Journal of the American Pomological Society 77(2): 103-109 2023

Effects of *Vaccinium arboreum* Rootstocks on Yield and Fruit Quality of 'Patrecia' Southern Highbush Blueberry Grown with Minimum Soil Amendment

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Additional Index words: grafting, pine bark, sparkleberry

Abstract

Blueberry plants (*Vaccinium corymbosum* interspecific hybrids) need soils with acidic pH and high organic matter. This leads growers to use soil amendments like pine bark and soil acidifying agents like sulfur. These inputs raise agricultural production costs and compromise the economic sustainability of blueberry production. Sparkleberry (*Vaccinium arboreum*) seedlings have been utilized experimentally as rootstocks for blueberry in Florida, but the impact of clonal sparkleberry rootstocks on blueberry productivity and quality is unknown. The objective of this study is to evaluate the performance of southern highbush blueberry (SHB) cv. 'Patrecia' grafted onto three clonal sparkleberry rootstocks. Based on previous research, we hypothesized that grafted blueberries have higher yield and fruit quality than own-rooted blueberries in minimally amended soil. 'Patrecia' SHB was grafted onto three different clonal rootstocks (R1, R2, and R3). Plants with their own roots were used as a control. Fruits were harvested in the springs 2019, 2021 and 2022. For each harvest season, fruit yield and quality (average fruit size, total soluble solids, titratable acidity, and firmness) were measured. Grafted plants exhibited equal or higher yields than own-rooted plants in 2021 and 2022. Grafted plants produced larger berries, and the quality of the fruit was similar among treatments. These results suggest that clonal sparkleberry rootstocks can be used to grow blueberries in soils with higher pH and less pine bark than is currently used.

Florida soils are not ideal for southern highbush blueberry (*Vaccinium corymbosum* interspecific hybrids) production. Blueberry plants require acidic soil and high soil organic matter, leading to the need for soil amendments like pine bark and acidifying products like sulfur (Williamson et al., 2018). These inputs increase the cost of production. For example, pine bark accounts for more than one quarter of the total establishment cost of a new blueberry field and additional applications of pine bark are usually needed at 3-year intervals (Singerman et al., 2019). Additionally, blueberries are mostly hand-harvested, which is the single greatest annual expense for southern highbush blueberry production in Florida.

Blueberry plants have shallow and fibrous root systems (Retamales and Hancock, 2018; Strik et al., 2014), and rigorous soil demands, such as low pH (4.0–5.5), high organic matter (above 1%), proper aeration and drainage (above 30% air filled porosity), and readily available iron and ammonium (Darnell and Hiss, 2006; Fang et al., 2022; Nunez et al., 2015; Williamson et al., 2018). In contrast, sparkleberry (*Vaccinium arboreum*) is a small tree native to Florida that is closely related to blueberry. It is known for its coarse, deep roots, which might contribute to drought tolerance (Nunez et al., 2016). Additionally, sparkleberry can grow on sandy, and sandy clay soils with a pH of up to 6.5, as well as in soils with little organic matter (Lyrene, 1997).

Sparkleberry was used in blueberry breeding to introduce root architecture traits (Nunez et al., 2016) and features that may be advantageous for machine harvesting (Olmstead et al., 2013). However, breeding is a

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long-term process and growers are searching for more immediate alternatives to improve cultivation and profitability. This provided the motivation to trial sparkleberry as a rootstock for blueberry production in Florida (Casamali et al., 2016a; Casamali et al., 2016b; Darnell et al., 2020).

Grafting blueberry onto sparkleberry rootstocks has shown great potential as a tool to reduce blueberry establishment and harvest costs. Casamali et al. (2016a) found that grafting onto sparkleberry seedlings can increase blueberry yield compared to own-rooted blueberry plants in soils not amended with pine bark. The use of pine bark accounts for approximately 25% of the overall costs associated with blueberry establishment in Florida (Singerman et al., 2019).

Additionally, Casamali et al. (2016b) reported that the use of sparkleberry rootstock can facilitate mechanical harvest by promoting a single-trunk architecture on grafted plants, compared to own-rooted plants with multiple canes (Fig.1). Furthermore, manual labor is a significant expense, accounting for approximately 45% of the total production expenses of one season.

Figure 1. Grafted blueberry plants develop a monopodial architecture (left) compared to the usual bush architecture of own-rooted blueberry plants (right).

Since sparkleberry is a native plant that is recalcitrant for propagation (Bowerman et al., 2013; Li et al., 2021), previous studies have used seedling sparkleberry rootstocks. However, clonally propagated rootstocks might offer a better opportunity to assess the impact of specific sparkleberry-blueberry combinations. The objective of this study was to evaluate the performance of southern highbush blueberry cv. 'Patrecia' grafted onto three clonal sparkleberry rootstocks in a field with minimally amended soil. Based on the results from Casamali et al., 2016a; Casamali et al., 2016b; Darnell et al., 2020, we hypothesized that grafted blueberry plants outperform own-rooted blueberry plants in this scenario.

Materials and Methods

Field. The experiment was conducted at the University of Florida Plant Science Research and Education Unit in Citra Fla. (29º40'N latitude and 82º14' W longitude). The soil was a well-drained Arredondo sand with a native pH of ~5.8. Before planting in spring 2018, the soil was amended with half of the amount of pine bark that is normally used in a blueberry field, resulting in approximately 269 m^3/ha. No additional pine bark was added to the field during the study. Soil pH was monitored biannually, and pH adjustments were made using granular sulfur (Tiger 90CR Sulphur, Tiger-Sul Products, Shelton, CT, USA), resulting in a soil pH range between 5.1 to 6.0 during the trial period.

Grafting and planting. Dormant blueberry scion wood of cv. 'Patrecia' was collected from a commercial farm and stored at 4° C until grafting. The scion wood was from previous season flushes and sized to approximately match the diameter of the rootstock plants at approximately 20 cm from the crown. Plants were grafted in Mar. 2017 by a professional grafter, using the whip graft method onto three different clonal rootstock selections (R1, R2, and R3, n = 5 per rootstock-scion combination). Rootstocks R1, R2, and R3 were selected from the original population of sparkleberry seedlings used in Casamali et al., 2016a; Casamali et al., 2016b, and Darnell et al., 2020. These selections exhibited vigorous growth, lower than average propensity to develop root/crown suckers, and successful propagation from root cuttings. The selections were made and propagated in spring 2015 using root cuttings and were grafted approximately 22 months later in 2017. Grafted plants were maintained in a greenhouse and screenhouse nursery for an additional year to ensure adequate scion growth and strong graft unions before planting in the field. The planting was established in spring 2018. Own-rooted plants from cv. 'Patrecia' were used as controls (n = 5). Irrigation, fertilization, pest, and disease control followed standard commercial practices (Williamson et al., 2018). Other SHB cultivars planted nearby serving as potential sources of pollen for crosspollination included 'Farthing', 'Keecrisp' and 'Optimus'.

Fruit yield and quality. Plants were harvested in the springs of 2019, 2021 and 2022. The 2020 season was skipped due to restrictions related to the COVID-19 pandemic. Yield data were collected by hand-harvesting ripe berries every week until no berries were left on the bush. For each harvest, the amount of fruit was weighted on a benchtop scale and summed for total yield per plant. Fruit quality data were collected early and late in the season in 2021 and 2022. Fruit quality was not measured in 2019 because plants were young and had low yields. Average berry weight was estimated by randomly selecting and weighing 25 berries. These same berries were stored at 2° C for 24 h after harvest to perform firmness measurements. Firmness was measured by determining the pressure (N) required to disrupt one millimeter of the surface of the fruit (FirmTech II, Bioworks, Wamego, KS, USA). A second batch of 25 berries was frozen at -30° C for subsequent analysis of internal fruit quality, consisting of total soluble solids (TSS) and titratable acidity (TTA). Frozen berries were thawed, blended, centrifuged, and the supernatant was filtered through cheese cloth to extract a clarified juice. TSS was measured through refractometry (Digital refractometer HI96801, Hannah instruments Inc., Woonsocket, RI, USA) and expressed as soluble solids concentration (%) TTA was measured using an automated titrator (Easy pH, Mettler Toledo, OH, USA) and expressed as percentage of citric acid. These two measurements were used to calculate TSS:TTA ratio to express the maturation index of the berries.

Statistical analysis. The experiment was a completely randomized design with five single-plant replications per treatment. There were four treatments, corresponding to three rootstock-scion combinations and an own-rooted control. Data were analyzed with a one-way analysis of variance (ANOVA). To overcome the limitations of small-plot research (n=5 per scion/rootstock combination), data were also analyzed using Kruskal-Wallis (KW) non-parametric ANOVA. Results from both tests were similar and both *P*-values are reported. Treatment means were compared using Fisher's Least Significant Difference (LSD) test at $P < 0.05$. Data analyses and illustration were performed in R (Version 1.4.1717; R Foundation for Statistical Computing, Vienna, Austria).

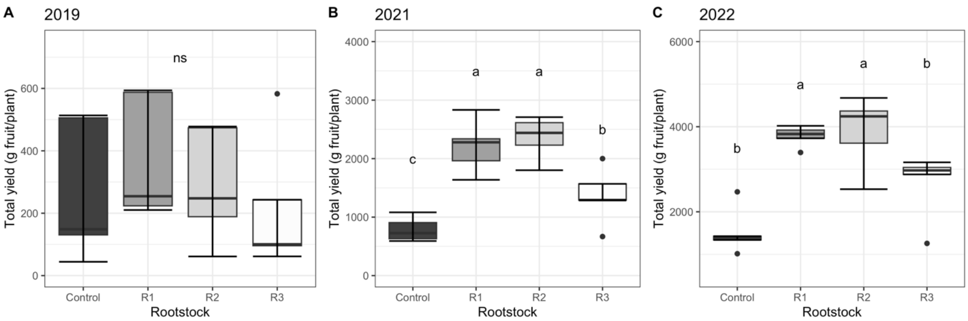

Figure 2. Seasonal yield for grafted and own-rooted 'Patrecia' SHB grown in a minimally amended field in Citra, FL. Control = own-rooted 'Patrecia'. R1, R2, R3 = 'Patrecia' grafted on Rootstock 1, Rootstock 2, or Rootstock 3, respectively. Boxes topped by the same letter were not significantly different according to the least significant difference test at $P < 0.05$, by LSD. Range of Y axis (Total yield) is different for each graph.

Results and Discussion

Yield. In this experiment, total yield and average berry weight were low and not different among treatments during the 2019 season (Fig. 2). Low yields during the 2019 season were likely due to the long establishment period blueberry plants typically have in a production field. Casamali et al. (2016a) found that in the first fruiting season after grafting, yields of grafted plants were lower than yields of own-rooted plants. The clonal rootstocks evaluated in this trial followed the same trend. Yields increased substantially in 2021 and 2022 (Fig. 2) and yields of plants grafted onto R1 and R2 were significantly higher than the own-rooted controls in both seasons. Yields of plants grafted onto R3 were higher than the control only in 2021. Results from R1 and R2 agree with previous findings where grafted plants exhibited higher yields than own-rooted plants in soils considered suboptimal for blueberry (Darnell et al., 2020). However, rootstock R3 did not always follow this trend, suggesting further work is necessary to identify optimum scion-rootstock combinations. Considering grafted plants were equally or more productive than own-rooted plants in minimally amended soils, it is possible that grafting reduces the need for pine bark and sulfur during establishment and cultivation. This reduction may lower establishment and production cost for blueberry production. Economic analysis is necessary to establish if using more costly plant material (grafted plants) while reducing establishment and production costs can make blueberry production more economically sustainable. Longer-term testing of grafted blueberry production is also necessary to establish if eventual addition of organic matter and acidifying agents is necessary.

Berry weight. In 2019 and 2021, average berry weight was not affected by the treatments (Fig. 3). In 2019, fruits in all treatments had lower berry weights than the 3 g average for the cultivar 'Patrecia' (Munoz, 2016) (avg = 1.95 g), likely due to plant age as detailed above. In 2021, the average berry weight achieved the 3 g standard for 'Patrecia'. In 2022, the average berry weight was similar to 2021, but own-rooted plants had significantly lower average berry weight than grafted plants. Casamali et al. (2016a) also reported greater mean berry weight from SHB grafted on sparkleberry seedlings versus same age own-rooted SHB.

Firmness. Berry firmness was not affected by the treatments in early or late season in 2021 or early season in 2022 (Table 1). However, in 2022, late season fruit from grafted

Table 1. Firmness, total soluble solids (TSS), titratable acidity (TTA), and sugar to acid ratio (TSS:TTA) of fruit harvested from grafted and own-rooted 'Patrecia' SHB grown in a minimally amended field in Citra, FL.

Year	Treatment[Z]	Firmness (N)		TSS (%)		TTA		TSS:TTA	
		Season		Season		Season		Season	
		Early	Late	Early	Late	Early	Late	Early	Late
2021	R1	2.65	2.44	10.78	12.36	0.48	0.55	20.46	22.63
	R2	2.92	2.57	10.72	11.92	0.52	0.48	20.34	29.40
	R3	2.63	2.44	11.14	12.20	0.61	0.52	21.37	21.12
	Control	2.76	2.44	11.36	12.06	0.57	0.48	21.54	28.36
	p-value ANOVA	***0.1654***	***0.1754***	***0.2824***	***0.5182***	***0.345***	***0.788***	***0.9546***	***0.1703***
	p-value KW	***0.1997***	***0.1476***	***0.2411***	***0.4657***	***0.162***	***0.765***	***0.7957***	***0.1176***
2022	R1	2.22	1.87 b[y]	15.04	11.22	0.80	1.02	49.32	13.82
	R2	2.24	1.89 b	15.92	11.42	0.73	1.29	16.87	9.63
	R3	2.39	1.99 b	22.96	12.04	1.23	1.74	29.17	7.41
	Control	2.38	2.23 a	16.00	12.64	1.02	1.56	23.82	8.72
	p-value ANOVA	***0.1829***	***0.0057***	***0.2444***	***0.07174***	***0.709***	***0.121***	***0.1674***	***0.2771***
	p-value KW	***0.2055***	***0.0126***	***0.3371***	***0.07128***	***0.446***	***0.159***	***0.176***	***0.4282***

[z] Control = own-rooted 'Patrecia'. R1, R2, R3 = 'Patrecia' grafted on Rootstock 1, Rootstock 2, or Rootstock 3, respectively.
[y] Means within columns and year followed by common letters do not differ at the pair-wise error rate of 5%, by LSD.

plants was softer than fruit from the own-rooted controls, and a general decrease in berry firmness was observed with late-season berries for all treatments in both years. Even with lower firmness in 2022, values were still considered acceptable for marketable blueberry fruit. Ehlenfeldt and Martin (2002) considered values greater than 1.57 N as being very good two decades ago. Nowadays, new cultivars are expected to have firmness not lower than 1.47 N (Cappai et al., 2018). 'Patrecia' (released in 2016) has an expected firmness of 2.07 – 2.6 N with an average 2.27 N (Munoz, 2016), which the berries from all treatments achieved in 2021. In 2022, only berries from control plants had average firmness close to that expected for 'Patrecia' in late season. Previous studies reported the same pattern of reduced firmness on grafted blueberry plants (Casamali et al., 2016a). Grafted plants produced larger berries, which tend to be less firm.

Internal fruit quality. Internal fruit quality was determined by total soluble solids (TSS), total titratable acidity (TTA), and maturation index (TSS:TTA), which define the percep-

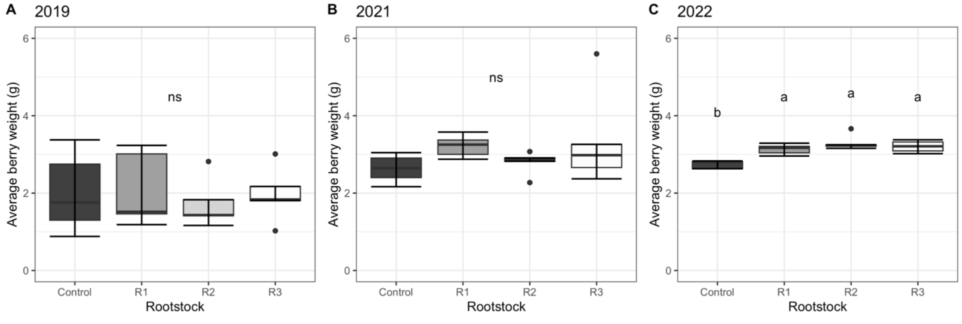

Figure 3. Average berry weight of grafted and own-rooted 'Patrecia' SHB grown in a minimally amended field in Citra, FL. Control = own-rooted 'Patrecia'. R1, R2, R3 = 'Patrecia' grafted on Rootstock 1, Rootstock 2, or Rootstock 3, respectively. Boxes topped by the same letter were not significantly different according to the least significant difference test at P < 0.05, by LSD.

tion of sweetness of the fruit (Sater et al., 2021). Neither trait was affected by grafting in any of the studied seasons. All fruit reached the expected TSS values for SHB 'Patrecia' (higher than 10 %) (Munoz, 2016). However TSS:TTA declined drastically during late season in 2022, below the expected values (higher than 14 units). This decline in fruit quality (lower TSS and higher TTA) might be related to increasing night temperatures during the late spring in Florida. Similar declines have been documented in strawberry (Menzel, 2022) and grape (Gaiotti et al., 2018). This was the only occasion when fruit quality was below the commercial standard. Since fruit from all treatments was affected, these results suggest that sparkleberry rootstocks did not have an influence on the internal fruit quality of blueberry fruits.

Summary

Blueberry plants grafted onto sparkleberry rootstocks R1 and R2 produced more fruit than own-rooted blueberry plants starting three years after planting in a minimally amended (50% of the recommended amount of pine bark) soil. Grafting reduced berry firmness, but fruit quality attributes met or exceeded commercial standards. These findings suggest that grafting SHB onto sparkleberry clonal rootstocks is a promising approach to producing commercial yields of high-quality fruit in soils with higher pH and lower amounts of pine bark. Additional research is necessary to identify rootstock-scion combinations that maximize productivity and longevity while minimizing soil input requirements.

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