# Dihydrochalcones and Apple Russeting

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## Introduction

Apple russeting is a result of genetic and environmental factors, presenting as a partial or completely brown, corky peel (Gutierrez et al. 2018). Although it is acceptable in pear, russeting in apple is considered undesirable to consumers in the United States. Research has identified an association with russeting and nutraceuticals, including phloridzin, an apple specific nutrient (Gutierrez et al. 2018). Improved health benefits related to russeting may be important to promote consumer acceptance of more diverse apple varieties.

Though thousands of apple varieties exist, apple production in the United States only favors a few dozen cultivars. The USDA Apple Collection in Geneva, NY maintains close to 6,000 unique cultivars of apple including wild apple relatives. This diversity collection helps to ensure the sustainability of the apple production in the United States with varieties suited to broad environments, and novel fruit qualities for consumers. The goal of this study is to continue a study by Gutierrez et al. (2018) and measure phloridzin content during onset of russeting in diverse apples. Additionally, we want to determine if increases of other dihydrochalcones, including sieboldin and trilobatin are associated with russeting.

Genetic russet (left, ‘St. Edmund’s Russet’) and environmental russet (right, ‘Imperial Stayman’)

## Methods and Materials

Sample Collection

Fruit samples were taken from the USDA Apple Collection in Geneva, NY and from a population of *Malus domestica* × *Malus prunifolia* (PRU-13427) maintained by Cornell University. Accessions with diverse levels of russeting and sieboldin, phloridzin, and trilobatin were selected for the study. The USDA samples were collected on two dates (5/26/21 and 6/23/21).

Sample Prep and Chromatography

Peels were separated from the flesh directly into liquid nitrogen. Individual PRU-13427 fruit were separated into russet vs. waxy samples. All samples were stored at -80℃, then transferred to a smaller container with two ball bearings and placed in the Genogrinder for one minute at 1350 RPM’s. 300mg of tissue (or as much as possible) was suspended in 1.5 mL 70% methanol and 2% formic acid, mixed at 1350 RPM for 30 minutes, and centrifuged 10 minutes at 15,000 RPM. The supernatant was used to measure the dihydrochalcones. Sample injection was 10 or 40 µL. Compounds were separated and measured using an Agilent 1260 Infinity II system with a diode array detector and an isocratic 20% acetonitrile, 10% formic acid mobile phase.

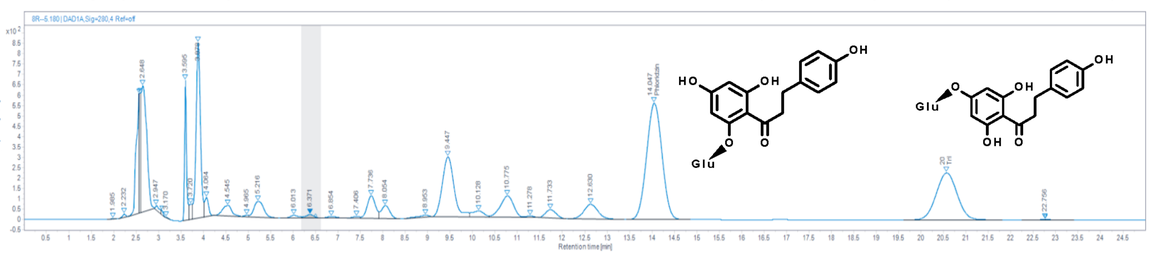
Data Analysis

The peak area data, collected from chromatograms in Open Lab CDS Version 2.4 was exported to Excel and analyzed in R. The concentrations of chlorogenic acid, phloridzin, and trilobatin were calculated using the calibration curves and the units converted to milligrams of each compound per gram of sample tissue:

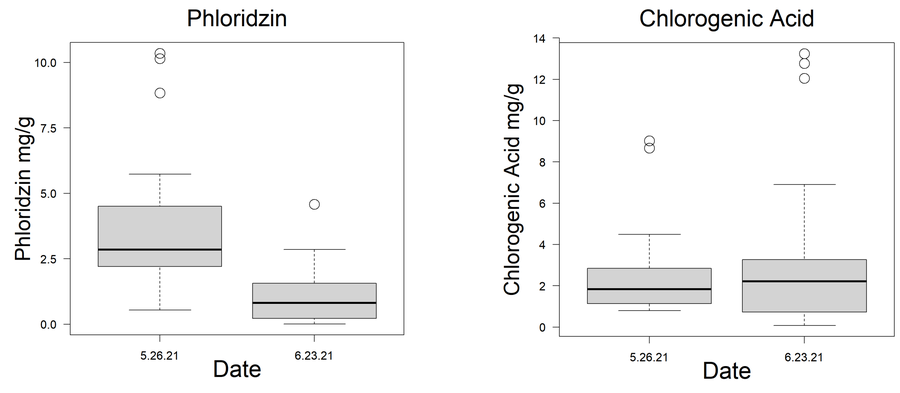
(Peak Area/ Slope of Calibration Curve) × vol. solvent/weight of tissue

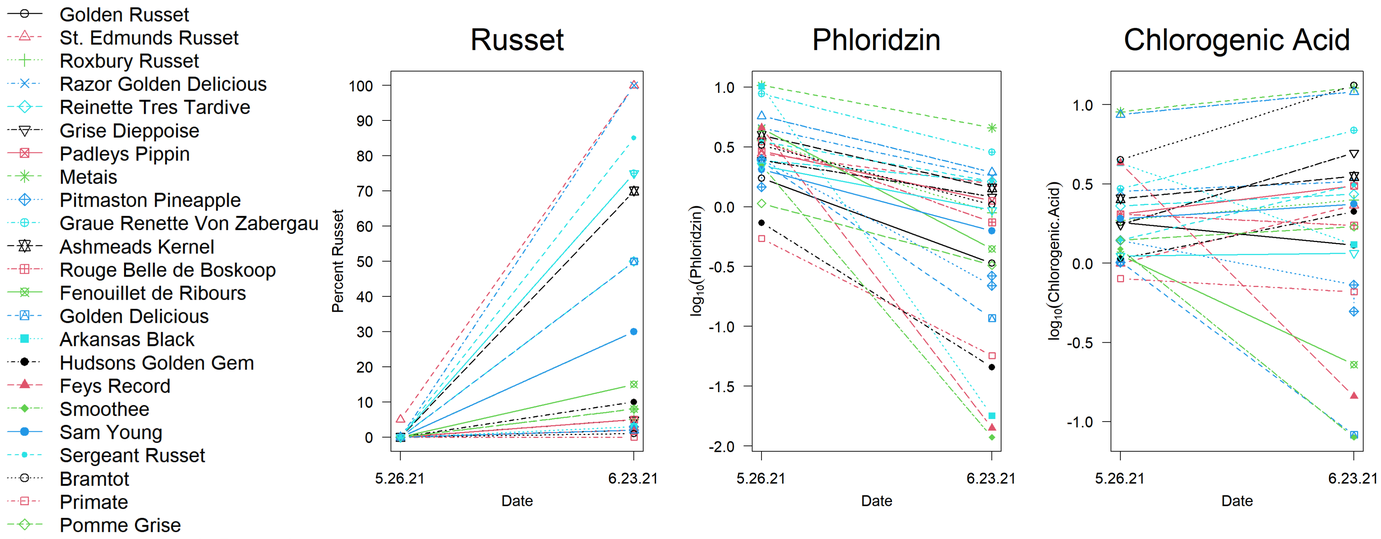
## Results

Chromatograph of sample 5.180 from PRU-13427. Molecular structure of phloridzin (14 min) and trilobatin (20.5 min)




Boxplots comparing phloridzin (left) and chlorogenic acid (right) in the USDA apple collection across two dates




Line plots showing change in russet (left) and phloridzin (center) and chlorogenic acid (right) concentrations in peels of diverse apple varieties (legend) during early fruit development

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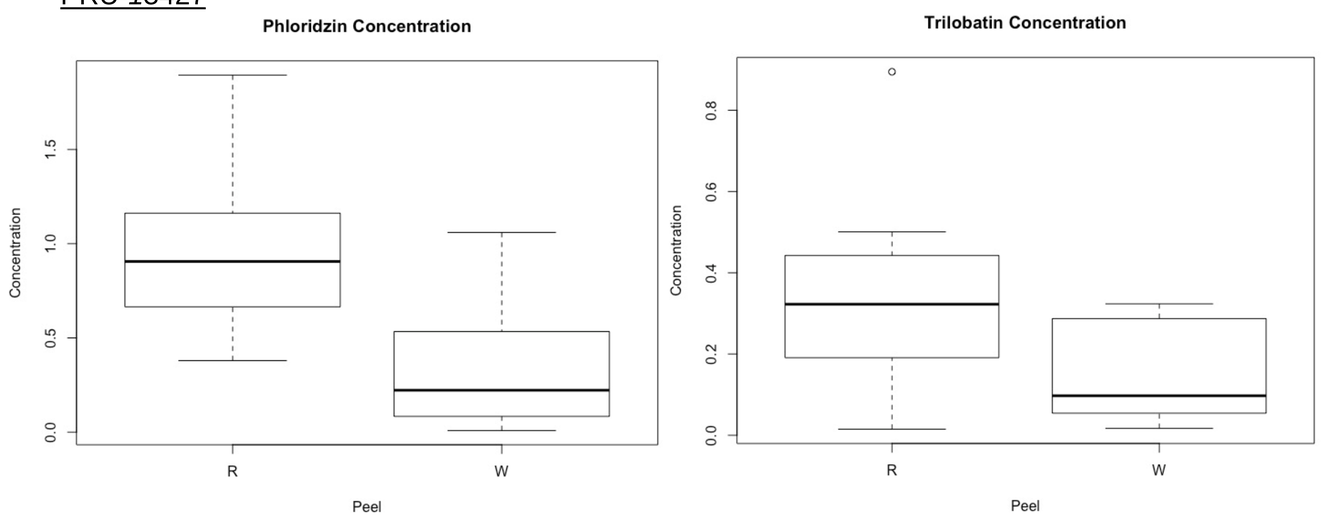


## Discussion

Most results of this study were aligned with those of previous papers. This includes the positive relationship between russeting and phloridzin concentration as well as the negative relationship between phloridzin concentration and time. Additionally, the russeted peel had higher concentrations of dihydrochalcones. Since the russeted apple peel resembles the texture and function of bark (which also has high levels of phloridzin), it is logical to posit that phloridzin is present in both locations but has a lower concentration in the waxy peel.

Conversely, the positive relationship between russeting and chlorogenic acid was unexpected. This could be due to the fact that the samples were taken at different years which leaves them susceptible to unique environmental factors, and a smaller sample size was chosen for the project. Additionally, a large amount of variation in fruit russeting may exist on an individual tree. Furthermore, results showed that chlorogenic acid decreases as russet increases. It is unclear why this occurred. Lastly, chlorogenic acid increased as a function of time and a significant difference in CA concentration was found between Set 1 and 2. Because most of the chlorogenic apple measurements do not seem to change a great amount between the two time points, this may suggest that the outliers are contributing to the significant difference between CA concentration in Set 1 and 2.

From the data above, one could conclude that phloridzin and trilobatin play a significant role in the process of apple russeting. However, chlorogenic acid, along with sieboldin require further investigation.

On set of russet during fruit development in Golden Delicious sport mutations. Left to right, ‘Razor’, Sergeant Russet’, ‘Golden Delicious’, and ‘Smoothee’.

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