Sensitive Detection and Characterization of Viroid, Virus, and Virus-like Agents of Avocado

Continuing Project: Year 3 of 5

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Benefit to the Industry

Avocado sunblotch viroid (ASBVd) causes fruit to develop discolorations and physical distortions that make them unmarketable. Our research has resulted in a rapid and cost effective screening test for the presence of ASBVd. It has been used to test for ASBVd in symptomatic or suspect field trees, and in research trees at South Coast Field Station (SCFS) and UCR, including existing and new rootstock and scion varieties. This testing has already helped prevent the distribution of infected sources of plant material and should continue to be of value for this purpose. It has also guided crop management decisions leading to the removal of infected trees from the field. Identification of symptomless carrier trees that are infected with ASBVd, but do not exhibit obvious symptoms is a research priority for us. These trees represent potential threats to the industry through the distribution of infected seed, pollen, and budwood. The more information we gain about how these strains of ASBVd differ from symptomatic strains and how they interact with specific avocado varieties will help in the overall effort to eliminate ASBVd in the avocado industry.

Objectives

A. Continue to improve the RT-PCR test for ASBVd and develop methods to characterize strains.
B. Study symptomless carrier trees and determine rate of transmission of ASBVd to fruit/seed.
C. Determine the incidence of dsRNAs in the Avocado Foundation Block at UCR and evaluate any correlation with variety.

Summary

A. Continue to improve the RT-PCR test for ASBVd and develop methods to characterize strains.

In the past 12 months we have tested 176 avocado trees for the presence of ASBVd. Seventeen trees (9.7%) were positive for the pathogen. This is a slightly higher incidence of infection than found in previous years (7%). Most of this increase in detection however can probably be attributed to sampling procedures that use "educated guesses" for the selection of trees that are tested and not just random collection. Centering collections around trees previously identified as positive has resulted in more infected trees being found and removed. Pooling of samples has been implemented and is useful for screening large numbers of young seedlings at the same time. When the incidence of infection is low, an 80% savings in testing costs can be realized through the use of sample pooling. Pooling is not possible using large field trees due to the size of the canopy and the ability of the pathogen to be sectored into individual branches making it possible to miss the infected areas of the tree during sample collection. One commercial grower and 3 UCR researchers provided samples for testing in the last year.

After testing by RT-PCR, the positive samples can be characterized by a gel-based method called single-stranded conformational polymorphisms (SSCP) to look for probable genetic differences between strains or isolates of ASBVd. We have found small variations between isolates of ASBVd (Figure 1A). These variations can be grouped by geographic source of the trees for the most part, however different patterns can also be found in the same grove. Eleven different VC239 trees and a single VC 49 tree were found to be PCR positive and appear to contain the same strain of ASBVd (Figure 1B). These 12 trees from the Volcani Center in Israel were non-symptomatic and would not have been detected as ASBVd positive without screening by RT-PCR. These results demonstrate the value of this research program to the avocado industry both in California and in Israel

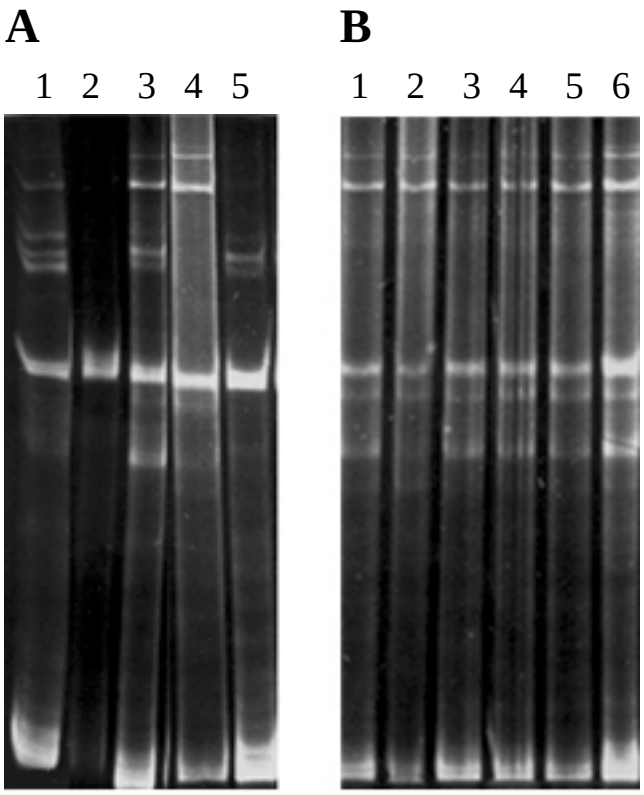

Figure 1. Characterization of ASBVd isolates by SSCP analysis of RT-PCR products. Panel A shows samples from: UCR (lane 1); Riverside (lane 2); San Diego Co. (lane 3); Israel (lane 4); and Ventura (lane 5). Panel B shows 6 representative samples from Israel from either variety VC 239 (lanes 1-5) or VC49 (lane 6).

B. Study symptomless carrier trees and determine rate of transmission of ASBVd to fruit/seed.

Four symptomless carrier trees were identified and all contained high levels of ASBVd, but showed no obvious symptoms which is typical for this class of trees. Another characteristic of these trees is that they set low levels of fruit. Ten fruit were collected from each tree (varieties Fuerte, Hass, Todd, and Topa Topa), along with an additional 10 fruit from the Fuerte tree with the small "cuke" morphology, which are seedless. Five fruit were also collected from a healthy Reed tree (foliage is negative for ASBVd after multiple tests) which is adjacent to the symptomless carrier trees, as a control. Samples of the flesh and skin from each fruit were tested for ASBVd by RT-PCR and 100% (50/50) of those from the 4 symptomless carrier trees were positive (Table 1).

Table 1. Summary of ASBVd transmission to fruit from symptomless carrier trees

Variety	# positive fruit/total # fruit	% fruit infected	% infected seedlings grown out from seed
Todd	10/10	100%	Not tested
Hass	10/10	100%	Not tested
Topa-Topa	10/10	100%	100%
Fuerte	20/20	100%	Not tested

Several of the 5 Reed fruit tested as ASBVd positive. Since this tree is immediately adjacent to the 4 symptomless carrier trees and some of its branches intermingle with and touch the Fuerte tree (ASBVd positive), these fruit probably became infected by pollen transmission from one or more of the other infected trees. This new result suggests that pollen from symptomless carrier trees may be able to cause infection of fruit on otherwise healthy trees. We are anxious to follow up on this result and test more of the Reed fruit to obtain a more statistically significant rate of transmission since only 5 were tested here.

Approximately 2 years ago several seeds from the symptomless carrier Topa-Topa tree (same as above) were germinated and grown in the greenhouse (courtesy Dr. John Menge's laboratory). Seven seedlings made it to maturity and we tested them by RT-PCR to determine the rate of seed transmission of ASBVd from symptomless carriers, which has previously been reported to be high (up to 100%) as compared to low transmission for symptomatically infected trees. All 7 of the seedlings were positive (100%, Table 1). We are waiting for the current crop of fruit to mature before collecting seeds for additional testing of all 4 varieties.

RT-PCR products from the 4 symptomless carrier trees were analyzed by SSCP to look for obvious differences between the ASBVd strains they harbor. There were slight differences in the pattern of the Fuerte isolate, but all were quite similar (Figure 2). These strains are being cloned and direct sequence comparisons will be made to help determine why these strains do not cause symptoms in these particular trees. It is possible that the symptomless nature of the infections has more to do with the host plant than with the strain of viroid.

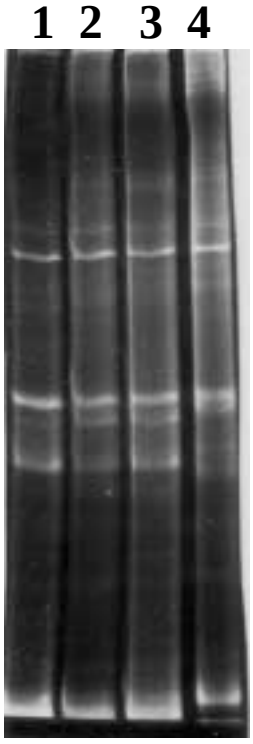

Figure 2. Comparison of SSCP patterns from 4 symptomless carrier trees. Tree varieties are: Todd (lane 1); Hass (lane 2); Topa-Topa (lane 3); and Fuerte (lane 4). Note slight differences in Fuerte pattern.

C. Determine the incidence of dsRNAs in the Avocado Foundation Block at UCR and evaluate any correlation with variety.

The Avocado Foundation Block at UCR contains 55 trees of diverse varieties and rootstock/scion combinations. Most avocado trees are known to contain double-stranded RNA segments representing 1 or more of 3 characteristic patterns. Examples of these patterns are shown in Figure 3 from four trees tested in the past 12 months. Note that one tree contains all three patterns.

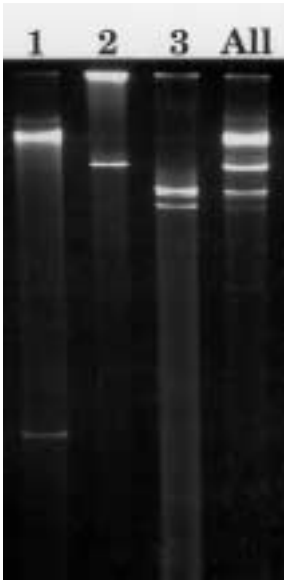

Figure 3. Double-stranded RNAs extracted from 4 different avocado trees in the Foundation Block at UC Riverside. Patterns shown are: Type 1 alone (lane 1); type 2 alone (lane 2); type 3 alone (lane 3); and types 1, 2, and 3 in a mixed infection.

Eighty-five percent of the trees in the Foundation Block contained one or more of these patterns (Table 2). These trees were tested previously in 1983 and the results for intact trees are identical except for one tree which is now being retested to confirm the current result. Several of the trees have been topworked and have undergone a shift in dsRNA pattern due to the new variety, which is an expected result. There was no obvious correlation between variety and dsRNA pattern. Large amounts of dsRNA from individual trees with each of the dsRNA types (type 1, 2, or 3 alone, no mixed infections) have been purified and cloned. Sequence data on the individual clones will be ready in the next few months. Comparisons will be made with known virus and plant sequence to try and determine the origin of these dsRNA molecules.

Table 2. Types of dsRNA patterns found in the 55 trees within the Foundation Block at UC Riverside. Basic patterns can be seen in Figure 3

DsRNA pattern	# of trees	% of total
0	8	14.5
1	2	3.6
2	23	42
3	4	7
1+2	2	3.6
1+3	4	7
2+3	6	11
All 3	6	11