California Avocado Society 1997 Yearbook 81: 91-96

DETECTION OF AVOCADO SUNBLOTCH VIROID BY POLYMERASE CHAIN REACTION (PCR)

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Abstract

Several avocado trees were identified which had been previously biologically indexed as either negative or positive for avocado sunblotch viroid (ASBVd). Each was then tested by RT-PCR for the presence of ASBVd. Of the 52 biologically indexed negative trees, all were subsequently scored as negative for ASBVd by PCR. All positive controls were scored as ASBVd positive by PCR. When testing multiple samples from single trees, all replications were scored as ASBVd positive with one exception, which was scored as a weak positive with further analysis. Two ASBVd positive field trees were identified from approximately 40 that were tested from South Coast Field Station. We have made improvements to the existing PCR test such that extraction time and handling are greatly reduced.

INTRODUCTION

Avocado sunblotch viroid (ASBVd) is a small RNA-based pathogen which causes sunken yellow or red areas on the fruit, stem discoloration, and bleaching, variegation, and distortion on the leaves of avocado ***(Figure*** I)[1,2,3]. It is transmitted by grafting of infected budwood, pollen, and seed; but it has no known vector. The incidence of ASBVd in commercial trees is low at this time due to a successful certification program of budwood that was performed in the past. ASBVd was traditionally tested for by performing multiple graft inoculations (up to 40 grafts per tree), and observing for 1-4 years for the development of characteristic symptoms. Due to retirement of key personnel, coupled with the requirements for greenhouse space and maintenance, etc., the ability to biologically index avocado trees for the presence of ASBVd no longer exists at UCR. We were approached last year to develop the polymerase chain reaction (PCR) for the detection of ASBVd. PCR is a highly sensitive molecular assay which is relatively quick (days) and inexpensive compared to biological indexing, as well as potentially more accurate. We report here the use of PCR to screen avocado trees for the presence of ASBVd in commercial and research trees both from the field and the greenhouse.

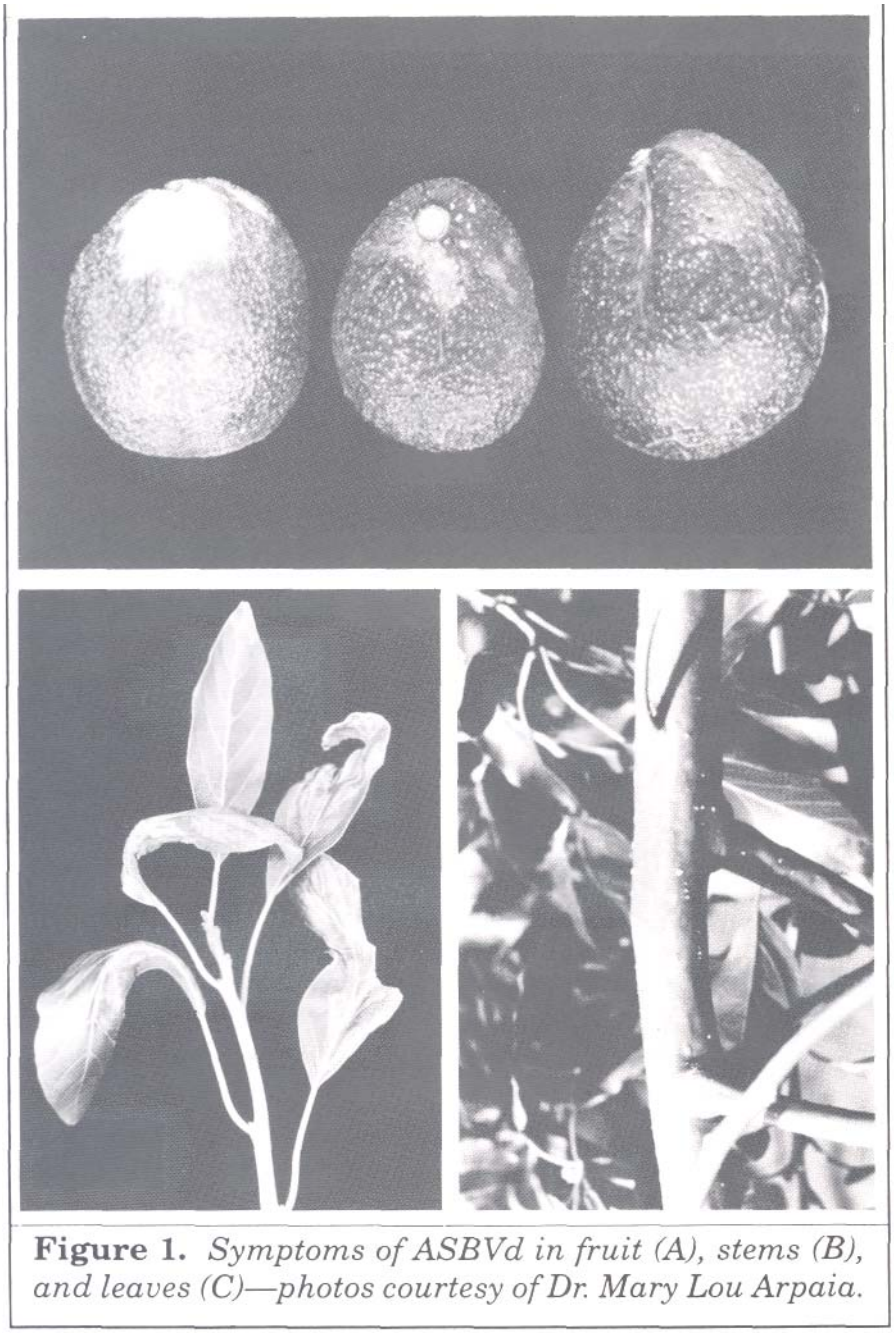

Figure 1. *Symptoms of ASBVd in fruit (A), stems (B), and leaves (C)—photos courtesy of Dr. Mary Lou Arpaia.*

MATERIALS AND METHODS

Sample collection. For positive and negative controls, three leaves were collected from greenhouse grown seedlings. For the registration block trees at UCR and the South Coast Field Station (SCFS) trees, five leaves were collected from each of the four compass points. Leaves were pooled into two samples; *e.g.,* N plus E (10 leaves) and S plus W (10 leaves), and each pool was tested independently. Five samples of five leaves each were collected from each of four trees previously biologically indexed as

ASBVd positive/symptomless carriers and were processed such that each tree had five replications performed on it.

Sample processing and PCR analysis. Leaves from each tree were stacked, and a razor blade was used to remove a slice of tissue measuring approximately 4 cm x 1 cm, and weighing 1 g. Tissue was ground in liquid nitrogen, buffer was added, and an aliquot was applied to a small filter. The filter was washed and dried, then placed in a sterile microfuge tube. Forty-five microliters of sterile water were added to the tube, and the sample was boiled for three minutes. Fifty-five microliters of an RT-PCR cocktail containing ASBVd specific primers were added, and the following reverse transcription and PCR cycles were performed: 45 min/42°C, 2 min/94°C, 40 cycles of 1 min/94° C, 2 min/55°C, 3 min/72°C, and one cycle of 5 min/72°C. Ten microliters of PCR product was analyzed by 2% agarose gel electrophoresis, and in some cases additionally analyzed by dot-spot hybridization with an ASBVd-specific oligonucleotide probe.

RESULTS AND DISCUSSION

Avocado trees in the UCR registration block which were indexed several years ago as negative for ASBVd were tested by the PCR method in the summer of 1997. All 52 trees tested negative for ASBVd, while all infected control plants produced a strong positive result (data not shown). This establishes that the incidence of false positives, a common criticism of PCR for use in field testing, is not a valid concern as long as due care is taken in processing the samples.

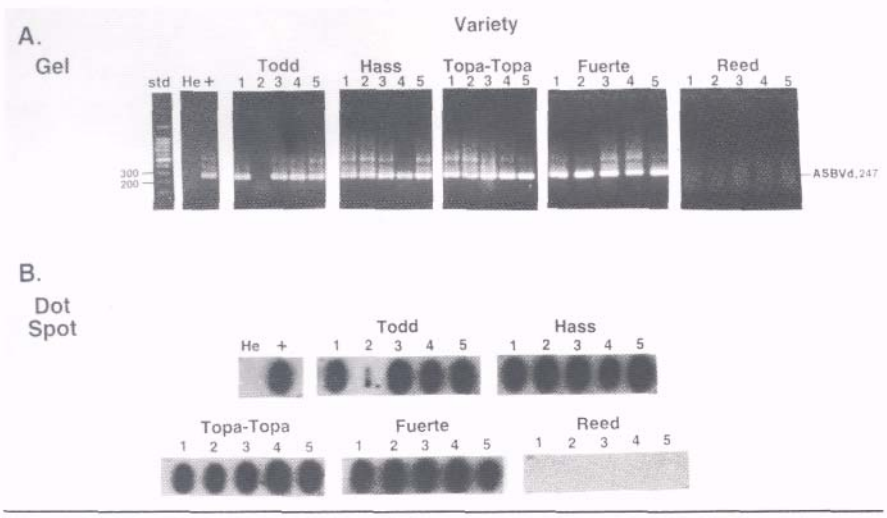

Figure 2. *PCR analysis of avocado trees. Panel A: Agarose gel electrophoresis of PCR products. A positive result is scored by the presence of a 247 bp DNA fragment. Panel B: Dot spot hybridization of PCR products with an ASBVd–specific radioactive probe. Samples for both assays included previously biologically indexed healthy (He) and ASBVd positive trees (+) used as controls, five replications from single trees of 'Todd', 'Hass', 'Topa–Topa', and 'Fuerte' varieties previously biologically indexed as ASBVd positive, symptomless carriers, and five replications from a 'Reed' avocado tree adjacent to the other four trees.*

Four trees at UCR which were previously indexed as ASBVd positive, but which do not exhibit any symptoms (so-called "symptomless carriers") were also analyzed by PCR in replicates of five tests per tree to check for consistency of the method.

One additional tree adjacent to these four, but which was not a symptomless carrier, was also included in the test as a negative control tree. PCR results from three of the four trees scored all five replicates as strong ASBVd positives visualized as a 247 base pair DNA fragment ***(Figure 2, Panel A, 'Hass', 'Topa-Topa', and 'Fuerte'),*** while for the fourth tree, only four out of five tests were scored as positive when analyzed by gel electrophoresis, the standard PCR assay method ***(Figure 2, Panel A, 'Todd').*** However, when the samples were analyzed by dot-spot hybridization of the PCR products, a more sensitive but time consuming assay, the fifth sample was scored as a weak positive ***(Figure 2, Panel B, 'Todd' #2).*** All five of the tests performed on the adjacent field tree were scored as negative for ASBVd ***(Figure 2, Panels A and B, 'Reed').*** This research, along with other results we have obtained, points out that collection and sampling of tissue is quite important, and that a positive result is not guaranteed even when the tree is known to be infected. We currently recommend the collection of 20 leaves per tree, 5 from each quadrant of the tree facing N, S, E, & W, each from separate branches or twigs, which are then pooled into two samples such

that each tree has two replications performed on it. This gives the greatest chance of harvesting at least one leaf that contains the viroid, in case the tree is not uniformly infected. Research is planned to test the sensitivity levels of PCR and to determine at what level leaves can be pooled into larger lots; *e,g.,* can one infected leaf be detected in the presence of 20, 50, or 100 non-infected leaves? For smaller nursery trees, sample collection is also often an issue because of the small number of leaves available and because the viroid may not have had a sufficient period of time to accumulate throughout the tree even if symptoms are present.

Several field trees have been tested from South Coast Field Station (courtesy of Dr. Mary Lu Arpaia) and two ASBVd positive trees were detected (data not shown).

Using previously published methods[4] with modifications developed in our laboratory, we have produced an assay which enables indexing of materials within several days of sample collection. The PCR test costs approximately $25.00 per tree, which includes two replications of each sample, the minimum we recommend at this time for confident diagnosis. The short time period now required for ASBVd diagnosis by PCR, along with the relatively low cost of the assay, will allow avocado growers and researchers to identify which trees harbor the viroid and eliminate them from cultivation. Our results have been reviewed by CDFA, and approval of the use of PCR for ASBVd detection is pending.

We would like to note that while the PCR test is quite promising and trees that test positive are most definitely infected with ASBVd, trees that test negative could still harbor an undetectable, low titre of the viroid. This of course is the problem with any screening assay, that limits of detection always are at the mercy of current technology. We believe, however, that the PCR test is a practical and valid method and is available now for the detection of ASBVd. Our laboratory is willing to test for ASBVd in nursery or field trees. For details and cost, call Dr. Deb Mathews or Jim Heick at (909) 787-3864.

We wish to acknowledge the financial support from Mr. Hank Brokaw for funds received to conduct the initial experiments in this project.

LITERATURE CITED

1. **Dale, J. L., R. H. Symons, and R. N. Allen.** 1982. Avocado sunblotch viroid. CMI/AAB. Descriptions of Plant Viruses, No. 254.
2. **Desjardins, P. R.** 1987. Avocado sunblotch. In: The Viroids, pp 299-313. Edited by T. O. Diener. New York: Plenum Press.
3. **Home, W. T., and E. R. Parker.** 1931. The avocado disease called sunblotch. Phytopathology 21: 235-238.
4. **Schnell, R. J., D. N. Kuhn, C. M. Ronning, and D. Harkins.** 1997. Applications of RT-PCR for indexing avocado sunblotch viroid. Plant Disease 81: 1023-1026.