AVOCADO SUNBLOTCH VIROID (ASBVd) SYMPTOM IDENTIFICATION AND DETECTION IN AVOCADO ORCHARDS

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ABSTRACT

Since the first observations of avocado sunblotch disease (ASBD) in South Africa in 1954, the economic impact of the disease has been underestimated. At that time, the causal agent of the disease was not clearly understood and it was only identified in 1979 as avocado sunblotch viroid (ASBVd). With the recent expansion of the avocado industry, it is important to recognise the disease as a threat to the growth of the industry. Therefore, it is important to understand the mechanisms of infection of the viroid to enable the industry to implement management strategies to limit the spread of the viroid and to achieve an ASBVd-free avocado industry in South Africa. The presence of asymptomatic trees in orchards is a major concern for the industry and accurate and reliable detection for ASBVd is crucial. Typical symptoms are found on leaves, fruit and bark of the tree, however, some trees do not display any visible symptoms and these are referred to as symptomless carrier trees. The distribution of ASBVd within a single plant was studied and an uneven distribution of ASBVd between branches and in the fruit were detected. This finding has huge implications for optimising detection methods and sampling strategies for avocado tree indexing. For example, a tree displaying no symptoms on the leaves or on the fruit tested positive in all branches and in all symptomless fruit. These symptomless carrier trees are currently the main concern for the avocado industry and precise sampling strategies and detection systems need to be in place to reduce the spread of ASBVd. The most important control measure for ASBD is careful selection of pathogen-free bud wood and seed sources used for propagation, which is achieved through indexing.

INTRODUCTION

Avocado sunblotch disease (ASBD) was first discovered in Southern California in 1914 (Whitsell, 1952; Horne and Parker, 1932). The green skin of the fruit displayed yellow sunken areas resembling sunburn which later turned brown (Whitsell, 1952). This was therefore confused for the physiological disorder caused by sunburn (Coit, 1928). Later the disease was formerly described as graft transmissible that also led to ASBD being mistaken again for a viral disease (Horne and Parker, 1932). Due to failed attempts to detect ASBVd using virus detection methods, Thomas and Mohamed (1979) investigated the possibility of the causal agent being a viroid. These researchers reported the presence of a low molecular weight (60 - 70 000 g/mol) molecule which they compared to other viroid diseases such as Potato Spindle tuber viroid (PStVd) (50 000 g/mol), Citrus exocortis viroid (CEVd) (50 - 60 000 g/mol) and Coconut cadang-cadang viroid (CCCVd) (84 000 g/mol).

However, Palukaitis *et al*. (1979) was the first to report the causal agent of ASBD as a viroid, namely Avocado sunblotch viroid (ASBVd). The first primary and a secondary structure of ASBVd were proposed to be 247 base pairs long (Symons, 1981). Symons (1981) compared ASBVd to the structures of viroids Potato spindle tuber viroid (PSTV) and Chrysanthemum stunt viroid (CSV) which were already known. The length of ASBVd appeared to be much smaller than the other two viroids. Furthermore, ASBVd shared a homology of only 18% with the two viroids, whereas the two viroids shared a homology of 69% with each other (Symons, 1981). In South Africa, the disease was only discovered in 1954, however, it was believed to have been present way before (Loest and Stofberg, 1954; Da Graca and Van Vuuren, 2003). In 1983, ASBV was reported to be present in all then commercial cultivars in South Africa (Da Graca and Mason, 1983).

Avocado sunblotch viroid (ASBVd) causes chronic infection in avocado and is well established in South African avocado orchards. Typical ASBVd symptoms are detected from all plant parts including the leaves, seed, roots, flowers and stem. Symptom expression on fruit is the most characteristic and trees showing fruit symptoms will not be marketable. Asymptomatic infection also occurs and these trees are referred to as


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symptomless carrier trees (Thomas and Mohamed, 1979). Symptomless carrier trees are common in avocado orchards; these trees remain undetected and the fruit will probably be harvested and sold in markets. Symptomless carrier trees contain higher ASBVd concentrations compared to symptom bearing trees (Mathews, 2011).

The identification of ASBVd symptoms in young trees can sometimes be problematic and visual detections of an infected stem or fruit symptom on a tree do not always yield a positive molecular detection result when leaves of the same tree are tested. Therefore, the importance of accurate symptom identification and the distribution of ASBVd in symptomatic and asymptomatic plants will be discussed.

SYMPTOM IDENTIFICATION

The symptoms of ASBD manifest in two ways, symptomatic and asymptomatic. Symptoms are evident on the young green stem, leaves and the fruit of the infected tree. Alternatively, trees can display no symptoms and are termed symptomless carriers (Thomas and Mohamed, 1979). ASBVd-infected trees may appear stunted, with branches spreading unevenly to the sides and sprawling of the lateral branches (Dodds, 2001), exposing the tree to sunburn (Acheampong *et al*., 2008). These trees may develop abnormal growth, growing in a flattened shape with limbs bending toward the ground (Wallace, 1958). These may be signs that trees are infected and material must be sent for indexing to confirm infection. Thinning of the tree canopy has also been described as a sign of ASBD (Dodds, 2001).

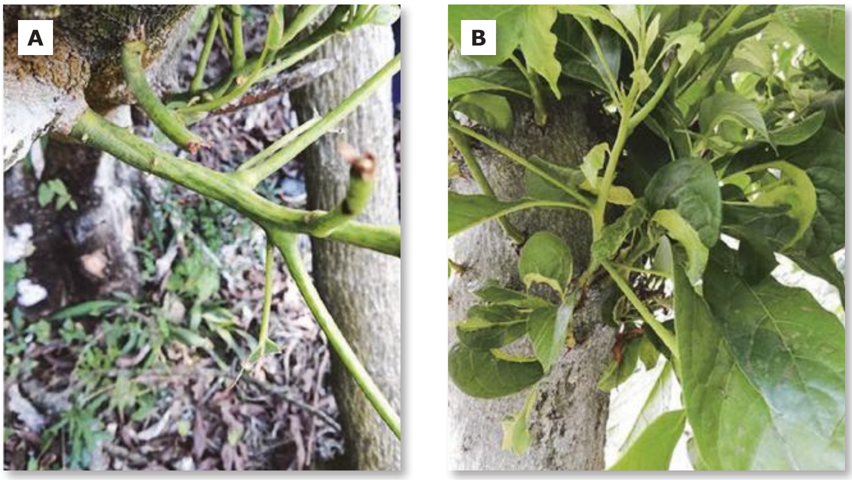

Figure 1: Symptoms on young emerging growth from an old infected 'Fuerte' tree showing yellow streaks on stem (A) and stem and leaf symptoms on the emerging growth (B) (Photos: Zanele Zwane).

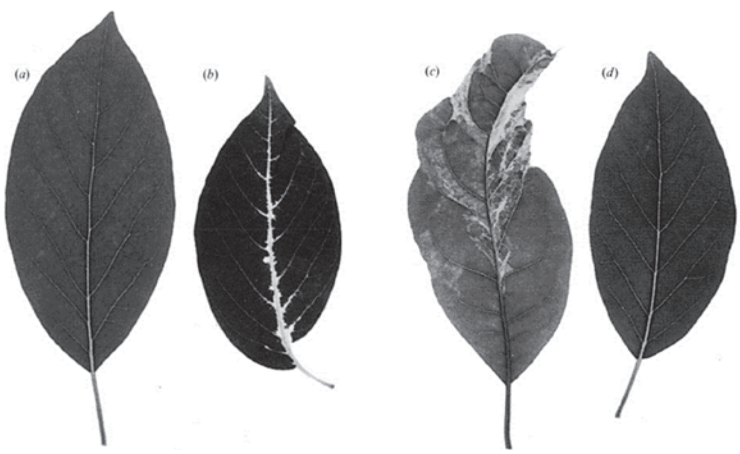

Figure 2: Avocado sunblotch leaf symptoms associated with different variants, (a) a healthy avocado leaf, (b) the variegated symptom associated with the ASBVd-V variant, (c) a bleached symptom associated with the ASBVd-B variant and (d) a symptomless carrier symptom associated with ASBVd-SC variant (Semancik and Szychowski, 1994).

Stem symptoms

Symptoms caused by ASBVd on infected young stems appear as yellow or colourless, sometimes reddish sunken longitudinal streaks (Fig. 1A). Early detection of these stem symptoms can be an indication of the presence of ASBVd. Trees displaying these symptoms do not always test positive in a diagnostic test, due to a low viroid titre in the leaves on a symptom-bearing branch. Infected trees showing stem symptoms should be marked and will not be suitable for propagation. On older trees, the trunk usually develops rectangular cracking, also referred to as alligator bark.

Leaf symptoms

Leaf symptoms are expressed as white/yellow variegation and bleaching of the leaves, however, these are very rare in the field (Fig. 1B; Fig. 2) (Semancik and Szychowski, 1994). These symptoms are associated with three ASBVd variants, namely ASBVd-B associated with bleached symptoms, ASBVd-V associated with the variegation symptom and ASBVd-SC associated with symptomless carriers (Palukaitis *et al*., 1979; Dann *et al*., 2013). Molecular variation between ASBVd variants is caused by small nucleotide changes between their sequences resulting in differences in the molecular weight (Semancik and Szychowski, 1994).

Fruit symptoms

Fruit affected by ASBVd develops streaks similar to those on the stem, depressed streaks with yellow or pink colour, which reduce fruit marketability (Vallejo-Perez *et al*., 2014).

Streaks extend from the stem end to the entire fruit, and sometimes fruits appear small and misshapen (Wallace, 1958). ASBD-affected fruit symptoms are caused by anatomical and chemical changes in the structure of the exocarp and mesocarp cells. This results from cellular disorganisation, accumulation of phenolic compounds in the cytoplasm and cell walls and reduction in cytoplasmic content leading to cell collapse and death (Vallejo-Perez *et al*., 2014). A study by Vallejo-Perez *et al.* (2014) showed an increase of up to 62% in phenolic compounds of symptomatic fruit compared to asymptomatic fruit, and reductions of up to 28% of both chlorophyll A and B. Chlorophyll reduction and the increase in phenolic compounds lead to the development of yellow and pink symptoms on the rind (Vallejo-Perez *et al*., 2014). Fruit symptoms appear as indicated in Figure 3.

Scouting of avocado orchards on a regular basis is critical to identify the subtle symptoms detected on the stems and the more prominent fruit symptoms. When any of these symptoms are detected, the tree should be marked with paint on the stem to avoid sampling from the tree for budwood or as a seed source.

ASBVd DETECTION IN SYMPTOMATIC AND ASYMPTOMATIC PLANT MATERIAL

The distribution of ASBVd was studied in infected trees that were selected from three nurseries in the Limpopo province. Plants were either symptom-bearing or had been previously diagnosed as positive for ASBVd, based on visual inspection of symptoms and molecular detection. In a single tree, each branch was indexed separately. ASBVd was extracted from leaf and fruit material using a cellulose column chromatography extraction method (Luttig and Manicom, 1999). The dsRNA was then used as the template in an optimised SYBR Green RT-qPCR reaction (Jooste, unpublished).

Variations in the distribution of ASBVd were observed in all the symptom-bearing trees during ASBVd amplification using quantitative RT-PCR (qRT-PCR) (Table 1). Fruits from trees that were only displaying fruit symptoms tested positive and the leaves from the tree tested negative (Trees 2, 5 and 7). An uneven distribution of ASBVd between the branches of the same tree was observed in trees showing symptoms all over (fruits, leaves and stem). Tree 1 had two branches

Figure 3: A typical fruit symptom caused by ASBVd (Photo: Tracey Campbell).

Table 1: Infected ASBVd trees with known indexing status from three nurseries in the Limpopo and Mpumalanga provinces in South Africa

Tree number	Symptom description	qPCR results	Number of branches	Number of infected branches	Number non-infected branches	Fruit	Number of infected fruit
1	Leaf symptoms on infected branch	Positive	2	1	1	0	
2	Fruit symptoms	Positive	3	0	3	3	3
3	Cracking of bark	Positive	6	1	5	0	
4	Fruit and leaf symptoms	Positive	2	1	1	0	
5	Fruit symptoms only	Positive	7	0	7	4	4
6	Symptomless	Positive	2	2	0	0	
7	Fruit symptoms	Positive	2	0	2	2	2
8	Symptomless	Positive	5	5	0	2	2
9	Symptomless	Negative	4	0	4	2	0
10	Symptomless	Positive	7	7	0	0	
11	Symptomless next to tree 10	Negative	6	0	6	0	
12	Symptomless	Negative	5	0	5	0	
13	Symptomless	Positive	2	0	2	2	2
13a	Recovery growth on tree 14	Positive	1	1	0	0	
14	Leaf symptoms	Positive	3	1	2	0	
15	Offspring 15 (symptomless)	Negative	4	0	4	0	
16	Dwarf	Positive	3	1	2	0	
17	Creeping growth	Negative	4	0	4	0	
18	Dwarf	Positive	4	2	2	0	
19	Creeping	Negative	4	0	4	0	
20	Symptomless	Negative	2	0	2	0	
21	Bark cracking	Positive	3	1	2	3	3

and symptoms were displayed on one of the branches and the other branch had no visual fruit or stem symptoms. The symptom-bearing branch tested positive for ASBVd and the other branch tested negative. Tree 3 displayed bark cracking on the older stem, and five of the six branches tested negative for ASBVd and only one tested positive. Tree 4 had leaf symptoms on all branches, however, only one branch tested positive for ASBVd and the other branch tested negative. Tree 15 displayed leaf symptoms on all the tree branches. Out of three branches sampled only one tested positive while the other two remained negative. Trees 16 and 18 were dwarfed (dwarfing is regarded as one of ASBVd symptoms in the field), both trees tested positive for ASBVd and ASBVd was found unevenly distributed between the branches of both trees. Tree 16 tested positive in one branch and Tree 18 tested positive in two branches and the other two branches tested negative. Trees 17 and 19 had creeping branches, also considered one of the ASBV symptoms in the field, and both trees tested negative for ASBVd.

Trees 9 and 20 were symptomless carriers that were previously indexed and tested positive for ASBVd, but later tested negative. These trees tested negative in this study. Tree 11 was tested as a possible symptomless carrier tree as it was growing next to tree 10 for more than 30 years; it was sampled to investigate the possibility of root grafting or other transmission from the neighbouring infected tree. This tree, however, tested negative for ASBVd. Trees 6, 8, 10 and 12 were all symptomless carrier trees that tested positive. In these trees all the branches and fruit tested positive, displaying an even distribution of ASBVd.

Furthermore, the distribution within a single fruit was investigated by separating the infected, yellow part of the skin from the green part within the same fruit. The fruits were divided into three groupings, i.e. infected symptomless fruits, slightly infected- and severely infected fruits. Fruits were further tested individually for ASBVd distribution (Table 2) and symptoms were separated into two categories, i.e. symptoms on the yellow infected part of the fruit and the seemingly green part of the fruit. For the slightly infected fruits, the yellow part tested positive while the green part tested negative for ASBVd. Results from the severely infected fruits showed that the green and the yellow infected parts of the fruits tested positive. Similar results were obtained with the symptomless fruits where every fruit tested positive all around the skin showing an even distribution of ASBVd.

CONCLUSIONS

Distribution studies indicated that ASBVd is unevenly distributed between the branches of a symptom-bearing tree and evenly distributed between the branches of the symptomless carrier trees. The latter also had a higher concentration of the viroid compared to the symptom-bearing trees.

Table 2: ASBVd distribution within a single infected avocado in trees with symptomless- and symptom-bearing fruit

Tree number	Fruit number	Type of infection	Skin description	RT-PCR results
Tree 1	1	Symptomless	Green	Positive
	2	Symptomless	Green	Positive
	3	Symptomless	Green	Positive
Tree 2	1	Slight infection	Yellow	Positive
			Green	Negative
	2	Slight infection	Yellow	Positive
			Green	Negative
	3	Severe infection	Yellow	Positive
			Green	Positive

The results further indicated that symptomless carrier trees can test positive one year and test negative again the following year. However, this is also true for the symptom-bearing trees (data not shown). It is recommended to immediately remove positively indexed trees from the orchard as they may pose threats for disease spread.

Trees displaying symptoms on the fruit only tested positive for the fruit and negative for the leaves. This is usually observed when healthy avocado trees are pollinated by infected pollen; in this case only the fruit exhibit symptoms while the rest of the tree remains disease-free (Dodds, 2001). It was suggested that symptomless carrier trees may be the source of pollen transmission in the field since they maintain higher concentrations of ASBVd (Mathews, 2011). It is therefore crucial to index all trees before they are used for propagative material, regardless of whether they bear symptoms or not. Symptomless trees appear healthy and are the main cause of inoculum in avocado orchards.

The ASBVd indexing technique used at the ARC-TSC is sensitive enough to detect even the lowest viroid concentrations in infected avocado trees. However, it is evident that ASBVd is unevenly distributed in symptomatic trees. Therefore, care should be taken during sampling to ensure that leaves from all the branches of the tree are represented in the sample to reduce the risk of false negative results. When the number of ASBVd negative leaves in a sample containing one ASBVd infected leaf was increased, the sensitivity of detection was not affected. Correct sampling and a quality assured indexing method will contribute to improved ASBV management strategies and production of quality avocados in South Africa.

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