Final Report for Southern Region Small Fruit Consortium Grant for SRSFC Project 2022-R-22

Title: Evaluation of Advanced Southern Highbush Selections for Splitting, Self-Fertility, and Fruit Quality Traits

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Public Abstract

Some blueberry cultivars are prone to splitting (also known as cracking) when it rains during harvest; these rain events are becoming more common with climate change. The NCSU blueberry breeding team is trying to find an optimal way to measure this trait outside the field so that this trait can be weeded out of their elite breeding germplasm.

Introduction:

Changing weather patterns have led to record-breaking rain events in the Southern US over the past decade. This has meant several challenges for blueberry growers, including pollination difficulties and fruit splitting. The latter has been compounded by the demand for machine-harvestable cultivars with the firmness to withstand rougher treatment than the cultivars of a decade ago. While highly desirable, firmness has been suspected in increased amounts of cracked and split fruit[1]. An estimated 14-30%[2] of a single harvest may be discarded as unmarketable as these imperfect fruit invite the ingress of mold and decay during shipping. During the 2020 growing season in our Ideal Tract experimental station (Castle Hayne, NC) and growers' farms (Bladen County, NC) we observed an unprecedented number of splits across multiple harvest dates, and that trend has continued into the 2021 season. Breeding for split-resistant fruit has thus become a priority for our program.

Irregular rain and field effects such as soil composition mean that a fruit splitting is not always observed, or that the splitting trait is minimized as observations are averaged over the years and locations of evaluation. A reliable method for screening new blueberry selections for splitting is needed. Studies show that while the tendency to split is related to firmness, it is not a reliable predictor since some firm fruited cultivars show low percentages of splitting[2]. The reasons for this are primarily unknown but suspected to be related to skin elasticity and the cellular matrix of the berry[5] (M. Dossett ongoing research Pers. Comm.). A simple laboratory assay[3] of soaking berries overnight was performed in 2021, and while it showed there is a correlation between field and assay, there was error introduced by the fruit evaluator, and by the field conditions themselves. To control for error, the % split was used to determine the 5 most and least likely to split cultivars and used for off-season berry analysis in controlled greenhouse conditions. Additional data was taken using the TAXTplus texture analyzer (TAXT) as a potential tool for predicting berry splits.

Objective

Determine if a texture analyzer can be used to select for non-splitting cultivars.

Materials and Methods

In 2019, fruit was hand harvested from one bush of each replicated plot in a trial planted at the Castle Hayne Horticultural Crops Research Station "Ideal" Field site (Castle Hayne, NC) in 2016 and 2017. 100g of berries from each bush harvested were counted and sorted to give berry weights, %tear, %split, %soft and %good, and firmness. Those meeting certain criteria in yield and firmness were advanced to stage 3. In 2020, these stage 3 and all commercial lines had at least 2 bushes per plot harvested for a minimum of 4 bushes total, while those that were not advanced had only 2 bushes total harvested for evaluations. All selections were evaluated for yield, size, firmness, Brix and acidity; however due to Covid-19 concerns, hand sorting of berries did not occur.

In 2021, bushes were harvested in the same manner as 2020, but as in 2019, berries were sorted into good, tear, split, and soft. When sufficient berries were available, 25 whole ripe berries were selected from the "good" category, placed with a label in a clean Magenta™ box and submerged in distilled water (Figure 1). After sitting overnight, berries were drained and categorized into "good", "soft", and "split" by lightly rolling each berry between thumb and index fingers to check for softness while visually looking for splits. Pictures were taken and splits were rated for severity at a later date. The first iteration of our protocol called for firmness testing to measure potential water retention, but split berries would often stick to the probe and create issues for subsequent tests. The protocol was changed for later assays so that firmness was tested only on those accessions with more than 15 whole berries. Notes were taken on whether any splits were seen after firmness testing. Overall, 416 splits assays were performed.

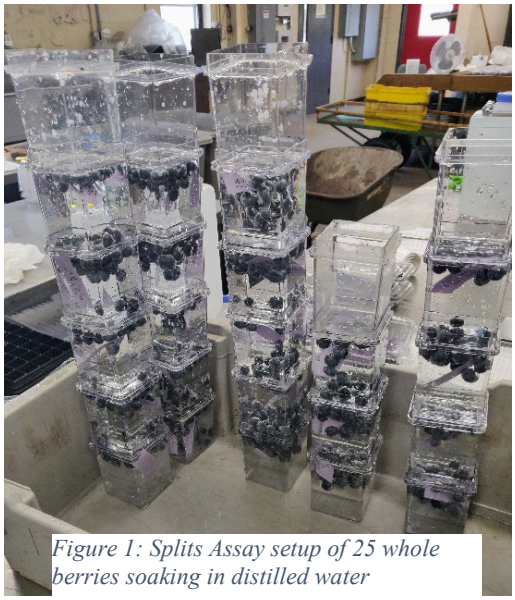

Figure 1: Splits Assay setup of 25 whole berries soaking in distilled water

From data collected from 2019-2021, 10 bushes were selected that showed the most and least number of splits. These were dug from the field, potted, and placed in a cooler at 5C until 1200+ chill hours were accumulated, then placed in the greenhouse in January and used for crossing. Flowers were emasculated and hand pollinated. Once ripe, fruit from each bush were carefully detached in the morning after watering and split into 2 groups. Each group was tested for firmness, and one group had the stem scar covered in quick-dry nail polish. Berries were then subjected to the same overnight soaking protocol for splits. Berries were then tested for firmness, examined for splits, and photographed. Once a week, a sample of harvested berries were taken to NCSU main campus for texture analysis.

In the 2022 harvest season, one pint of berries from advanced selections and checks were harvested from the 2016 and 2017 planted fields; one pint was also harvested from fields planted in 2019 and

2020. All were subjected to firmness, split, and acid/Brix/pH testing, but due to the larger amount of berries to process, they were not hand sorted. Once a week, berry samples were transported to the main NCSU campus for texture analysis.

Individual fruit were positioned equatorially for all firmness and texture analyses. Firmness testing was done using a Firmtech 2 (Bio-works, Inc, Wamego, KS). Texture analysis was performed using a TAXTplus texture analyzer (Stable MicroSystem Ltd., Godalimng, UK) fitted with a 2 mm flathead probe as previously described (Giongo, Poncetta et al. 2013). The speed of decompression of the instrument was set to a speed of 2.5 mm·sec-1 with a retraction speed of 5 mm·sec-1. The flathead probe applied a maximum force of 5 g, indicating completion of fruit puncture and platform contact, after which probe retraction was initiated. Points measured were Force at Target (g) (2 seconds), Break Point (g), Absolute Positive Force (g), Absolute Positive Distance (mm), Peak Force (g), Distance at Positive Force (mm), Area F-T 1:2 (g.s), and Area F-T 3:4 (g.s). All statistics were performed using JMP® Pro 16.0.0 (SAS Institute, Cary NC).

Results and Discussion

From our data from 2019-2021, multivariate analysis found that while the split assay correlates to the number of splits seen in the field, its strength is diluted by factors such as evaluator error, for example mistaking stem tears for splits and vice versa, and counting soft berries as split (figure 2). Additionally, In 2021, rainfall over the season totaled almost 25”, and more fruit splitting was seen from the field than usual; however this also led to and a higher mean % split and more outliers in the split assay than the mean splits seen in 2022 from field fruit, where season rain totaled 16.4” (figure 3).

Texture analysis of field harvested fruit in 2021 did not show any specific parameter that correlated to fruit splitting, although sample size was also fairly small (n=15). To increase sample size and help exclude confounding field factors, 5 low and high splitting cultivars were selected and pollinated by hand in the greenhouse during the off season, along with 16 other cultivars chosen for other studies. Over 12,000 pollinations were performed, and 2,600 fruit were used for the split assay, and approximately 260 fruit were used for texture analysis. Additionally, to see how large a role the stem scar plays in fruit splitting, half the fruit had the stem scar covered in quick dry nail polish similar to an experiment in post-harvest water loss by Moggia et al (2017)[4]. As expected, sealing the stem scar reduced splitting greatly in most cultivars, but only partially in others (figure 4).

Preliminary analysis by multivariate of greenhouse harvested fruit show that Distance at Positive Force (DPF) and and Area F-T 3:4 are inversely correlated to fruit split (table 1). DPF is the distance the probe travels once it strikes the blueberry until it punctures and so can be inferred to be a function of the elasticity of the skin. Area F-T 3:4 is the amount of force over time to puncture fruit and so is also related to elasticity, but may be less correlated because it will also be influenced by the size of the berry.

To test whether these two factors can predict splitting, all fruit that was texture analyzed and also had split assays performed were given a prediction value of 1-9 based on each factor, and a third prediction value was tallied as the sum of these 2 prediction values. The results show promise that these factors can be used as a means of predicting fruit split. More work if forthcoming in clarifying these results.

Conclusion

Several methods have been shown to be effective at screening for fruit splitting, but each has its constraints concerning time, equipment, and potential errors. Work still needs to be done in cleaning up the data and future work will use the analyzer software to explore the data further, as it was not accessible to the author until only recently.

Data from these assays will be used for future analyses and new experiments. One analysis will characterize splits by location – stem scar, equator, or calyx end- to compare texture analysis results. We will also be comparing post-harvest fruit weight loss to the sealed and unsealed splits data. Additionally, fruit from Reveille x Arlen population has been analyzed on the TAXT system as part of the VACCAP project-we hope that in the future these data may be used to explore the genetics behind fruit split.

Impact Statement

Data from 2019-2021 will provide valuable information for ongoing and future studies related to blueberry fruit quality and improving germplasm. Findings from this study will be presented at the SE Regional Fruit and Vegetable Conference in 2023 and regional grower meetings.

Literature Cited

1 Marshall, D. A., Spiers, J. M., & Stringer, S. J. (2008). Blueberry Splitting Tendencies as Predicted by Fruit Firmness, *HortScience horts*, *43*(2), 567-570.

2 Marshall, D.A., Spiers, J.M. and Curry, K.J. (2002). INCIDENCE OF SPLITTING IN 'PREMIER' AND 'TIFBLUE' RABBITEYE BLUEBERRIES. Acta Hortic. 574, 295-303

3 Marshall, D. A., Spiers, J. M., Stringer, S. J., & Curry, K. J. (2007). Laboratory Method to Estimate Rain-induced Splitting in Cultivated Blueberries, *HortScience horts*, *42*(7), 1551-1553.

4 Moggia C, Beaudry RM, Retamales JB, Lobos GA. 2017. Variation in the impact of stem scar and cuticle on water loss in highbush blueberry fruit argue for the use of water permeance as a selection criterion in breeding. Postharvest Biology and Technology. 132:88-96.

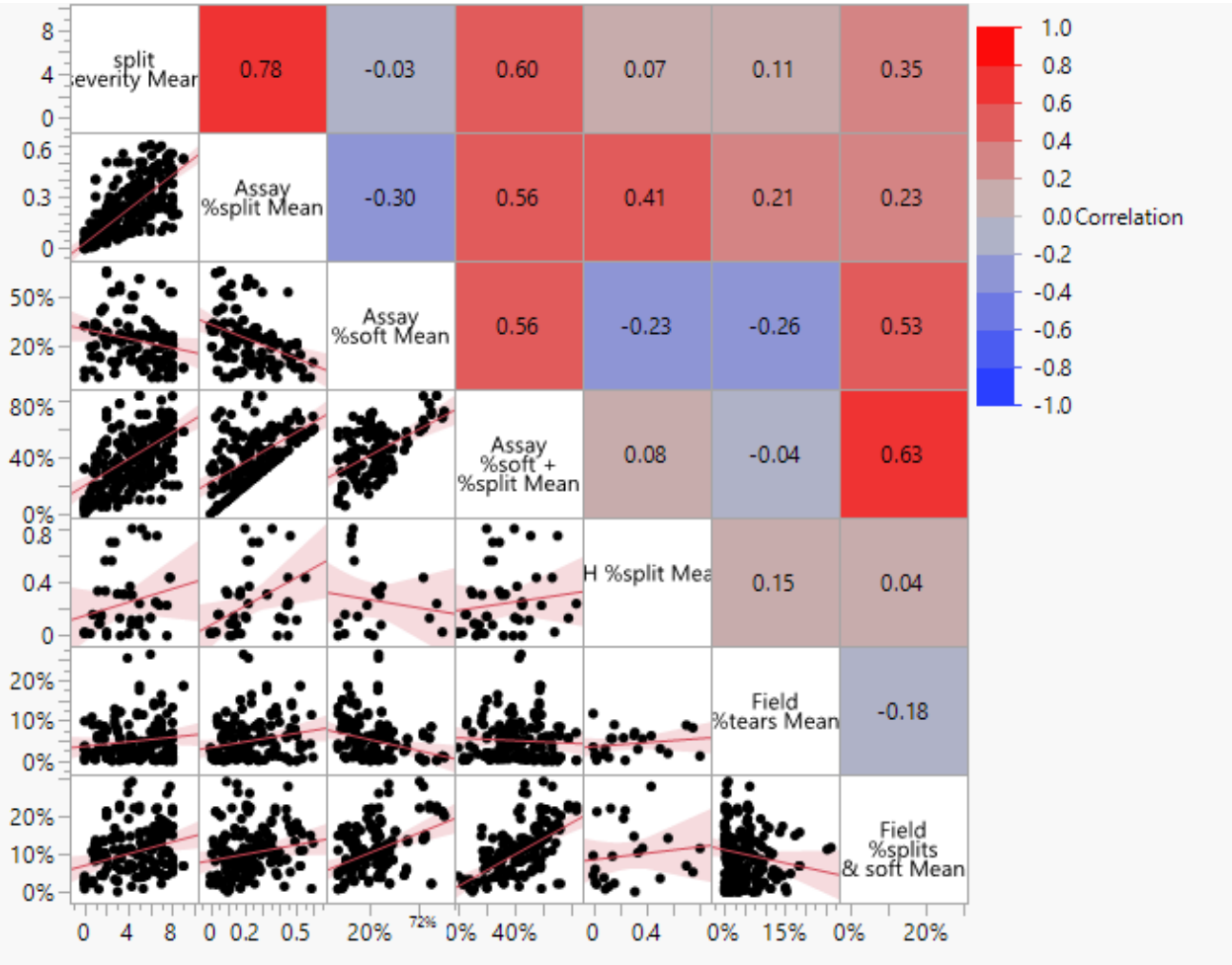

Figure 2: Correlation Matrix of Means of Split Severity, %Split Assay, %Soft Assay, %Soft+%Split Assay, GreenHouse (GH) % Splits, Field % Tears, and Field %Splits & Soft. Tears are tearing at the stem scar and can look similar to splits. Soft fruit can be caused by fruit splitting but can also be caused by rot. Both of these may be miscategorized.

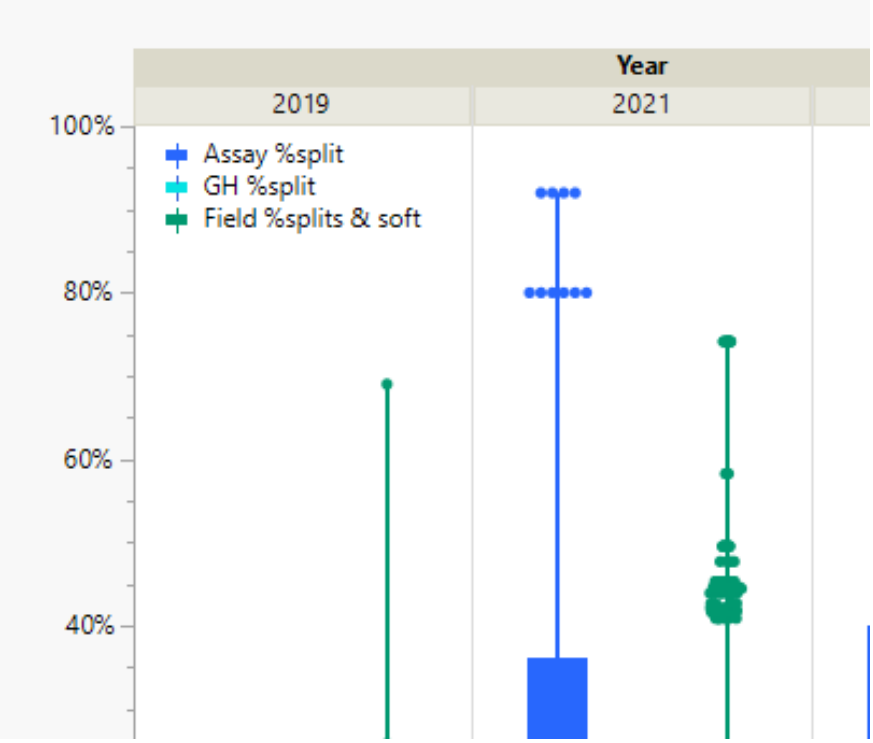

Figure 3: % Split counted from the field (2019 & 2021) compared to %split of berries assayed in 2021 & 2022 harvested from the field and greenhouse. Heavier rains in 2021 caused more fruit split seen in both the field and assay.

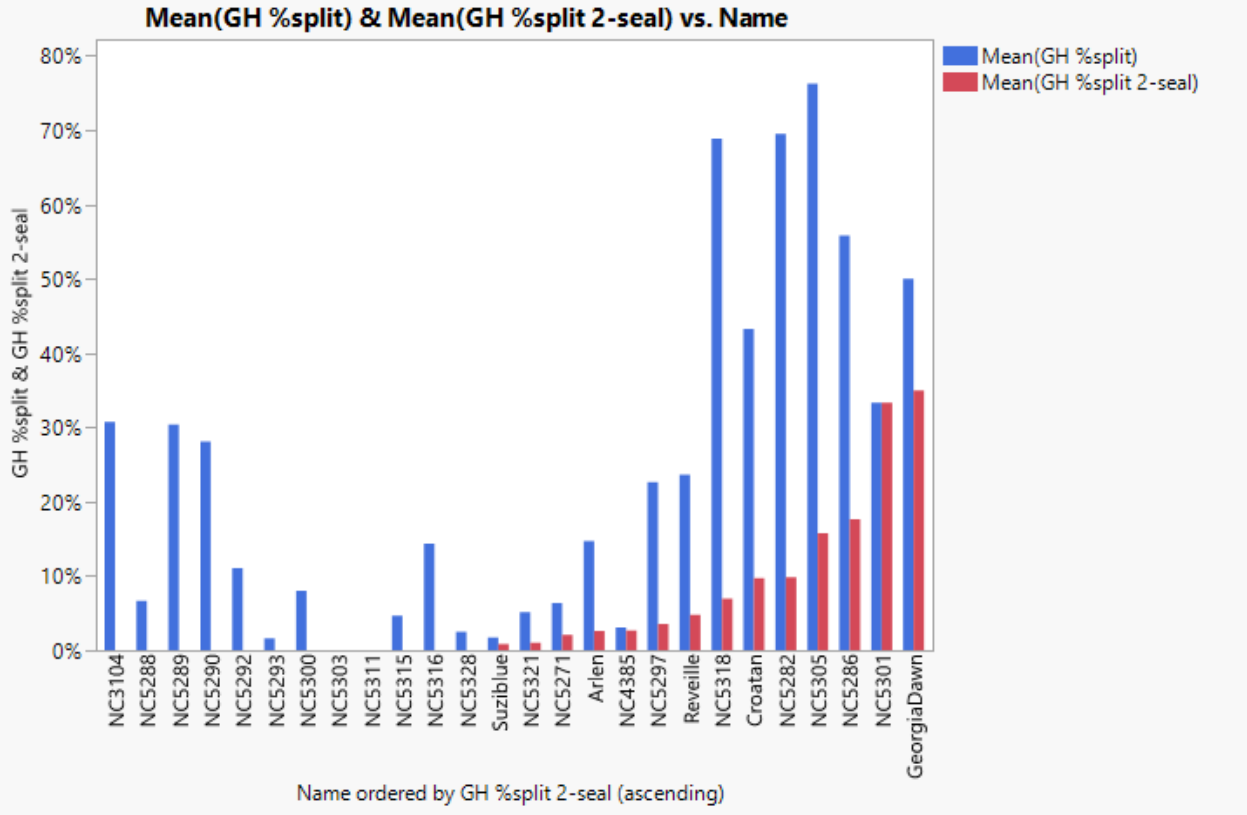

Figure 4: Results of greenhouse (GH) split experiment where stem scar was sealed on half the berries. Fewer splits were seen on sealed berries overall.

Table 1:Pairwise correlation of % split from greenhouse grown fruit and texture analysis variables.

Variable	by Variable	Correlatio	Coun	Lower 95%	Upper 95%	Signif Pro
GH %split 2-seal	GH %split	0.5024	86	0.3251	0.6455	<.0001
N(Force at Target (Cycle: 1) (g))	GH %split	0.2407	101	0.0475	0.4166	0.0153
Mean(Force at Target (Cycle: 1) (g))	GH %split	0.2179	101	0.0234	0.3964	0.0286
Mean(Absolute Positive Distance (Cycle: 1) (mm))	GH %split	0.1322	101	-0.065	0.3193	0.1877
Mean(Area (Traditional) F-T 3:4 (g.sec))	GH %split	-0.3967	101	-0.5495	-0.2181	<.0001
Mean(Distance At Positive Force 1) (mm))	GH %split	-0.4518	101	-0.5947	-0.2812	<.0001
N(Force at Target (Cycle: 1) (g))	GH %split 2-seal	0.1609	86	-0.0528	0.3605	0.1389
Mean(Absolute Positive Distance (Cycle: 1) (mm))	GH %split 2-seal	0.1056	86	-0.1087	0.3106	0.333
Mean(Area (Traditional) F-T 3:4 (g.sec))	GH %split 2-seal	-0.3095	86	-0.4893	-0.1045	0.0037
Mean(Distance At Positive Force 1) (mm))	GH %split 2-seal	-0.3433	86	-0.5175	-0.1417	0.0012

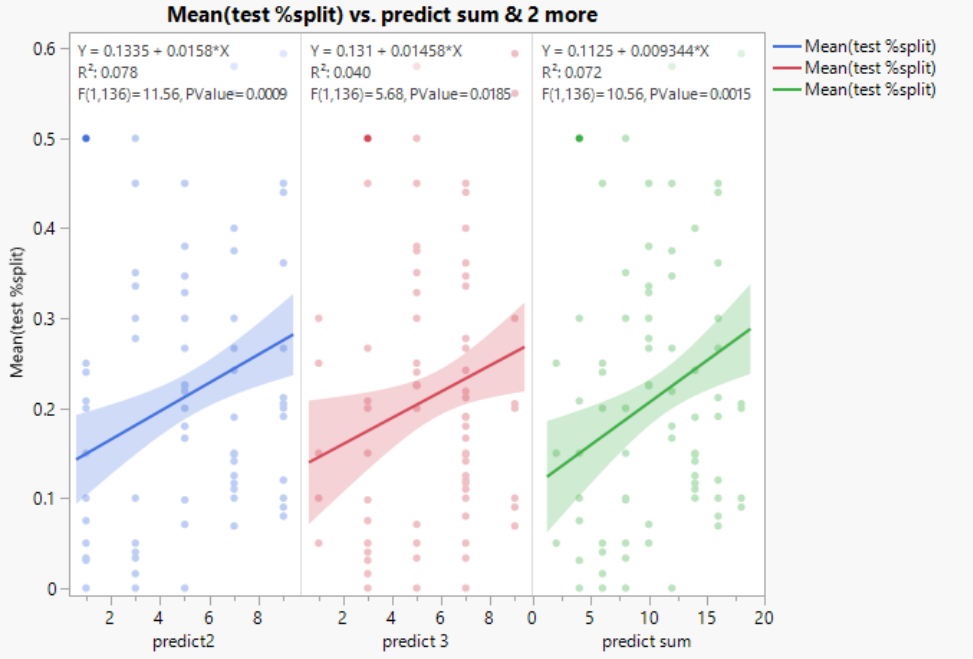

Figure 5: Predicted splitting on all texture and split assayed fruit compared to assayed % splits. Predict2 is based on Distance at Positive Force (mm) and predict 3 is based on Area F-T 3:4 (g.s); Predict sum is sum of predict2 and predict 3.