

ADOPTED: 27 June 2023

doi: 10.2903/j.efsa.2023.8116

Pest categorisation of the *avocado sunblotch viroid*

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Abstract

The EFSA Panel on Plant Health conducted a pest categorisation of the avocado sunblotch viroid (ASBVd) for the EU. The identity of ASBVd, a member of the genus *Avsunviroid* (family *Avsunviroidae*) is clearly defined and detection and identification methods are available. The pathogen is not included in the EU Commission Implementing Regulation 2019/2072. 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 in the EU wherever avocado (*Persea americana*) 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 and quality are severely diminished. ASBVd infects under experimental conditions a few more species in the family Lauraceae. The viroid is naturally transmitted at an extremely high rate by seeds (up to 100% in asymptotically infected trees), but with a low efficiency by pollen (only to the produced seeds), and possibly through root grafts. Plants for planting, including seeds, and fresh avocado fruits were identified as the most relevant pathways for further entry of ASBVd into the EU. Avocado crops are cultivated in southern EU countries. Should the pest further enter and establish in the EU, impact on the production of avocado is expected. Phytosanitary measures are available to prevent entry and spread of the viroid in the EU. ASBVd fulfils the criteria that are within the remit of EFSA to assess for it to be regarded as a potential Union quarantine pest.

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Keywords: asymptomatic infection, avocado sunblotch disease, pest risk, plant health, plant pest, quarantine, seed transmission

Requestor: European Commission

Question number: EFSA-Q-2022-00395

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Declaration of interest: If you wish to access the declaration of interests of any expert contributing to an EFSA scientific assessment, please contact interestmanagement@efsa.europa.eu.

Acknowledgements: EFSA wishes to acknowledge the contribution of Oresteia Sfyra (ISA expert) to this opinion.

Suggested citation: EFSA PLH Panel (EFSA Panel on Plant Health), Bragard, C., Baptista, P., Chatzivassiliou, E., Di Serio, F., Gonthier, P., Miret, J. A. J., Justesen, A. F., MacLeod, A., Magnusson, C. S., Milonas, P., Navas-Cortes, J. A., Parnell, S., Potting, R., Stefani, E., Thulke, H.-H., Van der Werf, W., Vicent Civera, A., Yuen, J., Zappalà, L., ... Reignault, P. L. (2023). Scientific Opinion on the pest categorisation of the *avocado sunblotch viroid*. *EFSA Journal*, 21(7), 1–25. <https://doi.org/10.2903/j.efsa.2023.8116>

ISSN: 1831-4732

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The EFSA Journal is a publication of the European Food Safety Authority, a European agency funded by the European Union.



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1. Introduction

1.1. Background and Terms of Reference as provided by the requestor

1.1.1. Background

The new Plant Health Regulation (EU) 2016/2031, on the protective measures against pests of plants, is applying from 14 December 2019. Conditions are laid down in this legislation in order for pests to qualify for listing as Union quarantine pests, protected zone quarantine pests or Union regulated non-quarantine pests. The lists of the EU regulated pests together with the associated import or internal movement requirements of commodities are included in Commission Implementing Regulation (EU) 2019/2072. Additionally, as stipulated in the Commission Implementing Regulation 2018/2019, certain commodities are provisionally prohibited to enter in the EU (high risk plants, HRP). EFSA is performing the risk assessment of the dossiers submitted by exporting to the EU countries of the HRP commodities, as stipulated in Commission Implementing Regulation 2018/2018. Furthermore, EFSA has evaluated a number of requests from exporting to the EU countries for derogations from specific EU import requirements.

In line with the principles of the new plant health law, the European Commission with the Member States are discussing monthly the reports of the interceptions and the outbreaks of pests notified by the Member States. Notifications of an imminent danger from pests that may fulfil the conditions for inclusion in the list of the Union quarantine pest are included. Furthermore, EFSA has been performing horizon scanning of media and literature.

As a follow-up of the above-mentioned activities (reporting of interceptions and outbreaks, HRP, derogation requests and horizon scanning), a number of pests of concern have been identified. EFSA is requested to provide scientific opinions for these pests, in view of their potential inclusion by the risk manager in the lists of Commission Implementing Regulation (EU) 2019/2072 and the inclusion of specific import requirements for relevant host commodities, when deemed necessary by the risk manager.

1.1.2. Terms of Reference

EFSA is requested, pursuant to Article 29(1) of Regulation (EC) No 178/2002, to provide scientific opinions in the field of plant health.

EFSA is requested to deliver 53 pest categorisations for the pests listed in Annex 1A, 1B, 1D and 1E (for more details see mandate M-2021-00027 on the [Open.EFSA](#) portal). Additionally, EFSA is requested to perform pest categorisations for the pests so far not regulated in the EU, identified as pests potentially associated with a commodity in the commodity risk assessments of the HRP dossiers (Annex 1C; for more details see mandate M-2021-00027 on the [Open.EFSA](#) portal). Such pest categorisations are needed in the case where there are not available risk assessments for the EU.

When the pests of Annex 1A are qualifying as potential Union quarantine pests, EFSA should proceed to phase 2 risk assessment. The opinions should address entry pathways, spread, establishment, impact and include a risk reduction options analysis.

Additionally, EFSA is requested to develop further the quantitative methodology currently followed for risk assessment, in order to have the possibility to deliver an express risk assessment methodology. Such methodological development should take into account the EFSA Plant Health Panel Guidance on quantitative pest risk assessment and the experience obtained during its implementation for the Union candidate priority pests and for the likelihood of pest freedom at entry for the commodity risk assessment of High Risk Plants.

1.2. Interpretation of the terms of reference

Avocado sunblotch viroid is one of the pests listed in Annex 1C to the terms of reference (ToR) to be subject to pest categorisation to determine whether it fulfils the criteria of a potential Union quarantine pest for the area of the EU excluding Ceuta, Melilla and the outermost regions of Member States referred to in Article 355(1) of the Treaty on the Functioning of the European Union (TFEU), other than Madeira and the Azores, and so inform EU decision-making as to its appropriateness for potential inclusion in the lists of pests of Commission Implementing Regulation (EU) 2019/ 2072. If a pest fulfils the criteria to be potentially listed as a Union quarantine pest, risk reduction options will be identified.

1.3. Additional information

This pest categorisation was initiated following the commodity risk assessment of avocado (*Persea americana*) plants from Israel performed by EFSA (EFSA PLH Panel, 2021), in which avocado sunblotch viroid was identified as a relevant non-regulated EU pest which could potentially enter the EU on *P. americana*.

2. Data and methodologies

2.1. Data

2.1.1. Information on pest status from NPPOs

In the context of the current mandate, EFSA is preparing pest categorisations for new/emerging pests that are not yet regulated in the EU. When official pest status is not available in the European and Mediterranean Plant Protection Organization (EPPO) Global Database (EPPO, online), EFSA consults the NPPOs of the relevant MSs. To obtain information on the official pest status for *Avocado sunblotch viroid*, EFSA has consulted the NPPOs of Greece and Spain. The results of this consultation are presented in Section 3.2.2.

2.1.2. Literature search

A literature search on *Avocado sunblotch viroid* was conducted at the beginning of the categorisation in the ISI Web of Science bibliographic database, using the scientific name of the pest as search term. Papers relevant for the pest categorisation were reviewed, and further references and information were obtained from experts, as well as from citations within the references and grey literature.

2.1.3. Database search

Pest information, on host(s) and distribution, was retrieved from the European and Mediterranean Plant Protection Organization (EPPO) Global Database (EPPO, online), the CABI databases and scientific literature databases as referred above in Section 2.1.1.

Data about the import of commodity types that could potentially provide a pathway for the pest to enter the EU and about the area of hosts grown in the EU were obtained from EUROSTAT (Statistical Office of the European Communities).

The Europhyt and TRACES databases were consulted for pest-specific notifications on interceptions and outbreaks. Europhyt is a web-based network run by the Directorate General for Health and Food Safety (DG SANTÉ) of the European Commission as a subproject of PHYSAN (Phyto-Sanitary Controls) specifically concerned with plant health information. TRACES is the European Commission's multilingual online platform for sanitary and phytosanitary certification required for the importation of animals, animal products, food and feed of non-animal origin and plants into the European Union, and the intra-EU trade and EU exports of animals and certain animal products. Up until May 2020, the Europhyt database managed notifications of interceptions of plants or plant products that do not comply with EU legislation, as well as notifications of plant pests detected in the territory of the Member States and the phytosanitary measures taken to eradicate or avoid their spread. The recording of interceptions switched from Europhyt to TRACES in May 2020.

GenBank was searched to determine whether it contained any nucleotide sequences for *Avocado sunblotch viroid* which could be used as reference material for molecular diagnosis. GenBank® (www.ncbi.nlm.nih.gov/genbank/) is a comprehensive publicly available database that as of August 2019 (release version 227) contained over 6.25 trillion base pairs from over 1.6 billion nucleotide sequences for 450,000 formally described species (Sayers et al., 2020).

2.2. Methodologies

The Panel performed the pest categorisation for *Avocado sunblotch viroid*, following guiding principles and steps presented in the EFSA guidance on quantitative pest risk assessment (EFSA PLH Panel, 2018), the EFSA guidance on the use of the weight of evidence approach in scientific assessments (EFSA Scientific Committee, 2017) and the International Standards for Phytosanitary Measures No. 11 (FAO, 2013).

The criteria to be considered when categorising a pest as a potential Union quarantine pest (QP) is given in Regulation (EU) 2016/2031 Article 3 and Annex I, Section 1 of the Regulation. Table 1 presents the Regulation (EU) 2016/2031 pest categorisation criteria on which the Panel bases its conclusions. In judging whether a criterion is met the Panel uses its best professional judgement (EFSA Scientific Committee, 2017) by integrating a range of evidence from a variety of sources (as presented above in Section 2.1) to reach an informed conclusion as to whether or not a criterion is satisfied.

The Panel's conclusions are formulated respecting its remit and particularly with regard to the principle of separation between risk assessment and risk management (EFSA founding regulation (EU) No 178/2002); therefore, instead of determining whether the pest is likely to have an unacceptable impact, deemed to be a risk management decision, the Panel will present a summary of the observed impacts in the areas where the pest occurs, and make a judgement about potential likely impacts in the EU. While the Panel may quote impacts reported from areas where the pest occurs in monetary terms, the Panel will seek to express potential EU impacts in terms of yield and quality losses and not in monetary terms, in agreement with the EFSA guidance on quantitative pest risk assessment (EFSA PLH Panel, 2018). Article 3 (d) of Regulation (EU) 2016/2031 refers to unacceptable social impact as a criterion for quarantine pest status. Assessing social impact is outside the remit of the Panel.

Table 1: Pest categorisation criteria under evaluation, as derived from Regulation (EU) 2016/2031 on protective measures against pests of plants (the number of the relevant sections of the pest categorisation is shown in brackets in the first column)

Criterion of pest categorisation	Criterion in Regulation (EU) 2016/2031 regarding Union quarantine pest (article 3)
Identity of the pest (Section 3.1)	Is the identity of the pest clearly defined, or has it been shown to produce consistent symptoms and to be transmissible?
Absence/presence of the pest in the EU territory (Section 3.2)	Is the pest present in the EU territory? If present, is the pest in a limited part of the EU or is it scarce, irregular, isolated or present infrequently? If so, the pest is considered to be not widely distributed.
Pest potential for entry, establishment and spread in the EU territory (Section 3.4)	Is the pest able to enter into, become established in, and spread within, the EU territory? If yes, briefly list the pathways for entry and spread.
Potential for consequences in the EU territory (Section 3.5)	Would the pests' introduction have an economic or environmental impact on the EU territory?
Available measures (Section 3.6)	Are there measures available to prevent pest entry, establishment, spread or impacts? If already present in the EU are measures available to slow spread or facilitate eradication?
Conclusion of pest categorisation (Section 4)	A statement as to whether (1) all criteria assessed by EFSA above for consideration as a potential quarantine pest were met and (2) if not, which one(s) were not met.

3. Pest categorisation

3.1. Identity and biology of the pest

3.1.1. Identity and taxonomy

Is the identity of the pest clearly defined, or has it been shown to produce consistent symptoms and/or to be transmissible?

Yes, the identity of avocado sunblotch viroid is clearly defined. The pathogen has been shown to produce consistent symptoms and to be transmissible.

Avocado sunblotch viroid (ASBVd) is a well-characterised viroid, classified in the species *Avocado sunblotch viroid* of the monotypic genus *Avsunviroid*, family *Avsunviroidae* (<https://ictv.global/taxonomy>).

As a viroid, ASBVd consists of a circular, non-coding RNA that replicates autonomously and invades systemically its hosts (Flores et al., 2005). It is transmissible and causes the economically important 'sunblotch disease' in avocado (*Persea americana* Miller), severely affecting fruit quality and yield (Saucedo Carabez et al., 2019). EPPO also uses the common name 'sun blotch of avocado' (EPPO, online).

The EPPO code¹ (Griessinger and Roy, 2015; EPPO, 2019) for this species is ASBVD0 (EPPO, online).

3.1.2. Biology of the pest

ASBVd is one of the smallest viroids (247 nt) and the only one with a base composition rich in Adenine and Uracil (62%). Its RNA folds into a rod-like conformation due to extensive internal base-pairing, and replicates in the chloroplast through a symmetric double rolling circle using the host nuclear-encoded polymerase (Navarro et al., 2000; Flores et al., 2005).

ASBVd affects avocado and possibly some other members of the Lauraceae family (da Graça and van Vuuren, 1980, 1981a). ASBVd systematically invades avocado plants, causing the 'sunblotch disease' characterised 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 (Figure 1; for details, see Section 3.1.5). The expression of symptoms can be affected by multiple factors including host cultivar, age of plants, environmental conditions and possibly the predominance of specific viroid sequence variants (Desjardins et al., 1987; Semancik and Szychowski, 1994; Saucedo Carabez et al., 2014). The infection may also remain symptomless or progress from severe to mild expression of symptoms (see below, Semancik and Szychowski, 1994). Under experimental conditions, in indexing programmes, symptoms may appear up to 36 months after grafting (CABI, 2021).

Foliar symptoms, when present, are also more pronounced and they appear earlier in seedlings kept at high (30°C) temperature or light intensity (da Graça and van Vuuren, 1981b; ICTV, 2023). The symptoms of the disease are associated with anatomical and chemical changes in the structure of the fruit exocarp and mesocarp cells (Vallejo-Pérez et al., 2014) or the leaf tissues (da Graça and Martin, 1981c). Efforts to eliminate ASBVd infection by micrografting and somatic embryo generation have been unsuccessful (Suarez et al., 2005, 2006).

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 (Wallace and Drake, 1962; Wallace, 1967). This recovery phenomenon is associated with changes in the viroid population interacting with the avocado host (Semancik and Szychowski, 1994). Asymptomatically infected trees also arise from seeds produced by these recovered trees (Wallace and Drake, 1962). Both recovered trees and their seedlings were in the past called 'symptomless carriers' or 'symptomless carrier trees' to stress that although asymptomatic they contribute significantly in the spread of ASBVd via mechanical and pollen transmission (Desjardins et al., 1979). Recovered plants show a dramatic increase in the rate of seed transmission of the viroid (from less than 5% to 90–100%), while symptoms cannot be (re)induced in the recovered plants or asymptomatic seedlings (Desjardins et al., 1987).

The transition of the infection from the acute form, identified by severe symptoms, to the recovery stage via a chronic infection with irregular and infrequent bleached and variegated symptoms, has been associated with a change in the viroid population. Distinct variants have been suggested to dominate in foliar tissues exhibiting different types of symptoms; bleached (ASBVd-B) and variegated (ASBVd-V) variants isolated from symptomatic leaf tissues and the 'symptomless carrier' (ASBVd-Sc) variant from asymptotically infected trees (Semancik and Szychowski, 1994). However, this association of possible variants with the different symptoms needs to be further investigated (Saucedo Carabez et al., 2019).

¹ An EPPO code, formerly known as a Bayer code, is a unique identifier linked to the name of a plant or plant pest important in agriculture and plant protection. Codes are based on genus and species names. However, if a scientific name is changed the EPPO code remains the same. This provides a harmonised system to facilitate the management of plant and pest names in computerised databases, as well as data exchange between IT systems (Griessinger and Roy, 2015; EPPO, 2019).



Figure 1: Symptoms of avocado sunblotch disease; (A) yellowish sunken areas on fruits; (B) discoloured and necrotic depressions on infected twigs; (C) distortion and variegation on leaves; (D) cracked bark ('Alligator skin') appearance on some mature branches; (E) fruits with reddish colour areas; (F) necrosis on severely affected fruits; (G) multiple yellowish sunken areas in fruits (from Saucedo Carabez et al., 2014)

ASBVd has no known insect vector and the major means for ASBVd dispersion is via infected propagative material (Vallejo Pérez et al., 2017). Transmission most often occurs by grafting infected budwood (Wallace, 1958) or growing rootstock seedlings from infected seeds, often asymptomatic (Saucedo-Carabez et al., 2019). This is a major characteristic that led to the evolution and perpetuation of ASBVd (Duran-Vila et al., 2008) and resulted in a frequent infection of genetic material held in national germplasm collections (Ronning et al., 1996; Olano et al., 2002; Tondo et al., 2010; Schnell et al., 1997, 2011).

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). Seed transmission may result from infection of either the ovule (from the infected mother plant) or from a pollen transmission at a low rate (1–4%) (vertical pollen transmission; Desjardins et al., 1984). Most of the infected progeny seedlings produced from symptomatic trees may exhibit symptoms on emergence. However, plants growing from infected seeds derived from recovered trees remain asymptomatic; symptoms may only appear on the scion of a susceptible variety grafted on these asymptomatic seedlings (Desjardins et al., 1984). Other than a reduction in their size, there is no visible indication of the presence of ASBVd in seeds (CABI, 2021).

Pollen from both symptomatic and asymptomatic infected trees can transmit ASBVd in field trees at a low rate of 1–4%. However, pollen transmission contributes only to the seed and fruit infection and no horizontal transmission (i.e. infection of the pollen-recipient tree during pollination) has been

recorded for ASBVd (Desjardins et al., 1979, 1984). Pollinating bees may facilitate viroid transmission via infected pollen (Desjardins et al., 1979; Roberts et al., 2023).

In nature, root-to-root grafts are suspected to transmit ASBVd between infected and healthy avocado trees (Whitsell, 1952). However, this mode of transmission results in a rather slow dissemination from plant to plant and it is restricted only to neighbouring trees (Tondo et al., 2010; Schnell et al., 2011).

In the laboratory, ASBVd was transmitted by slashing healthy avocado seedlings with a razor moistened either with extracts from infected trees or symptomatic fruit (Desjardins et al., 1980) or with highly purified ASBVd eluted from gels (Desjardins et al., 1980; Utermohlen et al., 1981; Desjardins and Drake, 1983). ASBVd reaches extremely high titres in avocado tissues (Semancik and Desjardins, 1980). The viroid is reported to be very stable at any heat treatment regime that avocado tissue could withstand (Desjardins et al., 1980). As a result, it can remain infective in plant debris and on sap-contaminated cutting equipment such as grafting, pruning or harvesting tools, and on surfaces where avocado plants are propagated (Desjardins et al., 1980, 1987). Therefore, mechanical transmission through wounds caused by contaminated tools is also expected to facilitate the spread of the viroid in the field (Desjardins et al., 1980). However, the frequency of this transmission has to be evaluated and its contribution to viroid spread under natural conditions is unclear.

3.1.3. Host range/species affected

ASBVd has one of the narrowest host ranges among the viroids (Kuhn et al., 2017). It infects only avocado in nature, while few plant species of the family Lauraceae, namely *Cinnamomum zeylanicum* (Ceylon cinnamon), *C. camphora* (camphor tree), *Persea schiedeana* (coyo) and *Ocotea bullata* (black stinkwood or cape walnut), have been confirmed as experimental hosts, using a bark-patch grafting technique (da Graça, 1978; da Graça and van Vuuren, 1980, 1981a; Aviña-Padilla et al., 2022).

ASBVd has been reported to replicate in the yeast *Saccharomyces cerevisiae* (Latifi et al., 2016), and the cyanobacterium *Nostoc* sp. (Delan-Forino et al., 2011) without causing any apparent effect and having no epidemiological significance. ASBVd was also reported to experimentally infect *Nicotiana benthamiana* (Wei et al., 2019) and the phytopathogenic fungi *Cryphonectria parasitica*, and *Valsa mali* (Wei et al., 2019) however, these results are considered doubtful (Serra et al., 2020).

3.1.4. Intraspecific diversity

Viroids occur in nature as complex populations of closely related sequences (Domingo et al., 1996). ASBVd sequence variants, differing in size from 247 to 250 nt, are sometimes observed within a single tree, between trees with different symptoms, or from a single tree when leaves and flowers were sampled over a period of years (Pallas et al., 1988; Rakowski and Symons, 1989; Semancik and Szychowski, 1994; Schnell et al., 2001a).

ASBVd variants recovered from specific diseased avocado tissues, which have similar nucleotide substitutions in specific regions of the molecule, are named ASBVd-B, ASBVd-V and ASBVd-Sc from their association with bleached, variegated or symptomless infected foliar tissues, respectively. The dominance of each of these variants is suggested to represent the transition in sunblotch disease from a severe acute to a persistent mild form of infection (Semancik and Szychowski, 1994). However, Suarez et al. (2005) and Schnell et al. (2001a) reported a higher number of ASBVd sequence variants, with the latter authors suggesting that this number is positively correlated with the years the tree has been infected.

3.1.5. Detection and identification of the pest

Are detection and identification methods available for the pest?

Yes, detection and identification methods are available for ASBVd.

Historically, viroids were detected by the specific symptoms they induce in their natural hosts. However, field symptoms are not reliable for their detection as they may be affected by several factors, resembling also abiotic disease (e.g. efficiency or excess of nutrients, phytotoxicity symptoms or sunburn for ASBVd).

For ASBVd, a distinctive sign of infection may be that the trees develop a characteristic flattened shape with limbs bending toward the ground (Wallace, 1958).

The most consistent symptom of the disease, and usually the initial one observed, is the appearance of narrow discoloured streaks or stripes and spots on the base of green twigs and limbs. Sometimes small side shoots or growth flushes are completely chlorotic. On the other hand, the most striking and recognisable symptom appears on the fruits of susceptible varieties (e.g. cultivars Hass, Bacon and Fuerte) that exhibit irregular superficial or sunken areas that can be white, yellow or reddish in colour and can later become necrotic. Fruits are fewer and smaller. Bark of trunks and old branches can have a scaly appearance with rectangular cracking referred to as 'alligator skin'. Leaf symptoms are uncommon in the field, but when present they can also be distinctive. In the most severe cases leaves appear distorted with discrete bleached areas associated with midveins and vascular tissues or with a more generalised variegated pattern (similar to a genetic aberration) associated with distortions. Leaves may also appear small in size, crinkled on one side of the midrib, giving a scimitar-shaped blade. The expression of foliar symptoms is erratic and appears in an irregular and unpredictable manner. ASBVd can also cause premature leaf and flower fall (Flores et al., 2000; Saucedo Carabez et al., 2019; CABI, 2021).

In general, the distribution of symptoms is irregular, and trees may not develop all the symptoms. Trees with severe symptoms may also appear stunted with a flattened shape having a disproportionate amount of horizontal growth or sprawling of the lateral low limbs (Saucedo Carabez et al., 2014, 2019). Some ASBVd-infected trees may recover from the disease, therefore appear symptomless, but may still have a reduced fruit yield. These trees produce a high number of infected seeds, the progeny seedlings of which never develop symptoms (Desjardins et al., 1987).

ASBVd was shown to induce typical sunblotch stem symptoms on *C. zeylanicum*, yellow depressed stem streaks on *P. schiedeana* and curly leaves and twig dieback on *O. bullata*, when graft-inoculated with infected avocado bark (da Graça, 1978; da Graça and van Vuuren, 1980, 1981a).

However, asymptomatic field ASBVd infections are also common (see Section 3.1.2). In the past, disease diagnosis was based on biological indexing, using graft transmission to indicator seedlings; however, this method is laborious and time- (up to 36 months) and space-consuming (Burns et al., 1968; Da Graça, 1979; Da Graça and van Vuuren, 1981a).

Diagnostic techniques have become available and progressively improved, for the detection and identification of ASBVd. Conventional diagnostic methods such as polyacrylamide gel electrophoresis indexing (Da Graça and Mason, 1983; López-Herrera et al., 1987), or dot-blot hybridisation with labelled complementary cDNA (Palukaitis and Symons, 1980; Allen and Dale, 1981; Rosner et al., 1983; Spiegel et al., 1984; Bar-Joseph et al., 1985; Lima et al., 1994) have been extensively used in the past.

Later, molecular detection methods became a common practice due to their high sensitivity compared with conventional methods. Routine protocols for the detection of ASBVd by reverse transcription polymerase chain reaction (RT-PCR) were developed for ASBVd (Semancik and Szychowski, 1994; Mathews et al., 1997; Schnell et al., 1997, 2001b) and were accepted for testing the health status of avocado propagating stocks. However, the conventional RT-PCR may sometimes fail to detect the viroid when in low concentration in the early stage of infection or due to its uneven distribution in the plants that for ASBVd may reach a 1,000-fold variation in the leaves on the same tree (Allen and Dale, 1981). To deal with this issue, RT-PCR may be coupled with capillary electrophoresis by incorporating fluorescent detection (Schnell et al., 2001a). The use of capillary electrophoresis and single-strand conformation polymorphism analysis also allows the detection of ASBVd variants (Schnell et al., 2001a).

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).

Flower buds are preferable tissues over leaves for diagnosis (da Graça and Mason, 1983). Commercial RT-PCR kits for ASBVd detection are also available.

In Genbank, 150 full sequences of ASBVd and the Reference genome GCF_000853625.1 (<https://www.ncbi.nlm.nih.gov/datasets/taxonomy/12896/>) are available.

3.2. Pest distribution

3.2.1. Pest distribution outside the EU

Outside the EU, ASBVd occurs in most avocado-producing countries on all continents; Americas: Guatemala, Mexico, Peru, USA (California, Florida) and Venezuela; Asia: Israel; Africa: Ghana and South Africa; and Australia. It is likely that the geographical distribution of ASBVd is wider than reported, especially in areas where untested avocado germplasm was imported before highly sensitive testing methods became available (Kuhn et al., 2017).

The global distribution of ASBVd is shown in Figure 2, with details and related references summarised in Appendix B.

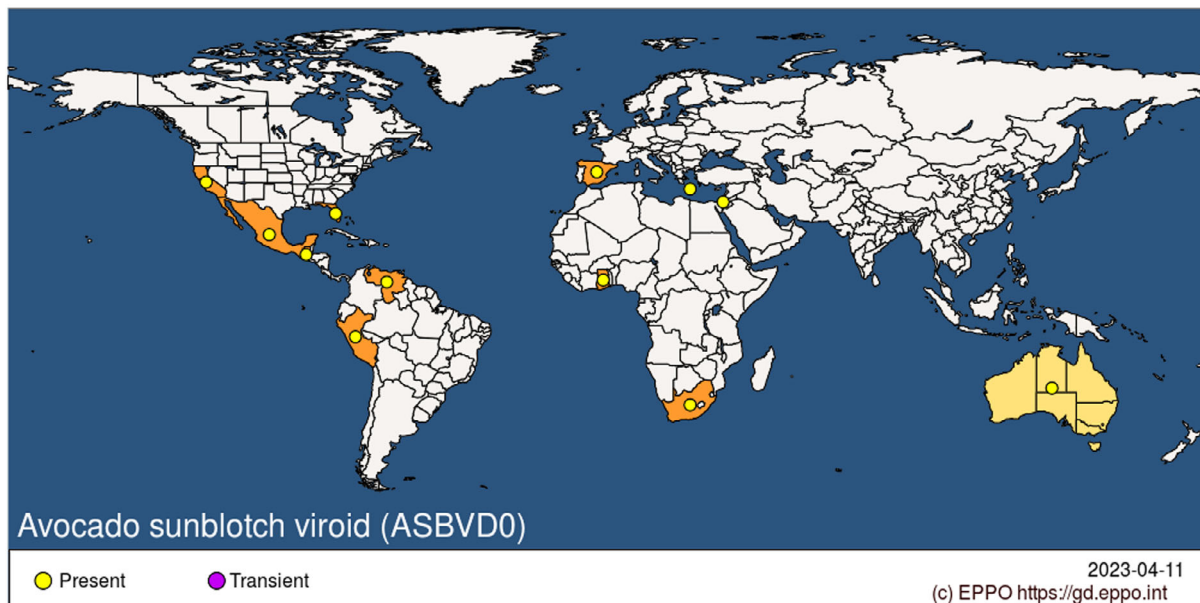


Figure 2: Global distribution of Avocado sunblotch viroid (Source: EPPO Global Database accessed on 4 November 2023)

3.2.2. Pest distribution in the EU

Is the pest present in the EU territory? If present, is the pest in a limited part of the EU or is it scarce, irregular, isolated or present infrequently? If so, the pest is considered to be not widely distributed.

Yes, ASBVd has been reported in Greece (Crete Island) and Spain.

ASBVd was reported to infect a single symptomatic avocado tree in an orchard in Greece (Crete Island) in 2016. However, only this plant was tested and no survey was performed (Lotos et al., 2018). The viroid was identified in seven of the 11 trees analysed of the cultivar 'Hass' and in the three trees investigated of the cultivar 'Fuerte' coming from one and three different (but not specified) locations in Southern Spain, respectively (López-Herrera et al., 1997). The status of the pest in Greece is considered as '*present, few occurrences*', while in Spain, it is reported as '*present, no details*' (EPPO GD). The Spanish NPPO declared in June 2023 that the pest status in Spain is '*Present, restricted distribution*' and no measures are carried out against ASBVd.

3.3. Regulatory status

3.3.1. Commission implementing regulation 2019/2072

Avocado sunblotch viroid is not listed in Annex II of Commission Implementing Regulation (EU) 2019/2072, an implementing act of Regulation (EU) 2016/2031, or in any emergency plant health legislation.

3.3.2. Hosts or species affected that are prohibited from entering the union from third countries

Persea americana and *P. schiedeana* are recognised as high-risk plants whose import into the EU is prohibited pending risk assessment (EU 2018/2019). However, there is a derogation on *P. americana* plants for planting from Israel (EU 2021/1936).

3.4. Entry, establishment and spread in the EU

3.4.1. Entry

Is the pest able to enter into the EU territory? If yes, identify and list the pathways.

YES, ASBVd could potentially further enter into the EU via plants for planting, including avocado seeds, and fresh avocado fruits.

Comment on plants for planting as a pathway.

Plants for planting, including avocado seeds, is the main pathway for further entry of the pathogen into the EU.

The Panel identified the trade of host plants for planting (including seeds for avocado) originating in infested third countries as the main pathway for the further entry of the viroid in the EU. The current global spread of ASBVd is considered to be closely related to the trade of propagating plant material of avocado (Duran-Vila et al., 2008). Avocado is considered a high-risk plant; therefore, import of plants for planting into the EU is prohibited pending a risk assessment (Commission Implementing Regulation EU 2018/2019). However, there is a derogation on plants from Israel (where the pest is present); thus, this pathway is regulated (EFSA PLH Panel, 2021).

Moreover, the viroid has additional hosts recorded under experimental conditions. *Persea schiedeana* is an endangered tree species native to southern Mexico and Central America (Wegier et al., 2020) and cultivated for the fruit and used as rootstock for avocado (Schroeder, 1974). *P. schiedeana* is also considered as a high-risk plant; therefore, this pathway is closed. As for the others recorded hosts in the *Lauraceae* family (e.g. *Cinnamomum zeylanicum*, *C. camphora* and *Ocotea bullata*), there are no specific requirements that target ASBVd for import of plants for planting, thus providing an entry pathway with generic requirements (phytosanitary certificates). However, *O. bullata* is a protected tree native to South Africa (Harris, 2004) and it is unlikely to be imported into the EU. Similarly, it is unlikely that the other two experimental hosts are imported into the EU.

ASBVd can be transmitted by seeds, and especially those produced by trees having recovered disease symptoms infected in high rates. A phytosanitary certificate is needed to import seeds for sowing from third countries (2019/2072, Annex XI, Part B). Up to 100% of the seeds produced by asymptotically infected avocado trees can be infected. However, there are no specific requirements for ASBVd.

Asymptomatic fresh fruits for consumption may also come from recovered infected plants producing seeds infected in high rates. Since germinating seeds of commercially purchased avocado fruit is easy for anyone, e.g. for the home garden, the entry of the viroid from an infected area by fresh fruits is possible. In addition, considering ASBVd high infectivity and the possibility of mechanical transmission, infected fruits as such may also represent an entry pathway. A phytosanitary certificate is required to import fruits of hosts from third countries other than Switzerland (Commission Implementing Regulation 2019/2072, Annex XI, Part A). However, there are no specific requirements for ASBVd.

Avocado pollen possibly used for breeding purposes may also represent an entry pathway (Fetters and Ashman, 2023). However, the Panel considers this pathway to be unlikely.

The potential pathways for further entry of ASBVd in the EU are listed in Table 2, together with the relevant mitigation measures in place.

Table 2: Potential pathways for ASBVd into the EU

Pathways (e.g. host/intended use/source)	Relevant mitigations [e.g. prohibitions (Annex VI), special requirements (Annex VII) or phytosanitary certificates (Annex XI) within Implementing Regulation 2019/2072]
Host plants for planting (other than seeds)	<i>Persea americana</i> and <i>P. schiedeana</i> are recognised as high-risk plants whose import into the EU is prohibited pending a risk assessment (EU 2018/2019). However, there is a derogation on <i>P. americana</i> plants for planting from Israel (Commission Implementing Regulation (EU) 2021/1936). Plants for planting (other than seeds) of <i>Cinnamomum zeylanicum</i> , <i>C. camphora</i> , and <i>Ocotea bullata</i> must be accompanied by a phytosanitary certificate.
Avocado seeds for sowing	A phytosanitary certificate is required for the introduction into the EU from 3rd countries, other than Switzerland, of seeds (2019/2072, Annex XI, Part B). However, no requirements are specified for ASBVd.
Fresh avocado fruits	A phytosanitary certificate is required for the introduction into the EU from 3rd countries, other than Switzerland, of avocado fruits (fresh or chilled) (Annex XI, Part A of Commission Implementing Regulation (EU) 2019/2072). However, no requirements are specified for ASBVd.
Avocado pollen	Plants, plant products or other objects for trial or scientific purposes or for work on varietal selections: a letter of authorisation needs to be issued, in line with Regulation (EU) 2019/829.

The quantity of fresh or dried avocados imported into the EU from countries where ASBVd is present is provided in Table 3. Volume of imported host plants for planting, avocado seeds and pollen is unknown.

Table 3: EU annual imports of fresh or dried avocados (CN Code: 0804 40 00) from countries where Avocado sunblotch viroid is present, 2017–2021 (in 100 kg). Source: Eurostat, accessed on 5 November 2023

	2017	2018	2019	2020	2021
South Africa	315,854.56	652,817.98	401,352.79	416,290.22	418,962.17
Mexico	445,611.06	463,741.28	767,878.48	716,113.14	751,530.02
Peru	1,353,466.49	2,009,222.64	1,584,511.63	2,132,092.95	2,670,248.50
Ghana	134.58	22.64	40.45	21.88	19.43
Venezuela	233.40	111.12	71.29	:	:
Guatemala	4,291.98	7,487.42	17,084.09	15,383.92	24,717.32
Israel	424,267.97	370,378.23	437,318.01	345,664.24	453,364.24
Australia	:	:	0.01	:	0.31
United States	1.19	2546.86	0.02	4.66	45.38

;, no data available.

Notifications of interceptions of harmful organisms began to be compiled in Europhyt in May 1994 and in TRACES in May 2020. As of May 2023, there were no records of interception of Avocado sunblotch viroid in the Europhyt and TRACES databases.

3.4.2. Establishment

Is the pest able to become established in the EU territory?

Yes, ASBVd has been reported in Greece (Crete Island) and Spain (see Section 3.2.2). The pathogen could establish wherever avocado is grown in the EU.

ASBVd has been reported to be present in Greece (Crete Island) and Spain (see Section 3.2.2). There is uncertainty on whether it has established in those two countries. However, the pathogen could establish wherever avocado is grown in the EU (see Section 3.4.2.1). Transfer of ASBVd from the pathway of entry to avocado grown in the EU can occur via contaminated tools, root grafts and avocado pollen and fruits. The panel notes that ASBVd reaches very high titres in avocado tissues

(Semancik and Desjardins, 1980) where it is quite stable (CABI, 2021) therefore, debris or waste of infected plants and fruits may further contribute to its establishment.

3.4.2.1. EU distribution of main host plants

Avocado, the only natural host of ASBVd, is cultivated in the EU countries of the Mediterranean basin, with Spain being the largest producer. Details on avocado crop production areas in individual EU MSs are provided in Table 4.

Table 4: Harvested area of avocados, natural host of the avocado sunblotch viroid, in the EU, 2017–2021 (1,000 ha). Source: Eurostat (accessed 5 November 2023)

Avocado	2017	2018	2019	2020	2021
EU*	12.72	13.22	17.50	19.69	22.85
Greece	0.60	0.72	1.08	1.10	1.93
Spain	11.81	12.16	14.10	15.85	18.06
France	0.23	0.24	0.24	0.24	0.13
Cyprus	0.08	0.10	0.10	0.16	0.16
Portugal	0.00	0.00	1.98	2.34	2.57

*: Eurostat does not provide data on Italy production.

3.4.2.2. Climatic conditions affecting establishment

Climatic conditions in the EU are not a limiting factor for ASBVd establishment, because establishment is dependent only on the availability of host plants in the EU. Therefore, the viroid is expected to thrive wherever avocado trees grow (Everett and Siebert, 2018).

3.4.3. Spread

Describe how the pest would be able to spread within the EU territory following establishment?

ASBVd could potentially spread further within the EU territory by natural (pollen and root grafts) and human-assisted means (trade of plants for planting including seeds, and tools) (see Section 3.1.2).

Trade of infected host plants for planting, including seeds (for avocado), is the main means of spread of the pathogen.

ASBVd systemically invades its hosts (see Section 3.1.2) and therefore can be transmitted through vegetative propagation of infected material of its hosts (budwood and scions) or grafting healthy scions on asymptotically infected avocado seedlings used as rootstock (Saucedo Carabez et al., 2019; CABI, 2021). Therefore, trade of host plants for planting is the main means of ASBVd spread.

ASBVd is also transmitted via avocado seed in high transmission rates (Wallace and Drake, 1953, 1962), pollen (but only to the produced seed) (Desjardins et al., 1984) and possibly root grafts (Tondo et al., 2010; Schnell et al., 2011; Hadidi et al., 2022). Transmission via contaminated tools during cultural practices is also expected to contribute to the field spread (Desjardins et al., 1980, 1987).

3.5. Impacts

Would the pests' introduction have an economic or environmental impact on the EU territory?

Yes, the further introduction and spread of ASBVd in the EU would potentially cause economic impact.

ASBVd is the causal agent of the 'avocado sunblotch disease' (Figure 1) initially attributed to physiological causes (solar irradiation) (Coit, 1928) or to a genetic disorder (Geering, 2018). The symptoms of the disease may vary from severe fruit bleaching to mild variegation, 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 (Desjardins et al., 1987; Saucedo Carabez et al., 2014). All known avocado cultivars have been reported as susceptible to the sunblotch

disease (Saucedo Carabez et al., 2019). There are no known methods to cure avocado trees of infection (Kuhn et al., 2019).

Sunblotch disease caused by ASBVd significantly impacts avocado yield at an economic level, decreasing fruit production but also affecting fruit morphology and quality (Flores et al., 2000; Saucedo Carabez et al., 2019; Zwane et al., 2023). An overall reduction of 30% in cultivar Fuerte yield has been observed (da Graça and Mason, 1983), while the yield reduction of cultivar Edranol could reach 82% (da Graça, 1985). Desjardins et al. (1987) reported a fruit yield reduction of up to 95% in the asymptomatic ASBVd-infected trees of cultivars Caliente and Reed. In more recent studies, symptomatic trees showed a decrease of total fruit yield of up to 75% and 83% for the cultivars Hass and Mendez, respectively. Even asymptotically infected trees showed a yield reduction of 30–58%, depending on the cultivar. More than half of the fruit from symptomatic trees were significantly downgraded due to the presence of skin distortions and chlorotic patches (da Graça and Mason, 1983; Saucedo Carabez et al., 2014).

ASBVd also affects the post-harvest quality of the symptomatic fruits of cultivar Hass, in regards of firmness, colour and weight loss, while the asymptomatic ones still satisfy the international quality standards. However, symptoms presence may not significantly affect dry matter, mineral and oil content of fruit (Saucedo Carabez et al., 2015).

No information is available on the impact of ASBVd in the EU. However, 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 of the southern EU countries, an impact can be expected.

3.6. Available measures and their limitations

Are there measures available to prevent pest entry, establishment, spread or impacts such that the risk becomes mitigated?

YES, although not specifically targeted against ASBVd, existing phytosanitary measures mitigate the likelihood of the pathogen's further entry into the EU territory on host plants for planting including avocado seeds and on avocado fruits (see Sections 3.3.2 and 3.4.1). Potential additional measures are also available to further mitigate the risk of further entry, establishment and spread as well as impacts of the pathogen in the EU (see Section 3.6.1).

3.6.1. Identification of potential additional measures

Phytosanitary measures (prohibitions) are currently applied to some host plants for planting (see Section 3.3.2).

Additional potential risk reduction options and supporting measures are shown in Sections 3.6.1.1 and 3.6.1.2.

3.6.1.1. Additional potential risk reduction options

Potential additional control measures are listed in Table 5.

Table 5: Selected control measures (a full list is available in EFSA PLH Panel, 2018) for pest entry/ establishment/spread/impact in relation to currently unregulated hosts and pathways. Control measures are measures that have a direct effect on pest abundance

Control measure/ Risk reduction option (<u>Blue underline</u> = Zenodo doc, Blue = WIP)	RRO summary	Risk element targeted (entry/establishment/spread/impact)
Require pest freedom	Use of plants for planting (including avocado seeds) but also avocado fruits originating from a country officially free from ASBVd or from a pest free area or from a pest free production site is effective	Entry/Spread

Control measure/ Risk reduction option (Blue underline = Zenodo doc, Blue = WIP)	RRO summary	Risk element targeted (entry/establishment/spread/impact)
<u>Roguing and pruning</u>	<p>Roguing is defined as the removal of infested plants and/or uninfested host plants in a delimited area, whereas pruning is defined as the removal of infested plant parts only without affecting the viability of the plant.</p> <p>Eradication of the infected, even symptomless trees may prevent further field spread however, a special care should be taken to chemically kill the stumps (especially for avocado) when removing symptomatic trees.</p>	Entry/Spread/Impact
<u>Cleaning and disinfection of facilities, tools and machinery</u>	<p>The physical and chemical cleaning and disinfection of facilities, tools, machinery, transport means, facilities and other accessories (e.g. boxes, pots, pallets, palox, supports, hand tools). The measures addressed in this information sheet are washing, sweeping and fumigation.</p> <p>Transmission via contaminated tools is expected (Section 3.1.2); therefore, cleaning and disinfection may contribute to reduce the probability of mechanical transmission to other plants.</p> <p>ASBVd can be effectively inactivated on pruning and propagating tools or cutting surfaces using a 1:1 mixture of 2% sodium hydroxide and 2% formaldehyde or a 6% solution of hydrogen peroxide or 10–20% commercial bleach (sodium hypochlorite) (Desjardins et al., 1987; Kuhn, 2017)</p>	Entry/Spread
<u>Waste management</u>	<p>ASBVd reaches very high titres in avocado tissues (Semancik and Desjardins, 1980) where it is quite stable (Desjardins et al., 1980). Therefore, the presence of the viroid on plant debris cannot be excluded, so proper waste (plants, fruits) management may contribute to reduce possible sources of infections.</p>	Establishment/Spread
<u>Post-entry quarantine and other restrictions of movement in the importing country</u>	<p>This information sheet covers post-entry quarantine (PEQ) of relevant commodities; temporal, spatial and end-use restrictions in the importing country for import of relevant commodities; prohibition of import of relevant commodities into the domestic country. 'Relevant commodities' are plants, plant parts and other materials that may carry pests, either as infection, infestation, or contamination.</p> <p>PEQ may be recommended for ASBVd host plants for planting. Nevertheless, this measure does not apply to avocado fruits.</p>	Establishment/Spread

3.6.1.2. Additional supporting measures

Potential additional supporting measures are listed in Table 6.

Table 6: Selected supporting measures (a full list is available in EFSA PLH Panel, 2018) in relation to currently unregulated hosts and pathways. Supporting measures are organisational measures or procedures supporting the choice of appropriate risk reduction options that do not directly affect pest abundance

Supporting measure (<u>Blue underline</u> = Zenodo doc, Blue = WIP)	Summary	Risk element targeted (entry/ establishment/ spread/impact)
Inspection and trapping	<p>Inspection is defined as the official visual examination of plants, plant products or other regulated articles to determine if pests are present or to determine compliance with phytosanitary regulations (ISPM 5).</p> <p>The effectiveness of sampling and subsequent inspection to detect pests may be enhanced by including trapping and luring techniques.</p> <p>Inspection can be useful, but not conclusive to identify diseased plants, as symptoms may appear several years after infection, while recovered or seedborne asymptomatic infections are also common (Section 3.1.2).</p>	Entry/establishment/spread
Laboratory testing	<p>Examination, other than visual, to determine if pests are present using official diagnostic protocols. Diagnostic protocols describe the minimum requirements for reliable diagnosis of regulated pests.</p> <p>Laboratory tests are available to detect and identify ASBVd presence in the host plants, even in the absence of symptoms (Section 3.1.5)</p>	Entry/Spread
Sampling	<p>According to ISPM 31, it is usually not feasible to inspect entire consignments, so phytosanitary inspection is performed mainly on samples obtained from a consignment. It is noted that the sampling concepts presented in this standard may also apply to other phytosanitary procedures, notably selection of units for testing.</p> <p>For inspection, testing and/or surveillance purposes the sample may be taken according to a statistically based or a non-statistical sampling methodology.</p> <p>Flower buds are preferable tissues over leaves for ASBVd diagnosis (da Graça and Mason, 1983).</p>	Entry
Phytosanitary certificate and plant passport	<p>An official paper document or its official electronic equivalent, consistent with the model certificates of the IPPC, attesting that a consignment meets phytosanitary import requirements (ISPM 5)</p> <p>a) export certificate (import) b) plant passport (EU internal trade)</p> <p>Recommended for ASBVd host plants for planting, avocado seeds and avocado fruits.</p>	Entry/Spread
Certified and approved premises	<p>Mandatory/voluntary certification/approval of premises is a process including a set of procedures and of actions implemented by producers, conditioners and traders contributing to ensure the phytosanitary compliance of consignments. It can be a part of a larger system maintained by the NPPO in order to guarantee the fulfilment of plant health requirements of plants and plant products intended for trade. Key property of certified or approved premises is the traceability of activities and tasks (and their components) inherent the pursued phytosanitary objective. Traceability aims to provide access to all trustful pieces of information that may help to prove the compliance of consignments with phytosanitary requirements of importing countries.</p>	Entry/Spread

Supporting measure (<u>Blue underline</u> = Zenodo doc, Blue = WIP)	Summary	Risk element targeted (entry/ establishment/ spread/impact)
	Certified and approved premises may reduce the likelihood of host plants for planting, avocado seeds and avocado fruits originating in those premises to be infected by ASBVd.	
<u>Certification of reproductive material (voluntary/official)</u>	Plants come from within an approved propagation scheme and are certified pest free (level of infestation) following testing; used to mitigate against pests that are included in a certification scheme. The risk of further entry and/or spread of ASBVd is reduced if host plants for planting and avocado seeds are produced under an approved certification scheme and tested free of the viroid.	Entry/spread
<u>Surveillance</u>	Surveillance to guarantee that plants and produce originate from a Pest Free Area could be an option (Bonnéry et al., 2023; Roberts et al., 2023). ASBVd has been reported to be present in the EU (Greece, Spain). Therefore, surveillance would be an efficient supporting measure to define pest-free areas or pest-free places of production as well as to prevent further spread of the pathogen.	Spread

3.6.1.3. Biological or technical factors limiting the effectiveness of measures

- Asymptomatic infection of recovered plants and of progeny seedlings of the infected seeds reduces the efficacy of inspections;
- Existence of a long asymptomatic phase of the ASBVd disease (recorded to be up to 36 months, under experimental conditions);
- Symptoms on the fruits of the sunblotch disease resemble those caused by abiotic stress or a genetic disorders;
- Low concentration in the early stage of infection and especially the uneven distribution of ASBVd within the infected trees may impair reliable detection;

3.7. Uncertainty

The Panel identified no key uncertainty potentially affecting the conclusions of this pest categorisation.

4. Conclusions

ASBVd has been reported in the EU (Greece, Spain), but with a restricted distribution. ASBVd satisfies the criteria that are within the remit of EFSA to assess for this viroid to be regarded as potential Union quarantine pest. Table 7 provides a summary of the PLH Panel conclusions.

Table 7: The Panel's conclusions on the pest categorisation criteria defined in Regulation (EU) 2016/2031 on protective measures against pests of plants (the number of the relevant sections of the pest categorisation is shown in brackets in the first column)

Criterion of pest categorisation	Panel's conclusions against criterion in Regulation (EU) 2016/2031 regarding Union quarantine pest	Key uncertainties
Identity of the pest (Section 3.1)	Yes, the identity of the pest is clearly defined. ASBVd has been shown to produce consistent symptoms and to be transmissible	None
Absence/presence of the pest in the EU (Section 3.2)	ASBVd has been reported in Greece and Spain but its presence in the EU is considered restricted	None

Criterion of pest categorisation	Panel's conclusions against criterion in Regulation (EU) 2016/2031 regarding Union quarantine pest	Key uncertainties
Pest potential for entry, establishment and spread in the EU (Section 3.4)	ASBVd is able to re-enter into the EU. The main pathways of entry is via infected plants for planting of its <i>Lauraceae</i> hosts, avocado seeds and fresh avocado fruits. If ASBVd were to further enter and establish in the EU territory, it could become established and spread	None
Potential for consequences in the EU (Section 3.5)	Further entry, establishment and spread of ASBVd may have an economic impact in areas in the EU, where avocado is grown	None
Available measures (Section 3.6)	Phytosanitary measures are available to reduce the likelihood of entry and spread of ASBVd in the EU	None
Conclusion (Section 4)	ASBVd satisfies all the criteria that are within the remit of EFSA to assess for this viroid to be regarded as potential Union quarantine pest	
Aspects of assessment to focus on/scenarios to address in future if appropriate:	Surveys to reduce the uncertainty about the distribution in the EU could be beneficial.	

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Abbreviations

ASBVd	Avocado sunblotch viroid
CABI	Centre for Agriculture and Bioscience International
EPPO	European and Mediterranean Plant Protection Organization
FAO	Food and Agriculture Organization
IPPC	International Plant Protection Convention
ISPM	International Standards for Phytosanitary Measures
MS	Member State
PEQ	Post-Entry Quarantine
PLH	EFSA Panel on Plant Health
PZ	Protected Zone
RT-PCR	Reverse Transcription Polymerase Chain Reaction
TFEU	Treaty on the Functioning of the European Union
ToR	Terms of Reference

Glossary

Containment (of a pest)	Application of phytosanitary measures in and around an infested area to prevent spread of a pest (FAO, 2022).
Control (of a pest)	Suppression, containment or eradication of a pest population (FAO, 2022).
Entry (of a pest)	Movement of a pest into an area where it is not yet present, or present but not widely distributed and being officially controlled (FAO, 2022).
Eradication (of a pest)	Application of phytosanitary measures to eliminate a pest from an area (FAO, 2022).
Establishment (of a pest)	Perpetuation, for the foreseeable future, of a pest within an area after entry (FAO, 2022).

Greenhouse	A walk-in, static, closed place of crop production with a usually translucent outer shell, which allows controlled exchange of material and energy with the surroundings and prevents release of plant protection products (PPPs) into the environment.
Hitchhiker	An organism sheltering or transported accidentally via inanimate pathways including with machinery, shipping containers and vehicles; such organisms are also known as contaminating pests or stowaways (Toy and Newfield, 2010).
Impact (of a pest)	The impact of the pest on the crop output and quality and on the environment in the occupied spatial units.
Introduction (of a pest)	The entry of a pest resulting in its establishment (FAO, 2022).
Pathway	Any means that allows the entry or spread of a pest (FAO, 2022).
Phytosanitary measures	Any legislation, regulation or official procedure having the purpose to prevent the introduction or spread of quarantine pests, or to limit the economic impact of regulated non-quarantine pests (FAO, 2022).
Quarantine pest	A pest of potential economic importance to the area endangered thereby and not yet present there, or present but not widely distributed and being officially controlled (FAO, 2022).
Risk reduction option (RRO)	A measure acting on pest introduction and/or pest spread and/or the magnitude of the biological impact of the pest should the pest be present. A RRO may become a phytosanitary measure, action or procedure according to the decision of the risk manager.
Spread (of a pest)	Expansion of the geographical distribution of a pest within an area (FAO, 2022)

Appendix A – Avocado sunblotch viroid host plants/species affected

Host status	Host name	Plant family	Common name	Reference
Cultivated hosts	<i>Persea americana</i>	Lauraceae	Avocado	EPPO (online)
Wild weed hosts	–	–	–	–
Artificial/experimental host	<i>Persea schiedeana</i> ,	Lauraceae	Coyo	CABI (2021)
	<i>Cinnamomum zeylanicum</i>	Lauraceae	Ceylon cinnamon	CABI (2021)
	<i>Cinnamomum camphora</i>	Lauraceae	Camphor laurel, camphor tree	CABI (2021)
	<i>Ocotea bullata</i>	Lauraceae	Black stinkwood, Cape walnut	CABI (2021)

Sources: EPPO Global Database (EPPO, online) and CABI (2021).

Appendix B – Distribution of *Avocado sunblotch viroid*

Distribution records based on EPPO Global Database (EPPO, online).

Region	Country	Sub-national (e.g. State)	Status	Reference	
North America	USA		Present, restricted distribution	EPPO (online)	
		California	Present, no details	EPPO (online)	
		Florida	Present, no details	EPPO (online)	
Central America	Guatemala		Present, no details	EPPO (online)	
	Mexico		Present, restricted distribution	EPPO (online)	
Africa	Ghana		Present, few occurrences	EPPO (online)	
	South Africa		Present, widespread	EPPO (online)	
South America	Peru		Present, restricted distribution	EPPO (online)	
	Venezuela		Present, no details	EPPO (online)	
Asia	Israel		Present, restricted distribution	EPPO (online)	
Europe	Greece		Present, few occurrences	EPPO (online)	
			Crete	Present, few occurrences	EPPO (online)
		Spain	Andalucía	Present, restricted distribution	Spanish NPPO
Oceania	Australia		Present, no details	EPPO (online)	