

CHARACTERIZATION OF *MALUS* GENOTYPES WITHIN THE USDA-PGRU
MALUS GERMPLASM COLLECTION FOR THEIR POTENTIAL USE WITHIN
THE HARD CIDER INDUSTRY

A Thesis

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ABSTRACT

In the United States, hard cider producers lack access to apple genotypes (*Malus* × *domestica* Borkh. and other *Malus* species) that possess higher concentrations of tannins (polyphenols that taste bitter and/or astringent) and acidity (described as having a sharp taste) than what is typically found in culinary apples. Utilizing the USDA-PGRU *Malus* germplasm collection, two projects were conducted to address these concerns. The first project characterized fruit quality and juice chemistry for a target population of 308 accessions with the goal of identifying accessions with desirable characteristics for hard cider production. The second project used the same sample population to explore the use of the Ma1 and Q8 genes as potential markers to predict the concentration of titratable acidity of cider apples. An initial target population of 308 accessions were identified and 158 accessions were assessed in 2017 for external and internal fruit characteristics along with juice chemistry. As per the Long Ashton Research Station (LARS) cider apple classification system where apples with tannin concentration (measured with the Löwenthal Permanganate Titration method) greater than 2.0 g·L⁻¹ are classified as bitter, and those with a malic acid concentration greater than 4.5 g·L⁻¹ are classified as sharp, 29% of the 158 accessions would be classified as bittersweet, 13% bittersharp, 28% sweet (neither bitter nor sharp), and 30% sharp. In addition, the polyphenol composition of 14 genotypes was measured using ultra high-performance liquid chromatography. and indicated that the variation in polyphenol levels measured by the Folin-Ciocalteu assay was largely due to procyanidins and phloretin compounds. In Chapter 3, the

accessions phenotyped in the 2017 harvest season were also genotyped for the Ma1 and Q8 genes and fit to a linear model where measured titratable acidity was the response variable and the Ma, Q8 and combined Ma1 and Q8 genotypes were used as predictor variables in two separate models. An ANOVA indicates that the Ma1 gene was found to be a reliable predictor of fruit titratable acidity ($P = < 0.0001$), but the Q8 gene was not ($P = 0.48$). Combining both genes in the analysis was not a better predictor than using the Ma1 gene alone ($P = 0.27$). From there an estimated marginal means model, was able to demonstrate a series of non-overlapping 99% confidence intervals for the means titratable acidity concentrations of each Ma1 allele.

Additionally, an estimated marginal means test of the Ma1 alleles was able to accurately predict the acidity classification as per the $4.5 \text{ g}\cdot\text{L}^{-1}$ threshold for the LARS cider apple classification system. Thus, a gene-based classification system of cider apples is proposed, with the titratable acidity levels defined by the Ma1 allele thresholds of 5.26 and $7.82 \text{ g}\cdot\text{L}^{-1}$. The advantage of a genetically based classification system is that it could be used for marker assisted breeding and it would not be subject to horticultural, seasonal, or geographic variability. Together, these two projects defined procedures for rapid phenotyping and genotyping of germplasm collections for addressing the needs of the emerging hard cider industry. Additionally, the characterization of cider apples for juice quality identified 49 bittersweet and 21 bittersharp genotypes that should be further evaluated for their suitability to perform in commercial orchards.

BIOGRAPHICAL SKETCH

Nathan Wojtyna was born to Stephen Wojtyna and Lisa Carey-Michaud January 1994 and was also raised by his step-mother Denise Wojtyna and step-father Robert Michaud. As a child, Nathan Wojtyna was enamored with reading, in particular with books about the nature that came to life exploring the 300 acres of woods surrounding his home in Willington, Connecticut. After graduating from Edwin O. Smith High School in 2012, he attended the University of Connecticut where he majored in Horticulture and became passionate about applied industry research and added a double major in Resource Economics and minors in both Biotechnology and Environmental Economic Policy, graduating in 2016. Once he finished his studies at the University of Connecticut he moved to Ithaca, NY to conduct his master's research at Cornell University.

To teachers who inspire their students and sow seeds of curiosity that last a lifetime.

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CHAPTER 1

Introduction

In the United States (U.S.) there has been a dramatic increase in hard cider production in recent years, with an annualized growth rate of 50% between 2009 and 2014, and revenue totaling \$1.3 billion in 2016 (Brager and Crompton, 2017). While cider producers continue to expand production, only one-third of cider producers surveyed in 2013 and 2014 at the national cider conference reported producing apples (*Malus ×domestica* Borkh.) within their own operation (Peck and Miles, 2015). A 2018 survey conducted in New York State reported that the availability and price of cider-specific apples were the greatest concerns in the hard cider supply chain (Pashow, 2018). Research indicates that cider producers are willing to pay an average of \$0.75 kg⁻¹ of cider specific apples compared to \$0.39 kg⁻¹ for culinary apples. The price premium for high-tannin cider apples is primarily driven by a lack of supply for this specialized fruit (Petrillo, 2014).

While the U.S. is second in total apple production globally, there are qualitative differences between current commercially grown culinary apples and high-tannin cider apples (Copas, 2013; United States Department of Agriculture, 2016). Specialized cider apples can have 5-10 times more tannin (a group of polyphenols which are perceived to possess an astringent and/or bitter taste by humans) along with higher levels of acidity (perceived as sharp or sour by humans) compared to culinary apples. The same study also illustrates that cultivars with sufficient acidity and sugars

are available commercially, but those with high tannin levels are not commonly grown in the U.S. (Thompson-Witrick et al., 2014).

Cider producers utilize apple classification systems to indicate expected quality attributes, including total polyphenol, titratable acidity, and sugar concentrations for different cultivars. Cider classification systems originally utilized descriptive terms for cider apple attributes (i.e., sweet, sharp, bittersweet, or bittersharp) (Barker and Etle, 1910). These terms were eventually given specific concentrations of tannins, as measured by the Löwenthal Permanganate Titration method, and titratable acidity (measured in malic acid equivalents). It should be noted that cider apple classification systems are primarily used to define juice quality attributes and do not include other important aspects of cultivar selection, such as horticultural performance, productivity, or disease resistance. Indeed, there are cider apples cultivars with horticultural flaws including fire blight (*Erwinia amylovora*) susceptibility, biennial bearing, and overly vigorous vegetative growth. Modern orchard production systems can cost over \$80,000·ha⁻¹ to plant and establish in the first year (Farris et al., 2013); thus the poor field performance for some European cider apple cultivars can have a detrimental impact on the long term profitability of an orchard planting.

Classification	Acid ($\text{g}\cdot\text{L}^{-1}$)	Tannins ($\text{g}\cdot\text{L}^{-1}$)
Sharp	>4.5	<2.0
Bittersharp	>4.5	>2.0
Bittersweet	<4.5	>2.0
Sweet	<4.5	<2.0

Table 1: The Long Ashton Research Station cider apple classification scheme authored by B.T.P. Barker and John Ertle in 1910. The classes sharp, sweet, bittersweet, and bittersharp are still used in the U.K. and U.S. industries for classifying cider apples.

The emerging cider industry in the U.S. has largely adopted the use of the Long Ashton Research Station (LARS) method for classifying cider apples which was first formalized by B.T.P. Barker and John Ertle in 1910 (Barker and Ertle, 1910). (Table 1). However, the Barker and Ertle report does not specify how the titratable acidity and tannin concentration thresholds of $4.5 \text{ g}\cdot\text{L}^{-1}$ and $2.0 \text{ g}\cdot\text{L}^{-1}$ were determined. Subsequent research has shown that model ciders with a procyanidin (a type of polyphenol) concentration of $750 \text{ mg}\cdot\text{L}^{-1}$ or greater were perceived to be more bitter and statistically different from the model cider without any procyanidins; in addition, perceived bitterness and astringency increased with increased procyanidin concentration and polymerization (Symoneaux et al., 2014). The threshold for sensory detection of procyanidin concentrations reported by Symoneaux is lower than the $2.0 \text{ g}\cdot\text{L}^{-1}$ tannin threshold established by the LARS classification system, although the

tannin component to the LARS classifications system relies upon the Löwenthal Permanganate Titration method which measures all phenolics, rather than just procyanidins, in juice, which would account for the difference in reported sensory thresholds.

Cider apple categorization systems have also been developed in France and Spain. The French method is similar to the LARS classification system, in that it uses titratable acidity and tannin concentrations to categorize cider apples. Within this system, cider apples are divided into six official categories (English translation in parenthesis): *amère* (bitter), *douce amère* (bittersweet), *douce* (sweet), *acidulée* (subacid), *aigre* (sharp), and *aigre amère* (bittersharp) (Table 2) (Institut Français Des Productions Cidricoles, 2009).

Polyphenol Concentration (g·L ⁻¹ Tannic Acid)	3	<i>Amère</i>		<i>Agire Amère</i>
	2	<i>Douce Amère</i>		
		<i>Douce</i>	<i>Acidulée</i>	<i>Aigre</i>
		4.5	6.75	
		Titratable Acidity (g·L ⁻¹)		

Table 2: Organizational grid for the French cider apple classification system where polyphenol concentration and titratable acidity are the two components which define the 6 classification categories (Adapted from: Institut Français Des Productions Cidricoles (2009)).

The Spanish cider classification system originally contained six designated classification categories as follows: sweet (<4.85 g malic acid equivalents $\cdot L^{-1}$, <1.45 g tannic acid $\cdot L^{-1}$), bittersweet (<4.85 g malic acid equivalents $\cdot L^{-1}$, >1.45 g tannic acid $\cdot L^{-1}$), semiacid (4.85 - 6.56 g malic acid equivalents $\cdot L^{-1}$, <1.45 g tannic acid $\cdot L^{-1}$), semiacid-bitter (4.85 - 6.56 g malic acid equivalents $\cdot L^{-1}$, >1.45 g tannic acid equivalents $\cdot L^{-1}$), acid (6.56 g malic acid equivalents $\cdot L^{-1}$, <1.45 g $\cdot L^{-1}$ tannic acid), and acid-bitter (>6.56 g malic acid equivalents $\cdot L^{-1}$, >1.45 g tannic acid $\cdot L^{-1}$) (Alonso-Salces et al., 2004). In 2009, the Spanish government created a “designation of origin” for Asturian ciders during which they defined 22 “elite” cider cultivars in nine distinct

categories, but the polyphenol and acidity metrics they used are not specified (Ministerio De Agricultura, Pesca y Alimentacion, 2003).

Market scarcity of cider-specific apples in the U.S. mirrors, and is compounded by, a broader lack of apple cultivar diversity among large-scale apple production. In the U.S., apples were the most economically important orchard fruit from 1880 to 1945, yet the apple went from having the greatest orchard genetic diversity in the 19th century to one of the largest cultivated orchard species losses in the 20th century (Dolan, 2009). The stark decline of apple diversity was also monitored at this time through repeated survey work initiated by the botanist Liberty Hyde Bailey of Cornell University. In 1892, Bailey conducted a survey of plant nurseries in the U.S. and found 735 apple cultivars available for sale to growers (Bailey, 1922). Horticulturalist Granville Lowther repeated the same survey in 1910 and found a 46% reduction in the number of apples cultivars available for sale at nurseries. Robert F. Carlson, a Michigan horticulturalist, surveyed nurseries in 1970 and found that roughly 10% of the cultivars initially documented by Bailey in 1892 were still being propagated 78 years later (Carlson et al., 1970). Further research in 2007 by Susan Dolan counted apple cultivars available in the Stark Brothers Nursery Catalogs (Louisiana, MO) found that 95 cultivars were available in 1918, 33 cultivars were available in 1928, and 19 cultivars were available in 1935 (Dolan, 2009). More recent industry surveys have identified that 15 cultivars comprise 90% of the commercially grown cultivars within the U.S., increasing the risk of a genetic improvement bottleneck due to a low diversity of subset cultivars used within commercial production (Gross et al., 2014). Among these 15 cultivars, none are cider-specific

bittersweet or bittersharp apple cultivars. By comparison, 56% of the 425,000 tonnes of apples grown in the United Kingdom (U.K.) are used to make cider; and of those, 80% of are cider-specific apples while the remaining 20% are cull dessert and culinary apples (Mitchell, 2012).

The USDA-PGRU *Malus* germplasm collection in Geneva, NY is among the largest in the world with 5,004 unique field accessions and 1,603 seed accessions across 33 *Malus* species and 15 hybrid species (Volk et al., 2015). The USDA-PGRU *Malus* germplasm collection plays a key role in the preservation of over 90% of the most popular historic apple cultivars grown in the United States (Volk and Henk, 2016). The USDA-PGRU *Malus* germplasm collection contains many known cider apple genotypes and potentially others that possess the high-tannin characteristics desired by cider producers.

Data collected on accessions within the USDA-PGRU *Malus* germplasm collection is stored and accessible within the USDA Germplasm Resources Information Network (GRIN) Global database which contains phenotypic and genotypic data on 106 phenotypic descriptors across seven categories: “Chemical”, “Cytologic”, “Disease”, “Growth”, “Morphology”, “Phenology”, and “Production”. The phenotypic and genotypic data on the accessions collected within the GRIN (and accessible online via the GRIN Global database) is research driven but is not complete for every accession. The data presented in this thesis will contribute data about the cider apple accessions in the collection, which have not previously been a major research focus for the USDA.

Phenotyping projects within the USDA-PGRU *Malus* germplasm collections have varied in focus and scope, with some projects emphasizing the search for genetic resistances to *Venturia inaequalis* (apple scab), *Erwinia amylovora* (fire blight), and *Gymnosporangium juniperi-virginianae* (cedar apple rust) among specific species and cultivars available in the collection (Fazio et al., 2009). Whereas other studies have place emphasis on collecting observations of a wide-ranging cross-section of the USDA-PGRU *Malus* germplasm by investigating specific polyphenol compounds present in the fruit or leaf tissue for to better understand the polyphenolic diversity within the USDA-PGRU *Malus* germplasm collection (Gutierrez et al., 2018; Sugimoto et al., 2015). A constant focus in *Malus* germplasm research is the consistent search for germplasm or related genes which can be utilized to create new and improved cultivars for commercial culinary apple production. However, to my knowledge, there have been no attempts to target the USDA-PGRU *Malus* germplasm collection for desirable fruit quality characteristics for cider.

Within this thesis, I describe research projects aimed at 1) phenotyping (i.e., evaluating the fruit and juice quality) of a targeted population of accessions within the USDA-PGRU *Malus* germplasm collection and 2) testing the robustness of using both the Ma1 and Q8 genes as genetic predictors for apple acidity in cider germplasm. In Chapter 2, I discuss how I identified a target population of 308 accessions from the USDA-PGRU *Malus* germplasm collection, of which 158 were phenotyped in 2017. Fruit weight, diameter, red peel percent or yellow-green background color, flesh firmness, and cortex starch pattern index (a 1-8 scale based on potassium iodine

staining) was measured on three 15-apple subsamples from each accession. Apple juice was evaluated for: soluble solid, titratable acidity, total polyphenol, sucrose, glucose, fructose, and sorbitol concentrations. Fifteen accessions were then selected for polyphenol composition analyses via Ultra High-Performance Liquid Chromatography – Mass Spectrometry; accessions were selected from the top, middle and bottom thirds of measured total polyphenol concentrations. In Chapter 3, I describe the identification of Ma1 and Q8 genotypes for 159 accessions. These data were then correlated with the accession's titratable acidity values to determine the statistical robustness of the two genetic markers for identifying potential cider apple genotypes.

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CHAPTER 2

Characteristics of 158 Hard Cider Apple Genotypes in the USDA-PGRU Malus Germplasm Collection

Abstract

In the United States, there is a limited supply of apples (*Malus* × *domestica* Borkh. and other *Malus* species) that possess high concentrations of tannins (polyphenols that are perceived as bitter and/or astringent) and acidity (described as having a sharp taste) that are desired by cider producers. In order to increase cider apple production, a targeted population of 158 accessions in the United States Department of Agriculture-Plant Genetic Resources Unit (USDA-PGRU) *Malus* germplasm collection in Geneva, NY was evaluated for desirable cider apple characteristics. Fruit weight (133; 31.09-385.59 g), diameter (65.6; 31.3-123.3 mm) red peel percent (49.9; 0-100%) or yellow-green background color (2.49; 1-4), flesh firmness (64; 4.5-140 N), and cortex starch pattern index, a 1-8 scale based on potassium iodine staining, (7.74; 5-8) was measured on three 15-apple subsamples from each accession (mean; range of measured values from the sample population is shown in parenthesis). Apple juice was evaluated for: soluble solid concentration (12.3; 4.93-18.80 °Brix), titratable acidity (4.27; 2.89-18.47 g·L⁻¹ malic acid equivalents), and total polyphenol concentration (1.41; 0.39-4.86 g·L⁻¹ gallic acid equivalents). Greater total polyphenol concentration was found to largely be related to increased procyanidins and phloretin concentrations. Sucrose (40.93; 4.11-86.13 g·L⁻¹), glucose (21.0; 3.02-60.55 g·L⁻¹), fructose (63.6; 8.80-130.49 g·L⁻¹), and sorbitol

(10.4; .23-28.67 g·L⁻¹) were also been measured on the apple juice samples. As per the Long Ashton Research Station cider apple classification system where a tannin concentration greater than 2.0 g·L⁻¹ are classified as bitter and a titratable acidity concentration of 4.5 g·L⁻¹ separates apples as sweet or sharp, 29%, of the 158 accessions were bittersweet, 13% bittersharp, 28% sweet (neither bitter nor sharp), and 30% sharp. This research identified 49 bittersweet and 21 bittersharp genotypes that should be further evaluated for their potential use by the emerging hard cider industry.

1.0 Introduction

Although the U.S. has one of the largest and most sophisticated apple industries in the world, there is a minimal volume of high-tannin apples desired by cider producers (Petrillo, 2014). When surveyed about their purchasing criteria for obtaining apples (*Malus ×domestica* Borkh.), the availability of cider specific apples was the number one concern of New York's cider producers followed by the high price of the cider specific apples (Pashow, 2018). The price premium for tannic cider apples is primarily driven by a lack of supply for this specialized fruit and market data indicated cider producers were willing to pay an average of \$0.75·kg⁻¹ for cider specific apples compared to \$0.39·kg⁻¹ for culinary apples (Raboin and Miller, 2016). Surveys conducted in the Northwestern and Northeastern U.S. also report a price premium of up to double for cider specific apples (Becot et al., 2016; Northwest Cider Association et al., 2016). Clearly, there is a supply-demand imbalance for cider apples in the U.S.

While all apples contribute some sweetness, sharpness, and bitterness to ciders, specialized cider apples are reported to have 5-10 times more tannin (a bitterness and astringent group of polyphenols) along with higher levels of acidity (sharpness) compared to culinary apples (Thompson-Witrick et al., 2014). This same study also highlights that apples with high concentrations of acidity and sweetness are commercially available in the Mid-Atlantic region of the U.S. apples, while high tannin apple cultivars are difficult to source (Thompson-Witrick et al., 2014).

The United States Department of Agriculture Plant Germplasm Resources Unit (USDA-PGRU) *Malus* germplasm collection in Geneva, NY is among the largest in the world with 5,004 unique field accessions and 1,603 seeds accessions across 33 *Malus* species and 15 hybrid species (Volk et al., 2015). This is especially important considering that 15 cultivars make up 90% of the commercially harvested apples within the U.S. (Gross et al., 2014). The GRIN Global database provides online access to phenotypic and genotypic data on 106 phenotypic descriptors across seven categories in the USDA-PGRU *Malus* germplasm collection. Data categories include: “Chemical”, “Cytologic”, “Disease”, “Growth”, “Morphology”, “Phenology”, and “Production” (Postman et al., 2010). Phenotyping projects within the USDA-PGRU *Malus* germplasm collections have varied in focus and scope, with some projects emphasizing the search for genetic disease and insect resistance whereas, other studies have place emphasis on collecting observations of a wide ranging cross-section of the USDA-PGRU *Malus* germplasm by investigating specific polyphenol compounds present in the fruit or leaf tissue for to better understand the polyphenolic diversity within the USDA-PGRU *Malus* germplasm collection (Fazio et al., 2009; Gutierrez et

al., 2018; Sugimoto et al., 2015). To my knowledge, there have been no published reports that assess germplasm within the USDA-PGRU *Malus* germplasm collection for desirable fruit quality characteristics for cider.

Within this chapter I describe fruit (external and internal) and juice quality attributes for 158 accessions within the USDA-PGRU *Malus* germplasm collection. Additionally, the polyphenol composition of 14 accessions were compared to understand the specific compounds that are found in a subset of low, medium, and high tannin genotypes. The goal of this project was to characterize a targeted population of accessions in the USDA-PGRU *Malus* germplasm collection for desirable cider apple characteristics.

2.0 Materials and Methods

2.1 Targeted Accession Selection

Two hundred and forty-seven accessions *M. ×domestica* and *M.* species were identified through information provided by Drs. Gayle Volk, Thomas Chao, and Ian Merwin (G. Volk, I. Merwin, and C. Chao, personal communication). In particular, Dr. Volk has conducted a literature search for apple cultivars that were historically used in cider production. Additional accessions were identified by Drs. Chao and Merwin based on their personal expertise and familiarity with cider apple cultivars.

An additional 61 accessions were identified through two multi-stepped queries within the USDA GRIN Global database. The first query started sorted all 4,867 *Malus* accessions for “Fruit Flesh Flavor”, which is defined by an anchored scale containing: “Aromatic Standard: Jonagold”, “Aromatic, Sweet”, “Sweet Standard:

Fuji”, “Subacid Standard: McIntosh”, “Acid Standard: Puritan”, and “Astringent Standard: *M. coronaria*”. Only accessions which were categorized as “Astringent: Standard *M. coronaria*” progressed to the next step, which removed 4,029 of the 4,867 accessions. The remaining accessions were then sorted by “Fruit Size (g)” (harvested fruit weight) and all accessions in the “<50 g” category were removed because they would likely be too small for commercial cider apple production. This step removed 611 of the 838 accessions. An additional 170 accessions were removed due to a lack of available fruit weight data, leaving 56 accessions that I added to the list of potential cider apple accessions.

The second multi-stepped query sorted the 4,867 accessions by the parameter “Fruit Flesh Flavor”, then accessions listed as “Acid Standard: Puritan”, “Sweet Standard: Fuji”, and “Sub-acid Standard: McIntosh” were removed since these categories represented accessions within the GRIN Global database that did not possess acidity characteristics desirable for cider production. This step removed 4,846 of the 4,867 accessions. The remaining 21 accessions were then sorted by the “Fruit Size (g)” parameter and accessions less than 50 g were removed. This final step removed 16 of the 21 accessions, resulting in five accessions that I added to the list of potential cider apple accessions.

2.2 Study Location and Sampling Design

Weekly sampling trips from Ithaca, NY to the USDA-PGRU *Malus* germplasm collection in Geneva, NY (42°53’40.3” N, 77°00’23.8” W) were made from 15 Aug. to the 17 Nov. All accessions phenotyped in 2017 were grown on virus-

free ‘Malling 7’ (M.7 EMLA) rootstocks. Accessions ranged in age from 4-18 years old. The date each accession was planted can be found in the supplemental tables (Supplemental Table 1).

Each accession was harvested at a similar maturity based upon the starch pattern index (SPI) assay. Cortex SPI was determined by staining the stem-side of an equatorial cross-section of the apples with iodine solution (0.22% w/v iodine, 0.88% w/v potassium iodine) and visual rating, where 1 = 100% staining and 8= 0% staining of the cortex (Blanpied and Silsby, 1992). As each accession approached the expected harvest date, two separate apples per accession were tested in the field. When an accession reached a SPI rating of six or higher out of eight, the accession was harvested.

Fifteen apples were randomly selected from each sample tree, with the only criteria being to avoid collecting more than one apple from the same branch. The unique identifying Plant Introduction (P.I.) number given to each accession in the USDA-PGRU collection was used to track the samples throughout the post-harvest analyses.

Fruit was stored at 4 °C under ambient atmospheric gases for 1 to 4 weeks in a commercial cooler at the Cornell University Agricultural Experiment Station-Cornell Orchards facilities (Ithaca, NY) before fruit analysis. The 15 fruit were then divided into three groups of five apples, as per Evans et al. (2012). Our sampling scheme, albeit without biological replicates, is similar to other *Malus* germplasm studies that have utilized the collection (Guo et al., 2016; Ma et al., 2015) and follows the

phenotyping guidelines developed by the RosBREED apple genetic breeding initiative (Evans et al., 2012).

2.3 External Fruit Characteristics

The phenotypic metrics used to assess the external fruit characteristics were based on established phenotyping guidelines developed by the RosBREED apple genetic breeding initiative (Evans et al., 2012). Fruit cracking was evaluated on an anchored 1-4 scale for each apple harvested, where 1 = no fruit cracking and 4 = severe fruit cracking. Fruit sunburn was also evaluated on an anchored 1-4 scale for each apple harvested, where 1 = indicated no sunburn present on the fruit and 4 = indicated severe fruit sunburn.

The percent surface area covered with a red blush was visually estimated from 0-100% for apples with a red peel. Apples with a green or yellow background peel color were scored on a 1-5 scale using the RosBREED system for green background color where 1 = yellow-green and 5 = dark green (Evans et al., 2012). Each apple was weighed and fruit diameter (mm) was measured at the widest point between the stem and calyx.

2.4 Internal Fruit Characteristics

At two opposite locations along the equator of each apple, the peel was removed, and fruit firmness was measured in Newtons (N) with a penetrometer fitted with a cylindrical 12.7 mm diameter tip (Fruit Texture Analyzer, GÜSS Manufacturing, South Africa). Cortex SPI was determined as described above.

2.5 Juice Extraction

The calyx half of each 5-apple pooled subsample was milled and pressed in a Norwalk 280 juicer (Bentonville, AR). Upon completion of juicing each subsample, the juice was stirred and aliquoted into 3-15 mL and 3-50 mL centrifuge tubes (VWR, Randor, PA). All juice extracting equipment was rinsed with water between samples to minimize cross-contamination. Juice samples were stored at -80 °C until the juice chemistry analyses were performed.

2.6 Juice Chemistry

Samples were thawed to room temperature, vortexed for 10 s, and then centrifuged for 8 s at 500 g. Soluble solids concentration (SSC) was measured using a PAL-1 BLT digital pocket refractometer (Atago U.S.A., Inc., Bellevue, WA) and reported as °Brix. Titratable acidity was measured by titrating a 5 mL juice aliquot against a standardized 0.1 N NaOH solution to an end-point of pH 8.1 with an Metrohm 809 Titrando autotitrator (Metrohm AG, USA). Total polyphenols, sucrose, D-fructose, D-glucose, and sorbitol were all measured using a Spectramax 384 Plus microplate spectrophotometer and SoftMax Pro 7 Microplate Data Acquisition & Analysis Software (Molecular Devices, San Jose, CA).

Total polyphenol concentration was measured using the Folin-Ciocalteu method (Waterhouse, 2012). Standards for the total polyphenol concentration were generated using an eight-point standard curve with gallic acid from 0-1.0 g·L⁻¹. The reaction was carried out in a Cellistar 96-well microplate (Greiner bio-one, Monroe, NC). The reaction mixture consists of 7.5 µL of standard or sample, 28.9 µL of water, and 90.9

μL of Folin-Ciocalteu reagent (Sigma-Aldrich, St. Louis, MO). Three minutes after the addition of the FC reagent, 72.7 μL of 7% (v/w) sodium carbonate (Sigma Aldrich, St Louis, MO) solution was added. The reaction mixture was incubated for 2 h at 21 °C in the dark. Samples were measured at 765 nm and total polyphenol content was determined by linear regression from the standard curve plot and multiplying by the dilution factor (for accessions which needed to be diluted to fit within the linear range). Tannic acid standards (Tannic Acid Powder, ACS-Alfa Aesar, Ward Hill, MA) were then analyzed via the Folin-Ciocalteu assay. A conversion factor between the Löwenthal Permanganate Titration method (0.005 M solution titrated against 1 mL of juice sample) and the Folin-Ciocalteu method was developed by creating a standard curve plotting the concentration of gallic acid standards from the Folin-Ciocalteu method compared to the tannic acid equivalents values measured against the same standards via the Löwenthal Permanganate Titration method to identify the linear relationship between the assays. Ten juice samples were measured for total polyphenol concentration via Löwenthal Permanganate Titration method and the Folin-Ciocalteu method to verify the linear relationship.

Sugars were measured using the Sucrose/D-Fructose/D-Glucose Assay Kit (K-SUFRG) (Megazyme International, Ireland). The amount of D-glucose present in the juice samples was determined by a two-stage reaction. Hexokinase catalyzes the phosphorylation of D-glucose by adenosine -5'-triphosphate (ATP) into glucose-6-phosphate (G-6-P) and ADP. In the presence of the enzyme glucose-6-phosphate dehydrogenase (GP-DH), the G-6-P (from step 1) is then oxidized by nicotinamide-adenine dinucleotide phosphate (NADP^+) to form a reduced nicotinamide-adenine

dinucleotide phosphate (NADPH). The NADPH from is stoichiometrically related to the amount of D-glucose present in the sample and is measured by an increase in absorbance at 340 nm (Knust et al., 1988).

The D-fructose assay was performed on the same samples immediately after the D-glucose assay as the subsequent difference in absorbance at 340 nm is also stoichiometrically related to the amount of D-fructose in the sample. Within the D-fructose assay hexokinase also catalyzes the phosphorylation of D-fructose to fructose-6 phosphate (F-6-P) and ADP by ATP. The F-6-P is then converted to gluconate-6-phosphate which reacts in turn with NADP⁺ forming G-6-P and NADPH which was again measured at 340 nm and the increase in absorbance indicates the amount of D-fructose present (Beutler, 1988).

Sucrose concentration was determined by the hydrolysis of sucrose by β -fructosidase (invertase) into the respective monomers D-glucose and D-fructose. The sucrose content is calculated from the difference in D-glucose concentration between hydrolyzed and non-hydrolyzed juice samples (Outlaw and Mitchell, 1988).

Sorbitol was measured using the D-Sorbitol/Xylitol Assay Kit (K-SORB) (Megazyme International, Ireland). Sorbitol is oxidized by nicotinamide-adenine dinucleotide (NAD⁺) to D-fructose in the presence of sorbitol dehydrogenase (SDH) with the parallel formation of NADH. In the presence of added diaphorase, NADH to reduces iodonitrotetrazolium (INT) into the NADNAD⁺ into an INT-formazan compound which is stoichiometrically related to the amount of D-sorbitol in the sample. The INT-formazan is measured by an increase in absorbance at 492 nm (Knust et al., 1988).

2.7 Polyphenol Composition

All 158 accessions were ordered by the total polyphenol concentration and accessions from each of the top, middle, and bottom range were selected for polyphenol composition analysis via Ultra High-Pressure Liquid Chromatography-Mass Spectrometry (UHPLC-MS) at the Cornell University Metabolomics Facility in Ithaca, NY (Table 3).

Table 3: The 14 accessions, harvested in the 2017 harvest season at the USDA-PGRU in Geneva, NY, identified for polyphenol composition analysis and measured totally polyphenol concentration. The 14 accessions were selected to represent the top, middle, and bottom of the accessions with regard to the total polyphenol concentrations measured using the Folin-Ciocalteu method.

PI Number	Cultivar Name	Total Polyphenols Gallic Acid Eq. (g·L ⁻¹)
588943	Liberty	0.56
590184	Golden Delicious	0.63
589690	Le Bret	0.95
589703	Kingston Black	1.08
588872	Northern Spy	1.19
589682	Improved Lambrook Pippin	1.37
589650	Ellis Bitter	1.44
589693	Stembridge Jersey	2.51
589219	Kola (<i>Malus coronaria</i>)	2.74
589614	Zapta	2.97
158731	Bramtot	3.53
594108	Medaille d'Or	4.29
162732	Launette	4.44
657039	Kaz 95-08-06 (<i>Malus serversii</i>)	4.86

For each of the 14 accessions, the three juice subsamples were analyzed separately and then averaged together. Samples were thawed to room temperature and then 100 µL was dried down and reconstituted in 90 mL of 1% acetic acid in methanol. Samples were then centrifuged at 17,000 g, for 5 min at 4 °C and then injected into a Thermo Vanquish UHPLC (Thermo Scientific, Waltham, MA) with an Accucore Vanquish C18+, 1.5 µm column (2.1 mm i.d. x 100 mm) and a column

temperature of 45 °C coupled to a Q Exactive™ Hybrid Quadrupole-Orbitrap High Resolution Mass Spectrometer (Thermo Fisher Scientific, San Jose, CA). Quality control samples were made from a pool of all samples and run at the beginning, middle, and end of the run sequence to normalize the chromatograms.

Sulfadimethoxine (5 ppm) was added to the samples as an internal standard. The UHPLC analysis was performed with an injection volume of 2 µL and binary elution was carried out with a 0.1% formic acid in water (phase A) and a 0.1% formic acid in acetonitrile (ACN, phase B). Linear gradient elution was performed with a flow rate of 320 µL·min⁻¹ as follows: 0-20 min (1% B), 2.0-20 min (1-30% B), 20-22 min (30-95% B), 22-22.5 min (95-1% B), and 22.5-25 min (1% B). The UHPLC effluent was analyzed by negative ionization electrospray (ESI) at a voltage of 3.5 kV, a maximum injection time of 100 ms, and a scan time of 67-1005 *m/z* along with a nitrogen sheath gas flow rate of 50 (AU), auxiliary gas flow rate of 10 (AU), and a sweep gas flow rate of 1 (AU). Capillary temperature was 275 °C and the auxiliary gas temperature was 375 °C. The acquired data set, composed of full MS and data-dependent MS-MS raw files, was processed using Compound Discoverer 2.1 and Xcalibur™ software (Thermo Scientific, Waltham, MA). An untargeted metabolomics workflow with putative identification through in-house mass list and databases. ChemSpider and mzCloud were used for processing the raw data. The software parameters for alignment were 5 ppm mass tolerance for the adaptive curve model and 0.5 min maximum shift for alignment. The software parameters for detecting unknown compounds were 5 ppm mass tolerance for detection, 30% intensity tolerance, 3 for the sensitivity and noise threshold, and 2×10^6 minimum peak height.

2.8. Statistical Analysis

Internal and external fruit quality and juice chemistry data were analyzed using R-Studio version 1.1.422 (R Studio, Boston, MA). Linear models were generated to assess the relationship between country of origin or species and juice quality measurements (titratable acidity and soluble solids, total polyphenol, glucose, fructose, sucrose, and sorbitol concentrations), were used as the predictor variable and each juice chemistry characteristic measured was used as a response variable. Statistical significance ($P < 0.05$) of the linear models was determined by ANOVA. Principal component data analysis of the polyphenol composition data was performed using Simca P (Sartorius Stedim Biotech, Malmö, Sweden). The PCA was performed using MetaboAnalyst (Xia Lab McGill University, Montreal) (Xia and Wishart, 2016).

3.0. Results

3.1. Targeted Accession Selection

Three hundred and eight putative cider apple accessions were identified in the USDA *Malus* germplasm collection for their potential to be used by the U.S. cider industry. Of those, 58% (180 accessions) were sampled in 2017, and the remaining 42% (128 accessions) were unable to be sampled due to fruit unavailability. An additional 12 accessions rotted in storage prior to juice extraction and eight other accessions were not included in the data analysis due to incomplete phenotypic data. One accession ‘Empire’ was removed from the data due to an accession sampling error or a mix up in fruit processing. Thus, 158 of the 308 accessions (52%) were analyzed in 2017.

3.2. External Fruit Characteristics

The mean value for fruit cracking score was a 1.01 ± 0.023 (1-1.17) while the mean value for fruit sunburn score was 1.01 ± 0.025 (1-1.10) and the data for both parameters was skewed towards 1.00 representing no cracking or fruit sunburn (Figs. 1-2). Peel red blush percentage had a mean of $49 \pm 22\%$ (1-93%) while peel green background color had a mean of 2.48 ± 1.29 (1-5) and the data distribution for both measurements followed a normal distribution (Figs. 3-4). Fruit weight had a mean of 135.2 ± 61.2 g (31.1-385.6 g) while the mean fruit diameter was 65.6 ± 2.4 mm (31.3-123.3 mm) and the data distribution for both fruit weight and fruit diameter followed a normal distribution (Figs. 5-6) (data presented as mean $\pm$ standard deviation with the range of measured values shown in parenthesis).

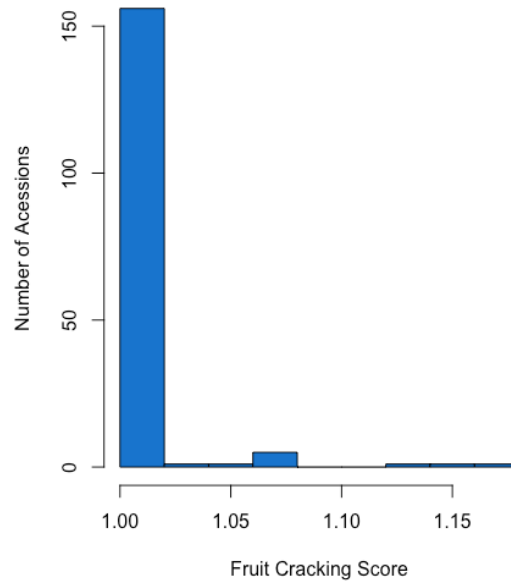


Figure 1: Histogram representing the fruit cracking data on 158 accessions harvested in 2017 at the USDA-PGRU Malus germplasm collection in Geneva, NY. The x-axis represents external fruit cracking score where 1=no fruit cracking and 4=severe fruit cracking.

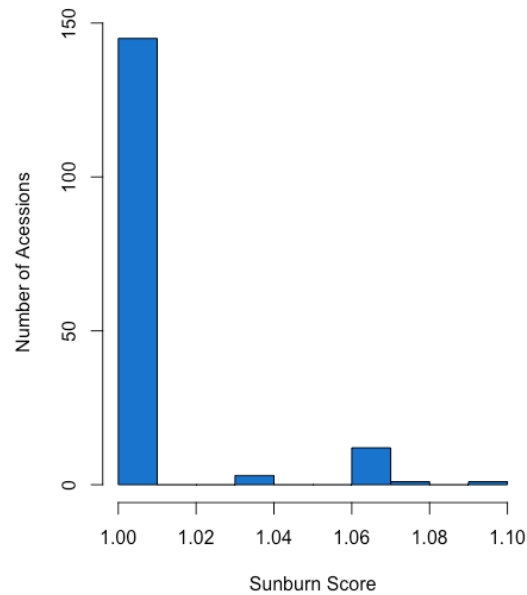


Figure 2: Histogram representing the fruit sunburn data on 158 accessions harvested in 2017 at the USDA-PGRU Malus germplasm collection in Geneva, NY. The x-axis represents external fruit sunburn score where 1=no fruit sunburn and 4=severe fruit sunburn.

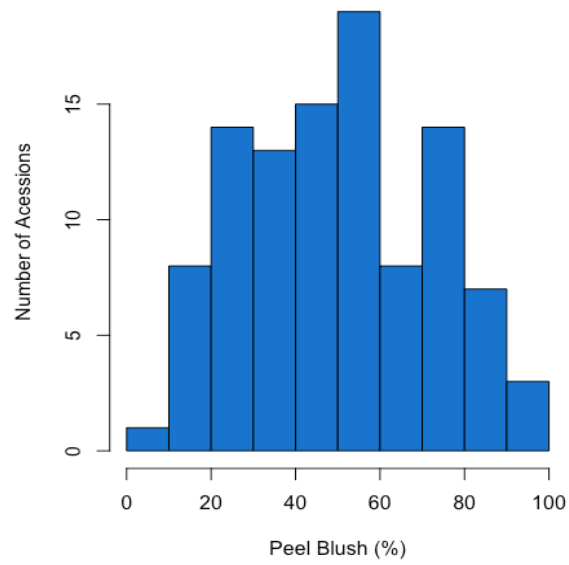


Figure 3: Histogram representing the percent peel blush data on 168 accessions harvested in 2017 at the USDA-PGRU *Malus* germplasm collection in Geneva, NY. The x-axis represents observed red peel blush (%).

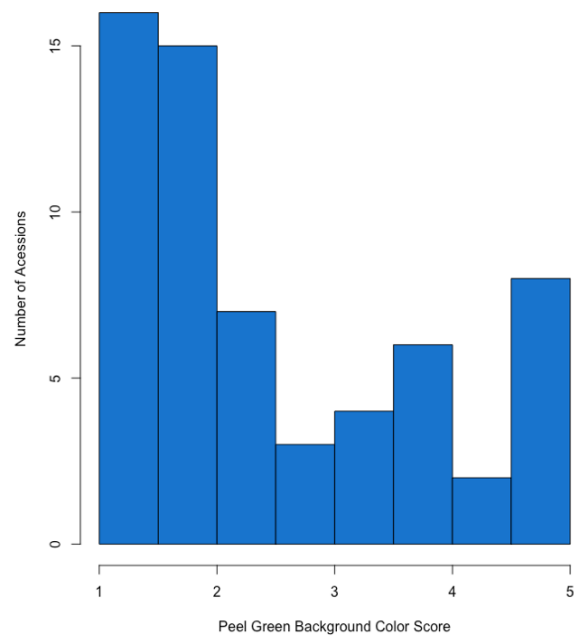


Figure 4: Histogram representing the peel green background color data on 158 accessions in 2017 at the USDA-PGRU *Malus* germplasm collection in Geneva, NY. The x-axis represents the peel green background color score where 1=yellow-green and 5=dark green.

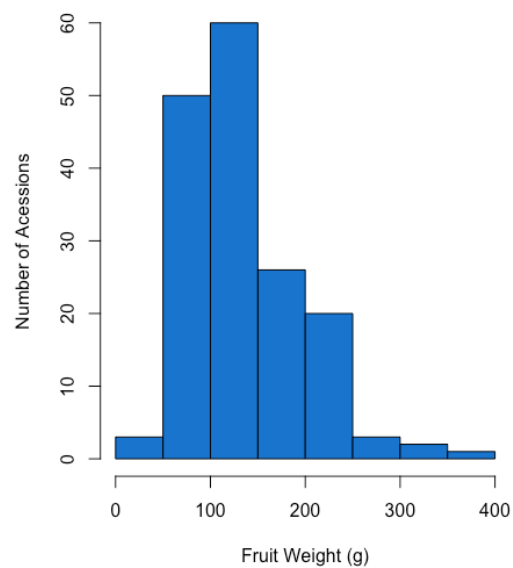


Figure 5: Histogram representing the fruit weight (g) data on 158 accessions harvested in 2017 at the USDA-PGRU Malus germplasm collection in Geneva, NY. The x-axis represents measured fruit weight (g).

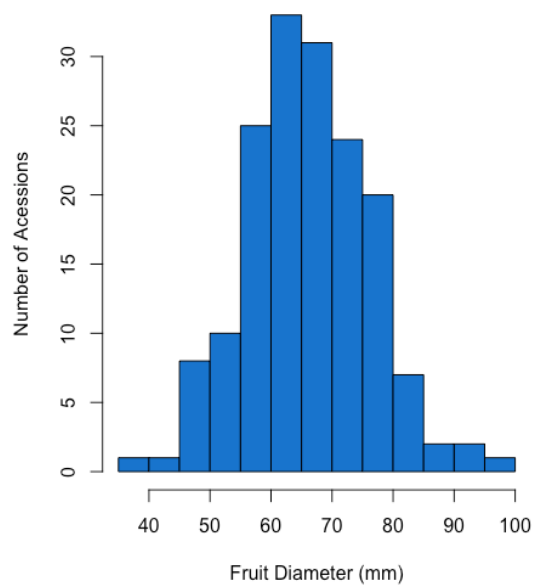


Figure 6: Histogram representing the fruit diameter data on 158 accessions harvested in 2017 at the USDA-PGRU Malus germplasm collection in Geneva, NY. The x-axis represents measured fruit diameter (mm).

3.3. Internal Fruit Characteristics

The cortex starch pattern index had a mean of 7.74 ± 0.05 (5-8) and the data was skewed towards the 8.0 score meaning that the starch had been fully hydrolyzed to sugars in most of the tested samples (Fig. 7). The mean fruit flesh firmness was 64.04 ± 21.94 N (19.74-136.94 N) and the data followed a normal distribution (Fig. 8) (data presented as mean $\pm$ standard deviation with the range of measured values shown in parenthesis).

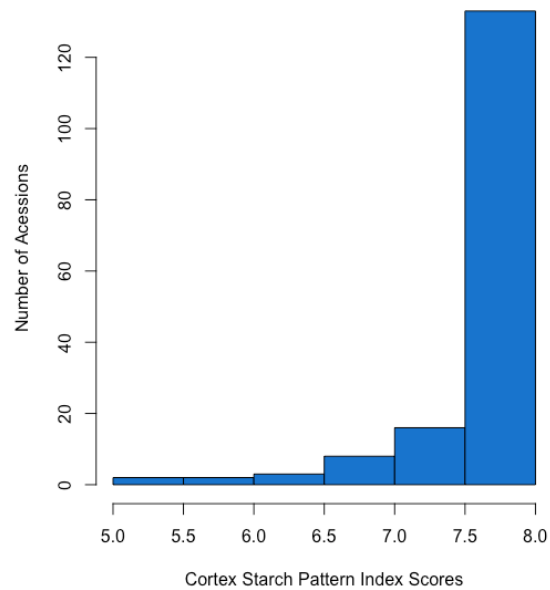


Figure 7: Histogram representing the cortex starch pattern index scores on 158 accessions harvested in 2017 at the USDA-PGRU Malus germplasm collection in Geneva, NY. The x-axis represents measured cortex starch pattern index scores where 1=no starch has been hydrolyzed and 8=all starch has been hydrolyzed.

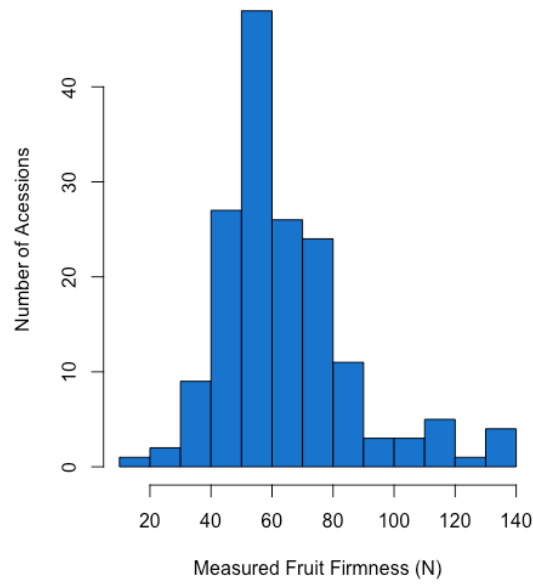


Figure 8: Histogram representing the fruit firmness (N) data on 158 accessions harvested in 2017 at the USDA-PGRU Malus germplasm collection in Geneva, NY. The x-axis represents measured fruit firmness data (N).

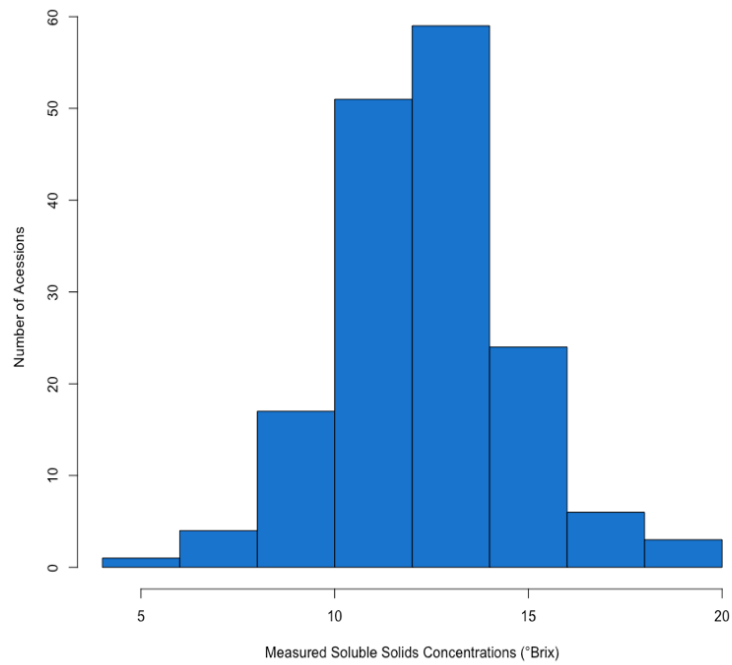


Figure 9: Histogram representing the measured soluble solids concentration data on 168 accessions harvested in 2017 at the USDA-PGRU Malus germplasm collection in Geneva, NY. The x-axis represents measured soluble solids concentrations (°Brix).

3.4. Juice Chemistry

The mean soluble solids content was 12.31 ± 2.3 °Brix (4.93-18.80 °Brix) and was normally distributed across the sample population (Fig. 9). Titratable acidity was 4.27 ± 2.44 g·L⁻¹ (2.8-18.47 g·L⁻¹) and the mean total polyphenols values were 1.41 ± 0.85 g·L⁻¹ (0.25-4.86 g·L⁻¹); the data for both variables were slightly skewed towards lower titratable acidity and total polyphenol concentrations (Figs 10-11). The mean glucose value of 21.02 ± 8.44 g·L⁻¹ (3.02-60.55 g·L⁻¹). The mean fructose value was 63.60 ± 18.11 g·L⁻¹ (8.80-130.49 g·L⁻¹). Sucrose was measured in the samples and had a mean value of 40.93 ± 17.69 g·L⁻¹ (4.11-86.13 g·L⁻¹). Sorbitol was also measured in the samples and the data had mean of 11.30 ± 5.37 g·L⁻¹ (0.23-28.67 g·L⁻¹) (data presented as mean $\pm$ standard deviation with the range of measured values shown in parenthesis). The data for glucose, fructose, sucrose and sorbitol concentration were all normally distributed (Figs. 12-15).

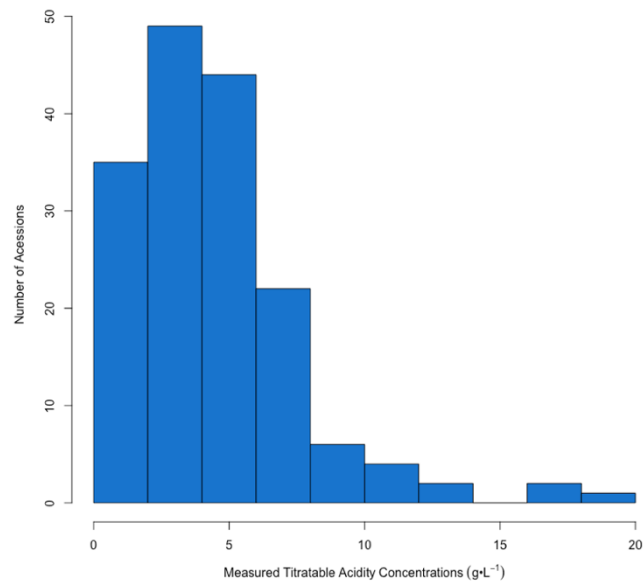


Figure 10: Histogram representing the titratable acidity concentration data on 158 accessions harvested in 2017 at the USDA-PGRU Malus germplasm collection in Geneva, NY. The x-axis represents measured titratable acidity concentration.

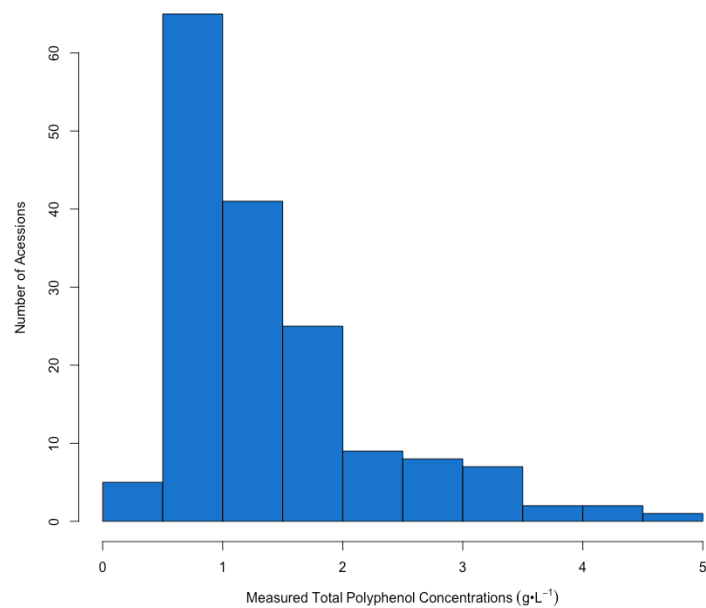


Figure 11: Histogram representing the total polyphenol concentration data on 158 accessions harvested in 2017 at the USDA-PGRU Malus germplasm collection in Geneva, NY. The x-axis represents measured total polyphenol concentrations as measured via the Folin-Ciocalteu spectrophotometric assay.

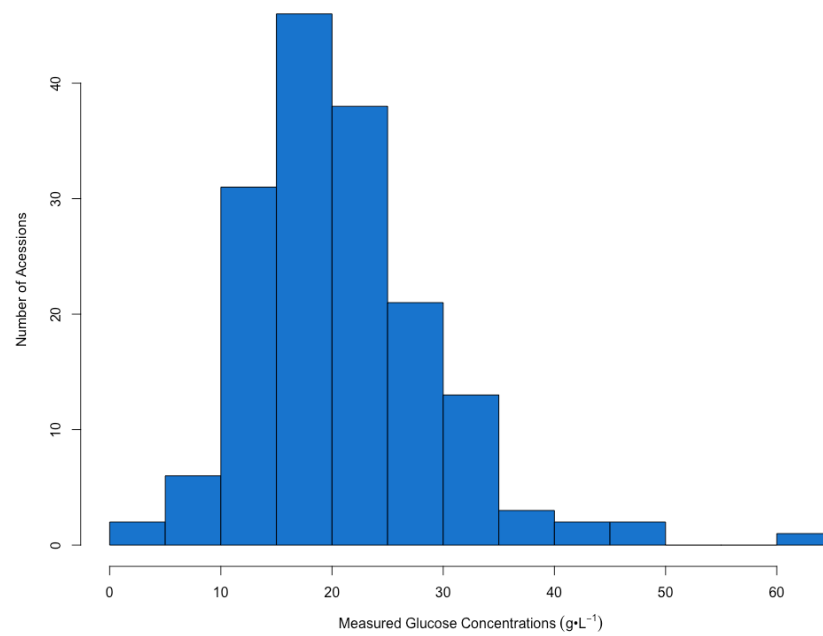


Figure 12: Histogram representing the measured glucose concentration data on 158 accessions harvested in 2017 at the USDA-PGRU Malus germplasm collection in Geneva, NY. The x-axis represents measured glucose concentrations.

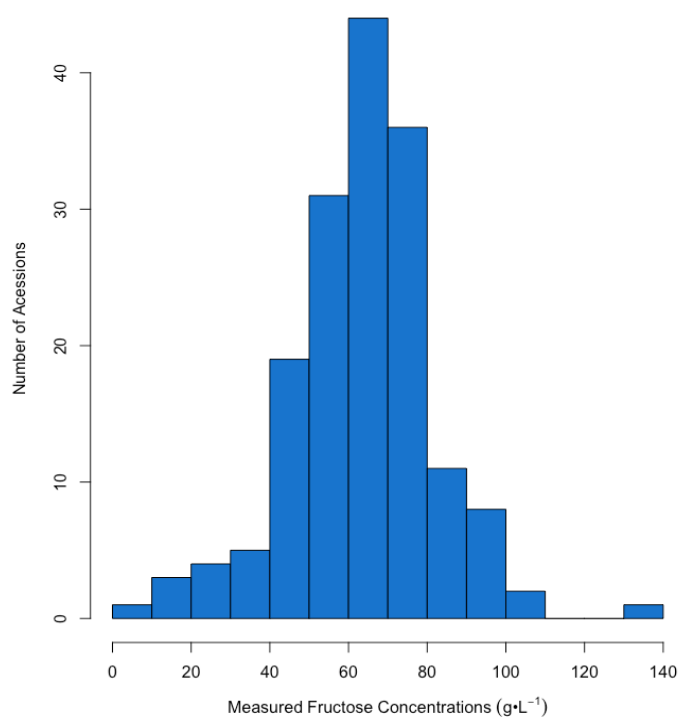


Figure 13: Histogram representing the measured fructose concentration data on 158 accessions harvested in 2017 at the USDA-PGRU Malus germplasm collection in Geneva, NY. The x-axis represents measured fructose concentrations.

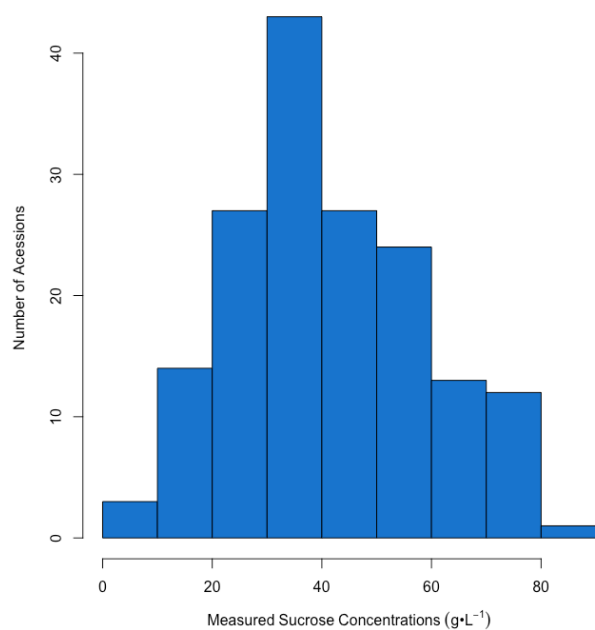


Figure 14: Histogram representing the measured sucrose concentration data on 158 accessions harvested in 2017 at the USDA-PGRU Malus germplasm collection in Geneva, NY. The x-axis represents measured sucrose concentrations.

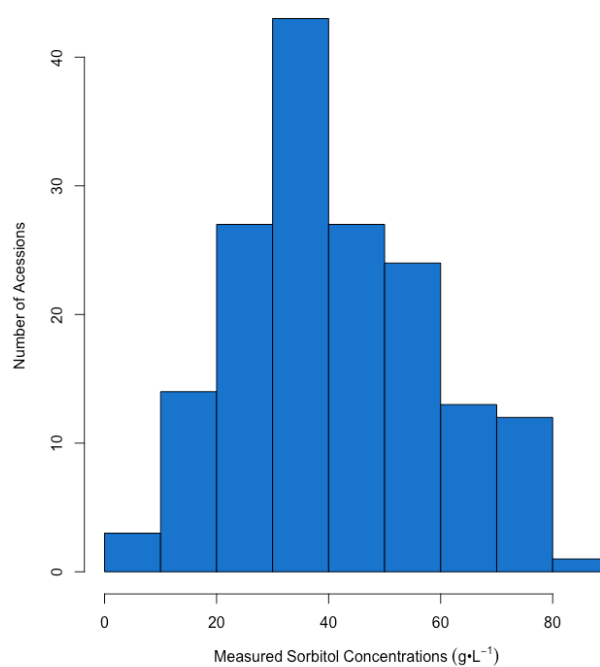


Figure 15: Histogram representing the measured sorbitol concentration data on 158 accessions harvested in 2017 at the USDA-PGRU Malus germplasm collection in Geneva, NY. The x-axis represents measured sorbitol concentrations.

The accessions with the five highest and lowest concentrations of soluble solids concentration, total polyphenols concentration, titratable acidity, glucose, fructose, sucrose, and sorbitol concentrations are presented in Table 4.

Table 4: The accessions with the highest and lowest concentrations of soluble solids concentration, titratable acidity in addition to total polyphenol, glucose, fructose, sucrose and sorbitol concentration. The measured values are noted within the parenthesis. Accessions were sampled in 2017 from the USDA-PGRU Malus germplasm collection in Geneva, NY.

Rank	Soluble Solids Concentration (°Brix)	Total Polyphenol Concentration (g·L ⁻¹ Gallic Acid Equivalent)	Titratable Acidity (g·L ⁻¹ Malic Acid Equivalent)	Glucose Concentration (g·L ⁻¹)	Fructose Concentration (g·L ⁻¹)	Sucrose Concentration (g·L ⁻¹)	Sorbitol Concentration (g·L ⁻¹)
1	'Blue Pearmain' (18.8)	'Kaz 95-08-06' (4.8)	'Kola' (18.6)	'Launette' (47.9)	'Red Ralls' (108.5)	'Stembridge Jersey' (86.1)	'Bramtot' (28.7)
2	'Bramtot' (18.7)	'Launette' (4.4)	'Kaz 95 08-06' (17.0)	'Red Ralls' (47.2)	'Improved Lambrook Pippin' (101.5)	'PRI 1744-1' (79.3)	'Nehou' (27.8)
3	'Launette' (18.2)	'Medaille d'Or' (4.3)	'Zapta' (16.9)	'Fillbarrel' (43.1)	'Twistbody Jersey' (97.1)	'Rott Jampple' (78.9)	'Launette' (27.3)
4	'GMAL 3232.g1' (17.5)	'Vagnon Ascher' (3.8)	'Eda' (13.8)	'Weidners Goldreinette' (42.8)	'Bella de Jardins' (95.6)	'Golden Delicious' (76.9)	'Binet Blanc Dore' (26.8)
5	'Cornish Aromatic' (16.9)	'Bramtot' (3.5)	'Forest King' (13.7)	'Belle de Crollon' (37.9)	'Jouveaux' (94.3)	'Reinette Thouin' (76.6)	'Margil' (25.7)
154	'Brown's Apple' (8.3)	'Court Pendu Rose' (0.6)	'Stembridge Jersey' (1.3)	'Wotanda' (8.7)	'Ellis Bitter' (25.6)	'Michelin' (11.3)	'Red Field' (4.37)
155	'Court Royal' (8.10)	'Reinette Jaegers' (0.5)	'American Forestier' (1.2)	'Pomme Raisin' (6.9)	'William Crump' (21.7)	'Le Bret' (10.2)	'GMAL 2996.c1' (3.8)
156	'Wotanda' (8.0)	'Reinette Jamin' (0.5)	'Ellis Bitter' (1.1)	Pethyre (6.2)	'Wotanda' (19.7)	'GMAL 2996.cl' (10.2)	'Nanot' (3.5)
157	'Nanot' (8.0)	'Pethyre' (0.5)	'Taylor's' (0.9)	'Brown's Apple' (4.0)	'Brown's Apple' (15.5)	'Nanot' (6.8)	'Twistbody Jersey' (3.0)
158	'Wamdesa' (7.1)	'Edelroter' (0.4)	'Freyberg' (0.99)	'GMAL 2996.c1' (3.0)	'GMAL 2996.c1' (8.8)	'Brown's Apple' (4.1)	'Brown's Apple' (0.2)

The measured total polyphenol concentration was converted from gallic acid equivalents (via the Folin-Ciocalteu assay) to tannic acid equivalents (via the Löwenthal permanganate Titration method) through an internal lab conversion factor of 1.6×. This conversion allows for the evaluated accessions to be classified as per the Long Ashton Research Station cider apple classification method that has been adopted by the U.S. cider industry. Of the 158 accessions, 29% would be classified as bittersweet, 13% as bittersharp, 28% as sweet (neither bitter nor sharp), and 30% as sharp (Figure 16).

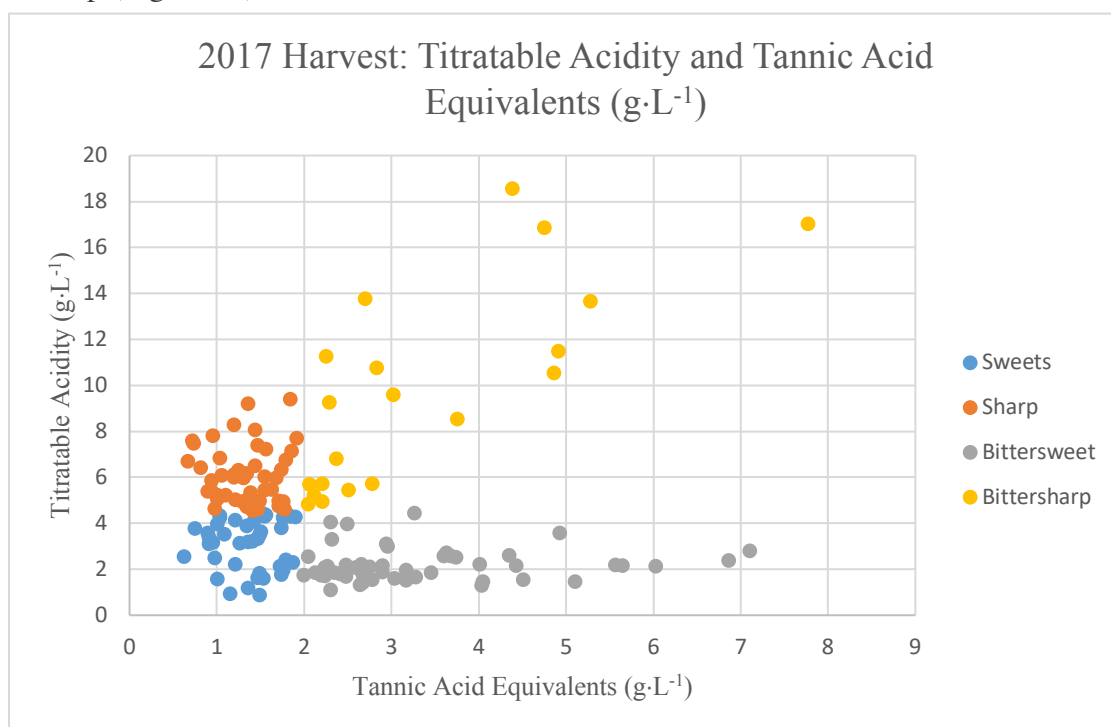


Figure 16: Scatterplot displaying the tannic acid equivalents and titratable acidity of all 158 accessions harvested in 2017 from the USDA-PGRU *Malus* germplasm collection in Geneva, NY. The accessions have also been categorized and color coded as per the LARS cider apple classification system.

The county of origin for the accession was not found to not be a statistically significant predictor for, soluble solids concentration ($P = 0.22$), titratable acidity ($P = 0.12$), total polyphenol concentration ($P = 0.15$), glucose concentration ($P = 0.23$),

fructose concentration ($P = 0.70$), sucrose concentration ($P = 0.48$), or sorbitol concentration ($P = 0.71$).

The accession's species was not found to be a predictor for soluble solids concentration ($P = 0.21$), total polyphenol concentration ($P = 0.12$), glucose concentration ($P = 0.29$), fructose concentration ($P = 0.07$), sucrose concentration ($P = 0.45$), or sorbitol concentration ($P = 0.71$). However, accession species did predict titratable acidity ($P < 0.0001$).

3.5. Polyphenol Composition

Of the 328 compounds identified from the polyphenol composition analysis via UHPLC-MS, 49 compounds (including isomers) were previously reported in the literature (Guo et al., 2016; Guyot et al., 2003; Hemingway and Laks, 1992; Mangas et al., 1999; Oszmianski and Lee, 1991; Ramírez-Ambrosi et al., 2015; Thompson-Witrick et al., 2014).

A heatmap depicting the normalized concentrations of the 25 most significant compounds [based upon P-values of statistical significance derived from a post hoc Tukey's honest significance difference (HSD) test] for each putative polyphenolic compound identified within the sample tested was generated (Figure 17). A dendrogram on the top of the figure depicts the clustering of samples via the 25 compound concentrations, while a dendrogram on the left side depicts the clustering of the compounds by their presence in the samples. 'Ellis Bitter', 'Kingston Black', 'Improved Lambrook Pippin', 'Le Bret', 'Golden Delicious', 'Northern Spy', and

‘Liberty’ possess relatively low levels of procyanidins and phloretins compared with ‘Launette’, ‘Medaille d’Or’, ‘Kaz 95-08-06’, ‘Bramtot’, and ‘Stembridge Jersey’.

Conversely, ‘Golden Delicious’, ‘Empire’, ‘Ellis Bitter’, ‘Le Bret’, ‘Improved Lambrook Pippin’, ‘Northern Spy’, and ‘Kingston Black’ exhibited higher relative concentrations of hydroxycinnamic acids, such as cinnamic and benzoic acids compared with ‘Launette’, ‘Medaille d’Or’, ‘Kaz 95-08-06’, ‘Bramtot’, and ‘Stembridge Jersey’. The data also indicated a cluster of compounds unique to both non-*M. ×domestica* accessions ‘Zapta’ (*M. hybrid*) and ‘Kola’ (*M. coronaria*), which were a mix of flavanols and hydroxycinnamic acid compounds.

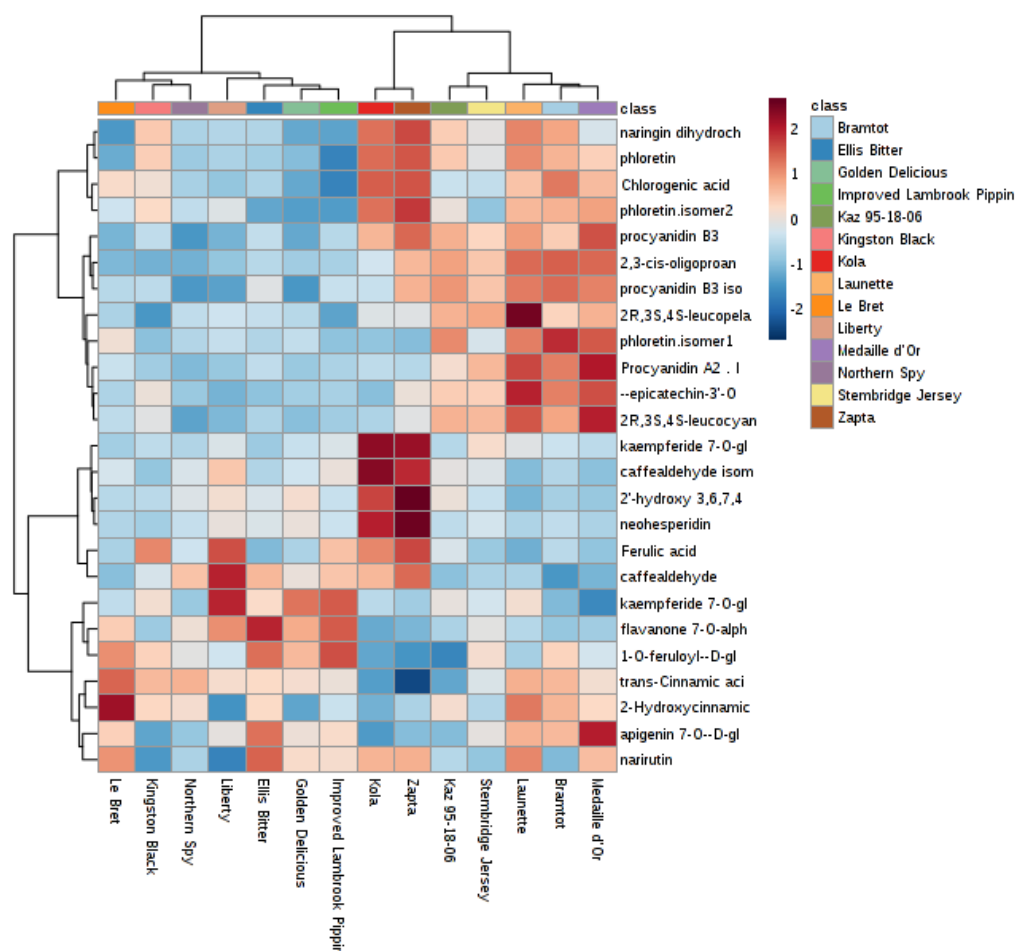


Figure 17: Heatmap of the normalized concentrations of the 25 most significant polyphenol compounds (based on p-values) for 14 accessions harvested from the USDA-PGRU Geneva NY collection during the 2017 harvest season. The heatmap also includes dendrograms on the top and left side of the figure representing the hierarchical clustering of the 14 accessions and 25 compounds respectively.

The principal component analysis revealed three distinct groupings of the 14 measured accessions (Figure 18). One group includes the low total polyphenol concentration accessions (left). A second group includes the high total polyphenol concentration accessions (upper right). The third group includes, ‘Medaille d’Or’, ‘Launette’, and ‘Kaz 95-08-06’, which also had the greatest total polyphenol concentrations.

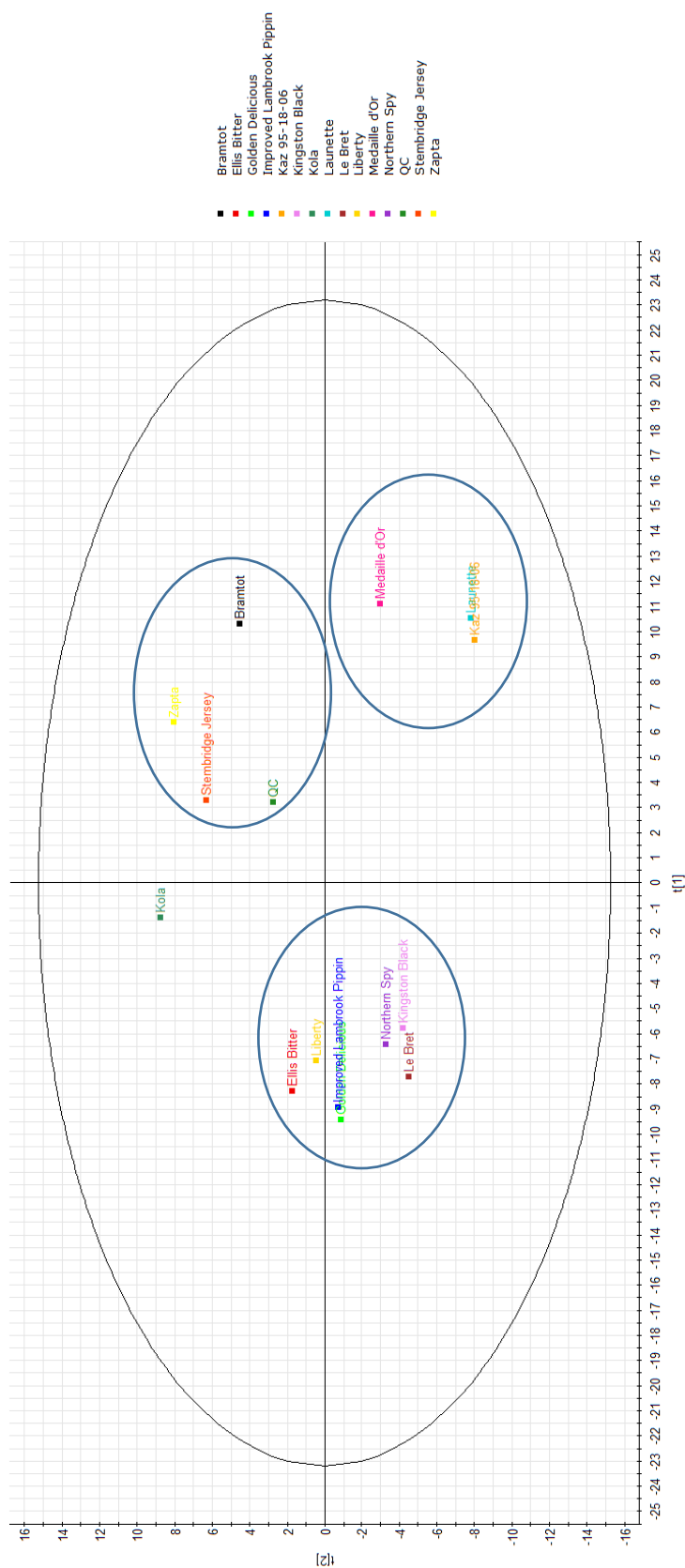


Figure 18: Principal component analysis cross-referencing the average of three observations for 14 accessions harvested from the USDA-PGRU in Geneva NY during the 2017 harvest season. Within these two scored indices, points with the relative distances indicate three grouped clusters among the 14 accessions evaluated for normalized polyphenol composition.

4.0 Discussion

4.1. External and Internal Fruit Characteristics

The external fruit characteristics of fruit cracking and fruit sunburn are important to consider when assessing the horticultural potential of a cultivar. Fruit cracking and sunburn can create the increased potential for secondary infections, such as black rot (*Botryosphaeria obtusa*) (Troncoso-Rojas and Tiznado-Hernández, 2014). The distribution of data for both fruit cracking and sunburn was skewed towards “No fruit cracking/sunburn present”. Severe fruit cracking and rot prevented sampling for ‘Royal Jersey’, ‘Tom Putt’, and ‘Lorna Doone’. It is unclear if the fruit for these accessions is genetically prone to pre-mature fruit cracking or if the fruit was overripe due to their early ripening.

Fruit weight and diameter are important characterization of cider fruit quality as most cider apples are smaller than dessert apples, which can result in up to a quadrupling in harvest time (Miles and King, 2014). The data indicated a wide range in fruit diameter (31.0-23.3 mm) and weight (31.09-385.59 g) with only three accessions (‘Midget Crab’, ‘GMAL 3232’, and ‘Kaz 95-08-06’) below the 50 g threshold. ‘Midget Crab’ was identified as a historically used cider apple accession and thus was not subjected to the greater than 50 g threshold imposed upon the accessions identified through querying the GRIN Global database. ‘GMAL 3232’ and ‘Kaz 95-08-06’ had a measured fruit weight of set 41.3 g and 41.4 g, respectively. Seasonal variability in fruit size could account for both accessions having a mean fruit weight greater than 50 g in the season they were measured for the GRIN database. Fruit size is a parameter that is directly influenced by crop load. Crop load

management is often employed as a method to improve fruit size, reduce preharvest drop, and prevent biennial bearing in commercial apple orchards (Racsó, 2006). The accessions within the USDA-PGRU *Malus* germplasm collection are not actively managed for crop load to allow the tree to exist in a more “natural” state. Thus, under commercial cultivation with crop thinning practices, fruit weight and diameter could increase for the accessions measured in this study.

Fruit firmness is used within the apple fresh market industry to ensure apples possess a minimum fruit firmness (44.5 N) or else they face the risk of rejection by the consumer for being too soft (DeEll et al., 2001). The fruit firmness values measured in this study had a mean of 64.04 N and 26 (15.5%) of the accessions phenotyped had a fruit firmness value below the 44.5 N fresh market standard. Although the firmness of the fruit is less critical for cider production as the fruit will just be pressed into juice rather than consumed as whole; firmer fruit may be more desirable for mechanical harvesting. For example, over-the-row mechanical harvesting has been reported to bruise 100% of the fruit harvested indicating that firmer fruit could be beneficial for mechanical harvesting (Miles and King, 2014). Although there is no research indicating an ideal fruit firmness for mechanical harvesting, future advances could reduce harvesting costs by 75%.

A notable difference between apples destined for fresh market consumption and cider production is the desirable level of fruit maturity. According to the cortex starch pattern index (SPI), culinary apples for long-term storage are commercially harvested between 2.8-3.0 on the SPI scale, while apples that are going to be sold soon after harvest are harvested when they are 4-6 on the SPI scale (Blanpied and Silsby,

1992). For cider production, apples are harvested when they reach 6-8 on the SPI scale. This is when most of the starch has been converted into sugar which yeast will convert into alcohol during the fermentation process. The mean cortex SPI score among the samples in my study was 7.5. This indicates that the fruit samples were evaluated at a similar maturity stage to each other. Interestingly, the *M. coronaria* ‘Kola’ and *M. hybrid* ‘Zapta’ had a unique fruit firmness and cortex SPI relationship. The fruit firmness values for these two accessions exceeded the maximum range on the penetrometer, yet they both had a SPI of 8.

4.2. Juice Chemistry

Soluble solid concentration and sugar composition (fructose, glucose, and sucrose) among all the accessions samples agree with other studies of apple juice (Karadeniz and Ekşi, 2002; Ma et al., 2015).

Malic acid, responsible for 90% of the organic acid present in apples (Zhang et al., 2010), had concentrations from 2.89 to 18.74 g·L⁻¹, a 6.5-fold difference. The relationship ($P < 0.001$) between *Malus* species and titratable acidity was present in the sample population. This relationship was first observed by Ma et al. (2015) who proposed that high fruit acidity was inadvertently selected against during domestication process towards a sweeter apple. This theory has also been observed with other *Malus* germplasm surveys (Zhang et al., 2010). More recently, Duan et al. (2017) proposed that cultivated apples originated from *M. sieversii* in modern day Kazakhstan with (intensive introgressions from *M. sylvestris*) and possesses two distinct genetic regions of substantially reduced genetic diversity in *M. ×domestica*

near a the Ma1 gene which has been characterized as playing a major role in the development of malic acid in apples.

The range ($0.4 \text{ g}\cdot\text{L}^{-1}$ to $4.86 \text{ g}\cdot\text{L}^{-1}$) of total polyphenol concentration was similar to other reports for apples (Guo et al., 2016; Guyot et al., 2003; Ramírez-Ambrosi et al., 2015; Thompson-Witrick et al., 2014). The 49 bittersweet accessions identified in this study indicate that the USDA *Malus* germplasm collection can be further investigated for other bittersweet accessions to address the reported lack of bittersweet cultivars in the hard cider industry.

Known limitations of the Folin-Ciocalteu assay includes that it measures reducing compounds, such as ascorbic acid, which provides a non-phenolic signal when present in high concentrations (Lester et al., 2012; Singleton et al., 1999). However, in the case of apple juice, ascorbic acid accumulation is low ($4.6\text{-}6 \text{ mg}\cdot\text{g}^{-1}$ of juice), and thus not a major source of interference (Kapur et al., 2012). The Folin-Ciocalteu assay also measures polyphenols that have minimal impact on flavor, such as anthocyanins. Within the measured genotypes, there was one accession ('Red Field', *Malus ×domestica*) where the fruit possessed red juice due to high anthocyanin content but did not possess any bitter or astringent properties when qualitatively sampled in the field.

Using geographic origin for the apple accessions did not predict total polyphenols, soluble solids, glucose, fructose, sucrose, or sorbitol concentration. Thus, broad searches of the USDA-PGRU *Malus* germplasm collection for cider apples should not place emphasis on any particular geographic origins.

4.3. Polyphenol Composition

The polyphenol composition data suggests that procyanidins and phloretin are responsible for the variation in total polyphenols measured by the Folin-Ciocalteu assay among the measured genotypes. High concentrations of procyanidins with a 2-5 degree of polymerization (dimers or pentamers) are particularly correlated to fruit bitterness (Lea and Arnold, 1978; Mangas et al., 1999; Ramírez-Ambrosi et al., 2015). Thus, procyanidins are generally deemed to be desired for cider production. The principal component analysis grouped the British cider apples ‘Ellis Bitter’ and ‘Kingston Black’ near the commercial culinary cultivars such as ‘Golden Delicious’. Additionally, currently used commercial cider apples ‘Ellis Bitter’ and ‘Kingston Black’ possess relatively low levels of procyanidins in comparison to some of the high polyphenol accessions (i.e., ‘Launette’ and ‘Kaz 95-08-06’).

In addition, the heatmap indicated a cluster of compounds unique to both non-*M. ×domestica* accessions ‘Zapta’ (*M. hybrid*) and ‘Kola’ (*M. coronaria*) which were a mix of flavanols and hydroxycinnamic acid compounds that were not prevalent in the other *M. ×domestica* accessions. Conversely, accessions within the lower total polyphenol group such as ‘Golden Delicious’, ‘Ellis Bitter’, and ‘Kingston Black’ exhibited higher relative concentrations of other types of hydroxycinnamic acids, such as cinnamic and benzoic acids. Hydroxycinnamic acids act as precursors of volatile phenols that contribute to cider aromas (Naish et al., 1993) and the contribution can be positive when the concentrations are low, but negative if the hydroxycinnamic acids are present past sensory thresholds (Hemingway and Laks, 1992).

While greater concentrations of hydroxycinnamic acids and flavanols are present in commercially grown cider apple cultivars such as ‘Kingston Black’ (one of the most commonly grown bittersharp cider apple cultivars in the U.S. (Miles and Peck, 2015), it had lower concentrations of the procyanidins desirable in bittersweet cider specific apples than accessions such as ‘Launette’ or ‘Bramtot’. The disparity between currently used cider cultivars and non-utilized cultivars within the USDA-PGRU germplasm collection indicates there is an opportunity for the U.S. cider industry to increase their cider apple cultivar diversity and therefore the diversity of cider styles that can be produced. Specifically, expanding the use of fruit that contains greater procyanidin concentration should be explored.

5.0. Conclusion

To my knowledge this is the first characterization of accessions within the USDA-PGRU *Malus* germplasm collection that emphasizes germplasm for the hard cider industry. The sample population included 49 bittersweet and 21 bittersharp accessions that might be of interest to the emerging hard cider industry. Future work will need to determine the seasonal variability in the fruit and juice characteristics for these 70 accessions, as well as other important horticultural performance traits such as: precocity, bearing habit, disease resistance, and overall compatibility with high-density apple orchard systems. In particular, accessions such as ‘Kaz 95-08-06’, ‘Launette’, ‘Medaille d’Or’, ‘Bramtot’, ‘Zapta’, ‘Red Rawls’ repeatedly appeared in the top five accessions based upon measured juice chemistry. Two of these accessions,

‘Medaille d’Or’ and ‘Bramtot’ have already started to see commercial field cultivation.

In the long term, this study provides a framework for future exploration of the USDA-PGRU *Malus* germplasm collection for cider apples by utilizing the methodologies from fundamental germplasm survey studies and applying them to address the demands of the emerging U.S. hard cider industry.

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CHAPTER 3

Using the Ma1 Gene as a Principal Component for Future Cider Apple Classification Systems

Abstract

The organic acid concentration in apple (*Malus ×domestica* Borkh.) juice is a critical component of hard cider flavor. Thus, rapidly classifying apple genotypes by their acid concentration is a useful tool for commercial cider producers, plant breeders, and horticulturalists. The existence of a malic acid (Ma1) gene on chromosome 16 in the ‘Golden Delicious’ genome has been reported to control 70% of the variation in acid concentration in apple fruit. Recently, a second malic acid gene (Q8) has been identified as controlling part of the remaining variation. The goal of this study was to measure the titratable acidity concentration in a target population from the USDA-Plant Germplasm Resources Unit *Malus* germplasm collection (n=159) and determine if the Ma1 and Q8 genes could predict the acid classification of these accessions according to the Long Ashton research station (LARS) cider apple classification system. The results indicate that the Ma1 gene is a strong predictor of acidity ($P < 0.0001$), but the Q8 gene, either alone ($P = 0.54$) or in combination with the Ma1 gene ($P = 0.27$) was not able to predict acidity concentrations within the sample population. Additionally, an estimated marginal means test of the Ma1 alleles was unable to accurately predict the acid classifications of accessions as per the LARS cider apple classification system that uses a $4.5 \text{ g} \cdot \text{L}^{-1}$ threshold. From this work, I propose a genetically based classification system for cider apples, with the acidity component

defined by the Ma1 allele. This system would use thresholds of 5.26 and 7.28 g·L⁻¹ of titratable acidity to categorize cider apples. Such a system would not be subject to geographic, horticultural, or seasonal variability.

1.0 Introduction

The United States (U.S.) has the second largest apple production (*Malus ×domestica*) industry in the world, but only recently have apple orchards been planted with cider-specific cultivars. Specialized cider apples can have 5 to 10 times more tannin (a bitter and astringent group of polyphenols) along with higher levels of acidity (sharpness) compared to culinary apples (Thompson-Witrick et al., 2014). However, while cultivars with high acidity and sweetness are readily available to cider makers, those with high tannin levels have limited commercial availability (Pashow, 2018).

The emerging cider industry in the U.S. has adopted a method for classifying cider apples that was originally developed at the Long Ashton Research Station (LARS) over a century ago. Although plant genetics and organoleptic science have progressed greatly since then, the LARS system remains in use today. The LARS classification system uses tannin concentration and titratable acidity measurements to classify an apple cultivar into one of four categories: sweet, bittersweet, sharp, or bittersharp. As originally proposed by B.T.P. Barker and John Ertle in 1910 (Barker and Ertle, 1910) three of the four categories; sweet, bittersweet, and sharp were subdivided into subclasses and groups within the subclasses, though these are not commonly used within the cider industry today. Acidity was, and still is, measured

using an acid-base titration and total tannin concentration, originally measured using the Löwenthal Permanganate Titration method. LARS classification system where apples with tannin concentration (measured with the Löwenthal Permanganate Titration method) greater than $2.0 \text{ g}\cdot\text{L}^{-1}$ are classified as bitter, and those with a malic acid concentration greater than $4.5 \text{ g}\cdot\text{L}^{-1}$ are classified as sharp.

Similar to the LARS system, a French cider apple classification system considers both acid and polyphenolic concentration to generate an organizational grid. Within the French system, cider apples are divided into six categories (English translation in parenthesis): *amère* (bitter), *douce amère* (bittersweet), *douce* (sweet), *acidulée* (subacid), *aigre* (sharp), and *aigre amère* (bittersharp) (Table 2) (Institut Français Des Productions Cidricoles, 2009). The acidity component of the French cider apple-classification method has three categories: *douce*, *acidulée*, and *aigre*. The *douce* category is defined as any fruit with less than $4.5 \text{ g}\cdot\text{L}^{-1}$ titratable acidity. This is the same threshold used by the LARS classification system. The *acidulée* category is defined as titratable acidity values between $4.5 \text{ g}\cdot\text{L}^{-1}$ and $6.75 \text{ g}\cdot\text{L}^{-1}$ titratable acidity, while the *aigre* category is defined as greater than $6.75\cdot\text{L}^{-1}$ titratable acidity (Institut Français Des Productions Cidricoles, 2009).

A Spanish cider classification system has undergone more recent changes than the 108-year-old LARS classification system. An initial classification system contained six technical groups following these criteria: sweet ($<4.85 \text{ g malic acid equivalents}\cdot\text{L}^{-1}$, $<1.45 \text{ g tannic acid}\cdot\text{L}^{-1}$), bittersweet ($<4.85 \text{ g malic acid equivalents}\cdot\text{L}^{-1}$, $>1.45 \text{ g tannic acid}\cdot\text{L}^{-1}$), semiacid ($4.85\text{-}6.56 \text{ g malic acid equivalents}\cdot\text{L}^{-1}$, $<1.45 \text{ g tannic acid}\cdot\text{L}^{-1}$), and sharp ($>6.56 \text{ g malic acid equivalents}\cdot\text{L}^{-1}$, $>1.45 \text{ g tannic acid}\cdot\text{L}^{-1}$) (Institut Français Des Productions Cidricoles, 2009).

tannic acid·L⁻¹), semiacid-bitter (4.85-6.56 g malic acid equivalents·L⁻¹, >1.45 g tannic acid equivalents·L⁻¹), acid (6.56 g malic acid equivalents·L⁻¹, <1.45 g·L⁻¹ tannic acid), and acid-bitter (>6.56 g malic acid equivalents·L⁻¹, >1.45 g tannic acid·L⁻¹) (Alonso-Salces et al., 2004). In 2009, in an effort to develop a designation of origin classification for Asturian ciders, the Spanish government created a classification system with nine distinct categories that officially classified 22 Asturian cider apple cultivars, but the chemical metrics used in this classification system are unclear (Ministerio De Agricultura, Pesca y Alimentacion, 2003).

The major organic acid in mature apple fruit is malic acid, although citric and quinnic acid are also detectable (Zhang et al., 2010). The genetic underpinnings of acidity in apples was first published in 1959 and subsequent studies have led to the identification and characterization of the Ma1 gene (Khan et al., 2013; Nybom, 1959; Visser and Verhaegh, 1978; Xu et al., 2012; Yao et al., 2008). The Ma1 gene has been located on chromosome 16 and is associated with one of the two aluminum-activated malate transporter-like genes (Bai et al., 2015a). The difference between Ma1 alleles has been attributed to the Single Nucleotide Polymorphism (SNP) location 1,455, where the guanine (G) is replaced with an adenine (A), causing a stop codon and terminating the rest of the gene sequence (Xu et al., 2012). Brown and Harvey (1971) reported that the variation between years was much less than the variation caused by allele differences for the Ma1 gene. However, the dominance of the Ma1 gene was found to be incomplete suggesting that there is both an additive and dominance effect of the Ma1 allele.

A second gene, temporarily named Q8, has been recently been reported to control acidity in apples (K. Xu, personal communication). The Q8 allelic variation is defined by a deletion in the promoter region spanning through the first intron, which prevents transcription of the Q8 gene. Full characterization of the Q8 gene is ongoing in the Xu Laboratory at Cornell University.

One issue with using the Ma1 gene as a predictor for acid classification is that the heterozygous Mama genotype displays a large variation in actual acidity which, in some cases, can be below the 4.5 g·L⁻¹ LARS threshold for sharp apples when previously observed in mapping populations (Xu et al., 2012).

The hypothesis of this study was that both the Ma1 and Q8 gene could be used as genetic markers to predict an accession's acidity classification as per the LARS cider classification system. The Ma1 and Q8 genotypes were identified for 159 accessions and then correlated with titratable acidity for the sample population using an estimated marginal means model. The null hypothesis was that neither the Ma1 nor Q8 genes, nor the combination of the two would be statistically significant predictors of titratable acidity concentrations.

2.0 Materials and Methods

2.1. Study Location and Accession Selection

The USDA-PGRU *Malus* germplasm collection in Geneva, NY (42°53'40.3" N, 77°00'23.8" W) holds the world's largest and most diverse cultivated collection of accessions within the *Malus* genus (Volk and Henk, 2016). Data collected on accessions within the USDA-PGRU *Malus* germplasm collection is stored and

accessible within the USDA Germplasm Resources Information Network (GRIN) Global database. This online database contains phenotypic and genotypic data on 106 descriptors across seven categories: “Chemical”, “Cytologic”, “Disease”, “Growth”, “Morphology”, “Phenology”, and “Production”. The GRIN Global database is research-driven but has incomplete data for many of the *Malus* accessions.

A sample population of 308 *M. ×domestica* and *M.* species genotypes were identified through a literature search for apple cultivars that were historically used in cider production and through a search of the GRIN-Global database. A list of 247 genotypes was compiled from information provided by Drs. Gayle Volk, Thomas Chao, and Ian Merwin who had each independently attempted to catalogue accessions within the USDA-PGRU *Malus* germplasm collection that have been reportedly used for hard cider production (G. Volk, I. Merwin, and C. Chao, personal communication).

An additional 61 accessions were identified through two multi-stepped queries of the USDA GRIN Global database. The first query started sorted all 4,867 *Malus* accessions for “Fruit Flesh Flavor”, which is defined by an anchored scale containing: “Aromatic Standard: Jonagold”, “Aromatic, Sweet”, “Sweet Standard: Fuji”, “Subacid Standard: McIntosh”, “Acid Standard: Puritan”, and “Astringent Standard: *M. coronaria*”. Only accessions which were categorized as “Astringent: Standard *M. coronaria*” progressed to the next step, which removed 4,029 of the 4,867 accessions. In the next step, the remaining accessions were sorted by the “Fruit Size (g)” (harvested fruit weight) and all accessions in the “<50 g” category were removed because they would likely be too small for commercial apple production, thus

removing 611 of the remaining 838 accessions. An additional 170 accessions were removed due to a lack of available fruit weight data, leaving 56 accessions.

The second multi-stepped query sorted the 4,867 accessions by the parameter “Fruit Flesh Flavor”, then accessions listed as “Acid Standard: Puritan”, “Sweet Standard: Fuji”, and “Sub-acid Standard: McIntosh” were removed since these categories represented accessions within the GRIN Global database that did not possess aromatic or astringent characteristics desirable for cider production. This step removed 4,846 of the 4,867 accessions. The remaining 21 accessions were then sorted by the “Fruit Size (g)” parameter and accessions less than 50 g were removed. This final step removed 16 of the 21 accessions, leaving five accessions.

2.2. DNA Extraction

For DNA extraction, 15-20 mg of plant leaf tissue was collected from each accession and ground for 1 min using a TissueLyserII (QIAGEN, Venio, Netherlands). Samples were incubated for 1 h in a hexadecyltrimethylammonium bromide (CTAB) extraction buffer containing polyvinylpyrrolidone (Fisher Catalogue number: BP431-500) and β -mercaptoethanol (Fisher catalogue number: BP176-100). A Nanodrop 1000 (Thermo Scientific, Waltham, MA) was used for DNA quantification.

2.3. Accession Genotyping

A cleaved amplified polymorphic sequence marker (CAPS₁₄₅₅) targeting base 1455 in the open reading frame of Ma1 gene was used to distinguish between the single nucleotide polymorphism difference between the Ma1 alleles. The polymerase

chain reaction (PCR) program included 2 min at 98 °C, 35 cycles of 10 s at 98 °C, 15 s at 55 °C, 90 s at 72 °C, and a final 5 min at 72 °C. The reactions were conducted in 20 µl volumes, containing 1× PrimeSTAR® MAX DNA Polymerase (R045A, Takara/Clontech, Mountain View, CA), 0.5 mM of each primer, and 30 ng of genomic DNA in an Eppendorf Mastercycler® EP Gradient Thermal Cycler (Eppendorf, Hamburg, Germany). Restriction digestion was performed overnight at 37 °C in 20 µl reactions that contained 10 µl PCR products, 2 U of BspHI (New England Biolabs, Ipswich, MA), and 1× NEBuffer 4 (New England Biolabs, Ipswich, MA). After sample incubation, 7 µl of sample and 3 µl of loading dye was injected into each well of a 1.5% (w/v) agarose gel. The samples were suspended in a 1 N Tris/Acetate/EDTA (TAE) buffer solution. After 1 h of electrophoresis, the gels were stained with ethidium bromide at a concentration of 2 µl to 100 mg of gel. The banding patterns in the gel were then illuminated with a 110-V UV light transilluminator (Thermo Scientific, Waltham, MA). The banding patterns in the gel images were then visually scored and the Ma1 alleles for each accession were recorded.

The PCR program for the Q8 gene included 2 min at 98 °C, 35 cycles of 30 s at 94 °C, 30 s at 54 °C, 45 s at 68 °C (primer sequence is unpublished, Xu, personal communication). The reactions were conducted in 20 µl volumes, containing 1× OneTaq® DNA Polymerase (New England BioLabs, Ipswich, MA), 0.5 mM of each primer, and 30 ng of genomic DNA in an Eppendorf Mastercycler® EP Gradient Thermal Cycler (Eppendorf, Hamburg, Germany). From there the 7 µl of sample and 3

µl of loading dye was injected into each well of a 1.5% (w/v) agarose gel and suspended in a 1 N Tris/Acetate/EDTA (TAE) buffer solution. After 1 h of electrophoresis, the gels were stained with ethidium bromide at a concentration of 2 µl to 100 mg of gel. The banding patterns in the gel were then illuminated with a 110-V UV light transilluminator (Thermo Scientific, Waltham, MA). The banding patterns in the gel images were then visually scored and the Q8 alleles for each accession were recorded.

2.4. Fruit Sampling Procedure

Weekly sampling trips from Ithaca, NY to the USDA-PGRU *Malus* germplasm collection in Geneva, NY were made from the 15 Aug. to 17 Nov. During each trip, two fruit per accession that were near the reported harvest date were field tested *in situ* for maturity using the cortex starch pattern index where an iodine solution (0.22% w/v iodine, 0.88% w/v potassium iodine) was applied to the stem-side of an equatorial cross-section of the apple (Blanpied and Silsby, 1992). A visual rating of the cortex flesh (hypanthium and mesocarp) stained was conducted and recorded on a 1-8 scale; where 1 = 100% staining and 8 = 0% staining. Fruit was harvested when they were rated to be a six or higher.

The 15 fruits were randomly harvested from different regions of the tree canopy avoiding selecting two or more fruit from the same branch. The unique identifying Plant Introduction (P.I.) number given to each accession in the USDA-PGRU collection was recorded and used to track the fruit throughout the phenotyping process. After harvest, the 15 fruits were divided into three groups of five apples to

allow for three subsamples of five fruit per sampled accession, as per Evans et al. (2012). The fruit were stored in a commercial storage room set to 4° C under ambient atmospheric gases for 1 to 4 weeks prior to fruit maturity analysis at the Cornell University Agricultural Experiment Station Research Orchards, Ithaca, NY.

2.5. Juice Extraction

Starch pattern index was determined on all sample apples, as described above (Blanpied and Silsby, 1992). Starch pattern index measurements were made to ensure fruit was at a score of 6 or greater prior to juicing.

The calyx half of each 5-apple pooled subsample was milled and pressed in a Norwalk 280 juicer (Bentonville, AR). Upon completion of juicing each subsample, was stirred and aliquoted into 3-15 mL centrifuge tubes and 3-50 mL centrifuge tubes. All juice extracting equipment was rinsed with water between samples to minimize cross-contamination. Juice samples were stored at -80° C until titrations were performed.

2.6 Titratable Acidity

Samples were thawed to room temperature and vortexed for 10 s. Titratable acidity was measured by titrating a 5 mL juice aliquot against a standardized 0.1 N NaOH solution to an end-point of pH 8.1 with a Metrohm 809 Titrando autotitrator (Metrohm AG, USA).

2.7 Statistical Analysis

Linear models were developed using Ma1, Q8, or both genotypes as predictor variables and titratable acidity as the response variable with RStudio version 1.1.442 (RStudio, Boston, MA). The linear models were used to predict 99% confidence intervals with the Estimated Marginal Means data analysis package. The data was not transformed prior to analysis and a p-value of less than or equal to 0.05 was considered statistically significant.

3.0. Results

3.1. Accession Genotypes

Of the 308 accessions originally identified as having the potential to be used for cider production, 159 were analyzed in 2017. The remaining 149 accessions were not sampled due to tree or fruit unavailability. An additional 8 of those 159 accessions were not sampled for genotyping during the 2017 harvest season. Of the 159 accessions, the mean starch pattern index for all the accessions evaluated post storage was 7.74 with a standard deviation of 0.5 and a range of 5 to 8.

For the 159 accessions genotyped, 12 (7.5%) had the MaMa gene, 96 (60%) had the Mama gene, and 51 (32%) had the mama gene. The 159 accessions contained 109 (68%) with the Q8Q8 gene, 44 (28%) with the Q8q8 gene, and 6 (4%) with the q8q8 gene (Table 5).

Table 5: Allele frequency from 159 cider apple accessions genotyped in 2017 from the USDA-PGRU Malus germplasm collection Geneva, NY. The number in each cell represents the number of accessions and the number in parenthesis is the representative percentage of the accessions phenotyped. In the top and left most column, the number in the parenthesis represents the mean titratable acidity measured in g·L⁻¹.

	MaMa (8.76)	Mama (5.25)	mama (2.14)
Q8Q8 (4.46)	11 (6.8%)	54 (33.8%)	44 (27.5%)
Q8q8 (4.27)	-	37 (23.1%)	7 (4.4%)
q8q8 (7.19)	1 (0.6%)	5 (3.1%)	-

3.2. Titratable Acidity

The mean titratable acidity for the sample population was 4.50 g·L⁻¹ with a standard deviation of 2.98 g·L⁻¹, and range of 0.86 to 18.56 g·L⁻¹. The MaMa allele had a titratable acidity mean of 8.76±3.97g·L⁻¹ (3.21-17.04 g·L⁻¹), while the Mama allele had a titratable acidity mean of 5.25±2.63 g·L⁻¹ (0.86-18.56 g·L⁻¹), and the mama allele had a mean of 2.14±1.05 g·L⁻¹ (0.926-6.27 g·L⁻¹) (data presented as mean ± standard deviation with measured sample range in parenthesis). The Q8Q8 allele had a titratable acidity mean of 4.46±3.22 g·L⁻¹ (1.01-18.56 g·L⁻¹), while the Q8q8 allele had a mean of 4.27±21.84 g·L⁻¹ (0.93-8.519 g·L⁻¹), and the q8q8 allele had a mean titratable acidity of 7.19±4.42 g·L⁻¹ (0.86-11.49 g·L⁻¹) (data presented as mean ± standard deviation with measured sample range in parenthesis).

When using a linear model with only the Ma1 gene as the predictor variable and titratable acidity as the response variable, a one-way ANOVA analysis indicated that the Ma1 gene is a statistically significant predictor of the titratable acidity ($P < 0.0001$). A series of non-overlapping mean confidence intervals for titratable acidity concentration for each Ma1 allele were calculated from utilizing the same linear model (Figure 19). The estimated marginal mean for the MaMa allele was 7.87±0.66 g·L⁻¹

titratable acidity. The estimated marginal mean for the Mama and the mama allele was $5.26 \pm 0.24 \text{ g} \cdot \text{L}^{-1}$, and $2.23 \pm 0.34 \text{ g} \cdot \text{L}^{-1}$ titratable acidity, respectively. The MaMa-Q8Q8, MaMa-Q8q8 and MaMa-q8q8 allele combinations had estimated marginal means of 8.11 ± 0.71 , 2.60 ± 2.34 and $10.544 \pm 2.34 \text{ g} \cdot \text{L}^{-1}$ titratable acidity, respectively. While the Mama-Q8Q8, Mama-Q8q8, and Mama-q8q8 allele combinations had estimated marginal means of 5.51 ± 0.32 , 4.74 ± 0.39 and $6.52 \pm 1.05 \text{ g} \cdot \text{L}^{-1}$ titratable acidity, respectively. The estimated marginal mean for the mama-Q8Q8 allele combination was $2.31 \pm 0.35 \text{ g} \cdot \text{L}^{-1}$ titratable acidity and $4.74 \pm 0.39 \text{ g} \cdot \text{L}^{-1}$ titratable acidity for the mama-Q8q8 allele combination titratable acidity (Figure 20) (data presented as mean $\pm$ standard error).

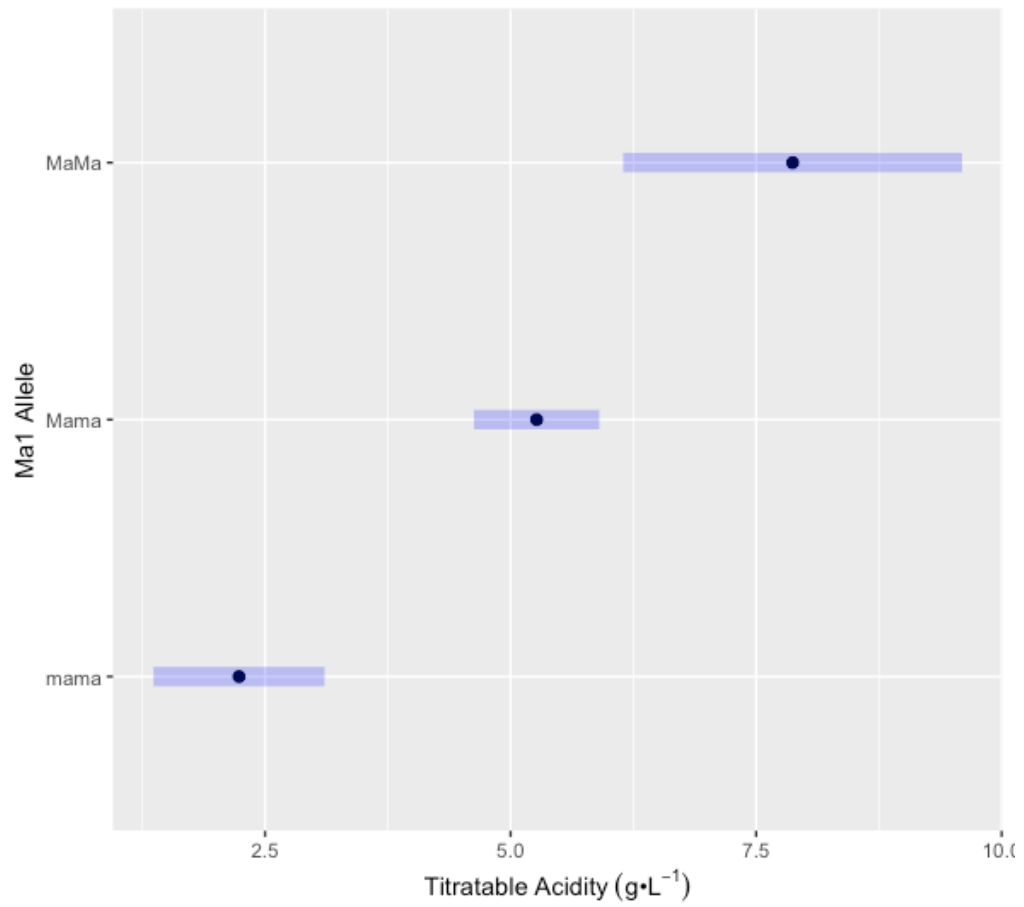


Figure 19: The variation in the estimated marginal mean of titratable acidity for each of the three Ma1 alleles combinations (MaMa, Mama, and mama) among 160 samples harvested in the 2017 harvest season from the Malus collection of the USDA-PGRU in Geneva, NY. The mean value is represented by the black circle. A 99% confidence interval was used to generate the estimated marginal means, which is represented by the blue bar.

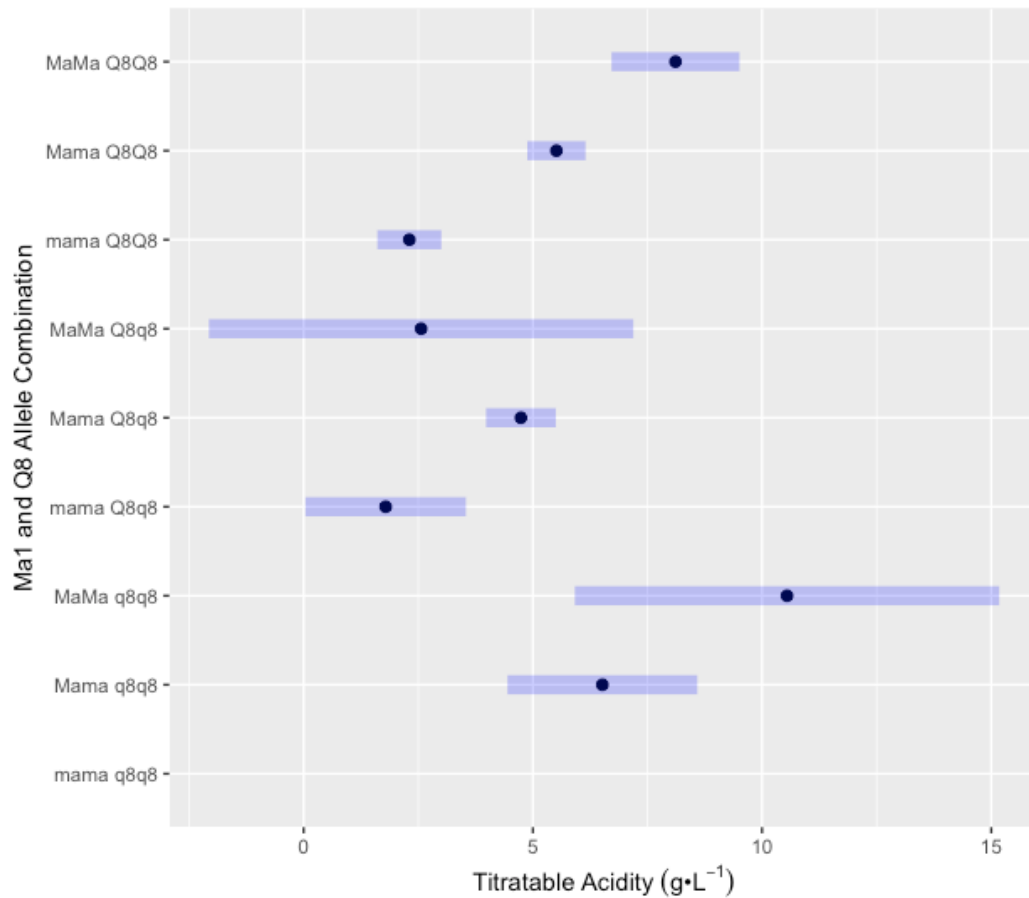


Figure 20: The variation in the estimated marginal mean of titratable acidity for each of the combinations of Ma1 and Q8 alleles among 159 samples harvested in the 2017 harvest season from the Malus collection of the USDA-PGRU in Geneva, NY. The MaMa-Q8q8 and MaMa-q8q8 gene combinations are not included as there were no accessions with that combination. The mean value is represented by the black circle. A 99% confidence interval was used to generate the estimated marginal means, which is represented by the blue bar.

When the Q8 gene was the only predictor within the linear model, an ANOVA analysis indicated that the Q8 gene is not a statistically significant predictor of the titratable acidity ($P = 0.81$). An ANOVA analysis using a linear model with both the Ma1 gene and the Q8 gene as predictors indicated the Ma1 gene is the predominant predictor ($P < 0.001$) while the Q8 gene was not a statistically significant ($P = 0.48$). There were no interaction effects present when both the Ma1 and Q8 gene were

predictors in the model ($P = 0.23$). Together, the Ma1 and the Q8 genes were unable to predict titratable acidity concentration in the sample population (Figure 20).

4.0. Discussion

4.1. Accession Genotypes

Using the USDA-PGRU *Malus* germplasm collection as a source for a wide range of plant genetic material for this experiment was ideal as it is instrumental in the preservation of over 90% of the most popular historic apple cultivars grown in the United States (Volk and Henk, 2016). One limitation to working within the USDA-PGRU *Malus* germplasm collection is the lack of cultivated replicates as most accessions are only represented by a single accession tree in the field. Nonetheless, the sheer diversity of the USDA-PGRU *Malus* germplasm collection has makes it an important resource to understand the relationships among genotype and phenotype (Gutierrez et al., 2018; Sugimoto et al., 2015).

4.2. Titratable Acidity

Titratable acidity can decrease during the ripening process (Ma et al., 2015). Thus, all accessions in this study were harvest at a similar maturity. Additionally, previous studies looking at the relationship between the Ma1 gene and titratable acidity evaluated the fruit at a mean SPI between 4-6 (Bai et al., 2012, 2015b; Xu et al., 2012). As the current study was focused on apple accessions for the hard cider industry, the goal was to test the fruit at a later stage in the maturity process when the SPI was between 6 and 8.

For the MaMa and mama alleles, Xu et al. (2012) reported measured mean titratable acidity concentrations of 10.383 and 2.063 g·L⁻¹, respectively, which agree with the values previously measured in this study. However, for the Mama allele, Xu et al. (2012) reported a mean titratable acidity of 8.46 g·L⁻¹, while our data indicates a mean titratable acidity concentration of 4.75 g·L⁻¹. The discrepancy of Mama alleles could be due to differences in sample population. The Xu et al. (2012) study used two mapping populations containing a maternal parent of ‘Royal Gala’ and two paternal parents of *M. sieversii* accessions, whereas the sample population in my dataset consist of almost entirely of *M. ×domestica* cultivars with only two *M. sieversii* accessions. Duan et al. (2017) proposed that cultivated apples *M. ×domestica* possess two distinct genetic regions of substantially reduced genetic diversity near a the Ma1 gene in comparison to progenitor species *M. sieversii*. The increased genetic diversity in these two regions along the Ma1 gene could be causing a higher mean titratable acidity concentration for the heterozygous Mama allele in the reported Xu et al (2012) study as it uses *M. sieversii* as the two paternal parents in the mapping population.

Similar to other studies that describe the Ma1 gene, our analyses indicate that the Ma1 gene is a reliable predictor of the acidity values ($P < 0.0001$). However, some measured values for the Mama allele spanned both sides of the LARS 4.5 g·L⁻¹ acidity threshold, thus hindering the effectiveness of the Ma1 gene as a predictor of the acidity component of the LARS cider apple classification system.

The estimated marginal means of titratable acidity for the Ma1 alleles within the data do align within the acid component within the French classification system.

Although, Xu et al. (2012) reported a mean titratable acidity of 8.46 g·L⁻¹ for the Mama allele indicating the titratable acidity measurements can range outside of the acid classification categories defined in the French cider apple classification system.

The targeted population of cider apple cultivars included in this study could have been improved if the sample population contained more accessions with the MaMa and q8q8 alleles. Evaluating a more diverse population would help determine if the Ma1 genotype remains a statistically significant predictor of titratable acidity concentration beyond the targeted cider apple germplasm used within this study.

4.3. Future Cider Apple Classification Recommendations

This study adds to the body of literature indicating that the Ma1 gene largely determines apple acidity (Bai et al., 2012; Brown and Harvey, 1971; Kouassi et al., 2009; Nybom, 1959; Visser and Verhaegh, 1978; Xu et al., 2012). Using this information, it is possible that Ma1 gene can be used for a cider apple classification system based upon genetic characteristics. Such a system would not be subject to geographic, horticultural, or seasonal variation. I propose three categories based on the three allelic combinations of the Ma1 gene. These would be: sweet (mama), semi-sharp (Mama), and sharp (MaMa). Our current data suggest that the mean titratable acidity thresholds for the three categories: 7.87, 5.26, and 2.23 g·L⁻¹ for the sharp, semi-sharp, and sweet classifications, respectively. However, sensory research would need to be used to confirm the effects on human perception for these acidity levels.

To establish the titratable acidity limits within each genotyped category, more of the USDA-PGRU *Malus* germplasm collection needs to be evaluated in order to

create a dataset that encompasses a wider range of genetic variability. Another factor needed within this larger dataset are occurrences of cultivars grown at different locations to observe variability of titratable acidity within geographic, climatic, and regional variability.

A second component to a cider apple classification system would be the inclusion of genetic markers for polyphenol concentration. However, the biochemical pathways and genetic controls for polyphenol development are much more complex than those for malic acid. Additionally, there is a large range of polyphenols that affect cider quality and human perception which would further complicated such an undertaking. Nonetheless, many genetic markers for polyphenol production in apples have been previously identified (Verdu et al., 2014).

5.0 Conclusion

The project described in this chapter is the first study to use genetic markers for acidity (Ma1 and Q8) as the basis for categorizing cider apples. The Ma1 and Q8 alleles were identified for 159 *Malus* accessions and correlated with the titratable acidity concentration. The results suggest that the Ma1 gene can be used in the development of a genetically based cider apple categorization system to determine acidity. The Q8 gene did not contribute enough additional information about acidity levels to be considered a useful tool for cider apple categorization. Continued evaluation of accessions within the USDA-PGRU *Malus* germplasm collection would provide more defined bounds for the titratable acidity concentrations that can be expected for each Ma1 allelic combination. Adding genetic markers for polyphenol

and sugar content would create a robust suite of markers for plant breeders, horticulturalists, and commercial cider producers to rapidly identify potential cider apple germplasm.

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CHAPTER 4

Conclusion

The research described in this thesis started with the idea of identifying and characterizing apples in the USDA-PGRU *Malus* germplasm collection that could be used for hard cider production. The work developed into creating a systematic approach to phenotype and genotype cider apples. This work represents the first step towards creating a genetically-based cider apple classification system.

The project described in Chapter Two is, to my knowledge, the most extensive survey of the USDA-PGRU *Malus* germplasm collection with the specific goal of searching for germplasm for the hard cider industry. I targeted 158 genotypes that were among a population of 308 potential cider apple accessions. The sample population was assembled from historical references of cider apples and executing a series of multi-stepped queries of the USDA-GRIN Global database. An intentional effort was made throughout this project to utilize established standardized phenotyping methods so that my data could be used by other researchers and apple breeders who are working with hard cider apples.

Of the 308 accessions, 158 were harvested in 2017 and characterized for external and internal fruit quality traits, along with an analysis of the juice chemistry. After measuring the total polyphenol concentrations, the accessions were ordered by measured total polyphenol values and fifteen accessions were then selected for polyphenol composition analyses via UHPLC-MS; of the 14 accessions selected, five were selected from the top, middle, and bottom tertials of the measured total polyphenol concentrations. Principal component analysis of the polyphenol composition data indicated that the variation in polyphenol concentration measured by

the Folin-Ciocalteu assay across the 14 samples is largely due to increased levels of procyanidins and phloretin compounds. Finding accessions within the USDA-PGRU *Malus* germplasm collection which possessed higher levels of procyanidins and phloretin than currently used cider apple accessions indicates room for improving the industry's access to cider specific cultivars with higher levels of these desirable compounds.

The sampled population of 158 included 49 bittersweet and 21 bittersharp genotypes [classified as per the Long Ashton research station (LARS) classification system] that might be of interest to the emerging hard cider industry. Future work will need to determine the seasonal variability in the fruit and juice characteristics for these 70 accessions, as well as other important horticultural performance traits such as: precocity, bearing habit, disease resistance, and overall compatibility with high-density apple orchard systems.

On occasion, when measuring the fruit maturity of an accession in the field, accessions were encountered which had fruit at dramatically different stages of maturity, including fruit falling from the tree while other fruit on the tree was under-ripe (scoring a two or three on the cortex starch pattern index scale). Thus, the fruit was allowed another one to two more weeks of maturation on the tree before sample collection to ensure a higher proportion of the sampled fruit was fully mature.

When arriving at an accession within the *Malus* germplasm collection, I also would occasionally encounter fruit with severe cracking, rendering it unusable. It is unclear if the accessions are genetically predisposed to fruit cracking or if the fruit was overripe. In the cases of accessions with severe fruit cracking, only fruit without any visible cracks were harvested.

The press used to juice samples was manually operated with no gauge to measure applied pressure on the pomace sample, thus I was unable to apply a consistent quantified amount of force on each pomace sample. In an attempt to exert a consistent amount of force on all pomace samples, all the juice was extracted until the pomace was dry to the touch; from there, another ten seconds of maximum pressing force was applied to the dry pomace to ensure all juice was extracted from the pomace.

When reviewing the phenotypic data for ‘Empire’ (PI Number: 588842), it became evident that the sample was likely not ‘Empire’, as the three subsample titratable acidity values were 1.9, 2.7, and 3.0 g·L⁻¹, rather than the 7-8 g·L⁻¹ reported in the literature. The ‘Empire’ juice samples also possessed the second to lowest fructose and sucrose concentrations of all the phenotyped accessions (11.51 and 6.58 g·L⁻¹, respectively), which are extremely low for a common eating apple especially since fructose is the most abundant sugar in apple. I repeated the titratable acidity analysis on the purported ‘Empire’ juice samples which confirmed the initial results and ruled out the possibility of a typographic error with the data. Thus, the ‘Empire’ phenotypic data was removed from the study as it became evident an incorrect accession was sampled in the tree or the fruit was incorrectly labeled during fruit processing.

The project described in Chapter Three focused on genotyping a targeted population within the USDA-PGRU *Malus* germplasm collection, followed by testing the robustness of the Ma1 and the Q8 gene as a genetic predictor for the acidic component of the LARS classification system. The Ma1 and Q8 genotypes were

identified for 136 accessions phenotyped during the 2017 harvest season. The data analysis showed that the Ma1 gene is a strong predictor of titratable acidity in the cider apples that I studied, but it is not a useful genetic marker to classify the acidity of apples as per the $4.5 \text{ g}\cdot\text{L}^{-1}$ threshold used in the LARS classification system. The Q8 and combined Ma1 and Q8 gene were also not shown to be useful predictors of titratable acidity. In light of these findings, I proposed a genetically based cider apple classification system which relies upon a permanent constant (Ma1 genotype) to define the acid classification principal of the classification system.

While this study is not the first to survey the USDA-PGRU *Malus* germplasm collection for fruit acidity concentration with respect to the Ma1 gene, it is the first study to do so using a targeted population of known cider specific apples. In addition, the project described in Chapter Three is the first study I know of which attempts to use genetic markers as the basis for the acid component of classifying cider apples into distinct classifications.

Overall, both of these research projects offer new approaches to using the USDA-PGRU *Malus* germplasm collection to benefit the rapidly growing hard cider industry. Together, both studies intentionally focused on the same target population of accessions to provide complementary phenotypic and genotypic datasets on cider specific cultivars of interest within the USDA-PGRU *Malus* germplasm collection. While Chapter Three in this thesis focused on the use of the Ma1 gene to define a genetically-based cider apple classification system, the phenotypic data collected within Chapter Two can, in future research, be used to assist in the identification of genetic markers for total polyphenol concentration that can be used as the other principal component for the genetically based cider apple classification system. Thus,

continued work phenotyping potential cider apples in the USDA-PGRU *Malus* germplasm collection is necessary to provide a better understanding of the phenotypic and genotypic diversity available within the collection, and how those phenotypes and genotypes might be leveraged to address concerns facing the rapidly expanding US hard cider industry.

Supplemental Table 1: List all 308 accessions within the USDA-PGRU Malus germplasm collection in Geneva NY, for the 2017 harvest season, which were identified as the target population of either historical cider apple accessions or accessions of interest from querying the USDA-GRIN Global database.

PI Number	Cultivar Name	<i>Malus</i> species	Origin	Sub-Origin	Date Planted
280022	Adams's Pearmain	<i>hybrid</i>	United Kingdom	England	6/27/2003
127311	Amere de Berthecourt	<i>domestica</i>	France	Maine-et-Loire	6/30/2003
173978	American Forestier	<i>domestica</i>	France		6/15/2000
589214	American Summer Pearmain	<i>domestica</i>	United States	New York	6/1/2005
127686	Amsib Crab	<i>hybrid</i>	United States	South Dakota	6/15/2000
136243	Amzr Gauthier	<i>domestica</i>	France	Eure	6/14/2004
107196	Antonovka 1.5 Pounds	<i>domestica</i>	(Former) Soviet Union		7/1/2005
295282	Antonovka Mitchurin	<i>domestica</i>	Finland		6/26/2002
588952	Arkansas	<i>domestica</i>	United States	New York	6/30/2003
589117	Arkansas Black	<i>domestica</i>	United States	New York	6/14/2004
589654	Ashmead's Kernel	<i>domestica</i>			6/15/2000
123733	Bedan des parts	<i>domestica</i>	France	Calvados	6/26/2002
105498	Bella de Jardins	<i>domestica</i>	Spain	Zaragoza	7/9/2004
102537	Bella di Pontoise	<i>domestica</i>	France		6/2/2005
162544	Belle de Crollon	<i>domestica</i>	France	Manche	6/15/2000
589584	Belle de Nordhaussen	<i>domestica</i>	Belgium		6/26/2002
162709	Belle Fille	<i>domestica</i>	France	Manche	5/14/2001
245048	Belle Fleur Rouge	<i>domestica</i>	France	Ville-de-Paris	5/10/2001
126495	Belle Imperiale	<i>domestica</i>	France	Calvados	7/7/2015
588951	Belle Sans Pepin	<i>domestica</i>	United States	New York	6/27/2003
588953	Ben Davis	<i>domestica</i>	United States	New York	6/15/2000
590027	Benham	<i>domestica</i>	United States	Oregon	6/1/2005
131964	Berglander	<i>domestica</i>	Belgium		6/26/2002
122598	Binet Blanc	<i>domestica</i>	France		6/2/2005
158729	Binet Blanc Dore	<i>domestica</i>	France	Maine-et-Loire	5/10/2001
158730	Binet Rouge	<i>domestica</i>	France	Maine-et-Loire	5/14/2001
589178	Black Ben Davis	<i>domestica</i>	United States	Arkansas	6/30/2003
341067	Blahova Oranzova Renetor	<i>domestica</i>	Chzech Republic	Central Bohemia	6/2/2005
590119	Blanc Dur (Orne)	<i>domestica</i>			8/26/2016
162719	Blanc Mollet	<i>domestica</i>	France	Hauts-de-Seine	5/21/2014
590180	Blue Pearmain	<i>domestica</i>			6/8/2012
162549	Boche	<i>domestica</i>	France	Manche	6/2/2005

PI Number	Cultivar Name	<i>Malus</i> species	Origin	Sub-Origin	Date Planted
590120	Bonne-Hotture	<i>domestica</i>			5/10/2001
162723	Boutteville	<i>domestica</i>	France	Hauts-de-Seine	5/10/2001
107171	Bramley's Seedling	<i>domestica</i>	United Kingdom	England	6/26/2002
158731	Bramtot	<i>domestica</i>	France	Maine-et-Loire	5/10/2001
590087	Breakwell's Seedling	<i>domestica</i>	United States	Oregon	5/21/2014
589685	Brown Snout	<i>domestica</i>	United Kingdom	England	6/7/2012
589677	Brown Thorn	<i>domestica</i>	United Kingdom	England	5/21/2014
589662	Brown's Apple	<i>domestica</i>	United Kingdom	England	6/26/2002
588808	Bulmer Norman	<i>domestica</i>	United Kingdom	England	5/10/2001
187297	C'Huero Biz Bras	<i>domestica</i>	France	Gironde	6/2/2005
187298	C'Huero Ru Bienn	<i>domestica</i>	France	Gironde	6/2/2005
161759	Caillouett	<i>domestica</i>	France	Indre	6/27/2003
589596	Calville Blanc	<i>domestica</i>	Belgium		7/1/2005
231939	Camuzat	<i>domestica</i>			6/2/2005
183961	Canavial-14	<i>domestica</i>	Portugal		5/1/2003
161830	Cap of Liberty	<i>domestica</i>	United Kingdom	England	6/2/2005
162710	Cartigny	<i>domestica</i>	France	Manche	6/1/2005
589451	CC-14-15	<i>domestica</i>	Canada	British Columbia	6/26/2002
264688	Champagne Reinette	<i>domestica</i>	Serbia		6/26/2002
589656	Cheddar Cross	<i>domestica</i>	United Kingdom	England	6/15/2000
588806	Chisel Jersey	<i>domestica</i>	United States	Washington	6/26/2002
589018	Cimitiere	<i>domestica</i>	United States	New York	6/2/2005
206022	Clear Heart	<i>domestica</i>	Ireland	Mayo	6/14/2004
589175	Coat Jersey	<i>domestica</i>	United States	New York	6/14/2004
162712	Colozette	<i>domestica</i>	France	Manche	6/2/2005
590121	Cornish Aromatic (Wakeley)	<i>domestica</i>			5/10/2001
588848	Cortland	<i>domestica</i>	United States	New York	7/1/2005
589601	Court Pendu	<i>domestica</i>	Belgium		6/15/00
589602	Court Pendu Gris	<i>domestica</i>			6/7/2012
123960	Court Pendu Plat	<i>domestica</i>			6/14/2004
589587	Court Pendu Rose	<i>domestica</i>			6/26/2002
589671	Court Royal	<i>domestica</i>	United Kingdom	England	6/26/2002
589057	Cowichan	<i>hybrid</i>	Canada	Ontario	6/27/2003

PI Number	Cultivar Name	<i>Malus</i> species	Origin	Sub-Origin	Date Planted
589180	Cranberry	<i>hybrid</i>	United States	North Dakota	6/14/2004
123735	Cremiere	<i>domestica</i>	France	Calvados	5/21/2014
158618	Crimson King	<i>domestica</i>	United Kingdom	England	6/26/2002
162721	Crollon	<i>domestica</i>	France	Hauts-de-Seine	6/20/2016
589196	Crow Egg	<i>domestica</i>	United States	Indiana	6/27/2003
590122	D'Arcy Spice	<i>domestica</i>			6/2/2005
589073	Dabinett	<i>domestica</i>	United States	New York	6/2/2005
162722	Damelot	<i>domestica</i>	France	Hauts-de-Seine	6/30/2003
162062	Daux Belan	<i>domestica</i>	France	Gironde	6/2/2005
188515	Dekkers Glorie	<i>domestica</i>	Netherlands		6/2/2005
264689	Djulabia	<i>domestica</i>	Serbia		6/30/2003
588870	Dolgo	<i>hybrid</i>	(Former) Soviet Union		7/1/2005
173979	Domaine	<i>domestica</i>	France		6/30/2003
131104	Double Bon Pommier	<i>domestica</i>	France	Aube	6/2/2005
161760	Doucet Rouge	<i>domestica</i>	France	Indre	6/2/2005
589667	Doux Normandie	<i>domestica</i>	United Kingdom	England	6/15/2000
162547	Doux Tardif	<i>domestica</i>	France	Manche	7/7/2015
122616	Doux-AMR	<i>domestica</i>	France	Maine-et-Loire	5/21/2014
131823	Drap d'Or Guemene	<i>domestica</i>	France		5/10/2001
175542	Dufflin	<i>domestica</i>	United Kingdom	England	6/14/2004
589666	Dunkerton Late Sweet	<i>domestica</i>	United Kingdom	England	6/15/2000
589642	Eda	<i>hybrid</i>	United States	South Dakota	6/15/2000
590125	Edelroter	<i>domestica</i>	United Kingdom	England	6/14/2004
392312	Edward VII	<i>domestica</i>	United Kingdom	England	5/1/2003
280401	Ein Shemer	<i>domestica</i>	Israel		7/1/2005
589650	Ellis Bitter	<i>domestica</i>	United Kingdom	England	6/26/2002
589440	Elstar	<i>domestica</i>	Netherlands	Gelderland	6/26/2002
588842	Empire	<i>domestica</i>	United States	New York	5/9/2006
588785	Esopus Spitzenburg	<i>domestica</i>	United States	Washington	6/26/2002
590126	Fenouillet de Ribours	<i>domestica</i>			6/14/2004
136591	Fenouillet Gris	<i>domestica</i>	France		5/11/2001
589679	Fillbarrel	<i>domestica</i>	United Kingdom	England	6/26/2002

PI Number	Cultivar Name	<i>Malus</i> species	Origin	Sub-Origin	Date Planted
651010	Finkenwerder Herbstprinz	<i>domestica</i>	Germany		4/30/2009
589626	Forest King	<i>hybrid</i>	United States	South Dakota	6/15/2000
589318	Foxwhelp	<i>domestica</i>	United Kingdom	England	6/15/2000
162503	Frequin	<i>domestica</i>	France	Maine-et-Loire	6/30/2003
161838	Frequin Audievre	<i>domestica</i>	United Kingdom	England	5/21/2010
247314	Frequin Lacaille	<i>domestica</i>	France	Gard	6/2/2005
589689	Frequin Tardive de la Sarthe	<i>domestica</i>	United Kingdom	England	6/14/2004
276299	Freyberg	<i>domestica</i>	New Zealand	North Island	6/15/2000
590127	Friandise	<i>domestica</i>	United Kingdom	England	5/8/2009
589182	Frostproof	<i>domestica</i>	United States	Virginia	5/20/2010
187352	Fuero Rous	<i>domestica</i>	France	Gironde	6/14/2004
392303	Gala	<i>domestica</i>	New Zealand	North Island	7/1/2005
589123	Geeveston Fanny	<i>domestica</i>	Australia	Tasmania	5/14/2001
589684	Gernadier	<i>domestica</i>	United Kingdom	England	6/15/2000
132225	Gewurzluiken	<i>domestica</i>	Germany		6/30/2003
613877	GMAL 2545.h1	<i>baccata</i>	United States	Oregon	5/11/2001
589878	GMAL 2720	<i>asiatica</i>	China		5/14/2001
613905	GMAL 3051.j1	<i>coronaria</i>	United States	Illinois	5/21/2014
613927	GMAL 3232.g1	<i>prunifolia</i>	China		5/14/2001
590184	Golden Delicious	<i>domestica</i>	United States	New York	7/1/2005
590128	Golden Harvey	<i>domestica</i>	United Kingdom	England	6/14/2004
590129	Golden Pippin	<i>domestica</i>	United States		6/14/2004
589892	Golden Russet	<i>domestica</i>			6/15/2000
588880	Granny Smith	<i>domestica</i>	Australia		5/15/2006
589233	Graue Renette Von Zabergau	<i>domestica</i>	Germany		6/14/2004
588791	Grimes Golden	<i>domestica</i>	United States	West Virginia	6/26/2002
173981	Gros Bois	<i>domestica</i>	France		5/19/2006
131105	Gros Frequin	<i>domestica</i>	France	Aube	5/1/2003
161761	Grosse Launette	<i>domestica</i>	France	Indre	6/14/2004
162545	Grosse Mouche	<i>domestica</i>	France	Manche	6/30/2003
681628	Harrison	<i>hybrid</i>	United States		6/17/2014
589653	Harry Masters Jersey	<i>domestica</i>	United Kingdom	England	5/23/2008
136001	Herring's Pippin	<i>domestica</i>	United Kingdom	England	6/15/2000
589585	Holaart Doux	<i>domestica</i>	Belgium		6/26/2002

PI Number	Cultivar Name	<i>Malus</i> species	Origin	Sub-Origin	Date Planted
590130	Hubbards Pearmain	<i>domestica</i>	United Kingdom	England	6/14/2004
589202	Hubbardston Nonsuch	<i>domestica</i>	United States	New York	5/20/2010
590157	Hudson's Golden Gem	<i>domestica</i>	United States	Oregon	6/2/2005
588841	Idared	<i>domestica</i>	United States		7/1/2005
589682	Improved Lambrook Pippin	<i>domestica</i>	United Kingdom	England	6/15/2000
589441	Ingol	<i>domestica</i>	Germany		7/1/2005
589072	Ingram	<i>domestica</i>	United States		6/30/2003
594103	Inuringo	<i>prunifolia</i>	Japan		7/1/2005
590131	Isle of Wight Pippin	<i>domestica</i>	United Kingdom	England	6/14/2004
589185	Jefferis	<i>domestica</i>	United States	Pennsylvania	6/14/2004
590185	Jonathan	<i>domestica</i>	United States	New York	5/6/2005
162731	Jouveaux	<i>domestica</i>	France	Hauts-de-Seine	6/2/2005
657065	Kaz 95-05-01P-22	<i>sieversii</i>	Kazakstan		5/21/2012
657013	Kaz 95-08-01	<i>sieversii</i>	Kazakstan		5/22/2012
657019	Kaz 95-08-06	<i>sieversii</i>	Kazakstan		5/23/2012
613955	KAZ 96-03-12	<i>sieversii</i>	Kazakstan		5/24/2012
657100	KAZ 96-09 02	<i>sieversii</i>	Kazakstan		5/25/2012
633920	Kaz 96-09-05	<i>sieversii</i>	Kazakstan		5/26/2012
633920	Kaz 96-09-15	<i>sieversii</i>	Kazakstan		7/1/2005
203814	Keegan's Crab	<i>domestica</i>	United Kingdom		6/26/2002
590173	Kelsey	<i>hybrid</i>	United States	New York	6/2/2005
589156	King David	<i>domestica</i>	United States	Arkansas	6/14/2004
175010	King Harry	<i>domestica</i>	United Kingdom		6/20/2010
589703	Kingston Black	<i>domestica</i>	United Kingdom	England	6/26/2002
589219	Kola	<i>coronaria</i>	United States	South Dakota	7/9/2003
589598	La Paix	<i>domestica</i>	Belgium		6/27/2003
589053	Lady	<i>domestica</i>	France		7/1/2005
161839	Lambrook Pippin	<i>domestica</i>	United Kingdom	England	6/2/2005
162724	Lande	<i>domestica</i>	France	Hauts-de-Seine	6/30/2003
589565	Landsberger Reinette	<i>domestica</i>	Germany	Brandenburg	6/15/2000
161851	Langworthy	<i>domestica</i>	United Kingdom	England	6/2/2005
162732	Launette	<i>domestica</i>	France	Hauts-de-Seine	6/15/2000

PI Number	Cultivar Name	<i>Malus</i> species	Origin	Sub-Origin	Date Planted
589690	Le Bret	<i>domestica</i>	United Kingdom	England	6/14/2004
161407	Lemoen	<i>domestica</i>	Netherlands		6/30/2003
588943	Liberty	<i>domestica</i>	United States	Washington	4/30/2009
589920	Lombart's Calville	<i>domestica</i>	Netherlands		7/6/2015
589681	Lord Derby	<i>domestica</i>	United Kingdom	England	5/21/2014
161840	Lorna Doone	<i>domestica</i>	United Kingdom	England	5/1/2003
589895	Macoun	<i>domestica</i>	United States	New York	6/26/2002
150649	Major	<i>domestica</i>			5/21/2010
162716	Manch Rouge	<i>domestica</i>	France	Manche	6/2/2005
264558	Margil	<i>domestica</i>			6/15/2000
173982	Marin Onfroy	<i>domestica</i>	France		5/10/2001
588998	Marshall McIntosh	<i>domestica</i>	United States	Massachusetts	7/1/2005
589714	Maude	<i>domestica</i>	United States		6/2/2005
162713	Maunerbe	<i>domestica</i>	France	Manche	6/30/2003
588817	McIntosh Summerland Red	<i>domestica</i>	Canada	British Columbia	6/26/2002
594108	Medaille d'Or	<i>domestica</i>	France		5/6/2005
589634	Mercer	<i>hybrid</i>	United States		5/10/2001
589709	Merton 778	<i>domestica</i>	Australia	Tasmania	6/1/2005
173983	Metais	<i>domestica</i>	France		6/27/2003
589670	Michelin	<i>domestica</i>	United Kingdom	England	5/21/2014
154164	Miron Sacharanij	<i>domestica</i>	Germany	Bavaria	6/14/2004
162735	Mottais	<i>domestica</i>	France	Hauts-de-Seine	6/30/2003
200780	Muscadet Bernay	<i>domestica</i>	France	Gironde	6/27/2003
589493	Muscadet de Dieppe	<i>domestica</i>	France		6/26/2002
173985	Muscadet de Lense	<i>domestica</i>	France		6/15/2000
223602	Mutsu	<i>domestica</i>	Japan	Aomori	5/9/2006
161763	Nanot	<i>domestica</i>	France	Indre	6/1/2005
175544	Nehou	<i>domestica</i>	United Kingdom	England	6/1/2005
161843	Neverblight	<i>domestica</i>	United Kingdom	England	5/21/2014
154166	Nitschners Erdbeerapfel	<i>domestica</i>	Germany		6/27/2003
173986	Noel Deschamps	<i>domestica</i>	France		6/30/2003
588872	Northern Spy	<i>domestica</i>	United States		7/1/2005
589647	Northwood	<i>domestica</i>	United Kingdom	England	6/26/2002
137094	Notaire	<i>domestica</i>	Belgium		6/2/2005

PI Number	Cultivar Name	<i>Malus</i> species	Origin	Sub-Origin	Date Planted
134809	Oetwiler Renette	<i>domestica</i>	Switzerland		6/2/2012
280027	Old Nonpareil	<i>hybrid</i>	United Kingdom	England	6/15/2000
590133	Old Pearmain	<i>domestica</i>	United Kingdom	England	6/1/2005
589562	Oldenburg	<i>domestica</i>	Germany		6/26/2002
589349	Ottawa 5	<i>hybrid</i>	Canada	British Columbia	6/26/2002
590178	Otterson	<i>hybrid</i>			6/2/2005
352653	P.2	<i>domestica</i>	Poland		6/26/2002
162740	Peau D'Ane	<i>domestica</i>	France		6/14/2004
136489	Peau de Vache	<i>domestica</i>	France		6/2/2005
589674	Pethyre	<i>domestica</i>	United Kingdom	England	6/7/2012
589597	Pigeonnet	<i>domestica</i>	Belgium		6/26/2002
132272	Pigeonnet Blanc	<i>domestica</i>	France		6/30/2003
132273	Pigeonnet Rouge	<i>domestica</i>	France		6/30/2003
157734	Pine Golden Pippin	<i>domestica</i>	United Kingdom		6/30/2003
383515	Poeltsama Winter Apple	<i>domestica</i>	Russian Federation		7/1/2005
588745	Pohorka	<i>domestica</i>	United States	New Jersey	6/30/2003
134668	Pomme Cloche	<i>domestica</i>	Switzerland	Vaud	6/30/2003
131975	Pomme Framboise	<i>domestica</i>	Belgium		6/27/2003
589242	Pomme Grise	<i>domestica</i>	United States	New York	5/11/2001
134669	Pomme Raisin	<i>domestica</i>	Switzerland	Vaud	5/25/2005
162548	Pomme Thoury	<i>domestica</i>	France	Manche	6/15/2000
240817	Pommier Llorca	<i>domestica</i>	Algeria	Oran	6/2/2005
589212	Porter	<i>domestica</i>	United States		6/15/2000
-	Porter's Perfection	<i>domestica</i>	United Kingdom	England	Dead
589789	PRI 1744-1	<i>hybrid</i>	United States	Indiana	7/1/2005
589250	Red Jacket	<i>hybrid</i>	United States	New York	6/30/2003
161845	Red Jersey (Loyal Drain)	<i>domestica</i>	United Kingdom	England	6/15/2000
437047	Red Ralls	<i>domestica</i>	Poland		6/2/2005
589087	Red Sauce	<i>domestica</i>	United States	New York	7/7/2015
589211	Redfield	<i>domestica</i>	United States	New York	6/14/2004
589010	Redflesh	<i>hybrid</i>	United States	South Dakota	6/14/2004
175543	Redstreak	<i>domestica</i>	United Kingdom	England	7/6/2015
158736	Reine des Hatives	<i>domestica</i>	France	Maine-et-Loire	6/30/2003

PI Number	Cultivar Name	<i>Malus</i> species	Origin	Sub-Origin	Date Planted
132571	Reine des Pommes	<i>domestica</i>	France		6/2/2005
279326	Reine des Reinettes x 1700	<i>domestica</i>	France		6/2/2005
279325	Reine des Reinettes x 82	<i>domestica</i>	France		6/2/2005
105524	Reineta do Caravia	<i>domestica</i>	Spain	Zaragoza	6/2/2005
589444	Reinette Clochard	<i>domestica</i>	Switzerland		6/26/2002
590135	Reinette d' Anjou	<i>domestica</i>	United Kingdom	England	5/14/2001
188606	Reinette d'Armorique	<i>domestica</i>	France		6/20/2016
322032	Reinette Da Mana	<i>domestica</i>	France		6/2/2005
590136	Reinette de Cuzy	<i>domestica</i>			6/7/2012
131828	Reinette do Chenee	<i>domestica</i>	France	Maine-et-Loire	5/25/2005
590137	Reinette Franche	<i>domestica</i>	United Kingdom	England	5/10/2001
589588	Reinette Grise	<i>domestica</i>	Belgium		5/25/2005
590138	Reinette Grise de Portugal	<i>domestica</i>	United Kingdom	England	5/14/2001
131561	Reinette Jaegers	<i>domestica</i>	Belgium		6/14/2004
135645	Reinette Jamin	<i>domestica</i>	France	Isere	5/11/2001
131978	Reinette Jaune de Butzel	<i>domestica</i>	Belgium		6/2/2005
590139	Reinette Ontz	<i>domestica</i>	United Kingdom	England	5/14/2001
590140	Reinette Thouin	<i>domestica</i>			5/10/2001
162741	Reinette Tres Tardive	<i>domestica</i>	France	Hauts-de-Seine	6/27/2003
188521	Reinette van Ekenstein	<i>domestica</i>	Netherlands		6/30/2003
104034	Renetta Dorata	<i>domestica</i>	Italy		6/15/2000
105528	Repinaldo do Liebana	<i>domestica</i>	Spain	Zaragoza	6/14/2004
589520	Rhode Island Greening	<i>domestica</i>			7/1/2005
588840	Ribston	<i>domestica</i>	United Kingdom	England	6/15/2000
437057	Roberts Crab	<i>hybrid</i>	United Kingdom	England	7/1/2005
588825	Robusta 5	<i>robusta</i>	Canada		7/1/2005
590141	Ross Nonpareil	<i>domestica</i>			6/14/2004
102148	Rott jarnpple	<i>domestica</i>	Sweden		6/30/2003
589143	Rouge Belle de Boskoop	<i>domestica</i>	United States	New York	6/14/2004
141243	Rougemont	<i>domestica</i>	United Kingdom		6/30/2003
136604	Rousse Latour	<i>domestica</i>	France	Aube	6/2/2005
588971	Roxbury Russet	<i>domestica</i>	United States	New York	6/26/2002
136605	Royal d'Angleterre	<i>domestica</i>	United Kingdom		6/27/2003

PI Number	Cultivar Name	<i>Malus</i> species	Origin	Sub-Origin	Date Planted
175545	Royal Jersey	<i>domestica</i>	United Kingdom	England	6/2/2005
161846	Skyrme's Kernel	<i>domestica</i>	United Kingdom	England	6/1/2005
589903	Smokehouse	<i>domestica</i>			6/26/2002
589699	Somerset Redstreak	<i>domestica</i>	United Kingdom	England	7/6/2015
231942	Spatbluehender	<i>domestica</i>	Germany		5/21/2014
588975	Stayman	<i>domestica</i>	United States	Kansas	6/15/2000
589692	Stembridge Cluster	<i>domestica</i>	United Kingdom	England	6/15/2000
589693	Stembridge Jersey	<i>domestica</i>	United Kingdom	England	6/7/2012
589697	Stoke Red	<i>domestica</i>	United Kingdom	England	6/26/2002
307382	Sturmer Pippin	<i>domestica</i>	United Kingdom	England	6/26/2002
589694	Sunset	<i>domestica</i>	United Kingdom	England	6/26/2002
125566	Surpasse Frequin	<i>domestica</i>	France		6/30/2003
589081	Sweet Alford	<i>domestica</i>	United States	New York	6/15/2000
589688	Sweet Coppin	<i>domestica</i>	United Kingdom	England	5/10/2001
589691	Tale Sweet	<i>domestica</i>	United Kingdom	England	9/26/2002
175548	Tardive Forestier	<i>domestica</i>	United Kingdom	England	6/14/2004
589663	Taylor's	<i>domestica</i>	United Kingdom	England	6/26/2002
175549	Teign Harvey	<i>domestica</i>	United Kingdom	England	6/2/2005
127370	Teint Fraise	<i>domestica</i>	France	Aube	6/2/2005
506361	Thorgauer Weinapfel	<i>domestica</i>	Switzerland		6/26/2002
125271	Tom Putt	<i>domestica</i>	United Kingdom	England	6/2/2005
589339	Trail	<i>hybrid</i>	Canada	Ontario	6/26/2002
175550	Trembletts Bitter	<i>domestica</i>	United Kingdom	England	7/9/2003
175551	Twistbody Jersey	<i>domestica</i>	United Kingdom	England	7/9/2003
629317	USSR 89-35-01	<i>sieversii</i>	Kazakstan		7/9/2003
175552	Vagnon Ascher	<i>domestica</i>	United Kingdom	England	5/1/2003
589060	Vandevere	<i>domestica</i>			6/27/2003
125273	Venus Pippin	<i>domestica</i>	United Kingdom		7/99/2003
588819	Vista Bella	<i>domestica</i>	United States		6/26/2002
589623	Wamdesa	<i>hybrid</i>	United States	South Dakota	6/14/2004

PI Number	Cultivar Name	<i>Malus</i> species	Origin	Sub-Origin	Date Planted
589635	Wecota	<i>hybrid</i>	United States	South Dakota	6/26/2002
590143	Weidners GoldreINETte	<i>domestica</i>	Germany		6/14/2004
590144	Weisser Winter Taffetapfel	<i>domestica</i>	United Kingdom	England	6/1/2005
589622	Wetonka	<i>hybrid</i>	United States	South Dakota	6/15/2000
175553	White Jersey	<i>domestica</i>	United Kingdom	England	6/2/2005
681625	White Winter Pearmain	<i>hybrid</i>	United States		6/17/2014
613818	Wickson	<i>domestica</i>	United States	California	6/2/2005
589309	William Crump	<i>domestica</i>	United Kingdom	England	6/14/2004
588799	Winesap	<i>domestica</i>	United States	Washington	6/26/2002
589632	Wotanda	<i>domestica</i>	United States	South Dakota	5/14/2001
589195	Yellow Bellflower	<i>domestica</i>	United States		6/15/2000
588773	Yellow Newtown	<i>domestica</i>	United States	New York	6/26/2002
589614	Zapta	<i>hybrid</i>	United States	South Dakota	6/14/2004
589569	Zuccalmaglio	<i>domestica</i>	Germany		6/7/2012

Supplemental table 2: The proportion of red blush on the peel (%) or peel green background color (1 = yellow-green, 5 = dark green) and associated standard deviations for all accessions phenotyped (n=158) in the 2017 harvest season from the USDA-PGRU *Malus* germplasm collection in Geneva, NY.

PI Number	Cultivar Name	Peel Blush (%)	Standard Deviation of Peel Blush (%)	Peel Green Background Color (1= Yellow-Green, 5 = Dark green)	Standard Deviation Peel Green Background Color
280022	Adam's Pearmain	52.67	23.06		
127311	Amere de Berthcourt			2.00	0.00
173978	American Forestier	63.33	21.27		
136243	Amzr Gauthier			1.13	0.35
588952	Arkansas	48.67	18.07		
589117	Arkansas Black	90.50	10.00		
589654	Ashmead's Kernel	16.00	6.99		
105498	Bella de Jardins	51.54	14.63		
162544	Belle de Crollon			2.53	0.83
589584	Belle de Nordhausen	20.00	14.64		
162709	Belle Fille	51.33	11.25		
588951	Belle Sans Pepin	20.33	19.22		

PI Number	Cultivar Name	Peel Blush (%)	Standard Deviation of Peel Blush (%)	Peel Green Background Color (1= Yellow-Green, 5 = Dark green)	Standard Deviation Peel Green Background Color
588953	Ben Davis	42.00	37.06		
122598	Binet Blanc			4.80	0.56
158729	Binet Blanc Dore	18.67	13.16		
158730	Binet Rouge	50.67	17.92		
590180	Blue Pearmain	81.43	8.19		
162549	Boche	38.00	11.46		
590120	Bonne-Hotture			3.20	1.01
107171	Bramley's Seedling			3.87	0.52
158731	Bramtot			1.00	0.00
589662	Brown's Apple	43.33	23.97		
588808	Bulmer Norman			1.50	0.76
187297	C'Huero Biz Bras	17.14	13.80		
187298	C'Huero Ru Bienn			4.73	0.46
161830	Cap of Liberty	72.00	21.78		
183961	Carnival-14			1.72	0.54
264688	Champagne Reinette			1.60	0.70
589175	Coat Jersey	55.00	21.73		
590121	Cornish Aromatic (Wakeley)	46.00	28.75		
589602	Court Pendu Gris				
589587	Court Pendu Rose	31.33	13.56		
589671	Court Royal			2.43	1.22
589196	Crow Egg	60.00	33.81		
162722	Damelot	51.33	15.52		
162062	Daux Belan	36.67	15.43		
173979	Domaine	73.33	17.29		
131104	Double Bon Pommier	88.00	8.37		
161760	Doucet Rouge	54.67	19.59		
589667	Doux Normandie	44.00	23.84		
162715	Doux Tardif	38.00	15.21		
122616	Doux-AMR			1.27	0.46
131823	Drap d'Or Guemene	77.67	14.13		
589666	Dunkerton Late Sweet			2.40	0.63
589642	Eda	52.67	20.17		
590125	Edelroter	16.67	14.96		
392312	Edward VII	47.14	24.31		

PI Number	Cultivar Name	Peel Blush (%)	Standard Deviation of Peel Blush (%)	Peel Green Background Color (1= Yellow-Green, 5 = Dark green)	Standard Deviation Peel Green Background Color
589650	Ellis Bitter				
588785	Esopus Spitzenburg	47.33	19.81		
590126	Fenouillet de Ribours			1.74	0.59
589679	Fillbarrel	26.67	23.20		
589626	Forest King			4.00	0.00
589318	Foxwhelp	58.42	30.96		
590127	Fraindisé				
162503	Frequin	83.00	12.22		
247314	Frequin Lacaille			1.00	0.00
589689	Frequin Tardive de la Sarthe	44.67	21.67		
276299	Freyberg			1.93	0.70
589123	Geeveston Fanny	77.33	11.63		
132225	Gewurzluiken	74.10	15.97		
613897	GMAL 2996.c1			1.93	1.03
613927	GMAL 3232.g1	91.43	8.52		
590184	Golden Delicious			3.73	0.59
590128	Golden Harvey			1.00	0.00
590129	Golden Pippin			1.13	0.35
589892	Golden Russet			5.00	0.00
589684	Grenadier			1.00	0.00
161761	Grosse Launette	31.00	23.92		
162545	Grosse Mouche	27.00	21.20		
681628	Harrison			3.13	0.74
589585	Holaart Doux	60.67	25.49		
590130	Hubbards Pearmain	44.33	29.09		
589682	Improved Lambrook Pippin	55.33	18.46		
590185	Jonathan	68.67	15.98		
162731	Jouveaux			1.53	0.52
657019	Kaz 95 08-06			4.13	0.64
589703	Kingston Black	69.63	31.75		
589219	Kola			4.00	0.95
589598	La Paix	52.00	18.97		
162724	Lande	27.67	12.08		
589565	Landsberger Reinette			1.60	0.74
161851	Langworthy	55.33	18.46		

PI Number	Cultivar Name	Peel Blush (%)	Standard Deviation of Peel Blush (%)	Peel Green Background Color (1= Yellow-Green, 5 = Dark green)	Standard Deviation Peel Green Background Color
162732	Launette			1.00	0.00
589690	Le Bret	75.33	15.06		
588943	Liberty	62.67	22.19		
264558	Margil	73.33	11.13		
173982	Marin Onfroy			4.47	0.83
588998	Marshall McIntosh	91.73	9.92		
588817	McIntosh Summerland Red	60.00	26.46		
594108	Medaille d'Or			1.00	0.00
589634	Mercer			2.20	0.86
589670	Michelin			2.07	0.96
588976	Midget Crab	55.53	28.84		
200780	Muscadet Bernay	21.67	18.87		
589493	Muscadet de Dieppe			1.93	0.53
173985	Muscadet de Lense	30.00	14.64		
223602	Mutsu			1.00	0.00
161763	Nanot			1.73	0.46
175544	Nehou	21.33	18.46		
173986	Noel Deschamps	28.00	11.46		
588872	Northern Spy	43.57	17.37		
280027	Old Nonpareil			2.00	1.05
590133	Old Pearmain	56.00	24.13		
589674	Pethyre			1.29	0.61
132272	Pigeonnet Blanc			3.87	0.74
132273	Pigeonnet Rouge	83.00	17.81		
588745	Pohorka	68.00	17.40		
134668	Pomme Cloche			1.86	0.77
131975	Pomme Framboise	84.00	11.21		
134669	Pomme Raisin	38.33	17.22		
162548	Pomme Thoury	38.67	13.02	1.67	0.71
240817	Pommier Llorca			1.20	0.56
589789	PRI 1744-1	76.00	16.39		
589211	Red Field	92.53	7.40		
437047	Red Ralls	42.67	13.87		
132571	Reine des Pommes			1.87	0.52
279326	Reine des Reinettes x 1600	31.15	22.74		

PI Number	Cultivar Name	Peel Blush (%)	Standard Deviation of Peel Blush (%)	Peel Green Background Color (1= Yellow-Green, 5 = Dark green)	Standard Deviation Peel Green Background Color
279325	Reine des Reinettes x 82	62.00	6.76		
105524	Reineta do Caravia			2.21	0.89
589444	Reinette Clochard			1.47	0.64
590135	Reinette d'Anjou	26.00	5.48		
590137	Reinette Franche			3.97	0.99
131561	Reinette Jaegers			2.40	0.99
135645	Reinette Jamin	26.92	7.51		
590140	Reinette Thouin			2.23	0.73
188521	Reinette van Ekenstain	1.17	0.41		
589520	Rhode Island Greening			4.53	0.64
588840	Ribston	75.60	12.27		
590141	Ross Nonpareil	36.67	24.69		
102148	Rott Jarnpple	74.33	19.99		
589143	Rouge Belle De Boskoop	16.25	9.16		
588971	Roxbury Russet			1.67	0.72
161846	Skyrme's Kernel	72.67	10.33		
588975	Stayman	76.67	11.13		
589692	Stembridge Cluster	27.33	7.04		
589693	Stembridge Jersey	76.33	11.29		
307382	Sturmer Pippin			4.87	0.35
125566	Surpasse Frequin			3.33	1.11
589691	Tale Sweet	33.33	19.88		
175548	Tardive Forestier			3.13	1.51
589663	Taylor's	31.25	13.56		
175549	Teign Harvey			1.00	0.00
127370	Teint Fraise	63.33	19.15		
506361	Thorgauer Weinapfel	52.00	23.05		
175551	Twistbody Jersey	13.33	4.88		
629317	USSR-89-35-01	53.33	11.75		
175552	Vagnon Ascher	47.33	13.35		
589060	Vandevere	34.29	18.69		
589623	Wamdesa	26.00	12.98		
589635	Wecota			4.86	0.53
590143	Weidners Goldreinette	41.43	30.60		
590144	Weisser Winter Taffetapfel			1.20	0.41

PI Number	Cultivar Name	Peel Blush (%)	Standard Deviation of Peel Blush (%)	Peel Green Background Color (1= Yellow-Green, 5 = Dark green)	Standard Deviation Peel Green Background Color
613818	Wickson	58.00	20.42		
589309	William Crump	44.67	13.56		
588799	Winesap	81.00	19.20		
589632	Wotonda			5.00	0.00
588773	Yellow Newtown	21.43	11.67		
589614	Zapta			2.73	0.80

Supplemental Table 3: The fruit cracking score (1=no fruit cracking and 4=severe fruit cracking), and sunburn score (1=no fruit sunburn, 4=severe fruit sunburn) and associated standard deviations for all accessions phenotyped (n=158) in the 2017 harvest season from the USDA-PGRU Malus germplasm collection Geneva, NY.

PI Number	Cultivar Name	Fruit Cracking Score (1=None, 4 = Severe)	Standard Deviation of Fruit Cracking Score	Sunburn Score (1= None, 4=Severe)	Standard Deviation of Sunburn Score
280022	Adam's Pearmain	1.00	0.00	1.00	0.00
127311	Amere de Berthcourt	1.00	0.00	1.00	0.00
173978	American Forestier	1.00	0.00	1.07	0.26
136243	Amzr Gauthier	1.00	0.00	1.00	0.00
588952	Arkansas	1.07	0.26	1.00	0.00
589117	Arkansas Black	1.00	0.00	1.07	0.26
589654	Ashmead's Kernel	1.00	0.00	1.00	0.00
105498	Bella de Jardins	1.00	0.00	1.00	0.00
162544	Belle de Crollon	1.00	0.00	1.00	0.00
589584	Belle de Nordhaussen	1.00	0.00	1.00	0.00
162709	Belle Fille	1.00	0.00	1.00	0.00
588951	Belle Sans Pepin	1.00	0.00	1.00	0.00
588953	Ben Davis	1.00	0.00	1.00	0.00
122598	Binet Blanc	1.00	0.00	1.00	0.00
158729	Binet Blanc Dore	1.00	0.00	1.00	0.00
158730	Binet Rouge	1.00	0.00	1.00	0.00
590180	Blue Pearmain	1.00	0.00	1.00	0.00
162549	Boche	1.00	0.00	1.00	0.00
590120	Bonne-Hotture	1.00	0.00	1.00	0.00
107171	Bramley's Seedling	1.00	0.00	1.00	0.00
158731	Bramtot	1.00	0.00	1.07	0.26

PI Number	Cultivar Name	Fruit Cracking Score (1=None, 4 = Severe)	Standard Deviation of Fruit Cracking Score	Sunburn Score (1= None, 4=Severe)	Standard Deviation of Sunburn Score
589662	Brown's Apple	1.00	0.00	1.00	0.00
588808	Bulmer Norman	1.00	0.00	1.00	0.00
187297	C'Huero Biz Bras	1.00	0.00	1.00	0.00
187298	C'Huero Ru Bienn	1.00	0.00	1.00	0.00
161830	Cap of Liberty	1.00	0.00	1.00	0.00
183961	Carnival-14	1.00	0.00	1.00	0.00
264688	Champagne Reinette	1.00	0.00	1.00	0.00
589175	Coat Jersey	1.00	0.00	1.00	0.00
590121	Cornish Aromatic (Wakeley)	1.00	0.00	1.00	0.00
589602	Court Pendu Gris	1.17	0.39	1.00	0.00
589587	Court Pendu Rose	1.00	0.00	1.07	0.26
589671	Court Royal	1.00	0.00	1.00	0.00
589196	Crow Egg	1.00	0.00	1.00	0.00
162722	Damelot	1.13	0.35	1.00	0.00
162062	Daux Belan	1.00	0.00	1.00	0.00
173979	Domaine	1.00	0.00	1.00	0.00
131104	Double Bon Pommier	1.00	0.00	1.00	0.00
161760	Doucet Rouge	1.00	0.00	1.00	0.00
589667	Doux Normandie	1.00	0.00	1.00	0.00
162715	Doux Tardif	1.00	0.00	1.00	0.00
122616	Doux-AMR	1.00	0.00	1.00	0.00
131823	Drap d'Or Guemene	1.00	0.00	1.00	0.00
589666	Dunkerton Late Sweet	1.00	0.00	1.00	0.00
589642	Eda	1.00	0.00	1.00	0.00
590125	Edelroter	1.00	0.00	1.07	0.26
392312	Edward VII	1.00	0.00	1.00	0.00
589650	Ellis Bitter	1.00	0.00	1.00	0.00
588785	Esopus Spitzenburg	1.00	0.00	1.00	0.00
590126	Fenouillet de Ribours	1.00	0.00	1.00	0.00
589679	Fillbarrel	1.00	0.00	1.00	0.00
589626	Forest King	1.00	0.00	1.00	0.00
589318	Foxwhelp	1.00	0.00	1.00	0.00
590127	Fraindise	1.00	0.00	1.00	0.00
162503	Frequin	1.00	0.00	1.00	0.00
247314	Frequin Lacaille	1.00	0.00	1.00	0.00

PI Number	Cultivar Name	Fruit Cracking Score (1=None, 4 = Severe)	Standard Deviation of Fruit Cracking Score	Sunburn Score (1= None, 4=Severe)	Standard Deviation of Sunburn Score
589689	Frequin Tardive de la Sarthe	1.00	0.00	1.00	0.00
276299	Freyberg	1.00	0.00	1.00	0.00
589123	Geeveston Fanny	1.00	0.00	1.00	0.00
132225	Gewurzluiken	1.00	0.00	1.00	0.00
613897	GMAL 2996.c1	1.00	0.00	1.00	0.00
613927	GMAL 3232.g1	1.00	0.00	1.00	0.00
590184	Golden Delicious	1.07	0.26	1.00	0.00
590128	Golden Harvey	1.00	0.00	1.00	0.00
590129	Golden Pippin	1.00	0.00	1.00	0.00
589892	Golden Russet	1.00	0.00	1.00	0.00
589684	Grenadier	1.00	0.00	1.00	0.00
161761	Grosse Launette	1.00	0.00	1.00	0.00
162545	Grosse Mouche	1.00	0.00	1.00	0.00
681628	Harrison	1.00	0.00	1.00	0.00
589585	Holaart Doux	1.00	0.00	1.00	0.00
590130	Hubbards Pearmain	1.00	0.00	1.03	0.18
589682	Improved Lambrook Pippin	1.00	0.00	1.00	0.00
590185	Jonathan	1.00	0.00	1.00	0.00
162731	Jouveaux	1.00	0.00	1.00	0.00
657019	Kaz 95 08-06	1.00	0.00	1.00	0.00
589703	Kingston Black	1.00	0.00	1.00	0.00
589219	Kola	1.03	0.18	1.03	0.18
589598	La Paix	1.00	0.00	1.00	0.00
162724	Lande	1.00	0.00	1.00	0.00
589565	Landsberger Reinette	1.00	0.00	1.00	0.00
161851	Langworthy	1.00	0.00	1.00	0.00
162732	Launette	1.00	0.00	1.00	0.00
589690	Le Bret	1.00	0.00	1.00	0.00
588943	Liberty	1.00	0.00	1.00	0.00
264558	Margil	1.07	0.26	1.00	0.00
173982	Marin Onfroy	1.00	0.00	1.00	0.00
588998	Marshall McIntosh	1.00	0.00	1.00	0.00
588817	McIntosh Summerland Red	1.00	0.00	1.00	0.00
594108	Medaille d'Or	1.00	0.00	1.00	0.00

PI Number	Cultivar Name	Fruit Cracking Score (1=None, 4 = Severe)	Standard Deviation of Fruit Cracking Score	Sunburn Score (1= None, 4=Severe)	Standard Deviation of Sunburn Score
589634	Mercer	1.00	0.00	1.00	0.00
589670	Michelin	1.00	0.00	1.00	0.00
588976	Midget Crab	1.07	0.26	1.00	0.00
200780	Muscadet Bernay	1.00	0.00	1.00	0.00
589493	Muscadet de Dieppe	1.00	0.00	1.00	0.00
173985	Muscadet de Lense	1.00	0.00	1.00	0.00
223602	Mutsu	1.00	0.00	1.00	0.00
161763	Nanot	1.00	0.00	1.00	0.00
175544	Nehou	1.00	0.00	1.00	0.00
173986	Noel Deschamps	1.00	0.00	1.00	0.00
588872	Northern Spy	1.00	0.00	1.00	0.00
280027	Old Nonpareil	1.00	0.00	1.10	0.32
590133	Old Pearmain	1.00	0.00	1.00	0.00
589674	Pethyre	1.14	0.36	1.00	0.00
132272	Pigeonnet Blanc	1.00	0.00	1.00	0.00
132273	Pigeonnet Rouge	1.00	0.00	1.00	0.00
588745	Pohorka	1.00	0.00	1.00	0.00
134668	Pomme Cloche	1.00	0.00	1.00	0.00
131975	Pomme Framboise	1.00	0.00	1.00	0.00
134669	Pomme Raisin	1.00	0.00	1.00	0.00
162548	Pomme Thoury	1.00	0.00	1.00	0.00
240817	Pommier Llorca	1.00	0.00	1.00	0.00
589789	PRI 1744-1	1.00	0.00	1.00	0.00
589211	Red Field	1.00	0.00	1.00	0.00
437047	Red Ralls	1.00	0.00	1.07	0.26
132571	Reine des Pommes	1.00	0.00	1.00	0.00
279326	Reine des Reinettes x 1600	1.00	0.00	1.00	0.00
279325	Reine des Reinettes x 82	1.00	0.00	1.07	0.26
105524	Reineta do Caravia	1.00	0.00	1.00	0.00
589444	Reinette Clochard	1.00	0.00	1.07	0.26
590135	Reinette d'Anjou	1.00	0.00	1.00	0.00
590137	Reinette Franche	1.00	0.00	1.00	0.00
131561	Reinette Jaegers	1.00	0.00	1.00	0.00
135645	Reinette Jamin	1.00	0.00	1.00	0.00

PI Number	Cultivar Name	Fruit Cracking Score (1=None, 4 = Severe)	Standard Deviation of Fruit Cracking Score	Sunburn Score (1= None, 4=Severe)	Standard Deviation of Sunburn Score
590140	Reinette Thouin	1.00	0.00	1.00	0.00
188521	Reinette van Ekenstain	1.05	0.22	1.00	0.00
589520	Rhode Island Greening	1.00	0.00	1.00	0.00
588840	Ribston	1.00	0.00	1.04	0.20
590141	Ross Nonpareil	1.00	0.00	1.00	0.00
102148	Rott Jarnpple	1.00	0.00	1.00	0.00
589143	Rouge Belle De Boskoop	1.00	0.00	1.00	0.00
588971	Roxbury Russet	1.00	0.00	1.00	0.00
161846	Skyrme's Kernel	1.00	0.00	1.00	0.00
588975	Stayman	1.00	0.00	1.07	0.26
589692	Stembridge Cluster	1.00	0.00	1.00	0.00
589693	Stembridge Jersey	1.00	0.00	1.00	0.00
307382	Sturmer Pippin	1.00	0.00	1.00	0.00
125566	Surpasse Frequin	1.00	0.00	1.00	0.00
589691	Tale Sweet	1.00	0.00	1.00	0.00
175548	Tardive Forestier	1.00	0.00	1.00	0.00
589663	Taylor's	1.00	0.00	1.00	0.00
175549	Teign Harvey	1.00	0.00	1.00	0.00
127370	Teint Fraise	1.00	1.00	1.00	0.00
506361	Thorgauer Weinapfel	1.00	0.00	1.00	0.00
175551	Twistbody Jersey	1.00	0.00	1.00	0.00
629317	USSR-89-35-01	1.00	0.00	1.00	0.00
175552	Vagnon Ascher	1.00	0.00	1.00	0.00
589060	Vandevere	1.00	0.00	1.07	0.27
589623	Wamdesa	1.00	0.00	1.00	0.00
589635	Wecota	1.07	0.27	1.00	0.00
590143	Weidners Goldreinette	1.00	0.00	1.00	0.00
590144	Weisser Winter Taffetapfel	1.00	0.00	1.07	0.26
613818	Wickson	1.00	0.00	1.07	0.26
589309	William Crump	1.00	0.00	1.00	0.00
588799	Winesap	1.00	0.00	1.00	0.00
589632	Wotonda	1.00	0.00	1.00	0.00
588773	Yellow Newtown	1.00	0.00	1.00	0.00
589614	Zapta	1.00	0.00	1.07	0.26

Supplemental table 4: The fruit weight (g), fruit diameter (mm) and associated standard deviations for all accessions phenotyped (n=158) in the 2017 harvest season from the USDA-PGRU Malus germplasm collection in Geneva, NY.

PI Number	Cultivar Name	Fruit Weight (g)	Standard Deviation of Weight (g)	Fruit Diameter (mm)	Standard Deviation of Fruit Diameter (mm)
280022	Adam's Pearmain	207.97	40.07	74.12	6.07
127311	Amere de Berthcourt	72.52	15.11	54.41	4.23
173978	American Forestier	83.98	17.36	58.52	4.97
136243	Amzr Gauthier	69.17	9.10	55.44	2.63
588952	Arkansas	107.72	19.92	59.17	4.27
589117	Arkansas Black	188.29	26.86	74.09	3.89
589654	Ashmead's Kernel	205.42	60.18	78.47	9.24
105498	Bella de Jardins	121.58	43.94	63.05	8.22
162544	Belle de Crollon	98.38	13.70	61.98	3.45
589584	Belle de Nordhaussen	161.82	40.60	72.75	6.91
162709	Belle Fille	107.80	15.29	65.03	3.85
588951	Belle Sans Pepin	176.95	40.34	72.48	6.26
588953	Ben Davis	231.36	37.36	82.14	5.57
122598	Binet Blanc	59.99	9.97	50.92	3.23
158729	Binet Blanc Dore	75.10	11.54	55.55	4.20
158730	Binet Rouge	73.99	15.24	55.97	4.25
590180	Blue Pearmain	197.68	33.70	77.04	5.42
162549	Boche	141.86	26.14	68.74	5.29
590120	Bonne-Hotture	111.67	36.14	63.50	7.39
107171	Bramley's Seedling	293.62	72.23	88.38	8.37
158731	Bramtot	81.98	29.58	57.09	7.56
589662	Brown's Apple	92.36	36.56	59.00	10.13
588808	Bulmer Norman	122.67	33.35	67.93	8.22
187297	C'Huero Biz Bras	127.69	18.80	63.08	2.77
187298	C'Huero Ru Bienn	79.51	16.04	53.15	3.60
161830	Cap of Liberty	57.34	15.32	49.20	4.43
183961	Carnival-14	226.30	61.75	81.02	8.74
264688	Champagne Reinette	184.55	42.30	77.25	6.29
589175	Coat Jersey	95.23	19.58	60.41	3.90

PI Number	Cultivar Name	Fruit Weight (g)	Standard Deviation of Weight (g)	Fruit Diameter (mm)	Standard Deviation of Fruit Diameter (mm)
590121	Cornish Aromatic (Wakeley)	179.27	45.31	72.34	7.37
589602	Court Pendu Gris	165.46	60.19	74.51	10.83
589587	Court Pendu Rose	126.07	22.88	67.83	4.93
589671	Court Royal	100.11	27.67	63.31	6.10
589196	Crow Egg	125.78	32.95	66.79	6.48
162722	Damelot	50.93	8.76	49.73	3.09
162062	Daux Belan	96.13	15.27	60.19	3.48
173979	Domaine	128.73	33.01	66.98	6.63
131104	Double Bon Pommier	250.30	89.97	82.18	10.20
161760	Doucet Rouge	140.56	18.32	68.16	3.67
589667	Doux Normandie	112.62	27.37	63.41	6.29
162715	Doux Tardif	122.42	32.76	64.71	2.61
122616	Doux-AMR	92.42	24.52	61.14	4.64
131823	Drap d'Or Guemene	126.09	27.91	66.06	5.50
589666	Dunkerton Late Sweet	118.33	35.19	64.53	6.72
589642	Eda	74.44	18.59	53.72	5.73
590125	Edelroter	88.13	14.84	58.35	3.90
392312	Edward VII	244.64	69.10	80.82	8.63
589650	Ellis Bitter	154.44	20.30	58.81	10.40
588785	Esopus Spitzenburg	124.04	25.48	64.50	4.52
590126	Fenouillet de Ribours	118.35	48.26	63.83	9.40
589679	Fillbarrel	108.08	25.76	63.95	6.14
589626	Forest King	90.34	26.39	56.65	5.64
589318	Foxwhelp	191.67	66.30	77.20	9.25
590127	Fraindise	129.73	29.12	62.29	4.94
162503	Frequin	52.18	7.69	49.77	2.83
247314	Frequin Lacaille	85.37	25.54	61.56	9.10
589689	Frequin Tardive de la Sarthe	108.01	24.40	62.89	5.64
276299	Freyberg	99.92	26.14	58.85	5.61
589123	Geeveston Fanny	125.95	27.88	67.24	5.22
132225	Gewurzluiken	182.90	39.20	75.42	6.81
613897	GMAL 2996.c1	223.30	65.75	77.49	8.95
613927	GMAL 3232.g1	41.27	9.73	42.97	4.10

PI Number	Cultivar Name	Fruit Weight (g)	Standard Deviation of Weight (g)	Fruit Diameter (mm)	Standard Deviation of Fruit Diameter (mm)
590184	Golden Delicious	214.62	51.21	75.41	6.46
590128	Golden Harvey	90.77	24.21	56.68	5.19
590129	Golden Pippin	88.94	24.74	57.56	6.25
589892	Golden Russet	111.36	21.20	61.09	4.55
589684	Grenadier	290.96	61.40	94.24	7.54
161761	Grosse Launette	127.28	17.49	66.00	3.30
162545	Grosse Mouche	142.52	28.74	70.04	5.02
681628	Harrison	91.28	17.29	57.47	3.85
589585	Holaart Doux	102.38	28.68	63.06	7.03
590130	Hubbards Pearmain	140.93	27.02	66.46	3.88
589682	Improved Lambrook Pippin	57.49	16.82	49.98	4.70
590185	Jonathan	173.70	24.07	74.45	3.92
162731	Jouveaux	116.87	29.30	66.89	5.38
657019	Kaz 95 08-06	41.36	6.59	45.89	2.98
589703	Kingston Black	131.12	60.27	65.81	13.04
589219	Kola	92.28	19.14	59.63	2.50
589598	La Paix	127.15	14.01	63.65	2.35
162724	Lande	127.53	24.54	66.93	4.35
589565	Landsberger Reinette	107.34	32.87	62.22	6.72
161851	Langworthy	126.55	14.42	66.66	2.70
162732	Launette	97.76	25.11	62.03	5.93
589690	Le Bret	163.19	33.32	70.34	8.78
588943	Liberty	142.20	23.17	67.05	3.84
264558	Margil	90.25	20.58	56.89	3.47
173982	Marin Onfroy	60.93	20.94	53.25	9.13
588998	Marshall McIntosh	138.51	28.55	70.74	4.64
588817	McIntosh Summerland Red	210.90	58.28	80.90	7.96
594108	Medaille d'Or	72.34	10.82	55.54	3.51
589634	Mercer	198.17	40.83	78.07	5.65
589670	Michelin	73.34	25.81	54.73	5.95
588976	Midget Crab	31.09	6.20	39.18	2.68
200780	Muscadet Bernay	87.88	13.74	60.89	3.89
589493	Muscadet de Dieppe	83.83	32.43	59.59	8.35
173985	Muscadet de Lense	81.70	14.40	58.58	4.03

PI Number	Cultivar Name	Fruit Weight (g)	Standard Deviation of Weight (g)	Fruit Diameter (mm)	Standard Deviation of Fruit Diameter (mm)
223602	Mutsu	244.91	50.00	79.13	7.04
161763	Nanot	66.44	14.67	55.84	9.27
175544	Nehou	125.31	29.72	64.35	6.19
173986	Noel Deschamps	53.98	11.82	50.59	4.48
588872	Northern Spy	155.21	22.81	71.02	3.72
280027	Old Nonpareil	134.48	44.05	67.11	8.41
590133	Old Pearmain	216.56	54.12	80.03	7.91
589674	Pethyre	138.92	37.39	66.98	6.43
132272	Pigeonnet Blanc	208.28	61.26	75.63	9.35
132273	Pigeonnet Rouge	94.44	17.73	57.51	4.64
588745	Pohorka	213.50	96.42	76.26	4.84
134668	Pomme Cloche	222.42	54.26	74.26	6.15
131975	Pomme Framboise	69.73	20.20	51.78	4.49
134669	Pomme Raisin	163.02	38.44	72.45	7.62
162548	Pomme Thoury	80.75	16.86	58.53	4.25
240817	Pommier Llorca	72.89	18.96	56.11	5.45
589789	PRI 1744-1	96.89	15.47	60.86	4.06
589211	Red Field	184.60	55.47	75.70	8.69
437047	Red Ralls	131.50	22.08	65.65	3.78
132571	Reine des Pommes	119.81	50.74	68.39	11.31
279326	Reine des Reinettes x 1600	202.57	85.37	77.72	13.62
279325	Reine des Reinettes x 82	121.48	24.38	62.98	4.25
105524	Reineta do Caravia	225.06	31.26	77.66	4.15
589444	Reinette Clochard	166.27	42.02	71.98	6.57
590135	Reinette d'Anjou	225.53	21.21	79.20	2.35
590137	Reinette Franche	138.59	33.87	67.20	5.92
131561	Reinette Jaegers	339.98	128.83	76.12	6.02
135645	Reinette Jamin	385.59	88.29	97.34	8.36
590140	Reinette Thouin	137.26	33.14	68.23	6.75
188521	Reinette van Ekenstain	180.46	40.76	73.28	4.85
589520	Rhode Island Greening	249.01	75.36	81.92	8.95
588840	Ribston	150.82	32.52	69.45	5.53
590141	Ross Nonpareil	139.06	35.06	67.72	6.50

PI Number	Cultivar Name	Fruit Weight (g)	Standard Deviation of Weight (g)	Fruit Diameter (mm)	Standard Deviation of Fruit Diameter (mm)
102148	Rott Jarnpple	133.53	21.62	65.25	3.39
589143	Rouge Belle De Boskoop	328.90	60.07	88.70	9.81
588971	Roxbury Russet	172.94	27.27	72.64	5.54
161846	Skyrme's Kernel	99.76	18.91	60.61	4.65
588975	Stayman	201.35	39.03	75.76	5.06
589692	Stembridge Cluster	89.07	17.42	57.20	4.09
589693	Stembridge Jersey	65.91	11.82	54.72	3.69
307382	Sturmer Pippin	119.43	27.16	65.10	4.10
125566	Surpasse Frequin	139.81	25.30	68.84	4.85
589691	Tale Sweet	128.68	21.84	70.11	4.10
175548	Tardive Forestier	122.31	47.27	64.88	9.12
589663	Taylor's	103.90	45.00	62.71	3.68
175549	Teign Harvey	138.00	28.10	72.09	4.76
127370	Teint Fraise	146.61	21.12	75.56	13.85
506361	Thorgauer Weinapfel	126.86	28.01	66.19	5.54
175551	Twistbody Jersey	91.32	8.58	61.02	2.35
629317	USSR-89-35-01	150.09	48.11	71.62	8.82
175552	Vagnon Ascher	110.21	37.57	59.25	7.29
589060	Vandevere	211.50	45.61	78.67	4.41
589623	Wamdesa	77.38	32.16	57.69	2.86
589635	Wecota	52.46	7.24	47.79	2.06
590143	Weidners Goldreinette	186.42	33.81	74.75	5.14
590144	Weisser Winter Taffetapfel	118.10	21.15	65.72	6.22
613818	Wickson	52.71	11.90	47.19	3.54
589309	William Crump	182.17	61.27	90.05	12.41
588799	Winesap	147.79	24.37	68.09	4.44
589632	Wotonda	95.02	17.18	54.12	9.73
588773	Yellow Newtown	170.54	52.67	71.58	7.47
589614	Zapta	107.89	15.24	62.57	3.28

Supplemental Table 5: The starch pattern index score (1-8 where 1=no hydrolyzation of starch and 8= all starch has been hydrolyzed), fruit firmness (N), and associated standard deviations for all accessions(n=158) phenotyped in the 2017 harvest season from the USDA-PGRU Malus germplasm collection in Geneva, NY.

PI Number	Cultivar Name	Starch Pattern Index Score	Standard Deviation of Starch Pattern Index Score	Fruit Firmness (N)	Standard Deviation of Fruit Firmness
280022	Adam's Pearmain	8.00	0.00	44.96	14.33
127311	Amere de Berthcourt	8.00	0.00	66.92	15.25
173978	American Forestier	8.00	0.00	55.76	10.48
136243	Amzr Gauthier	8.00	0.00	57.17	11.65
588952	Arkansas	7.00	1.00	67.87	8.82
589117	Arkansas Black	7.33	0.49	82.74	17.06
589654	Ashmead's Kernel	8.00	0.00	49.53	15.98
105498	Bella de Jardins	8.00	0.00	52.33	11.54
162544	Belle de Crollon	8.00	0.00	62.85	19.10
589584	Belle de Nordhaussen	8.00	0.00	57.13	10.49
162709	Belle Fille	8.00	0.00	52.65	13.70
588951	Belle Sans Pepin	8.00	0.00	60.20	14.78
588953	Ben Davis	7.90	0.32	77.92	13.27
122598	Binet Blanc	8.00	0.00	89.36	16.67
158729	Binet Blanc Dore	8.00	0.00	71.86	14.19
158730	Binet Rouge	7.00	0.00	70.83	4.36
590180	Blue Pearmain	7.71	0.61	50.51	8.61
162549	Boche	7.93	0.26	59.77	12.80
590120	Bonne-Hotture	8.00	0.00	69.39	10.09
107171	Bramley's Seedling	8.00	0.00	56.24	19.80
158731	Bramtot	7.67	0.49	71.12	11.34
589662	Brown's Apple	8.00	0.00	107.08	7.81
588808	Bulmer Norman	8.00	0.00	25.97	14.74
187297	C'Huero Biz Bras	6.64	2.17	61.97	16.85
187298	C'Huero Ru Bienn	7.87	0.35	90.43	12.84
161830	Cap of Liberty	8.00	0.00	49.90	10.26
183961	Carnival-14	7.48	0.87	52.45	6.14
264688	Champagne Reinette	8.00	0.00	51.30	6.01
589175	Coat Jersey	8.00	0.00	63.92	7.84

PI Number	Cultivar Name	Starch Pattern Index Score	Standard Deviation of Starch Pattern Index Score	Fruit Firmness (N)	Standard Deviation of Fruit Firmness
590121	Cornish Aromatic (Wakeley)	6.50	1.58	81.29	7.07
589602	Court Pendu Gris	6.25	1.14	85.91	19.96
589587	Court Pendu Rose	7.00	0.93	53.68	6.07
589671	Court Royal	8.00	0.00	50.37	5.82
589196	Crow Egg	8.00	0.00	44.50	9.46
162722	Damelot	8.00	0.00	38.05	9.84
162062	Daux Belan	7.30	0.94	85.85	13.27
173979	Domaine	7.73	0.70	62.88	9.21
131104	Double Bon Pommier	8.00	0.00	41.92	11.57
161760	Doucet Rouge	7.56	0.16	75.36	11.05
589667	Doux Normandie	8.00	0.00	64.04	6.76
162715	Doux Tardif	8.00	0.00	81.01	9.82
122616	Doux-AMR	8.00	0.00	40.69	9.31
131823	Drap d'Or Guemene	7.40	0.97	45.07	13.40
589666	Dunkerton Late Sweet	7.27	0.46	89.85	16.85
589642	Eda	8.00	0.00	57.88	8.27
590125	Edelroter	8.00	0.00	47.73	16.33
392312	Edward VII	8.00	0.00	53.61	23.65
589650	Ellis Bitter	8.00	0.00	57.29	8.74
588785	Esopus Spitzenburg	8.00	0.00	58.91	27.04
590126	Fenouillet de Ribours	7.53	0.52	78.82	12.16
589679	Fillbarrel	8.00	0.00	48.94	10.13
589626	Forest King	8.00	0.00	110.90	9.85
589318	Foxwhelp	8.00	0.00	51.38	18.30
590127	Fraindise	5.73	0.59	85.12	10.50
162503	Frequin	8.00	0.00	74.12	13.74
247314	Frequin Lacaille	8.00	0.00	57.03	15.05
589689	Frequin Tardive de la Sarthe	8.00	0.00	55.45	19.07
276299	Freyberg	7.87	0.35	83.22	11.94
589123	Geeveston Fanny	7.80	0.41	59.06	13.92
132225	Gewurzluiken	7.97	0.16	73.23	17.17
613897	GMAL 2996.c1	7.47	0.74	86.09	8.70

PI Number	Cultivar Name	Starch Pattern Index Score	Standard Deviation of Starch Pattern Index Score	Fruit Firmness (N)	Standard Deviation of Fruit Firmness
613927	GMAL 3232.g1	8.00	0.00	60.17	10.10
590184	Golden Delicious	7.27	0.46	79.11	7.21
590128	Golden Harvey	7.73	0.46	67.90	16.86
590129	Golden Pippin	7.67	0.49	42.18	4.49
589892	Golden Russet	5.85	0.90	83.70	19.35
589684	Grenadier	8.00	0.00	31.32	12.32
161761	Grosse Launette	8.00	0.00	44.21	11.73
162545	Grosse Mouche	8.00	0.00	50.90	11.99
681628	Harrison	7.00	0.00	114.12	12.67
589585	Holaart Doux	8.00	0.00	60.41	7.20
590130	Hubbards Pearmain	7.87	0.51	42.92	11.06
589682	Improved Lambrook Pippin	8.00	0.00	90.69	5.90
590185	Jonathan	8.00	0.00	54.65	9.68
162731	Jouveaux	8.00	0.00	78.79	16.90
657019	Kaz 95 08-06	8.00	0.00	56.32	7.16
589703	Kingston Black	7.89	0.24	41.79	11.84
589219	Kola	8.00	0.00	136.22	10.48
589598	La Paix	8.00	0.00	64.22	4.96
162724	Lande	8.00	0.00	75.22	11.08
589565	Landsberger Reinette	8.00	0.00	42.30	9.53
161851	Langworthy	8.00	0.00	55.35	18.28
162732	Launette	7.13	0.74	58.32	5.47
589690	Le Bret	8.00	0.00	52.20	6.64
588943	Liberty	7.40	0.51	66.84	10.18
264558	Margil	8.00	0.00	98.14	12.30
173982	Marin Onfroy	7.87	0.52	72.49	20.31
588998	Marshall	7.67	0.62	37.61	13.46
588817	McIntosh McIntosh	8.00	0.00	38.58	9.31
594108	Summerland Red Medaille d'Or	7.53	0.99	50.88	8.88
589634	Mercer	8.00	0.00	79.83	4.70
589670	Michelin	8.00	0.00	73.39	10.76
588976	Midget Crab	7.93	0.26	110.29	7.43

PI Number	Cultivar Name	Starch Pattern Index Score	Standard Deviation of Starch Pattern Index Score	Fruit Firmness (N)	Standard Deviation of Fruit Firmness
200780	Muscadet Bernay	7.73	0.59	52.20	16.35
589493	Muscadet de Dieppe	7.07	0.80	46.24	10.59
173985	Muscadet de Lense	7.73	0.46	64.05	28.64
223602	Mutsu	6.92	0.28	66.84	6.26
161763	Nanot	8.00	0.00	50.15	10.00
175544	Nehou	7.73	0.70	64.78	4.28
173986	Noel Deschamps	8.00	0.00	62.31	8.40
588872	Northern Spy	8.00	0.00	47.40	13.52
280027	Old Nonpareil	8.00	0.00	50.32	7.79
590133	Old Pearmain	7.70	0.48	39.72	17.85
589674	Pethyre	8.00	0.00	40.10	9.50
132272	Pigeonnet Blanc	6.40	1.35	36.87	17.88
132273	Pigeonnet Rouge	7.67	0.49	59.90	9.42
588745	Pohorka	8.00	0.00	46.43	16.20
134668	Pomme Cloche	8.00	0.00	66.40	10.92
131975	Pomme Framboise	8.00	0.00	114.12	6.75
134669	Pomme Raisin	8.00	0.00	50.42	15.54
162548	Pomme Thoury	8.00	0.00	74.14	4.87
240817	Pommier Llorca	8.00	0.00	40.32	13.46
589789	PRI 1744-1	8.00	0.00	42.57	4.52
589211	Red Field	8.00	0.00	42.10	12.53
437047	Red Ralls	8.00	0.00	69.44	8.69
132571	Reine des Pommes	8.00	0.00	69.17	17.23
279326	Reine des Reinettes x 1600	7.92	0.28	41.57	18.32
279325	Reine des Reinettes x 82	8.00	0.00	73.14	30.41
105524	Reineta do Caravia	6.77	0.66	75.30	18.41
589444	Reinette Clochard	7.60	1.30	58.20	14.16
590135	Reinette d'Anjou	8.00	0.00	41.75	13.00
590137	Reinette Franche	7.67	0.58	51.50	22.74
131561	Reinette Jaegers	8.00	0.00	39.30	9.89
135645	Reinette Jamin	8.00	0.00	19.74	7.44
590140	Reinette Thouin	8.00	0.00	57.76	15.78

PI Number	Cultivar Name	Starch Pattern Index Score	Standard Deviation of Starch Pattern Index Score	Fruit Firmness (N)	Standard Deviation of Fruit Firmness
188521	Reinette van Ekenstain	7.90	0.30	42.93	10.02
589520	Rhode Island Greening	8.00	0.00	51.18	10.22
588840	Ribston	8.00	0.00	45.95	8.03
590141	Ross Nonpareil	7.20	0.77	54.48	20.74
102148	Rott Jarnpple	7.07	0.26	108.90	12.70
589143	Rouge Belle De Boskoop	8.00	0.00	65.87	14.96
588971	Roxbury Russet	8.00	0.00	58.85	11.81
161846	Skyrme's Kernel	7.07	0.46	50.02	10.99
588975	Stayman	8.00	0.00	57.96	10.43
589692	Stembridge Cluster	8.00	0.00	78.31	4.23
589693	Stembridge Jersey	7.97	0.18	51.49	18.45
307382	Sturmer Pippin	7.07	0.46	73.08	13.47
125566	Surpasse Frequin	8.00	0.00	60.90	9.31
589691	Tale Sweet	7.40	0.72	70.25	4.56
175548	Tardive Forestier	7.93	0.26	58.73	8.71
589663	Taylor's	7.75	0.46	37.42	12.68
175549	Teign Harvey	7.75	0.45	26.04	8.22
127370	Teint Fraise	8.00	0.00	72.60	12.88
506361	Thorgauer Weinapfel	7.33	0.41	63.03	7.90
175551	Twistbody Jersey	8.00	0.00	65.21	5.61
629317	USSR-89-35-01	7.80	0.41	56.06	4.98
175552	Vagnon Ascher	8.00	0.00	76.37	15.52
589060	Vandevere	8	0	67.17	4.92
589623	Wamdesa	7.73	0.59	135.95	7.66
589635	Wecota	8	0	112.94	11.56
590143	Weidners Goldreinette	8	0	77.83	8.11
590144	Weisser Winter Taffetapfel	8	0	54.85	7.87
613818	Wickson	8	0	52.78	13.69
589309	William Crump	8	0	41.82	4.55
588799	Winesap	5.46	0.74	74.43	5.45
589632	Wotonda	8	0	136.94	8.41
588773	Yellow Newtown	8	0	49.29	14.89

PI Number	Cultivar Name	Starch Pattern Index Score	Standard Deviation of Starch Pattern Index Score	Fruit Firmness (N)	Standard Deviation of Fruit Firmness
589614	Zapta	8	0	135.95	4.87

Supplemental table 6: The glucose, fructose, sucrose, sorbitol concentrations, and associated standard deviations for all accessions phenotyped in the 2017 harvest season from the USDA-PGRU Malus germplasm collection in Geneva, NY.

PI Number	Cultivar Name	Glucose Concentration (g·L ⁻¹)	Standard Deviation of Glucose Concentration (g·L ⁻¹)	Fructose Concentration (g·L ⁻¹)	Standard Deviation of Fructose Concentration (g·L ⁻¹)	Sucrose Concentration (g·L ⁻¹)	Standard Deviation of Sucrose (g·L ⁻¹)	Sorbitol Concentration (g·L ⁻¹)	Standard Deviation of Sorbitol (g·L ⁻¹)
280022	Adam's Pearmain	19.4	3.6	68.4	8.4	60.5	13.9	8.1	2.0
127311	Amere de Berthcourt	28.3	5.2	51.5	27.6	20.6	5.7	12.4	6.3
173978	American Forestier	11.3	2.6	46.5	12.3	30.2	6.0	9.2	0.9
136243	Amzr Gauthier	26.6	16.4	93.2	14.6	42.7	22.3	9.9	2.5
588952	Arkansas	23.5	7.8	73.1	18.0	38.1	3.7	19.9	6.2
589117	Arkansas Black	16.8	1.9	61.3	8.6	65.3	1.0	11.8	0.3
589654	Ashmead's Kernel	18.6	2.6	36.4	7.2	26.7	7.6	4.5	1.1
105498	Bella de Jardins	23.2	2.1	95.6	8.5	61.8	4.6	14.5	1.8
162544	Belle de Crollon	37.9	14.3	77.5	13.3	33.1	4.2	9.4	1.4
589584	Belle de Nordhaussen	27.7	9.7	79.7	25.1	23.3	19.1	11.9	4.2
162709	Belle Fille	17.3	4.5	49.2	9.5	38.8	6.7	17.1	4.9
588951	Belle Sans Pepin	32.8	3.1	54.1	4.4	51.3	5.6	12.2	2.4
588953	Ben Davis	11.8	3.4	47.1	7.4	35.2	3.3	8.1	2.1
122598	Binet Blanc	25.3	21.5	53.5	46.3	43.2	39.4	7.1	1.8
158729	Binet Blanc Dore	12.3	1.4	63.5	5.2	72.1	17.9	26.8	5.3
158730	Binet Rouge	20.8	3.9	76.9	11.6	54.2	6.0	17.3	1.5
590180	Blue Pearmain	20.1	7.1	76.6	26.4	75.2	27.0	19.0	8.4
162549	Boche	10.1	2.5	40.8	12.1	32.5	1.4	7.5	1.7
590120	Bonne- Hotture	26.2	1.4	61.5	4.1	58.1	16.4	9.4	0.9
107171	Bramley's Seedling	17.8	3.2	80.8	28.9	40.4	18.1	6.1	3.8
158731	Bramtot	34.1	12.1	92.1	17.9	42.4	23.7	28.7	5.0
589662	Brown's Apple	4.0	3.0	15.5	3.0	4.1	3.0	0.2	4.0
588808	Bulmer Norman	17.1	2.2	65.2	3.9	27.9	7.9	18.7	0.7
187297	C'Huero Biz Bras	21.8	3.2	68.7	4.0	29.6	3.6	15.7	2.3
187298	C'Huero Ru Bienn	24.8	2.8	57.8	14.7	18.8	5.3	24.7	5.1

PI Number	Cultivar Name	Glucose Concentration (g·L ⁻¹)	Standard Deviation of Glucose Concentration (g·L ⁻¹)	Fructose Concentration (g·L ⁻¹)	Standard Deviation of Fructose Concentration (g·L ⁻¹)	Sucrose Concentration (g·L ⁻¹)	Standard Deviation of Sucrose (g·L ⁻¹)	Sorbitol Concentration (g·L ⁻¹)	Standard Deviation of Sorbitol (g·L ⁻¹)
161830	Cap of Liberty	27.1	2.6	71.3	10.4	32.7	5.3	6.4	0.8
183961	Carnival-14	20.2	11.4	58.3	17.8	43.1	30.4	16.3	6.9
264688	Champagne Reinette	16.3	1.0	61.4	9.4	28.3	5.2	8.2	1.0
589175	Coat Jersey	18.2	9.5	24.6	33.8	69.0	23.2	7.5	0.9
590121	Cornish Aromatic (Wakeley)	10.4	0.3	40.6	3.2	58.2	13.5	13.5	0.8
589602	Court Pendu Gris	19.6	7.7	66.6	28.6	48.4	23.8	8.7	2.2
589587	Court Pendu Rose	23.6	9.6	54.4	12.4	45.8	24.4	9.8	4.3
589671	Court Royal	25.6	2.1	65.7	13.5	55.2	14.5	9.2	2.3
589196	Crow Egg	18.5	1.7	66.5	22.9	27.6	22.3	24.6	5.9
162722	Damelot	13.6	6.9	46.9	28.8	54.9	25.6	15.5	3.6
162062	Daux Belan	22.7	16.9	76.2	29.8	39.9	23.0	10.7	2.4
173979	Domaine	20.6	6.7	68.3	24.2	38.1	12.2	18.0	6.8
131104	Double Bon Pommier	18.0	5.2	50.6	5.6	36.8	6.2	10.9	2.3
161760	Doucet Rouge	26.6	5.0	69.2	19.6	29.4	2.7	8.6	1.6
589667	Doux Normandie	20.5	4.1	66.2	23.1	32.9	16.5	5.6	3.0
162715	Doux Tardif	22.6	7.7	79.1	12.8	35.9	10.3	8.0	1.8
122616	Doux-AMR	16.8	8.3	52.6	15.2	57.2	13.3	14.6	4.4
131823	Drap d'Or Guemene	20.3	3.0	78.0	24.8	35.1	13.9	9.2	1.9
589666	Dunkerton Late Sweet	23.9	6.4	74.7	26.6	75.0	0.9	11.2	0.8
589642	Eda	17.2	6.7	59.2	8.9	35.9	18.1	15.2	1.2
590125	Edelroter	22.0	13.8	52.3	21.8	35.3	29.2	9.2	2.9
392312	Edward VII	21.0	4.5	71.6	14.7	33.3	10.2	6.2	1.5
589650	Ellis Bitter	12.3	3.7	25.6	4.0	21.2	13.8	5.5	1.8
588785	Esopus Spitzenburg	18.3	2.7	70.7	6.6	51.7	1.5	8.4	1.0
590126	Fenouillet de Ribours	25.6	4.5	71.6	13.0	26.4	10.6	11.2	4.8
589679	Fillbarrel	43.1	12.5	80.6	19.7	16.4	4.0	12.6	1.2
589626	Forest King	10.5	2.2	35.8	8.4	27.8	5.4	7.9	0.7
589318	Foxwhelp	15.6	2.1	61.1	3.1	22.0	9.8	5.4	1.6
590127	Fraindise	30.2	6.2	90.7	19.5	19.1	8.4	8.1	1.6
162503	Frequin	16.6	3.0	65.1	8.2	44.3	1.7	8.2	2.0
247314	Frequin Lacaille	11.0	4.7	38.8	16.1	53.9	21.6	12.4	1.0
589689	Frequin Tardive de la Sarthe	18.9	0.7	59.2	2.6	14.0	1.0	13.7	6.5
276299	Freyberg	11.2	5.0	78.9	33.6	63.6	6.1	5.0	2.6

PI Number	Cultivar Name	Glucose Concentration (g·L ⁻¹)	Standard Deviation of Glucose Concentration (g·L ⁻¹)	Fructose Concentration (g·L ⁻¹)	Standard Deviation of Fructose Concentration (g·L ⁻¹)	Sucrose Concentration (g·L ⁻¹)	Standard Deviation of Sucrose (g·L ⁻¹)	Sorbitol Concentration (g·L ⁻¹)	Standard Deviation of Sorbitol (g·L ⁻¹)
589123	Geeveston Fanny	22.4	4.1	74.4	11.0	32.9	0.9	9.7	7.0
132225	Gewurzluiken	19.1	5.8	61.3	13.6	24.2	5.3	11.1	5.3
613897	GMAL 2996.c1	3.0	3.0	8.8	3.0	10.2	3.0	3.6	4.6
613927	GMAL 3232.g1	17.4	1.9	46.8	9.3	21.5	9.8	8.2	1.2
590184	Golden Delicious	21.3	8.1	89.0	24.7	76.9	23.2	11.4	9.3
590128	Golden Harvey	18.2	4.4	72.0	14.6	48.7	13.0	10.8	1.8
590129	Golden Pippin	11.5	3.5	44.1	12.4	39.9	11.2	5.0	0.1
589892	Golden Russet	30.1	7.8	74.4	15.6	28.3	8.6	13.2	2.6
589684	Grenadier	12.7	1.9	43.0	2.6	66.0	2.3	10.6	3.2
161761	Grosse Launette	25.8	17.4	89.7	20.7	68.4	27.9	10.2	3.8
162545	Grosse Mouche	15.5	3.5	50.5	8.8	31.0	5.2	9.3	3.1
681628	Harrison	35.6	5.3	93.5	2.4	40.1	5.3	25.2	3.4
589585	Holaart Doux	14.6	1.6	63.8	21.9	32.4	10.1	5.3	0.7
590130	Hubbards Pearmain	11.2	6.0	45.0	10.0	30.1	8.0	4.0	.07
589682	Improved Lambrook Pippin	17.0	2.1	101.5	18.1	37.7	2.3	14.6	6.3
590185	Jonathan	14.4	1.4	62.6	9.5	52.7	17.0	8.2	2.8
162731	Jouveaux	32.1	8.7	94.3	33.0	53.3	23.2	19.1	5.8
657019	Kaz 95 08-06	12.0	2.5	58.8	7.0	24.3	4.0	6.6	0.3
589703	Kingston Black	28.9	12.8	82.8	14.3	42.7	21.4	11.9	0.6
589219	Kola	13.4	2.2	64.9	13.1	36.4	9.9	8.0	1.6
589598	La Paix	27.6	12.2	72.9	21.0	71.4	8.6	14.8	5.6
162724	Lande	18.7	0.9	60.5	4.4	30.0	4.5	16.0	5.8
589565	Landsberger Reinette	14.6	2.0	78.2	16.5	69.7	15.0	7.6	1.9
161851	Langworthy	16.3	4.5	79.2	28.8	51.9	30.4	5.1	6.3
162732	Launette	47.9	23.0	72.8	22.4	33.5	5.2	27.3	9.5
589690	Le Bret	12.8	5.0	27.2	2.3	10.5	7.0	13.4	5.3
588943	Liberty	14.2	5.1	59.5	39.5	41.6	15.0	6.6	1.7
264558	Margil	33.7	5.8	77.1	11.5	50.0	14.9	25.7	1.6
173982	Marin Onfroy	19.3	10.1	47.2	15.6	24.1	24.1	14.9	4.1
588998	Marshall McIntosh	28.9	4.2	47.6	6.7	75.3	14.4	13.9	6.1
588817	McIntosh Summerland Red	11.6	2.0	58.6	26.4	33.3	8.9	6.3	2.0
594108	Medaille d'Or	19.5	1.2	60.4	1.0	40.8	10.2	14.3	6.2
589634	Mercer	21.0	2.8	61.2	14.8	30.6	14.4	9.4	2.0

PI Number	Cultivar Name	Glucose Concentration (g·L ⁻¹)	Standard Deviation of Glucose Concentration (g·L ⁻¹)	Fructose Concentration (g·L ⁻¹)	Standard Deviation of Fructose Concentration (g·L ⁻¹)	Sucrose Concentration (g·L ⁻¹)	Standard Deviation of Sucrose (g·L ⁻¹)	Sorbitol Concentration (g·L ⁻¹)	Standard Deviation of Sorbitol (g·L ⁻¹)
589670	Michelin	23.0	6.1	52.5	3.2	11.2	12.0	12.0	0.5
588976	Midget Crab	23.0	9.2	54.8	6.4	50.6	15.5	8.3	1.5
200780	Muscadet Bernay	33.4	6.5	60.1	16.5	17.3	8.6	7.8	3.2
589493	Muscadet de Dieppe	19.8	6.1	67.9	22.1	43.4	11.2	10.0	4.7
173985	Muscadet de Lense	37.4	2.5	71.2	7.5	38.6	5.9	15.1	5.5
223602	Mutsu	18.0	3.0	85.5	22.3	43.4	39.1	18.7	4.0
161763	Nanot	18.6	1.4	46.1	11.4	6.8	2.7	3.5	0.8
175544	Nehou	31.9	9.2	73.3	20.8	41.4	11.2	27.9	4.7
173986	Noel Deschamps	28.5	6.2	56.2	12.8	11.2	0.9	8.5	2.1
588872	Northern Spy	19.7	3.3	72.2	2.5	39.0	3.6	6.8	1.4
280027	Old Nonpareil	17.9	2.9	87.9	18.8	46.0	15.8	6.4	1.7
590133	Old Pearmain	24.2	3.9	63.6	3.9	69.4	51.6	14.8	8.5
589674	Pethyre	6.2	2.1	49.1	17.0	70.4	37.9	11.7	5.9
132272	Pigeonnet Blanc	24.7	5.9	59.9	12.5	20.8	5.4	8.9	0.8
132273	Pigeonnet Rouge	20.9	7.0	73.5	3.7	26.3	13.0	8.3	1.6
588745	Pohorka	16.4	5.1	67.0	17.3	51.6	13.5	6.7	2.9
134668	Pomme Cloche	21.8	2.2	74.9	3.5	50.6	0.9	9.5	1.5
131975	Pomme Framboise	19.4	2.1	62.7	11.1	34.8	2.8	8.7	0.9
134669	Pomme Raisin	6.9	2.0	34.5	5.2	40.8	6.0	10.6	6.2
162548	Pomme Thoury	21.1	1.3	64.2	6.8	31.6	5.1	11.3	4.6
240817	Pommier Llorca	21.0	6.2	62.5	21.6	33.5	23.5	8.0	2.1
589789	PRI 1744-1	19.6	6.7	62.1	19.3	79.3	27.0	21.1	6.4
589211	Red Field	14.4	4.1	65.6	9.1	45.4	22.3	4.4	1.1
437047	Red Ralls	47.2	11.2	108.5	23.3	64.2	16.9	6.6	1.3
132571	Reine des Pommes	29.3	7.2	76.1	13.1	40.2	12.0	7.4	2.0
279326	Reinettes x 1600	21.8	15.3	46.4	9.5	31.8	7.4	8.6	1.7
279325	Reine des Reinettes x 82	24.9	6.9	80.1	33.4	46.7	9.3	16.5	2.8
105524	Reineta do Caravia	10.7	2.4	45.8	6.2	61.8	1.9	6.2	1.6
589444	Reinette Clochard	20.2	1.3	65.9	5.7	33.7	15.2	12.9	6.4
590135	Reinette d'Anjou	23.9	7.3	91.4	4.9	71.0	2.8	15.7	7.4
590137	Reinette Franche	15.0	4.1	40.0	6.2	34.1	5.2	4.0	2.0
131561	Reinette Jaegers	13.1	0.9	64.5	29.4	51.0	8.8	6.9	1.4

PI Number	Cultivar Name	Glucose Concentration (g·L ⁻¹)	Standard Deviation of Glucose Concentration (g·L ⁻¹)	Fructose Concentration (g·L ⁻¹)	Standard Deviation of Fructose Concentration (g·L ⁻¹)	Sucrose Concentration (g·L ⁻¹)	Standard Deviation of Sucrose (g·L ⁻¹)	Sorbitol Concentration (g·L ⁻¹)	Standard Deviation of Sorbitol (g·L ⁻¹)
135645	Reinette Jamin	15.3	3.5	63.3	13.9	69.0	8.0	13.2	1.1
590140	Reinette Thouin	18.3	4.9	59.1	12.6	76.6	11.6	10.5	3.4
188521	Reinette van Ekenstain	13.9	3.7	51.1	16.2	20.7	10.0	7.6	0.9
589520	Rhode Island Greening	21.3	11.2	71.5	7.6	40.1	42.5	8.8	5.2
588840	Ribston	19.5	3.0	66.0	14.5	57.1	14.5	10.3	1.7
590141	Ross Nonpareil	20.9	12.9	65.6	10.0	42.3	10.2	6.8	0.2
102148	Rott Jarnpple	29.1	29.0	77.9	40.9	78.9	32.6	12.2	5.7
589143	Rouge Belle De Boskoop	15.8	9.2	67.6	5.1	57.4	8.0	8.9	2.7
588971	Roxbury Russet	16.1	2.0	54.5	7.2	56.1	8.6	11.5	1.0
161846	Skyrme's Kernel	15.8	1.4	68.2	7.2	43.7	9.8	11.2	1.8
588975	Stayman	31.0	5.3	81.9	8.0	37.8	3.3	21.5	3.3
589692	Stembridge Cluster	22.0	7.6	56.6	31.9	10.5	2.8	7.7	2.6
589693	Stembridge Jersey	10.7	8.5	65.9	5.2	86.1	4.3	6.0	6.3
307382	Sturmer Pippin	12.6	0.4	57.5	2.1	54.8	2.0	19.7	0.1
125566	Surpasse Frequin	32.2	13.5	75.2	26.5	30.7	2.5	17.3	7.6
589691	Tale Sweet	27.9	6.1	59.8	20.9	37.0	22.0	10.7	3.8
175548	Tardive Forestier	14.2	4.0	74.3	4.6	34.4	10.5	10.1	1.5
589663	Taylor's	31.3	5.9	51.4	26.1	19.1	10.2	5.8	1.3
175549	Teign Harvey	17.8	4.2	49.5	6.9	26.3	1.9	13.4	0.8
127370	Teint Fraise	19.4	5.1	45.0	8.5	24.3	9.3	9.4	1.5
506361	Thorgauer Weinapfel	27.4	5.2	61.2	11.2	31.9	10.7	8.6	1.5
175551	Twistbody Jersey	23.8	17.2	97.1	37.1	34.2	10.9	3.0	1.1
629317	USSR-89-35-01	19.5	5.3	67.9	17.5	76.5	16.7	9.5	1.2
175552	Vagnon Ascher	25.8	7.3	54.5	13.0	14.7	8.3	17.4	5.3
589060	Vandevere	20.1	5.1	72.2	13.2	38.3	9.7	6.4	1.7
589623	Wamdesa	32.4	15.4	75.7	43.4	63.1	37.2	11.8	9.3
589635	Wecota	14.0	9.8	42.9	30.5	23.2	16.1	5.9	2.5
590143	Weidners Goldreinette	42.8	9.6	77.0	20.4	20.1	2.3	9.6	2.5
590144	Weisser Winter Taffetapfel	22.4	11.1	52.6	7.7	58.5	5.2	10.5	3.0
613818	Wickson	18.0	7.6	55.8	8.2	54.6	4.2	14.5	2.3
589309	William Crump	9.6	5.4	21.7	5.8	28.8	9.8	7.7	1.9
588799	Winesap	28.3	2.6	64.5	8.2	45.4	6.7	14.2	7.2

PI Number	Cultivar Name	Glucose Concentration (g·L ⁻¹)	Standard Deviation of Glucose Concentration (g·L ⁻¹)	Fructose Concentration (g·L ⁻¹)	Standard Deviation of Fructose Concentration (g·L ⁻¹)	Sucrose Concentration (g·L ⁻¹)	Standard Deviation of Sucrose (g·L ⁻¹)	Sorbitol Concentration (g·L ⁻¹)	Standard Deviation of Sorbitol (g·L ⁻¹)
589632	Wotonda	8.7	0.0	19.7	3.2	16.1	1.4	12.8	6.9
588773	Yellow Newtown	15.9	1.5	80.1	0.7	37.5	0.9	19.3	7.3
589614	Zapta	33.1	3.6	78.0	22.0	54.4	6.7	20.3	7.6

Supplemental table 7: The soluble solids concentration ($^{\circ}\text{Brix}$), total polyphenol concentration ($\text{g}\cdot\text{L}^{-1}$), titratable acidity concentration ($\text{g}\cdot\text{L}^{-1}$), initial pH measurement, and associated standard deviations for all accessions ($n=158$) phenotyped in the 2017 harvest season for accessions harvested within the USDA-PGRU Malus germplasm collection in Geneva, NY.

PI Number	Cultivar Name	Soluble Solids Concentration ($^{\circ}\text{Brix}$)	Standard Deviation of Soluble Solids Concentration ($^{\circ}\text{Brix}$)	Total Polyphenol Concentration ($\text{g}\cdot\text{L}^{-1}\text{GAE}$)	Standard Deviation of Total Polyphenol Concentration ($\text{g}\cdot\text{L}^{-1}\text{GAE}$)	Titratable Acidity Concentration ($\text{g}\cdot\text{L}^{-1}\text{Malic Acid Equivalents}$)	Standard Deviation of Titratable Acidity ($\text{g}\cdot\text{L}^{-1}\text{Malic Acid Equivalents}$)	Initial pH	Standard Deviation of Initial pH
280022	Adam's Pearmain	15.55	1.13	1.02	0.11	5.47	0.17	3.59	0.01
127311	Amere de Berthcourt	11.30	0.61	1.98	0.08	1.52	0.50	4.57	0.10
173978	American Forestier	10.50	1.01	0.85	0.06	1.18	0.23	4.72	0.03
136243	Amzr Gauthier	13.37	4.37	3.08	0.14	3.59	0.23	4.18	0.04
588952	Arkansas	11.90	1.40	1.81	0.19	1.87	0.04	4.56	0.07
589117	Arkansas Black	12.33	1.25	0.92	0.25	4.60	0.26	3.59	0.09
589654	Ashmead's Kernel	16.00	0.89	0.87	0.66	5.33	1.13	3.57	0.67
105498	Bella de Jardins	15.90	2.35	0.92	0.35	3.35	0.28	3.76	0.10
162544	Belle de Crollon	12.80	0.85	1.98	0.13	1.97	0.20	4.22	0.02
589584	Belle de Nordhaussen	11.33	4.42	0.75	0.03	6.11	2.04	3.26	0.07
162709	Belle Fille	13.17	1.57	1.51	0.15	1.80	1.39	4.63	0.06
588951	Belle Sans Pepin	11.53	1.36	0.79	0.06	3.14	0.66	3.83	0.04
588953	Ben Davis	10.05	2.01	0.68	0.17	3.51	3.13	3.77	0.43
122598	Binet Blanc	11.53	0.75	1.67	0.15	1.41	0.56	4.61	0.05
158729	Binet Blanc Dore	14.93	0.14	1.85	0.30	3.00	1.10	4.44	0.06
158730	Binet Rouge	14.57	0.81	1.65	0.07	1.32	0.10	4.87	0.03
590180	Blue Pearmain	18.80	2.34	1.09	0.32	6.32	0.42	3.65	0.05
162549	Boche	10.80	0.64	1.15	0.13	9.39	0.28	3.22	0.03
590120	Bonne-Hotture	13.10	0.64	0.90	0.65	6.51	52.00	3.52	0.31
107171	Bramley's Seedling	8.93	0.36	0.85	0.10	9.20	0.76	3.09	0.15
158731	Bramtot	18.73	0.57	3.53	0.03	2.17	3.32	4.53	0.74
589662	Brown's Apple	8.28	1.08	2.04	0.19	4.45	1.20	3.40	0.07
588808	Bulmer Norman	10.60	0.75	1.84	0.27	3.10	0.13	4.35	0.04
187297	C'Huero Biz Bras	11.70	2.31	2.16	0.56	1.84	0.13	4.55	0.06
187298	C'Huero Ru Bienn	12.80	0.14	2.51	0.24	2.22	0.05	4.34	0.01
161830	Cap of Liberty	13.53	3.39	1.74	0.25	5.73	0.09	3.50	0.04
183961	Carnival-14	12.32	0.57	0.82	0.08	5.97	0.71	3.41	0.03
264688	Champagne Reinette	11.55	0.55	0.75	0.16	8.29	0.83	3.29	0.08

PI Number	Cultivar Name	Soluble Solids Concentration (°Brix)	Standard Deviation of Soluble Solids Concentration (°Brix)	Total Polyphenol Concentration (g·L ⁻¹ GAE)	Standard Deviation of Total Polyphenol Concentration (g·L ⁻¹ GAE)	Titrateable Acidity Concentration (g·L ⁻¹ Malic Acid Equivalents)	Standard Deviation of Titrateable Acidity (g·L ⁻¹ Malic Acid Equivalents)	Initial pH	Standard Deviation of Initial pH
589175	Coat Jersey	14.55	1.31	3.19	0.22	1.45	0.73	4.63	0.07
590121	Cornish Aromatic (Wakeley)	16.90	0.07	1.05	0.06	5.96	0.13	3.55	0.01
589602	Court Pendu Gris	12.17	0.90	1.28	0.12	4.83	0.11	3.49	0.01
589587	Court Pendu Rose	16.17	2.04	0.59	0.67	5.39	0.19	3.70	0.12
589671	Court Royal	8.10	0.72	1.12	0.17	2.40	0.14	4.57	0.04
589196	Crow Egg	11.25	1.05	0.93	0.13	1.83	0.18	4.01	0.02
162722	Damelot	15.30	1.30	2.82	0.23	1.55	0.18	4.62	0.07
162062	Daux Belan	15.23	0.53	3.48	0.15	2.19	0.13	4.53	0.02
173979	Domaine	12.93	1.45	2.05	0.08	1.66	0.13	4.63	0.10
131104	Double Bon Pommier	11.70	0.25	0.60	0.14	3.16	0.15	4.04	0.14
161760	Doucet Rouge	12.40	1.65	1.39	0.21	1.94	0.20	4.30	0.07
589667	Doux Normandie	13.85	0.61	2.72	0.13	2.61	0.88	4.10	0.08
162715	Doux Tardif	11.43	0.71	1.55	0.12	2.19	1.11	4.08	2.28
122616	Doux-AMR	13.13	2.89	1.47	0.70	1.84	0.17	4.45	0.04
131823	Drap d'Or Guemene	14.03	1.84	1.10	0.01	4.24	0.11	3.78	0.06
589666	Dunkerton Late Sweet	13.43	1.19	0.63	0.03	1.57	0.10	4.65	0.06
589642	Eda	8.43	1.34	1.69	0.23	13.78	0.50	3.02	0.13
590125	Edelroter	12.45	2.23	0.42	0.25	6.70	1.44	3.49	0.06
392312	Edward VII	11.67	0.45	0.90	0.13	8.07	2.07	3.26	0.61
589650	Ellis Bitter	9.57	1.20	1.44	0.07	1.11	0.19	4.79	0.08
588785	Esopus Spitzenburg	10.67	1.63	0.85	0.09	5.06	1.30	3.54	0.01
590126	Fenouillet de Ribours	14.10	0.61	1.56	0.24	3.98	0.14	3.62	0.03
589679	Fillbarrel	11.77	0.97	1.81	0.48	2.16	0.10	4.25	0.11
589626	Forest King	13.07	2.58	3.30	0.22	13.66	0.81	3.00	0.06
589318	Foxwhelp	11.57	5.10	1.44	2.00	4.05	3.20	3.49	3.40
590127	Fraindise	13.33	0.30	1.29	0.06	5.68	0.95	3.57	0.67
162503	Frequin	12.70	2.19	2.53	0.22	1.47	2.31	4.83	0.86
247314	Frequin Lacaille	11.83	1.66	2.77	0.32	2.17	0.35	4.31	0.06
589689	Frequin Tardive de la Sarthe	14.20	0.95	1.40	0.08	2.07	1.84	4.37	0.35
276299	Freyberg	12.03	0.44	0.93	0.22	0.87	0.18	5.44	0.05
589123	Geeveston Fanny	9.93	4.02	0.61	0.74	2.49	0.65	3.90	0.19

PI Number	Cultivar Name	Soluble Solids Concentration (°Brix)	Standard Deviation of Soluble Solids Concentration (°Brix)	Total Polyphenol Concentration (g·L ⁻¹ GAE)	Standard Deviation of Total Polyphenol Concentration (g·L ⁻¹ GAE)	Titrateable Acidity Concentration (g·L ⁻¹ Malic Acid Equivalents)	Standard Deviation of Titrateable Acidity (g·L ⁻¹ Malic Acid Equivalents)	Initial pH	Standard Deviation of Initial pH
132225	Gewurzluiken	12.23	1.24	0.92	0.22	3.33	0.26	3.68	0.09
613897	GMAL 2996.c1	12.95	0.49	0.84	0.03	3.88	0.91	3.77	0.04
613927	GMAL 3232.g1	17.50	1.36	0.93	0.36	3.50	0.11	3.81	0.15
590184	Golden Delicious	12.97	0.78	0.63	0.06	5.22	0.71	3.60	0.29
590128	Golden Harvey	15.13	2.80	0.66	0.18	6.07	0.11	3.51	0.09
590129	Golden Pippin	13.05	1.48	1.16	0.14	7.13	1.85	3.34	0.14
589892	Golden Russet	16.27	1.14	1.07	0.23	4.96	0.15	3.52	0.04
589684	Grenadier	10.50	1.36	0.92	0.08	7.39	3.42	3.23	0.05
161761	Grosse Launette	10.03	0.35	1.33	0.21	1.84	0.18	4.59	0.05
162545	Grosse Mouche	10.73	0.28	0.98	0.01	7.23	0.15	3.39	0.03
681628	Harrison	15.70	1.65	0.96	0.25	4.42	0.58	3.72	0.03
589585	Holaart Doux	12.42	1.65	1.25	0.15	1.75	0.25	4.88	0.05
590130	Hubbards Pearmain	14.25	1.22	0.97	0.16	4.31	0.54	3.61	0.02
589682	Improved Lambrook Pippin	13.07	3.00	1.37	0.38	1.73	1.13	4.60	0.15
590185	Jonathan	12.43	0.71	0.78	0.15	6.27	0.14	3.43	0.01
162731	Jouveaux	15.43	0.42	1.74	0.13	1.54	1.75	5.59	0.01
657019	Kaz 95 08-06	11.23	2.26	4.86	0.08	17.04	0.24	3.00	0.03
589703	Kingston Black	12.53	1.23	1.08	0.27	2.14	0.00	4.23	0.01
589219	Kola	9.08	0.58	2.74	0.11	18.56	0.62	2.89	0.13
589598	La Paix	12.80	1.21	1.10	0.10	1.97	0.34	3.97	0.01
162724	Lande	9.80	1.80	0.57	0.13	3.37	0.69	3.72	0.02
589565	Landsberger Reinette	13.53	0.84	1.38	0.15	4.95	0.73	3.63	0.08
161851	Langworthy	11.25	1.54	1.09	0.18	3.80	0.29	3.59	0.24
162732	Launette	18.17	1.34	4.44	0.01	2.80	0.00	4.34	0.00
589690	Le Bret	15.95	0.21	0.92	0.17	1.64	1.73	4.78	0.95
588943	Liberty	10.57	2.10	0.56	2.20	5.40	3.20	3.44	4.20
264558	Margil	15.73	1.20	1.17	0.25	2.29	4.62	4.47	0.29
173982	Marin Onfroy	10.25	1.46	1.55	0.32	1.69	0.52	4.53	0.06
588998	Marshall McIntosh	12.17	0.17	0.89	0.09	4.08	0.83	3.48	0.05
588817	McIntosh Summerland Red	9.70	1.05	0.84	0.04	4.73	0.11	3.41	0.03
594108	Medaille d'Or	15.53	1.27	4.29	0.06	2.38	0.13	4.33	0.03

PI Number	Cultivar Name	Soluble Solids Concentration (°Brix)	Standard Deviation of Soluble Solids Concentration (°Brix)	Total Polyphenol Concentration (g·L ⁻¹ GAE)	Standard Deviation of Total Polyphenol Concentration (g·L ⁻¹ GAE)	Titrateable Acidity Concentration (g·L ⁻¹ Malic Acid Equivalents)	Standard Deviation of Titrateable Acidity (g·L ⁻¹ Malic Acid Equivalents)	Initial pH	Standard Deviation of Initial pH
589634	Mercer	9.73	1.63	1.41	0.03	11.26	2.20	3.07	0.21
589670	Michelin	11.90	2.19	1.66	0.15	2.20	0.27	4.35	0.02
588976	Midget Crab	16.30	2.36	1.43	0.22	9.25	0.12	3.28	0.07
200780	Muscadet Bernay	12.40	0.86	2.34	0.24	2.53	0.36	4.02	0.03
589493	Muscadet de Dieppe	13.60	1.01	1.90	0.25	1.60	4.11	4.81	0.06
173985	Muscadet de Lense	13.53	0.38	2.30	0.23	2.58	0.91	4.30	0.06
223602	Mutsu	16.00	3.45	1.07	0.36	4.74	0.60	3.76	0.01
161763	Nanot	8.00	1.25	1.48	0.13	6.81	0.54	3.24	0.03
175544	Nehou	14.55	1.39	1.67	0.36	1.79	0.08	4.60	0.03
173986	Noel Deschamps	11.67	0.64	2.27	0.02	2.72	2.06	4.20	0.48
588872	Northern Spy	12.67	1.91	1.19	0.13	4.26	0.36	3.54	0.08
280027	Old Nonpareil	10.10	0.97	0.88	0.12	3.21	1.51	3.85	0.14
590133	Old Pearmain	11.75	2.25	0.65	0.49	4.25	1.48	3.44	0.09
589674	Pethyre	11.23	0.12	0.46	0.10	7.48	0.21	11.43	0.03
132272	Pigeonnet Blanc	11.90	1.06	1.20	0.06	7.70	0.15	3.26	0.05
132273	Pigeonnet Rouge	13.23	5.39	1.32	0.26	5.22	0.80	3.55	0.03
588745	Pohorka	11.20	1.76	0.56	0.27	3.59	3.27	3.64	0.03
134668	Pomme Cloche	12.87	1.21	0.60	0.29	7.82	2.33	3.19	0.04
131975	Pomme Framboise	14.00	1.97	0.57	0.36	3.10	5.40	5.00	0.08
134669	Pomme Raisin	10.10	0.14	0.88	0.28	4.55	0.03	3.58	0.02
162548	Pomme Thoury	11.78	1.25	1.72	0.08	2.10	1.71	4.28	0.03
240817	Pommier Llorca	12.60	0.15	1.09	0.27	1.76	0.47	4.74	2.61
589789	PRI 1744-1	15.70	2.00	0.96	0.10	1.60	1.00	4.42	2.30
589211	Red Field	12.63	0.38	2.35	0.24	8.52	0.09	3.35	0.24
437047	Red Ralls	12.40	0.42	0.61	0.09	4.64	0.32	3.63	0.05
132571	Reine des Pommés	13.40	2.24	0.81	1.27	4.94	0.78	3.64	0.02
279326	Reinettes x 1600	12.53	1.84	1.15	0.27	4.30	0.05	3.95	0.69
279325	Reine des Reinettes x 82	12.30	2.63	0.63	0.18	3.98	0.10	3.76	0.16
105524	Reineta do Caravia	10.83	0.49	0.78	0.08	6.30	0.15	3.45	0.11
589444	Reinette Clochard	13.23	1.61	0.69	0.38	5.21	0.04	3.57	0.06

PI Number	Cultivar Name	Soluble Solids Concentration (°Brix)	Standard Deviation of Soluble Solids Concentration (°Brix)	Total Polyphenol Concentration (g·L ⁻¹ GAE)	Standard Deviation of Total Polyphenol Concentration (g·L ⁻¹ GAE)	Titrateable Acidity Concentration (g·L ⁻¹ Malic Acid Equivalents)	Standard Deviation of Titrateable Acidity (g·L ⁻¹ Malic Acid Equivalents)	Initial pH	Standard Deviation of Initial pH
590135	Reinette d'Anjou	13.20	1.48	0.59	0.34	5.86	1.81	3.44	0.23
590137	Reinette Franche	11.33	1.66	1.40	0.40	1.72	1.50	4.59	0.25
131561	Reinette Jaegers	10.70	3.10	0.51	0.46	6.41	0.47	3.49	0.08
135645	Reinette Jamin	12.30	0.55	0.47	0.03	3.78	0.91	3.70	0.09
590140	Reinette Thouin	9.07	1.50	0.75	0.25	6.01	0.20	3.55	0.40
188521	Reinette van Ekenstain	9.93	1.35	0.94	0.19	3.63	0.09	4.15	0.05
589520	Rhode Island Greening	13.43	2.62	0.98	0.15	4.37	0.07	3.76	0.05
588840	Ribston	12.80	1.11	0.85	0.13	3.20	0.20	3.81	0.03
590141	Ross Nonpareil	15.80	0.86	1.38	0.10	5.71	0.64	3.66	0.04
102148	Rott Jarnpple	14.50	4.55	1.14	0.60	4.35	0.53	3.47	0.48
589143	Rouge Belle De Boskoop	13.15	1.15	0.83	0.10	6.10	0.07	3.51	0.06
588971	Roxbury Russet	13.95	0.67	0.76	0.12	2.22	0.11	4.35	0.12
161846	Skyrme's Kernel	12.97	3.19	1.57	0.30	5.44	0.60	3.46	0.10
588975	Stayman	12.53	1.93	0.65	0.05	4.34	1.16	3.65	0.12
589692	Stembridge Cluster	11.33	0.25	0.93	0.03	4.97	1.20	3.41	0.60
589693	Stembridge Jersey	10.45	2.05	2.52	0.26	1.30	0.25	5.14	0.11
307382	Sturmer Pippin	11.93	0.98	0.65	0.10	6.84	0.04	3.28	0.02
125566	Surpasse Frequin	12.80	1.76	2.25	0.41	2.58	0.80	3.97	0.05
589691	Tale Sweet	12.70	3.21	1.42	0.14	2.12	0.68	4.37	0.05
175548	Tardive Forestier	11.40	1.06	0.84	0.12	6.18	0.48	3.45	0.02
589663	Taylor's	9.80	0.31	0.72	0.18	0.93	0.23	5.78	0.03
175549	Teign Harvey	9.85	0.78	1.45	0.15	3.29	1.19	3.71	0.04
127370	Teint Fraise	11.97	2.00	1.28	0.20	2.55	1.50	4.17	0.50
506361	Thorgauer Weinapfel	11.93	1.56	1.10	0.12	4.94	1.50	3.71	0.08
175551	Twistbody Jersey	10.70	0.74	1.62	0.12	2.08	0.86	4.35	0.07
629317	USSR-89-35-01	12.80	3.10	1.11	0.20	4.60	2.10	3.53	2.10
175552	Vagnon Ascher	12.97	2.66	3.77	0.22	2.12	1.81	4.25	0.01
589060	Vandevere	14.40	2.47	0.97	0.31	6.03	0.92	3.41	0.06
589623	Wamdesa	7.07	1.59	3.07	0.14	11.49	0.48	3.13	0.03
589635	Wecota	9.13	1.53	3.04	0.06	10.54	0.35	4.54	0.02
590143	Weidners Goldreinette	12.27	0.65	0.63	0.08	5.05	1.09	3.46	0.10

PI Number	Cultivar Name	Soluble Solids Concentration (°Brix)	Standard Deviation of Soluble Solids Concentration (°Brix)	Total Polyphenol Concentration (g·L ⁻¹ GAE)	Standard Deviation of Total Polyphenol Concentration (g·L ⁻¹ GAE)	Titrateable Acidity Concentration (g·L ⁻¹ Malic Acid Equivalents)	Standard Deviation of Titrateable Acidity (g·L ⁻¹ Malic Acid Equivalents)	Initial pH	Standard Deviation of Initial pH
590144	Weisser Winter Taffetapfel	10.05	3.93	0.76	0.47	5.02	0.76	3.49	0.02
613818	Wickson	12.83	2.89	0.45	0.14	7.59	1.88	3.52	0.10
589309	William Crump	10.43	0.35	1.12	0.09	6.76	0.44	3.24	0.09
588799	Winesap	11.70	1.20	0.76	0.23	4.12	0.48	3.64	2.00
589632	Wotonda	8.00	1.13	1.77	0.12	10.76	0.64	3.09	0.03
588773	Yellow Newtown	11.40	1.05	0.97	0.04	5.43	1.85	3.48	0.02
589614	Zapta	9.03	1.10	2.97	0.07	16.86	0.24	2.90	0.02

Supplemental Table 8: List the 45 putative compounds identified via UHPLC-MS, the molecular formula, molecular weight and the time of elution for ‘Liberty’ and ‘Golden Delicious’ accessions harvested in 2017 from the USDA-PGRU Malus germplasm collection in Geneva, NY.

Compound Name	Formula	Molecular Weight	RT [min]	Peak Area: ‘Liberty’	Peak Area: ‘Golden Delicious’
(-)-epicatechin-3'-O-glucoside_isomer4	C21 H24 O11	452.1318	8.775	3983.166	5084.734
2'-hydroxy 3,6,7,4'-tetramethylquercetagenin 3'-O-β-D-glucoside	C25 H28 O14	552.1466	16.126	5004.062	4882.424
flavanone 7-O-[α-L-rhamnosyl-(1->2)-β-D-glucoside]	C27 H32 O12	548.1892	13.17	57383.3	56401.27
(2R,3S,4S)-leucocyanidin or epigallocatechin	C15 H14 O7	306.073	7.892	8046.41	7365.346
Urolithin A-8-O-glucuronide	C19 H16 O10	404.0743	7.88	5025.888	5235.159
neohesperidin	C28 H34 O15	610.1883	13.874	4389.366	6047.786
1-O-caffeoyl-β-D-glucose	C15 H18 O9	342.094	8.704	108812.5	71037.22
(-)-epicatechin-3'-O-glucoside_isomer1	C21 H24 O11	452.1311	8.042	4633.199	7817.196
kaempferide 7-O-glucoside	C22 H22 O11	462.1156	16.217	814620.1	354949.4
oleuropein aglycone	C19 H22 O8	378.1308	16.956	4863.237	12646.59
kaempferide 7-O-glucoside isomer	C22 H22 O11	462.115	14.422	5718.119	4500.057
1-O-vanilloyl-β-D-glucose	C14 H18 O9	330.0947	5.703	124591.6	132650.5
phloretin	C15 H14 O5	274.083	14.203	13972.63	5641.755
phloretin.isomer1	C15 H14 O5	274.0838	10.982	4722.079	4196.65
(2E)-2-butyldiene-4-hydroxy-5-methyl-3(2H)-furanone	C9 H12 O3	168.0783	7.186	9099.053	11809.07
caffealdehyde isomer	C9 H8 O3	164.047	11.344	41713.94	17074.16
(-)-glycinol or Naringenin or Trihydroxyflavanone	C15 H12 O5	272.0677	17.969	7448.312	28037.55
naringin dihydrochalcone	C27 H34 O14	582.1937	15.08	12675.05	4070.057
procyanidin B3 isomer1	C30 H26 O12	578.1426	5.703	4482.918	4410.61
2,4',5,7-tetrahydroxyisoflavanone	C15 H12 O6	288.063	16.533	7465.601	6182.519
apigenin 7-O-β-D-glucoside	C21 H20 O10	432.105	13.439	49704.76	85983.09
2',3,4,4',6'-pentahydroxychalcone 4'-O-β-D-glucoside.isomer1	C21 H22 O11	450.1155	9.984	273940.8	123179

Compound Name	Formula	Molecular Weight	RT [min]	Peak Area: 'Liberty'	Peak Area: 'Golden Delicious'
2,3-cis-oligoproanthocyanidin	C45 H38 O18	866.2058	8.477	5002.091	6000.316
(-)-epicatechin-3'-O-glucoside_isomer2	C21 H24 O11	452.1321	7.34	12475.99	12185.3
(-)-epicatechin-3'-O-glucoside.	C21 H24 O11	452.1318	6.773	101087.2	27136.49
sinapaldehyde glucoside	C17 H22 O9	370.126	10.315	6213.993	5232.676
1-O-caffeoyl-β-D-glucose isomer	C15 H18 O9	342.094	7.821	712444.7	651065.7
(-)-epicatechin-3'-O-glucoside_isomer3	C21 H24 O11	452.1315	6.926	230816.9	50698.23
Catechin	C15 H14 O6	290.0783	13.711	29868.82	15494.02
2,4',5,7-tetrahydroxyisoflavanone. Isomer	C15 H12 O6	288.0627	7.513	5425.615	6027.533
Procyanidin A2	C30 H24 O12	576.1269	3.053	13505.49	13555.61
caffealdehyde	C9 H8 O3	164.0472	7.81	1524284	373000.8
narirutin	C27 H32 O14	580.1792	11.507	9702.028	309606.6
Procyanidin A2 . Isomer	C30 H24 O12	576.1263	17.084	4929.286	4564.045
procyanidin B3 isomer3	C30 H26 O12	578.1429	11.305	5681.719	4666.35
procyanidin B3 isomer4	C30 H26 O12	578.1422	7.235	16299.39	9733.178
procyanidin B3	C30 H26 O12	578.1425	8.905	32973.97	21025.86
procyanidin B3 isomer2	C30 H26 O12	578.1431	9.095	65588.89	29357.05
Chlorogenic acid	C16 H18 O9	354.0943	9.22	556906.6	414039.2
Neochlorogenic acid isomer	C16 H18 O9	354.0949	8.305	7360509	6425584
Rutin	C27 H30 O16	610.1529	12.76	719758.3	62055.69
phloretin.isomer2	C15 H14 O5	274.0836	15.308	462656.2	88828.05
phloretin.isomer3	C15 H14 O5	274.0837	19.642	6278.633	6353.92
Benzoic acid	C7 H6 O2	122.0368	6.91	644070.2	643431.7
2-Hydroxycinnamic acid	C9 H8 O3	164.0474	9.117	97631.16	140748.7
trans-Cinnamic acid	C9 H8 O2	148.0525	11.158	1124860	1306520
Neochlorogenic acid	C16 H18 O9	354.0951	9.755	144445.3	283815.1
Ferulic acid	C10 H10 O4	194.0574	8.932	438359.6	41289.28
Epicatechin	C15 H14 O6	290.0786	17.187	6464.506	5784

Supplemental Table 9: List the 45 putative compounds identified via UHPLC-MS, the molecular formula, molecular weight and the time of elution for 'Kingston Black', 'Northern Spy', 'Ellis Bitter' and 'Improved Lambrook Pippin' accessions harvested in 2017 from the USDA-PGRU Malus germplasm collection in Geneva, NY.

Compound Name	Formula	Molecular Weight	RT [min]	Peak Area: 'Kingston Black'	Peak Area: 'Northern Spy'	Peak Area: 'Improved Lambrook Pippin'	Peak Area: 'Ellis Bitter'
(-)-epicatechin-3'-O-glucoside_isomer4	C21 H24 O11	452.1318	8.775	1389.463	2909.615	2849.269	2663.358
2'-hydroxy 3,6,7,4'-tetramethylquercetagenin 3'-O-β-D-glucoside	C25 H28 O14	552.1466	16.126	2352.511	2983.541	3187.189	3429.435
flavanone 7-O-[alpha-L-rhamnosyl-(1->2)-beta-D-glucoside]	C27 H32 O12	548.1892	13.17	2025.67	14634.24	208348.2	524910
(2R,3S,4S)-leucocyanidin or epigallocatechin	C15 H14 O7	306.073	7.892	19675.94	5589.089	9434.219	5710.984
Urolithin A-8-O-glucuronide	C19 H16 O10	404.0743	7.88	3382.35	2959.148	3341.954	3139.637
neohesperidin	C28 H34 O15	610.1883	13.874	2369.152	3669.025	5383.414	4313.003

Compound Name	Formula	Molecular Weight	RT [min]	Peak Area: 'Kingston Black'	Peak Area: 'Northern Spy'	Peak Area: 'Improved Lambrock Pippin'	Peak Area: 'Ellis Bitter'
1-O-caffeoyl- β -D-glucose	C15 H18 O9	342.094	8.704	40057.71	42017.26	451039.9	110154.7
(-)-epicatechin-3'-O-glucoside_isomer1	C21 H24 O11	452.1311	8.042	15516.14	7685.67	7272.217	4052.5
kaempferide 7-O-glucoside	C22 H22 O11	462.1156	16.217	55689.91	18193.11	632324.8	70497.37
oleuropein aglycone	C19 H22 O8	378.1308	16.956	269081.9	465859.4	112278.1	88209.97
kaempferide 7-O-glucoside isomer	C22 H22 O11	462.115	14.422	4629.881	3597.754	10926.41	3109.102
1-O-vanilloyl- β -D-glucose	C14 H18 O9	330.0947	5.703	60821.65	94068.15	112412.6	397168
phloretin	C15 H14 O5	274.083	14.203	39339.99	5527.235	2775.587	5042.211
phloretin.isomer1	C15 H14 O5	274.0838	10.982	1944.985	3147.15	2305.902	3939.973
(2E)-2-butylidene-4-hydroxy-5-methyl-3(2H)-furanone	C9 H12 O3	168.0783	7.186	18758.53	5974.788	8143.591	23109.24
caffealdehyde isomer	C9 H8 O3	164.047	11.344	13173.67	23172.92	35852.76	5390.636
(-)-glycinol or Naringenin or Trihydroxyflavanone	C15 H12 O5	272.0677	17.969	6462.315	54657.52	47816.11	66355.87
naringin dihydrochalcone	C27 H34 O14	582.1937	15.08	71246.1	10921.35	2311.137	3665.403
procyanidin B3 isomer1	C30 H26 O12	578.1426	5.703	1651.321	2640.797	2461.675	2786.97
2,4',5,7-tetrahydroxyisoflavanone	C15 H12 O6	288.063	16.533	10815.45	7076.665	5634.141	59745.39
apigenin 7-O- β -D-glucoside	C21 H20 O10	432.105	13.439	10458.66	25366.37	99559.38	312832.5
2',3,4,4',6'-pentahydroxychalcone 4'-O- β -D-glucoside.isomer1	C21 H22 O11	450.1155	9.984	186494.4	214783.2	347874.2	167293.4
2,3-cis-oligoproanthocyanidin	C45 H38 O18	866.2058	8.477	3144.576	2999.196	7205.631	4268.171
(-)-epicatechin-3'-O-glucoside_isomer2	C21 H24 O11	452.1321	7.34	33677.06	20105.05	17335.43	9166.821
(-)-epicatechin-3'-O-glucoside.	C21 H24 O11	452.1318	6.773	117149.9	140083.2	64823.81	45966.16
sinapaldehyde glucoside	C17 H22 O9	370.126	10.315	15179.9	7351.237	96866.16	795463.4
1-O-caffeoyl- β -D-glucose isomer	C15 H18 O9	342.094	7.821	510983.7	552119.7	828524.3	313111.7
(-)-epicatechin-3'-O-glucoside_isomer3	C21 H24 O11	452.1315	6.926	221510.9	243562.1	81249.59	88225.88
Catechin	C15 H14 O6	290.0783	13.711	45055.02	21705.64	22288.75	12913.48
2,4',5,7-tetrahydroxyisoflavanone. Isomer	C15 H12 O6	288.0627	7.513	6696.491	4083.791	11408.68	4898.499
Procyanidin A2	C30 H24 O12	576.1269	3.053	7160.802	8096.014	10790.61	11878.97
caffealdehyde	C9 H8 O3	164.0472	7.81	246623.1	576712.2	857761.8	556774
narirutin	C27 H32 O14	580.1792	11.507	11563.28	69116.19	118318.2	1222834
Procyanidin A2 . Isomer	C30 H24 O12	576.1263	17.084	5789.646	2801.656	5474.334	4157.371
procyanidin B3 isomer3	C30 H26 O12	578.1429	11.305	2988.16	3652.961	5623.677	4028.001
procyanidin B3 isomer4	C30 H26 O12	578.1422	7.235	94191.9	6804.819	83662.91	182750.3
procyanidin B3	C30 H26 O12	578.1425	8.905	168616.2	10949.49	111561.1	78189.13
procyanidin B3 isomer2	C30 H26 O12	578.1431	9.095	253257.7	47374.7	184698.4	149311.5
Chlorogenic acid	C16 H18 O9	354.0943	9.22	1927989	817804.4	118215.4	508096.6
Neochlorogenic acid isomer	C16 H18 O9	354.0949	8.305	4140388	27488430	9221949	326617.8

Compound Name	Formula	Molecular Weight	RT [min]	Peak Area: 'Kingston Black'	Peak Area: 'Northern Spy'	Peak Area: 'Improved Lambrock Pippin'	Peak Area: 'Ellis Bitter'
Rutin	C27 H30 O16	610.1529	12.76	132412.8	1101136	3817159	778007.1
phloretin.isomer2	C15 H14 O5	274.0836	15.308	712918.1	239830.8	66725.65	59051.81
phloretin.isomer3	C15 H14 O5	274.0837	19.642	61518.65	5024.607	2682.992	4857.827
Benzoic acid	C7 H6 O2	122.0368	6.91	990084.3	1937079	5639907	2801769
2-Hydroxycinnamic acid	C9 H8 O3	164.0474	9.117	486806.6	612762	193372.6	623888.4
trans-Cinnamic acid	C9 H8 O2	148.0525	11.158	2500303	2970979	775289	1304316
Neochlorogenic acid	C16 H18 O9	354.0951	9.755	113497.5	112477.8	289710.7	507127.2
Ferulic acid	C10 H10 O4	194.0574	8.932	244142.3	67205.78	230941.2	18907.2
Epicatechin	C15 H14 O6	290.0786	17.187	1844.683	10883.36	2414.453	11413.58

Supplemental Table 10: List the 45 putative compounds identified via UHPLC-MS, the molecular formula, molecular weight and the time of elution for 'Kola', 'Stembridge Jersey', 'Zapta' and 'Bramtot' accessions harvested in 2017 from the USDA-PGRU Malus germplasm collection in Geneva, NY.

Compound Name	Formula	Molecular Weight	RT [min]	Peak Area: 'Kola'	Peak Area: 'Stembridge Jersey'	Peak Area: 'Zapta'	Peak Area: 'Bramtot'
(-)-epicatechin-3'-O-glucoside_isomer4	C21 H24 O11	452.1318	8.775	1105.086	6906.11	22196.76	9579.191
2'-hydroxy 3,6,7,4'-tetramethylquercetagenin 3'-O-β-D-glucoside	C25 H28 O14	552.1466	16.126	36222.91	1925.842	157449	1384.933
flavanone 7-O-[α-L-rhamnosyl-(1->2)-β-D-glucoside]	C27 H32 O12	548.1892	13.17	1085.866	4800.595	1003.185	1441.839
(2R,3S,4S)-leucocyanidin or epigallocatechin	C15 H14 O7	306.073	7.892	9073.823	135915.8	22327.95	61270.15
Urolithin A-8-O-glucuronide	C19 H16 O10	404.0743	7.88	10444.72	2508.877	27836.36	8873.032
neohesperidin	C28 H34 O15	610.1883	13.874	100521.3	3586.101	196196.2	3961.496
1-O-caffeoyl-β-D-glucose	C15 H18 O9	342.094	8.704	15357.08	109440.5	16954.4	29834.17
(-)-epicatechin-3'-O-glucoside_isomer1	C21 H24 O11	452.1311	8.042	4142.798	61360.6	16470.82	55886.27
kaempferide 7-O-glucoside	C22 H22 O11	462.1156	16.217	16912.23	60024.3	15610.28	11263.24
oleuropein aglycone	C19 H22 O8	378.1308	16.956	43468.81	21854.97	80453.04	108576
kaempferide 7-O-glucoside isomer	C22 H22 O11	462.115	14.422	219647.9	12423.99	165255.7	7908.67
1-O-vanilloyl-β-D-glucose	C14 H18 O9	330.0947	5.703	28543.7	201405.3	36376.56	228797.1
phloretin	C15 H14 O5	274.083	14.203	215180.9	31396.09	216299.9	61395.71
phloretin.isomer1	C15 H14 O5	274.0838	10.982	2869.116	6065.86	1184.763	365060.6
(2E)-2-butyldiene-4-hydroxy-5-methyl-3(2H)-furanone	C9 H12 O3	168.0783	7.186	1192.92	18511.15	3151.048	11741.01
caffealdehyde isomer	C9 H8 O3	164.047	11.344	318155.5	17518.13	164960.6	16603.18
(-)-glycinol or Naringenin or Trihydroxyflavanone	C15 H12 O5	272.0677	17.969	1165.267	64707.33	1143.523	11394.77
naringin dihydrochalcone	C27 H34 O14	582.1937	15.08	284613.5	61046.75	380982.6	95182.04
procyanidin B3 isomer1	C30 H26 O12	578.1426	5.703	3246.353	14754.19	19833.68	28962.5
2,4',5,7-tetrahydroxyisoflavanone	C15 H12 O6	288.063	16.533	2262.438	206285.5	5614.873	21920.28
apigenin 7-O-β-D-glucoside	C21 H20 O10	432.105	13.439	8322.509	148902.4	14472.3	126578.5
2',3,4,4',6'-pentahydroxychalcone 4'-O-β-D-glucoside.isomer1	C21 H22 O11	450.1155	9.984	6600.523	249021.9	13652.52	515963.6
2,3-cis-oligoproanthocyanidin	C45 H38 O18	866.2058	8.477	14811.74	191371	84536.67	394900.5

Compound Name	Formula	Molecular Weight	RT [min]	Peak Area: 'Kola'	Peak Area: 'Stembridge Jersey'	Peak Area: 'Zapta'	Peak Area: 'Bramtot'
(-)-epicatechin-3'-O-glucoside_isomer2	C21 H24 O11	452.1321	7.34	22291.64	178359.5	89401.69	261668.2
(-)-epicatechin-3'-O-glucoside.	C21 H24 O11	452.1318	6.773	4356.889	327570	13497.29	287245
sinapaldehyde glucoside	C17 H22 O9	370.126	10.315	938.8028	58016.83	1008.648	682057.6
1-O-caffeoyl-β-D-glucose isomer	C15 H18 O9	342.094	7.821	298663.9	668893.1	366218.9	79087.48
(-)-epicatechin-3'-O-glucoside_isomer3	C21 H24 O11	452.1315	6.926	6748.422	564672.8	22543.22	394266.4
Catechin	C15 H14 O6	290.0783	13.711	272085.4	67709.4	833934.8	134580.9
2,4',5,7-tetrahydroxyisoflavanone. Isomer	C15 H12 O6	288.0627	7.513	17804.89	214281.9	125852.6	402707.3
Procyanidin A2	C30 H24 O12	576.1269	3.053	21010.89	254851.8	176256.6	350554.9
caffealdehyde	C9 H8 O3	164.0472	7.81	444009.4	202813.6	953324.5	69154.65
narirutin	C27 H32 O14	580.1792	11.507	571636.9	74228.92	504391.2	23418.85
Procyanidin A2 . Isomer	C30 H24 O12	576.1263	17.084	7921.081	730893.9	10222.13	455012.5
procyanidin B3 isomer3	C30 H26 O12	578.1429	11.305	61500.57	137555.2	148617.7	271259.6
procyanidin B3 isomer4	C30 H26 O12	578.1422	7.235	169246.7	2455905	1561545	7745683
procyanidin B3	C30 H26 O12	578.1425	8.905	5220387	11156944	29603174	12783628
procyanidin B3 isomer2	C30 H26 O12	578.1431	9.095	9644307	17617802	47168706	50535760
Chlorogenic acid	C16 H18 O9	354.0943	9.22	16351884	3925323	13841515	9639421
Neochlorogenic acid isomer	C16 H18 O9	354.0949	8.305	8619707	6106876	4201453	10777015
Rutin	C27 H30 O16	610.1529	12.76	12319.2	710936.2	4128.303	155073.3
phloretin.isomer2	C15 H14 O5	274.0836	15.308	2394182	916605.5	4584418	1268854
phloretin.isomer3	C15 H14 O5	274.0837	19.642	123292.7	208370.7	116317.4	90124.21
Benzoic acid	C7 H6 O2	122.0368	6.91	381592.3	681658.4	78994.82	885951.7
2-Hydroxycinnamic acid	C9 H8 O3	164.0474	9.117	59011.33	822722	472315.8	849273.7
trans-Cinnamic acid	C9 H8 O2	148.0525	11.158	50686.65	1269234	11195.96	2511249
Neochlorogenic acid	C16 H18 O9	354.0951	9.755	34316.47	315622.8	264873.4	202474.2
Ferulic acid	C10 H10 O4	194.0574	8.932	264649.6	30591.36	467231	42138.07
Epicatechin	C15 H14 O6	290.0786	17.187	10057.12	43589.62	11617.1	22795.28

Supplemental Table 11: List the 45 putative compounds identified via UHPLC-MS, the molecular formula, molecular weight and the time of elution for 'Medaille d'Or', 'Launette', and 'Kaz 95-08-06' accessions harvested in 2017 from the USDA-PGRU Malus germplasm collection in Geneva, NY.

Compound Name	Formula	Molecular Weight	RT [min]	Peak Area: 'Medaille d'Or'	Peak Area: 'Launette'	Peak Area: 'Kaz 95-08-06'
(-)-epicatechin-3'-O-glucoside_isomer4	C21 H24 O11	452.1318	8.775	29575.84	20372.1	137858.6
2'-hydroxy 3,6,7,4'-tetramethylquercetagenin 3'-O-β-D-glucoside	C25 H28 O14	552.1466	16.126	1294.057	949.4685	5660.569
flavanone 7-O-[alpha-L-rhamnosyl-(1->2)-beta-D-glucoside]	C27 H32 O12	548.1892	13.17	1867.897	2946.786	1619.568
(2R,3S,4S)-leucocyanidin or epigallocatechin	C15 H14 O7	306.073	7.892	216771.4	135321	122530.3
Urolithin A-8-O-glucuronide	C19 H16 O10	404.0743	7.88	20794.2	6845.52	282684.2
neohesperidin	C28 H34 O15	610.1883	13.874	2668.789	2643.996	3774.845
1-O-caffeoyl-β-D-glucose	C15 H18 O9	342.094	8.704	30187.15	14693.46	13193.39
(-)-epicatechin-3'-O-glucoside_isomer1	C21 H24 O11	452.1311	8.042	100859.7	163604.5	52126.16

Compound Name	Formula	Molecular Weight	RT [min]	Peak Area: 'Medaille d'Or'	Peak Area: 'Launette'	Peak Area: 'Kaz 95- 08-06'
kaempferide 7-O-glucoside	C22 H22 O11	462.1156	16.217	1091.749	55705.16	113662.4
oleuropein aglycone	C19 H22 O8	378.1308	16.956	6129.25	126411.5	115280.8
kaempferide 7-O-glucoside isomer	C22 H22 O11	462.115	14.422	4405.554	6749.121	4688.565
1-O-vanilloyl-β-D-glucose	C14 H18 O9	330.0947	5.703	383839.2	149195.5	254281.5
phloretin	C15 H14 O5	274.083	14.203	46431.59	107250.1	128405.7
phloretin.isomer1	C15 H14 O5	274.0838	10.982	182283.4	100893	273942
(2E)-2-butyldene-4-hydroxy-5-methyl-3(2H)-furanone	C9 H12 O3	168.0783	7.186	271801.5	154469	2511.51
caffealdehyde isomer	C9 H8 O3	164.047	11.344	11405.58	11764.14	42691.34
(-)-glycinol or Naringenin or Trihydroxyflavanone	C15 H12 O5	272.0677	17.969	177983.5	176075.2	11573.46
naringin dihydrochalcone	C27 H34 O14	582.1937	15.08	47668.9	177666.8	179618.2
procyanidin B3 isomer1	C30 H26 O12	578.1426	5.703	175530.8	109232.9	481405.9
2,4',5,7-tetrahydroxyisoflavanone	C15 H12 O6	288.063	16.533	389716.6	229438.7	19333
apigenin 7-O-β-D-glucoside	C21 H20 O10	432.105	13.439	762736.7	146667.7	21597.64
2',3,4,4',6'-pentahydroxychalcone 4'-O-β-D-glucoside.isomer1	C21 H22 O11	450.1155	9.984	351970.4	134536.2	432580.7
2,3-cis-oligoproanthocyanidin	C45 H38 O18	866.2058	8.477	374557.3	327921.7	507449.7
(-)-epicatechin-3'-O-glucoside.isomer2	C21 H24 O11	452.1321	7.34	230845.9	353736	931232.1
(-)-epicatechin-3'-O-glucoside.	C21 H24 O11	452.1318	6.773	249040.1	248181.7	383008
sinapaldehyde glucoside	C17 H22 O9	370.126	10.315	13935.62	31788.64	4432.627
1-O-caffeoyl-β-D-glucose isomer	C15 H18 O9	342.094	7.821	92915.98	336873	280713.3
(-)-epicatechin-3'-O-glucoside.isomer3	C21 H24 O11	452.1315	6.926	312719.9	381141.5	928904.1
Catechin	C15 H14 O6	290.0783	13.711	238075.9	221776.1	337579.1
2,4',5,7-tetrahydroxyisoflavanone. Isomer	C15 H12 O6	288.0627	7.513	474539.3	667749.1	578304.4
Procyanidin A2	C30 H24 O12	576.1269	3.053	642419.7	483649.2	1710226
caffealdehyde	C9 H8 O3	164.0472	7.81	105008.4	148332.8	428675.4
narirutin	C27 H32 O14	580.1792	11.507	374453.9	959379.8	138307.4
Procyanidin A2 . Isomer	C30 H24 O12	576.1263	17.084	2591921	1248413	89986.48
procyanidin B3 isomer3	C30 H26 O12	578.1429	11.305	1361985	1075164	3972769
procyanidin B3 isomer4	C30 H26 O12	578.1422	7.235	5025898	6113242	10271351
procyanidin B3	C30 H26 O12	578.1425	8.905	65534096	18307193	53180269
procyanidin B3 isomer2	C30 H26 O12	578.1431	9.095	1.18E+08	1312969	1054530
Chlorogenic acid	C16 H18 O9	354.0943	9.22	4104267	3764856	3909554
Neochlorogenic acid isomer	C16 H18 O9	354.0949	8.305	13527559	2963470	23641662
Rutin	C27 H30 O16	610.1529	12.76	494458.2	47994.98	753868.1
phloretin.isomer2	C15 H14 O5	274.0836	15.308	1768331	1196895	2094709
phloretin.isomer3	C15 H14 O5	274.0837	19.642	39331.57	2640172	39236.76
Benzoic acid	C7 H6 O2	122.0368	6.91	383112.1	1173057	253857.8
2-Hydroxycinnamic acid	C9 H8 O3	164.0474	9.117	534873.8	1371441	1272172
trans-Cinnamic acid	C9 H8 O2	148.0525	11.158	1092068	2890410	250424.7
Neochlorogenic acid	C16 H18 O9	354.0951	9.755	678545.6	211465.5	262120.2

Compound Name	Formula	Molecular Weight	RT [min]	Peak Area: 'Medaille d'Or'	Peak Area: 'Launette'	Peak Area: 'Kaz 95-08-06'
Ferulic acid	C10 H10 O4	194.0574	8.932	26625.81	19421.49	100934.7
Epicatechin	C15 H14 O6	290.0786	17.187	57251.34	358129.7	41443.29

Supplemental Table 12: List the 45 putative compounds identified via UHPLC-MS, the molecular formula, and the percent sample variation (n=3) for 'Golden Delicious', 'Le Bret', 'Kingston Black', and 'Northern Spy' accessions harvested in 2017 from the USDA-PGRU Malus germplasm collection in Geneva, NY.

Compound Name	Formula	Sample Variation (%): 'Liberty'	Sample Variation (%): 'Golden Delicious'	Sample Variation (%): 'Le Bret'	Sample Variation (%): 'Kingston Black'	Sample Variation (%): 'Northern Spy'
(-)-epicatechin-3'-O-glucoside_isomer4	C21 H24 O11	20.48	20.48	3.08	50.00	12.61
2'-hydroxy 3,6,7,4'-tetramethylquercetagenin 3'-O-β-D-glucoside	C25 H28 O14	25.60	17.92	6.47	26.64	21.14
flavanone 7-O-[alpha-L-rhamnosyl-(1->2)-beta-D-glucoside]	C27 H32 O12	54.70	64.05	48.27	31.36	58.04
(2R,3S,4S)-leucocyanidin or epigallocatechin	C15 H14 O7	18.73	45.81	64.84	21.25	42.26
Urolithin A-8-O-glucuronide	C19 H16 O10	15.96	21.54	8.41	24.04	15.25
neohesperidin	C28 H34 O15	82.52	14.05	4.68	9.33	2.31
1-O-caffeoyl-β-D-glucose	C15 H18 O9	32.74	48.44	18.05	26.34	51.72
(-)-epicatechin-3'-O-glucoside_isomer1	C21 H24 O11	23.80	23.46	49.95	19.25	38.94
kaempferide 7-O-glucoside	C22 H22 O11	37.74	50.63	9.36	23.12	45.78
oleuropein aglycone	C19 H22 O8	24.41	22.85	99.54	28.30	59.79
kaempferide 7-O-glucoside isomer	C22 H22 O11	54.98	13.16	41.73	28.84	31.00
1-O-vanilloyl-β-D-glucose	C14 H18 O9	13.31	53.28	16.37	27.26	73.25
phloretin	C15 H14 O5	50.09	29.39	53.24	30.90	74.96
phloretin.isomer1	C15 H14 O5	24.50	15.71	104.31	26.06	15.45
(2E)-2-butyldene-4-hydroxy-5-methyl-3(2H)-furanone	C9 H12 O3	34.79	60.55	56.85	36.70	31.30
caffealdehyde isomer	C9 H8 O3	32.26	49.75	16.81	28.33	71.71
(-)-glycinol or Naringenin or Trihydroxyflavanone	C15 H12 O5	9.66	116.44	30.38	45.04	66.81
naringin dihydrochalcone	C27 H34 O14	16.70	18.59	47.79	26.56	101.39
procyanidin B3 isomer1	C30 H26 O12	22.53	20.38	4.37	36.11	19.57
2,4',5,7-tetrahydroxyisoflavanone	C15 H12 O6	13.46	29.66	63.18	21.40	77.55
apigenin 7-O-β-D-glucoside	C21 H20 O10	17.99	60.96	13.37	36.98	58.47
2',3,4,4',6'-pentahydroxychalcone 4'-O-β-D-glucoside.isomer1	C21 H22 O11	16.64	55.95	18.25	23.60	53.07
2,3-cis-oligoproanthocyanidin	C45 H38 O18	22.45	30.10	45.30	21.64	17.89
(-)-epicatechin-3'-O-glucoside_isomer2	C21 H24 O11	14.22	13.41	66.84	12.89	79.17

Compound Name	Formula	Sample Variation (%): 'Liberty'	Sample Variation (%): 'Golden Delicious'	Sample Variation (%): 'Le Bret'	Sample Variation (%): 'Kingston Black'	Sample Variation (%): 'Northern Spy'
(-)-epicatechin-3'-O-glucoside.	C21 H24 O11	21.11	87.92	27.36	21.75	86.20
sinapaldehyde glucoside	C17 H22 O9	51.51	43.72	11.84	27.46	21.97
1-O-caffeoyl-β-D-glucose isomer	C15 H18 O9	41.63	102.11	66.56	45.08	64.66
(-)-epicatechin-3'-O-glucoside_isomer3	C21 H24 O11	14.26	50.82	30.25	31.90	50.39
Catechin	C15 H14 O6	37.48	75.67	36.27	24.38	19.35
2,4',5,7-tetrahydroxyisoflavanone. Isomer	C15 H12 O6	22.10	19.29	78.00	70.18	65.61
Procyanidin A2	C30 H24 O12	22.88	19.49	4.62	33.53	20.38
caffealdehyde	C9 H8 O3	24.64	66.03	8.36	33.74	44.61
narirutin	C27 H32 O14	48.26	69.75	30.81	37.41	89.24
Procyanidin A2 . Isomer	C30 H24 O12	25.44	19.30	75.71	20.78	24.12
procyanidin B3 isomer3	C30 H26 O12	18.90	17.62	38.02	20.22	39.36
procyanidin B3 isomer4	C30 H26 O12	25.82	39.35	104.69	37.57	95.92
procyanidin B3	C30 H26 O12	24.55	39.74	91.46	32.73	17.27
procyanidin B3 isomer2	C30 H26 O12	28.69	51.69	67.36	30.74	12.01
Chlorogenic acid	C16 H18 O9	36.39	38.78	31.21	25.58	87.95
Neochlorogenic acid isomer	C16 H18 O9	26.08	41.08	67.60	38.79	51.42
Rutin	C27 H30 O16	36.31	40.98	29.62	24.04	59.50
phloretin.isomer2	C15 H14 O5	16.55	84.71	43.24	27.06	81.73
phloretin.isomer3	C15 H14 O5	27.40	27.82	90.77	23.31	27.46
Benzoic acid	C7 H6 O2	24.40	76.43	34.90	30.89	46.24
2-Hydroxycinnamic acid	C9 H8 O3	15.01	46.79	27.47	35.17	47.67
trans-Cinnamic acid	C9 H8 O2	8.82	35.31	18.86	27.14	65.19
Neochlorogenic acid	C16 H18 O9	13.25	49.50	0.66	24.08	50.98
Ferulic acid	C10 H10 O4	14.20	31.47	14.67	27.01	49.45
Epicatechin	C15 H14 O6	70.70	51.31	12.25	71.38	58.65

Supplemental Table 13: List the 45 putative compounds identified via UHPLC-MS, the molecular formula, and the percent sample variation (n=3) for 'Improved Lambrook Pippin', 'Kola', 'Stembridge Jersey', 'Zapta' and 'Bramtot' accessions harvested in 2017 from the USDA-PGRU Malus germplasm collection in Geneva, NY.

Compound Name	Formula	Group CV (%): 'Improved Lambrook Pippin'	Group CV (%): 'Ellis Bitter'	Group CV (%): 'Kola'	Group CV (%): 'Stembridge Jersey'	Group CV (%): 'Zapta'	Group CV (%): 'Bramtot'
(-)-epicatechin-3'-O-glucoside_isomer4	C21 H24 O11	70.03	44.75	57.50	57.34	24.77	77.36
2'-hydroxy 3,6,7,4'-tetramethylquercetagenin 3'-O-β-D-glucoside	C25 H28 O14	60.00	40.27	49.61	56.59	13.25	27.59
flavanone 7-O-[α-L-rhamnosyl-(1->2)-β-D-glucoside]	C27 H32 O12	55.38	48.61	46.41	89.26	10.36	25.51

Compound Name	Formula	Group CV (%): 'Improved Lambrook Pippin'	Group CV (%): 'Ellis Bitter'	Group CV (%): 'Kola'	Group CV (%): 'Stembridge Jersey'	Group CV (%): 'Zapta'	Group CV (%): 'Bramtot'
(2R,3S,4S)-leucocyanidin or epigallocatechin	C15 H14 O7	54.65	142.07	91.48	81.50	33.15	17.39
Urolithin A-8-O-glucuronide	C19 H16 O10	71.13	40.97	52.68	27.65	50.19	44.41
neohesperidin	C28 H34 O15	56.97	43.48	43.84	64.44	1.49	35.38
1-O-caffeoyl-β-D-glucose	C15 H18 O9	59.16	43.54	62.74	84.77	6.75	11.05
(-)-epicatechin-3'-O- glucoside_isomer1	C21 H24 O11	92.03	129.61	67.08	78.64	27.34	30.46
kaempferide 7-O-glucoside	C22 H22 O11	57.95	23.44	52.77	93.17	12.29	41.43
oleuropein aglycone	C19 H22 O8	76.66	39.60	129.41	123.39	99.99	39.48
kaempferide 7-O-glucoside isomer	C22 H22 O11	71.53	36.30	44.11	75.82	28.46	58.90
1-O-vanilloyl-β-D-glucose	C14 H18 O9	53.76	19.22	39.49	92.12	15.27	19.19
phloretin	C15 H14 O5	60.04	141.35	52.10	80.98	5.59	38.55
phloretin.isomer1	C15 H14 O5	60.39	44.98	57.48	52.93	66.29	11.92
(2E)-2-butyldiene-4-hydroxy-5- methyl-3(2H)-furanone	C9 H12 O3	74.14	26.43	59.92	74.26	11.60	26.06
caffealdehyde isomer	C9 H8 O3	90.36	156.31	45.96	66.16	35.54	21.41
(-)-glycinol or Naringenin or Trihydroxyflavanone	C15 H12 O5	75.52	53.98	37.99	118.28	42.37	45.23
naringin dihydrochalcone	C27 H34 O14	99.89	158.02	44.05	84.61	10.42	30.70
procyanidin B3 isomer1	C30 H26 O12	65.60	88.47	45.38	81.12	8.11	16.92
2,4',5,7-tetrahydroxyisoflavanone	C15 H12 O6	83.03	59.15	81.65	131.71	35.85	22.22
apigenin 7-O-β-D-glucoside	C21 H20 O10	60.39	29.52	69.00	93.91	9.14	23.91
2',3,4,4',6'-pentahydroxychalcone 4'- O-β-D-glucoside.isomer1	C21 H22 O11	61.03	20.16	64.88	102.50	13.85	27.62
2,3-cis-oligoproanthocyanidin	C45 H38 O18	66.89	159.52	109.97	95.47	46.43	14.80
(-)-epicatechin-3'-O- glucoside_isomer2	C21 H24 O11	79.00	133.30	21.40	86.68	13.50	19.23
(-)-epicatechin-3'-O-glucoside.	C21 H24 O11	78.13	114.96	99.67	87.29	33.63	59.34
sinapaldehyde glucoside	C17 H22 O9	62.26	36.15	47.37	85.69	14.94	30.67
1-O-caffeoyl-β-D-glucose isomer	C15 H18 O9	76.26	34.08	65.54	90.40	11.84	30.22
(-)-epicatechin-3'-O- glucoside_isomer3	C21 H24 O11	92.21	113.34	46.18	84.24	31.82	12.04
Catechin	C15 H14 O6	95.38	150.38	68.57	96.40	23.93	42.67
2,4',5,7-tetrahydroxyisoflavanone. Isomer	C15 H12 O6	112.85	146.53	72.72	99.18	20.86	41.10
Procyanidin A2	C30 H24 O12	79.68	137.85	112.28	98.16	65.60	39.62
caffealdehyde	C9 H8 O3	70.69	37.76	60.63	91.88	13.34	10.91
narirutin	C27 H32 O14	86.93	50.94	48.71	89.62	58.82	37.72
Procyanidin A2 . Isomer	C30 H24 O12	109.45	158.78	64.45	85.84	45.88	8.54
procyanidin B3 isomer3	C30 H26 O12	60.20	103.39	41.27	93.81	8.90	15.95
procyanidin B3 isomer4	C30 H26 O12	91.39	145.99	59.05	95.87	43.80	17.68
procyanidin B3	C30 H26 O12	84.63	158.75	69.76	105.73	39.24	102.74
procyanidin B3 isomer2	C30 H26 O12	56.06	159.58	60.19	110.60	39.33	86.31
Chlorogenic acid	C16 H18 O9	77.04	147.65	46.27	84.74	30.43	31.22
Neochlorogenic acid isomer	C16 H18 O9	83.33	47.85	14.37	113.55	46.95	10.80

Compound Name	Formula	Group CV (%): 'Improved Lambrook Pippin'	Group CV (%): 'Ellis Bitter'	Group CV (%): 'Kola'	Group CV (%): 'Stembridge Jersey'	Group CV (%): 'Zapta'	Group CV (%): 'Bramtot'
Rutin	C27 H30 O16	62.83	64.36	70.82	91.77	28.45	27.22
phlorethin.isomer2	C15 H14 O5	103.95	165.29	60.15	86.22	13.30	25.88
phlorethin.isomer3	C15 H14 O5	86.70	118.48	89.25	120.15	67.99	41.08
Benzoic acid	C7 H6 O2	63.09	50.08	48.52	58.23	16.23	13.82
2-Hydroxycinnamic acid	C9 H8 O3	89.63	117.83	143.93	109.50	77.43	4.91
trans-Cinnamic acid	C9 H8 O2	71.53	123.57	65.17	113.42	26.21	20.10
Neochlorogenic acid	C16 H18 O9	59.91	29.28	35.78	93.26	25.12	14.20
Ferulic acid	C10 H10 O4	75.65	46.02	36.50	51.16	16.42	10.31
Epicatechin	C15 H14 O6	112.69	64.60	85.90	97.24	51.32	17.19

Supplemental Table 14: List the 45 putative compounds identified via UHPLC-MS, the molecular formula, and the percent sample variation (n=3) for 'Medaille d'Or', 'Launette', and 'Kaz 95-08-06' accessions harvested in 2017 from the USDA-PGRU Malus germplasm collection in Geneva, NY.

Compound Name	Formula	Sample Variation (%): 'Medaille d'Or'	Sample Variation (%): 'Launette'	Sample Variation (%): 'Kaz 95-08-06'
(-)-epicatechin-3'-O-glucoside_isomer4	C21 H24 O11	19.01	65.06	92.41
2'-hydroxy 3,6,7,4'- tetramethylquercetagetin 3'-O-β-D- glucoside	C25 H28 O14	5.17	7.35	66.25
flavanone 7-O-[α-L-rhamnosyl-(1- >2)-β-D-glucoside]	C27 H32 O12	27.66	11.79	75.28
(2R,3S,4S)-leucocyanidin or epigallocatechin	C15 H14 O7	13.38	13.79	86.18
Urolithin A-8-O-glucuronide	C19 H16 O10	4.43	17.22	84.56
neohesperidin	C28 H34 O15	9.09	56.41	31.36
1-O-caffeoyl-β-D-glucose	C15 H18 O9	23.97	13.73	53.03
(-)-epicatechin-3'-O-glucoside_isomer1	C21 H24 O11	11.67	16.00	80.69
kaempferide 7-O-glucoside	C22 H22 O11	165.05	1.45	83.62
oleuropein aglycone	C19 H22 O8	64.61	41.28	88.88
kaempferide 7-O-glucoside isomer	C22 H22 O11	56.63	35.31	55.73
1-O-vanilloyl-β-D-glucose	C14 H18 O9	4.92	14.14	83.15
phlorethin	C15 H14 O5	12.46	14.75	80.76
phlorethin.isomer1	C15 H14 O5	18.90	2.97	88.84
(2E)-2-butylidene-4-hydroxy-5-methyl- 3(2H)-furanone	C9 H12 O3	75.39	10.86	52.56
caffealdehyde isomer	C9 H8 O3	10.43	22.24	77.12
(-)-glycinol or Naringenin or Trihydroxyflavanone	C15 H12 O5	92.77	38.64	88.44
naringin dihydrochalcone	C27 H34 O14	91.14	9.17	86.05
procyanidin B3 isomer1	C30 H26 O12	21.68	23.98	92.45
2,4',5,7-tetrahydroxyisoflavanone	C15 H12 O6	86.75	35.06	93.06
apigenin 7-O-β-D-glucoside	C21 H20 O10	1.71	15.04	75.84

Compound Name	Formula	Sample Variation (%): 'Medaille d'Or'	Sample Variation (%): 'Launette'	Sample Variation (%): 'Kaz 95-08-06'
2',3,4,4',6'-pentahydroxychalcone 4'-O-β-D-glucoside.isomer1	C21 H22 O11	6.67	7.75	88.20
2,3-cis-oligoproanthocyanidin	C45 H38 O18	14.83	26.90	85.32
(-)-epicatechin-3'-O-glucoside_isomer2	C21 H24 O11	13.41	3.60	85.34
(-)-epicatechin-3'-O-glucoside.	C21 H24 O11	6.92	5.34	109.42
sinapaldehyde glucoside	C17 H22 O9	8.56	8.61	19.21
1-O-caffeoyl-β-D-glucose isomer	C15 H18 O9	60.73	18.78	89.38
(-)-epicatechin-3'-O-glucoside_isomer3	C21 H24 O11	15.44	1.67	89.99
Catechin	C15 H14 O6	16.23	34.21	88.77
2,4',5,7-tetrahydroxyisoflavanone. Isomer	C15 H12 O6	26.34	30.04	98.82
Procyanidin A2	C30 H24 O12	9.62	3.17	84.78
caffealdehyde	C9 H8 O3	21.03	10.25	87.02
narirutin	C27 H32 O14	9.88	33.72	90.91
Procyanidin A2 . Isomer	C30 H24 O12	31.24	17.05	94.32
procyanidin B3 isomer3	C30 H26 O12	10.38	6.19	88.16
procyanidin B3 isomer4	C30 H26 O12	11.44	8.04	88.17
procyanidin B3	C30 H26 O12	19.54	78.77	90.06
procyanidin B3 isomer2	C30 H26 O12	86.53	155.49	120.57
Chlorogenic acid	C16 H18 O9	7.75	11.66	89.68
Neochlorogenic acid isomer	C16 H18 O9	14.17	5.87	102.32
Rutin	C27 H30 O16	34.77	28.37	87.41
phloretin.isomer2	C15 H14 O5	23.24	23.96	85.53
phloretin.isomer3	C15 H14 O5	145.67	75.57	103.99
Benzoic acid	C7 H6 O2	31.67	13.92	68.59
2-Hydroxycinnamic acid	C9 H8 O3	61.97	16.29	83.27
trans-Cinnamic acid	C9 H8 O2	28.62	3.21	82.56
Neochlorogenic acid	C16 H18 O9	7.55	11.58	89.53
Ferulic acid	C10 H10 O4	5.88	4.23	65.79
Epicatechin	C15 H14 O6	63.86	40.71	53.88

Supplemental Table 15: The accessions (n=160) phenotyped within the 2017 harvest season from the USDA-PGRU Malus germplasm collection in Geneva, NY with respective Ma1, Q8 genotypes, titratable acidity (g·L⁻¹) and initial pH values.

PI Number	Cultivar Name	Ma Genotype	Q8 Genotype	Titratable Acidity	Initial pH
280022	Adam's Pearmain	Mama	Q8q8	5.470	3.59
127311	Amere De Berthcourt	mama	Q8Q8	1.516	4.57
173978	American Forestier	mama	Q8Q8	1.176	4.72
136243	Amzr Gauthier	mama	Q8Q8	1.880	4.18
588952	Arkansas	mama	Q8Q8	1.874	4.56
589117	Arkansas Black	Mama	Q8Q8	4.596	3.59
589654	Ashmead's Kernel	Mama	Q8q8	5.329	3.57
105498	Bella de jardins	Mama	Q8Q8	3.346	3.76
589584	Belle de Nordhaussen	Mama	Q8Q8	6.111	3.26
162709	Belle Fille	mama	Q8Q8	1.803	4.63
588951	Belle Sans Pepin	Mama	Q8Q8	3.137	3.83
588953	Ben Davis	Mama	q8q8	3.508	3.77
122598	Binet Blanc	mama	Q8Q8	1.410	4.61
158729	Binet Blanc Dore	mama	Q8Q8	2.995	4.44
158730	Binet Rouge	mama	Q8Q8	1.316	4.87
341067	Blahova Oranzova Renetor	Mama	Q8q8	2.785	4.23
590180	Blue Pearmain	Mama	Q8q8	6.315	3.65
162549	Boche	Mama	Q8Q8	9.386	3.22
590120	Bonne-Hotture	Mama	Q8q8	6.509	3.52
107171	Bramley's Seedling	MaMa	Q8Q8	9.199	3.09
158731	Bramtot	mama	Q8Q8	2.170	4.53
589662	Brown's Apple	Mama	Q8Q8	4.447	3.40
588808	Bulmer Norman	mama	Q8Q8	1.880	4.35
187297	C'Huero Biz Bras	mama	Q8Q8	1.841	4.55
187298	C'Huero Ru Bienn	mama	Q8Q8	2.224	4.34
161830	Cap of Liberty	Mama	Q8q8	5.731	3.50
183961	Carnival-14	Mama	Q8Q8	5.975	3.41
264688	Champagne Reinette	Mama	Q8Q8	8.295	3.29
589175	Coat Jersey	mama	Q8Q8	1.450	4.63
590121	Cornish Aromatic (Wakeley)	Mama	q8q8	5.964	3.55
589602	Court Pendu Gris	Mama	Q8Q8	4.833	3.49
589587	Court Pendu Rose	Mama	Q8Q8	5.392	3.70
589671	Court Royal	mama	Q8Q8	2.398	4.57
589196	Crow Egg	Mama	Q8Q8	1.833	4.01
162722	Damelot	mama	Q8q8	1.554	4.62

PI Number	Cultivar Name	Ma Genotype	Q8 Genotype	Titrateable Acidity	Initial pH
162062	Daux Belan	mama	Q8Q8	2.187	4.53
264689	Djulabia	Mama	Q8Q8	2.557	3.77
173979	Domaine	mama	Q8Q8	1.656	4.63
131104	Double Bon Pommier	Mama	Q8q8	3.157	4.04
161760	Doucet Rouge	mama	Q8Q8	1.936	4.30
589667	Doux Normandie	mama	Q8q8	2.607	4.10
162715	Doux Tardif	mama	Q8Q8	2.185	4.08
122616	Doux-AMR	mama	Q8q8	1.838	4.45
131823	Drap d'Or Guemene	Mama	Q8q8	4.240	3.78
589666	Dunkerton Late Sweet	mama	Q8q8	1.567	4.65
590125	Edelroter	Mama	Q8q8	6.699	3.49
392312	Edward VII	MaMa	Q8Q8	8.068	3.26
589650	Ellis Bitter	mama	Q8Q8	1.111	4.79
588785	Esopus Spitzenburg	Mama	Q8Q8	5.061	3.54
590126	Fenouillet Ribours	Mama	Q8Q8	3.985	3.62
589679	Fillbarrel	mama	Q8Q8	2.156	4.25
589626	Forest King	Mama	Q8Q8	13.661	3.00
589318	Foxwhelp	Mama	Q8Q8	4.051	3.49
590127	Frandise	Mama	Q8Q8	5.678	3.57
162503	Frequin	mama	Q8Q8	1.468	4.83
247314	Frequin Lacaille	mama	Q8Q8	2.172	4.31
589689	Frequin Tardive de la Sarthe	mama	Q8Q8	2.072	4.37
276299	Freyberg	Mama	q8q8	0.865	5.44
589123	Geeveston Fanny	Mama	Q8Q8	2.493	3.90
132225	Gewurzluiken	Mama	Q8q8	3.331	3.68
590184	Golden Delicious	Mama	Q8q8	5.216	3.60
590128	Golden Harvey	Mama	Q8Q8	6.073	3.51
590129	Golden Pippin	Mama	Q8Q8	7.128	3.34
589892	Golden Russet	Mama	Q8q8	4.958	3.52
589684	Grenadier	Mama	Q8Q8	7.390	3.23
588791	Grimes Golden	Mama	Q8q8	4.310	3.69
161761	Grosse Launette	mama	Q8q8	1.835	4.59
162545	Grosse Mouche	Mama	Q8Q8	7.226	3.39
681628	Harrison	Mama	Q8q8	4.418	3.72
589585	Holaart Doux	mama	Q8Q8	1.755	4.88
590130	Hubbards Pearmain	Mama	Q8Q8	4.306	3.61
590185	Jonathan	mama	Q8Q8	6.268	3.43
162731	Jouveaux	mama	Q8Q8	1.545	5.59

PI Number	Cultivar Name	Ma Genotype	Q8 Genotype	Titrateable Acidity	Initial pH
657019	Kaz 96-08-06	MaMa	Q8Q8	18.560	3.00
589703	Kingston Black	Mama	Q8Q8	2.141	4.23
589219	Kola	Mama	Q8Q8	18.562	2.89
589598	La Paix	Mama	Q8q8	1.969	3.97
162724	Lande	Mama	Q8Q8	3.365	3.72
589565	Landsberger Reinette	MaMa	Q8Q8	4.945	3.63
161851	Langworthy	Mama	Q8Q8	3.796	3.59
162732	Launette	mama	Q8Q8	2.804	4.34
589690	Le Bret	mama	Q8Q8	1.639	4.78
588943	Liberty	Mama	Q8Q8	5.404	3.44
589895	Macoun	Mama	Q8Q8	3.710	3.51
173982	Marin Onfroy	mama	Q8Q8	1.685	4.53
588998	Marshall McIntosh	Mama	Q8Q8	4.075	3.48
588817	McIntosh Summerland Red	Mama	Q8Q8	4.733	3.41
589634	Mercer	Mama	Q8Q8	11.261	3.07
589670	Michelin	mama	Q8q8	2.195	4.35
588976	Midget Crab	MaMa	Q8Q8	9.25	3.28
200780	Muscadet Bernay	mama	Q8Q8	2.532	4.02
589493	Muscadet de Dieppe	mama	Q8Q8	1.604	4.81
173985	Muscadet de Lense	mama	Q8Q8	2.583	4.30
223602	Mutsu	Mama	Q8q8	4.737	3.76
161763	Nanot	Mama	Q8q8	6.805	3.24
175544	Nehou	mama	Q8Q8	1.793	4.60
173986	Noel Deschamps	mama	Q8Q8	2.721	4.20
588872	Northern Spy	Mama	Q8q8	4.261	3.54
137094	Notaire	Mama	Q8q8	1.346	4.71
280027	Old Nonpareil	MaMa	Q8Q8	3.210	3.85
590133	Old Pearmain	Mama	Q8q8	4.251	3.44
589674	Pethyre	mama	Q8Q8	1.006	4.74
132272	Pigeonnet Blanc	Mama	Q8q8	7.698	3.26
132273	Pigeonnet Rouge	Mama	Q8Q8	5.218	3.55
588745	Pohorka	Mama	Q8q8	3.592	3.64
131975	Pomme Framboise	MaMa	Q8Q8	10.66	4.6
134668	Pomme Cloche	MaMa	Q8Q8	7.817	3.19
134669	Pomme Raisin	Mama	Q8Q8	4.551	3.58
240817	Pommier Llorca	mama	Q8Q8	1.759	4.74
589789	PRI 1744-1	mama	Q8Q8	1.6	4.42
437047	Red Ralls	Mama	Q8Q8	4.637	3.63

PI Number	Cultivar Name	Ma Genotype	Q8 Genotype	Titrateable Acidity	Initial pH
589211	Redfield	Mama	Q8q8	8.519	3.35
132571	Reine des Pommes	mama	Q8Q8	4.937	3.64
279326	Reine des Reinettes x 1700	Mama	Q8Q8	4.295	3.95
279325	Reine des Reinettes x 82	Mama	Q8q8	3.982	3.76
105524	Reineta do Caravia	Mama	Q8Q8	6.303	3.45
589444	Reinette Clochard	Mama	Q8Q8	5.209	3.57
590135	Reinette d' Anjou	Mama	Q8q8	5.857	3.44
590140	Reinette Franche	Mama	Q8Q8	6.008	3.55
131561	Reinette Jaegers	Mama	Q8q8	6.415	3.49
135645	Reinette Jamin	Mama	Q8Q8	3.777	3.70
590137	Reinette Thouin	Mama	Q8Q8	8.681	3.38
162741	Reinette Tres Tardive	Mama	Q8Q8	10.68	3.77
188521	Reinette van Ekenstein	Mama	Q8Q8	3.634	4.15
589520	Rhode Island Greening	Mama	Q8Q8	4.369	3.76
588840	Ribston	Mama	Q8q8	3.204	3.81
590141	Ross Nonpareil	Mama	Q8q8	5.714	3.66
102148	Rott Jarnapple	Mama	Q8q8	4.353	3.47
589143	Rouge Belle de Boskoop	Mama	Q8q8	6.097	3.51
588971	Roxbury Russet	Mama	Q8Q8	2.217	4.35
161846	Skyrme's Kernel	Mama	Q8q8	5.440	3.46
589903	Smokehouse	Mama	Q8q8	4.448	3.44
588975	Stayman	Mama	Q8q8	4.340	3.65
589692	Stembridge Cluster	Mama	Q8q8	4.967	3.41
589693	Stembridge Jersey	Mama	Q8q8	1.297	5.14
307382	Sturmer Pippin	MaMa	Q8Q8	6.839	3.28
125566	Surpasse Frequin	mama	Q8Q8	2.583	3.97
589691	Tale Sweet	mama	Q8Q8	2.123	4.37
175548	Tardive Forestier	mama	Q8Q8	6.181	3.45
589663	Taylors	mama	Q8q8	0.926	5.78
175549	Teign Harvey	Mama	Q8Q8	3.293	3.71
506361	Thorgauer Weinapfel	Mama	Q8Q8	4.938	3.71
127370	Tient Fraise	mama	Q8Q8	2.554	4.17
175551	Twistbody Jersey	mama	Q8Q8	2.083	4.35
629317	USSR 89-35-01	MaMa	Q8Q8	4.604	3.53
589060	Vandevere	Mama	Q8Q8	6.026	3.41
175552	Vangnon Ascher	Mama	Q8Q8	2.116	4.25
589623	Wamdesa	Mama	q8q8	11.489	3.13
589635	Wecota	MaMa	q8q8	10.544	4.54

PI Number	Cultivar Name	Ma Genotype	Q8 Genotype	Titrateable Acidity	Initial pH
590143	Weidners Goldreinette	Mama	Q8Q8	5.054	3.46
590144	Weisser Winter Taffetapfel	Mama	Q8Q8	5.020	3.49
613818	Wickson	MaMa	Q8Q8	7.593	3.52
589309	William Crump	Mama	Q8Q8	6.762	3.24
588799	Winesp	Mama	Q8q8	4.121	3.64
589632	Wotanda	Mama	q8q8	10.756	3.09
588773	Yellow Newtown	Mama	Q8Q8	5.427	3.48
589614	Zapta	-	Q8Q8	17.13	2.9
613927	-	Mama	Q8q8	3.500	3.81
613897	-	Mama	Q8Q8	3.88	3.77