Variation in Pawpaw (Asimina triloba L. Dunal*) Productivity and Fruit Quality Among Cultivars and Orchards in Ohio*

THESIS

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Abstract

Natural environmental gradients affect crop productivity; while the same is true for genetic and agronomic factors these can be controlled by the grower. Growers have to choose cultivars, rootstocks, and agronomic inputs that account for environmental factors to gain consistent yields and high-quality produce. For many emerging fruit crops, such as pawpaw (*Asimina triloba* L. Dunal), factors controlling yield and quality still need to be properly understood. The pawpaw is a fruit tree native to the Eastern United States and has a rapidly growing market. How environmental gradients, cultivars, and agronomic inputs influence the yield and quality of a pawpaw fruit harvest has not been studied extensively. This study's aim was to investigate the relationship between environmental factors and cultivar identity in terms of fruit yield and quality. Eight commercial and semi-commercial orchards across the State of Ohio had flower counts and fruit counts performed on them for 24 cultivars. Fruits were counted by four size classes based on their length and width to estimate the yield. Allometric relationships between fruit size, total mass, and pulp mass were investigated to predict the total pulp and fruit masses for each tree by cultivar. Ten cultivars (Allegheny, NC-1, Overleese, Potomac, Shawnee Trail, Shenandoah, Sunflower, Susquehanna, Wabash, and Wells) were measured for 18 quality metrics. Linear mixed effects models demonstrated significant differences in fruit yield and quality between both cultivars and genetic related cultivar groups. Principal Component Analysis (PCA) was used to evaluate multivariate differences in fruit quality and showed strong gradients in quality associated with cultivars, sites, and ripeness scores. Site Valley View Farm had the smallest standard deviation (0.40) which demonstrates sites that are more proactively managed have the most consistent fruit quality. The lowest ripeness scores were associated with harder fruit with a higher pH; the highest ripeness scores were associated with higher browning potential, sugar content, greater *Phyllosticta* abundance, and increased pulp mass. Pawpaw quality is complex and more than 50% of the variance could not be explained with by cultivar, site, and ripeness scores measured within this study. Evaluating tools and techniques to reduce variance in quality to produce consistent, high quality fruit should be the objective of further research.

Acknowledgments

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Fields of Study

Major Field: Environment and Natural Resources

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*Chapter 1: Variation in Pawpaw (*Asimina triloba *L.* Dunal*) Cultivar Productivity Among Orchards in Ohio*

Introduction

Genetic, agronomic and environmental factors are key determinants of crop performance (Musacchi & Serra, 2018). Small changes to one of these individual factors can vary yield outcomes drastically (e.g. Dwire et al., 2004, Faust, 2000, Musacchi & Serra, 2018) even within small areas. Genetic factors affecting tree fruit crops include variety/cultivar and rootstock. These describe the variability within a species and growth patterns and development, such as dwarfing, that may affect the characteristics of the final crop (Musacchi & Serra, 2018). Agronomic factors involve conditions that are manipulated by the farmer, such as pollination management, pruning, thinning, training systems, irrigation, and nutrition (Musacchi & Serra, 2018). Finally, crop yield is strongly influenced by environmental gradients (Dwire et al., 2004) including light availability, temperature, humidity, wind, soil moisture and fertility.

Maximum yield can be expressed as the total number of fruits or total mass of fruit. While there are optimal conditions that enable fruit trees to produce maximum yields, most trees are not grown in such conditions (Musacchi & Serra, 2018). Understanding fruit tree yields across abiotic gradients allows growers to make appropriate adjustments to agronomic practices to compensate for deficiencies in growing conditions. Apple trees, for instance, have various recommendations for ideal agronomic production practices. In cool climates apple tree growth is moderate; (e.g. United Kingdom) and increasing planting density will produce ideal crop volume. In contrast, climates with warm days and cool nights (e.g. Washington, USA) naturally maintain ideal conditions for high yields (Faust, 2000). For emergent crops, there needs to be extensive research into how individual environmental factors affect yield but also how all factors (genetic, environmental, and agronomic) interact. For example, a two year experiment on the effects of organic mulch and irrigation on pomegranate (*Punica granatum* L.), a developing fruit crop for U.S. and India, recommended the sugarcane mulch for its water retentions effect (Mesharm et al., 2018). This study demonstrates determining best practices for producing a consistent yield; the grower needs to monitor environmental factors, adjust the agronomic inputs, and choose the best-suited genetic material (cultivars and rootstocks) to plant (Musacchi & Serra, 2018). The research and distribution of this information is critical for producers to make informed decisions.

Pawpaw (*Asiminia triloba* L.), in the family Annonanceae, is a small understory softwood tree that grows in forest bottomlands from north Florida to the southern regions of Canada (Pomper & Layne, 2005). Pawpaw fruits can weigh over 1kg each, have green to yellow skin, pulp from white to yellow to orange, and a row of brown to black 15-25 mm seeds (Pomper & Layne, 2005, Pomper et al., 2010). The fruit grows in clusters from one to nine from a single pollinated flower (Pomper & Layne, 2005). Pawpaw has a complex flavor profile, described as having notes of mango, pineapple, banana, or melon, with possible bitter and sour undertones, and caramel flavors as the ripening process progresses (Duffrin & Pomper, 2006). Two primary markets have developed for pawpaw fruit: the fresh marketed fruit is sold at farmers markets, while a market for pulp exists among customers such as brewers, bakers, ice cream makers as well as general consumers. Pawpaws have recently developed a passionate following which is partly explained by a resurgence of interest in its cultural and horticultural history (Moore, 2015, Peterson, 2003) and attention from producers involved in local food movements and foraging groups.

Prior to World War I, pawpaws experienced a surge in popularity and there was significant effort made to commercialize production. Large orchards were established, but the inability to find a cultivar with an adequate shelf life left the ventures unprofitable (Peterson, 2003). Renewed commercialization efforts began in the 1980s and started to bring the pawpaw to modern markets (Peterson, 2003). Commercialization of new pawpaw varietals/cultivars has followed a progression of stages including: selection of cultivars from wild populations, assessment of the newly chosen cultivars against others chosen from wild and curated populations, creation of a germplasm, horticultural and genetic research, testing of the selected cultivars, development of commercially viable orchards and markets, and scientific breeding for specific characteristics (Peterson, 2003). Currently there are many pawpaw cultivars that have been released but scientific breeding has yet to be undertaken. Horticultural and genetic research is making steady progress (e.g. Huang et al., 1997; 1998; 2000; Pomper et al., 2003a; Wang et al., 2005). Most notably, Pomper et al. (2010) studied 41 cultivars for genetic similarity utilizing microsatellites (simple sequence repeats (SSRs)) as DNA fingerprints for assesing genetic diversity. He found 39 unique fingerprints (SSRs) out of the 41 and was able to divide the cultivars into five groups (Pomper et al., 2010) based on their level of genetic relatedness. When compared to Peterson (2003), who looked at genealogical maps of the cultivars, there are some similarities (e.g. PA-Golden 1 and 3, and Zimmerman were all from G.L. Slate collection), but also notable differences (e.g. the cultivars Davis and Taylor, both from the C. Davis collection, are genetically dissimilar). Examining how genetically similar cultivars interact with environmental and agronomic factors allow for a broader generalization to be reached about a range of cultivars in reference to growing recommendations in diverse settings.

A small number of multi-site field studies have been previously completed. Twelve field sites across eleven states were established between 1995-1999 for the Pawpaw Regional Variety Trials (PRVT) (Pomper et al., 2003b). The PRVT investigated how 15 named varieties and 13 advanced selections performed across the twelve field sites but has, thus far, only reported on the difference between the two field sites in Kentucky (Pomper et al., 2008b). There was not a difference in the number of fruits per tree, but the size of fruits was greater at the site with irrigation. The named cultivars (Potomac, Susquehanna, Wabash, Overleese, NC-1, Shenandoah, and Sunflower) were recommended to be planted in Kentucky (USDA Zone 6) whereas the advance selections were not. The PRVT in Oregon, (U.S. Department of Agriculture) which was established in 1999, had to be removed in 2001 due to vascular wilt symptoms that caused a mortality rate of 50% over two years. The pathogen was not able to be identified

(Postman, Hummer, & Pomper, 2003). Iowa State University published a report on the PRVT maintenance at their site in 2008; the trees had started to produce fruit of marketable size, over 85 g (3 oz.) (O'Malley, 2008). Further reporting has yet to be released about the PRVT (Greenwalt, 2016). Separately from results from the PRVT, Cantaluppi (2016) studied four Peterson cultivars (Allegheny, Shenandoah, Susquehanna, and Potomac) in a randomized complete block design at one site in Oxford, North Carolina (USDA Zone 7) over nine years (planted 2007 as one-year old grafted seedlings to 2015). Unfortunately, two years of potential full fruit production (2012, 2014) had flowers set killed by an April frost. For the two years fruit was collected (2013, 2015), the yield increased for all cultivars, with Potomac increasing by four times from 2013 to 2015 (Cantaluppi, 2016). Greenwalt (2016) examined data collected over eight years by Dr. Ron Powell at three separate locations, in Ohio. This data set had fruit with an average weight 15 g larger than that of the fruit from PRVT but saw a shorter harvest duration. Growing year had a significant effect on the average weight of the fruit, yield, and harvest duration which indicates that environmental factors have a significant role in the outcome of pawpaw harvests (Greenwalt, 2016).

While improved information on how growing conditions, cultivar selections, and orchard management affect pawpaw productivity, making reliable yield estimates remains, in fact, difficult. Pawpaws range drastically in size (<50 to >500 g) even within clusters and this leads to fruit counts not encapsulating the yield of a tree accurately. Historically, this diversity has been captured by weighing all the fruits on a tree (Cantaluppi, 2016, Crabtree, 2004, Pomper et al., 2003b, Pomper et al., 2008a, Pomper et al., 2008b). This method gives an accurate representation of the yield and fruit count but has been carried out with work at a few locations. It is also unsuitable for on-farm research where growers wish to retain fruit on the tree until suitably ripe for marketing. Pawpaw fruits ripen independently over a two to three week period and even fruits within the same cluster may become ripe at different times. When ripe, the abscission layer forms at the peduncle of the fruit and releases the fruit to fall to the ground. Growers also have the perception that, to be of marketable quality, pawpaws must be picked when ripe but before the fruit releases from the peduncle. The non-synchronous ripening of the fruit, coupled with pawpaw physiology and grower's perceptions, leads to growers picking every two to three days. Within this study, we have attempted to develop a methodology for obtaining non-destructive fruit yield estimates and generating estimates of yield for both the fresh fruit and pulp markets. Developing allometric relationships can, however, allow simple measurements to estimate the yield and avoid destructive sampling (e.g. pulp mass) (Sliva et al., 1997). Allometric relationships can assist in grading fruit, yet to be attempted in pawpaw, for fresh market consumption (Koshman et al., 2018). Developing allometric relationships will assist in forming recommendations for cultivar selection for both the pulp and fresh markets of pawpaw fruit.

Given the relative paucity of information on pawpaw cultivar performance, the aim of this study was to investigate how environmental, agronomic, and genetic factors affect the total yield of pawpaw trees. Established commercial and semi-commercial orchards with identifiable cultivars were used to examine fruit characteristics. The research had three specific objectives: i) to determine allometric relationships between fruit dimensions and both fruit mass and pulp mass; ii) to examine how the total number of pawpaw fruit produced per tree was affected by cultivar, site, and environmental factors; and iii) to compare how the total mass of pulp produced varies by cultivar and site.

Methods

Site Selection

Eight commercial and semi-commercial pawpaw orchards in Ohio were identified in collaboration with the Ohio Pawpaw Grower's Association (Appendix A). Each orchard was at a different location (environment conditions, soil type, climate, etc.) and was managed uniquely (cultural practices). Each orchard had known cultivars that could be identified and were old enough to produce fruit (Appendix B). The eight sites spanned from the southern border of Ohio to suburbs of Cleveland. Orchards were monitored beginning in April 2018 through to October 2018. At each site, the number of trees was counted, and cultivars present were recorded along with diameter at breast height (DBH) for each tree.

Flower Counts

Starting in early May 2018, sites were visited to complete flower counts. Sites were visited from South to North to ensure all sites were recorded at approximately the same phenological stage. Flowers were counted by sampling primary branches (any branch originating at the trunk that had one or more flowers). All flowers were counted when there were less than ten primary branches. For trees with more than ten primary branches, flowers on five randomly selected branches were counted and the total number of primary branches used estimate total flowers per tree.

Fruit Counts

Owners are rarely able to pick all the pawpaw fruit on mature trees and on any given tree fruit can vary substantially in size. Fruit gathered in September 2017, along with 30 random fruits from a mixture of cultivars collected in August 2018, were characterized in terms of their length, width and mass. Length × width was chosen to define size classes, rather than volume or weight, for ease of fruit classification in the field. Quartile breaks in size (length × width) and mass were compared to size classes defined in the qualitative assessment worksheet devised by Peterson (1990). Four new size classes were created from the quartiles size class: 1) <4×7 cm, 2) 4×7 to 5×8 cm, 3) 5×8 cm to 6×10 cm, and 4)> 6×10 cm. Size classes where defined by length × width to allow for the simplest measurement possible to estimate yield.

Fruit counts were preformed starting in August before fruit fully ripened. Pawpaw fruit ripen in a window (2-3 weeks); counting before the fruit are fully ripe mitigates losses to wildlife and fruit drop. All fruit, and fruit clusters, on a tree were tallied, with the former quantified according to size class. Dropped fruits were not included in the cluster count but were accounted for in the total fruit assessment.

Yield Estimates

When fruit started to ripen, sites were revisited to collect fruits from as many cultivars and trees as possible. Under the grower's direction, fruits were chosen from selected trees and dropped fruits. At least three fruits of each size class for each cultivar were collected and unique identification codes assigned to them. The fruit were transported back to the laboratory for measurement, processing, and weighing. Fruit volume was calculated by assuming all the fruit collected were prolate ellipsoids ($4/3\pi ab^2$) where 'a' was equal to the length measurement and 'b' was equal to the width measurement.

To define allometric relationships for each cultivar between pulp mass, fruit mass, volume and length × width; I used data from all fruit that had more than five fruits per cultivar. A linear model (function: lm, package: stats (R Core Team, 2018)) was fitted for each model with the dependent variable square root transformed to produce a normal distribution of residuals. Volume was estimated based on cultivar and length × width (LTW) to check for efficacy of use of LTW as an index of volume. Pulp mass and fruit mass were estimated based on cultivar and LTW. The same models were run again using genetic groups (Pomper et al., 2010), rather than cultivar, as this allowed data from more cultivars to be included, though at a coarser level of specificity.

To estimate the total yield in terms of both fruit mass and pulp mass, the LTW quartile breaks were used for the upper three size classes (class 2= 24 cm^2, class 3=40 cm^2, and class 4= 60 cm^2) to gain a conservative estimate of the mass. Size class 1 was estimated at LTW of 12 cm^2 (half of size class two) (Appendix C). Each cultivar that had a minimum three trees at three or more different sites (Potomac, Shenandoah, and Wabash), had their fruit and pulp mass predicted by size class based on the previously established allometric relationship. The resulting predicted masses were by the fruit counts in the respective size classes and summed together. This process was repeated for Pomper et al.'s genetic groups (II: Zimmerman, III: Alice, and V: Sunflower).

Assessing Differences among Cultivars, Groups, and Sites

All data analysis was completed in R studio 3.3 (RStudio Team, 2016). Due to lack of replication of some cultivars between and within research sites, we were unable to include all the cultivars (24) sampled in the formal statistical analyses. Instead, statistical analysis focused on i) three cultivars, and ii) three genetic groups, that had three or more trees at three or more sites. The total number of fruit produced by each tree was modeled in two different ways; once as a function of cultivar (three were included) and once according to Pomper et al. (2010) genetic groups (eleven cultivars included).

To model the total number of fruit produced, a generalized linear mixed-effects model was used (function: glmer, package: lme4, Bates et al., 2015). A Poisson model form was used to account for the count data. DBH and estimated flower counts were included as covariates; cultivar was defined as a fixed effect. Site was included as a random effect within the model encompassing locational difference (climate) and cultural practices. The genetic groupings from Pomper et al. (2010) were modeled similarly but with the group substituted for cultivar. Post-hoc pairwise comparisons of differences in total fruit production between cultivar and genetic groupings were completed via least square means (function: lsmean, package: emmeans, Russell, 2019). Models were summarized using the “Anova” function with type III sums of squares (package: car, (Fox & Weisberg, 2011)). The marginal r-squared value explaining the variance related to the fixed effects and covariates (including DBH, estimated flower counts, and cultivar/group) and the conditional r-squared value, which quantifies the variance explained by the whole model, were calculated for both the cultivar and genetic group models (function: r.squaredGLMM, package: MuMin, Kamil, 2018).

A linear mixed-effects model of total tree pulp with DBH and estimated flower counts as covariates, cultivar as a fixed effect, and site as a random effect was used to model both pulp mass and total fruit mass. Total fruit mass and pulp mass was square root transformed to produce a more normal distribution of the residuals. The model was run a second time with the trees classified by genetic groups rather than cultivar. Simplification of all linear mixed-effects models was attempted by fitting models with maximum likelihood to compare changes in Akaike information criterion hereafter AIC and Bayesian information criterion hereafter BIC following removal of non-significant predictors. For all the models, the full models were retained as the changes in AIC/BIC were less than, or close to, two. The final selected model was then refitted using restricted maximum likelihood estimation. Post-hoc pairwise comparisons of cultivar/ genetic groups were completed using least square means (function: lsmean) to account for the role of the covariates within the model. The marginal r-squared and conditional r-squared were reported for all models (function: r. squaredGLMM, package: MuMin).

Results

Number of Fruit Produced

Graphical representation by cultivar, with five trees within the scope of all sites, was generated for total fruit (Figure 1) and demonstrated the positive relationship between the size (DBH) and productivity of trees (Table 1). A few cultivars, most notably PA-Golden #1, but also Susquehanna, Potomac and to a lesser extent Shenandoah, did not appear to conform to this trend. Wabash had the lowest average of fruit per cluster at 1.32 and KSU-Atwood had the highest at 3.35. Allegheny and Quaker's Delight had median fruit from size class one (Table 1). Chappell, NC-1, Overleese, Susquehanna, and Wabash had median fruit from size class three and the remaining cultivar were in size class two; no cultivar had median fruit from size class four.

Allometric Relationships of Fruit Mass

The first allometric relationship to be investigated was the relationship between volume and length × width (LTW) to assume that LTW is a tolerable replacement for volume. The relationship was found to be significant and that LTW was a very strong predictor of volume for both cultivar and group. LTW was used to model both pulp mass and fruit mass. The allometric model of pulp mass indicated LTW was a very strong predictor of pulp mass. The total mass allometric relationship produced a significant result (Table 2).

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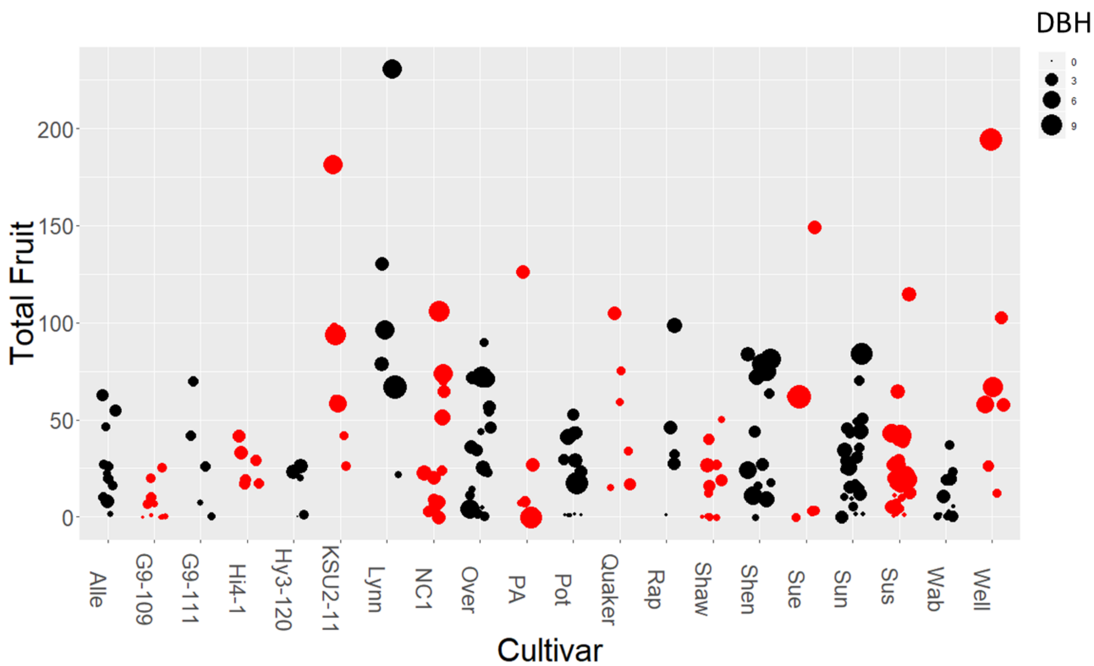


Figure 1: Total number fruit produced by individual trees across all sites for each cultivar. The size of the circles represents the size (DBH, cm) of the tree. The Cultivar abbreviation can be found in Appendix B.

Allometric Relationships of Fruit Mass

The first allometric relationship to be investigated was the relationship between volume and length × width (LTW) to assume that LTW is a tolerable replacement for volume. The relationship was found to be significant and that LTW was a very strong predictor of volume for both cultivar and group. LTW was used to model both pulp mass and fruit mass. The allometric model of pulp mass indicated LTW was a very strong predictor of pulp mass. The total mass allometric relationship produced a significant result (Table 2).

Total Number of Fruit Produced

With total number of fruit modeled by cultivar, Potomac and Wabash were significantly different from Shenandoah (Figure 2). For the genetic groups Sunflower was significantly different from Alice and Zimmerman within the post-hoc pairwise comparison (Figure 2). The r-squared values demonstrate that for both models conditional r-squared, which exemplified the whole model including site, explained nearly all of the variance present (ca. 0.9) whereas the marginal r-squared, explaining the fixed effects, only account for ca. 0.2 of the variance (Table 3).

Table 1: Cultivars investigated across the eight study sites. Cultivars had to have three or more trees to be reported.

Cultivar	***# trees***	***# sites***	***Average DBH (cm)***	***Average # fruit per tree***	***Average # fruit per cluster***	***Median size class***
Allegheny	10	4	1.83 ± 0.92	29.40 ± 19.06	1.91 ± 0.25	One
Chappell	6	2	2.70 ± 0.67	26.17 ± 10.25	2.24 ± 0.29	Three
G9-111	5	2	1.62 ± 0.68	36.25 ± 26.66	2.69 ± 0.76	Two
Hy3-120	5	2	2.12 ± 1.78	17.50 ± 11.27	2.33 ± 0.82	Two
Jenny's Gold	11	2	0.92 ± 0.74	11.50 ± 9.14	2.30 ± 0.75	Two
KSU 2-11	7	2	4.06 ± 3.32	80.00 ± 51.52	2.30 ± 0.63	Two
KSU Atwood	3	1	6.37 ± 3.12	41.33 ± 36.91	3.35 ± 1.16	Two
KSU Benson	4	1	0.70 ± 0.32	9.50 ± 2.12	1.09 ± 0.85	Two
Lynn's Favorite	6	3	5.78 ± 3.59	103.83 ± 71.25	2.06 ± 0.81	Two
NC-1	14	4	3.63 ± 2.16	35.62 ± 33.59	2.29 ± 0.60	Three
Overleese	17	4	2.98 ± 2.19	37.89 ± 26.76	2.01 ± 0.53	Three
PA-Golden #1	8	4	2.50 ± 3.10	42.00 ± 56.75	1.91 ± 0.85	Two
Potomac	8	2	2.72 ± 2.89	34.75 ± 12.12	1.82 ± 0.53	Two
Quaker's Delight	6	3	1.90 ± 1.11	50.83 ± 35.49	2.15 ± 0.72	One
Rappahannock	4	3	2.76 ± 1.75	51.00 ± 32.99	2.46 ± 073	Two
Shawnee Trail	12	3	1.77 ± 1.11	28.22 ± 13.45	2.06 ± 0.66	Two
Shenandoah	16	4	4.19 ± 2.74	41.57 ± 31.37	2.09 ± 0.48	Two
Sue	6	3	3.92 ± 3.46	55.80 ± 59.78	3.07 ± 0.72	Two
Sunflower	23	5	2.58 ± 1.98	31.00 ± 21.03	1.88 ± 0.63	Two
Susquehanna	25	5	3.35 ± 2.26	23.22 ± 23.12	1.98 ± 0.70	Three
Wabash	14	2	1.37 ± 1.10	14.88 ± 12.05	1.32 ± 074	Three
Wells	7	4	4.99 ± 3.25	72.50 ± 56.06	2.40 ± 1.04	Two
Wilson	4	2	5.60 ± 320	122.25 ± 27.04	2.46 ± 0.33	Two

Table 2: Summary of linear models defining allometric relationships between length × width (LTW) and fruit volume, total mass and pulp mass. Separate models were developed based on cultivars and genetic groups

Dependent variable	***Variable***	***DF***	***F***	***P***	R^2
Volume	Cultivar	22	246.94	<0.001	0.98
	LTW	1	15607.49	<0.001	
	Cultivar × LTW	22	6.23	<0.001	
Pulp Mass	Cultivar	22	20.88	<0.001	0.76
	LTW	1	805.02	<0.001	
	Cultivar ×LTW	22	2.84	<0.001	
Total Mass	Cultivar	22	80.31	<0.001	0.94
	LTW	1	4484.05	<0.001	
	Cultivar × LTW	22	6.49	<0.001	
Volume	Group	5	97.96	<0.001	0.97
	LTW	1	10202.98	<0.001	
	Group × LTW	4	7.77	<0.001	
Pulp Mass	Group	5	16.16	<0.001	0.72
	LTW	1	616.13	<0.001	
	Group × LTW	4	3.57	0.007	
Total Mass	Group	5	65.57	<0.001	0.94
	LTW	1	4288.29	<0.001	
	Group × LTW	4	8.19	<0.001	

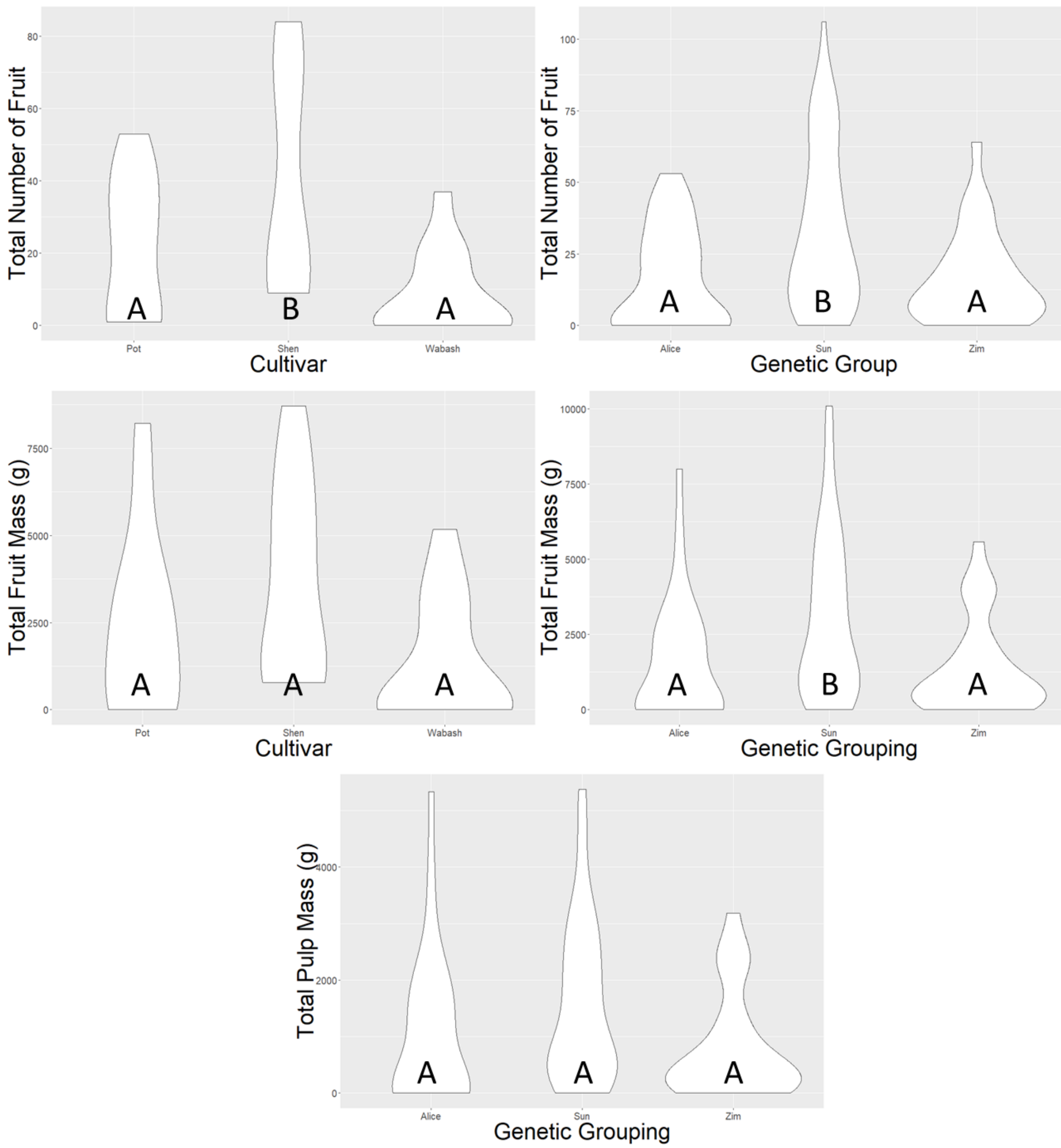


Figure 2: Variation in pawpaw yield as a fuction of cultivar. The letter above each of the cultivar/ group name indicates where significant differences in least squared means exist between cultivars or groups.

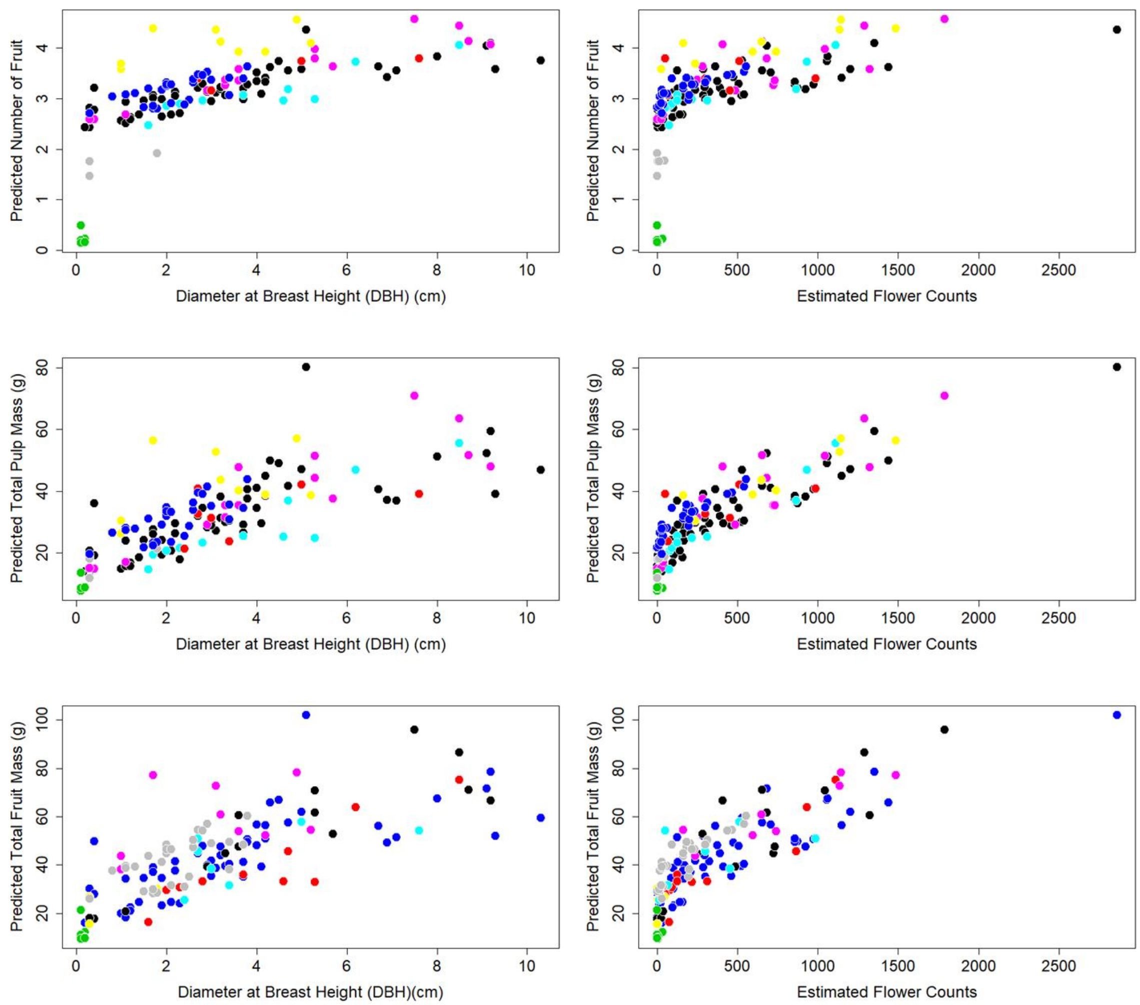


Figure 3: Outputs from the linear mixed effect models showing predicted total number of fruit, total fruit mass, and total pulp mass as a function of DBH and estimated flower count. Different colors represent site: Foxpaw (black), Butler (pink), Clinton (red), Dublin (green), Royalton (pink), Valley View (grey), and Urbana (yellow).

Table 3: Summary outputs of generalized linear mixed effect models for total number of fruit produced comparing results for cultivar versus genetic grouping.

Model	***Variable***	***D.F.***	***Chi Squared***	***P***	***R^2c***	***R^2m***
(Total Fruit~ DBH+ Cultivar+ Estimated Flower count+ (1| Site Code) Family= Poisson)	DBH	1	24.97	<0.001 *	0.35	0.98
	Cultivar	2	164.38	<0.001 *		
	Estimated Flower Counts	1	53.98	<0.001 *		
(Total Fruit~ DBH+ Group+ Estimated Flower count+ (1| Site Code) Family= Poisson)	DBH	1	127.30	<0.001 *	0.34	0.96
	Group	2	237.76	<0.001 *		
	Estimated Flower Counts	1	238.65	<0.001 *		

Total fruit mass and pulp mass models

Linear mixed effects models were used to examine total production of pulp and total mass production by cultivar and genetic group. The total mass models had more variance explained with the cultivar model (0.67) versus the genetic model (0.47) (Table 4). For the pulp production models, the cultivar model explained more variance (0.58) compared to the genetic model (0.45) and genetic group was not significant within the model whereas cultivar was significant (Table 5). But for the total mass models, cultivar was not significant within its model and genetic group was significant, inverse of the total pulp models. The cultivars Potomac and Wabash both fall into the genetic grouping of Alice and Shenandoah falls into the grouping Sunflower.

Table 4: Summary of linear mixed effects models of total fruit mass by cultivar and genetic group respectively. R-squared values return marginal r-squared (R^2m) which explains the variance that originates from the fixed effects and conditional r-squared (R^2c) which includes the fixed and random effect.

Model	***Variable***	***D.F.***	***Chia squared***	***P***	***R^2c***	***R^2m***
Square root (Total Mass) ~DBH+ Cultivar+ Estimated Flower counts+(1|Site Code)	DBH	1	4.37	0.03	0.44	0.66
	Cultivar	2	5.02	0.08		
	Estimated Flower Counts	1	9.72	0.002		
Square root (Total Mass) ~DBH+ Group+ Estimated Flower counts+(1|Site Code)	DBH	1	8.10	0.004	0.42	0.48
	Group	2	16.77	<0.001*		
	Estimated Flower Counts	1	28.21	<0.001*		

Table 5: Summary of linear mixed effects models of total pulp by cultivar and genetic group respectively. R-squared values return marginal r-squared (R^2m) which explains the variance that originates from the fixed effects and conditional r-squared (R^2c) which includes the fixed and random effect.

Model	***Variable***	***D.F.***	***Chia squared***	***P***	***R^2c***	***R^2m***
Square root (Total Pulp) ~DBH+ Cultivar+ Estimated Flower counts+(1|Site Code)	DBH	1	4.94	0.03	0.38	0.74
	Cultivar	2	1.00	0.61		
	Estimated Flower Counts	1	11.36	<1.00*		
Square root (Total Pulp) ~DBH+ Group+ Estimated Flower counts+(1|Site Code)	DBH	1	8.00	0.005	0.41	0.47
	Group	2	10.21	0.006		
	Estimated Flower Counts	1	29.29	<0.001		

Discussion

The trend from Figure 1, suggests as the diameter at breast height (DBH) increases the fruit production goes up. There are a few exceptions to this trend, most notably PA-Golden #1, which are large trees that produced relatively few fruit. The two trees in question, both on Foxpaw Farm, were > 20 years old which is about the life span of an orchard pawpaw tree. The other cultivars that do not follow the trend, Susquehanna, Potomac, and Shenandoah are commonly known to produce larger fruit but less of those larger fruit (Figure 2).

Figure 1 does not, however, account for differences in the size of the fruit produced. For example, Lynn's Favorite produced the most fruit, but the sizes of the fruit were mostly size class 1 and 2. This demonstrates that cultivar (genetics) controls the size of the fruit, age which is directly related to DBH, controls the number of fruit produced.

The trend of larger size of tree increasing production of number of fruit is also present in Table 1, furthermore it exposes that the average number of fruit per cluster does not follow any discernable trend. Regarding the numbers of fruit per cluster, one to two fruit per cluster is the most desirable for fresh market production. The number of fruit per cluster could be controlled by genetics or by pollinators. The decrease in the number of fruits lets the one to two fruit gather all resources for the cluster. The number of fruit per cluster can be as high of 12 (Pomper & Layne, 2005). Wabash had the lowest ratio (1.32) making it a good cultivar for fresh market production, whereas KSU-Atwood and Sue had ratios of over three. The KSU-Atwood trees surveyed were all older (indicated by their high average DBH) this could have been a factor in these trees bearing more fruit per cluster. In the Pawpaw Variety Regional Trails (PVRT), Wabash had a relatively high fruit per cluster ratio (2.5) (Pomper et al., 2008 b) which was greater than our finding. Half of the Wabash trees within the study are on the site Valley View, where the grower hand-thins his fruit (removes immature fruit in July to one to two fruit per cluster) which could have affected the average fruits per cluster drastically.

Most of the cultivars examined have median fruit in size class two; however, Quaker's Delight and Allegheny had median fruit in size class one. Some have claimed that fruit under 85 g (3 oz.), which is all of size class one is unmarketable (O'Malley, 2008), but the grower of Valley View has specifically sold Quaker's Delight and Shawnee Trail for their smaller fruit size. Chappell, NC-1, Overleese, Susquehanna, and Wabash had median fruit from size class three; these cultivars are often cited by growers, especially Susquehanna, as being producers of larger fruit. These cultivars would be good trees from a yield standpoint to grow if large fruit is desired.

The allometric relationships allow translation of total fruit production into more understandable, and market-relevant, total mass (fresh fruit production) and pulp mass (pulp production). Cultivar is a significant control on fruit size. The genetic groups from Pomper et al. (2010) were also used in the allometric relationships to gain a more general model which can be applied to a greater range of cultivars. Genetic groups demonstrate traits (genetics) that may be related to the groups rather than specific cultivars giving more information of how genetic traits differ across the population of cultivars. There was little difference in the allometric relationships based on cultivar and genetic groups. The adjusted r-square for the pulp models were not as strong as the total mass models (c.a. 0.7 and 0.9 respectively) (Table 2). The total mass models encapsulate some additional genetic differences, skin thickness and seed weight, which affect pulp weight along with human error when pulping the fruit. The allometric relationship for pulp production may need to be a more complex model to account for the internal difference of the pawpaw fruit.

An allometric relationship for predicting total production of pulp mass and total mass has not been attempted for pawpaw fruits as other studies have relied on total weight of all fruit (Crabtree, 2004, Pomper et al., 2008b) or grower picked fruit for total yield (Greenwalt et al., 2016). Cantaluppi (2016) used total fruit counts to measure production, fruit mass per tree, and mass per area but this data may have been influenced by precocity of the pawpaw trees; the first year of production is likely different from years of full production. Within that study, for the four Peterson cultivars examined there was only a significant difference between one of the cultivars (Potomac) for the total number of fruit in the first year of production (2013) and in 2015 no significant differences were found for total number of fruit. The advantage of the allometric models for both total fruit mass and pulp mass allow for simple measurement (fruit counts by size class) which provides early, rapid, and non-destructive assessment of orchard level production.

Due to the commercial and semi-commercial orchards we studied being non-experimental there was a low degree of replication. Only three cultivars (Shenandoah, Potomac, and Wabash) were replicated at three or more sites with three or more trees per site. The genetic groups allowed for eleven cultivars to be represented but there were still only three groups represented. These replication problems precluded broad comparison across the full diversity of cultivars available but did allow investigation into the cultivars present for differences in fruit counts, pulp mass and total fruit mass.

The generalized linear mixed models for total fruit production Potomac and Wabash (both within group III: Alice) were significantly different form Shenandoah demonstrating why genetic groups are important (Figure 2). Groups Alice and Zimmerman were significantly different from Sunflower in the pairwise comparison (Figure 2). Groups Alice and Zimmerman are more closely related within the genetic chart (Pomper et al., 2010) which suggests an even courser grouping of cultivars may adequately account for the number of fruit produced. A larger sample size and multiple years need to be observed to confirm this pattern. For both models DBH and estimated flower counts were significant; logically, the size of tree and flowering effort play a key role in how many fruits are produced (Crabtree 2004, Pomper et al., 2008a). Site explained over half of the variance in both models which leads to the conclusion that agronomic inputs or site condition play an important role in total number of fruit produced. Previous work by Pomper et al. (2008b) and Crabtree (2004) reported total number of fruits and the total mass produced by cultivars and found clones of a cultivar performed similarly in different locations and large trees produced more flowers and in turn more fruit. This study has added to these previous studies by explicitly considering the fruit pulp markets. This market makes up a notable portion of the total pawpaw market, so much so that some growers solely grow pawpaws for pulp.

Total mass of the pawpaw fruit was modeled by cultivar and genetic group. The cultivar within its model was not significant but the genetic group within its model was found to be significant. The cultivar may have been not significant due to two of the cultivars being within the same genetic group. The least-square means pairwise comparison takes the significance of the other co-variable (DBH and estimated flower counts) into account for the significance levels. This leads to the idea that genetic groups would be a better predictor of total mass and thus marketed fresh fruit. The marginal r-squared values for the genetic model are marginally lower (0.40) than the r-squared for the cultivar model (0.44) but the conditional r-squared for the genetic model (0.47) is larger and marginally lower than for the cultivar model (0.67) (Table 4). This trend was to a lesser degree in the other models (total number of fruit and pulp) but for the total mass the trend was more pronounced suggesting that cultivars are affected by site conditions which is not captured in the larger data of genetic group. A larger dataset containing increased number of cultivars should be used in future research. PVRT attempted to create a distributed cultivar network of plantings with limited success (Pomper et al., 2008b). Greenwalt et al. (2016) found significant difference in yield across three locations; these three sites were also within this paper but here were only broken into two sites. Greenwalt also used Pomper et al. (2010) genetic groups to compare yields and found significant differences between group II: Zimmerman and V: Sunflower which was not echoed within this study. This study was only over one year in comparison to seven years but across eight sites. Greenwalt's method of total mass from the tree was not a predicted figure but the total weight of pawpaws harvested per tree by the grower. The allometric relationship examines repeatable measurement whereas Greenwalt's methods were on a unique dataset; for future work an allometric relationship should be used to assist in yield calculations.

Lastly, the pulp production models demonstrate that cultivar should be used when determining which trees to plant for pulp production. With seed to pulp ratio and skin thickness varying among cultivars (Peterson, 2003); these internal factors are captured by cultivar better than genetic group (Pomper et al., 2008a). The variance explained by site in the pulp by cultivar model was 0.58 which was greater that the genetic model 0.45. These models demonstrate that yield for pulp is related to estimated flower counts and size of tree (DBH) regardless if the sample was broken down into cultivars or genetic groups. The pulp yield was not significantly different by cultivar or genetic group. Investigating a wider range of cultivars would encompass more of the genetic diversity within pawpaws and would confirm if this trend remains true throughout other genetic material.

Conclusions

To estimate pawpaw's potential yield for the pulp and fresh markets, an allometric relationship was developed between fruit mass, and easy, non-destructive field measurements (length × width) combined with cultivar or genetic group identity yielding repeatable results. The cultivar Wells had relatively low production of both total mass and pulp mass. Site explained most variance in both models of the total number of fruit produced. Pulp mass production models demonstrated cultivar should be used for measurement and that site explains a generous amount of variance in the models. Modeling total mass production suggests that genetic group should be used for estimating total mass of fruit harvest. But the site variance, explained by the marginal r-squared, explained over a third of the variance in the cultivar model, a trend not in the genetic grouping model. Estimating the total output for pawpaw trees for both fresh (total mass) and pulp (pulp mass) market will help grow this emergent industry.

Chapter 2: Site, cultivar, and ripeness controls pawpaw (Asimina triloba L. Dunal) on fruit quality

Introduction

Fruit quality is a complicated issue that mixes consumer perceptions, government regulations, and science-based measures (Porter et al., 2018). In general, decisions made by consumers are based on heuristics - when facts or information are traded-off in favor of making a faster decision instead of a time consuming, more informed decision (Gigerenzer & Garssmaier, 2011). When purchasing novel fruits consumers use uniformity of product and limited heuristics-based approaches to determine if they will buy (Shulte-Mecklenbeck et al., 2013).

Depending on the fruit and season, it may have to travel large distances (potential for damage or rot) and interact with automated packing machinery. Produce needs to be uniform in size, hardness, and appearance to meet the transport and packing requirements of grocery store markets which reaches most consumers. The intended market of the fruit determines which quality characteristic of the fruit are emphasized. Acceptable fruit quality is defined both by consumers and by governments (Porter et al., 2018, Kyriacou & Rouphael, 2018). For example, quality standards for fruit in the European Union (Commission Implementing Regulation, 2011) emphasize visual aspects, ensuring the product is homogenous and has ease of transport. Only two chemical properties are regularly measured for quality standards: soluble solids content (Brix) and titratable acidity (Kyriacou & Rouphael, 2018). Some consumer markets (e.g. high-end grocery stores, high dollar restaurants) demand higher quality goods that are more rigorously tested for quality as well as uniformity (Kyriacou & Rouphael, 2018). These uniformity standards can cause problems, mostly from food waste due to less visually appealing fruit being discarded (Porter et al., 2018). Fruit quality is a nebulous, multivariate concept, with various characteristics. Individual characteristics may have greater or lesser importance depending on the end product or target market. For example, if the end goal was the juice market, the fruit flavor and sugar levels would be important, whereas a fresh fruit market the appearance of the exterior of the fruit would be paramount to sell it (Caswell, 2009, Powell, 2018). Chemical properties are controlled by crop genetics, socioeconomic factors affecting consumers, market value, and post-harvest factors in the determination of fruit quality (Kyriacou & Rouphael, 2018).

Fruits with well-established markets have been studied for many years and often have clearly defined quality characteristics. For example, easy-peel mandarins, an established fruit with a market that is continuing to grow, rely on quality measurements based on appearance factors (color, size, shape) and nutritional factors (sugar/acid balance, vitamins, and phenolics) (Goldenberg et al., 2017). In blood oranges, quality has been defined according to vitamin C, polyphenol, flavonoid, and acid concentration (Pannitteri et al., 2017). In a study by Kleina et al. (2018) where the quality of plums was related to leaf scald disease (*Xylella fastidiosa*), skin color, pH, and total soluble solids were used as quality indicators. In general, the sugar/acid balance is a principle component of quality, as it determines how flavor is delivered in a fruit. The concentrations of sugars (sucrose, fructose, etc.) are expressed by total soluble solids (TSS, Brix) whereas fruit acids tend to be evaluated via titratable acid (TA) and pH (Erikson 1968, Goldenberg et al., 2017). Appearance quality characteristics are uniformity in color, size, and shape (Kyriacou & Rouphael, 2018) whereas, chemical characteristics are on a gradient with too much or too little being undesirable. This is one of the complexities of quality, where uniformity is critical for appearance, but gradients of internal and chemical characteristic have to be independently characterized. Appearance can also have gradients if the market calls for difference in color, size or shape, further complicating quality. For emerging fruit crops, the scope of individual characteristics needs to be investigated to find the range of natural variability and determine acceptable values.

Pawpaw (*Asimina triloba* L. Dunal) is a fruit not currently available in most chain grocers but, it has the potential to become a widely marketable fruit. The pawpaw is native to the east coast of the United States (USDA Zones 5A-9) where it naturally grows as an understory tree in woodlands and lowlands. The pawpaw fruit can grow to one kilogram in weight, with a skin that is green to yellow, a fleshy inside of white to yellow to orange and brown, 15-25 mm seeds (Pomper et al., 2008). The pawpaw fruit grows in clusters of one to nine fruit produced from a single pollinated flower (Pomper & Layne, 2005). Only the flesh of the pawpaw is consumed, and it has a tropical taste with notes of melon, banana, and pineapple. When overripe it can have a caramel-like flavor but may also develop off notes (Duffrin & Pomper, 2006, Pomper et al., 2008b, Powell, 2019).

Wild pawpaw trees that grow naturally tend to have low yields and small fruit. When grown in orchard settings, the pawpaw fruit become more consistent in size and weight (Crabtree et al., 2004). Grafted cultivars usually have superior and more consistent flavor, as compared to wild fruit (Duffrin & Pomper, 2006), but pawpaws are not usually marketed under their cultivar name. In addition to the known cultivars, wild fruit is sold which is more likely to have the bitter or off-notes in the taste (Peterson, 2003).

Pawpaws are being planted around the world due their tropical tasting fruit and hardiness. The Republic of Korea has trees that are producing fruit, and researchers there have reported the nutritional composition of the fruit, twigs, leaves and seeds of pawpaw grown in their country (Nam et al., 2017). The University of Florence, Italy, began researching the pawpaw tree in 1990, establishing a breeding program, and creating the cultivars Prima 1216 and Prolific (Bellini et al., 2003). Within the United States of America, pawpaws have been researched in Kentucky, Louisiana, Maryland, Michigan, Nebraska, New York, North Carolina, South Carolina, Oregon, and Ohio through the Pawpaw Regional Variety Trails (PRVT). This focused on total production of the fruit over a bio-gradient (Pomper et al., 2003b). Differences were found in the PRVT but only the two sites in Kentucky (Frankfort and Princeton) were reported out of the twelve sites. Within Ohio, there has been investigation with consumer and trained panels to develop a sensory wheel for pawpaw fruit (Brannan et al., 2012, Duffrin et al. 2001, Duffrin & Pomper, 2006). Sensory analysis attempts to define aromas, flavors, textures, and appearance within a sample of the fruit (Brannan et al., 2012). Ohio has growing production with a handful of productive commercial or semi-commercial orchards and more being planted every year (Powell, 2018).

Pawpaw fruit at farmers markets in the eastern United States have been observed to retail for $2-4 per kg ($4-10 per lbs.) (Powell, 2018). Producers also sell their fruit to local breweries, and a small number of processors, in volume and at much lower prices (approximately $0.23-0.68 per Kg.). Local markets dominate pawpaw sales for many reasons including the complications that they are labor-intensive to harvest, ripeness is difficult to determine, and fruit do not ripen at once (Powell, 2018). Pawpaw lack of color break when ripe thus visual inspection is not sufficient to judge ripeness as this requires the fruit to be individually handled to test softness of the fruit. Pawpaws ripen rapidly once picked, and the fruit has a short shelf life of ca. five days at room temperature, though this can be extended to 28 days with refrigeration (Archbold & Pomper, 2003, Kobayashi et al., 2008, McGrath & Karahadian, 1994). Ripe fruit are very tender and can be bruised easily, which makes transporting pawpaw even more challenging. Ripeness has been linked to firmness/hardness of the fruit, but that research was conducted on fruit disregarding cultivar differences (Archbold & Pomper, 2003). Given that firmness/ hardness of the fruit is a critical quality control on market accessibility, and linked to the ripeness of the fruit, it is vital that we better understand cultivar and ripeness differences to allow producers to optimize choices when planning and harvesting from orchards.

Another constant battle, especially for fresh fruit markets, is the disease *Phyllosticta asiminae* Ellis & Kellerm (hereafter *Phyllosticta*), that grows on the skin of the fruit and leaves of the pawpaw tree (Farr et al. 1989). The black dots of *Phyllosticta* are thought to only become a major problem when it causes the fruit to crack, but blemishes may make the fruit less attractive to commercial buyers. There are currently no disease management practices, due to lack of licensed chemicals approved to spray on pawpaws, in pawpaw orchard settings, making it critical to characterize fruit and tree differences in susceptibility among cultivars.

Given the complex range of variables influencing perception of quality it is not surprising that recommendations on which cultivars have superior quality has little consensus among growers. A general paucity of empirical evidence makes the situation even more challenging. Some quality characteristics of pawpaw fruit have been investigated in a small number of previous studies (e.g. Duffrin et al., 2001, Kobayashi et al., 2008), but only a few select cultivars have been investigated. Kobayashi et al. (2008) studied how ripeness affects the hardness (penetration force), soluble solid content (Brix), phenolic content, and antioxidant capacity of PA-Golden (#1) and 1-23, an advance selection from Kentucky State University (KSU). McGrath & Karahadian (1994) used advanced selections from the University of Maryland to investigate which quality measures (headspace volatiles, soluble solids content, hardness, skin color, and sensory attributes) were indicators of ripeness. Significant differences have been detected between cultivars in a number of quality characteristics. For instance, Kobayashi et al. (2008) found PA-Golden (#1) to be harder, have a lower Brix value, and higher phenolic content than the advance selection 1-23. With the range of cultivars present within pawpaw, quality factors across multiple cultivars have yet to be examined. Scientific recommendations have yet to be made for pawpaw either as a baseline, consistency that governing bodies would recommend, or superior quality characteristics that high-end markets would demand.

Measures of quality can be linked to biotic (genetics), abiotic (growing conditions, cultural practices- i.e. site), and ripeness factors. How these quality measures are controlled will push the understanding of how to produce pawpaw fruit. Objective one of this research was to characterize variation in multivariate fruit quality between and within cultivars, sites, and ripeness scores. Objective two was to quantify the relative importance of cultivar, site, and ripeness score in determining overall, multivariate fruit quality metrics. Finally, objective three was to model variation in individual fruit quality metrics as a function of cultivar and ripeness.

Method

Site Selection

Eight commercial and semi-commercial pawpaw orchards in Ohio were identified in collaboration with the Ohio Pawpaw Grower's Association (Appendix A). Each orchard was at a different location (climatic conditions) and was managed uniquely (cultural practices). Each orchard had known cultivars that could be identified and were old enough to produce fruit. The eight sites spanned from the southern border of Ohio to suburbs of Cleveland. Orchards were monitored beginning in April 2018 to October 2018. In each orchard, the numbers of trees were counted, and cultivars present were recorded along with diameter at breast height (DBH) for each tree (Appendix B).

Counting Pawpaw Fruit

Fruit counts were preformed starting in August before the fruit ripened. Pawpaw fruit ripen in a short window (2-3 weeks) and in that time wildlife likes to share in the harvest, counting before the fruit are ripe mitigates some of this error. Dropped fruits were not included in the cluster count, due to the fact that origin of the drop could not be determined but were accounted for in the total fruit assessment.

Harvesting and Disease Assessment of Pawpaw Trees and Fruit

During this count, trees were assessed for the presence of disease and pest damage. There are several leaf spot diseases of pawpaw, *Mycocentrospora aiminae* (Ellis et Kellerm.), *Rhopaloconidium asiminae*, (Ellis et Morg.) and *Phyllosticta asiminae* (Ellis et Morg.) (Farr et al., 1989). Only Phyollosticata has been described in depth and appears on the leaves and fruit. For each tree, the presence or absence of each type of damage (dead branches specifically any branches that were defoliated at the time of fruit counts, split trunks due to freeze and thaw cycles, *Phyllosticta* on leaves was counted as black spots on any leaves, Magnesium deficiency detected by yellow of the leaves from the edge in to the center, Japanese beetle damage categorized as leaves that were ‘lacy’ in appearance, and caterpillar damage was from swallowtail caterpillars and was fully or partially eaten leaves) was recorded.

When fruit started to ripen, sites were revisited to collect fruits from as many cultivars and trees as possible. Under the grower's direction, fruits were chosen from the trees selected. Some growers choose fruits both from the tree and from drops; I aimed to collect at least three fruits of each size class for each cultivar at each site. Each fruit was labeled with a unique identification code. Fruit weight, length, and width measurements were taken in the laboratory setting.

Fruit Phyllosticta Analysis

Two pictures were taken of opposite sides of the outsides of the fruit. Adobe Photoshop Version CS5 was used to analyze all pictures. The fruit was isolated from the image background and the number of pixels in the selected area was recorded. Then the contrast and brightness was increased to 100% to account for the differences in lighting when the pictures were taken. The *Phyllosticta* (black) was isolated based on a representative color set made from multiple pictures and loaded onto the picture using the color range function. Shadows and bruising were excluded. The pixel count of the areas defined as *Phyllosticta* was recorded to find the percent of disease on the fruit skin.

Characterization of Variation in Fruit Quality

Fruit quality first was assessed using the qualitative assessment devised by Peterson (1990). This evaluation ranges from "Good", to "Average", to "Bad" for fruitfulness, flavor, fleshiness, size of fruit, seed size and appearance characteristics; these fruits were characterized by one of two analysts (Appendix D). Fruit ripeness was assessed using the OPGA Ripening Chart (Appendix E); with "1" being the least ripe to "5" being most ripe (Powell, 2018).

Fruit were pulped by removing the seeds and skin, and the pulp was homogenized via hand mixing. The total weight of the pulp was recorded and sub-samples (~10 g) were separated and frozen. Samples were weighed and placed in an 80C oven for 24 hours to determine the moisture content.

Selected cultivars, those growing at more than three sites, were subject to additional quality analyses. Prior to dissection and pulping, the strength of the skin was tested with a force gauge, Accu-Force II (Ametek, Mansfield and Green Div., Berwyn, PA). Three readings were taken at randomly selected spots on the skin. The color of the skin was recorded at three random locations, avoiding damaged/bruised areas and *Phyllosticta* with a Minolta CR-300 chromameter (Konica Minolta, Osaka, Japan) utilizing CIE values. The Hunter Lab method (Setser, 1984) was used to record the reading. The Hunter Lab method reads color in three dimensions where: *L* is the light to dark ratio, *a* is the red to green scale, and *b* is the yellow to blue scale. The fruits were then cut open lengthwise and the pulp color was recorded immediately using the Minolta CR-300. Three readings were taken while avoiding the seeds and any areas with obvious discoloration (e.g. due to bruising). The hardness of the pulp was then tested in the cortex of the cut fruit with using three readings with the force gauge. To assess fruit browning rate a ca. 20 gram sample of the pulp was placed in a sample cup. The color of the pulp was estimated as the mean of three chromameter readings. Readings were repeated twenty-four hours later. The zero (L_1) and twenty-four hour (L_2) readings for each sample used the Delta E formula which expresses the total difference in the two colors (Identifying, 2019). ($\Delta E = \sqrt{(L_2 - L_1)^2 + (a_2 - a_1)^2 + (b_2 - b_1)^2}$)

Following pulping seeds were extracted, weighed, and a subsample saved and frozen. Seed subsamples were weighed and were placed in an 80C oven for 24 hours to determine the moisture content.

A subsample of pulp was used to test total soluble solids (Brix) with a refractometer (PAL-1, ATAGO, Japan) and pH. The sub-samples were thawed, a pin-hole made in the bag, and a drop of pawpaw liquid squeezed onto the refractometer; this process was replicated twice. The pH probe was placed in the thawed sub-sample and two separate readings were recorded.

A further subsample was used to test total phenolics using a Folin-Ciocalteu assay (Singleton & Rossi, 1965, Singleton et al., 1999). Samples were freeze-dried, ground, and were tested for phenolics using a 0.5 gram sample of the freeze-dried tissue. The tissue was extracted with 20 ml acidified methanol (MeOH) for half hour in a falcon tube then centrifuged for fifteen minutes at 7800 g_n. The supernatant was decanted through grade 1Whatman filter paper leaving the substrate intact. A second extraction with 30 ml acidified methanol (MeOH) was carried out with half hour extraction, fifteen minutes centrifuge, and filtered. The resulting extracted samples were brought to 50 ml in volumetric flasks. Samples were frozen until analysis one ml of Folin-Ciocalteu reagent with one ml of extracted sample in 23 mL of deionized water was reacted for eight minutes. Folin-Ciocalteu reagent reacts with the phenolics compounds changing from yellow to blue; the intensity of the blue indicates the concentration of phenolic compounds. The reaction was stopped using ten ml of 7% $NaCO_3$ solution and 20 mL of deionized water. After an incubation of two hours, the samples were measured in a spectrophotometer (Beckman Coulter DU 730). Concentrations were estimated based on a Gallic acid standard curve (0,100,200,300,400, and 500) using the wavelength 700mm. Samples were expressed as milligrams of gallic acid equivalents per gram of sample and two laboratory replication were performed for each sample.

Statistical Analysis

All data was analyzed in R studio 3.3 (RStudio Team 2016). Color data was converted from the Hunter Lab system into CIE L×C×h color space. The value L remains the same representing light to dark. Chroma (C) is the intensity of the color, bright to duller. Hue angle (h) represents differences in spectrum. The data was transformed in CIE L×C×h because these values mimic how the human eye interprets color. The Lab coordination for color is a rectangular system and the L×C×h system is cylindrical. When transformed this makes the Chroma and hue angle hard to fit into linear models subsequently only the *L* values were used in the statistical analysis.

All quality metrics were checked for normality with histogram and QQ plots. Where metrics had obviously non-normal distributions, they were transformed using either the cube root, square, cube, or log functions. Length to width ratio, weight of pulp, skin hardness, flesh hardness, DeltaE, phenolics, and volume were transformed by the log function as they were strongly left skewed. The seed to pulp ratio data was transformed using a square root transformation since the data was left skewed. *Phyllosticta* abundance showed substantial zero inflation and both it and the flesh *L* score were cube root transformed. Since the pH data was highly skewed to the right, a cube transformation was used. Brix, fruit moisture, and skin *L* were not transformed.

To assess the differences between cultivars for the individual quality metrics, a liner mixed model (function: lmer, package: Testlmer, Kuznetsova et al., 2017) was used. Cultivar and ripeness score were specified as fixed effects and site was defined as a random effect. The marginal r-squared value, relating to the variance associated with the fixed effects of each model, and the conditional r-squared value, which explains the variance related to the whole model, were calculated for each quality metric (function: r.squaredGLMM, package: MuMin, Kamil, 2018). Post-hoc pairwise comparisons of differences between cultivars were performed via least square means which reports the effect of the variable in question whilst accounting for differences in other variables in model (function: lsmean, package: emmeans, Russell, 2019).

Principal Components Analysis (PCA) was used to evaluate multivariate patterns in fruit quality among cultivars and sites. The quality metrics were standardized (function: scale) and analysis completed using the rda function, package: vegan; (Oksanen et al., 2019). To determine the appropriate number of principal components to further analyze, a screeplot was generated. Bi plots of PC1 and PC2 were generated for cultivar, site, and ripeness with the function ordiellipse representing the standard deviation of PC scores for all the points within cultivar, site or ripeness categories (function: ordiellipse, package: vegan).

To explore the relative importance of site, cultivar and ripeness on overall variation in fruit quality variance partitioning was used. First, the variance explained for each variable (cultivar, site, and ripeness) was determined for cultivar and site interaction, (cultivar, site) and for all three variables (cultivar, site, ripeness) (function: varpart, package: vegan). Partial Redundancy Analyses were performed for each variable, cultivar, site and ripeness, to determine the independent effect of the predictor variable (function: rda, package: vegan).

A disease susceptibility index was calculated for each tree by summing the presences of dead branches, split trunks, *Phyllosticta* on leaves, Magnesium deficiency, beetle damage, and caterpillar damage. A classification tree was used to model disease index scores as a function of site and cultivar and was pruned (function: rpart, package: rpart, (Therneau and Atkinson, 2018)).

Results

Qualitative Assessment of Fruit Quality

The proportion of Peterson's (1990) scores of "Bad", "Average", and "Good" are reported for each criteria and cultivar in Appendix E but demonstrate few clear patterns. Shawnee Trail had lower portion in the fleshiness scores and Allegheny, NC-1, Overleese, and Susquehanna had a proportion over 0.5 in the "Good" category for flavor. The standard deviation for the flavor evaluations were displayed on the graph of Principal Component one and two (Figure 3). Fruit rated as "Bad" was associated with harder fruit, high seed to pulp ratio, and to a lesser extent *Phyllosticta* abundance. The “Average” and “Good” scores were centrally located on the graph with fruit classified as having a “Good” flavor with the smallest standard deviation (0.46). These fruit were associated with higher Brix values and lower pH values.

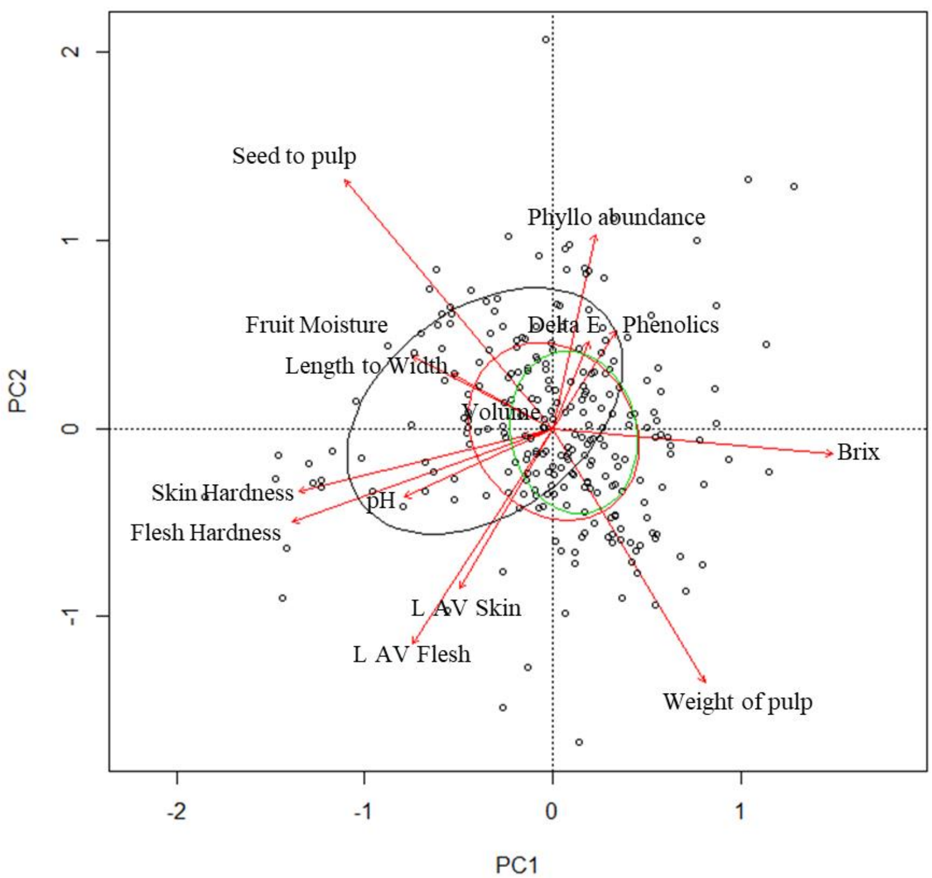


Figure 4: Principal Components Analysis of multivariate patterns in fruit quality metric displayed as a function of flavor: “Bad” (black) 1.40, “Average” (red) 0.66, and “Good” (green) 0.46.

Multivariate Assessment of Overall Fruit Quality

Four principal components accounted for 53% of the variance in the fruit quality data (Table 7). Principal component one (PC1) was strongly associated with Brix and pulp weight and negatively correlated with seed to pulp ratio and skin and flesh hardness. Principal component two (PC2) explained a relationship between a lower proportion of seeds in the fruit, greater *Phyllosticta* abundance, lighter flesh and skin color, and heavier pulp weight. Principle component three (PC3) loaded as the relationship describing greater *Phyllosticta* abundance on the skin with darker color skin colors and reduced fruit pH, hardness, length to width ratio and moisture. For principle component four (PC4) the gradient between fruit moisture and Brix, more moisture lead to less concentrated Brix. Phenolics and volume were not strongly associated with the first four gradients in the principle components analysis but associated strongly with component five and six (not reported).

Table 6: Principal Components Analysis (PCA) Loading for the four principal components from the Principal Components analysis (PCA) on the fruit quality metrics. Bolded for strong correlation.

Variable	*C1*	*C2*	*C3*	*C4*
Length to Width ratio	0.51	.29	0.68	.94
Fruit Moisture	0.75	.39	0.93	**1.33**
Weight of Pulp	.80	**1.35**	.08	0.40
Seed to Pulp ratio	**1.10**	**.32**	0.36	.49
Fruit *Phyllosticta* Percent	.22	**.03**	.82	0.36
Skin Hardness	**1.35**	0.33	**.26**	.14
Flesh Hardness	**1.38**	0.50	**.09**	.12
Brix	**.49**	0.13	.29	**.00**
pH	0.78	.36	**0.85**	.75
L Average for Flesh	0.74	**1.14**	.09	.47
L Average for Skin	0.50	0.85	**1.10**	0.07
Delta E	.19	.46	0.44	.77
Phenolics	.34	.52	.24	.11
Volume	0.16	0.04	0.27	0.28
Cumulative proportion	.17	.31	.43	.53

In terms of overall, multivariate fruit quality, cultivars Overleese, NC-1, Wells and Sunflower were all centrally located with standard deviations between 0.34-.59 (Figure 4). Allegheny was also centrally located but more associated with higher seed to pulp ratio and had the lowest standard deviation of all cultivars investigated i.e. more consistent in the fruit quality metrics. Wabash was associated with higher Brix as was Susquehanna which was also associated with higher weight of pulp. Potomac was associated with higher weight of pulp and lighter skin and flesh. Shawnee trail was the only cultivar associated with higher *Phyllosticta* abundance, Delta E, and Phenolics. Lastly Shenandoah was associated with higher fruit moisture, length to width ratio, and hardness of fruit. Susquehanna, Potomac, and Shawnee Trail all had large standard deviation (1.02, 0.88, and 0.94 respectively) indicating less consistent quality across all sites.

The visualization of quality by site (Figure 5) showed site Clinton to be strongly associated with skin and flesh hardness and higher fruit moisture and it only overlapped with one other site (Hamilton). Valley View farm had the lowest standard deviation (0.40) and was associated with higher Brix and weights of pulps. Foxpaw Farm and Royalton were centrally located with Foxpaw being more associated with the Phenolics and Delta E and Royalton with heavier weights of pulp. Butler was also centrally located but was linked to lighter skin and flesh values, harder fruit and higher pH. Lastly, Hamilton was associated with higher seed to pulp ratio, fruit moisture and Length to width ratio.

The ripeness scores displayed a clear gradient from scores one to scores five (Figure 6). Score one was affiliated with higher pH, and harder fruit. Fruit with scores of five were associated with heavier pulp mass, higher Brix, phenolics, Delta E, and *Phyllosticta* abundance.

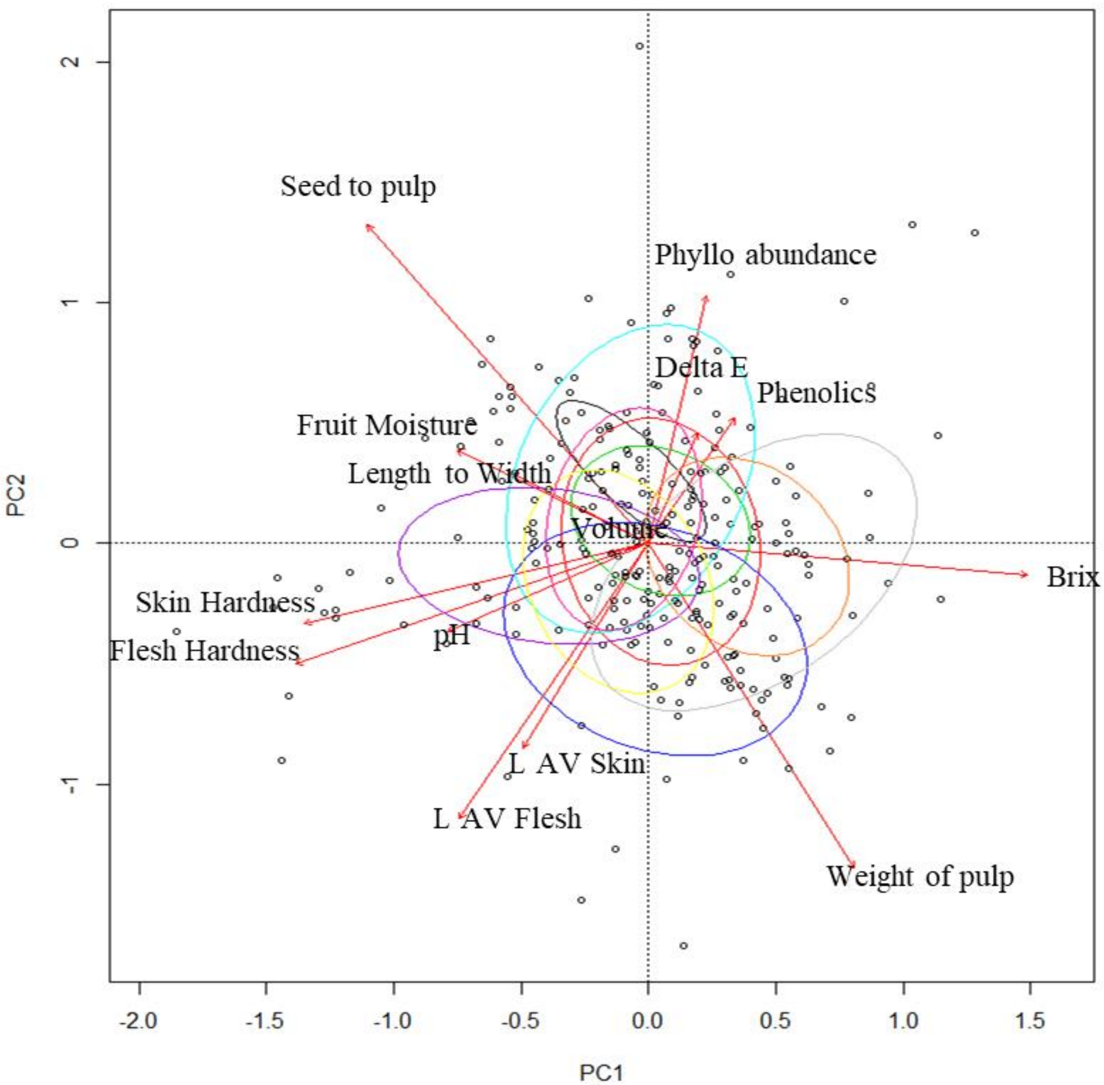


Figure 5: Principal Components Analysis of multivariate patterns in fruit quality metric displayed as a function of cultivar: Allegheny (black) 0.17, NC-1(red) 0.63, Overleese (green) 0.34, Potomac (blue) 0.88, Shawnee Trail (light blue) 0.94, Shenandoah (purple) 0.59, Sunflower (yellow) 0.50, Susquehanna (gray) 1.02, Wabash (orange) 0.51, Wells (pink) 0.43.

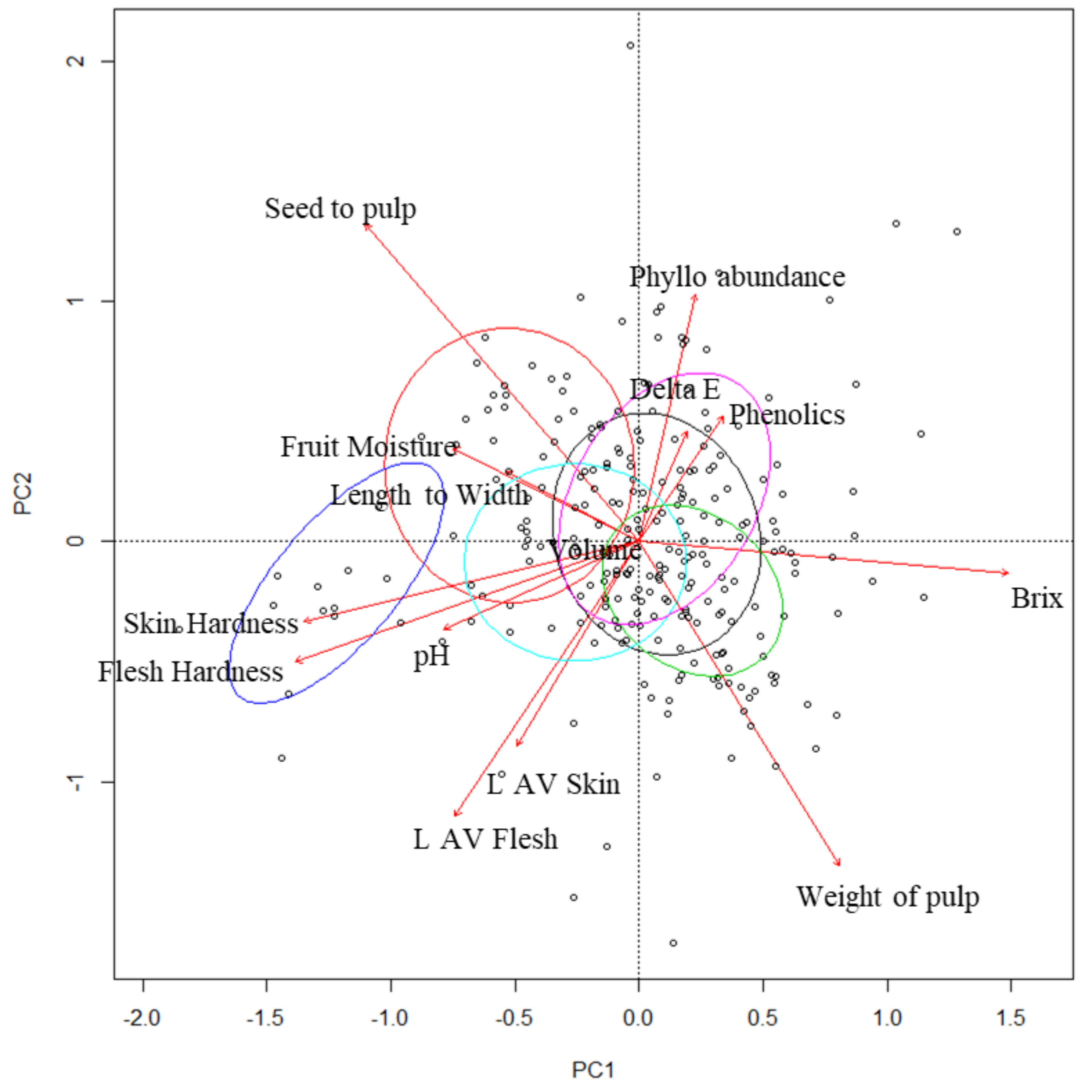


Figure 6: Principal Components Analysis of multivariate patterns in fruit quality metric displayed as a function of site: Foxpaw (black) 0.66, Hamilton (red) 0.90 , Valley (green) 0.40, Clinton (blue) 0.50, Butler (light blue) 0.58, Royalton (magenta) 0.66.

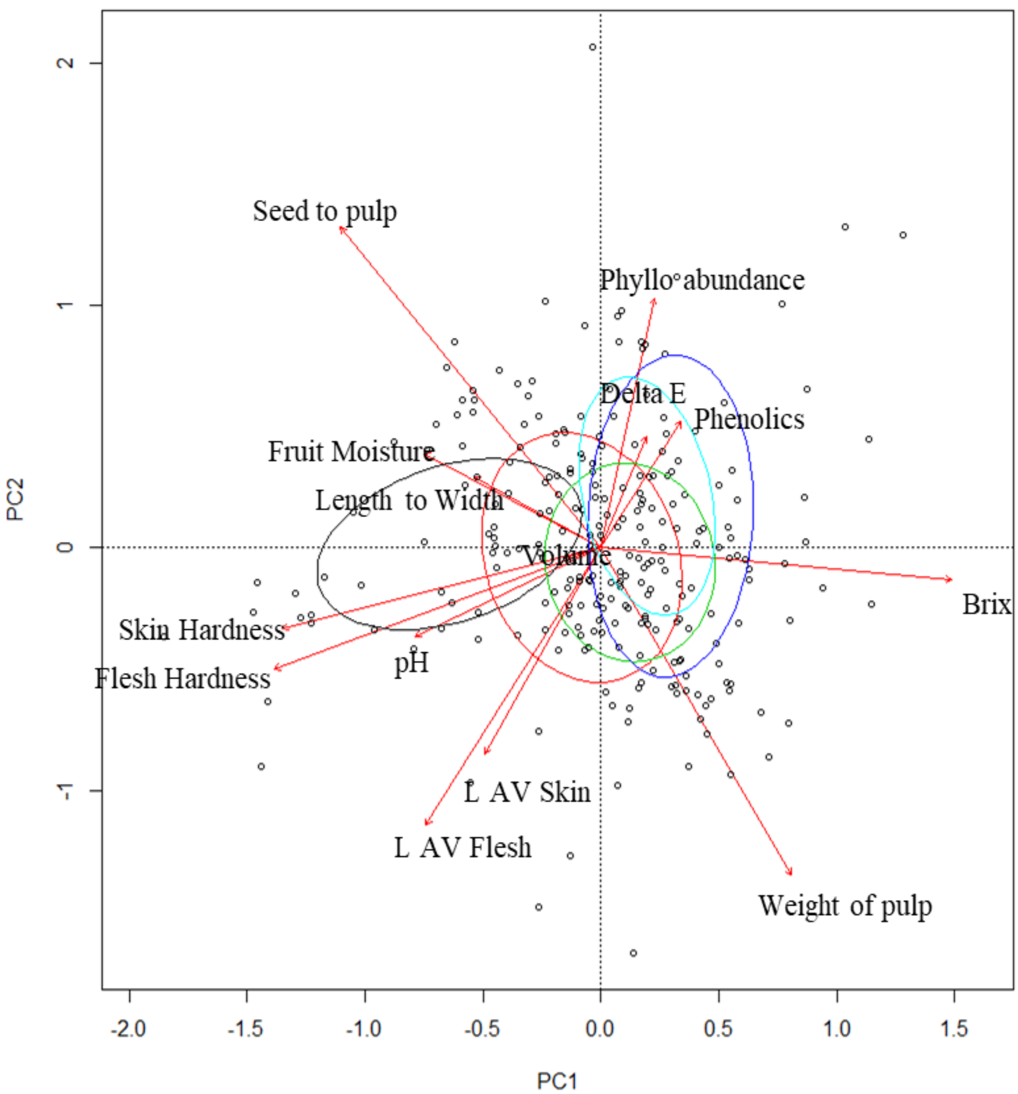


Figure 7: Principal Components Analysis of multivariate patterns in fruit quality metric displayed as a function of ripeness scores: 1(least ripe, black) 0.58, 2(red) 0.67, 3(green) 0.46, 4(blue) 0.73, 5(most ripe, cyan) 0.42.

When examining the interaction of cultivar, site, and ripeness, the variance explained increased marginally (over a quarter) (Figure 7). A conditional redundancy analysis was performed for each of the variables, considering variance of the other two variables (Table 7).

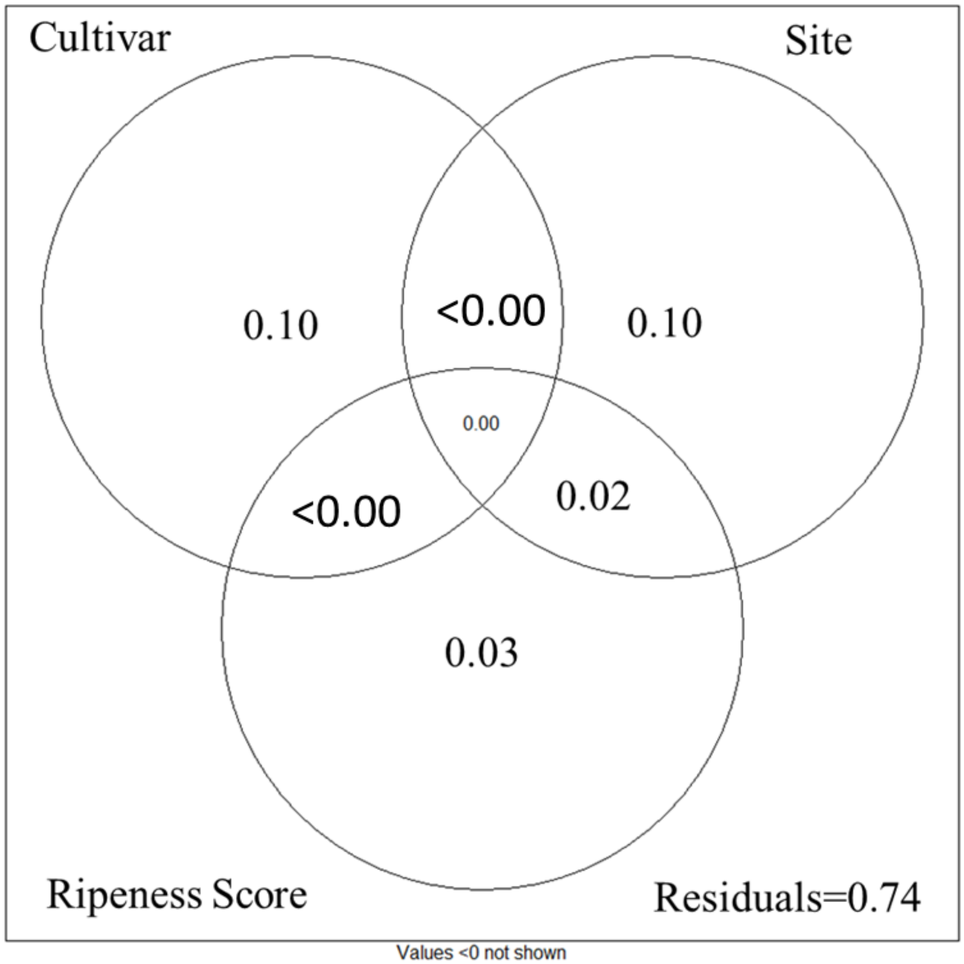


Figure 8: Explanation of variance for cultivar, site and ripeness score; partitioning the variance from each variable and their interactions.

Table 7: Partial redundancy analysis of fruit quality as a function of cultivar, site, or ripeness score. Models partialed out the variance associated with the other two variables.

Variable	***DF***	***Variance***	***F***	***P***
Cultivar	9	1.70	4.59	0.001
Site	5	1.27	6.36	0.001
Ripeness Score	1	0.35	9.53	0.001

Univariate Assessment of Individual Fruit Quality Metrics

The mixed effects models showed significant differences exist between cultivars and ripeness scores for multiple quality metrics (Table 6). Out of the fourteen quality metrics measured, ten were significantly different for cultivar (fruit moisture, length to width ratio, weight of pulp, seed to pulp ratio, fruit *Phyllosticta* abundance, flesh hardness, Brix, *L* average for Flesh, *L* average for skin, pH, and Delta E). Ripeness scores were associated with significant differences in quality for eight out of the fourteen quality metrics (weight of pulp, seed to pulp ratio, skin hardness, flesh hardness, Brix, L average for flesh, pH, and Delta E). Phenolics and fruit volume were not found to have significant differences between cultivars or ripeness scores.

Table 8: Summary of linear mixed effects models examining variation in fruit quality metrics as a function of cultivar and ripeness scores. Site was included as a random effect in all models. Marginal R squared (fixed effects) and conditional R-squared (fixed and random effects). NDF is the numerator degrees of freedom and DDF is the denominator degrees of freedom.

	Cultivar				***Ripeness Score***				***R-squared***	
Quality metrics	NDF	DDF	F	P	NDF	DDF	F	P	R^2m	R^2c
Fruit Moisture	9	238.31	7.65	<0.001*	1	240.97	1.18	0.28	0.14	0.53
Length to Width ratio	9	235.97	5.65	<0.001*	1	144.51	1.78	0.18	0.18	0.20
Weight of Pulp	9	238.87	6.92	<0.001*	1	222.93	20.18	<0.001*	0.22	0.36
Seed to Pulp ratio	9	238.37	13.29	<0.001*	1	229.72	12.69	<0.001*	0.30	0.46
Fruit *Phyllostica* Abundance	9	237.76	3.30	<0.001*	1	240.99	2.07	0.15	0.07	0.52
Skin Hardness	9	238.39	1.55	0.13	1	239.66	137.67	<0.001*	0.36	0.59
Flesh Hardness	9	238.14	2.69	<0.001*	1	229.63	81.92	<0.001*	0.30	0.46
Brix	9	238.3	5.14	<0.001*	1	239.48	9.62	0.002	0.13	0.43
L Average Flesh	9	238.33	5.79	<0.001*	1	231.09	23.14	<0.001*	0.21	0.40
L Average Skin	9	239.02	5.52	<0.001*	1	190.46	0.01	0.93	0.16	0.21
pH	9	239.38	9.85	<0.001*	1	224.39	7.18	0.008	0.26	0.37
DeltaE	9	234.56	1.74	0.08	1	129.5	4.84	0.03*	0.07	0.09
Phenolics	9	239.39	1.26	0.26	1	216.11	1.05	0.31	0.04	0.31
Volume	9	238.66	1.68	0.10	1	195.65	1.74	0.19	0.06	0.15

The quality metrics of fruit moisture, fruit *Phyllosticta* abundance, and length to width ratio were only significantly different between cultivars and not between ripeness score. The other quality metrics of skin hardness and Delta E were significantly different for ripeness score and not for cultivar. Weight of pulp, seed to pulp ratio, flesh hardness, Brix, and pH were significantly different for cultivar and ripeness score.

The marginal r-squared values included cultivar and ripeness scores whereas the conditional r-squared values include the full model (cultivar, ripeness scores and site). The models did not perform particularly strongly barring skin hardness, *Phyllosticta* abundance, seed to pulp ratio and fruit moisture. *Phyllosticta* abundance and fruit moisture stand out among this cohort as those were the random effect of site explained most of the variance within the model. The model of fruit quality metric Delta E (browning), phenolics, and volume performed poorly. The Brix model did not perform well but did show that site explained twice the variance from cultivar and ripeness score.

The pairwise comparisons were visualized on strip and violin charts for each quality metric barring phenolics and volume which were not significant (Figure 8). Within fruit moisture there was a distinct difference in site especially Foxpaw Farm and the majority of cultivars (eight out of the ten) were in one group, Shenandoah and Susquehanna were the cultivars not included. Wells stands out in the length to width ratio as having the greatest variability in fruit size. Within weight of pulp Allegheny had the most consistent yield but there were few differences in mean yield between cultivars. Potomac, Susquehanna, and Wabash had the lowest seed to pulp ratio. Fruit *Phyllosticta* abundance had distinct differences in site with Valley View Farm having low abundances barring Susquehanna. The *Phyllosticta* abundance was broken into only two categories with six cultivars being in both demonstrating smaller amount of variation within the dataset. Skin hardness had no significant difference between cultivar which was mirrored in Table 1. Shenandoah was an outlier in flesh hardness. For Brix the pairwise comparison with Allegheny, Overleese, Potomac, and Wabash were in all the categories. Susquehanna had an outlier that was much lighter than all others for the readings for L average of flesh. The L averages for the skin were only broken into two categories and only Susquehanna being solely in the other. The pairwise comparison for pH exhibits the small range of pH within pawpaw; Susquehanna had a distinct group from Royalton which had lower pH. Finally, Delta E had only two categories with Alleghany solely in one and Susquehanna solely in the other; all other cultivars were in both categories. Within cultivars there is often a relatively clear separation in sites throughout the quality metrics.

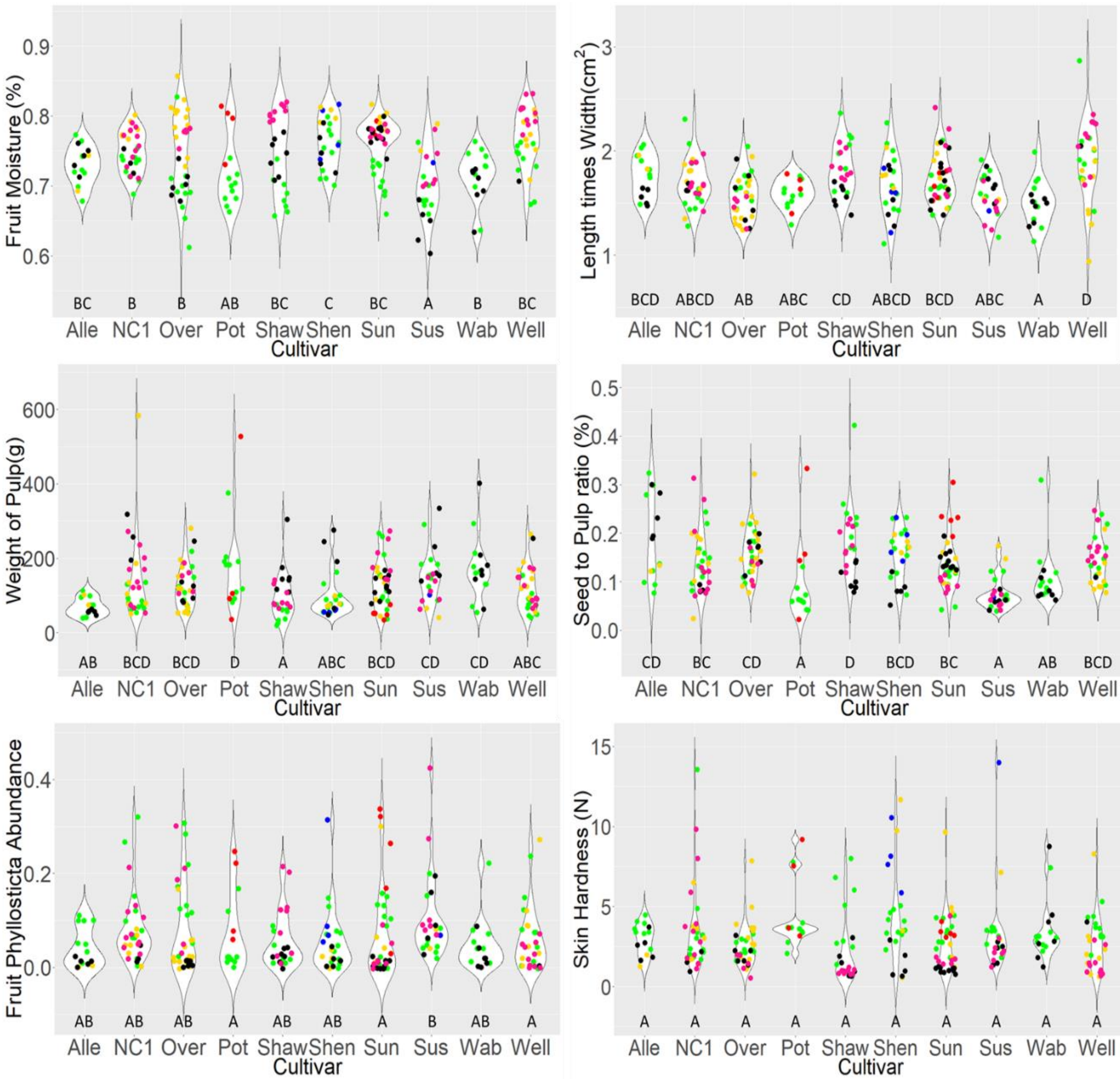
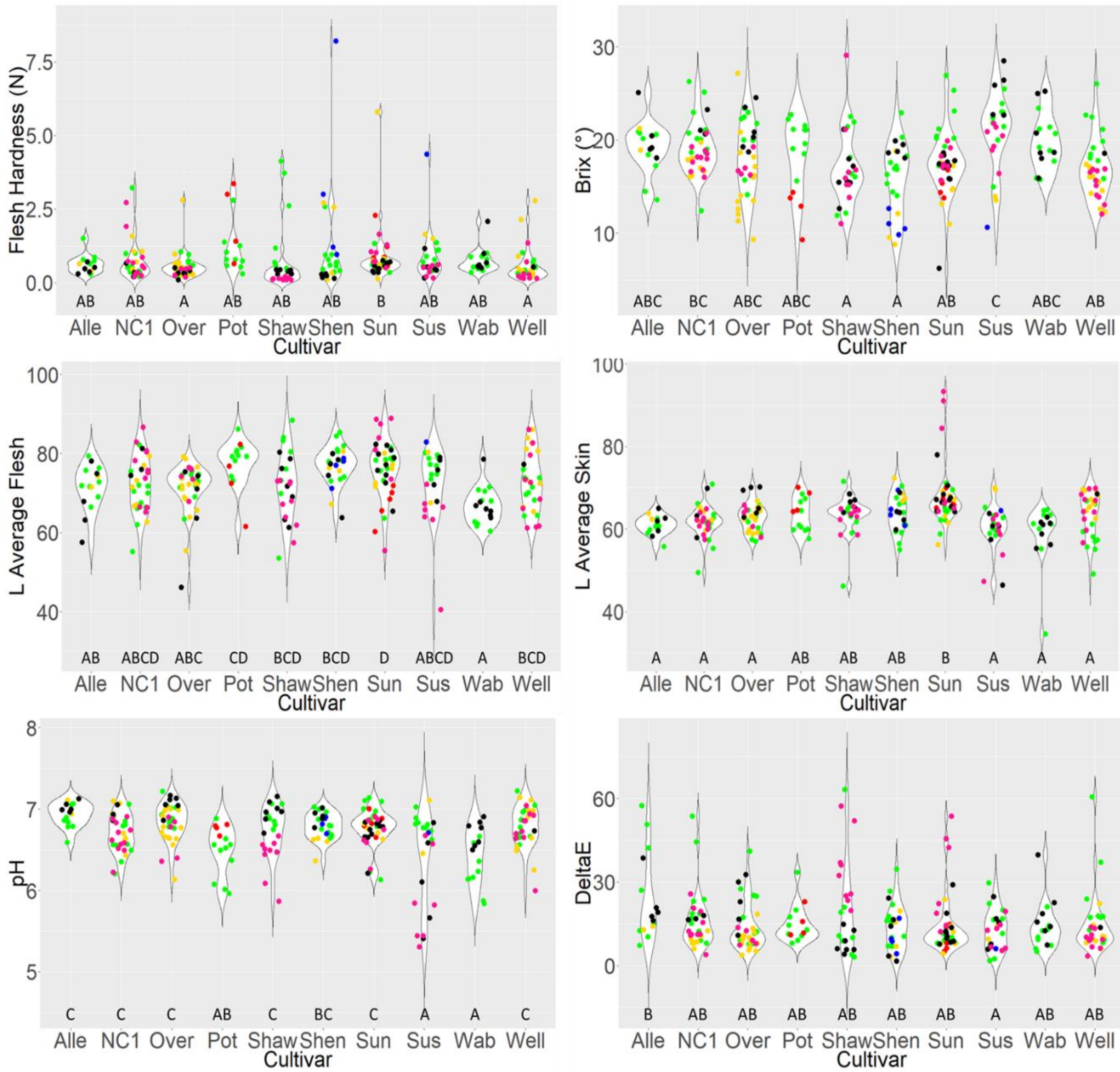


Figure 9: Pairwise comparison for fruit quality metrics by cultivar. The letter under each vilion indicates the significant difference in least squared means between cultivar accounting for ripeness scores. Sites are differentiated by color: Foxpaw (green), Hamilton (red), Valley (black), Clinton (blue), Butler (gold), Royalton (pink).

Figure 9 continued



Color Assessment by Cultivar

Due to their cylindrical nature, the hue angle and Chroma for both flesh and skin were not analyzed with the other quality metrics (Figures 9). The strip and violin graphs of the individual metrics demonstrated some groupings by site within the cultivars.

Sunflower's Chroma and hue angle for the flesh and hue for skin were distinctly different form the other cultivars. There was a similar trend for Potomac and Wabash, but fruit was only available from two sites. Overall the site Foxpaw had a distinctly small range throughout all flesh color readings. Cultivars Shawnee trail, Sunflower, and Susquehanna had larger ranges for skin color and for flesh Chroma Potomac, Shenandoah and Sunflower had larger ranges. For the Hue Angle, which determines the color, the readings were not consistent.

Disease Presence

Pruning the regression tree for the disease index score at four branches minimized the cross-validated error rate (Figure 11). The decision tree generated a group of sites (Hamilton, Clinton, Valley, Butler, and Royalton) with smaller average of disease index scores (1.3), and a second group (Foxpaw, Dublin, and Urbana) with a higher mean scores 2.5. The three cultivars NC-1, PA-Golden #1, and Sue had a lower average disease index scores (mean 1.4) even when they occurred at sites that otherwise had high an incidence of disease.

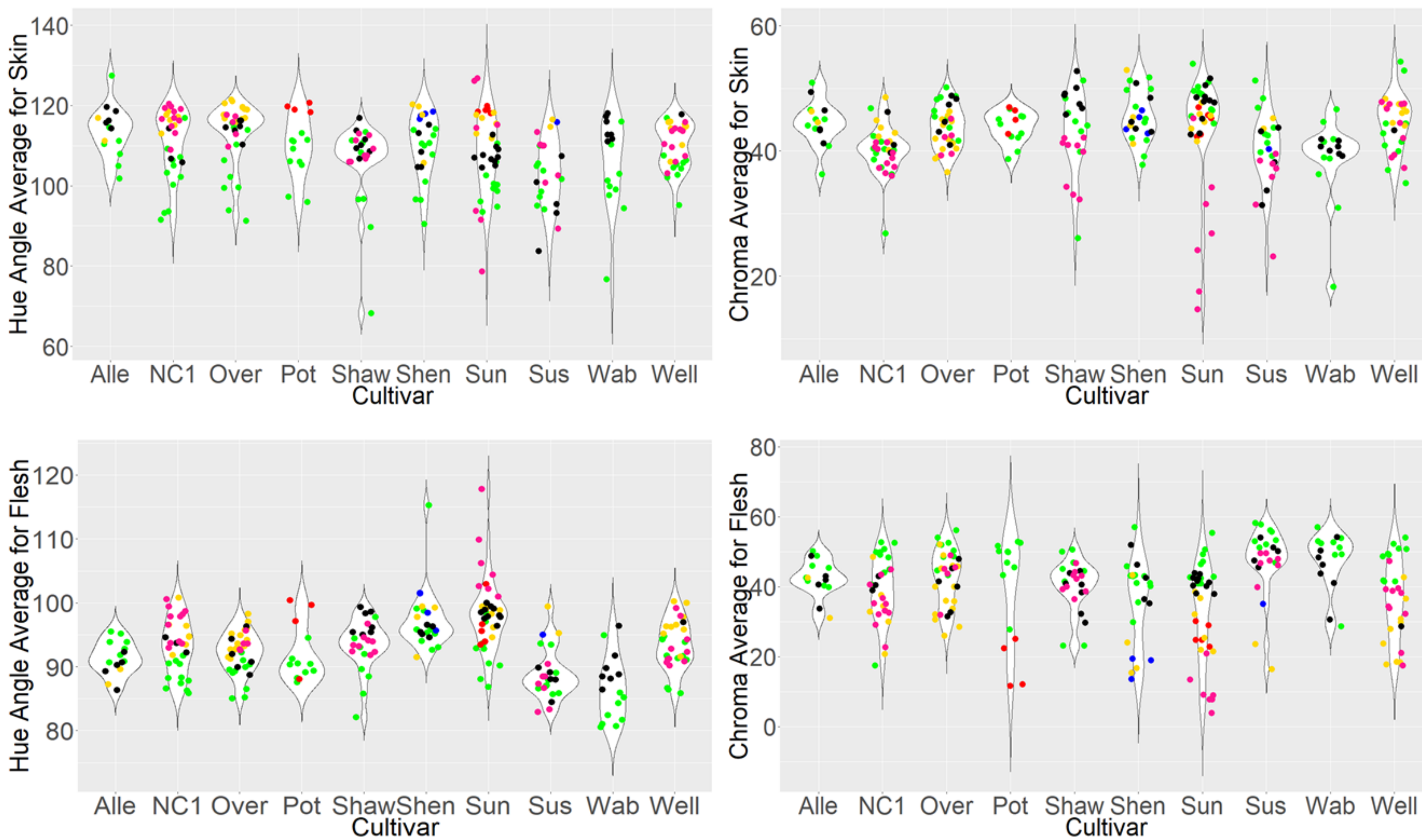


Figure 10: Average Chroma and Hue Angle for flesh and skin of pawpaw fruit by cultivar. Site are differentiated by color: Foxpaw (green), Hamilton (red), Valley (black), Clinton (blue), Butler (gold), Royalton (pink).

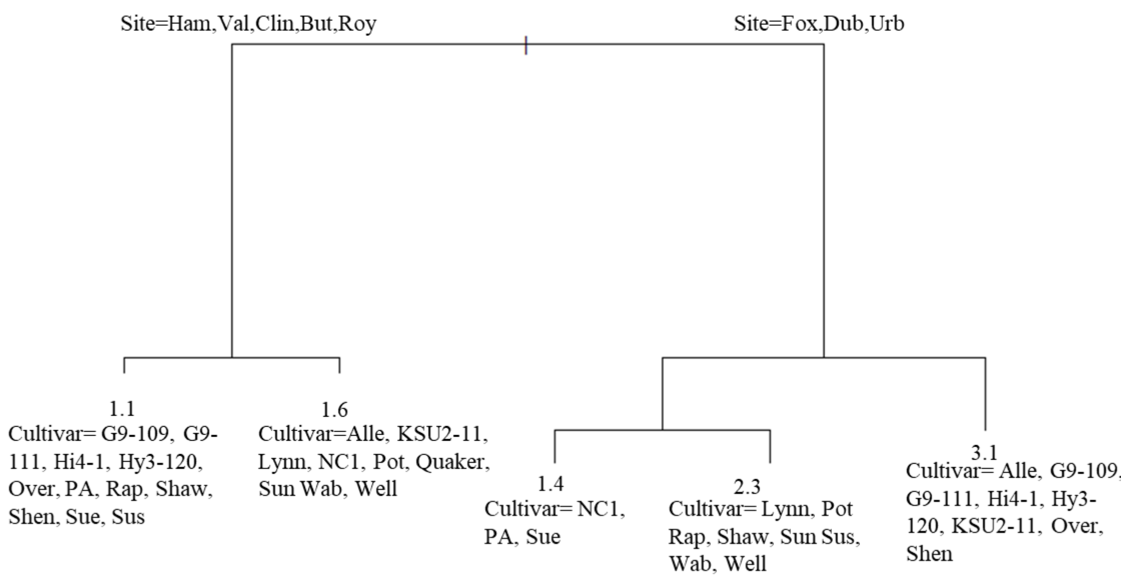


Figure 11: Decision Tree in relation to disease index. Cultivars abbreviation can be found in Appendix A.

Discussion

Qualitative Assessment of Fruit Quality

The visualization of the standard deviation of flavor shows preliminary data that there are discernable differences in quality for pawpaw fruit within this basic level of qualitative analysis. Though this data was taken by only two analysts (small sample size) the trend that "Bad" fruit was related to hard fruit with more seed may suggest that fruits that are focused on reproductive efforts (seeds) do not make quality fruit. The standard deviation of the "Good" fruit was the smallest (0.46), a trend that suggest these fruits were not as variable. In depth sensory analysis must be performed to explore the correlation between flavor and quality metrics.

Multivariate Assessment of Overall Fruit Quality

The cultivar Shawnee Trail was associated with greater *Phyllosticta* abundance and higher browning potential (Delta E and phenolics). This correlation could be a causal relationship with the stress of *Phyllosticta* increasing production of phenols. Susquehanna and Wabash had higher Brix values and the greatest pulp weights suggesting larger, sweeter fruit which would make these cultivars candidates for pulp production. Five cultivars (Overleese, Sunflower, Wells, Allegheny and NC-1) were very central to the bi-plot which suggested similarity in overall quality. Potomac was not clustered with other cultivars and was particularly associated with higher L values, lighter flesh and skin color. The implication of such differences is, however, unclear as consumer color preference has yet to be investigated. Shenandoah had higher fruit moisture, length to width ratio, and hardness which could lend this cultivar to be shipped and stored well. These associations may have been skewed due to fruits from the site Clinton being less ripe; further studies would need to confirm this finding. Three cultivars (Susquehanna, Potomac, and Shawnee Trail) had large standard deviations which means that these fruits were inconsistent in overall quality. Though inconsistent quality is often less desirable in commercial fruit (Abbott, 1999), these cultivars still possessed metrics that might makes them higher quality in some circumstances. For instance, Susquehanna was associated with sweeter and bigger fruit which are both quality metrics that would be prized by the pulp industry.

When examining differences in overall quality between sites, Valley View Farm was associated with higher Brix readings and greater weights of pulp which are both indicators of higher quality fruit. In addition, this site's standard deviation was the lowest indicating that site condition (cultural practices or abiotic factors) play a role in producing consistent fruit with two metrics that are desired. Valley View Farm is rigorously maintained with trees pruned yearly for crossing branches, top pruned when reached 10 feet, and clusters are hand-thinned to one to two fruits after the trees abort fruit in early July; no other site within this study hand-thins their fruit. Contrasting site Clinton, grown under shade and with little intervention, had the next lowest standard deviation but was associated with harder fruit and higher fruit moisture; harder fruit is not a prized quality metric unless it is for transportation and more water fruit dilutes flavor and sugar.

The gradient of the ripeness scores shows low scores are associated with higher pH and harder fruit, a result in line with previous studies (McGrath & Karahadian, 1994). Scores four and five were overlapped substantially in overall quality but score five (highest ripeness) had a larger standard deviation. High ripeness was associated with large pulp masses, higher Brix, phenolics, Delta E, and *Phyllosticta* abundance. The higher Brix and weights of pulp are quality characteristic that are wanted by both the fresh and pulp markets. But the browning potential and *Phyllosticta* abundance are metrics that for either high quality fruit or for homogenous fruit lower values are desired. As fruit ripens the phenolics will build up and this will in turn increase the Delta E (browning potential) which is a less desirable especially in the pulp market.

Exploring the interactions between site, cultivar, and ripeness, the work showed only a quarter of the variance in the combined quality metrics could be constrained by these factors. These findings suggest that 75% of the variance is due to factors not measured within the scope of this study. These factors could include cultural practices, tree to tree variability, or within cluster variability. Site accounted for a substantial proportion of the explained variance (Appendix A); this includes both abiotic differences (e.g. climate, soil fertility) and cultural practices. Orchards ranged from no management (Hamilton) to traditional orchard management i.e. mowed straight rows, intense side pruning, top pruning, glyphosate weed control, and managing number of fruit on each tree by hand thinning (Valley View) to almost ornamental management (Royalton). These finding suggests cultural practices, such as hand-thinning, could produce more consistent fruit quality.

Univariate Assessment of Individual Fruit Quality Metrics

Within the univariate assessment of the fruit quality metrics, *Phyllosticta* abundance, fruit moisture, and Brix to a lesser extent had strong models where most of the variance was explained by the random effect of site. Fruit *Phyllosticta* abundance is a biotic factor that could be controllable with fungicide in the future, but none are labeled for pawpaws. Less *Phyllosticta* has generally been accepted to indicate quality due to its effects on homogeneity in appearance. Fruit moisture is an abiotic factor that may be associated with rainfall and soil moisture. Due to the distance between sites, and the pawpaw ripening from south to north, the fruit was picked in a long-time window. Thus, fruit moisture cannot be teased apart to determine if the rainfall or soil moisture was more influential. Soils with clay or mostly rock, e.g. Foxpaw Farm, produce fruit with less moisture. Preferences of moisture concentration has yet to be investigated and would require a sensory panel to determine what constitutes higher quality in this metric. Brix is an internal characteristic of the pawpaw measuring the sugar content, findings here suggest that site conditions influence the Brix. Brix levels in pawpaws ranged from 6.2 to 29° Brix. Compared to other fruit, pomegranates concentration is 16° Brix (Chater et al., 2018), apples concentration is between 8-15° Brix (Zhang et al., 2015), and grapes concentration can be over 20° Brix (Gomes et al., 2017). Above 20° Brix is considered very sweet. Previous research (McGrath & Karahadian, 1994) proposed that a ripe pawpaw should be over 20° Brix but that may be cloying sweet (like over ripe bananas) for consumers in fresh markets. Sensory panels need to investigate where the ideal Brix levels for pawpaw fall.

To capture the browning potential of pawpaw flesh (Delta E), the change of the color reading over a 24-hour period, was used. Delta E was too coarse of a measurement to capture all of the browning of the fruit. Furthermore, from the data collection certain structures within the fruit appear to brown considerably faster (within 15 minutes of the fruit being cut open) than the majority of the pulp. Homogenizing the pulp and taking readings of a series of subsamples might not be capturing the problem of browning. In addition, the samples used were fresh pulp. Informal observations suggested frozen pulp appeared to brown more uniformly and quickly when thawed. Brannan et al. (2012) found that after pulp was frozen for 12 months that the color was darker which will have implications for the pulp industry. Brannan has also attempted to develop a method for storing frozen pulp without browning (Brannan & Wang, 2017).

Fruit moisture and Brix had trends of site being distinctly different when the metrics were graphed by cultivar which was reflected inTable8. Lower length to width ratios indicates rounder fruit while large ratios are longer fruit. Cultivar Wells had the lowest and highest value suggesting a potential for shapes that are not consistent within the cultivar thus making Wells potentially less marketable from a homogeneity of appearance aspect. Seed to pulp ratio has previously been recommend to be as low as possible (Peterson, 2003) with Potomac and Susquehanna having the lowest ratio. Zero *Phyllosticta* would be ideal on a fruit; the site Valley View had very low abundance barring Susquehanna which is commonly thought to be more susceptible to *Phyllosticta* (Powell, 2019). The skin and flesh hardness were above average for Clinton which could be related to ripeness or cultural practices grown under shade. Brix levels were for the cultivars Allegheny, NC-1, Overleese, Potomac, Susquehanna, and Wabash and these would be recommended for cultivars for planting if maximum sugar levels are a desirable quality metric. There was little variation in pH (5.31-7.22); Potomac, Susquehanna, and Wabash in had the lowest pH, notably many Susquehanna from Royalton had lower pH than observer values.

Color Variation by Cultivar

The Chroma (intensity of color) and hue angle (color) for the skin appeared to show some separation between sites. Though areas of *Phyllosticta* were avoided when the color readings were taken. The flesh L color reading demonstrated that different cultivars exhibit broad different colors. The L, Chroma, and hue angle all show separation between sites by cultivar. The separation seen in all the color measurements leads to the conclusion that the site's environmental factors or cultural practice impact the color of both the skin and flesh. Appearance is a main factor of fruit quality (Kyriacou & Rouphael, 2018). Further work is needed to determine if consumers have a preference in skin and flesh color and if the black spots of the *Phyllosticta* can be tolerated in general marketplaces. The mechanisms by which fruit of the same cultivar varies between sites also needs to be understood.

Disease Presence

Disease presence was strongly related to site identity. Within quality this is important due to the fact that disease or pests can stress the trees, which in turn leads to lower quality fruit (Pomper & Layne, 2004). There is little to no information on how to control pests or disease within pawpaws and there are no labeled chemical controls. None of the orchard within the study performed any disease control but one of the sites (Valley View) were planted next to apple trees that are treated regularly which could assist in fungal control. Further research is needed to determine if age of the orchard or other biotic or abiotic factors caused the disease to be greater in Foxpaw, Urbana, and Dublin. Cultivars of interest are NC-1, PA-Golden #1, and Sue (mean 1.4) due to the fact that these cultivars were at sites that had relatively high levels of disease (mean 2.5 overall) but were comparable to the sites with less disease. These cultivars show preliminary resilience to the pests and diseases of pawpaw trees; further work will need to test if this trend extents throughout the whole of the cultivar or if only at particular sites.

Conclusions

This study quantified variation in a broader range of quality metrics and cultivars (10) than previous studies of pawpaw. Though we did not try to define quality classes or thresholds for pawpaw fruit, the information gathered gives context to the range of quality present within and between pawpaw cultivars. The current market defines as 'quality', including low seed to pulp ratio, as little as possible *Phyllosticta* on the skin, larger, and sweeter fruit. We have defined that several quality metrics vary greatly within the sample set (Brix, color, seed to pulp ratio, and fruit moisture) and those which have a smaller range (pH, phenolics). The context of the end market for the fruit will be the deciding factor for which quality metrics are prioritized. For a fresh market, appearance, flavor (Brix, pH), and *Phyllosticta* abundance will likely be the most important. In contrast for the pulp market seed to pulp ratio, Brix, pH, and phenolics will likely be the metrics of interest. Generally, as a pawpaw fruit ripens the Brix, pH, and phenolics increase as the overall hardness of the fruit decrease. Even though pawpaw fruit differed by site, cultivar, and ripeness, these three variables only explained about a quarter of the overall variance in quality. This increased to 50% for some individual quality metrics. Understanding the tree and intra-site drivers of the remaining variance should be a research priority. The overall aim of our study was to gain a greater understanding of what constitutes quality within pawpaw fruit. Color was influenced by cultivar and ripeness for the flesh but *Phyllosticta* was an influential factor for many of the readings of the fruit. Determining if quality or homogeneity is needed for pawpaw fruit to be marketable, and testing if cultural practice can decrease variability of quality metrics are logical next steps to move the pawpaw fruit further toward commercialization.

References

Abbott, J. A. (1999). Quality measurement of fruits and vegetables. *Postharvest Biology and Technology*, *15*(3), 207–225. https://doi.org/10.1016/S0925-5214(98)00086-6

Archbold, D. D., & Pomper, K. W. (2003). Ripening pawpaw fruit exhibit respiratory and ethylene climacterics. *POSTEC Postharvest Biology and Technology*, *30*(1), 99–103.

Bellini, E., Nin, S., & Cocchi, M. (2003). The Pawpaw Research Program at the Horticulture Department of the University of Florence. *HortTechnology*, 455–457. https://doi.org/10.21273/HORTTECH.13.3.0455

Brannan, R.G., Salabak, D.E., & Holben, D.H. (2012) Sensory analysis of pawpaw (*Asimina triloba*) pulp puree: consumer appraisal and descriptive lexicon. *Journal of Food Research,* 179-192.

Brannan, R.G., & Wang, G. (2017) Effect of frozen storage on polyphenol oxidase, antioxidant content, and color of pawpaw (*Asimina triloba* [L.] Dunal) fruit pulp. *Journal of Food Research, 6,* 93-101.

Cantaluppi, C. J. (2016). The Pawpaw: An Emerging Specialty Crop. *Journal of the NACAA*, *9*(1). Retrieved from https://www.nacaa.com/journal/index.php?ji=582

Caswell, H. (2009). The role of fruit juice in the diet: an overview. *Nutrition Bulletin*, *34*(3), 273–288. https://doi.org/10.1111/j.1467-3010.2009.01760.x

Chater, J. M., Merhaut, D. J., Jia, Z., Mauk, P. A., & Preece, J. E. (2018). Fruit quality traits of ten California-grown pomegranate cultivars harvested over three months. *Scientia Horticulturae*, *237*, 11–19. https://doi.org/10.1016/j.scienta.2018.03.048

Commission Implementing Regulation (EU) No543/2011 of 7 June 2011 laying down detailed rules for the application of Council Regulation (EC) No 1234/ 2007 in respect of the fruit and vegetables and processed fruit and vegetables sectors (OJ L 157, 15.6.2011, p. 1).

Crabtree, S., Pomper, K., & Lowe, J.. (2010). Within-cluster hand-thinning increases fruit weight in North American pawpaw [*Asimina triloba* (L.) Dunal. *Journal-American Pomological Society*, *64*(4), 234–240.

Croxall, H. E., Gwynne, D. C., & Jenkins, J. E. E. (1952). The rapid assessment of apple scab on fruit. *Plant Pathology*, *1*(3), 89–92. https://doi.org/10.1111/j.1365-3059.1952.tb00038.x

Duffrin, M. W., Holben, D. H., & Bremner, M. J. (2001). Consumer acceptance of pawpaw (*Asimina Triloba*) fruit puree as a fat-reducing agent in muffins, compared to muffins made with applesauce and fat. *FCSR Family and Consumer Sciences Research Journal*, *29*(3), 281–287.

Duffrin, M. W., & Pomper, K. W. (2006). Development of flavor descriptors for pawpaw fruit puree: a step toward the establishment of a native tree fruit industry. *FCSR Family and Consumer Sciences Research Journal*, *35*(2), 118–130.

Dwire, K. A., Kauffman, J. B., Brookshire, E. J., & Baham, J. E. (2004). Plant biomass and species composition along an environmental gradient in montane riparian meadows. *Oecologia*, *139*(2), 309–317.

Erickson, L. C. (1968). The general physiology of citrus. *The Citrus Industry*.

Farr, D.F., G. F. Bills, G. P. Chamuris, and A. Y. Rossman. (1989). Fungi on plants and plant products in the United States. P. 26 St. Paul, MN: APS Press.

Faust, M. (2000). Physiological considerations for growing temperate-zone fruit crops in warm climates. *Amom Erez Kluwer Academic Publishers*, 137–156.

Fox, J. & Weisberg, S. (2011). An {R} Companion to Applied Regression, Second Edition. Thousand Oaks CA: Sage. URL: http://socserv.socsci.mcmaster.ca/jfox/Books/CompanionGigerenzer, G., & Gaissmaier, W. (2011). Heuristic decision making. *Annual Review of Psychology*, *62*, 451–482.

Goldenberg, L., Yaniv, Y., Porat, R., & Carmi, N. (2018). Mandarin fruit quality: a review. *Journal of the Science of Food and Agriculture*, *98*(1), 18–26.

Gomes, V. M., Fernandes, A. M., Faia, A., & Melo-Pinto, P. (2017). Comparison of different approaches for the prediction of sugar content in new vintages of whole Port wine grape berries using hyperspectral imaging. *Computers and Electronics in Agriculture*, *140*, 244–254. https://doi.org/10.1016/j.compag.2017.06.009

Greenawalt, L., Brannan, R., & Leite, R. (2016). *Comparative Analysis of Pawpaw Production Data from 2005-2012*. Ohio University.

Huang, H., Layne, D. R., & Kubisiak, T. L. (2000). RAPD inheritance and diversity in pawpaw (*Asimina triloba*). *Journal of the American Society for Horticultural Science*, *125*(4), 454–459.

Huang, H., Layne, D. R., & Peterson, R. N. (1997). Using isozyme polymorphisms for identifying and assessing genetic variation in cultivated pawpaw [*Asimina triloba* (L.) Dunal]. *Journal of the American Society for Horticultural Science*, *122*(4), 504–511.

Huang, H., Layne, D. R., & Riemenschneider, D. E. (1998). Genetic diversity and geographic differentiation in pawpaw (*Asimina triloba* (L.) Dunal) populations from nine states as revealed by allozyme analysis. *Journal of the American Society for Horticultural Science.*, *123*(4), 635.

Identifying Color Differences Using L*a*b* or L*C*H* Coordinates. (n.d.). Retrieved May 31, 2019, from https://sensing.konicaminolta.us/blog/identifying-color-differences-using-l-a-b-or-l-c-h-coordinates/

Kamil, Barton (2018). MuMIn: Multi-Model Inference. R package version 1.42.1. https://CRAN.R-project.org/package=MuMIn

Khoshnam, F., Tabatabaeefar, A., Varnamkhasti, M. G., & Borghei, A. (2007). Mass modeling of pomegranate (*Punica granatum* L.) fruit with some physical characteristics. *Scientia Horticulturae*, *114*(1), 21–26. https://doi.org/10.1016/j.scienta.2007.05.008

Kleina, H. T., Pádua, T., Jacomino, A. P., & De Mio, L. L. M. (2018). Postharvest quality of plums in response to the occurrence of leaf scald disease. *Postharvest Biology and Technology*, *143*, 102–111.

Kobayashi, H., Wang, C., & Pomper, K. W. (2008). Phenolic content and antioxidant capacity of pawpaw fruit (Asimina triloba L.) at different ripening stages. *HortScience*, *43*(1).

Kuznetsova, A., Brockhoff, P.B., & Christensen, R. H. B. (2017). "lmerTest Package: Tests in Linear Mixed Effects Models." *Journal of Statistical Software*, *82*(13), 1-26. doi:10.18637/jss.v082.i13 (URL: http://doi.org/10.18637/jss.v082.i13).

Kyriacou, M. C., & Rouphael, Y. (2018). Towards a new definition of quality for fresh fruits and vegetables. *Scientia Horticulturae*, *234*, 463–469.

Lenth, Russell (2019). emmeans: Estimated Marginal Means, aka Least-Squares Means. R package version 1.3.3. https://CRAN.R-project.org/package=emmeans.

McGrath, M. J., & Karahadian, C. (1994). Evaluation of physical, chemical, and sensory properties of pawpaw fruit (*Asimina triloba*) as indicators of ripeness. *Journal of Agricultural and Food Chemistry*, *42*(4), 968.

Meshram, D. T., Gorantiwar, S. D., Sharma, J., & Babu, K. D. (2018). Influence of organic mulches and irrigation levels on growth, yield and water use efficiency of pomegranate (Punica granatum L.). *Journal of Agrometeorology*, *20*(3), 196–201.

Moore, A. (2015). *Pawpaw: In Search of America's Forgotten Fruit*.

Musacchi, S., & Serra, S. (2018). Apple fruit quality: overview on pre-harvest factors. *Scientia Horticulturae*, *234*, 409–430.

Nam, J. S., Jang, H. L., & Rhee, Y. H. (2017). Antioxidant activities and phenolic compounds of several tissues of pawpaw (*Asimina triloba* [L.] Dunal) crown in Korea. *JFDS Journal of Food Science*, *82*(8), 1827–1833.

Oksanen, J. F., Blanchet, G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P R., O'Hara, R. B., Simpson, G. L., Peter, S., Henry, M., Stevens, H., Szoecs, E. & Wagner, H. (2019). vegan: Community Ecology Package. R package version 2.5-4. https://CRAN.R-project.org/package=vegan.

O'Malley, P. (2008). Pawpaw trial maintenance. *Leopold Center Completed Grant Reports*. Retrieved from https://lib.dr.iastate.edu/leopold_grantreports/311.

Pannitteri, C., Continella, A., Cicero, L. L., Gentile, A., La Malfa, S., Sperlinga, E., … Siracusa, L. (2017). Influence of postharvest treatments on qualitative and chemical parameters of Tarocco blood orange fruits to be used for fresh chilled juice. *Food Chemistry*, *230*, 441–447.

Peterson, N. (1990). Guide to evaluating pawpaws. Retrieved April 17, 2018, from http://www.pawpaw.kysu.edu/ppf/evalguid.htm.

Peterson, R. N. (1991). Pawpaw (Asimina). *Acta Horticulturae, ISHS*.

Peterson, R. N. (2003). Pawpaw variety development: a history and future prospects. *HortTechnology*, 449–454. https://doi.org/10.21273/HORTTECH.13.3.0449

Pomper, K. W., Crabtree, S. B., Layne, D. R., & Peterson, R. N. (2008) (a). Flowering and fruiting characteristics of eight pawpaw [*Asimina triloba* (L.) Dunal] selections in Kentucky. *Journal-American Pomological Society*, *62*(3), 89–97.

Pomper, K.W., Crabtree, S. B., Layne, D. R., Peterson, R. N., Masabni, J., & Wolfe, D. (2008) (b). The Kentucky pawpaw regional variety trial. *Journal of the American Pomological Society*, *62*(2), 58–69.

Pomper, K. W., & Layne, D. R. (2005). The North American Pawpaw: Botany and Horticulture (Vol. 31). *Horticulture Review*.

Pomper, Kirk W., Layne, D. R., & Jones, Snake C. (2003) (a). Container production of pawpaw seedlings. *HortTechnology*, *13*(3), 434–438.

Pomper, K. W., Layne, D. R., Peterson, R. N., & Wolfe, D. (2003) (b). The pawpaw regional variety trial: background and early data. 6.

Pomper, K. W., Lowe, J. D., Li Lu, Crabtree, S. B., Dutta, S., Schneider, K., & Tidwell, J. (2010). Characterization and identification of pawpaw cultivars and advanced selections by simple sequence repeat markers. *Journal of the American Society for Horticultural Science*, *135*(2).

Porter, S. D., Reay, D. S., Bomberg, E., & Higgins, P. (2018). Avoidable food losses and associated production-phase greenhouse gas emissions arising from application of cosmetic standards to fresh fruit and vegetables in Europe and the UK. *Journal of Cleaner Production*, *201*, 869–878.

Postman, J. D., Hummer, K. E., & Pomper, K. W. (2003). Vascular decline in the Oregon pawpaw regional variety trial. *HortTechnology*, *13*(3), 418–420.

Powell, R. (2018). *NAPGA& OPGA Educational Publications*.

Powell, R. (2019, May 23).

RStudio Team (2016). RStudio: Integrated Development for R. RStudio, Inc., Boston, MA URL http://www.rstudio.com/

R Core Team (2018). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Schulte-Mecklenbeck, M., Sohn, M., de Bellis, E., Martin, N., & Hertwig, R. (2013). A lack of appetite for information and computation. Simple heuristics in food choice. *Appetite*, *71*, 242–251.

Setser, C. S. 1984. Color: reflections and transmissions. *Food Quality*. 6:183

Silva, H. N. D., Tustin, D. S., Cashmore, W. M., Stanley, C. J., Lupton, G., & McArtney, S. J. (1997). Fruit fresh mass—diameter relationship for `Royal Gala' apple across seasons and among fruit production regions of New Zealand. *HortScience*, *32*(7), 1169–1173. https://doi.org/10.21273/HORTSCI.32.7.1169

Singleton, V. L., and Rossi, J.A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture*, *16*, 144-151

Singleton, V.L., Orthofer, R., and Lamuela-Raventos, R.M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods in Enzymology, 299*, 152-178.

Soil Survey Staff, Natural Resources Conservation Service, United States Department of Agriculture. Web Soil Survey. http://websoilsurvey.nrcs.usda.gov/. Accessed [04/07/2019].

Therneau, T. & Atkinson, B. (2018). rpart: Recursive Partitioning and Regression Trees. R package version 4.1-13. https://CRAN.R-project.org/package=rpart

TNM Elevation. (n.d.). Retrieved May 29, 2019, from https://viewer.nationalmap.gov/theme/elevation/##bottom

Wang, Y., Reighard, G. L., Layne, D. R., Abbott, A. G., & Huang, H. (2005). Inheritance of AFLP markers and their use for genetic diversity analysis in wild and domesticated pawpaw [*Asimina triloba* (L.) Dunal]. *Journal of the American Society for Horticultural Science*, *130*(4), 561–568.

Wickham, H.. (2016). ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York.

Zhang, Y., Zheng, L., Li, M., Deng, X., & Ji, R. (2015). Predicting apple sugar content based on spectral characteristics of apple tree leaf in different phenological phases. *Computers and Electronics in Agriculture*, *112*, 20–27.https://doi.org/10.1016/j.compag.2015.01.006

Appendix A: Site Information

Table 9:Table of Site information. Latitude (LAT) and Longitude (LONG) in WGS84 coordinate system (degrees). Frost abbreviation is the frost free period in days. Elevation was found on USGS, National Map (TNM Elevation, 2019). Management practices, denoted by present (X) or absent (O): Herbicide control around the base of the tree with glyphosate; Fertilize applied in early spring; Pruned side branch removal in late winter occurs every other year unless marked; Hand pollinated is a process of manually pollinating flowers to increase production of tree; Hand-thinning is a process of thinning premature clusters in early July after the tree aborts some of the premature fruit to increase the size of the fruit, cluster are thinned to one to two fruit.

Site	LAT	LONG	# of trees	# Cultivars	Age of planting	Soil type	Frost(day)	Mean rain(mm)	USDA Zone	Elevation (m)	Herbicide	Fertilize	Pruned	Hand pollinated	Hand thinning	Mulched
Foxpaw	38.68018	-83.7249	91	21	25	FaC2	178	1160	6b	216.92	X	X	X	O	O	O
Butler	39.29473	-84.3618	30	16	20	CnC2& JoR1B1	170	1120	6a	227.72	X	X	X	O	O	X
Hamilton	39.13164	-84.34017	7	4	~ 10	UADXC	170	1120	6a	181.93	O	O	O	O	O	O
Clinton	39.45179	-83.9116	16	10	~10	MhC2	170	1040	6a	277.24	O	X	O	O	O	O
Dublin	40.07218	-83.06858	14	7	2	Ku	167	990	6a	273.33	O	X	O	O	O	X
Valley	40.13603	-83.83297	68	17	6	MIB	158	1020	6a	317.79	X	X	X	O	X	X
Urbana	40.03519	-83.78073	6	4	5	WrA	158	1020	6a	307.25	X	X	O	O	O	X
Royalton	41.32804	-81.74073	15	10	~10	MgB	177	970	6a	374.76	X	X	X	X	O	X

Appendix B: Cultivars within the Study

Table 10: Table of all cultivars within the study. Breeders were taken from Peterson 2003 and KSU website. Genetic group was based off Pomper et al. (2010) genetic analysis

Cultivar	***Abbreviation***	***Breeder***	***Genetic Group***	***# of trees***	***# of sites***	***Mean DBH (cm)***	***Mean # fruit per tree***
Allegheny	Alle	Peterson	NA	10	4	1.83 ± 0.9	29.40 ± 19.06
Chappell	Hi41	KSU	NA	6	2	2.7 ± 0.67	26.17 ± 10.25
Davis	Davis	Davis	V	1	1	0.1	1
G9-111	G9111	Lehman	NA	5	2	1.62 ± 0.68	36.25 ± 26.66
Green River Belle	Green	Fiedman	III	1	1	1.6	0
Hy3-120	Hy3120	KSU	NA	5	2	2.12 ± 1.78	17.5 ± 11.27
Jenny's Gold	G9109	Lehman	NA	11	2	0.92 ± 0.74	11.50 ± 9.14
KSU 2-11	KSU211	KSU	NA	7	2	4.06 ± 3.32	80.00 ± 51.52
KSU Atwood	At	KSU	NA	3	1	6.37 ± 3.12	41.33 ± 36.91
KSU Benson	Ben	KSU	NA	4	1	0.70 ± 0.32	9.50 ± 2.12
Lynn's Favorite	Lynn	Davis	NA	6	3	5.78 ± 3.59	103.83 ± 71.25
NC-1	NC1	Campbell	V	14	4	3.63 ± 2.16	35.62 ± 33.59
Overleese	Over	Ward	V	17	4	2.98 ± 2.19	37.89 ± 26.76
PA-Golden #1	PA	Gordon	II	8	4	2.50 ± 3.10	42.00 ± 56.75
Potomac	Pot	Peterson	III	8	2	2.72 ± 2.89	34.75 ± 12.12
Quaker's Delight	Quak	Glaser	NA	6	3	1.90 ± 1.11	50.83 ± 35.49
Rappahannock	Rapp	Peterson	III	4	3	2.76 ± 1.75	51.00 ± 32.99
Shawnee Trail	Shaw	Glaser	NA	12	3	1.77 ± 1.11	28.22 ± 13.45
Shenandoah	Shen	Peterson	V	16	4	4.19 ± 2.74	41.57 ± 31.37
Sue	Sue	Unknown	IV	6	3	3.92 ± 3.46	55.80 ± 59.78
Sunflower	Sun	Gibson	V	23	5	2.58 ± 1.98	31.00 ± 21.03
Susquehanna	Sus	Peterson	II	25	5	3.35 ± 2.26	23.22 ± 23.12
Taylor	Tay	Davis	I	3	2	3.30 ± 2.71	3.30 ± 2.71
Wabash	Wab	Peterson	III	14	2	1.37 ± 1.10	14.88 ± 12.05
Wells	Well	Callaway	IV	7	4	4.99 ± 3.25	72.50 ± 56.06
Wilson	Wil	Creech	I	4	2	5.60 ± 3.20	122.25 ± 27.04

Appendix C: Predicted Pulp Mass and Total Fruit Mass by Cultivar and Genetic Grouping

Table 11: Table of predicted pulp mass and total fruit mass by cultivar and genetic group

	Pulp Mass (g)				**Total Mass (g)**			
	Class 1	Class 2	Class 3	Class 4	Class 1	Class 2	Class 3	Class 4
CULTIVAR								
Allegheny	10.24	26.63	60.37	121.66	32.95	60.22	109.41	191.27
Chappell	14.21	34.29	68.39	132.25	40.83	70.56	122.77	208.22
G9-111	74.82	89.11	110.04	139.48	109.62	132.48	166.41	214.33
Hy3-120	59.91	71.57	88.92	113.00	84.64	103.63	131.56	171.35
Jenny's Gold	16.81	34.11	66.59	122.10	38.07	65.14	112.78	190.16
KSU 2-11	49.28	54.91	63.04	73.79	84.09	99.80	123.21	155.75
KSU Atwood	37.09	51.70	74.82	109.83	50.13	75.52	117.29	182.25
Lynn's Favorite	5.66	18.84	48.30	104.24	27.88	52.56	97.81	173.71
NC-1	22.37	40.20	72.08	72.08	41.47	69.72	118.81	198.25
Overleese	13.40	28.62	57.76	108.37	30.58	57.76	107.33	190.99
PA- Golden #1	9.73	24.80	55.50	111.09	21.53	47.89	99.00	188.79
Potomac	68.56	82.99	108.58	145.44	56.25	83.72	128.60	198.25
Quaker Delight	25.91	36.97	54.76	82.08	35.40	59.44	101.40	169.26
Rappahannock	17.39	34.57	66.59	121.00	27.67	56.40	110.46	203.63
Shawnee Trail	12.96	28.30	57.91	109.62	30.91	58.06	107.45	190.44
Shenandoah	13.36	27.36	53.73	99.16	42.77	68.72	113.42	184.69
Sue	6.10	18.92	47.06	100.00	48.44	74.30	117.51	185.23
Sunflower	12.32	27.67	57.76	110.46	37.45	64.32	111.51	188.51
Susquehanna	18.58	37.45	72.76	133.40	40.32	69.06	119.46	201.36
Wabash	13.91	33.47	72.67	142.56	36.72	67.90	124.10	218.15
Wells	34.81	48.58	70.39	103.43	47.47	72.93	115.35	181.98
Wilson	33.18	41.47	54.02	72.08	64.64	81.72	107.54	144.72
GROUP								
I: Taylor	14.75	29.92	58.22	106.92	46.51	77.62	131.33	218.45
II: Zimmerman	11.36	28.41	63.20	126.11	28.52	56.10	107.54	195.16
III: Alice	26.42	45.70	79.57	135.26	39.44	68.39	119.46	202.78
IV: Wells	21.25	34.81	58.22	95.84	37.58	62.73	106.30	176.62
V: Sunflower	14.90	30.58	60.22	111.51	38.07	65.29	113.21	191.27

Appendix D: Qualitative Fruit Quality Analysis

Table 12: Proportion of sample for each cultivar that were in the Bad: “1”, Average: “2”, and Good “3” categories. N is equal to sample size. Bolded are proportion over 0.50.

	N	Seeds			Appearance			Fleshiness			Fruit Size			Flavor		
		1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Allegheny	17	0.18	0.41	0.41	0.00	**0.82**	0.18	0.35	0.47	0.18	0.47	**0.53**	0.00	0.06	0.35	**0.59**
Chappell	8	0.00	**1.00**	0.00	**0.50**	0.25	0.25	0.00	0.38	**0.62**	0.00	0.38	**0.62**	0.13	0.38	0.49
G9-111	5	0.00	**1.00**	0.00	0.40	**0.60**	0.00	0.00	**0.60**	0.40	0.00	**0.80**	0.20	0.20	0.20	**0.60**
Hy3-120	8	0.00	**0.75**	0.25	0.25	**0.62**	0.13	0.00	**0.62**	0.38	0.25	**0.62**	0.13	0.00	0.00	**1.00**
Jenny’s Gold	14	0.00	**0.71**	0.29	0.14	**0.57**	0.29	0.00	**0.57**	0.43	0.14	**0.79**	0.07	0.14	0.43	0.43
KSU 2-11	12	0.25	**0.58**	0.17	0.00	**0.75**	0.25	0.00	**0.83**	0.17	0.42	**0.58**	0.00	0.17	0.25	**0.58**
KSU Atwood	9	0.22	**0.67**	0.11	0.00	**0.56**	0.44	0.22	**0.56**	0.22	0.11	**0.78**	0.11	0.22	**0.56**	0.22
Lynn’s Favorite	19	0.00	**0.84**	0.16	0.11	**0.52**	0.37	0.21	0.32	0.47	0.16	**0.84**	0.00	0.11	0.26	**0.63**
NC-1	33	0.15	**0.70**	0.15	0.09	0.30	**0.61**	0.18	**0.58**	0.24	0.09	**0.70**	0.21	0.15	0.24	**0.61**
Overleese	36	0.28	**0.58**	0.14	0.03	**0.61**	0.36	0.17	**0.55**	0.28	0.06	**0.63**	0.31	0.03	0.25	**0.72**
PA-Golden #1	13	0.00	**0.69**	0.31	0.15	0.31	**0.54**	0.23	**0.62**	0.15	**0.61**	0.31	0.08	0.15	0.47	0.38
Potomac	14	0.21	**0.58**	0.21	0.00	**0.79**	0.21	0.07	0.43	**0.50**	0.00	0.29	**0.71**	0.14	0.43	0.43
Quaker Delight	10	**0.80**	0.10	0.10	0.00	0.20	**0.80**	0.30	**0.60**	0.10	0.30	**0.70**	0.00	0.10	**0.70**	0.20
Rappahannock	11	0.09	**0.55**	0.36	0.00	**0.82**	0.18	0.09	**0.55**	0.36	**0.55**	0.45	0.00	0.00	0.36	**0.64**
Shawnee Trail	25	0.20	**0.48**	0.32	0.08	**0.68**	0.24	**0.60**	0.32	0.08	0.20	**0.72**	0.08	0.32	0.36	0.32
Shenandoah	26	0.15	**0.70**	0.15	0.04	0.42	**0.54**	0.15	**0.70**	0.15	0.19	**0.69**	0.12	0.27	0.35	0.38
Sue	20	0.00	**0.75**	0.25	0.00	0.40	**0.80**	0.10	**0.75**	0.15	**0.55**	0.45	0.00	0.15	**0.50**	0.35
Sunflower	40	0.18	**0.67**	0.15	0.05	**0.65**	0.30	0.15	**0.52**	0.33	0.08	**0.59**	0.33	0.05	**0.52**	0.43
Susquehanna	25	0.00	0.40	**0.60**	0.28	**0.68**	0.04	0.12	**0.52**	0.36	0.08	**0.80**	0.12	0.12	0.28	**0.60**
Wabash	16	0.31	**0.56**	0.13	0.25	**0.50**	0.25	0.13	**0.69**	0.18	0.13	**0.56**	0.31	0.38	**0.56**	0.06
Wells	30	0.17	**0.70**	0.13	0.13	**0.54**	0.33	0.27	**0.50**	0.23	0.13	0.47	0.40	0.10	**0.57**	0.33
Wilson	14	0.21	**0.65**	0.14	**0.64**	0.00	0.36	0.36	0.35	0.29	**0.50**	**0.50**	0.00	0.14	**0.57**	0.29

Appendix E: Ripening Chart

Table 13: Pawpaw Ripening Chart published in NAPGA& OPGA Educational Publications.

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	Stage 1	Stage 2	Stage 3	Stage 4	Stage 5
Firmness	Very firm – yields slightly to moderate pressure	Firm – yields more to moderate pressure	Slightly soft – yields easily to moderate pressure	Moderately soft – yields easily to moderate pressure	Very soft – skin breaks under light pressure
Fragrance of uncut fruit	Light floral/fruity	More pronounced floral/fruity	Very pronounced floral/fruity	Very pronounced distinctive floral/fruity	Loss of most fragrance
Flesh	Opaque, light cream to deep gold	Opaque, light cream to deep gold	Opaque, light cream to deep gold. Pulp sac around seed turning translucent	Opaque, more translucent areas, brown just under the skin	Opaque, many areas of translucence. Mostly brown or pink/tan and becoming mushy
Flavor	Delicate, underdeveloped	More developed with mango, melon, banana flavors	Fully developed with mango, melon, banana flavors	Fully developed with mango, melon, banana flavors	Loss of characteristic fruity flavor; sometimes develops a caramel-like flavor
Skin	Green with light blue/green florescence on unhandled fruit. Need peeler to remove	Green. Need peeler to remove or cut in half and scoop flesh	Green. Need peeler to remove or cut in half and scoop flesh	Green or yellowish with predominant brown areas. Can be removed without peeler	Discolored – mostly dark brown. Can be easily removed without peeler but pulls flesh with it
Seeds	Firmly imbedded in flesh	Firmly imbedded in flesh	Loosening within the pulp sac	Loose and free from sac, or pulp sac containing seeds have separated from flesh	Dislodge from cut fruit with no effort
Usage	Eating out of hand but not much flavor	Eating out of hand, fruit salad	Eating out of hand, fruit salad, ice cream, smoothies, baking, wine-making	Eating out of hand, fruit salad, ice cream, smoothies, baking (the product will be darker), wine-making	Baking (product will be dark), wine-making