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

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Public Abstract:

The NC State blueberry breeding program is dedicated to developing high quality blueberries for North Carolina and other South regional growers. Changing weather patterns has led to record-breaking rain events in the Southern US over the past decade. This has meant several challenges for blueberry growers such as pollination difficulties and fruit splitting. In 2021, two assays were trialed as methods to help breeders select cultivars that are more self-fruitful and less prone to splitting. One-gallon paint straining bags were trialed as pollinator exclusion bags on unopen flowers of cultivars known to be either self-fertile or pollination dependent. Half of the bags were shook on a weekly basis until corolla drop or flowers were no longer present. Preliminary observations showed that the shook treatment were more likely to contain more fruit overall and more ripe fruit than unshook treatments when a genotype was known to be self-fertile, while the known non-self-fertile genotypes had no fruit in either treatment, however definitive data was not obtained this season. The other assay tested the likelihood of fruit splitting when soaked overnight in distilled water. A strong positive correlation was seen between the fruit soft and split in the lab and soft and split fruit from the field. Splitting was very genotype dependent but not positively correlated to firmness as previously hypothesized. Further research is being done to explore the causes of split fruit and the relationship to rain events, which may have the additional bonus of creating a model to estimate fruit yields by cultivar in the likelihood of rain events.

Introduction:

Our specific objective in this project is to examine the self-fertility and fruit quality attributes, especially firmness and splitting/cracking, of NCSU southern highbush advanced breeding selections alongside named cultivars, with the end goal of identifying elite germplasm with superior fruit quality for release as named cultivars and use as parents in crossing as well as informing growers on current cultivar characteristics.

In the past several years, growers in North Carolina and elsewhere have faced unprecedented rain events throughout both the flowering and harvesting seasons that have caused issues with pollination and split fruit. Populations of many native pollinators were decimated by hurricane Florence in 2018 while rain events in early 2019 prevented honeybees, the preferred pollinator for growers, from flying. These were estimated to be a large contributing factor for the lower-than-average yields in 2019 (NC growers Pers. Comm.). While many modern blueberry cultivars are semi self-fertile which allows for mono-cultural fields and easier management, pollinated flowers produce berries that mature faster and are larger in size. Rain events later in the season can cause fruit splitting and an estimated 14-30%[1] of a single harvest to be discarded as unmarketable as these imperfect fruit invite the ingress of mold and decay during shipping. Increased demand for machine-harvestable cultivars with the firmness to withstand rougher treatment have caused firmness to be suspected in playing a role in fruit splitting. However, studies have shown that while the tendency to split is related to firmness, it cannot be predicted by it since some firm cultivars show low percentages of splitting[2]. The reasons for this are mostly unknown but suspected to be related to skin elasticity and the cellular matrix of the berry (M. Dossett ongoing research Pers. Comm).

It is therefore imperative to blueberry breeders to breed for traits that increase self-fruitfulness and fewer splits while maintaining firmness and resistance to bruising. To help easily identify and quantify these traits, we've tested two assays. The first was testing 1-gallon paint straining bags as pollinator exclusion bags on unopen flowers of cultivars known to be either self-fertile or pollination dependent. The other assay tested the likelihood of fruit splitting when soaked overnight in distilled water.

Materials and Methods:

Pollinator exclusion/self-fertility: Four cultivars known to be self-fertile, one advanced selection known to be pollination dependent, and 2 advanced selections with unknown self-fertility were chosen to trial. One-gallon paint straining bags were secured with flagging tape to 4 branches that either had open flowers removed or were in a pre-bloom state for each accession. Half of the bags were shook on a weekly basis (identified by flagging tape color) until corolla drop or flowers were no longer present. Bags were later removed to allow


Figure 1: Pollinator exclusion bags on NC3104

leaf expansion and prevent potential disease after green fruit set and/or flower senescence. The flagging tape was left on to identify the branches later in the season.

Fruit Splitting:

In 2021, 89 cultivars and advanced selections were harvested from single bushes in replicated plots. After total yield was taken for each bush a portion of berries were weighed and sorted into good, soft, split, stem tears, and other damage categories while another portion was tested for Brix, titratable acidity, berry size and firmness using a Firmtech 2 (Bio-works, Inc, Wamego, KS). When sufficient berries were harvested, 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 so splits could be rated on 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 2: Splits Assay setup of 25 whole berries soaking in distilled water

Results and Discussion:

Pollinator exclusion/self-fertility:

Differences in green fruit-set were seen as expected between each cultivar as well as between shook and unshook branches, however, blue fruit was not collected this season due to labor shortage, and only brief observational notes were taken. For 2022, we propose continuing this assay with more accessions and adding a 1-9 rating for selected branches for flower abundance. Similarly rated branches will be selected for treatment. A control branch will also be added to each genotype and open flowers removed so that differences in ripening time can be clearly seen. Although we often test self-fertility and parthenocarpy in the greenhouse while performing crosses in the winter, a proven and simple field assay using easily acquired materials is useful for future research when greenhouse testing is not feasible.

Fruit Splitting:

Statistical analysis was performed using JMP® Pro 16.0.0 software (SAS Institute Inc., Cary, NC, 1989–2021). Several parameters were analyzed by linear regression models for effects on %

splits and % soft from both in the field and by laboratory assay. Genotype accounted for the most effect on splits and softness (Table1). When genotype was removed, some effect of firmness can be seen (Table 2). Significant firmness effects, however, are negatively correlated to soft fruit and non-significantly correlated to splits (Figure 3). If there is any relationship between splits and firmness, it would need to be revealed by deeper analysis into berry anatomy, possibly by puncture testing. Some anecdotal observations were made during the 2021 season. While every effort was made to only assay whole perfect fruit, berries with scarring (a hailstorm pre-harvest left many fruit with scars of various sizes) appeared to have fewer instances of splitting. In addition we observed that larger, and theoretically more mature fruit, were more prone to splitting, and vice versa. These observations and their role in the significant effects seen below await further analysis at this time.

Table 1: Prob > F comparison of effects on fruit softness and splits in field and lab assay (Test)

Source of Effect	DF	Field % splits	Test % splits	Field % soft	Test % soft
Genotype	82	**0.0002****	**<.0001****	**<.0001****	**<.0001****
prior harvest accum precip.	1	0.8795	**0.0064****	0.1328	0.0182*
days since last rain event	1	0.6206	0.3059	0.2769	**0.0003****
days since last harvest	1	0.4103	0.7946	0.0491*	0.3586
firm	1	0.1438	0.8403	0.6588	0.2352
size	1	**0.0179***	0.1004	0.5862	0.2273
%acid	1	0.0583	0.2061	0.8537	0.9372
%ss	1	0.7047	0.5427	0.7675	0.8965
pH	1	0.3299	0.5918	0.796	0.4808

Table 2: Prob > F comparison of effects on fruit softness and splits in field and lab assay (Test) without Genotype

Source of Effect	Field % split	Test % split	Field % soft	Test % soft	Field %soft+ %split	Test %soft+ %split
prior harvest accum precip.	0.779	0.2019	0.1193	**0.0075****	0.2824	**0.0004****
days since last rain event	0.8881	0.0193*	0.0036*	**0.0047****	0.0241*	0.7502
days since last harvest	0.8989	**0.0007****	0.1181	0.0421*	0.1827	0.9444
firm	0.8857	0.097	**<.0001****	**<.0001****	**0.0003****	**<.0001****
size	0.1877	0.3143	0.7153	0.8507	0.5958	0.5085
%acid	0.021*	0.7147	0.566	0.7867	0.3247	0.8507
%ss	0.6902	0.6127	0.0508	0.0118*	0.1868	0.0483*
pH	0.0338*	0.3473	0.6145	0.9338	0.3553	0.209

The correlations between splits seen in the field and those seen in the laboratory assay were also explored. As multiple people helped to sort soft and split berries from the field, there is reason to believe error was introduced. Splits from the field could easily be mistaken as soft, and similarly, splits from the assay could often be subtle and present as soft. Multivariate regression analysis showed improved correlation between field splits and assay splits when % soft berries were also accounted for (Figure 4). Further analysis and data are needed to correlate rain events to field splits and assay splits. Within these analyses is the possibility of creating a predictive model to estimate fruit yields by cultivar in the likelihood of rain events.

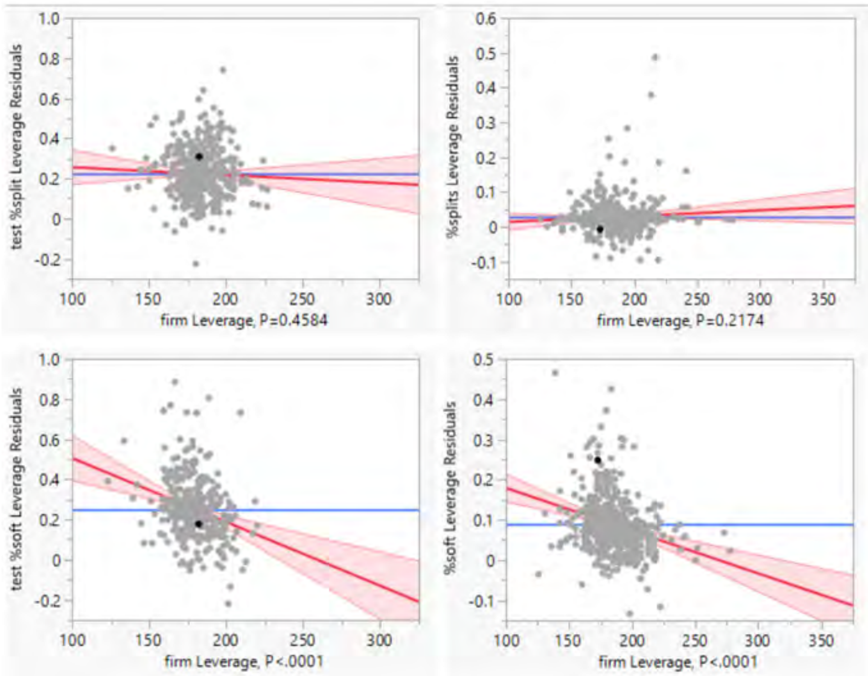

Figure 3 Test, Field % Split and % Soft x Firm

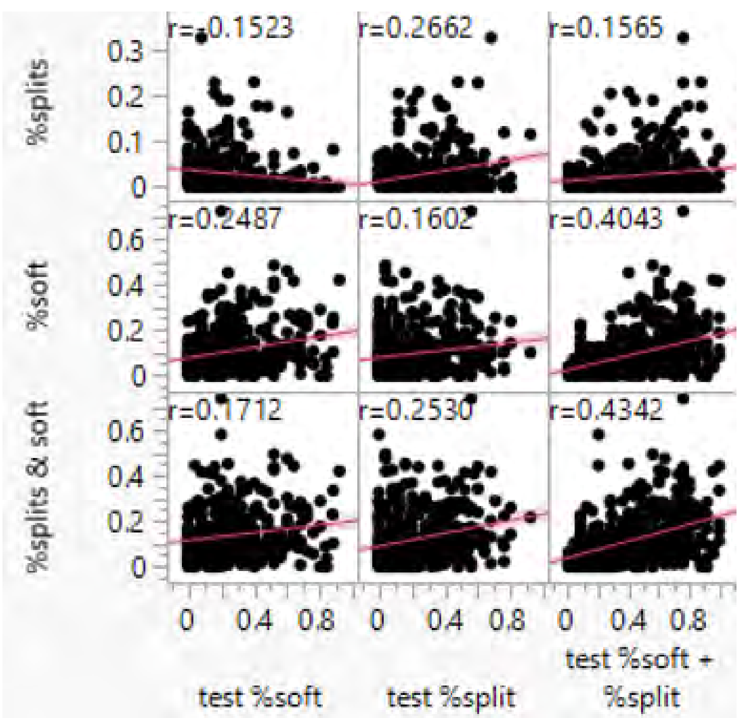

Figure 4 Multivariate regression analysis of Test and Field % Soft and % Split

Lastly, Tukey's HSD test was run on the % splits assay. In Table 3, the top and bottom 10 advanced selections with are presented alongside commercial cultivars from the same trial.

Table 3: Least Squares Means Differences by Tukey HSD Test: %Split Assay

Genotype	Least Sq Mean	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
NC5313	-0.0431298				D	E	F	G	H	I	J	K	L	M	N	O
NC4725	-0.0167175	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
NC4385	-0.015388													M	N	O
NC5293	-0.0042172										J			M		O
MissJackie	-0.0019986													M		O
NC5314	-0.0019243															O
NC5330	-0.0010974	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
NC4976	-0.0006599	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
Summit	0.00127	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
NC5295	0.0152296								H	I	J			M	N	O
Gupton	0.0301873						F		H	I	J			M	N	O
NC5327	0.0381304								H	I	J			M	N	O
Pender	0.0415344						F		H	I	J			M	N	O
NC4002	0.052082								H	I	J			M	N	O
Megasblue	0.1051974	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
Titanium	0.1190538	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
Paloma	0.1638185	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
NewHanover	0.1762638				D	E	F		H	I	J	K		M	N	O
Huron	0.1812573		B	C	D	E	F		H	I	J	K	L	M	N	O
Croatan	0.2262611	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
Legacy	0.2572071	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
Suziblue	0.2692134	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
O'Neal	0.2711518	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
NC5298	0.3749733	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
NC5280	0.3817364	A	B	C	D	E	F	G	H	I	J	K	L		N	
Krewer	0.38269	A	B	C	D	E	F	G	H		J	K	L		N	
NC5325	0.3923593	A	B	C	D	E	F	G	H	I	J	K	L	M	N	
NC5297	0.4247856	A	B	C	D	E	F	G	H	I		K	L		N	
Onslow	0.4874611	A	B	C	D	E	F	G	H	I	J	K	L	M	N	
NC3168	0.5092454	A	B					G					L			
Columbus	0.5106838	A	B	C	D	E	F	G	H	I	J	K	L			
NC5324	0.5246636	A	B	C	D			G				K	L			
NC2930	0.5285881	A	B	C	D	E	F	G	H	I	J	K	L			
MissLilly	0.600238	A						G								
NC5282	0.6762702	A	B	C												
NC5332	0.7177922	A	B	C	D	E	F	G				K	L			
NC5319	0.7342165	A	B	C	D	E		G					L			

References:

1 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

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