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Assessing Fruit Maturity and Quality of 'Buckeye Gala' Grown on a Diverse Panel of Apple (*Malus domestica* Borkh.) Rootstocks in Western Maryland

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Abstract: Apple (*Malus domestica* Borkh.) is usually produced in the form of a rootstock grafted scion. Rootstocks have important effects on several horticultural attributes. However, the results are not consistent regarding sites and scion–rootstock combinations. The aim of this research was to characterize the horticultural performance of 'Buckeye Gala' apple scion grafted onto ten rootstocks grown in Western Maryland during two harvest seasons. Our results demonstrated that, on average, tree size and yield in G.935, M.26 and G.969 rootstocks were 5–40% higher, but weight per fruit was 2–15% lower than in all other rootstocks. Fruit maturity was significantly delayed with increasingly vigorous rootstocks. There were no crop load differences. Overall, the assessed rootstocks were discriminated into seven significantly distinct clusters characterized by marked differences in vigor, yield, and fruit maturity. Moreover, significant correlations were obtained amongst all assessed variables. Rootstock impact must be considered when making management decisions in 'Buckeye Gala' fruit grown under Western Maryland conditions as they are critical in modulating fruit maturity and quality.

Keywords: *Malus domestica* Borkh.; rootstocks; fruit quality; ethylene; tree growth

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1. Introduction

Apple (*Malus domestica* Borkh.), one of the most commercially important horticultural crops worldwide, is usually produced in the form of a rootstock grafted scion [1]. In modern apple orchard production systems, particularly in high-density systems, appropriate rootstock selection is as critical as the choice of scion cultivar to ensure success and economic viability of the operation [2,3]. Rootstocks have been reported to have important effects on a number of horticultural attributes, including precocity, productivity, winter hardiness, tree vigor, pest and disease resistance, drought tolerance, nutrient uptake, cropping efficiency, as well as fruit maturity and quality [4–15].

Fruit quality is determined by multiple irreversible physiological and biochemical modifications that take place as fruit matures [16,17]. These include modifications in fruit color (degradation of chlorophyll and non-photosynthetic pigment accumulation), texture (flesh softening), and flavor (increase in sugar contents, decrease in organic acids, and changes in aroma volatile compounds) [18,19]. Furthermore, apple fruits are classified as climacteric, indicative of fruit characterized by presenting an upsurge in respiration rates and internal ethylene concentration (IEC) as it matures [20,21]. Quality-related attributes have been reported to be strongly influenced by ethylene production as well as to be interrelated among each other [19,22–24]. Several studies have reported that rootstock genotype can influence key aspects of fruit quality such as fruit size/weight, flesh firmness, soluble solids contents (SSC), color, starch contents, as well as fruit maturity by affecting respiration rate and IEC in different apple cultivars [6,15,25–32]. For example, 'Pacific Gala' fruit on B.9 and G.30 rootstocks had the highest SSC and starch degradation patterns compared to other rootstocks [33], while 'Delicious' apples were reported to develop yellow background color earlier when grown on M.26 than on M.7 [29], and fruit from trees on M9.T337 had the lowest percentage of red color with 'Fuji' and the highest with 'Gala' when compared with other rootstocks [34]. Furthermore, the fruit of 'Starkspur Golden Delicious' was shown to have higher IEC when grafted on M.26 rootstock [26]. However, the effects in fruit maturity and quality of most of the rootstocks studied have not been consistent from site to site as well as among scion–rootstock combinations [35,36].

Rootstocks also play a crucial role in controlling tree size and yield. The size of the tree, and consequently tree vigor, is generally expressed as trunk cross-sectional area (TCA) [37,38], and there have been several studies reporting how TCA and yield can be influenced by rootstock genotype [4,10,11,32–34,39,40]. The use of dwarfing rootstocks in apple orchards significantly reduces tree size and yield, allowing for an increased planting density, production efficiency, reduction in input costs and improved apple fruit quality [41,42]. Trees on dwarfing rootstocks usually have better sunlight distribution throughout the canopy, and therefore can positively impact overall fruit quality [30,35,43] as fruits developed in the shade have generally less sugars and are less mature compared to fruit exposed to full sun [41,44]. Nevertheless, the effects of rootstock genotypes on tree size and yield can also be influenced by the specific environmental conditions and scion cultivars.

The number of rootstocks available commercially has been steadily increasing due to the presence of breeding programs worldwide [4]. The most widely used apple rootstock in the world is the 'Malling 9′ (M.9) and its sport mutations (novel phenotypes generated by somatic cell mutations in the meristematic from which a new shoot is derived) [45,46]. Other commercially important rootstocks include the Cornell-Geneva series and the Budagovsky rootstocks series [3]. Novel rootstock genotypes continue to arise as a response to the climatic stresses affecting different apple production areas globally, and several of these still need to be evaluated. In terms of apple scions, 'Gala' is one of the most planted cultivars worldwide. In areas such as Western Maryland, where climatic conditions are not ideal for suitable red color development at harvest, the use of strains such as 'Buckeye Gala' has been widely adopted due to their enhanced red skin coloration [47].

Based on the above, the aim of the present work was to characterize the horticultural performance of 'Buckeye Gala' apple scion grafted onto ten rootstocks (including novel types which have not yet been described in the literature) grown in Western Maryland during two harvest seasons.

2. Materials and Methods

2.1. Plant Material

A rootstock trial was planted in 2018 at the Western Maryland Research and Education Center (39.5100° N, 77.7334° W; Keedysville, MD, USA) using 'Buckeye Gala' as the scion cultivar. Trees were planted in three rows in a randomized complete block design, with a total of five three-tree replicates per each rootstock genotype (therefore a total of 15 trees per rootstock). Tree spacing was 1.0 m x 3.5 m and trees were trained in tall spindle. Rootstock genotypes included two Malling, M.9T337 and M.26; one Budagovsky, B.10; five Geneva, G.11, G.41, G.935, G.814, G.969; and two novel rootstocks from New Zealand, NZ.1 and NZ.2. For all assessed rootstock genotypes, crop load was adjusted to a 6–7 fruit cm^{-2} trunk cross-sectional area (TCA) [48].

'Buckeye Gala' apples were harvested from each rootstock during two consecutive production seasons (2021 and 2022). Fruit maturity indices were monitored throughout the season to harvest fruit at the optimal commercial maturity stage each year, corresponding to changes in background color from green to yellow, flesh firmness of 75–80 N, starch contents between 3 and 4 and soluble solid contents (SSC) > 12%. We used 'Buckeye Gala' scion grafted on M9.T337 as reference rootstock for defining harvest time for all assayed genotypes. For each of the five replications, a total of twenty-five fruit were harvested. Per replication, five fruits were used for the analysis of internal ethylene concentration, while the rest of the fruits were used to assess physicochemical properties (described below). Fruits with absence of visual blemishes, bruises and/or diseases were chosen. After harvest, fruits were quickly transported to the laboratory.

2.2. Tree Performance Measurements

Trunk circumference was measured 30 cm above the graft union between leaf senescence in fall and bloom each year (November–April). Trunk cross-sectional area (TCA) was then calculated. Additionally, yield (kg fruit/tree) and number of fruits per tree were assessed each year. Crop load (fruit/cm^2 TCA) was then calculated.

2.3. Fruit Internal Ethylene Concentration

At commercial harvest, the internal ethylene concentration (IEC) of each fruit was measured on 1 mL samples of internal gas from the core cavity using a gas chromatograph (GC-2014C, Shimadzu Co., Kyoto, Japan) equipped with an activated alumina column attached to a flame ionization detector as previously described [24,49]. Nitrogen (N_2) was used as the carrier gas at a flow rate 30 mL min^{-1} while O_2 and H_2 were used to create the flame of the detector at flow rate of 300 and 30 mL min^{-1}, respectively. Injector, detector, and oven temperatures were set at 140, 150 and 80 °C, respectively.

2.4. Fruit Physicochemical Measurements

Fruit weight, skin and flesh color, index of absorbance difference (I_{AD}), red blush percentage, flesh firmness, starch pattern index (SPI), SSC and titratable acidity (TA) were measured. Fruit weight was quantified using an electronic balance (Sartorius, AG Gottingen, Germany). Skin and flesh color were assayed on the two opposite sides of each fruit along the equatorial axes and the red-green (*a**) and yellow-blue (*b**) values were measured using a colorimeter (Konica Minolta CR400 Chroma Meter, Konica Minolta Sensing, Inc., Osaka, Japan). Hue angle (*hue°*), representing changes in primary colors, was calculated as $h = arctan(a^*/b^*)$ [50]. The index of absorbance difference ($I_{AD} = A_{670} - A_{720}$; DA-Meter, TR Turoni, Forli, Italy) was measured on fruit skin by averaging the values recorded on three spots on each apple fruit [51]. Flesh firmness was measured on the two opposite peeled sides of each fruit using an TA.XT Plus Connect texture analyzer (Texture Technologies Corp., Scarsdale, NY, USA) equipped with a 50 kg loadcell and analyzed with the Exponent TE32 (v6.0, Texture Technologies Corp., Scarsdale, NY, USA) software fitted with an 11.1 mm diameter probe. The SPI of each fruit cut at the equator was assessed using the Cornell generic chart where 1 = 100% iodine stained starch and 8 = 0% stained starch [52]. To determine SSC and TA, a wedge from each fruit was removed and pooled to create a composite sample of each biological replication. Juice was extracted from these composite samples with a hand press and filtered through cheesecloth. SSC was determined by using a digital hand-held refractometer (Atago, Tokyo, Japan) and expressed as %, whereas TA was computed by automatic titration (855 Robotic Titrosampler; Metrohm, Riverview, FL, USA) with a 0.1 N sodium hydroxide solution to an end point to pH 8.2, expressed as % malic acid [19,20].

2.5. Climate Data

Climate variables including maximum and minimum temperature (Tmax, Tmin, respectively), relative humidity (RHmax, RHmin, respectively), and total rainfall were recorded daily during the study period (for each production season). Data were downloaded from the Western Maryland Research and Education center automatic weather station. The data were recorded automatically in a datalogger and downloaded daily to a central server.

2.6. Statistical Analysis

Response variables were modeled using generalized linear mixed models including rootstock genotype as a fixed factor, block as a random factor and production season as repeated measures. When this analysis was statistically significant, Tukey's HSD test was used to compare between rootstock genotypes for the different assessed variables. The data met normality assumptions.

Pearson's correlation coefficients, using mean-centered data, were calculated for each pairwise combination of assessed parameters. PCA, which was applied to reduce the dimensionality of the data, was visualized through a 'biplot' graph, thus representing the relationships among the variables (internal ethylene production, physicochemical measurements, yield, and TCA) and rootstock genotypes. The Scree test was used to select the number of principal components that captured most of the variation, defined by a plot of the magnitude of an eigenvalue (=the variance of the PC) versus its number. The number of components can be taken to be the point at which a distinct bend in the scree plot occurs, such that the remaining eigenvalues are then relatively small and all about the same size [53]. Furthermore, we performed linear discriminant analysis (LDA) to define the statistically significant number of clusters into which fruit from the 10 rootstock genotypes were classified, as well as the discriminant ability of the analyzed variables (internal ethylene production, physicochemical measurements, yield, and TCA). The LDA was visualized through a 'biplot' graph, representing the two dimensions that provide maximum separation among the ten assessed rootstocks. The software package JMP (ver 15.2, SAS Institute) and a significance level of 5% were used for all the statistical analyses.

3. Results

3.1. Climactological Parameters during the Two Production Seasons of the Study

Climatological parameters for Western Maryland between the two production seasons in which this study was carried out were very consistent. There were no statistically significant differences among the two production seasons assayed in this study. Both production seasons in Western Maryland exhibited minimum yearly temperatures (Tmin) ~8.5–8.7 °C and maximum yearly temperatures (Tmax) ~18.2–18.4 °C. In the case of relative humidity, minimum values were ~44–45%, while maximum values were ~82–83% for both years. Regarding total rainfall, both production seasons presented ~775–785 mm of rainfall (Supplementary Figure S1).

3.2. Effect of Roostock Genotypes on 'Buckeye Gala' Tree Size, Yield and Crop Load

Rootstock genotype significantly influenced tree size (F = 22.7; $p \leq 0.0001$) and fruit yield (F = 32.3; $p \leq 0.0001$), but not crop load (F = 0.03; $p = 0.99$) consistently throughout the two seasons (Figure 1A,B).

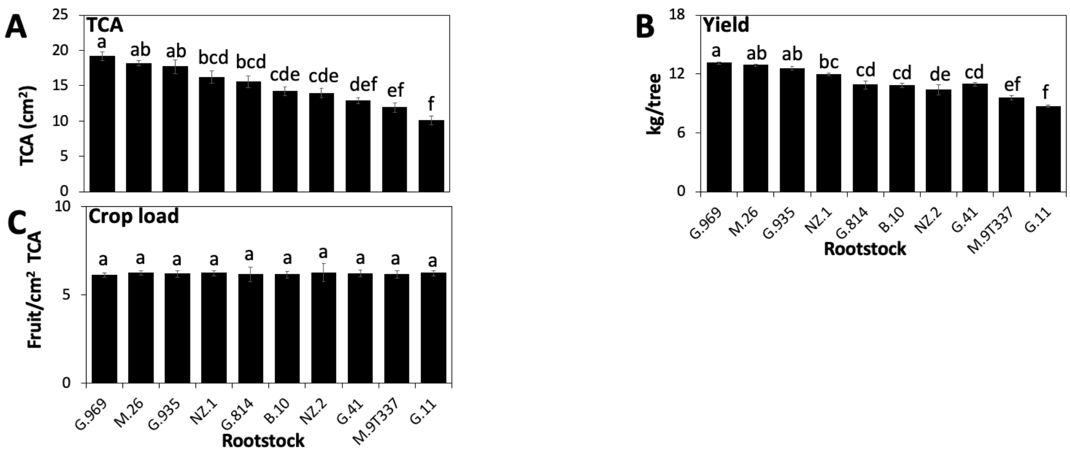

Figure 1. Tree performance measurements evaluated on 'Buckeye Gala' grafted on 10 rootstock genotypes. (**A**) Trunk cross-sectional area (TCA); (**B**) yield; (**C**) crop load. Analysis was performed using mean data of the two production seasons of study. Values are means ± standard error ($n = 5$). Different letters indicate significant differences ($p \leq 0.05$, Tukey's HSD) among genotypes.

'Buckeye Gala' trees on G.969 had significantly larger TCA, except for M.26 and G.935. These were followed by NZ.1, G.814 and subsequently by B.10 and NZ.2 with no significant differences among them. The smallest or less vigorous rootstock was G.11, which presented no statistical differences compared to M.9T337 and G.41. In terms of yield, results followed the same trends as for tree size, with G.969 displaying the significantly highest yield and G.11 the lowest, with the latter not statistically differing only from M.9T337 (Figure 1B).

3.3. Internal Ethylene Concentration and Physicochemical Properties of 'Buckeye Gala' Apple Fruit Grafted on Ten Different Rootstocks

Internal ethylene concentration was significantly influenced by the rootstock genotype (F = 85.8; $p \leq 0.0001$). Highest ethylene concentration occurred with fruit from the scion cultivar 'Buckeye Gala' grafted on G.11, M.9T337, and G.41, followed by NZ.2, and subsequently by B.10, G.814, NZ.1. Fruit from 'Buckeye Gala' on G.935 and G.969 were significantly different from M.26, which had the lowest ethylene concentration values (Figure 2A).

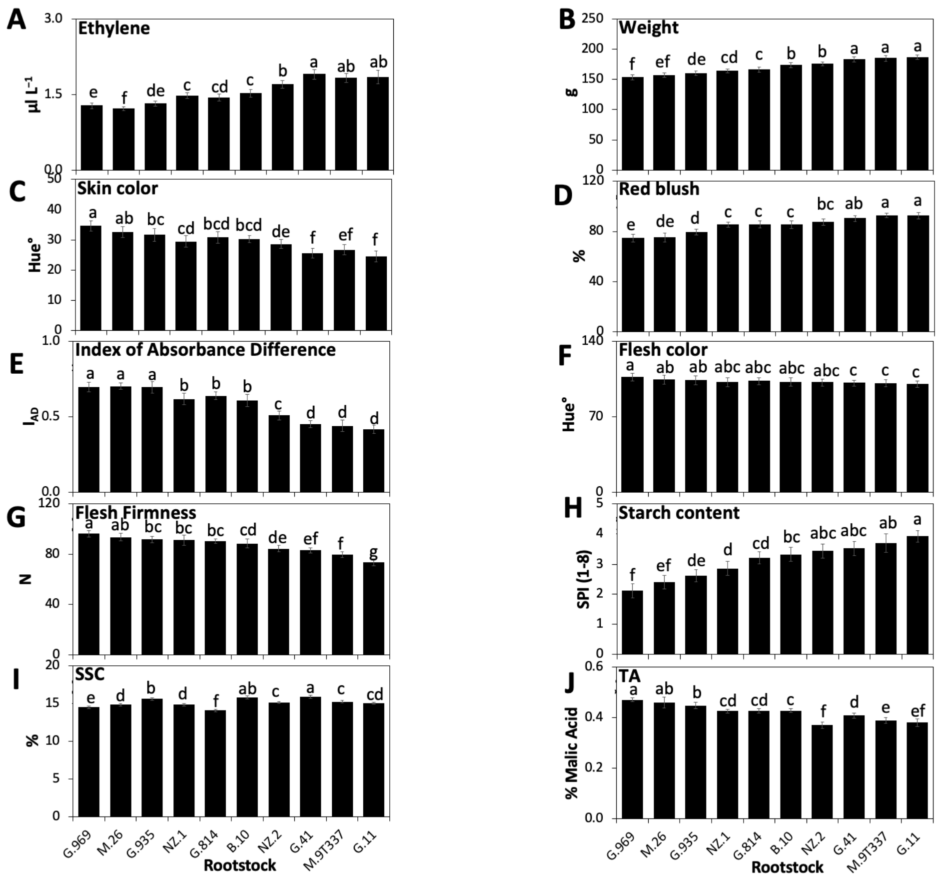

Figure 2. Internal ethylene concentration and physicochemical parameters evaluated at commercial harvest of 'Buckeye Gala' apple fruit grafted on 10 rootstock genotypes. (**A**) Internal ethylene concentration; (**B**) fruit weight; (**C**) skin color; (**D**) skin blush; (**E**) Index of absorbance difference (I_{AD}); (**F**) flesh color; (**G**) flesh firmness; (**H**) starch pattern index (SPI); (**I**) soluble solids contents (SSC); (**J**) titratable acidity (TA). Analysis was performed using mean data of the two production seasons of study. Values are means ± standard error ($n = 5$). Different letters indicate significant differences ($p \leq 0.05$, Tukey's HSD) among genotypes.

Fruit weight displayed similar trends to what was observed for internal ethylene concentration, as well as significant differences (F = 80.5; $p \leq 0.0001$) (Figure 2B). In both years, the significantly highest weight values (>180 g) for 'Buckeye Gala' fruit were obtained from trees grafted on G.11, M.9T337 and G.41. Fruit from NZ.2 and B.10 followed by exhibiting weight values between 173 and 175 g, which were significantly higher than those from fruit grafted on G.814 and NZ.1 (164–166 g). The lowest weight values (154–156 g) occurred with G.969 and M.26 rootstocks.

Skin coloration of the 'Buckeye Gala' fruit was also significantly affected by the rootstock genotype in terms of skin hue angle (F = 31.3; $p \leq 0.0001$), red blush (F = 47.9; $p \leq 0.0001$) and index of absorbance difference (F = 113.7; $p \leq 0.0001$). Skin hue angle values were highest in fruit grafted on G.969 and M.26, followed by G.935 (Figure 2C). The latter did not significantly differ from G.814, B.10 or NZ.1. The statistically lowest values for skin hue angle (indicative of an increase in skin red coloration) were observed for G.11, G.41 and M.9T337. Fruit from the scion cultivar 'Buckeye Gala' grafted on G.11, M.9T337 and G.41 displayed > 90% skin red blush, followed by NZ.2, B.10, G.814 and NZ.1 (Figure 2D). The 'Buckeye Gala' fruit on G.969, M.26 and G.935 rootstock genotypes presented the lowest values with ~75% red blush. Furthermore, evaluation of the index of absorbance difference (I_{AD}) showed, in both assessed production seasons, that fruit grafted on G.969, M.26 and G.935 displayed the statistically highest values (~0.7), indicative of a lower rate of chlorophyll disappearance (Figure 2E). Subsequently, rootstock genotypes NZ.1, G.814 and B.10 followed with values ~0.6, while fruit on NZ.2 displayed statistically lower values of ~0.5. The significantly lowest values for I_{AD} (~0.4) amongst all rootstock genotypes were exhibited by 'Buckeye Gala' fruit grown on G.41, M.9T337 and G.11, evidencing the highest rate of chlorophyll disappearance. Finally, flesh hue angle displayed significant differences only between G.969 (with the highest values) and G.41, M.9T337 and G.11, which displayed the significantly lowest values (Figure 2F).

Fruit flesh firmness was different amongst the ten assayed rootstock genotypes (F = 57.5; $p \leq 0.0001$) (Figure 2G) consistently throughout the two production seasons. Fruit grown on G.969 and M.26 exhibited the highest firmness values (~95 N), while the latter displayed no statistically significant differences with G.935, NZ.1, and G.814, followed by B.10. Subsequently, lower flesh firmness occurred with 'Buckeye Gala' fruit on NZ.2 and G.41 (~84 N), followed by M.9T337 (~80 N). The significantly lowest firmness values for all assayed rootstocks were recorded for G.11 (~74 N).

Rootstock genotype also affected the starch pattern index (F = 35.7; $p \leq 0.0001$) (Figure 2H). In both years, the highest SPI values, indicative of a lower starch content, were exhibited by G.11, M.9T337 followed by G.41 with values ranging between 3.5 and 4; the lowest SPI values (<2.5), indicative of a higher starch content, were obtained for fruit grown on G.969 and M.26. The latter did not differ statistically from G.935. The rest of the evaluated rootstocks displayed SPI values in between the above-mentioned ranges. Soluble solid contents also differed amongst rootstocks (F = 97.6; $p \leq 0.0001$) and were highest (>15.5%) in fruit grown on G.41 and B.10, with the latter presenting no significant differences to G.935 (Figure 2I). Fruit on NZ.2, M.9T337 and G.11 followed with SSC values ~15%, and significantly differed from G.969 which presented values ~14.5%. Fruit on G.814 exhibited the significantly lowest SSC values (~14%) amongst all assayed rootstocks.

Titratable acidity (TA) values also displayed significant differences amongst fruit grown on the ten evaluated rootstocks (F = 65.6; $p \leq 0.0001$) (Figure 2J) consistently throughout the two production seasons. Fruit on G.969 and M.26 exhibited the highest TA values (>0.45), with the latter displaying no differences with respect to G.935. Fruit grafted on B.10, NZ.1 and G.814 presented values ~0.4, followed by fruit on G.41. Finally, fruit in M.9T337, G.11 and NZ.2 presented TA values < 0.4, with the latter presenting the significantly lowest TA values of all evaluated genotypes.

3.4. Relationships among Tree Perdomance Measurements, Ethylene Concentration and Physicochemical Properties of 'Buckeye Gala' Apple Fruit Grafted on Ten Different Rootstocks

Correlation coefficients were calculated (Table 1) and Principal Component Analysis (PCA) was performed to visualize the relationships among all the parameters that presented statistically significant differences amongst rootstock genotypes and described below (Figure 3) during the two assayed production seasons. Additionally, linear discriminant analysis (LDA; Figure 4) was performed to define how the ten rootstock genotypes were clustered as well as to test differences among the defined rootstock genotype clusters.

Table 1. Pearson correlation coefficients among all assessed features in 'Buckeye Gala' fruit including trunk cross-sectional area (TCA), yield, internal ethylene concentration (IEC) and physicochemical parameters throughout two production seasons.

Feature	TCA	Yield	Weight	IEC	SkinHue	FleshHue	I_{AD}	Skinblush	Firmness	SPI	SSC	TA
TCA	1.00	0.97 *	−0.98 *	−0.94 *	0.95 *	0.93 *	0.95 *	−0.95 *	0.97 *	−0.97 *	NS	0.93 *
Yield		1.00	−0.92 *	−0.85 *	0.86 *	0.87 *	0.88 *	−0.93 *	0.93 *	−0.95 *	NS	0.84 *
Weight			1.00	0.97 *	−0.95 *	−0.91 *	−0.96 *	0.95 *	−0.94 *	0.96 *	NS	−0.96 *
IEC				1.00	−0.95 *	−0.89 *	−0.98 *	0.93 *	−0.91 *	0.90 *	NS	−0.97 *
SkinHue					1.00	0.95 *	0.95 *	−0.93 *	0.94 *	−0.92 *	NS	0.97 *
FleshHue						1.00	0.89 *	−0.95 *	0.89 *	−0.92 *	NS	0.92 *
I_{AD}							1.00	−0.92 *	0.94 *	−0.89 *	NS	0.95 *
Skinblush								1.00	−0.89 *	0.96 *	NS	−0.95 *
Firmness									1.00	−0.92 *	NS	0.89 *
SPI										1.00	NS	−0.92 *
SSC											1.00	NS
TA												1.00

All correlations shown are significant (*; $p \leq 0.05$) except NS (non-significant) (index of absorbance difference (I_{AD}), starch pattern index (SPI), soluble solids contents (SSC), titratable acidity (TA)).

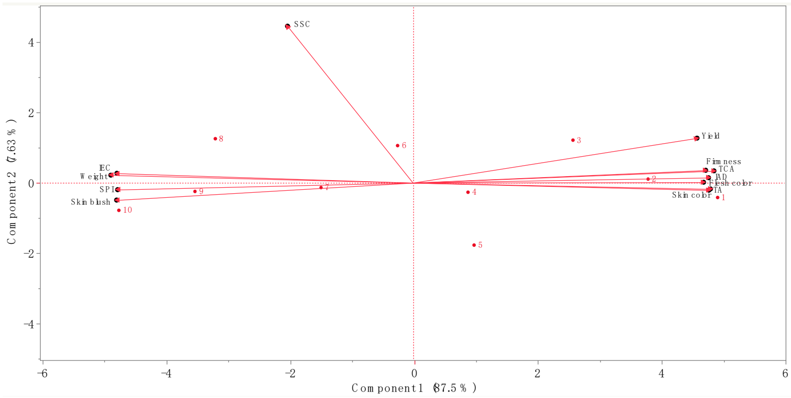

Figure 3. Biplot from Principal Component Analysis of data obtained from trunk cross-sectional area (TCA), yield, internal ethylene concentration (IEC) and physicochemical parameters of 'Buckeye Gala' apple fruit grafted on 10 rootstock genotypes. Analysis was performed using mean data of the two production seasons of study. Physicochemical parameters include weight, skin and flesh color (skin and flesh hue angle), index of absorbance difference (I_{AD}), skin red blush, flesh firmness, starch pattern index (SPI), soluble solids contents (SSC) and titratable acidity (TA). Numbers correspond to each of the 10 assayed rootstock genotypes (1 (G.969), 2 (M.26), 3 (G.935), 4 (NZ.1), 5 (G.814), 6 (B.10), 7 (NZ.2), 8 (G.41), 9 (M.9T337), 10 (G.11)).

TCA significantly and positively correlated with yield (r = 0.97) as well as with the color-related features of skin and flesh hue angle (r = 0.95 and r = 0.93, respectively) and I_{AD} (r = 0.95) as well as with with fruit flesh firmness (r = 0.97) and TA (r = 0.93), but was negatively associated with fruit weight (r = −0.98), IEC (r = −0.94), skin blush (r = −0.95) and SPI (r = −0.97).

Yield displayed significant and positive correlations with skin and flesh hue angles (r = 0.86 and r = 0.87, respectively) and I_{AD} (r = 0.88) as well as with fruit flesh firmness (r = 0.93) and TA (r = 0.84). On the other hand, and as observed for TCA, yield negatively correlated with fruit weight (r = −0.92), IEC (r = −0.85), skin blush (r = −0.93) and SPI (r = −0.95).

IEC was significantly and positively correlated with fruit weight (r = 0.97), skin blush (r = 0.93) and SPI (r = 0.90) (Table 1), while it was negatively correlated with flesh firmness (r = −0.91) and titratable acidity (r = −0.97) as well as with skin and flesh hue angle (r = −0.95 and r = −0.89, respectively) and I_{AD} (r = −0.98).

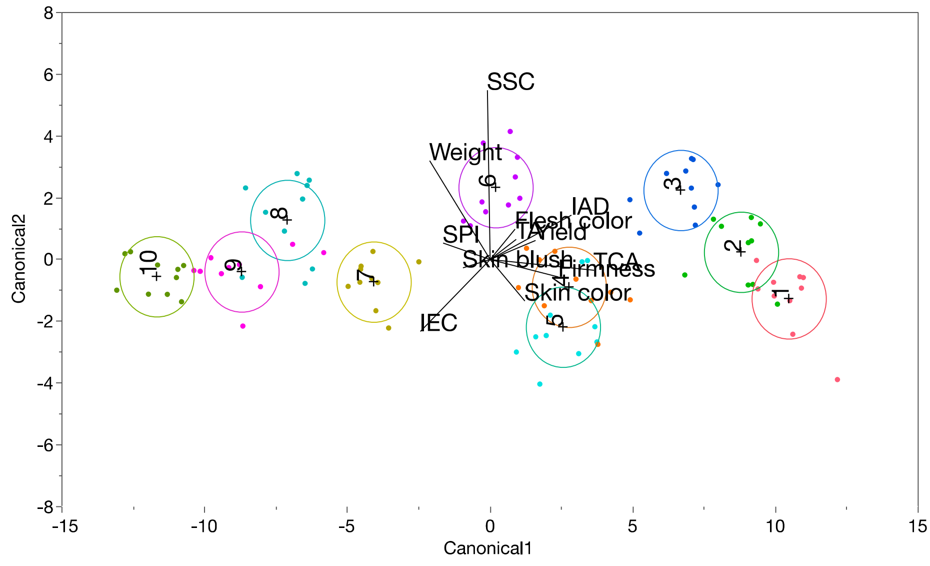

Figure 4. Biplot from Linear Discriminant Analysis of data obtained from trunk cross-sectional area (TCA), yield, internal ethylene concentration (IEC) and physicochemical parameters of 'Buckeye Gala' apple fruit grafted on 10 rootstock genotypes. Analysis was performed using mean data of the two production seasons of study. Physicochemical parameters include weight, skin and flesh color (skin and flesh hue angle), index of absorbance difference (I_{AD}), skin red blush, flesh firmness, starch pattern index (SPI), soluble solids contents (SSC) and titratable acidity (TA). Numbers correspond to each of the 10 assayed rootstock genotypes (1 (G.969), 2 (M.26), 3 (G.935), 4 (NZ.1), 5 (G.814), 6 (B.10), 7 (NZ.2), 8 (G.41), 9 (M.9T337), 10 (G.11)). Each rootstock genotype is represented by data points for each of the five biological replications assayed throughout two production seasons and the plus ("+") sign marker denotes the multivariate mean. A 95% confidence level ellipse is plotted for the mean for each rootstock genotype. If two rootstock genotypes differ significantly, the confidence ellipses tend not to intersect.

Amongst the color-related parameters, skin hue angle was positively correlated with flesh hue angle (r = 0.95) as well as to I_{AD} (r = 0.95), and negatively correlated with skin blush (r = −0.93). On the other hand, skin blush displayed significantly negative correlations with flesh hue angle (r = −0.95) and I_{AD} (r = −0.92) (Table 1).

The parameter of flesh firmness was positively associated with assessed fruit physicochemical properties such as the index of absorbance difference (r = 0.94), skin and flesh color hue values (r ≥ 0.89), as well as with titratable acidity (r = 0.89). Furthermore, there was a significant negative correlation between flesh firmness and fruit weight (r = −0.94), skin blush (r = −0.89) and SPI (r = −0.92) (Table 1).

The starch pattern index presented positive correlations with fruit weight (r = 0.96) and skin blush (r = 0.96) but was negatively associated with skin and flesh color hue (r = −0.92), I_{AD} (r = −0.89) and titratable acidity (r = −0.92) (Table 1). The latter, on the other hand, correlated positively with skin and flesh color hue angle (r ≥ 0.85) and with the index of absorbance difference (r = 0.88), although negatively with fruit weight (r = −0.87) and skin blush (r = −0.89). SSC displayed no significant correlations with any of the assessed parameters in this study.

The PCA showed that the first and second principal components explained 87.5% (Component 1) and 7.63% (Component 2) of the observed variation (95.1% total), respectively (Figure 3). Along the first principal component, separation of the rootstocks was driven by IEC, weight, SPI and skin blush on the negative side of the axis (associated with rootstocks G.11, M.9T337, G.41, NZ.2 and B.10) and by yield, TCA, firmness, skin and flesh hue angle, I_{AD}, and titratable acidity on the positive side of the axis (associated with rootstocks G.969, M.26, G,935, G.814 and NZ.1) (Figure 3).

Furthermore, to test the significant number of clusters into which the ten assayed rootstock genotypes were classified, LDA was performed (Figure 4). The LDA represents the ten rootstock genotype locations in the two dimensions that provide maximum separation among the genotypes (Canonical 1 and Canonical 2). A lack of intersection between the confidence ellipses (significance level of 5%) between rootstock genotypes indicates that these genotypes differ significantly from each other and thus conform to independent clusters. The LDA results show that the ten rootstock genotypes were discriminated into seven statistically significant distinct clusters: Genotype #10 (G.11); Genotypes #8 (G.41) and #9 (M.9T337); Genotype #7 (NZ.2); Genotype #6 (B.10); Genotypes #4 (NZ.1) and #5 (G.814); Genotype #3 (G.935) and Genotypes #1 (G.969) and #2 (M.26) (Figure 4). This result supports the distribution of the rootstock genotypes throughout Component 1 of the PCA (Figure 3).

Genotype #10 (G.11) constituted the first cluster and displayed the closest association with IEC, SPI, skin blush and weight. A second cluster was composed of Genotype #9 (M.9T337) in combination with Genotype #8 (G.41) and located close to the same parameters as the first cluster. Genotype #7 (NZ.2) clustered on its own with a lower association with IEC, SPI, and skin blush. Genotype #6 (B.10) constituted a fourth cluster, displaying an intermediate positioning, and closer association with the parameters of fruit weight and SSC. The fifth cluster included Genotypes #4 (NZ.1) and #5 (G.814) associated with TCA, firmness, and skin hue angle, while Genotype #3 (G.935) constituted an independent cluster with a closer location to the parameters of flesh hue angle, I_{AD}, TA and yield, as well as to TCA, firmness, and skin hue angle. Finally, the last cluster comprised Genotypes #1 (G.969) and #2 (M.26) and displayed the closest association with the parameters of skin and flesh hue angles, TCA, firmness, as well as to yield, I_{AD} and TA, and the furthest positioning with respect to the parameters of IEC, SPI, skin blush and weight (Figure 4).

4. Discussion

Rootstocks are a key component of apple production systems and can significantly influence scion cultivar physiology. Although there are several reports on the effects of rootstocks on apple maturity and quality characteristics [6,14,15,28,30–33,40,54,55], the results are inconsistent from site to site and/or for different scion cultivars. Particularly, there is limited information about rootstock effects on maturity and quality of apples grown in Western Maryland. Although in some studies, rootstock effects have also been reported to vary across years, climatological parameters in this study were consistent. In the present study, there was a general trend for delayed fruit maturity, lower fruit weight and higher yield with increasingly vigorous rootstocks.

Tree size can be affected by rootstock genotype as estimated using TCA [4,10,32–34,39]. Consistent with our results, M.26 has been reported to be significantly more vigorous than the dwarfing M.9T337 and to have similar vigor to the semidwarf rootstocks G.935 and G.969 when using 'Gala' as a scion [34,56–58]. Furthermore, G.41 was shown to present the lowest vigor together with M.9T337 when grafted with 'Buckeye Gala' in a 7-year trial in Northern Mexico [59]. Nevertheless, in the latter work, G.11 displayed an intermediate vigor, differing from G.41 and M.9T337, and thus from our results. These differences in rootstock performance can be explained by climatic variations between the arid conditions in Northern Mexico and the humid conditions in Western Maryland. On the other hand, in studies under New York climactic conditions, G.11 exhibited the smallest trees [11], and was classified as a dwarfing rootstock together with G.41, M.9T337, and B.10, coinciding with our observations [4]. B.10 has been described to have a tree size between M.9T337 and M.26 rootstocks [60], which supports our findings. The novel NZ.1 and NZ.2 rootstocks, which have not yet been described in the literature, could be categorized as semidwarf and dwarf, respectively, under our specific conditions.

Regarding yield, this parameter has also been shown to be impacted by rootstock genotype [11,32,34,40,56]. In agreement with our results, trees on G.935 and G.969 displayed significantly higher yields than M.9T337 rootstocks using 'Gala' 'Fuji', and 'Golden Delicious' as scions [11,34,57]. The positive correlation obtained between TCA and yield support the similar result trends obtained for both parameters for all assessed rootstocks and could be explained by the increased bearing surface that more vigorous rootstocks have compared to more dwarfing rootstocks.

Rootstock genotypes have also been reported to have an influence on fruit weight, but this influence may differ from site to site and between scion cultivars [40]. Nevertheless, and consistent with our results, several studies conducted in different locations showed that there were significant effects of rootstock on 'Gala' fruit weight [2,11,27,33]. Previous work showed that 'Gala' fruit grown on G.41 displayed larger fruit than on M.26 [14], in agreement with this study, suggesting that dwarfing rootstocks, with lower yield, increase fruit weight. This supports the negative correlation obtained between fruit weight and both TCA and yield in this work and agrees with previous studies [32]. Although it has been shown that crop load is negatively related to fruit weight [37,61,62] as a result of fruit-to-fruit competition [63], in this study, there were no differences in crop load, suggesting that rootstock genotype is an important factor in determining fruit weight in 'Buckeye Gala'.

Apple fruit display a climacteric fruit ripening behavior; thus, IEC has been reported to play a key role in controlling fruit maturity in apples [21]. Previous results in the influence of rootstock genotypes on IEC are contradictory, as some have found no relationships [6] while others have found significant effects of rootstocks on fruit scion IEC [14,25,26,54,55]. In agreement with the latter, our results show a consistent effect of rootstocks on IEC of 'Buckeye Gala' fruit at harvest. Furthermore, the positive correlations of IEC with fruit weight, skin blush and SPI, as well as the negative associations of IEC with flesh firmness, TA, skin and flesh hue angle and index of absorbance difference (I_{AD}), are in agreement with other studies [24,55] and are indicative of IEC promoting an increase in fruit maturity. The highest IEC values in 'Buckeye Gala' fruit at harvest were associated with fruit from the dwarfing and lower yielding G.11, M9.T337 and G.41 rootstocks. Studies reporting that scion cultivars grafted in dwarfing rootstocks have an advanced fruit maturity [30,54,64] are consistent with our findings, and support the negative correlation obtained between IEC and both TCA and yield. The capacity of rootstocks to modify internal hormone levels in xylem sap of grafted scions in apples [39] also support our results.

Fruit coloration is of key importance for fruit quality, as it is directly tied to consumer preference and market value [35]. On the other hand, changes from green to yellow in the background color of apples, through a decrease in the values of chlorophyll disapearance (I_{AD}), have been shown to be associated with increased fruit maturity [51]. These results support the significantly negative correlations between I_{AD} and IEC obtained for fruit in all trees on all assessed rootstocks in this work, and is consistent with other studies in apples [55] and peaches [24,51]. Furthermore, in the present study, rootstocks dramatically affected 'Buckeye Gala' fruit red coloration and chlorophyll disappearance, in agreement with other authors using 'Gala' and 'Fuji' as scion cultivars [11,14,32,55]. Consistent with our results, 'Gala' fruit grafted on dwarfing rootstocks such as M9.T337 displayed the highest percentage of red skin coloration compared to more vigorous rootstocks such as G.969 [34]. It has been reported that differences in coloration can be a consequence of the reduced canopy size of dwarfing rootstocks, which allows better sunlight distribution across the canopy that consequently increases red skin coloration [30,35,41,43]. Furthermore, it has been shown that rootstocks can have significant effects on the metabolic composition of the phenylpropanoid and flavonoid pathways, which are crucial for the development of red coloration of the scion cultivar [65].

Fruit flesh firmness impacts fruit softening ability and is directly associated with cell wall modifications along with changes in turgor pressure [66,67]. Several authors have shown significant effects of rootstocks on fruit flesh firmness, indicating these can vary from site to site [14,15,25,32,33,40,55,68]. Consistent with the positive correlation obtained between TCA and yield with flesh firmness in this study, higher flesh firmness in the 'Gala' fruit from the more vigorous rootstocks such as M.26 compared to the dwarfing G.41 has been reported [14]. These results can be partially explained by the higher IEC displayed by fruit from dwarfing rootstocks, as negative correlation between IEC and flesh firmness has been widely shown in different fruit [19,21,24,69,70]. Furthermore, the negative correlation between flesh firmness and fruit weight obtained in this study agrees with the findings of previous authors assessing effects of different apple rootstocks on various apple scion cultivars, including 'Gala' [26,32,33,40,55]. Additionally, the higher flesh firmness displayed by fruit grafted in the more vigorous and higher yielding rootstocks in this work can also be supported by their higher I_{AD} values, as a positive correlation between these parameters is consistent with studies in apples [55] and peaches [24,71,72]. The differences in flesh firmness detected in this study may be noticeable by consumers at harvest, as it has been reported that consumers can detect differences in firmness that are greater than 6 N [73]. However, these differences might not be maintained in fruit after postharvest storage, as has been shown in previous studies [14]. Fruit texture, and overall quality, is affected by numerous variables during storage [74] that have not been accounted for in this study, but are currently under investigation.

Starch pattern index (SPI) and soluble solid contents (SSC), which provide energy and carbon structural sources during fruit growth and, together with titratable acidity contribute to apple overall taste [75], were significantly influenced by rootstock genotypes, in agreement with previous studies [6,25,30,31,33]. The negative correlations between SPI with both TCA and yield, together with the significant positive correlations between IEC and SPI, suggest that the dwarfing and more vigorous rootstocks can be advancing and delaying fruit maturity in 'Buckeye Gala', respectively. Although starch in the fruit flesh is converted to sugars, and thus it would be expected that there was an increase in SSC as starch content decreases, no significant correlation between SPI and SSC was detected in this study. This result can be explained by the fact that sugars present in the fruit are not only supplied by the degradation of starch, but are also translocated to fruit from other sources. Sugars can be synthesized in leaves and translocated to fruit [20,69,76]. Particularly in apples and other members of the *Rosaceae* family, the sugar–alcohol sorbitol is translocated to the fruit along with sucrose [77], contributing to the fruit SSC. The lack of significant correlations of SSC with other variables in this study could be due to the particular environmental conditions of Western Maryland.

Particularly in this study, the ten assessed rootstock genotypes were discriminated into seven statistically distinct clusters by LDA. The clusters composed of rootstock G.11 as well as M.9T337 and G.41 correspond with these genotypes displaying the lowest TCA values (i.e., displaying the most dwarfing trees), lowest yield, as well as presenting the highest IEC and thus the most advanced maturity for 'Buckeye Gala' fruit, impacting fruit quality. In contrast, the two clusters comprising G.935 as well as M.26 and G.969 are characterized by exhibiting the highest TCA, highest yield, and the least mature fruit of all the assessed genotypes. This is in agreement with previous studies indicating that apple fruit in the most dwarfing rootstocks resulted in the earliest ripening [30,54,64]. The novel NZ.1 and NZ.2 rootstocks clustered separately, with NZ.1 grouping together with the semidwarf G.814 and characterized by displaying a more vigorous tree, higher yield, and less mature 'Buckeye Gala' fruit with respect to NZ.2 under the conditions of this study. Rootstock B.10 represented a transition-like genotype in terms of vigor, yield, and maturity, with a closer association with SSC supported by the highest values it displayed for this parameter. However, the resulting clustering of 'Buckeye Gala' fruit may be only applicable for fruit grown under Western Maryland hot and humid conditions. Thus, this study needs to be replicated in major production regions of 'Gala' with different environmental conditions, such as the Pacific Northwest (hot and dry climate) to assess the transferability of these results between regions. Furthermore, these results are specific for 'Buckeye Gala' as scion cultivar, and future work is required to include a wide range of scion cultivars to assess their robustness of these results.

5. Conclusions

'Buckeye Gala' scion grafted in a diverse panel of ten rootstock genotypes under Western Maryland environmental conditions showed that there was a trend for delayed fruit maturity, lower fruit weight and higher yield with increasingly vigorous rootstocks. Significant differences existed amongst rootstocks with regard to their impacts on vigor, yield, ethylene concentration, weight, color, firmness and starch and sugar contents, as well as acidity at harvest. Strong correlations were also detected amongst most of the assessed variables. Consequently, rootstock impact must be considered when making preharvest and postharvest management decisions in 'Buckeye Gala' fruit grown under Western Maryland conditions. Further research is warranted for assessing the consistency of the influence of rootstocks across contrasting environments, different scion cultivars, as well as after postharvest storage.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy13102528/s1. Figure S1: Climatological parameters during the two production seasons of the study.

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