al., 2012). Dwarfing interstocks that significantly reduce vegetative growth and canopy size usually produce lower yield per apple tree than those from vigorous plants grafted onto interstocks or rootstocks. However, the effect on scion yield efficiency is more critical because the high-density planting of dwarf apple trees in orchards could more than compensate for the reduction in yield per tree (Webster, 1995; Losciale et al., 2008). The dwarf apple plants had the lowest yield per

tree but had the highest yield efficiency in this study, thus given the above consideration, the Mark interstock should be considered. Martínez-Alcántara et al. (2013) pointed out that the high-yield efficiency characteristic of dwarf plants could be attributed to a decline in the translocation of carbon to the interstock and rootstock, resulting in greater carbohydrate availability above the graft union. Therefore, the reduced photosynthetic assimilation found in this experiment, induced by grafting onto dwarfing interstocks, may provide insight into the mechanism underlying the dwarfing effect related to biomass and fruit yield.

Conclusions

In summary, the dwarfing effect induced by interstocks was associated with reduced growth vigour, leaf photosynthetic rate, and yield per tree, but improved yield efficiency and fruit quality of 'Red Fuji' apple trees. The interstock Mark had a greater dwarfing effect than the other interstocks. The application of interstocks with a greater dwarfing effect decreased the translocation of photoassimilates belowground and led to smaller roots, which might limit the uptake of mineral nutrition, and further resulted in early fruiting and increased fruit quality. Furthermore, interstocks with greater dwarfing effect (i.e., interstock Mark) greatly reduced yield per tree, but the 34.9% shorter plant, 50.0% lower TCSA, and 25.5% smaller canopy size (compared to the interstock 77-34) simultaneously yield high potential for future high-density apple orchard planting regimes, which is recommended to obtain higher yield efficiency.

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References

An, H.S., Luo, F.X., Wu, T., Wang, Y., Xu, X.F., Zhang, X.Z., and Han, Z.H. (2017). Effect of rootstocks or interstems on dry matter allocation in apple. *Eur. J. Hortic. Sci.* 82, 225–231. <https://doi.org/10.17660/eJHS.2017/82.5.1>.

Atkinson, C., and Else, M. (2001). Understanding how rootstocks dwarf fruit trees. *Compact Fruit Tree* 34, 46–49.

Autio, W.R. (1994). Rootstock affects apple ripening, quality and storability. *Compact Fruit Tree* 27, 41–47.

Baughner, T.A., Singha, S., Leach, D.W., and Walter, S.P. (1994). Growth, productivity, spur quality, light transmission and net photosynthesis of 'Golden Delicious' apple trees on four rootstocks in three training systems. *Fruit Var. J.* 48, 251–255.

Berdeni, D., Cotton, T.E.A., Daniell, T.J., Bidartondo, M.I., Cameron, D.D., and Evans, K.L. (2018). The effects of arbuscular mycorrhizal fungal colonisation on nutrient status, growth, productivity, and canker resistance of apple (*Malus pumila*). *Front. Microbiol.* 9, 1461. <https://doi.org/10.3389/fmicb.2018.01461>.

Cookson, S.J., Hevin, C., Donnart, M., and Ollat, N. (2012). Grapevine rootstock effects on scion biomass are not associated with large modifications of primary shoot growth under nonlimiting conditions in the first year of growth. *Funct. Plant Biol.* 39, 650. <https://doi.org/10.1071/FP12071>.

Corelli-Grappadelli, L., Musacchi, S., and Magnanini, E. (2001). Single leaf and whole canopy gas exchange of pear as affected by graft incompatibility. *Acta Hortic.* 557, 377–384. <https://doi.org/10.17660/ActaHortic.2001.557.50>.

Costes, E., Salles, J.C., and Garcia, G. (2001). Growth and branching pattern along the main axis of two apple cultivars grafted on two different rootstocks. *Acta Hortic.* 557, 131–138. <https://doi.org/10.17660/ActaHortic.2001.557.16>.

Croft, H., Chen, J.M., and Zhang, Y. (2014). The applicability of empirical vegetation indices for determining leaf chlorophyll content over different leaf and canopy structures. *Ecol. Compl.* 17, 119–130. <https://doi.org/10.1016/j.ecocom.2013.11.005>.

Dayer, S., Peña, J.P., Gindro, K., Torregrosa, L., Voinesco, F., Martínez, L., Prieto, J.A., and Zufferey, V. (2017). Changes in leaf stomatal conductance, petiole hydraulics and vessel morphology in grapevine (*Vitis vinifera* cv. Chasselas) under different light and irrigation regimes. *Funct. Plant Biol.* 44, 679. <https://doi.org/10.1071/FP16041>.

De Souza, A.P.D., Grandis, A., Arenque-Musa, B.C., and Buckeridge, M.S. (2018). Diurnal variation in gas exchange and nonstructural carbohydrates throughout sugarcane development. *Funct. Plant Biol.* 45, 865. <https://doi.org/10.1071/FP17268>.

Di Vaio, C.D., Cirillo, C., Buccheri, M., and Limongelli, F. (2009). Effect of interstock (M.9 and M.27) on vegetative growth and yield of apple trees (cv 'Annurca'). *Sci. Hortic.* 119, 270–274. <https://doi.org/10.1016/j.scienta.2008.08.019>.

Fallahi, E., Chun, I.-J., Neilsen, G.H., and Colt, W.M. (2001). Effects of three rootstocks on photosynthesis, leaf mineral nutrition, and vegetative growth of 'BC-2 Fuji' apple trees. *J. Plant Nutr.* 24, 827–834. <https://doi.org/10.1081/PLN-100103776>.

Fallahi, E., Arzani, K., and Fallahi, B. (2013). Long-term leaf mineral nutrition in 'Pacific Gala' apple (*Malus × domestica* Borkh.) as affected by rootstock type and irrigation system during six stages of tree development. *J. Hortic. Sci. Biotechnol.* 88, 685–692. <https://doi.org/10.1080/14620316.2013.11513025>.

FAO (2017). Food and Agriculture Organization of the United Nations. <http://www.fao.org/faostat/en/#data/QC> (accessed December 12, 2018).

Fazio, G., Chang, L., Grusak, M.A., and Robinson, T.L. (2015). Apple rootstocks influence mineral nutrient concentration of leaves and fruit. *N.Y. Fruit Q.* 23, 11–15.

Ferree, M.E., and Barden, J.A. (1971). Influence of strains and rootstocks on photosynthesis, respiration, and morphology of delicious apple trees. *J. Am. Soc. Hortic. Sci.* 96, 453–457.

Foster, T.M., McAtee, P.A., Waite, C.N., Bolding, H.L., and McGhie, T.K. (2017). Apple dwarfing rootstocks exhibit an imbalance in carbohydrate allocation and reduced cell growth and metabolism. *Hortic. Res.* 4, 17009. <https://doi.org/10.1038/hortres.2017.9>.

Foster, T.M., Watson, A.E., Van Hooijdonk, B.M., and Schaffer, R.J. (2013). Key flowering genes including *FT*-like genes are upregulated in the vasculature of apple dwarfing rootstocks. *Tree Genet. Genom.* 10, 189–202. <https://doi.org/10.1007/s11295-013-0675-z>.

Glenn, D.M. (2010). Canopy gas exchange and water use efficiency of 'Empire' apple in response to particle film, irrigation, and microclimatic factors. *J. Am. Soc. Hortic. Sci.* 135, 25–32. <https://doi.org/10.21273/JASHS.135.1.25>.

Gonçalves, B., Moutinho-Pereira, J., Santos, A., Silva, A.P., Bacerlar, E., Correia, C., and Rosa, E. (2005). Scion-rootstock interaction affects the physiology and fruit quality of sweet cherry. *Tree Physiol.* 26, 93–104. <https://doi.org/10.1093/treephys/26.1.93>.

Greer, D.H. (2015). Seasonal changes in the photosynthetic response to CO₂ and temperature in apple (*Malus domestica* cv. 'Red Gala')

- leaves during a growing season with a high temperature event. *Funct. Plant Biol.* 42, 309. <https://doi.org/10.1071/FP14208>.
- Greer, D.H. (2018). Photosynthetic light responses of apple (*Malus domestica*) leaves in relation to leaf temperature, CO₂ and leaf nitrogen on trees grown in orchard conditions. *Funct. Plant Biol.* 45, 1149. <https://doi.org/10.1071/FP18093>.
- Iwanami, H., Moriya-Tanaka, Y., Honda, C., Hanada, T., and Wada, M. (2018). A model for representing the relationships among crop load, timing of thinning, flower bud formation, and fruit weight in apples. *Sci. Hortic.* 242, 181–187. <https://doi.org/10.1016/j.scienta.2018.08.001>.
- Karlidağ, H., Aslantaş, R., and Eşitken, A. (2014). Effects of interstock (M9) length grafted onto MM106 rootstock on sylleptic shoot formation, growth and yield in some apple cultivars. *Tarım Bilimleri Dergisi – J. Agric. Sci.* 20, 331–336. https://doi.org/10.1501/Tarimbil_00000001291.
- Kviklys, D., Čeidaitė, A., Lanauskas, J., Uselis, N., and Samuolienė, G. (2016). The effect of rootstock on apple tree bearing stability in a cooler climate. *Agric. Food Sci.* 25, 81–88. <https://doi.org/10.23986/afsci.53381>.
- Liu, B.H., Cheng, L., Liang, D., Zou, Y.J., and Ma, F.W. (2012). Growth, gas exchange, water-use efficiency, and carbon isotope composition of 'Gala Gala' apple trees grafted onto 9 wild Chinese rootstocks in response to drought stress. *Photosynthetica* 50, 401–410. <https://doi.org/10.1007/s11099-012-0048-0>.
- Losciale, P., Zibordi, M., Manfrini, L., and Corelli Grappadelli, L. (2008). Effects of rootstock on pear photosynthetic efficiency. *Acta Hortic.* 800, 241–248. <https://doi.org/10.17660/ActaHortic.2008.800.28>.
- Martínez-Alcántara, B., Rodríguez-Gamir, J., Martínez-Cuenca, M.R., Iglesias, D.J., Primo-Millo, E., and Forner-Giner, M.A. (2013). Relationship between hydraulic conductance and citrus dwarfing by the Flying Dragon rootstock (*Poncirus trifoliata* L. Raft var. *monstruosa*). *Trees* 27, 629–638. <https://doi.org/10.1007/s00468-012-0817-1>.
- Martínez-Cuenca, M.-R., Primo-Capella, A., and Forner-Giner, M.A. (2016). Influence of rootstock on citrus tree growth: Effects on photosynthesis and carbohydrate distribution, plant size, yield, fruit quality, and dwarfing genotypes. In *Plant Growth (IntechOpen)*. <https://doi.org/10.5772/64825>.
- Reig, G., Lordan, J., Fazio, G., Grusak, M.A., Hoying, S., Cheng, L., Francescatti, P., and Robinson, T. (2018). Horticultural performance and elemental nutrient concentrations on 'Fuji' grafted on apple rootstocks under New York State climatic conditions. *Sci. Hortic.* 227, 22–37. <https://doi.org/10.1016/j.scienta.2017.07.002>.
- Robinson, T., Hoying, S., Sazo, M.M., DeMarree, A., and Dominguez, L. (2013). A vision for apple orchard systems of the future. *New York Fruit Q* 21, 12–16.
- Rufato, L., Rossi, A.D., Piccolotto, L., and Fachinello, J.C. (2006). Cover crops manure in the peach orchard (*Prunus persica* L. Batsch) on integrated crop management. *Ciência Rural* 36, 814–821. <https://doi.org/10.1590/S0103-84782006000300014>.
- Samuolienė, G., Viškelienė, A., Sirtautas, R., and Kviklys, D. (2016). Relationships between apple tree rootstock, crop-load, plant nutritional status and yield. *Sci. Hortic.* 211, 167–173. <https://doi.org/10.1016/j.scienta.2016.08.027>.
- Seleznova, A.N., Stuart, T.D., and Thorp, T.G. (2008). Apple dwarfing rootstocks and interstocks affect the type of growth units produced during the annual growth cycle: Precocious transition to flowering affects the composition and vigour of annual shoots. *Ann. Bot.* 101, 679–687. <https://doi.org/10.1093/aob/mcn007>.
- Skrzyński, J. (2007). Growth and productivity of apple trees and fruit quality at harvest as affected by rootstocks. *Acta Hortic.* 732, 151–154. <https://doi.org/10.17660/ActaHortic.2007.732.17>.
- Sotiropoulos, T.E. (2008). Performance of the apple (*Malus domestica* Borkh.) cultivar Imperial Double Red Delicious grafted on five rootstocks. *Hortic. Sci.* 35, 7–11. <https://doi.org/10.17221/645-HORTSCI>.
- Twooski, T., and Miller, S. (2007). Rootstock effect on growth of apple scions with different growth habits. *Sci. Hortic.* 111, 335–343. <https://doi.org/10.1016/j.scienta.2006.10.034>.
- Twooski, T., Fazio, G., and Glenn, D.M. (2016). Apple rootstock resistance to drought. *Sci. Hortic.* 204, 70–78. <https://doi.org/10.1016/j.scienta.2016.01.047>.
- Vemmos, S.N., Petri, E., and Stournaras, V. (2013). Seasonal changes in photosynthetic activity and carbohydrate content in leaves and fruit of three fig cultivars (*Ficus carica* L.). *Sci. Hortic.* 160, 198–207. <https://doi.org/10.1016/j.scienta.2013.05.036>.
- Vercammen, J., Van Daele, G., and Gomand, A. (2007). Can fruit size and colouring of 'Jonagold' be improved by an interstock? *Acta Hortic.* 732, 165–170. <https://doi.org/10.17660/ActaHortic.2007.732.20>.
- Watson, A.E., Seleznova, A.N., Dayatilake, G.A., and Tustin, D.S. (2012). Rootstocks affect pear (*Pyrus communis*) tree growth through extent of node neof ormation and flowering with key differences to apple. *Funct. Plant Biol.* 39, 493. <https://doi.org/10.1071/FP12031>.
- Webster, A.D. (1995). Rootstock and interstock effects on deciduous fruit tree vigour, precocity, and yield productivity. *N. Z. J. Crop Hortic. Sci.* 23, 373–382. <https://doi.org/10.1080/01140671.1995.9513913>.
- Zhao, D., Yuan, J., Xu, K., Cheng, C., and Li, H. (2016). Selection of morphological, physiological and biochemical indices: Evaluating dwarfing apple interstocks in cold climate zones. *N. Z. J. Crop Hortic. Sci.* 44, 291–311. <https://doi.org/10.1080/01140671.2016.1220398>.
- Zhou, Y.Q., Qin, S.J., Ma, X.X., Zhang, J.E., Zhou, P., Sun, M., Wang, B.S., Zhou, H.F., and Lyu, D.G. (2015). Effect of interstocks on the photosynthetic characteristics and carbon distribution of young apple trees during the vigorous growth period of shoots. *Eur. J. Hortic. Sci.* 80, 296–305. <https://doi.org/10.17660/eJHS.2015/80.6.5>.

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SUPPLEMENTAL INFORMATION

SUPPLEMENTAL INFORMATION – TABLE S1. Background information on the interstocks and rootstock used in this study.

Rootstock	Origin	Parentage
Mark	Michigan State University	Open-pollinated seedling of M.9
GM.310	Jilin Academy of Agricultural Sciences Pomology Institute	Hongtaiping × M.9
SH.6	Shanxi Academy of Agricultural Sciences Pomology Institute	Rails × <i>M. honanensis</i>
SH.38	Shanxi Academy of Agricultural Sciences Pomology Institute	Rails × <i>M. honanensis</i>
77-34	Institute of Pomology, CAAS, Liaoning	M.9 × <i>M. xiaohuangensis</i>
Baleng	Huailai County, Hebei Province	Natural seedlings