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## Determination of Chemical, Physical and Sensory Characteristics of Apricot Jam from Winter-Hardy Genotypes

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**Additional index words:** Fruit jams, chemical characterization, flavor, color, sensory evaluation, texture analysis, *Prunus armeniaca*.

### Abstract

Apricots are highly desirable aromatic fresh fruits, although their high respiration rates as climacteric fruit limits their shelf life. Thus, they are often preserved as dried fruits or jams for enjoyment throughout the year. Winter hardy apricots that survive in USDA Zone 4 have never been tested for physicochemical properties and sensorial profiles of their jams; this was the objective for the present study. Fresh fruit from eight winter hardy apricot genotypes were harvested and made into jam; these apricot jams, along with three comparative jam controls were tested for soluble solids, pH, titratable acidity, and L\*a\*b\* CIELAB chromaticity coordinates, hue angle, and chrome values. Sensorial profiles were determined in a sensory evaluation panel using the following traits: color, spreadability, texture, fruit pieces, flavor, off-flavor, sweetness, bitterness, overall quality, and desire to purchase. ‘Sungold’, ‘Westcott’ and the tart cherry jam control had greater than 60% soluble solids (°Brix). MN 604, MN203, ‘Brookcot’ and ‘Sungold’ apricot jams had the lowest pH levels. The lightest color jam (L\*) was ‘Brookcot’ with ‘Debbie’s Gold’ having the yellowest color (b\*). The darkest jams were made from MN206 and MN203 similar to the tart cherry control. Panelists were able to discern differences among apricot jams for spreadability, texture, fruit pieces, flavor, off-flavor and overall quality but could not distinguish differences in sweetness and bitterness across cultivars. Results from this study provided much-needed information on sensorial profiles and physicochemical qualities of apricot jams made from these winter-hardy genotypes. We concluded that the best apricot for use in jam making is ‘Sungold’.

Along with a number of other fruit and nut crops the apricot (*Prunus armeniaca* L.) belongs to the large, economically important genus, *Prunus* L., part of the Rosaceae family (Potter, 2012). *Prunus armeniaca* are native to Asia (China) and have been bred and adapted for cultivation in areas that fulfills the chilling requirements (Touati et al., 2014). World production of apricots was 4.04 M metric tonnes in 2012 and ranked 16<sup>th</sup> in cultivated fruit worldwide (FAOSTAT 2013).

Apricots are aromatic, nutritionally rich fruits (Gutierrez-Martinez et al., 2007; Mehlenbacher et al., 1991) with a high fiber content, and a source of vitamins, minerals and sugars (Sartaj et al., 2011) as well as carotenoids and phytochemicals, e.g. ferulic,

caffeic, chlorogenic and *p*-coumaric acids (Dragovic-Uzelac et al., 2007; Rababah et al., 2011). However, since apricots are climacteric fruit, high respiration rates, fast ripening and soft texture limit shelf life (Touati et al., 2014). Thus, apricots are frequently processed into dried fruits, jams, marmalades, jellies or nectars (Touati et al., 2014).

The production of jellies and jams is a method used to preserve perishable fruits, which allows for consumption during periods of the year when fresh fruit is not available (Touati et al., 2014). Jams are classified as intermediate moisture foods, created by boiling whole fruit or pulp with pectin, acid, and sugars to a thick but spreadable consistency (Touati et al., 2014; Vidhya and Nara-

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in, 2011; Kurz et al., 2008; Wicklund et al., 2005). Today, jam is a common and popular food product with 92% of households consuming jams, jellies, and preserves (Agriculture and Agri-Food Canada, 2012).

Consumers' perception of jam quality is affected by a number of physical, chemical, and sensory characteristics (Grujić et al., 2007). Sensory attributes perceived by the consumer during purchasing and consumption influence whether or not the product will be bought. According to Lawless and Heymann (2010), color is one of the most important sensory factors that consumers perceive when evaluating a food product's quality. Other important sensory characteristics that have been examined when evaluating jam quality include taste, sweetness, sourness, spreadability, and overall quality (Culetu et al., 2014; Sandulachi and Tatarov, 2012; Touati et al., 2014). Previous studies examining *Prunus* jam quality have also analyzed chemical and physical characteristics such as pH, soluble solids, titratable acid, and color parameters (Culetu et al., 2014; Sandulachi and Tatarov, 2012). Gelation, flavor, and shelf life of a jam are all affected by pH, which measures the amount of organic acid present in the sample (Culetu et al., 2014). The amount of sugar present in a jam is quantified via soluble solid content, which affects the gelation and stability of a jam (Culetu et al. 2014). Sucrose, pH and pectin are critical components of jams to ensure gelling for spreadability and are routinely manipulated in jam recipes to ensure adequate gel structure (Culetu, et al., 2014). Sugar binds water molecules, removing water away from pectin molecules which allows them to chemically link with each other and form polymeric network.

Although apricots are cultivated and enjoyed throughout the world, damage due to spring frosts and the lack of winter-hardy cultivars with good fruit quality limit the production of apricots in northern climates such as USDA Zones 3 and 4 (Mehlenbacher et al., 1991). Early breeding programs, includ-

ing the University of Minnesota, developed winter-hardy apricot hybrids by crossing commercial cultivars with hardy wild species (Anderson and Weir, 1967; Hoover et al. 2015). A number of hardy apricot hybrids, most notably 'Moongold' and 'Sungold', were developed using the Manchurian apricot (*P. mandshurica* [Maxim.] Koehne) as a male parent (Anderson and Weir, 1967). The apricots 'Brookcot', 'Debbie's Gold', and 'Westcot' are also considered winter-hardy cultivars (Ames, 2013). Although a number of hardy apricot selections and cultivars were introduced decades ago (Hoover and Zins, 1998), little is known about the quality of jam made from the fruits of these genotypes.

The objective of this paper was to quantify attributes of jams made from select USDA Zone 4 winter-hardy apricot genotypes from the University of Minnesota breeding program along with named comparisons. Specifically, physicochemical properties and sensory profiles were examined to determine quantitative genotypic differences. Qualitative data, including the desire to purchase jams, were also evaluated.

## Materials and Methods

*Genotypes and fruit harvest.* During weeks 31-32 (2013) mature fruits from apricots *P. armeniaca* 'Brookcot', 'Debbie's Gold', 'Sungold', 'Westcot' and unnamed selections MN604, MN206, MN203, MN202 were harvested from trees at the University of Minnesota research plots in Excelsior, MN (44°52'06.5" N lat., -93°38'03.9" W long.). Week number is defined as the number of weeks from January 1<sup>st</sup>, 2013. All trees in the research plots were managed for fruit production. Fruits were stored at 3-5°C no more than one week prior to pitting and jam preparation. All apricot fruits were cut along the suture line with a pairing knife to remove the pit prior to jam preparation.

*Jam preparation.* Sugar and pectin were added to increase the concentrations in the harvested fruit mixture (Culetu, et al., 2014). Jams were made in sterilized dishes us-

ing sterilized wooden, glass or non-reactive metal utensils in a semi-commercial, private kitchen (Kurz et al. 2008). All jams were made according to a standard recipe of 1.5 L (6.33 US cups) pitted fruit, 74 ml (5 US tablespoons) fresh-squeezed lemon juice, 14.2 g (1 US tablespoon) unsalted butter, 56.8 g (4 US tablespoons) Ball® RealFruit® Classic Pectin (Hearthmark, LLC dba Jarden Home Brands) and 1350 g (6 US cups) sugar. Pitted fruit were macerated using a hand-held puree machine (KitchenAid® 2-Speed Immersion Hand Blender, #KHB1231) until fruit and skins were thoroughly pureed. Fruit, lemon juice, butter, and pectin were combined in an uncovered, non-reactive Revere® copper-clad base stainless steel pot (4.26 L or 4.5 US quart), stirring constantly with a flat wooden spoon. The mixture was allowed to vigorously boil for 1 minute. Sugar was then added, again stirring constantly until the jam began sheeting off from the flat, wide spoon. Each mixture was then removed from the heat source. The jam surface was skimmed to remove any impurities and immediately poured into sterilized 0.24 L (0.5 US pint) glass jars and lids/rings were attached to the jars. Jars were inverted for 5 minutes and then reverted to upright position and cooled under a towel for 24 hours until sealed. Jars were labeled with the cultivar name and fruit type and stored at 12.8°C (55°F) in darkness for up to 6 months to maximize color retention and stability (García-Viguera, et al., 1999; Touati et al. 2014). Minimums of three jars of each cultivar were made for sensory evaluations.

*Chemical analyses.* Sugar content of the jams was measured in °Brix using an Atago Digital Hand-held "Pocket" Refractometer PAL-2 (Cole-Parmer, Court Vernon Hills, IL). All measurements were made in triplicate (n=3 replications) with new samples placed on the refractometer each time. The refractometer was washed in between measurements with deionized water and dried with a Kimwipe (KIMWIPES™ Delicate Task Wipers, 11.2 cm x 21.3 cm or 4.4" x 8.4"). Between cultivars, the refractometer

was washed with mild detergent and dried with a Kimwipe.

Titrateable acidity (g/L) citric acid equivalent, a measure of the total amount of protons available, was determined by titrating a solution containing 5 mL of jam and 50 mL of deionized water with 0.1 M NaOH (sodium hydroxide) to the endpoint of pH=8.20 using an Thermo Scientific Orion 950 ROSS® FAST QC™ Titrator with a Thermo Scientific Orion ROSS Sure-Flow pH electrode. Titrations were done in duplicate with all materials rinsed in between with deionized water. The pH of each sample was measured in triplicate using a Thermo Scientific Orion 950 ROSS® FAST QC™ Titrator with a Thermo Scientific Orion ROSS Sure-Flow pH electrode. The electrode was rinsed with deionized water between measurements of the same sample, between samples the junction was flushed and the electrode rinsed with deionized water.

Hue, lightness and color saturation angles for each sample were measured in triplicate for each jam sample using a Konica Minolta CR-400 chroma meter; data were expressed as L\* a\* b\* color space or CIELAB where L\* indicates lightness, higher values are lighter in color, and a\* and b\* are the chromaticity coordinates (Konica Minolta Sensing, Inc., 2003). Chromaticity coordinates a\* and b\* indicate the directions of color: +a\* (red), -a\* (green), +b\* (yellow) and -b\* (blue) with the center being "achromatic" (Konica Minolta Sensing, Inc., 2003). Color saturation increases as a\* and b\* values increase in size. Chroma or saturation ( $C_{ab}^*$ ) values were calculated using  $\sqrt{a^{*2} + b^{*2}}$  and are expressed as distance between the center, the "achromatic point", and color (Gulrajani, 2010). Medium to high values of  $C_{ab}^*$  indicate bright or saturated color whereas lower values indicate duller or less saturated colors (Gulrajani, 2010). Hue angle ( $H_{ab}$ ) expresses the angle measured beginning at the +a\* axis (Konica Minolta Sensing, Inc., 2003).  $H_{ab}$  was calculated using  $\text{Arctan}(\frac{b^*}{a^*})$  (Gulrajani, 2010).

*Sensory evaluation.* Jams were evaluated

by a sensory panel made up of  $n=33$  individuals of which 45% were female and 55% male, aged from their early 20s to 70s. Some of the panel members had sensory training and others had little to no sensory panel experience.

For the sensory session, all eight apricot jams along were randomized and assigned an alphanumeric code. While avoiding duplication, four codes were assigned to each evaluator's seat. Each seat was also assigned each of three commercial jam standards for comparisons: Bonne Maman® apricot preserves (apricot control; <http://www.bonnemaman.us/preserves-jellies/apricot-preserves/>), Bonne Maman® cherry preserves (tart cherry control; <http://www.bonnemaman.us/preserves-jellies/>

cherry-preserves/) and Bonne Maman® plum preserves (plum control; <http://www.bonnemaman.us/preserves-jellies/plum-preserves/>) for a total of seven samples / evaluator. The tart cherry and plum controls were included to provide diversity of flavor and color. For statistical purposes, individual evaluators were considered incomplete blocks.

Jam jars were labeled with their corresponding code, and then approximately 15 g of each sample was placed in a neutral colored, 29.6 mL plastic, disposable, odor-free cup (Culetu, et al., 2014) labeled with the jam code, along with a 7.62 cm plastic taster spoon. These samples were placed at their corresponding seats along with one instruction (Fig. 1) and seven evaluation

Name \_\_\_\_\_

***Taste the Difference! Sensory Evaluation***

**26 February 2014**

**Jams from the University of Minnesota *Prunus* Collection**

You will be evaluating individual jam samples based on the ten criteria below. Please adhere to the following instructions as closely as possible and evaluate characteristics in the numbered order.

- Please taste the samples in order, from left to right
- Watch for pits!
- Write the code (on the side of sample cup) at the top of the evaluation sheet.

1. Color:

Align the spectrum card with the bar scale on the scoring sheet - blue on the left, red on the right. Using the blank sheet of paper supplied, examine closely the color of the jam, and to the best of your ability match that color on the spectrum card. Draw a vertical mark through the bar scale at the point corresponding to the color on the spectrum card.

2. Spreadability:

Using the spoon, move the jam back and forth in the cup, gauging its' resistance to your movement. Using water as the thin extreme and frozen ice cream as the thick extreme, draw a vertical mark through bar scale at the point most accurately reflecting your impression.

The following characteristics all require the jam to be placed in your mouth. You will not have enough of each sample to evaluate each characteristic with a separate mouthful. Therefore, please read through the instructions for criteria 3-10 before starting, so that you can evaluate numerous characteristics with each mouthful. You do not have to swallow the jam. The paper cup next to your water glass is a spit cup, if needed.

3. Texture:

Move a small amount of jam around in your mouth. With your tongue, push the jam against the inside of your mouth paying close attention to the texture of the jam. Using pudding as a smooth extreme and gritty as the opposite extreme, make a vertical mark on the bar scale corresponding to your impression of the texture of the jam.

4. Fruit Pieces (if present):

With a small amount of jam in your mouth, take note of the texture of any fruit pieces in the jam; bite down on one of the pieces. With melting as a soft extreme and citrus rind as a firm extreme, make a vertical mark on the bar scale corresponding to your impression of the texture of the fruit pieces.

5. Flavor:

While moving a small amount of jam around in your mouth, take note of the intensity of fruit flavor. Is the flavor extremely strong and pronounced (intense) or is it barely perceptible or absent (none)? Make a vertical mark on the bar scale corresponding to your impression of the fruit flavor.

6. Off-Flavor:

While moving a small amount of jam around in your mouth, take note of the intensity of any distracting or unpalatable flavor you would not normally associate with the corresponding fruit (cherry or plum). Is the flavor extremely strong and pronounced (intense), or is it barely perceptible or absent (none)? Make a vertical mark on the bar scale corresponding to your impression of the off-flavor.

7. Sweetness:

While moving a small amount of jam around in your mouth, take note of the intensity of sweetness. Is the sensation of sweetness strong and overpowering (intense) or is it barely perceptible or absent (dry)? Make a vertical mark on the bar scale corresponding to your impression of the sweetness.

8. Bitterness:

While moving a small amount of jam around in your mouth, take note of the intensity of bitterness. Is the sensation of bitterness strong and overpowering (intense) or is it barely perceptible or absent (none)? Make a vertical mark on the bar scale corresponding to your impression of the bitterness.

9. Overall Quality:

What is your overall impression of the jam? Is it an enjoyable, well-balanced product, or do you find it distasteful or unpalatable? Make a vertical mark on the bar scale corresponding to your impression of the quality of the jam.

10. Would you buy this?

Exactly that - please circle either YES or NO.

Once you have finished evaluating this sample, cleanse your palate with water and eat one unsalted cracker and then proceed to the next jam. Rinse your mouth again, if necessary. Once you are finished please double check that you have written your name on the top of the packet and that the code for each jam is written on the top of each evaluation sheet.

Thank you for your participation.



Jam Code \_\_\_\_\_

1. Color  
Blue Red

--	--	--	--	--	--	--

2. Spreadability  
Easy (water) Moderate Firm (ice cream)

--	--	--	--	--	--	--

---

Starting here, you will need to put the jam in your mouth.

3. Texture  
Smooth (pudding) Gritty

--	--	--	--	--	--	--

4. Fruit Pieces (if present)  
Soft (melting) Moderate Firm (citrus rind)

--	--	--	--	--	--	--

5. Flavor  
None Moderate Intense

--	--	--	--	--	--	--

6. Off-Flavor  
None Moderate Intense

--	--	--	--	--	--	--

7. Sweetness  
Dry Moderate Intense

--	--	--	--	--	--	--

8. Bitterness  
None Moderate Intense

--	--	--	--	--	--	--

9. Overall Quality  
Poor Average Excellent

--	--	--	--	--	--	--

10. Would you buy this jam?

Yes	No
-----	----

**Fig. 1.** Sensory evaluation panel instructions used with the apricot jam taste tests.

sheets (Fig. 2), one color reference card, one neutral white and unlined 7.62 x 12.7 cm card, water cup, spit cup, and unsalted crackers (Fig. 3; Halat et al., 1997). All sensory evaluation panels were conducted at room temperature

to match the predominant conditions for jam consumption and conditions for previous panels (Culetu, et al., 2014).

Each group was given a brief, oral introduction on how to taste jams, palette cleans-

Jam Code \_\_\_\_\_

1. Color

Blue Red

--	--	--	--	--	--	--

2. Spreadability

Easy (water) Moderate (Syrup) Firm (ice cream)

--	--	--	--	--	--	--

3. Mouth Feel

Thin (water) Thick (cream)

--	--	--	--	--	--	--

4. Fruit Pieces (if present)

Soft (descriptor) Moderate Firm (citrus rind)

--	--	--	--	--	--	--

5. Flavor

None Moderate Intense

--	--	--	--	--	--	--

6. Off-Flavor

None Moderate Intense

--	--	--	--	--	--	--

7. Sweetness

Dry Moderate Intense

--	--	--	--	--	--	--

8. Bitterness

None Moderate Intense

--	--	--	--	--	--	--

9. Overall Quality

Poor (unpalatable) Average Excellent

--	--	--	--	--	--	--

10. Would you buy this?

YES NO

**Fig. 2.** Example score sheet used by each evaluator during the sensory evaluations.

ing procedures, use of the color chart (*cf.* Fig. 3) and a review of the instruction (Fig. 1) and evaluation (Fig. 2) sheets. A modification of the standard Hedonic 9-point (Lawless and Heymann, 2010; Basu et al. 2011) to 7-point scale (Grujić, et al., 2007) was im-

plemented with an unnumbered scalar range of seven boxes for recording scores (Fig. 2). All members of each group taste-tested the first sample (apricot control) together using the instructions (Fig. 1) and, once they had recorded their evaluative assessments of the

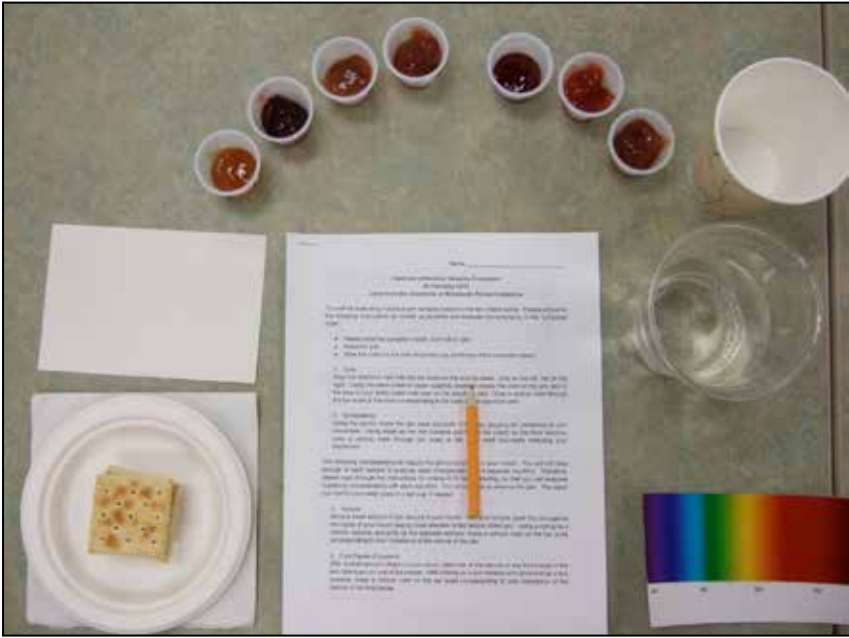


Fig. 3. Setup of all items used in the sensory evaluation panels (see text)

apricot control, discussed the potential data points for each of the ten factors for each jam (Fig. 2). Sensory sessions took place in classroom settings with overhead cool white florescent lighting (538 Lux) and room temperature conditions (21°C). Each panelist was provided adequate space to evaluate his or her samples. However, physical barriers did not separate panelists.

Since evaluators marked the first nine sensory characteristics in the linear box plots (Fig. 2), these were transformed into quantitative data points, based on measuring (mm) from the beginning (far left-hand side) of the scale to wherever the panelist made their mark. This value was then divided by the total length of the scale and then multiplied by ten to give data points on a ten-point scale.

**Data Analyses.** One-way Analysis of Variance (ANOVA) as well as mean separations with Tukey's Honest Significant Difference (HSD) tests  $\alpha=0.05$  were carried out for all quantitative data. Quantitative chemical analysis and ratings data were also analyzed

using principle components analysis. Qualitative data, specifically desire to purchase, was analyzed using a Chi-square test with equal distribution across the two classes (1:1  $\chi^2$ ). Since there was only 1 degree of freedom for the Chi-square test, the Chi-square correction of  $(\text{Observed}-\text{Expected}-0.5)^2$  was used. Pearson's Rank Correlations were carried out between variables.

## Results

**Chemical analyses.** Mean soluble solid concentration of jams ranged from 48.87° Brix for the tart cherry control to 68.47° Brix for MN604 (Table 1). The tart cherry control and 'Westcot' differed significantly for soluble solid concentration from all other jams (Table 1). In addition, MN604 differed significantly from both 'Sungold' and the cherry control (Table 1).

The range in mean pH values was 3.00 for MN604 and 'Sungold' to 3.35 for the tart cherry control (Table 1). The tart cherry control pH differed significantly from all other

**Table 1.** Mean soluble solids (S.S.; °Brix), pH, titratable acidity (T.A.; g/L citric acid equivalent), L\*a\*b\* color space or CIELAB (where L\* indicates lightness; a\* and b\* are the chromaticity coordinates), hue angle ( $H_{ab}^* = \arctan(b^*/a^*)$ ) and chrome ( $C_{ab}^* = \sqrt{a^{*2}+b^{*2}}$ ) for apricot, tart cherry and plum jams used in the sensory evaluation panel. Mean separations within traits (columns), based on Tukey's 5% HSD.

Jam	S.S. (°Brix)	pH	T.A.	Color				
				L*	a*	b*	$H_{ab}^*$	$C_{ab}^*$
Tart cherry control	48.87 c	3.35 a	7.29 f	29.93 bc	13.18 abc	4.11 d	0.32 e	13.84 d
Plum control	65.87 ab	3.23 b	8.44 f	24.62 c	17.70 a	10.13 d	0.52 d	20.39 cd
Apricot control	67.30 ab	3.20 bc	12.37 de	41.24 a	16.44 ab	38.71 a	1.17 bc	42.06 a
MN604	68.47 a	3.00 f	16.23 ab	39.71 ab	13.22 abc	31.05 abc	1.17 bc	33.79 ab
MN206	65.50 ab	3.15 cd	11.74 e	38.44 ab	14.16 ab	27.65 bc	1.10 c	31.07 abc
MN203	61.57 ab	3.02 ef	15.41 abc	36.89 ab	14.83 ab	30.71 abc	1.10 c	34.55 ab
MN202	66.50 ab	3.12 d	14.17 cd	44.88 a	8.53 c	29.89 abc	1.29 a	31.10 abc
'Brookcot'	64.93 ab	3.01 f	14.54 bc	46.25 a	8.71 c	31.49 abc	1.30 ab	32.68 ab
'Debbie's Gold'	64.37 ab	3.11 d	15.17 bc	42.06 a	13.51 abc	38.78 a	1.24 ab	41.07 a
'Sungold'	59.77 b	3.00 f	14.41 c	41.45 a	12.27 bc	35.93 ab	1.24 a	38.00 a
'Westcot'	50.70 c	3.06 e	17.60 a	42.73 a	8.93 c	23.57 c	1.21 ab	25.09 bc

jam types whereas the plum control differed significantly from all jams except for the apricot control (Table 1). MN206 was significantly different from the majority of other jams' pH values except for the apricot control, MN202, and 'Debbie's Gold' (Table 1). 'Westcot' was significantly different than the majority of jams except for MN203. Apricot jams from MN604, MN203, 'Brookcot', and 'Sungold' were not significantly different from each other but differed from the remaining jams for pH (Table 1).

There was significant variation among the apricot jams for titratable acidity. The titratable acidity ranged widely, from 7.29 ml (tart cherry control) to 17.60 ml for 'Westcot' (Table 1). Both the plum and tart cherry controls were significantly different than all of the other jams in this study.

Color lightness ( $L^*$ ) ranged from the darkest  $L^*=24.62$  (plum control) to the lightest ( $L^*=46.25$  for 'Brookcot'; Table 1). As would be expected with lighter colored or yellower apricots, the darkest jams (plum and tart cherry controls) did not differ from each other in  $L^*$  values or most other jams tested (Table 1). The only exceptions were MN604, MN206, and MN203 (Table 1), which were

significantly lighter than the plum control but overlapped with the tart cherry control.

The chromaticity coordinates for green-red ( $a^*$  values) ranged from  $a^*=8.53$  units for MN202 to  $a^*=17.70$  units for the plum control, which had the "reddest" color (Table 1). The plum control differed significantly from MN202, 'Brookcot', 'Sungold' and 'Westcot' for the green-red coloration; the apricot control, MN206, and MN203 were significantly different than MN202, 'Brookcot' and 'Westcot' for  $a^*$  (Table 1). All other jams had intermediate  $a^*$  values (Table 1). Chromaticity coordinates for blue-yellow ( $b^*$ ) varied from  $b^*=4.11$  (tart cherry control) to  $b^*=38.78$  ('Debbie's Gold'; Table 1). The jam with the "yellowest" or least coloration saturation chromaticity coordinates was 'Debbie's Gold', which was significantly different than both the tart cherry and plum controls as well as MN206 and 'Westcot' (Table 1). The plum and tart cherry controls differed for  $b^*$  from all apricot accessions, including the apricot control (Table 1).

Hue angles,  $H_{ab}^*$ , were distributed from 0.32 (tart cherry control) to 1.30 ('Brookcot'; Table 1) with significant variation among genotypes. The plum and tart cherry controls

were significantly different than all apricot jams for hue angles (Table 1). There was significant variation among the apricot jams for hue angle with MN202 and 'Sungold' differing significantly from the majority of other genotypes except for 'Brookcot', 'Debbie's Gold', and 'Westcot' (Table 1). In addition, 'Brookcot' and 'Debbie's Gold' were significantly different than about half of the other genotypes (Table 1).

Mean chrome ( $C_{ab}^*$ ) values ranged widely from 13.84 for the tart cherry control to 42.06 for the apricot control (Table 1). The tart cherry and plum controls did not differ significantly from each other; the tart cherry control chrome values differed significantly from all jams with the exception of the plum control. The plum control  $C_{ab}^*$  values differed significantly from the apricot control, MN604, MN203, 'Brookcot', 'Debbie's Gold', and 'Sungold' (Table 1). 'Westcot' jam differed significantly from the apricot control, 'Debbie's Gold', and 'Sungold' (Table 1).

**Sensory evaluations.** There was significant variation among jams for color, spreadability, texture, fruit pieces, flavor, off-flavor, and overall quality in this study ( $p < 0.05$ ). In contrast, for the sweetness and bitterness ratings there was no significant variation among genotypes ( $p = 0.09$  and  $p = 0.48$ , respectively).

The pooled mean rating for sweetness was 6.2 and 1.9 for bitterness (data not shown).

Mean ratings for color in the sensory evaluations (10 point scale) ranged from 5.3 for MN206 to 8.8 for the tart cherry control; all apricot jams differed significantly from the plum and cherry controls (Table 2). Spreadability mean ratings ranged from 4.2 (plum control) to 8.5 (MN206) with the tart cherry and plum controls differing significantly from only MN206 (Table 2).

Texture ratings ranged from 3.4 for the plum control to 8.7 for 'Brookcot' jams (Table 2). 'Brookcot' jam differed significantly from all other jams except for MN604; likewise, MN604 differed significantly from all other jams except for MN206 (Table 2). In addition to being significantly different from 'Brookcot', MN206 also differed from both the tart cherry and plum control jams (Table 2).

The mean ratings for fruit pieces in the jams ranged from 3.9 for the apricot control to 7.1 for MN604 (Table 2) with a higher presence of solids. MN604's mean fruit pieces rating was significantly different than the apricot and tart cherry controls as well as 'Westcot' (Table 2). 'Brookcot' differed significantly from the tart cherry and apricot controls (Table 2).

**Table 2.** Mean color, spreadability, texture, fruit pieces, flavor, off-flavor, and overall quality ratings (10 point scale) for apricot jams and tart cherry/plum controls as determined by sensory panelists ( $n = 33$ ). Mean separations within significant traits (columns), based on Tukey's 5% HSD.

Jam	Color	Spreadability	Texture	Fruit Pieces	Flavor	Off Flavor	Overall Quality
Tart cherry Control	8.8 a	4.8 b	3.5 d	4.0 c	6.4 bc	0.9 c	7.0 a
Plum Control	8.2 a	4.2 b	3.4 d	5.2 abc	6.0 c	1.7 abc	5.6 b
Apricot Control	5.7 b	5.9 ab	3.9 cd	3.9 c	6.4 bc	1.7 abc	6.3 ab
MN604	6.0 b	6.0 ab	6.9 ab	7.1 a	7.4 bc	2.8 a	5.7 ab
MN206	5.3 b	8.5 a	5.3 bc	5.1 abc	6.3 abc	1.7 abc	5.8 ab
MN203	6.0 b	5.7 ab	4.9 cd	5.2 abc	7.3 abc	2.4 ab	6.7 ab
MN202	5.5 b	5.6 ab	4.7 cd	5.9 abc	7.1 abc	1.4 abc	6.1 ab
'Brookcot'	5.4 b	6.4 ab	8.7 a	6.4 ab	6.0 c	2.2 abc	3.6 c
'Debbie's Gold'	5.4 b	5.8 ab	4.8 cd	5.5 abc	7.6 ab	1.7 abc	6.4 ab
'Sungold'	5.6 b	5.2 ab	4.7 cd	5.5 abc	7.1 abc	1.0 bc	6.8 ab

Sensory evaluation ratings for flavor varied from 6.0 for 'Brookcot' and the plum control to 8.1 for 'Westcot', with the latter differing significantly from the cherry, plum, and apricot controls as well as MN604 and 'Brookcot' (Table 2). The mean rating for flavor of 'Debbie's Gold' jam differed significantly from the plum control and 'Brookcot'.

Off-flavor ratings for all jams were relatively low with mean ratings ranging from 0.9 for the cherry control to 2.7 for 'Westcot' (Table 2). Apricot jams from MN604 and 'Westcot' differed significantly from 'Sungold' and the tart cherry control (Table 2). MN203 apricot jam also was significantly different than the cherry control (Table 2).

The wide range in sensory evaluation values for overall quality was 3.6 for 'Brookcot' to 7.0 for the cherry control (Table 2). The tart cherry control differed significantly from the plum control, 'Westcot', and 'Brookcot' jams for overall quality (Table 2). 'Brookcot'

had significantly lower overall quality ratings than all other jams, particularly the control comparisons (Table 2).

**Correlations.** The correlation matrix (Table 3) shows chemical and sensory evaluation trait combinations that were either positively or negatively correlated. Color ratings were positively and significantly correlated with overall quality, desire to purchase, pH, and negatively but significantly correlated with texture, fruit pieces, titratable acidity,  $L^*$ ,  $b^*$ ,  $H_{ab}^*$ , and  $C_{ab}^*$  (Table 3).

Texture ratings were positively and significantly correlated with fruit pieces, off-flavor, soluble solids,  $H_{ab}^*$  but texture was negatively correlated with overall quality, desire to purchase, and pH (Table 3). Fruit pieces were positively and significantly correlated with flavor, off-flavor, bitterness, and soluble solids whereas fruit pieces were negatively, but significantly correlated, with overall quality (Table 3).

**Table 3.** Correlations between parameters color, texture (Text.), fruit pieces (Pieces), flavor (Flav.), off-flavor (Off-Flav.), sweetness (Sweet.), bitterness (Bitter.), and overall quality (Quality) ratings, desire to purchase (Purch.) and soluble solids (S.S.), pH, titratable acidity (TA), hue directions  $L^*$ ,  $a^*$ ,  $b^*$ ,  $H_{ab}^*$ , and  $C_{ab}^*$  for all jams tested. An asterisk (\*) indicates a significant correlation coefficient (<0.05).

	Color	Text.	Pieces	Flav.	Off Flav.	Sweet.	Bitter.	Quality	Purch.	S.S.	pH	TA	$L^*$	Hue directions				
														$a^*$	$b^*$	$H_{ab}^*$	$C_{ab}^*$	
Color	1.00																	
Text.	-0.25*	1.00																
Pieces	-0.15*	0.47*	1.00															
Flav.	-0.06	0.04	0.14*	1.00														
Off-Flav.	-0.06	0.26*	0.16*	0.18*	1.00													
Sweet.	0.10	0.02	0.00	0.11	-0.03	1.00												
Bitter.	0.00	0.26*	0.32*	0.20*	0.37*	-0.25*	1.00											
Quality	0.18*	-0.29*	-0.15*	0.29*	-0.26*	0.12	-0.23*	1.00										
Purch.	0.17*	-0.20*	-0.11	0.28*	-0.19*	0.13	-0.15*	0.73*	1.00									
S.S.	-0.33	0.44*	0.64*	-0.24	0.34	-0.10	0.27	-0.33	-0.16	1.00								
pH	0.70*	-0.39*	-0.30	-0.10	-0.23	-0.07	-0.16	0.02	0.09	-0.30	1.00							
TA	-0.82*	0.40	0.22	0.09	0.27	0.10	0.11	-0.07	-0.04	0.34	-0.86*	1.00						
$L^*$	-0.76*	0.28	-0.07	0.13	0.18	0.09	0.21	-0.02	0.04	0.15	-0.59*	0.66*	1.00					
$a^*$	0.34	-0.29	0.26	-0.14	-0.09	-0.20	-0.08	0.02	0.08	0.25	0.35*	-0.32	-0.73*	1.00				
$b^*$	-0.82*	0.27	0.29	0.05	0.22	-0.11	0.21	-0.02	0.10	0.50*	-0.65*	0.75*	0.67*	-0.06	1.00			
$H_{ab}^*$	-0.90*	0.37*	0.15	0.11	0.22	0.04	0.15	-0.05	-0.01	0.39*	-0.79*	0.86*	0.86*	-0.45*	0.88*	1.00		
$C_{ab}^*$	-0.71*	0.23	0.34	0.00	0.20	-0.17	0.20	-0.01	0.11	0.54*	-0.54*	0.67*	0.48	0.15*	0.90*	0.75*	1.00	

Flavor ratings were positively correlated with off-flavor, bitterness, overall quality, and desire to purchase. Off-flavor rating was positively correlated with bitterness and negatively correlated with overall quality and the desire to purchase (Table 3). As would be expected, sweetness ratings were negatively correlated with bitterness. The bitterness ratings were negatively correlated with overall quality and desire to purchase (Table 3). Overall quality was also positively correlated with desire to purchase.

Unexpectedly, soluble solid concentration was positively correlated with hue directions  $b^*$ ,  $H_{ab}^*$ , and  $C_{ab}^*$ . pH was positively correlated with  $a^*$  but negatively correlated with titratable acidity,  $L^*$ ,  $b^*$ ,  $H_{ab}^*$ , and  $C_{ab}^*$  (Table 3). In addition, titratable acidity was positively correlated with  $L^*$ ,  $b^*$ ,  $H_{ab}^*$ , and  $C_{ab}^*$  (Table 3). Hue  $L^*$  was positively correlated with  $b^*$ ,  $H_{ab}^*$ , and negatively correlated with  $a^*$  (Table 3). In addition,  $a^*$  was positively correlated with  $C_{ab}^*$ . Hue direction  $b^*$  was positively correlated with  $H_{ab}^*$  and  $C_{ab}^*$ . Finally,  $H_{ab}^*$  and  $C_{ab}^*$  were positively correlated with each other (Table 3).

*Chi-square.* The expected  $\chi^2$  ratio of willingness to purchase or not (yes:no) was 1:1. For the majority of jams, the ratio did not differ significantly from the expected. Only the tart cherry control differed significantly from the expected ratio with 81.8% individuals

stating they would purchase and 18.2% stating they would not ( $\chi^2$  value=6.1; Table 4).

*Chemical Analysis PCA.* The first two principal components for the chemical analysis data, PC1 and PC2, had eigenvalues  $\geq 1.0$  and, together, accounted for 80.9% of the variation. PC1 accounted for 59.1% of the variation and was positively associated with  $a^*$ , soluble solid content,  $C_{ab}^*$ ,  $b^*$ , and pH (Fig. 4A). PC1 was negatively associated with  $L^*$ ; PC2 accounted for 21.8% of the variation and was positively associated with soluble solids,  $C_{ab}^*$ ,  $b^*$ , titratable acid,  $H_{ab}^*$ , and  $L^*$  (Fig. 4A). PC2 was negatively associated with  $a^*$  and pH. The majority of apricot jams were positively associated with PC1 and PC2; 'Westcot' was negatively associated with PC1 (Fig. 4A). The plum control was positively associated with PC1 and negatively associated with PC2; the tart cherry control was negatively associated with both principle components (Fig. 4A).

*Sensory Evaluation Ratings PCA.* The first four principal components (PC1, PC2, PC3, and PC4) had eigenvalues  $\geq 1.0$  and accounted for 64.9% of the variation. PC1 accounted for 25.2% of the variation and was positively associated with all ratings except for texture and spreadability (Fig. 4B). The fruit pieces, bitterness, and off-flavor variable vectors were closely clustered on the PCA biplot (Fig. 4B). Flavor, off-flavor, fruit pieces,

**Table 4.** Chi-square tests of the desire to purchase (sensory evaluation) for each jam type tested (1:1 $\chi^2$ ). Chi-square ( $\chi^2$ ) was corrected by (Observed-Expected-0.5)<sup>2</sup> due to the fact there was only 1 degree of freedom (df=1).

Jam tested	% Yes	% No	$\chi^2$
Tart cherry Control	81.8	18.2	6.1*
Plum Control	45.5	54.5	0.2
Apricot Control	60.6	39.4	0.5
MN604	37.5	62.5	0.8
MN206	66.7	33.3	0.5
MN203	83.3	16.7	3.4
'Brookcot'	25.0	75.0	2.5
'Debbie's Gold'	50.0	50.0	0.0
'Sungold'	73.3	26.7	1.2
'Westcot'	50.0	50.0	0.0

bitterness, texture, and spreadability ratings were positively associated with PC2 whereas overall quality, sweetness, and color were negatively associated with PC2 (Fig. 4B). Most jams, except for the apricot control, 'Brookcot', MN206, and the plum control, were positively associated with PC1 (Fig. 4B). Most jams were positively associated with PC2 except for the three controls (Fig. 4B).

### Discussion

The pH values for the apricot jams and comparisons in the present study (Table 1) were similar to those reported by Aslanova et al. (2010). Apricot jams tested by Touati et al., (2014) had higher values (pH=3.54) prior to storage of the jams. Titratable acidity levels in the jams tested herein were similar to previous reports as well (Touati et al., 2014;

Aslanova et al., 2010). The significantly lowest pH values found in MN 604, MN203, 'Brookcot' and 'Sungold' apricot jams could mean increased protection against the development of microorganisms over time (Touati et al. 2014), although this was not tested. Lightness ( $L^*$ ) is also an important factor in non-enzymatic browning (Touati et al., 2014), although  $L^*$  and pH were negatively and significantly correlated for the 11 tested jam samples (Table 3).

'Sungold', 'Westcot' and the tart cherry jam control all had <60% soluble solids ( $^{\circ}$ Brix; Table 1), which is the minimal level required by the Codex Alimentarius Standard (CODEXSTAN, 2009). All other apricot jams tested met the CODEXSTAN minimum soluble solid level and were similar to previous findings for other apricot (Touati et al., 2014) and quince jams (Ferreira et al., 2014).

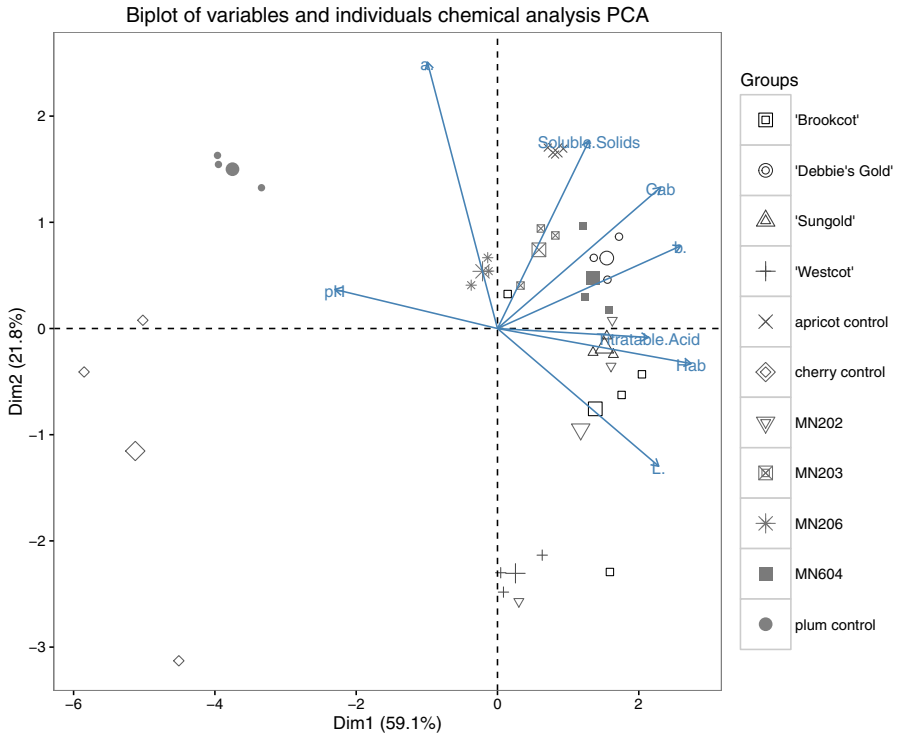
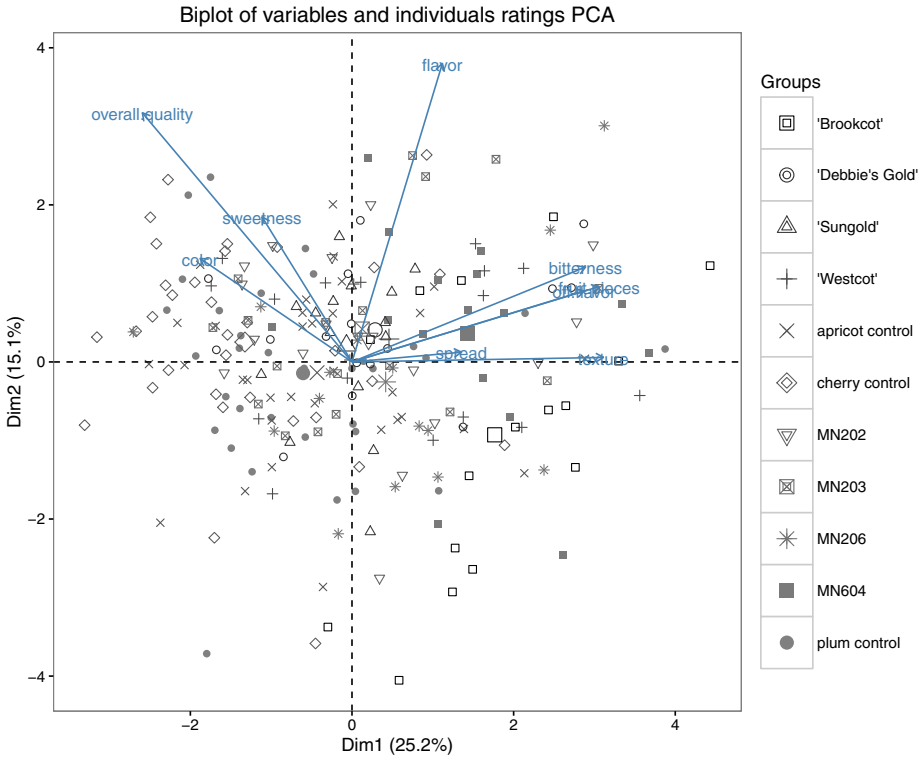


Fig. 4A.





**Fig. 4.** Biplots from principal components analyses measured variables for the apricot jams and comparisons used in the (A) chemical analysis for mean soluble solids, pH, titratable acidity (g/L citric acid equivalent)  $L^*a^*b^*$  color space or CIELAB (where  $L^*$  indicates lightness;  $a^*$  and  $b^*$  are the chromaticity coordinates), hue angle ( $H_{ab}^* = \arctan(b^*/a^*)$ ) and chrome ( $C_{ab}^* = \sqrt{a^{*2} + b^{*2}}$ ) and (B) sensory evaluation panels for fruit pieces, flavor, off-flavor, sweetness, color and overall quality (10-point scale); spreadability, texture and bitterness ratings on a 0 to 10 scale.

Apricot jam color, as gauged by color lightness ( $L^*$ ), is one of the most important consumer selection criteria (Touati et al., 2014).  $L^*$  was the lightest for 'Brookcot' jam (Table 1). The panelists in the sensory evaluation were also able to discern differences among the apricot jams and their comparisons for color (Table 2). Since apricot fruits range in colors of yellow to orange and red when ripe, chromaticity coordinates of blue-yellow ( $b^*$ ) indicate that 'Debbie's Gold' was the yellowest apricot jam and significantly yellower than MN206, 'Westcott', the tart cherry and plum controls (Table 1). In contrast to 'Brookcot', apricot jams made

from MN604, MN206 and MN203 were significantly darker in color and were statistically similar to the tart cherry control. Such darker-colored apricot jams, changing from yellower to more reddish tones may be due to the Maillard reaction whereby brown pigmentation is formed or enzymatic browning occurs. The browning of jams has been observed in previous studies of apricot (Touati et al., 2014) and strawberry jams (Wicklund et al., 2005; Patras et al., 2011).

The panelists in the sensory evaluation were able to discern differences among the apricot jams and comparisons for spreadability, texture, fruit pieces, flavor, off-flavor

and overall quality (Table 1). For the spreadability scores, MN206 had the most similar value (8.5) reported in other studies (range of 7.0-8.11; Touati et al. 2014). Panelists could not distinguish differences for sweetness and bitterness ratings for any of the jams (data not shown). Thus, even if fructose or glucose levels in the fresh fruit differed, the addition of comparative sucrose levels during the jam making process may have masked such differences, if they existed. Future chemical research could identify whether or not fructose and/or glucose levels differ in the apricot cultivar jams tested herein. Likewise, future studies could include testing storage effects on all parameters to determine whether jam quality changes over time.

Oftentimes panelists in sensory evaluations are unable to discriminate for specific traits among jam samples. For example, some apricot jams are admixtures with undeclared additives such as apples (Drugovic-Uzelac et al., 2005b), pumpkin (Drugovic-Uzelac et al., 2005a) or sugar and water (Fuchs and Koswig, 1997; Hammond, 1997). Such additions occur due to the high cost of fresh apricot fruit, limited production or crop failures. Sensory evaluation panelists could not detect these adulterations in apricot jams (Drugovic-Uzelac et al., 2005b).

One unnamed apricot selection, MN 206, had the highest number of traits (5 in total) that differed significantly from other tested apricot jams. MN 206 had low T.A. and high scores for spreadability, texture, fruit pieces, flavor and overall quality. However, since MN 206 is not on the market and unavailable to consumers, the second tier of high quality apricot jams were made from 'Sungold' and 'Brookcot'. Both of these cultivars had significantly lower pH, which ensures long-term storage and has a lower likelihood of browning from the Maillard reaction, while 'Sungold' had <60% soluble solids as required by the Codex Alimentarius Standard. 'Sungold' also rated high in overall quality, T.A., and 73.3% of the sensory evaluation panelists said they would purchase this apricot

jam. This is in contrast to 'Brookcot' where 75% of the panelists would not purchase it (Table 4). Thus, we recommend 'Sungold' as the best apricot for making jam with the currently available winter hardy trees for purchase.

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## Potential of New *Prunus* Rootstocks for Managing *Armillaria* Root Rot Disease in Peach Production

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### Abstract

*Armillaria* root rot (ARR) pathogen is currently one of the most important diseases affecting peach [*Prunus persica* (L.) Batsch] production in the southeastern United States causing high plant mortality. This soil-borne disease affects the roots of the plant, producing subsequent symptoms in the canopy, and finally killing the host. No chemical control is currently available for ARR. To overcome this disease, rootstock use is an option; however, resistant rootstocks are fairly new and their availability is limited. The objective of this review is to describe the sources of resistance against the pathogen, the rootstock breeding procedures for peaches, and the management tools for fighting the infection and reducing symptoms. Multiple peach and plum accessions have been evaluated for ARR resistance over the last few decades. The main sources of resistance were identified in plum hybrids of native North American plum species. These resistance sources were used as the foundation for breeding peach rootstocks with resistance to ARR. Resistant plum lines were hybridized with peach germplasm to develop rootstocks resistant to ARR. Two rootstock cultivars were developed and released: ‘Sharpe’ and ‘MP-29’. Although some ARR disease management practices have been examined, rootstocks are still a good option to reduce losses induced by ARR in peaches.

*Armillaria fungi overview.* *Armillaria* root rot (ARR) is naturally present in forests (Wargo and Shaw III, 1985). The disease is mainly found in temperate and tropical areas of the world, and in almost every state in the United States (Williams et al., 1986). It is caused by different species within the fungal genus *Armillaria*, such as *Armillaria tabescens* (Scop) Emel, *Armillaria mellea* (Vahl:Fr) Kummer, *Armillaria ostroya* (Romagn.) Herink, *Armillaria gemina* Bérubé & Dessureault, *Armillaria calvescens* Bérubé & Dessureault, *Armillaria sinapina* Bérubé & Dessureault, *Armillaria gallica* Marxmüller & Romagn., *Armillaria nabsnona* Volk & Burdsall, and *Armillaria cepistipes* Velenovsky (Williams et al., 1986; Cox et al., 2005; Volk and Burdsall, 2016). In the southeastern United States, *A. tabescens* is the main species causing ARR, followed by *A. mellea* (Schnabel et al., 2005). Classified as basidiomycetes (Smith et al., 1990), these

fungi can behave as primary pathogen, negatively affecting plant growth, leaving plants susceptible to attack by various pathogens and insects. This behavior occurs mainly in inland coniferous forests of the Western United States, a relatively dry region (Williams et al., 1986). Besides acting as a primary pathogen, ARR can be a secondary pathogen in stressed plants (because of competition, pests, and adverse climatic conditions for example) and even behave as a saprophyte in decomposing dead trees (Wargo and Shaw III, 1985).

The life cycle of most *Armillaria* species involves a parasitic phase, which is characterized by the fungi invading the host, and the saprophytic phase, which is characterized by utilizing the host as food for its development (Morrison, 1976). The parasitic phase of ARR starts by spreading through rhizomorphs which are root-like fungal structures (Wargo and Shaw III, 1985; Williams et al.,

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1986). Rhizomorphs start the colonization process by penetrating the outer layers of the host's root, mainly in root sections that have suffered stress or necrosis. Further, as the mycelial fans grow during the saprophytic phase and the necrotic area increases, the infection may reach the cambial zone inducing the decay of the root. After colonizing one plant, the rhizomorphs will grow and reach other plants. These additional plants may be affected by the fungi depending on the specific health and conditions of the new plant (Morrison, 1976; Wargo and Shaw III, 1985). However, some differences in the life cycle are seen in the southeastern United States. Rhizomorphs are rarely produced and the disease spreading is primarily through contact among peach roots and old infected root pieces left in the soil from previous orchards/forests. Mushroom spores coming from adjacent forests contribute little to the disease spreading (Cox et al., 2005)

The detection of an *Armillaria* infection is difficult because the initial plant symptoms occur underground (Williams et al., 1986). However, as the infection progresses, the plant canopy starts to display symptoms like foliage discoloration (chlorosis, sometimes bronzing of foliage and branches), branch dieback, and plant growth reduction (Cox et al., 2005; Morrison, 1976; Williams et al., 1986).

*Importance of ARR in peach production.* One of the main causes of premature tree mortality in stone fruit orchards in the southeastern United States is ARR (Cox et al., 2005) (Fig. 1), followed by peach tree short life (PTSL) (Fig. 2) (Clemson Cooperative Extension, 2015). ARR is a devastating disease (Fig. 3); however, no chemical control is feasible because of the high persistence of ARR in the soil (Myers and Bennett, 1989; Evert and Bertrand, 1993; Beckman, 1998), leaving few options to control the disease (discussed below). The high disease persistence inhibits the establishment of new



**Fig. 2:** Peach tree killed by PTSL. Courtesy of T. Beckman.



**Fig. 1:** Peach tree plans collapsing due to ARR infection. Courtesy of T. Beckman.



**Fig. 3:** Commercial peach orchard devastated by ARR. Courtesy of T. Beckman.

orchards in previously cultivated land, adding additional costs for the peach industry (Clemson Cooperative Extension, 2015).

The first symptom of ARR infection is below the soil's surface with root necrosis causing roots to have a spongy consistency. White to yellow fungi mycelial fans can be observed by cutting through the bark (Fig. 4). Rhizomorphs may grow in infected tissues. Under favorable environmental conditions, the reproductive fungal structures (basidiocarps) may emerge from the base of the trunk or from shallow roots around the infected trees. After severe infection of the root system and plant crown, cracks or wounds in the bark can exude gum, and leaves can become chlorotic, underdeveloped, curled, and wilted. Subsequently, individual limbs and branches will die as the disease progresses. Eventually, the entire plant will die (Cox et al., 2005).

*Breeding for ARR resistance: Possible germplasm sources and its utilization.* The genus *Prunus* L. is composed of approximately 100 species, subspecies, and varieties of peaches, plums, cherries, almonds, nectarines, and apricots (USDA Natural Resources Conservation Service, 2015). Members of this genus can be found in most of the United States (Ramming and Cociu, 1991).

Native *Prunus* species are potential sources of beneficial genetic material with inherit variation for disease and insect resistances, which could be beneficial for the improvement of either fruiting cultivars or rootstocks (Blažek, 2007; Hancock, 2008). Additionally, these materials may also offer useful contrasts in chilling requirement and cold hardiness (Beckman and Okie, 1994).

At the beginning of the 19<sup>th</sup> century, native North American plum species, such as *Prunus americana* Marsh., *P. hortulana* Bailey, *P. angustifolia* Marsh., *P. besseyi* Bailey, *P. nigra* Ait., and *P. munsoniana* Wight & Hedrick and their hybrids, were commonly utilized as fruiting cultivars (Beckman and Okie, 1994). However, following the introduction of Japanese and European lines with



Fig. 4: Mycelial mat beneath bark in ARR infected peach tree. Courtesy of T. Beckman.

their perceived superior handling and eating qualities, the utilization of cultivars developed from native North American species declined (Ramming and Cociu, 1991). This trend has recently reversed, and now, in addition to the species utilized at the beginning of the 19<sup>th</sup> century, additional germplasm is also used, such as *P. salicina* Lindley, *P. cerasifera* Ehrhart, *P. pumila* L., *P. subcordata* Benth, and *P. mexicana* S. Watson (Beckman and Okie, 1994). These different species provide distinct useful traits that are not found elsewhere (Norton et al., 1990, 1991a, 1991b; Okie et al., 1992; Layne, 1994; Nicotra and Moser, 1997; Grzyb et al., 1998; Lu et al., 1998; Lecouls et al., 1999; Stefani, 2010)

Trait characterization in different species has helped identify the best germplasm for use in breeding programs with the aim to generate lines and cultivars with new and superior characteristics. For example, efforts have been made over the last two decades to develop an ARR-tolerant rootstock for peach production (Beckman et al., 1998, 2008; Beckman and Pusey, 2001; Reighard, 2002; Beckman, 2011).

Reighard et al., (1997) evaluated 37

*Prunus* rootstock cultivars and advanced selections in six locations in South Carolina over multiple years. Various species and sources of germplasm were used, such as peach and hybrid plum rootstocks. The objective of the research was to evaluate tree vigor, longevity, disease resistance, and yield of commercial cultivars grafted onto different rootstocks. As expected, there were useful variations within the rootstocks. Rootstocks bred to tolerate non-fumigated replant PTSL areas performed better than the others. However, European rootstocks did not perform well in South Carolina soils. These results illustrated the effect of environmental variation and the genotype by environment interaction on many commercial traits.

A large cooperative regional trial was established in 1983 (Beckman et al., 1998) to test the survival of more than 100 lines of *Prunus*, including peaches and plums (Fig. 5). They reported that the main cause of plant mortality was PTSL (50%), followed by ARR (35%). Further examination of the results indicated that some plums were the least affected by ARR. Plum hybrids with North American plum species in their genetic background were among “the best lines”, while the lines without North American plum ancestry were among “the worst lines”. In the same report, the authors stated that although



**Fig. 5:** High density trial to evaluate peach trees resistance to PTSL and ARR. Courtesy of T. Beckman.



**Fig. 6:** Bronzing of foliage due to the grafting incompatibility of peach on a hybrid plum rootstock. Courtesy of T. Beckman.

some plums showed potential as rootstocks for peach, most of the plums displayed variable grafting compatibility with commercial peach cultivars, thereby limiting their direct use as rootstocks (Fig. 6). Efforts were undertaken to utilize the resistant plum germplasm via crossbreeding with peach lines in order to improve graft compatibility.

Several other sources of resistance for ARR were reported. Thomas et al. (1948), detected resistance to ARR in different plum lines in California. Proffer et al. (1988) tested different cherry rootstocks in Michigan for ARR infection. Guillaumin et al. (1991) investigated the level of ARR resistance in different rootstocks originated from plums. Loreti (1997), recommended plum rootstocks based on several traits, including resistance to ARR.

*Rootstock development.* Historically, peach seedlings have been used as rootstocks for commercial peach production (Layne, 1987); however, seedlings are not uniform. Breeding programs have started to focus on developing rootstocks adapted for specific regions and conditions in the United States (Reighard, 2002). For example, in an effort to understand the genetics of PTSL, Blenda et al. (2007) crossed a PTSL resistant rootstock (Guardian) with a susceptible rootstock



**Fig. 7:** Greenhouse grown rootstock seedlings destined for field. Courtesy of T. Beckman.



**Fig. 8:** Nursery grown rootstock seedlings being prepared for tests in the field. Courtesy of T. Beckman.

(Nemaguard). The objective was to evaluate the segregating population for PTSL syndrome, and to develop a genetic linkage map for peach rootstocks.

The United States Department of Agriculture, Agricultural Research Service (USDA-ARS), located in Byron, GA houses the peach rootstock breeding program for the southeastern United States. The first evidence of resistance to ARR was reported by Beckman et al. (1998) in this breeding program. The resistant lines were used as parents in crosses, and with the addition of other sources of resistance, superior parents were generated and utilized to develop new hybrids resistant to ARR (Beckman, 2011) (Fig. 7, 8, and 9).

One of the first ARR-resistant rootstocks released for peach production was ‘Sharpe’, a clonal plum rootstock (Beckman et al., 2008) (Fig. 10). The pedigree of ‘Sharpe’ is unknown. ‘Sharpe’ appears to be a hybrid of *P. angustifolia* with an unknown plum species. Furthermore, this rootstock is also resistant to PTSL and some root-knot nematodes. Despite that, as trees aged, yields of ‘Redhaven’ peach on ‘Sharpe’ declined when compared with trees grafted onto ‘Guardian’ (Fig. 11) (Beckman et al., 2008). ‘Sharpe’ is a potential source of disease resistant genes for peach rootstock breeding (Beckman and Chaparro, 2015). ‘Sharpe’ can be propagated by softwood or hardwood cuttings. ‘Sharpe’



**Fig. 9:** High density field trial of advanced rootstock selections. Courtesy of T. Beckman.



**Fig. 10:** ‘Sharpe’ clonal plum rootstock for peach. Courtesy of T. Beckman.





**Fig. 11:** Guardian peach seedling rootstock. Courtesy of T. Beckman.



**Fig. 12:** ‘MP-29’ clonal interspecific hybrid peach rootstock. Courtesy of T. Beckman.

was not patented and is publicly available for research, cultivar development (Beckman et al., 2008), and homeowner production.

The most recent rootstock release resistant to ARR was ‘MP-29’, a clonal interspecific plum-peach hybrid rootstock for peach (Beckman et al., 2012). ‘MP-29’ was selected in a 1994 cross of a hybrid plum species (‘Edible Sloe’) and an advanced peach rootstock selection (‘SL0014’) (Beckman et al., 2013). ‘MP-29’ was released as a superior ARR, PTSL, and nematode resistant rootstock (Beckman and Chaparro, 2015). ‘MP-29’ induces equal if not superior yields of ‘Redhaven’ peach, compared with trees grafted onto ‘Guardian’ rootstock (Beckman et al., 2012). ‘MP-29’ can be propagated through softwood or hardwood cuttings and tissue culture. ‘MP-29’ was patented in 2013 using The Florida Foundation Seed Producers, Inc. as the licensing agent. Peach trees grafted on ‘MP-29’ are currently commercially available in small numbers due to its recent release and due to its different propagation and grafting scheme from the traditional seed propagated rootstock. Commercial trials comparing ‘Guardian’ and ‘MP-29’ in ARR infested soils can be located across southeastern United States. Until now, ‘MP-29’ trials show increased survival and comparable performance to trees grafted onto ‘Guardian’ rootstocks. ‘Sharpe’ and ‘MP-29’



**Fig. 13:** ‘MP-29’ clonal interspecific hybrid peach rootstock grafted with ‘Julyprince’ peach. Courtesy of D. Chavez.

rootstocks have been tested for graft compatibility with several scions other than ‘Redhaven’, and have shown no signs of incompatibility (Beckman et al., 2008, 2012).

*Disease management.* The use of rootstocks resistant to ARR is a feasible avenue for disease management. Two rootstock cultivars have been released - ‘Sharpe’ and ‘MP-29’ – and are an excellent alternative for cultural management for ARR. ‘Sharpe’ trees

are currently recommended for homeowner production due to its yield decline as trees aged in comparison with standard rootstocks. 'MP-29' is recommended for commercial production; however, commercial trials are still in early stages of evaluation. No known adverse characteristics have been identified in 'MP-29' compared with 'Guardian' rootstocks (Beckman, personal communication).

There are only a few cultural management options for ARR, and most are not effective or need more study in commercial settings. Baldi et al. (2015) tested the effects of *Brassica* seed meal on *A. mellea* growth *in vitro* and *in vivo*. *A. mellea* growth was reduced *in vitro*; however, there was not enough infection symptoms in potted trees (*in vivo*) to conduct the experiment. The authors suggested that *Brassica* derivatives have a potential activity against *A. mellea* (based on the *in vitro* studies). Schnabel et al. (2012) tested root collar excavation in peach trees planted in two ARR infested sites. Peach trees were initially planted directly in the ground (as the standard growers' method) or in open-bottom Smart Pot (fabric pot of 45 cm height by 60 cm diameter). Eight months later, roots were excavated in order to expose and evaluate the root collar. Five years after planting, approximately 50% of the plants grown as the standard growers' method died due to ARR infection and only 5% of the plants grown with the excavated root collar died. The authors indicated this prototype as a potential option for ARR management, maintaining vigorous plants as the control plots. In another study, Schnabel et al. (2011) drenched *Trichoderma spp.* onto peach trees after planting and biannually (spring and fall) for three years. Plants were grown in commercial orchards on replant sites previously infected with ARR. No significant differences were found on tree survival between the treated and non-treated plants, and trunk diameter was greater for treated plants compared to non-treated plants three and four years after planting. The results indicate that *Trichoderma spp.* is ineffective to control ARR infection in peaches.

Cox and Scherm (2006) tested five species of saprobic (*Ganoderma lucidum*, *Hypholoma fasciculare*, *Phanerochaete velutina*, *Schizophyllum commune*, and *Xylaria hypoxylon*) in combination with *A. tabescens* and *A. mellea* with the objective of assess if the five species would exclude *Armillaria* from peach roots. The experiments were conducted using glass slides, wood blocks, and root pieces in controlled conditions in the laboratory. *G. lucidum*, *S. commune*, and *X. hypoxylon* reduced *Armillaria* growth above and below the bark. The authors speculated that these three species are good candidates for future field tests in peach orchards.

Chemical treatment to fight ARR infection is not feasible in commercial orchards due to the nature of the disease. Research on soil fumigation and drenches produced inconclusive results and field tests were not extensively conducted (Clemson Cooperative Extension, 2015). Amiri et al. (2008) tested six different chemical groups of fungicides to control ARR, showing some promising results. The objectives were to evaluate the fungicides' efficiency against *A. tabescens* isolates *in vitro*, and the activity of these fungicides in peach roots and trunk after intravascular infusion. Propiconazole was the most effective group inhibiting mycelial growth of the isolates. Furthermore, propiconazole was detected in primary roots and trunk segments of peach plants, indicating that after infusion, the fungicide was able to move in the plant. These results suggested that propiconazole can be used as a management option against *A. tabescens*. Adaskaveg et al. (1999) tested different therapeutic treatments of sodium tetrathiocarbonate (STTC) and propiconazole to manage ARR in almond plants grafted onto peach rootstocks in laboratory and field conditions. Single-season treatments of STTC in infected mature trees did not prevent tree mortality caused by ARR. ARR infected trees treated with propiconazole had a 2-year life span, whereas plants not treated died within 4 months. Propiconazole reduced mycelial growth of *A. mellea* by 50%, in laboratory studies.

### Summary

The use of ARR resistant rootstocks remains the main option to control ARR infection in peaches. The development of these rootstocks is an important step towards sustainable peach production in the southeastern United States, increasing tree longevity in peach orchards. Through the use of native plum lines in hybridizations, ARR resistant rootstocks were released and have been used with proven ability to produce high yields while avoiding ARR infection. Breeding efforts targeting ARR are currently in place in public and private institutions, foreseeing the production and availability of resistant material for future tests and uses.

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## Phenolic Content and Antioxidant Capacity of American Persimmon (*Diospyros virginiana* L.) Teas

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**Additional index words:** Antioxidants, chemoprevention, phenolics, teas, persimmon

### Abstract

Regular consumption of green tea, a rich source of phenolic compounds, has been linked to various health benefits. Since most green tea on the market in the U.S. is imported, there have been concerns for contamination with heavy metals and pesticides. Leaves and other plant parts of various species such as American persimmon (*D. virginiana* L.), which are native to the eastern U.S., have been similarly used to make beverages in the past. Unfortunately, the health benefits of these teas have not been studied. The objectives of this study were to examine phenolic content and antioxidant capacity of American persimmon infusions (tea) made from the leaves of different cultivars. Leaves from five cultivars of American persimmon were harvested in May of 2012. Folin-Ciocalteu assay was performed to determine phenolic content of teas. The phenolic content of green tea was 209.7, and that of American persimmon teas ranged from 136.8 to 166.2 in mg of gallic acid equivalent per ml. The Ferric Reducing Antioxidant Power assay was performed to determine antioxidant capacity, revealing that the antioxidant capacity of the persimmon tea was roughly a half of that of green tea. Teas made from American persimmon leaves are a caffeine-free healthy alternative to regular or green tea.

Tea, *Camellia sinensis*, especially green tea, has long been claimed to be helpful for prevention of hypercholesterolemia, atherosclerosis, Parkinson's disease, Alzheimer's disease, and other aging-related disorders (Zaveri, 2006). Along with, *C. sinensis*, various plant species have been used as a source for teas, and some have been reported to be beneficial to human health.

Despite ever increasing tea consumption in U.S., domestic tea production is a very limited endeavor. Currently, there is only one tea plantation in South Carolina and the magnitude of tea production is very small compared to the amount consumed in the U.S. This may be partly due to the fact that tea plants require specific cultural conditions. They are also prone to various disease and insect problems aside from the climatic requirement. Because of the significant shortage of, and possible indifference to tea culti-

vation in this country, the vast majority of tea consumed in the U.S. is imported (Meeberg, 1992).

As is often the case with imported food-stuffs, there is always a concern for food safety. In the case of tea, residual pesticide and mineral contamination are of particular concern. For example, one study revealed polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans, chlorinated pesticides and polynuclear aromatic hydrocarbons (Fielder et al., 2002) in tea leaves. Similarly, a relatively high concentration of heavy metal elements, including lead (Jin et al., 2005a, 2005b; Han et al., 2006; Han et al., 2007), chromium (Seenivasan et al., 2008), and copper (Jin et al., 2008), have been detected in different kinds of tea. Furthermore, tea is also known to contain concentrations of aluminum, fluoride and oxalate, which may pose a potential health threat to some

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consumers (Flaten, 2002). Therefore, it is advisable to seek an alternative with equal or greater health benefits and fewer undesirable characteristics.

American persimmon (*Diospyros virginiana* L.) is a native species that is found throughout the eastern half of the U.S. and Canada, ranging from New England to Florida and west to Kansas, Oklahoma and Texas (NRCS, 2013). This tree grows wild, but has been cultivated for its fruit and wood by Native Americans. However, this species is most commonly grown for fruit, which is high in vitamin C. The unripe fruit is noted for its astringency, but the ripe fruit may be eaten raw, cooked or dried. Additionally, tea can be made from the leaves, and the roasted leaves were used as a coffee substitute during the Civil War (Lee and Gordon, 1993).

The Asian counterpart of the American persimmon, the Asian persimmon (*Diospyros kaki* Thun.) has been extensively studied for its medicinal and health ameliorating properties. Asian persimmon fruit is particularly rich in vitamin C, carotenoids and polyphenols (Giordani et al., 2011), all of which are considered powerful antioxidants that protect against free-radicals and prevent the risk of cardiovascular disease, diabetes and cancer (Georgé et al., 2011). The antioxidant activity of persimmons has been chemically assessed by determining the radical scavenging activity through various chemiluminescent assays, including the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method, or 2,2'-azino-bis(3-ethyl-benzoathiazoline-6-sulfonic acid) diammonium salt (ABTS), the measuring of ferric reducing antioxidant power (FRAP) and low-density-lipoprotein (LDL) oxidation. It has been suggested that proanthocyanidins found in persimmon may reduce blood pressure and platelet aggregation and therefore exert a beneficial effect on coronary diseases (Giordani et al., 2011).

In addition to fruit, leaves of the Asian persimmon have been used for human consumption (Kotani et al., 2000, Sakanaka et al., 2005, Weijian et al., 2005, Lee et al.,

2006). The most notable example would be persimmon teas. Similar to fruit, persimmon leaves are rich in phenolic constituents, particularly tannins (Weijian et al., 2005), and persimmon tea has health promoting properties, including inhibiting development of dermatitis (Kotani et al., 2000); improving the lipid profile of rats fed a high-fat diet (Weijian et al., 2005); and reduction of hydrogen peroxide-induced injury of NG108-15 cells. While there is a recorded use of American persimmon leaves for tea (Lee et al., 2006), and Asian persimmon teas have been studied, it appears that an investigation on health benefits of American persimmon tea has not been conducted.

While the medicinal and health promoting properties of Asian relatives have been studied, very little information is presently available for American persimmon on its health benefits and medicinal components. As the Asian species is known for health promoting properties, it is natural to hypothesize that American counterparts may also possess health beneficial properties. A chemical characterization of compounds found in teas of these plants may lead to further investigation on health amelioration with underutilized common species found in many parts of Kentucky and the surrounding region. Thus a thorough and detailed investigation of the properties of American persimmon tea could lead to a wider usage of these teas.

### Materials and methods

**Samples.** Leaves from five American persimmon cultivars 'Early Golden', 'Evelyn' (Orleans, KY), 'Evelyn' (Upton, KY), 'John Rick', 'Valeene Beauty', and 'Yates' with three replicates were collected from a commercial orchard in Orleans, IN. Additionally, leaves of 'Evelyn' with three replicates were collected from two nurseries. Locations, and a list of cultivars with descriptions are depicted in Table 1. Lipton® Green Tea was purchased to compare its phenolic content and antioxidant capacity to those of persimmon teas.

**Table 1.** Cultivars of American persimmon used. Compiled from Reich (2004) and Raymond (2006).

Cultivar	Description
'Early Golden'	Origin: Alton, Illinois in the late 1800's. Probably the oldest cultivar known.
'Evelyn'	Origin: North Tonowonda, NY.
'John Rick'	Origin: A seedling of 'Killen,' which was a seedling of 'Early Golden'
'Valeene Beauty'	Origin: Bred by James Claypool and released by Don Compton. A seedling of 'Lena' x 'Early Golden.' Reddish leaf color when leaves emerge and expand.
'Yates'	Origin: Southern Indiana. Probably same as 'Juhl.'

Fresh young leaves from 50 shoots were first weighed and thoroughly washed to remove debris, insects, etc. Excess moisture was removed with paper towels, and leaves were placed in a Ziploc® Zip'n Steam® Microwave Cooking bag. Leaves were microwaved for 30 sec/50 g of samples in a 750w Whippoorwill counter top microwave. Leaves were then roasted on an electric skillet (Hamilton Beach, Southern Pines, NC) at 400°F, immediately after removal from the bag.

*Preparation of teas.* American persimmon tea was prepared in the same manner previously described for green tea (Chandra and de Mejia, 2004). After boiling 140 ml of double distilled water (DDH<sub>2</sub>O), 1.4 mg of roasted American persimmon leaves were added and brewed for 5 min. with heat. The tea was left to cool down for another 5 min, and then vacuum filtered through fiberglass microfiber filter paper (Whatman, Piscataway, NJ).

*Measurement of phenolic content.* The amount of soluble phenolic content was quantified by a modified protocol for 96 well plates (Dicko et al., 2002). To each well of the 96-well plate, 10 ml of either DDH<sub>2</sub>O, standard, or sample was added, followed by dispensing of 25 ml Folin-Ciocalteau reagent (Sigma, St. Louis). After 10 min. incubation, 25 ml of 20% (w/v) Na<sub>2</sub>CO<sub>3</sub> was added to each well. Immediately after addition of Na<sub>2</sub>CO<sub>3</sub>, 140 ml of DDH<sub>2</sub>O was added to the wells. The final volume of the reaction mixture in each well was 200 ml. Absorbance of the mixture was measured at 760 nm with a microplate reader (Infinite®200 Pro, Tecan, Raleigh, NC) and analyzed with i-Control™.

Kinetics of the reaction were observed for two hrs. to determine the total PC, expressed in g of gallic acid equivalent per ml of tea (mg GAE/ml).

*Ferric reducing antioxidant power (FRAP) assay.* Antioxidant capacity of the teas was quantified by a modified ferric reducing antioxidant power (FRAP) assay for 96 well plates (Firuzi et al., 2005). Working FRAP solution was freshly made by mixing 15 ml of acetate buffer (300 mM) and 1.5 ml ea. of TPTZ (10 mM) and ferric chloride solution (20 mM). Both acetate buffer and FRAP solution were warmed to 37°C prior to adding to the well. In each well, 25 ml of either standard or sample of different concentrations was be dispensed, and the equal amount of solvents used to dissolve standards was used as blank. Plates was incubated after adding 175 ml of FRAP solution. Absorbance of the mixture was measured at 595 nm. Temperature was kept at 37°C for the whole period of experiments, and kinetics of the reaction were observed for two hrs. Antioxidant power is then expressed in mol of Trolox equivalent (mM TE).

*Data analysis.* Gallic acid and Trolox equivalent values of teas were obtained by using the equations for these standard curves. The equation and the value were obtained after plotting absorbance readings. Results were analyzed using one-way ANOVA followed by Student's least significant difference test with the general linear model (LSD,  $P < 0.05$ ), and correlation coefficients between phenolic content and antioxidant capacity was determined. All statistical

analyses were performed using the statistical software package CoStat Version 6.400 (Co-Hort, Pacific Grove, CA).

### Results and Discussion

This is the first study to report a comparison of the phenolic and antioxidant capacity of American persimmon and *C. sinensis* teas. Phenolic content of green tea was significantly higher than that of American persimmon teas (209.7 mg GAE/ml) (Table 2). Phenolic content of American persimmon teas ranged from 136.8 to 166.2 mg GAE/ml, approximately 65.2% to 79.2% of green tea tested in this study. Of American persimmon cultivars tested, the tea made of 'John Rick' leaves had the greatest phenolic content. 'Valeene Beauty' had the second highest phenolic content, followed by 'Evelyn,' 'Yates,' and 'Early Golden.' The current finding suggests that there is a great deal of diversity in the amounts of phenolics contained in leaves of different cultivars examined in this study.

Similarly, antioxidant capacity of green tea was significantly higher than that of persimmon teas at 1015.9 mM TE (Table 2). Antioxidant capacity of American persimmon was roughly half of green tea's, ranging from 577.5 to 437.2 mol TE/g FW. Similar to phenolic content result, teas made from 'John Rick' had the greatest antioxidant capacity (575.5 mM), followed by 'Valeene Beauty' (500.6 mM TE). However, there was no statistically significant difference among

cultivar samples with the exception of 'John Rick' in antioxidant capacity.

Leaves of 'Evelyn' were collected at both Upton, KY and Orleans, IN sites. This was the only cultivar available in this study with triplicate trees at both sites. Phenolic content of 'Evelyn' teas were very similar for both Orleans (149.0 mg GAE/ml) and Upton (146.4 mg GAE/ml) samples. Similarly, a small difference in antioxidant capacity was observed for 'Evelyn' samples collected in Orleans (495.6 mM TE) and Upton (437.2 mM TE), but it was not statistically significant. This may be due to a relative proximity between the two sites (app. 160 km), but it appears that differences in location had little effect on phenolic content and antioxidant capacity of teas made of this cultivar.

Of all cultivars tested, three were genetically related. 'Early Golden' is probably the oldest cultivar available, and has sired other well-known cultivars. 'John Rick' is a seedling of 'Killen,' which is a seedling of 'Early Golden.' In addition, 'Valeene Beauty' is a seedling of 'Lena' ('Mitchellena') and 'Early Golden' originated in Claypool breeding (Raymond, 2006). Despite their genetic relatedness, phenolic content and antioxidant of these cultivars, especially, 'John Rick' was significantly different from 'Early Golden.'

The foliage of 'Valeene Beauty' has a reddish tinge when leaves emerge in spring, indicating the presence of phenolics such as anthocyanins. This cultivar also seems more

**Table 2.** Phenolic content and antioxidant capacity of American persimmon teas

Species	Cultivar	Phenolic content (mg GAE/ml)	Antioxidant capacity (mM TE)
<i>Camellia sinensis</i>	NA	209.7 a	1015.9 a
<i>Diospyrus virginiana</i>	'Early Golden'	136.8 e <sup>2</sup>	474.4 c
	'Evelyn' (Orleans)	149.0 cd	495.6 c
	'Evelyn' (Upton)	146.4 cd	437.2 c
	'John Rick'	166.2 b	577.5 b
	'Valeene Beauty'	156.8 c	500.6 c
	'Yates'	143.9 d	495.2 c

<sup>2</sup>Means followed by the same letters are not significantly different within the same column ( $P < 0.05$ )



prone to diseases than others based on visual observation. As is known, plants produce phenolics and other secondary metabolites in response to both abiotic and biotic stress, including fungal infection (Latouche, 2013). Elicitation by fungal pathogens, along with anthocyanins in leaves, might have also contributed to the higher amount of phenolic found in 'Valeene Beauty' teas.

In conclusion, while teas made of American persimmon had lower phenolic content and antioxidant capacity, consumption of such tea may be beneficial to human health. The phenolic content and antioxidant capacity of American persimmon tea is comparable to more commercially available black tea. In one report, phenolic content and antioxidant capacity of black tea were reported to be approximately 75.2% and 54.8% of green tea respectively (Lee and Lee, 2002). In this study, phenolic content and antioxidant capacity of American persimmon tea was 68.3~77.8% and 39.3~51.7%. Furthermore, Yerba mate (*Ilex paraguariensis* A. St. Hil.) or Ardisia tea (*Ardisia compressa* Kunth.), and other teas known for their chemopreventive properties also have lower phenolic content and antioxidant capacity compared to those of green tea (Chandra and de Mejia, 2004).

Aside from health benefits, teas made from American persimmon may prove to be a valuable alternative as people become more aware of importance in local food production. In spite of local abundance, American persimmon is relatively unexploited as a foodstuff. Likewise, production of teas with its leaves may provide an additional income source for wildcrafters or source limited farmers. Finally, consumers may prefer safer alternatives such as American persimmon teas due to lack of caffeine or contaminants such as pesticides and heavy metals that have been reported in commercially available teas.

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## Yield and Quality Characteristics of Several Table Apricot (*Prunus armeniaca* L.) Cultivars in the Silifke/Mersin Ecological

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**Additional index words:** Turkey, fruit quality, earliness

### Abstract

This research was conducted between 2009-2012 in Silifke, Turkey utilizing 8 different apricot cultivars, five from non-domestic origins 'Aurora', 'Ninfa', 'Bebeco', 'Precoce De Tyrinthe', 'Priana' and three from domestic origins 'Alyanak', 'Tokaloglu', 'Cagataybey'. In material cultivars some phenological and pomological characters such as flowering, yield, fruit weight, fruit dimensions, flesh/seed ratio, acidity and total soluble solids concentration (TSC) were examined. In terms of fruit yield, 'Ninfa'(21.37 kg/tree; 39.55 kg/tree; 45.81 kg/tree; 79.11kg/tree), 'Priana'(20.97 kg/tree; 36.08 kg/tree; 44.76 kg/tree; 77.61 kg/tree) and 'P.De Tyrinthe'(18.74 kg/tree; 31.52 kg/tree; 38.13 kg/tree; 64.58 kg/tree) were most productive in 2009-2012 respectively; 'Tokaloglu', 'Bebeco' and 'P.De Tyrinthe' had the largest fruit in all years. Due to their precocity and yield, 'Ninfa', 'Priana' and 'P.De Tyrinthe' were the most promising cultivars for the Silifke area.

Apricot (*Prunus armeniaca* L.) is grown around the world and in Turkey, and can be consumed as fresh or dried fruit. The total amount of apricot production in the world is more than 4,000,000 tons, and 811,609 tons are supplied by Turkey. Turkey is the largest producer of apricots in the world (Fao, 2015). Apricot can be grown in cold regions of Siberia, in subtropical North Africa, desert in Central Asia, in the humid climate of Japan and East China. Although Turkey is one of the leading countries and has expertise for production of dried apricot, the production of fresh apricot is quite small (Paydas et al., 1992; Bas et al., 2001). More than 80% of world trade in table apricots are early season. Mediterranean countries greatly benefit from this situation. Spain, Greece, Italy, France and Hungary are among the apricot leading exporting countries. Although Turkey is located in the same climate zone, there is almost no exportation of fresh apricots; most Turkish apricots are exported as dried product (Kaska, 2006).

Apricot cultivation in subtropical areas, decreases late spring frost risk (Rodrigo and Julian, 2006). Looking at the number of trees and the production of apricots in Turkey in recent years, adverse climatic events some years cause annual fluctuations in production. However, overall the number of trees and production of apricots are on a steady rise in Turkey (Durgac 2001). Apricots are grown almost everywhere in Turkey except the very moist areas near the Black Sea and in mountainous areas of Eastern Anatolia Region where the winters are too cold. Turkey ranks first in the world production of fresh and dried apricots (Anonymous, 2007). Mediterranean and Aegean regions have great potential for growing early table apricots but to achieve this it is important to increase the number of quality early-season cultivars. In recent years, due to adaptation of early and table apricots production in the Mediterranean region has rapidly increased.

In a study in Erdemli/Alata conditions, the cultivars 'Precoce De Colomer', 'San

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Castrese', 'Boccucia', 'Sakit 2', 'Cigli' and 'Fracasso' were promising in terms of earliness and fruit quality (Ayanoglu and Saglamer, 1986). Aegean and Mediterranean regions have a high potential for cultivation of early and table apricot varieties. To achieve this potential, increasing of earliness, fruit quality and the long distance transport of cultivars have great importance (Onal et al., 1995).

The aim of this study was to evaluate the phenology, productivity, and fruit quality characteristics of several early and high quality table apricot types in the region of Silifke, Turkey.

### Materials and Methods

The experiment was carried out between 2009-2012 in Silifke-Mersin, with 3 year-old 'Alyanak', 'Aurora', 'Bebeco', 'Cagataybey', 'Ninfa', 'Priana', 'Tokaloglu' and 'Precoce De Tyrinthe' apricot trees budded on wild apricot rootstock. Soil texture is sandy loam, medium in organic matter, with neutral pH, no soluble salt problem and sufficient total nitrogen. Trees were trained to a vase shape and spaced 4 m apart both between and in rows (625 trees/ha<sup>1</sup>). In the trial there were 6 trees of each apricot cultivar. Trees were replicated three times with two trees per plot in a randomized complete block design.

*Phenological observations.* Dates for first bloom, full bloom, end of bloom and harvest were recorded. Date of full bloom

was recorded as the date when 90% of the flowers were open and the harvest date was determined by visual observations and color changes (from green to yellow and red). The ripening period was the period between the first and the final harvest. Fruits were harvested at maturity based on appearance and taste, and 15 fruits were randomly sampled from each tree to evaluate fruit quality characteristics. Fruit diameter was measured with digital calipers, and fruit and seed weights, and flesh/seed ratios were recorded. Total soluble solids concentration (SSC) of the fruit juice were determined by hand refractometer, and titratable acidity (malic acid) was calculated by titrating fruit juice with 0.1 N NaOH). Yield per tree was obtained annually.

*Statistical Analysis.* The experimental design was completely randomized with six trees per cultivar, and 2 trees were treated as a replicate. Therefore, there were 90 fruits and three replicates per cultivar. Data were analyzed with analysis of variance and means were compared with Tukey's test using Costat software (Duzgunes, 1963).

### Results and Discussion

*Phenological observations.* Harvest dates and phenological data are presented in (Table 1). Full bloom was earlier for 'Ninfa', 'Priana' and 'Precoce De Tyrinthe' than the other cultivars (Table 1). The latest flowering cultivars were 'Cagataybey', 'Tokaloglu' and

**Table 1.** Average date of phenological stages of eight apricot cultivars (2009-2012).

Cultivar	First bloom	Full bloom	End of bloom	Harvest date
Alyanak	08 March	13 March	20 March	8 June
Aurora	11 March	19 March	26 March	12 May
Bebeco	01 March	4 March	10 March	5 June
Cagataybey	12 March	16 March	21 March	3 June
Ninfa	20 February	22 February	26 February	11 May
Priana	21 February	24 February	28 February	12 May
Tokaloglu	14 March	16 March	23 February	16 June
Tyrinthe	26 February	01 March	06 March	19 May

'Aurora'. These results correspond with those reported by Paydas et al. (1995) working in Adana conditions with the cultivars of 'Bebeco', 'Beliana', 'Canino', 'Feriana', 'Precoce De Colomer', 'Precoce De Tyrinthe', 'Priana' and 'Trewatt'. Time of maturity ranged from 11 May to 16 June (Table 1). 'Ninfa', 'Priana' and 'Aurora' ripened earliest, on 11 and 12 May respectively. These results are in accord with those of other studies done in Mut ecological conditions of Turkey (Son and Kuden, 2001). 'Bebeco', 'Alyanak' and 'Tokaloglu' were the latest ripening cultivars and matured on 5-16 June (Table 1). These findings are similar to the results of adaptation studies performed in different areas (Seferoglu and Gulsen, 2003; Ayanoglu et al., 1995).

*Yield per tree (kg/tree)*. The difference between the varieties in terms of yield per tree was statistically significant at 5% level. 'Ninfa', 'Priana' and 'Precoce De Tyrinthe' were the most productive cultivars in all trial years, whereas 'Tokaloglu', 'Aurora' and 'Alyanak' ranked last in productivity in all years (Table 2). The yield differences between for 'Ninfa' and 'Priana' were statistically non-significant (Table 2). Our findings in terms of yield are in agreement with the those of Son and Kuden (2001). Also, Ayanoglu et al. (1995) and Son's (2004) research support results.

*Fruit characteristics*. Fruit characteristics differed significantly for the different cultivars (Tables 3-6). For fruit weight, 'Tokaloglu' (52 g), 'Bebeco' (51.3 g) and

**Table 2.** Average yield of eight apricot cultivars (kg/tree) (2009-2012).

Cultivar	2009	2010	2011	2012
Alyanak	5.98e	11.01d	12.54d	23.20e
Aurora	2.56f	4.63e	7.39e	15.58f
Bebeco	14.96c	25.02c	35.18b	60.45c
Cagataybey	12.59d	20.61c	31.39c	55.31d
Ninfa	21.37a	39.55a	45.81a	79.11a
Priana	20.97a	36.08a	44.76a	77.61a
Tokaloglu	2.14f	3.78e	7.35e	14.85f
Tyrinthe	18.74b	31.52b	38.13b	64.58b
LSD (P=0.05)	2.02	4.51	3.06	3.43

Means within columns followed by the same letter are not significantly different according to LSD,  $P < 0.05$ .

**Table 3.** Fruit quality characteristics of eight apricot (*Prunus armeniaca* L.) cultivars (2009).

Cultivar	Fresh Fruit weight (g)	Seed weight (g)	Flesh/Seed Ratio	SSC (%)	Acidity
Alyanak	42.41d	2.42cd	16.52d	16.20a	1.01e
Aurora	39.98e	2.40d	15.65e	15.86a	1.02e
Bebeco	51.55a	3.65b	13.11f	15.06bc	1.24b
Cagataybey	46.67c	2.37e	18.66b	15.33b	1.18c
Ninfa	43.36d	2.40d	17.04c	14.86c	1.02e
Priana	38.20f	2.35e	15.23e	15.13bc	1.02e
Tokaloglu	51.82a	3.82a	12.55g	14.13d	1.07d
Tyrinthe	49.36b	2.43c	19.26a	11.13e	1.46a
LSD (P=0.05)	1.42	0.02	0.47	0.36	0.03

Means within columns followed by the same letter are not significantly different according to LSD,  $P < 0.05$ .

'Precoce De Tyrinthe' (49.6 g) were superior to the others. These data agree with the results of Seferoglu and Gulsen (2003). The smallest fruits were obtained from 'Priana' and 'Aurora' (Tables 3-6). Fruit flesh/seed ratio was greatest for 'Precoce De Tyrinthe',

**Table 4.** Fruit quality characteristics of eight apricot (*Prunus armeniaca* L.) cultivars (2010).

Cultivar	Fresh Fruit weight (g)	Seed weight (g)	Flesh/Seed Ratio	SSC (%)	Acidity
Alyanak	43.84d	2.44c	16.91b	16.26a	1.01f
Aurora	40.33e	2.41e	15.71c	15.86b	1.02ef
Bebeco	51.37ab	3.66b	13.01d	14.93d	1.24b
Çagataybey	47.99c	2.38f	19.14a	15.40c	1.19c
Ninfa	43.88d	2.41de	17.15b	14.93d	1.03e
Priana	38.62f	2.36f	15.32c	15.04d	1.02ef
Tokaloglu	52.42a	3.83a	12.67d	14.06e	1.07d
Tyrinthe	50.20b	2.43cd	19.60a	11.07f	1.46a
LSD (P=0.05)	1.36	0.02	0.51	0.20	0.01

Means within columns followed by the same letter are not significantly different according to LSD,  $P < 0.05$ .

**Table 5.** Fruit quality characteristics of eight apricot (*Prunus armeniaca* L.) cultivars (2011).

Cultivar	Fresh Fruit weight (g)	Seed weight (g)	Flesh/Seed Ratio	SSC (%)	Acidity
Alyanak	44.10d	2.44c	17.03b	16.06a	1.01e
Aurora	40.88e	2.42d	15.89c	15.80a	1.02e
Bebeco	51.89b	3.67b	13.12d	14.80c	1.24b
Çagataybey	48.60c	2.38e	19.36a	15.33b	1.19c
Ninfa	44.87d	2.42d	17.51b	14.93c	1.03e
Priana	39.85e	2.37e	15.71c	15.06bc	1.03e
Tokaloğlu	53.11a	3.84a	12.81d	14.06d	1.07d
Tyrinthe	51.02b	2.45c	19.80a	11.13e	1.46a
LSD (P=0.05)	1.20	0.01	0.53	0.28	0.01

Means within columns followed by the same letter are not significantly different according to LSD,  $P < 0.05$ .

**Table 6.** Fruit quality characteristics of eight apricot (*Prunus armeniaca* L.) cultivars (2012).

Cultivar	Fresh Fruit weight (g)	Seed weight (g)	Flesh/Seed Ratio	SSC (%)	Acidity
Alyanak	44.17c	2.46b	16.95b	16.52a	1.01f
Aurora	39.21e	2.41b	15.26c	15.92b	1.02ef
Bebeco	50.39a	3.66a	12.77e	14.65f	1.24b
Çagataybey	46.58b	2.39b	18.49a	15.26c	1.16c
Ninfa	42.53d	2.41b	16.64b	14.72e	1.04e
Priana	36.65f	2.36b	14.53d	15.01d	1.01f
Tokaloglu	50.65a	3.83a	12.22e	13.99g	1.07d
Tyrinthe	47.82b	2.45b	18.52a	10.90h	1.46a
LSD (P=0.05)	1.63	0.23	0.58	0.04	0.02

Means within columns followed by the same letter are not significantly different according to LSD,  $P < 0.05$ .

**Table 7.** Fruit quality characteristics of eight apricot (*Prunus armeniaca* L.) cultivars (average of years)(2009-2012).

Cultivar	Fresh Fruit weight (g)	Seed weight (g)	Flesh/Seed Ratio	SSC (%)	Acidity
Alyanak	43.63d	2.44c	16.85b	16.26a	1.01c
Aurora	40.10e	2.41cd	15.63c	15.86b	1.02c
Bebeco	51.30ab	3.66b	13.00d	14.86d	1.24b
Cagataybey	47.46c	2.38d	18.91a	15.33c	1.18b
Ninfa	43.66d	2.41cd	17.09b	14.86d	1.03c
Priana	38.33e	2.36d	15.20c	15.06c	1.02c
Tokaloglu	52.00a	3.83a	12.56d	14.06e	1.07c
Tyrinthe	49.6bc	2.44c	19.30a	11.06f	1.46a
LSD (P=0.05)	1.31	0.03	0.52	0.23	0.03

Means within columns followed by the same letter are not significantly different according to LSD,  $P < 0.05$ .

followed by 'Cagataybey' and 'Ninfa' whereas 'Tokaloglu' had the lowest ratio (Tables 3-6), which confirmed previous work (Durgac, 1995). The highest SSC was obtained from 'Alyanak', it was followed by 'Aurora' and 'Cagataybey' in all trial years (Tables 3-6). The findings of Seferoglu and Gulsen(2003), and Son (2004) were similar to ours.

### Conclusions


'Ninfa', 'Priana' and 'Aurora' were the earliest cultivars in the trial. Early maturing cultivars are very advantageous in terms of launching them in the market. But 'Aurora' had unacceptable low yields. 'P. De Tyrinthe' despite later maturation than 'Ninfa' and 'Priana' had a higher market value due to firmer fruit flesh and better appearance. Silifke's fruit growing potential is quite high because it is in an extremely convenient location for growing fresh apricots, but the region has low chilling. Desirable cultivar characteristics for the region include long shelf life, high yield and high fruit quality. According to the results of this study five cultivars have potential in the Silifke-Mersin region. 'Ninfa', 'Priana' and 'P. De Tyrinthe' are early and high-yielding cultivars. 'Cagataybey' and 'Bebeco' ripen later, and have moderate yields, but produce large fruit with high SSC.

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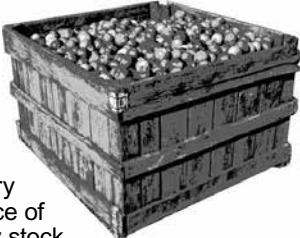
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## Effect of 1-MCP on Persimmon Fruit Quality and Expression of Ethylene Response Genes During Ripening

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**Additional index words:** Astringent, *Diospyros kaki* Thunb, Shelf-life, Softening

### Abstract

This study was conducted to investigate the effects of 1-MCP on the quality and ethylene response gene expression in astringent persimmon 'Bansi' during ripening. Ethylene production was reduced from 0.59  $\mu\text{L}\cdot\text{kg}^{-1}\cdot\text{hr}^{-1}$  immediately after harvest to 0.14  $\mu\text{L}\cdot\text{kg}^{-1}\cdot\text{hr}^{-1}$  and 0.04  $\mu\text{L}\cdot\text{kg}^{-1}\cdot\text{hr}^{-1}$  within five days in the control and 1-MCP treated fruit, respectively. Firmness was 13.8N immediately after harvest and declined rapidly to 7.4N within 1 day for control fruit, on the other hand 1-MCP fruit softened slightly to 11.1N up to the 7 day. Treatment with 1-MCP did not influence soluble solids concentrations. Soluble tannin declined significantly from 399.5  $\text{mg}\cdot 100\text{g}^{-1}$  to 248.5  $\text{mg}\cdot 100\text{g}^{-1}$  in control fruit but tannin level for 1-MCP treated fruit was 357.8  $\text{mg}\cdot 100\text{g}^{-1}$  one day after harvest and did not change significantly through the ripening period. Expression of all the ethylene response genes during ripening was lower in 1-MCP treated fruit than in the control fruit. These results indicate that the inhibition of expression of ethylene receptor genes by 1-MCP treatment resulted in extended shelf life of astringent persimmons. The ethylene response genes mainly associated with this 1-MCP effect appear to be DKERF1, DKERF3, and DKERF8.

The genus of *Diospyros* consists of about 400 species and is distributed in Africa, Asia, and America. Of these a few species can be cultivated in the temperate region, and the best known one is the persimmon (*Diospyros kaki* Thunb.). Persimmon is mainly grown in East Asia, including China, Japan and Korea. In Korea, persimmon ranks the fourth in fruit production following apple, pear and citrus; thus, it is an important fruit crop. Persimmon fruit contains mainly glucose and fructose, beta-carotene and high levels of functional materials, such as vitamin C, gallic acid and catechin (Hiroshi and Akira, 2007). Persimmon cultivars can be classified into two groups based on dissimilarity in flesh coloration as affected by seed formation during pollination (Miller, 1984). The first one is pollination-constant (PC), and the other is

pollination-variant (PV). The fruit flesh in PC persimmon cultivars does not change color by seed formation while the fruit flesh in PV persimmon cultivars has dark coloration. In addition, PC and PV persimmon cultivars have astringent and non-astringent types depending on fruit loss (non-astringent type) or no fruit loss (astringent type) of astringency at maturation. Based on these two classification methods, persimmon cultivars are classified into four types: pollination-constant non-astringent (PCNA), pollination-variant non-astringent (PVNA), pollination-constant astringent (PCA), and pollination-variant astringent (PVA) (Xue-ren et al, 2012). The main native and cultivated types of East Asia are astringent (Xue-ren et al, 2012; Yamada et al, 1994). Astringent persimmon is one of the most important fruit due to its high eco-

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conomic value in major producing countries such as Korea, Japan and China (Pang et al., 2007; Qinggang et al., 2013).

Various aspects of ripening and astringency removal of persimmon have been studied, including ethylene treatment (Lim et al., 2015), CO<sub>2</sub> treatment (Arnal and Rio, 2003; Salvador et al., 2007), ethanol treatment (Ortiz et al., 2005), and high temperature treatment, etc. Kato (1987) reported that ethylene effectively removed astringency. However, after astringency removal and ripening, the fruits became softer and sensitive to damage resulting in shorter shelf life (Guinevere, 2005; Akira, et al., 2011). Therefore, Korea's export is limited regardless of production quality and intensity of management. 1-Methylcyclopropene (1-MCP) is a material that blocks the effects of ethylene by binding to the ethylene receptor in plants and is used to study the mechanisms of the ripening process (Sisler et al., 1995; Zisheng, 2007).

1-MCP delays ripening of climacteric fruits and has been used on various fruits including persimmon (Luo, 2007), banana (Pathak et al., 2003), and tomato (Opiyo and Ying, 2005; Wang et al., 2010). The effect of 1-MCP has been reported recently on pear fruit as it delays softening and reduced respiration and ethylene production (Villalobos-Acuna et al., 2011; Liu et al., 2013; Ioannis et al., 2013; Hanxu et al., 2016). These results suggest that 1-MCP treatment may extend the shelf life of 'Bansi' astringency persimmon. Effects of 1-MCP, however, may vary depending on the genetic ability of cultivars to coordinate physiological, biochemical and molecular responses. Thus, it is important to test the efficacy of 1-MCP for extending persimmon shelf life. In this study, we investigated the effect of 1-MCP treatment on the shelf life of 'Bansi' persimmon by observing physiological and molecular changes of fruits.

### Material and Methods

*Plant material and 1-MCP treatment.* Astringent persimmon fruit (*Diospyros*

*kaki* Thunb. 'Bansi'), an astringent persimmon cultivar (PCA), were harvested from Gyeongsangnam-do, Miryang, Korea on 14 Oct. 2014. The fruit were transported to Kangwon national University horticulture laboratory within 24 h of harvest. A total of 135 persimmon fruits were treated with 1-MCP on the same day after harvest. 1-MCP was generated from commercial powder (EthylBloc, Bio Technologies for Horticulture, IL, USA). The treatment was applied at 1.0  $\mu\text{L}\cdot\text{L}^{-1}$  in a sealed 62.0 L container for 12 h at 20°C. Six containers were used for this study, and each container contained 45 fruits. The 1-MCP concentration chosen as optimal from preliminary experiments was from 0.1  $\mu\text{L}\cdot\text{L}^{-1}$  to 100  $\mu\text{L}\cdot\text{L}^{-1}$  (Zisheng, 2007). Control fruit were treated similarly but without 1-MCP. After 1-MCP treatment, all fruit were ripened with ethylene, at 100  $\mu\text{L}\cdot\text{L}^{-1}$  in a sealed 62L container at 20°C (Akaura, 2010), generated from an ethylene producing tablet. *Measurement of ethylene production.* Persimmon fruit samples were placed in air tight 4.0 L volume containers for three hours and ethylene concentration was analyzed using GC2010 Shimadzu (Shimadzu Corporation, Japan) equipped with BP 20 Wax column (30 m x 0.25 mm x 0.25  $\mu\text{m}$ , SGE analytical science, Australia) and a flame ionization detector (FID). The rate of ethylene production was expressed as  $\mu\text{L}\cdot\text{kg}^{-1}\cdot\text{hr}^{-1}$ .

*Measurement of fruit quality.* Effect of 1-MCP application on fruit quality was evaluated by measuring fruit firmness, total soluble solids concentration, fruit skin color and soluble tannin concentration. Fruit firmness was measured in an equatorial end area using a Rheo meter (Sun Scientific Co. Ltd., Japan), fitted with a 3mm diameter head (Agusti et al., 2004). Fifteen fruits were measured for each treatment and firmness was expressed in newton (N). Total soluble solids concentration was measured on each fruit by expressing juice from each side of the fruit onto a digital refractometer (Model-Atago, USA). Fruit skin color was evaluated on the most colored parts of 15 fruit from

**Table 1.** Real-time PCR primers of ethylene signaling related genes.

Gene	Primary PCR (5'-3')	Secondary PCR (5'-3')
DkCTR1	GGCTTGTAACCCACCAATA	CCATTGAAGCCCAGAGAAAC
DkEIL1	GCCTACCCCTGGTCAAGTGAA	GAGACCAGCATGGGACAAGT
DkERF1	GCTGCTGTCGGAGAGTGAT	TCTCGGGCCTTACAAAGAAG
DkERF2	AAGCCCGACTTGAACGAATA	AAGGTCACAATCCCTTTGGA
DkERF3	AAGAGGCGGTGACAAACAAG	TCACCACATTCATCATCCA
DkERF5	GGCCGTAGACAGGTTCTTGA	AAAAAGGGAAACTCCTCAACG
DkERF7	GACGACGGAGATGGAGACAT	ATCAACATCAGAGGCGAAGG
DkERF8	ATCTGGAAGGGGACAATTC	AGAGTAGCGCGGCAAAATTA

each treatment using a Minolta Colorimeter (Model CR-400, Japan) and calibrated with a white and black standard tile. Result were expressed in Hunter 'L' and 'a' values. Soluble tannin concentration was measured according to the method of Folin-Dennis method described by Taira (1995). 5.0 g of the sample were placed directly into a solution of 25 mL of 80% methanol. 1 mL of this sample solution and 6 mL of distilled water were mixed. Then, 0.25 mL of 2N Folin-Ciocalteu reagent was added and vortexed. After 3min, 1mL of saturated Na<sub>2</sub>CO<sub>3</sub> plus and 1.5 mL of distilled water was added. After incubation for 1 h at 25 °C, the solution was measured using a spectrometer by reading absorbance at 725 nm. The results were expressed as mg/100g F-W.

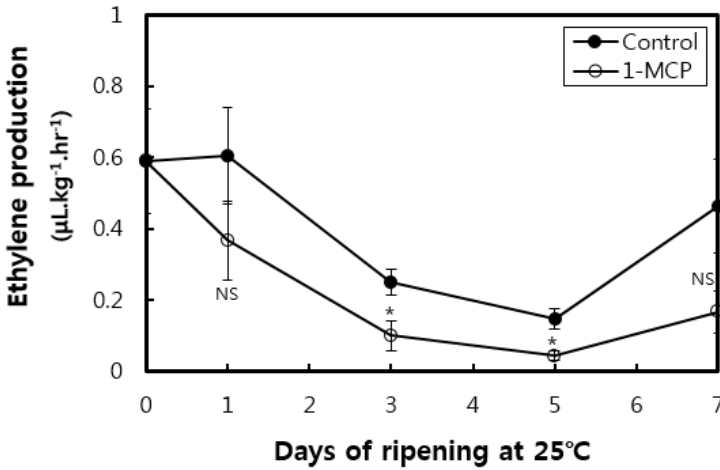
**Gene expression analysis.** Transcript accumulation of DkCTR1, DkEIL1, DkERF1, DkERF2, DkERF3, DkERF5, DkERF7 and DkERF8 was evaluated via quantitative real-time PCR (RT-PCR). Total RNA was isolated from frozen fruit samples with the Robospin Plant TM Kit (GeneAll, Korea) according to the manufacturer's instructions, and treated with RNA-free DNAase I to remove genomic DNA. The quality and concentration of the extracted RNA were measured using a Nano-drop and then cDNA was synthesized with oligo d(T)<sub>18</sub> primer and SuperScript® III Reverse Transcriptase (Life Technologies, USA) from 5 µg of total RNA. Subsequently, the cDNA was utilized to conduct

real time PCR using gene-specific primers. Specific primers were as reported in Table 1 and adapted from an earlier study (Xueren et al, 2012). 1µl of cDNA template was amplified using the Platinum SYBR Green qPCR supermix-UDG (Invitrogen, the Netherlands) in a 20µl qPCR reaction according to the manufacturer's protocol. The samples were amplified with PCR as follows: 3min 50°C, 3min 95°C, 45 cycles of 10 sec at 95°C followed by 30 sec at 60°C. Melting curve analyses were performed on the PCR products. DtActin was used as the reference gene to calculate relative expression levels, using the ΔΔCt method (Livak and Schmittgen, 2001). Three RT-PCR runs were performed per each treatment.

**Statistical Analysis.** All results were presented as means ± standard errors and differences between treatment groups were tested for significance using t-test. Statistical analyses were performed with SPSS statistics program (Version 21, SPSS, USA).

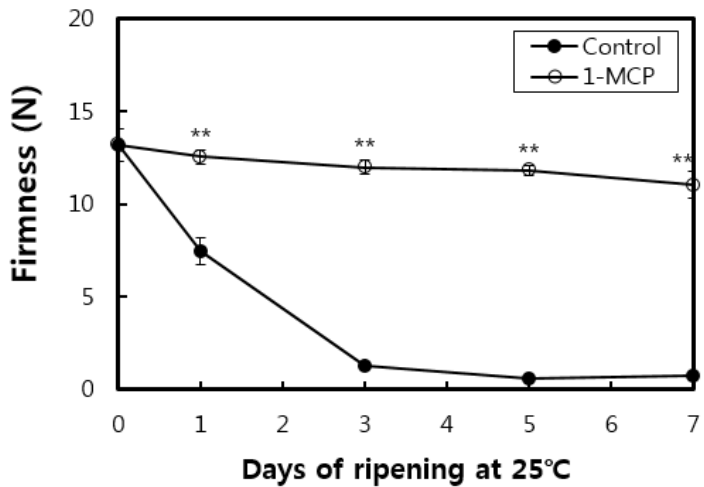
## Results and Discussion

Ethylene production immediately after harvest was 0.59 µL·kg<sup>-1</sup>·hr<sup>-1</sup>; this changed to 0.60 µL·kg<sup>-1</sup>·hr<sup>-1</sup> for the control group and 0.36 µL·kg<sup>-1</sup>·hr<sup>-1</sup> for 1-MCP treatment group after one day of ripening (Fig. 1). At day 5 of ripening, levels of ethylene production decreased to 0.14 µL·kg<sup>-1</sup>·hr<sup>-1</sup> and 0.04 µL·kg<sup>-1</sup>·hr<sup>-1</sup>, for the control and 1-MCP treatment groups, respectively. Persimmon is a climac-



**Fig 1.** Effect of 1-MCP treatment on ethylene production in 'Bansi' persimmon fruit during ripening at 25°C. Vertical bars represent standard errors of the means (n=15). NS,\*,\*\* indicate nonsignificant at  $P>0.05$ , significant at  $P<0.05$  and  $P<0.01$  probability level, respectively.

**Fig 2.** Effect of 1-MCP treatment on firmness in 'Bansi' persimmon fruit during ripening at 25°C. Vertical bars represent standard error of the means (n=15). NS,\*,\*\* indicate non significant at  $P>0.05$ , significant at  $P<0.05$  and  $P<0.01$  probability level, respectively.



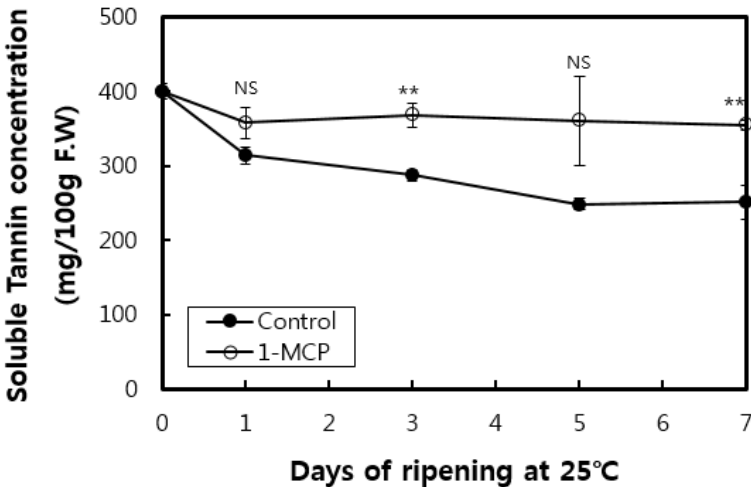
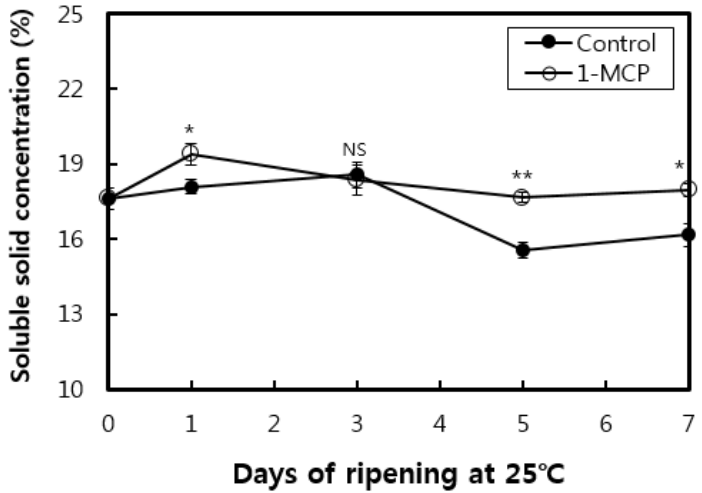
teric fruit, but shows low ethylene production during ripening (Nakano et al. 2002; Pang et al. 2007). The results demonstrated that 1-MCP treatment can reduce ethylene production, similar to previous reports for persimmon fruit (Shinji et al. 2003).

Fruit firmness, a typical fruit ripening indicator, decreased after harvest, from 3.8 N to 7.4 N in the control group at day 1 after ethylene treatment, followed by a steady decrease to 0.7 N over 7 days (Fig. 2). The 1-MCP fruit, softened only a slightly, from

13.8 N to 12.5 N at day 1, with an overall decrease to 11.1 N over 7 days. This inhibition of fruit ripening and softening by 1-MCP treatment agrees with previous reports for other fruits including apple (Watkins et al., 2000), banana (Pelayo et al., 2003), kiwifruit (Boquete et al., 2004) and plums (Menniti et al., 2004).

Total Soluble Solids (TSS) concentration at harvest was 18.1% for both treatments and changed to only 18.3% by day 3 (Fig. 3). After 7 days soluble solids decreased to 17.9%

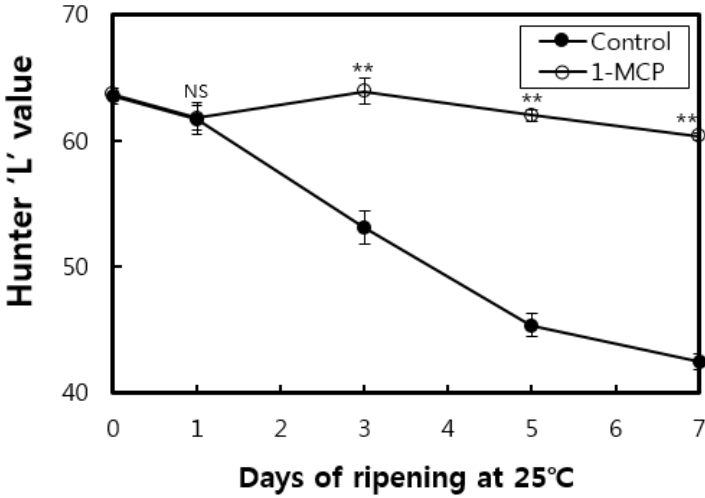
**Fig 3.** Effect of 1-MCP treatment on soluble solid contents in 'Bansi' persimmon fruit during ripening at 25°C. Vertical bars represent standard error of the means (n=15). NS,\*,\*\* indicate non significant at  $P>0.05$ , significant at  $P<0.05$  and  $P<0.01$  probability level, respectively.



**Fig 4.** Effect of 1-MCP treatment on soluble tannin content in 'Bansi' persimmon fruit during ripening at 25°C. Vertical bars represent standard error of the means (n=15). NS,\*,\*\* indicate non significant at  $P>0.05$ , significant at  $P<0.05$  and  $P<0.01$  probability level, respectively.

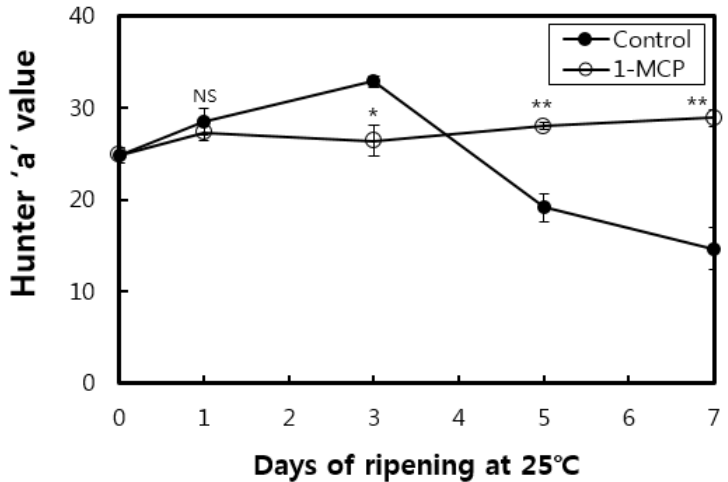
and 16.1% for the 1-MCP and control fruit, respectively. Persimmon fruits contain free sugars such as fructose, glucose and sucrose, and Yoshihiro et al. (1985) reported that sucrose can break down to fructose and glucose through enzyme activities during storage. The reduction in TSS as the ripening period progressed is related to this breakdown. The 1-MCP group retained higher levels of TSS than the control group due to the inhibition of ripening by 1-MCP.

At harvest, the Hunter 'L' value was 63.68 for control fruit and decreased rapidly to 42.51 by day 7 (Fig. 5). 1-MCP treated fruit decreased slightly to 60.37 within 7 days. The Hunter 'a' value at harvest was 24.86. The control fruit showed a color development value up to 32.87 within 3 days, which declined to 14.67 by day 7 day, and only a slight change in the Hunter 'a' value during the ripening period (Fig. 6). Ethylene treatment of persimmon before harvest can



**Fig 5.** Effect of 1-MCP treatment on Hunter 'L' value in 'Bansi' persimmon fruit during ripening at 25°C. Vertical bars represent standard error of the means (n=15). NS,\*\* indicate non significant at P>0.05, significant at P<0.05 and P<0.01 probability level, respectively.

**Fig 6.** Effect of 1-MCP treatment on Hunter 'a' value in 'Bansi' persimmon fruit during ripening at 25°C. Vertical bars represent standard error of the means (n=15). NS,\*\* indicate non significant at P>0.05, significant at P<0.05 and P<0.01 probability level, respectively.



therefore promote maturation and pigment development, resulting in increased Hunter 'a' values, which explains the results for control fruit (Lee and Chujo, 1991; Park and Kim, 2002a, 2002b). According to No et al. (2014), a longer astringency removal treatment period decreases the Hunter 'a' value, probably due to the dissolution of water-soluble tannins. Our results were similar, as the control fruit had lower Hunter 'a' values by day 3.

Immediately after harvest, the soluble tan-

nin concentration was 399.5 mg/100g. For control fruit, the tannin level decreased significantly to 248.5 mg/100g by day 5, with no significant subsequent change (Fig. 6). The 1-MCP fruit declined to 357.8 mg/100g at day 1, and did not change during the subsequent ripening period. Tannins are mainly associated with astringency; the loss of astringency is due to reactions between the acetaldehyde produced in the fruit and the soluble tannins (Seo et al., 1999; Plaza et al., 2012). Treatment of astringent persim-

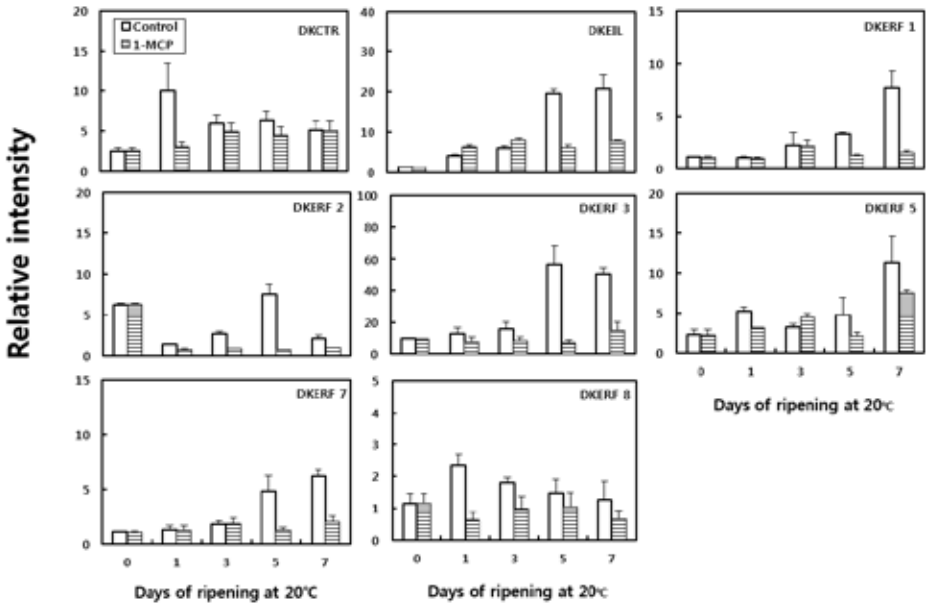


Fig. 7. Effect of 1-MCP treatment on transcript accumulation of targeted genes in 'Bansi' persimmon fruit.

mons with ethylene reduced the soluble tannin concentration from 1.45% to 0.39% in 3 days (Xue-ren et al., 2012); a similar trend occurred in the present study for the control fruit. The changes in soluble tannins were smaller in the 1-MCP treatment fruit than in the control fruit, possibly because ripening of persimmon is controlled by ethylene action, which is inhibited by 1-MCP treatment.

Ethylene action is achieved by regulating ethylene receptors and triggering of signal transduction reactions, and ultimately by controlling relevant gene expression in the fruits (Solano et al., 1988; Bleecker and Kende, 2000). Therefore, real-time PCR was used to evaluate the expression of eight ethylene receptor genes to determine the molecular mechanism of 1-MCP on ethylene production and fruit quality. Expression of all the ethylene response genes during ripening was lower in the 1-MCP treatment group than in the control group (Fig. 7). The control fruit showed strong expression increases during ripening, but the 1-MCP treated fruit

showed strong suppression of the increases in DkERF1, DkERF3 and DkERF7 transcript levels toward the end of the ripening period. The increase in some transcript levels, such as DkCTR and DkERF8, found in the control fruit at day 1 was also inhibited by 1-MCP. The expression of the DKEIL and DkERF5 ethylene receptor genes was significantly inhibited by 1-MCP treatment at days 5 and 7, again confirming a likely association between the transcript increases and ethylene production.

We also found that DkERF1 and DkERF3 expression was associated with soluble tannin content, while DkERF8 expression was associated with fruit firmness and ethylene production. Xue-ren et al. (2012) reported that fruit ripening and softening in astringent persimmon were associated with the DkCTR, DkEIL, and DkERF1-8 gene families. In addition, DkERF8 expression was highly related to fruit ripening and softening. These results indicate that the inhibition of expression of ethylene receptor genes by

1-MCP treatment resulted in extended shelf life of astringent persimmons. The 1-MCP treatment reduced ethylene production and delayed ripening, as indicated by inhibition of fruit softening and expression of ethylene response genes. The ethylene response genes mainly associated with this 1-MCP effect appear to be DKERF1, DKERF3, and DKERF8.

This result suggests that 1-MCP application blocks ethylene receptors, resulting in the reduction of the softening during postharvest of astringent persimmon as does on non-astringent persimmon (Kim and Lee, 2005).

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# The Effect of Heat Stress on the Reproductive Structures of Peach

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**Additional index words:** *Prunus persica*, pollen, pistil, blooming, high temperature

## Abstract

As in other areas of the world, global warming is also a reality in Southern Brazil, where the occurrence of temperatures above 25°C prior to blooming is becoming common, which is detrimental to the production of temperate climate fruit species. The aim of this work was to evaluate the effect of 30°C, during blooming, on pistil length, pollen number and viability of peach genotypes. Different genotypes as well as male and female parts of the flowers, responded differently to temperature. Among the assayed genotypes, 'BR1', 'Chimarrita', 'Tropic Beauty' and 'Atenas' showed higher tolerance to the high temperature condition.

In warm geographic zones, high temperature is the main environmental stress that limits growth, metabolism, and plant productivity worldwide (Hasanuzzaman et al., 2013). The most sensitive phase of plant development to extreme temperatures that dramatically affects the productivity of grains, vegetables and fruit crops is the flowering stage. As the flower is the organ that develops into a fruit, abiotic stress affects its capacity for fruit and seed production, leading to productivity loss (Hedhly, 2011). Very low temperatures during winter can damage buds by freezing, while high temperatures during pre-flowering and flowering leads to poor flower quality, a shortened flowering period and reduced effective pollination period (Hedhly et al., 2005). Poor fruit set is a serious problem for peach production under tropical and subtropical climatic conditions mainly due to warm temperatures during dormancy and bloom (Kozai et al., 2004). The reduced number of chilling hours associated with mild winter conditions, results in abnormal shoot growth patterns and poor plant development of temperate climate fruit trees in these regions. In addition, high temperatures,

especially those above 25°C, before and during bloom can cause poor fruit set and low productivity.

Studies involving sexual reproduction are difficult because gamete development and fertilization are complex processes that occur in a short period of time and are mostly hidden by flower tissues (Zinn and Harper, 2010). Nevertheless, it is important to understand the effect of temperature on the reproductive phase of peach, since maximum temperatures above 25°C during the pre-flowering and flowering phases have been observed in peach production areas of Brazil.

The objective of this study was to evaluate the effect of two different temperatures during the pre-flowering stage on pistil length, number of pollen grains per anther (NPGA), and pollen viability in different peach genotypes.

## Materials and Methods

The experiment was carried out over a three-year period (2011, 2012 and 2014) at Embrapa Clima Temperado, Pelotas, Rio Grande do Sul, Brazil (2013 was not included due to data loss). Twelve peach genotypes

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**Table 1.** Chill hours (CH), average full bloom (FBD) and harvest dates (HD), average cycle (C), flesh color (FC) and purpose (PUR) of 12 peach genotypes, when grown in Pelotas, RS, Brazil.

Genotype	CH <sup>x</sup>	FBD	HD	C <sup>z</sup>	FC	PUR
Atenas	250	05/08	21/11	108	Y	D
Aurora 1	< 50 <sup>y</sup>	22/07	20/11	120	Y	F
BR 1	< 300	22/08	06/12	116	W	F
Cascata 1303	< 250	05/08	14/11	101	W	F
Chimarrita	350	22/08	04/12	104	W	F
Conserva 594	< 250	25/07	06/12	134	Y	P
Diamante	200	06/08	06/12	122	Y	D
Granada	300	12/08	18/11	108	Y	P
BRS Libra	< 200	10/07	24/10	106	Y	P
Maciel	< 300	27/07	10/12	135	Y	D
Tropic Beauty	< 50 <sup>y</sup>	18/07	09/11	114	Y	F
Turmalina	350	03/08	22/11	111	Y	P

<sup>x</sup> Chilling requirement in hours below 7.2°C (CH) data from the Embrapa peach breeding program.

<sup>y</sup> As reported by Pedro Junior et al., (2007).

<sup>z</sup> Average cycle calculated based on number of days from full bloom to harvest date (C); Flesh color yellow (Y) or white (W); Dual purpose (D) (processing and fresh), fresh market (F), or Processing (P).

were used in this study (Table 1). They were grafted on ‘Aldrighi’ peach rootstock and established in pots. All genotypes are from the peach breeding program of Embrapa except ‘Tropic Beauty’ which was released in a partnership between Texas A&M University and the University of Florida, and ‘Aurora 1’, which originated from the Instituto Agrônômico de Campinas breeding program, São Paulo, Brazil. ‘Tropic Beauty’ was chosen because of its adaptation to warm areas, ‘Aurora 1’ was developed for planting in subtropical areas, Cascata 1303 and Conserva 594 are selections from the Embrapa breeding program, considered as very low chill and being adapted to subtropical regions like ‘Turmalina’. The other tested cultivars are largely planted in Southern Brazil.

Before bud swelling (June), eight to 10 plants of each genotype, were placed in a cold room at 4°C, 70% average humidity and no light, for 360 h, aiming to accumulate enough chill hours (hours below 7.2°C) for dormancy completion of all the genotypes. After this period, plants were kept in a greenhouse at 14°C, until buds reached the

desirable flowering stage. It is interesting to note that due to genetic differences, the phenological behavior of the genotypes were different so the temperature treatments started at different times for each genotype. When most of the buds in each genotype began to swell, or reached the B stage, according to the Baggioolini scale (Baggioolini, 1952), four to five plants of that genotype were placed in a heat chamber at 30°C, for 48 h, in absence of light, whereas others remained at 14°C in a greenhouse under natural light. Both environments were kept at 70% relative humidity. After the 48 h in the heat chamber, the plants were returned to the greenhouse (with natural light) until bloom. Four replications of five flowers recently opened were randomly collected from each genotype and treatment, and in random positions of the plant, and their pistil lengths were measured in mm, with a ruler.

For number of pollen grains per anther (NPGA), the experimental design was completely randomized with four replications and five flowers per plot. These flowers were randomly collected from the plants exposed

to 14°C or 30°C. From each plot, five anthers were detached, giving a total of 25 anthers per replication. The anthers were placed in vials and, when they were dry, 1 ml of lactic acid was added to the vial. The number of pollen grains per anther (NPGA) was counted according to Tuite (1969), using a Neubauer chamber.

For in vitro pollen viability, remaining anthers of the same flowers used for NPGA were removed and dried on a piece of paper, at room temperature for two days. Immediately after drying, the viability was measured by scattering the pollen on a solidified germination medium (sucrose 10%, agar 1% dissolved in distilled water) on slides adapted to this purpose, and left to germinate during three hours at 24°C (Couto et al., 2010). The pollen was considered germinated when the pollen tube length exceeded the pollen grain size.

NPGA had two years of data whereas the other parameters were observed for three years.

For statistical analysis, NPGA and pol-

len viability data were transformed to the proportion of the square root of the arc sin respectively. The experimental design was completely randomized with a 12 x 2 factorial treatment structure (genotype-temperature) with four replications. Data were analyzed by analysis of variance (ANOVA), and means were compared by Scott-Knott test using the SISVAR statistical software (Ferreira, 2011).

### Results and Discussion

Significant genotype-temperature interaction was observed for NPGA in 2011 and 2012 (Table 2). Two cultivars, Tropic Beauty and Chimarrita, were not affected either year whereas the selections Cascata 1303, Conserva 594 and 'BRS Libra' had lower NPGA when the plants were exposed to the 30°C temperature, compared to 14°C, for both years of evaluation. The selection Conserva 594 had the highest reduction, 52.9% and 68.8% in 2011 and 2012, respectively. Other cultivars had reduced NPGA in only one of the two years. These included 'Diamante'

**Table 2.** Number, percentage loss and average number of pollen grains per anther for 12 peach genotypes exposed to 14°C and 30°C, during pre-bloom in Years 2011 and 2012, Pelotas, RS, Brazil.

Genotype	2011				2012			
	14 °C	30 °C	Loss (%)	Average	14 °C	30 °C	Loss (%)	Average
Atenas	800 bA <sup>z</sup>	560 bA	30.0	680 b	1260 bA	530 cB	57.9	895 c
Aurora 1	570 bA	400 bA	29.8	485 b	1220 bA	800 bB	34.4	1010 b
BR 1	840 bA	1240 aA	- 47.6	1040 a	1850 aA	1420 aB	23.2	1635 a
Cascata 1303	1090 aA	610 bB	44.0	850 b	1340 bA	790 bB	41.0	1065 b
Chimarrita	780 bA	570 bA	26.9	675 b	210 eA	190 eA	9.5	200 d
Conserva 594	1020 aA	480 bB	52.9	750 b	160 eA	50 gB	68.8	105 e
Diamante	980 aA	400 bB	59.2	690 b	170 eA	260 eA	- 52.9	215 d
Granada	930 aA	490 bB	47.3	710 b	130 eB	250 eA	- 92.3	190 d
BRS Libra	1470 aA	960 aB	34.7	1215 a	670 dA	400 dB	40.3	535 d
Maciel	980 aA	1030 aA	- 5.1	1005 a	230 eA	60 gB	73.9	145 e
Tropic Beauty	1270 aA	840 aA	33.9	1055 a	910 cA	700 bA	23.1	805 c
Turmalina	1120 aA	370 bB	67.0	745 b	175 eA	140 fA	20.0	157 d
Average	988 A	663 B	31.1	825.0	694 A	466 B	20.6	579.8
CV (%)	17,2				13,3			

<sup>z</sup> Means followed by the same lowercase letters in the column and uppercase letters in the row do not differ by Scott-Knott test at  $p < 0.05$ . Mean comparisons were made only within years.

(59.2%), ‘Granada’ (47.3%) and ‘Turmalina’ (67.0%) with decreases in NPGA in 2011, and ‘Atenas’ (57.9%), ‘Aurora 1’ (34.4%), ‘BR1’ (23.2%) and ‘Maciel’ (73.9%), in 2012. Interesting to note that ‘Granada’ and ‘Diamante’ had higher NPGA at 30°C, in 2012.

‘BR 1’ had a reduction in 2012, for plants exposed to 30°C, but still had the highest pollen production among the evaluated genotypes.

The production and germination of pollen is affected by both genetic and environmental factors (Camposeo et al., 2008; Mert, 2009). Differences in pollen grain production among years were found in other *Prunus* species such as sour cherry (Davarynejad et al., 2008), peach (Nava et al., 2009) and apricot (Gallotta et al., 2014). The NPGA differences between years could also be due to the pretreatment conditions of the potted plants (which were grown outside before the cold room treatment, thus exposed to natural conditions). The temperatures (maximum, average and minimum) in May, were higher in 2012 than in 2011 (Table 3).

High temperatures during dormancy to the pre-bloom period can negatively influence the production of pollen grains or lead to male gametophyte sterility (Kozai et al., 2004). In our case, May temperatures in 2012 were warmer than in 2011, by 2.7°C, 1.6°C and 0.7°C for the maximum, average and minimum temperature respectively.

Genotype-temperature interaction was significant for pollen viability for the three stud-

ied years (Table 4). ‘Atenas’ and ‘BR1’ were not negatively affected by exposure to 30°C, whereas ‘Aurora 1’, ‘BRS Libra’, ‘Maciel’, ‘Turmalina’ and the selection Cascata 1303 had pollen viability reduced in two out of three years, indicating that these genotypes were more sensitive to high temperatures. A similar temperature effect on pollen germination with varied cultivar response was reported for citrus (Distefano et al., 2012) and strawberry (Ledesma and Sugiyama, 2005).

The most adapted cultivars to subtropical-tropical climates should produce 1000-2000 pollen grains per anther with a viability ranging usually from 60% to 95% (Barbosa et al., 1989). Only ‘BR1’ fulfilled these requirements. In general, an average percentage of germination over 50%, regardless of the year and temperature, is considered satisfactory (Scorza and Sherman, 1995). None of genotypes exposed to high temperature treatment had pollen viability lower than 50% for the three years of evaluation, except ‘Diamante’ and ‘Turmalina’, with the latter one in two out of three years of study. ‘Diamante’ did not have reduced pollen viability from the temperature stress in the second and third years of evaluation, but had the lowest average viability among the studied genotypes in all years except ‘Chimarrita’ in 2012.

This fact did not appear to have much consequence since a single pollen grain can fertilize the ovule. However, it may also indirectly serve as an indicator of higher or lower tolerance of genotypes to high temperatures at the pre-bloom stage.

**Table 3.** Average maximum (Max.), medium (Med.) and minimum (Min.) temperature (°C) occurred on May, June, July and August of 2011, 2012, and 2014 at the Embrapa Clima Temperado, Pelotas, RS, Brazil.

Year	Average Temperature (°C) <sup>a</sup>								
	May			June			July		
	Max.	Med.	Min.	Max.	Med.	Min.	Max.	Med.	Min.
2011	19.0	14.4	10.9	15.6	11.7	8.3	14.9	10.6	7.1
2012	21.7	16.0	11.6	17.2	12.0	7.7	14.5	9.7	5.5
2014	19.9	15.6	12.3	12.3	18.0	14.4	18.7	14.3	11.2

<sup>a</sup> Data collected from the Agrometeorological Station of Embrapa Clima Temperado, Pelotas, RS, Brazil.

Table 4. Pollen viability (%) and average pollen viability for 12 peach genotypes exposed to 14°C and 30°C during pre-blooming time in the years of 2011, 2012 and 2014, Pelotas, RS, Brazil.

Genotype	2011			2012			2014		
	14 °C	30 °C	Average	14 °C	30 °C	Average	14 °C	30 °C	Average
Atenas	29 dB <sup>z</sup>	55 bA	42 c	63 aA	67 aA	65 b	77 bA	73 aA	75 a
Aurora 1	89 aA	54 bB	72 b	62 aA	55 bA	59 b	85 aA	74 aB	80 a
BR 1	74 bA	63 bA	69 b	78 aA	75 aA	77 a	48 dA	60 aA	54 c
Cascata 1303	84 bA	56 bB	70 b	76 aA	76 aA	76 a	84 aA	72 aB	78 a
Chimarrita	87 bA	40 cB	64 b	29 bA	36 bA	33 d	67 bA	62 bA	65 b
Conserva 594	80 bA	46 cB	63 b	37 bA	49 bA	43 c	61 cA	68 bA	65 b
Diamante	51 cA	28 cB	39 c	39 bA	31 bA	35 d	35 eA	30 cA	33 d
Granada	89 aA	56 bB	73 b	48 bA	51 bA	50 c	74 bA	65 bA	70 b
BRS Libra	88 aA	84 aA	86 a	71 aA	50 bB	61 b	90 aA	67 bB	79 a
Maciel	82 bA	67 bB	75 b	75 aA	50 bB	63 b	79 bA	75 aA	77 a
Tropic Beauty	84 bA	62 bB	73 b	55 bA	49 bA	52 c	78 bA	72 aA	75 a
Turmalina	89 aA	39 cB	64 b	70 aA	44 bB	57 b	72 bA	78 aA	75 a
Average	77.2 A	54.2 B	66	58.6 A	52.8 B	56	70.8 A	66.3 B	69
CV(%)	10.9			16.4			11.5		

<sup>z</sup> Means followed by the same lowercase letters in the column and uppercase letters in the row do not differ by Scott-Knott test at  $p \leq 0.05$ . Mean comparisons were made only within years.

Table 5. Pistil length and average pistil length (cm) of peach genotypes exposed to the temperatures of 14°C and 30°C, during pre-blooming time in the years 2011, 2012 and 2014, Pelotas, RS, Brazil.

Genotype	2011			2012			2014		
	14 °C	30 °C	Average	14 °C	30 °C	Average	14 °C	30 °C	Average
Atenas	1.49 aA <sup>NS</sup>	1.37 aA	1.43 a	1.91 aA <sup>z</sup>	1.94 aA	1.93a	1.73 aA	1.67 aA	1.7 a
Aurora 1	1.37 aA	1.26 aA	1.32 a	1.63 cA	1.51 cA	1.57 c	1.26 cA	1.32 cA	1.29 d
BR 1	1.57 aA	1.52 aA	1.55 a	1.59 cA	1.28 dB	1.44 c	1.60 aA	1.31 cB	1.46 c
Cascata 1303	1.48 aA	1.52 aA	1.50 a	1.71 bA	1.74 bA	1.73 b	1.57 aA	1.45 bB	1.51 b
Chimarrita	1.36 aA	1.25 aA	1.31 a	1.45 cA	1.57 cA	1.51 c	1.47 bA	1.47 bA	1.47 c
Conserva 594	1.52 aA	1.61 aA	1.57 a	1.45 cA	1.58 cA	1.52 c	1.47 bA	1.50 bA	1.49 c
Diamante	1.51 aA	1.15 aA	1.33 a	1.58 cA	1.42 cA	1.50 a	1.45 bA	1.36 cA	1.41 c
Granada	1.31 aA	1.17 aA	1.24 a	1.64 cA	1.47 cA	1.56 c	1.46 bA	1.35 cA	1.41 c
BRS Libra	1.47 aA	1.34 aA	1.41 a	1.55 cA	1.50 cA	1.53 c	1.63 aA	1.45 bB	1.54
Maciel	1.59 aA	1.35 aA	1.47 a	1.92 aA	1.73 bB	1.83 c	1.57 aA	1.56 aA	1.57 b
Tropic Beauty	1.53 aA	1.51 aA	1.52 a	1.65 cA	1.62 bA	1.64 c	1.53 aA	1.60 aA	1.57 b
Turmalina	1.54 aA	1.52 aA	1.53 a	1.76 bA	1.65 bA	1.71 b	1.64 aA	1.48 bB	1.56 b
Average	1.48 A	1.37 B		1.65 A	1.58 B		1.53 A	1.46 B	
CV (%)	19.6			7.8			5.7		

<sup>NS</sup> Non significant at  $p \leq 0.05$ .

<sup>z</sup> Means followed by the same lowercase letters in the column and uppercase letters in the row do not differ by Scott-Knott test at  $p \leq 0.05$ . Mean comparisons were made only within years.

For pistil length, the genotype-temperature interaction was significant for the years 2012 and 2014 but not for 2011 (Table 5). Overall the 30°C treatment caused a shortening of the pistils, in this study. This shortening along with abnormal development of ovarian tissue was also observed in apricot during the last week of flower development when temperature was increased (Rodrigo and Herero, 2002), and may be related to an acceleration of anthesis (Zinn et al., 2010) which does not allow the reproductive structures to completely develop before the flower opens.

In our study, pistil length of 'Atenas', 'Aurora 1', 'Chimarrita', 'Conserva 594', 'Diamante', 'Granada' and 'Tropic Beauty' were not negatively affected by high temperature.

Analyzing the data together, for the male flower parts there was no reduction in NPGA for 'Chimarrita' and 'Tropic Beauty', and the cultivars Atenas and BR1 had no reduction in pollen viability, when plants were exposed to 30°C for 48 hours. 'BR1', even with the reduction in the number of pollen grains per anther, in 2012, produced more pollen grains than those produced by other genotypes.

For the female part of the flower evaluated, in this case the pistil length, genotypes not negatively affected by high temperature were 'Atenas', 'Aurora 1', 'Chimarrita', 'Conserva 594', 'Diamante', 'Granada' and 'Tropic Beauty'. However, there are other important variables not considered in this study such as stigma receptivity and ovule longevity, among others.

Genotypes that were superior to the others in at least two of the variables studied were 'Chimarrita', 'Atenas', and 'Tropic Beauty'.

Overall, there was a reduction in number of pollen grains per anther, pollen viability, and pistil length for plants subjected to 30°C as compared to those maintained at 14°C. However, peach genotypes differed dramatically in their responses with the most tolerant of the genotypes assayed, being 'BR1', 'Chimarrita', 'Tropic Beauty' and 'Atenas'. In spite of the differences between years, this indicates that it is possible to develop peach

cultivars with enhanced tolerance to high temperatures during blooming.

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## About The Cover:



There is rich diversity in the genus *Actinidia*, as show in the cover photograph. Considerable variation exists in internal color as well as size, external color and shape. Breeding for improved size, flavor, color and storage life is progressing but the crop is based on only a very few cultivars internationally. Commercially, kiwifruit was originally reliant on only the green-fleshed type but gold-fleshed cultivars have recently become very popular, especially in Asian markets.

*Photo courtesy of* Dr A. R. Ferguson,  
The New Zealand Plant and Food Research  
Institute Ltd.



## Kiwifruit: The Genus *Actinidia*

IAN J. WARRINGTON

Hongwen Huang. 2016. Academic Press (an imprint of Elsevier Inc.). 334pp. Hardcover. ISBN: 978-0-12-803066-0. \$236.95.

Kiwifruit is one of the few new fruit to be commercialised over the past century – others being macadamia, avocado and blueberry. Much of the early commercial development of this crop occurred in New Zealand, particularly during the latter half of the 20<sup>th</sup> century, and production rapidly followed into other countries around the world, including Italy, France, Greece, Chile, Japan and the USA. These developments were based on a very limited range of germplasm and almost entirely on one cultivar, ‘Hayward’.

The origin of kiwifruit is, in fact, China which has a rich diversity of species within the genus *Actinidia*. However, access to germplasm and understanding of the diversity of this genus outside of China has been very limited until recently. Further, the successful commercialization of kiwifruit in other countries has resulted in Chinese fruit-growers and scientists being more aware of the value of this germplasm within China, along with the opportunity to establish a commercial industry within that country. Accordingly both scientific and commercial activities have accelerated over the past 30-40 years. Nonetheless, much of the information that has resulted from such developments has been published mainly in Chinese and has been difficult to access elsewhere.

‘Kiwifruit: The Genus *Actinidia*’, has, for the first time, summarised in English much of the published scientific knowledge secured on this crop in China along with details about the Chinese industry. It includes references to research elsewhere in the world, espe-

cially in New Zealand and in Italy. The book is authored by Professor Hongwen Huang, Director, Wuhan Botanical Garden, Chinese Academy of Sciences, Wuhan, China in association with 14 other contributors from a number of other research institutes and universities across the country.

The book is presented in eight chapters: Systematic and Genetic Variation of *Actinidia*; Species; Natural Distribution of Genus *Actinidia*; Domestication and Commercialization of *Actinidia*; Biology, Genetic Improvement, and Cultivar Development; Main Cultivars in Commercial Production; Cultivation and Management; Harvest and Storage.

The real value of this text is the comprehensive information that is presented on the different *Actinidia* taxa, their distributions, the relationships between them and their commercial potential (Chapters 1, 2 and 3). This includes detailed discussion about the taxonomic and nomenclatural changes that have recently occurred (and will no doubt continue to occur in this extensive genus).

Chapter 1 provides an excellent presentation about the challenges involved in the taxonomic treatment of the genus. It includes very good summaries of the previous attempts at classification of species and of the revisions that have recently occurred. Topics such as ploidy variation, pollen characteristics, flower morphology and sex variation (all *Actinidia* taxa are functionally dioecious) and the evolution of particular species are very well covered.

Chapter 2 in particular is richly illustrated with color photographs showing details of the vegetative, floral and fruit characteristics of each of 106 species and varieties within

the genus, and detailed maps showing their current distribution within China. Chapter 3 further develops the information about species distribution by defining, in detail, the ecological characteristics of each of the regions where the species are located naturally. This information would have been enhanced had some photographs been included showing the *Actinidia* germplasm in these natural locations (noting that three such images are included later on in the text in Chapter 5). Chapter 6, which describes in detail characteristics of main cultivars that are currently used in commercial production, is also very informative in that it includes descriptions of those involved within the industry in China (which differ somewhat from those used in other countries). This section too is well illustrated with excellent color photographs.

The other chapters involving domestication, commercialization and the management of commercial crops, although important, are of less value than the chapters outlined above. More detailed information on those topics is available from other countries in more comprehensive texts on the pre- and post-harvest management of this crop. Nonetheless, the information is valuable in that it provides detail of production practices in China, including information about some of the pests and diseases that are not present in other kiwifruit-producing countries.

Overall the text is very well presented with rich augmentation using many color photographs, colored graphs and a large number of tables. It is well laid out and there are few errors. Translation into English from Chinese has been well managed and the text is mostly easy to read. There are, nonetheless, some issues that should be addressed in any revision: single and not double quotes should be used

around cultivar names such as ‘Hayward’; the key shown in many of the maps (starting with Figure 2.122, page 131) is not explained until page 185; words such as monsoon (not moonson – pages 181, 182), talk instead of stalk (pages 130, 132 and elsewhere) and maritime (not marital, pages 181, 182) need to be corrected; “plum blooming rain significance” (page 178) is not understood; Vc (chapter 2) and VC (chapter 6) should be standardised. The excellent color photographs of the different species shown on the cover and on pages 218 and 224 would be considerably enhanced if a key was included naming the different species. Finally, the index needs to be arranged in alphabetical order and not date order within each family name (the current order does not follow accepted scientific convention).

This text is essential reading for anyone involved with the science and management of kiwifruit. In many of the topics covered it greatly adds to prior knowledge about this genus and provides valuable information about the industry in China. It is also a valuable text for those involved with the breeding of other fruit species and with interests in plant ecology, taxonomy and botany.

A previous version of ‘Kiwifruit: The Genus *Actinidia*’ was published in China in 2014 by the Scientific Press, Beijing. The 2016 version includes some additional material, such as descriptions of the latest cultivars from New Zealand, and has different page numbers.

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## W. G. Brierley: Pioneering Pomologist of the Prairie

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Despite newspaperman Horace Greeley's purported proclamation that he "would not live in Minnesota because you can't grow apples there," Minnesota produced almost 25 million pounds of apples in 2014 (Luby, 1991; NASS, 2015). The University of Minnesota's fruit breeding program has worked since the 1860s to prove Greeley wrong and produce new cultivars of apples, as well as many other fruits, that could survive the variable and often difficult Minnesota winter. Much of the University's success in understanding the winter behavior and hardiness of fruit crops can be traced to one man: Wilfred Gordon Brierley (Figure 1). Brierley's career at the University of Minnesota lasted over forty years, in which time he made significant contributions to the Department of Horticulture, the fruit breeding program, and the field of pomology as a whole.

This paper will review some of Brierley's most significant findings and will publish, for the first time, a consolidated bibliography of Brierley's works in Table I. The paper, as well as the bibliography are organized by crop, as Brierley's research focused on winter hardiness but covered many different species of fruit. Digitized versions of Brierley's publications that are currently in the public domain will also be made available through the University of Minnesota's Digital Conservancy (<https://conservancy.umn.edu>). In publishing Brierley's complete bibliography, it is our hope that researchers can recognize his significant contributions to the field of horticulture and honor him as the Pioneering Pomologist of the Prairie.

Wilfred Gordon Brierley was born in Dover, New Hampshire in 1885. He left New



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Minn.**

Fig. 1. Image of W. G. Brierley (from Brierley, 1916)

Hampshire for his studies, receiving a B.S. in 1906 from Cornell University and an M.S. from the State College of Washington (now Washington State University) in 1913. Following the completion of his master's thesis, 'Modern Marketing and Storage for Fruits and Vegetables,' Brierley began working in the Division of Horticulture at the University of Minnesota, where he remained until his retirement in 1954. Unlike the typical faculty member today, Brierley was able to work as a professor for seventeen years before com-

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pleting his Ph.D. in 1930. His dissertation, 'A Study of Senescence in the Red Raspberry Cane,' reflects his expertise in just one of the many fruit crops he studied during his time at the University of Minnesota—in addition to his work on raspberries, Brierley published significant findings about apples, strawberries, grapes, blueberries, plums, and even nut trees. In his long career at the University of Minnesota, Brierley published over 60 reports, bulletins, and journal articles.

Many of Brierley's findings found their way to the public through the *Minnesota Horticulturist* magazine, a precursor to *Northern Gardener* magazine, which is still published today by the Minnesota State Horticultural Society. A disclaimer at the head of Brierley's *Minnesota Horticulturist* publications reminded readers that the articles only "recite the experience and opinions of the writers, and this must be kept in mind in estimating their practical value" (Brierley and Child, 1926). Despite the caveat, Brierley was a trusted and respected pomologist whose work has been cited as recently as 2014 (Read and Gamet, 2014).

In addition to being a highly respected scholar, Brierley was known as a kind and gentle man who went out of his way to support of his students. In 1970, an announcement to the University of Minnesota's Senate of Brierley's passing described him as "never too busy to discuss personal or academic problems with his students," and as a mentor who "did his utmost to smooth the bumpy roads that students have to travel." He had a particular affinity for athletes, having been one in college, and attracted graduate students from all around the United States and Canada to work with him (University of Minnesota, 1969).

As mentioned, Brierley's primary area of interest was winter hardiness. In his paper "The Winter Hardiness Complex in Deciduous Woody Plants", published in the *Proceedings of the American Society for Horticultural Science*, Brierley

explained the many factors influencing woody plant survival of winter, asserting that hardiness is the ability to survive not only cold temperatures, but also the other numerous difficult environmental conditions of the winter months (Figure 2; Brierley, 1947a). In addition to publishing in the *Proceedings of the American Society for Horticultural Science*, Brierley made the same information available to industry groups via their publications, showing his dedication to public outreach (Brierley 1947b, 1948).

A 1948 Brierley paper published in the *Minnesota Horticulturist* gives a thorough description of a 'test winter,' and is a particularly interesting look back at how horticulturists' ideas about test winters have developed over time (Brierley 1948). Using the framework of the 1947 paper, Brierley described the injuries that resulted from the winter of 1947-1948, and the ways in which the factors of the hardiness complex for apples, plums, grapes, raspberries, evergreens, strawberries, and apple nursery stock were lacking and thus resulted in severe damage to the crop. Brierley's thorough analysis of winter damage mirrors the work of horticulturists in Minnesota today who

TABLE I—FACTORS OF THE HARDINESS COMPLEX

I. *Basic Factors*

1. Condition of plant
2. Variety
3. Maturity
4. Exposure

II. *Water Relations*

5. Winter desiccation

III. *Temperature Relations*

6. Rest period
7. Dormancy
8. Time of development of cold resistance
9. Rate of development of cold resistance
10. Ultimate or absolute cold resistance
11. Retention or loss of cold resistance
12. Ability to regain cold resistance

Fig. 2. Table of Factors of the Hardiness Complex (from Brierley, 1947b)

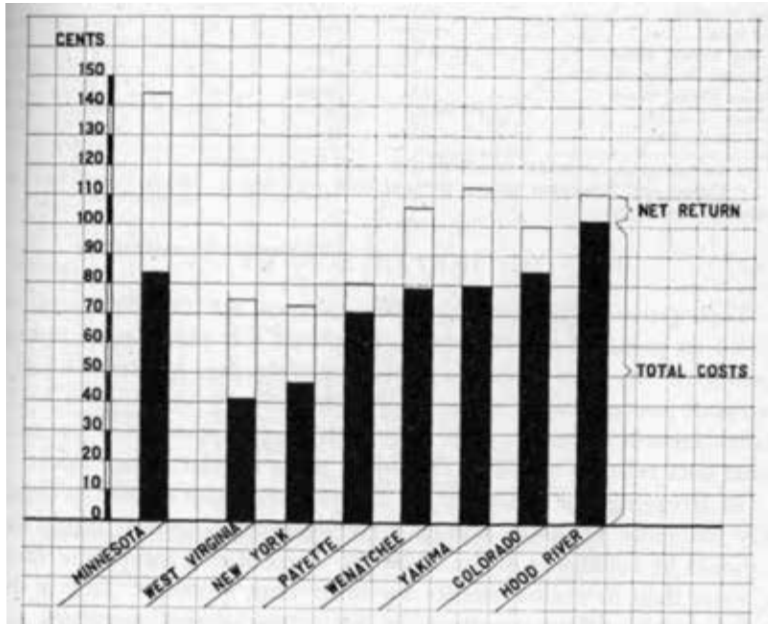


Fig. 3. Comparison of Total Costs and Net Returns per Bushel in Minnesota and Other Sections (from Brierley et al., 1924)

still discuss test winters and try to understand factors causing plant death, guided by the desire to have plants survive Minnesota's variable climate.

Brierley is perhaps best known at the University of Minnesota for his work on apples. His master's thesis from the State College of Washington focused on the marketing and storage of apples and, when he arrived at the University of Minnesota in 1913, he immediately began searching for the best Minnesota apple cultivars for cider and vinegar. His search eventually led to his first published paper, "Cider-and vinegar-making qualities of Minnesota apples" (Brierley, 1919). As his work began shifting towards winter hardiness and general survivability, Brierley published data on the longevity of apple trees growing in Minnesota (Brierley, 1921). In his 1921 paper, Brierley addressed the "wide variation in length of life" of apple trees, and the difficulty of separating climate from the other factors that affect how long a tree lives, a question still considered today.

The 1921 survey of orchards throughout the state indicated that most orchards were planted between 1900 and 1906, and that older orchards were few in number; few if any orchards had trees older than 25 years. It is interesting to note that there were very few orchards planted prior to 1900, as there were many fewer people in Minnesota, and fewer still cold hardy apple cultivars.

Using data from a large survey of orchardists, Brierley published an apple longevity study that concluded that the high net return for apples in Minnesota made up for the large total cost of growing the fruit (Figure 3; Brierley et al., 1924). Brierley also happily concluded that Minnesota growers were averaging ¢196 per bushel between 1915 and 1920, which was at least 30 to 80 cents above the earnings of growers in Idaho, Illinois, Colorado, Michigan, West Virginia, Oregon, Washington, and New York. Today, a bushel of apples, assuming forty pounds (approximately 18 kilograms) per bushel, grown in Minnesota could make a grower on average

\$33, which is still higher than growers in neighboring Michigan, Illinois, Missouri and Wisconsin (NASS, 2015). Brierley reported that while Minnesota growers had among the highest total costs compared to other regions, the net return was much higher (Brierley et al., 1924).

By 1925, Brierley had begun to make a name for himself in studying the various aspects of survivability of apple trees and his focus on winter hardiness solidified. He examined the healing of pruning wounds on apple trees, concluding that vigorous apple trees could have limbs thinned from November into the following spring with no impact on tree survival (Brierley 1925, 1932). Brierley's focus soon turned to winter hardiness issues in other crops, but he continued to publish research on apples until 1955.

Brierley took a hiatus in the 1920s to pursue his Ph.D. at Michigan Agricultural College (now Michigan State University). In 1930, he published his thesis work on raspberry cane senescence, in which he reported that cambial activity in second year canes developed xylem and phloem only when associated with lateral bud development (Brierley, 1930). Following his Ph.D. research, Brierley spent several years focusing on the physiology and production practices in raspberry. Though he received his Ph.D. in Michigan, it appears that Brierley conducted his research at the University of Minnesota and continued working in Minnesota while pursuing his final degree.

Brierley's work centered on the 'Latham' red raspberry, released from the Minnesota Experiment Station in 1920. Brierley used this cultivar in many of his studies, including the effect of pruning height on yield and berry size (Brierley, 1931a), growth habits of old, new, and lateral flower producing canes (Brierley, 1931b), transpiration rates of raspberry cane (Brierley, 1931c), the impact of cane tipping to increase lateral bud formation (Brierley, 1934), and numerous articles on winter survival, including studies of cold resistance in raspberry canes and roots (Brierley and Landon, 1946a; Brierley and Landon 1946b; Brierley et al., 1952).

Brierley also spent significant time in the 1930s and 1940s studying winter hardiness in strawberries. In 1937, Brierley and his colleagues examined plant metabolism and gas exchange in overwintering strawberry plants, concluding that while respiration slows significantly when the soil temperature falls below 0° C, it never completely ceases, showing that the plants respire even when the soil is frozen (Brierley and Landon, 1937). Brierley also examined strawberry plants' ability to survive 'smothering' under ice (Brierley and Landon, 1942), the impact of cooling and warming cycles (Brierley and Landon, 1944), the physiology of hardening (Brierley, 1943), and the minimum temperatures at which plants could survive (Brierley and Landon, 1943). In addition, Brierley published recommendations for local growers on mulching techniques and the best cultivars for the Upper Midwest. 'Burgundy,' 'Catskill,' 'Gem,' and 'Wayzata' topped the recommendations in 1943; none of these cultivars are recommended today (Brierley and Landon, 1944; Hoover et al., 2016). Brierley, working in conjunction with the Division of Home Economics, also released cultivar recommendations and technique tips for strawberry canning and jam making (Brierley and Child, 1926).

Brierley is perhaps best known for his work with apples, raspberries, and strawberries, but he did not stop there. During his long career at the University of Minnesota, Brierley, like many horticulturists, had broad expertise and many interests. He published research and reports on cherries, plums, grapes and blueberries that focused on cold hardiness and adaptability to Minnesota winters (Brierley and Alderman, 1938; Brierley and Angelo, 1934; Brierley and Hildreth, 1928; Brierley and Kenety, 1920; Brierley et al., 1952; Brierley and McCartney, 1950). As he approached his retirement, Brierley also began studying walnuts, hickory nuts, and hazelnuts, and published recom-

mendations for cold hardy nut cultivars. In addition to a review of noted apple breeder Peter Gideon's contributions to nut breeding, he seemed particularly interested in grafting techniques, and wrote several assessments of apple and nut graft trials as his final publications (Brierley, 1944).

Brierley dedicated half a century to exhaustively researching cold hardiness in numerous fruit crops and in doing so, significantly contributed to the body of knowledge on fruit crop dormancy and low temperature survival. His findings and publications "laid the groundwork for the University of Minnesota to develop into an internationally known center for cold hardiness research," as his University of Minnesota Senate obituary announced. The obituary also stated that with Brierley's help "the University of Minnesota gained a reputation as a center of excellence in the studies of the nature of the problems of winter survival of fruit plants" (University of Minnesota, 1969). The thousands of acres of apples, strawberries, raspberries, and other fruit crops that now grow in the Minnesota landscape serve as proof of Brierley's contributions, and to his ultimate success in proving Horace Greeley wrong.

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**Table 1.** Complete W. G. Brierley Bibliography, arranged by topic then chronologically.

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