South African Avocado Growers' Association Yearbook 1996. 19:68-69

Simplification and Improved Sensitivity of Avocado Sunblotch Viroid Detection

B.Q. Manicom M. Luttig
Institute for Tropical and Subtropical Crops, Private Bag XI1208, Nelspruit 1200

ABSTRACT

Indexing for avocado sunblotch viroid in South Africa has passed through the stages of biological indexing, polyacrylamide gel electrophoresis, radio-labelled oligonucleotide probes and DNA hybridization with DIG labelled probes. The latter test was commercialized and could detect sunblotch viroid when 1 infected leaf is present in a sample of 10 leaves. This test has been changed to an RNA hybridization and the process modified to achieve a sensitivity of 1 infected leaf in 1 000 leaves. PCR methods are being investigated.

INTRODUCTION

The symptoms, and negative effects, of avocado sunblotch viroid (ASBV) infection have been sufficiently discussed in this forum and in a large number of publications, and do not require repetition. The purpose of this paper is to report on improvements in the commercial indexing of ASBV.

Indexing for the disease goes back to the 1950s (Wallace, 1958; Burns *et al.,* 1968; 1969), before the causal organism was known. Buds or bark strips were grafted onto at least ten 3-5 month old, viroid-free seedlings of a sensitive variety such as Collinson. Seedlings were cut back regularly and in 12-18 months symptoms developed. Freedom from symptoms for 18 months to two years was regarded as a negative indexing result.

By 1980 the viroid nature of ASBV, which had been suspected for some years, was accepted (Palukaitis *et al.,* 1979; Mohamed & Thomas, 1980). A critical technique in this work was the use of polyacrylamide gel electrophoresis (PAGE), which naturally became the indexing method used. Variants of the technique were elaborated by various authors (Utermohlen, 1981; Da Graca & Mason, 1983; Lopez-Herrera *et al.,* 1987). For a time this was used as an indexing method, originally with toluidine blue staining, later with silver staining, which considerably boosted the sensitivity, but it often missed identifying known positives and never truly went commercial.

As early as 1979, molecular techniques were used in ASBV investigations (Palukaitis *et al.,* 1979), and in 1981 cDNA hybridization methods were suggested as a rapid indexing method (Palukaitis *et al.,* 1981; Allen & Dale, 1981). This became used in Australia on a semi-commercial basis. In South Africa, the technique of Bar-Joseph was introduced in the mid-80s (Bar-Joseph *et al.,* 1986). This comprised the use of oligonucleotides which specifically hybridized with ASBV. For a while the test was used commercially (Korsten

et al., 1986), but only 47-55 % of known positives were detected (Bar-Joseph *et al.,* 1986).

All the molecular methods mentioned required the use of radioactive labels. These are expensive, short lived, and decidedly eco-unfriendly for anyone within range. In the 90s we became interested in this problem and started looking into the possibility of providing a commercial service for sunblotch indexing.

MATERIALS AND RESULTS

After a study of the literature it was decided that DNA hybridization offered the best option. Boehringer had by this time marketed a kit based on the antibody detection of a digoxigenin label (DIG) as a substitute for radioactive labelling. A dimeric clone of ASBV was obtained, the dimer excised and DIGS labelled and used as a probe in a DNA-RNA dot-blot hybridization test. Using 4 g of leaf tissue in a 3-day process, and after some fine tuning of the system, we were able to detect sunblotch viroid where 1 infected leaf was present in a sample of 10 leaves. Once we were able to detect 100 % of known positive trees in blind samples (thanks to Westfalia for sending coded samples), the test was commercialized.

Funding was obtained from the South African Avocado Growers' Association to improve the test and we used a 'kaizen' approach in which every step of the process was analysed to see if it could be improved upon.

The first major change was in the extraction buffer. Avocado tissue is particularly difficult in that it has a high level of phenols and phenol oxidases which tend to form brown glue when the tissue is processed. Some 15 viroid extraction buffers to be found in the literature were tested, and finally that of Laulhere & Rosier (1976) was selected as a base. Antioxidants were added and phenol was eliminated from the initial grinding step as this tended to produce substances inhibitory to hybridization (Allen & Dale, 1981).

The second major change was that of the probe. A T3 polymerase runoff was made of the clone using DIG labelled nucleotides. This gave a highly labelled RNA probe, and the hybridization was thus RNA-RNA which is more stable than the previous DNA-RNA binding. Washing could then be more severe which reduced background staining of the blots.

Almost every step in the process was modified in some way — centrifugation steps were moved to a cheaper and quicker micro-centrifuge, formaldehyde was substituted for heating to denature the viroid before blotting, hybridization is done at a higher than normal temperature and various blotting membranes were investigated.

DISCUSSION AND CONCLUSIONS

Currently, using a l g leaf sample, the process can be completed in a day and a half and will detect sunblotch when 1 infected leaf is present in a leaf sample of 1 000 under optimal conditions. The concentration of ASBV in leaves from various sources can vary by a factor of 10 000 (Palukaitis *et al.,* 1981) and in one tree was measured as varying

from 2-2000 ng/g leaf by Allen & Dale (1981).

The detection limit of PAGE is 30-90 ng/g leaf and that of DNA hybridization about 1 ng. It appears to be detecting at this range or better.

Notwithstanding these improvements, the test is not and might never be 100 %. Growers submitting samples are asked to include a positive unknown and on occasion this is missed. However, not all growers do this, and in the interests of quality control it is suggested that the South African Avocado Growers' Association arranges to send a blind sample every three months as a quality control check. It is also known that at least two trees which show symptoms of sunblotch, do not test positive (one tests positive on bark and fruit but only sometimes on leaves). It might be that strains of ASBV are present which do not hybridize very well to the probe used, or it might be that a different disease is involved.

Currently we are confident of detecting one diseased tree in a pool of 20. Our aim is to be able to detect one diseased plant in 500 with confidence, so that a bulk sample of nursery trees can be screened. Initial work with PCR methods have shown promise, but will require some refinement as yet, and cost would be about double that at present.

REFERENCES

ALLEN, R.N. & DALE, J.L. 1981. Application of rapid biochemical methods for detecting avocado sunblotch disease. *Ann. Appl. biol.* 98: 451 - 461

BAR-JOSEPH, M., YESODI, V., FRANCK, A., ROSNER, A. & SEGEV, D. 1986. *South African Avocado Growers' Association Yearbook* 9: 75 - 77

BURNS, R.M., DRAKE, R.J., WALLACE, J.M. & ZENTMEYER, G.A. 1968. Testing Duke avocado seed sources for sunblotch. *Calif. Avocado Soc. Yearbook* 52: 109 - 112.

BURNS, R.M., DRAKE, R.J., WALLACE, J.M. & ZENTMEYER, G.A. 1969. Resistance to sunblotch virus in seed source trees of Duke avocado. *Calif. Agr.* 23: 7 - 8.

DA GRACA, J.V. & MASON, T.E. 1983. Detection of avocado sunblotch viroid in flower buds by polyacrylamide gel electrophoresis. *Phytopath. Z.* 108: 262 - 266.

KORSTEN, L., BAR-JOSEPH, M., BOTHA, A.D., HAYCOCK, L.S. & KOTZÉ, J.M. 1986. Commercial monitoring of avocado sunblotch viroiod. *South African Avocado Growers' Association Yearbook* 9: 63.

LAULHERE, J.P. & ROSIER, C. 1976. One-step extraction of plant nucleic acids. *Plant Sci. Lett.* 6: 237 - 242.

LOPEZ-HERRERA, C., PLIEGO, E & FLORES, R. 1987. Detection of avocado sunblotch viroid in Spain by double polyacrylamide gel electrophoresis. *J. Phytopathology* 119: 184 - 189.

MOHAMED, N.A. & THOMAS, W. 1980. Viroid-like properties of an RNA species associated with the sunblotch disease of avocados. *J. Gen. Virol.* 46: 157 - 167.

PALUKAITIS, P., HATTA, T, ALEXANDER, D.McE. & SYMONS, R.H. 1979 Characterization of a viroid associated with avocado sunblotch disease. *Virology* 99: 145 - 151.

PALUKAITIS, P., RAKOWSKI, A.G., ALEXANDER, D.McE. & SYMONS, R.H. 1981. Rapid indexing of the sunblotch disease of avocados using a complementary DNA

probe to avocado sunblotch viroid. *Ann. Appl. biol.* 98: 439 - 449.
UTERMOHLEN, J.G. 1981. A polyacrylamide gel electrophoresis index method for avocado sunblotch. *Plant disease.* 65: 800 - 802.
WALLACE, J.M. 1958. The sunblotch disease of avocados. *J. Rio Grande Vall. Hort. Soc.* 12: 69 - 74.