

Review

The Avocado Sunblotch Viroid: An Invisible Foe of Avocado

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Abstract: This review collects information about the history of avocado and the economically important disease, avocado sunblotch, caused by the avocado sunblotch viroid (ASBVd). Sunblotch symptoms are variable, but the most common in fruits are irregular sunken areas of white, yellow, or reddish color. On severely affected fruits, the sunken areas may become necrotic. ASBVd (type species *Avocado sunblotch viroid*, family *Avsunviroidae*) replicates and accumulates in the chloroplast, and it is the smallest plant pathogen. This pathogen is a circular single-stranded RNA of 246–251 nucleotides. ASBVd has a restricted host range and only few plant species of the family *Lauraceae* have been confirmed experimentally as additional hosts. The most reliable method to detect ASBVd in the field is to identify symptomatic fruits, complemented in the laboratory with reliable and sensitive molecular techniques to identify infected but asymptomatic trees. This pathogen is widely distributed in most avocado-producing areas and causes significant reductions in yield and fruit quality. Infected asymptomatic trees play an important role in the epidemiology of this disease, and avocado nurseries need to be certified to ensure they provide pathogen-free avocado material. Although there is no cure for infected trees, sanitation practices may have a significant impact on avoiding the spread of this pathogen.

Keywords: avocado; *Persea americana*; avocado sunblotch viroid

1. The Origin of the Avocado and the Avocado Sunblotch Viroid

Mesoamerica is considered the center of origin of the avocado, principally the highlands of México and Guatemala [1]. The oldest avocado fossils, dating back more than 8000 years ago [2], were found in the Caves of Coxcatlán, in the Tehuacán Valley of the State of Puebla, México. Human pilgrimages extended it to the South of México and North of Central America, down to Colombia, Venezuela, Ecuador, and Perú [3]. Ahuacatl (aguacate) in the Nahuatl language means “testicle”, from the shape of the fruit. Domesticated avocado seeds were found buried with Incan mummies in Perú dating back to 750 B.C., and there is evidence that avocados were cultivated in México as early as 500 B.C. [4]. The Mendoza Codex was painted by Mexican painters with the intent by México’s first virrey, Antonio de Mendoza, to send it to Carlos I, King of Spain (also Charles V of the Holy Roman Empire). This codex is a pictographic script, which in the first chapter describes life in México from 1325 (the foundation of Tenochtitlan, the great Mexica Empire Capital) to the arrival of the Spanish

invasion (1521). The second chapter lists all the towns in Mexica's domain and the tributes they should periodically deliver. The folio (page 39) lists 14 towns, the fifth of which (from the top left) is Ahuacatlan, represented by an avocado tree (Figure 1). The teeth drawing on the trunk means "place", and Ahuacatlan means "place where aguacate (avocado) grows". There are several Ahuacatlan towns in the States of México, Nayarit (Ahuacatlan), and Puebla, and in Guatemala and Colombia. The 14th month in the Mayan calendar (K'ank'in) is represented by an avocado glyph [3]. During the reign of Carlos I, the Crown of Spain (Castilla) expanded its territories over much of America, and avocado was probably spread during this expansion: Hernán Cortés conquered the Mexica Empire in 1521, which would result in the Kingdom of New Spain (Spaniards tried avocados for the first time), and Nuño de Guzmán conquered the Tarascan Empire and founded the Kingdom of Nueva Galicia. The first written document that mentions aguacate is by Francisco Fernández de Enciso, who found and tasted aguacate in Yaharo, close to Santa Marta, Colombia. He described the fruit: "it looks like an orange and the pulp is yellow, like butter with a delicious, soft and marvelous taste" [3]. Palta is another name in Spanish used in South America for aguacate. Pedro de Cieza described in 1532–1550 the presence of avocado in several countries. He mentioned aguacate as the fruit that belongs to Panama. Then, he mentioned it as palta in Colombia (Cártago, Cali, and Cauca Valley) and Ecuador. Garcilaso de la Vega in "Comentarios regios de los Incas" mentioned Tupac Inco Yupanqui advancing and conquering several towns (1450–1475), one of which was Palta, mentioning that exquisite fruit named palta. This is the approximate time when the palta tree was brought from Ecuador to Perú. In Europe, the avocado was first mentioned by Clusius, who referred to avocado trees of a Mexican type growing in Valencia, Spain. Now, avocados are cultivated and consumed on the five continents of our planet [3].

The sunblotch disease is economically important in avocado (*Persea americana* Miller) [3,5,6] and is a quarantine pest in some countries [7]. The sunblotch was observed first in Southern California in 1914 and Palestine in 1924 [8]. The first official report of sunblotch was published in 1928 in the USA, and the symptoms were attributed to physiological causes (solar irradiation) [9] or to a genetic disorder [7], and to a virus following graft-transmissibility [8,10–12]. Since virus particles were not observed by electron microscopy in sections or extracts from tissue affected by sunblotch and since low-molecular-weight RNA was isolated from these tissues, a viroid was suggested as the potential causal agent of this disease [13–15]. A viroid was indeed purified from avocado leaves infected by sunblotch disease and named avocado sunblotch viroid [16,17]. ASBVd was found to be associated with avocado sunblotch disease and was characterized as a covalently closed circular RNA molecule with a molecular size lower than the chrysanthemum stunt viroid and citrus exocortis viroid, while hybridization analysis with ³²P-labeled complementary DNA indicated that the viroid consists of a single RNA species [18]. ASBVd was confirmed in 1981 as the causal agent of the disease when healthy avocado seedlings developed symptoms of avocado sunblotch disease 2–5 months after being inoculated with bark from infected trees or 4–8 months later when inoculated with filter paper pieces moistened with the purified viroid. Infection by ASBVd was confirmed in seedlings inoculated by both methods by PAGE and cDNA probe [16]. In 1948, in a tour to México, in the Rodiles grove near Atlixco, State of Puebla, a commission of The California Avocado Society noted avocado trees with sunblotch [19]. Actually, the ASBVd origin has not been confirmed, but it is known that the avocado industry in Southern California began with trees and seeds from México and Guatemala, and that the cities of Atlixco and Queretaro, México, were two important sources of seeds [8]. This germplasm could have been infected with ASBVd. This evidence suggests that the ASBVd origin is the same as that of its natural host [7,8]. Considering the molecular nature of ASBVd and its dependence on its natural host, the avocado, we postulate that the viroid evolved together with the avocado plant a long time ago.

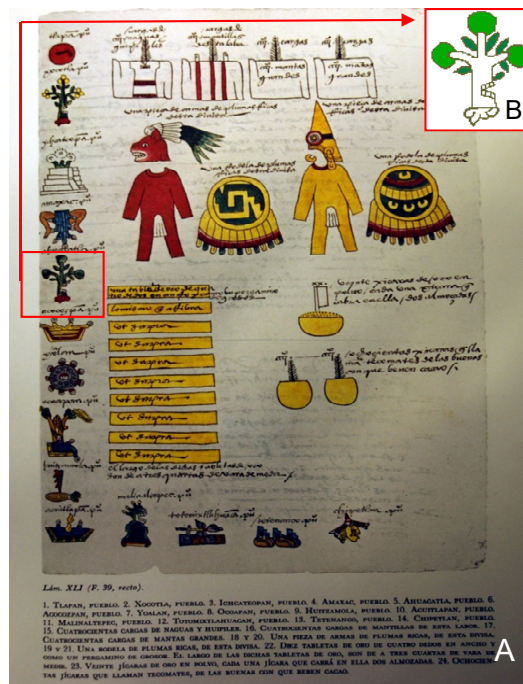


Figure 1. Pictographic script from the Mendoza Codex (A) with an illustration of Ahuacatlan town represented by an avocado tree (B) (fifth from top left). The teeth drawing on the trunk means “place”. Ahuacatlan means “place where avocados grow”.

2. Symptoms

The alterations caused by ASBVd vary and are influenced by the cultivar, environmental conditions, and the variants of the viroid that predominate in the host [20–24]. The most typical symptom on fruits is sunken crevices of white, yellow, or reddish color (Figure 2A) [25,26]. On severely affected fruits, the sunken areas may become necrotic (Figure 2F) [27,28]. The bases of some shoots and young branches of infected trees may show discolored streaks or stripes (Figure 2B). On some leaves of infected trees, distorted and variegated areas develop from the central vein that may progress and deform the entire leaf blade (Figure 2C). The bark of some trunks and old branches of some infected trees develop a cracked appearance also known as “alligator skin” (Figure 2D). The distribution of sunblotch symptoms is irregular, and infected trees may develop only one or multiple symptoms. In some cases, infected trees are fruitless and remain stunted [26,29,30]. The most reliable ASBVd symptom in México is the sunken crevices of white, yellow, or reddish color on fruits (Figure 2E). In some cases, multiple fruits with initial symptoms may occur on the same tree (Figure 2G). Remarkably, some infected trees are asymptomatic but may develop symptoms under stress conditions. Likewise, symptomatic trees may become asymptomatic for underexplored reasons. More research is needed to understand the distribution and possible variants associated with the different symptoms.



Figure 2. Symptoms of avocado sunblotch disease. Yellowish sunken areas on fruits (A); discolored and necrotic depressions on infected twigs (B); distortion and variegation on leaves (C); cracked bark (“Alligator skin”) appearance on some mature branches (D); fruits with reddish color areas (E); necrosis on severely affected fruits (F); multiple yellowish sunken areas in fruits (G).

3. Taxonomy and Structure of ASBVd

As stated by Flores et al., viroids are subviral plant pathogens at the frontier of life [31]. They are solely composed by a single-stranded circular RNA of 246–434 nt with a compact secondary structure. Some properties of viroids, particularly the presence of ribozymes in members of the family *Avsunviroidae*, suggest that they might have appeared very early in evolution and could represent “living fossils” of the precellular RNA world that presumably preceded our current world based on DNA and proteins [31–33]. Viroids replicate autonomously when inoculated into their host plants and incite, in most of them, economically important diseases. The characterized viroids are exclusive to the plant kingdom, and analysis of their structural and functional properties has grouped them into two families: *Pospiviroidae* (type species *Potato spindle tuber viroid*) and *Avsunviroidae* (type species *Avocado sunblotch viroid*) (ASBVd) [31]. ASBVd is a plant pathogen that affects avocado and other members of the *Lauraceae* [23]. ASBVd replicates and accumulates in the chloroplasts, and the single-unit nuclear-encoded polymerase (NEP) is the RNA polymerase required in ASBVd replication [34]. ASBVd also presents the smallest genomic size (246–250 nt) and an A + U content (62%) above that of any other viroid (40–47%) [35], with this characteristic suggesting a polyphyletic viroid origin [31]. Recently, Giguère et al. [36] studied the structure features of the ASBVd with a high-throughput technique of selective 2'-hydroxyl acylation analyzed by primer extension (hSHAPE) [36]. Although they did not investigate the structure of multiple variants of the ASBVd, with this approach they provided some features not elucidated before: A loop in the left and the terminal domain as well as a central loop with the conserved hammerhead nucleotides in the rod-like structure [36]. The mechanism of ASBVd replication that operates is the symmetric rolling circle, where the monomeric circular form (mc) (+) of RNA serves as a template for the synthesis of the oligomeric head-to-tail (-) RNA intermediates that self-cleavage into monomeric linear forms (ml) (-) and are subsequently circularized to act as the initial template for the second half of the cycle [37] involving the single-unit nuclear-encoded

polymerase [34]. The self-cleavage is mediated by cis-acting hammerhead ribozymes embedded in both polarity RNAs [38,39].

4. Host range of ASBVd

ASBVd belongs to the *Avsunviroidae* family and has one of the narrowest host ranges among the viroids [23,24]. Experimentally, ASBVd was transmitted first to *Cinnamomum zeylanicum* plants that expressed typical symptoms of yellow depressed streaks on the stem [40,41] and subsequently to *Persea schiedeana*, *Ocotea bullata*, and *Cinnamomum camphora* [42], all belonging to the family *Lauraceae*. Additionally, other studies showed that ASBVd could replicate in unicellular organisms from other kingdoms, such as eukaryotic cells of the yeast *Saccharomyces cerevisiae* [43] and prokaryotic cells of the cyanobacterium *Nostoc* sp. [44]. The replication of ASBVd in both unicellular organisms does not induce any phenotype, and any of these organisms represent a risk for avocado production.

5. Transmission

ASBVd can be transmitted to avocado via different routes. The use of diseased propagative material is the major route responsible for ASBVd spread [12]. Grafting is the principal and more effective infection technique to transmit the pathogen [11,12,45,46], as well as micrografting [47]. Natural and artificial root graft transmission between infected and healthy avocado trees have been reported [8,11], although the frequency of this transmission is unclear [12,23]. The ASBVd seed transmission was first presumed from observations of two parallel cases in California [8,48], and then in avocado seeds from symptomless carrier trees at a high rate (86–100%) of ASBVd transmission observed in symptomless seedlings. However, a low transmission rate (0–5.5%) was observed in seedlings generated from symptomatic trees [46]. The presence of the viroid was detected in the skin and pulp of avocado fruits with symptoms of sunblotch [49]. Pollen transmission of ASBVd was experimentally demonstrated using honeybees with a low transmission rate (1.8–3.1%), difficult to detect in infected orchards [49]. However, the spread of infection is more likely due to the reintroduction of infected materials than to natural processes, with an average annual growth rate of 2.3% to 4.7% of disease incidence [50]. Experiments using pruning knives indicated that ASBVd was not mechanically transmissible [8], but razor-slash inoculation was later able to successfully transmit ASBVd using sap extracted from infected trees [14]. It is important to consider that nurseries play an important role in the dissemination of ASBVd, and it is necessary to establish regulations for the production of ASBVd-free avocado plants. ASBVd uninfected avocado trees that are seed and scion donors should be the first step to obtain healthy plants free of ASBVd in order to protect the growing avocado industry.

6. Economic Importance

The most relevant economic impact of sunblotch disease is the effect on avocado yield. Likewise, the fruit quality and the tree growth are affected [51]. Up until today, all cultivars have been reported as susceptible to this disease. Infected trees may have a significant yield reduction when compared with healthy trees. Moreover, symptomatic fruits are of low quality, being discarded during harvesting, and the selection processes exacerbate the economic impact of this disease. Yield losses of 14% in symptomatic “Fuerte” trees and 80% in asymptomatic “Edranol” have been reported [52,53]. On the other hand, both asymptomatic “Caliente” and “Reed” trees showed yield reductions of 95% [21]. More recently, it has been reported that asymptomatic “Hass” trees have reductions of avocado yield in the range of 15–30%, while symptomatic trees are more severely affected with a reduction of 67–76%. In addition, the cost of managing this disease is very high. Removal of infected trees is expensive and, usually, the machinery is not accessible. Clearly, more information about the economic impact of this disease and its management is needed in more cultivars and in different areas. It is worth emphasizing that prevention practices are the principal way to avoid the effects of the disease, and these include the use of seeds and vegetative material free of ASBVd, the establishment of donor orchards for seed and vegetative scion production, and the continuous disinfestation of pruning, harvesting, and grafting tools [51].

7. Postharvest and Histological Effects of ASBVd

ASBVd symptomatic fruits are disqualified for human consumption during the packing process, generating important postharvest losses. Postharvest physiology studies about the effects of ASBVd in fruits indicate a reduction in ethylene and CO₂ production, which causes a delay in fruit maturation, an effect that becomes more evident for symptomatic fruits [28]. Nevertheless, the weight loss, coloration changes, fruit size, mineral content and proximate analysis (protein, carbohydrates dietary fiber, and total ash) were similar in asymptomatic and control fruits. The lipid content is affected by ASBVd, but these fruits exceed the minimum required value for the quality standards (22% of dry matter and 8% oil). Symptomatic avocado fruits still fulfill the minimum requirements to be industrialized [28,54]. The ASBVd may induce anatomical and chemical changes in the cellular structure, more evident in the symptomatic tissues, including cellular disorganization, accumulation of phenolic compounds in the cytoplasm and cell walls, and reduction of chlorophyll and cytoplasmic content that may be conducive to cell collapse (Figure 3) [27,28]. Histological observations have showed organizational and chemical changes correlated to symptom severity: The parenchyma exocarp (rind) cells walls exhibited a higher accumulation of reddish polyphenols and apparent lower chloroplast content; the mesocarp parenchyma (pulp) cells reduced their cell size, and they showed disorganization and an increase in the cell number with phenolic content. Finally, the vascular tissue presented hyperplasia, phloem cells collapsed, and xylem vessels were probably occluded with phenolic compounds (Figure 3) [27,28].

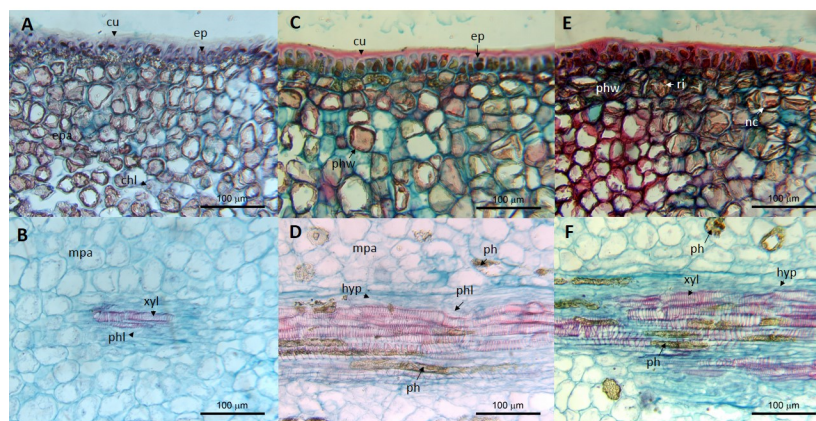


Figure 3. Cross-sectional micrographs of the exocarp and mesocarp tissues of avocado fruits infected with the avocado sunblotch viroid (ASBVd): (A,B) Asymptomatic; (C,D) yellowish sunken spot and (D,E) yellowish sunken crack. Cuticle (cu), epidermal cells (ep), exocarp parenchyma (epa), mesocarp parenchyma (mpa), chloroplasts (chl), phloem cells (phl), xylem vessels (xyl), phenol accumulation in cell wall (phw), hypertrophy (hyp), accumulation of red inclusions (ri), and necrotic cells (nc).

8. Geographic Distribution

The avocado sunblotch disease occurs in areas of the five continents where avocados are grown [26,55] (Figure 4). This disease was firstly reported in the Western Hemisphere, in California, United States, as a physiological [56] or genetic [10] disorder. In 1941, Stevens and Piper reported the sunblotch disease occurred in Florida [57]. Subsequently, ASBVd was reported in the Eastern Hemisphere in New South Wales, Australia [13,58], in Venezuela [59], in the African continent (South Africa) [52], the Middle East (Israel) [60], and in Europe (Spain) [61]. Afterwards, ASBVd was confirmed in South America (Perú) [62] and in Africa (Ghana) [63], in México [64], and more recently in Greece (Crete) [65]. Although Guatemala does not have official reports of this pathogen and Costa Rica has declared its absence, The World Trade Organization (Notification of emergency measures G/SPS/N/CRI/160, 5 May 2015) and the European Plant Protection Organization [55] have reported its presence in both Guatemala and Costa Rica. Therefore, more information is needed about the official geographical distribution of ASBVd as well as more reliable and sensitive techniques to detect the pathogen.



Figure 4. Geographical distribution of ASBVd in the five continents: America (USA, Venezuela, Perú, México), Europe (Spain, Greece), Asia (Israel), Africa (Ghana, South Africa), and Australia. 1. California 1928, 2. Florida 1939, 3. Australia 1970, 4. Venezuela 1976, 5. South Africa 1983, 6. Israel 1984, 7. Spain 1987, 8. Perú 1991, 9. Ghana 2008, 10. México 2009, 11. Greece 2018. The updated status of ASBVd in the different countries based on EPPO (2016) and Lotos et al. [65] are as follows: United States—Present, no details; Australia—Present, no details; Venezuela—Present, no details; South Africa—Present, widespread; Israel—Present, restricted distribution; Spain—Present, no details; Perú—Present, restricted distribution; Ghana—Present, few occurrences; México—Present, restricted distribution; and Greece—Present, no details. * No official reports in Guatemala or Costa Rica.

The actual status of ASBVd in México is considered as “*Present, Restricted Distribution*” [55] according to the ISPM No. 8 “*Determination of pest status in an area*” [66]. Recent reports confirmed the presence of ASBVd in the Michoacán State in orchards located in the Tingambato Municipality (four orchards) [17,28,67] and Uruapan Municipality (one orchard) [26,54,68]. Michoacán and Jalisco are the principal states which make up the geographic region where the “Hass” avocado is cultivated (avocado belt), and the area shows high entropy values (Trans-Mexican Volcanic Belt) for the establishment of avocado, so it is important to implement major measures to prevent viroid dispersion [67] (Figure 5).

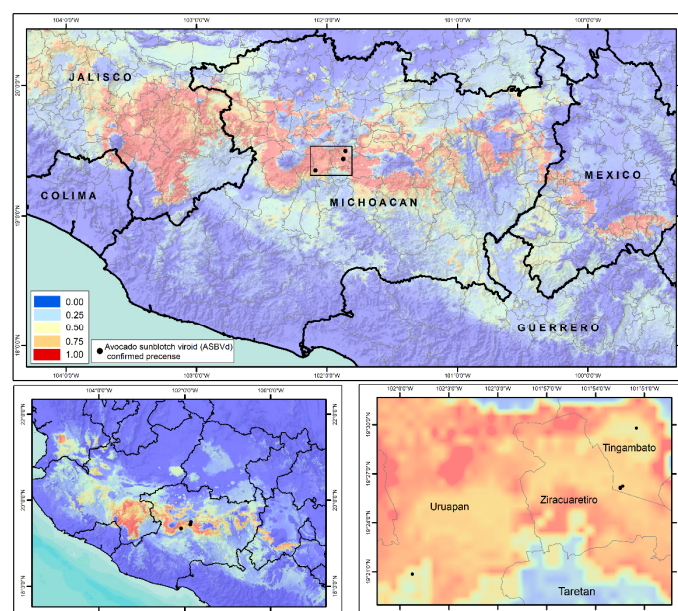


Figure 5. Geographical zones of greater adaptability according to maximum entropy modeling (Maxent) for establishing and developing avocado (*Persea americana* Miller) var. “Hass” in México [67] and location points (orchards) of the actual ASBVd distribution considering the reliable reports according to the ISPM 8 (International Standards for Phytosanitary Measures) [66].

9. Diagnostic Methods

The sunblotch disease can be detected in avocado trees by identifying the typical symptoms in fruits; however, this approach is not applicable to infected asymptomatic trees. Therefore, diagnosis based on symptoms is not reliable and other sensitive diagnostic techniques are necessary to determine the health status of an avocado tree. Once the infectious nature of sunblotch disease was demonstrated through graft transmission [11,45], successful indexing experiments were done with avocado seedlings that showed typical symptoms in a period up to three years [8,12,23,46,69,70]. Because viroids do not encode any proteins, ELISA testing is not an option for a proper diagnosis. Instead, molecular techniques are the most reliable for a correct diagnosis. With the development of protocols to obtain preparations enriched in low-molecular-weight RNAs and the characterization of ASBVd [16,18], new and faster techniques such as polyacrylamide gel electrophoresis (PAGE) were developed [33,60]. Nevertheless, this technique was not completely reliable due to inconsistent results because known positives were often missed [71]. Improved and more reliable sensitive molecular techniques such as Reverse Transcription Polymerase Chain Reaction (RT-PCR) and dot blot hybridization have been recently adapted as better techniques to diagnose ASBVd-infected avocado plants.

Molecular techniques such as dot blot hybridization with radioactively labelled complementary cDNA [72] and digoxigenin-labelled complementary RNA [37] have been developed as alternative methods to detect ASBVd. Likewise, digoxigenin- and biotin-labelled probes were also used, coupled with electron microscopy, for detecting ASBVd in the chloroplasts [73–75]. In situ hybridization using RNA complementary probes both digoxigenin- and biotin-labelled [74] is an additional tool to detect ASBVd in the chloroplast. Although these techniques of dot blot hybridization are more sensitive than PAGE, they are not completely reliable because known positives were often not detected.

RT-PCR performed in one [18] or two steps [24,76] has long been used as a routine laboratory test for ASBVd. More recently, a more sensitive method based on real-time RT-PCR was developed [71]. Non-destructive massive detection of avocado trees showing symptoms of sunblotch disease was performed using satellite spectral imaging [68] with an accuracy of 70% of actual ASBVd infection with respect to parallel analyses performed using RT-PCR [68]. Although this technique provides an additional tool to detect ASBVd-infected plants, more research in different regions is needed to corroborate these results.

10. Management of ASBVd

Avocado cultivars might have a different response against the ASBVd infection, but there are no therapeutic or curative methods to control sunblotch disease [51]. Therefore, 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 the pathogen. Asymptomatic infected trees play an important role in the epidemiology of the pathogen because they are the principal source for viroid dispersion through budding or grafting practices [51]. Additionally, neighboring trees (15 m radius) also need to be destroyed to impede root-grafting [30]. The agronomic management of avocado is the principal route for disease dispersion in commercial orchards. In contrast, the native populations of Mexican avocado (*P. americana* var. *drymifolia*) remain healthy due to the scarce manipulation or vegetative propagation [67]. Despite ASBVd being transmitted by seed, asymptomatic fruits can be exported since they are for human consumption rather than for propagation. Thus, the material used to establish new orchards needs to be obtained from certified ASBVd-free sources. Reliable methods to diagnose ASBVd from trees providing seeds and scions must be used in all nurseries [51]. Disinfestation of pruning tools and harvesting and grafting material with sodium hypochlorite (1.5%) is a simple but crucial step to maintain uninfected avocado trees and avoid the spread of an invisible foe of avocado.

11. Future perspectives

This review emphasizes the importance of ASBVd in the avocado industry. Strategies for ASBVd management need to be implemented. The use of ASBVd-free avocado plants from certified nurseries needs to be encouraged, and regulatory actions must regulate the movement of uncertified avocado material to avoid ASBVd spread.

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References

- Williams, L.O. The avocados, a synopsis of the genus *Persea*, subg. *Persea*. *Econ. Bot.* **1977**, *31*, 315–320. [CrossRef]
- Smith, C.E. Archeological evidence for selection in avocado. *Econ. Bot.* **1966**, *20*, 166–175. [CrossRef]
- Téliz, D.; Mora, G.; Morales, L. Importancia histórica y socioeconómica del aguacate. In *El Aguacate y su Manejo Integrado*; Téliz, D., Mora, A., Eds.; Mundi-Prensa: México, D.F, México, 2000; p. 231. ISBN 968-7462-15-9.
- California Avocado History. Available online: <https://www.californiaavocado.com/avocado101/the-california-difference/avocado-history> (accessed on 17 March 2019).
- Luttig, M.; Manicom, B.Q. Application of highly sensitive indexing method. *South Afr. Avocado Grow. Assoc. Yearb.* **1999**, *22*, 55–60.
- Suárez, I.E.; Litz, R.E.; Schnell, R.J.; Kuhn, D.N. El viroide de la mancha de sol (ASBVd) es persistente en cultivos nucelares de aguacate (*Persea americana* Mill.). *Rev. Colomb. Biotecnol.* **2005**, *7*, 10–18.
- Geering, A.D. A review of the status of *Avocado sunblotch viroid* in Australia. *Australas. Plant Pathol.* **2018**, *47*, 555–559. [CrossRef]
- Whitsell, R. Sunblotch disease of avocados. *Calif. Avocado Soc. Yearb.* **1952**, *37*, 215–240.
- Coit, J.E. Sunblotch of the avocado. *Calif. Avocado Soc. Yearb.* **1928**, *20*, 27–32.
- Horne, W.T. Progress in the study of certain disease of avocado. *Phytopathology* **1929**, *19*, 1144.
- Horne, W.T.; Parker, E.R.; Rounds, M.B. The nature of Sun-blotch and its practical control. *Calif. Avocado Soc. Yearb.* **1941**, *26*, 35–38.
- Wallace, J.M. The sun-blotch disease of avocados. *Calif. Avocado Soc. Yearb.* **1958**, *42*, 86–89.
- Dale, J.L.; Allen, R.N. Avocado affected by sunblotch disease contains low molecular weight ribonucleic acid. *Australas. Plant Pathol.* **1979**, *8*, 3–4. [CrossRef]
- Desjardins, P.R.; Drake, R.J.; Swiecki, S.A. Infectivity studies of avocado sunblotch disease causal agent, possibly a viroid rather than a virus. *Plant Dis.* **1980**, *64*, 313–315. [CrossRef]
- Thomas, W.; Mohamed, N.A. Avocado sunblotch—a viroid disease? *Australas. Plant Pathol.* **1979**, *8*, 1–3. [CrossRef]
- Allen, R.; Palukaitis, P.; Symons, R. Purified *Avocado sunblotch viroid* causes disease in avocado seedlings. *Australas. Plant Pathol.* **1981**, *10*, 31–32. [CrossRef]
- Lopez-Rivera, L.A.; Ramírez-Ramírez, I.; Gonzalez-Hernandez, V.A.; Cruz-Huerta, N.; Teliz Ortiz, D. Differential gene expression of avocado defense genes in response to avocado sunblotch viroid infection. *Revista Mexicana de Fitopatología* **2017**, *36*, 151–161.

18. MacKenzie, D.J.; McLean, M.A.; Mukerji, S.; Green, M. Improved RNA extraction from woody plants for the detection of viral pathogens by reverse transcription-polymerase chain reaction. *Plant Dis.* **1997**, *81*, 222–226. [[CrossRef](#)]
19. Trask, E.E. Observations on the avocado industry in México. *Calif. Avocado Soc. Yearb.* **1948**, *33*, 50–53.
20. Dale, J.L.; Symons, R.H.; Allen, R.N.; Avocado sunblotch viroid. CMI/AAB Descriptions of Plant Viruses. 1982, p. 254. Available online: <http://www.dpvweb.net/dpv/showdpv.php?dpvno=254> (accessed on 11 February 2019).
21. Desjardins, P.R.; Sasaki, P.J.; Drake, R.J. Chemical inactivation of avocado sunblotch viroid on pruning and propagation tools. *Calif. Avocado Soc. Yearb.* **1987**, *71*, 259–262.
22. Schnell, R.J.; Kuhn, D.N.; Olano, C.T.; Quintanilla, W.E. Sequence diversity among avocado sunblotch viroids isolated from single avocado trees. *Phytoparasitica* **2001**, *29*, 451–460. [[CrossRef](#)]
23. Semancik, J.S. Avocado viroids: Avocado Sunblotch viroid. In *The Viroids*; Hadidi, A., Flores, R., Randles, J.W., Semancik, J.S., Eds.; CSIRO Publishing: Melbourne, Australia, 2003; pp. 171–177.
24. Semancik, J.S.; Szychowski, J.A. Avocado sunblotch disease a persistent viroid infection in which variants are associated with differential symptoms. *J. Gen. Virol.* **1994**, *75*, 1543–1549.
25. Kuhn, D.N.; Geering, D.W.; Dixon, J. Avocado sunblotch viroid. In *Viroids and Satellites*; Hadidi, A., Flores, R., Randles, J., Palukaitis, P., Eds.; Academic Press: Cambridge, MA, USA, 2017; pp. 299–300. ISBN 9780128017029.
26. Saucedo-Carabez, J.R.; Téliz-Ortiz, D.; Ochoa-Ascencio, S.; Ochoa-Martinez, D.; Vallejo-Pérez, M.R.; Beltrán-Pena, H. Effect of *Avocado sunblotch viroid* (ASBVd) on avocado yield in Michoacan, México. *Eur. J. Plant Pathol.* **2014**, *138*, 799–805. [[CrossRef](#)]
27. Vallejo-Pérez, M.R.; Téliz-Ortiz, D.; De La Torre-Almaraz, R.; Valdovinos-Ponce, G.; Colinas-León, M.T.; Nieto-Ángel, D.; Ochoa-Martínez, D.L. Histopathology of avocado fruit infected by avocado sunblotch viroid. *J. Agric. Sci.* **2014**, *6*, 158–165. [[CrossRef](#)]
28. Vallejo-Pérez, M.R.; Téliz-Ortiz, D.; Colinas-León, M.T.; De La Torre-Almaraz, R.; Valdovinos-Ponce, G.; Nieto-Ángel, D.; Ochoa-Martínez, D.L. Alterations induced by Avocado sunblotch viroid in the postharvest physiology and quality of avocado Hass fruit. *Phytoparasitica* **2015**, *43*, 355–364. [[CrossRef](#)]
29. Ploetz, R.C.; Zentmyer, G.A.; Nishijima, R.T.; Rohrbach, K.G.; Ohr, D. *Compendium of Tropical Fruit Diseases*; APS Press: St Paul, MN, USA, 1998; p. 88.
30. Ploetz, R.C.; Dann, E.; Pegg, K.; Eskalen, A.; Ochoa, S.; Campbell, A. Pathogen exclusion: Options and implementation. In *Proceedings of the 7th World Avocado Congress*, Cairns, Queensland, Australia, 5–9 September 2011.
31. Flores, R.; Gago-Zachert, S.; Serra, P.; Sanjuán, R.; Elena, S.F. Viroids: Survivors from the RNA world? *Annu. Rev. Microbiol.* **2014**, *68*, 395–414. [[CrossRef](#)]
32. Diener, O.T. Circular RNAs: Relics of precellular evolution? *Proc. Natl. Acad. Sci. USA* **1989**, *86*, 9370–9374. [[CrossRef](#)]
33. Flores, R. A naked plant-specific RNA ten-fold smaller than the smallest known viral RNA: The viroid. *C. R. Acad. Sci. Paris Sci. de la vie/Life Sci.* **2001**, *324*, 943–952. [[CrossRef](#)]
34. Navarro, J.A.; Vera, A.; Flores, R. A chloroplastic RNA polymerase resistant to tagetitoxin is involved in replication of Avocado sunblotch viroid. *Virology* **2000**, *268*, 218–225. [[CrossRef](#)]
35. Flores, R.; Hernandez, C.; Martinez de Alba, A.E.; Daros, J.A.; di Serio, F. Viroids and viroid-host interactions. *Annu. Rev. Phytopathol.* **2005**, *43*, 117–139. [[CrossRef](#)] [[PubMed](#)]
36. Giguère, T.; Adkar-Purushothama, C.R.; Bolduc, F.; Perreault, J.P. Elucidation of the structures of all the members of the *Avsunviroidae* family. *Mol. Plant Pathol.* **2014**, *15*, 767–779. [[CrossRef](#)]
37. Daros, J.A.; Marcos, J.F.; Marcos, J.F.; Hernández, C.; Flores, R. Replication of avocado sunblotch viroid: Evidence for a symmetric pathway into two rolling circles and hammerhead ribozyme processing. *Proc. Natl. Acad. Sci. USA* **1994**, *91*, 12813–12817. [[CrossRef](#)]
38. Hutchins, C.J.; Rathjen, P.D.; Foster, A.C.; Symons, R.H. Hammerhead ribozymes structure and function in plant RNA replication. *Nucleic Acids Res.* **1986**, *14*, 3627–3640. [[CrossRef](#)]
39. López, C.A.; Flores, R. The predominant circular form of avocado sunblotch viroid accumulates *in planta* as a free RNA adopting a rod-shaped secondary structure unprotected by tightly bound host proteins. *J. Gen. Virol.* **2017**, *98*, 1913–1922.
40. Da Graca, J.V. Avocado sunblotch research in South Africa. *S. Afr. Avoc. Grow. Avocado Res. Rep.* **1978**, *2*, 53.

41. Da Graca, J.V.; Van Vuuren, S.P. Transmission of avocado sunblotch disease to cinnamon. *Plant Dis.* **1980**, *64*, 475. [[CrossRef](#)]
42. Da Graca, J.V.; Van Vuuren, S.P. Host range studies on avocado sunblotch. *S. Afr. Avocado Grow. Assoc. Yearb.* **1981**, *4*, 81–82.
43. Delan-Forino, C.; Maurel, M.C.; Torchet, C. Replication of Avocado sunblotch viroid in yeast *Saccharomyces cerevisiae*. *J. Virol.* **2011**, *85*, 3229–3238. [[CrossRef](#)]
44. Latifi, A.; Bernard, C.; da Silva, L.; Andéol, Y.; Elleuch, A.; Riouls, V.; Vergne, J.; Maurel, M.C. Replication of Avocado sunblotch viroid in the cyanobacterium *Nostoc* sp. PCC7120. *J. Plant Pathol. Microbiol.* **2016**, *7*, 4. [[CrossRef](#)]
45. Horne, W.T.; Parker, E.R. The avocado disease called sun-blotch. *Phytopathology* **1931**, *21*, 235–238.
46. Wallace, J.M.; Drake, R.J. The high rate of seed transmission of avocado sun-blotch virus from symptomless trees and the origin of such trees. *Phytopathology* **1962**, *52*, 237–241.
47. Suarez, I.E.; Schnell, R.A.; Kuhn, D.N.; Litz, R.E. Micrografting of ASBVd-infected avocado (*Persea americana*) plants. *Plant Cell Tissue Organ Cult.* **2005**, *80*, 179–185. [[CrossRef](#)]
48. Barret, C.; Rounds, M.B.; Coit, J.E.; Shepard, S.; Hazzard, A.G.; Adams, W.; Biery, E.; Griswold, H.B.; Thille, J.N.; Trask, E.E.; et al. Report of the variety committee on avocados California Avocado Society-1945. *Calif. Avocado Soc. Yearb.* **1945**, *30*, 12–18.
49. Desjardins, P.R.; Drake, R.J.; Atkins, E.L.; Bergh, B.O. Pollen transmission of avocado sunblotch virus experimentally demonstrated. *Calif. Agri.* **1979**, *33*, 14–15.
50. Pegg, K.G.; Coates, L.M.; Korsten, L.; Harding, R.M. Foliar, fruit and soilborne disease. In *The Avocado: Botany, Production and Uses*; Whiley, A.W., Schaffer, B., Wolstenholme, B.N., Eds.; CABI Publishing: Oxon, UK, 2002; pp. 299–358.
51. GIIIA (Grupo Interdisciplinario e Interinstitucional de Investigación en Aguacate. La Mancha de Sol del Aguacate. In *El Aguacate en Michoacán: Plagas y Enfermedades*; APEAM AC-SENASICA, México; López Impresores: Morelia, Michoacán, México, 2013; pp. 40–42. ISBN 978-607-715-103-6.
52. Da Graca, J.V.; Moon, T.E. Detection of avocado sunblotch viroid in flower buds by polyacrylamide gel electrophoresis. *Phytopathology. Z.* **1983**, *108*, 262–266. [[CrossRef](#)]
53. Da Graca, J.V. Sunblotch associated reduction in fruit yield in both symptomatic and symptomless carrier trees. *South Afr. Avocado Grow. Assoc. Yearb.* **1985**, *8*, 59.
54. Saucedo-Carabez, J.R.; Téliz-Ortiz, D.; Ochoa-Ascencio, S.; Ochoa-Martinez, D.; Vallejo-Pérez, M.R.; Beltrán-Peña, H. Effect of Avocado sunblotch viroid (ASBVd) on the postharvest quality of avocado fruits from México. *J. Agric. Sci.* **2015**, *7*, 85–92. [[CrossRef](#)]
55. European Plant Protection Organization (EPPO). Geographical Distribution of Avocado sunblotch viroid (ASBVd). 2016. Last Updated: 16 December 2016. Available online: <https://gd.eppo.int/taxon/ASBVd/distribution> (accessed on 14 October 2018).
56. Coit, J.E. Sunblotch of the avocado, a serious physiological disease. *Calif. Avocado Soc. Yearb.* **1928**, *12*, 26–29.
57. Stevens, H.E.; Piper, R.B. *Sunblotch in Avocado Disease in Florida*; USDA: Washington, DC, USA, 1941; pp. 40–46.
58. Trochoulías, T.; Allen, R.N. Sunblotch disease of avocado in New South Wales. *Agric. Gaz. N. S. Wales* **1970**, *81*, 67.
59. Rondón, A.; Figueroa, M. Mancha de sol (sunblotch) de los aguacates (*Persea americana*) en Venezuela. *Agron. Trop.* **1976**, *26*, 463–466.
60. Spiegel, S.; Alper, M.; Allen, R.N. Evaluation of biochemical methods for the diagnosis of the avocado sunblotch viroid in Israel. *Phytoparasitica* **1984**, *12*, 37–43. [[CrossRef](#)]
61. López-Herrera, C.; Pliego, F.; Flores, R. Detection of avocado sunblotch viroid in Spain by double polyacrylamide gel electrophoresis. *J. Phytopathol.* **1987**, *119*, 184–189. [[CrossRef](#)]
62. Vargas, C.O.; Querci, M.; Salazar, L.F. Identificación y estado de diseminación del viroide del manchado solar del palto (*Persea americana* L.) en el Perú y la existencia de otros viroides en palto. *Fitopatología* **1991**, *26*, 23–27.
63. Acheampong, A.K.; Akromah, R.; Ofori, F.A.; Takrama, J.F.; Zeidan, M. Is there Avocado sunblotch viroid in Ghana? *Afr. J. Biotechnol.* **2008**, *7*, 3540–3545.
64. De La Torre-Almaraz, R.; Téliz, O.D.; Pallás, V.; Sánchez, N.J.A. First Report of Avocado sunblotch viroid in avocado from Michoacán, México. *Plant Dis.* **2009**, *93*, 202. [[CrossRef](#)] [[PubMed](#)]

65. Lotos, L.; Kavroulakis, N.; Navarro, B.; Di Serio, F.; Olmos, A.; Ruiz, G.A.; Katis, N.I.; Maliogka, V.I. First report of Avocado sunblotch viroid (ASBVd) naturally infecting Avocado (*Persea americana*) in Greece. *Plant Dis.* **2018**, *102*, 1470. [[CrossRef](#)]
66. International Plant Protection Convention (IPPC). Determination of Pest Status in an Area. Last Updated: 29 May 2017. Available online: <https://www.ippc.int/en/publications/612/> (accessed on 13 November 2018).
67. Vallejo-Pérez, M.R.; Téliz-Ortiz, D.; De La Torre Almaraz, R.; López-Martínez, J.O.; Nieto-Ángel, D. Avocado sunblotch viroid: Pest risk and potential impact in México. *Crop Prot.* **2017**, *99*, 118–127. [[CrossRef](#)]
68. Beltrán-Peña, H.; Soria-Ruiz, J.; Téliz-Ortiz, D.; Ochoa-Martínez, D.L.; Nava-Díaz, C.; Ochoa Ascencio, S. Molecular and satellite spectral imaging detection of Avocado sunblotch viroid (ASBVd). *Rev. Fitotec. Mex.* **2014**, *37*, 21–29.
69. Moll, J.N.; Hussey, K.M.; Van Vuuren, S.P. Sunblotch indexing for plant impromevent scheme. *South Afr. Avocado Grow. Assoc. Yearb.* **1984**, *7*, 24.
70. Palukaitis, P.; Hatta, T.; Alexander, D.M.; Symons, R.H. Characterization of a viroid associated with avocado sunblotch disease. *Virology* **1979**, *99*, 145–151. [[CrossRef](#)]
71. Morey-León, G.; Ortega-Ramírez, E.; Julca-Chunga, C.; Santos-Chanta, C.; Graterol-Caldera, L.; Mialhe, E. The detection of *Avocado sunblotch viroid* in avocado using a real-time reverse transcriptase polymerase chain reaction. *BioTechnologia* **2018**, *99*, 99–107. [[CrossRef](#)]
72. Palukaitis, P.; Rakowski, A.G.; Alexander, M.; Symons, R.H. Rapid indexing of the sunblotch disease of avocados using a complementary DNA probe to avocado sunblotch viroid. *Ann. Appl. Biol.* **1981**, *98*, 439–449. [[CrossRef](#)]
73. Bonfiglioli, R.G.; McFadden, G.I.; Symons, R.H. In situ hybridization localizes avocado sunblotch viroid on chloroplast thylakoid membranes and coconut cadang cadang viroid in the nucleus. *Plant J.* **1994**, *6*, 99–103. [[CrossRef](#)]
74. Lima, M.I.; Fonseca, M.E.N.; Flores, R.; Kitajima, E.W. Detection of avocado sunblotch viroid in chloroplasts of avocado leaves by in situ hybridization. *Arch. Virol.* **1994**, *138*, 385–390. [[CrossRef](#)] [[PubMed](#)]
75. Sanchez, N.; Aparicio, F.; Rowhani, A.; Pallas, V. Comparative analysis of ELISA, nonradioactive molecular hybridization and PCR for the detection of prunus necrotic ringspot virus in herbaceous and *Prunus* hosts. *Plant Pathol.* **1998**, *47*, 780–786. [[CrossRef](#)]
76. Schnell, R.J.; Kuhn, D.N.; Ronning, C.M.; Harkins, D. Application of RT-PCR for indexing avocado sunblotch viroid. *Plant Dis.* **1997**, *81*, 1023–1026. [[CrossRef](#)] [[PubMed](#)]



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