Report on *Avocado sunblotch viroid* studies in South Africa

AEC Jooste and ZR Zwane

Agricultural Research Council – Tropical and Subtropical Crops,
Private Bag X11208, Mbombela 1200, SOUTH AFRICA
E-mail: JoosteE@arc.agric.za

ABSTRACT

Avocado sunblotch disease, caused by *Avocado sunblotch viroid* (ASBVd), is one of the important diseases of avocado that affects yield and quality worldwide. 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 sunblotch disease is careful selection of pathogen-free bud wood and seed that are used for propagation, which are identified by indexing. In this study, we validated the sensitivity of ASBVd detection techniques used for indexing, studied the distribution of ASBVd in a single infected tree, conducted field surveys on commercial farms to determine the occurrence of ASBVd and studied the molecular variation of ASBVd in infected plants. The distribution of ASBVd in avocado orchards in two provinces, Limpopo and Mpumalanga, was investigated. A total of 30 commercial farms and 4 nurseries were visited and 316 trees were sampled randomly at these sites and tested for the presence of ASBVd. In this survey, 11.2% of the trees sampled, tested positive for ASBVd. Uneven distribution of ASBVd within a single plant was detected, between different tree branches and in fruit. The uneven distribution within a plant highlights the importance of proper sampling strategies for ASBVd indexing. 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 in avocado orchards.

INTRODUCTION

Avocado sunblotch disease 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 and fruit being degraded on quality standards (Acheampong *et al*., 2008). Avocado sunblotch-infected trees may appear stunted, with branches spreading unevenly to the sides and the sprawling of the lateral branches (Dodds, 2001). The most prominent symptoms are seen on the fruit (Fig. 1). ASBVd 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, namely; 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). ASBVd can be present in symptomless carrier trees that are 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).

Objectives of the study

The main objectives of the study were:

- To study the distribution of ASBVd in branches and fruit of symptomatic and asymptomatic trees
- To conduct a survey to determine the current occurrence of ASBVd in commercial avocado orchards

Figure 1: Typical ASBVd symptoms on fruit (Source: Tracey Campbell).

- To do a population study of ASBVd variants from field samples
- To validate the ASBVd diagnostic method used by the ARC-TSC laboratory.

MATERIALS AND METHODS

Sample collection

The distribution of ASBVd in branches and fruit was studied using infected trees selected from three nurseries in the Limpopo province. Plants were either symptom-bearing or were previously diagnosed as positive for ASBVd. In a single tree, each branch was indexed separately. Furthermore, the distribution within a single fruit was investigated by testing symptomatic and asymptomatic sections of the fruit. Fruit were divided into three categories: infected symptomless, slightly symptomatic and severely symptomatic.

The sensitivity of the ARC-TSC diagnostic ASBVd test was determined using leaf material collected from an ASBVd infected tree maintained in a glasshouse at the ARC-TSC, Nelspruit, showing typical ASBVd symptoms on the leaves and stem. An ASBVd infected leaf was added to 9, 19, 29, 39 and 49 healthy avocado leaves. These samples therefore represented dilutions of 10, 20, 30, 40 and 50 times of a single infected leaf.

Field survey

Field samples were collected from avocado orchards in the Mpumalanga and Limpopo provinces. Eight farms were visited in the Mpumalanga province, including farms in the Nelspruit, White River, Hazyview and Kiepersol regions. Six farms were selected from the Levubu and six from the Tzaneen regions in Limpopo province. A random sampling strategy was followed on each farm. No specific cultivar was sampled, since ASBVd infects all commercial avocado cultivars (Da Graca and Mason, 1983). In a single block, ten trees were selected and the total number of trees sampled per farm depended on the number of blocks present. Both young and old leaves were sampled from each tree and fruit samples were included, if available. Samples were kept in sealed plastic bags in cooler boxes and transported to the ARC-TSC Pathology laboratories for processing.

Online survey

An online survey was created using Google sheets and the survey link was posted on the Subtrop website for famers and nurserymen to complete. Questions included in the survey aimed to determine the level of awareness regarding symptom identification and disease management practises.

ASBVd RNA extraction

Two different extraction methods were compared; an RNA extraction method using Cetyltrimethylammonium bromide (CTAB) (White *et al*., 2008) and a cellulose column purification (Luttig and Manicom, 1999) currently used in the ARC-TSC laboratory.

Molecular detection

A total of 111 ASBVd sequences from GenBank® were aligned in BioEdit 7.2.5 (Hall, 1999). Primers currently used in the ASBVd indexing were checked against these variants to determine whether primers would detect all variants. Three primer sets were compared to assess ASBVd variant detection and included primer sets published by Bar-Joseph *et al*. (1985) and Luttig and Manicom (1999).

RESULTS AND DISCUSSION

Distribution of ASBVd between branches and fruit of symptomatic and asymptomatic trees

The presence of ASBVd in fruit displaying a range in symptom severity was investigated. The study found that from a tree bearing asymptomatic fruit, all the fruit tested positive for the presence of ASBVd. Results of fruit obtained from a second symptomatic tree were varied, depending on whether symptomatic or asymptomatic fruit sections were tested. Mildly symptomatic infected fruit tested positive for ASBVd in the symptomatic part of the fruit and negative in the asymptomatic sections. However, fruit that showed severe symptoms, tested positive in both the symptomatic and asymptomatic sections.

The study demonstrated that symptoms on fruit is not a reliable indicator for the presence of ASBVd. Symptomless fruit were also found to be infected with ASBVd. Testing of 4 symptomatic trees showed an uneven distribution of ASBVd in the trees where ASBVd detection differed between branches and between fruit in a tree. Even within a tree displaying symptoms throughout the canopy, some leaf samples can be ASBVd free. In certain instances, leaves of a tree tested negative, but fruit samples tested positive. This uneven distribution of ASBVd in a tree is a major concern and highlights that incorrect sampling procedures can lead to false negative results. Leaf samples from all branches of a symptomless tree and all fruit tested from this tree, were positive for ASBVd. These symptomless carrier trees are a concern for the avocado industry, as they can be inadvertently propagated if not indexed.

Online survey

There were 20 respondents to the online survey and although more interaction was expected, the feedback described here gave an indication of the understanding of respondents towards disease identification and management strategies.

Most respondents were familiar with sunblotch disease (83%) and 78% could identify symptoms in the field. Approximately half of the respondents had trained workers to identify field symptoms; however, this leaves a high percentage of respondents which have not trained field workers. The lack of trained field workers to identify symptoms, limits the containment of disease transmission between farms by mechanical means with infected tools. Tool sterilisation after use on visibly infected trees can limit spread.

There were 26% respondents who had not observed symptoms in their orchards, 52% had seen symptoms occasionally and 21% reported regular detection of ASBVd symptoms.

The most common field symptoms detected by respondents were fruit symptoms and 85% could identify the symptoms on fruit. Stem symptoms were detected by 14% and no leaf symptom were reported in orchards. Severely infected trees show most of their symptoms on young new leaves and fruits, which quickly die and fall off the tree which then appear as normal. Semancik and Szychowski (1994) mentioned that ASBVd leaf symptoms are rare in the field. Fruit symptoms could be an indication that there is a symptomless carrier tree around, acting as infection source, spreading the disease via pollen in the field. These trees are known to maintain higher ASBVd concentrations (Mathews, 2011).

It is highly recommended that infected trees are removed immediately from the field to avoid further infections; even trees within a 15 m radius from the infected trees should be removed (Schnell *et al*., 1997). This practise is not always feasible for farmers, but care should be taken to remove infected plants and monitor the neighbouring plants. From the survey results, only 31% participants removed infected trees immediately from orchards, 37% removed the infected trees after some time and 31% never removed the infected trees. This is poor disease management practises and could lead to ASBVd spread in South African avocado orchards. It is important to create disease management awareness and to establish a culture of disease management and control. Where trees were removed from the orchards, 42% of participants still experienced new infections and 57% never experience any new infections. Again, the symptomless carrier trees could be around causing those new infections.

Field survey

A total of 316 trees were randomly sampled from commercial orchards in the Mpumalanga and Limpopo provinces and 11.2% of the trees tested positive for ASBVd.

In the Limpopo province, a total of 198 trees were collected from twelve farms, where six farms were sampled from the Tzaneen growing region and six from the Levubu region. From the total number of trees from Limpopo province, 19 tested positive for ASBVd. However, 16 of the total infected trees were detected in the Tzaneen region and three positive trees in the Levubu region (Fig. 2). In the Tzaneen region, older avocado orchards were included in the survey and this can explain the higher ASBVd detection rate in Tzaneen.

In the Mpumalanga province, 112 trees were collected from eight farms, and 13 of the collected trees tested positive for ASBVd (Fig. 2). The most infected trees were detected at two farms and the rest of the farms had none or very few infections. Management and handling of injection and harvesting tools is very important. ASBVd can easily be transmitted by sap-contaminated injection material, harvesting clippers and pruning blades, which were found to have an 8-30% transmission rate (Dodds, 2001; Semancik, 2003).

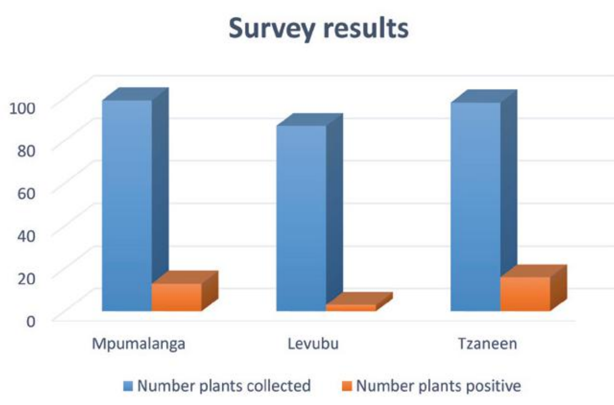

Figure 2: Summary of survey results from three regions.

Molecular analyses of ASBVd

Phylogenetic analysis of the sequences indicated similarities with known ASBVd variants from GenBank®. Numerous (32) variants were identified due to minor nucleotide changes throughout the ASBVd genome and not limited to any genome region.

Analyses showed that the primers used in the ASBVd detection protocol could detect all known ASBVd variants.

Validation of ARC-TSC detection technique

The cellulose column extraction method yielded the best quality RNA. The primer pair yielding a 99 bp amplification product gave optimal detection. The sensitivity of the diagnostic real-time ASBVd RT-PCR was determined by a dilution series of a positive sample. We showed a detection ability of a 1 in 50 dilution.

RECOMMENDATIONS

The distribution of ASBVd in avocado trees is erratic; therefore, representative sampling from all main branches is vital for indexing. Trees used as seed sources should be tested individually. It is important to apply the following best practises:

- Use source trees from reputable nurseries with proven indexing records
- Infected trees should be removed immediately
- Avoid mechanical transmission: pruning tools, injecting equipment and harvesting implements should be thoroughly disinfected with 1% active ingredient of a commercial bleach (sodium hypochloride) solution.

SAMPLING STRATEGY

Currently, there is an option to send individual trees for indexing or to send pooled leaf samples. The pooling of samples is effective when there is a requirement to determine the status of an orchard or motherblock. If a pooled sample then tests positive, individual trees of the pooled sample should be tested.

Proposed sampling protocol to increase representation of each tree per sample:

- Sample from all main branches of a tree
- Pool seven leaves from three trees for a pooled sample to have a total of 21 leaves per sample or collect 20 leaves from individual trees.

Propagation material should be screened as individual samples, especially seed trees.

Please contact the authors for additional information regarding ASBVd indexing.

Indexing of propagation material is extremely important and correct sampling protocols should be followed. Care should be taken to avoid ASBVd spread by tools and harvesting equipment. Infected plants should be removed immediately from orchards, especially the symptomless carrier trees that can act as sources of transmission between trees. ASBVd infected trees will have decreased yields yearly and keeping these trees in orchards will cause economic losses.

Acknowledgements

We would like to thank SAAGA and the ARC-TSC for their financial support. Furthermore, a special thanks to the personel of Subtrop that assisted with field surveys and all the producers and nurserymen for their assistance during this study.

REFERENCES

ACHEAMPONG, A.K., AKROMAH, R., OFORI, F.A., TAKRAMA, J.F. & ZEIDAN, M. 2008. Is there Avocado sunblotch Viroid in Ghana? *African Journal of Biotechnology* 7: 3540-3545.

DA GRACA, J.V. & MASON, T.E. 1983. Field indexing for avocado sunblotch disease. *South African Growers' Association Yearbook* 6: 83-85.

DODDS, J.A., MATHEWS, D., ARPAIA, M.L. & WITNEY, G.W. 2001. Recognizing avocado sunblotch disease. Avoresearch: http://www.avocadosource.com/Journals/AvoResearch/avoresearch_01_03_2001_Dodds_Sunblotch.pdf

HALL, T.A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Ser*. 41: 95-98.

LUTTIG, M. & MANICOM, B.Q. 1999. Application of a Highly Sensitive Avocado Sunblotch Viroid Indexing Method. *South African Avocado Growers' Association Yearbook* 22: 55-60.

MATHEWS, D.M. 2011. Avocado sunblotch viroid testing by RT-PCR. Dept. of Plant Pathology, University of California, Riverside. http://mathewslab.ucr.edu/handout/Avo_handout.pdf

SEMANCIK, J.S. & SZYCHOWSKI, J.A. 1994. Avocado Sunblotch disease: A persistent viroid infection in which variants are associated with differential symptoms. *Journal of General Virology* 75: 1543-1549.

SEMANCIK, J.S. 2003. '*Avocado sunblotch viroid*', in Hadidi, A., Flores, R., Randles, J.W. and Semancik, J.S (eds.). Viroids. Science Publishers, Inc. United States of America. Pp 171-176.

SCHNELL, R.J., KUHN, D.N., RONNING, C.M & HARKINS, D. 1997. Application of RT-PCR for indexing avocado sunblotch viroid. *Plant Disease* 81: 1023-1026.

WALLACE, J.M. & DRAKE, R.J. 1962. A high rate of seed transmission of avocado sun-blotch virus from symptomless trees and the origin of such trees. *Phytopathology* 52: 237-241.