UNDERSTANDING FIELD TRANSMISSION OF AVOCADO SUNBLOTCH VIROID (ASBVd)

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ABSTRACT

Avocado sunblotch disease (ASBD), caused by the avocado sunblotch viroid (ASBVd), is one of the most 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. Symptomless carrier trees are currently the main concern for the avocado industry. Precise sampling strategies and detection systems need to be in place for nurseries and avocado growers to reduce the spread of ASBVd. A recent study on symptomless 'Hass' trees showed the impact on tree morphology, fruit maturity, yield and quality of 'Hass' avocado when infected with a symptomless ASBVd strain. The most important control measure for ASBD is careful selection of pathogen-free budwood and seed that are used for propagation, which is achieved through indexing. The uneven distribution of ASBVd in an avocado tree between branches of symptomatic trees was described and this finding has significant implications on optimising detection methods and sampling strategies for avocado tree indexing. Other management strategies include the removal of infected trees from orchards, to source trees from certified nurseries and to avoid mechanical transmission in orchards with thorough sanitation protocols. The importance of sanitation is supported by an experiment that tested the stability of ASBVd on different surfaces. The involvement of honeybees, as potential carriers of pollen from infected to healthy trees, was also studied. Pollen and bees collected from hives were tested for the presence of ASBVd and the implications thereof will be discussed.

INTRODUCTION

Symptomless carrier trees play an essential role in the epidemiology of ASBVd, as they have been described as the primary sources of infection spreading the disease through budding and grafting practices (Saucedo Carabez *et al*., 2019). Studies have shown that ASBD can significantly reduce the yield and quality of avocado fruit (Da Graça, 1985; Running *et al*., 1996; Randles, 2003; Tondo *et al*., 2010; Saucedo Carabez *et al*., 2014). Avocado sunblotch viroid-infected symptomless carrier trees could have between 50% to 80% yield reduction compared with symptom bearing trees, which cause a yield reduction between 15% to 30% (Saucedo Carabez *et al*., 2014). Cultivar type has no influence on ASBVd severity, as demonstrated in a recent study conducted in South Africa on 'Hass' trees that showed a yield loss of approximately 90% from infected symptomless carrier trees (Z.R. Zwane, pers. comm.).

Wallace and Drake (1962) demonstrated that symptomless carrier trees develop from an infected symptomatic tree. The tree produces new shoots that appear healthy; and the leaves dominate the tree and replace all the symptomatic leaves. However, symptomless carriers can also establish from symptomless carrier parent trees. These trees can exhibit symptoms when they are subjected to stress, for instance fire, or when the trees are cut back and when a healthy scion is grafted onto a symptomless carrier tree (Dodds, 2001). In 1953, Wallace and Drake indicated that seed obtained from symptomless carriers could transmit up to 100% of the disease to the seedlings. The progeny of the symptomless carriers are all symptomless carriers (Wallace, 1958). Mathews (2011) mentioned that symptomless carrier trees have been found to maintain high levels of the viroid in leaves, fruit and seed, which could explain the high transmission rate.

Therefore, the modes of transmission that are critical in the management of ASBVd spread from symptomless carrier trees in orchards include seed transmission, graft transmission, mechanical transmission, pollen transmission and, in older established orchards, root transmission. Experiments to illustrate some factors contributing to field spread, with regard to mechanical and pollen transmission, will be discussed here.

MATERIALS AND METHODS

Mechanical transmission – investigate the impact of cutting tools on plant-to-plant transmission

The stability of ASBVd on different surfaces were tested using three treatments, including:

i) extracted RNA,
ii) infected plant sap macerated in avocado extraction buffer (ARC-TSC protocol) and
iii) infected plant sap macerated in water. The surfaces included metal (silver knife), a latex glove and cotton surfaces.

The three surfaces were subsequently treated with extracted RNA and the two plant sap treatments. 1 µl of either plant sap or extracted RNA was pipetted onto different surfaces. The samples were left to dry for one, five or 15 minutes and one or 24 hours, respectively. After drying, 20 µl of deionized water was used to re-suspend dry samples into 2 ml reaction tubes, and the tubes were kept at -20 °C. Samples were analysed using a qRT-PCR assay developed at the ARC-TSC.

Pollen transmission – investigate the involvement of bees

Pollen was collected from flowers of three symptomless 'Fuerte' plants (Block 4A) and one 'Ryan' plant (Block C) that previously tested positive for ASBVd at a commercial farm in KwaZulu-Natal (-29,5445538; 30,3735709). The pollen was collected from positive trees by removing anthers with unshed pollen. Anthers were carefully removed from the flowers using forceps and transferred to small Petri dishes, sealed with parafilm and cold stored until use. Within a few metres to as far as 1.7 km from positive trees, three beehives were identified with the help of professional beekeepers. Pollen was collected from beehives by identifying hive frames with the most pollen. Pollen was then scooped from parts of the frame with light yellow to white pollen using a spatula and transferred to 2 ml reaction tubes. From a single hive, ten 2 ml tubes were sampled. A minimum of 10 bee samples were collected from hives by opening the hives and rolling a 50 ml tube over the bees (Fig. 1). The extraction method optimised at the ARC-TSC was used to extract ASBVd dsRNA from leaves, pollen and bees.

RESULTS AND DISCUSSION

Mechanical transmission – the stability of ASBVd on different surfaces

Binding of ASBVd to different surfaces was demonstrated for all treatments. The treatment with plant sap macerated in water was the closest to simulating the use of metal pruning equipment in the field. After 24 hours, the ASBVd particles remained intact on the metal surface. Therefore, if equipment used on an infected tree is used for the next tree, there is a chance that ASBVd will be transmitted to the next tree and lead to ASBVd spread in an orchard. This finding demonstrates the importance of sanitation practices in the field and in nurseries to mitigate against the risk of the spread of ASBVd. All equipment, including drill bits, syringes, handsaws, hand clippers or loppers, pole pruners and chainsaws

Figure 1: Sampling from beehives.

Table 1: Detection of ASBVd in leaf and pollen samples from positive symptomless carrier trees

Block-plant number	Cultivar	Leaf sample		Pollen	
		Ct value	Copy number	Ct value	Copy number
Block 4A-04	'Fuerte'	11.94	1 210 397	14.80	155 702
Block 4A-06	'Fuerte'	14.38	210 096	12.23	964 180
Block 4A-07	'Fuerte'	10.98	1 304 7292	21.63	1215
Block C- 35	'Ryan'	16.83	36 723	15.58	182 471

should be sterilised between operations. If the ASBVd status of an orchard is unknown, cleaning of equipment between plants and between rows is recommended. In ASBVd-infected orchards, growers should avoid pruning with all cut pruners. It is crucial to remove ASBVd-infected trees from orchards, although it is not always easy to convince growers to remove infected trees when they are still bearing fruit. One should keep in mind that over time, the yield of symptomless carrier trees will decline and infected trees will be a source of infection in orchards.

ASBVd transmission via pollen and the role of bees

Pollen was collected from 'Fuerte' and 'Ryan' symptomless carrier trees that previously tested positive for ASBVd (Table 1). Double stranded RNA was extracted from leaf and pollen samples and used as template in the qRT-PCR reaction. Low Ct values were detected in the trees, indicating high ASBVd titres. The results showed the presence of ASBVd in pollen derived from symptomless carrier trees with similar levels of infection in leaf and pollen samples.

Pollen and bee samples collected from three beehives, that were located close to infected trees, tested positive for ASBVd. The spatial position of the infected hives in relation to the ASBVd-positive trees (not shown) was determined and ranged between 100 m from an infected tree to 1.7 km. Results showed that bees can carry ASBVd from pollen of symptomless carrier trees to beehives. Although transmission of ASBVd via pollen is known to be relatively low (1.8% and 3.125% according to Desjardins *et al*., 1979), this is a concern as it represents yet another avenue for transmission of the disease. When symptomatic fruit is detected on a tree and occurs as single infected fruit, it is most likely that the infection was derived from pollen transmission. The rest of the tree will remain negative upon indexing and only the fruit will be infected. These trees should be marked and monitored over time. Symptomatic fruit that are derived from infected pollen should be removed from the tree to ensure that these fruit are not used as a seed source.

CONCLUSION

To mitigate against the risk of field transmission of ASBVd, a comprehensive suite of management strategies are needed. This includes, firstly, to know the ASBVd status of trees in orchards and to remove infected trees. Secondly, it is important to understand the modes of transmission of ASBVd and to include sanitation as a critical strategy to prevent the spread of ASBVd in orchards and nurseries. Equipment should be sanitised between plants and rows. If the ASBVd status of an orchard is known, it will be easier to use equipment without the risk of spreading ASBVd. Trees showing fruit symptoms should be marked and the fruit should not be used as a seed source. A tree with only a few symptomatic fruit was probably infected through pollen and the rest of the tree will remain healthy. These trees will test negative with molecular detection methods. When trees with fruit symptoms are detected, the probability of finding symptomless carrier trees in close proximity are high. Systematic testing of orchards is recommended.

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REFERENCES

DA GRAÇA, J.V. 1985. Sunblotch-associated reduction in fruit yield in both symptomatic and symptomless carrier trees. *SAAGA Yearb.* 8: 59-60.

DESJARDINS, P.R., DRAKE, R.J., ATKINS, E.L. & BERGH, B.O. 1979. Pollen transmission of avocado sunblotch virus experimentally demonstrated. *Calif. Agric.* 33: 14-14.

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

MATHEWS, D.M. 2011. Avocado sunblotch viroid testing by RT-PCR. Dept. of Plant Pathology, University of California, Riverside. https://mathewslab.ucr.edu/sites/g/files/rcwecm5606/files/2021-06/Avo_handout.pdf

RUNNING, C.M., SCHNELL, R.J. & KUHN, D.N. 1996. Detection of avocado sunblotch viroid and estimation of infection among accessions in the national germplasm collection for avocado. *Proc. Fla. State Hortic. Soc.* 109: 235-237.

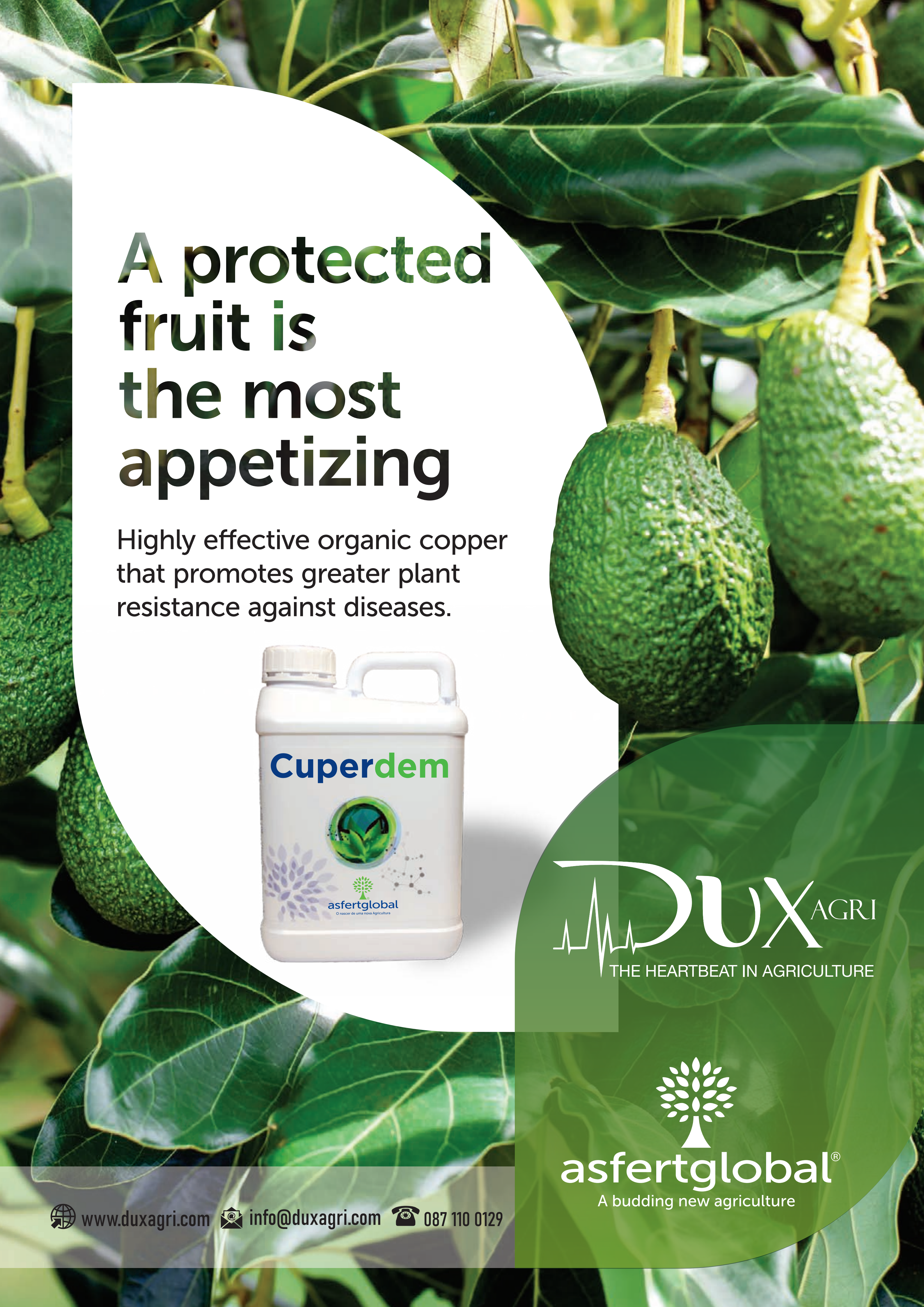
SAUCEDO CARABEZ, J.R., TÈLIZ ORTIZ, D., VALLEJO PÈREZ, M.R. & BELTRÁN PEÑA, H. 2019. The Avocado Sunblotch Viroid: An Invisible Foe of Avocado. *Viruses* 11: 1-12.

SAUCEDO CARABEZ, J.R., TÈLIZ ORTIZ, D., OCHOA-ASCENCIO, S., OCHOA-MARTINEZ, D., VALLEJO-PÉREZ, M.R. & BELTRÁN PEÑA, H. 2014. Effect of Avocado sunblotch viroid (ASBVd) on avocado yield in Michoacan, Mexico. *Eur. J. Plant Pathol.* 138: 799-805.

TONDO, C.L., SCHNELL, R.J. & KUHN, D.N. 2010. Results of the 2009 ASBVd Survey of Avocado Accessions of the National Germplasm Collection in Florida*. Proc. Fla. State Hortic. Soc.* 123: 5-7.

WALLACE, J.M. 1958. The Sun-Blotch Disease of Avocado. *Proc. Rio Grande Valley Hortic. Soc.* 12: 69-74.

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. *Phytopathol.* 52: 237-241.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