

1 **Conventional and alternative pre-harvest treatments affect the quality of ‘Golden Delicious’**  
2 **and ‘York’ apple fruit**

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18

19 **Abstract**

20 Apple trees cv. ‘Golden Delicious’ and ‘York’ were sprayed from bloom to fruit maturity  
21 with different products to evaluate the effect of pre-harvest treatments on fruit quality, including  
22 insect/disease damage and physicochemical fruit traits. Apple trees were assigned to five treatments:  
23 unsprayed (control), holistic solution (foliar nutrients and probiotics), insecticides, antimicrobials  
24 (fungicides and antibiotics), and a combination of antimicrobials + insecticides. The treatments  
25 started soon after bloom and were carried out every two weeks until fruit were ready to harvest.

26 Diseases such as sooty blotch (complex of several fungi) and flyspeck (*Zygothiala jamaicensis*  
27 Mason) were the major source of damage on fruits. ‘Golden Delicious’ trees had a higher percentage  
28 of undamaged fruit than ‘York’, but all trees had some percentage of damaged fruit. Damage was  
29 most severe in the control (unsprayed) and insecticide treatments, intermediate in the holistic  
30 treatment, and much lower in the antimicrobial and antimicrobial + insecticide treatments ( $p < 0.003$   
31 for all comparisons). There was also a significant interactive effect ( $p < .0001$ ) of cultivars and pre-  
32 harvest spray treatment on apple fruit mass. For both cultivars there was a strong effect of spray  
33 treatment on size, with larger apples produced in the antimicrobial and antimicrobial + insecticide  
34 treatments, but when apple trees were not sprayed (control) or sprayed with holistic and insecticides  
35 treatments, the fruit mass was higher in ‘Golden Delicious’ than ‘York’. ‘Golden Delicious’ trees  
36 produced 1.4-fold heavier and bigger fruits compared to ‘York’ and ‘Golden Delicious’ fruit were  
37 more mature than ‘York’ at harvest. Pre-harvest treatments also affected other quality parameters of  
38 apple fruit, such as soluble solids content (SSC) and starch-iodine index. Using partial least squares  
39 discriminant analysis (PLS-DA), ‘Golden Delicious’ fruit could be well classified according to the  
40 holistic, antimicrobial, and antimicrobial + insecticide treatments. Control and insecticide samples  
41 clustered together, indicating similarities between fruit quality. Overall, pre-harvest spray treatment  
42 affected the quality of ‘Golden Delicious’ and ‘York’ apples, mainly the fruit mass and disease  
43 infection.

44 **Keywords:** *Malus x domestica* Borkh., fungicides, antibiotics, insecticides, probiotics,  
45 physicochemical composition.

46

## 47 1. Introduction

48 Apple is the fourth most consumed fruit in the world and the apple annual production in 2016  
49 reached 89,329,179 tons (FAOSTAT, 2018). Apples are popular with consumers due to their  
50 convenience and nutritional value, and the iconic image of the apple as a health-promoting fruit has

51 stimulated extensive research surrounding the health benefits of apple phytochemicals (Boyer and  
52 Liu, 2004). Various quality parameters, including pest damage and other traditional quality  
53 parameters such as soluble solids content (SSC) and firmness, are all very important attributes for  
54 apple acceptance (McCluskey et al., 2013).

55 According to Abbott (1999), quality is a term that implies “the degree of excellence of a  
56 product or its suitability for a particular use”. It is a flexible concept and can involve various  
57 properties or characteristics. The quality of fresh produce comprises many attributes, such as  
58 appearance (size, shape, color, gloss, presence of defects and decay), texture (firmness, crispness,  
59 juiciness, mealiness, and toughness), flavor (sweetness, acidity, astringency, aroma, and off-flavors),  
60 and nutritive value (vitamins, minerals, dietary fiber, phytonutrients) (Kader 2001). According to  
61 Vanoli and Buccheri (2012), consumers first evaluate produce by its appearance (presence of  
62 damage, color, size and shape) and then its eating quality. Although appearance typically determines  
63 the purchase of produces, flavor is an important quality parameter for apple consumers' acceptance  
64 (Aprea et. al., 2012) and consumers satisfaction will influence the repeat purchases (Kader, 2001).  
65 Regarding flavor, the sweet and acid taste of apple fruits are key sensory attributes for consumer  
66 preference (Jaeger et al., 1998) and SSC might be used as a predictor of sweetness while the acid  
67 taste may be predicted based on the titratable acidity (Harker et al., 2002). The relationships between  
68 SSC and titratable acidity (TA) commonly called ratio (Kader, 2001) presents good relationships  
69 between apple fruit quality and consumer acceptability, therefore it is an important quality attribute  
70 for apple evaluation.

71 Commercial quality standards for apples are based on aspects such as size, color, integrity,  
72 and presence and/or absence of defects (Musacchi and Serra, 2018). Although post-harvest  
73 management can affect the quality of apples, various pre-harvest practices can also affect and modify  
74 fruit quality attributes. The pre-harvest factors that can affect apple quality might be grouped into  
75 genetic (rootstock and cultivars), environmental (soil, light, temperature, humidity, wind), and

76 agronomic (nutrition, irrigation, training system, pruning, crop load/thinning, plant growth regulator,  
77 pollination, etc.) factors (Musacchi and Serra, 2018).

78 Apples, in particular, are heavily attacked by insects and disease and require intensive pre-  
79 harvest management to produce marketable fruit (Beers et al., 2003). For a considerable period,  
80 chemicals have been used to control pests and diseases. Starting several decades ago, apples have  
81 received more pesticides than any other fruit crop in the United States of America (Huffaker and  
82 Croft, 1978), a trend that continues today (USDA, 2018). Applying pesticides can have unanticipated  
83 effects on various components of fruit quality (Schuphan, 1961), although pesticides can be highly  
84 effective at controlling disease and insect pressure. Therefore, pre-harvest spray programs might not  
85 just control pest and diseases, but also influence apple quality parameters, such as firmness, SSC, TA  
86 and aroma (Róth et al., 2007), and these impacts should be taken in consideration in pre-harvest  
87 practices. Many studies can be found comparing the apple quality produced in conventional farming,  
88 integrated pest management systems, and organic systems (Weibel et al., 2000; Peck et al., 2006;  
89 Róth et al., 2007; Amarante et al., 2008; Jönsson et al., 2010); however, few have specifically  
90 examined the effect of pre-harvest spray procedures on fruit quality (Hutcheon et al., 1986; Palmer et  
91 al., 2003). Therefore, the objective of this study was to investigate how different pre-harvest spray  
92 programs could affect ‘Golden Delicious’ and ‘York’ apple fruit quality.

93

## 94 **2. Material and methods**

### 95 **2.1. Plant material**

96 The experiment was carried out at Virginia Polytechnic Institute and State University  
97 (Virginia Tech), Kentland Farm, Blacksburg, Virginia, the United States of America (USA). The  
98 orchard is located at 37° 11’ 23’’ North and 80° 34’ 35’’ West, 516 meters above sea level. The  
99 Köppen climate classification subtype is humid subtropical climate (Cfa).

100 The apple orchard was 16 years old and the ‘Golden Delicious’ and ‘York’ apples were  
101 grafted on M.26 rootstock apples and planted at 2.5 x 4.5 m spacing in a soil classified as Braddock  
102 loam, fine, mixed, semi active, mesic Typic Hapludults (Penn et al., 2004). The orchard has  
103 historically been managed with conventional spray programs. The orchard was not irrigated, and the  
104 fertilization program was the same for both cultivars.

## 105 **2.2. Experimental setup**

106 During the 2018 summer/fall season, 36 ‘Golden Delicious’ and 33 ‘York’ apple trees were  
107 selected for uniformity and divided into five groups, related to the following treatments: 1, control –  
108 unsprayed trees, 2, trees sprayed with a ‘holistic’ solution, 3, trees sprayed with insecticides, 4, trees  
109 sprayed with antimicrobials (fungicides and antibiotics), and 5, trees sprayed with antimicrobials +  
110 insecticides. Details on the components of each treatment are provided in Table 1. The holistic  
111 treatment was a combination of products developed and recommended by Phillips (2012) and it is  
112 currently commercially marketed from organic grower supply companies (e.g. Fedco Seeds). It  
113 includes macronutrients and trace minerals that can act as foliar fertilizers, probiotics and nutrients  
114 intended to support microorganisms on the plant surface, and neem oil extracts that can act as  
115 botanical insecticides. To our knowledge, this spray has not been evaluated in a scientific context for  
116 its effects on fruit quality. The treated apple trees were separated by one guard tree and a buffer row  
117 to avoid the effect of spray drift. Apple trees were hand thinned, aiming to remove excessive fruitlets  
118 from the plants. The chemical products sprayed were applied on the trees every two weeks beginning  
119 at bloom and continuing through mid-September (Table 1).

120 The experiment was laid down according to a randomized complete block design (RCBD)  
121 with two blocks (cultivars – ‘Golden Delicious’ and ‘York’) containing all five treatments (1,  
122 control, 2, holistic, 3, insecticides, 4, antimicrobials, and 5, combination antimicrobials +  
123 insecticides) with 6-8 replicate apple trees per treatment/cultivars combination. ‘Golden Delicious’  
124 fruit harvest was carried out from October 3<sup>rd</sup> to 12<sup>th</sup> and ‘York’ from October 12<sup>th</sup> to 19<sup>th</sup> 2018.

### 125 **2.3. Fruit damage evaluation**

126 At maturity, apple samples were collected for fruit damage evaluation. Fruits from all  
127 treatments were harvested, with up to 20 fruits per tree and totaling 684 ‘Golden Delicious’ and 395  
128 ‘York’ fruit. The apple fruit were evaluated according to the following pest and diseases:

129 *Pests.* Insect damage was identified following the descriptions reported by Agnello et al.  
130 (2006). The most common insect damage was caused by plum curculio beetles (*Conotrachelus*  
131 *nenuphar* Herbst) and the number of fruits with the typical crescent-shaped blemishes were counted  
132 and the data transformed using the square root of  $x+1$ .

133 *Diseases.* The symptoms of the most common diseases were identified following the  
134 description of Agnello et al. (2006). The presence of sooty blotch, attributed to a complex of  
135 different fungi, and flyspeck (*Zygothiala jamaicensis* Mason), were evaluated using a five-point  
136 scale, 0 = undamaged fruit, 1 = < 5%, 2 = 5 – 25%, 3 = 25 – 50%, 4 = 50 – 75%, and 5 = > 75% skin  
137 coverage. Cedar apple rust disease (*Gymnosporangium juniperi-virginianae*) was also observed and  
138 the number of fruits with the typical pale-yellow pinhead sized spots were counted and the data  
139 transformed using the square root of  $x+1$ .

### 140 **2.4. Quality evaluations**

141 At maturity, apple samples were also collected for quality evaluation. Three apples were  
142 harvested per plant from all treatments, totaling 207 fruit. The apple fruit were evaluated according  
143 to the following quality parameters:

144 *Fruit mass.* The mass was determined for all harvested fruit using an analytical balance  
145 (Radwag, model AS 60/220-R2, Miami Beach, USA) and the mass results were expressed in grams  
146 (g).

147 *Firmness.* The pulp firmness was determined using a penetrometer (Fruit Hardness Tester,  
148 model FHT-1122, Merit Technology, Shahekou, China) with a 11.0 mm tip. The determinations  
149 were performed on each fruit after removing the peel. The results were expressed in Newton (N).

150 *Fruit maturity.* The harvest maturity was determined using the Cornell starch-iodine index  
151 (Blanpied and Silsby, 1992). To assess maturity, apple fruit were cut, and the slices were placed into  
152 iodine solution for 30 seconds and allowed to dry for 20 minutes. The color of the slices was  
153 compared to the Cornell starch-iodine chart and the maturity recorded from 1 (immature) to 8 (ripe).

154 *Soluble solids content.* Freshly squeezed apple juice was used to determine the soluble solids  
155 content (SSC). A handheld refractometer BX-20 (Veegee Analytical Instruments, Kirkland, USA)  
156 was used and the SSC was expressed as mass percentage (%) in the solution, A.O.A.C. (1997).

157 *Moisture.* The moisture content of the apple pulp was determined by the sample's loss in  
158 mass after drying for 70 hours at 65 °C in an oven/incubator (Type 19200, Thermolyne, Thermo  
159 Fisher Sci. Inc., Waltham, USA), which allowed samples to reach constant mass (A.O.A.C., 1997).

160 *Dry matter.* The dry matter (DM) content was determined using the formula  $DM = 100 - M$ ,  
161 where: DM = dry matter and M = moisture content. DM content was expressed as gram per kilogram  
162 (A.O.A.C., 1997).

## 163 **2.5. Univariate statistical analysis**

164 To compare individual quality parameters among treatments, the data were subjected to  
165 analysis of variance (ANOVA) using the PROC GLM procedure of the Statistical Analysis System  
166 (SAS, 1999). Treatment and cultivars were included as fixed effects, tree was included as a random  
167 effect, and the treatment means were compared using the Tukey's test at a significance level of  $p <$   
168 0.05.

## 169 **2.6. Multivariate statistical analysis**

170 Multivariate analyses were carried out first using the physicochemical traits and then with all  
171 quality parameters (including damage) to assess the overall differences among treatments. The  
172 discriminant models were developed separately for each cultivar and combining the results of both  
173 averaging the data per tree within each pre-harvest treatment as the data was obtained from different  
174 fruit numbers.

175 The data were processed using MATLAB® R2014b software (MathWorks, USA) with PLS  
 176 Toolbox version 7.9.3 (Eigenvector Research, Inc., USA). The data were auto scaled before analysis.  
 177 Samples were divided into training (70 %) and test (30 %) sets using the Kennard-Stone uniform  
 178 sample selection algorithm (Kennard and Stone, 1969). The training set was used for model  
 179 construction and the test set for final model evaluation. Cross-validation venetian blinds with eight  
 180 data splits was employed for model optimization.

181 Initially, principal component analysis (PCA) was employed for exploratory analysis of the  
 182 data (Bro and Smilde, 2014). Sample classification was then performed using the partial least squares  
 183 discriminant analysis (PLS-DA) algorithm (Brereton and Lloyd, 2014). The main difference between  
 184 PLS-DA and PCA is that PLS decomposes the data in an interactive process involving both the  
 185 experimental observations and category information, therefore generating scores and loadings for  
 186 both data sets as follows:

$$187 \quad \mathbf{X} = \mathbf{TP} + \mathbf{E} \quad (01)$$

$$188 \quad \mathbf{y} = \mathbf{Tq} + \mathbf{f} \quad (02)$$

189 Where  $\mathbf{X}$  is a matrix containing the experimental observations;  $\mathbf{y}$  is a vector containing the  
 190 sample's category (e.g., 0/1);  $\mathbf{T}$  is a common scores matrix;  $\mathbf{P}$  is matrix containing the loadings of the  
 191 experimental observations;  $\mathbf{E}$  are the data residuals;  $\mathbf{q}$  represents the loadings of the category  
 192 variables; and  $\mathbf{f}$  the category residuals. In PLS-DA, a linear classifier is employed to the predicted  
 193 PLS response  $\hat{\mathbf{y}}$  separating the data into groups, where  $\hat{\mathbf{y}}$  is estimated using the regression coefficients  
 194  $\mathbf{b}$  as follows:

$$195 \quad \hat{\mathbf{y}} = \mathbf{Xb} = \mathbf{X}[\mathbf{W}(\mathbf{PW})^{-1}\mathbf{q}] \quad (03)$$

196 In which  $\mathbf{W}$  is the weight matrix (Brereton and Lloyd, 2014).

197

198 One of the most popular measures is the area under the ROC curve (AUC). AUC is a  
 199 combined measure of sensitivity (Equation 4) and specificity (Equation 5), respectively. AUC is a

200 measure of the overall performance of a diagnostic test and is interpreted as the average value of  
 201 sensitivity for all possible values of specificity. It can take on any value between 0 and 1, since both  
 202 the x and y axes have values ranging from 0 to 1.

$$203 \text{ sens (\%)} = \frac{TP}{TP+FN} \times 100 \quad (04)$$

$$204 \text{ spec (\%)} = \frac{TN}{TN+FP} \times 100 \quad (05)$$

205 TP is true positive, FP is false positive, TN is true negative and FN is false negative.

206

### 207 3. Results

#### 208 3.1. Fruit damage evaluation

209 All fruit presented some sort of damage (disease and/or insect) and it was more severe in the  
 210 control (unsprayed), holistic and insecticide treatments, which resulted in 0.0% undamaged fruit. The  
 211 antimicrobial treatments resulted in 15.0% and 6.67% undamaged fruit for ‘Golden Delicious’ and  
 212 ‘York’, respectively. This percentage was increased to 48.57% when ‘Golden Delicious’ trees were  
 213 sprayed with the antimicrobial + insecticide treatment, compared to only 10.71% for ‘York’ (Figure  
 214 1). In the ANOVA assessing how pre-harvest spray treatment and cultivars affected the percentage  
 215 of undamaged fruit, there was a significant interaction between cultivars and treatment ( $F_{9,62}=6.32$ ,  
 216  $p=0.0003$ ), a significant difference among treatments ( $F_{9,62}=29.84$ ,  $p<.0001$ ) and a significant  
 217 difference between cultivars ( $F_{9,62}=8.19$ ,  $p=0.0060$ ; Figure 1). ‘Golden Delicious’ presented the  
 218 highest average percentage of undamaged fruit (12.71%) compared to ‘York’ (4.11%). Based on the  
 219 interaction between treatment and cultivars, differences among treatments were also analyzed  
 220 separately for each cultivar (Figure 1). The major difference between cultivars was that in ‘Golden  
 221 Delicious’ apples the antimicrobial + insecticide treatment was more clearly distinguishable from the  
 222 antimicrobial only treatment, resulting in a higher percentage of undamaged fruit (Figure 1).

223 Fruits from both cultivars were severely infected by sooty blotch and flyspeck, but the  
 224 severity varied among treatments and cultivars (Figure 2). Infection was strongly reduced when

225 plants were sprayed with antimicrobials or antimicrobials + insecticides (Table 2). There was also a  
226 significant reduction in infection severity in the holistic treatment compared to the insecticide  
227 treatment and controls (Table 2). In addition, the severity of both flyspeck and sooty blotch was  
228 higher in ‘York’ apples compared to ‘Golden Delicious’ (Table 2). Cedar apple rust disease was also  
229 present on leaves and fruits and caused minor damages. There were no differences in this disease  
230 among treatments, but ‘York’ fruit were more affected than ‘Golden Delicious’ (Table 2).

231         Regarding insect damage, the most common blemish was caused by plum curculio beetles,  
232 but no significant differences ( $p>0.1526$ ) were observed between cultivars or among pre-harvest  
233 treatments ( $p>0.0688$ ; Table 2).

### 234         **3.2. Fruit quality evaluation**

235         Apple fruit mass was significantly affected by the pre-harvest treatments, the cultivars, and  
236 the interaction between treatment and cultivars (Table 3; Figure 3). Both cultivars produced heavier  
237 fruit in the antimicrobial + insecticide treatment, intermediate size fruit in the antimicrobial  
238 treatment, and the smallest fruits in the control, holistic, and insecticide treatments (Figure 3);  
239 however, the differences among treatments were more pronounced for ‘York’ apples than for  
240 ‘Golden Delicious’. ‘Golden Delicious’ trees also produced 1.4-fold heavier and bigger fruits  
241 ( $110.61\pm 22.80$  g) compared to ‘York’ ( $76.67\pm 40.42$  g).

242         For the physicochemical aspects of fruit quality, the different spray treatments affected only  
243 the SSC and maturity (Table 3). The highest SSC was observed in apples from the holistic spray  
244 treatment and the lower starch-iodine index was obtained in apples sprayed with antimicrobials  
245 (Table 3). In addition, most of these variables differed between the two cultivars (Table 3). At  
246 harvest, ‘Golden Delicious’ apples were more mature than ‘York’ based on the starch-iodine index  
247 (Table 3). ‘Golden Delicious’ fruit also had higher mean SSC, fruit firmness, and dry-matter content,  
248 but lower moisture content than ‘York’ fruit (Table 3).

### 249         **3.3. Multivariate analysis**

250 *3.3.1. Physicochemical discrimination models*

251 Using separate PCAs for ‘Golden Delicious’ and ‘York’ apple samples, a tendency was  
252 observed of separation between two clusters, one representing the control, holistic, and insecticide  
253 treatments and another representing the antimicrobial and antimicrobial + insecticide treatments  
254 (Figure 1S-A, Figure 1S-C). Samples mainly separated along PC2, which was associated with  
255 increasing fruit maturity and decreasing fruit mass (Figure 1S-B, Figure 1S-D). However, a clear  
256 separation between clusters was not observed as superposition of samples was observed along PC1  
257 and PC2.

258 Better separation between the two clusters was obtained by using PLS-DA (Table 1S). For  
259 ‘Golden Delicious’ samples, the antimicrobials had the best discriminatory values with an area under  
260 the curve (AUC) of 0.97 (almost perfect classification), indicating that this class was highly different  
261 from the others. The holistic, insecticide and the combination of antimicrobial + insecticides have  
262 fair classification results (AUC ranging from 0.69 to 0.76). The control samples had the worst  
263 classification result (AUC=0.57). For ‘York’ samples, the classification performance was slightly  
264 better. The antimicrobials treatment still had the best classification (AUC=0.89), and the AUC values  
265 improved for the control, insecticide, and the combination of antimicrobial + insecticides.

266 The discriminant function (DF) and PLS-DA coefficients for ‘Golden Delicious’ and ‘York’  
267 samples can be seen in Figure 2S. The fruit mass and fruit maturity in ‘Golden Delicious’ samples  
268 were the main parameters associated with the good classification of antimicrobial and insecticide  
269 pre-harvest sprayed samples; while for the holistic treatment, SSC, moisture, and DM were the most  
270 important parameters (Figure 2S-B). For ‘York’ samples, mass and fruit maturity were also the main  
271 parameters responsible for all class differentiations. SSC and moisture had little influence, except for  
272 the holistic and control samples; and firmness influenced only the antimicrobial and antimicrobial +  
273 insecticide samples.

274 A second analysis was performed combining all ‘Golden Delicious’ and ‘York’ samples into  
275 the same dataset. The classification rate remained similar to the previous results, indicating that  
276 differences between apple cultivars did not influence the pre-harvest treatment classification  
277 outcome (Table 2S).

### 278 3.3.2. *Physicochemical + damage discrimination models*

279 The inclusion of the fruit damage evaluation improved the discrimination power of the PCA  
280 models. Better separation between the two clusters (control, holistic, and insecticide treatments  
281 *versus* antimicrobial and antimicrobial + insecticide treatments) were observed for ‘Golden  
282 Delicious’ samples (Figure 4A). There was strong separation of these groups along PC1, with the  
283 antimicrobial and antimicrobial + insecticide treatments associated with increasing fruit mass,  
284 percent undamaged fruit, and cedar apple rust disease, and decreasing fruit maturity, flyspeck, and  
285 sooty blotch (Figure 4B). A better separation was also observed for ‘York’ samples. Patterns were  
286 similar to those for ‘Golden Delicious’, however, the antimicrobial and antimicrobial + insecticide  
287 treatments were also associated with decreasing plum curculio damage (Figure 4D).

288 When PLS-DA was used to develop the discrimination models it was possible to get an  
289 excellent separation between some pre-harvest treatments (Table 4). A perfect classification  
290 (AUC=1.00) was observed in ‘Golden Delicious’ samples from the holistic, antimicrobial and  
291 antimicrobial + insecticide treatments, and a value of 0.96 (almost perfect classification) was found  
292 for the insecticide treatment (Table 4). Again, the control samples had the worst classification result  
293 (AUC=0.81), indicating that the model was not able to clearly distinguish these samples from the  
294 others. The sensitivity values for all classes reached 100%, and the best specificity (97%) was  
295 observed for the antimicrobial + insecticide treatment. The classification performance for ‘York’  
296 samples was slightly inferior. The antimicrobial treatment had the best classification (AUC=0.87,  
297 sensitivity=100%, specificity=80%), and the AUC values decreased for the control (AUC=0.77),  
298 insecticide (AUC=0.84), and antimicrobial + insecticide (AUC=0.83) treatments. A sensitivity of

299 100% was observed for the antimicrobial and antimicrobial + insecticide treatments, indicating that  
300 these classes can be clearly differentiated from the others (Table 4). The best specificity (100%) was  
301 observed for the holistic treatment.

302 The main quality parameters associated with the good classification of each pre-harvest  
303 treatment can be evaluated based on the discriminant function (DF) and PLS-DA coefficients (Figure  
304 5). Overall, classification of ‘Golden Delicious’ fruit quality was related to fruit mass, fruit maturity  
305 and the number of undamaged fruit (Figure 5B). On the other hand, damage caused by flyspeck,  
306 sooty blotch and cedar apple rust had the largest influence on the unsprayed, holistic, and insecticide  
307 treatments. However, fruit mass and SSC were also important parameters (Figure 5B). A similar  
308 trend was observed for ‘York’ samples (Figure 5C and 5D).

309 When ‘Golden Delicious’ and ‘York’ samples were combined, the PLS-DA classification  
310 performance was inferior (Table 5) compared to the previous attempt with each cultivar separately  
311 (Table 4). This indicates that each cultivar responded differently to the pre-harvest treatments. An  
312 almost perfect classification (AUC=0.98) was obtained for the antimicrobial + insecticide treatment,  
313 but lower accuracy values were observed for the control (AUC=0.83), holistic (AUC=0.88),  
314 insecticide (AUC=0.85), and antimicrobial (AUC=0.85) treatments (Table 5). However, a sensitivity  
315 of 100% was observed for the insecticide, antimicrobial, and antimicrobial + insecticide treatments.  
316 The control (sensitivity=80%, specificity=78%) and holistic samples (sensitivity=80%,  
317 specificity=70%) were misclassified (Table 5), but with better performance when compared to the  
318 models that used only physicochemical parameters (Table 1S and 2S).

319

#### 320 **4. Discussion**

321 Pre-harvest spray treatments can affect various quality parameters of fruit, with downstream  
322 consequences for market value, agricultural sustainability, and human health. As appearance  
323 determines the purchase intention of produce (Vanoli and Buccheri, 2012), the presence of damage

324 caused by diseases and/or insects, even if primarily cosmetic, is an important quality parameter.  
325 Other physicochemical parameters are also critical for determining market value and consumer  
326 acceptance of apples. This study showed that pre-harvest treatments can impact various aspects of  
327 fruit quality, including disease incidence, mass, soluble solid content, and maturity and that the  
328 magnitude of these effects varies among apple cultivars.

329         The presence of blemishes mainly caused by cosmetic diseases (sooty blotch and fly speck) in  
330 both cultivars severely impaired the fruit quality, which resulted in a low percentage of undamaged  
331 fruits. Overall, better fruit quality was observed when antimicrobials were used as pre-harvest  
332 treatments, as the fungicides used were very effective to control sooty blotch and flyspeck  
333 (Williamson and Sutton, 2000). Sooty blotch and flyspeck are among the most common diseases of  
334 pome fruits in humid temperate growing regions of the world, such as Virginia, USA (Williamson  
335 and Sutton, 2000). These diseases are particularly severe in the southeastern USA and are considered  
336 of great economic importance as fruit become unsuitable for fresh market due to the reduced fruit  
337 quality. The overall quality of untreated fruit and fruit sprayed with holistic and insecticide  
338 treatments was severely affected by the presence of these two diseases. Although the infection levels  
339 were significantly lower in the holistic treatment (Table 2), these fruits still all had some level of  
340 damage (Figure 1). These fruits may be useful for processing, but fruit quality was not satisfactory  
341 for the fresh market.

342         Other damaging agents observed in the orchard, including cedar apple rust and plum curculio,  
343 were more minor. The fungicide spray program used in this study was not optimized to control cedar  
344 apple rust, as the typical spray during tight cluster was missed and the bloom spray included only  
345 captan as a fungicide, which provides only slight protection against rusts (Pfeiffer et al. 2018).  
346 Interestingly, the treatment with insecticides did not result in lower damage caused by plum curculio  
347 beetles, although Imidan, which was first applied at petal fall, is rated as excellent for control of  
348 these insects (Pfeiffer et al. 2018). It is worth noting that we observed limited insect pressure, which

349 may have been unique to this season and/or the result of low insect populations due to a long history  
350 of conventional management in this orchard.

351 Both cultivars produced heavier and bigger fruit when plants were sprayed with antimicrobial  
352 or antimicrobial + insecticide treatments. However, the effects on size were more pronounced in  
353 ‘York’ apples (Figure 3). Both ‘Golden Delicious’ and ‘York’ cultivars bear medium size to large  
354 fruits (Ingle and D’Souza, 2000; Burford, 2013; Ornelas-Paz et al., 2018), yet ‘Golden Delicious’  
355 trees produced heavier and bigger fruit compared to ‘York’ (Figure 3). Hatcher (1995) reported that  
356 in infected leaves the overall photosynthesis declines and the transport of photoassimilate is also  
357 affected. The infected leaf exports less photoassimilate (Walters and Ayres, 1982) and exports can  
358 almost cease as the infection develops (So and Thrower, 1976). Therefore, it is likely that the  
359 antimicrobials controlled fungal infection in the canopy and on the fruit surface, allowing the plant to  
360 translocate more photoassimilates to fruit and bear heavier and bigger fruits. The same trend was  
361 observed by Hutcheon et al. (1986) in ‘Cox’s Orange Pippin’ apple trees sprayed with different  
362 fungicides.

363 The other main fruit quality parameter that was affected by the pre-harvest treatments was the  
364 soluble solid content, which was significantly higher in the holistic treatment compared to other  
365 treatments. The holistic treatment contained a variety of products, including fish and kelp-based  
366 fertilizers and certain microorganisms that might directly benefit the plant host by stimulating plant  
367 immune responses and/or acting as biocontrol agents (Song et al., 2012; Phillips, 2012). Past work  
368 has shown that foliar fertilization with certain nutrients such as Zn, B, P, and Ca can increase the  
369 content of sucrose, glucose, fructose, and sorbitol in apples (Stampar et al. 1999); however, in  
370 another study, fertilization with N and Zn decreased soluble solid content (Amiri et al. 2008).  
371 Considering the mix of products in the holistic treatment, the mechanism of observed changes in  
372 fruits is unclear, but overall this treatment did improve the quality of fruits somewhat relative to the  
373 controls by both increasing sugar content of fruit and reducing the severity of damage from disease.

374 It is important to note that, in this study, the holistic spray was taken out of the context of a larger  
375 holistic program in which it is recommended (Phillips 2012) and applied over a single growing  
376 season, thus additional or different effects on quality may be seen in a different agroecological  
377 context.

378 Fruit quality parameters also differed strongly between the two cultivars (Table 3). ‘Golden  
379 Delicious’ is an early season cultivar which bears apples with green to yellow skin. On the other  
380 hand, ‘York’ is a late season cultivar with light red blush to full red skin apples (Virginia Apples,  
381 2018). Consequently, differences in fruit quality might be related to these physiological differences,  
382 especially fruit maturity. ‘Golden Delicious’ is an early-season cultivar and was harvested ahead of  
383 ‘York’ apples. Still, ‘Golden Delicious’ apples were more mature than ‘York’ at the time of harvest.  
384 Therefore, fruits were sweeter, with a higher SSC and a higher starch-iodine index, indicating starch  
385 degradation into soluble sugars (Doerflinger et al., 2015). The more advanced maturity of ‘Golden  
386 Delicious’ apples was also confirmed by the higher moisture and lower DM contents. These findings  
387 agree with Ornelas-Paz et al. (2018), who also reported increases in moisture content, and  
388 consequent DM reduction, in ‘Golden Delicious’ apples during on-tree development. Fruit firmness  
389 was higher than ‘York’, but were in the range of what is commonly reported for ‘Golden Delicious’  
390 produced in other regions (Felicetti and Mattheis 2010). On the other hand, as ‘York’ is a late season  
391 cultivar, the fruit were less mature, with lower SSC, starch-iodine index, and moisture content, and  
392 higher DM content. Fruit firmness was lower than ‘Golden Delicious’, though. According to Ingle  
393 and D’Souza (2000), due to the local commercial importance of ‘York’ apple, few publications are  
394 available regarding maturation and storage of this cultivar. Our results provide additional  
395 information on the factors affecting fruit quality in this cultivar.

396 The multivariate analysis confirmed the quality differences observed in the univariate  
397 analysis and it was possible to obtain a clear separation between pre-harvest treatments, mainly when  
398 the fruit damage evaluation was incorporated into the dataset. Using PCA, which is an unsupervised

399 exploratory data analysis (Bro and Smilde, 2014), it was possible to observe the formation of just  
400 two clusters representing the main quality differences. On the other hand, using a supervised  
401 algorithm PLS-DA (Brereton and Lloyd, 2014) it was possible to improve the discrimination  
402 between classes. With this algorithm, apple samples of ‘Golden Delicious’ cultivar could be  
403 correctly classified according to the holistic, antimicrobial, and antimicrobial + insecticide  
404 treatments, and the proportion of positive samples correctly identified (sensitivity) reached 100% for  
405 all pre-harvest treatments. The performance of the PLS-DA models for ‘York’ samples was not as  
406 accurate as ‘Golden Delicious’, possibly because we used fewer samples from ‘York’ to develop the  
407 models. Therefore, the inclusion of more data into the dataset improved the robustness and increased  
408 the classification accuracy.

409

## 410 **5. Conclusions**

411 Pre-harvest spray programs affected ‘Golden Delicious’ and ‘York’ apple fruit quality mainly  
412 by controlling in-field disease development which ultimately affected fruit mass. Apple trees sprayed  
413 with antimicrobial and antimicrobial + insecticide treatments had less damage caused by diseases  
414 and produced bigger and heavier fruit compared to unsprayed (control) trees and those treated with  
415 holistic and insecticide sprays. The holistic spray also reduced the severity of sooty blotch and  
416 flyspeck somewhat relative to controls.

417 Pre-harvest spray treatments also affected the soluble solids content (SSC) and fruit maturity  
418 with the holistic treatment resulting in fruit with higher SSC and the antimicrobial treatment resulting  
419 in fruit with lower starch-iodine index.

420 PCA-LDA and especially PLS-DA were both useful to discriminate fruit quality, but better  
421 pre-harvest spray treatment discrimination was achieved using PLS-DA with ‘Golden Delicious’  
422 fruit. Fruits were well classified according to the holistic, antimicrobial, and antimicrobial +

423 insecticide pre-harvest treatments. Unsprayed and insecticide treated fruit clustered together,  
424 indicating similarities between fruit quality, especially for ‘York’ fruit.

425         These results contribute to a broader understanding of the factors impacting fruit quality in  
426 one of the most economically important fruit crops in temperate regions.

427

#### 428 **Acknowledgments**

429         The authors would like to thank the Virginia Tech Kentland Farm personnel, especially  
430 Brooks Saville, Brent Stewart, and Ricky Hughes, for their support during the field spray treatments.  
431 This project was supported by start-up funds to S.R. Whitehead from Virginia Tech. K.M.G. Lima  
432 acknowledges the CNPq (grant 303733/2017-9) for financial support.

433

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524

525

526 **Tables**

527

528 **Table 1.** Spray dates and products used as pre-harvest treatments on ‘Golden Delicious’ and ‘York’

529 apple trees\* during 2018 season in Blacksburg, Virginia, USA.

<b>Date</b>	<b>Phenological event</b>	<b>Treatment</b>	<b>Product(s)</b>
23 April 2018	Bloom	Untreated	None
		Holistic	Liquid fish, effective microbes, molasses, liquid kelp
		Insecticides	None
		Antimicrobials	Captan® <sup>1</sup> and streptomycin
		Antimicrobials+fungicides	Captan® and streptomycin
07 May 2018	Petal fall	Untreated	None
		Holistic	Neem oil, soap emulsifier, liquid fish, effective microbes, molasses, liquid kelp
		Insecticides	Imidan® <sup>2</sup>
		Antimicrobials	Mancozeb® <sup>3</sup> and Oxytetracycline
		Antimicrobials+fungicides	Imidan®, Mancozeb®, and Oxytetracycline
21 May 2018	First cover	Untreated	None
		Holistic	Neem oil, soap emulsifier, liquid fish, effective microbes, molasses, liquid kelp
		Insecticides	Altacor® <sup>4</sup>
		Antimicrobials	Captan® and streptomycin
		Antimicrobials+fungicides	Altacor®, Captan®, and streptomycin
04 June 2018	Second cover	Untreated	None
		Holistic	Neem oil, soap emulsifier, liquid fish, effective microbes, molasses, liquid kelp
		Insecticides	Imidan®
		Antimicrobials	Mancozeb®, and Oxytetracycline
		Antimicrobials+fungicides	Imidan®, Mancozeb®, and Oxytetracycline
18 June 2018	Third cover	Untreated	None
		Holistic	Neem oil, soap emulsifier,

			liquid fish, effective microbes, molasses, liquid kelp Altacor® Captan® and streptomycin Altacor®, Captan®, and streptomycin
02 July 2018	Fourth cover	Untreated Holistic  Insecticides Antimicrobials Antimicrobials+fungicides	None Neem oil, soap emulsifier, liquid fish, effective microbes, molasses, liquid kelp Imidan® Mancozeb®, and Oxytetracycline Imidan®, Mancozeb®, and Oxytetracycline
16 July 2018	Fifth cover	Untreated Holistic  Insecticides Antimicrobials Antimicrobials+fungicides	None Neem oil, soap emulsifier, liquid fish, effective microbes, molasses, liquid kelp Altacor® Captan® and streptomycin Altacor®, Captan®, and streptomycin
30 July 2018	Sixth cover	Untreated Holistic  Insecticides Antimicrobials Antimicrobials+fungicides	None Neem oil, soap emulsifier, liquid fish, effective microbes, molasses, liquid kelp Imidan® Mancozeb®, and Oxytetracycline Imidan®, Mancozeb®, and Oxytetracycline
13 August 2018	Seventh cover	Untreated Holistic  Insecticides Antimicrobials Antimicrobials+fungicides	None Neem oil, soap emulsifier, liquid fish, effective microbes, molasses, liquid kelp Altacor® Captan® and streptomycin Altacor®, Captan®, and streptomycin
27 August 2018	Eighth cover	Untreated Holistic	None Neem oil, soap emulsifier,

				liquid fish, effective microbes, molasses, liquid kelp
		Insecticides		Imidan®
		Antimicrobials		Mancozeb®, and Oxytetracycline
		Antimicrobials+fungicides		Imidan®, Mancozeb®, and Oxytetracycline
10 2018	September Ninth cover	Untreated		None
		Holistic		Neem oil, soap emulsifier, liquid fish, effective microbes, molasses, liquid kelp
		Insecticides		Altacor®
		Antimicrobials		Captan® and streptomycin
		Antimicrobials+fungicides		Altacor®, Captan®, and streptomycin

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530 \*Sprays started from bloom to fruit maturity and the whole apple plants were sprayed using a tow  
531 behind sprayer. <sup>1</sup>N-(trichloromethylthio) cyclohex-4-ene-1,2-dicarboximide, <sup>2</sup>N-(Mercaptomethyl)  
532 phthalimide, S-(O,Q-dimethyl phosphorodithioate, <sup>3</sup>Manganese ethylenebis(dithiocarbamate)  
533 (polymeric) complex with zinc salt, <sup>4</sup>3-Bromo-N-[4-chloro-2-methyl-6-  
534 [(methylamino)carbonyl]phenyl]-1-(3-chloro-2-pyridinyl)-1H-pyrazole-5-carboxamide.

535

536 **Table 2.** Fruit damage evaluation of ‘Golden Delicious’ and ‘York’ apples submitted to different  
 537 pre-harvest spray treatments during the 2018 growing season in Blacksburg, Virginia, USA.

Parameters	Flyspeck (0 to 5) <sup>1</sup>	Sooty blotch (0 to 5) <sup>2</sup>	Cedar apple rust (%) <sup>3</sup>	Plum curculio (%) <sup>4</sup>
<b>Cultivars (C)</b>				
‘Golden Delicious’ <sup>35 trees, 684 fruits</sup>	2.32±1.87 b	2.40±1.78 b	3.86±5.30 b	7.29±8.43
‘York’ <sup>28 trees, 394 fruits</sup>	2.70±1.63 a	3.14±1.71 a	12.11±12.21 a	12.18±18.41
F <sub>9,62</sub>	4.85	47.40	14.15	2.11
p-value	0.0320	<.0001	0.0004	0.1526
<b>Treatments (T)</b>				
Control <sup>11 trees, 191 fruits</sup>	4.43±0.78 a	4.48±0.60 a	8.91±14.46	14.55±19.39
Holistic <sup>12 trees, 176 fruits</sup>	3.15±0.64 b	3.47±0.73 b	3.75±7.42	16.42±19.20
Insecticides <sup>13 trees, 172 fruits</sup>	4.07±1.11 ab	4.64±0.51 ab	8.08±11.24	6.00±9.11
Antimicrobials <sup>13 trees, 259 fruits</sup>	0.86±0.62 c	0.92±0.53 c	9.31±7.66	7.00±9.37
Insecticides+antimicrobials <sup>14 trees, 280 fruits</sup>	0.63±0.50 c	0.87±0.61 c	2.50±8.26	5.00±7.34
F <sub>9,62</sub>	84.51	250.60	0.61	2.32
p-value	<.0001	<.0001	0.6553	0.0688
<b>Interaction (C x T)</b>				
F <sub>9,62</sub>	1.88	1.15	2.78	0.79
p-value	0.1280 <sup>NS</sup>	0.3436 <sup>NS</sup>	0.0359 <sup>NS</sup>	0.5360 <sup>NS</sup>
CV (%)	27.82	15.42	114.99	140.56

538 <sup>1</sup>Mean (±SD) flyspeck (*Zygophiala jamaicensis* Mason) damage rated on a scale from 0 (no damage)  
 539 to 5 (> 75% coverage of fruit). <sup>2</sup>Mean (±SD) sooty blotch damage rated on a scale from 0 (no  
 540 damage) to 5 (> 75% coverage of fruit). <sup>3</sup>Mean (±SD) cedar apple rust (*Gymnosporangium juniperi-*  
 541 *virginianae*) damage. <sup>4</sup>Mean (±SD) plum curculio (*Conotrachelus nenuphar* Herbst). Values with the  
 542 same letter within the columns are not statistically different by Tukey’s test (p<0.05). Values in the  
 543 column without letter are not statistically different by Tukey’s test (p<0.05). NS = not significant.  
 544 CV = coefficient of variation.

545

546

547 **Table 3.** Fruit quality parameters of ‘Golden Delicious’ and ‘York’ apples submitted to different pre-harvest spray treatments during the 2018

548 growing season in Blacksburg, Virginia, USA.

Parameters	Mass (g)	SSC <sup>1</sup> (%)	Firmness (N)	Maturity <sup>2</sup> (1 to 8)	Dry matter (g / kg)	Moisture (g / kg)
<b>Cultivars (C)</b>						
‘Golden Delicious’ <sub>36 trees, 108 fruits</sub>	110.61±22.80 a	13.72±1.18 a	74.63±7.34 a	7.70±0.52 a	143.5±17.3 a	856.5±17.3 b
‘York’ <sub>33 trees, 98 fruits</sub>	76.67±40.42 b	11.01±0.84 b	57.74±10.25 b	6.06±1.93 b	124.3±18.4 b	875.6±08.4 a
F <sub>9,68</sub>	78.94	154.48	65.95	28.69	20.80	20.80
p-value	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
<b>Treatments (T)</b>						
Control <sub>13 trees, 38 fruits</sub>	77.93±29.61 b	12.17±1.61 b	69.02±8.69	6.86±1.80 b	130.9±20.3	869.1±20.3
Holistic <sub>15 trees, 45 fruits</sub>	75.53±30.49 b	13.16±1.92 a	66.63±13.55	7.62±0.51 ab	142.0±25.0	858.0±25.0
Insecticides <sub>14 trees, 42 fruits</sub>	64.50±28.52 b	11.69±1.59 b	67.61±13.53	7.36±1.47 ab	127.3±18.9	872.7±18.9
Antimicrobials <sub>13 trees, 39 fruits</sub>	130.31±16.84 a	12.77±1.68 ab	66.19±13.28	5.84±1.80 b	139.1±19.3	860.9±19.3
Insecticides+antimicrobials <sub>14 trees, 42 fruits</sub>	122.29±19.81 a	12.00±1.37 b	61.53±11.44	6.60±1.76 b	129.6±13.2	870.3±13.2
F <sub>9,68</sub>	46.19	6.37	1.43	3.92	19.3	19.3
p-value	<.0001	0.0002	0.2365	0.0069	0.1169	0.1169
<b>Interaction</b>						
F <sub>9,68</sub>	<.0001	0.4525 <sup>NS</sup>	0.2274 <sup>NS</sup>	0.3352 <sup>NS</sup>	0.4818 <sup>NS</sup>	0.4818 <sup>NS</sup>
p-value	7.49	0.93	1.46	1.17	0.86	0.86
CV (%)	17.01	7.26	12.97	175.10	129.7	20.1

549 <sup>1</sup>SSC = soluble solids content expressed as a percentage in solution by mass. <sup>2</sup>Cornell starch-iodine index on a scale from 1 (immature) to 8

550 (fully mature). Average values with the same letter within the columns are not statistically different by Tukey’s test (p&lt;0.05). Values in the

551 column without letter are not statistically different by Tukey’s test (p&lt;0.05). NS = not significant. CV = coefficient of variation.

552 **Table 4.** PLS-DA results for discriminating the pre-harvest treatments five groups (1, control; 2,  
553 holistic; 3, insecticide; 4, antimicrobials; and 5, antimicrobial + insecticides treatments) of apple  
554 samples from two cultivars ('Golden Delicious' and 'York') based on the physicochemical  
555 parameters and fruit damage evaluation (flyspeck, sooty blotch, undamaged, plum curculio, cedar  
556 apple rust) separately by cultivar.

<b>Accuracy</b>	<b>'Golden Delicious'</b>					<b>'York'</b>				
	1	2	3	4	5	1	2	3	4	5
Training (%)	91	83	91	94	91	96	96	100	96	74
Cross-validation (%)	63	77	69	66	91	42	63	57	42	69
Test (%)	88	92	88	92	96	57	50	73	90	85
AUC <sup>1</sup>	0.81	1.00	0.96	1.00	1.00	0.77	0.59	0.84	0.87	0.83
Sensitivity (%)	100	100	100	100	100	50	0	67	100	100
Specificity (%)	75	83	75	83	92	64	100	80	80	70

557 <sup>1</sup>AUC = area under the curve.

558

559

560 **Table 5.** PLS-DA results for discriminating the preharvest treatments five groups (1, control; 2,  
 561 holistic; 3, insecticide; 4, antimicrobials; and 5, antimicrobial + insecticides treatments) of apple  
 562 samples from two cultivars ('Golden Delicious' and 'York') based on the physicochemical  
 563 parameters and fruit damage evaluation (flyspeck, sooty blotch, undamaged, plum curculio, cedar  
 564 apple rust) combining the two cultivars.

<b>Accuracy</b>	<b>'Golden Delicious' + 'York'</b>				
	1	2	3	4	5
Training (%)	73	70	84	89	91
Cross-validation (%)	76	70	65	75	70
Test (%)	79	75	84	86	89
AUC <sup>1</sup>	0.83	0.88	0.85	0.86	0.98
Sensitivity (%)	80	80	100	100	100
Specificity (%)	78	70	68	73	77

565 <sup>1</sup>AUC = area under the curve.

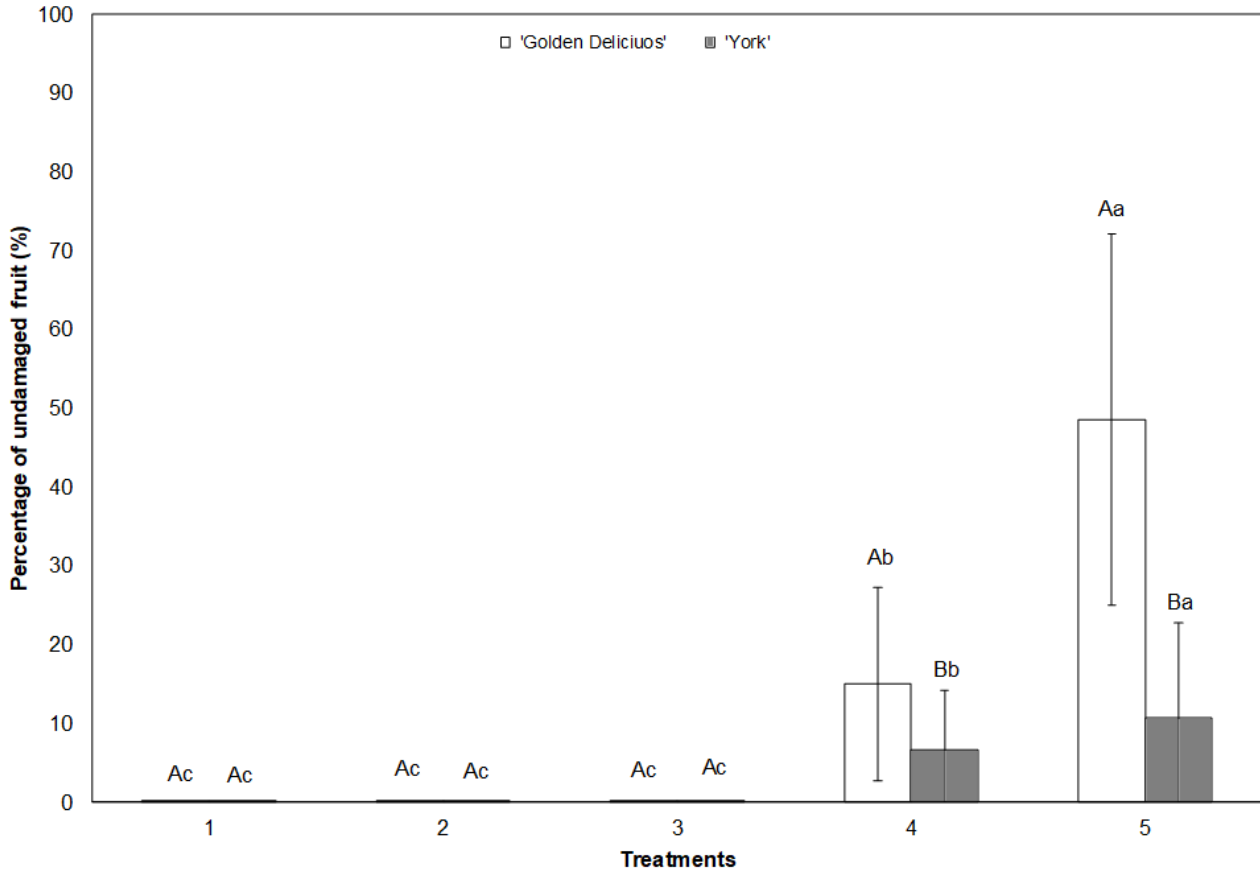
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569 **Figures**

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572 **Figure 1.** Percentage of undamaged fruit (%) of 'Golden Delicious' (GD) and 'York' (Y) submitted  
 573 to different pre-harvest treatments: 1, control – unsprayed trees (7 trees, 133 fruits GD; 4 trees, 57  
 574 fruits Y), 2, trees sprayed with holistic (8 trees, 161 fruits GD; 4 trees, 16 fruits Y), 3, trees sprayed  
 575 with insecticides (6 trees, 110 fruits GD; 7 trees, 62 fruits Y), 4, trees sprayed with antimicrobials (7  
 576 trees, 140 fruits GD; 6 trees, 119 fruits Y), and 5, trees sprayed with antimicrobials + insecticides (7  
 577 trees, 140 fruits GD; 7 trees, 140 fruits Y). Treatments with the same lowercase letters are not  
 578 statistically different by Tukey's test ( $p < 0.05$ ) within cultivars. Cultivars with the same capital letters  
 579 are not statistically different by Tukey's test ( $p < 0.05$ ) within treatments. The bars represent the  
 580 standard deviation of each plant repetitions.

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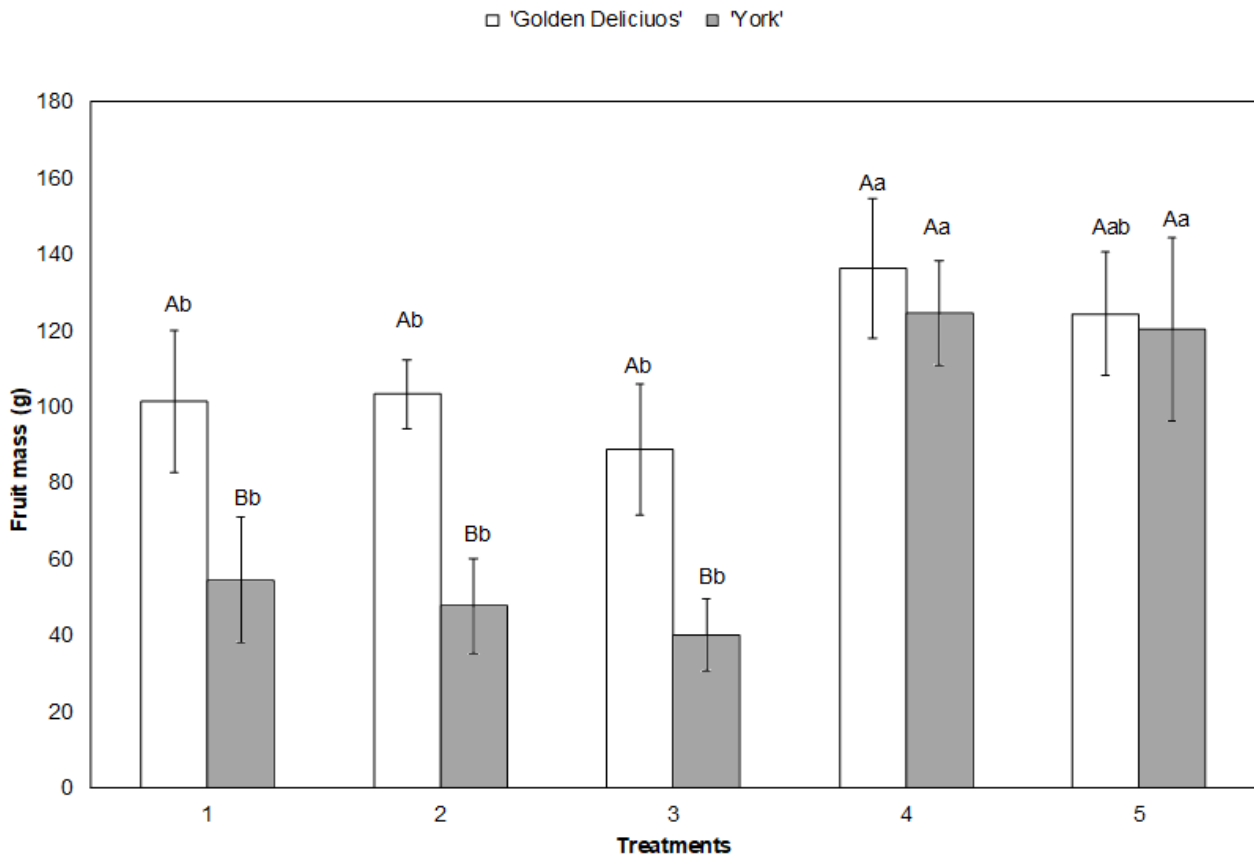
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584 **Figure 2.** 'Golden Delicious' (top) and 'York' (bottom) apples submitted to different pre-harvest  
585 treatments: 1, control – unsprayed trees, 2, trees sprayed with holistic, 3, trees sprayed with  
586 insecticides, 4, trees sprayed with antimicrobials, and 5, trees sprayed with antimicrobials +  
587 insecticides.

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592 **Figure 3.** Fruit mass (g) of 'Golden Delicious' and 'York' submitted to different pre-harvest  
 593 treatments: 1, control – unsprayed trees (7 trees, 21 fruits GD; 6 trees, 17 fruits Y), 2, trees sprayed  
 594 with holistic (8 trees, 24 fruits GD; 7 trees, 21 fruits Y), 3, trees sprayed with insecticides (7 trees, 21  
 595 fruits GD; 7 trees, 21 fruits Y), 4, trees sprayed with antimicrobials (7 trees, 21 fruits GD; 6 trees, 18  
 596 fruits Y), and 5, trees sprayed with antimicrobials + insecticides (7 trees, 21 fruits GD; 7 trees, 21  
 597 fruits Y). Treatments with the same lowercase letters are not statistically different by Tukey's test  
 598 ( $p < 0.05$ ) within cultivars. Cultivars with the same capital letters are not statistically different by  
 599 Tukey's test ( $p < 0.05$ ) within treatments. The bars represent the standard deviation of each plant  
 600 repetitions.

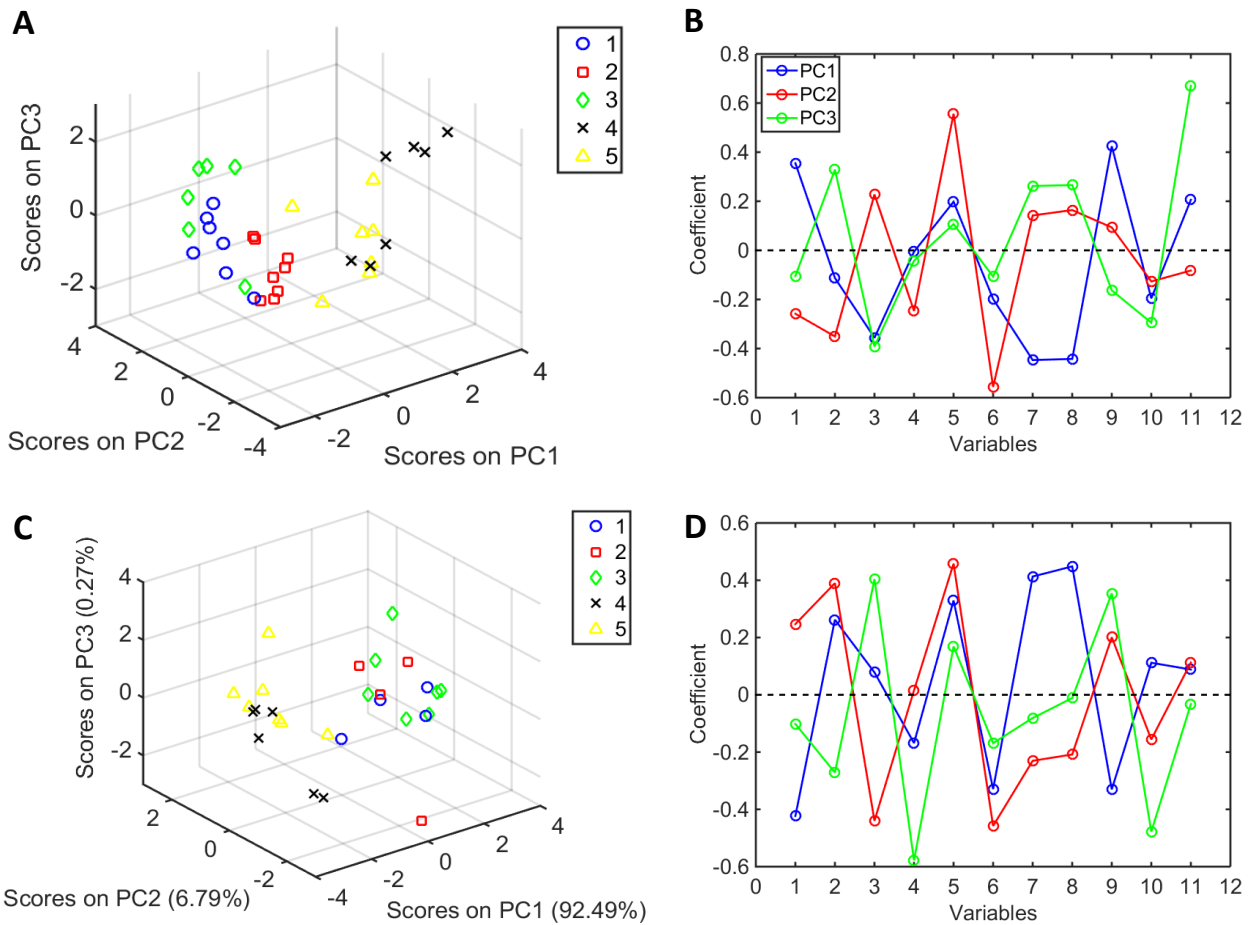
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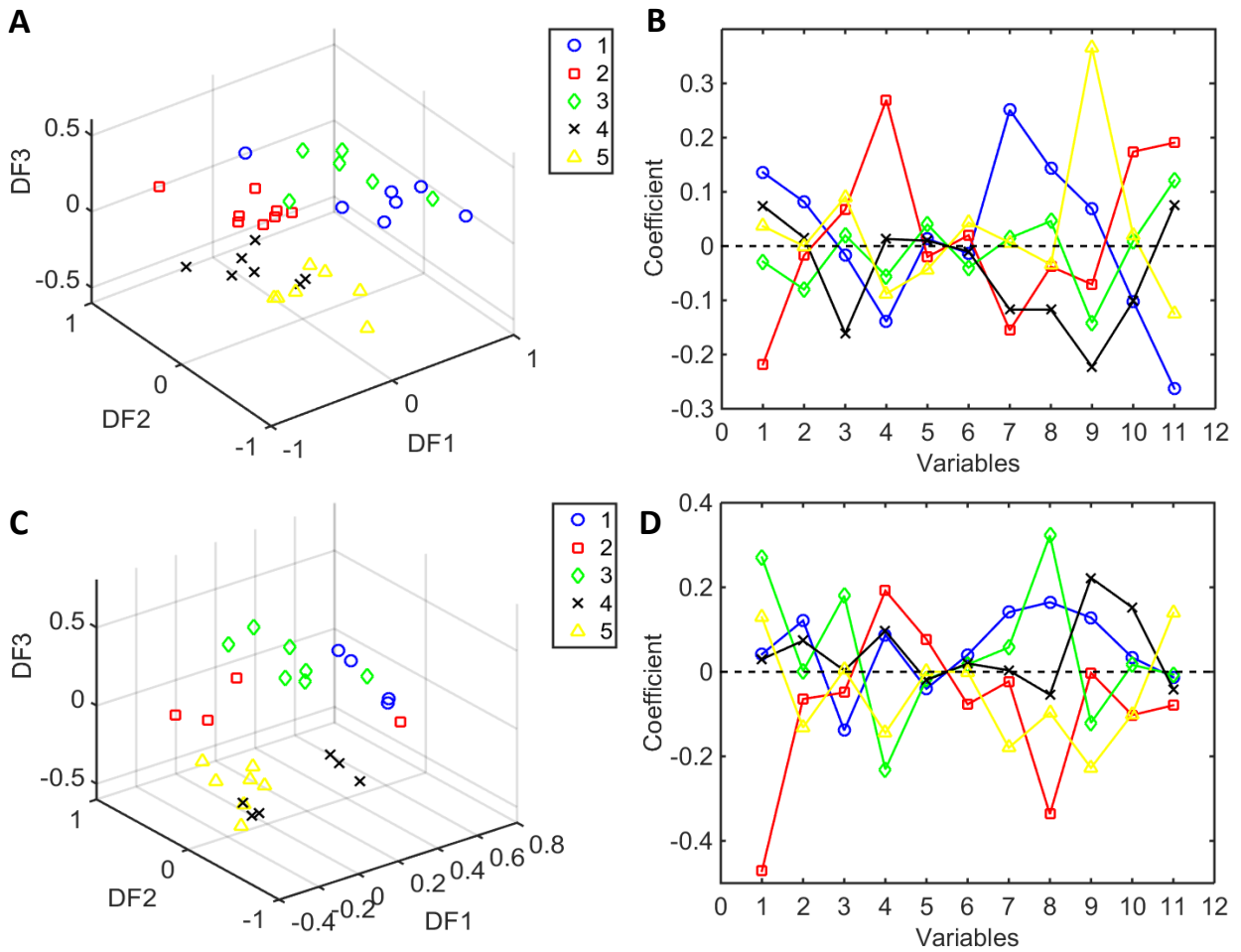
607 **Figure 4.** PCA scores (A) and loadings (B) for ‘Golden Delicious’ and PCA scores (C) and loadings  
 608 (D) for ‘York’ cultivars. Class legend (A & C): 1, control; 2, holistic; 3, insecticide; 4,  
 609 antimicrobials; and 5, antimicrobial + insecticides treatments. Variables legend (B & D): 1, fruit  
 610 mass; 2, firmness; 3, maturity; 4, soluble solids content; 5, moisture; 6, dry matter; 7, flyspeck level;  
 611 8, sooty blotch level; 9, undamaged; 10, plum curculio; and 11, cedar apple rust.

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618 **Figure 5.** Discriminant function (DF) represented by the predicted PLS-DA categories (A) and PLS-  
 619 DA coefficients for ‘Golden Delicious’ (B). DF represented by the predicted PLS-DA categories (C)  
 620 and PLS-DA coefficients for ‘York’ (D) cultivars. Class legend (A & C): 1, control; 2, holistic; 3,  
 621 insecticide; 4, antimicrobials; and 5, antimicrobial + insecticides treatments. Variables legend (B &  
 622 D): 1, fruit mass; 2, firmness; 3, maturity; 4, soluble solids content; 5, moisture; 6, dry matter; 7,  
 623 flyspeck level; 8, sooty blotch level; 9, undamaged; 10, plum curculio; and 11, cedar apple rust.

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