



Submissions for Diagnostic Protocols

I. General information

Submission number	2023-026
Title of Proposal	Diagnostic protocol for Avocado sun blotch viroid
Submitted by (Country or Organization)	IPPC Contracting Party
IPPC Official Contact Point or RPPO	Kenya
Supported by	Kenya

2. Contact information

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3. Summary of proposal

Summary of justification for the proposal	<p>Avocado (<i>Persea Americana</i>) industry is expanding exponentially. It is estimated that by 2030, global production of avocado fruit will be about 12Mt valued at about \$25B. With this expansion of trade, there are risks of introduction of pests via fresh fruit and propagation material. One such pathogen is Avocado sunblotch viroid (ASBVd). ASBVd has been reported in Australia, Ghana, Guatemala, Israel, Mexico, Peru, South Africa, USA (California, Florida) and Venezuela. In the EU, it has been reported in Greece (Crete Island) and Spain. The pathogen could establish wherever avocado (<i>Persea americana</i>) is grown. The only known natural host of ASBVd is avocado to which it causes the severe 'avocado sunblotch' disease, characterised by white, yellow, red or necrotic depressed areas or scars on the fruit surface, bleached veins and petioles of the leaf, and rectangular cracking patterns in the bark of the old branches. Fruit yield losses (30-83%) have been reported coupled with poor quality. Under experimental conditions, ASBVd infects a few more species in the family Lauraceae. The viroid is naturally transmitted at an extremely high rate by seeds, pollen, through root stock grafts and fresh avocado.</p>
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	Exclusion is the most effective way to manage the disease, and the availability of ASBVd-free plant material from certified nurseries is the most important requirement to avoid spreading of the pathogen. Several NPPOs like Australia, USA, South Africa have developed guidelines for indexing of orchards, nurseries and routine surveillances. However, these guidelines are varied in terms of sampling methodology and diagnostic assays. Hence, the need for a harmonized standard to be adopted by regulatory systems in ensuring ASBVd free commodities are traded thus preventing introduction of the viroid to new environments.
Proposed priority	I (high)
Comments	There is high likelihood of introduction of this pathogen to many countries, hence need for harmonized diagnostic protocol

4. Literature review

Literature review	<p>The Avocado industry is one of the world's growing tropical fruit industries and the pathogen Avocado sunblotch viroid is a major threat to both production and access to international markets (Roberts et al., 2022). ASBVd is one of the smallest viroids (247 nt) and the only one with a base composition rich in Adenine and Uracil (62%). It affects avocado and possibly some other members of the Lauraceae family. ASBVd systematically invades avocado plants, causing the 'sunblotch disease' characterized by discoloured depressed areas on the fruit surface that turn necrotic over time, bleached veins and petioles of the leaf, and rectangular cracking patterns in the bark of older branches. 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 (Saucedo-Carabez et al., 2019). Mixed variants are sometimes observed within a single tree and between trees with different symptoms, (Semancik and Szychowski, 1994; Schnell et al., 2001). Asymptomatic infections also often occur in nature. Trees of many varieties exhibiting symptoms may occasionally grow new asymptomatic vegetation showing a recovery phenomenon. These plants almost fully recover from visible symptoms. However, they still suffer a great reduction in fruit yield. This recovery phenomenon is associated with changes in the viroid population interacting with the avocado host (Semancik and Szychowski, 1994). Although asymptomatic, they contribute significantly in the spread of ASBVd via mechanical and pollen transmission. ASBVd is reported to be transmitted at extremely high rates of 86–100% in avocado seeds from trees with asymptomatic infections after recovery, but much lower (0–5.5%) in those from symptomatic trees (Wallace and Drake, 1953, 1962). ASBVd has no known insect vector and the major means for ASBVd dispersion is via infected propagative material (Vallejo Perez et al., 2017). Transmission most often occurs by grafting infected budwood</p>
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	<p>or growing rootstock seedlings from infected seeds, often asymptomatic (Saucedo-Carabez et al., 2019). Contaminated tools also facilitate the spread of the viroid in the field. Through transboundary trade of planting material, seeds and fresh fruits, the viroid is likely to enter into new territories. Avocado sunblotch viroid disease has been reported in many avocado producing countries such as Australia, Ghana, Guatemala, Israel, Mexico, Peru, South Africa, USA (California, Florida) and Venezuela. In the EU, it has been reported in Greece (Crete Island) and Spain (Carabez et al., 2019). Recently, the viroid has been reported in Kenya (Kibwage et al., 2023). This demonstrates expansion of the viroid to new environments. To ensure importation of ASBVd free planting materials, countries may prescribe measures such as Pest free area, areas of low pest prevalence or pest free production sites to countries where the viroid has been reported. To enhance implementation of these measures the exporting country should have scientific evidence which can be achieved through the use of harmonized sampling and testing standards. At the moment, sampling methodologies adopted by NPPOs are varied as well as the diagnostic procedures.</p>
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5. Criteria for prioritization of Diagnostic Protocols

Criteria	Information provided by Submitter
<p>1. Need for international harmonization of the diagnostic techniques for the pest (e.g. due to difficulties in diagnosis or disputes on methodology)</p>	<p>As the expansion in production and trade of avocado occurs so does the spread of some avocado pathogens such as ASBVd to new areas. To prevent introduction of ASBVd to new territories, importing countries may decide on Phytosanitary measures such as indexing of propagation material, pest free areas or areas of low pest prevalence or pest free production sites may be imposed to countries where ASBVd has been officially reported. There is provision for an exporting country to declare an area as being pest free or a place of low pest prevalence or pest free production site but this claim must be supported by survey data (ISPM 10). Though this viroid has been reported in several countries, there is no harmonized sampling and testing protocol. In New Zealand, the diagnostic standard specifies that when testing asymptomatic trees, a total of ten leaves should be collected from the four compass points of the tree at the height of a standing person, ideally taking single leaves from separate branches (MAF New Zealand, 2009). In Florida, six leaves are sampled from each tree, four from around the base and two from the top of the tree (Kuhn et al, 2019). The sampling protocol in South Africa specifies that 20 to 24 leaves should be collected from all the main branches of a tree when testing individual asymptomatic trees, and eight leaves per tree when pooling three trees in one sample. Though Reverse transcription-(RT) qPCR is now the preferred diagnostic method for ASBVd in many countries around the world, there are variations and the assay is not harmonized (Kuhn et al., 2017).</p>

<p>2. The relevance of the diagnosis to the protection of plants including measures to limit the impact of the pest.</p>	<p>A harmonized sampling and testing protocol will be useful in limiting the introduction of ASBVd through infected seed and propagation material in international trade as well as within a country or territory. Approaches such as adoption of pest free areas, areas of low pest prevalence or pest free production sites and certification schemes may be required when ASBVd is present in exporting countries. In countries where the viroid is present, certification schemes may be adopted to ensure scions, seedlings and orchards are free from the viroid. A harmonized protocol to be used in optimizing sampling and testing strategies to establish pest free status from ASBVd in avocado orchards will assist regulatory bodies designate orchards and/or nurseries as ASBVd free.</p>
<p>3. Importance of the plants protected on the global level (e.g. relevant to many countries or of major importance to a few countries).</p>	<p>Avocado is an economically important tropical fruit globally. Currently, avocado production is valued at about \$10.27B, and it is projected to increase to \$19.9B by 2026 and to \$ 25B in 2030 (FAO, 2021). The economic and social importance of avocado resides in the benefit that its cultivation gives to producers, marketers, processors, and consumers. The orchards create jobs by demanding labor for farming operations, harvest, packinghouse operations, transportation, and marketing (Téliz, 2000). Largest producers of avocado are Mexico, Dominican Republic, Peru, Colombia, Netherlands, Spain, Chile. In Africa, South Africa is the leading producer though other countries like Kenya, Tanzania, Ghana, Burundi are increasing their production.</p>
<p>4. Volume / importance of trade of the commodity that is subjected to the diagnostic procedures (e.g. relevant to many countries or of major importance to a few countries).</p>	<p>Avocado is a major tropical fruit whose worldwide production has increased tremendously in the recent years. Global production is projected to reach 12Mt by 2030 valued at approximately \$25B, this represents a threefold increase since 2010 (FAO, 2021). This increase in production is driven by the ever increasing consumer demand, particularly in developed countries where the fruit is viewed as nutrient rich. With increase in production, there is a increase in demand for planting material which will require stringent quarantine measures particularly pertaining to ASBVd.</p>
<p>5. Other criteria for topics as determined by CPM that are relevant to determining priorities</p>	<p>Avocado sunblotch viroid requires urgent attention. Prioritization is guided by the increase in production and trade of avocado globally. Countries need to prevent introduction of this viroid into their territories as this will significantly affect avocado fruit production and also may pose trade restrictions. To prevent trade disputes, exporting countries should be guided by a harmonized protocol that can be adopted and fulfilled to ensure safe trade. Literature on ASBVd is available and there is expertise available for the development of a harmonized diagnostic protocol that will be adopted by majority avocado producing countries.</p>
<p>6. The balance between pests of importance in different climatic zones</p>	<p>ASBVd is the causal agent of the 'avocado sunblotch disease' initially attributed to physiological causes (solar irradiation) or to a genetic disorder (Geering, 2018). The symptoms of the disease may vary from severe fruit bleaching to mild variegation,</p>

<p>(temperate, tropics etc) and commodity classes.</p>	<p>while asymptomatic infections are also common, depending on the host cultivar, age of plants, environmental conditions and possibly the predominance of specific viroid sequence variants. All known avocado cultivars have been reported as susceptible to the sunblotch disease (Saucedo Carabez et al., 2019). ASBVd disease significantly impacts avocado yield at an economic level, decreasing fruit production but also affecting fruit morphology and quality yield losses ranging from 30% to 83% have been reported depending on affected cultivars. There are no known methods to cure avocado trees of infection (Kuhn et al., 2019). Considering that all avocado cultivars are susceptible and the climatic conditions do not affect the ASBVd establishment, if ASBVd would become widespread in the avocado cultivation areas and impact on production.</p>
<p>7. Number of labs undertaking the diagnosis.</p>	<p>Diagnostic techniques of ASBVd have become available and progressively improved, for the detection and identification of ASBVd. Conventional diagnostic methods such as polyacrylamide gel electrophoresis indexing or dot-blot hybridisation were used in the past. Later, molecular detection methods became a common practice due to their high sensitivity. Routine protocols for the detection of ASBVd by reverse transcription polymerase chain reaction (RT-PCR) were developed for ASBVd and were accepted for testing the health status of avocado propagating stocks. Recently, a SYBR green-based real-time RT-PCR (RT-qPCR) assay was developed and it is reported to significantly increase (~ 100x) detection sensitivity (Morey-León et al., 2018). A protocol with the pre-amplification of the entire viroid cDNA followed by detection using real-time PCR and a TaqMan assay, has improved sensitivity and specificity in ASBVd detection and has been used to create a viroid-free backup of the USDA avocado germplasm collection in Miami (Kuhn et al., 2019). Improvements in the RNA extraction e.g. using the filter paper method have further improved the cost effectiveness and the labour efficiency of qPCR, to be suited for large-scale surveys for ASBVd (Mathews et al., 2022; Pretorius et al., 2022; Pretorius and Geering, 2023). In addition, satellite techniques using spectral images have also been developed to support the detection of infected trees (Beltrán-Peña et al., 2014). South Africa and Australia have ASBVd reference diagnostic labs. Recently Kenya initiated testing of seedlings and mother blocks as a phytosanitary measure to limit the spread of the disease in the country. In summary, several laboratories are undertaking indexing for certification programs.</p>
<p>8. Feasibility of production of a protocol, including availability of knowledge and expertise.</p>	<p>There is varied expertise on ASBVd available to guide on developing sampling and detection guidelines. Over the years, scientists have continued to optimize the diagnosis of ASBVd, and currently highly sensitive real time PCR assay are available that are able to detect low titres of the viroid from symptomatic and asymptomatic plants. Recently, Mathews et al. (2022) published a protocol for 'Detection of avocado sunblotch and other viroids using RNA filter paper capture and RT-PCR'. South Africa, Australia, US, Kenya and other</p>

	countries have expertise and knowledge that can be used develop a protocol.
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