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Avocado Sunblotch Viroid Disease and Challenges to Tackle

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Fungi, bacteria, and viruses are three major types of pathogens causing plant diseases. In addition to these, there are viroids, which are like viruses and are infectious agents, but much smaller than viruses, contain single-stranded circular RNAs that lack a protein coat. Viruses have the necessary components to highjack the biological machinery and translation system of plant host, produce proteins and make copies of themselves, spreading throughout the plant. In contrast, viroids are much smaller and do not have all the components that a virus has to infect and replicate in a plant host. The viroid has a naked strand of RNA with 246-301 nucleotides, without a protein coat or genetic materials to code proteins (Flores, et al. 2009). Due to the lack of any proteins in their system, they cannot recognize or bind to the healthy cells for entry and use passive mechanisms of transmission.

For replication, viroids are able to take advantage of the rolling-circle replication process which is used to duplicate DNA. The details of the mechanism are beyond the scope of the current article. Briefly, they use host's DNA-dependent RNA polymerase (DdRPs) enzymes to make long, attached chains of their genomes that needs to be cleaved at the correct places before they become entirely functional. The replication mechanism and cleavage methods that viroid's use, classify them into two main families: the *Pospiviroidae* which uses host's RNase III to cleave and RNA Ligase to fuse the fragments; and the *Avsunviroidae* which are self-sufficient and carry their own tool for cleavage, hammerhead ribozyme (Figure 1) (Flores, et al. 2009).

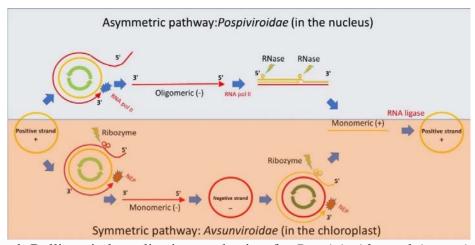


Figure 1. Rolling-circle replication mechanism for *Pospiviroidae* and *Avsunviroidae*.

Unlike other plants, avocado (*Persea americana* Mill.), is not a host for very many viral diseases. The occurrence of viral and viroid diseases in avocado is limited to two viroids: Avocado Sunblotch Viroid (ASBV), the smallest known viroid (246-251 nt) belonging to the *Avsunviroidae* and Potato Spindle Tuber Viroid (PSTV) belonging to the *Pospiviroidae*. The first, ASBV, only infects avocado and is economically very important and damaging to the avocado industry. While the latter has a wider range of plant hosts and is much more important for

vegetables and ornamentals. ASBVd symptoms have been documented in California since the early 1900's and the name of sunblotch was given to this disease because symptoms resemble those of sunburn. Despite the first description of sunblotch in early twentieth century in California (Coit, 1928), distribution and economic impact of ASBVd on California avocado industry is not well-known yet. Currently, the disease is well-distributed in most of the avocado growing regions in the world and where it impacts yield and fruit quality. The viroid is unevenly distributed in the tree and sometimes the infected tree does not show symptoms. Symptomless trees play a significant role in the epidemiology and latent spread of the pathogen. The full impact of ASBVd is difficult to estimate because of the symptomless nature of the disease; however, researchers have documented known symptomatic and asymptomatic 'Hass' and found up to 75% and 30% yield reduction, respectively (Saucedo-Carabez et al., 2014). Additionally, ASBVd symptoms may vary under the influence of different environment, avocado cultivar, and variant of the viroid. viroid is a stable molecule which can survive outside the host cell environment for almost 7 weeks (Mehle et al. 2014), ASBV has been documented to remain viable for 3 months in bee pollen taken from beehives (Roberts et al. 2023).

Symptoms. ASBVd causes symptoms on all tree parts including fruit, leaves, branches, and twigs. While symptoms on leaves e.g., discoloration and physical distortion may rarely be seen, fruit typically show depressed/sunken scars in white, yellow, and red color which are dominantly seen at the pedicle end (Dodds et al. 2001; Jordan et al. 1983). Sometimes severely infected fruit show necrotic areas at the center of the crevices (Figure 2, a-c). Contradictory reports exist regarding the impact of ASBVd on yield, some reports state that infected trees produce abundant but small fruit in size while in some, tree yield was reported to be severely reduced. Small and misshaped fruit resulting from the viroid impact fruit marketing value. As seen in Figure 2 (d-e), alligator skin/bark is another typical symptom of ASBVd which is described as rectangular cracking appearance on bark of large branches and trunk of old trees. Infected trees may look stunted and old trees grow into a low and flattened shape with branches bowing toward the ground.



Figure 2. Typical symptoms of Avocado Sunblotch Viroid in fruit and old branches. Sunken crevices on fruit in white, yellow, and red color (a-c); Alligator skin on old branches (d-e).

Transmission. Viroid is transmitted through infected budwood and seed from both symptomatic or asymptomatic trees (Eskalen and Faber, 2016). Natural root grafting is another mechanism of ASBVd transmission in groves (Whitsell, 1952). It has been shown that infective pollen can only infect the seed and not pass into the tree the fruit is attached to (Desjardins et al., 1979, 1980). Seeds from asymptomatic trees spread the disease up to 100% (Desjardins, 1987). There is no evidence of direct transmission from insects other than the assumption that honeybees might be involved in spreading the pathogen through moving infective pollen (Desjardins, et al., 1979; Eskalen and Faber, 2016). In Queensland, Australia researchers found the viroid in pollen samples taken from beehives placed in avocado orchards, but the viroid was not found in the actual honeybee, while in South Africa, the viroid was detected in both pollen and the insect (Roberts et al. 2023). As mentioned above, viroids are reliant on passive mechanical transmission such as wounds caused by contaminated grafting or pruning tools and injection equipment rather than insect vectors (Desjardins et al., 1980). More research is needed to shed light on understanding the transmission mechanisms by pollen, seed, and roots of the viroid in avocado orchards.

Control. There are no treatments to cure plants from ASBVd except preventive such as disease-free nursery materials and sanitation measures such as disinfecting tools used for pruning, grafting, and injecting trees. Additionally, any tools used in the nursery should be disinfected before use on the next plant. Removing infected plants and roots is the best course of field. Removing adjacent trees, although expensive, is necessary since adjacent trees may be infected. Nurseries need to ensure that seed for rootstocks or as nurse-seeds for clonal rootstocks as well as budwood is free of ASBVd.

Future Explorations. The pace of the global avocado production is remarkedly high compared to other tropical fruits. Such increase in production is driven by high consumer demand and requires expansion of the industry and may involve the exchange of plant materials. To meet this goal, we first need to optimize reliable, sensitive, fast, and, if possible, affordable techniques to detect the pathogen. However, detection is challenging due to the uneven and irregular distribution of the viroid in avocado trees as well as the presence of asymptomatic trees.

Another interesting and challenging point that will require the attention of researchers is that some symptomatic trees spontaneously become asymptomatic and vice versa. It is assumed that stress plays a part in symptomology, but more research is needed to determine this.

Observations show that all avocado cultivars are susceptible to ASBVd but more information about the susceptibility of rootstocks and their roles in root-to-root transmission or combination of rootstock-scions is needed to be explored. Our next steps on the status of this disease will be focused on understanding the distribution and economic impacts, developing fast and reliable detection techniques with ability to detect the pathogen in any infected part of the tree, and the possible variants associated with the different symptoms. Lastly, it would be ideal if we could develop methods to control the disease.

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Citrus yellow vein clearing virus, a new citrus pathogen identified in the San Joaquin Valley of California

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The California Department of Food and Agriculture (CDFA) regularly performs surveys across the San Joaquin Valley (SJV) to monitor for potential insect and pathogen issues for California agricultural producers. In October of 2021, CDFA inspectors began performing a multi pest survey for citrus in addition to the routine inspections for the Asian citrus psyllid and Huanglongbing in residential properties. In March of 2022, a surveyor came across some unique leaf symptoms on a residential lemon tree in the city of Tulare, CA (Figure 1). Samples from the initial find in Tulare County were sent to CDFA's Plant Pest Diagnostic Center in Sacramento, California (https://www.cdfa.ca.gov/plant/PPD/) and tested positive for the citrus yellow vein clearing virus (CYVCV), a putative new member of the genus *Mandarivirus* associated with the yellow vein clearing disease of citrus. This result was subsequently confirmed by the United States Department of Agriculture, Plant Pathogen Confirmatory Diagnostics Laboratory (https://www.aphis.usda.gov/aphis/ourfocus/planthealth/ppq-program-overview/science-technology/plant-pathogen-confirmatory-diagnostics-laboratory).

After consulting with the United States Department of Agriculture regarding sampling strategies, delimitation surveys are continuing to determine the extent and distribution of CYVCV in California. Once a positive tree has been identified, CDFA returns to the property and surveys all citrus trees within a 1-mile delimitation radius around the detection, additionally CDFA conducts delimitation surveys in arcs around the delimitation core. Around the initial

detection core in the city of Tulare, CDFA has already conducted 7-mile and 6-mile arcs and around the new core CDFA is conducting a 4-mile arc delimitation survey. All samples are sent to CDFA's Plant Pest Diagnostic Center for molecular testing. A total of 578 trees have tested positive so far for CYVCV in the city of Tulare and recently two trees tested positive in the city of Visalia, but no findings in commercial citrus groves in the SJV have been reported thus far.

Symptoms of CYVCV disease include vein clearing when viewed from the top of the leaves (Fig. 1) and water soaking when viewed from the bottom of the leaves (Fig. 2). Symptomatic leaves may also be curly or have some crinkling. Researchers from other parts of the world have reported that the virus is vectored by the citrus white fly (*Dialeurodes citris*) and several aphid species (*Aphis spiraecola*, *A. craccivora*, and *A. gossypii*.), all of which occur in California. Currently scientists are performing experiments to determine vector transmission under California conditions. There have also been single reports of virus detection on some weed species and CYVCV can also spread by grafting techniques as well as by pruning tools.

The disease was first identified in Pakistan in 1988 on lemon and sour orange trees. The virus disease was then found in India on 'Etrog' citron, 'Rangpur' lime, sour orange, and lemon trees in India in 2003. The disease was subsequently found in Turkey, Iran, and China. Lemons and sour orange seem to be the most susceptible types to this disease, but most citrus species, varieties, and hybrids tested thus far developed the disease with varying symptomatology. Finally, there has also been one report of CYVCV infecting wild grapes in Turkey in 2020. In the study from Turkey, the infected wild grapevine was climbing on an infected citrus tree. There was no evidence of serious impacts on the wild grapevine.

It is also important to note here that the citrus yellow vein <u>clearing</u> virus (CYV<u>C</u>V) is not related to and should not be confused with the citrus yellow-vein <u>associated</u> virus-like RNA (CYV<u>a</u>V). CYV<u>a</u>V was recently identified to be associated with the citrus yellow-vein disease reported once in California in the 1950s in a few limequat trees, but since then, it has not been reported again in California.

The report of CYVCV from the city of Tulare, California was the first account of this virus in North America, and the impacts of this disease on the citrus economy are not clearly known at this time. Researchers in China have reported up to 80% loss in production in some lemon groves but the losses are usually less in most instances. CDFA continues with delimitation surveys around the positive finds to define the extend of the infestation and if any commercial citrus operations, groves or nurseries, are infected or are at risk. For updates on this and other citrus pests in California visit 'Citrus Insider' at https://citrusinsider.org/.

Further readings:

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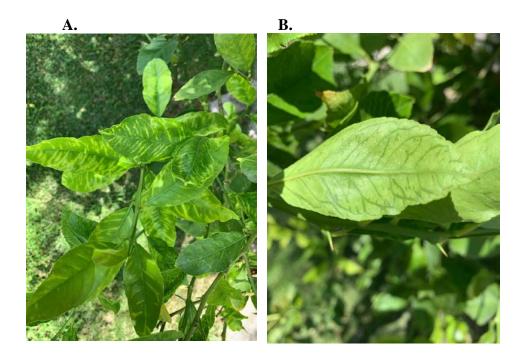


Figure 1. (A) An example of CYVCV symptoms when looking at the top of the leaves showing vein clearing and leave curling/crinkling. (B) An example of CYVCV symptoms when looking at the bottom of the leave showing water soaking symptoms on the veins. Photo courtesy of CDFA.

A pictorial guide to rind scarring damage on mandarins and sweet orange published!

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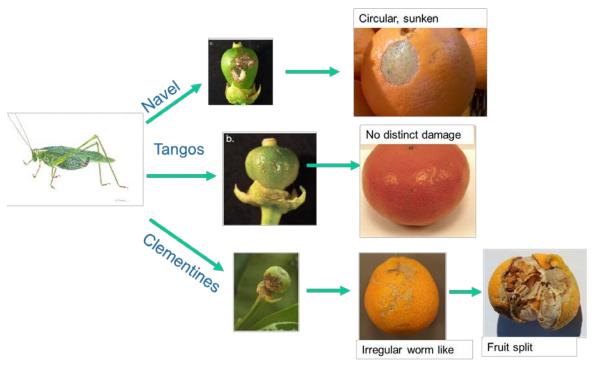
Did you know rind scarring damage caused by the same pest could look different in mandarins and sweet oranges? A NEW extension article <u>UCANR publication 8708</u>, brings you a pictorial guide to help differentiate between the damages caused by the same pest on mandarin species and sweet oranges.

Early-season insect pests, such as katydids, earwigs, and citrus thrips feeding on newly developing fruit can cause rind scarring damage. Resulting scar damages can lead to the downgrading of fruit in packinghouses causing huge economic losses for growers. Highly valued for the fresh citrus fruit (unblemished), managing these surface-feeding pests and minimizing their damage is vital to California citrus growers. Several resources, such as "Photographic Guide to Citrus Fruit Scarring" UCANR publication 8090, published in 2003 exist to help identify various types of damages. But previous work was mainly based on work with sweet oranges.

With the increasing acreage of mandarins in California, the need for identifying early season rind scarring damage caused by several pests was recognized. Dr. Bodil Cass led a group of researchers from UC Davis in evaluating grower data and conducting experiments at the Lindcove Research and Extension Center. The results of this research are now published as a photographic guide that provides information on how three early-season pests cause damage to mandarin species compared to sweet orange.

What are the main findings?

- Damages caused by early-season pests are different in tango mandarin and clementine mandarin.
- Katydids and earwigs do not cause feeding damage on tango or Afourer mandarins.
- Katydid damage on clementine looks like worm damage on sweet orange. It can cause maturing fruit to split and then drop.



Pictorial summary showing the different types of damages caused by katydid feeding on young and mature fruit.

Insects in Common to Avocado and Introduced Habitat Gardens

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Habitat gardens were installed in three avocado orchards in the Santa Barbara / Ventura region of Southern California to evaluate differences in native bee/insect diversity and abundance between habitat gardens (treatment sites) and surrounding orchards and wildlands (control sites). Native bees, honeybees, wasps and flies were monitored at each orchard over 6 years from 2014-2019, with supplemental collections done in 2020-2021. Habitat garden sites had greater bee species diversity, and slightly greater bee abundance. At the three orchards, 105 bee taxa were recorded from spring standardized monitoring sessions during avocado flowering season (March-May).

Honeybees were found to visit many garden plants, and growers indicated the importance of having floral resources year-round for the bees. Syrphid flies were significant visitors to both garden plants and avocados. Lisbon lemons are part of the avocado orchard landscape, as they are often grown in close proximity, and control of pest problems, especially the Asian Citrus Psyllid, were factored into variables impacting avocado visitors. At least 60 non-Apis insect taxa were recorded visiting avocado flowers. Factors impacting long-term bee monitoring results

included unusual weather conditions, wildfires, and possible pesticide drift. You can read more about these gardens at:

https://ucanr.edu/blogs/blogcore/postdetail.cfm?postnum=41435&sharing=yes



Composition of Avocado Pollinators

Wasps
11.6%
Bees
30 species
7 species

Beetles
20 species
3 species

The proportion of avocado visitors near the habitat gardens

Topics in Subtropics

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