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# Mineral nutrient profiles and relationships of ‘Honeycrisp’ grown on a genetically diverse set of rootstocks under New York climatic conditions

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## Abstract

We measured mineral nutrient concentrations in leaves, fruit flesh and fruit peel from field grown apple trees grown on a genetically diverse group of rootstocks from the fifth leaf through the eighth leaf (2014-2017) and identified relationships between nutrients in leaves and fruit of ‘Honeycrisp’ apple. We also evaluated the constancy of rootstock influence on scion nutrient profiles over the four years. Boron concentration was consistent in different seasons, with rootstocks M.9 (several clones), B.9, M.26EMLA, PiAu51-11 and B.71-7-22 resulting in consistently low boron levels, while G.935, CG.2034, CG.4004, G.222 and CG.5087 provided consistently high boron levels. Unlike boron, calcium concentration in scion tissues was somewhat variable in different seasons suggesting a role for climate and perhaps management factors in the rootstock induced absorption and translocation of calcium. We identified a group of rootstocks that yielded high calcium levels in scion tissues (G.214, B.10, G.41, B.9, G.935, G.11, CG.4003) while another group yielded low calcium levels (M.26, M.9-T337, G.202, CG.4004, G.814). We also identified a group of rootstocks that induced low potassium values in scion tissues (CG.4003, G.935, G.222, and G.202), whereas B.70-20-21, B.67-5-32, B.70-20-20 yielded consistently higher values. Rootstock B.10 and CG.2034 induced consistently higher potassium values in fruit tissues. The ratio of K/Ca in leaves and fruit was the best predictor of bitter pit in ‘Honeycrisp’ apples, suggesting that rootstock induced levels for both nutrients play a role in this disorder and that the addition of potassium fertilizers on potassium-efficient rootstocks might increase the susceptibility to bitter pit. Rootstocks CG.4003, B.10, G.41N, and G.214 seemed to produce fruit with lower levels of bitter pit. Our data indicates that some rootstocks are more efficient at absorbing nutrients than others, which implies that fertilizer recommendations developed using one rootstock, such as M.26 or M.9, might be too high for other rootstocks which are more efficient and could thus be causing tree and fruit physiological disorders. Newer rootstocks might be already supplying the needed amounts of minerals to scion tissues.

**Keywords:** calcium, potassium, magnesium, boron, nitrogen, K/Ca ratio, bitter pit

## 1. Introduction

One of the most critical functions of a root system is to gather nutrients from a soil substrate and shuttle them to different sinks in the plant where they will be used for varied cellular functions. Nutrient deficiencies detected in orchard soils by either soil or foliar analyses are normally addressed by the addition of fertilizer formulations designed to compensate for the deficiencies and can be delivered by multiple means (ferti-irrigation, foliar, soil pellets, mulches, etc.) (Chun *et al.*, 2002; Fallahi *et al.*, 1984a; Fallahi *et al.*, 1984b; Milosevic and Milosevic, 2015). These amendments to the orchard ecosystem have been designed based mostly on the performance of a few, genetically restricted, traditional rootstocks, which have been shown to absorb more or less of a particular nutrient contained in the rhizosphere. Rootstocks are embedded in a complex environment where interactions with pH, soil particles, fungi, bacteria, insects, soil water status (Kang *et al.*, 2011), scion cultivar, and cover crops

(and their competing roots) all play into their performance as foragers of nutrients. However, most fertilizer recommendations have not been tailored to a specific rootstock-scion-soil interaction. This raises the possibility that such applications will be less than optimum (more or less than specifically needed by the rootstock-scion-soil combination) and potentially wasteful. Genotypic differences in the ability to absorb and shuttle nutrients to the scion have been observed in apple rootstocks (Fallahi *et al.*, 2014; Fallahi *et al.*, 1984a; Neilsen and Hampson, 2014; Rom and Rom, 1991; Rom *et al.*, 1991; Tukey *et al.*, 1962), peach rootstocks (Reighard *et al.*, 2013), citrus (Dubey and Sharma, 2016; Hippler *et al.*, 2016; Kumar *et al.*, 2018; Toplu *et al.*, 2012), cherry (Hrotko *et al.*, 2014), and watermelon (Nawaz *et al.*, 2017). While most research on nutrient uptake by apple rootstocks has focused on developing the best management practices for nutrient application on a genetically restricted set of rootstocks, efforts to include a large set of genetically diverse rootstocks in different soils and pH treatments allowed the physiological diversity of apple roots to be revealed (Fazio *et al.*, 2012). In that study, varying soil pH caused different genotype dependent responses of the absorption and translocation of nutrients, while soil affected by replant disease was associated with differences in nutrient absorption. The ability to uncouple root (rootstock) functions from scion functions by grafting the same scion cultivar on a diverse panel of apple rootstocks and breeding populations used for genetic mapping produced the first set of data aimed at breeding for improved nutrient efficiency in apple rootstocks (Fazio, 2014; Fazio *et al.*, 2015a; Fazio *et al.*, 2013; Fazio *et al.*, 2015b). Several quantitative trait loci were identified for scion leaf mineral concentrations of potassium (K), sodium (Na), phosphorus (P), calcium (Ca), zinc (Zn), magnesium (Mg) and molybdenum (Mo), with the most significant ones on chromosome 5 for potassium, chromosome 17 for sodium and lower significance QTLs for calcium, copper, zinc, and phosphorus. The influence of rootstocks on various sinks in the scion, including fruit, has the potential to modify organoleptic, post-harvest qualities and disease resistance (Chun and Chun, 2004; Kim *et al.*, 2004; Lockard, 1976; West and Young, 1988; Westwood and Bjornstad, 1980). The analysis of scion nutrient concentration in leaves and fruit in several rootstock comparison trials in New York State have indicated the possibility that specific rootstocks may affect fruit quality of ‘Honeycrisp’ apples showing that certain rootstocks are able to transfer higher calcium levels to the fruit and that the calcium-linked disorders typical of ‘Honeycrisp’ are a result of intrinsic scion challenges in the movement of calcium into the fruit (Fazio *et al.*, 2015a). In these studies, concentrations of some nutrients were highly correlated (K and P, S and P), possibly indicating commonalities in the pathway that take these nutrients from the soil through the rootstocks to diverse sinks in the scion. Similar findings were observed in young trees grafted on a series of diverse rootstocks which were part of an NC-140 coordinated rootstock trial in Canada (Neilsen and Hampson, 2014). The very different mechanisms (interaction with soil biota, active and passive transport, vessel composition and size, etc.) that impact absorption and transport of nutrients and the fact that crop load and irrigation can also influence mineral concentrations (Labidi *et al.*, 2012; Mazzola and Manici, 2012; Neilsen *et al.*, 2015; Zhu *et al.*, 2017; Zribi *et al.*, 2012) makes these traits difficult to improve through breeding without the aid of a robust understanding of molecular genetic factors involved. Modeling those factors to achieve a particular balance of nutrients in selected scions is therefore very complicated.

The objective of this study was to identify relationships between nutrients in leaves and fruit of mature ‘Honeycrisp’ trees and to investigate the constancy of rootstock influence on scion nutrient profiles over four years.

## 2. Materials and Methods

### 2.1. Trees and design

A rootstock field trial was planted in 2010 at the New York State Agricultural Experiment Station (Geneva, NY, USA), using ‘Honeycrisp’ as the scion cultivar (Autio *et al.*, 2017). Trees were planted in a randomized complete block design, with 4 replications and with each block containing 2-3 trees of each rootstock. Blocking was done by initial tree diameter. Tree spacing was 1.2 m × 3.5 m. Rootstocks included three from the Malling series: M.26

87 EMLA, M.9T337, and M.9 Pajam2; nine from the Budagovsky series: B.10 (B.62-396), B.64-194, B.67-5-32,  
 88 B.7-20-21, B.7-3-150, B.70-20-20, B.70-6-8, B.71-7-22 and B.9 (Kazlouskaya and Samus, 2011); six Cornell  
 89 Geneva<sup>®</sup> released rootstocks: G.11, G.41N, G.41TC, G.935N, G.935TC, G.202N, G.202TC, G.214, G.222 and  
 90 G.814 where the “N” and “TC” distinctions refer to normal propagation and tissue culture propagation  
 91 respectively; six experimental Geneva rootstocks: CG.2034, CG.3001, CG.4003, CG.4004, CG.4013, CG.5087;  
 92 and three rootstocks from the Pillnitz series: Piau51-11, Piau9-90, Supporter 3 (Supp. 3) See the companion paper  
 93 (Lordan et al., 2018) for a description of the origin, parentage and vigor class of each rootstock.

## 94 2.2 Nutrient analysis

95 For the 2014 and 2015 growing seasons, ten mid position leaves on new extension growth and ten fruit randomly  
 96 distributed throughout the tree canopy were harvested 80-90 days after bloom on all tree replicates of each  
 97 rootstock. Fruit was cored and processed retaining a fruit tissue sample composed only of a 0.5 cm thick  
 98 horizontal doughnut shaped section obtained two cm from the calyx end of fruit. The ten doughnut shaped  
 99 sections obtained from ten fruit harvested from one tree were bulked into one sample for mineral nutrient analysis.  
 100 Leaves and fruit were oven dried, ground into powder and shipped to the USDA-ARS Children's Nutrition  
 101 Research Center in Houston, TX for mineral analysis of several macro- and micro-mineral nutrients via  
 102 inductively coupled plasma optical emission spectrometry. For the 2016 and 2017 seasons fifteen mid position  
 103 leaves on new extension growth randomly distributed throughout the tree canopy were harvested 90 days after  
 104 bloom on all tree replicates of each rootstock. At harvest, a 20-fruit sample for each rep was collected. From that  
 105 sample, a 10-fruit sample for each rep was processed for nutrient analysis. Each fruit was cut longitudinally twice  
 106 to produce two opposite, seedless, stemless, wedge-shaped segments which constituted the fruit sample and  
 107 amounted to approximately 1/16th of the whole fruit mass. Each wedge-shaped segments were separated in flesh  
 108 and peel samples, cutting the inner part below the skin. The segments from each sample were combined and  
 109 immediately weighed prior to drying. Leaves and fruit were oven dried, ground into powder and shipped to the  
 110 A&L Great Lakes laboratories in Fort Wayne, IN for mineral analysis of several macro- and micro-mineral  
 111 nutrients via inductively coupled plasma optical emission spectrometry. For ease of distinguishing the nutrient  
 112 data, labels include element, year collected, abbreviated tissue (Fr= Fruit, Fl =Flesh, Pe = Peel, Lf = Leaf), and  
 113 concentration in  $\mu\text{g/g DW}$  (Dry Weight),  $\text{mg/g DW}$ , percent (%) or parts per million (PPM).

## 114 2.3 Data analysis

115 Statistical analyses of the data were performed with a one-way ANOVA with rootstock genotype as the main  
 116 effect and replicate as a random effect in a complete block analysis. Mean separation was determined using least  
 117 significant difference test (LSD) with a  $P$  value of 0.05. Pearson correlation was carried out to study correlations  
 118 among all the traits evaluated. Rootstock genotype means were used in multivariate analysis to generate two-way  
 119 similarity cluster diagrams based on genotype similarity and variable similarity. The Ward's minimum variance  
 120 criterion was used. Data were analyzed using the JMP statistical software package (Version 12; SAS Institute  
 121 Inc., Cary, North Carolina) for the calculation of genotypic means, multivariate cluster analysis and correlation.

## 122 3. Results

### 123 3.1 Rootstock modulated nutrient analysis by element

#### 124 3.1.1 Boron

125 Boron concentration in leaves and fruit appeared highly rootstock dependent as genotypic mean pairwise  
 126 correlation between years was as high as 0.78 in leaf samples (2014 and 2015) and 0.7 in fruit samples (2014 and  
 127 2017) (Figure 1). Correlation was also very high between the same tissues collected in the same season, as fruit  
 128 flesh and fruit peel values were 0.93 and 0.94 for the 2016 and 2017 season respectively. Correlation between  
 129 leaf and fruit tissues was as high as 0.87 in the 2017 season. Two-way clustering of the same data revealed four

main groups of rootstocks enabling low, medium-low, medium and high levels of boron in shoot tissue. In the low boron transmitter group we found B.9, two M.9 clones, a very dwarf B series rootstock (B.71-7-22) and PiAu51-11. In the high group we found the two versions of G.935, CG.2034, CG.4004, G.222 and CG.5087. G.214, G.41, G.11 and B.10 appeared in the medium cluster.

### 3.1.2 Calcium

Calcium concentration in leaves and fruit were mildly correlated and varied season to season, as samples collected during the same season were better correlated than between seasons with some exceptions where leaf calcium pairwise correlation was 0.73 between seasons 2015 and 2017 (Figure 2). While the within season correlation between fruit and leaf tissues was on average close to 0.5 for seasons 2014, 2015 and 2016, year 2017 showed a deviation from this trend where correlation between leaf and fruit tissues averaged 0.06, possibly due to the influence of some outlier genotypic means for G.11, CG.2034, and B.71-7-22 in flesh and peel samples. Two way clustering analysis revealed that rootstock influence on calcium concentration was mildly dependent on genotype: two main groups (medium-high and medium-low) were discerned. The medium-high featured G.214, B.10, G.41, B.9, G.935, G.11, CG.4003 and others. The medium-low group featured M.26, M.9-T337, G.202, CG.4004, G.814 and others.

### 3.1.3 Copper

Copper seemed substantially regulated by rootstock genotype as season to season correlation between genotypic means was generally higher than 0.45 (Figure 3). Correlation was similar between leaves and fruit sample means. Two way clustering revealed three groups (low, medium and high) of rootstocks with similar transfer capabilities of copper to leaves and fruit. Among the high copper rootstocks were B.10, M.9T337, G.814, G.222, Supp.3 and CG.234. Rootstocks CG.4003, CG.4004, M.26 and G.214 conferred some of the lowest concentrations of copper in ‘Honeycrisp’ fruit and leaves. M.9-Pajam2, G.41, G.935, G.11 and G.202 were among the rootstocks that conferred medium values for copper.

### 3.1.4 Iron

Rootstock influenced iron levels in leaves and fruit tissues of ‘Honeycrisp’ were not consistently correlated across years (Figure 4). Strong correlations between same season fruit and leaf tissues in the 2014 season ( $r=0.69$ ) and between leaf concentration values measured in 2014 and 2017 ( $r=0.68$ ) were detected. The two way clustering analysis identified two main equal size clusters that contained rootstocks that conferred medium-high and medium-low values. Rootstocks CG.4013, G.214, B.9, G.202, and M.9T337 appeared in the medium-low cluster and G.935, B.10, CG.5087, M.26 and CG.4004 were in the medium-high cluster.

### 3.1.5 Potassium

Rootstock influenced leaf potassium concentration was tightly correlated between years (Figure 5). However, fruit concentrations displayed different behavior where fruit samples clustered together and were more dissimilar than leaf measurements from the same season. Notably fruit potassium concentration of samples in the 2014 season were very similar to peel ( $r=0.75$ ), and flesh ( $r=0.59$ ) concentrations in 2016 and flesh ( $r=0.66$ ) concentrations in 2017. Two way clustering revealed two major groups of high and low potassium rootstocks. In the low group were, CG.4003, G.935, G.222, and G.202 which had consistently lower values, whereas B.70-20-21, B.67-5-32, B.70-20-20 had consistently higher values. Rootstock B.10 and CG.2034 induced consistently higher values in fruit tissues.

### 3.1.6 Magnesium

Leaf magnesium values across years were highly positively correlated, while leaf magnesium values were somewhat negatively correlated with fruit tissue values (Figure 6). Fruit tissue magnesium values across years

as a group displayed correlation coefficients as high as 0.64 (between seasons 2014 and 2017). Leaf values between the 2015 and 2017 seasons had a correlation coefficient of 0.71. Two way clustering analysis revealed two major clusters (high and medium-low magnesium rootstocks) where the medium-low cluster featured a subgroup with very low values. The high Mg cluster contained the rootstocks G.11, CG.3001, B.10 and M.9-Pajam2, whereas the low Mg cluster contained rootstocks that had consistently relatively low values in both fruit and leaves (B.9, G.202, G.214, M.9-T337 and CG.4003). Rootstocks G.41, CG.2034, G.814 displayed relative higher values in fruit than leaves.

### 3.1.7 Manganese

Rootstock influenced levels of manganese seemed to be variably correlated across years and tissue types (Figure 7). High correlation was detected between leaf levels in seasons 2014 and 2015 ( $r=0.61$ ) and between fruit tissues collected in 2015 and leaf tissues in 2017. Negative correlation coefficients were found between fruit flesh measurements in 2017 and leaf 2015 ( $r=-0.42$ ) and peel measurements for the same year; 2017 fruit flesh data seemed to perform differently than the rest of the tissue/years. Two way clustering analysis revealed two major manganese groups, one that displayed more consistently high values of manganese in most tissues and years and the other that contained rootstocks that seemed to have variable values in the medium to low range. The first group displaying high values contained B.9, B.10, B.71-7-22. The group with consistently low values contained G.935, G.202, and G.214. All other rootstocks seemed to confer variable manganese levels across years and tissues.

### 3.1.8 Sodium

Sodium was measured only in leaves and fruit collected in 2014 and 2015 (Figure 8). Sodium levels in 'Honeycrisp' leaves were significantly correlated between 2014 and 2015 ( $r=0.52$ ) and the levels in leaves for both years were positively correlated with fruit collected in 2015. Fruit sodium levels examined in 2014 were negatively correlated with the rest of the data. Two-way clustering analysis revealed three sodium clusters which were influenced by the inconsistent 2014 fruit data. The first cluster containing G.935 and CG.5087 possessed somewhat higher levels of sodium. The second cluster containing M.9 clones, G.202 and CG.4004 displayed medium levels of sodium. The third cluster with B.9, G.222, G.214 and G.11 displayed very variable levels ranging from medium-low to high.

### 3.1.9 Phosphorus

Of all the mineral nutrients measured, rootstock influenced phosphorus levels were highly consistent across years and tissues (Figure 9). The highest correlation value ( $r=0.93$ ) was detected between leaf measurements made in 2015 and 2017. Other correlation coefficients between leaf and fruit tissues across years were for the most part higher than 0.6. Two-way cluster analysis revealed three phosphorus groups. One group with consistently high phosphorus values contained mostly B series of rootstocks (B.70-20-20, B.7-20-21, B.70-6-8, B.7-3-150, B.67-5-32 and B.64-194) and one CG series (CG.4013). The second cluster was consistently low in phosphorus levels and contained both M.9 clones, G.214, B.9, G.222, CG.4003 and M.26EMLA. The third cluster contained rootstocks that conferred variable medium levels of phosphorus and featured rootstocks G.41, G.11, B.10, and G.935.

### 3.1.10 Sulfur

Levels of sulfur influenced by rootstock on 'Honeycrisp' fruit were more consistently similar in fruit tissues than leaves (Figure 10). Correlation coefficients between fruit tissues across years were in the range of  $r=0.4$  to  $r=0.6$  while low negative correlation coefficients were detected between fruit and leaves across years ( $r=-0.25$  to  $r=-0.31$ ). Two-way clustering displayed two groups (medium-high and medium-low) and an outlier (CG.2034). The

214 medium-high group included G.814, B.10, G.41 and G.11. The medium-low group featured M.26, M.9Pajam2,  
215 G.202 and G.214.

### 216 *3.1.11 Zinc*

217 Rootstock influenced zinc levels seemed to be more closely associated within seasons than between seasons,  
218 where correlation coefficients of leaf and fruit tissues were more similar for the same year than across years  
219 (Figure 11). Data from the 2017 season was negatively correlated with data from the 2014-2016 seasons. Because  
220 of the seasonal variation, the two-way cluster analysis discerned three clusters of unequal size. One cluster,  
221 featuring mostly high levels of zinc, contained rootstocks B.71-7-22, B.9 and G.814. Another cluster, featuring  
222 medium-low values for seasons 2014-2016 and higher values for 2017, contained G.11, G.41N and CG.4013. A  
223 third cluster featuring higher levels in 2014-2016 and lower levels in 2017 included rootstocks B.10, G.222,  
224 G.214, Supp.3 and CG.4004.

### 225 *3.1.12 Nitrogen*

226 Nitrogen levels were measured only in data collected in 2016 and 2017 on selected rootstocks (Figure 12). Leaf  
227 nitrogen concentration was measured only in 2017 and was not correlated to fruit tissue measurements of 2016  
228 and 2017. Fruit peel 2017 levels of nitrogen were highly correlated ( $r=0.80$ ) to fruit flesh 2017 levels and peel  
229 levels in 2016 ( $r=0.63$ ). Two way clustering revealed an outlier rootstock (CG.2034) with very high concentration  
230 of nitrogen in fruit tissues and low in leaves. The other two groups included medium-low rootstocks (G.214,  
231 CG.5087, CG.4004, and G.222) and a medium-high group (G.11, G.41, B.10 and M.9-Pajam2).

### 232 *3.2 Nutrient relationships in leaf tissues.*

233 The relationships between rootstock modulated nutrient concentrations in leaf tissues are summarized in Figure  
234 13. Warm colors (reds) represent positive correlation coefficients, cold colors (blues) represent negative  
235 correlation coefficients and light colors (white or gray) represent coefficients closer to zero (no relationship). Leaf  
236 boron displayed consistent negative relationships with leaf calcium, magnesium, manganese and zinc and  
237 consistent positive relationships with potassium and somewhat phosphorus. Leaf calcium displayed consistent  
238 negative relationships with leaf potassium and boron. Leaf copper displayed mostly minor and variable  
239 correlation coefficients with other nutrients. Leaf iron values were consistently negatively correlated with leaf  
240 magnesium and loosely positively correlated with boron and phosphorus. Leaf potassium was consistently  
241 positively correlated with leaf boron and consistently negatively correlated with leaf calcium and leaf magnesium.  
242 All other potassium relationships were variable across years. Leaf magnesium was consistently negatively  
243 correlated with leaf boron, iron, and potassium. Leaf manganese was consistently negatively correlated with leaf  
244 boron. All other relationships were variable across years. Leaf phosphorus was inconsistently associated with  
245 boron and potassium where strong associations were detected only between the 2014 and 2015 seasons. All other  
246 relationships were variable across years. Leaf sulfur exhibited variable and opposite relationships with other leaf  
247 nutrients. Leaf zinc featured consistent negative relationships with leaf boron and potassium. All other  
248 relationships were variable across years.

### 249 *3.3 Nutrient relationships in fruit tissues*

250 The relationships between rootstock modulated nutrient concentrations in fruit tissues are summarized in Figure  
251 14. Rootstock influenced fruit tissue levels of boron were consistently negatively associated with copper and  
252 zinc. Fruit boron was also mostly negatively associated with fruit iron, potassium, manganese, and phosphorus.  
253 Fruit boron was mostly positively associated with fruit sulfur. The relationship between fruit boron and calcium  
254 was very variable with mixed polarities. Rootstock induced levels of calcium were mostly negatively associated  
255 with potassium, manganese, and phosphorus. Fruit calcium was mostly positively associated with fruit sulfur.  
256 All other calcium relationships were somewhat minor and displayed different polarities across years. Fruit copper

was positively associated with fruit potassium, magnesium, phosphorus and sulfur. Fruit copper was negatively associated with fruit boron. Fruit copper relationships with fruit iron, manganese and zinc were variable across years and displayed different polarities. Fruit iron exhibited minor relationships with other nutrients and displayed different polarities across years. Fruit potassium showed consistently positive associations with fruit copper, magnesium, phosphorus and sulfur. All other relationships with fruit potassium were minor, variable and displayed opposite polarities across years. Fruit magnesium featured consistent positive associations with fruit potassium, phosphorus and sulfur. Fruit magnesium showed mostly negative relationships with calcium and zinc and variable associations with iron and manganese. Fruit manganese displayed mostly negative relationships with boron and calcium. All other fruit manganese relationships were variable across years and displayed different polarities. Fruit phosphorus showed consistently positive associations with copper, potassium, and magnesium. Fruit phosphorus had mostly negative associations with boron and calcium. All other fruit calcium relationships were variable across years and displayed different polarities. Fruit sulfur displayed consistently positive associations with copper and potassium. It also displayed mostly positive associations with fruit calcium, boron, magnesium and manganese. All other fruit sulfur relationships were variable across years and displayed different polarities. Fruit zinc displayed mostly negative associations with fruit boron. All other fruit zinc relationships were minor, variable across years and displayed different polarities.

### 3.3 Nutrient relationships between fruit and leaf tissues

We present only a few of 8,000+ pairwise correlation coefficients between all fruit and leaf nutrient data collected. More pairwise comparisons can be found in the clustered coefficients graphic in supplementary Figure 15. A tightly correlated group of variables containing leaf boron, potassium, phosphorus showed consistent negative associations with flesh, peel and whole fruit calcium, iron, copper, magnesium and zinc. Another tightly correlated group of variables containing flesh, peel and whole fruit boron was negatively correlated with whole fruit, peel iron, manganese, calcium and zinc.

### 3.4 Nutrient concentration and horticultural traits

Our companion paper (Lordan et al., 2018) reported the horticultural performance of the trees we used in the present study to evaluate mineral nutrient relationships. The data from the companion paper and this paper revealed some important relationships between mineral nutrient concentrations and horticultural traits. First is the relationship of nutrient concentrations and leaf zonal chlorosis. Leaf zonal chlorosis in 2015 was slightly negatively associated with leaf concentration of boron and sulfur, and positively associated with the concentration of sodium (Figure 16) and that although there was some seasonal variation some of the same apple rootstocks (CG.2034, G.814) displayed higher values consistently. Interestingly levels of sodium and sulfur were also influenced by seasonal climate.

Rootstock induced concentration of fruit and leaf phosphorus, leaf potassium and leaf boron were all significantly positively correlated with higher incidence of bitter-pit and fruit size (Figure 17). Concentration of fruit zinc in the previous year (2014) seemed to be negatively correlated with incidence of bitter-pit (2015). When comparing bitter pit and nutrient levels the best predictor of the level of rootstock induced bitter pit in fruit was the ratio of fruit potassium to calcium (K/Ca) as evidenced in Figure 18 where the confidence intervals (95%) boundaries were much closer in K/Ca regression lines than K or Ca individually.

Rootstock induced soluble solids (brix) values were positively correlated with leaf phosphorus, potassium and tree size and negatively correlated with leaf-fruit calcium levels, crop load and yield efficiency (Figure 19).

## 4. Discussion

The connection between yield components and nutrient concentration as modulated by apple rootstocks is becoming clearer as more data is collected from replicated field trials. In this case, the trial used in this study was



part of a larger set of trials organized by the NC-140 group and for the most part the tree growth and yield data after 8 years at the Geneva, NY location (Lordan, *et al.*, 2018) coincided with the assessments published after 5 years of multi-site data collection (Autio *et al.*, 2017). In addition to yield components, tree size potential, fruit size and physiological disorders measured at all locations of this multi-location trial, the site in British Columbia (BC) collected three years of mineral nutrient concentration data that coincided with the orchard establishment and first year of fruit production (Neilsen and Hampson, 2014) where some correlations and tendencies were reported between nutrients and rootstock performance attributes. That data with our data will allow us to begin to unravel the interaction of soil x rootstock x scion x climate with regards to nutrient uptake and orchard performance.

Our mineral nutrient data collected from the 4<sup>th</sup> through 7<sup>th</sup> leaf allowed us to assess the steady state rootstock performance at the site rather than experiencing changes from the nutrient status observed during establishment experienced at the BC site, where leaf nutrient concentration values were generally higher in the first year and decreased overall in subsequent years. An example is the steady state of boron concentration experienced in NY where the data was consistent in different seasons, identifying the M.9 clones, B.9, M.26 EMLA, PiAu51-11 and B.71-7-22 as low boron rootstocks, while the BC data showed the same rootstocks having lower values only in the third season. This indicates that there is a transition phase from the vegetative growth phase started in the nursery and continued for the first few years in the orchard where nutrients are maximized for maximum growth to a more stable phase fruiting phase when some nutrients are lower. Remarkably, high boron rootstocks were similarly consistent in all years and included G.222, G.935TC, G.935N, CG.4004, G.41N, G.41TC and CG.4003. This would suggest that rootstock genotypic control of boron concentration seems to be consistent at least with the scion ‘Honeycrisp’ and that fertilizer recommendations made using antiquated low boron rootstocks like M.26 and M.9 (Smith *et al.*, 1987; Wojcik, 2000; Wojcik *et al.*, 1999; Wojcik *et al.*, 1997; Wojcik *et al.*, 2003) might be causing tree and fruit physiological disorders (Wojcik *et al.*, 2000) where newer rootstocks might be already supplying the needed amounts (Fazio *et al.*, 2015a; Reig *et al.*, 2018). Boron concentration was correlated with phosphorus and potassium and negatively correlated with calcium, furthering the possibility that excess boron might exacerbate calcium deficiency related fruit disorders like bitter pit.

Unlike boron, calcium concentration was somewhat variable in different seasons suggesting the role of climate and perhaps management factors in the rootstock induced absorption and translocation of calcium. Calcium concentration in apple tree tissues is tied to very important processes that affect apple tree and fruit physiology and disease resistance (Fallahi *et al.*, 1997b; Freitas *et al.*, 2012; Raese, 2000; Raese and Drake, 2000; Solhjoo *et al.*, 2017). Similar to our findings, correlation data by Neilsen and Hampson (2014) showed that rootstock induced fruit calcium was negatively correlated with tree size, yield per tree and fruit size suggesting that the availability of calcium ions might be a limiting factor to productivity and that efforts to improve calcium uptake and distribution might result in higher yields and quality improvements. Scion varieties seem to vary significantly in their calcium uptake and transport physiology (Korban and Swiader, 1983, 1984; Volz *et al.*, 2006) and ‘Honeycrisp’ seems to be a good example of calcium absorption and transport inefficiency when compared to other varieties as it is especially susceptible to bitter pit (Biggs and Peck, 2015; Jarolmasjed *et al.*, 2016). The relationship between fruit size, crop load, calcium concentration and incidence of bitter pit has been well characterized (Drazeta *et al.*, 2001; Fallahi *et al.*, 2010; Hopfinger and Poovaiah, 1979; Perring and Jackson, 1975; Perring and Pearson, 1986; Robinson and Lopez, 2012; Rosenberger *et al.*, 2004). Our data emphasized the role of potassium concentration and the ratio of K/Ca in leaves and fruit as one of the best predictors of bitter pit in ‘Honeycrisp’ apples, suggesting that rootstock induced levels for both nutrients play a role in this disorder and that the addition of potassium fertilizers on potassium-efficient rootstocks might increase the susceptibility to bitter pit. Small apples and low potassium levels with B.9 and other similar rootstocks are probably the reason for the low bitter pit witnessed with this rootstock where the distance from petiole to the calyx end of the fruit may be a factor in calcium movement (Kalcsits *et al.*, 2017; Serra *et al.*, 2016).

346 Rootstock induced levels of phosphorus were highly consistent throughout the seasons and were significantly  
347 positively correlated to potassium and boron. In *Prunus* rootstocks, increasing phosphorus applications increased  
348 the concentration of K, Ca, Mg, N and Mn concentrations (Balal *et al.*, 2011) suggesting some similarities in the  
349 transport mechanisms in the phloem for those nutrients, which might occur with apple rootstocks as well. A  
350 major quantitative trait locus (QTL) for potassium was co-located with a QTL for magnesium concentration on  
351 chromosome 5 of apple (Fazio *et al.*, 2013). Interestingly this QTL was in the same region of one of the dwarfing  
352 loci (*Dw1*) where plants that inherited the vigorous allele displayed higher levels of both magnesium and  
353 potassium (Fazio *et al.*, 2017). Two major rootstock clusters were discerned in the potassium concentration data  
354 where the high potassium cluster displayed mostly vigorous rootstocks with the exception of sub-dwarfing  
355 rootstock B.71-7-22 and CG.2034 which had the most consistent higher fruit potassium concentration of all  
356 rootstocks tested but low to medium levels in leaves. The tendency of more vigorous rootstocks to display higher  
357 potassium levels in leaves and fruit is congruent with findings by Neilsen and Hampson (2016) where a larger  
358 portion of potassium might travel through passive components similar to sodium (Kronzucker and Britto, 2011)  
359 where evapotranspiration is the main driver of movement. Rootstock induced leaf sodium levels were similar in  
360 different seasons but not consistent with fruit sodium levels, with G.935 and CG.5087 displaying some of the  
361 highest leaf levels which were not realized in fruit tissues. G.935 was also one of the higher leaf sodium rootstocks  
362 with 'Fuji' scion (Reig *et al.*, 2018). Similarly to Neilsen and Hampson (2017), rootstock B.10 displayed higher  
363 levels of magnesium along with G.11 and M.9-Pajam2, but B.9 and CG.4003 consistently displayed the lowest  
364 levels. Interestingly, seasonal leaf magnesium levels were positively correlated but very different (opposite) to  
365 fruit magnesium concentration. These opposite leaf-fruit nutrient trends were similar to sulfur where all fruit  
366 tissues over seasons displayed similarities. There is paucity of literature relating the role of sulfur in apple tree  
367 growth and physiology, however in peach it was recognized as a limiting factor in sandy soils and certain  
368 rootstocks (Finch *et al.*, 1997; Reighard *et al.*, 2013). A positive correlation between fruit sulfur and fruit calcium  
369 was reported in a similar replicated rootstock field trial grown in a higher soil pH in the Champlain valley in New  
370 York (Fazio *et al.*, 2015a). That relationship was not observed in the current experiment. Perhaps the sulfur  
371 amendments in the Champlain valley experiment were making calcium more available to some rootstocks. More  
372 positive associations were displayed between fruit sulfur, fruit magnesium, and fruit copper levels.

373 Rootstock induced copper levels were positively associated across seasons and tissues where rootstocks CG.4003  
374 and CG.4004 consistently displayed the lowest levels and Supporter 3 displayed consistently higher levels.  
375 Perhaps rootstocks with low copper uptake might be useful in soil conditions where copper toxicity is an issue  
376 (Liu *et al.*, 2011). Similar to observations made by Neilsen and Hampson (2014) and regardless of vigor potential,  
377 all Budagovsky rootstocks with the exception of B.9, B.10 and B.71-7-22 displayed the highest levels of  
378 phosphorus in leaf and fruit tissues over all seasons indicating a selection pressure for more efficient phosphorus  
379 uptake in that germplasm. The most dwarfing rootstock (B.7-20-21) and the most vigorous (B.70-20-20)  
380 consistently displayed the highest concentration of phosphorus and a related rootstock B.9 (dwarfing) was one of  
381 the lowest, suggesting a mechanism for phosphorus uptake efficiency that is uncoupled from dwarfing properties.  
382 This is consistent with the observation that the genetic components for phosphorus were on different  
383 chromosomes than the dwarfing loci *Dw1* and *Dw2* (Fazio *et al.*, 2013; Fazio *et al.*, 2014). Rootstock induced  
384 phosphorus levels were highly correlated across years and tissues and associated with potassium levels. The  
385 significant relationship between leaf P and leaf K was also reported by Neilsen and Hampson (2014), suggesting  
386 a robust physiological tie between the two nutrients that transcends climatic and soil conditions.

387 Rootstock induced levels of zinc were more related within seasons than across tissues, suggesting that the seasons'  
388 climate plays a bigger role in the uptake and transport of this nutrient in 'Honeycrisp'. This is exemplified by the  
389 levels displayed by rootstocks G.11 and G.935 which were in the lowest category in 2014 and 2016 and were  
390 among the highest in 2015 and 2017. The trends observed in fruit zinc were unique and did not correlate

391 significantly with other nutrients, however there was a significant negative correlation between fruit and leaf zinc  
392 concentration in year 2014 and the mean fruit size of next season (2015).

393 Nitrogen data was collected only in the 2016 and 2017 seasons on a subset of rootstocks. Nitrogen levels were  
394 more similar in fruit tissues than leaf. Rootstocks G.41N, B.10 and G.11 displayed the highest levels, whereas  
395 G.214 conferred low values on 'Honeycrisp'. Nitrogen is essential for vegetative growth and is a nutrient that  
396 needs to be present in a balanced way where too little will inhibit growth, fruit set and too much will induce  
397 problems with fruit quality, excessive vegetative growth and diseases (Fallahi *et al.*, 1997a; Fallahi and Mohan,  
398 2000; Raese and Drake, 1997). Knowing the nitrogen uptake potential of rootstocks will allow for more precise  
399 application and utilization when combined with modern recommendations that include methods, timing and  
400 amounts (Nielsen and Nielsen, 2002).

401 Fertilizer recommendations based on leaf nutrient level among rootstocks are not always consistent with fruit  
402 trends. For example, it is clear that while potassium and magnesium were negatively correlated in leaf samples  
403 (Figure 13-14) fruit samples showed a positive trend suggesting a rootstock  $\times$  sink interaction that should be  
404 monitored for certain nutrients. This seems to also be true for the relationship between calcium and potassium  
405 (Figure 15) where there is a clear negative association in leaves, but when fruit calcium is compared to leaf and  
406 fruit potassium the negative relationship is somewhat lost suggesting the influence of other variables, like crop  
407 load and fruit size may play a role.

408 'Honeycrisp' trees are sensitive to a leaf physiological disorder caused by over-loading of carbohydrates in leaves  
409 that causes damage to the photosynthesis systems (Chen *et al.*, 2010; Fleck *et al.*, 2011; Snyder-Leiby and Wang,  
410 2008) and often referred to as leaf zonal chlorosis. Its impact on tree performance is unknown. Rootstocks have  
411 also been shown to have an influence on this physiological disorder, with the degree of influence affected by  
412 season and perhaps management practices (Autio *et al.*, 2017). Our data showed this disorder was slightly  
413 negatively associated with leaf concentration of boron and sulfur, and positively associated with the concentration  
414 of sodium while some rootstocks (CG.2034, G.814) displayed higher values consistently.

415 Bitter-pit incidence has a large impact on marketable yield (Lordan *et al.*, 2018). When comparing bitter pit and  
416 nutrient levels the best predictor of the level of rootstock induced bitter pit in fruit was the ratio of fruit potassium  
417 to calcium (K/Ca) and was better than either K or Ca individually. Often Ca concentration is not related to bitter  
418 pit incidence (Robinson and Lopez, 2012). They have shown that often nitrogen concentration and fruit  
419 phosphorus and fruit boron concentration are better related than calcium concentration.

## 420 **5. Conclusion**

421 Our data indicates that some rootstocks are more efficient at absorbing nutrients than others which implies that  
422 fertilizer recommendations developed using older rootstocks like M.9 and M.26 might be too high for other newer  
423 rootstocks which are more efficient and could thus be causing tree and fruit physiological disorders. Newer  
424 rootstocks might be already supplying the needed amounts. With boron, current rootstocks like M.9 (several  
425 clones), B.9, M.26EMLA, appear to be low absorbing genotypes while some of the newer Geneva stocks like  
426 G.935, CG.2034, CG.4004, G.222 and CG.5087 had consistently high boron. Adding boron fertilizer to these  
427 stocks likely would create fruit quality problems. Likewise a group of rootstocks had high calcium values (G.214,  
428 B.10, G.41, B.9, G.935, G.11, CG.4003) while another group had low calcium values (M.26, M.9-T337, G.202,  
429 CG.4004, G.814). Also some rootstocks are low potassium absorbers like CG.4003, G.935, G.222, and G.202  
430 whereas B.70-20-21, B.67-5-32, B.70-20-20 had consistently higher values. The ratio of K/Ca in leaves and fruit  
431 was the best predictor of bitter pit in 'Honeycrisp' apples, suggesting that rootstock induced levels for both  
432 nutrients might play a role in this disorder and that the addition of potassium fertilizers on potassium-efficient  
433 rootstocks might increase the susceptibility to bitter pit. Rootstocks CG.4003, B.10, G.41N, and G.214 seemed to  
434 produce fruit with lower levels of bitter pit. Given the strong involvement of nutrient levels on tree performance

435 and fruit quality, future research should focus on developing “Designer rootstocks” which are high yielding but  
436 have low levels of bitter pit incidence for ‘Honeycrisp’.

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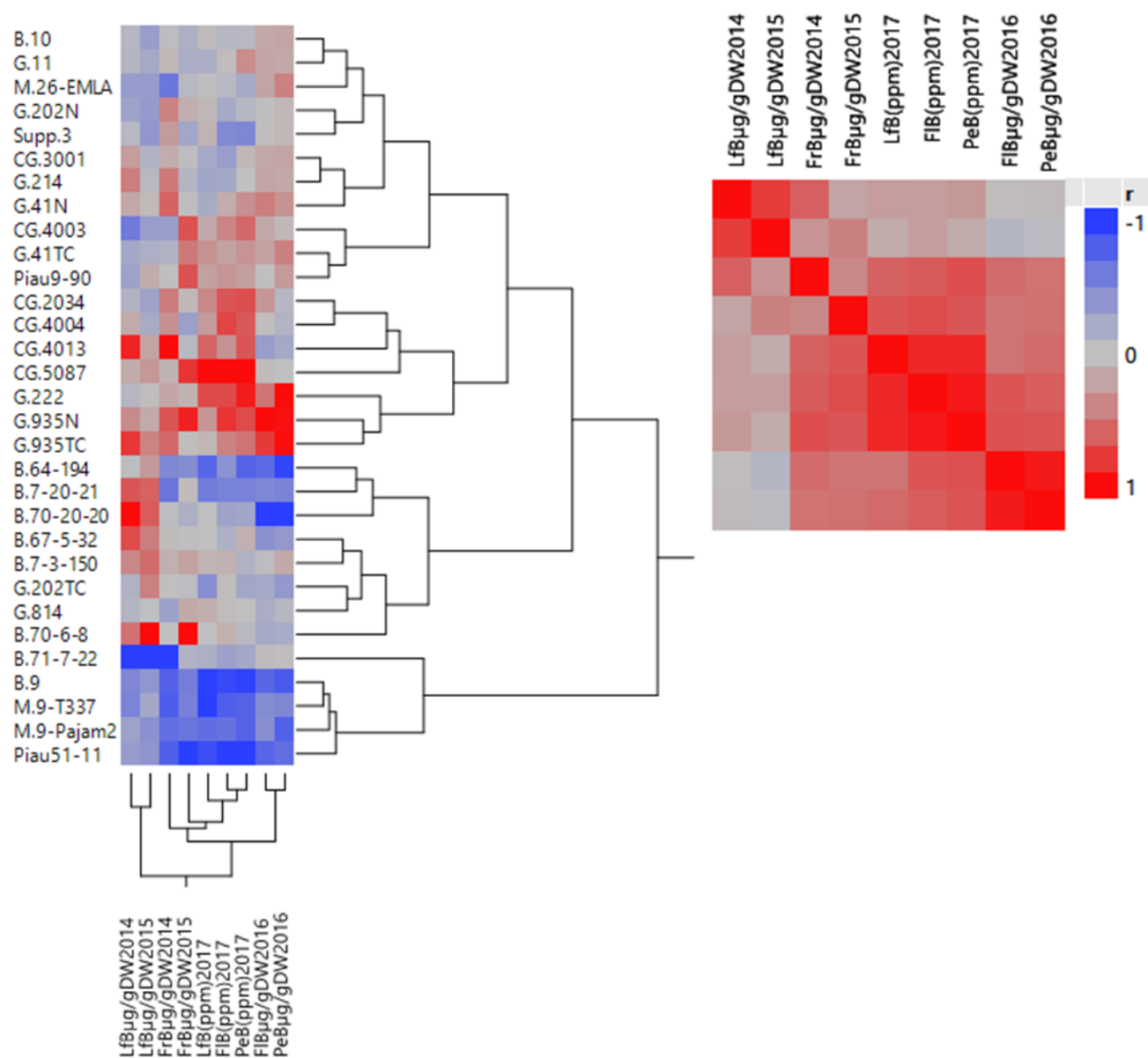
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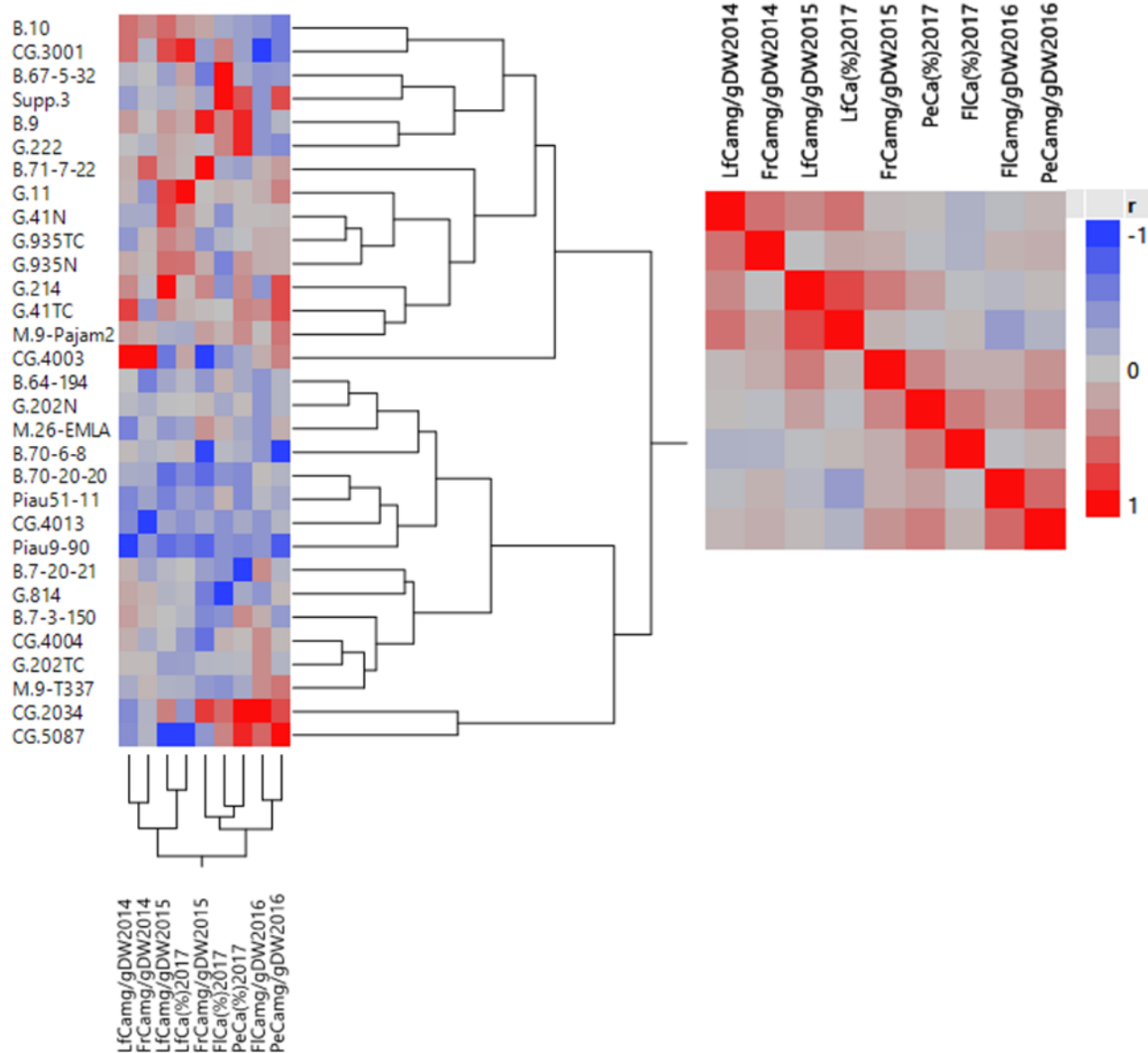
617 Figures

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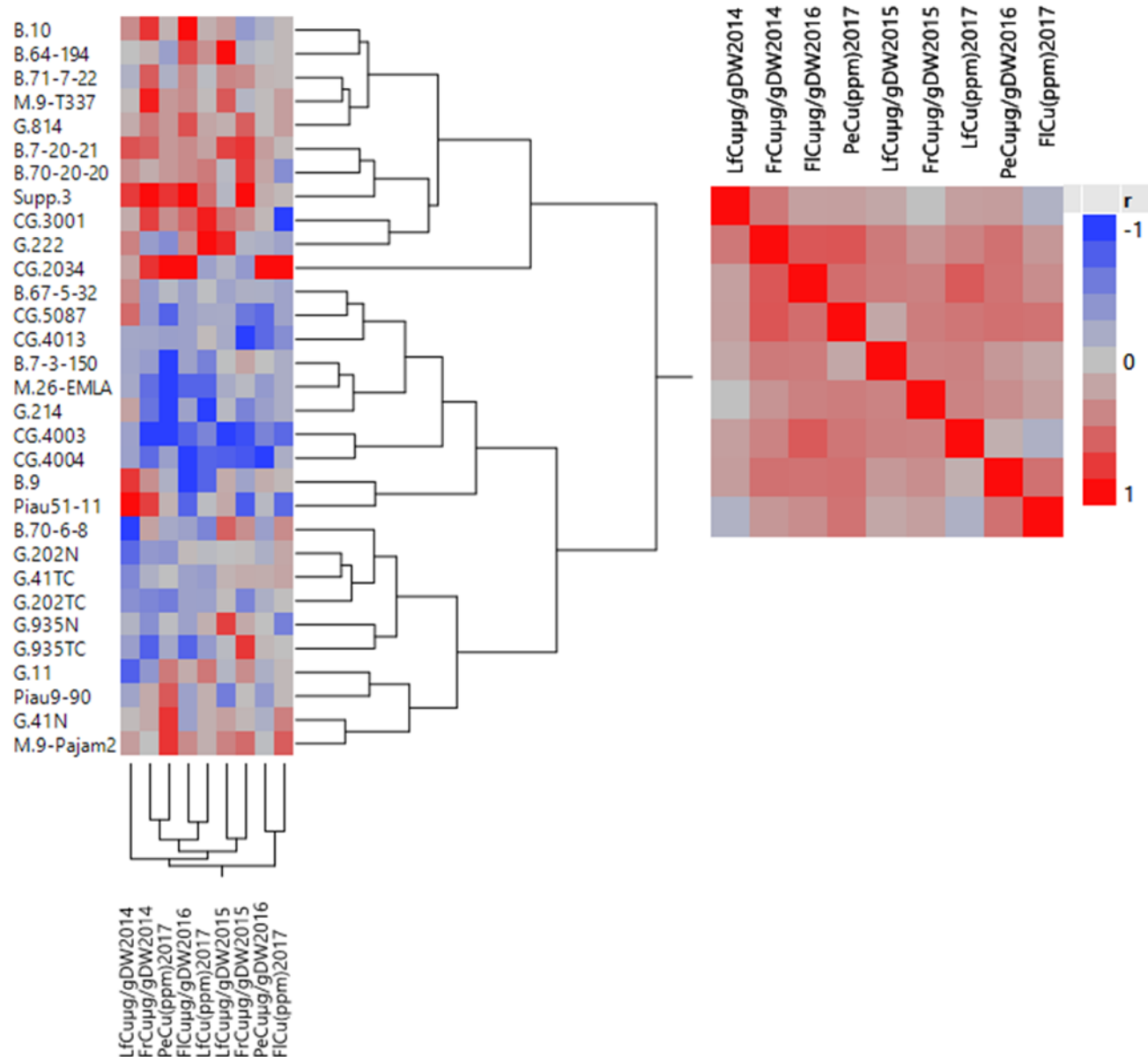
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620 Figure 1. Two-Way similarity cluster analysis (Left) and Clustering Pearson's correlation coefficient (Right) for  
 621 leaf and fruit boron concentration of 'Honeycrisp' grown on 31 rootstocks at Geneva, NY. Rootstocks and year-  
 622 year-tissue are grouped by similarities in mineral boron concentration mean values (gradient from low (blue), medium  
 623 (gray), and high (red)). Pearson's correlation coefficients  $r$  are clustered by similarity where red represents  
 624 positive values, gray neutral values and red negative values. Genotypic means for these data are available in  
 625 Supplementary Table 1.



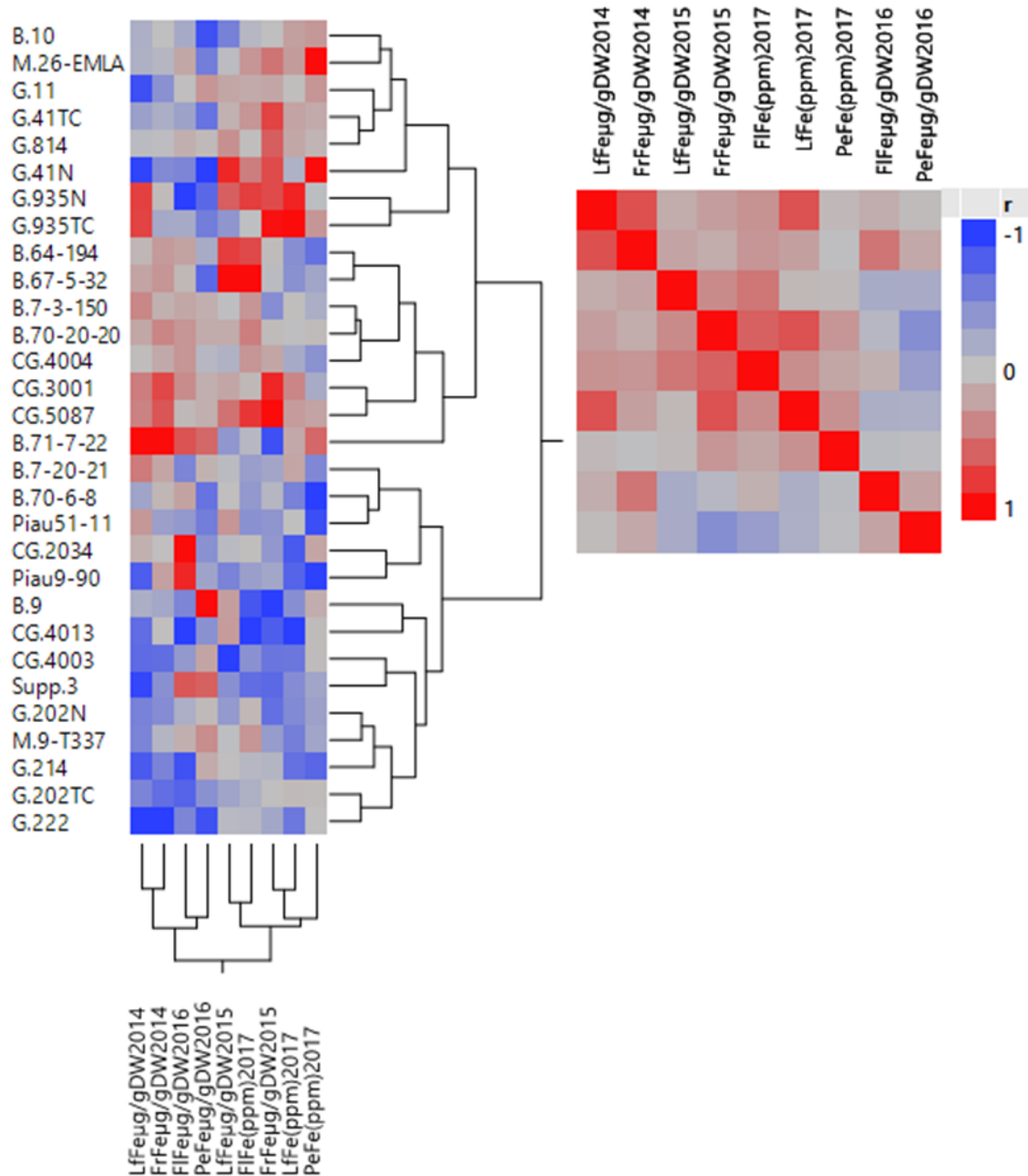
626

627 Figure 2. Two-Way similarity cluster analysis (Left) and Clustering Pearson's correlation coefficient (Right) for  
 628 leaf and fruit calcium concentration data of 'Honeycrisp' grown on 31 rootstocks at Geneva, NY. Rootstocks and  
 629 year-tissue are grouped by similarities in mineral calcium concentration mean values (gradient from low (blue),  
 630 medium (gray), and high (red)). Pearson's correlation coefficients  $r$  are clustered by similarity where red  
 631 represents positive values, gray neutral values and blue negative values. Genotypic means for these data are  
 632 available in Supplementary Table 1.



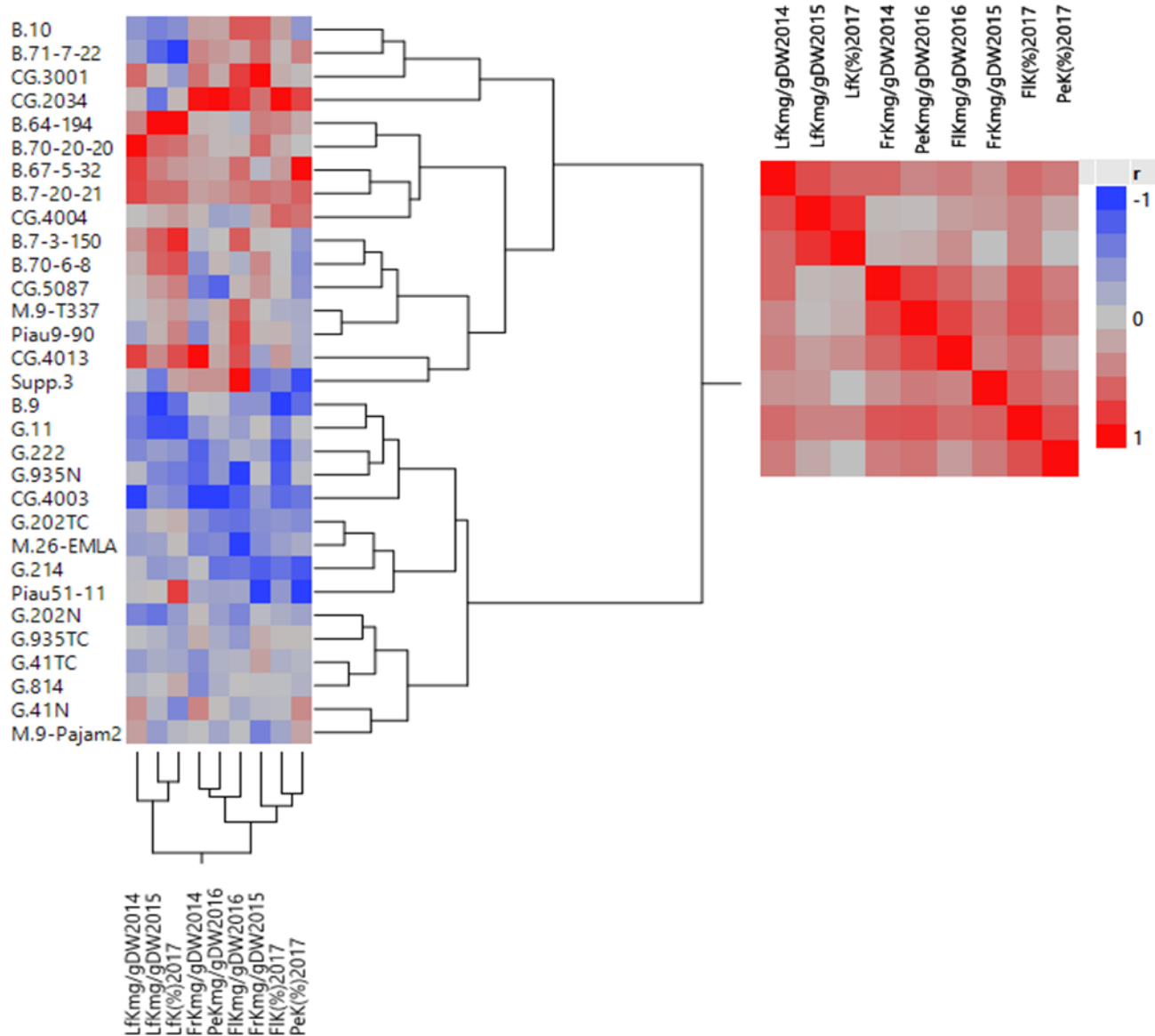
633

634 Figure 3. Two-Way similarity cluster analysis (Left) and Clustering Pearson's correlation coefficient (Right) for  
 635 leaf and fruit copper concentration data of 'Honeycrisp' grown on 31 rootstocks at Geneva, NY. Rootstocks and  
 636 year-tissue are grouped by similarities in mineral Copper concentration mean values (gradient from low (blue),  
 637 medium (gray), and high (red)). Pearson's correlation coefficients  $r$  are clustered by similarity where red  
 638 represents positive values, gray neutral values and red negative values. Genotypic means for these data are  
 639 available in Supplementary Table 1.



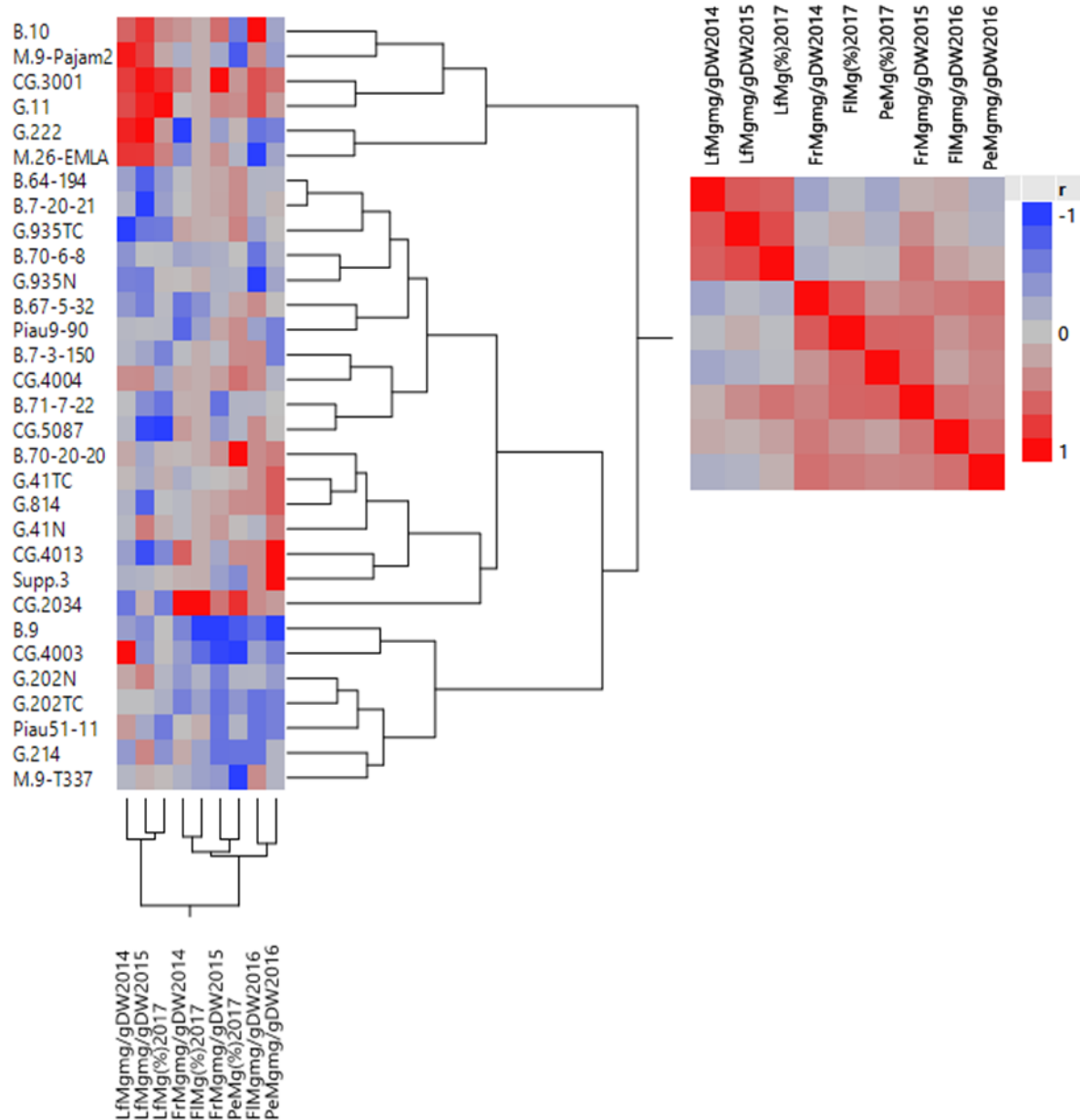
640

641 Figure 4. Two-Way similarity cluster analysis (Left) and Clustering Pearson's correlation coefficient (Right) for  
 642 leaf and fruit iron concentration data of 'Honeycrisp' grown on 31 rootstocks at Geneva, NY. Rootstocks and  
 643 year-tissue are grouped by similarities in mineral iron concentration mean values (gradient from low (blue),  
 644 medium (gray), and high (red)). Pearson's correlation coefficients  $r$  are clustered by similarity where red  
 645 represents positive values, gray neutral values and red negative values. Genotypic means for these data are  
 646 available in Supplementary Table 1.



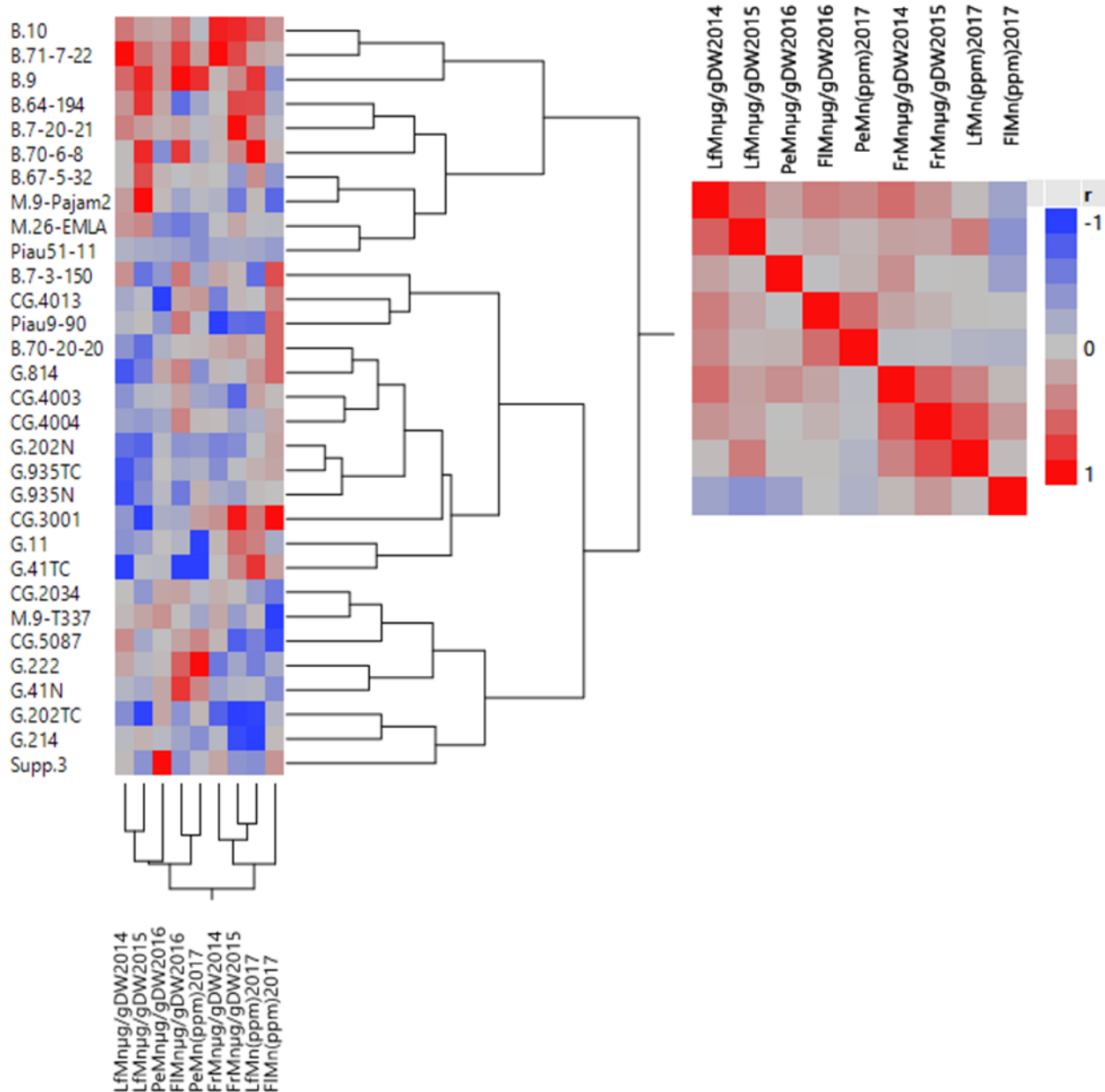
647

648 Figure 5. Two-Way similarity cluster analysis (Left) and Clustering Pearson's correlation coefficient (Right) for  
 649 leaf and fruit potassium concentration data. Rootstocks and year-tissue are grouped by similarities in mineral  
 650 potassium concentration mean values (gradient from low (blue), medium (gray), and high (red)). Pearson's  
 651 correlation coefficients  $r$  are clustered by similarity where red represents positive values, gray neutral values and  
 652 red negative values. Genotypic means for these data are available in Supplementary Table 1.



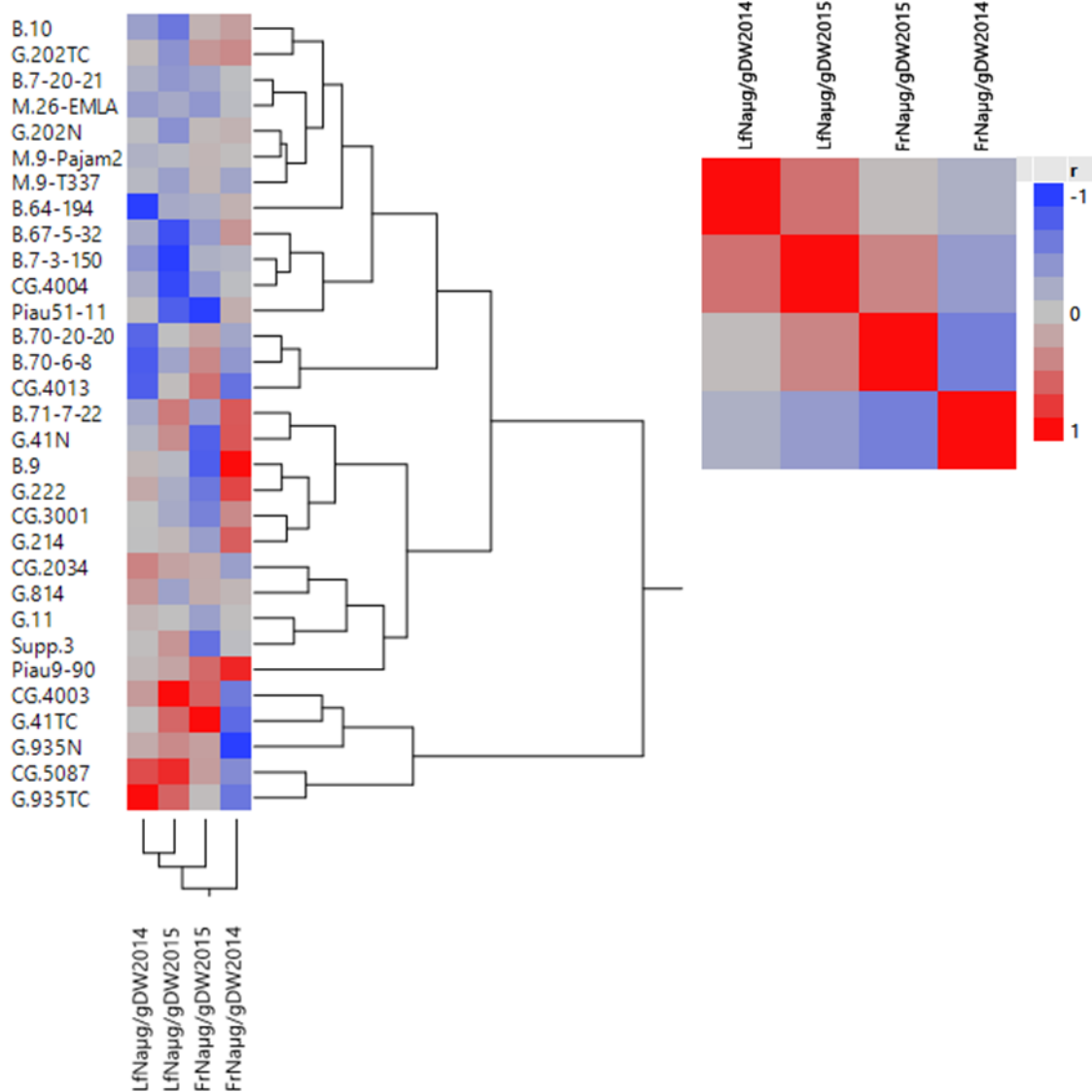
653

654 Figure 6. Two-Way similarity cluster analysis (Left) and Clustering Pearson's correlation coefficient (Right) for  
 655 leaf and fruit magnesium concentration data of 'Honeycrisp' grown on 31 rootstocks at Geneva, NY. Rootstocks  
 656 and year-tissue are grouped by similarities in mineral magnesium concentration mean values (gradient from low  
 657 (blue), medium (gray), and high (red)). Pearson's correlation coefficients  $r$  are clustered by similarity where red  
 658 represents positive values, gray neutral values and red negative values. Genotypic means for these data are  
 659 available in Supplementary Table 1.



660

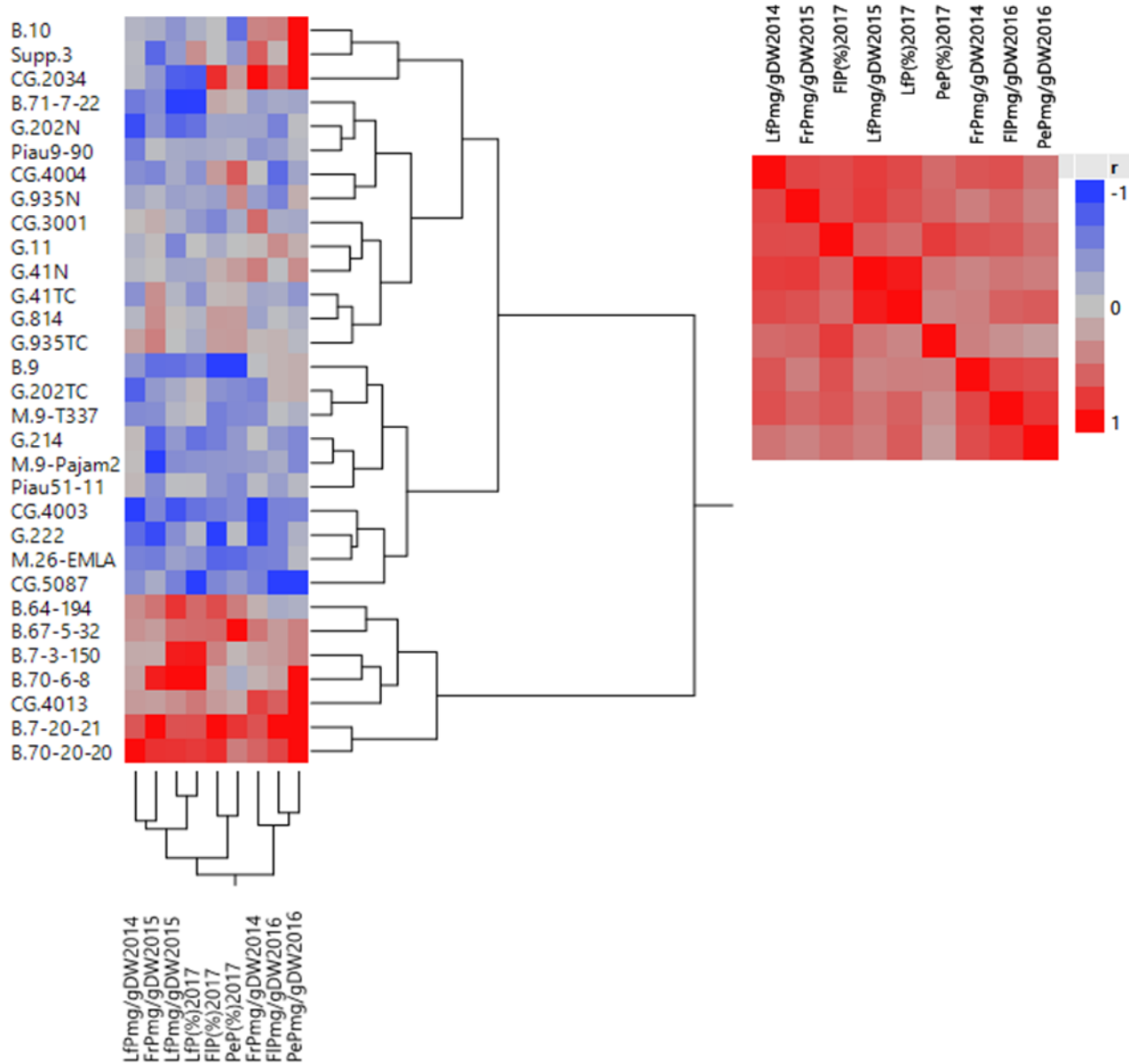
661 Figure 7. Two-Way similarity cluster analysis (Left) and Clustering Pearson's correlation coefficient (Right) for  
 662 leaf and fruit manganese concentration data of 'Honeycrisp' grown on 31 rootstocks at Geneva, NY. Rootstocks  
 663 and year-tissue are grouped by similarities in mineral manganese concentration mean values (gradient from low  
 664 (blue), medium (gray), and high (red)). Pearson's correlation coefficients  $r$  are clustered by similarity where red  
 665 represents positive values, gray neutral values and red negative values. Genotypic means for these data are  
 666 available in Supplementary Table 1.



667

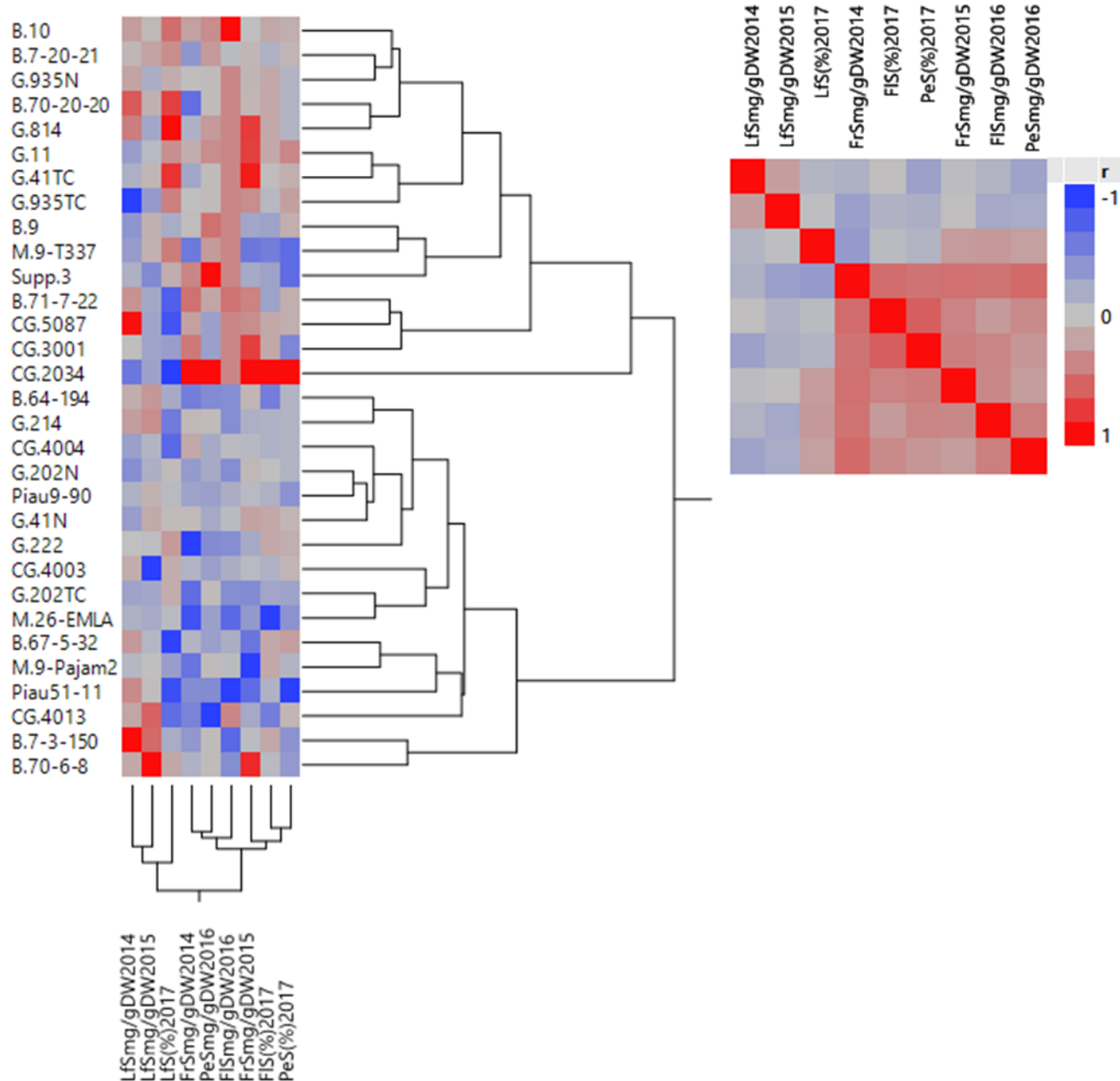
668 Figure 8. Two-Way similarity cluster analysis (Left) and Clustering Pearson's correlation coefficient (Right) for  
 669 leaf and fruit sodium concentration data of 'Honeycrisp' grown on 31 rootstocks at Geneva, NY. Rootstocks and  
 670 year-tissue are grouped by similarities in mineral sodium concentration mean values (Gradient from low (blue),  
 671 medium (gray), and high (red)). Pearson's correlation coefficients  $r$  are clustered by similarity where red  
 672 represents positive values, gray neutral values and red negative values. Genotypic means for these data are  
 673 available in Supplementary Table 1.





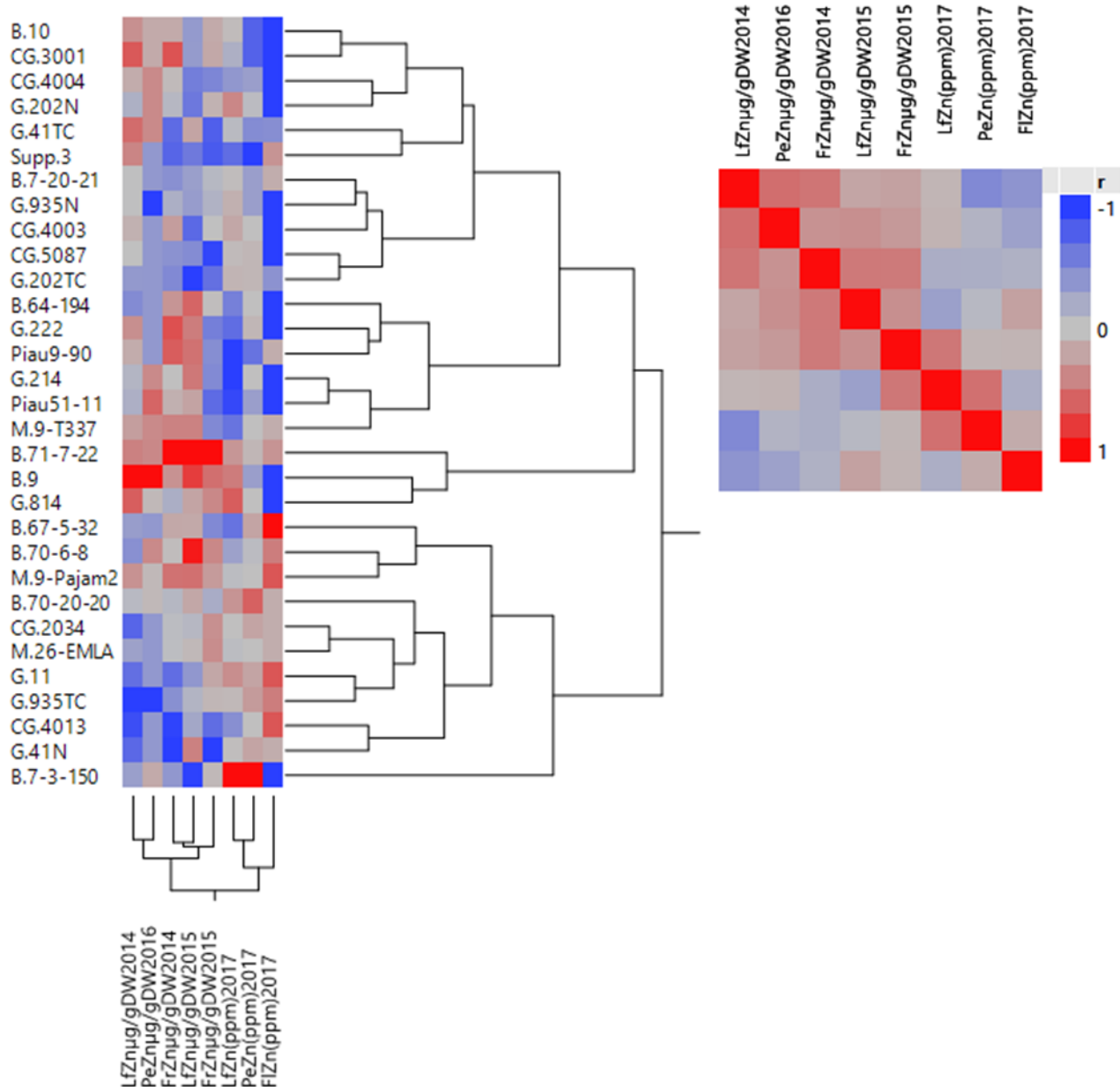
674

675 Figure 9. Two-Way similarity cluster analysis (Left) and Clustering Pearson's correlation coefficient (Right) for  
 676 leaf and fruit phosphorus concentration data of 'Honeycrisp' grown on 31 rootstocks at Geneva, NY. Rootstocks  
 677 and year-tissue are grouped by similarities in mineral phosphorus concentration mean values (Gradient from low  
 678 (blue), medium (gray), and high (red)). Pearson's correlation coefficients  $r$  are clustered by similarity where red  
 679 represents positive values, gray neutral values and red negative values. Genotypic means for these data are  
 680 available in Supplementary Table 1.



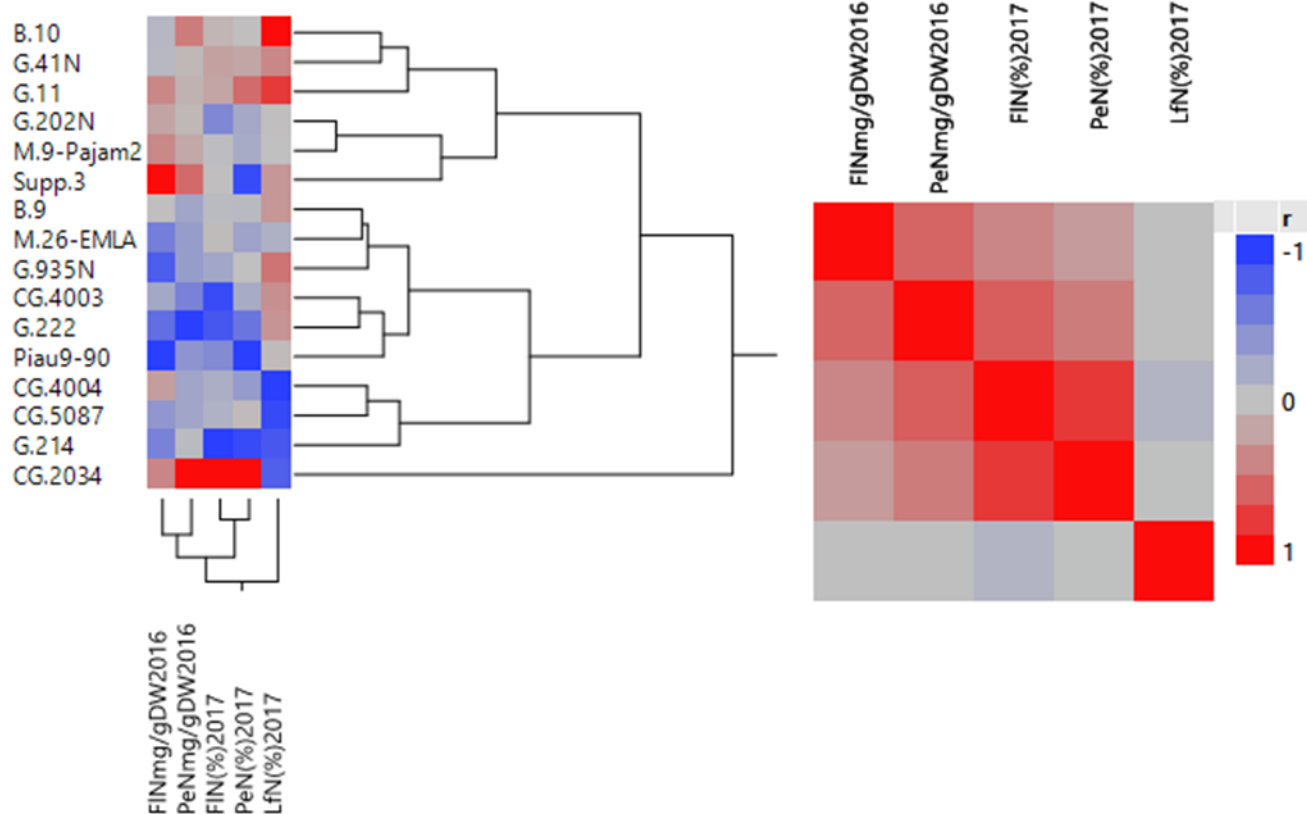
681

682 Figure 10. Two-Way similarity cluster analysis (Left) and Clustering Pearson's correlation coefficient (Right) for  
 683 leaf and fruit sulfur concentration data of 'Honeycrisp' grown on 31 rootstocks at Geneva, NY. Rootstocks and  
 684 year-tissue are grouped by similarities in mineral sulfur concentration mean values (Gradient from low (blue),  
 685 medium (gray), and high (red)). Pearson's correlation coefficients  $r$  are clustered by similarity where red  
 686 represents positive values, gray neutral values and red negative values. Genotypic means for these data are  
 687 available in Supplementary Table 1.



688

689 Figure 11. Two-Way similarity cluster analysis (Left) and Clustering Pearson's correlation coefficient (Right) for  
 690 leaf and fruit zinc concentration data. Rootstocks and year-tissue are grouped by similarities in mineral zinc  
 691 concentration mean values (Gradient from low (blue), medium (gray), and high (red)). Pearson's correlation  
 692 coefficients  $r$  are clustered by similarity where red represents positive values, gray neutral values and red negative  
 693 values. Genotypic means for these data are available in Supplementary Table 1.



694

695 Figure 12 Two-Way similarity cluster analysis (Left) and Clustering Pearson's correlation coefficient (Right) for  
 696 leaf and fruit nitrogen concentration data. Rootstocks and year-tissue are grouped by similarities in mineral  
 697 nitrogen concentration mean values (Gradient from low (blue), medium (gray), and high (red)). Pearson's  
 698 correlation coefficients  $r$  are clustered by similarity where red represents positive values, gray neutral values and  
 699 red negative values. Genotypic means for these data are available in Supplementary Table 1.

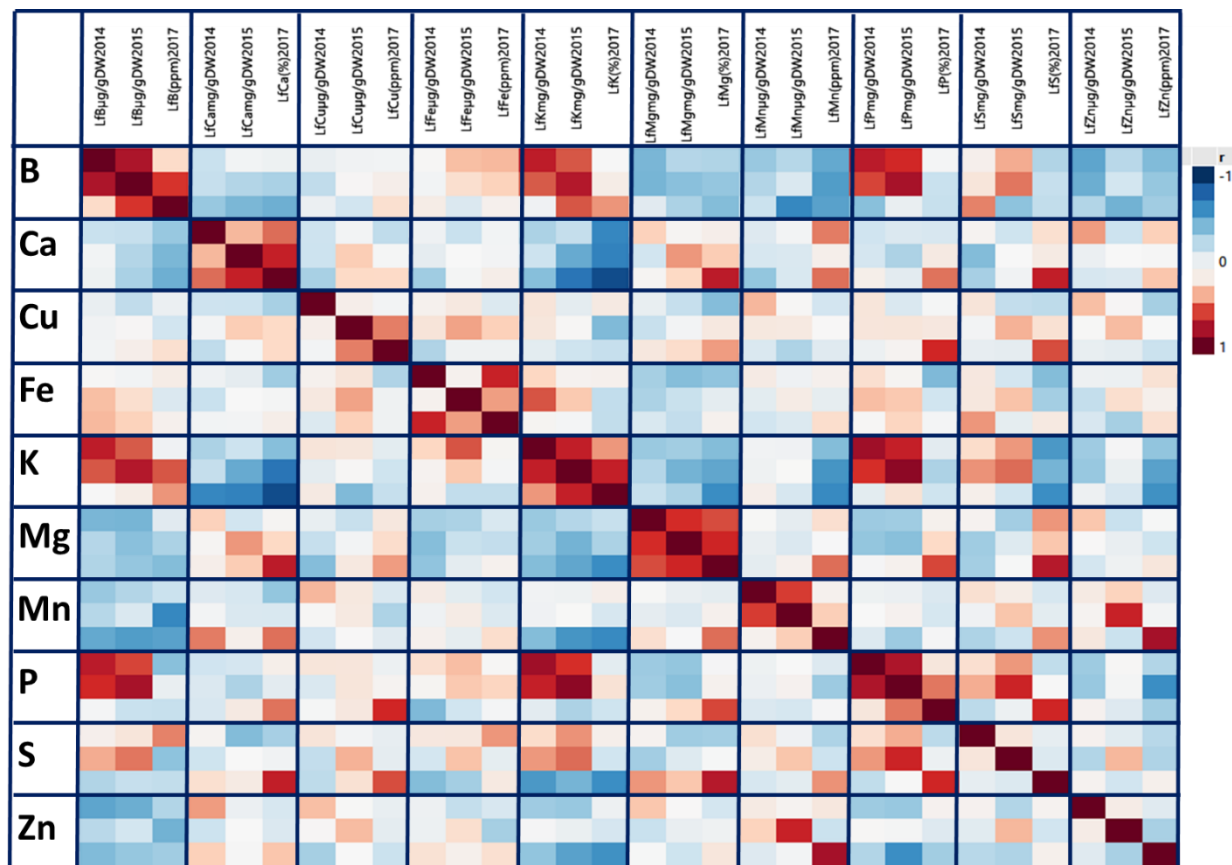


Figure 13. Pairwise correlation coefficients for rootstock induced mineral nutrient concentration in leaf tissues of Honeycrisp collected in August 2014, 2015, 2016, and 2017 of ‘Honeycrisp’ grown on 31 rootstocks at Geneva, NY. The color gradient for the correlation coefficients changes from  $r=-1$  (blue) to  $r=0$  (white) to  $r=1$  (red). Different seasons for the same nutrient are grouped together in the same rectangle. Notable relationships are the consistently negative coefficients between calcium and potassium, potassium and magnesium, and calcium and boron.

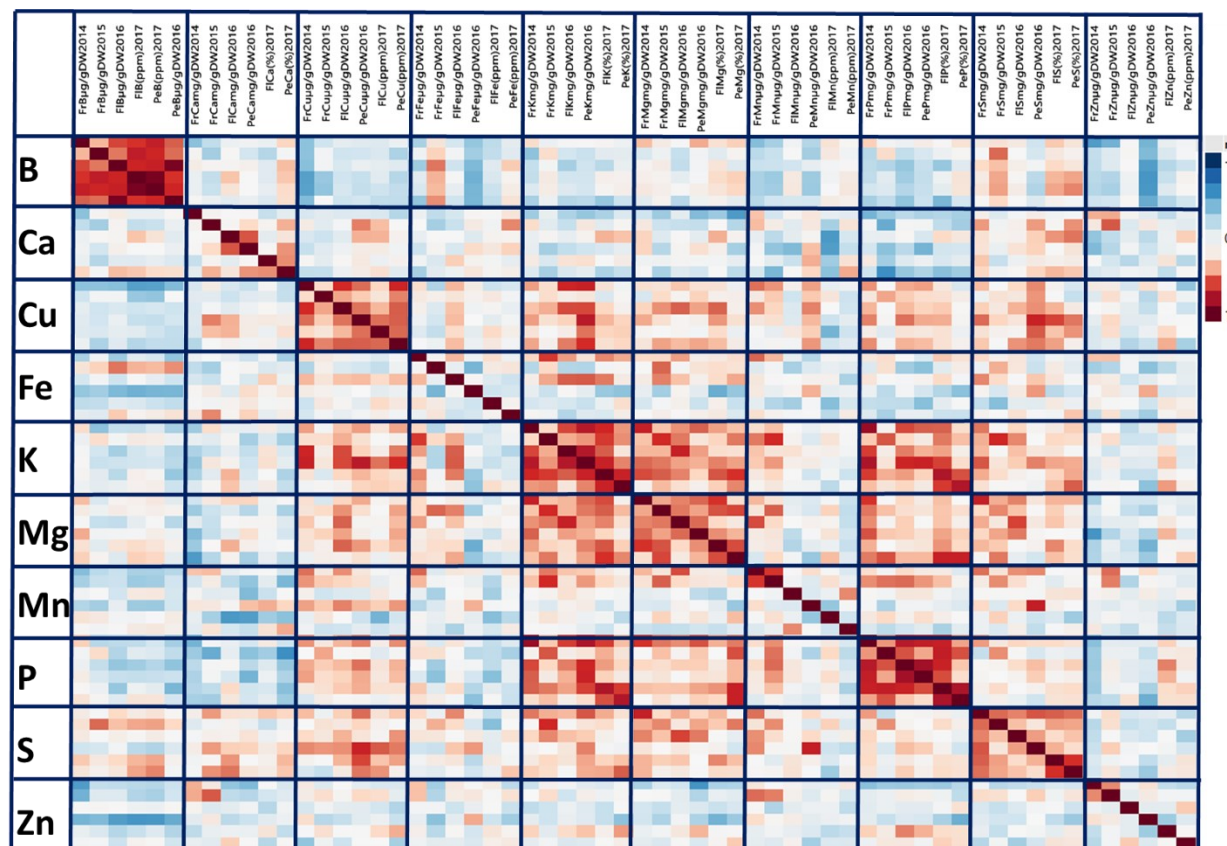
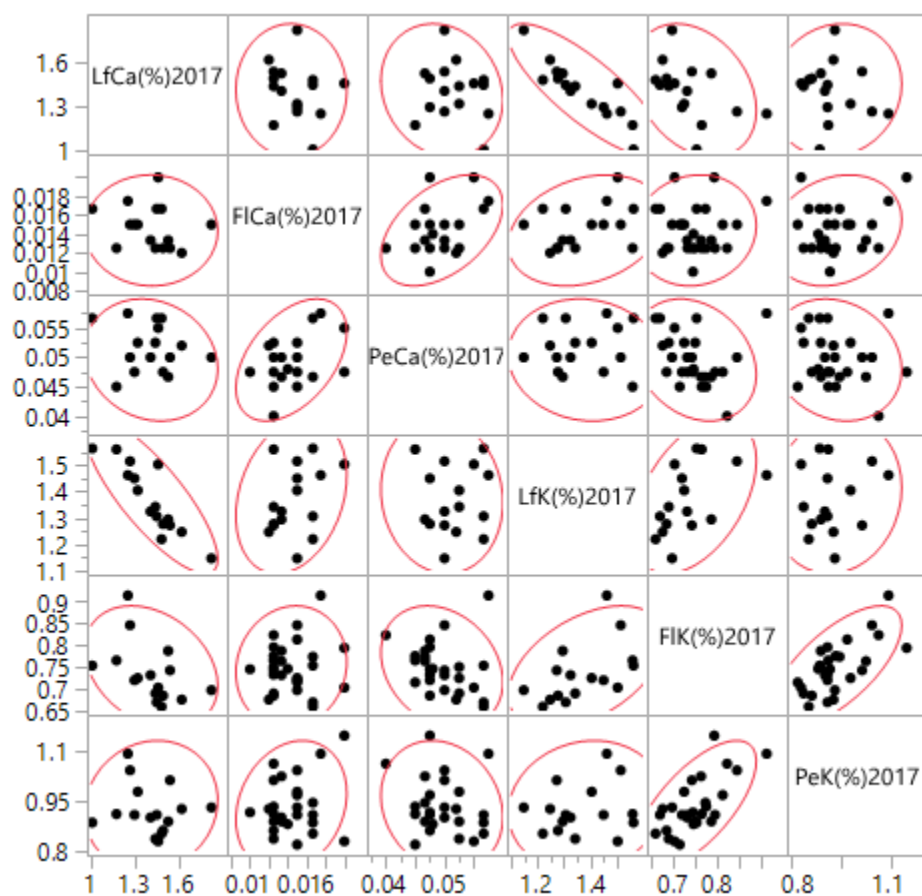


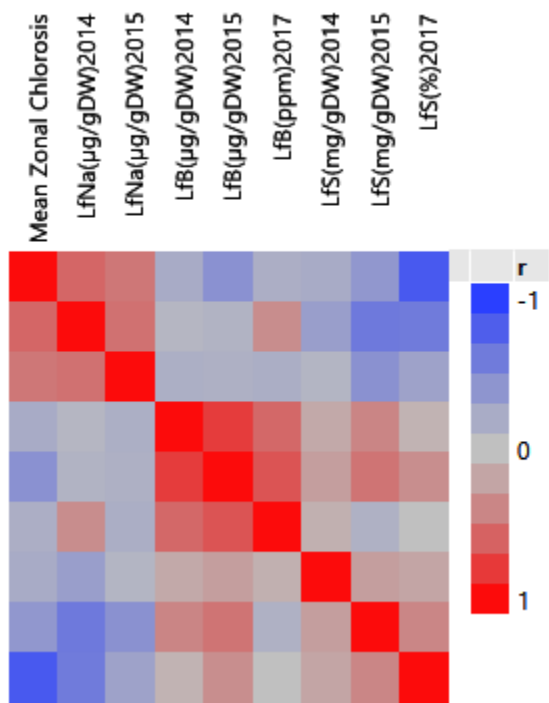
Figure 14. Pairwise correlation coefficients for rootstock induced mineral nutrient concentration in fruit tissues of Honeycrisp collected in August 2014, 2015, 2016, and 2017 of ‘Honeycrisp’ grown on 31 rootstocks at Geneva, NY. The color gradient for the correlation coefficients changes from  $r=-1$  (blue) to  $r=0$  (white) to  $r=1$  (red). Different seasons for the same nutrient are grouped together in the same rectangle. Notable relationships are the generally positive correlation coefficients between fruit sulfur and calcium, copper, potassium and magnesium. The fruit calcium-sulfur relationship may hint at a possible role of sulfur in calcium metabolism that has not been highlighted in the past and may provide some possible avenue of treatment for low calcium fruit disorders.



716

717 Figure 15. Scatterplot of genotypic means of rootstock induced Fruit (F), Leaf (L), and Peel (Pe) concentration  
 718 of calcium and potassium for samples collected in the 2017 season of 'Honeycrisp' grown on 31 rootstocks at  
 719 Geneva, NY. While there is clearly a very tight negative relationship between leaf calcium and leaf potassium,  
 720 the relationship is somewhat lost when fruit is compared. This might indicate that other variables like crop load,  
 721 and other factors are influencing the relationship at the fruit level.

722



723

724 Figure 16. Clustered Pearson's correlation coefficients of 'Honeycrisp' grown on 31 rootstocks at Geneva, NY  
 725 indicating a strong positive correlation between genotypic means for zonal chlorosis and the concentration of  
 726 sodium and negative correlations with the concentration of boron and sulfur.

727



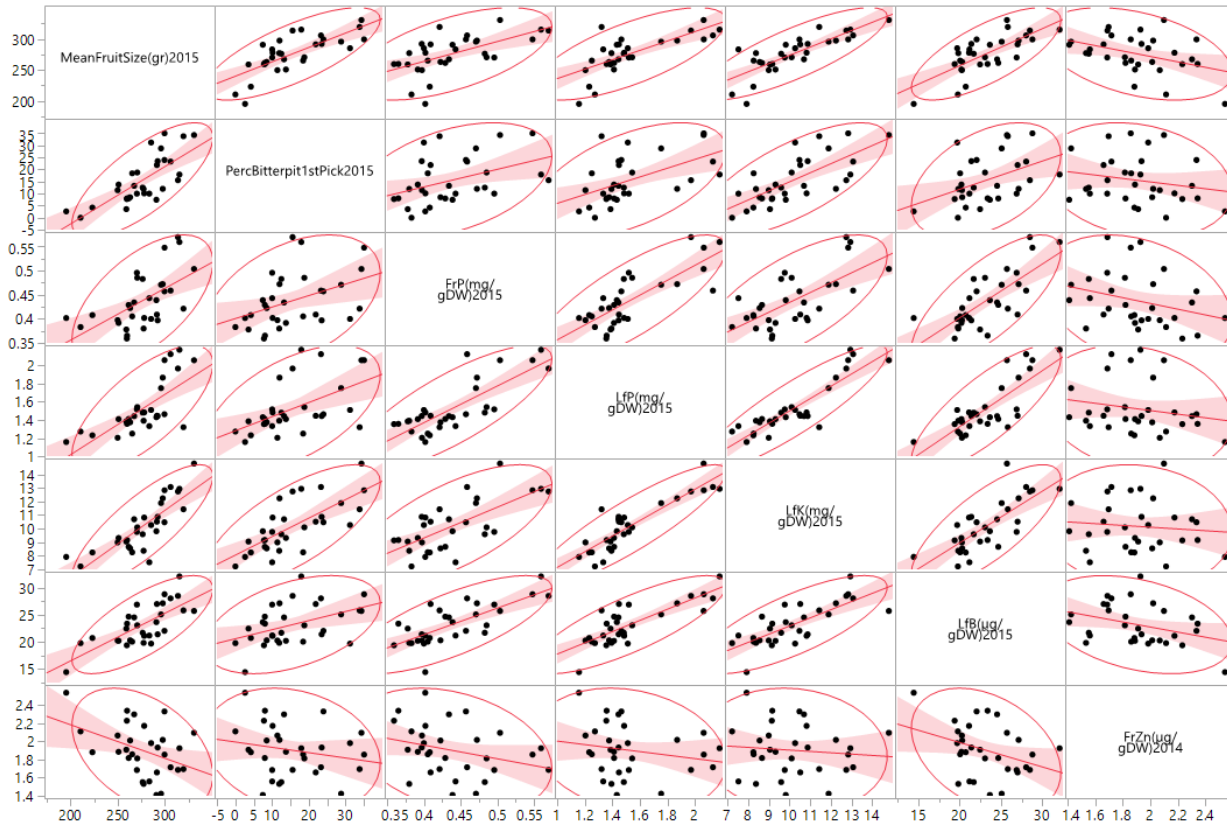
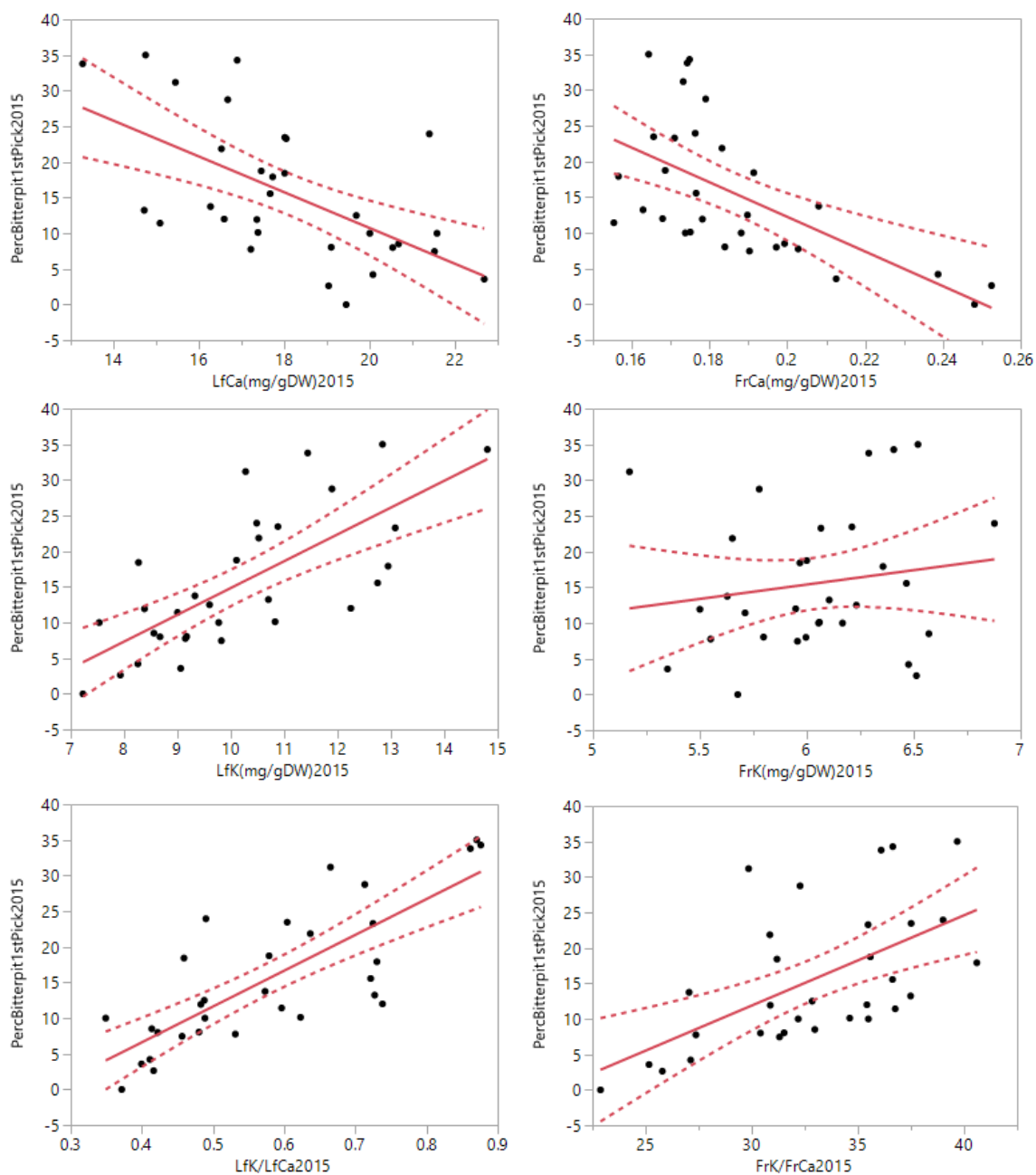


Figure 17. Relationship between rootstock induced mean fruit size (2015), percent bitter pit (2015) and several correlated elemental concentration in leaves and fruit of 'Honeycrisp' grown on 31 rootstocks at Geneva, NY. Rootstocks that induced larger fruit tended to show more bitter-pit. Rootstock induced concentration of fruit and leaf phosphorus, leaf potassium and leaf boron were all significantly positively correlated with higher incidence of bitter-pit and fruit size. Concentration of fruit zinc in previous year (2014) seemed to be negatively correlated with incidence of bitter-pit (2015).

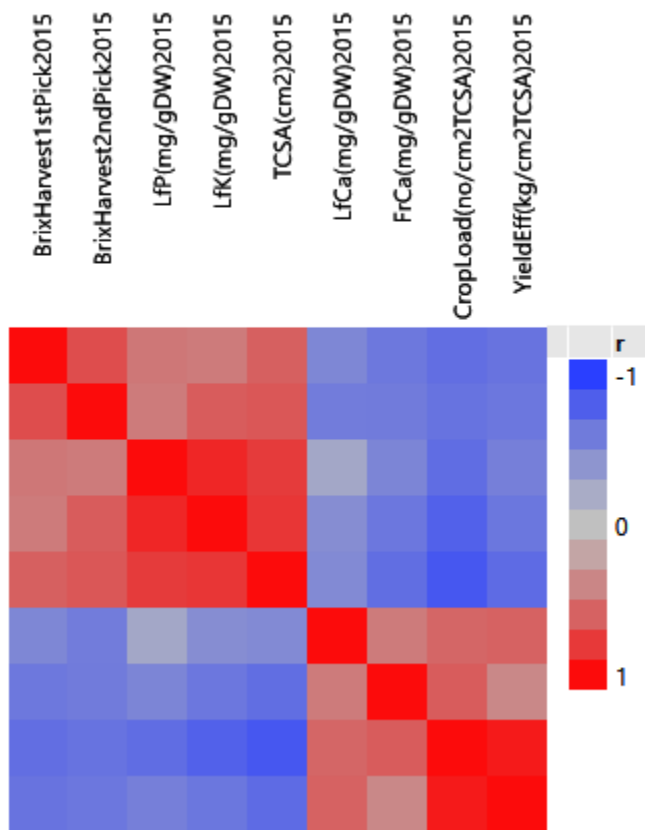


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737

738

Figure 18. Relationship between leaf and fruit calcium and potassium and their ratios (LfK/LfCa and FrK/FrCa) of 'Honeycrisp' grown on 31 rootstocks at Geneva, NY with 95% confidence intervals for the regression lines.



739

740 Figure 19. Clustered correlation coefficients for 2015 data on crop load, brix, TCSA, yield efficiency and several  
 741 nutrient concentrations of 'Honeycrisp' grown on 31 rootstocks at Geneva, NY. Higher brix was positively  
 742 associated with leaf phosphorus and potassium and with tree vigor and negatively associated with leaf and fruit  
 743 calcium, crop load (number of fruit/TCSA) and yield efficiency (kg of fruit/TCSA).