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Evaluation of harvest time/temperature and storage temperature on postharvest incidence of red drupelet reversion development and firmness of blackberry (Rubus L. subgenus Rubus Watson)

Jack E. McCoy
University of Arkansas, Fayetteville

John R. Clarke
University of Arkansas, Fayetteville

Alejandra A. Salgado
University of Arkansas, Fayetteville

Andrew Jecmen
University of Arkansas, Fayetteville

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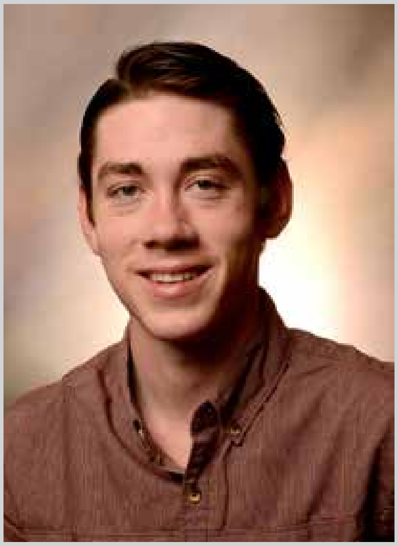
Jack E. McCoy, John R. Clark†, Alejandra A. Salgado§, and Andrew Jecmen‡*

Abstract

Since 1964, the University of Arkansas blackberry breeding program has worked to improve fruit quality and shipping capabilities. A major limitation in blackberry fruit is postharvest handling potential for the shipping market. Maintaining fruit firmness in storage is crucial. Red drupelet reversion (or simply reversion) is also an important postharvest disorder in which drupelets change from black to red during storage. It is hypothesized that reversion is increased when fruit is picked at hot temperatures and exposed to a rapid change of temperature. These studies evaluated harvest time/temperature, as well as storage temperature, on berry firmness and the incidence of reversion. In Study One, eight genotypes were evaluated. Fruit was harvested at four harvest times (7:00 AM, 10:00 AM, 1:00 PM and 4:00 PM) and then stored for 7 d at 5 °C before evaluation. Results indicated significant sources of variation were genotype and time of harvest for the variables compression (a measure of firmness) and incidence of reversion. Breeding selection A-2453T maintained high firmness and low incidence of reversion after storage compared to other genotypes. Reversion was also significantly lower at the 7:00 AM harvest time compared to later harvests. Study Two included two genotypes harvested at 7:00 AM and 1:00 PM which were evaluated at different storage temperatures (5 and 1 °C). No significant effects were found; however, trends suggested that A-2453T maintained higher firmness despite storage temperature. These studies confirm differences in firmness and reversion among genotypes as well as reveal harvest time impact on reversion.

* Jack E. McCoy is a May 2016 graduate with a major in Horticulture.
† John R. Clark, the faculty mentor, is a fruit breeder and distinguished professor of Horticulture.
§ Alejandra A. Salgado is a former Ph.D. graduate in Horticulture.
‡ Andrew Jecmen is a program associate, Department of Horticulture.

Meet the Student-Author



Jack McCoy

I grew up in Little Rock, Arkansas and came to Fayetteville to attend the University in 2012. Upon arriving, I was unsure of what I wanted to study. After taking an introductory plant science course my freshman year, I decided to declare a major in Horticulture and have not looked back since. The more I learned about the horticulture sciences the more my interests grew. During my undergraduate career I have had the opportunity to work on a variety of research projects, was the program assistant for the National Strawberry Sustainability Initiative, a Teaching Assistant for Principles of Horticulture and served as the President of the Horticulture Club. In the Fall of 2014 I had the opportunity to study and conduct research in Santiago, Chile. This solidified my interest in research. I learned about the wonders of plant breeding through my academic and research mentor, John Clark and now know this is the field where I can make a significant impact. I graduated from the University of Arkansas in May of 2016 and will be pursuing a graduate degree under the direction of Paul Bosland, a world renowned chile pepper breeder at New Mexico State University.

Introduction

Blackberry (*Rubus* L. subgenus *Rubus* Watson) is an important fruit crop in the Rosaceae family. It plays an important role in both the fresh and processed market and its interest to growers and consumers has increased greatly in recent years. In the early 1990s, blackberry markets were small, localized operations found mainly in pick-your-own and local fresh markets. Poor postharvest handling attributes prevented the fruit from being shipped long distances (Clark, 2005). With significant cultivar improvements came a great increase in production from the later 1990s on. According to the Agricultural Marketing Resource Center (Geisler and Morgan, 2012), blackberry production in the United States was valued at $30.8 million in 2009 and just two years later it was estimated at $43.2 million. With expanding interests from both growers and consumers, improvements in breeding and postharvest handling are crucial.

The blackberry is a perennial plant with biennial canes where vegetative canes (primocanes) are produced in the first year and are followed by the flower/fruiting growth period (floricanes). Blackberry produces an aggregate fruit that consists of a number of drupelets, each containing a seed (pyrene), which form around the torus (Moore and Skirvin, 1990).

A major concern in fresh market blackberries is the retention of color in drupelets (Clark and Finn, 2011). Known as “red drupelet reversion” or just “reversion”, blackberry often develops red drupelet color after harvest. It is thought that when fruits are exposed to a drastic change in temperature, cell organelle membranes, specifically the vacuole, break apart. The vacuole is a large organelle that can occupy 90% of a mature cell. It accumulates sugars, organic acids, aromas, flavors, ions, and water and rupturing in the membrane can cause changes in the pH of the fruit (Fontes et al., 2011). This contributes to color change (reversion) in the drupelets, resulting in an unattractive berry that is not desirable in the market. Retention of color in blackberry can be selected for, but cannot be evaluated in the field (Clark and Finn, 2011).

The University of Arkansas System Division of Agriculture’s Breeding Program utilizes a standard postharvest protocol in evaluating breeding selections and cultivars for storage potential (Clark and Perkins-Veazie, 2011). The protocol evaluates berry firmness, leakage, and reversion. The program has released several cultivars with improved postharvest capabilities. This protocol is usually conducted using berries that are harvested prior to 10:00 AM. ‘Natchez’ is a popular cultivar, and postharvest trials in Arkansas performed well in storage, usually with low reversion observed. However, when it was grown in warmer climates such as southern Georgia, ‘Natchez’ fruit had high levels of reversion and required harvesting prior to mid-morning before high heat was experienced. This highlighted a need to evaluate harvests of cultivars and advanced selections in the breeding program later in the day, when berries are exposed to higher temperatures. This could allow further confidence in identifying genotypes with greater postharvest storage potential that are harvested under less optimum conditions such as high heat.

The objectives of this study were to (1) determine the impact of time of harvest/fruit temperature at harvest on the development of red drupelet reversion and firmness of blackberry fruits during postharvest storage on various cultivars and advanced breeding selections and (2) determine the effect of postharvest storage temperature on the red drupelet reversion development and firmness on a very firm, low-reversion breeding selection compared to a standard commercial cultivar.

Materials and Methods

The studies were conducted at the University of Arkansas System Division of Agriculture's Fruit Research Station, in Clarksville, on berries harvested in June and July, 2015. Fruit for the studies was harvested from one to three 3.3-m plots with the number of plots harvested varying by genotype from replicated selection trials of advanced breeding selections and commercial standard cultivars. The plants were managed according to routine blackberry production practices, including annual dormant pruning, summer tipping of canes, trickle irrigation, fertilization, and control of spotted wing drosophila (*Drosophila suzukii* Matsumura). No fungicides were applied to the plants during the harvest season. Plants were grown on a four-wire, horizontal trellis with black plastic mulch.

For both studies, fruit temperature was measured at every harvest time across all genotypes using an infrared crop temperature meter (Spectrum Technologies Inc., Aurora, Ill.). Mean fruit temperatures averaged across all genotypes and standard deviations for the means were calculated at each harvest time in order to show the use of harvest time as an appropriate indication of fruit temperature.

Study One

Study One evaluated the impact of field temperature at harvest on firmness and the development of red drupelet reversion on blackberry fruits during postharvest storage. Shiny-black fruit free of defects of eight blackberry cultivars/selections were harvested into 0.24-L commercial plastic, vented clamshells at 7:00 AM, 10:00 AM, 1:00 PM, and 4:00 PM each day with two replicates at each harvest time. Genotypes evaluated included two breeding selections, A-2453T (crispy texture) and A-2450T, as well as the commercially available cultivars Black Magic™/APF-77, Natchez, Ouachita, Osage, Prime-Ark® 45, and Prime-Ark® Traveler. Harvest was repeated twice for each genotype. Fruit was immediately stored for 7 d in cold storage at 5 °C prior to evaluations. After storage, the fruit was evaluated for firmness and reversion.

Firmness was evaluated using an iCon Texture Analyzer (Texture Technologies Corp. Hamilton, Mass.) in Newtons (N) measuring both compression and drupelet skin penetration. For each compression measurement, 10 individual fruit were placed on a flat surface and measured using a cylindrical plane probe 7.6 cm in diameter (Fig. 1). Drupelet penetration measured the skin firmness using a probe 1 mm in diameter (Fig. 2). Three

Fig. 1. Fruit compression measurement procedure utilizing a flat surface and cylindrical plane probe of 7.6 cm in diameter.

Fig. 2. Skin drupelet penetration measurement procedure utilizing a probe of 1 mm in diameter.

drupelets were measured on each of the 10 berries evaluated. Reversion was assessed on every berry harvested. Fruit was recorded for the presence of reversion or having no reversion. Percent berries showing no reversion was used in data analysis.

Data were analyzed by analysis of variance as a split-plot design using SAS v. 9.3 (SAS Institute, Inc., Cary, N.C.). Genotype served as the whole plot, split plot was time of harvest, and clamshells were the experimental unit or replication. Least square means were separated using the least significant difference procedure.

Study Two

Study Two evaluated storage temperature on postharvest handling on the firm, crispy breeding selection A-2453T and the commercial cultivar Osage. Shiny-black fruits were harvested into 0.24-L commercial plastic, vented clamshells at 7:00 AM and 1:00 PM with two replicates at each harvest. The harvest was repeated twice for each genotype. Fruits were then divided into two groups and stored for 7 d at 1 and 5 °C. Firmness and color reversion were evaluated using the same procedures as Study One.

Data were analyzed by SAS v. 9.3 as a split-split plot design with the whole plot being the genotype, split plot harvest time, split-split plot storage temperature, and clamshell as the experimental unit.

Results and Discussion

Harvest Time and Fruit Temperature

Results of average temperature of fruits for the four harvest times confirmed the differences in harvest-time temperatures (Table 1). The earlier harvest time had a cooler fruit temperature and the temperature increased throughout the day. It is important to note the large increase in temperature from the 7:00 AM to 10:00 AM harvest time of 6.1 °C with only small temperature changes from 10:00 AM onward.

Study One

Firmness. The analysis of variance of the data indicated no significant interaction effects for any sources of variation for any firmness variables measured. Main effect of genotype was significant for compression, but not penetration (Table 2). Black Magic/APF-77 had the lowest mean compression value of 4.2 N indicating the softest-fruited genotype (Table 2), although statistically it was similar to all named cultivars except 'Prime-Ark Traveler'. The firm, crispy breeding selection A-2453T had the highest firmness compression value of 9.4 N and this value was significantly higher than all other genotypes evaluated with the exception of A-2450T, another firm but not crispy breeding selection.

Table 1. Mean fruit temperature and standard deviation for all harvests and genotypes at each harvest time.

Harvest time	Mean fruit temp (°C)	Std. dev.
7:00 AM	23.1	1.68
10:00 AM	29.2	3.45
1:00 PM	31.1	3.32
4:00 PM	31.8	2.35

Table 2. Main effect means of genotypes for fruit firmness.

Genotype	Compression (N)†	Penetration (N)†
Black Magic™/APF-77	4.2a‡	0.21
Ouachita	5.8ab	0.12
Osage	6.0ab	0.12
Natchez	6.3ab	0.11
Prime-Ark® 45	6.3ab	0.13
Prime-Ark® Traveler	6.9b	0.19
A-2450T	7.8bc	0.22
A-2453T	9.4c	0.21
P value	0.03	0.75

†Mean compression and penetration values (N = Newtons).

‡Least square means separated using least significant difference procedure.

Main effect means of harvest time were also found to be significantly different for compression, but not penetration. The 4:00 PM harvest time had the highest compression value (7.1 N) indicating firmer fruit, but it was only significantly different from the 10:00 AM harvest time (Table 3). Harvest times of 7:00 AM, 10:00 AM, and 1:00 PM were not different from each other. Firmness values fluctuated slightly throughout the day, but ultimately showed highest level at the latest harvest time.

The results for firmness were not as expected. It was anticipated that there would be an interaction of genotype and time of harvest since it had been shown in unpublished research that the genotypes varied in postharvest variables including firmness, and it was thought that the firmest selections would maintain greater firmness while softer genotypes would get softer at later harvest times that had warmer temperatures. It is not fully clear why this expected result was not seen. A possible reason is that the harvest season in 2015 was wetter than normal, as rains occurred one or more times each week during harvest, and might have reduced the potential firmness of the firmer genotypes in the study. Additionally, more replications or harvest dates could have reduced variation in the data resulting in more significant differences, although the means were not that greatly different in practical values.

It was of note that there were differences among means for compression but not penetration. A similar finding was reported by Salgado (2015). This indicates that compression is a more useful firmness measurement compared to penetration and would likely be the only measurement recommended in further investigations.

The time of harvest results were unexpected also. It was anticipated that berries would become softer as temperatures rose during the day. The opposite was found. There were no reports located in the literature that measured firmness during the day or as temperatures increased. Possibly berries became firmer due to reduced water content later in the day. However, water content was not measured in the study.

Red Drupelet Reversion. No significant interaction effects were found with incidence of reversion; however, main effect means were significant for genotype and time of harvest. Breeding selection A-2453T had very little incidence of reversion with a mean of 74.4% of fruit showing no reversion and was significantly lower than all other genotypes (Table 4). Similar to firmness evaluations,

Table 3. Main effect means of harvest time for fruit firmness averaged across all eight genotypes.

Harvest time	Compression (N)†	Penetration (N)†
7:00 AM	6.6ab‡	0.17
10:00 AM	6.0a	0.16
1:00 PM	6.6ab	0.16
4:00 PM	7.1b	0.17
P value	0.03	0.23

†Mean compression and penetration values (N = Newtons).

‡Least square means separated using least significant difference procedure.

Table 4. Main effect means of genotype for percent fruit showing no red drupelet reversion (RD_0).

Genotype	RD_0 (%)†
Black Magic™/APF-77	27.1a‡
A-2450T	40.8ab
Ouachita	43.2ab
Prime-Ark® Traveler	43.2ab
Natchez	54.2b
Prime-Ark® 45	54.6b
Osage	59.3b
A-2453T	74.4c
P value	0.01

†Percent fruit with no red drupelets.

‡Least square means separated using least significant difference procedure.

Black Magic/APF-77 was on the opposite end of the spectrum with the highest incidence of reversion with only 27.1% of berries showing no reversion. The other genotypes ranged between the two extremes, although A-2450T, 'Ouachita' and 'Prime-Ark Traveler' were statistically similar to 'Black Magic'/APF-77.

Incidence of reversion was also significant for harvest time (Table 5). The 7:00 AM harvest time had an average of fruit showing no incidence of reversion of 56.9%, significantly different than all other harvest times. An increase in reversion development can be seen at harvest times after 7:00 AM although there were no differences among other times.

The findings for reversion for main effects of genotype and harvest time were largely as expected, although it was anticipated there would be an interaction of main effects for reversion. Among genotypes, the crispy, firm A-2453T performed as expected and, as had been found in previous research (Salgado, 2015), as well as 'Black Magic'/APF-77 which had been reported to have high reversion (Clark et al., 2014). It was surprising that the firm-fruited cultivar Ouachita as well as 'Prime-Ark Traveler' were not different from 'Black Magic'/APF-77. However, environmental effects as well as number of samples and harvest dates might have impacted results, as discussed for compression.

It was anticipated that the earlier harvest time would result in lower reversion, and this was confirmed in the findings. It was also anticipated that reversion might increase with later harvest times at least for some genotypes. This was not found however, but then when one examines fruit mean temperatures for harvest time (Table 1), it is seen that fruit temperatures did not increase as much with later harvests (increase of approximately 2 °C from 10:00 AM to the later harvests) compared to 7:00 AM and 10:00 AM where a 6.1 °C increase in temperature was seen. Therefore, the findings indicate there may be a relationship between fruit temperature increase and increased reversion of blackberries. Further research is needed to confirm this result, however. The finding of increased reversion with later harvest times parallels that of the Georgia grower with 'Natchez' (J.R. Clark, pers. comm.). This finding does not fully support the idea that later harvests at higher temperatures are needed in the breeding program to evaluate a genotype's postharvest potential, since the interaction of genotype and time of harvest was not significant, indicated by parallel performance of genotypes in reversion with later harvests.

Study Two

Firmness. Analysis of variance showed no significance for either firmness measurement for main effect or interaction sources. This was surprising because prior observations of 'Osage' and A-2453T indicated the possibility of different firmness levels at harvest and in storage (J.R. Clark, unpublished data). Although no significant differences were found, there were trends in the data which suggest that A-2453T had a higher overall firmness than 'Osage' for both measurements and harvest times as well as storage temperatures (Table 6).

It was anticipated that significant differences in firmness would be observed between cultivars. Salgado (2015) found that crispy textures, such as A-2453T, were significantly higher in both compression and penetration values than their non-crispy counterparts. Study One also showed significant differences between 'Osage' and A-2453T. The trend towards A-2453T showing higher overall firmness suggests that increasing replications could increase the likelihood of finding significant differences. It is also important to note that June and July 2015 made for an unusually wet harvest season and could have affected postharvest data collection.

Red Drupelet Reversion. Incidence of reversion also showed no significant differences and no clear trends (Table 6). It is thought that if the study were repeated with a larger set of replications, significant differences could be observed for storage temperature, harvest time, and genotype.

Salgado (2015) reported significant differences in incidence of reversion between crispy and non-crispy textures. Once again, it is suspected that significant differ-

Table 5. Main effect means of harvest time for percent fruit showing no red drupelet reversion (RD_0) averaged across all eight genotypes.

Time	RD_0 (%)[†]
7:00 AM	56.9a[‡]
10:00 AM	49.1b
1:00 PM	47.4b
4:00 PM	45.2b
P value	0.001

[†]Percent fruit with no red drupelets.

[‡]Least square means separated using least significant difference procedure.

ences would likely be found with increased replications and that the unusually wet harvest season affected results. However, when comparing these results with Study One, it can be seen that 59.3% of fruit collected from 'Osage' in Study One had no incidence of reversion, second to that of the lowest genotype, A-2453T. Overall reversion values were close to this mean value for 'Osage' in Study Two, although the reversion values were significantly different for these two genotypes in Study One.

Interestingly, storage temperature played a small role in firmness and the development of reversion. Previous studies indicated that storage temperature can have a significant effect on compression and reversion, but not penetration (Salgado, 2015). It is possible that a five degree difference between storage temperatures is not large enough for observable effects.

These results do not support the idea that storage temperature and harvest time affect firmness and incidence of reversion because of lack of significance both in main effects and interaction, but trends in the data as well as results of Study One suggest a need for continued research.

Acknowledgments

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Table 6. Interaction means of storage temperature and harvest time for berry firmness and incidence of red drupelet reversion across the two genotypes. No significant differences were found in the data.

Genotype	Storage temperature	Compression (N)†	Penetration (N)†	RD_0 (%)‡
		Harvest 7:00 AM (21.0 °C)§		
Osage	1°C	4.6	0.09	64.9
	5°C	4.4	0.09	65.1
A-2453T	1°C	7.7	0.18	63.4
	5°C	8.1	0.27	65.1
		Harvest 1:00 PM (27.8 °C)§		
Osage	1°C	5.7	0.11	56.4
	5°C	4.7	0.11	55.4
A-2453T	1°C	8.8	0.23	55.7
	5°C	8.1	0.25	65.3

†Mean compression and penetration values (N = Newtons).

‡Percent berries with no reversion.

§Mean fruit temperature of all harvests at this time.