Avocado sunblotch viroid in South Africa: Present status and future prospects

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ABSTRACT

Avocado sunblotch disease (ASBD), caused by *Avocado sunblotch viroid* (ASBVd), is one of the important diseases of avocado worldwide that affects yield and quality. 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 most important control measure for ASBD is careful selection of pathogen-free bud wood and seed that are used for propagation, which is achieved through indexing. The distribution of ASBVd within a single plant was studied and an uneven distribution of ASBVd between branches and in the fruit was 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. A summary on the indexing status of nurseries and commercial growers and the strategies towards an ASBVd-free avocado industry in South Africa will be discussed. Research currently underway include the risk of cutting tools in the spread of ASBVd, root graft transmission from infected root trees to neighbouring trees, investigation of transmission through pollen, and comparison of the productivity of ASBVd-infected symptomless trees to healthy trees by measuring yield and fruit size. Experiments towards these research goals are underway. The ultimate aim is to mobilise all role players in the South African avocado industry to ensure an ASBVd-free industry that will lead to optimal production.

INTRODUCTION

Avocado sunblotch disease (ASBD) is a chronic, infectious disease of avocado induced by *Avocado sunblotch viroid* (ASBVd). Sunblotch disease is the only viroid disease of economic value infecting avocados worldwide, leading to losses as a result of fruit degradation and subsequent poor fruit quality. Avocado sunblotch-infected trees may appear stunted, with branches spreading unevenly to the sides and sprawling of lateral branches. The most prominent symptoms are seen on the fruit. Fruit symptoms are caused by anatomical and biochemical changes in the structure of the exocarp and mesocarp cells which 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. Fruit develop streaks, similar to those on the stem, which are depressed and yellow or pink in colour and which reduce fruit marketability. Streaks extend from the fruit stem end to the entire fruit and sometimes fruit are small and misshapen.

Tree symptoms are either yellow or colourless, sometimes reddish, sunken longitudinal streaks on the green stems of young growth. On older trees, the trunks can develop rectangular cracking, also referred to as alligator bark, one of the more common sunblotch disease symptoms observed in the field, which is diagnostic of the disease.

Leaf symptoms include white or yellow variegation and bleaching of leaves, but these symptoms are rarely observed in the field. Varied symptoms are associated with three different ASBVd variants, *viz* ASBVd-B with bleached symptoms, ASBVd-V with variegation and ASBVd-SC with no symptoms. The ASBVd-SC variant is therefore associated with symptomless carrier trees (Semancik and Szychowski, 1994). The presence of ASBVd in symptomless carrier trees is very common in avocado orchards. Research demonstrated that symptomless carrier trees could arise from an infected symptomatic tree by producing new shoots that appear healthy to replace all the symptomatic leaves (Wallace and Drake, 1962). These symptomless carriers are the main concern for the avocado industry and correct, representative sampling strategies are crucial, together with sensitive detection methods to identify infections and reduce the spread of ASBVd. ASBVd spread is a threat to the avocado industry, as the disease can cause up to 80% of yield losses if uncontrolled (Da Graca and Mason, 1983).

The use of infected propagative material is the most important mode of spreading ASBD. ASBD can be transmitted via seed from the infected tree used for propagative rootstock, scion used for grafting and *via* root grafts and pollen. ASBVd can be transmitted on sap-contaminated pruning blades, injection material and harvesting clippers, therefore care should be taken to disinfect tools.

In this document, the present status of ASBVd indexing will be discussed, including the optimised sampling procedure, a discussion of the best practices and the current research focus.

Present status of ASBVd indexing

The increased demand for ASBVd indexed material is a direct result of the growing avocado industry in South Africa. The Agricultural Research Council – Tropical and Subtropical Crops (ARC-TSC) indexing laboratory increased its output capacity drastically in 2018 and accurate results were provided to nurseries, commercial growers and growers beyond the borders of South Africa. The indexing method used at ARC-TSC has been developed and perfected over years of research and development. The most crucial part of the method is to have a pure template for molecular detection of ASBVd. As reported previously (Jooste and Zwane, 2018), the technique, including extraction and primer sequences, were compared with various other protocols. The extraction method and primers that is currently used provides for accurate detection of all published ASBVd variants.

In the last three years, the average percentage of positive samples has decreased (Fig. 1). The average percentage of positive samples received from the Limpopo province in 2017 was more than 15%, while in 2018 less than 10% was recorded. The average percentage of positive samples received in 2016 from the Mpumalanga region was more than 10%, whereas in 2018 it was less than 5%. This is a result of accurate detection of ASBVd and precise selection of propagation material by nurseries. The established nurseries are all testing regularly to ensure that clean material is provided to commercial growers.

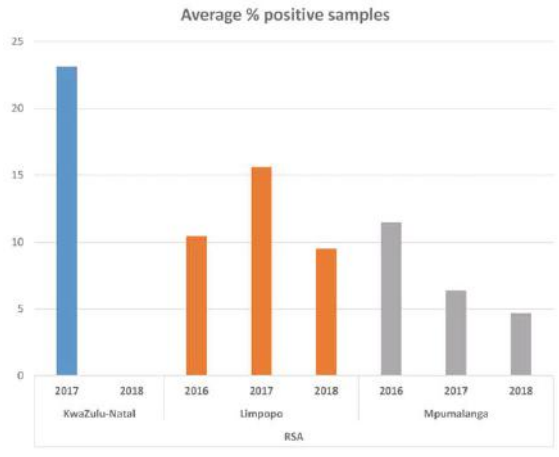

Figure 1. Average percentage positive samples received in three years from different localities.

Sampling strategy

Currently there is an option of either sending in individual samples or pooled samples for testing. When pooled samples test positive, the entire pool has to be tested to determine which plant was positive. The proposed sampling protocol includes the following:

- Sample from all main branches of a tree;
- Pool leaves from 3 trees to maximum of 21 leaves per sample (3 trees with 7 leaves per tree);
- Propagation material should be screened as individual samples, especially seed trees.

ASBVd is a systemic pathogen but its concentration can vary widely between the branches, leaves and flowers within a single avocado tree (Running *et al.*, 1996). This variation can be influenced by temperature and growing season (Running *et al.*, 1996). Damage caused by viroid diseases is known to be more severe in hotter climates compared with cooler climatic regions (Singh, 1983). Increasing the temperature (28-30°C) throughout the day and night can accelerate symptom development in ASBV indicator avocado trees (Graca and Van Vuuren, 1981). However, a combination of higher temperatures with consistent light, day and night, is more effective in symptom development (Desjardins, 1987). Cutting back trees can also effectively accelerate symptom development in the infected avocado seedlings (Da Graca and Van Vuuren, 1980). The uneven distribution of ASBVd in symptomatic 'Fuerte' trees was investigated where leaves and branches that contained visibly infected fruit were tested for ASBVd titre. Infected leaves, leaves close to infected fruit, young leaves close to infected fruit, a branch without symptoms and leaves from a branch with deformed fruit all showed a weak reaction in an ASBVd real-time PCR test. However, the infected fruit showed a high titre in the molecular test. This result confirmed earlier findings that symptomatic trees have a lower titre of ASBVd and detection without seeing the symptoms on the fruit can be easily overlooked. Generally, ASBVd-infected trees are lower yielding than non-infected trees and the infected fruits are discarded (Randles, 2003).

The formation of cukes, or cucumber-like fruit, on 'Fuerte' trees is common. Cuke formation is an indication of the presence of female sterile flowers on avocado trees (Steyn *et al.*, 1993). The presence of ASBVd in cukes on symptomatic 'Fuerte' trees was tested. All cukes tested positive and later in the season, symptoms developed on the small fruits (Campbell, personal communication). Therefore, the cukes are also an infection source of ASBVd.

Disease management practises

It is important for growers to source avocado trees from reputable nurseries and to inspect avocado orchards for fruit symptoms regularly. Nurseries should take care not to use infected propagation material, especially in the production of clonal trees. All seed sources should be tested to ensure ASBVd-free status. Infected seed from symptomless trees can be 100% infected with ASBVd. Infected trees should be removed from orchards by either removing trees

mechanically or injecting trees with, e.g. Roundup to die off and then remove the tree. It is important to ensure that all root material has decomposed and is removed before re-planting. Scouting for symptomatic fruits in orchards is crucial and farm scouts should be trained in symptom identification (Fig. 2). Avocado trees surrounding infected trees should be monitored by regular indexing to ensure their ASBVd-free status. Top working should be avoided if the ASBVd status of the rootstock is unknown.

Current research

Although eradication of ASBVd from nurseries and field orchards is the best management strategy, further investigations are currently being conducted to understand the different modes of transmission of ASBVd and the yield losses of the symptomless trees. Current research includes determination of the risk of cutting tools in the distribution of ASBVd and the study of root graft transmission of ASBVd from trees with an infected root system to healthy trees. Another objective is to investigate pollen transmission and to compare the productivity of ASBVd-infected symptomless carrier trees. These experiments are currently underway and will be reported on once data is available.

Future prospects: An ASBVd-free industry in South Africa

All role-players in the South African avocado industry have a responsibility to secure an ASBVd-free industry. The role players include: 1) The South African Avocado Growers' Association (SAAGA), 2) Avocado Nurserymen's Association (ANA), 3) Nurseries providing clonal and seedling rootstock trees, 4) Commercial farmers, 5) Small-scale farmers, 6) Farm workers and 7) Research organisations.

For each role-player the focus is different but in general, it will be crucial to ensure that motherblocks are ASBVd-free and that only certified material is used for propagation. Nurseries and avocado growers have the responsibility to remove infected trees from orchards and to follow the disease management guidelines listed above. Established and developing nurseries should be members of ANA to comply with expected requirements. Scouting strategies in orchards is crucial to detect symptomatic fruit and trees. Farm scouts should be trained to inspect orchards regularly. The removal of infected symptomless plants is essential for managing the disease and this will require indexing of trees and keeping records of indexing results.

The ultimate goal is the creation and maintenance of an ASBVd-free industry in South Africa. However, in order to work towards achieving this goal, it will be necessary for all stakeholders to play an active role in supporting ongoing research efforts towards improving methods for detection, preventing transmission and developing strategies for eradication of the disease in future.

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Figure 2. Farm scout identifying infected fruit in an orchard.

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