

Effect of *Avocado sunblotch viroid* (ASBVd) on the Postharvest Quality of Avocado Fruits from Mexico

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Abstract

The effect of *Avocado sunblotch viroid* (ASBVd) on the postharvest quality was studied in avocado (*Persea americana* Miller) Hass fruits from five classes: healthy fruits from healthy trees (C1), asymptomatic fruits from asymptomatic but infected trees (C2), asymptomatic fruits from symptomatic trees (C3), symptomatic fruits severity rating of 1 (C4) and symptomatic fruits severity rating of 2 (C5) harvested in 2011 and 2012. The postharvest parameters evaluated were: firmness, color, weight loss, dry matter, mineral and oil content. C4 and C5 were significantly different (Tukey, $p = 0.05$) in delayed ripening, less dark coloration and less weight loss compared to C1, C2 and C3. Firmness in C4 and C5 was harder (2-5.5 Newtons) on the eighth day, while C1, C2 and C3 was softer (< 2 Newtons) and reached it on the sixth day. Coloration in C1, C2 and C3 was black on the eighth day, whereas in C4 and C5 remained 75% obscure. Weight loss in C4 and C5 was 1.4 g/day whereas in C1, C2 and C3 was 2 g/day. Dry matter, mineral and oil content were similar in the five classes. ASBVd affected the postharvest quality of symptomatic fruits. Asymptomatic fruits satisfied the international quality standards.

Keywords: avocado, postharvest parameters, fruit quality, ASBVd

1. Introduction

Avocado is a tropical fruit native from Mexico and Central America, as well as having considered one of the most economically important fruits in Mexico with positive effects on human health (high content of High Density Lipoprotein, folic acid, potassium, Vit A, B, C, D, E, K) (Téliz & Mora, 2007). The high nutritional content and its dietetic properties make avocado one of the most attractive fruits to worldwide consumers (Ochoa, 2009). The international avocado trade requires high quality fruits to achieve acceptable prices in the market; additionally, the quality characteristics must be preserved until the final consumer (Ochoa, 2009). The postharvest fruit quality can be affected by biotic and abiotic factors (Kader, 2002). The first official, molecularly verified report in Mexico of *Avocado sunblotch viroid*, which causes deep white, yellow, red or necrotic streaks on avocado fruits was published in 2009. (De La Torre et al., 2009). Fruits with these symptoms are rejected for marketing. ASBVd disease occurs worldwide: United States (Coit, 1928), Peru (Vargas et al., 1991), Venezuela (Rondón & Figueroa, 1976) and Mexico (De La Torre et al., 2009); Spain (López et al., 1987); Israel (Spiegel et al., 1984); Ghana and South Africa (Acheampong, 2008; Da Graca & Van Vuuren, 1977; Da Graca et al., 1983; Luttig & Manicom, 1999), and Australia (Dale & Allen, 1979). Symptomatic fruits are routinely culled at the packinghouses, and symptomatic trees are removed from the field, although sometimes symptomatic fruits are commercialized in some markets. However, there is no precise data on ASBVd effect on the postharvest quality of avocado fruits. Therefore, the aim of this study was to determine the effect of ASBVd on the postharvest quality of Hass avocado fruits.

2. Materials and Methods

2.1 Location of the Study Area and Sampling

The experiment was performed in a Hass avocado grove, located at 19°20'51.095"N and 102°5'14.107"W at 1503 masl in the county of Uruapan, Michoacan, Mexico. Symptomatic and asymptomatic infected trees as well as healthy trees were identified and selected through leaves collected from four points on the tree (according to the four cardinal directions) from each of 50 trees and RT-PCR analyzed.

Fifty trees of similar age (20-25 years old) and vigor were analyzed by RT-PCR; leaf samples collected from each cardinal point from each tree were molecularly analyzed. Four trees with symptomatic fruits (symptomatic), four RT-PCR positive trees with asymptomatic fruits (asymptomatic) and four healthy trees free of ASBVd (healthy) were identified and selected.

2.2 Molecular Identification of ASBVd

Total RNA extraction (MacKenzie et al., 1997) was performed in a MM400® grinder macerator (Retsch, Haan, Germany). Samples of RNA were analyzed by RT-PCR to determine the presence or absence of ASBVd. RT-PCR was performed with Superscript™ III One-Step RT-PCR. Reaction mixture per sample was composed of 2.5 µl of 2X buffer, 0.25 µl of forward primer 10 µM (5'-AAGTCGAAACTCAGAGTCGG-3'), 0.25 µl of reverse primer 10 µM (5'-GTGAGAGAAGGAGGAGT-3') (Bar-Joseph et al., 1985; Schnell et al., 1995; Schnell et al., 1997), 0.5 µl PVP 40 (10%), 1 µl of nuclease free water, 0.2 µl of the enzyme Superscript™ III RT/Platinum® Taq Mix* and 0.3 µl of total RNA. Final reaction volume was 5 µl. RT-PCR was performed in an ABI 2720 thermocycler (Applied Biosystems, USA) with the following conditions: reverse transcription (50 °C 32 min, 94 °C 2 min), PCR amplification repeated in 30 cycles (94 °C 15 s, 53 °C 15 s and 68 °C 15 s) with a final extension (68 °C 5 min). RT-PCR products were checked on agarose gel 1% stained with ethidium bromide and visualized in a 1000/26MX Xpress® photodocumenter (Vilber Lourmat, Valley of Marne, France).

2.3 Fruit Samples

Hass avocado fruits in the physiological ripening stage were harvested in each selected tree during December 2011 (main flowering in Mexico) and then again during December 2012 from the same trees. The fruits were classified in five classes (Table 1). Symptomatic fruits with different severity (Figure 1) were determined with a diagrammatic scale adjusted by Horsfall and Barratt (1945) by 2LOG v.1.0 program (Mora et al., 2003). Twenty fruits of each class were collected in December 2011 and again in December 2012. Firmness, weight loss and color were evaluated every twenty-four hours after harvest, until fruits reached their commercial ripening. Fruits were kept under laboratory conditions at 23±2 °C and 85% relative humidity. Each variable was evaluated in two different harvests (2011 and 2012) and evaluated until the first class reached ripening.

Table 1. Avocado fruit classes for evaluation of the effect of ASBVd on postharvest quality variables (2011-2012)

Class	Tree Category	Fruit Category
C1	Healthy ASBVd -	Healthy
C2	Asymptomatic ASBVd positive	Asymptomatic
C3	Symptomatic ASBVd +	Asymptomatic Severity 0
C4	Symptomatic ASBVd +	Severity rating of 1
C5	Symptomatic ASBVd +	Severity rating of 2



Figure 1. Scale of 4 classes used to assess the severity of ASBVd symptoms on Hass avocado fruits: Class 0 (asymptomatic), Class 1 (0-7% severity), and Class 2 (8-24% severity). *Fruits of Class 3 (25-40% severity) were very few in number and were not evaluated

2.4 Mineral Content of Fruits

For each class, ten mesocarp samples from five fruits were processed to determine the content of N, P, K, Ca, Mg, Na, Zn and Mn in physiological and commercial ripening stages. The process was carried out with the methodology of digestion in tubes containing sulfuric acid, salicylic acid and selenium (Walinga et al., 1995) in a digestion unit (Bloc Digest 12).

2.5 Assessment of Firmness

Firmness was measured using a non-destructive method. Twenty fruits from each class were measured in a Fruit Texture Analyzer model GS-14 (Güss, South Africa) equipped with a semispherical tip of 8 mm. Fruit firmness was determined with the device applying a constant force to three equidistant points from the equatorial region until 2 mm of deformation. The values were expressed in Newtons under the following four categories: I >10, II 5.6 - 10, III 2 - 5.5, and IV <2 (Ochoa et al., 2009), whereby the highest value represents the highest firmness.

2.6 Exocarp Color

The color evolution of the exocarp was observed and recorded every twenty-four hours using the following scale: 1). Emerald green, 2). Forest green, 3). 25% dark coloration, 4). 75% dark coloration, 5). Purple, 6). Black (White et al., 2009). Fruit color was evaluated in each fruit of each class.

2.7 Weight Loss

The weight loss average of 40 fruits from each class during both assessments (December 2011 and December 2012) was measured with an EB15 electronic scale (OHAUS®, 0.1-15,000 g), every 24 hours during 8 days.

2.8 Dry Matter and Oil Content

During both harvests (2011 and 2012), ten fruits from each class were measured to determine dry matter and oil content according to the AOAC (1990) method. Dry matter and oil content were measured the same day of harvest. Ten g of pulp (wet weight) were cut into thin longitudinal slices and placed on a 15 × 10 cm filter paper. The samples were dehydrated for 30 s in a MS1140SL microwave (LG®, 120V~60Hz 650W) with a power of 30% and subsequently each sample was weighed. The process was repeated again for 3.5 min with a power of 25% and for 2 min with a power of 20% until reaching a stable weight (dry weight). Oil content was determined by a regression relationship to dry matter (Lee et al., 1983). The dry matter percentage was determined as follows:

$$\text{Dry weight} - \text{Paper weight} \times 100 / \text{Wet weight} = \text{Dry matter} \quad (1)$$

2.9 Statistical Analysis

Analysis of variance and means comparison by Tukey test from two harvests (2011 and 2012) were performed with SAS® V9.0 statistical package.

3. Results

3.1 Molecular Diagnostics

Molecular diagnosis by RT-PCR differentiated the healthy and asymptomatic fruit classes from those that were symptomatic.

3.2 Color Changes during Fruit Ripening

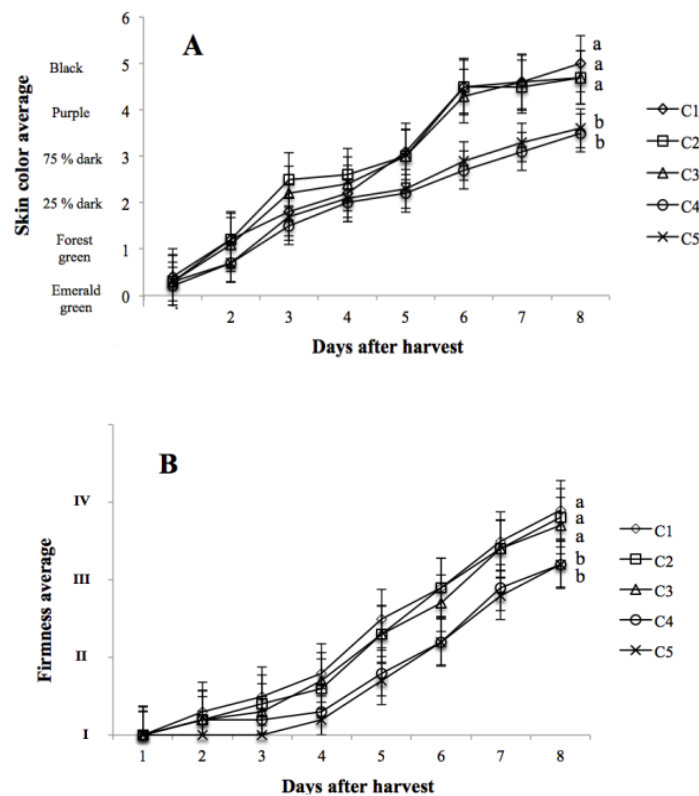
Two days after harvest, the emerald green color of asymptomatic C1, C2, and C3 fruits reverted to forest green. Four days after harvest, the forest green turned to black. Symptomatic fruits remained 25% darker in color with a reddish, pink atypical color in areas with symptoms. Color changes in C1, C2, and C3 were similar, turning black on the eighth day. C4 and C5, however, were significantly (Tukey, $p = 0.05$) slower to change color and did not become fully black even after commercial ripening (Figure 2A).

3.3 Firmness Assessments

Firmness was statistically higher (Tukey, $p = 0.05$) in C4 and C5 symptomatic fruits when compared to C1, C2, and C3 asymptomatic fruits. On the sixth day, symptomatic fruits developed category II firmness, whereas asymptomatic fruits obtained category III and reached category II on the fifth day. Asymptomatic fruits reached category IV firmness on the eighth day and had uniform ripening, whereas the symptomatic fruits reached category IV on day eight and did not ripen (Figure 2B).

3.4 Weight Loss

C4 and C5 symptomatic fruits had a weight loss of 1.4 g/day, significantly lower than the weight loss of 2 g/day of C1 and C2 asymptomatic fruits. C3 had a regular ripening period and had not significant difference in weight loss compared with symptomatic fruits (Figure 2C). Symptomatic fruits from C4 and C5 classes had delayed weight loss, delayed color change, and delayed firmness with a deteriorated appearance.



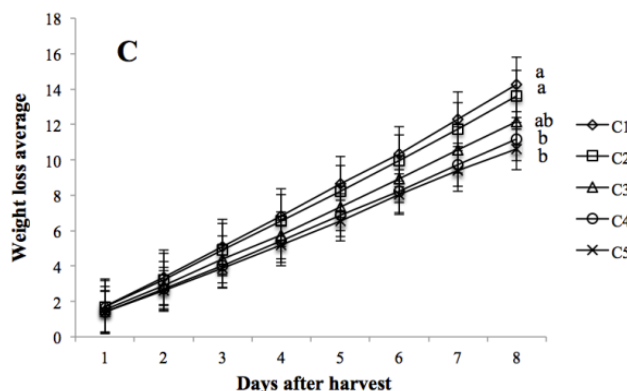


Figure 2. Average changes in skin color (A), firmness (B), weight loss rate (C) of Hass fruits during ripening (C1, C2 and C3 = ASBVd asymptomatic fruits; C4 and C5 = ASBVd symptomatic fruits). Lines with the same letter are not significantly different (Tukey, $p = 0.05$)

3.5 Dry Matter and Oil Content

There were no significant differences in dry matter and oil content between ASBVd symptomatic and asymptomatic fruits (Tukey, $p = 0.05$). There was a percent range of 28.5 to 29.3 and 16.9 to 17.8 in dry matter and oil content, respectively.

3.6 Mineral Content of Fruits

There were no significant differences in the mineral content of ASBVd asymptomatic (C1, C2 and C3) fruits and symptomatic C4 and C5 fruits (Tukey, $p = 0.05$) at 2 stages (physiological and commercial ripening) (Table 2).

Table 2. Percent (%) and parts per million (ppm) of minerals in 5 classes of avocado Hass fruits, ASBVd asymptomatic (C1, C2, C3) and symptomatic fruits (C4, C5) at the physiological and commercial ripening stages

Ripening stages	N	P	K	Ca	Mg	Na	Zn	Mn
	-----%*-----						-----ppm**-----	
<i>Physiological</i>								
C1	0.99 ^a	0.18 ^a	1.15 ^a	0.20 ^a	0.13 ^a	0.04 ^a	12 ^a	6 ^a
C2	0.92 ^a	0.16 ^a	1.43 ^a	0.32 ^a	0.12 ^a	0.01 ^a	17 ^a	4 ^a
C3	0.80 ^a	0.15 ^a	1.25 ^a	0.24 ^a	0.13 ^a	0.03 ^a	10 ^a	6 ^a
C4	0.87 ^a	0.17 ^a	1.50 ^a	0.29 ^a	0.15 ^a	0.02 ^a	17 ^a	6 ^a
C5	0.99 ^a	0.16 ^a	1.13 ^a	0.17 ^a	0.12 ^a	0.01 ^a	9 ^a	4 ^a
<i>Commercial</i>								
C1	0.96 ^a	0.16 ^a	1.43 ^a	0.15 ^a	0.16 ^a	0.02 ^a	11 ^a	4 ^a
C2	1.32 ^a	0.21 ^a	1.91 ^a	0.25 ^a	0.20 ^a	0.02 ^a	14 ^a	6 ^a
C3	0.98 ^a	0.17 ^a	1.73 ^a	0.18 ^a	0.12 ^a	0.01 ^a	12 ^a	6 ^a
C4	0.99 ^a	0.18 ^a	1.55 ^a	0.26 ^a	0.10 ^a	0.03 ^a	12 ^a	5 ^a
C5	1.25 ^a	0.19 ^a	1.70 ^a	0.13 ^a	0.10 ^a	0.02 ^a	8 ^a	4 ^a

Note. Percent and parts per million were calculated with average of 2 assessments (2011 and 2012). Mineral content was significantly similar in all fruit classes. *N, P, K, Ca, Mg and Na are percent values. **Zn and Mn are ppm.

4. Discussion and Conclusions

Deep, yellow streaks on fruits skins from ASBVd trees are typical symptoms of the disease (Saucedo et al., 2014; GIIIA, 2013). There is, however, variety of symptoms in the field. Fruits with symptoms (C4 and C5) developed

delayed ripening on all variables in 2011 and 2012. These delays in ripening were due to changes in their respiratory process (Vallejo et al., 2011) probably caused by ASBVd. Three and four days after harvest, asymptomatic fruits became darker while most symptomatic fruits remained a forest green color. The symptomatic fruits epidermis did not become black, the typical color of normal commercial ripening. Discoloration was slower than asymptomatic and healthy fruits even when ripening indicators, such as dry matter and oil content, were similar. These early changes in avocado fruit color may be due to chlorophyll degradation (Cox et al., 2004), from the effect of the viroid replication in chloroplasts and on respiration process (Vallejo et al., 2011). Firmness, as an important characteristic and the most reliable method for determining whether a fruit is ripe and ready to eat (White et al., 1999), remained regular and similar in asymptomatic C1, C2, and C3 fruits. Symptomatic fruits (C4 and C5), however, did not develop homogeneous firmness eight days after harvest due to delayed ripening. Weight loss rate is related to physical deterioration and quality loss (Kader, 2002). Symptomatic fruits (C4 and C5) had a slower and delayed weight loss rate, whereas asymptomatic C1, C2, and C3 fruits showed regular weight loss. These results might explain the ASBVd effect on symptomatic fruits during ripening (Vallejo et al., 2011). Dry matter percentage is widely accepted as an indicator of fruit ripeness (Arpaia et al., 2001) and is strongly linked to oil content and avocado fruits quality (Lee et al., 1983; Brown, 1984; Ranney, 1991). Dry matter content was similar in all assessed classes and greatly exceeded the minimum values prescribed by Mexican (22% dry matter and 8% oil) (Téliz & Mora, 2007) and American quality standards (18.4-21.9% dry matter) (Kader, 2002). Oil and mineral content were similar in symptomatic and asymptomatic fruits and fulfilled the nutritional recommendations (Pérez et al., 2005). ASBVd affected firmness, weight loss, and color change in symptomatic fruits (C4 and C5) in 2011 and 2012. Asymptomatic fruits were not affected on the variables evaluated and developed a similar condition to healthy fruits from healthy trees (C1), fulfilling quality standards required by national and international markets. Symptomatic fruits developed delayed ripening based on firmness, skin color, and weight loss rate due to alterations in respiration process. The dry matter and oil content of the five fruit classes fulfilled minimum requirements for ripeness. The mineral analysis of symptomatic fruits was not affected, so these fruits could be used in industry to ensure total uptake of nutritional content. Symptomatic trees and fruits should be removed from the field and packinghouses, respectively, to prevent dispersal of the pathogen. Furthermore, collecting plants from uncertified sources remains a high risk. Therefore, the only method to control ASBVd is in certified nurseries using viroid-free seed for seedlings and bud cuttings for cultivars propagation.

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