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1 **Highlights:**

- 2 • Honeycrisp tree productivity is drastically affected by rootstock choice.
- 3 • Fruit size, bitter pit, soluble solids, biennial bearing and zonal chlorosis are all affected by
- 4 rootstocks.
- 5 • Rootstock choice can have very large economic impact when considering tree density, productivity
- 6 parameters and fruit quality.

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10

11 **II. Horticultural performance of ‘Honeycrisp’ grown on a genetically diverse**

12 **set of rootstocks under Western New York climatic conditions**

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18

19 **Abstract**

20 A field experiment with 31 rootstocks representing a genetically diverse group of rootstocks

21 featuring ‘Honeycrisp’ as the scion was planted in 2010 at Geneva, NY USA. Rootstocks included

22 three from the Malling series (UK), nine from the Budagovsky series (Russia), 16 from the Cornell

23 Geneva series (USA) and three from the Pillnitz series (Germany). Over the first 8 years (2010-

24 2017) we measured final tree size (trunk cross-sectional area: TCA) and cumulative yield. In the

25 last 4 years we measured fruit soluble solids, bitter pit incidence, biennial bearing, and leaf zonal

26 chlorosis. Tree size varied dramatically with the largest trees on B.70-20-20 and smallest trees on

27 B.71-7-22. Setting the most vigorous rootstock at 100% we categorized rootstocks into 5 size

28 categories: sub-dwarfing class (10-25%), dwarfing class (25-35%), semi-dwarfing class (35-50%),

29 semi-vigorous category (50-70%) and vigorous class (70-100%). Cumulative yield varied 8 fold

30 between the highest yielding rootstock (CG.3001) and the lowest yielding rootstock (B.71-7-22).

31 We calculated theoretical yield per ha by multiplying cumulative yield per tree by a theoretical

32 optimal tree density (trees/ha) based on tree size (TCA). The dwarfing rootstocks G.814, G.41TC,

33 G.11 and B.10 had the highest yields per hectare while the most vigorous rootstocks B.70.20.20

34 and B.71-7-22 were the least productive. Theoretical cumulative yields varied from a high of 400

35 t/ha to a low of 50 t/ha, an 8-fold difference. Rootstock also influenced the incidence of bitter pit

36 with the lowest levels of bitter pit with the rootstocks B.10, CG.2034, B.71-7-22, G.41N, CG.4003,

37 G.202N, G.214, and Supporter 3. Considering bitter pit, yield, and optimum tree density, the

38 theoretical yield of bitter pit free fruit varied from a high of 340 t/ha to a low of 35 t/ha, almost a
39 10-fold difference. The dwarfing rootstocks B.10, G.11, G.41TC, G.214 and G.814 had the highest
40 yields per hectare of bitter pit free fruit. Rootstocks B.9 and M.26 had significantly lower
41 cumulative bitter pit free yield/ha. These data indicate that rootstock not only has a large influence
42 on mature tree cumulative yield but also bitter pit incidence which combine to create a large
43 economic impact of rootstock choice on the long-term economic result of an orchard. This leads
44 to the need for “designer rootstocks” which combine the rootstock characteristics needed to
45 maximize the economic potential of each scion cultivar.

46 **Keywords:** yield, yield efficiency, biennial bearing, tree vigor, leaf zonal chlorosis, fruit soluble
47 solids, bitter pit

48 1. Introduction

49 The large majority of apple trees planted in modern orchards are composites of two or three
50 genotypes (rootstock, interstem and scion) combined by grafting. Most commercial orchards make
51 use of clonally propagated rootstocks and interstems to induce dwarf trees and to increase
52 productivity per unit of land. The most widely used clonal rootstock in the world is ‘Malling 9’
53 (M.9) and its sport mutations, it is also the oldest dwarfing rootstock technology to be adopted by
54 apple growers as some of the original clones may have been planted as early as the 1700’s under
55 the name of ‘Paradis Jaune de Metz’ (Wertheim, 1998). Other apple rootstocks, derivatives of M.9,
56 are also popular but suffer from some of the same drawbacks as M.9 clones such as susceptibility
57 to the apple replant disease complex, fire blight caused by *Erwinia amylovora* Burill, and insect
58 pests such as the woolly apple aphid (*Eriosoma lanigerum* Hausmann) (Fazio, 2014; Fazio et al.,
59 2015). A set of more contemporary rootstocks emerging from the Budagovsky and Geneva®
60 breeding programs are being evaluated in the NC-140 collaborative rootstock testing project in
61 North America (Autio et al., 2013; Autio et al., 2017). One such trial that includes 31 apple
62 rootstocks with ‘Honeycrisp’ as the grafted scion was planted in the year 2010 at 11 sites in the
63 U.S. and Canada. ‘Honeycrisp’ apple has become an important new cultivar but it has a low vigor
64 level in the scion which has caused problems filling allotted space in many growing areas of the
65 world (Robinson et al., 2011; Rosenberger et al., 2001). The 2010 NC140 rootstock trial was
66 designed to identify rootstocks that had sufficient vigor and productivity to maximize the
67 performance of ‘Honeycrisp’. Results of the developmental years (1-5) of this study from all 11
68 sites were included in the report by Autio et al., (2017). In addition individual cooperators have
69 used this trial to evaluate rootstock induced phytohormone concentration changes in the scion
70 (Lordan et al., 2017), and to measure leaf and fruit nutrient concentration during the initial orchard
71 growth and establishment (Nielsen and Hampson, 2014). Here we present results on the
72 horticultural performance of the 31 rootstocks at the mature phase (years 5-8) from only one
73 location, the Geneva trial. Bitter pit is a physiological disorder of some apple varieties including
74 ‘Honeycrisp’ and ‘Fuji’ which manifests itself by small indentations (pits) that can be distributed
75 uniformly on the surface of the apple (Prange et al., 2011; Raese and Drake, 1997). Sometimes the
76 incidence of these pits is greater in areas more distant from the stem end (lower 1/3 of the apple or
77 calyx end) (Jarolmasjed et al., 2016). The incidence and size of bitter pits may increase after apple
78 are cold stored for weeks or months (Wargo and Watkins, 2004). Mineral nutrient concentrations

79 in apple fruit have been associated with the formation of bitter pits, where lower levels of calcium,
80 higher levels of potassium and nitrogen may increase the incidence (Baughner et al., 2017; Ford,
81 1979; Perring and Jackson, 1975). Larger fruit size which is positively correlated with low crop
82 load is also associated with increase in bitter pit (Robinson and Lopez, 2012). Apple rootstocks,
83 given their diverse effect on the nutrition of the canopy have been implicated in the physiology of
84 bitter pit (Fazio et al., 2013; Fazio et al., 2018).

85 Leaf vein chlorosis also referred to as zonal chlorosis is a physiological disorder of ‘Honeycrisp’
86 that appears as blotchy yellow colorations on leaves with no distinct border likely caused by
87 overloading of sorbitol and starch products (Chen and Cheng, 2004). Low crop load and vegetative
88 growth have been linked to the incidence of this leaf disorder (Fleck et al., 2011; Snyder-Leiby
89 and Wang, 2008). Inheritance of this disorder in ‘Honeycrisp’ progenies was localized to linkage
90 group 9 of the apple genome near the MdMYB1 locus that controls red fruit color (Howard et al.,
91 2019).

92 Alternate bearing also referred to as biennial bearing is a phenomenon common to some apple
93 cultivars like ‘Fuji’ and ‘Honeycrisp’ where high crop load one year affects negatively flower
94 induction and fruit set of the next year which is then followed by another high crop load year
95 (Wunsche and Ferguson, 2005) thus resulting in trees that bear fruit on alternate years giving good
96 yields biennially instead of annually (Samuoliene et al., 2016). Phenotypic and genetic differences
97 in the tendency of some scion varieties to fall into alternate bearing mode were described in
98 progeny from ‘(Red) Delicious’ (biennial) and ‘Granny Smith’ (strongly annual) where
99 quantitative trait loci (QTLs) were detected on linkage groups 4, 8 and 10 of the apple genome
100 (Durand et al., 2017; Guitton et al., 2012). The exact physiological pathway that controls alternate
101 bearing is not yet known and may involve timing of flower initiation, the availability of
102 carbohydrate and nutrient resources and the hormonal status of the tree when meristems are ready
103 to change from vegetative to reproductive modes. Rootstocks from a diverse genetic background
104 have been recently implicated in the ability to influence alternate bearing of some cultivars perhaps
105 through the induction of different hormone levels (Lordan et al., 2017) or by changes in crop load
106 and carbohydrate storage (Reig et al., 2019).

107 Tree vigor in commercial apple orchards is largely controlled by the choice of rootstocks where
108 some rootstocks can dwarf the tree all the way down to 10% of the growth of a normal tree on its
109 own roots. The ability of rootstocks to dwarf apple trees has been leveraged to increase the
110 productivity of orchards two to three-fold and reduce inputs (fertilizers, water, pesticides) and
111 human accidents drastically. The dwarfing effect of apple rootstocks has been ascribed to 2-3 loci
112 Dw1 and Dw2 and possibly a third locus (Fazio et al., 2014; Foster et al., 2015; Harrison et al.,
113 2016). Dwarfing rootstocks have been shown to have a dramatic effect on the cumulative yield
114 and yield efficiency (tree productivity/tree size) which determine the optimal spacing to capture
115 light energy and convert it to fruit (Autio et al., 2013; Fallahi et al., 2018).

116 The objective of this study was to study the effect of a genetically diverse set of 31 rootstocks for
117 their potential to maximize the performance of ‘Honeycrisp’ apple with emphasis on mature tree
118 size, cumulative yield, cumulative yield efficiency, biennial bearing, leaf zonal chlorosis, fruit size
119 and, bitter pit incidence.

120 2. Materials and Methods

121 2.1. Trees and design

122 A rootstock field trial was planted in 2010 at the New York State Agricultural Experiment Station
123 (Geneva, NY, USA), using ‘Honeycrisp’ as the scion cultivar (Autio et al., 2017). Trees were
124 planted in a randomized complete block design, with 4 replications and with each block containing
125 2-3 trees of each rootstock. Blocking was done by initial tree diameter. Tree spacing was 1.2 m ×
126 3.5 m. Rootstocks included three from the Malling series: M.26EMLA, M.9T337, and M.9Pajam2;
127 nine from the Budagovsky series: B.10 (B.62-396), B.64-194, B.67-5-32, B.7-20-21, B.7-3-150,
128 B.70-20-20, B.70-6-8, B.71-7-22, and B.9 (Kazlouskaya and Samus, 2011); six Cornell Geneva®
129 released rootstocks: G.11, G.41N, G.41TC, G.935N, G.935TC, G.202N, G.202TC, G.214, G.222
130 and G.814 where the “N” and “TC” distinctions refer to normal propagation and tissue culture
131 propagation respectively; six experimental Geneva rootstocks: CG.2034, CG.3001, CG.4003,
132 CG.4004, CG.4013, CG.5087; and three rootstocks from the Pillnitz series: Piau51-11, Piau9-90,
133 Supporter 3 (Supp. 3). Table 1 describes the origin, parentage and vigor class of each rootstock.

134

135 2.2. Tree performance measurements and fruit quality analysis

136 Tree trunk circumference (30 cm above the graft union), yield, and number of fruits were assessed
137 every year. Trunk-cross-sectional area (TCA) and fruit size were then calculated. We calculated a
138 theoretical yield/ha by multiplying cumulative yields per tree by a theoretical optimal tree density
139 (trees/ha) coefficient based on tree size (TCA) (500 trees/ha for seedling size rootstocks to 5,000
140 trees/ha for sub-dwarfing rootstocks). Tree height from the ground and tree spread (average of tree
141 spread in the alley and in the row) were measured in the Fall of 2017. Biennial bearing was
142 calculated as follow: $(\text{year 1 yield}) - (\text{year 2 yield}) / (\text{year 1 yield} + \text{year 2 yield})$, where 0 indicates
143 no alternate bearing and 1 complete alternate bearing. Yield efficiency represents yield (kg) by
144 TCA (cm²). Near harvest the severity of leaf zonal chlorosis was evaluated by visually assessing
145 the percentage of leaves with chlorosis.

146 At harvest for the 1st and 2nd picks, a 30-fruit sample was collected for each rootstock replicate,
147 from 2014-2017. Ten of the fruits in each sample were then used to assess soluble solids (Brix).
148 The remaining 20 apples of each sample were preconditioned 1 week at 10°C and then stored at
149 3°C for six months. After storage, all of the apples contained in each sample were individually
150 examined for any external signs of superficial bitter pit. The incidence of bitter pit of each sample
151 was calculated as the percentage of fruit with bitter pit symptoms.

152 Climatic data were recorded for each year from the closest automatic weather station. Tree
153 transpiration from May through October was calculated using a modified Penman-Monteith
154 equation (NEWA.org) (Robinson et al. 2017). The trees were trickle irrigated as needed during the
155 growing season using the Cornell apple irrigation model (NEWA.org).

156 2.4 Data analysis

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

166 **3. Results**

167 *3.1 Tree vigor*

168 'Honeycrisp' tree size measured by the size of the trunk-cross-sectional area (TCA) in the fall of
 169 2017 (end of 8th year) was strongly influenced by rootstock genotype (Figure 1). Using the size of
 170 the largest trees (B.70-20-20 rootstock) as 100% we categorize rootstocks into 5 size categories.
 171 Rootstocks B.71-7-22, CG.2034 and B.9 were in the smallest group (sub-dwarfing class - 10-25%
 172 of B.70-20-20), while G.11, G.41, B.10, M.9-T337, Supporter 3, G.935, M.9-EMLA, M.9-Pajam2,
 173 CG.4003 and G.214 were larger (25-35%) and fell into the dwarfing class. In the semi-dwarfing
 174 class (35-50%) were CG.5087, G.814, G.222, G.202 and CG.3001, while rootstocks Piau51-11,
 175 CG.4013, B.67-20-21, Piau-9-90, B.7-3-150, B.70-6-8 fell in the semi-vigorous category (50-
 176 70%). Rootstocks B.64-194 fell into the vigorous range (70-100% of B.70-20-20). Tree height
 177 and tree canopy spread measured in the fall of 2017 were 0.83 ($P<0.001$) and 0.96 ($P<0.001$)
 178 correlated respectively to the TCA (data not presented).

179 *3.2 Cumulative yield and yield efficiency*

180 Cumulative yield per tree over the first 8 years was greatest for CG.3001(120 kg/tree) followed by
 181 G.814 (110 kg/tree) (Figure 2). Following the top 2 rootstocks was a large group of rootstocks that
 182 had a cumulative yield between 80 and 100 kg/tree (B.7-3-150, CG.4004, B.64-194, CG.5087,
 183 B70-6-8, G.214, G.202N, B.70-20-20, G.41N, G.202TC, B.7-20-21, B.10, G.935N, PiAu51-11,
 184 G.41TC, G.222, G.11, M.9T337, Supp3, G.935TC). There was a smaller group of rootstocks that
 185 yielded 70-80 kg/tree (M.9Pajam2, PiAu9-90, CG.4013, B.67-5-32, CG.4003 and M.26EMLA),
 186 while the lowest yielding rootstocks were B.71-7-22, CG.20134 and B.9 with less than 55 kg/tree.
 187 Cumulative yield efficiency was highest with rootstocks G.11, B.9 and G.41TC, followed by B.10,
 188 G.41N, M.9T337, G.214 and G.814 (Figure 3). The lowest yield efficiencies were measured on
 189 rootstocks B.70-20-20, Piau9-90, B.67-5-32, B.64-194, B.70-6-8, B.70-20-21, B.7-3-150,
 190 CG.4013 and Piau51-11. The intermediate yield efficiency group in this experiment featured well
 191 known rootstocks like M.9-Pajam2, and both G.935 and G.202 either N or TC, and experimental
 192 rootstocks such as CG.4004, CG.5087 and CG.4003.

193 The calculated theoretical yield per ha obtained by multiplying cumulative yields per tree by a
 194 theoretical optimal tree density (trees/ha) coefficient based on tree size (TCA) showed that the
 195 dwarfing rootstocks G.814, G.41TC, G.11 and B.10 had the highest yields per hectare while the
 196 most vigorous rootstocks B.70.20.20 and B.71-7-22 were the least productive (Figure 4).

197 Theoretical cumulative yields varied from a high of 400 t/ha to a low of 50 t/ha, an 8-fold
198 difference.

199 *3.3 Biennial bearing*

200 Mean biennial bearing index (BBI) measured over six years was lowest (<0.5) in G.41TC,
201 CG.4003, G.814 and G.202N (Figure 5). It was highest (>0.7) in rootstocks B.67-5-32, Piau9-90,
202 B.71-7-22 and CG.4013. The intermediate group (>0.5 and <0.7) featured the rest of the rootstocks
203 in this trial. Biennial bearing indices, were not very correlated with tree vigor as dwarfing
204 rootstocks Supporter 3, M.9 Pajam2 and B.9 exhibited fairly high yield fluctuations.

205 *3.4 Leaf zonal chlorosis*

206 Leaf zonal chlorosis measured over three years (2014, 2015 & 2017) was influenced by apple
207 rootstocks (Figure 6). While environmental conditions influenced the overall rating for this
208 physiological disorder, there was strong correlation between years according to rootstocks
209 (between 2014 and 2017 $r=0.59$; $P<0.001$). Higher values (red in Figure 6) were observed in the
210 lower cluster of rootstocks (G.814, CG.2034, PiAu9-90, and CG.5087), whereas lower values of
211 zonal chlorosis (blue in Figure 6) were observed by the rootstocks cluster of B.7-20-21, B.70-6-8.
212 While higher rootstock vigor seems to be slightly associated with the lack of zonal chlorosis, there
213 are dwarfing rootstocks that also displayed low to average (gray in Figure 6) levels of this disorder
214 (G.222, M.9-T337, B.10 and CG.4003).

215 *3.5 Fruit size, and soluble solids*

216 Rootstock had a significant effect on fruit size (Figure 7). Fruit size was small (blue in Figure 7)
217 for rootstocks B.71-7-22, B.9, CG.2034 and CG.4003, while CG.5087 and B64-194 had larger
218 fruit size (red in Figure 7). Years 2015 and 2017 were more similar than 2016 which was a very
219 dry year, cumulated rainfall from May through October was ~ 4000 m³/ha vs 1400 m³/ha, 2015-
220 2017 vs 2016 respectively (Figure 8).

221 Rootstock influenced fruit soluble solids (brix) values (Figure 9). Certain rootstocks (G.11
222 CG.2034, B.9, G.935TC, G.41N, G.202N CG4004 and Supp. 3) seemed to induce consistently
223 lower soluble solids (blue in Figure 9) over 3 harvest years, whereas CG.4003 PiAu51-11, and
224 PiAu9-90 had consistently high soluble solids (red in Figure 9). There was generally good
225 consistency between results in 2015 and 2016 but often opposite results with 2017. In the first 2
226 years, B.71-7-22, CG.3001, CG.5087, G.41N and G.935TC had low soluble solids but in 2015 and
227 2016 they all had high soluble solids in 2017.

228 *3.6. Fruit bitter pit incidence*

229 Rootstock influenced fruit bitter pit incidence (Figure 10). One cluster with somewhat consistent
230 low bitter-pit (blue in Figure 10) featured rootstocks B.10, CG.2034, B.71-7-22, G.41N, CG.4003,
231 G.202N, G.214, and Supp. 3. A second cluster of low to medium bitter-pit levels featured
232 rootstocks M.9-T337, G.814, G.11 and G.935N. Consistently high bitter-pit levels (red in Figure
233 10) were observed on rootstock B.70-20-20, B.7-3-150, CG.3001, CG.4013, and PiAu9-90. B.9

234 generally had low incidence of bitter pit but in the very dry year of 2016 it had high incidence of
235 bitter pit.

236 Using the percent bitter pit incidence we calculated yield of bitter pit free fruits. Rootstock had a
237 significant influence on yield of bitter pit free fruits (Figure 2) Cumulative yield of bitter pit free
238 fruit over the first 8 years was greatest for G.814, followed by G.214, G.202N, B.10, G.202TC,
239 G.41TC, G.11 and CG.5087 (red bars in Figure 2). Following the top rootstocks was a large group
240 of rootstocks that had a cumulative yield of bitter pit free fruit between 60 and 80 kg/tree. The
241 rootstocks with the lowest yields of bitter pit free fruit were B.71-7-22, CG.2034 and B.9 which
242 were also the smallest trees.

243 Considering both tree size and bitter pit incidence we calculated the theoretical yield of bitter pit
244 free fruit per ha (obtained by multiplying cumulative yields per tree by percentage of bitter pit free
245 fruit and by the theoretical optimal tree density (trees/ha) coefficient based on tree size (TCA).
246 Theoretical cumulative yields/ha of bitter pit free fruit varied from a high of 340 t/ha to a low of
247 35 t/ha, almost a 10-fold difference (Figure 4). The dwarfing rootstocks B.10, G.11, G.41TC,
248 G.214 and G.814 had the highest yields per hectare of bitter pit free fruit (blue bars in Figure 4).
249 A second group included G.41N, G.935TC and N, M.9T337, CG.5087, Supp. 3, M.9Pajam2,
250 G.202TC and N, CG.4004 and G.222. Rootstocks B.9 and M.26 had lower cumulative bitter pit
251 free yield/ha along with several other un-released rootstock selections. The most vigorous
252 rootstocks B.70.20.20 and B.71-7-22 had the lowest yield of bitter pit free fruit.

253 **4. Discussion**

254 *4.1 Tree size and yield*

255 Our results of mature tree size were similar to what Autio et al. 2017 reported at the end of year 5,
256 where sub-dwarfing rootstock B.71-7-22 and vigorous (standard seedling size) B.70-20-20
257 represented the ends of the spectrum of rootstocks. The classes of dwarfing potential described in
258 Autio et al. 2017 are congruent with the findings in this experiment. The highest cumulative yield
259 per tree (2011-2017) was obtained on rootstock CG.3001, however the highest bitter pit free yield
260 was produced by G.814 with G.214 and B.10 as second and third respectively. When the per tree
261 yields were adjusted to how many trees could be planted per hectare of land based on the dwarfing
262 capacity the theoretical cumulative yield per hectare for each rootstock, G.814, G.11, G.41TC,
263 B.10, G.41N were the superior rootstocks where in the meta-analysis performed in Autio *et al.*
264 2017, G.814 was the most efficient also. The low theoretical cumulative yield with the most
265 vigorous rootstocks B.70-20-20 and B.71-7-22 indicates that it is counterproductive to try to
266 increase vigor of a weak scion cultivar ('Honeycrisp') with non-precocious, vigorous rootstocks.
267 Our results also indicate that if the rootstock is too dwarfing for a weak growing scion like
268 'Honeycrisp' that even when planted very close theoretical yields are less than the best semi-
269 dwarfing rootstocks. These results show that when evaluating rootstocks it is not enough to rank
270 them by yield efficiency which is the common way to rank rootstocks. Although cumulative yield
271 efficiency (kg of apples per cm² of the trunk cross sectional area) is a way to capture how well the
272 rootstock is partitioning photosynthesis product toward producing fruit instead of vegetative
273 growth (vigorous rootstocks that produce less apples per TCA are considered yield inefficient

274 whereas dwarfing rootstocks that are able to produce more apples per TCA are yield efficient), it
275 is also important to assess if the vigor of the rootstock is sufficient to fill the space and if the
276 rootstock has high yield efficiency. A specific case was B.9 which was one of the most yield
277 efficient rootstocks but because it was excessively dwarfing, its theoretical yield was less than half
278 of the best rootstock's theoretical yield (G.814). Further affecting the ranking of rootstocks for
279 theoretical yield per ha was the incidence of bitter pit. Once that characteristic of 'Honeycrisp'
280 was considered, the relative ranking of B.9 improved substantially, since it had low incidence of
281 bitter pit, but it still had significantly less yield/ha of bitter pit free fruit (205 t/ha) compared to the
282 best rootstocks (~330 t/ha) which were B.10, G.11, G.41TC, G.214 and G.814. Considering the
283 high farm gate fresh market price for 'Honeycrisp' apples (\$1.65/kg) (Lordan et al., 2018), the
284 extra yield of the best rootstocks compared to B.9 would be worth ~\$222,000 per ha. This large
285 economic impact illustrates the practical importance of rootstock evaluation to find the rootstocks
286 which have the best combination of vigor, productivity and fruit quality to match specific scions
287 to maximize yield of bitter pit free fruit. It is likely that in each geographic region different
288 rootstocks may be the optimum for 'Honeycrisp'. Our results unfortunately are limited to the
289 climate in the Northeastern USA.

290 The tendency of some apple varieties to bear fruit heavily in one season and poorly the next season
291 referred to as alternate or biennial bearing is dependent on the genetic components of each cultivar
292 where 'Honeycrisp' and 'Fuji' are strongly biennial while 'Gala' is much less biennial (Guitton et
293 al., 2012). Apple rootstocks can influence bienniality (Barritt et al., 1997) by means of influencing
294 crop load, vigor, hormone concentration and gene expression of the scion (Jensen et al., 2012;
295 Lordan et al., 2017; Tworkoski and Fazio, 2016). In this trial, G.41TC, CG.4003 and G.814
296 displayed the lowest levels of alternate bearing, and CG.4013 (semi-dwarf) and B.71-7-22 (sub-
297 dwarf) displayed the highest levels of alternate bearing, indicating as in Barritt *et al.* 1997, that
298 vigor control is not always related to the suppression of alternate bearing. More research is needed
299 to fully understand all the variables that influence this trait that causes large losses in production
300 in apple orchards worldwide.

301 *4.2 Fruit quality*

302 Rootstock did influence fruit soluble solids (brix) but the results varied between years. Fruit in the
303 2015 season were harvested in two picks according to color maturity, suggesting that the trends
304 observed in rootstocks were not due to differences in maturity, rather more correlated with crop
305 load (Robinson and Lopez, 2012) and may be one of the reasons why apples may taste different
306 when grown on different rootstocks.

307 *4.3 Zonal leaf chlorosis*

308 'Honeycrisp' trees are sensitive to a leaf physiological disorder caused by over-loading of
309 carbohydrates in leaves that causes damage to the photosynthesis systems (Chen et al., 2010; Fleck
310 et al., 2011; Snyder-Leiby and Wang, 2008) and often referred to as leaf zonal chlorosis.
311 Rootstocks have also been shown to have an influence on this physiological disorder, with the
312 degree of influence affected by season and perhaps management practices (Autio et al., 2017). In
313 our study zonal chlorosis is less prevalent with the more vigorous rootstocks. This indicates that

314 in conditions where vegetative growth is more active these vegetative sinks can utilize
315 carbohydrates produced in the leaves thus limiting the buildup of starch granules which cause cell
316 rupture and the zonal chlorosis (Cheng and Robinson, 2006). However, some semi-dwarfing
317 rootstocks (G.222, M.9-T337, B.10 and CG.4003) also had low values for zonal chlorosis
318 indicating an unknown rootstock mediated mechanism to prevent the buildup of starch granules in
319 the leaf.

320 **5. Conclusion**

321 This experiment using a genetically diverse set of rootstocks showed dramatic differences in tree
322 size, yield, yield efficiency, biennial bearing and bitter pit incidence on the fruit which were
323 induced by the rootstock. However, in ranking rootstocks for a particular climate and a particular
324 scion variety, it is important to not only consider yield efficiency but also the vigor of the rootstock
325 which must be sufficient to fill the space rapidly and combined with high yield efficiency. Our
326 data also indicate that rootstock not only has a large influence on mature tree cumulative yield but
327 also bitter pit incidence which combine to create a large economic impact of rootstock choice on
328 its long-term economic result of an orchard. The best rootstocks had theoretical yields of bitter pit
329 free fruit of 10 times the poorest performing rootstocks. A final important factor is that when scion
330 cultivar vigor is low as with ‘Honeycrisp’ the best rootstock may not be the same as for a more
331 moderate vigor scion cultivar or even a vigorous scion cultivar which need more dwarfing power
332 from the rootstock compared to weak scion cultivars (Reig et al., 2018). This leads to the need for
333 “designer rootstocks” which combine the rootstock characteristics needed to maximize the
334 potential of each scion cultivar in a particular climate.

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341 **References**

- 342 Autio, W., Robinson, T., Archbold, D., Cowgill, W., Hampson, C., Quezada, R.P. and Wolfe, D.
343 2013. 'Gala' apple trees on Supporter 4, P.14, and different strains of B.9, M.9 and M.26
344 rootstocks: Final 10-year report on the 2002 NC-140 apple rootstock trial. *Journal of the*
345 *American Pomological Society* 67:62-71.
- 346 Autio, W., Robinson, T., Archbold, D., Cowgill, W., Hampson, C., Quezada, R.P., Wolfe, D.,
347 2013. 'Gala' apple trees on supporter 4, P 14, and different strains of B 9, M 9 and M 26
348 rootstocks: final 10-year report on the 2002 NC-140 apple rootstock trial. *Journal of the*
349 *American Pomological Society* 67, 62-71.
- 350 Baugher, T.A., Marini, R., Schupp, J.R., Watkins, C.B., 2017. Prediction of Bitter Pit in
351 'Honeycrisp' Apples and Best Management Implications. *Hortscience* 52, 1368-1374.
- 352 Autio, W., Robinson, T., Black, B., Blatt, S., Cochran, D., Cowgill, W., Hampson, C., Hoover, E.,
353 Lang, G., Miller, D., Minas, I., Quezada, R.P., Stasiak, M., 2017. Budagovsky, Geneva,

- 354 Pillnitz, and Malling apple rootstocks affect 'Honeycrisp' performance over the first five years
355 of the 2010 NC-140 'Honeycrisp' apple rootstock trial. *Journal American Pomological Society*
356 71, 149-166.
- 357 Barritt, B.H., Konishi, B.S., Dilley, M.A., Kappel, F., 1997. Tree size, yield and biennial bearing
358 relationships with 40 apple rootstocks and three scion cultivars. *Acta Hort.* 451, 105-112.
- 359 Chen, L., Cheng, L.L., Chen, L.S., 2010. The acceptor side of photosystem II is damaged more
360 severely than the donor side of photosystem II in 'Honeycrisp' apple leaves with zonal
361 chlorosis. *Acta Physiologiae Plantarum* 32, 253-261.
- 362 Chen, L.-S., Cheng, L., 2004. CO₂ assimilation, carbohydrate metabolism, xanthophyll cycle, and
363 the antioxidant system of 'Honeycrisp' apple leaves with zonal chlorosis. *Journal of the*
364 *American Society for Horticultural Science* 129, 729-737.
- 365 Cheng, L. and Robinson T.L. 2006. Zonal chlorosis of Honeycrisp leaves: Causes and
366 implications. *Compact Fruit Tree* 39(1):28-29.
- 367 Durand, J.-B., Allard, A., Guitton, B., van de Weg, E., Bink, M.C.A.M., Costes, E., 2017.
368 Predicting Flowering Behavior and Exploring Its Genetic Determinism in an Apple Multi-
369 family Population Based on Statistical Indices and Simplified Phenotyping. *Frontiers in Plant*
370 *Science* 8:858.
- 371 Fallahi, E., Kiester, M.J., Fallahi, B., Mahdavi, S., 2018. Rootstock, Canopy Architecture, Bark
372 Girdling, and Scoring Influence on Growth, Productivity, and Fruit Quality at Harvest in 'Aztec
373 Fuji' Apple. *Hortscience* 53, 1629-1633.
- 374 Fazio, G., 2014. Breeding apple rootstocks in the 21st century – what can we expect them to do to
375 increase productivity in the orchard? *Acta Horticulturae* 1058, 421-428.
- 376 Fazio, G., Robinson, T.L., Aldwinckle, H.S., 2015. The Geneva apple rootstock breeding program.
377 *Plant Breeding Reviews* 39, 379-424.
- 378 Fazio, G., Kviklys, A., Grusak, M.A., Robinson, T.L., 2013. Phenotypic diversity and QTL
379 mapping of absorption and translocation of nutrients by apple rootstocks. *Aspects of Applied*
380 *Biology* 119, 37-50.
- 381 Fazio, G., Lordan, J., Francescato, P., Cheng, L., Wallis, A., Grusak, M.A., Robinson, T.L., 2018.
382 'Honeycrisp' apple fruit nutrient concentration affected by apple rootstocks. *Acta Hort* 1228,
383 223-228.
- 384 Fazio, G., Wan, Y.Z., Kviklys, D., Romero, L., Adams, R., Strickland, D., Robinson, T., 2014.
385 Dw2, a New Dwarfing Locus in Apple Rootstocks and Its Relationship to Induction of Early
386 Bearing in Apple Scions. *Journal of the American Society for Horticultural Science* 139, 87-
387 98.
- 388 Fleck, S., Embree, C.G., Nichols, D.S., 2011. The influence of crop load, shoot type, canopy
389 structure, and leaf zonal chlorosis on leaf photosynthesis of 'Honeycrisp' apple trees. *Acta*
390 *Hortic.* 903, 767-774.
- 391 Ford, E.M., 1979. Distribution of Calcium in Mature Apple Fruits Having Bitter Pit Disorder.
392 *Journal of Horticultural Science* 54, 91-92.
- 393 Foster, T.M., Celton, J.M., Chagne, D., Tustin, D.S., Gardiner, S.E., 2015. Two quantitative trait
394 loci, Dw1 and Dw2, are primarily responsible for rootstock-induced dwarfing in apple. *Hortic*
395 *Res* 2, 15001.
- 396 Guitton, B., Kelner, J.J., Velasco, R., Gardiner, S.E., Chagne, D., Costes, E., 2012. Genetic control
397 of biennial bearing in apple. *Journal of Experimental Botany* 63, 131-149.
- 398 Harrison, N., Harrison, R.J., Barber-Perez, N., Cascant-Lopez, E., Cobo-Medina, M., Lipska, M.,
399 Conde-Ruiz, R., Brain, P., Gregory, P.J., Fernandez-Fernandez, F., 2016. A new three-locus

- 400 model for rootstock-induced dwarfing in apple revealed by genetic mapping of root bark
401 percentage. *J Exp Bot* 67, 1871-1881.
- 402 Howard, N.P., Tillman, J., Vanderzande, S., Luby, J.J., 2019. Genetics of zonal leaf chlorosis and
403 genetic linkage to a major gene regulating skin anthocyanin production (MdMYB1) in the
404 apple (*Malus x domestica*) cultivar Honeycrisp. *Plos One* 14.
- 405 Jarolmasjed, S., Espinoza, C.Z., Sankaran, S., Khot, L.R., 2016. Postharvest bitter pit detection
406 and progression evaluation in 'Honeycrisp' apples using computed tomography images.
407 *Postharvest Biology and Technology* 118, 35-42.
- 408 Jensen, P.J., Halbrecht, N., Fazio, G., Makalowska, I., Altman, N., Praul, C., Maximova, S.N.,
409 Ngugi, H.K., Crassweller, R.M., Travis, J.W., McNellis, T.W., 2012. Rootstock-regulated
410 gene expression patterns associated with fire blight resistance in apple. *Bmc Genomics* 13.
- 411 Lordan, J., Fazio, G., Francescato, P., Robinson, T., 2017. Effects of apple (*Malus x domestica*)
412 rootstocks on scion performance and hormone concentration. *Scientia Horticulturae* 225, 96-
413 105.
- 414 Lordan, J., Wallis, A., Francescato, P. and Robinson, T.L. 2018. Long-term effects of training
415 systems and rootstocks on 'McIntosh' and 'Honeycrisp' performance, a 15-year study in a
416 northern cold climate - part 2: Economic analysis. *HortScience* 53, 978-992.
- 417 Neilsen, G., Hampson, C. 2014. 'Honeycrisp' apple leaf and fruit nutrient concentration is
418 affected by rootstock during establishment. *Journal of the American Pomological Society* 68,
419 178-189.
- 420 Perring, M.A., Jackson, C.H., 1975. Mineral-Composition of Apples - Calcium Concentration
421 and Bitter Pit in Relation to Mean Mass Per Apple. *Journal of the Science of Food and*
422 *Agriculture* 26, 1493-1502.
- 423 Prange, R., DeLong, J., Nichols, D., Harrison, P., 2011. Effect of fruit maturity on the incidence
424 of bitter pit, senescent breakdown, and other post-harvest disorders in 'Honeycrisp'(TM)
425 apple. *Journal of Horticultural Science & Biotechnology* 86, 245-248.
- 426 Raese, J.T., Drake, S.R., 1997. Nitrogen fertilization and elemental composition affects fruit
427 quality of 'Fuji' apples. *Journal of Plant Nutrition* 20, 1797-1809.
- 428 Reig, G., Lordan, J., Sazo, M.M., Hoying, S.A., Fargione, M.J., Hernan Reginato, G., Donahue,
429 D.J., Francescato, P., Fazio, G., Robinson, T.L., 2019. Effect of tree type and rootstock on
430 the long-term performance of 'Gala', 'Fuji' and 'Honeycrisp' apple trees trained to Tall Spindle
431 under New York State climatic conditions. *Scientia Horticulturae (Amsterdam)* 246, 506-
432 517.
- 433 Reig, G., Lordan, J., Fazio, G., Grusak, M.A., Hoying, S., Cheng, L.L., Francescato, P., Robinson,
434 T., 2018. Horticultural performance and elemental nutrient concentrations on 'Fuji' grafted on
435 apple rootstocks under New York State climatic conditions. *Scientia Horticulturae* 227, 22-37.
- 436 Robinson, T.L., Fazio, G., Hoying, S.A., Miranda, M. and Iungerman, K. 2011. Geneva rootstocks
437 for weak growing scion cultivars like 'Honeycrisp'. *New York Fruit Quarterly* 19(2), 10-16.
- 438 Robinson, T., Lopez, S., 2012. Crop load affects 'Honeycrisp' fruit quality more than nitrogen,
439 potassium, or irrigation. *Acta Hort.* 940, 529-537.
- 440 Robinson, T., Lopez, S., 2012. Crop load affects 'Honeycrisp' fruit quality more than nitrogen,
441 potassium, or irrigation. *Acta Hort.* 940, 529-537.
- 442 Robinson, T. L., J. Lordan, D. Dragoni, A. N. Lakso and P. Francescato (2017). "Precision
443 irrigation management of apple with an apple-specific Penman-Monteith model." *Acta*
444 *Horticulturae* **1150**: 245-250.

- 445 Rosenberger, D., Schupp, J., Watkins, C.B., Iungerman, K., Hoying, S., Straub, D. and Cheng, L.
446 2001. Honeycrisp: promising profit maker or just another problem child? . New York Fruit
447 Quarterly 9, 9-13.
- 448 Samuoliene, G., Ceidaite, A., Sirtautas, R., Duchovskis, P., Kviklys, D., 2016. Effect of crop load
449 on phytohormones, sugars, and biennial bearing in apple trees. *Biologia Plantarum* 60, 394-
450 400.
- 451 Snyder-Leiby, T.E., Wang, S.X., 2008. Role of crop load in chloroplast ultra-structure and zonal
452 chlorosis, a physiological disorder in 'Honeycrisp' apple trees. *HortScience* 43, 1819-1822.
- 453 Tworkoski, T., Fazio, G., 2016. Hormone and growth interactions of scions and size-controlling
454 rootstocks of young apple trees. *Plant Growth Regulation* 78, 105-119.
- 455 Wargo, J.M., Watkins, C.B., 2004. Maturity and storage quality of 'Honeycrisp' apples.
456 *Horttechnology* 14, 496-499.
- 457 Wertheim, S.J. 1998. Rootstock guide: Apple, pear, cherry, European plum. Publication nr. 25.
458 Wilhelminadorp Research Station, The Netherlands.
- 459 Wunsche, J.N., Ferguson, I.B., 2005. Crop load interactions in apple. *Horticultural Reviews* 31,
460 231-290.

Tables

Table 1. Apple rootstocks in the NC-140 Trial planted in 2010 with ‘Honeycrisp’ as the scion in Geneva, NY.

Rootstock	Type	Origin	Parentage	Tree size	References
B.62-396	Dwarf	Michurinsk College, Russia	B.13-14 x B.9	M.9-M-26	U.S. PP 21223P3
B.64-194	Semi-Dwarf	Michurinsk College, Russia	Unknown	M.7-MM.106	Kazlouskaya, Z.A. and V.A. Samus, 2011
B.67-5-32	Semi-Dwarf	Michurinsk College, Russia	Unknown	M.7	Kazlouskaya, Z.A. and V.A. Samus, 2011
B.7-20-21	Semi-Dwarf	Michurinsk College, Russia	Unknown	M.7	Kazlouskaya, Z.A. and V.A. Samus, 2011
B.7-3-150	Semi-Dwarf	Michurinsk College, Russia	Unknown	M.7-MM.106	Kazlouskaya, Z.A. and V.A. Samus, 2011
B.70-20-20	Vigorous	Michurinsk College, Russia	57-469 x 57-344	Seedling	U.S. PP 25500P3
B.70-6-8	Semi-Dwarf	Michurinsk College, Russia	Unknown	M.7-MM.106	Kazlouskaya, Z.A. and V.A. Samus, 2011
B.71-7-22	Super Dwarf	Michurinsk College, Russia	Unknown	M.27	Kazlouskaya, Z.A. and V.A. Samus, 2011
B.9	Dwarf	Michurinsk College, Russia	Unknown	B.9	Kazlouskaya, Z.A. and V.A. Samus, 2011
CG.2034	Dwarf	Geneva Res. Station, New York, USA	Dolgo crab x Malling 27	M.27	<i>Personal communication (Fazio G.)</i>
CG.3001	Dwarf	Geneva Res. Station, New York, USA	P.2 x Robusta 5	M.9	<i>Personal communication (Fazio G.)</i>
CG.4003	Dwarf	Geneva Res. Station, New York, USA	(Antonovka Kamienaja x Ottawa 3)xRobusta 5	M.26	Norelli et al., 2003
CG.4004	Dwarf	Geneva Res. Station, New York, USA	722506-004 x OP	M.26	<i>Personal communication (Fazio G.)</i>
CG.4013	Dwarf	Geneva Res. Station, New York, USA	Ottawa 3 x Robusta 5	M.26	Norelli et al., 2003
CG.5087	Dwarf	Geneva Res. Station, New York, USA	Ottawa 3 x Robusta 5	M.26 to M.7	Norelli et al., 2003
G.11	Dwarf	Geneva Res. Station, New York, USA	M.26 x Robusta 5	M.9	Norelli et al., 2003
G.202N	Dwarf	Geneva Res. Station, New York, USA	M.27 x Robusta 5	M.26	Norelli et al., 2003
G.202TC	Dwarf	Geneva Res. Station, New York, USA	M.27 x Robusta 5	M.26	Norelli et al., 2003
G.214	Dwarf	Geneva Res. Station, New York, USA	Ottawa 3 x Robusta 5	M.26	Norelli et al., 2003
G.222	Semi-dwarf	Geneva Res. Station, New York, USA	M.27 x Robusta 5	M.26	<i>Personal communication (Fazio G.)</i>
G.41N	Dwarf	Geneva Res. Station, New York, USA	M.27 x Robusta 5	M.9	<i>Personal communication (Fazio G.)</i>
G.41TC	Dwarf	Geneva Res. Station, New York, USA	M.27 x Robusta 5	M.9	<i>Personal communication (Fazio G.)</i>
G.814	Dwarf	Geneva Res. Station, New York, USA	Ottawa 3 x Robusta 5	M.26	Norelli et al., 2003
G.935N	Dwarf	Geneva Res. Station, New York, USA	Ottawa 3 x Robusta 5	M.26	Norelli et al., 2003
G.935TC	Dwarf	Geneva Res. Station, New York, USA	Ottawa 3 x Robusta 5	M.26	Norelli et al., 2003
M.26	Dwarf	HRI-East Malling, UK	M.16 x M.9	M.26	Preston, 1954, 1970; Rogers, 1958; Proctor et al., 1974
M.9-Paj2	Dwarf	Reselected at HRI-East Malling, UK	Unknown	M.9	Halton, 1917; Van Oosten, 1977, 1986; Webster and Hollands, 1999
M.9-T337	Dwarf	Reselected at HRI-East Malling, UK	Unknown	M.9	Halton, 1917; Van Oosten, 1977, 1986; Webster and Hollands, 1999
PiAu.51-11	Semi-Dwarf	Pillnitz, Germany	M 4 open pollinated	M.7	Norelli et al., 2003
PiAu.9-90	Semi-dwarf	Pillnitz, Germany	Unknown	M.7	
Supp.3	Semi-dwarf	Pillnitz, Germany	Unknown	M.9	

Figures

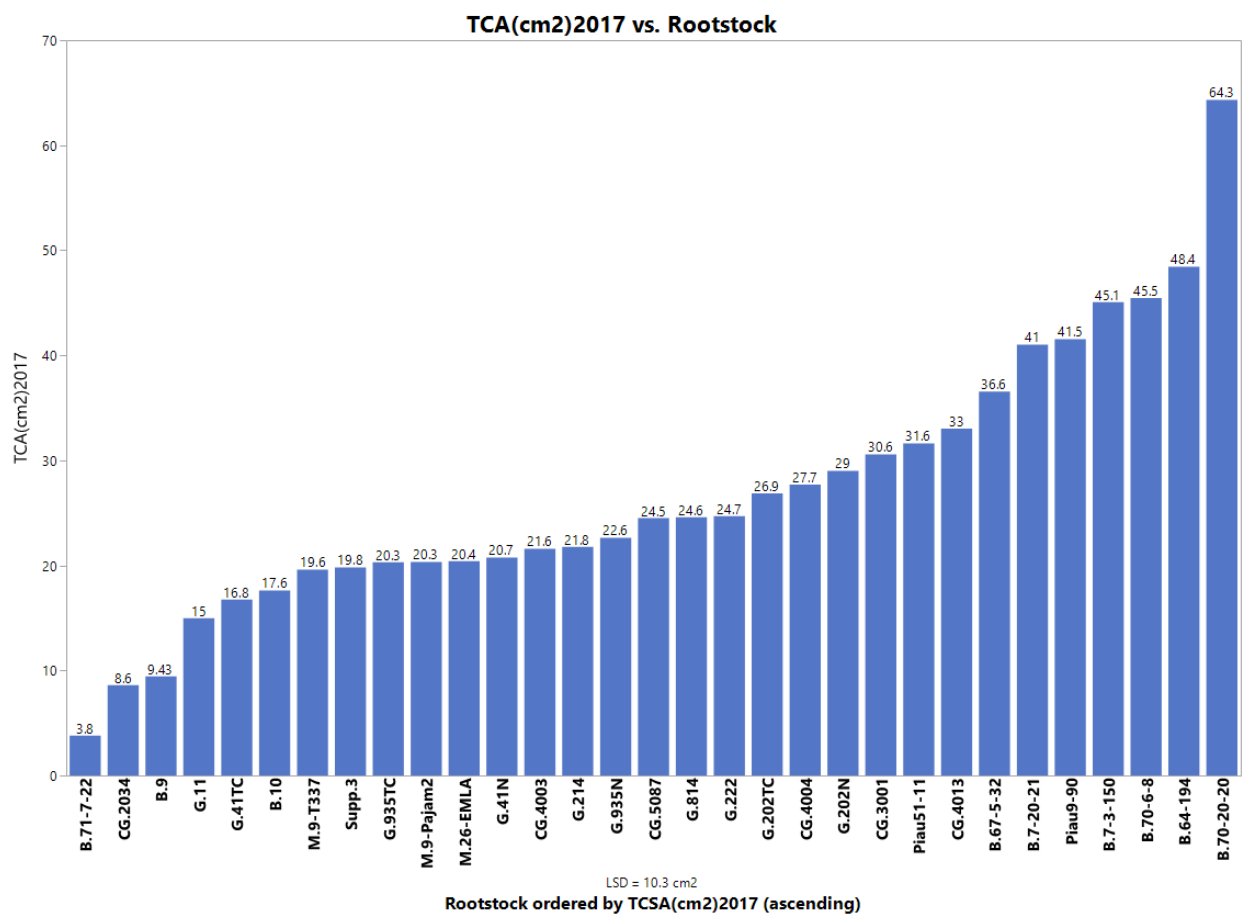


Figure 1. Genotypic means for the trunk cross sectional area (TCA) (which correlate to the whole tree size) for 31 rootstocks after 8 years when grown at Geneva, NY. LSD for TCA = 10.3.

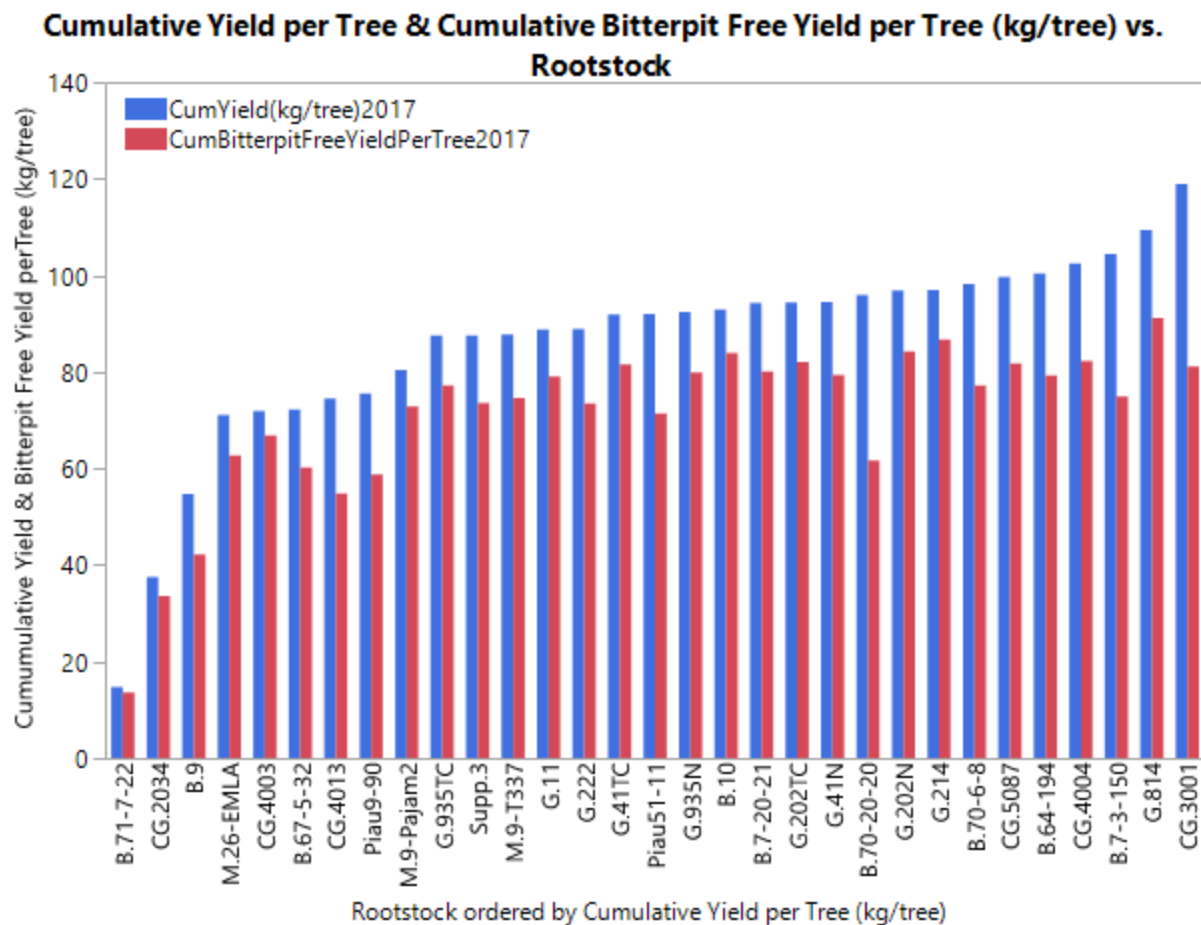


Figure 2. Genotypic means for cumulative yield (blue) and bitter-pit free cumulative yield per tree (red) for 31 rootstocks after 8 years when grown at Geneva, NY. LSD for Yield/tree=18.6. LSD for Bitter Pit free Yield/tree=18.1.

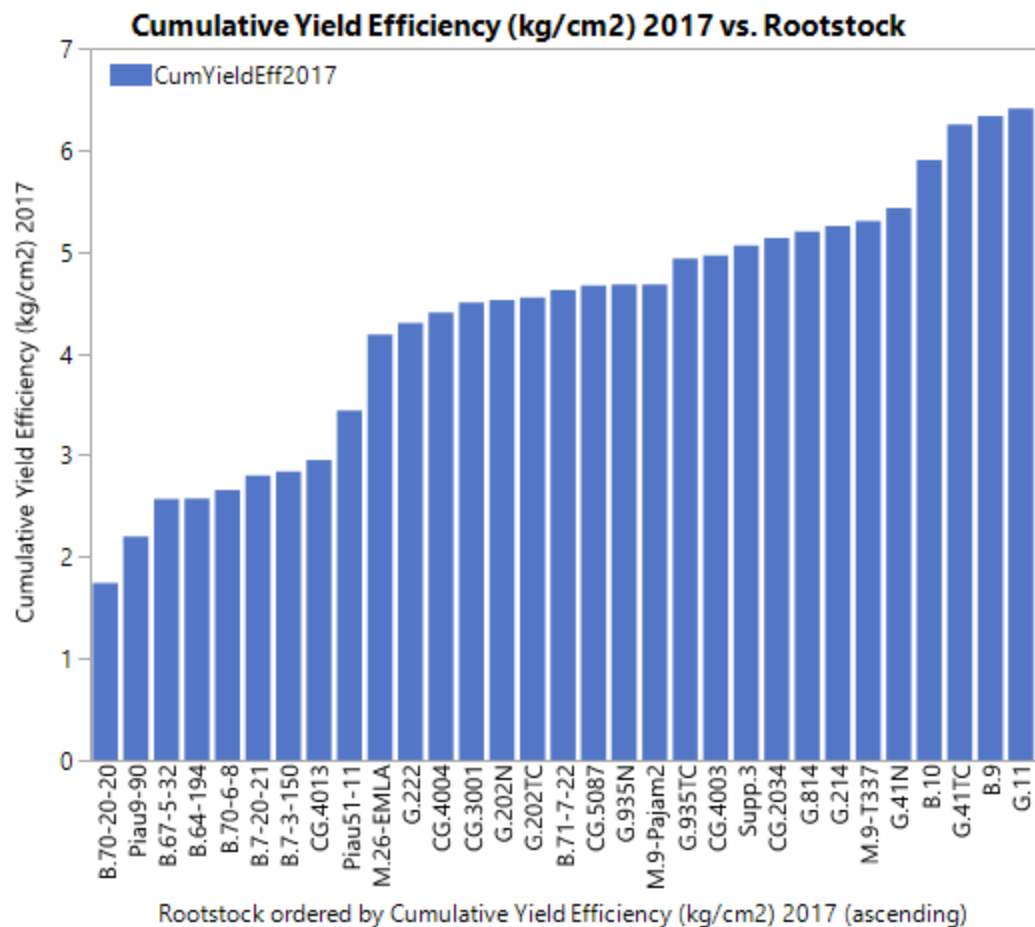


Figure 3. Genotypic means for cumulative yield efficiency (kg of apples per cm² of the trunk cross sectional area) for 31 rootstocks after 8 years when grown at Geneva, NY. LSD for Yield Efficiency=1.05.

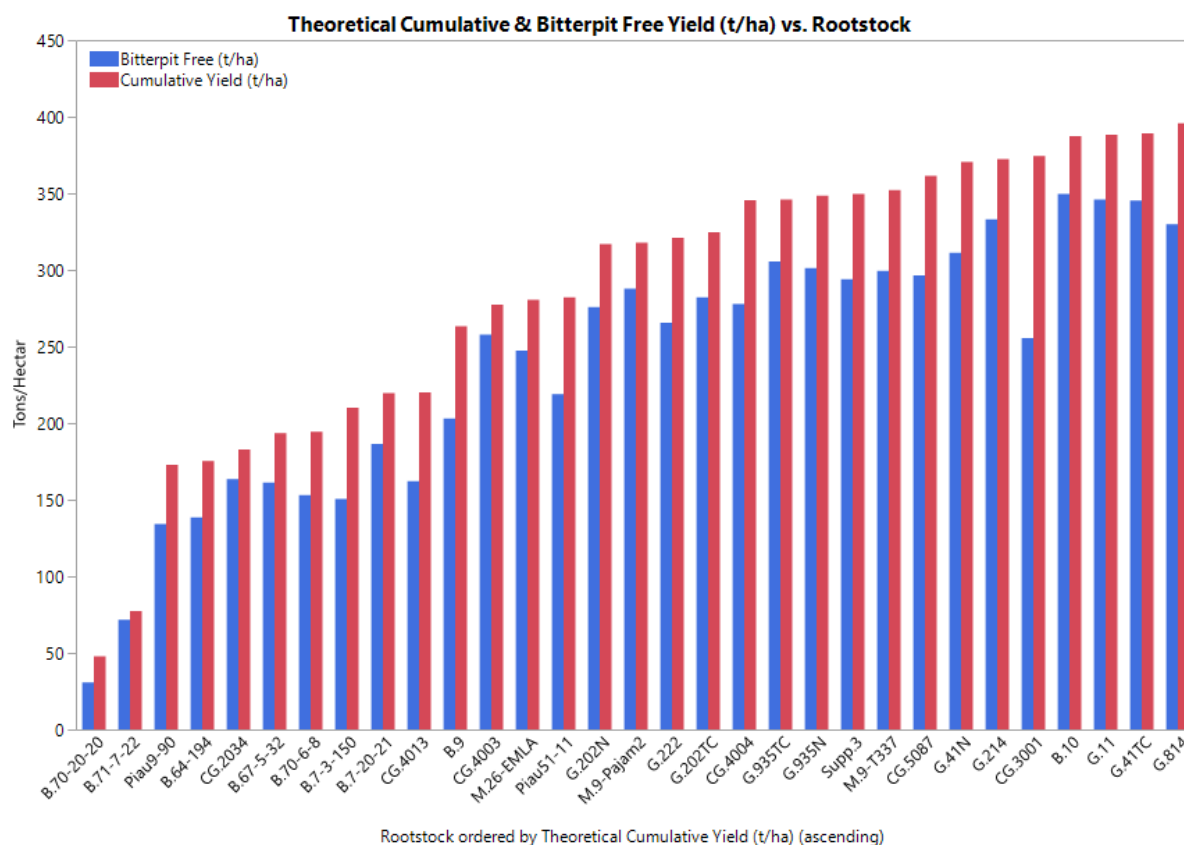


Figure 4. Genotypic means for cumulative yield and bitter pit free yield (t/ha) (calculated by multiplying cumulative yields per tree by a theoretical optimal tree density (trees/ha) based on tree size potential (500 trees/ha for seedling size rootstocks to 5,000 trees/ha for sub-dwarfing rootstocks) of ‘Honeycrisp’ apple grown on 31 rootstocks after 8 years at Geneva, NY. LSD for Theoretical cumulative yield/ha=59.4 and LSD for Theoretical Bitter Pit free yield/ha=52.3.

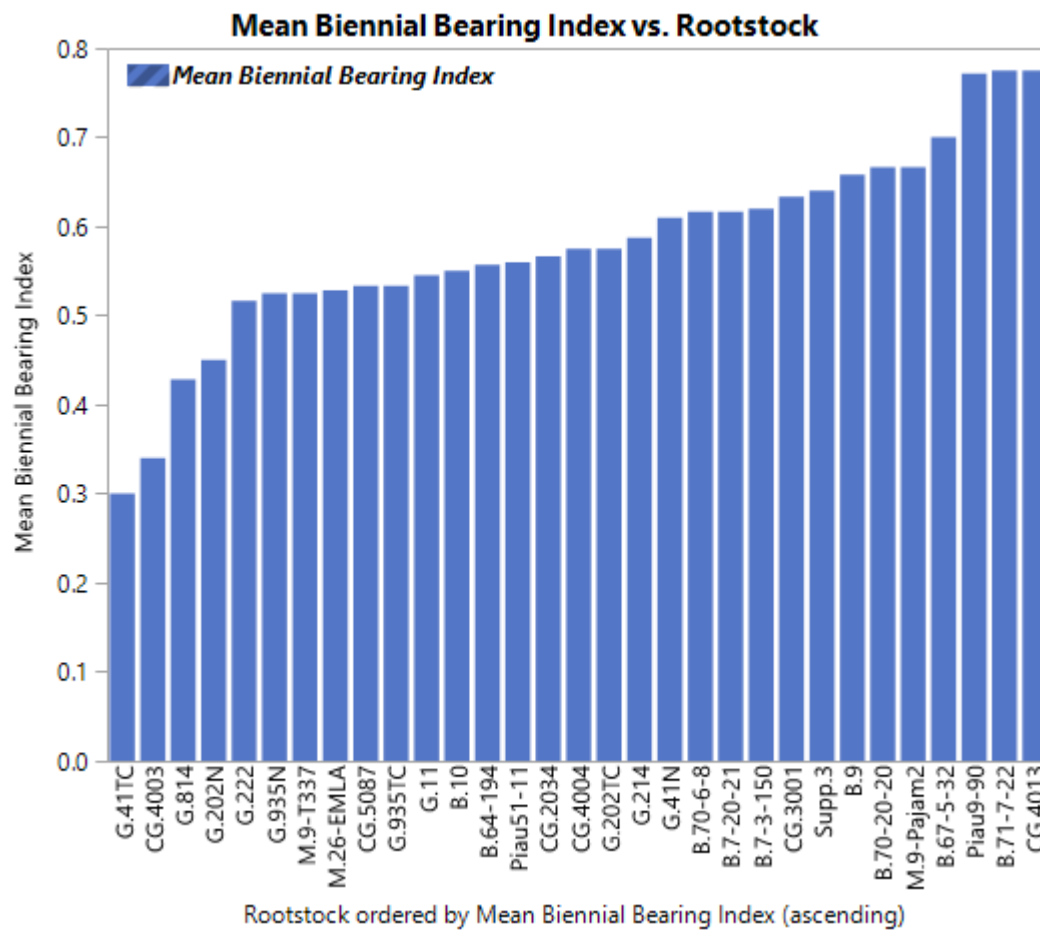


Figure 5. Genotypic means for biennial bearing index (BBI) of ‘Honeycrisp’ apple grown on 31 rootstocks over 6 years (3rd-8th years) at Geneva, NY. LSD for Biennial Bearing Index=0.21.

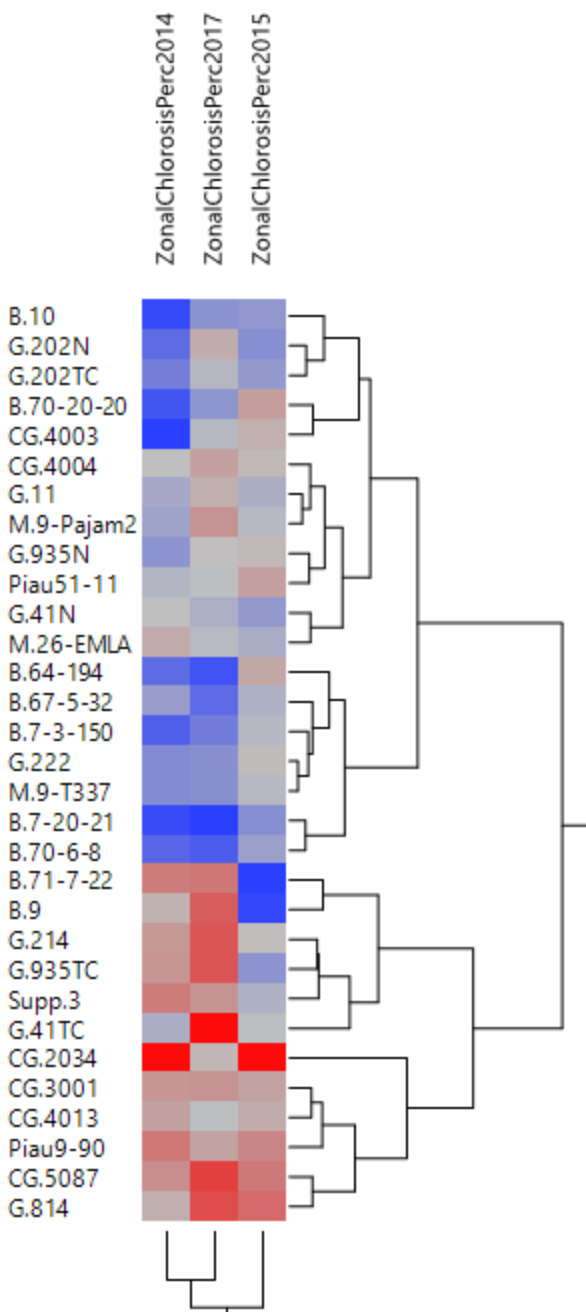


Figure 6. Two-Way similarity cluster analysis of zonal chlorosis genotypic means over three growing seasons of ‘Honeycrisp’ apple grown on 31 rootstocks at Geneva, NY. Higher values are red whereas lower genotypic means are blue and average values are gray.

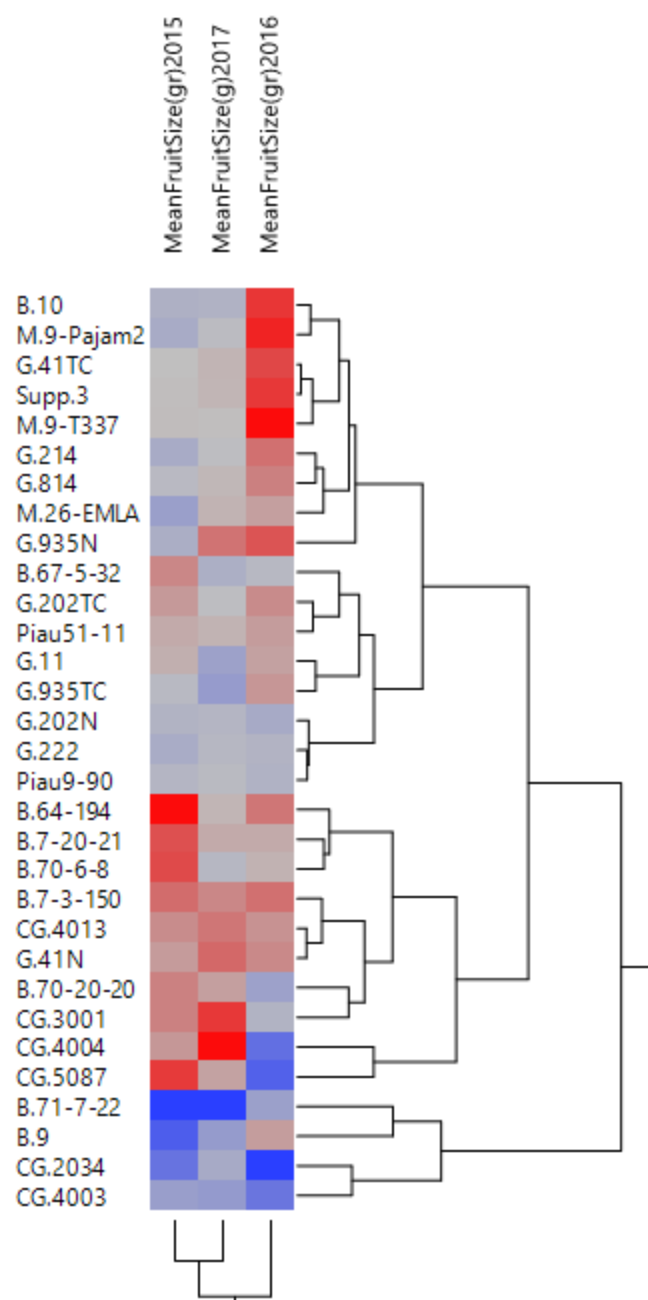


Figure 7. Two-Way similarity cluster analysis of fruit size as affected by apple rootstocks of 'Honeycrisp' apple grown on 31 rootstocks at Geneva, NY. Higher values are red whereas lower genotypic means are blue and average values are gray.

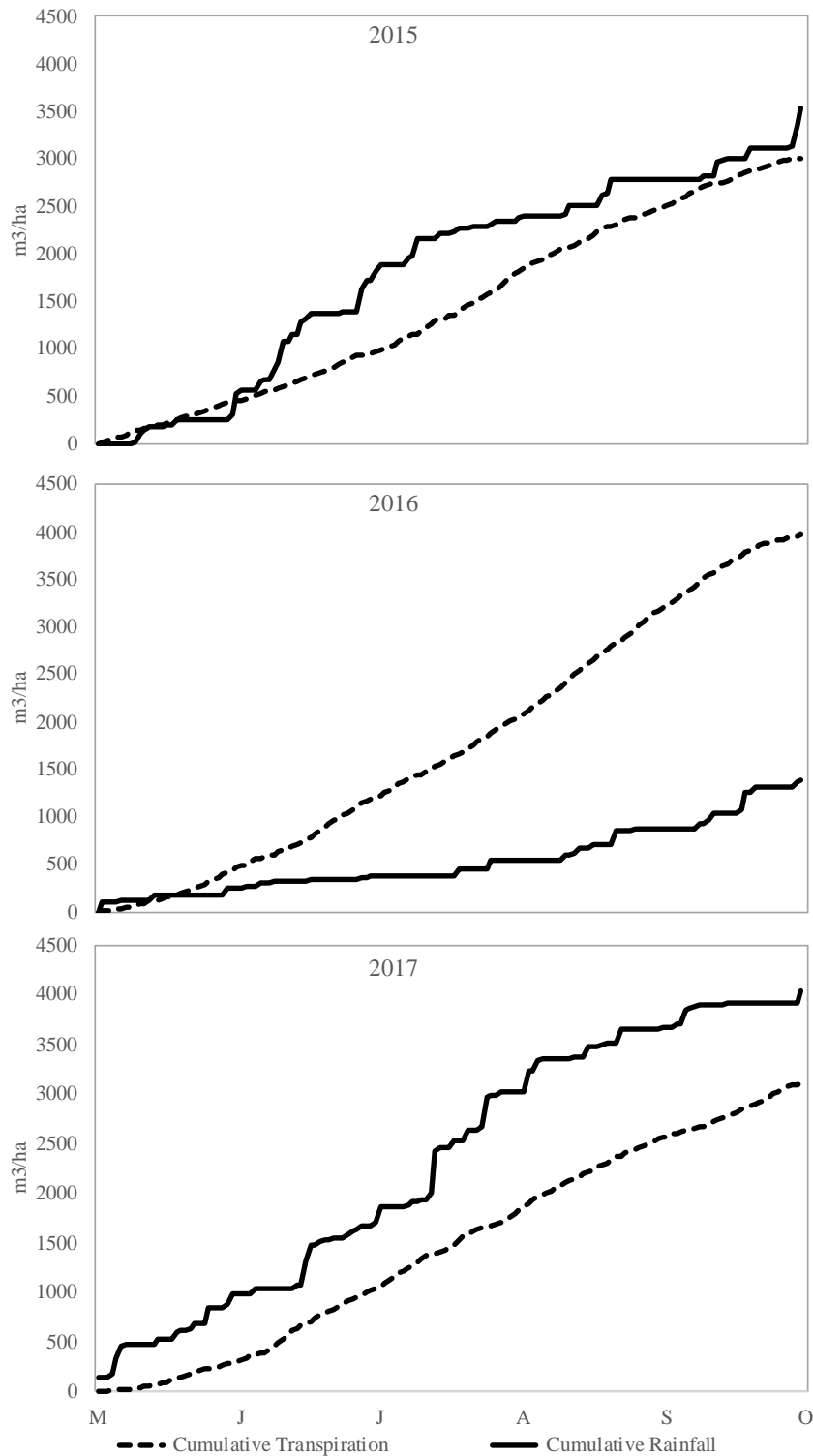


Figure 8. Cumulative rainfall and tree transpiration (m^3/ha) from May through October at Geneva Ny. Tree transpiration was calculated using a modified Penman-Monteith equation (NEWA.org) (Robinson et al. 2017).

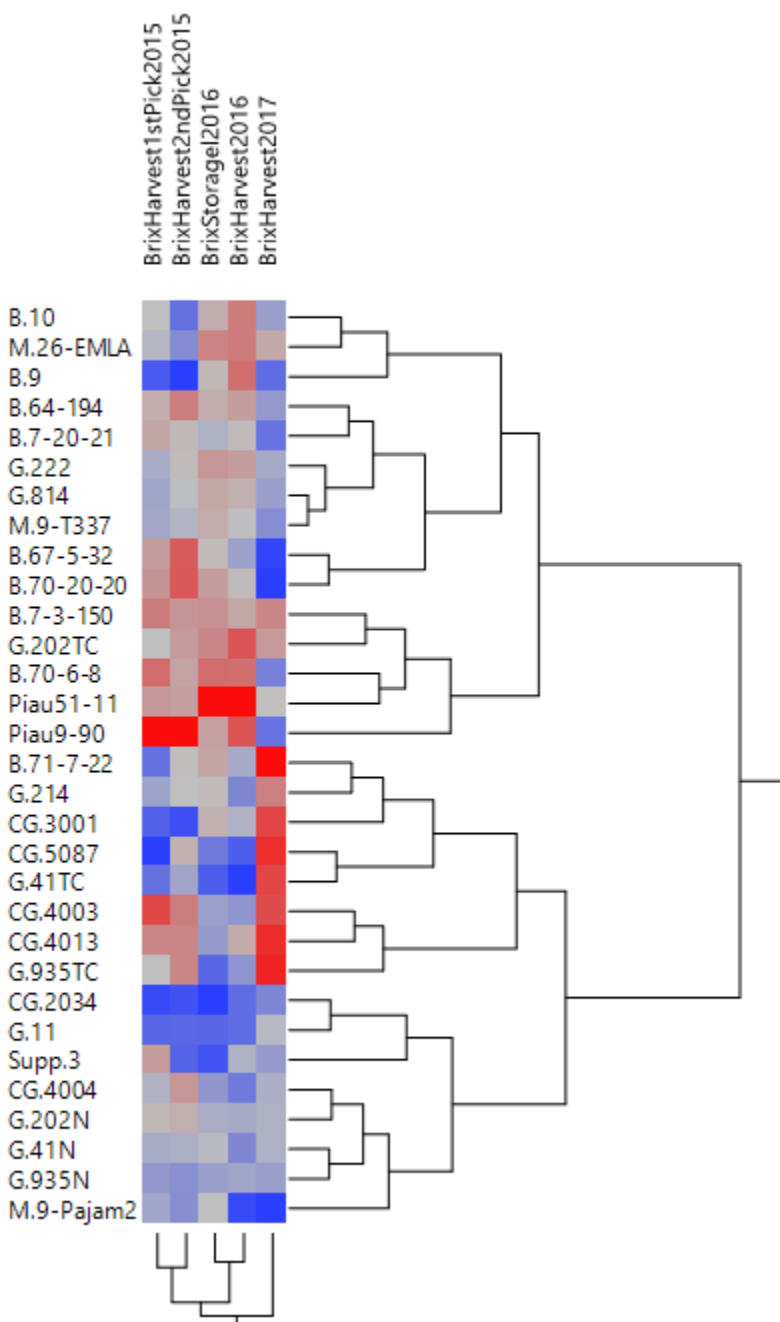


Figure 9. Two-Way similarity cluster analysis of genotypic means for fruit brix or soluble solid content of different harvest years and storage conditions of 'Honeycrisp' apple grown on 31 rootstocks at Geneva, NY. Higher values are red whereas lower genotypic means are blue and average values are gray.

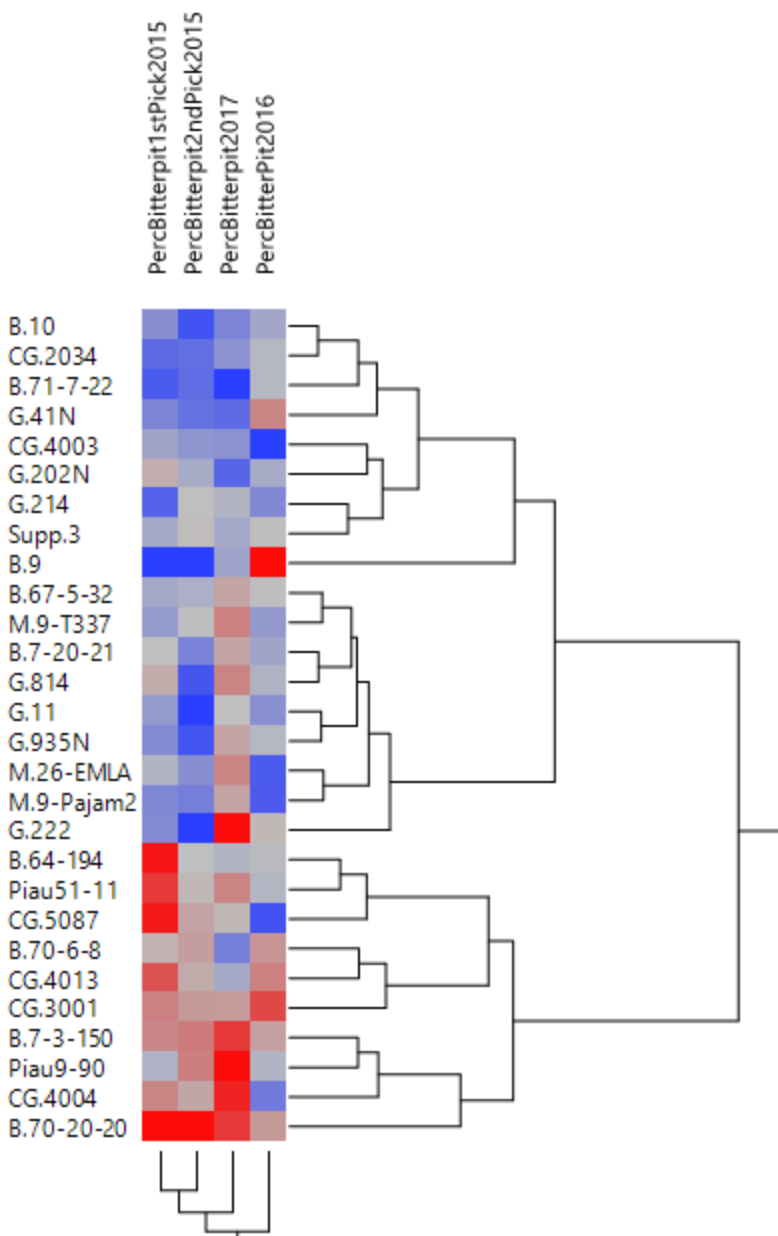


Figure 10. Two-Way similarity cluster analysis of rootstock induced mean percent bitter-pit incidence for years 2015-2017 of ‘Honeycrisp’ apple grown on 31 rootstocks at Geneva, NY. Higher values are red whereas lower genotypic means are blue and average values are gray.