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Rootstock vigor dictates the canopy light environment that regulates metabolite profile and internal fruit quality development in peach

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Abstract:	Five rootstock cultivars of differing vigor: vigorous ('AtlasTM' and 'Bright's Hybrid® 5'), standard ('Krymsk® 86' and 'Lovell') and dwarfing ('Krymsk® 1') grafted with 'Redhaven' as the scion were studied for their impact on productivity, mid-canopy photosynthetic active radiation transmission (i.e., light availability) and internal fruit quality. Average yield (kg per tree) and fruit count increased significantly with increasing vigor (trunk cross sectional area, TCSA). A detailed peach fruit quality analysis on fruit of equal maturity (based on the index of absorbance difference, IAD) coming from trees with equal crop load (no. of fruit cm-2 of TCSA) characterized the direct impact of rootstock vigor on peach internal quality [dry matter content (DMC) and soluble solids concentration (SSC)]. DMC and SSC increased significantly with decreasing vigor and increasing light availability, potentially due to reduced intra-tree shading and better light distribution within the canopy. Physiologically characterized peach fruit mesocarp was further analyzed by non-targeted metabolite profiling using gas chromatography mass spectrometry (GC-MS). Metabolite distribution was associated with rootstock vigor class, mid-canopy light availability and fruit quality characteristics. Fructose, glucose, sorbose, neochlorogenic and quinic acids, catechin and sorbitol were associated with high light environments and enhanced quality traits, while sucrose, butanoic and malic acids related to low light conditions and inferior fruit quality. These outcomes show that while rootstock genotype and vigor are influencing peach tree productivity and yield, their effect on manipulating the light environment within the canopy also plays a significant role in fruit quality development.
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December 6th, 2023

Dear Dr. De Tullio editor-in-chief of 'Plant Physiology and Biochemistry',

Please find enclosed our manuscript titled '**Rootstock vigor dictates the canopy light environment that regulates metabolite profile and internal fruit quality development in peach**' by Jeff Pieper, Brendon Anthony, Jacqueline Chaparro, Jessica Prenni, and Ioannis Minas.

We are pleased to submit this manuscript for consideration to be published in the '**Plant Physiology and Biochemistry'**. In this manuscript we demonstrate: **that while rootstock genotype and vigor are influencing peach tree productivity and yield, their effect on manipulating the light environment within the canopy also plays a significant role in fruit quality development.**

A study on tree vigor as a biological system to investigate the role rootstock selection, a critical preharvest factor, has on fruit quality development and metabolism. Care was taken to eliminate the confounding factors of crop load and maturity, two factors that are often ignored in fruit quality studies. The trees were thinned based on trunk cross sectional area to ensure the number of fruit per centimeter of trunk was not different across vigor profiles. Additionally, the use of Vis/NIRS technology allowed for sampling of equal maturity fruit for destructive fruit quality, and non-targeted GC-MS analysis. This novel approach enabled comparisons between rootstocks with differing vigor profiles, without the confounding influence of maturation. Physiologically, when fruit of equal maturity coming from dwarfing trees were compared to vigorous, standard, and semi-dwarfing trees, superior quality enhancements were noted, underscoring the direct impact of rootstock vigor on fruit internal quality and primary metabolism.

Our results demonstrated that fruit from dwarfing rootstock canopies which also had greater light availability in the canopy, had higher dry matter content, soluble solid concentrations and an enhanced primary metabolism. Metabolite distribution was associated with rootstock vigor class, mid-canopy light availability and fruit quality characteristics. Fructose, glucose, sorbose, neochlorogenic and quinic acids, catechin and sorbitol were associated with high light environments and enhanced quality traits, while sucrose, butanoic and malic acids related to low light conditions and inferior fruit quality.

Ultimately, the results of this study suggest that that optimization of preharvest factors, where quality and the assimilation of carbohydrates occurs, can facilitate the up-accumulation of several primary metabolites that prime and enhance the taste, flavor, aroma and quality of the fruit. These results yield implications for proper rootstock selection to ensure superior quality, as well as future molecular signatures that could be used to target enhanced quality fruit.

As an original manuscript related to the fruit quality development and metabolism, we believe this technical research approach and the significance of findings fit the aims and scope of the 'Plant Physiology and Biochemistry'. This manuscript has not been published and is not under consideration for publication elsewhere and all authors have read and agreed in this final version of the manuscript.

A list of six potential reviewers' experts in the field of pomology, tree fruit physiology, horticulture chemistry, peach pre- and postharvest physiology, plant and fruit metabolism, metabolomic analysis, fruit and food quality is provided bellow:

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Thank you for your consideration!

Sincerely,

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Highlights

- Peach rootstocks of varying vigor assessed for productivity, light availability, fruit quality and metabolism.
- Fruit quality increased with decreasing vigor and increasing light availability.
- Enhanced quality associated with sorbitol, monosaccharides, neochlorogenic and quinic acids and catechin.
- Inferior quality associated with sucrose, butanoic and malic acids.
- Rootstock-manipulated light environment drives fruit quality development.

Rootstock vigor dictates the canopy light environment that regulates metabolite profile and internal fruit quality development in peach

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Abstract

Five rootstock cultivars of differing vigor: vigorous (‘Atlas™’ and ‘Bright’s Hybrid® 5’), standard (‘Krymsk® 86’ and ‘Lovell’) and dwarfing (‘Krymsk® 1’) grafted with ‘Redhaven’ as the scion were studied for their impact on productivity, mid-canopy photosynthetic active radiation transmission (i.e., light availability) and internal fruit quality. Average yield (kg per tree) and fruit count increased significantly with increasing vigor (trunk cross sectional area, TCSA). A detailed peach fruit quality analysis on fruit of equal maturity (based on the index of absorbance difference, I_{AD}) coming from trees with equal crop load (no. of fruit cm^{-2} of TCSA) characterized the direct impact of rootstock vigor on peach internal quality [dry matter content (DMC) and soluble solids concentration (SSC)]. DMC and SSC increased significantly with decreasing vigor and increasing light availability, potentially due to reduced intra-tree shading and better light distribution within the canopy. Physiologically characterized peach fruit mesocarp was further analyzed by non-targeted metabolite profiling using gas chromatography mass spectrometry (GC-MS). Metabolite distribution was associated with rootstock vigor class, mid-canopy light availability and fruit quality characteristics. Fructose, glucose, sorbose, neochlorogenic and quinic acids, catechin and sorbitol were associated with high light environments and enhanced quality traits, while sucrose, butanoic and malic acids related to low light conditions and inferior fruit quality. These outcomes show that while rootstock genotype and vigor are influencing peach tree productivity and yield, their effect on manipulating the light environment within the canopy also plays a significant role in fruit quality development.

Keywords: *Prunus persica*, dry matter content, index of absorbance difference, gas chromatography mass spectrometry, light availability, non-targeted metabolomics, near-infrared spectroscopy

Abbreviations: FF, flesh firmness; DMC, dry matter content; SSC, soluble solids concentration I_{AD}, index of absorbance difference; HDPs, high-density plantings; TCSA, trunk cross sectional area.

1. Introduction

Proper orchard design is critical for maximizing yield and fruit quality in peach (*Prunus persica* Batsch L.) (Anthony and Minas, 2021). Rootstock selection is an important factor when considering orchard design and planting densities (Minas et al., 2018; Anthony and Minas, 2021). Historically, few peach rootstocks have been used in production systems, with the majority being peach seedlings. An effort to increase the number of rootstock selections available to combat biotic and abiotic stress has led to a drastic increase in peach rootstock availability. These breeding efforts have revolved around identifying traits tolerant to various soil related issues (Minas et al., 2023a). For example, rootstock breeding efforts in Europe have focused on interspecific hybrids as they possess superior traits for tolerance to high pH, drought, salinity, water logging, and fungal diseases (Reighard, 2000; Reighard and Loreti, 2008; Minas et al., 2023a). Such efforts led to the widespread adoption of the peach-almond hybrid rootstock 'GF 677' in peach growing areas across Europe. More recent rootstock investigations have shown plum, and plum hybrid, rootstocks to be more tolerant of replant conditions (Jimenez et al., 2011). The continued focus on peach rootstock breeding has produced a variety of potential selections, however, their adaptation to biotic and abiotic stressors as well as the physiological traits imparted to the scion remain largely unknown (Rubio-Cabetas, 2009). Interspecific hybrid rootstocks from Europe have potential to provide the US with rootstock traits that have shown a superior ability to tolerate many of the pedoclimatic and disease issues growers grapple with (Manganaris et al., 2022). The NC-140 project is a United States Department of Agriculture (USDA) multistate research effort examining the suitability of various peach rootstocks across different growing regions in the US (Reighard et al., 2020; Minas et al., 2022; 2023a). In addition to providing tolerance to biotic and abiotic stressors, rootstock selection also has the ability to impact orchard design by manipulating the scion's physiological factors.

Rootstocks influence the size of the canopy, thus dictating orchard design and planting densities (Webster, 1995). Rootstock can affect tree growth/vigor, precocity, productivity, fruit size and above ground dry matter accumulation (Caruso et al. 1997). More vigorous rootstocks can bear a higher number of flowers per tree as they generate larger fruiting areas (Fournier et al., 1998). However, vigorous rootstocks have also shown delayed precocity and fruit maturation and can be more expensive for growers to manage as they require more labor for pruning, thinning, and harvesting (Webster, 2002; Iglesias et al., 2022). Vigor-limiting rootstocks are widely available for apple and cherry, and have enabled successful high-density plantings (HDPs), while the production and evaluation of suitable size-controlling rootstocks for peach have recently come into focus (Gao et al., 1994; Reighard, 2002; 2020; DeJong et al., 2004; Minas et al., 2022; 2023a).

Few studies have investigated the impact of rootstock on fruit quality characteristics beyond fruit size (Albas et al., 2004). With those that have, few controlled for confounding variables that influence fruit quality such as crop load and physiological maturity (Anthony et al., 2020; Anthony and Minas, 2022). Throughout on-tree ripening and maturation, fruit undergo several organoleptic and quality transitions (Minas et al., 2023b). These include sensorial and textural changes, such as flesh softening, aromatic volatilization, pigment accumulation, increasing dry matter content (DMC) and soluble solids concentration (SSC); parameters that relate well to consumer satisfaction (Crisosto and Costa, 2008). Vigor-limiting rootstocks have shown enhanced fruit quality characteristics across a range of canopy positions (Gullo et al., 2014). Vigor-limiting rootstocks have also been shown to enhance DMC and SSC compared to other rootstocks in Mediterranean and Western USA climates (Fonti i Forcada et al., 2012; Reig et al., 2020; Minas et al., 2023c). Overall, previous rootstock studies demonstrated that reduced vigor increases fruit quality characteristics (e.g., DMC, SSC, and overcolor), but have been limited in their ability to characterize the direct impact these genotypic differences have on fruit quality due to their lack of maturity control in their experimental approaches (Anthony et al., 2020; 2021; Anthony and Minas, 2022). In other words, it is difficult to understand whether the observed impact of these vigor-limiting rootstocks on fruit quality can be attributed to the canopy environment resulting from the rootstock and/or the maturity status of the sampled fruit (Anthony and Minas, 2022).

Another important aspect for investigation is how rootstock selection (i.e., vigor control) manipulates the light environment within the canopy and how those microclimates influence fruit quality development (Gullo et al., 2014). Carbon partitioning differences between various rootstocks show reduced shoot extension in dwarfing genotypes (Basile et al., 2003; Solari and DeJong., 2006). In apple, dwarfing rootstocks have also been shown to alter structural tree development by growing fewer and shorter, axillary shoots, which subsequently grow shorter shoots with increased levels of return bloom (Seleznyova et al., 2008). The reductions in canopy development (i.e., shoot extension) alter light environments by reducing intra-canopy shade for the developing fruit and lead to enhanced and homogenous fruit quality (Gullo et al., 2014). This is critical as fruit quality appears to be directly linked to the light environment, rather than just the position in the canopy or rootstock genotype alone (Lewallen and Marini, 2003; Anthony et al., 2021). Therefore, optimal selection and adoption of vigor-limiting, dwarfing or semi-dwarfing rootstocks in peach, can increase canopy zone light availability and light distribution uniformity (Anthony and Minas, 2021). Maintaining uniform light distribution throughout the canopy can lead to more homogenous fruit maturation and quality across the tree (Anthony et al., 2021), which yields fruit that can be harvested with enhanced quality characteristics and with a reduced number of picks.

As mentioned, the maturity status of the fruit influences quality parameters, but it also affects the fruit's biology. This is because fruit ripening and maturation is a highly regulated process at the molecular level (Giovannoni et al., 2017). Without selecting for fruit of uniform maturity, biological investigations on preharvest factor manipulation (e.g., rootstock selection) are limited (Anthony et al., 2020). With the development of non-destructive technologies that can accurately and reliably predict physiological maturity and quality in a single scan (Minas et al., 2021; 2023c), across different cultivars (Anthony et al., 2023a) maturity control and biological investigations into the role preharvest factors have on fruit metabolism are enabled (Anthony et al., 2020; Anthony and Minas, 2022).

Previous studies investigating the role of rootstock on metabolomic characteristics in peach fruit are limited (Albás et al., 2002; Tavarini et al., 2011; Gullo et al., 2014) and none controlled for fruit maturity. Precise metabolomic investigations across rootstock genotypes may provide insight into how quality is developed and influenced by this critical preharvest factor. Previous -omics studies in peach have identified critical pathways that may be involved with quality development, such as the phenylpropanoid, shikimic and glycolytic pathways, which synthesize primary and secondary metabolites that relate to quality, including catechin, shikimic acid, sucrose, and sorbitol (Anthony et al., 2020; 2021; 2023b). The present study seeks to identify biological targets and metabolic processes that correspond to peach fruit quality development that may be affected as a result of the canopy environment generated by rootstocks of variable vigor. In this study, fruit of equal maturity and from uniform canopy position, from trees with equal crop loads, across five rootstock genotypes, were analyzed for their internal quality and primary metabolome as analyzed by gas-chromatography-mass spectrometry (GC-MS). This study examines the relationship between preharvest factors and their impact on fruit quality parameters. In this case, detailing how rootstock vigor affects the internal quality and metabolic profiles of fruit harvested at equal maturity.

2. Materials and Methods:

2.1. Plant material and experimental approach

Research was conducted during the 2019 season at Colorado State University's experimental orchard at the Western Colorado Research Center-Orchard Mesa, Grand Junction, CO (39°02'31.3"N, 108°27'56.8"W). The semi-arid site is located at roughly 1430 m in elevation and consists primarily of Turley clay loam, featuring 30 % clay, 1.3 % organic matter and a pH of 8.3. The block used for the study was planted in 2009 as part of a United States Department of Agriculture (USDA) North Central (NC) 140 (NC-140) Regional Project's peach [*Prunus persica* (L.) Batsch] rootstock evaluation trial using 'Redhaven' as the scion cultivar (Reighard et al., 2020). Trees were planted in a randomized complete block design (RCBD) at a spacing of 4 x 5 m (509 trees ha^{-1}) and trained to an open-vase system. Standard local commercial practices for irrigation, fertilization and pest management were used to manage the trees. Within this plot, five rootstocks in three distinct classes of vigor (vigorous, standard, dwarfing) were identified for further investigation. These rootstock genotypes included: 'Bright's Hybrid® 5' (BH5) and 'Atlas™' (ATL) (vigorous), 'Krymsk® 86' (K86) and 'Lovell' (LOV) (standard), and 'Krymsk® 1' (K1) (dwarfing). Five healthy trees of uniform vigor were selected from each rootstock genotype for a total of 25 trees for the entire experiment.

Trunk cross sectional area (TCSA, cm^2) was used to distinguish differences in rootstock vigor. TCSA was calculated after measuring the trunk circumference at 15 cm above the graft union. Crop load (fruit cm^{-2} TCSA) was standardized for all rootstock genotypes by hand thinning trees to 1.4 fruit cm^{-2} of TCSA, on average. An effort to balance fruit distribution throughout the canopy was made while thinning. Canopy volume was also determined by measuring the canopy height, width, and length (m^3).

One day post-harvest (13 August 2019; 125 DAFB), photosynthetic active radiation (PAR) was measured to determine canopy zone light availability for each tree at 0.5 and 1.5 m, using a line quantum sensor (LI-191, LI-COR Biosciences, Lincoln, NE, USA). Measurements were taken ± 1 hr of solar noon in each cardinal direction, according to the methods laid out in Anthony et al. (2021). An incident PAR measurement was taken at the beginning of each row, prior to measuring light at each tree, using the Li-Cor 190R Quantum Sensor (Li-Cor Biotechnology, Lincoln, NE, USA). All data was logged with the Li-Cor LI-1500 Light Sensor Logger (Li-Cor Biotechnology, Lincoln, NE, USA). Light availability (LA, %) was calculated as 100 x (average PAR at each position / average total PAR).

2.2. Fruit quality analyses

To characterize the direct impact of rootstock genotype on peach fruit quality canopy height measurements across all rootstocks were used to establish an average optimal fruiting zone at a canopy height of 1.5 m. At harvest, five fruit at 1.5 m from each tree were selected for equal optimal maturity using a pre-calibrated non-destructive Vis-NIRS sensor (DA meter T.R. Turoni, Sinteleia, Bologna, Italy). This tool assesses peach physiological maturity based on the chlorophyll levels (index of absorbance difference, I_{AD}) of the background color underneath the skin (Costa et al., 2009; Ziosi et al., 2008). In this trial, fruit were selected within a range of 0.40 – 0.60 I_{AD}.

Destructive fruit quality analyses were conducted on fruit from each rootstock genotype (five reps × five fruit; n=25).

Fruit of equal maturity were analyzed to understand the direct impact of rootstock vigor on internal fruit quality. Each fruit was evaluated for size (mm), fresh weight (FW) and overcolor blush percent coverage (%). Fruit exocarp color measurements were conducted with a portable colorimeter (Minolta CR-20, Minolta, Osaka, Japan), on the sun exposed, blushed and the shaded portions of each fruit. Lightness coefficient (L^*), which ranges from black = 0 to white = 100, and hue angle (h^o), which describes color that is closest to human perception numerically, were used to determine differences in fruit overcolor (Minas et al., 2015). Additional destructive analyses were conducted to evaluate fruit flesh firmness (FF, N), dry matter content (DMC), soluble solids concentration (SSC) and titratable acidity (% malic acid) according to Minas et al. (2021).

2.3. Non-targeted metabolite profiling using gas chromatography mass spectrometry (GC-MS)

Following quality analysis, five biological replicates (i.e., tree) consisting of five homogenized fruit mesocarp samples coming from the selected equally mature fruit from each rootstock were sampled, flash frozen with liquid nitrogen (i.e., quenched) and stored at -80 ^{o}C until analysis. Prior to -omics analyses, peach mesocarp was freeze-dried (Freezone 4.5, Labconco, Kansas City, MO, USA) at -40 ^{o}C for 12 h. Lyophilized peach mesocarp samples (n=25) of equal maturity were homogenized with a bead beater (Bullet Blender Storm, Next Advance, Troy, NY, USA) for five minutes. Mesocarp extraction and derivatization were performed according to Anthony et al. (2020), by suspending 25 ± 1 mg of each sample tissue in a two mL autosampler glass vial (VWR, Radnor, PA, USA) with one mL of 80 % methanol (MeOH) in LC-MS grade water solution. After centrifuging samples, 800 µL of each sample's supernatant was transferred into a new vial. A pooled quality control (QC) was created by transferring 10 µL of each sample into a separate glass vial. A total of 11 QCs were created by transferring 5 µL of the pooled QC into 11 new vials. Five µL of each of the samples' supernatant were also transferred into new vials for derivatization. All 25 samples and 11 QCs were then centrifuged and dried down with nitrogen gas prior to derivatization.

Immediately prior to running the samples, derivatization (methoximation and silylation) occurred according to Anthony et al. (2020), by suspending dried down samples in 50 µL pyridine containing 15mg mL^{-1} of methoxyamine hydrochloride (prewarmed to 60 ^{o}C) and 50 µL of N-Methyl-M (trimethylsilyl) trifluoroacetamide (MSTFA) + 1 % trimethylchlorosilane (TMCS) (ThermoFisher Scientific, Waltham, MA, USA) (Chaparro et al., 2018). Samples were loaded (~90 µL) into glass inserts within glass autosampler vials and centrifuged prior to GC-MS analysis (Anthony et al., 2020).

GC-MS was performed on a Clarus 690 GC coupled to a Clarus SQ 8S Mass Spectrometer (PerkinElmer, Waltham, MA, USA). A 30 m TG-5MS column (Thermo Scientific, 0.25 mm i.d. 0.25 µm film thickness) was used to separate metabolites. The GC program scanned masses between 50-620 m/z at four scans s^{-1} after electron impact ionization following protocols from Anthony et al. (2020). A slit control of 12 mL min^{-1} was used. QC samples were run after every 6th sample to account for analytical variation.

Processing for metabolomic data followed procedures detailed in Chaparro et al. (2018) and Anthony et al. (2020). GC-MS files were converted to .cdf format and processed by XCMS in R (Smith et al., 2006; Mahieu et al., 2016; R Core Team, 2015). Total ion current (TIC) was used to normalize all samples. Peak deconvolution into spectral clusters occurred in RAMClust to facilitate metabolite annotation (Broeckling et al., 2014). Metabolites were annotated in

RAMSearch (Broeckling et al., 2016) using retention time, retention index and spectral matching against external spectral databases including Golm Metabolome Database (Hummel et al., 2007; Hummel et al., 2013) and NIST (http://nist.gov).

2.4. Statistical analyses

Mean comparisons across rootstock genotypes for tree physiological and agronomical characteristics, fruit quality, light availability, and metabolite abundances were performed in JMP (SAS Inc., Cary, NC, USA) using Tukey's HSD. Different lettering groups were assigned where the model was significant (P<0.05). Figures were created using Prism 9 for Windows OS (GraphPad Inc., San Diego, CA, USA). Principal component analyses (PCA) were run on tree physiology, fruit quality and mesocarp metabolomics data using SIMCA (Umetrics, Umea, Sweden). Heat maps were developed using the z-score of mesocarp metabolite profiles across rootstocks. Prism 9 for Windows OS (Graph Pad Inc., San Diego, CA, USA) was used to create figures and heat maps.

3. Results

3.1. Influence of rootstock vigor on tree physiology, yield, light availability, and internal fruit quality.

The TCSA of the vigorous (ATL and BH5) and standard (K86 and LOV) rootstocks were 3.1-fold and 2.3-fold greater, on average, than the dwarfing rootstock (K1) (Fig. 2B). Canopy volume (m^3) as a secondary measurement of vigor followed the same trend as TCSA, with the vigorous and standard rootstocks being 3.9 and 2.4-fold larger than the dwarfing rootstock, respectively (Fig. 2A). These differences of tree vigor were reflected in canopy zone light availability (LA) that exhibited a trend of increase with decreasing tree vigor (Fig. 2C). The dwarfing rootstock, K1, had the highest LA (85 %) at 1.5 m (Fig 2C). The standard rootstocks K86 and LOV had light availability levels of 49 and 38 %, respectively, which was a 2-fold decrease from K1, on average (Fig. 2C). The vigorous rootstocks BH5 and ATL had a 3.5-fold decrease in LA compared to K1, each had 24 % LA at 1.5 m (Fig. 2C).

Vigorous rootstocks maintained the highest yields (kg $tree^{-1}$), on a five-year average, which were followed by the standard and dwarfing rootstocks (Fig. 2E). This resulted in a significant positive relationship (R^2=0.99) between cumulative yield (MT ha^{-1}) and tree vigor, as expressed as TCSA (Fig. 2I). The 5-year cumulative yield for ATL (vigorous) was 84 MT ha^{-1}, which was a 3-fold increase in yield when compared to the dwarfing K1 rootstock (27.9 MT ha^{-1}) (Fig. 2E). Both standard rootstocks also produced significantly greater than K1, with 60.3 (K86) and 58.7 MT ha^{-1} (LOV), respectively (Fig. 2E). While yield was significantly different by vigor classification, crop loads were controlled by adjusting the number of fruits per cm^2 of TCSA (Fig. 2G). To minimize these differences in source-sink relationships, the crop load for each rootstock was adjusted to an average of 1.4 fruit cm^{-2} of TCSA (Fig. 2G). As a result, with equal crop loads adjusted per rootstock, fruit weight (g) was not significantly different across rootstocks on a five-year average basis (Fig. 2H). Overall, average fruit weight over the 5-year period, across all rootstocks was 178 g.

Detailed fruit quality analyses were conducted on 5 fruits per tree rep (25 in total per rootstock) on 9 August 2019, 121 days after full bloom. (Fig. 3). Average maturity (I_{AD}) across rootstock genotypes was 0.5 I_{AD} and was not significantly different across rootstocks (Fig. 3A). Quality analyses on fruit of equal maturity revealed the impact of rootstock vigor on internal quality of peach fruit. In respect to flesh firmness, LOV was the firmest (39 N) and was firmer than K86, which had the lowest firmness (31 N) (Fig. 3B). Flesh firmness for ATL, BH5, and K1 (37, 35, and 36, respectively) were not statistically different from either LOV or K86 (Fig. 3B).

Titratable acidity (TA) demonstrated minimal differences between rootstocks at harvest (Fig. 3E). Only the two most vigorous rootstocks demonstrated a significant difference, with ATL having higher levels than BH5 (Fig. 3E). In addition to internal quality, overcolor blush evaluations and colorimetric scans for skin (i.e., exocarp) lightness (L^*) and hue angle (h^o) were conducted (Figs. 3F-H). Fruit overcolor blush (%) was highest in LOV (62 %) and least in ATL (49 %) (Fig 3F). Lightness (L^*) values followed a similar trend to vigor, with lightness decreasing with decreased rootstock vigor and increased light availability (Fig. 3G). Hue angle (h^o) values across rootstocks were not significantly different from one another (Fig. 3H).

With respect to important consumer acceptance related parameters, the vigorous rootstocks had the lowest DMC and SSC levels of all rootstocks (Figs. 3C-D). Vigorous rootstocks BH5 and ATL demonstrated the poorest internal quality, in respect to exhibiting the lowest DMC (14.1 and 14.9 %, respectively) and SSC values (13.6 and 14.0 %, respectively). Standard rootstocks (K86 and LOV) demonstrated increased levels of internal quality (DMC: 15.6 and 16.1 %; SSC: 15.6 and 16.2 %, respectively). However, these values were still significantly less than K1. The dwarfing rootstock K1 had the highest DMC (17.3 %) and SSC levels (16.8 %), which were significantly higher than all other rootstocks. Characteristics of tree vigor (TCSA and canopy volume) as well as light availability (LA) at 1.5 m were highly correlated with internal quality parameters such as DMC and SSC (Figs. 3I-L).

To fully encapsulate the global physiological impacts of rootstock vigor on fruit quality parameters, a principal component analysis (PCA) was conducted with all the tree physiology, yield, and destructive fruit quality data (Fig. 4). Crop load and fruit maturity were excluded from the PCA given they were not significantly different across rootstocks as a result of the experimental design. The PCA shows a strong separation between rootstock vigor classes, primarily along PC1, which explains ~72 % of the total variation. Minimal separation was also noted along PC2 (~17 %), noting genotypic variation within each vigor class. A total of 89 % of the model's variability was explained by these two components (Fig. 4). Overall, fruit quality and light availability was strongly related with the dwarfing rootstock (K1), while yield and tree size relate to the most vigorous rootstocks (ATL and BH5) (Fig. 4).

3.2 Global metabolic changes of peach fruit mesocarp primary metabolome in response to rootstock vigor.

Analysis of peach mesocarp by GC-MS resulted in a total of 358 detected metabolites of which 29 were confidently annotated. The 29 metabolites, organized by chemical class in a heatmap, show notable metabolic shifts between vigorous and dwarfing rootstock classes (Fig. 5). Positive shifts towards the dwarfing rootstock, K1, are observed in soluble sugars (SS), sucrose withstanding, flavonoids (FL), chlorogenic acids (CHL), and cyclitols (CYC). While positive shifts towards BH5, a vigorous rootstock, are seen in amino acids (AA), fatty acids (FA), and classified unknown chemical classes (Fig 5). Of the five organic acids (OA) annotated, two (citric acid and tartronic acid) showed positive shifts towards size-controlling rootstocks, while three (malic acid, threonic acid and 2-imidezolidone-4-carboxylic acid (ICA)) shifted positively towards the vigorous BH5 (Fig. 5).

Principal component analysis (PCA) was conducted to evaluate the global variation of these annotated metabolites across the five rootstocks. In total, the PCA explained (38 %) of the total variation in the data (Fig. 6). Along PC1, the separation was related to differing levels of rootstock vigor (Fig. 6). Additional variation was noted along PC2, which accounted for 17 % of the variation and appears to be related to separations within the standard vigor rootstock class. Along PC1, wide separation was observed between the dwarfing (K1) and most vigorous genotype (BH5). Several monosaccharides and metabolites from the shikimate pathway (e.g., quinic acid, catechin and neochlorogenic acid) associated with the dwarfing rootstock K1 separating it from the other rootstock classes. Amino acids, fatty acids, and the organic acids: malic, threonic and ICA, drive the separation of BH5 (vigorous) from the other rootstocks (Fig. 6). Increased sugar alcohols (e.g., sorbitol, and myo-inositol) were associated with the LOV rootstock, which appeared to be responsible for the vertical separation found in PC2 (Fig. 6).

3.3 Unique metabolites influenced by vigor and light reveal fruit quality related trends.

Of the 29 peach mesocarp metabolites annotated from the GC-MS spectral analysis, 10 showed significant differences between the rootstock classes. The most notable significant differences in metabolite abundances were observed between the most vigorous (BH5) and dwarfing (K1) rootstocks. Saccharide composition varied by rootstock vigor with monosaccharides [glucose (Glu), fructose (Fru) and sorbose (Sor)] exhibiting the highest abundances in K1 (Figs. 7B-D) and lowest abundances in the most vigorous rootstock, BH5. Glucose, fructose, and sorbose levels in K1 were significantly greater (29, 30, and 26 %, respectively) than BH5 (Figs. 7B-D). Conversely, sucrose, a disaccharide, demonstrated the greatest abundance in the most vigorous rootstock, BH5. K1 had 23 and 17 % less sucrose than BH5 and ATL, respectively (Fig. 7A).

Four additional metabolites quinic acid, catechin, neochlorogenic acid and butanoic acid, appeared to be influenced by light availability, as an artefact of vigor classification (Figs. 7E-H). Much like the monosaccharides, these metabolites showed significant differences between BH5 and K1. Quinic acid, catechin, and neochlorogenic acid all showed up accumulation with decreasing vigor, while butanoic acid increased with increasing vigor. Quinic acid in BH5, ATL and K86 was 26 % less than K1 levels, on average, while LOV did not demonstrate significant difference from K1 (Fig. 7F). Catechin, a flavonoid, followed a similar trend with abundances peaking in K1, which was significantly higher than LOV, K86, and BH5 (by 48, 43, and 44 %, respectively). However, catechin abundance was not statistically different between K1 and ATL (Fig. 7G). Neochlorogenic acid abundance was highest in K1 significantly more than BH5 and ATL (148 and 78 %, respectively), but was not significantly different from K86 or LOV (Fig. 7H). The fatty acid, butanoic acid, demonstrated an inverse trend, showcasing decreased abundance with decreasing vigor. Butanoic acid was 81 % greater in BH5 when compared to the lowest abundance found in K1 (Fig. 7E).

3.4. Sorbitol and malic acid represent inverse relationships with fruit quality parameters across rootstocks of variable vigor.

At harvest, two metabolites demonstrated significant trends with two critical fruit quality parameters, SSC, and DMC, across rootstock genotypes characterized by variable vigor. In general, sorbitol abundance increased with decreasing vigor and increasing light availability (Fig. 8A). Sorbitol abundance peaked in LOV, with statistically similar levels in K86 and K1 (Fig. 8A). The vigorous genotypes (ATL and BH5) demonstrated the lowest levels of sorbitol (Fig. 8A). When assessing the relationship between sorbitol abundance and DMC and SSC, moderate relationships were identified with R^2 values of 0.61 and 0.71, respectively (Figs. 8B-C). Apart from LOV, sorbitol abundance and fruit quality trends appear to follow the gradient of vigor and light availability (Figs. 8A-C). Inversely, malic acid demonstrated the opposite trend, with decreasing abundance of this organic acid in association with reduced rootstock vigor and enhanced light availability (Fig. 8D). Malic acid abundance was 41 % higher in BH5, the most vigorous rootstock, when compared to K1, the most dwarfing rootstock (Fig. 8D). As a result, negative relationships were noted between malic acid abundance and DMC and SSC, with R^2 values of -0.85 and -0.77, respectively (Figs. 8E-F). In short, malic acid abundance appears to increase with elevated rootstock vigor and reduced light availability in the canopy, underscoring inferior fruit quality (i.e., reduced DMC and SSC) at harvest (Figs. 8D-F).

4. Discussion

4.1 Rootstock vigor influences yield, light availability, and fruit quality.

Rootstock selection poses economic tradeoffs for growers. Increased rootstock vigor has been shown to increase yields (Reighard et al., 2020; Font i Forcada et al., 2012), however, maintenance of more vigorous trees may also coincide with additional labor costs such as pruning, thinning, and harvesting (Webster, 2002; Iglesias and Echeverria, 2022). Conversely, dwarfing rootstocks have higher light availability and invest a greater percentage of photosynthates towards fruit development (Chalmers et al., 1981), which contributes to increased fruit quality profiles (Marini and Sowers 1990; Anthony et al., 2020). Fruit from reduced-vigor rootstocks with higher light availability in the canopy have enhanced sugar and phenolic profiles (Chalmers et al 1981; Gullo et al., 2014; Anthony et al., 2020). However, reduced vigor rootstocks used in peach production have previously been associated with small fruit size (Reighard et al., 2020). Additionally, many rootstock studies failed to control confounding factors such as crop load or fruit maturity status. The conflicting results have made it difficult for peach growers to discern the most economically sound option. To gain further insight on the effect of rootstock vigor on peach production and fruit quality, we evaluated five rootstocks in three distinct classes of vigor from 11-year-old trees that used 'Redhaven' as the scion.

A nine-year NC-140 rootstock trial consisting of a broad range of rootstock vigor profiles conducted across 16 North American sites found seedling rootstocks like 'Lovell', 'KV010127', 'Guardian®' and vigorous hybrid rootstocks 'Atlas™' and 'Bright's Hybrid® 5' had the highest cumulative yields (Reighard et al., 2020). Our results with five years (2015 - 2019) of data concur that vigor is positively correlated with increases in yield and fruit count (Figs. 2E-F and I), as larger trees can support larger numbers of fruit (Reighard et al., 2020; Minas et al., 2023). Giorgi et al. (2005) concluded that while total yield related to vigor, fruit weight was more closely tied to genotype than vigor. While crop load was cited as a potential factor in determining fruit size, more vigorous rootstocks ('Atlas™', 'Bright's Hybrid® 5', 'Guardian®') have been associated with larger fruit (Reighard et al., 2020). Contrarily, Gullo et al., (2014) found that 'Penta', a vigor-limiting rootstock, produced larger fruit than the more vigorous rootstock 'GF-677.' The five years of agronomic data used for this experiment show no significant differences in fruit weight between the selected rootstocks (Fig. 2H).

Caruso (1996) reported that rootstock did not affect SSC levels in a high-density planting. In contrast, our results from a low-density planting demonstrate TCSA, and canopy volume did affect SSC, which increased with decreased vigor (Figs. 3D and L). Contradictory findings such as these may be due to a failure to account for additional physiological factors that affect fruit quality, such as crop load and fruit maturity status. In fact, Anthony et al. (2020) demonstrated that crop load greatly impacted fruit quality characteristics, even on fruit of equal maturity. Therefore, in this study, fruit numbers were adjusted according to tree TCSA, to eliminate crop load (fruit per cm^2 of TCSA) as a confounding variable (Anthony et al., 2020; Minas et al., 2018; Fig. 2G). In addition to the crop load, rootstock vigor also influences the light environment within the canopy. Increased levels of light availability for developing fruit may hasten maturity and result in more advanced physiological maturity at harvest (e.g., reduced firmness, more yellow background color, lower I_{AD} values) (Marini et al., 1991; Anthony et al., 2021; Minas et al., 2021). Therefore, to accurately understand how vigor and the light environment are affecting fruit quality, fruit of equal maturity were evaluated (Anthony et al., 2021; Anthony and Minas, 2022).

To ensure fruit were in similar states of maturity, a handheld Vis-NIRS sensor that was pre-calibrated to accurately assess physiological maturity (I_{AD}) (Costa et al., 2009) was used to select fruit for destructive internal quality comparisons as well as for further metabolomic investigations (Fig. 3A). The results presented herein demonstrate that a decrease in vigor significantly increased light availability throughout the canopy, thus improving illumination of developing fruit in the canopy and resulting in enhanced quality attributes at harvest (Figs. 2C and 3C-D). Increased light availability better exposes canopy, which increases leaf nitrogen content and photosynthetic efficiency, thus generating a higher amount of photosynthates for fruit located in close proximity to these sources (Rosati et al., 1999; Myers, 1993; Marini and Sowers, 1990). Similar to Marini et al. (1991) who found that canopies with higher light availability produce fruit with increased DMC and SSC levels, the dwarfing rootstock in this trial had significantly higher light availability, and fruit with higher DMC and SSC than the standard and vigorous rootstock classes (Figs. 2C and 3C-D).

There have been differing reports on the relationship between light availability and fruit color development. Marini et al. (1991) determined that fruit exposed to more light on the exterior of the canopy had redder overcolor blush than shaded interior fruit. Others have reported that poor light distribution across the canopy resulted in lower portions of the canopy not receiving enough light for optimal fruit quality development (e.g., skin overcolor, SSC) (Bible and Singha, 1993). However, Corelli-Grappadelli and Coston (1991) found that low light levels did not reduce red pigment development. Here, we observed that skin overcolor blush was highest in LOV and lowest in ATL (Fig. 3F). Exocarp hue angle (Fig. 3H) and chroma (data not shown) did not show significant differences across rootstocks. Although BH5, with the lowest light availability, demonstrated significantly higher exocarp lightness values (L^*), when compared to K1 (Fig. 3G). These results suggest that rootstock genotype may play a role in pigment development, although this may be more related to scion characteristics than the fruit's growing environment.

Overall, the three distinct classes of vigor manifested physiological differences in three distinct ways. The first, as expected, is that the vigorous rootstock class had the largest TCSA and canopy volumes (Figs. 2A-B), resulting in increased yields (Figs. 2E and I). Secondly, different levels of vigor created distinct light environments for the developing fruit (Fig. 2C) impacting internal fruit quality characteristics (Figs. 3C-D and 3I-L). Lastly, by controlling for equal crop loads and fruit physiological maturity, our results showcase the direct impact of rootstock vigor on internal fruit quality. The district vigor/light environments generated variable levels of fruit quality across trees of the same age and scion cultivar providing an excellent opportunity to study the biological mechanisms involved in peach fruit quality development.

4.2 Peach mesocarp primary metabolome at harvest relates to rootstock vigor and light availability.

A recent metabolomic study investigated the role of carbon supply (i.e., crop load) on peach quality development and found minimal difference at harvest in primary metabolism of fruit in two distinct carbon supply treatments (Anthony et al., 2020). In the present study, fruit of equal maturity displayed global metabolic shifts and associations (Figs. 5-6), revealing the influence of rootstock vigor and light availability on the peach mesocarp metabolome at harvest. The most vigorous rootstock, BH5, had the lowest light availability in the canopy and generated positive shifts (i.e., up-accumulation) in AA and FA (Fig. 5). The inferior quality observed in the vigorous rootstock is likely correlated with increased shading (Marini et al., 1991), which leads to a cooler micro-climate for fruit in this canopy zone. Reduced canopy temperatures can inhibit protein synthesis, contributing to increased abundances of amino acids, which has been shown to correlated with inferior quality in both apple and peach (Feng et al., 2014; Wang and Feng, 2011; Anthony et al., 2021). Excess shading also reduces net photosynthesis (Marini and Sowers, 1990) which supports our results demonstrating a negative shift (i.e., down-accumulation) of SS in BH5 (Fig. 5).

Contrarily, the increased canopy light availability in K1, the dwarfing rootstock, showed an up accumulation in soluble sugars (SS), cyclitol (CYC), flavonoid (FL) and chlorogenic acid (CHL) (Fig. 5). Increased SS have been associated with lower vigor rootstocks in previous studies (Kubota et al., 1992; Giorgi et al., 2004), and are commonly associated with enhanced fruit quality (Anthony et al., 2020; 2021). This is perhaps due to the increased light availability, contributing to increased photosynthetic activity and carbon exportation to nearby developing fruits (Anthony et al., 2021; Marini and Sowers, 1990; Marini et al., 1991). Monosaccharides (primarily Fru and Glu) are intermediate compounds that can be used in the biosynthesis of metabolites in the CYC, FL and CHL chemical classes, as part of the phenylpropanoid pathway (Lara et al., 2020). Thus, the authors hypothesize that the increased light availability in K1, which led to the up accumulation of monosaccharides via enhanced photosynthesis, contributed to the up accumulation of phenylpropanoid compounds (intermediates and products) such as quinic acid, catechin and chlorogenic acid (i.e., CYC, FL, CHL; Figs. 5-7).

The annotated metabolites found in this study demonstrated separation in the heat map and PCA based on rootstock vigor class and the light environment they create for the developing fruit (Figs. 5-6). Thus, the canopy light availability dictated by the rootstock vigor appears to be fundamental in determining the fate of metabolite profiles and fruit quality at harvest (Fig. 9; Anthony et al., 2021).

4.3 Rootstock vigor influences the light environment and metabolite upregulation.

In the present study, levels of monosaccharides (Glu, Fru, Sor) increased with decreasing vigor, while levels of Suc, a disaccharide, increased with increasing vigor (Fig. 7). Sorbitol, a sugar alcohol, is one of the main sugars translocated via the peach phloem from sources (leaves) to sinks (developing fruit) and is readily converted to Fru and Glu (Cirilli et al., 2016). Glucose and Fru can be phosphorylated to glucose-6-phosphate (G6P) and fructose-6-phosphate (F6P) via enzymes such as hexokinase and fructokinase (Cirilli et al., 2016). After G6P has been converted to UDP-glucose (UDPG), it can be synthesized to form sucrose with F6P by sucrose phosphate synthase (SPS) (Cirilli et al., 2016). In short, SPS generates sucrose from Glu and Fru, and has been shown to be heavily inhibited by drought stress conditions and extreme transpirational losses, leading to increased hexose concentrations in apple and peach (Yang et al., 2019; Escobar-Gutierrez et al., 1998). In this study, Suc was lowest in the most dwarfing, and most illuminated canopy, K1 (Fig. 7A), which may have been experiencing water stress conditions (i.e., increased transpirational losses). This could have been a result of excessive light availability in the canopy (Anthony et al., 2021) and/or a primary dwarfing mechanism in peach rootstocks: xylem anatomy restriction and reduced stem water conductance (Tombessi et al., 2009). Therefore, with increased light and potentially reduced stem water conductance, SPS activity could have been inhibited resulting in increased monosaccharide composition and reduced sucrose abundance in the dwarfing rootstock

(Figs. 7 and 9). Further, increased light has been shown to also increase soluble solids concentration in peach fruit (Marini et al., 1991). Thus, with increased light availability associated with decreased rootstock vigor (Fig. 2C), increased photosynthate creation and transport to sink tissues is possible, as evidenced by increased SSC, DMC, and monosaccharides with decreasing rootstock vigor (Figs. 3 and 7).

Alternatively, upon reaching sink tissues, Suc, can also be rapidly cleaved to Glu and Fru, which can then be utilized in the synthesis of other compounds, such as secondary metabolites (Morandi et al., 2008). These metabolites can be further utilized in the formation of secondary metabolites, such as phenolic compounds, terpenoids, and sulfur or nitrogen containing compounds, contributing a fundamental role in the plant's defensive and quality enhancing mechanisms (Anthony et al., 2023). One fundamental pathway that connects the primary metabolism with the secondary metabolism is the shikimate pathway.

Two metabolites in our study associated with the shikimate pathway, quinic acid and neochlorogenic acid, increased with decreasing rootstock vigor (Figs. 7F-H). These organic acids can be synthesized using monosaccharides, especially Glu (Lara et al., 2020). Our results agree with previous work by Anthony et al. (2020), which reported increased quinic acid levels in fruit developing in a carbon sufficient environment. Levels of quinic acid have also been suggested to be an indicator of peach maturity as they were found to negatively correlate with fruit maturity (Chapman et al., 1991). Quinic acid combines with caffeic acid to form caffeoylquinic acids (CQA). Neochlorogenic acid, an isomer of chlorogenic acid is formed by bonding hydroxycinnamic acid to quinic acid (Infante et al., 2011). Part of the hydoxycinnamic acid pathway, they are two of the most abundant secondary metabolites found in peach flesh that contribute to plant defense mechanisms and the organoleptic profiles of ripe fruit (Teixeira et al., 2013; Lara et al., 2020). The increased levels of light in the canopy associated with vigor-limiting rootstocks may contribute to enhanced synthesis of both primary and secondary metabolites that are associated with alleviating plant stress and contributing to higher fruit quality (Anthony et al., 2021; Fig. 9).

Another phenolic compound class, anthocyanins, are responsible for fruit color differentiation in *Prunus* species. Anthocyanins are members of the flavonoid group formed in the cytosol and stored in vacuoles (Lara et al., 2020). A member of a subgroup of flavonoids, catechins are condensed tannins found in many fruits (Lara et al., 2020). Catechin readily oxidizes to other phenolic compounds such as chlorogenic and neochlorogenic acid (Lara et al., 2020). It was reported that both carbon sufficient fruit and fruit exposed to increased light showed increased levels of catechin and CQAs (Anthony et al., 2020; 2021). In this study, fruit on dwarfing rootstocks were exposed to more light and demonstrated elevated levels of catechin, further supporting the hypothesis that these flavonoids, along with other phenylpropanoid pathway products, are up-regulated under optimal growth conditions (e.g., enhanced carbon supply and canopy zone light availability) (Anthony et al., 2020; 2021; 2023b).

As previously discussed, increased light availability in low vigor canopies is likely to result in increased transpiration and heat, thus reducing SPS activity and maintaining higher levels of monosaccharides (Figs. 7B-D and 9). The excess monosaccharides can then be used in phenol synthesis as a stress response to the increased light and/or heat in the canopy. Further support for this hypothesized relationship is observed in the phenolic compound abundance across rootstock genotypes in this study, as K1 phenolic compounds are in greater abundance than those of BH5 (Figs. 7F-H). Tavarini et al., (2011) found total phenolic compounds and hydroxycinnamic acids were significantly higher in dwarfing rootstocks. However, when these same rootstocks were exposed to drought stress, an inverse relationship was shown, suggesting that the dwarfing rootstocks may already be concentrating both primary and secondary metabolites in the fruits, as a stress response, due to higher transpirational loss, than their more vigorous counterparts.

Peach fruit is comprised of many volatile ester compounds, including acetic acid butyl esters (Sanchez et al., 2012), contributing to the aroma profile in peach (Ortiz et al., 2009). The fatty acid butanoic acid is one of these known esters and has previously been associated with inferior quality (Anthony et al., 2020). It has been suggested that butanoic acid may be volatized in high light environments (Anthony et al., 2020; Campbell et al., 2020). This would reflect our findings as butanoic acid levels decreased with decreasing rootstock vigor and increased light availability (Fig. 7E).

4.4. Sorbitol and malic acid serve as metabolic signatures of rootstock dictated vigor and canopy environment for superior or inferior peach fruit quality at harvest.

As mentioned, sorbitol, along with sucrose, are primary sugars translocated throughout the phloem of peach trees and have consistently served as a metabolic indicators of optimal fruit growth conditions in previous experiments (i.e., sufficient carbon supply, elevated available light, enhanced photosynthetic conditions) (Anthony et al., 2020; 2021; Morandi et al., 2008). Rootstocks that create less vigorous canopies facilitate increased light availability in the canopy, contributing to enhanced photosynthesis and fruit quality/nutritional characteristics (Gullo et al., 2014). When available light is reduced dramatically in the interior of vigorous canopies, photosynthetic rates diminish, restricting the translocation of photosynthates (e.g., sorbitol) to nearby carbon sinks (e.g., developing fruits) (Marini and Sowers, 1990). Therefore, as light availability increases within less vigorous canopies, like LOV, K86 and K1 (Fig. 2C), sorbitol levels, along with monosaccharide composition, may increase (Fig. 7). This may contribute to elevated levels of DMC and SSC (Figs. 3, 8), which are parameters characterized by the saccharide content in the fruit and are critical to consumer preference. This relationship is further supported with the elevated levels of monosaccharides like Fru and Glu in the less vigorous genotypes (Figs. 7B, C), as sorbitol is readily converted to Fru and Glu via sorbitol dehydrogenase (SDH) and sorbitol oxidase (SOX) in the fruit, respectively (Morandi et al., 2008). In this study, the less vigorous rootstocks appear to generate these optimal canopy conditions for fruit quality development and facilitate the up-accumulation of sorbitol, a metabolic signature for optimal light conditions and high fruit quality (Anthony et al., 2021).

In contrast, malic acid was observed to be up-accumulated with increased vigor and reduced canopy zone light availability and was related to inferior fruit quality at harvest (Fig. 8). Malic acid is a fundamental organic acid in peach fruit development (Walker and Faminai, 2018), although its behavior in fruit development appears to be cultivar-specific (Lobit et al., 2006). In a previous peach study, malic acid demonstrated a strong inverse relationship (r^2=-0.95) with sorbitol throughout peach fruit development (Anthony et al., 2020). Similarly, malic acid and quinic acid have demonstrated negative relationships in peach (Bae et al., 2014). These reports are supported with the results herein, with malic acid increasing in abundance in the reverse trend (up-accumulation with increased vigor) as sorbitol and quinic acid (up-accumulation with decreasing vigor) (Figs. 7-8). Elevated malic acid levels were also associated with peach fruits developing on canopies with high total leaf area (i.e., elevated canopy vigor) and minimal light exposure in the morning (Génard and Bruchou, 1992). Further, low malic acid levels were also associated with increased sun exposure and reduced sucrose content (Génard and Bruchou, 1992), similar to K1 canopy conditions and fruit quality attributes (Figs. 2, 7). This is again perhaps due to elevated temperatures, as a result of increased light availability within the canopy, inhibiting enzymatic activity of SPS forming Suc (Génard and Bruchou, 1992; Anthony et al., 2021; Cirilli et al., 2016). Malic acid, and its derivative malate, are also affected by temperature, with reduced accumulations under increased temperatures, especially at the beginning of ripening (Lobit et al., 2006). In sum, reduced rootstock vigor promotes the generation of canopies that enhance light relations within the canopy, which have the potential to increase canopy temperatures, thus decreasing SPS activity, reducing Suc and malic acid abundance and increasing Glu, Fru abundance. These biological dynamics underscore the role environmental conditions play in the regulation of metabolite accumulations, and not just the vigor of the tree alone (Anthony et al., 2021). After all, metabolites are the biological response to physiological stimuli in the tree or fruit. Ultimately, it is these environmental conditions within the canopy that heavily influence and contribute to peach fruit quality development and metabolic shifts.

5. Conclusion

Rootstock selection is a critical choice in orchard design. By controlling confounding factors in rootstock studies, such as crop load, fruit physiological maturity and fruit position in the canopy, the impact of rootstock vigor on internal fruit quality and the mesocarp metabolome is better determined. This approach showed that increasing rootstock vigor increased yield, but decreased canopy light availability. This genetic modification impacts the environment where fruit development occurs. As rootstock vigor decreased, light availability increased, resulting in fruit from the dwarfing rootstock exhibiting superior fruit quality (DMC and SSC) compared to the other vigor classes at harvest. Primary metabolites demonstrated differences based on vigor class and canopy light availability, which in turn, mirrored fruit quality distinctions. Metabolic signatures of the dwarfing rootstock, Krymsk®1, related to increased light availability and enhanced fruit quality included monosaccharides (glucose, fructose, sorbose), catechin, neochlorogenic acid and quinic acid. Conversely, amino acids, malic acid and butanoic acid were associated with inferior quality, and were metabolic signatures of the more vigorous rootstock, 'Bright's Hybrid® 5'. To maximize fruit quality, growers should select rootstocks with a vigor classification that suits their orchard design, with special consideration being paid to inter- and intra-tree spacing and training system. Selecting a combination that optimizes land efficiency while allowing for adequate light penetration through the canopy is of upmost importance to capitalize on high yield and fruit quality.

Declaration of Interest
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability
GC-MS data have been deposited to the MassIVE database (DOI: 10.25345/C5VD6PG07) with the identifier MSV000093207. The complete dataset can be accessed here: ftp://massive.ucsd.edu/v02/MSV000093207.

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Tables

Table 1. Rootstock cultivars and their country and genetic origin and vigor classification. Vigor classification is bracketed as follows: vigorous rootstocks are >110% the size of 'Lovell' with the size estimated by trunk cross-sectional area (TCSA); standard size rootstocks are 110-90% of Lovell size; semi-dwarfing rootstocks are 60-90% of Lovell and dwarfing rootstocks are <60% the size of Lovell (Minas et al., 2023c).

Rootstock	**Abbreviation**	**Breeder, Country of Origin**	**Species and interspecific hybrids**	**Vigor Classification**
Atlas	ATL	Zaiger Genetics, USA	complex interspecific hybrid of peach, almond, plum, apricot (*Prunus persica*, *P. amygdalus*, *P. cerasifera*, *P. mume*)	Vigorous
Bright's Hybrid®#5 (BH-5)	BH5	Bright's Nursery, Inc., USA	almond × peach interspecific hybrid (*P. amygdalus* × *P. persica*)	Vigorous
Krymsk®86 (Kuban 86)	K86	KEBS*, Russian Federation	plum x peach interspecific hybrid *P. cerasifera* × *P. persica*	Standard
Lovell	LOV	G.W. Thissell, USA	peach seedling (*P. persica*)	Standard
Krymsk®1 (VVA-1)	K1	KEBS*, Russian Federation	cherry x plum interspecific hybrid (*P. tomentosa* × *P. cerasifera*)	Dwarfing

*Krymsk Experimental Breeding Station, Krasnodar Region

Figure Captions

Fig. 1. Determining how rootstock vigor impacts fruit quality profiles. Five distinct rootstock cultivars were selected to determine the impact of differing vigor profiles on fruit internal quality and metabolite profiles. Based on trunk cross sectional area (TCSA) and canopy volume, the five rootstocks segregated into three vigor profiles. Light availability was determined at 1.5 m for each rootstock. Crops loads were standardized for each rootstock genotype based on (TCSA). Fruit of equal maturity were selected based on the index of absorbance difference (I_{AD}). Each fruit was assessed for weight, color, blush, flesh firmness, dry matter content and soluble solid concentration shortly after harvest. Mesocarp tissue from each fruit was quenched using liquid nitrogen directly after internal quality parameters were obtained. Frozen tissues were freeze dried and derivatized for non-targeted metabolite analysis using gas chromatography mass spectrometry (GC-MS).

Fig. 2. The impact of rootstock on vigor, yield, and light availability. The influence of rootstock on vigor canopy volume (A), and trunk cross sectional area (TCSA, B); mid-canopy light availability (C); light interception (D); cumulative 5-year yield (E); five year (2015 – 2019) average yield (F) and fruit weight (H). In 2019, crop load was standardized across rootstocks by hand thinning according to TCSA (G). Colored bars indicate rootstock and are displayed by decreasing vigor; BH5 (Bright's Hybrid® 5), ATL (Atlas™), K86 (Krymsk®86), LOV (Lovell), and K1 (Krymsk®1). Mean values ± S.E. are displayed. Means followed by the same letter are not statistically different according to Tukey's HSD test ($P < 0.05$). Regression analyses of trunk cross-sectional area (TCSA, cm^2) in 2019 and cumulative yield (MT ha^{-1}) (I); of TCSA in 2019 and mid-canopy light availability (J); of canopy volume (m^3) and mid-canopy light availability (K) and of cumulative yield (MT ha^{-1}) and mid-canopy light availability (L) with five replicated samples from each rootstock treatments are plotted. R^2 values are displayed to demonstrate the linearity of the relationships.

Fig. 3. The impact of rootstock on internal fruit quality characteristics and exocarp pigment development. Fruit harvested from a canopy height of 1.5 m ± 30 cm in 2019 were segregated for equal maturity (I_{AD}, A) and assessed by internal fruit quality characteristics: flesh firmness (B), dry matter content (DMC, C), soluble solids concentration (SSC, D), titratable acidity (TA, E); as well as exocarp color development: skin over color blush (F), skin lightness (L^*, G), and hue angle (h^o, H). Colored bars indicate rootstock and are displayed by decreasing vigor; BH5 (Bright's Hybrid® 5), ATL (Atlas™), K86 (Krymsk®86), LOV (Lovell), and K1 (Krymsk®1). Mean values ± S.E. are displayed. Means followed by the same letter are not statistically different according to Tukey's HSD test ($P < 0.05$). Regression analyses of parameters characterizing or affected by tree vigor like canopy volume (m^3, I), trunk cross-sectional area (TCSA, cm^2, J) or mid-canopy light availability (K) and internal fruit quality parameters like DMC (I, J and K) or SSC (L) with five replicated samples from each rootstock treatments are plotted. R^2 values are displayed to demonstrate the linearity of the relationships.

Fig. 4. Principal component analysis biplot of rootstock on vigor, light availability, and internal fruit quality characteristics. Large symbols indicate the scores for the rootstock treatments [colored by rootstock; and are pareto scaled (-1.0 – 1.0)] with vigor (TCSA), light availability (LA %), internal fruit quality (DMC, SSC), yield (loadings, grey diamonds). Principal component analysis (PCA) of the five reps per rootstock were averaged in the biplot. The PC1 (85.2 %) demonstrates that rootstock vigor class [dwarfing (K1), standard (K86 and LOV), and

vigorous (BH5 and ATL)] is driving the separation between internal fruit quality, light availability, yield and exocarp color.

Fig. 5. Heat map of metabolite profiles across rootstocks of variable vigor. Profiles of metabolism changes at harvest in 'Redhaven' peach fruit mesocarp. Figure shows comparisons of the metabolite abundance by rootstock vigor, displayed with vigor decreasing from left (most vigorous) to right (dwarfing). Each of the 29 annotated metabolites were transformed z-scores and shown with the following color scale (green to red) according to Lombardo et al. (2011). Fruits were harvested from a canopy height of 1.5 m ± 30 cm and were of equal maturity according to the I_{AD} measured by the DA meter. Annotated metabolites are organized by chemical class: sugar alcohols (SA), soluble sugars (SS), organic acids (OA), cyclitols (CYC), flavonoids (FL), fatty acids (FA), amino acids (AA), other (O) and classified un-knowns (UK).

Fig. 6. Principal component analysis biplot of rootstock vigor on peach fruit mesocarp metabolism. Metabolite profiles across five rootstocks at harvest in peach fruit mesocarp in 'Redhaven' fruit. Figure shows comparisons of mesocarp metabolite profiles across five rootstocks. The rootstocks are as follows: BH5 (Bright's Hybrid® 5), ATL (Atlas™), K86 (Krymsk®86), LOV (Lovell), and K1 (Krymsk®1). Large symbols indicate the scores for the rootstock treatments [colored by rootstock; and are pareto scaled (-1.0 – 1.0)] with the 29 annotated metabolites detected in the peach mesocarp (loadings, grey diamonds). Principal component analysis (PCA) of the five reps per rootstock were averaged in the biplot. The PCA demonstrates that rootstock vigor (PC1 20.5 %) was a contributor to metabolome variation with rootstock separation occurring by vigor class.

Fig. 7. Accumulation trends of metabolite abundances by rootstock vigor in peach mesocarp. Mean peak area (AU) of selected metabolites that are influenced by vigor, soluble sugars: sucrose (A), glucose (B), fructose (C), sorbose (D); phenylpropanoid pathway: butanoic acid (E), quinic acid (F), catechin (G), neochlorogenic acid (H) in the peach mesocarp of 'Redhaven' fruit at harvest. Colored bars indicate rootstock and are displayed by decreasing vigor; BH5 (Bright's Hybrid® 5), ATL (Atlas™), K86 (Krymsk®86), LOV (Lovell), and K1 (Krymsk®1). Samples were controlled for equal maturity (I_{AD}) at harvest and harvested from similar canopy heights (1.5 m ± 30 cm). Mean values ± S.E. are displayed with the low vigor presented on the left of each graph, while the high vigor is displayed on the right. Means with the same letter displayed above the bar are not statistically different according to Tukey's HSD test ($P \leq 0.05$).

Fig. 8. Abundance of two metabolites and their relationship with peach internal quality parameters at harvest. Mean peak area (AU) of sorbitol (A) and malic acid (D), respectively, at harvest by rootstock vigor, BH5 (Bright's Hybrid® 5), ATL (Atlas™), K86 (Krymsk®86), LOV (Lovell), and K1 (Krymsk®1). Mean values ± S.E. are displayed. Means followed by the same letter are not statistically different according to Tukey's HSD test ($P < 0.05$). The relationships between the mean peak area of sorbitol and malic acid with dry matter content (DMC, %; B and E, respectively) and soluble solids concentration (SSC, %; C and F, respectively) at harvest with five replicated samples from each rootstock treatments are plotted. R^2 values are displayed to demonstrate the linearity of the relationships.

Fig. 9. The impact of rootstock vigor on light availability and metabolite abundance in peach mesocarp. Up- and down-accumulation trends are presented for chemical classes and specific metabolites in peach mesocarp as a result of various canopy volumes and thus differing light availability profiles. Metabolites related to development and maturity are also displayed. A gradient of advanced maturity from the bottom of the canopy to the top is displayed, although quality analysis and metabolite profiling was conducted on fruit of equal maturity. Light availability generally increases as well, from the bottom of the canopy towards the top, especially in the canopy of higher vigor rootstocks.

Supplementary Materials

Table. S1. Relative abundances of 29 annotated metabolites by class in peach mesocarp by rootstock. Statistical analysis presented as one-way ANOVA by rootstock assessed for significance at $P < 0.05$. Mean values are displayed. Means followed by the same letter are not statistically different according to Tukey's HSD test ($P<0.05$).

Author Contributions

Conceptualization, I.S.M., J.R.P., J.E.P.; methodology, J.R.P., B.M.A., J.M.C., I.S.M.; software, J.R.P., B.M.A., J.M.C. validation, J.R.P., B.M.A., I.S.M., J.M.C., J.E.P.; formal analysis, J.R.P., B.M.A., I.S.M.; investigation, J.R.P., B.M.A., I.S.M.; resources, I.S.M., J.E.P.; data curation, J.R.P., I.S.M.; writing-original draft preparation, J.R.P., B.M.A., I.S.M.; writing-review and editing, J.R.P., B.M.A., I.S.M., J.M.C., J.E.P.; visualization, J.R.P., I.S.M.; supervision, I.S.M.; project administration, I.S.M.; funding acquisition, I.S.M. All authors have read and agreed to the published version of the manuscript.

Figure1

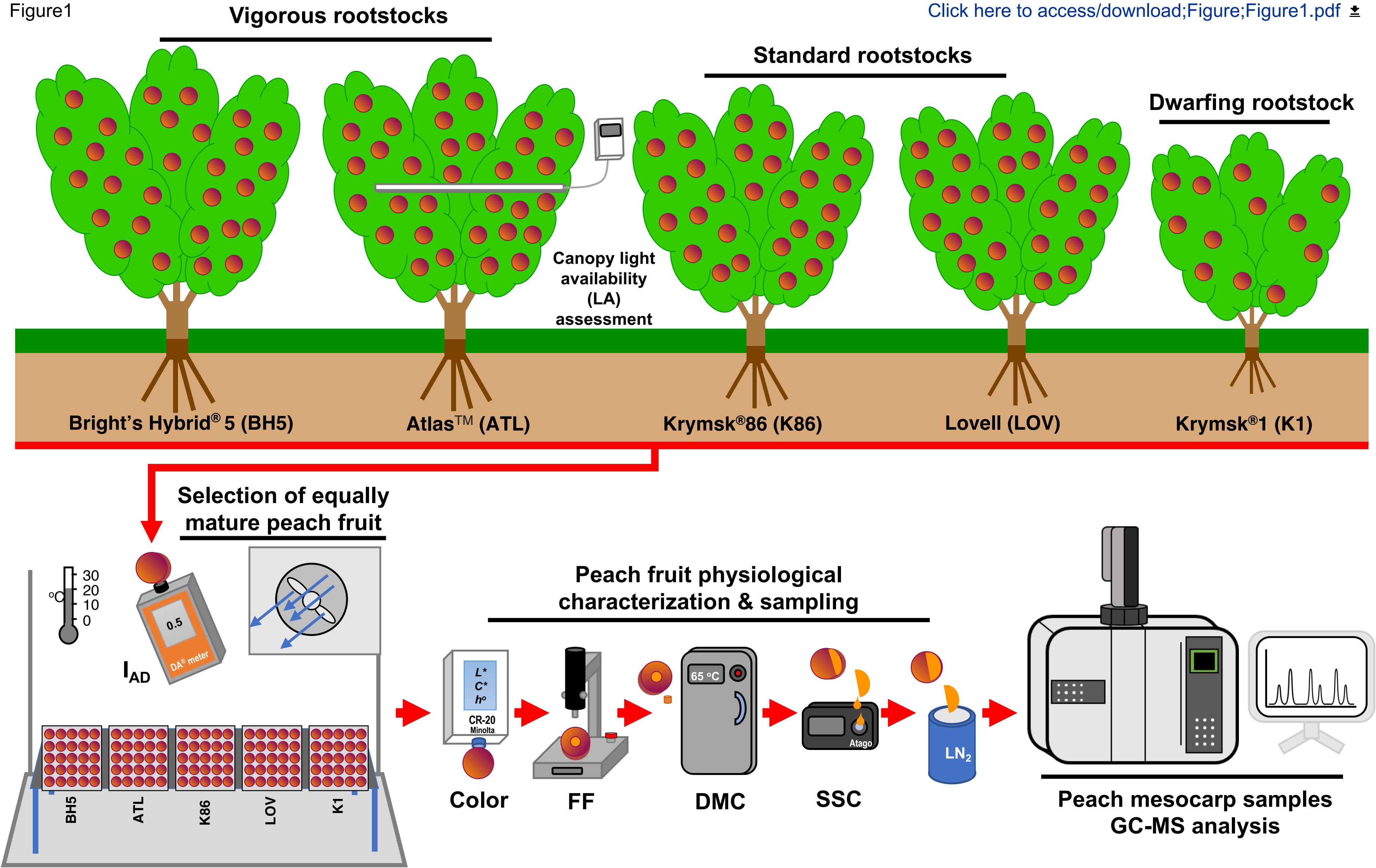

Vigorous rootstocks
Standard rootstocks
Dwarfing rootstock
Canopy light availability (LA) assessment
Bright's Hybrid® 5 (BH5)
Atlas™ (ATL)
Krymsk®86 (K86)
Lovell (LOV)
Krymsk®1 (K1)
Selection of equally mature peach fruit
°C
30
20
10
0
0.5
DA® meter
I_AD
BH5
ATL
K86
LOV
K1
Peach fruit physiological characterization & sampling
L*
C*
h°
CR-20
Minolta
Color
FF
65 °C
DMC
Atago
SSC
LN2
Peach mesocarp samples GC-MS analysis

Figure2

Click here to access/download;Figure;Figure2.pdf

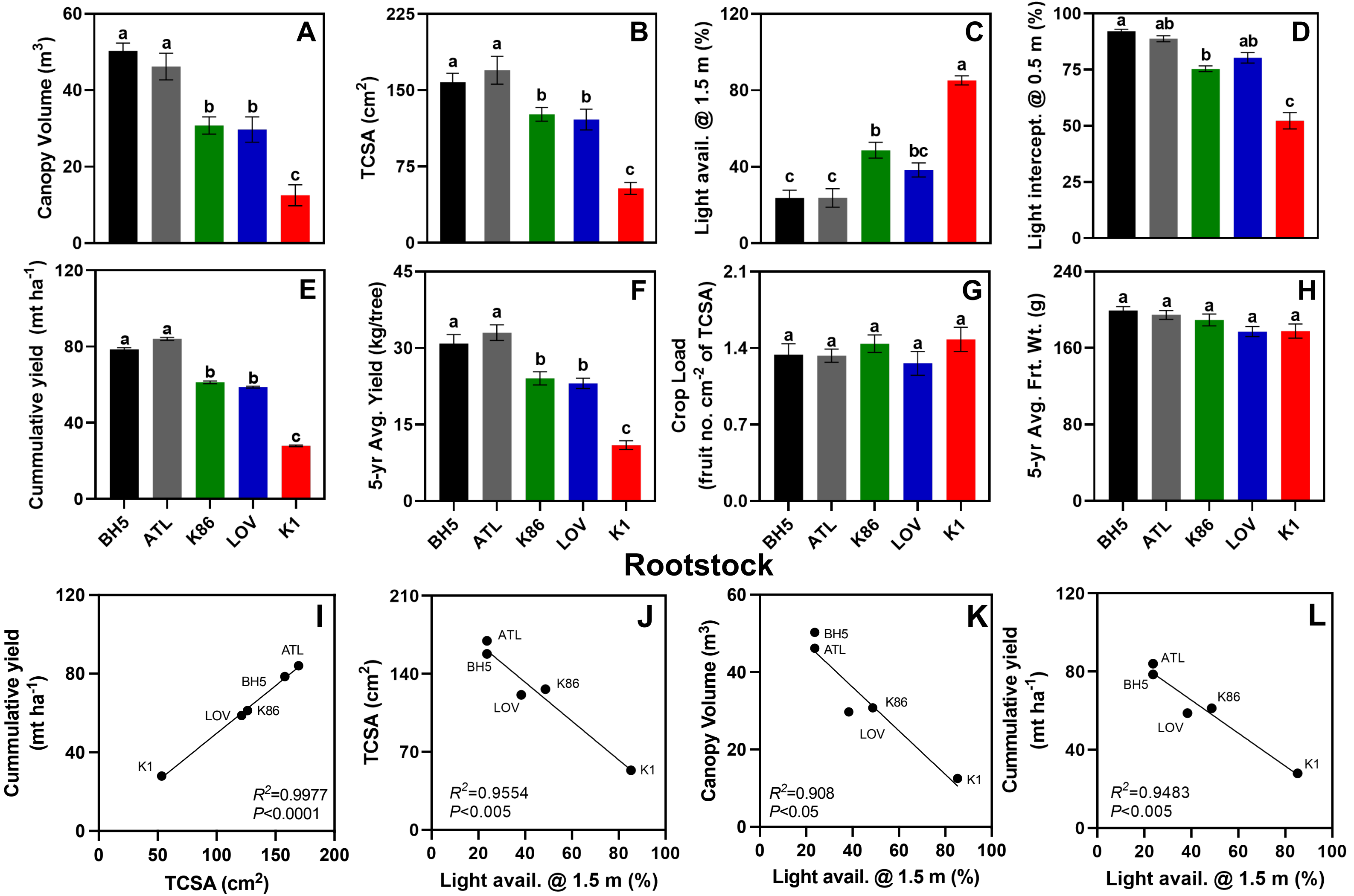


Figure3

Click here to access/download;Figure;Figure3.pdf

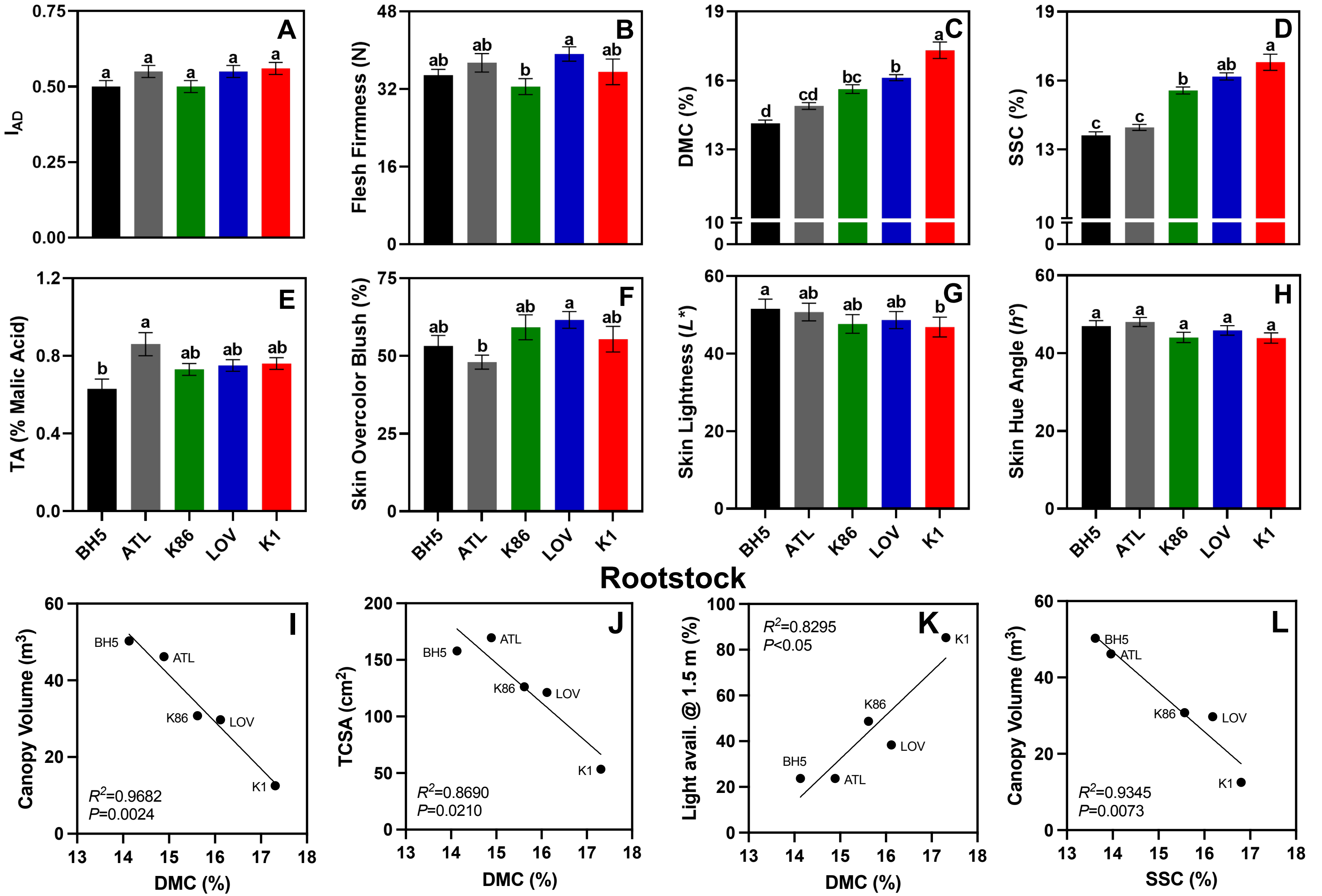


Figure4

Click here to access/download;Figure;Figure4.pdf

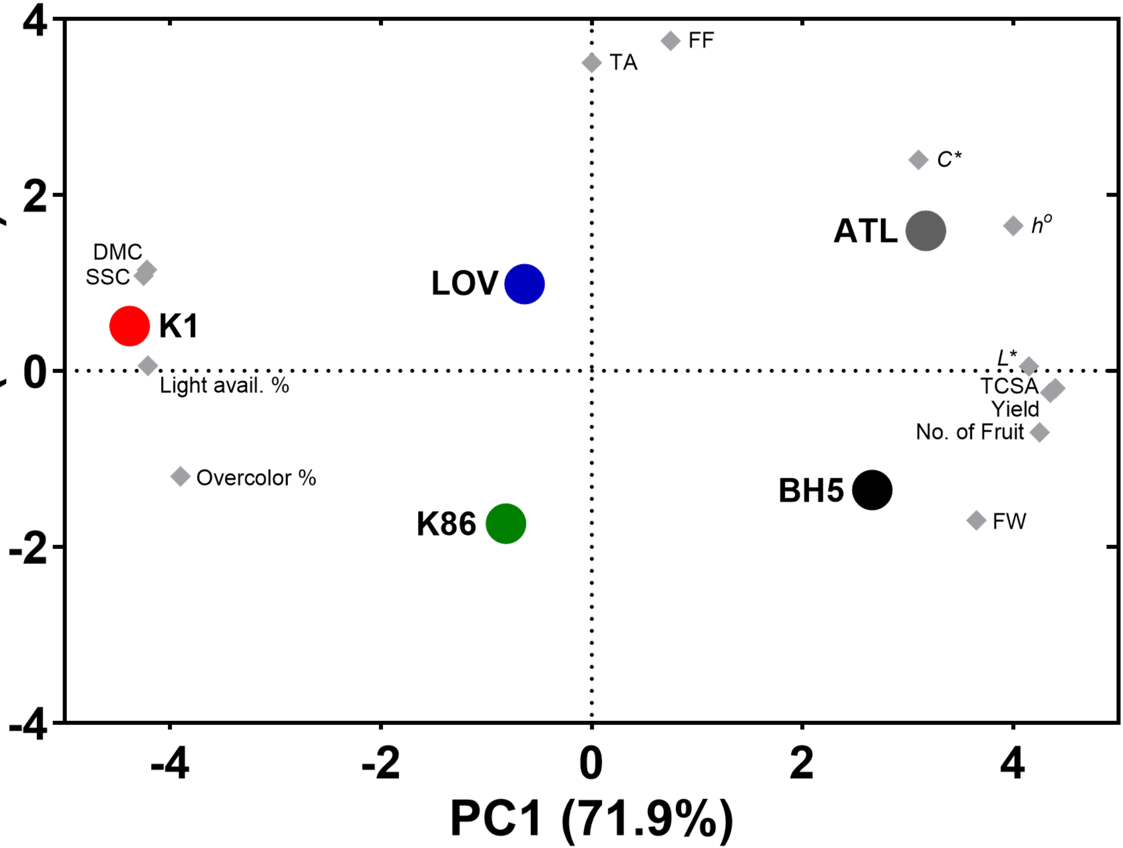


Figure5

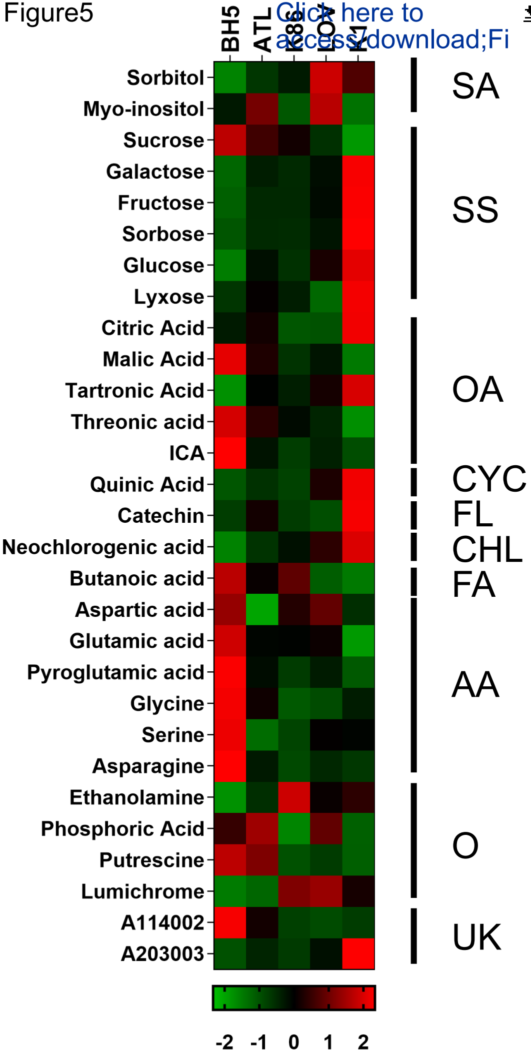

BH5
ATL
K86
LOV
K1
Sorbitol
Myo-inositol
Sucrose
Galactose
Fructose
Sorbose
Glucose
Lyxose
Citric Acid
Malic Acid
Tartronic Acid
Threonic acid
ICA
Quinic Acid
Catechin
Neochlorogenic acid
Butanoic acid
Aspartic acid
Glutamic acid
Pyroglutamic acid
Glycine
Serine
Asparagine
Ethanolamine
Phosphoric Acid
Putrescine
Lumichrome
A114002
A203003
SA
SS
OA
CYC
FL
CHL
FA
AA
O
UK
-2
-1
0
1
2

Figure6

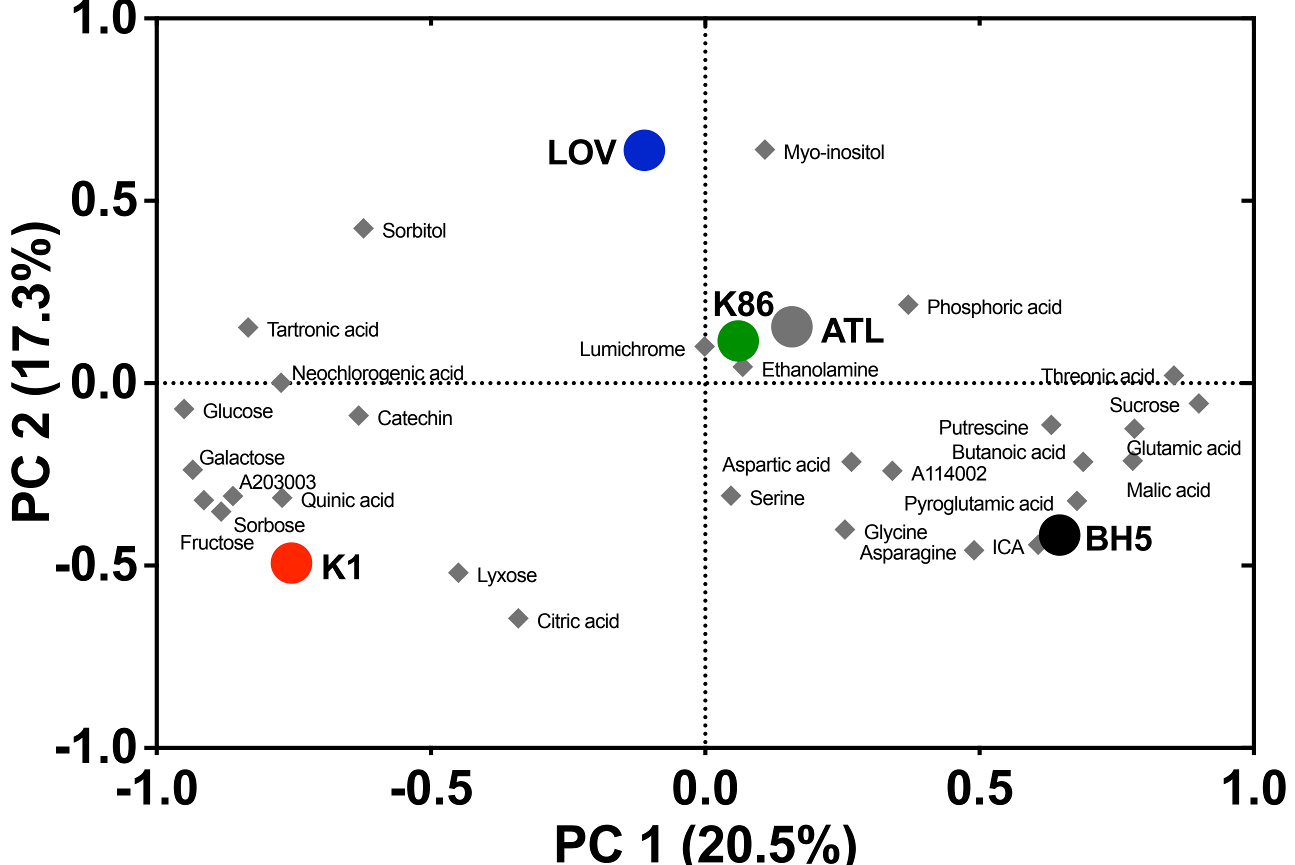

1.0
0.5
0.0
-0.5
-1.0
PC 2 (17.3%)
-1.0
-0.5
0.0
0.5
1.0
PC 1 (20.5%)
LOV
Myo-inositol
Sorbitol
K86
ATL
Phosphoric acid
Tartronic acid
Lumichrome
Ethanolamine
Neochlorogenic acid
Threonic acid
Glucose
Catechin
Sucrose
Putrescine
Butanoic acid
Glutamic acid
Aspartic acid
A114002
Galactose
A203003
Quinic acid
Serine
Malic acid
Pyroglutamic acid
Sorbose
Fructose
Glycine
Asparagine
ICA
BH5
K1
Lyxose
Citric acid

Figure7

Click here to access/download;Figure;Figure7.pdf

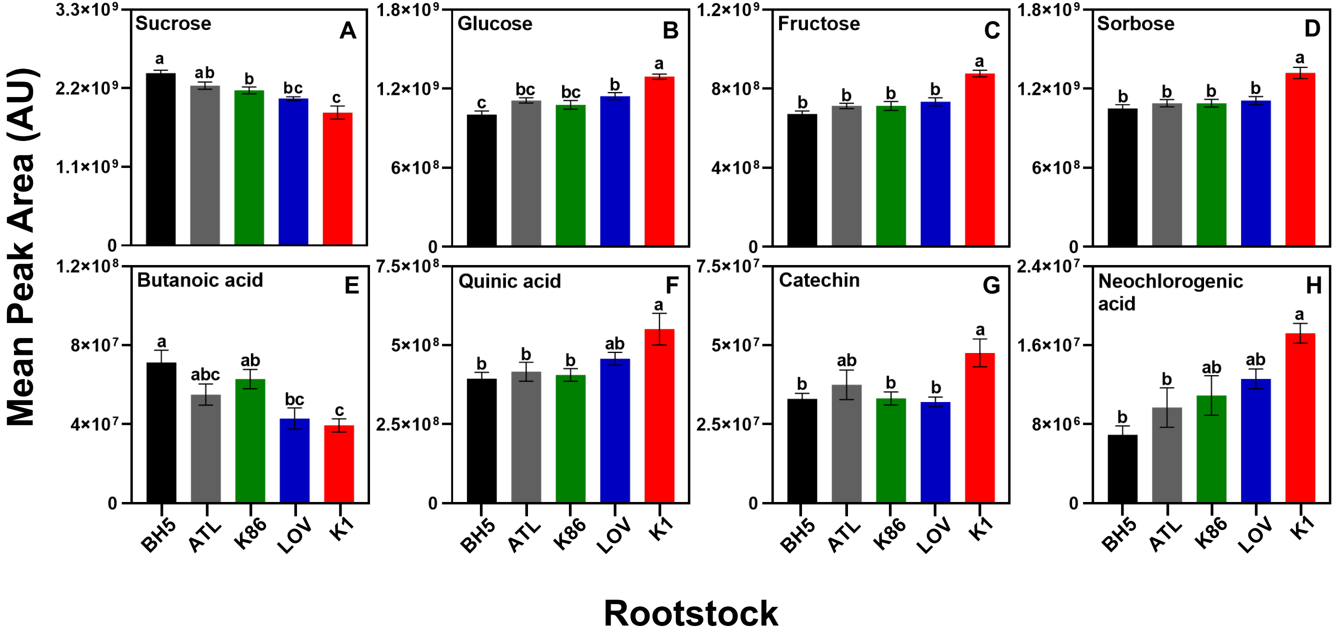


Figure8

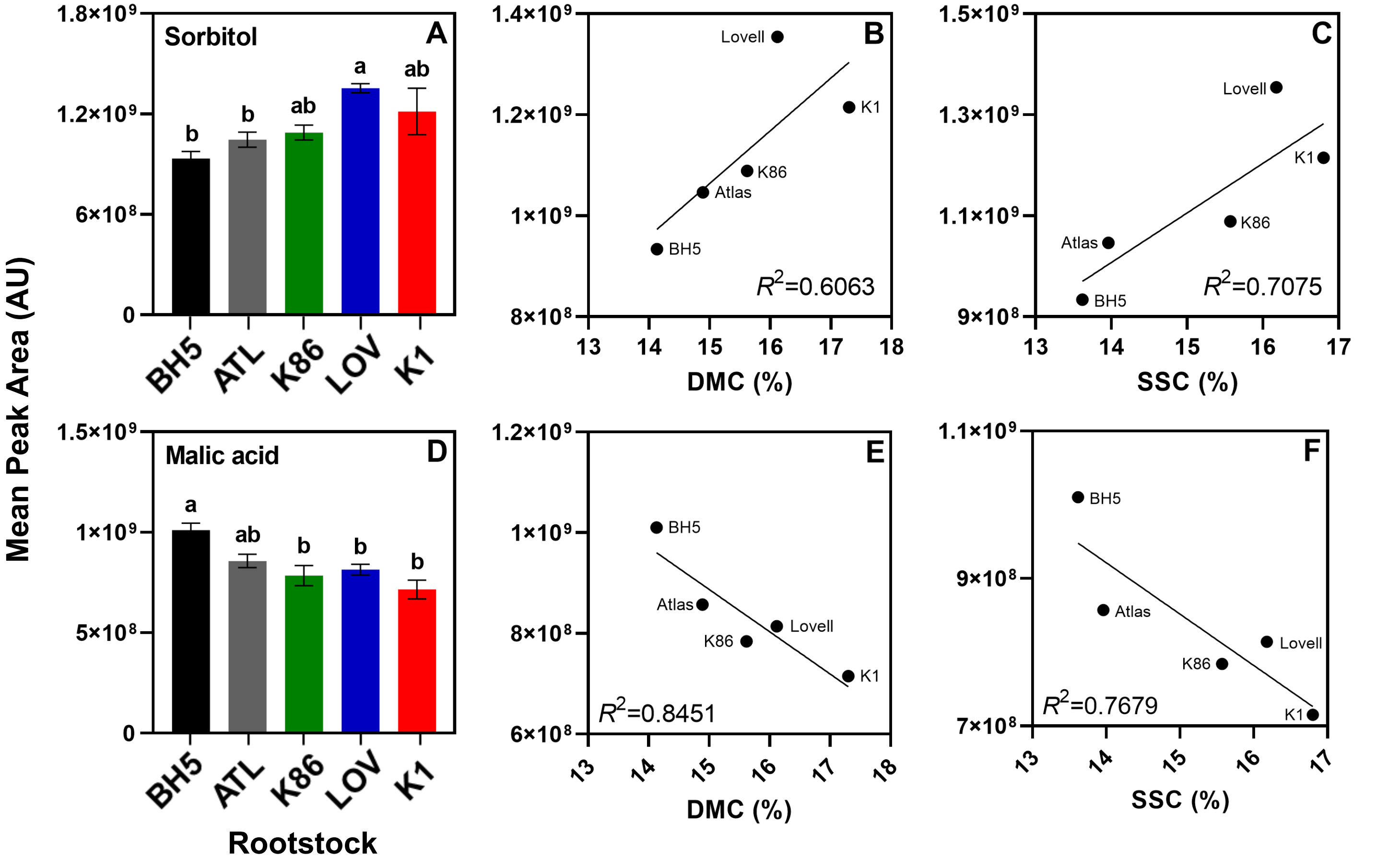
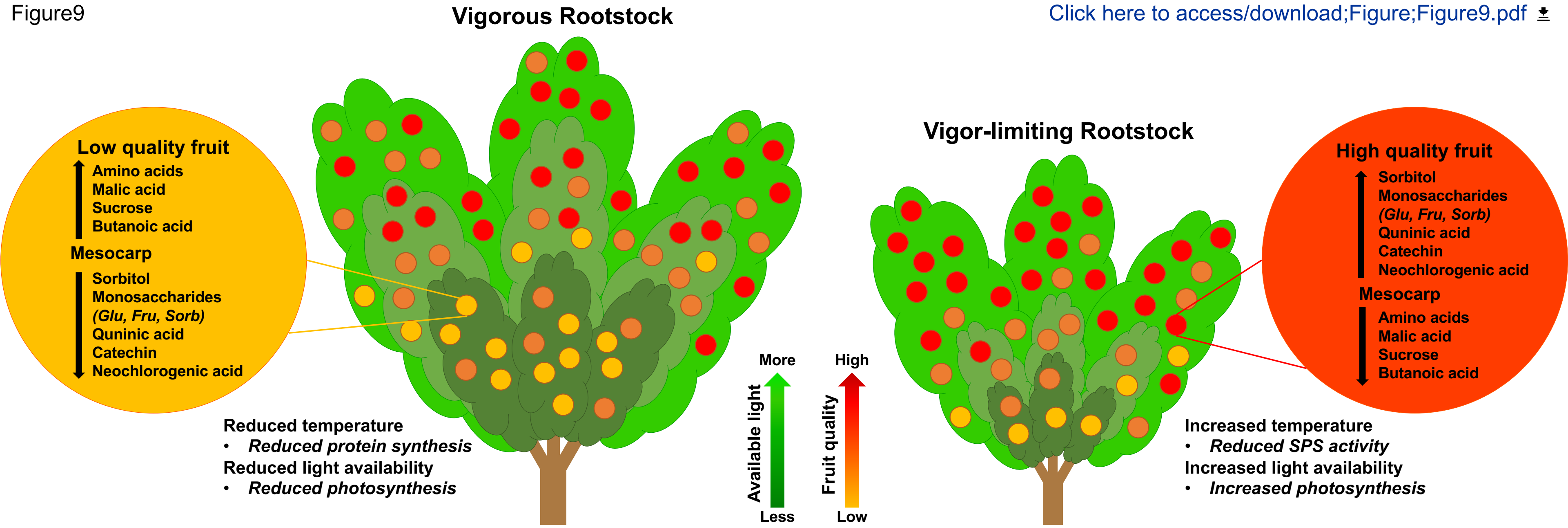

Mean Peak Area (AU)
Sorbitol
A
a
ab
b
b
ab
1.8×10⁹
1.2×10⁹
6×10⁸
0
BH5
ATL
K86
LOV
K1
B
Lovell
K1
K86
Atlas
BH5
R²=0.6063
1.4×10⁹
1.2×10⁹
1×10⁹
8×10⁸
13
14
15
16
17
18
DMC (%)
C
Lovell
K1
K86
Atlas
BH5
R²=0.7075
1.5×10⁹
1.3×10⁹
1.1×10⁹
9×10⁸
SSC (%)
Malic acid
D
1.5×10⁹
1×10⁹
5×10⁸
Rootstock
E
BH5
Atlas
Lovell
K86
K1
R²=0.8451
1.2×10⁹
1×10⁹
8×10⁸
6×10⁸
DMC (%)
F
BH5
Atlas
Lovell
K86
K1
R²=0.7679
1.1×10⁹
9×10⁸
7×10⁸
SSC (%)

Figure9


Vigorous Rootstock
Low quality fruit
Amino acids
Malic acid
Sucrose
Butanoic acid
Mesocarp
Sorbitol
Monosaccharides
(Glu, Fru, Sorb)
Quninic acid
Catechin
Neochlorogenic acid
Reduced temperature
• Reduced protein synthesis
Reduced light availability
• Reduced photosynthesis
More
Available light
Less
High
Fruit quality
Low
Vigor-limiting Rootstock
High quality fruit
Sorbitol
Monosaccharides
(Glu, Fru, Sorb)
Quninic acid
Catechin
Neochlorogenic acid
Mesocarp
Amino acids
Malic acid
Sucrose
Butanoic acid
Increased temperature
• Reduced SPS activity
Increased light availability
• Increased photosynthesis

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.