DISSERTATION

THE IMPACT OF ROOTSTOCK ON PEACH TREE VIGOR, LIGHT ENVIRONMENT, FRUIT QUALITY, AND METABOLISM

Submitted by

Jeffrey Ross Pieper

Department of Horticulture and Landscape Architecture

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Doctoral Committee:

Advisor: Ioannis Minas

Marisa Bunning
Horst Caspari
Jessica Prenni

ABSTRACT

THE IMPACT OF ROOTSTOCK ON PEACH TREE VIGOR, LIGHT ENVIRONMENT, FRUIT QUALITY, AND METABOLISM

The key to Colorado's successful peach industry is superb fruit quality. The fruit quality growers achieve allows for the highest premium 'farm-gate' price per pound in the nation. Fruit quality is created in the orchard via the interaction of several pre-harvest factors. One critical pre-harvest factor that has several knock-on effects for orchard management decisions is rootstock selection. Rootstock selection has the potential to impact the longevity, productivity, efficiency, and profitability of an orchard, and is dependent on climatic and edaphic environments as well as the soil microbiome. Rootstock selection may also allow growers to augment orchard design through vigor manipulation. In Colorado, growers are faced with relatively short growing seasons, sudden fall and spring frost events, and calcareous soils which limit the availability of certain nutrients. The unique growing environments coupled with the need for high quality fruit production makes rootstock selection limited. Identifying rootstocks suitable for production in Colorado and determining how they impact fruit quality is paramount. While previous studies have evaluated rootstocks for their performance and relationship to fruit quality, few have limited confounding factors such as crop load, canopy position, and or physiological maturity when assessing fruit. The following experiments evaluated twenty-one genetically diverse rootstocks for their phenotypic and agronomic performance and potential use in Colorado production systems. The nine-year performance review, in chapter one, details the productivity and suitability of seventeen genetically diverse peach rootstocks in Colorado growing conditions. The trial determined rootstock vigor strongly correlates with cumulative yield. However, vigor also showed an inverse relationship with internal fruit quality development measured as dry matter content (DMC) and soluble solids concentration (SSC). The trial showed interspecific peach and non-peach hybrids outperformed peach seedling rootstocks. One interspecific peach rootstock in particular, ‘Krymsk® 86’, performed exceptionally well and has since been widely adopted by industry. By controlling for several confounding factors, the rootstock vigor trial, chapter two, demonstrated the true impact of vigor and light availability on fruit quality enhancement and primary metabolite profiles. Fruit developing in reduced vigor canopy of the dwarfing rootstock ‘Krymsk® 1’ had increased light availability and enhanced internal fruit quality parameters (DMC and SSC) at harvest. Mesocarp metabolites relating to internal quality showed they are up and down accumulated by rootstock vigor and the light environment. Several metabolite classes including soluble sugars, cyclitols, flavanols, and chlorogenic acids were associated with ‘Krymsk® 1’, a low vigor rootstock that had high light availability and enhanced fruit quality profiles. ‘Atlas™’ and ‘Bright’s Hybrid® 5’, both vigorous rootstocks, showed low light availability and reduced fruit quality. The vigorous rootstocks also showed an increase of amino and fatty acids compared to the standard and dwarfing rootstocks. The six-year physiological and agronomic performance of modern semi-dwarfing rootstocks trial, chapter three, reiterated the impact of vigor on yield, light availability, and fruit quality development. Furthermore, the trial showed increased vigor was related to an increase of gummosis incidence and severity. Also, intra-specific *Prunus* hybrids had increased rates of proleptic shoot formation, however, some showed they were susceptible to iron chlorosis. Overall, the rootstock trials identify key parameters of performance and suitability in Colorado production systems. The outcomes indicate that rootstocks with increased vigor resulted in higher yields per tree, however, lower light availability in the canopy decreased DMC and SSC. While rootstock genotype and vigor are influencing peach fruit development and quality, their effect on light availability may play a more significant role in achieving optimal yield and fruit quality and augmented metabolite profiles. Additionally, this work demonstrates the importance of controlling for confounding variables when evaluating preharvest factors for their impact on internal fruit quality and metabolite profiles.

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DEDICATION

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CHAPTER ONE

PEACH ROOTSTOCK EVALUATIONS FOR INTERMOUNTAIN TREE FRUIT GROWERS: A NINE-YEAR PERFORMANCE REVIEW OF SEVENTEEN ROOTSTOCKS GRAFTED TO THE CULTIVAR REDHAVEN

1.1. Introduction

Pre-harvest orchard management decisions can affect fruit growth and quality development (Minas et al., 2018). When planting an orchard, the choice of rootstock is an important decision that has cascading effects and/or ramifications for the design and management of the orchard. A poor performing rootstock can result in reduced efficiency and lower the overall profitability of an orchard. Various rootstock genotypes offer growers the ability to overcome biotic and abiotic pressures of a given growing region, allowing production in adverse soil conditions, such as those with parasitic nematodes, disease pressure, or high pH. Peach production in Colorado is concentrated in Western Colorado near the Utah border. While this arid climate offers a unique growing environment that is low in disease and insect pressure, its sudden temperature swings, cold winters, and calcareous soils can adversely affect peach tree performance and survival.

The data presented in this fact sheet was collected as part of the US Department of Agriculture (USDA) multi-state project North Central (NC)-140 2009 Peach Rootstock Trial and was the 5th NC-140 peach rootstock trial conducted in Colorado. Combined data from the multi-state trial was published by Reighard et al. in 2020. The 2009 rootstock trial was designed to evaluate the commercial viability of seventeen newly commercialized rootstocks for peach trees (Table 1.1). The novel rootstocks were compared to the industry standard rootstock ‘Lovell’, which was also planted in the trial. The trial spanned nine growing seasons, and evaluated rootstock traits such as vigor, root suckering, yield (average weight per tree, and cumulative yield), fruit size, and fruit quality. The rootstocks were budded to ‘Redhaven’, an early to mid-season freestone cultivar of commercial importance in Colorado. The trees were planted at 206 trees per acre (509 trees per hectare), at a spacing of 13 × 16 feet (4 × 5 m) and trained in an open vase system. Eight single tree replicates of each rootstock genotype were planted in a randomized complete block design.

1.2. Rootstock performance

Vigor – Rootstock vigor was determined by calculating trunk cross sectional area 15 cm (6 inches) above the graft union. Vigor class was determined using ‘Lovell’ as the reference standard size rootstock (Figure 1). Other standard sized rootstocks, those within 10% of ‘Lovell’s trunk cross sectional area (TCSA), were ‘KV-010127’, ‘Krymsk® 86’, ‘Controller™ 7’, and ‘Empyrean® 2’. Rootstocks greater than 10% larger than ‘Lovell’ were considered vigorous. The vigorous rootstock class included ‘Atlas™’, ‘Bright’s Hybrid® 5’, ‘Guardian®’, ‘Viking®’, and ‘Imperial California’. The vigor class of 60 – 90% of ‘Lovell’ were considered semi-dwarfing and included ‘KV-010123’, ‘Controller™ 8, ‘Rootpac® R’, and ‘Fortuna’. Rootstocks less than 60% of ‘Lovell’ were considered to be dwarfing and included ‘Controller™ 5’ and ‘Krymsk® 1’, and ‘*P. americana’*.

Survival – Rootstock survival was largely determined in two phases (Table 1). The first being within one year of planting (2009 – 2010), and the other between the fourth and fifth year after planting (2013 – 2014). Nine of the seventeen rootstocks lost at least one replicate tree within the first year after planting. ‘Viking®’ had the lowest rate of survival during this period with half of the replicates dying prior to the end of the first year. The high losses were attributed to root zone drying as a result of inadequate watering after planting (Pokharel and Reighard, 2015). After the first year, ‘Viking®’ replicates had 100% survival rates over the next eight seasons. The rootstocks ‘Krymsk® 1’, ‘Rootpac® R’, and ‘Empyrean® 2’, showed strong survival with each losing one tree during the first year and none the remainder of the trial. In 2013 and 2014, eleven of the seventeen rootstocks lost one replicate. Three ‘Bright’s Hybrid® 5’ trees were lost from 2013 - 2014. Determinations as to why these periods coincided with mortality were inconclusive, however, cold weather that resulted in crop loss may have played a part in 2014. No rootstock experienced additional mortality after 2014.

Results across all trial sites showed ‘Fortuna’ may be incompatible with peach scions (Reighard et al., 2020), and should be avoided as a rootstock for peach. ‘Fortuna’ has been excluded from the analysis.

Suckering – Root suckering is a common trait of many *Prunus* species. While suckers can often be removed with herbicides, prolific amounts create hazards, limiting mobility and reducing the efficiency of laborers in the field. General trends of root suckering from each rootstock can be found in Table 1. The rootstocks producing the most root suckers in this trial were ‘Rootpac® R’, ‘Bright’s Hybrid® 5’, and ‘Krymsk® 1’. The *P. persica* rootstocks ‘Lovell’, ‘Guardian®’, ‘KV-010123’, ‘KV-010123’, and ‘Controller™ 7’ had moderate suckering. The hybrid rootstocks ‘Atlas™’, ‘Imperial California’, and ‘*P. americana’,* also had moderate suckering. ‘Viking®’, ‘Empyrean® 2’, ‘Krymsk® 86’, ‘Controller™’ 5 and 8, produced none to a few root suckers over the course of the trial.

Iron chlorosis – While the arid climate of Colorado’s Western Slope limits many soil borne diseases and organisms, high abundance of lithogenic and secondary carbonates in the soil mineral matrix create calcareous alkaline soils with high calcium carbonate content. Calcareous soils can reduce nutrient uptake and limit availability of iron (Fe), manganese (Mn), copper (Cu), zinc (Zn) and boron (B). Symptoms related to these nutrient deficiencies include interveinal chlorosis, reduced photosynthesis, reduced fruit size, reduced leaf area, and diminished shoot growth (Tagliavini and Rombola, 2001; Minas et al., 2023b).

Visual observations of interveinal chlorosis were found in some *P. persica* rootstocks such as 'Controller^{TM}' 7 and 8, 'KV-010127', 'KV-010123'. However, chlorosis was not found in the *P. persica* rootstocks 'Lovell' and 'Guardian®'. Generally, the *P. persica* rootstocks appeared more chlorotic than the interspecific hybrids 'Viking®', 'Rootpac® R' and 'Atlas^{TM}' (Pokharel and Reighard, 2015). The remaining rootstocks showed varying trends, the European plum rootstock, 'Imperial California' showed signs of chlorosis while 'Krymsk® 86' and 'Controller^{TM} 5', both interspecific plum hybrids, 'Krymsk® 1', a complex hybrid, 'Empyrean® 2', a European plum, and '*P. americana*' did not (Pokharel and Reighard, 2015). Susceptibility to iron chlorosis of each rootstock can be found in Table 1.1.

Two additional arid high-altitude sites in Utah provided an opportunity to further determine the impacts of alkaline soils on rootstock performance in the Intermountain West. While the soil pH at the Colorado site was 8.3, one of the Utah sites had a soil pH of 8.5 and the other was closer to neutral, 7.5.

The average trunk cross sectional area (TCSA) at the two high pH sites were quite similar, with Colorado only slightly smaller on average across all rootstocks (Black et al., 2021). However, the average TCSA of *P. persica* rootstocks were more than two times larger at the low pH site than high pH sites (Black et al., 2021). The interspecific peach hybrid and non-*P. persica* rootstocks at the low pH site were also larger than the high pH sites, but the size differences were not as significant (Black et al., 2021). Growers wanting to utilize *P. persica* rootstocks in high pH soils should be aware that vigor profiles in Colorado may be reduced from what nurseries are promoting.

Replant tolerance – Replanting orchards with the same fruit species (i.e., peach after peach) can result in replant disease. The specific mechanisms of peach replant disease are not widely understood and vary based on soil chemistry, physical properties, and microbiota (Bent et al., 2009). Common traits of replant disease are stunted growth, and lowered productivity and longevity of the orchard. Given the small geographic region that is suitable for peach production in Colorado, and the economic penalty associated with rotating fields out of peach production, growers are often forced into replant conditions. With the geographic and economic limitations in mind, it is important to determine a rootstock's tolerance of replant conditions. While not a specific focus of this field trial, initial results from Colorado greenhouse study suggests that *P. persica* seedling rootstocks are the most susceptible to replant conditions (Minas et al., 2023b). Conversely, long term trials in South Carolina suggest European bred, interspecific rootstocks, were outperformed by *P. persica* rootstocks 'Guardian®' and 'Lovell' and 'MP-29' a plum peach hybrid, in severe PTSL replant sites (Reighard et al., 2022). Alternatively, *Prunus* hybrid rootstocks such as 'Krymsk® 86', 'Bright's Hybrid® 5' have been shown to tolerate replant conditions (Minas et al., 2023b).

Cold Hardiness and Acclimation – Identifying rootstocks that can tolerate the cold winters of Colorado is important to successful peach production. During this trial, bud pairs from 'Redhaven' scion wood were selected from several rootstock vigor classes to determine fall acclimation and winter cold hardiness. The investigations during the 2016/17 and 2017/18 dormant seasons showed that the overall least hardy rootstocks were 'Atlas™' and 'Krymsk® 1' and the hardiest were 'Guardian®' and 'Krymsk® 86'. Interestingly peach seedling rootstocks like 'Guardian®', 'Lovell', 'Controller™' 7 and 8 exhibited early acclimation in fall, whereas *Prunus* hybrids such as 'Krymsk® 86', 'Atlas™' and 'Bright's Hybrid® 5' exhibited late acclimation.

Additionally, it was observed that ‘Krymsk® 86’ exhibited late deacclimation in early spring (Minas personal communication).

Interestingly, during the dormant season of 2022/23 similar studies showed no significant differences over several time periods over the winter across rootstocks. Prominent temperature swings during fall in Colorado make cold acclimation equally important to growers. Trees that are slow to acclimate are subject to frost cracking which can cause orchard dieback and create possible entry wounds for disease such as *cytospora* canker. Trials in Europe have shown vigorous interspecific hybrid rootstocks, such as peach x almond hybrids, as slow to acclimate in fall (Massai and Loretti, 2002). Several vigorous interspecific hybrid rootstocks were trialed in this study and could be susceptible to freezing due to slow acclimation. Preliminary observations may suggest ‘Krymsk® 86’, an interspecific hybrid, acclimates slower than other rootstocks. Given the importance of cold acclimation in Colorado, future trials involving interspecific hybrids should incorporate this aspect into their investigations.

1.3. Agronomic performance

Yield – When evaluating production performance across rootstocks, the general trend was yield and number of fruit per tree increased with increasing rootstock vigor (Fig. 1.2A). There was a strong linear relationship between rootstock vigor and cumulative yield (mt / ha), with ‘Atlas™’, the biggest tree producing the most and ‘*P. americana’,* ‘Controller™ 5’, and ‘Krymsk® 1’, the most dwarfing rootstocks, producing the least (Fig. 1.2B). However, within each vigor class, some rootstocks showed higher yields. For instance, ‘KV-010127’ and ‘Viking®’ had high cumulative yields for their respective TCSA while ‘Imperial California’, ‘Controller™ 7’ and ‘Controller™ 5’ had low cumulative yields compared to other rootstocks in their vigor class (Figs 1.2, and 1.4). While the most vigorous trees had the highest yields, as rootstock vigor decreased, yield efficiency (kg / TCSA) increased with 'Krymsk® 1' recording the highest yield efficiency in the trial (Fig. 1.4). Additionally, not all rootstocks within each rootstock class performed the same. The contradiction between yield per tree and yield efficiency has both pros and cons for growers to consider. Selecting a high vigor rootstock will result in higher yields per tree, however it will also require lower density plantings and result in lower returns per unit of area. Conversely, selecting a dwarfing rootstock with a higher yield efficiency per unit of area will require higher density plantings which raises the cost of establishing the orchard. While this trial did not experiment with different planting densities, growers should be aware that rootstock selection has significant implications on cost and field practices. The differing vigor profiles will impact upfront cost, cost of labor, and overall return on investment.

Fruit weight – One of the greatest concerns when evaluating peach rootstock performance is the tendency for less vigorous rootstocks to produce smaller fruit. Fruit weight can be influenced by several factors, including length of growing season, crop load, and rootstock vigor. Theories on how dwarfing occurs in rootstocks may also play a role in reducing fruit size. Several of these theories are associated with reduced conductance and transportation via the xylem. An example of reduced xylem function is poor graft compatibility. The reduced xylem flow restricts water, nutrient, and hormone movement which could also reduce fruit size.

In this trial, crop loads were adjusted based on TCSA, to eliminate excessive fruit loads that may result in reduced fruit weights. While yield per tree was positively related to vigor (Fig. 2A), fruit weight and vigor showcased less direct trends. The average fruit size across all rootstocks from 2011 – 2017 was 176 g (6.2 oz). Each rootstock's fruit size was categorized based on the average fruit size (176 g) and is noted in Table 1.1. The three most vigorous rootstocks 'Atlas™', 'Bright's Hybrid® 5', and 'Guardian®' obtained the largest fruit size averaging 195 g (6.7 oz).

‘KV-010127’, ‘Krymsk® 86’ and ‘Empyrean® 2’, three standard sized rootstocks averaged 180 g (6.3 oz) fruit while the ‘*P. americana’* and ‘Controller™ 5’ had the smallest average fruit weight at 154 g (5.4 oz). Despite being in reduced vigor rootstock classes, ‘Krymsk® 1’ (dwarfing) and ‘Rootpac® R’, and ‘KV-010123’ (semi-dwarfing) produced average sized fruit with means of 177 g (6.2 oz), and 170 g (6.0 oz), respectively.

Internal fruit quality – The differing rootstock genetics in this trial created various vigor profiles that created distinct canopy environments for fruit development. To characterize the impact of the distinct canopies on fruit quality, our lab developed a non-destructive tool to determine fruit maturity, dry matter content (DMC %) and soluble sugar content (SSC %) quickly and effectively in a single rapid scan (Minas et al., 2023a).

With the accurate predictive models for maturity, it was possible to select fruit of equal maturity and determine the direct relationship between the canopy environment and internal fruit quality development, as maturity impacts fruit quality attributes. Overall, internal fruit quality profiles were reduced by the increased internal shading and/or cooler temperatures found in more vigorous rootstock canopies (Figs. 1.3 and 1.5). Conversely, as canopy size is reduced, higher levels of light are available for developing fruit and photosynthetic leaves, which can lead to enhanced fruit quality attributes, such as DMC (Figs. 1.3 and 1.5).

1.4. Summary of individual rootstock performance

Rootstock summaries encompass data and observations from the 2009 NC-140 multisite trial as well as specific rootstock performance at the Western Colorado Research Station. Given the results were captured in multiple settings, specific attributes listed in the rootstock descriptions below may not align with Table 1.1 which only represents results observed in the Colorado planting.

‘Fortuna’ is an interspecific plum x peach hybrid with semi-dwarfing characteristics. It is also highly susceptible to *cytospora*. It has been shown to have incompatibility with ‘Redhaven’ (Reighard et al., 2013) which could extend to other cultivars as well. Given its issues with compatibility and its susceptibility to *cytospora*, it would be a poor choice for Colorado peach production systems.

‘KV-010123’ was developed at USDA Kearneysville, WV. This *P. persica* was similar to standard vigor rootstocks with minor suckering. While fruit size and SSC were average cumulative yield per tree was 11 kg (24 lbs.) less than ‘Lovell’. It was reported as having severe chlorosis.

‘KV-010127’ was developed at USDA Kearneysville, WV and is a standard sized *P. persica* rootstock with moderate suckering and prominent chlorosis. It had higher cumulative yields and increased yield efficiency when compared to other standard rootstocks. Fruit size was reported to be the largest across all sites. In Colorado fruit size was more similar to vigorous rootstocks, and quality was a bit reduced, but probably not to a level that would be noticeable in relation to other standard rootstocks.

Prunus americana is a wild American plum that is commonly used successfully as a plum rootstock. This Minnesotan selection has also been used, with good success, by hobby peach farmers. However, in Colorado, it resulted in the third most dwarfing rootstock. The small tree size also led to reduced fruit size. Like many plum rootstocks it is associated with excessive root suckering.

‘Viking®’ was developed by Zaiger genetics in California. It is a complex hybrid consisting of four *Prunus* species: almond, plum, apricot and peach. Compared to a standard rootstock, it is reported as being vigorous to moderately more vigorous. This rootstock is very resistant to chlorosis, root knot nematode, tolerant of saline and alkaline soil, and does not produce a lot of suckers (Reighard et al., 2020). When selecting for your orchard, the roots should be kept moist as it does not tolerate dying down during establishment, which was a possible problem for their survival in this trial. In this trial it was the third most vigorous, with a vigor profile similar to ‘Guardian®’. Yields were high and very consistent from 2015–2017, averaging higher per tree yields than all other rootstocks.

‘Imperial California’ is a European plum (*P. domestica*) rootstock that was developed in Italy. The rootstock is slightly smaller than standard size, and resistant to chlorosis. Trials in California found it lacked uniformity in vigor and spread. In the Southern US it was found to be highly susceptible to bacterial canker. In this trial it was more vigorous than ‘Lovell’, however, yields and fruit weights were similar. This rootstock produced some to few suckers. Compared to the other rootstocks it had the lowest incidence of *cytospora* cankers (Pokharel and Reighard, 2015).

‘Lovell’ was the standard peach (*P. persica*) seedling rootstock in the US for many years. It was selected in California in 1882 and was widely available due to its prominent use as a canning peach. ‘Lovell’ produces standard vigor trees with good root anchorage. It is not widely used in major peach producing regions anymore as it is not suitable for replant conditions and is susceptible to *Armillaria* root rot (ARR) and root-knot nematodes. However, it does offer excellent resistance to bacterial canker. ‘Lovell’ prefers well drained soils as it shows low tolerance to waterlogging and poor tolerance to calcareous soil expressed as iron chlorosis. ‘Lovell’ exhibits good acclimation performance in fall and moderate mid-winter hardiness.

‘Guardian®’ is a vigorous peach *(P. persica*) seedling rootstock that was released by USDA due to its tolerance to peach tree short life (PTSL). It is less susceptible to bacterial canker and PTSL and shows resistance to root-knot and ring nematode thus it is widely planted in southeastern USA. It is highly susceptible to ARR (Beckman and Pusey, 2001). It is more vigorous than ‘Lovell’ and had consistent yields and fruit size, and moderate to high levels of suckering. There were no visual observations of chlorosis.

‘Controller™ 5’ (K146-43) is a dwarfing interspecific plum x ‘Favorcrest’ peach (*P. salicina x P. persica*) hybrid rootstock developed by UC Davis and USDA in California. It is a dwarfing rootstock, 60% of the size of ‘Lovell’ after 9 years. No root suckering was observed with this rootstock. It flowers heavily and has high crop loads; however, fruit size is small. Its reduced vigor profile is not well suited to an open vase training system. In two sites with alkaline soils (Utah and Colorado) it had high mortality, but the surviving trees did not show iron chlorosis (Black et al., 2021). It is intolerant to waterlogged soil and has performed poorly in root-knot, lesion and ring nematode and bacterial canker field sites (Reighard et al., 2006).

‘Controller™ 7’ (HBOK 32) is a semi-dwarfing intraspecific hybrid coming from the F2 of an open pollination of ‘Harrow Blood’ x ‘Okinawa’ peach (*P. persica*) cultivars. The rootstock imparts dwarfing characteristics and is resistant to root-knot nematodes. It performs poorly in alkaline or calcareous soils showing severe chlorosis. Compared to ‘Lovell’ yields and vigor are reduced. Due to its issues with iron chlorosis, this rootstock is not recommended for use in Colorado without applications of chelated iron.

‘Controller™ 8’ (HBOK 10) was developed by UC Davis breeding program. It is an open pollinated *P. persica* cross between the cultivars ‘Harrow Blood’ and ‘Okinawa’. Trees are semi-dwarfing to standard vigor profiles with good root anchorage and no suckering. It has shown root-knot nematode resistance but is not tolerant to calcareous soils, showing symptoms of iron chlorosis. In Colorado cumulative yields from this trial were roughly 10 kg (22 lbs.) per tree less than ‘Lovell’.

‘Empyrean® 2’ (Penta) is a European plum (*P. domestica*) semi-dwarf rootstock, which is resistant to root-knot nematode, phytophthora, waterlogged, and heavy soils. It appears to be very susceptible to ring nematode, so it could be highly susceptible to bacterial canker as well. It was released by the Istituto Sperimentale per la Frutticoltura (ISF) of Rome, Italy in 1997 (Nicotra and Mosser, 1997). In Europe, it is reported to be very vigorous, similar to peach-almond hybrids and peach x *P. davidiana*, such as ‘Cadaman’ (Reig et al., 2020). In the NC-140 trials where it was evaluated, it survived well, however it was the earliest blooming which may not be desired in Colorado. The trees that survived were significantly less vigorous than the standard rootstock, ‘Lovell’, which were approximately 70% of its size after 9 years from planting (Reighard et al., 2020). While its yields were similar to other reduced vigor rootstocks such as ‘Controller™ 8’, average fruit size in Colorado was more similar to ‘Viking®’ however, at other sites in the trial it was reported as being reduced (Reighard et al., 2020).

‘Atlas™’ is a vigorous complex almond (*P. dulcis x P. blierianna*) x ‘Nemaguard’ peach (*P. persica*) interspecific hybrid that was introduced by Zaiger Genetics in the USA in 1994. It results in vigorous trees with high survival rates and high cumulative yields with large fruit size. It is not cold hardy, acclimates slowly in fall, and is intolerant to waterlogged soils. It is resistant to root-knot, but susceptible to lesion and ring nematodes. It was the most vigorous rootstock in this trial and had reduced light availability in the canopy, which reduced fruit quality parameters. Little to no visual chlorosis. However, it was highly susceptible to *cytospora* canker with 100% of trees showing symptoms (Pokharel and Reighard, 2015). It is likely too vigorous for a pedestrian style orchard using a modern training system unless vigor was diffused by increasing scaffold numbers, such as in a Hex-V training system.

‘Bright’s Hybrid® 5’ is a vigorous almond (*P. dulcis*) x peach (*P. persica*) interspecific hybrid rootstock that was introduced by Bright's Nursery in California. It is very vigorous, nematode resistant, deep rooting, well anchored, drought and replant tolerant. This rootstock needs deep well drained soil. Selection 5 was a survivor in an orchard with high pH due to excess calcium carbonate. It shows poor resistance to ARR; however, moderate survival rates have been reported (Reighard et al., 2020). It was the second most vigorous rootstock in this trial producing a large and dense canopy. However, pre-harvest fruit maturity was increased when compared to other less vigorous rootstocks in this trial suggesting there may be some genetic control of maturation with this rootstock. It had moderate suckering, and large fruit size. Cumulative yields per tree were nearly 25 kg (55 lbs.) more than ‘Lovell’.

'Krymsk® 86' (Kuban 86) is a standard vigor myrobalan plum (*P. cerasifera*) x peach (*P.persica*) interspecific hybrid rootstock that was developed by Krymsk Experimental Breeding Station (KEBS) in Russia. It is susceptible to root-knot and lesion nematodes, crown gall (Pinochet et al., 2012) and Phytophthora but has exhibited good levels of resistance to ARR. Its standard vigor profile, graft compatibility with both peach and almond, as well as its ability to tolerate a plethora of abiotic soil factors has led it to becoming one of the most universally used rootstocks in the US. Its extensive rooting and root strength help make it tolerant to dry conditions and have outstanding anchorage. It is cold hardy in mid-winter with slower acclimation rates in fall. It performs well in heavy calcareous soils and is tolerant to iron chlorosis and root asphyxia from waterlogged soil. It also has good productivity, fruit size, and quality (Minas et al., 2023a; Pieper et al., 2024). Trials in Spain found it does not perform well with flat peaches, or in areas with lower chilling.

'Krymsk® 1' (VVA-1) is a dwarfing myrobalan plum (*P. cerasifera x P. tomentosa*) interspecific hybrid rootstock developed by KEBS in Russia. In Colorado, 'Krymsk® 1' was a dwarfing rootstock with many suckers, high yield efficiency, average yields, and average fruit size of superior internal quality characteristics (Minas et al., 2023a, Pieper et al., 2024). The vigor is too low for an open vase training system and may be better suited in a single leader or "V" training system. An important note from other 2009 NC-104 sites, and in different trials, this rootstock has shown incompatibility with several scion varieties. Symptoms include rolled leaves, weak graft unions and high mortality rates (Zarrouk et al. 2006; Ben Yahmed et al., 2016).

'Rootpac® R' was developed by Agromillora in Iberia, Spain. It is an open pollinated hybrid of a Myrobalan plum (*P. cerasifera*) and almond, both of unknown origin (Pinochet, 2010). It is reported as vigorous, however, at the western sites of Colorado and Utah which are characterized by cold winters, hot and dry conditions, and calcareous soils, ‘Rootpac® R’ behaved as a semi-dwarfing rootstock with reduced vigor and yields, little suckering, and generally smaller fruit size, and without any visual symptoms of iron chlorosis (Pokharel and Reighard, 2015; Black et al., 2021). It was highly susceptible to *cytospora* canker with 100% of trees showing symptoms (Pokharel and Reighard, 2015).

* Rootstock performance evaluations adapted from Minas et al. (2023b), as well as individual observations made at the Western Colorado Research Station, Grand Junction, CO.

1.5. Takeaways from the NC-140 2009 ‘Redhaven’ rootstock trial

This long-term rootstock trial provided an opportunity to develop a greater understanding of the performance of seventeen novel rootstocks in Colorado’s growing conditions. Throughout this 12-year rootstock trial, several key takeaways were revealed. Firstly, larger canopies produce and support more fruit. However, the larger canopy also has a negative impact on light availability, which negatively impacts fruit quality. Together this information poses some economic tradeoffs for growers. On one hand more, vigorous rootstocks support more fruit and thus have the potential for higher returns. On the other hand, vigorous trees can delay and cause unequal fruit maturity leading to an increased number of harvests. Furthermore, the increased shading in vigorous trees reduces internal quality parameters that may lead to a decrease in consumer acceptance. Additionally, the increase in vigor increases canopy complexity slowing worker productivity and increasing labor costs. As peach production systems shift to higher density with planar canopies and simplified training systems, high vigor rootstocks will likely outgrow their allotted space in the orchard design and require harder pruning to maintaining adequate light for fruit development and flower bud initiation. While increasing density may reduce vigor through root competition, finding rootstocks with reduced vigor profiles that are able to yield high amounts of large, high quality, peaches is of greater importance. Here we trialed seventeen rootstocks, many with reduced or standard vigor profiles that may be suitable for modern peach production systems. Future trials in Colorado should determine how rootstocks perform when grown in high density plantings with modern planar canopy training systems. While many of the peach growing regions around the world are trying to reduce vigor through the use of dwarfing rootstocks, peach production in the Intermountain West may be ideal for high density plantings as previous multiyear peach rootstock trials conducted across North America have shown Colorado to have some of the lowest vigor profiles and slowest growth rates. However, the reduced vigor profiles Colorado growers are accustomed to may be perfectly suited to modern high-density plantings which have replaced traditional production methods in many temperate orchards systems.

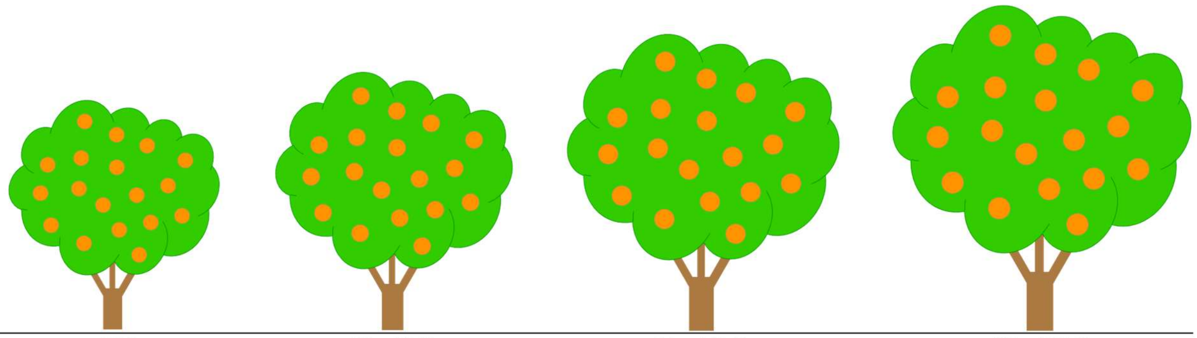
Tables.

Table 1.1. Characteristics of the seventeen rootstock cultivars in the 2009 NC-140 trial. Genetic background and breeding origin for the seventeen rootstocks in the NC-140 multistate trial. Each rootstock is characterized by attributes observed at the Orchard Mesa Research Station in Grand Junction, CO. Final evaluation and characterization was determined in 2017, nine years after planting. Rootstocks were evaluated for vigor, survival, root suckering, leaf chlorosis, replant tolerance, cold hardiness, fruit size, and fruit quality. Each individual rootstock attribute was compared to 'Lovell' a longtime industry standard.

Rootstock	Breeder, Origin Genetics	Genetics	Vigor[a]	Survival[b]	Suckering[c]	Chlorosis[d]	Replant tolerant	Fruit size[e]	Fruit Quality[f]
Atlas™	Zaiger Genetics, CA	Complex Prunus hybrid, *Prunus persica x (P. dulcis x (P. cerasifera x P. armeniaca))*	Vigorous	Good	Some	No	Yes	Large	Reduced
Bright's Hybrid® 5	Bright's Nursery, CA	Peach almond hybrid, *P. persica* x *P. dulcis*	Vigorous	Poor	Many	No	Yes	Large	Reduced
Guardian®	Clemson/USDA, SC	*P. persica*	Vigorous	Good	Some	No	No	Average	Average
Viking®	Zaiger Genetics, CA	Complex Prunus hybrid, *Prunus persica x (P. dulcis x (P. cerasifera x P. armeniaca))*	Vigorous	Poor*	None	No	Yes	Large	Average
Imperial California	Istituto Sperimentale per la Frutticoltura, Italy	European plum, *P. domestica*	Vigorous	Good	Some	Yes	Yes	Large	Average
KV-010127	USDA, WV	*P. persica*	Standard	Good	Some	Yes	No	Average	Reduced
Krymsk® 86 (Kuban 86)	Krymsk Experimental Breeding Station, Russia	Peach x myrobalan plum, *P. persica x P. cerasifera*	Standard	Good	None	No	Yes	Large	Average
Lovell	G.W. Thissell, CA	Peach seedling, *P. persica*	Standard	Good	Some	No	No	Avearge	Average
Controller™ 7 (HBOK 32)	UC Davis, CA	*P. persica* x *P. persica*	Standard	Good	Some	Yes	No	Reduced	Average
Empyrean® 2 (Penta)	Istituto Sperimentale per la Frutticoltura, Italy	European plum, *P. domestica*	Standard	Good	None	No	Yes	Large	Average
KV-010123	USDA, WV	*P. persica*	Semi-dwarfing	Good	Some	Yes	No	Average	Reduced
Controller™ 8 (HBOK 10)	UC Davis, CA	*P. persica* x *P. persica*	Semi-dwarfing	Good	None	Yes	No	Average	Improved
Rootpac® R (Replantpac)	Agromillora, Spain	Myrobalan plum x Almond, *P. cerasifera x P. dulcis*	Semi-dwarfing	Good	Many	No	Yes	Average	Improved
Prunus americana	Bailey's Nursery, MN	American plum, *P. americana*	Dwarfing	Good	Some	No	Yes	Reduced	Improved
Controller™ 5	UC Davis, CA	Peach x Japanese plum hybrid, *P. persica x P. salicina*	Dwarfing	Good	None	No	Yes	Reduced	Improved
Krymsk® 1 (VV1-1)	Krymsk Experimental Breeding Station, Russia	Nanking cherry x myrobalan Plum, *P. tomentosa x P. cerasifera*	Dwarfing	Good	Many	No	Yes	Average	Improved
Fortuna§	Krymsk Experimental Breeding Station, Russia	Myrobalan Plum x Peach, *P. cerasifera x P. persica*	Semi-dwarfing	Poor	-	-	-	-	-

Data from 2009 NC-140 'Redhaven' rootstock trial presented in descending order by rootstock vigor. *Viking low survival likely due to root zone drying out. §Poor survival, no additional data collected. [a]Vigor as trunk corss sectional area (TCSA cm2) in 2017. [b]Survival over 9 seasons. [c]Average number of root suckers produced per tree. [d]Visual observation of iron chlorosis in 2011. [e]Fruit size (g). [f]Fruit quality as % dry matter content (DMC).

Figures.



< 60 %	60 – 90 %	90 – 110 %	110 – 120 %
Dwarf	**Semi-dwarf**	**Standard**	**Vigorous**
Controller™ 5	Controller™ 8	Lovell	Atlas™
Krymsk® 1	KV-010123	Krymsk® 86	Bright's Hybrid® 5
P. americana	Fortuna	KV-010127	Viking®
	Rootpac® R	Empyrean® 2	Guardian®
		Controller™ 7	Imperial California

Figure 1.1. Rootstock genotypes and vigor classifications. Rootstock vigor profiles from the Colorado 2009 NC-140 'Redhaven' trial. Rootstocks were classified by their relative vigor compared to 'Lovell'; a commercial standard growers are familiar with. Vigor profiles presented here were from the ninth year of the trial. This figure is adapted from Anthony and Minas (2022) and shows average results of trunk cross sectional area from Colorado in 2017.

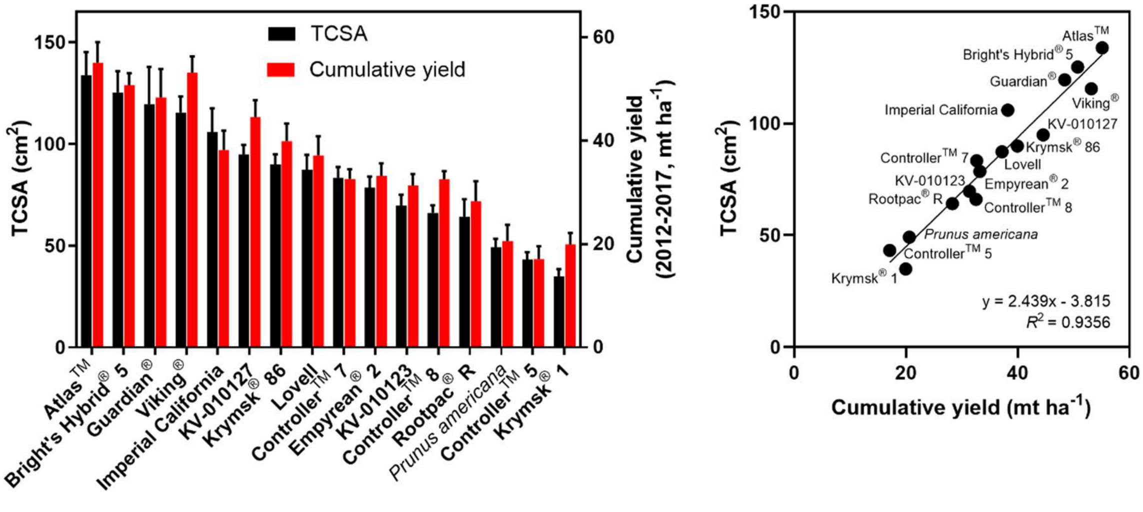


Figure 1.2. Vigor profiles and cumulative yields of sixteen rootstocks from the 2009 NC-140 'Redhaven' trial. Rootstocks are presented by vigor profile (TCSA cm^2), left to right, starting with the most vigorous, and represented in black (A). Cumulative yield (mt ha^{-1}) from 2012-2017 is presented by rootstock in red (A). A strong linear relationship between rootstock vigor (TCSA) and cumulative yield (mt ha^{-1}) is presented in figure B. As rootstock vigor increases so too does yield.

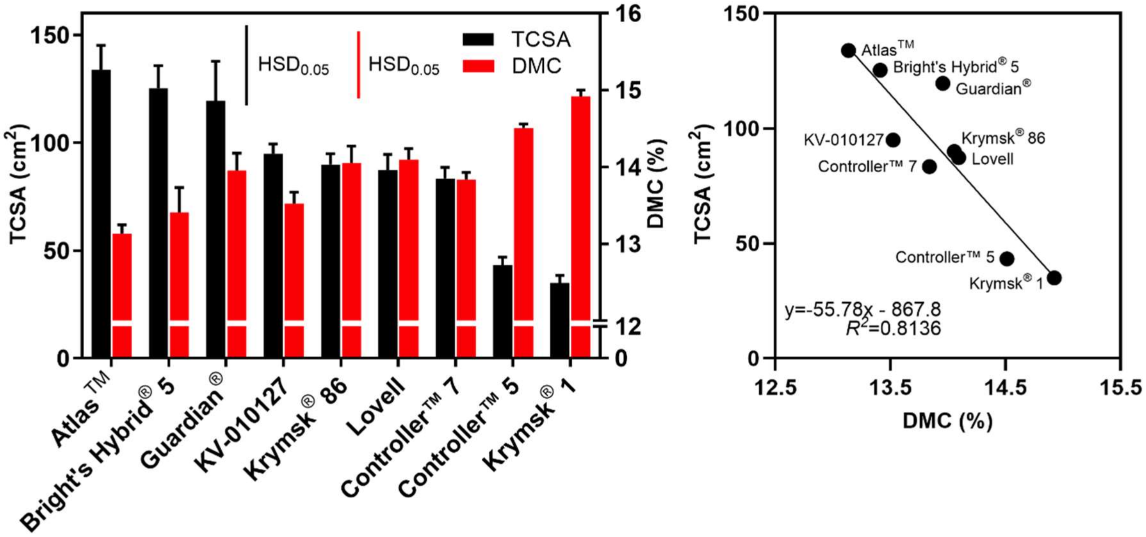


Figure 1.3. The impact of rootstock vigor on peach fruit dry matter content. Fruit from nine promising rootstocks with differing vigor profiles were selected for evaluation of dry matter content (DMC %). Rootstocks are presented in order of decreasing vigor (TCSA, cm^2, black), with the most vigorous, 'Atlas$^{\text{TM}}$', on the left and most dwarfing, 'Krymsk$^{®}$ 1', on the right (A). DMC (red) was averaged across two growing seasons (2016 -2017, A). Columns represent mean values ± SE (A). The linear relationship between TCSA and DMC (B). This figure was previously published in Minas et al., 2023a.

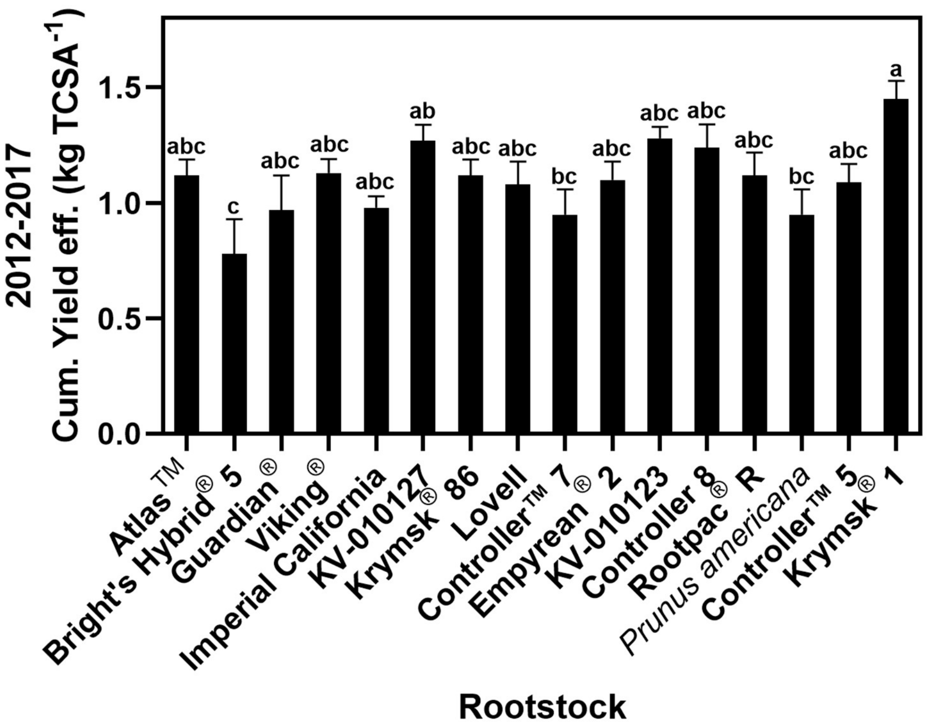


Figure 1.4. Cumulative yield efficiency of the sixteen rootstocks from the 2009 NC-140 ‘Redhaven’ trial. Rootstocks are presented in order of decreasing vigor (TCSA, cm^2), with the most vigorous, ‘Atlas™’, on the left and most dwarfing, ‘Krymsk® 1’

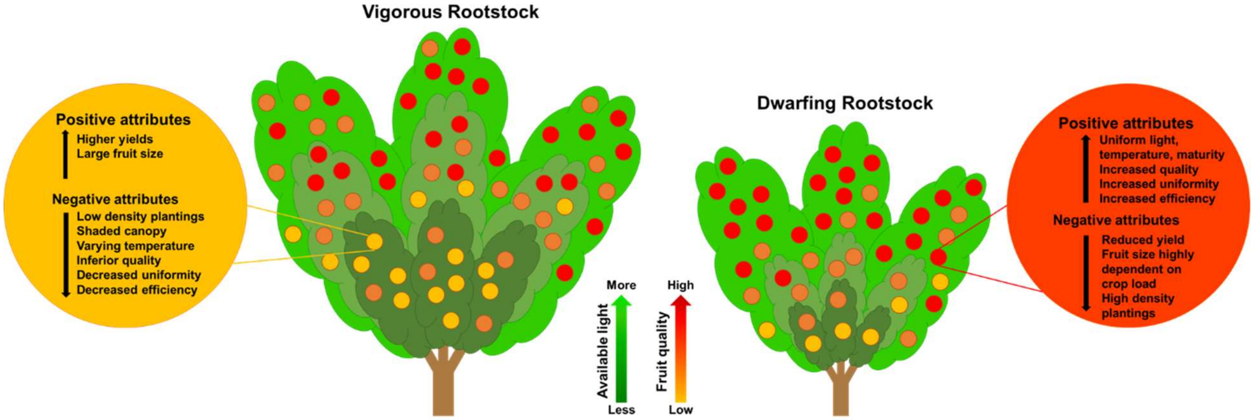


Figure 1.5. Attributes of vigorous and dwarfing rootstocks. The two vigor profiles are compared for the positive and negative attributes each provide to a production system. Vigor affects yield per tree, as well as the canopy light environment which impacts internal fruit quality parameters. Both vigor profiles offer important decisions growers need to contemplate prior to rootstock selection. This figure was adapted from Pieper et al., 2024.

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CHAPTER TWO

ROOTSTOCK VIGOR DICTATES THE CANOPY LIGHT ENVIRONMENT THAT REGULATES METABOLITE PROFILE AND INTERNAL FRUIT QUALITY DEVELOPMENT IN PEACH

2.1. 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 performance.

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 and Echevarria, 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.

2.2. Materials and Methods:

2.2.1. 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. The selected rootstocks included various breeding origins and genetic makeups and are as follows: 'Bright's Hybrid® 5' (BH5) and 'Atlas™' (ATL) (vigorous), ‘Krymsk® 86’ (K86) and ‘Lovell’ (LOV) (standard), and ‘Krymsk® 1’ (K1) (dwarfing) (Table 2.1). Five healthy trees of uniform vigor were selected from each rootstock genotype for a total of twenty-five trees for the entire experiment. While several trials have found ‘Krymsk® 1’ to have low survival rate due to bacterial canker (Reighard et al., 2020), graft incompatibility (Zarrouk et al., 2016) and high mortality and bud failure (Ben Yahmed et al., 2016) it has performed well in Colorado with 88% survival rate in 2019.

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).

2.2.2. Fruit quality analyses

To provide a more accurate depiction of how rootstock vigor impacts agronomic performance, five year (2015–2019) mean cumulative (mt ha^{-1}) and per tree yields (kg $tree^{-1}$) as well as fruit size (g) were calculated. Cumulative yield was calculated as means across replicates by year. Yearly mean values were summed to represent five year means. Average per tree yields and fruit weight were determined by counting and weighing all marketable fruit each season. Mean five-year yield per tree and fruit weight were averaged by rep by year and then across years.

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, tree scans were conducted to identify five fruit from the 1.5 m canopy height. All fruit 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 (Ziosi et al., 2008; Costa et al., 2009). In this trial, fruit were selected at stage 4-I, or commercial harvest stage that correlates 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).

Physiological characterization and sampling was conducted on fruit of equal maturity to understand the direct impact of rootstock vigor on internal fruit quality (Fig. 2.1). 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 ($h°$), 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).

2.2.3. 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 ºC until analysis (Fig. 2.1). Prior to -omics analyses, peach mesocarp was freeze-dried (Freezone 4.5, Labconco, Kansas City, MO, USA) at -40 ºC 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 ºC) 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; R Core Team, 2015; Mahieu et al., 2016). 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, 2013) and NIST (http://nist.gov).

2.2.4. 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.

2.3. Results

2.3.1. 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. 2.2B). 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. 2.2A). These differences of tree vigor were reflected in canopy zone light availability (LA) that exhibited a trend of increase with decreasing tree vigor (Fig. 2.2C). The dwarfing rootstock, K1, had the highest LA (85%) at 1.5 m (Fig. 2.2C). 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. 2.2C). 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. 2.2C).

Vigorous rootstocks maintained the highest yields (kg tree^{-1}), on a five-year average, which were followed by the standard and dwarfing rootstocks (Fig. 2.2E). 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. 2.2I). 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. 2.2E). Both standard rootstocks also produced significantly greater than K1, with 60.3 (K86) and 58.7

MT ha^{-1} (LOV), respectively (Fig. 2.2E). While yield was significantly different by vigor classification, crop loads were controlled by adjusting the number of fruits per cm^2 of TCSA (Fig. 2.2G). 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. 2.2G). 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. 2.2H). 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. 2.1). Average maturity (I_{AD}) across rootstock genotypes was 0.5 I_{AD} and was not significantly different across rootstocks (Fig. 2.3A). 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. 2.3B). Flesh firmness for ATL, BH5, and K1 (37, 35, and 36, respectively) were not statistically different from either LOV or K86 (Fig. 2.3B).

Titratable acidity (TA) demonstrated minimal differences between rootstocks at harvest (Fig. 2.3E). Only the two most vigorous rootstocks demonstrated a significant difference, with ATL having higher levels than BH5 (Fig. 2.3E). 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. 2.3F-H). Fruit overcolor blush (%) was highest in LOV (62%) and least in ATL (49%) (Fig 2.3F). Lightness (L^*) values followed a similar trend to vigor, with lightness decreasing with decreased rootstock vigor and increased light availability (Fig. 2.3G). Hue angle (h^o) values across rootstocks were not significantly different from one another (Fig. 2.3H).

With respect to important consumer acceptance related parameters, the vigorous rootstocks had the lowest DMC and SSC levels of all rootstocks (Figs. 2.3C-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. 2.3I-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. 2.4). 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. 2.4). 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. 2.4).

2.3.2 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. 2.5). 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. 2.5). 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. 2.5).

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. 2.6). Along PC1, the separation was related to differing levels of rootstock vigor (Fig. 2.6). 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. 2.6). 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. 2.6).

2.3.3 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. 2.7B-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. 2.7B-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. 2.7A).

Four additional metabolites butanoic acid, quinic acid, catechin, and neochlorogenic acid, appeared to be influenced by light availability, as an artefact of vigor classification (Figs. 2.7E-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. 2.7F). 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. 2.7G). 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. 2.7H). 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. 2.7E).

2.3.4. 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. 2.8A). Sorbitol abundance peaked in LOV, with statistically similar levels in K86 and K1 (Fig. 2.8A). The vigorous genotypes (ATL and BH5) demonstrated the lowest levels of sorbitol (Fig. 2.8A). 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. 2.8B-C). Apart from LOV, sorbitol abundance and fruit quality trends appear to follow the gradient of vigor and light availability (Figs. 2.8A-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. 2.8D). Malic acid abundance was 41% higher in BH5, the most vigorous rootstock, when compared to K1, the most dwarfing rootstock (Fig. 2.8D). 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. 2.8E-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. 2.8D-F).

2.4. Discussion

2.4.1 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', 'KV-010127', '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. 2.2E-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^{TM}’, ‘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. 2.2H).

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. 2.3D 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. 2.2G). 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. 2.3A). 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. 2.2C and 2.3C-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. 2.2C and 2.3C-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. 2.3F). Exocarp hue angle (Fig. 2.3H) 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. 2.3G). 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. 2.2A-B), resulting in increased yields (Figs. 2.2E and I). Secondly, different levels of vigor created distinct light environments for the developing fruit (Fig. 2.2C) impacting internal fruit quality characteristics (Figs. 2.3C-D and 2.3I-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 distinct 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.

2.4.2 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. 2.5 and 2.6), 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 amino acids and fatty acids (Fig. 2.5). 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 soluble sugars in BH5 (Fig. 2.5).

Contrarily, the increased canopy light availability in K1, the dwarfing rootstock, showed an up accumulation in soluble sugars, cyclitol (CYC), flavonoid (FL) and chlorogenic acid (CHL) (Fig. 2.5). Increased soluble sugars 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 fructose and glucose) are intermediate compounds that can be used in the biosynthesis of metabolites in the cyclitol, flavonoid and chlorogenic acid 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. 2.5-2.7).

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. 2.5 and 2.6). 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. 2.9; Anthony et al., 2021).

2.4.3 Rootstock vigor influences the light environment and metabolite upregulation.

In the present study, levels of monosaccharides (glucose, fructose, sorbose) increased with decreasing vigor, while levels of sucrose, a disaccharide, increased with increasing vigor (Fig. 2.7). 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 fructose and glucose (Cirilli et al., 2016). Glucose and fructose 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 glucose-6-phosphate has been converted to UDP-glucose (UDPG), it can be synthesized to form sucrose with fructose-6-phosphate by sucrose phosphate synthase (SPS) (Cirilli et al., 2016). In short, sucrose phosphate synthase generates sucrose from glucose and fructose, 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, sucrose was lowest in the most dwarfing, and most illuminated canopy, K1 (Fig. 2.7A), 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. 2.7 and 2.9). 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. 2.2C), increased photosynthate creation and transport to sink tissues is possible, as evidenced by increased SSC, DMC, and monosaccharides with decreasing rootstock vigor (Figs. 2.3 and 2.7).

Alternatively, upon reaching sink tissues, sucrose can also be rapidly cleaved to glucose and fructose, 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. 2.7F-H). These organic acids can be synthesized using monosaccharides, especially glucose (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 hydroxycinnamic 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. 2.9).

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 caffeoylquinic acids (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 sucrose phosphate synthase activity and maintaining higher levels of monosaccharides (Figs. 2.7B-D and 2.9). 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. 2.7F-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. 2.7E).

2.4.4. 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 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. 2.2C), sorbitol levels, along with monosaccharide composition, may increase (Fig. 2.7). This may contribute to elevated levels of DMC and SSC (Figs. 2.3, 2.8), 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 fructose and glucose in the less vigorous genotypes (Figs. 2.7B, C), as sorbitol is readily converted to fructose and glucose 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).

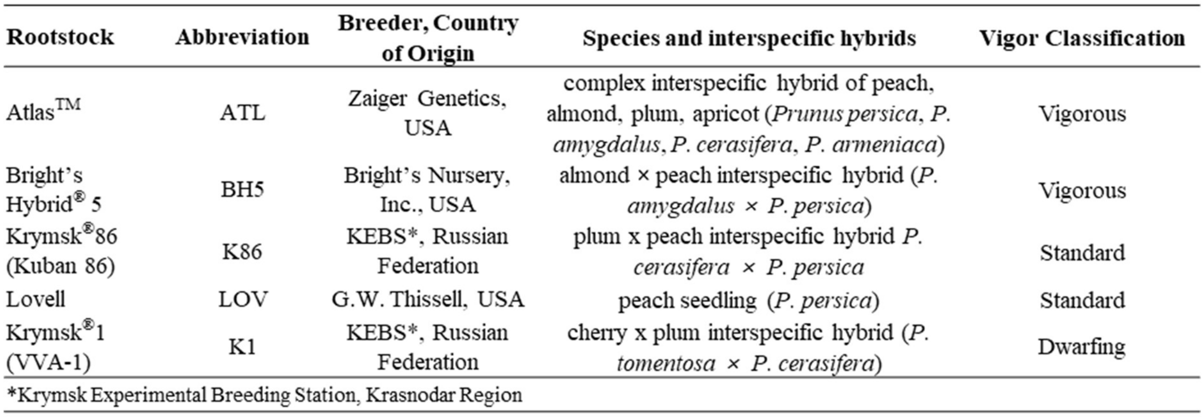
In contrast, malic acid was observed to be up-accumulated with increased vigor and reduced canopy zone light availability and was related to inferior fruit quality at harvest (Fig. 2.8). Malic acid is a fundamental organic acid in peach fruit development (Walker and Faminai, 2018), although its behavior 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. 2.7-2.8). 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. 2.2, 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 sucrose (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 sucrose phosphate synthase activity, reducing sucrose and malic acid abundance, and increasing glucose, fructose 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.

2.5. 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.

Tables.

Table 2.1 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; 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. armeniaca*)	Vigorous
Bright's Hybrid® 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

Figures.

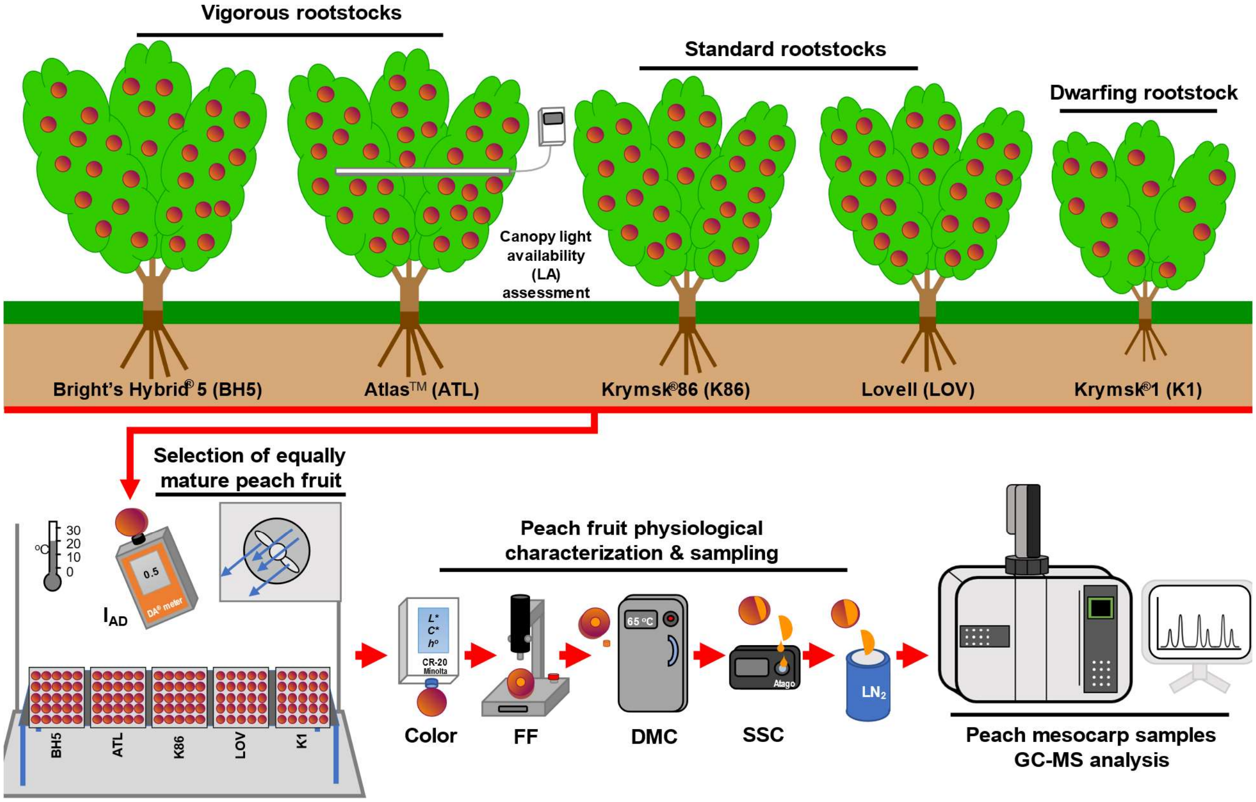


Figure 2.1. Determining how rootstock vigor impacts fruit quality profiles. Five distinct rootstock cultivars were selected to determine the impact of differing vigor profiles on fruit internal quality and metabolite profiles. Based on trunk cross sectional area (TCSA) and canopy volume, the five rootstocks 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).

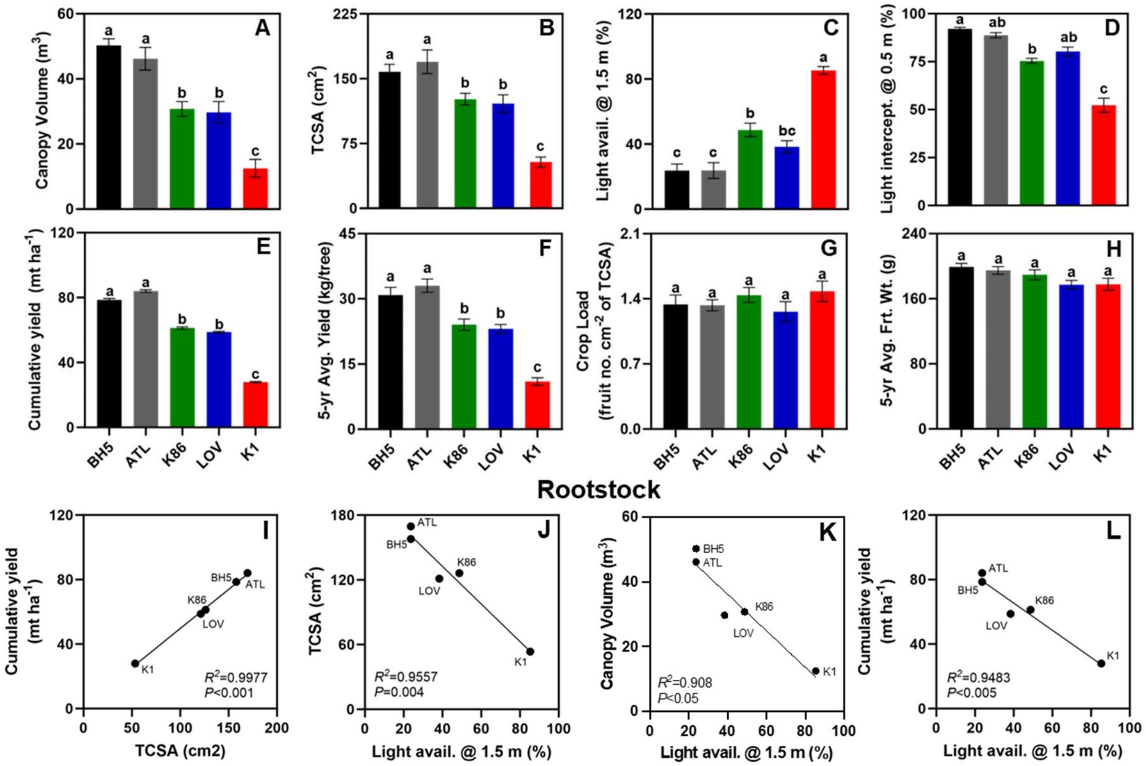


Figure 2.2. The impact of rootstock on vigor, yield, and light availability. The influence of rootstock on vigor canopy volume (A), and trunk cross sectional area (TCSA, B); mid-canopy light availability (C); light interception (D); cumulative 5-year yield (E); five year (2015 – 2019) average yield (F) 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.

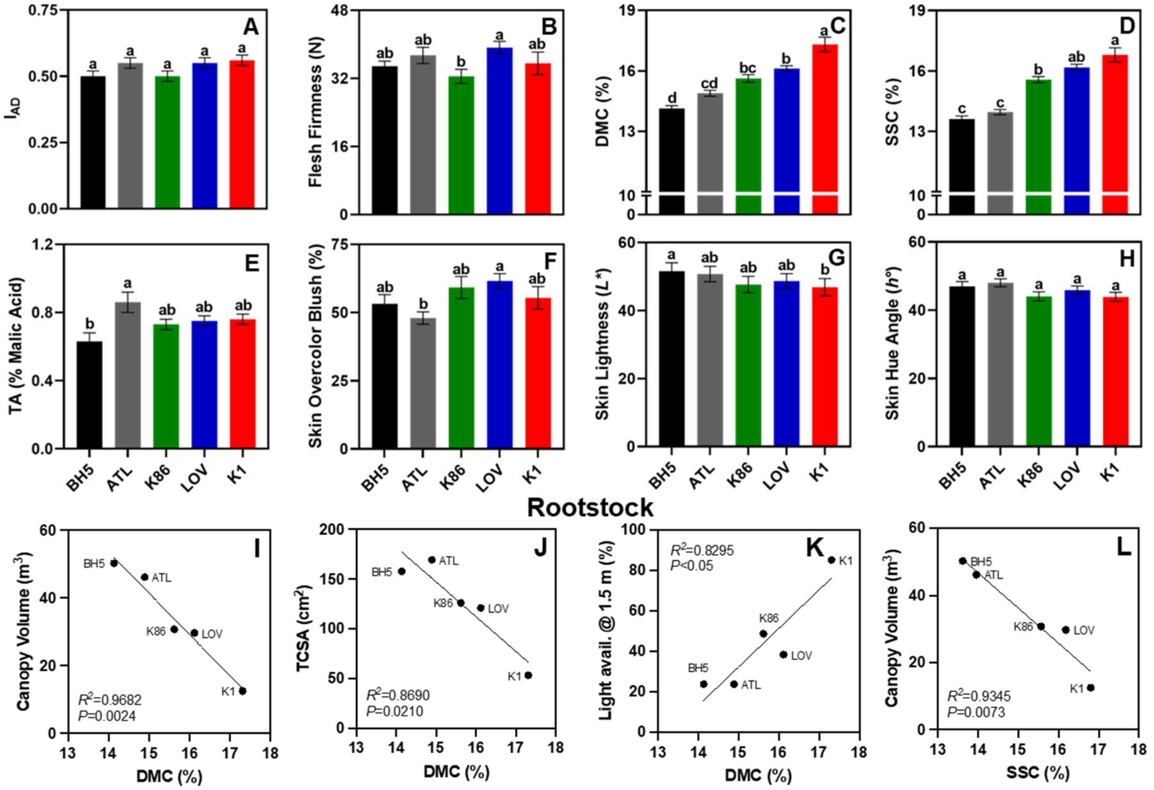


Figure 2.3. The impact of rootstock on internal fruit quality characteristics and exocarp pigment development. Fruit harvested from a canopy height of 1.5 m ± 30 cm in 2019 were segregated for equal maturity (I_{AD}, A) and assessed by internal fruit quality characteristics: flesh firmness (B), dry matter 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.

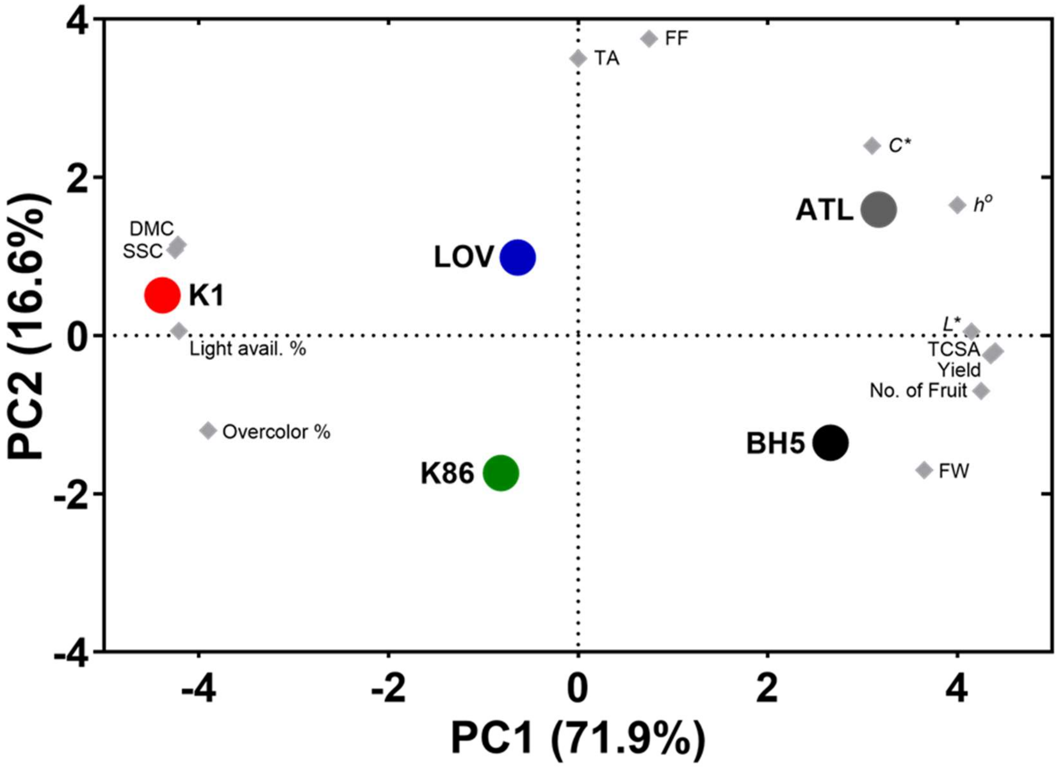


Figure 2.4. Principal component analysis biplot of rootstock on vigor, light availability, and internal fruit quality characteristics. Large symbols indicate the scores for the rootstock treatments [colored by rootstock; and are pareto scaled (-1.0 – 1.0)] with vigor (TCSA), light availability (LA%), 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 (71.9%) 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.

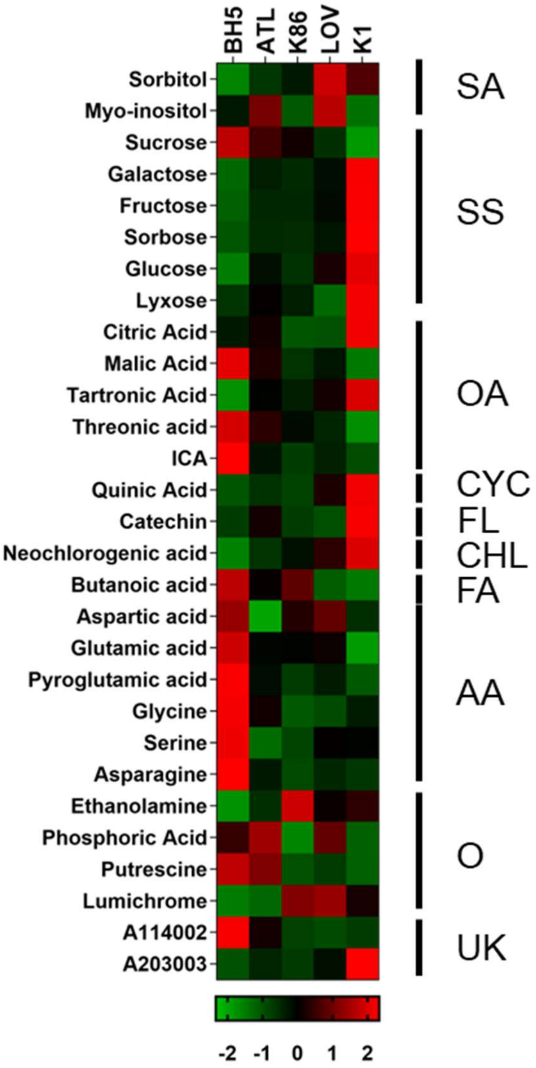


Figure 2.5. Heat map of metabolite profiles across rootstocks of variable vigor. Profiles of metabolism changes at harvest in 'Redhaven' peach fruit mesocarp. Figure shows comparisons of the metabolite abundance by rootstock vigor, displayed with vigor decreasing from left (most vigorous) to right (dwarfing). 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), chlorogenic acids (CHL), fatty acids (FA), amino acids (AA), other (O) and classified un-knowns (UK).

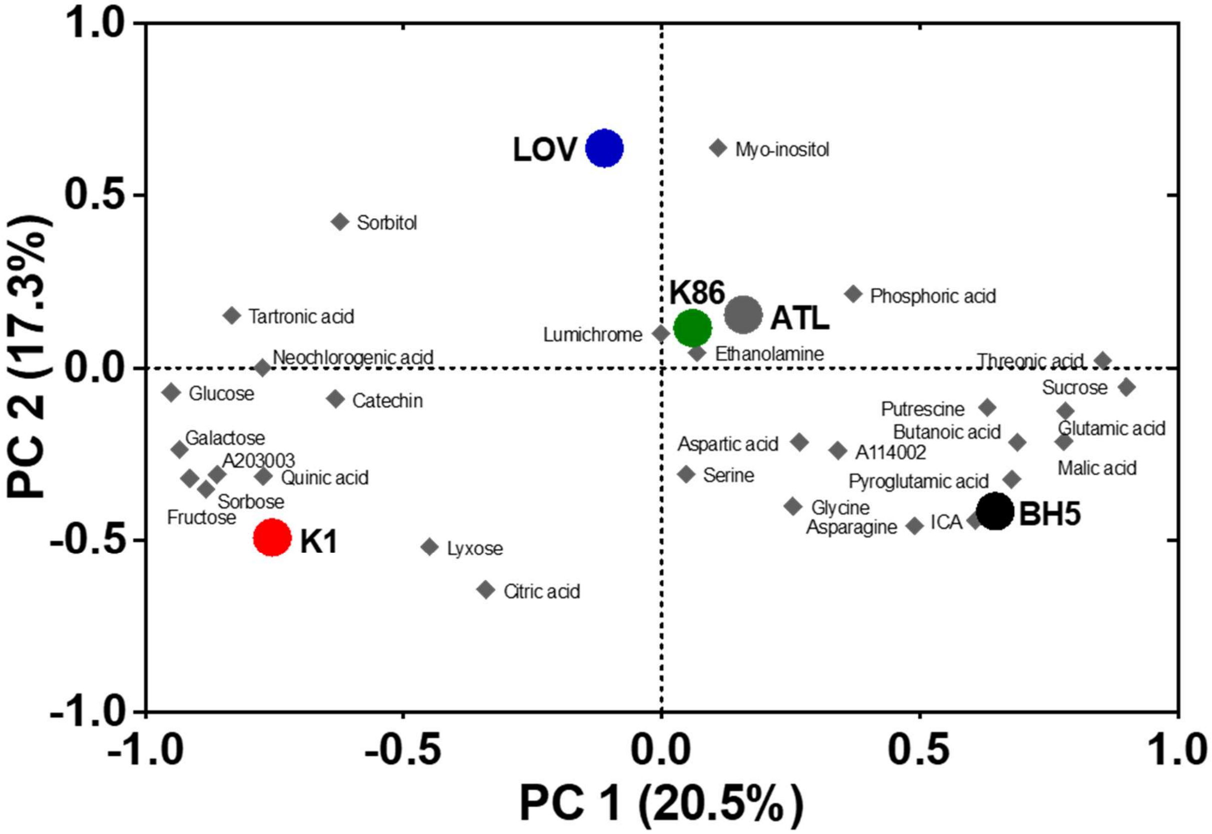


Figure 2.6. Principal component analysis biplot of rootstock vigor on peach fruit mesocarp metabolism. Metabolite profiles across five rootstocks at harvest in peach fruit mesocarp in 'Redhaven' fruit. Figure shows comparisons of mesocarp metabolite profiles across five rootstocks. The rootstocks are 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.

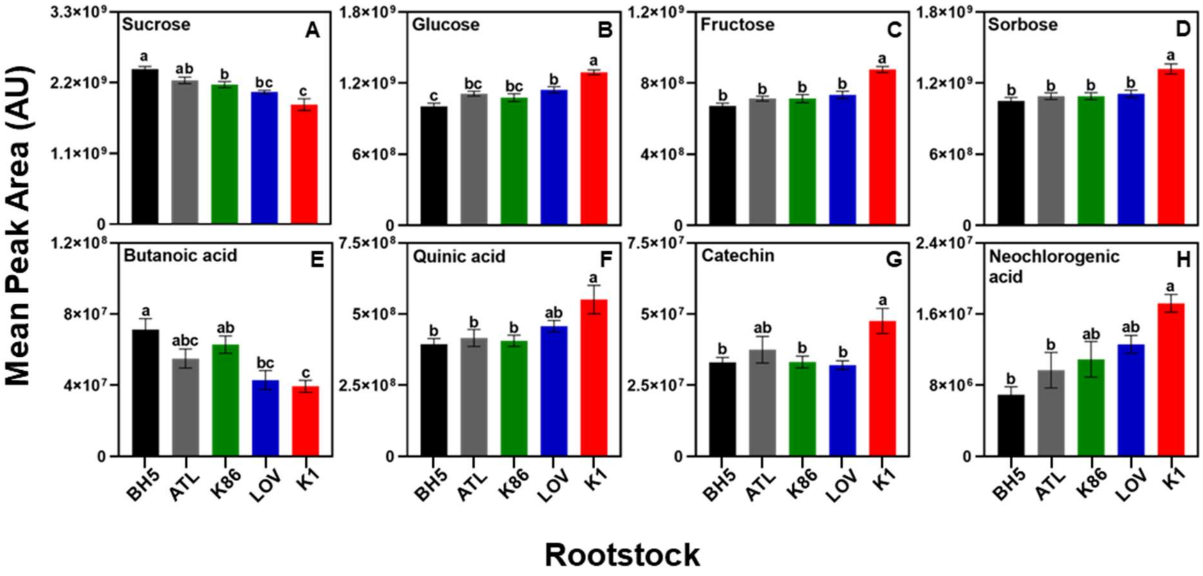


Figure 2.7. Accumulation trends of metabolite abundances by rootstock vigor in peach mesocarp. Mean peak area (AU) of selected metabolites that are influenced by vigor, soluble sugars: sucrose (A), glucose (B), fructose (C), sorbose (D); phenylpropanoid pathway: butanoic acid (E), quinic acid (F), catechin (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 ($P \leq 0.05$).

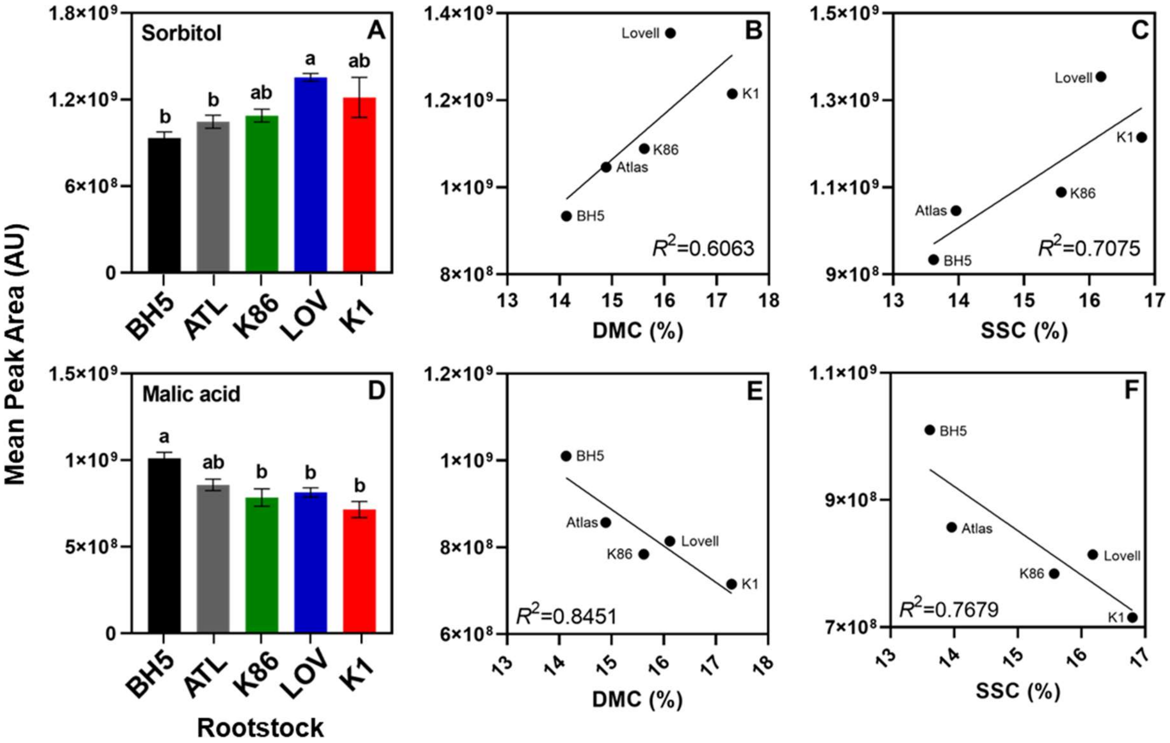


Figure 2.8. Abundance of two metabolites and their relationship with peach internal quality parameters at harvest. Mean peak area (AU) of sorbitol (A) and malic acid (D), respectively, at harvest by rootstock vigor, BH5 ('Bright's Hybrid® 5'), ATL ('Atlas™'), K86 ('Krymsk® 86'), LOV ('Lovell'), and K1 ('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.

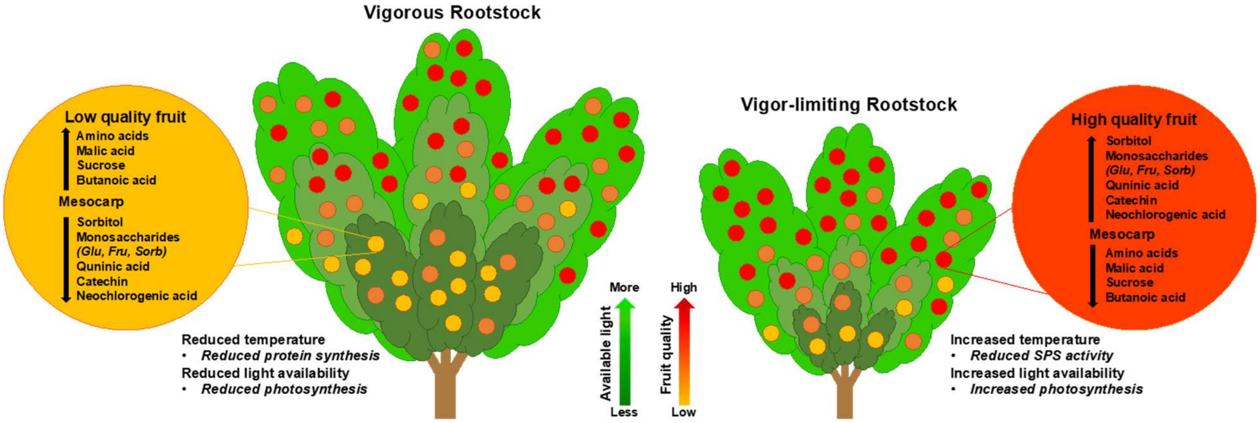


Figure 2.9. The impact of rootstock vigor on light availability and metabolite abundance in peach mesocarp. Up- and down-accumulation trends are presented for chemical classes and specific metabolites in peach mesocarp as a result of various canopy volumes and thus differing light availability profiles. 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.

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CHAPTER THREE

SIX YEARS OF PHYSIOLOGICAL EVALUATION AND PERFORMANCE OF MODERN ROOTSTOCK GENOTYPES FOR PEACH PRODUCTION IN WESTERN COLORADO

3.1 Introduction

Pre-harvest factors play a key role in developing fruit quality in the orchard (Minas et al., 2018a; Anthony and Minas 2022). Rootstock selection is a key pre-harvest factor and represents a crucial decision for growers as rootstock vigor will dictate final tree size, impacting other management decisions including orchard spacing, design and training system selection (Loreti and Massai, 2001). Rootstock manipulation via genetic vigor control creates the possibility for high density plantings (HDPs) and two-dimensional canopy development (i.e., planar) (Anthony and Minas 2021; Iglesias and Echeverria, 2022). Given the successful incorporation of vigor limiting rootstocks into apple and sweet cherry production systems, recent breeding and evaluation programs have devoted substantial efforts to identifying similar rootstock traits for peach [*Prunus persica* (L.) Batsch]. Rootstock selection, however, is not limited to vigor profiles, they also impart a tree's tolerance to abiotic and biotic soil conditions such as alkalinity (Black et al., 2021; Reig et al., 2020), water logging (Zhang et al., 2023), replant disease (Newberger et al., 2023), nematodes (Pinochet et al., 2012), *Armillaria* spp. (Beckman and Pusey, 2001), *Phytophthora* (Browne, 2017) and peach tree short life (PTSL) (Okie et al., 2001; Reighard et al., 2002; Blenda et al., 2007).

Historically, multi-site rootstock trial results within the United States showed Colorado as being among the least vigorous sites (Perry et al., 2000; Reighard et al., 2004; Johnson et al., 2011; Reighard et al., 2011; Reighard et al., 2020). Colorado is characterized by a short growing season; however, edaphic conditions could also contribute to vigor reduction. Peach is highly susceptible to high pH and soil bicarbonate levels, which can reduce the availability of macro and micronutrients to the trees and lead to, for example, iron (Fe) deficiency (Rombola and Tagliavini, 2006). Iron deficiency in peach causes interveinal chlorosis at the apical end of shoots reducing photosynthetic efficiency and the total carbon pool (Lucena, 2000; Larbi et al., 2006), hindering shoot growth and ultimately reducing yields and fruit quality (Alvarez-Fernandez et al., 2003, 2006; Rombola and Tagliavini, 2006). Observations three years post planting in a peach rootstock trial in Colorado revealed that *P. persica* rootstocks had a greater proclivity for iron chlorosis than interspecific hybrid rootstocks (Pokharel and Reighard, 2013). Rootstock trials hosted on alkaline sites in Colorado and Utah found *Prunus persica* rootstocks were generally outperformed by interspecific *Prunus* hybrids and non-*P. persica* selections (Reighard et al., 2020; Black et al., 2021). Given the ubiquitous calcareous alkaline soils in Western Colorado, identifying rootstocks tolerant to calcareous alkaline soils is paramount for sustainable peach production.

Peach has graft compatibility with several species, which provides breeders with flexibility in choosing potential crosses. Recent focus on breeding interspecific and complex hybrids or plum rootstocks for peach production in alkaline and saline soils has shown enhanced performance of these rootstocks when compared to seedling rootstocks (Mestre et al., 2017; Font i Forcada et al., 2020). However, many of these selections have yet to be evaluated in North America. Prior to commercial development, new breeding selections need to be field tested to determine suitability for the various peach production regions in North America. Several new rootstock genotypes have been historically evaluated across different peach growing regions in the U.S., under the guidance of the USDA's multistate project North Central (NC)-140 (Minas et al., 2023)

Previously, Colorado growers relied on 'Lovell', a peach seedling, as their primary rootstock. Due to its historic use and grower familiarity, 'Lovell' is often used as a standard for novel rootstock comparison. More recently however, Colorado growers have turned to 'Krymsk®

86’. ‘Lovell’ and ‘Krymsk® 86’ share similar vigor profiles, and internal fruit quality characteristics (Pieper et al., 2024). ‘Krymsk® 86’ is also a popular rootstock among almond growers in California due to its resistance to root pathogens (Brown, 2017), making it suitable for replant sites in Colorado where land available for peach production is limited. ‘Krymsk® 86’ is also widely accepted as having excellent anchorage (Milliron et al., 2021), another desirable trait for Colorado peach production systems. However, Colorado growers are interested in finding additional rootstocks to fortify their already limited options (personal communication, Bruce Talbott and Harrison Topp).

The success of high-density planar production systems in apple and sweet cherry has created desire to identify vigor limiting rootstocks that would increase orchard productivity in peach production (Iglesias and Echeverria, 2022). Given the limited land with available irrigation water located in the correct climatic zone for peach production in Colorado, increasing orchard productivity on a per-unit area basis is of utmost importance. A potential solution can be achieved through the utilization of vigor limiting rootstocks that promote smaller, uniform, precocious and productive canopies (Loreti and Massai, 2001; Iglesias and Echeverria, 2022). However, given the low vigor and productivity of rootstocks in Colorado, a reduction of vigor may not be preferable to growers who realize the significantly reduced vigor profiles most rootstock genotypes incur. Alternatively, Colorado may be uniquely positioned to facilitate HDPs with standard vigor rootstock genotypes.

Of additional interest to Colorado peach growers is susceptibility to gummosis. Often manifesting in relation to *Cytospora plurivora* infections, gummosis reduces xylem conductance causing girdling shoots and/or scaffolds (Hampson and Sinclair, 1973) leading to full tree decline and reducing orchard productivity and longevity (Miller et al., 2019). A 2009 trial of seventeen rootstocks of various vigor profiles at Colorado State University's Western Colorado Research Center found C*ytospora plurivora* incidence was related to rootstock genotype with several rootstocks showing 100% infection rates (Pokharel and Reighard, 2013), meaning continued observations of the detrimental qualities in orchards is incredibly important to growers in this current trial.

This trial was designed to compare physiological and production traits of eight rootstock genotypes. Two of the rootstocks are industry standard *Prunus persica* rootstocks while the remaining six rootstocks were deemed novel intra- and inter-specific or complex hybrid rootstocks with a semi-dwarfing vigor profile. The aim of the trial is to determine each rootstock's potential for use in modern orchard designs in Western Colorado. In this paper the results from the first six years after planting are being presented.

3.2. Experimental Design and Rootstock Evaluation

3.2.1. Plant material and experimental approach

An orchard block was planted in 2017 as part of the USDA's NC-140 semi-dwarfing rootstock trial that was conducted at ten sites across the US and Canada. The trees were planted in a randomized complete block design at a spacing of 1.8 m x 4.5 m or 1,195 trees per hectare. Each rootstock was replicated five times except for 'Rootpac® 40' which was replicated four times. The trees were planted in four tree sub-blocks with the two central trees serving as data trees. At planting, the trees were headed to a height between 0.5 – 0.7 m. Subsequent pruning was conducted to train the trees in a perpendicular "V" system (DeJong et al., 1994). The trial investigated eight rootstocks, six of which were classified as semi-dwarfing while the remaining two, 'Guardian®' and 'Lovell', were used as industry standard *P. persica* controls. The semi-dwarfing rootstocks were a mix of peach x peach, interspecific, and complex hybrids, listed as: 'Rootpac® 20' (*P.*

besseyi x P. cerasifera), 'Rootpac® 40' (*P. persica x P. dulcis* by *P. persica x P. dulcis*), 'Controller™ 6' (*P. persica x P. persica*), 'Controller™ 7' (*P. persica x P. persica*), 'Controller™ 8' (*P. persica x P. persica*) and 'MP-29' (*P. umbellata* x *P. persica*) (Table 3.2). The rootstocks were budded to 'Cresthaven', a cultivar of economic importance for growers in Colorado. Irrigation for the first year was provided by microjet sprinklers while in subsequent years the trees were irrigated using two lines of drip tubing per tree row based on local commercial grower practice. Soil and foliar nutrients were applied annually based on standard commercial grower practices and leaf tissue analysis.

3.2.2. Rootstock survival, vigor profiles and field performance

Rootstock survival was based on a tree's ability to perform adequately under the stresses of Colorado's alkaline soils, arid environment, and cold winter growing conditions. Inferior performance was rated similar to mortality in that if a tree showed an inability to tolerate these conditions it was deemed unsuitable. Tree vigor was measured as trunk cross sectional area (TCSA, cm^2) 15 cm above the graft union at planting, and each subsequent fall post leaf drop. Tree height, as an additional measurement of vigor was recorded as the point at which the highest lateral branched from the main scaffold. Root sucker counts were tallied prior to removal in July of each year and averaged by rootstock. Proleptic shoot formation was tallied for each rootstock in 2022 and 2023. One-year old proleptic shoots arising from the scaffold were counted for the top (> 1.5 m) and bottom (< 1.5 m) canopies. Additionally, three representative proleptic shoots at a height between 1.2 and 1.7 m were measured for length (mm). Pruning weights (g) were collected in 2020 and 2023. In 2023, the number of cuts were also tallied by canopy positions top and bottom as well as by tree and averaged by rootstock.

Photosynthetic active radiation (PAR) was measured to determine canopy zone light interception for each replicate tree at 0.5 m, using a 1 m line quantum sensor (LI-191, LI-COR Biosciences, Lincoln, NE, USA). Measurements were taken 7 days (2021) and 6 days (2022) post 10 % ripe fruit dates ± 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 interception (LI, %) was calculated as 100 x (average PAR at each position / average total PAR).

To determine rootstock performance in Colorado's alkaline soils, chlorosis was observed. In 2018 and early 2019 chlorosis severity was done by presence-absence monitoring with any sign of interveinal chlorosis deemed as present. From 2019 – 2022 additional chlorosis monitoring was conducted using a soil plant analysis development (SPAD) meter (Minolta SPAD-502DL, Konica Minolta, Osaka, Japan). SPAD data was collected from two shoot positions (basal and distal) in mid-July of each year. Three readings, on individual leaves, were conducted from fully expanded leaves in both positions. To determine differences in SPAD readings data were analyzed by rootstock and shoot position.

The occurrence of gummosis was evaluated by visual inspection post leaf drop. Individual incidences were recorded by location (top > 1.5 m or bottom < 1.5 m), cause of wound (pruning wound or non-pruning), severity (1 – 5), size (< 25 mm, 25 – 75 mm, and > 75 mm), type of wood (fruiting shoot, scaffold, trunk, or crotch), and age of wood (1 yr., 2 yr., 3+ yrs.). Tallies were summed by tree and averaged by rootstock.

3.2.3. Flowering habits and fruit quality analyses

Prior to flowering, three shoots representing average shoot size were selected for each rootstock replicate. The shoots were measured, and a midpoint was determined. Flower buds and nodes for the basal and distal sections were counted prior to bloom. Day of year was noted for first bloom and 90% bloom on each branch. At bloom and fruit set, flowers and fruitlets were counted in each section (basal and distal) of the branch.

Fruit counts and weights were determined by harvesting and weighing each tree individually. Both data trees were averaged by replicate and served as the average yield for each replicated genotype. At harvest, five fruit from the same canopy height (1.5 m) across rootstocks, were segregated 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 beneath the skin (Ziosi et al., 2008; Costa et al., 2009). After selecting for equal maturity, within a range of 0.40 – 0.50 I_{AD}, the fruit were further analyzed to understand the direct impact of rootstock vigor on internal fruit quality. The segregated fruit were assessed for internal quality values: dry matter content (DMC, %) and soluble solids concentration (SSC, %), by using a non-destructive handheld Vis-NIRS scanner that accurately predicts both values using models developed by the Minas Lab (Minas et al., 2021; 2022; 2023). Fruit was also evaluated for size (mm), weight (g) and overcolor blush 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).

3.2.4. Statistical Analyses

Mean comparisons across rootstock genotypes for tree physiological and agronomical characteristics, fruit quality, and light availability 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).

3.3. Results

3.3.1. The impact of rootstock genotype on survival, vigor, and field performance

Rootstocks have shown an ability to influence tree physiology and fruit quality and biochemical composition in nectarine and peach (Iglesias et al., 2019; Minas et al., 2023, Pieper et al., 2024). Here our results show several physiological, agronomic, and fruit quality variables are impacted by year and rootstock as well as their interactions (Table 3.1). Three variables not affected by rootstock were the lethal temperatures from cold hardiness data acquisition, day for 10% ripe fruit, and fruit maturity (Table 3.1).

Since planting in 2017, all rootstocks besides 'Controller^{TM} 7', have shown the ability to survive Colorado's cold winters, hot summers, alkaline soils, and intense solar radiation. 'Controller^{TM} 7' revealed issues early on with iron chlorosis (Fig. 3.3) and had basal and distal SPAD values of 34 and 27 (2019), and 25 and 16 (2020), respectively. The 2019 and 2020 'Controller^{TM} 7' SPAD was 80 to 47% of the mean SPAD value from the other rootstocks. Due to its inferior performance with SPAD values and visible iron chlorosis, 'Controller^{TM} 7' was deemed unsuitable for production in Colorado and omitted from further analysis. All other rootstocks had a 100% survival through the sixth leaf.

Root suckers were counted on the remaining rootstocks prior to removal each July. Overall, the rootstock with the highest sucker count was 'Rootpac® 20', which produced an average of five to twelve suckers per tree (Fig. 3.2d). The rootstocks with the second and third highest average root suckers were ‘Lovell’ (1.8) and ‘Guardian®’ (1.4) most of which occurred from 2018-2020. ‘Lovell’ had two years (2018-19) where the number of suckers averaged four suckers per tree, however, outside of those years root suckers averaged one or less per tree (Fig. 3.2d). Similarly, ‘Guardian®’ had an average of 3 and 5 root suckers per tree in 2019 and 2020, respectively. Outside of these years, root sucker production on ‘Guardian®’ averaged less than one per tree per year. To date, the ‘Controller™’ series and ‘MP-29’ produced very few root suckers in this trial (Fig. 3.2d). Interestingly, sucker production increased dramatically in the 4th leaf period, with root sucker counts per tree increasing by an average of 375% for all rootstocks, except ‘Lovell’ which averaged less than one per tree (Table 3.2; Fig. 3.2d). This period was preceded by severe fall and spring frosts that caused damage to scaffolds and fruiting wood tissue.

Rootstock vigor profiles were determined each fall by measuring TCSA. After the 6th leaf, vigor profiles were classified as standard (‘Guardian®’, ‘Rootpac® 20’, ‘Lovell’, and ‘Controller™ 8’), semi-dwarfing (‘Controller™ 6’ and ‘Rootpac® 40’), and dwarfing (‘MP-29’). The various vigor classes were averaged and compared to TCSA measurements of the standard vigor class rootstock: ‘Lovell’. The averaged TCSA from each class were 110 - 90% (standard), 90 - 60% (semi-dwarfing), and less than 60% (dwarfing) of ‘Lovell’ TCSA (Table 3.2). Regardless of overall genetic differences in vigor profiles, total growth from planting, represented as percent change, shows the smallest (TCSA) rootstocks at planting experienced the highest growth rates. The two smallest TCSA measurements at planting were ‘Rootpac® 40’ and ‘Controller™ 6’. Over the six-year growing period they averaged a TCSA change of over 7000% (Fig. 3.2a). Conversely, the largest TCSA at planting was the dwarfing rootstock, ‘MP-29’, which experienced a 515% change from planting (Fig. 3.2a).

Another proxy for rootstock vigor is tree height which was collected each fall post leaf drop. In 2022, ‘Guardian®’ produced the tallest trees (3.5 m, on average) (Fig. 3.4a). Height was largely a reflection of TCSA vigor profiles with ‘Rootpac® 20’, ‘Lovell’, ‘Controller™ 8’, ‘Rootpac® 40’, and ‘MP-29’ following their vigor classes (Table 3.2). One exception was ‘Controller™ 6’ which was 10% taller than ‘Rootpac® 40’, and closer in height to the standard vigor class rather than its semi-dwarfing TCSA class (Fig. 3.4a). ‘Rootpac® 40’, and ‘MP-29’, the sole dwarfing genotype in this study produced the shortest trees (2.9 and 2.5 m, on average, respectively) (Fig. 3.4a).

Pruning weights (kg) were collected for each of the rootstocks in the winter of the fourth (2020) and sixth leaf (2022). Combined average pruning weight across all rootstocks increased 2.7 times from the fourth to the sixth leaf. ‘Guardian®’ and ‘Controller™ 8’ had the highest while ‘Rootpac® 40’ and ‘MP-29’ had the lowest average pruning weights in both years (Fig. 3.4c). In 2020, the average pruning weight for ‘Guardian®’ and ‘Controller™ 8’ (1.16 kg) was over 400 g heavier than the average pruning weight of ‘Lovell’, ‘Controller™ 6’, and ‘Rootpac® 20’ (0.73 kg), and a 2.4-fold greater than ‘Rootpac® 40’ (0.48 kg) (Fig. 3.4c). Pruning weights for 2022 were more similar across rootstocks than in 2020. ‘Guardian®’ had the highest average pruning weight at 2.88 kg, however, the average pruning weight from ‘Controller™ 8’, ‘Rootpac® 20’ and ‘Controller™ 6’, the next three highest, was 500 g (2.33 kg) less than ‘Guardian®’ (Fig. 3.4c). The average 2022 pruning weight for ‘Lovell’ (1.95 kg) was nearly 1 kg less than ‘Guardian®’. ‘Controller™ 8’ had the highest pruning weight in 2020 and second highest in 2022 (Fig. 3.4c). The pruning weight for ‘Controller™ 6’ was similar to ‘Rootpac® 20’ and ‘Lovell’ in both years. Both ‘Controller™’ series rootstocks produced higher levels of pruning weights compared to the other rootstocks in their vigor class.

Proleptic shoot production was highest in ‘Controller^{TM} 6’, which produced 89 and 82 shoots per tree in 2021 and 2022, respectively (Fig. 3.4d). The rootstocks with the fewest proleptic shoots were ‘MP-29’ and ‘Rootpac® 40’, both of which had an average of 36 fewer proleptic shoots than ‘Controller^{TM} 6’. The two most vigorous rootstocks in the trial, ‘Guardian®’ and ‘Rootpac® 20’, had the second lowest average number of proleptic shoots with 60 and 56 shoots, respectively (Fig. 3.4d). In respect to proleptic shoot length ‘Rootpac® 20’, ‘Controller^{TM} 8’, and ‘Controller^{TM} 6’ had the longest shoots, while ‘MP-29’ had the shortest proleptic shoots (Fig. 3.5a). Shoot lengths of ‘Guardian®’, ‘Lovell’ and ‘Rootpac® 40’ were similar, and not significantly different than any of the other rootstocks (Fig. 3.5a).

Light interception measurements were performed roughly one week post the 10% ripe period. The average percentage of light interception had a strong linear relationship with TCSA (Fig 3.7h). In 2021, ‘Guardian®’ had the highest while ‘MP-29’ had the lowest light interception (Fig. 3.4b). Exceptions to this vigor trend were ‘Lovell’ and ‘Controller^{TM} 6’. Average light interception for ‘Lovell’ was the same as ‘Guardian®’, while ‘Controller^{TM} 6’ was only slightly less than ‘Guardian®’ in 2021. In 2022, ‘Controller^{TM} 6’ and ‘Lovell’ had the highest light interception, however this was not significantly different from ‘Rootpac® 20’, ‘Controller^{TM} 8’, ‘Guardian®’, or ‘Rootpac® 40’. ‘MP-29’ (64%) had the lowest light interception levels in 2022 (Fig. 3.4b).

3.3.2. Rootstock tolerance to abiotic and biotic stressors

In the fall and winter of 2022-2023, five to eight proleptic shoots were removed from each rootstock over three time periods (acclimation (mid-November), peak cold hardiness (early January), and de-acclimation (late January)) related to floral bud developmental stages and assessed for cold hardiness. Cold hardiness assessments were performed to determine the lethal temperatures (LT) to kill 10, 50 and 90% of the buds. Across the three time periods, results showed there were no significant differences in LT across rootstocks (Table 3.4). It should be noted that previous studies on the cultivar 'Cresthaven' have shown that it performs very well in Colorado winters (Minas and Sterle, 2018).

The presence of iron chlorosis in the 2nd leaf led to visual presence/absence monitoring in 2018 and 2019. The presence of iron chlorosis was highest in 2018 with 100% of 'Controller™ 7' rootstocks showing prominent chlorosis. Additionally, the standard and vigorous rootstock classes also showed elevated levels of iron chlorosis with an average of 50% of trees showing signs of chlorosis. Additional measurements using a SPAD meter were conducted in early July of 2019 and 2020. Mean SPAD values showed 'Controller™ 7' had significantly lower levels of chlorophyll in two shoot positions across both years (Fig. 3.3). 'Controller™ 7' basal and tip SPAD values reduced nearly ten points between 2019 and 2020. Compared to mean SPAD values from all other rootstocks, the basal and tip leaf SPAD values were nearly ten points less in 'Controller™ 7' (Fig. 3.3). Visual observations of iron chlorosis, SPAD results, and general poor performance led to 'Controller™ 7' being labeled as unsuitable for production in Colorado. Mean base and tip values varied by year, with mean values dropping across years. Base and tip mean values across all rootstocks minus 'Controller™ 7' were 43 and 38 (2019), and 35 and 34 (2020), respectively. Across both years, 'MP-29' had the highest SPAD levels in both base and tip positions, however differences from the next highest SPAD values were more pronounced in 2019 than in 2020 (Fig. 3.3).

Detailed monitoring of gummosis began in 2021 and continued in 2022. Gummosis incidence was tracked by severity, placement on the tree, height in the canopy and whether the gummosis was related to pruning or not (Table 3.3). The majority of gummosis observations occurred below 1.5 m in height and were a result of pruning wounds on scaffold wood that were three years or older (i.e., dormant cuts of vigorous water sprouts). Gummosis evaluations in 2022 showed that the most vigorous rootstocks, ‘Guardian®’ and ‘Rootpac® 20’, had the highest number of cankers, 19 and 21 per rootstock, respectively (Table 3.3). Conversely, the semi-dwarfing (‘Rootpac® 40’ and ‘Controller™ 6’) and dwarfing (‘MP-29’) rootstocks had a significantly reduced number of cankers (9, 6, and 4), respectively (Table 3.3). Severity of gummosis was also evaluated and followed a similar trend to that of canker incidence. The highest rates of the most severe cankers (i.e., 4 and 5) occurred in the most vigorous rootstocks (‘Guardian®’ and ‘Rootpac® 20’), while reduced severity was observed in ‘Controller™ 6’ and ‘MP-29’. One exception was ‘Rootpac® 40’, which had a higher proclivity for more severe gummosis than ‘Controller™ 8’, ‘Controller™ 6’ and ‘MP-29’ (Table 3.3). ‘MP-29’ had the lowest observed occurrence of gummosis across all categories (Table 3.3).

3.3.3. Rootstock effect on flowering habit, fruit set, yield, and internal fruit quality

To ascertain a deeper understanding of how vigor or genetics affected shoot formation or flowering habit, shoot length, fruit set, internode length and number of nodes per shoot were recorded in 2021 and 2022 (Fig. 3.5). The mean proleptic shoot length from 2021 – 2022 in this trial was roughly 60 cm (Fig. 3.5a). The average internode length was largely dictated by tree vigor with internode length decreasing with decreasing vigor. The most vigorous rootstock, ‘Guardian®’, averaged 2.1 cm between nodes while the dwarfing rootstock, ‘MP-29’, averaged 1.6 cm between nodes (Fig. 3.5c). The mean number of nodes per shot was not significantly different between all rootstocks across both years (Fig. 3.5d). ‘Lovell’ shoots had the highest average set fruit (7), while ‘Rootpac 40’ had the lowest (4) (Fig. 3.5b). The high fruit set meant that on average, ‘Lovell’ had one more fruit per shoot than ‘Guardian®’, ‘Rootpac® 20’, ‘Controller™ 8’ and ‘Controller™ 6’, all of which averaged six fruit per shoot. ‘Rootpac® 40’, which had the lowest percentage of floral buds setting fruit at 18%, also had the lowest mean fruit set at 3 per shoot (Fig. 3.5b).

Since planting in 2017, there have been three harvests (2019, 2021, 2022). The 2020 crop was lost to due to hard spring and fall frosts. To obtain non-confounded internal fruit quality data crop loads were set for each rootstock using TCSA measurements. However, reduced precocity from some rootstocks in 2019 lead to variable fruit set across rootstocks (data not shown). Crop loads were established at 1.1 and 1.75 fruit per cm^2 of TCSA for 2021 and 2022, respectively (Fig. 3.7a). A reduced crop, due to frost in 2021, showed the rootstock ‘Rootpac® 40’ unable to set enough fruit to meet crop load thresholds resulting in significant differences between rootstocks (Fig. 3.7a). With ‘Rootpac® 40’ removed from the means analysis, there were no significant differences between the remaining rootstocks in 2021 (Fig 3.7a).

The first harvest (2019) showed that ‘Rootpac® 20’ had strong early precocity, producing a yield of 4.6 kg $tree^{-1}$ (Fig. 3.2c). The next highest yielding rootstocks were ‘Controller™ 6’ with 3.1 and ‘Guardian®’ with 2.6 kg $tree^{-1}$, respectively (Fig. 3.2c). The remaining rootstocks were below 1.6 kg $tree^{-1}$ (Fig. 3.2c). A frost in 2021 reduced yield potential, however, an average of 6.8 kg $tree^{-1}$ were harvested from the two most vigorous rootstocks ‘Guardian®’ and ‘Rootpac® 20’ (Fig. 3.6b). ‘Lovell’ and the two ‘Controller™’ rootstocks averaged 5.5 kg $tree^{-1}$, while ‘Rootpac® 40’ and ‘MP-29’ yielded 2.5 and 1.5 kg $tree^{-1,}$ respectively (Fig. 3.6b).

In 2022, the first year with a full harvest, yields mirrored vigor profiles with the most vigorous rootstocks producing the highest yields (Figs. 3.6b). ‘Guardian®’ and ‘Rootpac® 20’, the two most vigorous trees had yields of 21.5 and 21.7 kg $tree^{-1}$, respectively (Fig. 3.6b). ‘Lovell’ had the third highest cumulative yield at 18.2 kg $tree^{-1}$ (Fig. 3.6b). ‘Controller™ 6’ continued to produce high yields for its semi-dwarfing vigor profile (16.4 kg $tree^{-1}$) averaging 1 kg $tree^{-1}$ more than ‘Controller^{TM} 8’, and 1.8 kg tree^{-1} less than ‘Lovell’ (Fig. 3.6b). ‘Controller^{TM} 8’, the other standard vigor rootstock, yielded 85% of ‘Lovell’ (Fig. 3.6b). ‘Rootpac$^{®}$ 40’ and ‘MP-29’ continued to have the lowest yields (Fig. 3.6b). Yields from ‘Rootpac$^{®}$ 40’ and ‘MP-29’ in 2022 were 75% (13.6 kg tree^{-1}) and 31% (5.7 kg tree^{-1}) of ‘Lovell’, respectively (Fig. 3.6b). Fruit count per tree mirrored yield results. ‘Guardian$^{®}$’ and ‘Rootpac$^{®}$ 20’ had the highest fruit counts while ‘MP-29’ had the lowest (Fig. 3.6d). The 2022 growing season produced respectable yields for Colorado which typically range roughly 20 kg tree$^{-1,}$ which at the spacing in this trial equates to between 23 – 26 metric tons ha^{-1}. Two rootstocks, ‘Rootpac$^{®}$ 20’ and ‘Guardian$^{®}$’ produced yields in this range for 2022 with ‘Lovell’ and ‘Controller^{TM} 6’ producing 1.8 and 3.6 kg tree^{-1}, respectively, less than optimal yields (Fig. 3.6b). Interestingly, the yield efficiency of 2022 was not significantly different between rootstocks (Fig. 3.6c). ‘Rootpac$^{®}$ 20’ had the heaviest mean fruit weight over the first three years (Table 3.2). Mean fruit weight from all three harvests showed ‘Controller^{TM} 6’ and ‘Lovell’ fruit weight was second heaviest (239 g) followed by ‘Guardian$^{®}$’ (235 g), ‘Controller^{TM} 8’ (224 g), and ‘Rootpac$^{®}$ 40’ (218 g) (Table 3.2). Cumulative fruit size data from ‘MP-29’ showed the rootstock had the lowest mean fruit weight (201 g) and size (72 mm). The average fruit weight from 2021 and 2022 for ‘MP-29’ was not significantly reduced in 2021 but was significantly lighter and smaller than ‘Rootpac$^{®}$ 20’ in 2022 (Fig. 3.7c). Contrary to its semi-dwarfing vigor classification, ‘Controller^{TM} 6’, had the largest mean fruit size (79 mm) in 2021.

Cumulative yields were also strongly correlated to vigor profiles ($r^2 = 0.83$; Fig. 3.7g), with ‘Rootpac$^{®}$ 20’ and ‘MP-29’ having the highest and lowest cumulative yields, respectively (Fig. 3.2c). Cumulative yields were a result of production consistency with ‘Rootpac$^{®}$ 20’ having high yields year over year. One exception to the trend is the semi-dwarfing rootstock, ‘Controller^{TM} 6’, which had cumulative yields similar to that of the standard rootstock, 'Lovell'. While yield efficiency was not significantly different in 2022 (Fig. 3.6c), the high cumulative yield in 'Controller^{TM} 6' resulted in the second highest yield efficiency among all rootstocks. 'Rootpac$^{®}$ 20' (0.95 kg TCSA^{-1}) and 'Controller^{TM} 6' (0.93 kg TCSA^{-1}) have the highest cumulative yield efficiencies in the trial while 'Controller^{TM} 8' and 'Rootpac$^{®}$ 40' have the lowest, an average of 0.65 kg TCSA^{-1} (Fig. 3.2d).

In order to compare fruit size and quality profiles between differing rootstock vigor classes, confounding variables were controlled for. Fruit were harvested from consistent canopy heights, and uniform crop loads and fruit maturity (I_{AD}) thresholds were established (Fig. 3.7a and b). Averaged across both 2021 and 2022, fruit quality parameters, DMC, and SSC, were strongly correlated to vigor and light interception (Fig. 3.7k – l). Across 2021 and 2022 harvests, 'MP-29', the least vigorous rootstock with the highest light availability, had the highest DMC and SSC, respectively (Fig. 3.7e and f). The 'MP-29' fruit quality parameters are 11% more than 'Guardian$^{®}$', the most vigorous rootstock, which averaged 14.6% DMC and 12.7% SSC over the same harvests (Fig. 3.7e and f). 'Rootpac$^{®}$ 40' had the second lowest light interception and the second highest 2021 and 2022 average DMC (15.6%) and SSC (13.6%) (Fig. 3.7e and f). The remaining rootstocks ('Controller^{TM} 6' and 8, 'Lovell', and 'Rootpac$^{®}$ 20') had similar fruit quality parameters and were 92% of the DMC and SSC measured in 'MP-29' (Fig. 3.7e and f). Linear regression analysis showed strong positive relationships between vigor, yield, and light interception. As noted above, rootstock vigor dictated yield and the level of light interception (Fig. 3.7g and h). Light interception and yield also had a positive linear relationship (Fig. 3.7i). However, as rootstock vigor increased along with yields and light interception dry matter content diminished (Fig. 3.7j). Rootstocks with decreased vigor profiles, and thus higher light availability had increased fruit quality parameters (DMC and SSC) (Fig. 3.7k and l).

3.4 Discussion

3.4.1. Rootstock physiology and field performance

This trial was designed as a semi-dwarfing rootstock trial, and vigor profiles for all but 'MP-29' were within 14% of the vigor of 'Lovell' potentially making them attractive candidates for use in Colorado. Previously, rootstock vigor classes have been suggested as comparisons to 'Lovell', a widely used rootstock in the industry. The vigor classes were established in widely spaced trials with trees trained in open center systems with the following guidelines: vigorous (>110%), standard (90 – 110%), semi-dwarfing (60 – 90%), dwarfing (<60%) (Reighard et al., 2020). Following all these guidelines, the rootstocks in this trial segregated into three distinct vigor classes standard ('Guardian', 'Rootpac® 20', 'Lovell', and 'Controller™ 8'), semi-dwarfing ('Rootpac® 40' and 'Controller™ 6') and dwarfing ('MP-29'). However, this may lack nuance given the trees planted in this trial are higher density and trained to a "V" system. Two of the rootstocks performed differently than previously reported. Previous data from almond and peach suggested 'Rootpac® 40' is larger than 'Rootpac® 20' (Lordan et al., 2019; Iglesias and Echeverria, 2022). In this trial, 'Rootpac® 20' was found to be 20% larger than 'Rootpac® 40'. Contrarily, a subsequent two-dimensional trial planted in 2019, at the Western Colorado Research Center, has shown 'Suncrest' budded to 'Rootpac® 40' and trained as Bi-axis "V" (narrow crotch) is 22% larger while Bi-axis "U" (wide crotch) and SSA (super slender axe) trees are an average of 2 – 3% larger than 'Rootpac® 20'. Leaf tissue analysis using 10 Single Sequence Repeats (SSR) DNA markers from the two 'Rootpac®' rootstocks in this trial revealed both were genetic matches for their respective Agromillora breeding stock. The reasoning for the vigor anomalies in this trial have yet to be determined, further investigation is necessary prior to making further recommendations.

Along with the various morphologies and vigor profiles this trial allowed for an assessment of various intra and inter specific, as well as complex hybrid rootstocks which may impart specific genotypic characteristics. Rootstocks with plum heritage, such as 'Rootpac® 20', have been previously associated with high suckering (Pinochet et al., 2010; Torrents et al., 2009; Johnson et al., 2011; Reighard et al., 2011; Reig et al., 2020). Typically, sucker removal is an effective way to lower future occurrences, however, rootstocks with plum heritage seem more resistant to removal, and often resprout in more densely formed clusters when compared to other rootstocks where suckers are often individual and easily removed. For instance, it was noted that 'Rootpac® 20' suckers grow in thick clumps and readily multiply from previously removed suckers making manual removal more difficult. While suckers are typically easy to remove, a large planting of 'Rootpac® 20' may lead to additional labor and herbicide costs to control or remove the high number of suckers. Spring frosts in 2020 resulted in a complete crop loss for the year, and while no trees died, the excessive sucker production observed could have been triggered by cold temperatures or damage to above-ground tissue. Root sucker production diminished after the 5th leaf, showing that proper removal during the growing season can eliminate further sucker production in select rootstocks.

Rootstock genotype plays an important role in shoot formation and has been shown to augment tree architectures in almond (Montesinos et al., 2022) and peach (Caruso et al., 1997). Pruning weights from this trial largely followed the rootstock vigor class profiles, with two exceptions. The 'Controller™' series rootstocks 6 and 8 produced larger than average pruning weights. 'Controller™ 8' had the highest average pruning weight in 2020 and was similar to

‘Rootpac® 20’ in 2022. ‘Controller™ 6’ had pruning weights similar to that of the more vigorous ‘Lovell’ in 2020. However, in 2022 ‘Controller™ 6’ pruning weight was similar to both ‘Rootpac® 20’ and ‘Controller™ 8’. Likely this is due to the high number of proleptic shoots these two rootstocks produce. Dry matter allocation has mirrored rootstock vigor profiles, with higher vigor rootstocks accumulating more vegetative dry matter (Caruso et al., 1997; Inglese et al., 2002). However, due to physical manipulations caused by pruning, it could be difficult to determine the canopy architecture modifications rootstocks impose. In this case, tree height (m) was an accurate reflection of vigor classification for most rootstocks with one exception. Measurements of TCSA indicated that ‘Controller™ 6’ was less vigorous than ‘Lovell’. However, when evaluating height, ‘Controller™ 6’ was 10% taller than ‘Rootpac® 40’, and more similar in height to ‘Lovell’ and ‘Controller™ 8’ despite the reduced TCSA. In both years (2021 – 2022) of proleptic shoot counts, ‘Controller™ 6’ had the most proleptic shoots averaging 43% more proleptic shoots than ‘Guardian®’, ‘Rootpac® 20’, and ‘Lovell’. Rootstock genotype influenced canopy architecture in this trial by promoting/inhibiting proleptic shoot production, which has strong implications on productivity and fruit quality. While the mechanism here is not fully understood, it may be related to vigor, growth habit, and/or genetic composition. Regardless, the number and length of proleptic shoots has crop load implications and should be considered when selecting an appropriate rootstock. Regarding the two most dwarfing rootstocks in the trial (‘Rootpac® 40’ and ‘MP-29’), perhaps these two rootstocks would be better suited in a single leader training system, as to not diffuse the already reduced vigor further across multiple uprights.

3.4.2. Rootstock genotype effect on tolerance to abiotic and biotic stressors

Alkaline soils are common in peach production areas with as much as 30% of peach production across the world occurring in high pH soils. Alkaline soils such as those in Colorado, limit mineral nutrient availability of phosphorus, iron, copper, zinc, manganese, and boron. Additionally, Colorado soils are calcareous, which can further exacerbate nutrient deficiency issues due to the high level of buffering bicarbonates in the soil (i.e., bioavailability). Peach is highly susceptible to iron chlorosis with apical growing points showing interveinal yellowing early in the season. The Colorado planting of the 2009 NC-140 rootstock trial using 'Redhaven' as the scion found *P. persica* rootstocks including 'Controller^{TM} 8', 'KV-010123', and 'KV-010127' to have visual signs of iron chlorosis (Pokharel and Reighard, 2013). An additional investigation comparing Colorado to two sites in Utah, which has a similar altitude and growing season to Colorado, observed the sites with high soil pH (8.5) having greater incidence of iron chlorosis than a neighboring site with reduced soil pH (7.5) (Black et al., 2021). In this trial, 'Controller^{TM} 7' was observed to have increased iron chlorosis in both 2nd and 3rd leaf seasons. Leaf chlorophyll concentrations have been used as an indicator of iron chlorosis in peach (Lleó et al., 2023; Jimenez et al., 2008). Measurements of iron chlorosis were made in the 3rd and 4th leaf using a SPAD meter which showed 'Controller^{TM} 7' as having greatly reduced SPAD readings compared to the other rootstocks (Fig. 3.3). Previously reported levels of tree health have been suggested for SPAD readings in peach with > 32 suggesting good health and < 24 representing high levels of visible chlorosis (Lourdes et al., 2023). Similarly, Pinochet (2010) determined peach trees with SPAD levels < 30 were susceptible, low to mid 30's tolerant and > 37 tolerant to chlorosis. 'Controller^{TM} 7' SPAD scores were less than 30 in three of four samplings across 2019 and 2020 with both scores 25 or less in 2020. The severe iron chlorosis and inferior performance led to 'Controller^{TM} 7' being removed from the trial. A peach and nectarine trial on calcareous soils associated plum rootstocks with high SPAD values (Zarrouk et al., 2006). Our results support the observation by Zarrouk et al. (2006) as ‘MP-29’, which has *Prunus umbellata* (sloe plum) in its genetics had the highest SPAD values in this trial.

Gummosis is a response to wounding and may be caused by pathogens including the pathogen of greatest concern in Colorado, *Cytospora spp.* Gummosis sites often clog vascular tissues and can result in die back of shoots or even scaffolds and often lead to the removal of large, infected sites taking out limbs or removing full trees, which lowers yields and overall orchard productivity. Ultimately, this leads to replanting of entire orchards, or orchards with trees of various ages in numerous states of production making management difficult for growers. Compounding this issue is the sensitivity peach rootstock genotypes have shown in replant conditions, however, plum based rootstocks have displayed increased survival rates in replant soils (Jimenez et al, 2011). Visual monitoring for gummosis occurrence was conducted in 2021 and 2022. ‘Guardian®’, ‘Rootpac® 20’ and ‘Controller™ 8’ averaged the highest amount of gummosis sites in 2022 with an average of 4 per tree (Table 3.3). The most severe gummosis lesions, likely to lead to the removal of a scaffold or the full tree, were reported in the rootstocks with the highest vigor, ‘Guardian®’, ‘Rootpac® 20’ and ‘Lovell’. Results suggest gummosis sites in this planting are associated with pruning wounds below 1.5 m in the canopy. Many of these sites were likely the result of pruning of vigorous water sprouts when developing tree architecture during establishment. How this may be related to rootstock genetics or vigor profiles was not determined in this trial. When using high vigor rootstocks in Colorado, care should be taken to treat training and pruning wounds to discourage the occurrence of gummosis in key components of architectural structures such as trunks and lower portions of scaffolds as trees are establishing. Several studies have suggested Captan (Reilly and Okie, 1984; Miller et al., 2019), and Topsin (Miller et al., 2019) as being effective after pruning to reduce gummosis incidence.

3.4.3. Vigor reflects yields and dictates light environments impacting internal fruit quality

It has been repeatedly demonstrated that rootstock vigor is closely related to production (Font i Forcada et al., 2012; Reighard et al., 2020; Minas et al., 2023; Pieper et al., 2024). Yield followed TCSA vigor classes in this trial apart from ‘Controller^{TM} 6’, which has shown an ability to consistently produce high yields for a semi-dwarfing rootstock. With early precocity in 2019, and similar yields in 2021, ‘Controller^{TM} 6’ maintained 99% of ‘Lovell’ cumulative yields. However, differences in 2022 showed a reduction in yield potential as ‘Controller^{TM} 6’ averaged 90% of ‘Lovell’. In full crop years, these variations may further illustrate the differences in vigor profiles between the two rootstocks. The crop load protocol for these three harvests was based on TCSA, and ‘Controller^{TM} 6’TCSA was 83% of ‘Lovell’. Thinning by TCSA in 2022 resulted in ‘Lovell’ having a 14% higher fruit count than ‘Controller^{TM} 6’ (Fig 3.6b). Even though crop loads have not been significantly different in the trial, ‘Controller^{TM} 6’ has had the highest crop loads, while at the same time maintaining fruit weights and sizes that ranked among the highest in the trial (Fig. 3.5c and d). The differences in TCSA between ‘Controller^{TM} 6’ and ‘Lovell’ resulted in fewer fruit per tree in ‘Controller^{TM} 6’. Given ‘Controller^{TM} 6’ mean fruit weight (260 g) this would amount to 2.86 kg reduction in yield (kg tree^{-1}), which when added to the mean yield of 2022 would equal ‘Lovell’ yield. While TCSA measurements placed ‘Controller^{TM} 6’ in the semi-dwarfing category, as previously noted, tree heights were not significantly different than ‘Lovell’ (Fig. 3.3a). Vigorous rootstocks have been shown to allocate a greater percentage of carbohydrates going to vegetative tissues than less vigorous rootstocks (Inglese et al., 2002). Despite its semi-dwarfing class, ‘Controller^{TM} 6’ had pruning weights that were not significantly different from vigorous and standard rootstocks (Fig. 3.3c), however, the rootstock produced many more proleptic shoots than all other rootstocks (Fig. 3.3d). This may suggest ‘Controller^{TM} 6’ is capable of producing larger yields without the negative consequences related to excessive crop load (Anthony et al., 2021). Obtaining higher yields could also decrease vigor as more carbohydrates would be allocated to fruit and internal quality development rather than vegetative tissue expansion (Miller and Walsh, 1988). The reduction in carbohydrates reduces shoot extension and lowers tree height to one similar to that of other semi-dwarfing rootstocks (Basile et al. 2003). While an increased crop load may also reduce the number of proleptic shoots formed, increasing 'Controller^{TM} 6' yields to those of more vigorous rootstocks to determine if the rootstock could support a higher crop load should be investigated in follow-up trials. Additionally, an increase in planting density could increase per area yields of semi to dwarfing rootstocks. Reig et al. (2020) and Lordan et al. (2019) have conducted trials to determine optimal orchard densities and economic efficiencies for several rootstock and scion combinations in apple. Similar density trials should be conducted for promising semi-dwarfing and dwarfing rootstocks in peach.

While rootstock vigor dictates yield, vigor also has a strong relationship to the canopy light environment. Sugar accumulation in peach depends on genotype and environmental interactions (Cirilli et al., 2016). Canopy position augments light availability, playing a role in maturity status and fruit quality development (Anthony et al., 2021; Pieper and Minas 2022). As light interception decreases percentages of dry matter content (DMC) and soluble solid concentration (SSC) increase (Anthony et al., 2020; Pieper and Minas 2022; Pieper et al., 2024). Previous work in our lab has suggested that increased vigor decreases light availability, which has a negative relationship with internal fruit quality (Anthony et al., 2020, 2021, 2023; Minas et al., 2023; Pieper et al., 2024). Similar results were found here showing light availability had a positive linear relationship to internal fruit quality in 2021 and 2022 (Fig. 3.7k and l). The semi-dwarfing rootstock 'Controller^{TM} 6' averaged the highest proleptic shoot formation, producing on average 25 more proleptic shoots than ‘Guardian®’, the most vigorous rootstock. Light availability and canopy position is a major factor in determining fruit quality parameters at harvest (Marini 1991). Average DMC and SSC from 2021 and 2022 show a strong linear relationship with light availability (Fig. 3.7k and l) with ‘MP-29’ having the highest quality profile. The increase in proleptic shoots decreased the light availability above that of ‘Rootpac® 40’, the other semi-dwarfing rootstock in this trial, making it more similar to ‘Controller™ 8’, ‘Lovell’, and ‘Guardian®’, three rootstocks with greater vigor than ‘Controller™ 6’. However, this change in light environment did not affect the internal fruit quality of ‘Controller™ 6’, which had the third highest mean DMC and SSC in the trial. However, the differences in SSC observed here may be beneath, less than 1 °Brix, what has been previously reported as a consumer’s perceptible observance as reported by Harker et al. (2002) in apple.

Semi-dwarfing rootstocks allow for high density plantings (HDP) which have the potential to increase orchard uniformity and fruit homogeneity while lowering costs (Iglesias and Echeverria, 2022). Coupling vigor to yield efficiency helps to identify rootstocks suitability for HDP production systems (Jimenez et al., 2008). ‘Controller™ 6’ and ‘Rootpac® 20’ had the highest yield efficiency. However, ‘Rootpac® 20’ displayed discrepancies to previously reported vigor profiles (Iglesias and Echevarria, 2022), and in subsequent trials at this Colorado site (data unpublished).

3.5 Conclusion

The eight rootstocks assessed in this trial segregated into three vigor classes by TCSA: standard (‘Guardian®’, ‘Rootpac® 20’, ‘Lovell’, and ‘Controller™ 8’), semi-dwarfing (‘Rootpac® 40’ and ‘Controller™ 6’), and dwarfing (‘Controller™ 7’ and ‘MP-29’). ‘Guardian®’, in terms of TCSA and height, was the most vigorous. Tree vigor was highly correlated to yield, thus ‘Guardian®’ and ‘Rootpac® 20’ had the highest yields, however, this came at the expense of fruit quality. Conversely, the least vigorous rootstocks, ‘Rootpac® 40’ and ‘MP-29’, had the lowest yields, but superior fruit quality characteristics. Increased vigor was also related to higher occurrences of, and more severe, gummosis in ‘Guardian®’ and ‘Rootpac® 20’. The opposite was true of the least vigorous rootstocks, ‘MP-29’, and ‘Controller™ 6’, which showed less severe and fewer occurrences of gummosis than the other rootstocks. Two rootstocks, ‘Rootpac® 20’ and ‘Controller™ 6’, stood out as potential ideal candidates for Colorado’s growing conditions. As the second most vigorous rootstock in the trial, ‘Rootpac® 20’ had the highest cumulative yield. However, ‘Rootpac® 20’ had a proclivity for severe gummosis, profuse root suckers, poor proleptic shoot formation, and has shown vigor discrepancies across sites and trials. Conversely, ‘Controller™ 6’ produced the most proleptic shoots, and had the second lowest gummosis occurrences. While the proleptic shoot formation reduced light availability, perhaps hindering DMC and SSC levels, these internal quality attributes were not significantly different from the standard rootstocks. ‘Controller™ 6’ was found to be a semi-dwarfing rootstock based on TCSA measurements, however, its height was not different than the standard rootstock ‘Lovell’. Through three harvests, ‘Controller™ 6’ produced large heavy fruits that resulted in yields similar to that of the standard rootstock ‘Lovell’. The large fruit size and abundant proleptic shoots could signal the rootstock has an ability to hold more fruit, possibly without incurring drastic reductions to fruit size (i.e., increased crop loads). Additionally, given the semi-dwarfing TCSA vigor profile of ‘Controller™ 6’, growers may be able to increase planting density. Combined, the semi dwarfing vigor profile, the high proleptic shoot production and large fruit size of ‘Controller™ 6’ merits further investigation to determine if the rootstock maintains these positive qualities with greater crop loads and is able to produce yields similar or greater than that of the other, more vigorous, rootstocks in this trial.

Tables.

Table 3.1 Two-way ANOVA of the effect of rootstock, date, and their interaction on tree physiology, agronomic and fruit quality characteristics of seven *Prunus* spp. rootstock cultivars in the 2017 'Cresthaven' trial. Physiology, agronomic and fruit quality parameters were affected by rootstock, date, and their interaction. Significant differences were not found in rootstock lethal temperatures, harvest day, or fruit maturity.

Tree physiology data						Agronomic and fruit quality data				
Source of variation	Unit	Date^{a} (D)	Rootstock (R)	D x R		Source of variation	Unit	Date^{a} (D)	Rootstock (R)	D x R
Height	m	***	***	ns		Crop load	Fruit TCSA^{-1}	***	**	*
TCSA	cm^{2}	***	***	***		Harvest 10 %	Julian day	***	ns	*
Suckers	count	***	***	***		Yield	kg tree^{-1}	***	***	***
Pruning weight	kg	***	**	***		Fruit ct.	count	***	***	***
Proleptic shoots	count	*	***	***		Fruit wt.	g	***	**	ns
Light interception	%	***	***	ns		Fruit size	mm	***	**	ns
Lethal temperature	°C	***	ns	ns		Yield eff.	yield TCSA^{-1}	***	**	*
SPAD base	na	***	***	*		Matuiry I_{AD}	na	***	ns	ns
SPAD tip	na	***	***	***		DMC	%	***	***	**
Bloom 90 %	Julian day	***	***	**		SSC	%	***	***	**

Level of significance noted as follows: ns (no significance), significant at *p-value* * < 0.05, ** < 0.01, *** < 0.0001. ^{a}Across years data was collected for all

Table 3.2 Rootstock classification after 6th leaf (2017-2022) for semi-dwarfing genotypes trained to a KAC-V at Western Colorado Research Center, Grand Junction, CO.

Rootstock	Breeding Origin	Genetics	TCSA (cm^{2}) ^{a}	TCSA % of Lovell	Height (m)	Vigor Class	Proleptic Inc^{b}	Gumm. Inc.^{c}	Sucker^{d}	Cum.yield^{e} (t ha^{1})	Fruit wt. (g)^{f}	Cum. YE^{g}	90 % bloom^{h}	10 % mature^{i}
Guardian	USDA – Clemson	*P. persica*	51.3a	109	3.5a	Vigorous	Medium	High	Low	36.7ab	235ab	0.76bc	99	232
Rootpac® 20†	Agromillora	*P. besseyi* x *P. cerasifera*	49.3ab	105	3.4a	Vigorous	Medium	High	High	39.8a	248a	0.95a	98	231
Lovell		*P. persica*	46.9abc	-	3.3a	Standard	Medium	Medium	Low	31.0bc	239a	0.74bc	99	233
Controller® 8	USDA – UC Davis	*P. persica x P. persica*	44.1abc	94	3.2a	Standard	Medium	Medium	Low	27.3cd	224ab	0.68bc	99	233
Rootpac® 40†	Agromillora	*P. persica x P. dulcis* by *P. persica x P. dulcis*	41bc	87	2.9a	Semi-dwarfing	Low	Medium	Low	20.8d	218ab	0.62c	98	231
Controller® 6	USDA – UC Davis	*P. persica x P. persica*	40.2c	86	3.2a	Semi-dwarfing	High	Low	Low	29.6bcd	239a	0.93ab	100	232
MP-29	USDA – Georgia	*P. umbellata* x *P. persica*	17d	36	2.5b	Dwarfing	Low	Low	Low	9.9e	201b	0.75bc	98	230
Controller® 7*	USDA – UC Davis	*P. persica x P. persica*	-	-	-	Dwarfing	-	-	-	-	-	-	-	-
													ns	*ns*

*Data not shown as genotype was not suitable for CO conditions due to iron chlorosis

†Data reflects results from 2017 NC140 ‘Cresthaven’ trial

^{a} Trunk cross sectional area (cm^{2}) fall 2022; ^{b} Proleptic shoot incidence rating; ^{c} Gummosis incidence rating; ^{d} Sucker incidence rating; ^{e} Cumulative yield; ^{f} Mean fruit weight to date in trial;

^{g}Cumulative yield efficiency (kg·cm^{2} TCSA^{-1}); ^{h}Average Day of Year (DOY) for 90% bloom; ^{i}Average DOY for 10% fruit maturity date.

Table 3.3 Gummosis incidence and severity by rootstock genotype after 6th leaf (2022) of the 'Cresthaven' trial at the Western Colorado Research Center in Grand Junction, CO.

Rootstock	Avg. Gum. ct. tree^{-1}[a]	Total Gum.[b]	Gum. severity[c]			Prune[d]	No Prune[e]	Above 1.5 m[h]	Below 1.5 m[g]
			3	4	5				
Guardian®	3.8^{ab}	19	9	4	6	18	1	3	16
Rootpac® 20	4.2^{a}	21	13	4	4	20	1	3	18
Lovell	1.4^{bcd}	10	3	2	5	9	1	1	6
Controller™ 8	3.6^{abc}	18	12	4	2	17	1	3	15
Rootpac® 40	2.25^{abcd}	9	5	1	3	9	0	2	7
Controller™ 6	1.2^{cd}	6	3	2	1	5	1	1	5
MP-29	0.60^{d}	4	2	1	1	4	0	0	3
Estimated LSD	2.4								

*Data from 2017 NC-140 rootstock trial, collected in the fall of the 6th leaf (2022).

[a] Mean gummosis count per tree; [b] Total gummosis summed across replications; [c] Gummosis severity, 3 – sign of gum, 4 – large active site not encircling wounded area, 5 – infection site encircling area, will likely lead to removal; [d] Gummosis resulting from pruning wound; [e] Gummosis not associated with pruning wound; [f] Gummosis incidence occurring above 1.5 m; [g] Gummosis incidence occurring below 1.5 m. Means followed by the same letter are not statistically different according to Tukey's HSD test ($P < 0.05$).

Table 3.4 Rootstock cold hardiness by genotype over three dates in the winter of 2022–2023. Temperatures were obtained using differential thermal analysis on three replicates of five bud pairs.

Rootstock	Nov. 15, 2022			Jan. 9, 2023			Jan. 29, 2023		
	LT10a	LT50b	LT90c	LT10	LT50	LT90	LT10	LT50	LT90
Guardian	-17.66	-19.56	-20.88	-19.34	-21.5	-23.01	-16.80	-20.63	-23.88
Rootpac® 20	-15.65	-18.73	-21.05	-19.08	-21.14	-22.53	-16.97	-20.53	-23.56
Lovell	-18.17	-20.29	-21.8	-17.34	-21.05	-24.16	-17.48	-20.68	-23.25
Controller® 8	-17.76	-19.85	-21.33	-18.92	-21.38	-23.08	-18.10	-21.10	-23.43
Rootpac® 40	-17.18	-19.26	-20.73	-19.1	-21.12	-22.57	-17.16	-20.30	-22.65
Controller® 6	-17.14	-19.52	-21.22	-18.68	-21.19	-22.96	-17.68	-20.68	-23.05
MP-29	-17.71	-19.75	-21.17	-19.67	-21.56	-22.86	-18.08	-21.25	-23.63
Significance	ns	ns	ns	ns	ns	ns	ns	ns	ns

[*]Data reflects results from fall and winter 2022–2023, of the 2017 NC–140 rootstock trial. [a]Lethal temperature to kill 10 % of the buds; [b]Lethal temperature to kill 50 % of the buds; [c]Lethal temperature to kill 90 % of the buds. Level of significance noted as follows: ns (no significance), significant at p-value * < 0.05, ** < 0.01, *** < 0.0001.

Figures.

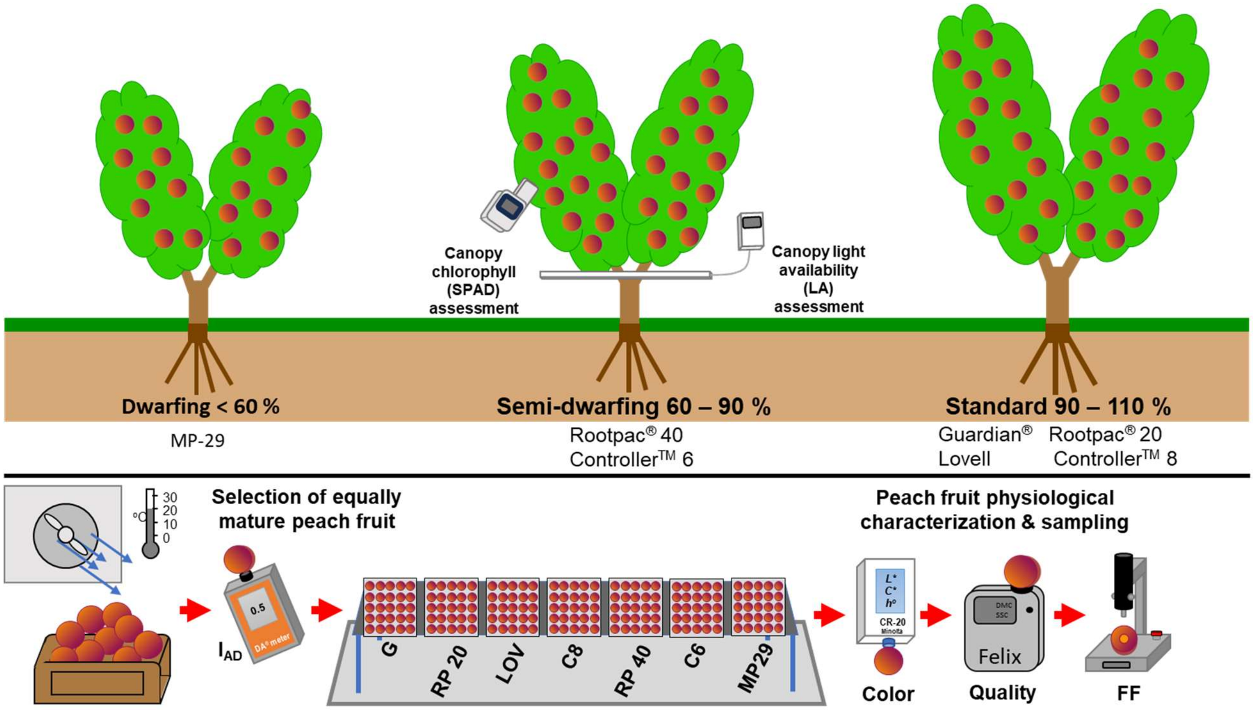

Figure 3.1. Seasonal workflow and data collection protocols from the NC-140 2017 'Cresthaven' trial. Trunk cross sectional area (TCSA cm^2) was measured 15 cm above the graft union at planting and each fall post leaf drop. In spring, three representative branches were tagged in each tree and measured for length. Each shoot was flagged in the center and the number of nodes, flower buds, and fruit set were counted in the distal and proximal sections of each shoot. Midsummer, root suckers were counted and removed. SPAD values were collected at 1.5 m from fully expanded distal and proximal leaf tissue. At harvest, fruits were counted, weighed, and non-destructively scanned for maturity and internal fruit quality using a XLSOR handheld scanner. Fruit of equal maturity was compared to determine the impact of rootstock vigor on internal fruit quality. Light availability was collected at 0.5 m in the four cardinal directions one-week post-harvest. After leaf drop. Tree height and width were recorded. Branch tissue was inspected for gummosis lesions. Lesion data was separated by canopy height (top >1.5 m, bottom < 1.5 m), and ranked by severity. Prior to pruning, proleptic shoot counts were conducted. Pruning weights were collected and weighted by canopy positions (top >1.5 m, bottom < 1.5 m).

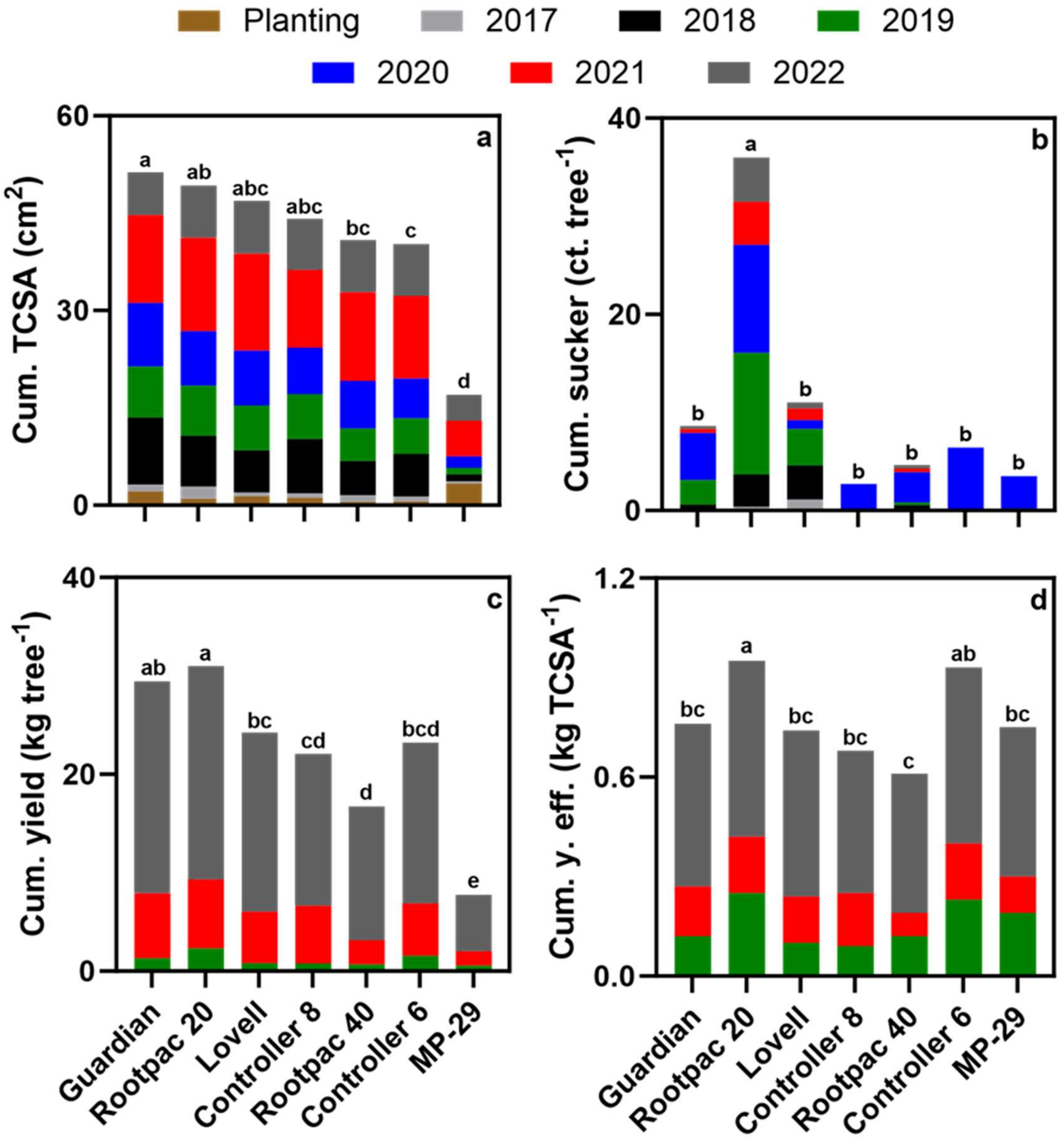

Figure 3.2. Cumulative results of vigor, root suckers, yield, and yield efficiency from planting through the 6th leaf. Vigor as trunk cross sectional area (TCSA cm^2) shows annual trunk expansion by year (a). Rootstock vigor class profiles were established as follows; standard ('Guardian®', 'Rootpac® 20', 'Lovell', and 'Controller™ 8') semi-dwarfing ('Rootpac® 40', 'Controller™ 6'), and dwarfing ('MP-29'). Cumulative root sucker occurrence (b), several rootstocks showed very little suckering. Hard fall and spring frosts in 2019 and 2020 increased suckering in many rootstocks. Trees began bearing fruit in 2019 (c), with two additional harvests in 2021 and 2022. The first full crop was 2022. High cumulative yield efficiency (kg TCSA^{-1}) in 'Rootpac® 20' and 'Controller™ 6' was partially due to early precocity in 2019 (d).

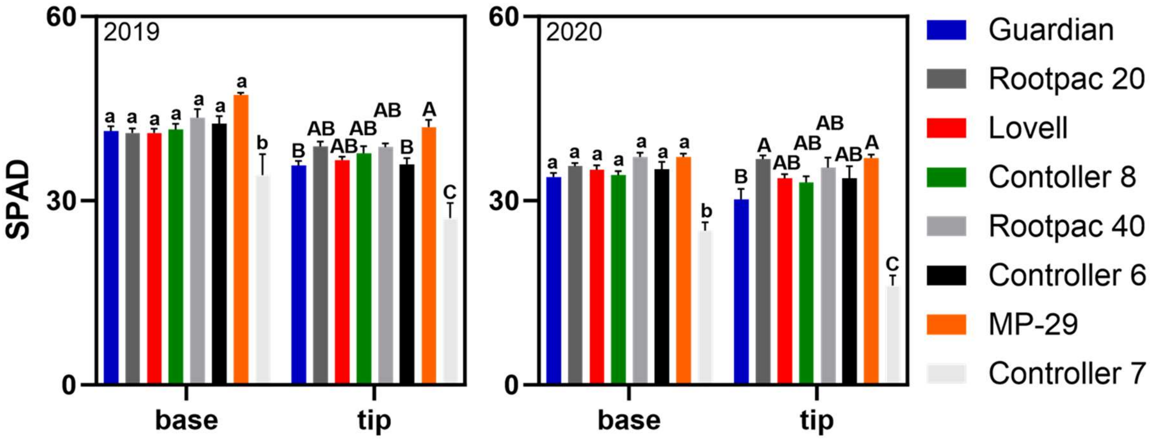

Figure 3.3. Leaf SPAD values from all rootstocks at two shoot positions across two seasons. Fully expanded leaves on the basal and distal sides of proleptic shoots were scanned with a SPAD meter at 1.5 m in the canopy. Three individual leaves were scanned on three separate shoots for each position in summer of 2019 and 2020. Values were compared to several previously conducted studies on SPAD levels in peach. 'Controller^{TM} 7' showed poor growth, with visible chlorosis and significantly diminished SPAD values and was removed from further observation. Rootstocks are presented by vigor starting with the most vigorous ('Guardian$^{®}$') to the least ('MP-29'). Mean values ± S.E. are displayed. Means followed by the same letter are not statistically different according to Tukey's HSD test ($p < 0.05$).

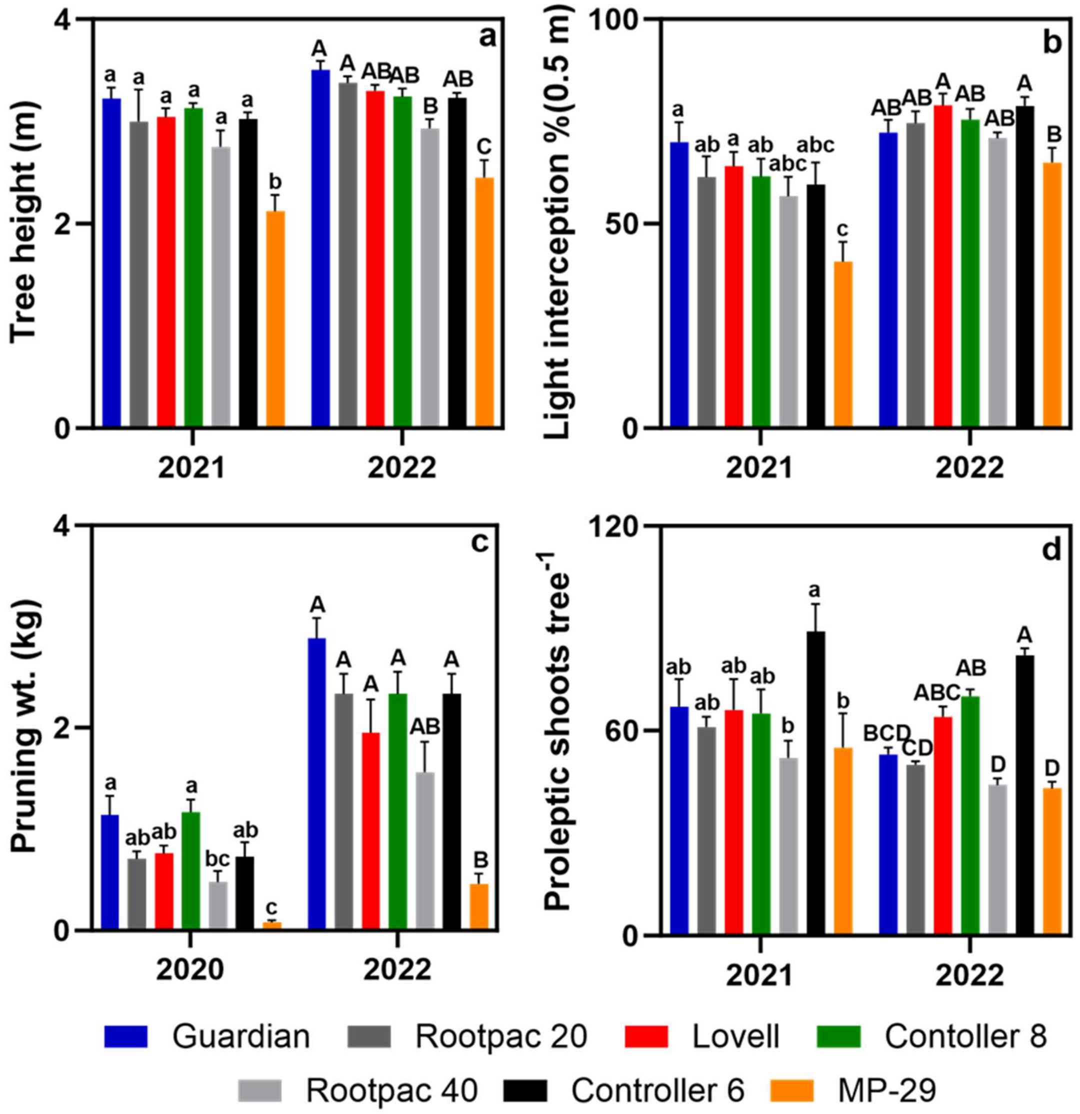

Figure 3.4. The impact of rootstock on vigor, light interception, proleptic shoot formation, and pruning weight. The influence of rootstock on proleptic shoot production (a), and tree height (b), light interception (c), and pruning weight (d) in the 5th (2021) and 6th (2022) leaf. Rootstocks are presented by vigor starting with the most vigorous (‘Guardian®’) to the least (‘MP-29’). Mean values ± S.E. are displayed. Means followed by the same letter are not statistically different according to Tukey’s HSD test ($p < 0.05$).

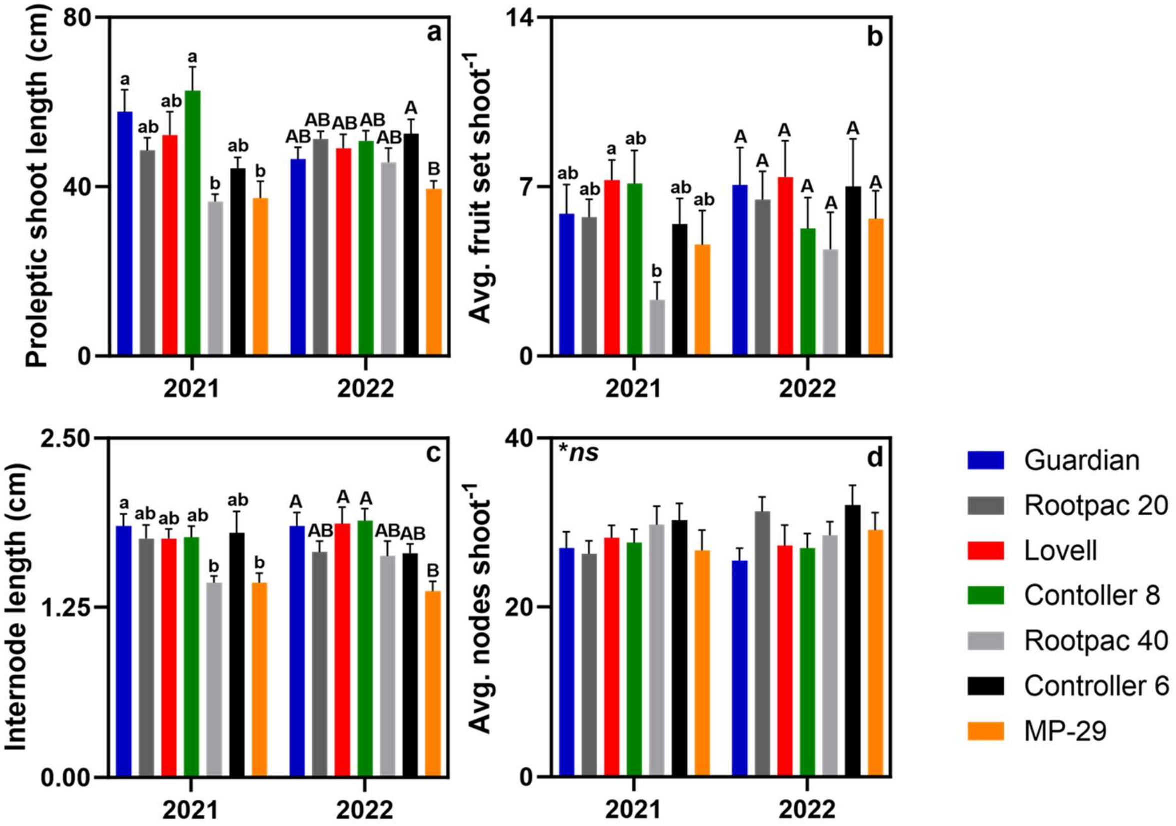

Figure 3.5. Proleptic shoot length and flowering habits from the 2017 semi-dwarfing rootstock trial. Proleptic shoot length (a) number of fruit set per branch (b) internode length (c) and average number of nodes per shoot (d) were collected in the spring of the 2021 and 2022 growing seasons to characterize the effect of rootstock on physiological and flowering traits. Significant Rootstocks are presented by vigor starting with the most vigorous (‘Guardian®’) to the least (‘MP-29’). Mean values ± S.E. are displayed. Means followed by the same letter are not statistically different according to Tukey’s HSD test ($p < 0.05$).

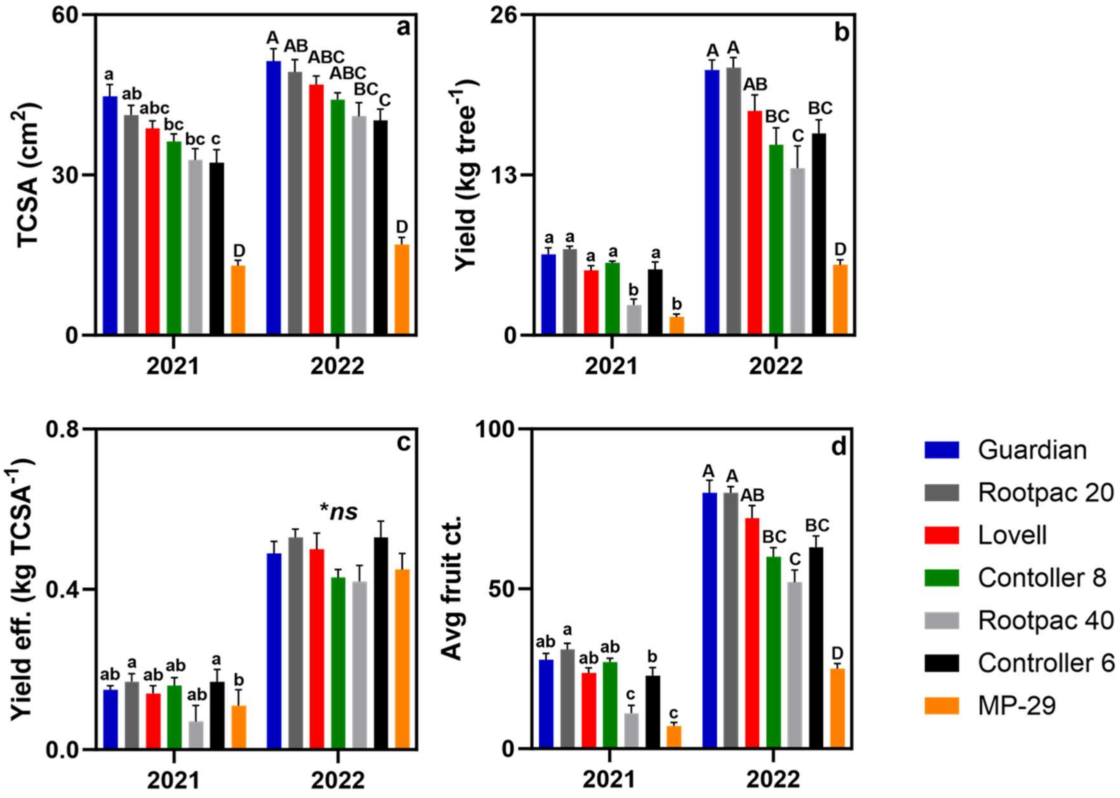

Figure 3.6. Rootstock vigor and the impact on yield, yield efficiency and fruit count. Average TCSA (a), yield (b), yield efficiency (c), and fruit count (d) from the 5th (2021) and 6th (2022) leaf seasons. The first full crop was harvested in 2022. Rootstocks are presented by vigor starting with the most vigorous (‘Guardian®’) to the least (‘MP-29’). Mean values ± S.E. are displayed. Means followed by the same letter are not statistically different according to Tukey’s HSD test ($p < 0.05$).

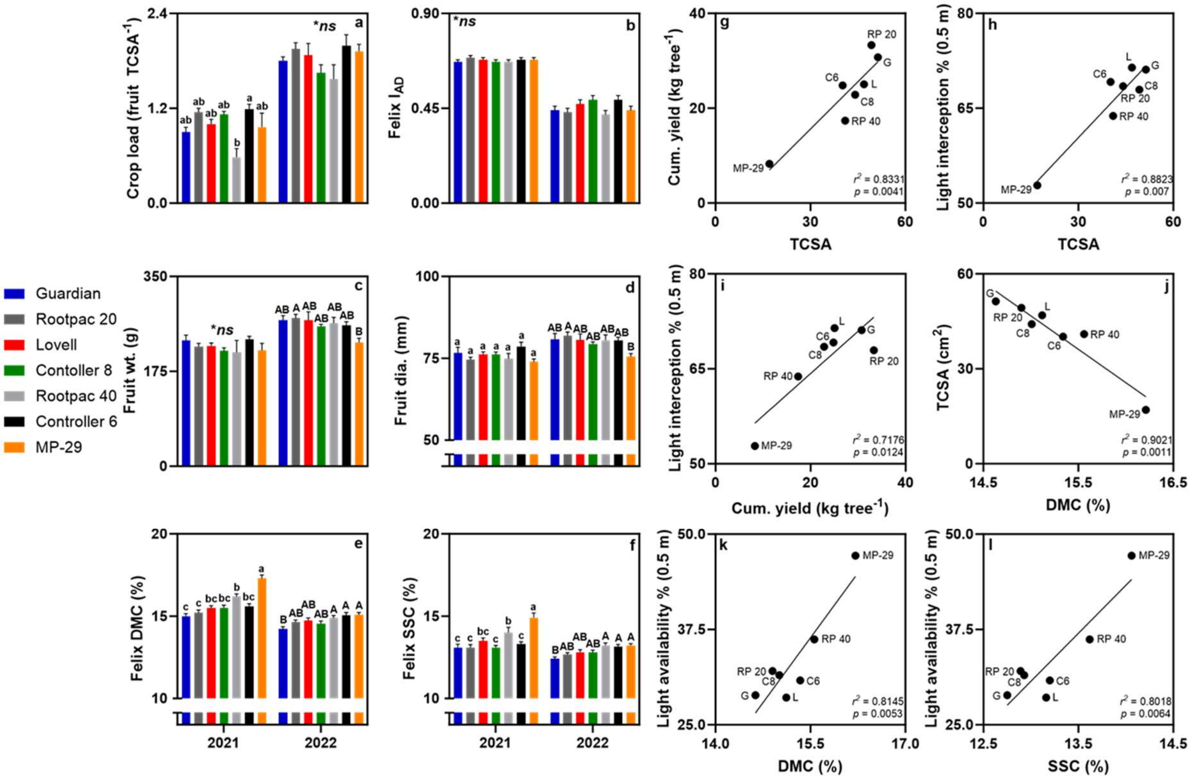

Figure 3.7. The impact of rootstock on fruit quality parameters and relationships between vigor, light interception, and internal fruit quality. Crop load was standardized across rootstocks by hand thinning according to TCSA (a). Fruit harvested in 2021 and 2022 from a canopy height of 1.5 m ± 30 cm and segregated for equal maturity (b) and assessed by internal fruit quality characteristics: fruit weight (c), fruit diameter (d), dry matter content (e), and soluble solids concentration (f). Rootstocks are presented by vigor starting with the most vigorous (‘Guardian®’) to the least (‘MP-29’). Mean values ± S.E. are displayed. Means followed by the same letter are not statistically different according to Tukey’s HSD test ($p < 0.05$). Linear regression analysis of rootstock vigor, light interception, and internal quality (g-l) are as follows: vigor and yield (g), vigor and light interception (h), light interception and yield (i), vigor and DMC (j), light interception and DMC (k), and light interception and SSC (l). Details of the linear relationships (r^2 and *p-value*) between the variables and the seven rootstocks are presented within their respective figures.

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