Studies

Apple rootstocks affect functional leaf traits with consequential effects on carbon isotope composition and vegetative vigour

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Abstract

Composite trees combine optimal traits from both the rootstock and the scion. Dwarfing rootstocks are commonly used to reduce shoot vigour and improve fruit quality and productivity. Although growth habits of different rootstocks have been clearly described, the underlying physiological traits affecting scion vigour are not well understood. Plant water status and stem water potential are strongly influenced by water supply and demand through the soil–plant–atmosphere continuum. In the scion, stomata regulate water loss and are essential to prevent hydraulic failure. Stomatal conductance influences leaf carbon isotope composition. Combined, the effects of reduced stomatal conductance and, consequently, carbon fixation may affect tree growth. These differences could also correspond to differences in scion vigour controlled by rootstock genotype. Here, vegetative growth, gas exchange, stem water potential and leaf \( \delta^{13}C \) were compared to determine how rootstocks affect scion water relations and whether these differences correspond to shoot vigour. There was a range in vigour among rootstocks by almost 2-fold. Net leaf carbon assimilation rates were lower in rootstocks with lower vigour. Rootstock vigour was closely associated with leaf gas exchange and stem water potential in the scion and was reflected in leaf \( \delta^{13}C \) signatures. Dwarfing was strongly affected by changes to plant water status induced by rootstock genotype and these changes are distinguishable when measuring leaf and stem \( \delta^{13}C \) composition. These observations indicate that scion water relations and leaf carbon isotope discrimination were affected by rootstock genotype. These results have implications for better understanding dwarfing mechanisms in apple rootstocks and the relationship with water-use traits.

Keywords: Carbon assimilation; carbon isotope composition; dwarfing rootstock; \textit{Malus domestica}; plant water relations; shoot growth.

Introduction

Apples (\textit{Malus domestica}), like many horticultural crops, are clonally propagated and are comprised of a rootstock and a scion. Apple production relies on dwarfing rootstocks which enhance yield efficiency, fruit quality, disease resistance and tolerance to freezing conditions (Mika et al. 1981; Palmer and Wertheim 1981; Sansavini et al. 1981). In the last 50 years, the adoption of dwarfing rootstocks has transformed apple production because they reduce vigour and increase precocity (Czynczyk 1981; Webster 2001; Marini and Fazio 2018). Currently, there are four size categories for apple rootstocks; dwarfing, semi-dwarfing, semi-vigorous and vigorous (Roper 2001). Despite extensive characterization of rootstock traits in the last century, the mechanisms by which rootstocks control scion growth are still not well defined (Westwood 1970; Jones 1971, 1984).

When plants are grafted, fusing of vascular tissue occurs and can be affected by both rootstock and scion genotype. Dwarfing is the key rootstock-induced trait in apple. Differences in growth among rootstocks can often be seen immediately upon grafting (Roberts 1927) and rootstocks can change the healing of the graft union and stem xylem morphology, both of which can potentially impact scion water relations (Olien and Lakso 1984, 1986; Albacete et al. 2015). Several potential mechanisms of dwarfing have been proposed involving hormonal regulation, mechanical limitations or genomic control (Beakbane and Thompson 1940, 1947; Spangelo 1974; Tubbs 1980; Higgs and Jones 1990; Soumelidou et al. 1994; Webster 1995; Selezniova et al. 2003; Tworkoski and Miller 2007; Dolgun et al. 2009; Gregory et al. 2013; Harrison et al. 2016). However, phenotypic variation in these traits is difficult to characterize in composite plants. Higher-throughput approaches are needed that capture the effect of rootstocks on scion water relations and downstream effects on plant growth and development.

Changes to stem water potential are normally induced via changes in soil water availability (Suter et al. 2019). Stem water potential contributes to the control of stomatal...
conductance (Buckley 2019). Stomatal behaviour depends on the physiological traits of the plant and the host environment. At night, water is taken up and stored within the plant (Knipfer et al. 2019). In the morning, stomata open and transpiration rates increase, reducing leaf water potential and turgor pressure (Domínguez et al. 2019). Drought stress forces earlier stomatal closure due to loss of leaf turgor and, consequently, a decline in leaf xylem conductance to avoid excessive negative pressure in xylem (Knipfer et al. 2020). A reduction in stomatal conductance is, in part, an effect of a decline in root hydraulic conductance and these effects are mediated partially by responses of stomata to leaf water status (Knipfer et al. 2020). Although environmental conditions are key contributors to these responses, limitations in water supply from the soil via intraspecific variation in root traits can also impact stem water potential.

Measurements of stable carbon isotope ratios in plant material can be used to study plant water relations (Saugier et al. 2012). Carbon isotope ratios are used to calculate intrinsic water-use efficiency (iWUE) and these ratios can reflect the balance between carbon acquisition and water consumed from transpiration (Cernusak et al. 2003; Seibt 2008; Ma et al. 2010; Bchir et al. 2016). Carbon isotope discrimination occurs during diffusion of CO₂ to the site of fixation by Rubisco and then, by Rubisco itself. Since carbon discrimination by Rubisco is much greater than that of diffusion and diffusion to the site of fixation by Rubisco is proportional to the ratio of internal CO₂ concentrations and atmospheric CO₂ (c/i), less discrimination occurs when stomatal conductance is low (Guy et al. 1993). Atmospheric and soil conditions are known to affect iWUE and carbon isotope discrimination in plants (Cernusak 2020). However, in comparative experiments, environmental conditions should be similar for all plants. Differences in stomatal conductance and thus, carbon isotope composition differences should, therefore, be more affected by internal changes in water supply or demand. Vigorous plants, where water supply may be less limited, may have depleted isotopic values compared to water-limited species or cultivars (Lehmann et al. 2018). Carbon isotope discrimination can be an effective seasonally integrated proxy for gas exchange and water relations throughout the season (Mills et al. 1998). Since these effects on stomatal function and gas exchange are downstream from changes in water relations, the effect that rootstocks have on root water uptake, limitations at the graft union or induced hydraulic limitations in the scion stem or leaves should be clearly reflected in the isotopic signature.

The objective for this study was to assess rootstock-mediated variability in water relations for a common apple scion grafted onto nine rootstocks. We hypothesized that phenotypic differences in vigour among rootstocks will be reflected in their effect on gas exchange and water relations of the scion. Consequently, these effects should have downstream effects on carbon isotope composition of leaves and stems as a carbon source and sink, respectively. These findings will help understand the relationship between water relations and rootstock dwarfing capacity in the scion. Furthermore, clear rootstock-mediated effects on carbon isotope ratios will improve selection efficiency for dwarfing capacity. The knowledge acquired from this study can be applied to better pair rootstocks with specific cultivars, environment conditions, and improve efficiencies for rootstock breeding programs.

### Materials and Methods

#### Site description and experimental design

The experiment was conducted using ‘Honeycrisp’ as scion grafted onto nine different rootstocks at Washington State University (WSU) Sunrise Research Orchard in Rock Island, WA, USA (47°18′35.6″N, 120°03′59.5″W). The rootstocks in this experiment were: Budagovsky 9 (B.9), CG.4292, CG.5257, M.9-T337, Geneva 210 (G.210), Geneva 814 (G.814), Geneva 87 (G.87), Geneva 890 (G.890) and Geneva 969 (G.969). The dwarfing capacity of these rootstocks ranged from dwarving (B.9) to semi-vigorous (G.890). The experiment was planted in 2017 and each rootstock had three replicated plots arranged in a completely randomized design. The soil is a shallow sandy loam soil with good drainage. The orchard was oriented north to south and rows spaced 3.6 m apart and 0.9 m between trees which were trained to a spindle system and irrigated using at approximately 110 % replacement of estimated evapotranspiration during the growing season using a combination of drip and micro-sprinklers. Trees were managed and pruned according to commercial tree fruit management practices. Experiments took place in 2018 and 2019. In both years, flowers were completely removed from the trees to eliminate any cropping during early growth. Soil volumetric water content and soil temperature were measured using an ECH2O 5TM soil moisture and temperature probe (Meter Group, Pullman, WA, USA) (Table 1). Three soil moisture sensors were placed in different locations in the herbicide strip equidistant between two trees at 20 cm depth. Relative humidity and air temperature were measured with a VP-3 sensor (Meter Group, Pullman,

| Year | Mean temperature (°C) | Solar radiation (MJ m⁻²) | Relative humidity (%) | Soil temperature (°C) | Soil moisture (m³ m⁻³) |
|------|----------------------|-------------------------|----------------------|----------------------|-----------------------|
| 2018 |                      |                         |                      |                      |                       |
| June | 19.9                 | 737                     | 41                   | 20.06                | 0.22                  |
| July | 26                   | 817                     | 31.6                 | 23.93                | 0.27                  |
| August | 23.9               | 583                     | 38.1                 | 23.06                | 0.27                  |
| 2019 |                      |                         |                      |                      |                       |
| June | 21.1                 | 756                     | 37.4                 | 21.07                | 0.23                  |
| July | 23.5                 | 728                     | 37.8                 | 23.88                | 0.19                  |
| August | 24.5               | 630                     | 41.3                 | 23.68                | 0.24                  |

Table 1. Mean environmental data from June to August in 2018 and 2019 at Washington State University Sunrise Research Orchard in Wenatchee, WA, USA. Data were recorded from 1 June until 20 August with interval of 30 min between measurements.
Physiological measurements

Physiological measurements were made monthly in 2018 and 2019 starting from June through August on days that were cloud-free and with temperatures not exceeding 30 °C. Means were calculated for all sampling times during the season. Maximum stomatal conductance of water vapour ($g_s$) and net CO$_2$ assimilation were measured on two sun-exposed mature leaves in one tree per plot replicate between 10 am and 12 pm using a LI-6400XT infrared gas analyser (Li-COR, Lincoln, NE, USA). During the experiment the air flow was constant at 400 µmol s$^{-1}$, reference CO$_2$ concentration was set at 400 ppm, leaf temperature at 25 °C and photosynthetic photon flux density inside the chamber was set to 1500 µmol m$^{-2}$ s$^{-1}$. After placing the leaf in the gas exchange chamber, the leaf was allowed to equilibrate until reference and sample values stabilized. Intrinsic water-use efficiency ($\mu$mol CO$_2$ mol H$_2$O$^{-1}$) (Equation (1)) was obtained by calculating the ratio of net photosynthetic rate ($\mu$mol CO$_2$ m$^{-2}$ s$^{-1}$) to stomatal conductance of water vapour (mol m$^{-2}$ s$^{-1}$).

\[
i_{WUE} = \left( \frac{\text{Net Photosynthetic Rate}}{\text{Stomatal Conductance}} \right)
\]  

(1)

Morphological measurements

Morphological measurements took place once a month from June to August in 2018 and 2019.

Current-year shoot growth (cm) was measured from the bud scar that developed at bud break to the apical meristem. For this measurement, six shoots were measured on two trees per replicate. In these same trees, trunk diameter was measured at 10 cm above the graft union and trunk cross-sectional area (TCSA) was calculated.

Carbon isotope composition ($\delta^{13}$C)

The entire portion of new annual growth was excised from three terminal stems per replicate, then leaves and stems were separated for carbon isotope composition analyses. The samples were placed in a paper bag and dried in a chamber with constant air flow. After the samples were completely dry, subsamples of 2 g were taken from shoots and leaves, respectively, and ground to a fine powder for each replicate using a VWR Homogenizer (VWR, Radnor, PA, USA). Once each sample was transformed into fine powder, 3 mg were weighed for leaves and 4 mg were weighed for stems using a high-precision analytical balance (XSE105 DualRange, Metler Toledo, Greifensee, Switzerland) and placed into 5 mm × 9 mm tin capsules (Costech Analytical Technologies, Inc., Valencia, CA, USA). Prepared capsules were shipped for analysis to the Stable Isotope Core Laboratory at Washington State University. Samples were analysed using an elemental analyser (ECG 4010, Costech Analytical, Valencia, CA, USA) coupled with a continuous flow isotope ratio mass spectrometer (Delta PlusXP, Thermofinnigan, Bremen, Germany). Carbon isotope ratios were determined using the standard Vienna PeeDee belemnite and the values reported in ‘delta’ notations as δ values in permil (‰) (Sharp 2017) as described in Equation (2).

\[
\delta^{13}C = \left( \frac{\left( \frac{^{13}C}{^{12}C} \right) \text{sample}}{\left( \frac{^{13}C}{^{12}C} \right) \text{standard}} - 1 \right) \times 1000
\]  

(2)

where the ratio of the heavy ($^{13}$C) over the lighter ($^{12}$C) isotope was calculated for the sample and then was divided by the $^{13}$C/$^{12}$C ratio of the standard.

Statistical analysis

Composite seasonal means were calculated for net carbon assimilation, stomatal conductance, iWUE and midday stem water potential since there were no interactions between the measurement periods during each season. Data were analysed using a two-way analysis of variance (ANOVA) with rootstock and year as main factors for shoot growth, TCSA, net carbon assimilation, stomatal conductance, iWUE, midday stem water potential, leaf and stem carbon isotope assimilation using PROC GLM (SAS Campus, Cary, NC, USA). Since stem water potential showed an interaction between years, the data were presented for each year, 2018 and 2019 separately. For other data, when no interactions were present, years were pooled together and only the effects of rootstock were presented. Mean separation tests were performed with Tukey’s HSD test (α = 0.05). Linear regression was used to test the relationships between individual variables. Figures were prepared using OriginPro 2021 Data Analysis and Graphing Software (OriginLab Corporation, Northampton, MA, USA). Non-linear relationships as well as regression analysis between variables were calculated only for stem and leaf isotope discrimination with SigmaPlot 12.5 (Systat Software Inc., San Jose, CA, USA).

Results

Tree growth

Rootstock affected scion growth rates that were reflected in both TCSA and shoot growth. One year after planting, TCSA ranged from 2.14 to 6.56 cm$^2$ in 2018 and then, 3.55 to 8.90 cm$^2$ in 2019 (Table 2). In 2018, TCSA was lower for B.9 among all other rootstocks. In 2019, B.9 had lower TCSA compared to M.9, CG.4292, G.210, CG.5257, G.814, G.890. Rootstock genotype significantly affected terminal shoot growth and annual growth ranged from approximately 20 to 50 cm (Fig. 1). Shoot growth was significantly greater for G.890 compared to B.9, M.9 and G.969. Greater growth was also observed for G.210, G.87, CG.5257 and G.814.
Scion water relations and leaf gas exchange

Rootstock affected midday stem water potential in both years (Fig. 2). In this irrigated orchard, mean midday stem water potential (Ψ_m) did not range beyond −0.8 to −1.0 MPa. B.9 had the lowest stem water potential of all rootstock genotypes. However, in 2018, Ψ_m for B.9 was only significantly different from CG.5257. In 2019, differences among rootstocks were greater but trends were similar to 2018. Ψ_m was significantly lower for B.9 than more vigorous rootstocks like G.87, CG.5257 and G.890. Midday stem water potential was strongly correlated with seasonal shoot growth (Fig. 3) indicating that rootstocks that confer higher vigour in the scion also contribute to greater stem water potential. Leaf gas exchange traits measured mid-morning differed less among rootstocks than shoot growth or trunk diameter. Net carbon assimilation was the lowest for B.9 at approximately 9 µmol CO2 m⁻² s⁻¹ and the highest for CG.5257 at approximately 13.5 µmol CO₂ m⁻² s⁻¹ (Fig. 4). There was also a significant relationship between net carbon assimilation and seasonal shoot growth (r = 0.923; P < 0.001) (Fig. 5). Stomatal conductance ranged from 0.10 to 0.19 mol m⁻² s⁻¹ among rootstocks (Fig. 4). G.969 presented the lower stomatal conductance values and was significantly different from G.4292, G.210, G.87 and CG.5257. Intrinsic water-use efficiency was lowest for G.210 but was higher for G.969 (Fig. 4). Midday stem water potential also strongly correlated with water relations indicated that rootstocks limiting water and maintaining a lower inherent stem water potential will also have less vigour.

Table 2. Mean TCSA of ‘Honeycrisp’ apple trees on B.9, M.9, G.969, CG.4292, G.210, G.87, CG.5257, G.814 and G.890 rootstocks measured in 2018 and 2019. Different letters indicate significant differences among rootstocks means determined using a Tukey’s mean separation test (α = 0.05). SEM, standard error of the mean.

| Rootstock | 2018         | 2019         |
|-----------|--------------|--------------|
|           | TCSA (cm²)   | SEM          | TCSA (cm²)   | SEM          |
| B.9       | 2.14a        | 0.10         | 3.55a        | 0.29         |
| M.9       | 5.07d        | 0.26         | 8.17b        | 0.53         |
| G.969     | 3.80b        | 0.44         | 5.81ab       | 0.28         |
| CG.4292   | 3.96cd       | 0.45         | 7.96b        | 0.96         |
| G.210     | 6.56d        | 0.51         | 8.19b        | 0.98         |
| G.87      | 2.71bc       | 0.30         | 6.12ab       | 0.41         |
| CG.5257   | 3.72cd       | 0.47         | 7.83b        | 0.93         |
| G.814     | 4.14d        | 0.51         | 8.53b        | 0.96         |
| G.890     | 5.15d        | 0.24         | 8.90b        | 0.74         |
Carbon isotope composition

Rootstock affected leaf and terminal stem carbon isotope composition (Table 3). In 2018, the rootstocks M.9 and G.969 had less depleted values for leaves when compared to CG.5257. Leaf δ¹³C for all rootstocks was approximately −25 ‰ and ranged by approximately 1 ‰ among rootstock genotypes for both 2018 and 2019. Stem δ¹³C values were closer to −24.5 ‰ and ranged by approximately 2 ‰. At the stem level, although there were no statistical differences, δ¹³C for B.9 was the most enriched and δ¹³C for G.210 was the most depleted. In 2019, for stems, B.9 was more enriched than G.210, G.87, CG.5257, G.814 and G.890. There was a positive non-linear relationship between δ¹³C in leaves and stems (P = 0.05, r² = 0.8914) (Fig. 7). The data demonstrate that the carbon isotope composition of non-photosynthetic tissues from stem tissue is more enriched compared with leaves of the same branch but converge when values are both on the high or low end of the range in variation observed in this experiment. Carbon isotope composition in leaves was also strongly and negatively correlated with seasonal shoot growth (P < 0.001, r = −0.924) (Fig. 8). Rootstocks with lower vegetative vigour had higher δ¹³C in both stems and leaves. Net CO₂ assimilation and stomatal conductance correlated strongly and negatively with δ¹³C (P < 0.01, r =
to the scion rather than simply access to water within the soil profile.

The degree of scion dwarfing by rootstocks affected water relations and leaf physiology. These rootstock-induced changes in leaf function also translated to differences in carbon isotope composition in both leaves and stems. In this study, rootstocks modulated scion leaf water relations by affecting stomatal conductance and leaf gas exchange which had downstream effects on δ13C composition. There have been previous studies which have reported differences in water relations when different rootstocks were used in woody plants (Bongi et al. 1994; Padgett-Johnson et al. 2000; Gibberd et al. 2001; Cohen and Naor 2002; Ma et al. 2010; Liu et al. 2012a; Galibignani et al. 2016; Peccoux et al. 2018; Villalobos-Gonzales et al. 2019; Frioni et al. 2020). However, these studies did not show the linear relationship between these traits among many phenotypically diverse rootstocks. More often, differences were reported among scion cultivars or among treatments that affect water supply to the roots. Liu et al. (2012b) described how rootstocks manipulated stomatal conductance and photosynthetic capacity in ‘Gala Gala’. Here, carbon isotope composition was different for ‘Honeycrisp’ when grafted to apple rootstocks with differences in dwarfing capacities. A reduction in discrimination against 13CO2 may indicate an increase in water-use efficiency (Jones 1984; Farquhar et al. 1989). Differences in carbon isotope composition were not consistent with measured differences in water-use efficiency; carbon isotope composition integrates gas exchange over a much longer period, thus not necessarily surprising to see such inconsistency.

In apples, carbon isotope composition was reported to be lower for Fuji compared to Braeburn apples because of limitations in stomatal and leaf respiration rates (Massonnet et al. 2007). In this study, less-negative δ13C for more dwarfing rootstocks compared to more vigorous rootstocks indicates increased conservation of water through lower stomatal conductance in leaves, but was not related to iWUE. These effects on water relations had downward effects on scion vigour and were consistent with our knowledge of dwarfing capacity of the rootstocks tested in this study (Seleznyova et al. 2008; Kosina 2010; Tworkoski and Fazio 2015; Foster et al. 2017).

The positive relationship between carbon isotope composition between leaf and stem demonstrates short-term and long-term impacts of rootstocks on scion water relations and carbon assimilation. We observed enrichment of stems compared to leaves and this is well aligned with previous studies (Arndt and Wanek 2002; Cernusak et al. 2003). Specifically, B.9 showed a clear divergence in carbon isotope composition between stem and leaves where leaves were depleted relative to stems. Cernusak et al. (2009) presented six hypotheses for this effect. One of the outlined hypotheses to explain enrichment of non-photosynthetic tissues compared with their leaves rely on post-photosynthetic carbon fractionation due to seasonal separation of growth (Cernusak et al. 2009). Development and growth of leaves occur in spring with plentiful soil water content. Since variability in carbon discrimination is mainly determined by the ratio of intercellular to ambient CO2 partial pressure, the photosynthetic carbon assimilation is heavily discriminating against 13C at this time (Werner and Gessler 2011). Wood growth occurs later in the season, when temperatures and vapor pressure deficit (VPD) are higher which can reduce stomatal conductance producing
less discrimination against $^{13}$C at the time carbon is used for synthesis of non-photosynthetic tissues (Cernusak et al. 2009). Differences in $\delta^{13}$C between leaves and stems were less pronounced when discrimination and vegetative vigour were also greater. Since more vigorous rootstocks extend growth into later parts of the season, we could expect a greater agreement in $\delta^{13}$C between leaves and stems than for less vigorous rootstocks like B.9.

The most obvious influence of dwarfing rootstock is the ability to produce lower vegetative biomass relative to more vigorous rootstocks. Trunk diameter and shoot extension measurements are two of the main traits used to characterize tree growth (Lauri et al. 2006). In both years, B.9 produced the smallest scion compared to all other rootstocks and our observations were similar to Gjamovski and Kiprijanovski (2011). Corresponding reductions in stem water potential, an

**Figure 6.** (A) The relationship stem water potential (MPa) and stomatal conductance (mol m$^{-2}$ s$^{-1}$) in 2018 ($P < 0.05$, $r = -0.708$) and (B) the relationship between stem water potential (MPa) and stomatal conductance 2019 ($P < 0.001$, $r = 0.974$) for 'Honeycrisp' apple trees on nine rootstock genotypes. The line indicates the adjusted linear regression for the combined data points.
accurate measure of plant water status (Shackel et al. 1997), were observed among rootstock cultivars.

The rootstock-modulated effect on scion water relations represents an important component in the understanding of rootstock–scion water use (Higgs and Jones 1990). When net carbon assimilation was measured among rootstocks, we observed that less conservative rootstocks like semi-dwarfing G.890 had higher photosynthetic rates. These results follow similar patterns with those reported by Fallahi et al. (2001), who reported lower net photosynthetic rates for B.9 compared to more vigorous rootstocks like Ottawa 3 and M.7. Clearly, the capacity of the tree to improve net carbon assimilation and thus, increase carbon fixation is related to the ability of the rootstock to supply water to the leaves (Koepke and Dhingra 2013). Therefore, the hypothesis that reduced growth of dwarfing rootstocks may be associated with rootstock water restrictions at a particular point in the tree was supported by this study as has been suggested by past research (Atkinson and Else 2001; Cohen and Naor 2002; Atkinson et al. 2003; Cohen et al. 2003; Cohen et al. 2007; Tworkoski and Fazio 2015).

These restrictions could either occur at the root or the graft union, or in the impact of rootstocks on stem and leaf hydraulic traits. We acknowledge that this is not completely addressed by this study and more work is needed to better discern how rootstocks might affect each point of resistance within the plant.

In conclusion, we report the effect of rootstocks on carbon isotope composition in leaves and stems and show the close association between leaf gas exchange and stem water potential. Rootstocks were able to strongly affect water-use efficiency and carbon assimilation for the same apple scion. Furthermore, there was a close association between rootstock-influenced gas exchange and overall shoot vigour under uniform soil moisture conditions. Rootstocks that were more vigorous have more negative carbon isotope composition indicating lower water restrictions in transport to above-ground parts compared to rootstocks with lower vigour. While there have been numerous studies comparing water relations for dwarfing and non-dwarfing apple rootstocks, this is the first time that scion water relations were associated with carbon isotope discrimination across a range of dwarfing rootstocks. Finally, these results increase our understanding of the physiological mechanisms underlying

### Table 3. Mean stem and leaf carbon isotope composition (‰) for 2018 and 2019 of ‘Honeycrisp’ apple trees on B.9, M.9, G.969, CG.4292, G.210, G.87, CG.5257, G.814, G.890 rootstocks measured in 2018 and 2019. Different letters indicate significant differences among rootstock means determined using a Tukey’s mean separation test ($\alpha = 0.05$). SEM, standard error of the mean.

| Rootstock | Leaf $\delta^{13}$C (‰) | SEM | Stem $\delta^{13}$C (‰) | SEM | Leaf $\delta^{13}$C (‰) | SEM | Stem $\delta^{13}$C (‰) | SEM |
|-----------|-------------------------|-----|-------------------------|-----|-------------------------|-----|-------------------------|-----|
| B.9       | -24.37ab                | 0.30| -24.41a                 | 0.04| -24.62a                | 0.09| -23.67a                 | 0.32|
| M.9       | -24.18a                 | 0.13| -24.91a                 | 0.05| -25.53bc                | 0.26| -24.81ab                | 0.10|
| G.969     | -24.14a                 | 0.44| -24.67a                 | 0.17| -25.02abc               | 0.46| -24.30ab                | 0.42|
| CG.4292   | -24.82ab                | 0.26| -24.76a                 | 0.17| -25.09abc               | 0.16| -25.27ab                | 0.17|
| G.210     | -25.33ab                | 1.20| -25.47a                 | 0.34| -25.04abc               | 1.18| -25.25b                 | 0.4  |
| G.87      | -25.10ab                | 0.36| -24.71a                 | 0.21| -25.66c                 | 0.03| -25.2b                  | 0.16|
| CG.5257   | -25.72b                 | 1.07| -24.97a                 | 0.08| -25.40bc                | 0.47| -25.44b                 | 0.04|
| G.814     | -24.79ab                | 0.19| -24.94a                 | 0.11| -25.54bc                | 0.12| -25.45b                 | 0.19|
| G.890     | -24.76ab                | 0.06| -24.70a                 | 0.07| -24.92ab                | 0.16| -25.36b                 | 0.17|

**Figure 7.** The relationship between leaf $\delta^{13}$C (‰) isotope and stem $\delta^{13}$C isotope (‰) for ‘Honeycrisp’ apple trees on nine rootstock genotypes ($P = 0.05, r = 0.8914$). Each data point represents the mean for both years for each rootstock. The line indicates the best-fit adjusted non-linear regression for the combined data points.

**Figure 8.** The relationship between mean leaf $\delta^{13}$C isotope (‰) composition and shoot growth (cm) ($P < 0.001, r = -0.924$) for ‘Honeycrisp’ apple trees on nine rootstock genotypes. Each data point represents the mean for both years for each rootstock. The lines indicate the adjusted linear regression for the combined data points.
dwarfing in composite woody plants like apple and show how below-ground traits imparted from rootstocks can affect water-use efficiency and carbon isotope composition in leaves and stems.

Sources of Funding

E.C.B. and L.A.K. were supported by the USDA National Institute of Food and Agriculture Specialty Crop Research Initiative project ‘AppleRoot2Fruit: Accelerating the development, evaluation and adoption of new apple rootstocks’ (2016-51181-25406). L.A.K. was also supported by the USDA National Institute of Food and Agriculture, Hatch/State project 1014919 and NC-140 Multistate Project ‘Improving Economic and Environmental Sustainability in Tree-Fruit Production Through Changes in Rootstock Use’, accession no. 1013833.

Contributions by the Authors

L.K. acquired the funding. E.C.B. and L.K. designed the experiment. E.C.B. collected, analyzed the data, and prepared the manuscript draft. E.C.B. and L.K. edited and finalized the manuscript for submission.

Conflict of Interest

None declared.

Acknowledgements

We would like to acknowledge the support of Michelle Reid, Katie Mullin and Hector-Camargo-Alvarez for technical assistance and the Washington State University Stable Isotope Laboratory for isotope analysis and support.

Data Availability

Please contact the author for data requests, and the data will be made available upon your request.

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