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RAPID TESTING FOR AVOCADO SUNBLOTCH

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OPSOMMING

'n Eenvoudige, vinnige metode vir die waarneming van A vokadosonvleksiekte word verduidelik. Hierdie metode neem slegs 6 uur en is gebaseer op poly-akrylamied gel elektroforese van die RNA verkry van blaarweefsel ekstrak.

SUMMARY

A rapid method for the detection of avocado sunblotch disease by polyacrylamide gel electrophoresis of RNA's extracted from leaf tissue is presented. The entire test can be completed in only 6 hours.

INTRODUCTION

Until recently, the standard method used to index avocado trees for sunblotch disease was to inoculate indicator seedlings and observe for symptom development for at least 18 months (Burns etal, 1968), but by conducting such indexing in a warm glasshouse, the time was reduced to 8 months (da Graca, 1979; da Graca & van Vuuren, in press).

In 1979, evidence for the presence of a low molecular weight RNA in infected avocados was published, and it was suggested that the causal agent of sunblotch is a viroid (Thomas & Mohamed, 1979; Dale & Allen, 1979). Further characterization of this RNA showed it to be considerably smaller than any known viroid (Palukaitis et al., 1979; Mohamed & Thomas, 1980). Recently Semancik & Desjardins (1980) reported the detection of five new RNA species in infected avocado, and suggested that the smallest, which coincides with the single species detected by others, may be a non-infectious viroid-associated RNA. All these workers employed polyacrylamide gel electrophoresis (PAGE). As it now appears certain that sunblotch is caused by a viroid, PAGE offers a quick, sensitive test for the disease. The techniques described in the literature take from two (Semancik & Desjardins, 1980) to four days (Thomas & Mohamed, 1979) to complete.

The following paper reports a modified detection method which enables the test to be completed in two to three days depending on the gel stain used, and another method which takes only 6 hours to complete.

MATERIALS AND METHODS

The first method was based on that used by Morris & Smith (1977) for potato spindle tuber viroid. Fifteen grams each of healthy and sunblotch-infected (with symptoms and symptomless carrier) avocado leaves were macerated in 50 ml buff er (0,2 M glycine, 0,1 M Na_2HPO_4, 0,6 M NaCl, 1% SDS, 0,1% sodium dimethyldithiocarbamate, pH 9,5), 12 mi chloroform, 12 mℓ n-butanol, 2,5g PVP and 10 mℓ water saturated phenol (with 0,1% 8-hydroxyquinoline). After centrifugation at 10000 x g for 20 min the supernatant was removed, and 1/5 vols. 10 M LiCl added, and the mixture placed on ice for 2 h. This was then centrifuged at 10000 x g for 15 min, and 2 vols. 95% ethyl alcohol (+ a few drops of 4 M sodium acetate) added to the supernatant and kept on ice for 2 h to precipitate the nucleic acids. After centrifugation at 10000 x g for 15 min the pellet was re-suspended in 5 mℓ distilled water, un-dissolved material removed by low speed centrifugation and this supernatant dialyzed overnight against distilled water in the cold. The nucleic acids were again precipitated with ethyl alcohol as above, and the pellet re-suspended in 0,1 mℓ water.

Electrophoretic separation of the RNA's in this solution was performed on 5% polyacrylamide gels (Adesnik, 1972). The gels were then stained overnight in 0,01% toluidine blue, and destained with several changes of 5% acetic acid, or they were stained for 15 min with 7,5 ug/ml ethidium bromide in 0,001 M EDTA, then destained in 0,001 M EDTA for 15 min, and viewed under UV light.

The second, more rapid test involved combining the LiCl fractionation with the maceration step, and eliminating dialysis (Pfannenstiel *et at.,* 1979). The following method was a modified one of that used by Pfannenstiel (Personal communication) for potato spindle tuber viroid.

Five grams of each healthy and diseased avocado leaf tissue were macerated in 5 mℓ distilled water, 2 mℓ 4 M NH_4OH, 2 mℓ 0,1 M EDTA, 6 mℓ 10 M LiCf and 20 mℓ water-saturated phenol (0,1% 8-hydroxyquinoline) on ice. This was centrifuged for 15 min at 10000 x g, and to the supernatant was added two volumes of 95% ethyl alcohol. This was incubated at — 10°C for 30 min and then centrifuged at 10000 x g for 15 min. The pellet was re-suspended in 5 mℓ water and alcohol precipitation repeated again in the presence of sodium acetate. The final pellet was re-suspended in 0,1 mℓ water, and PAGE was performed as above using a third of the suspension. The gels were stained in ethidium bromide.

RESULTS

Using toluidine blue to stain the gels, one new RNA species appeared about halfway down the gel of infected samples. In some cases a host RNA band (9S) appeared in both healthy and diseased samples above the position of the new species (Fig. 1), but it was more frequently absent (Fig. 2).

Five additional bands were visible in gels of infected samples stained in ethidium bromide (Fig. 3). With the rapid, 6-h method, only two of the new species appeared (Fig. 4).

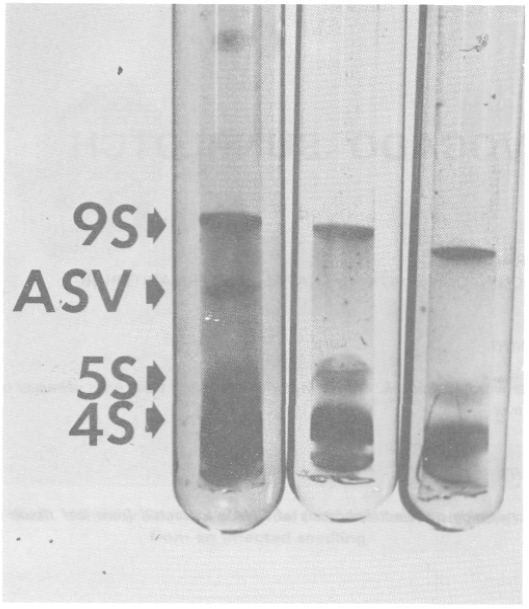

FIG. 1: PAGE OF AVOCADO LEAF RNA's stained with 0,01% toluidine blue. From left to right: sunblotch-infected (symptomless carrier), infected (with symptoms), healthy

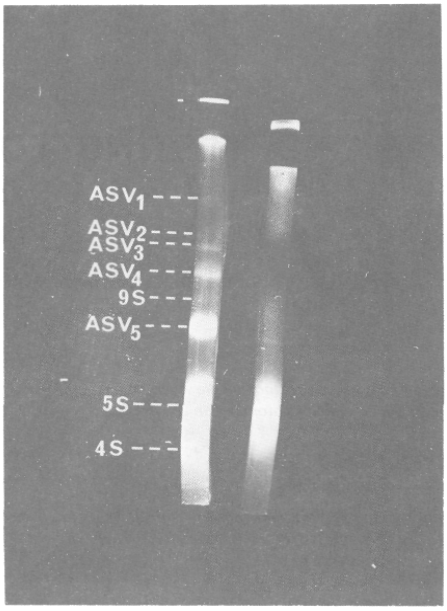

FIG. 3: PAGE of avocado leaf RNA's stained with ethidium bromide (Two-day method). Left, sunblotch-infected (symptomless carrier); right, healthy

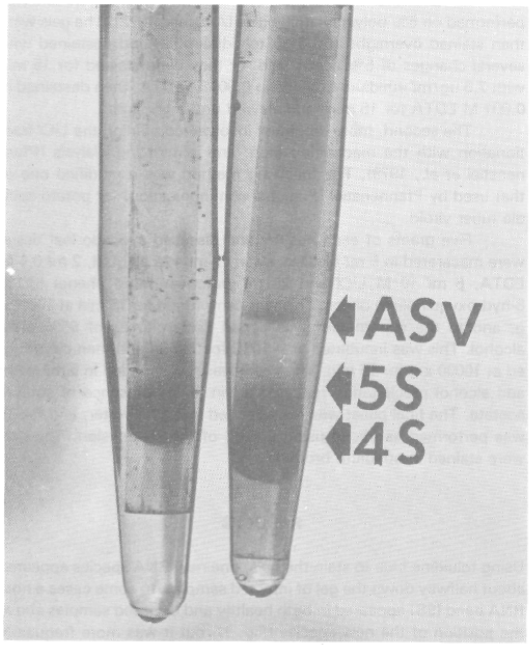

FIG. 2: PAGE of avocado leaf RNA's stained with 0,01% toluidine blue. No 9S RNA is visible. Left, healthy: right, sunblotch-infected (symptomless carrier)

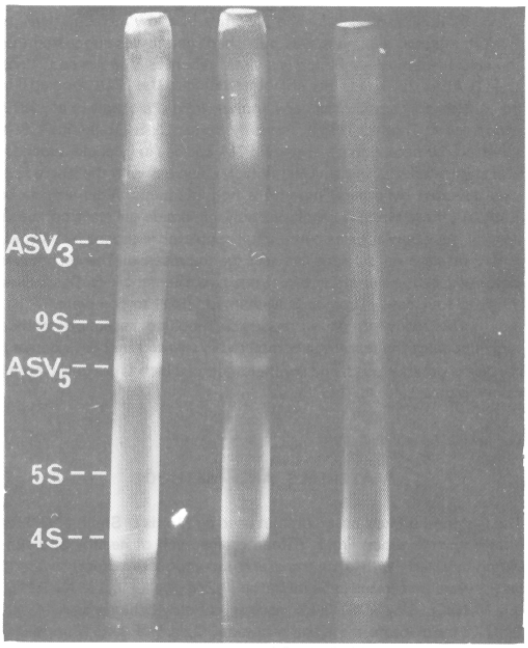

FIG. 4: PAGE of avocado leaf RNA's stained with ethidium bromide (6-h method). From left to right: sunblotch-infected (symptomless carrier), infected (with symptoms), healthy

DISCUSSION

It is now generally accepted that the avocado sunblotch agent is a viroid. However, the RNA species originally detected by Thomas & Mohammed (1979) and Dale & Allen

(1979) appears to be a nonpathogenic sunblotch associated molecule (Semancik & Desjardins, 1980), similar to those associated with some other viroid diseases. Semancik & Desjardins (1980) suggest that one of the larger new species, which they designated ASV_4, is probably the viroid. The results presented in this paper confirm that ethidium bromide is a more sensitive gel stain than toluidine blue, and that its quick reaction is an added advantage.

The need for a rapid, and straight forward, indexing method is essential if nurserymen are to be certain of producing sunblotch-free trees. While a two-day test is very quick, compared with the earlier 18-month (Burns *et al.,* 1968) or eight-month (da Graca, 1979; da Graca and van Vuuren, in press) methods, the application of the even faster test reported here will have obvious advantages in a future avocado improvement programme for South Africa.

Although only two new RNA species, probably ASV_3 and ASV_5, were detected by the more rapid technique, this is considered to be quite adequate for indexing. However, further attempts are to be made to obtain better resolution.

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REFERENCES

1. ADESNIK, M. 1972. Polyacrylamide gel electrophoresis of viral RNA. In: Methods in Virology, vol. 5 (eds. K. Maramorosch & H. Koprowski). *Academic Press* 125 - 129.
2. BURNS, R.M., R.J. DRAKE, J.M. WALLACE & G.A. ZENTMYER. 1968. Testing Duke avocado seed source trees for sunblotch. *Calif. Avo. Soc. Yearbook* 52: 109 - 112.
3. DA GRACA, J.V. 1979. Avocado sunblotch research report. *S. Afr. Avo. Grower's Assoc. Res. Rept.* 3: 65 - 66.
4. DA GRACA, J.V. & S.P. VAN VUUREN. The use of high glasshouse temperature to increase the rate of avocado sunblotch symptom development in indicator seedlings. *Plant Dis.* (in press).
5. DALE, J.L. & R.N. ALLEN. 1979. Avocado affected by sunblotch disease contains low molecular weight ribonucleic acid. *Australasian Plant Pathol.* 3: 3 - 4.
6. MOHAMED, N.A. & W. THOMAS. 1980. Viroid-like properties of an RNA species associated with the sunblotch disease of avocados. *J. Gen. Virol.* 46: 157 - 167.
7. MORRIS, T.J. & E.M. SMITH. 1977. Potato spindle tuber disease: procedures for the detection of viroid RNA and certification of disease free potato tubers. *Phytopathology* 67: 145 - 150.
8. PALUKAITIS, P., T. HATTA, D.M. ALEXANDER & R.H. SYMONS. 1979. Characterization of a viroid associated with avocado sunblotch disease. *Virology* 99: 145 - 151.
9. PFANNENSTIEL, M.A., S.A. SLACK & L.C. LANE. 1979. Detection of potato

spindle tuber viroid in field-grown potatoes by a modified polyacrylamide gel electrophoresis procedure. *Abstr. 9th Intern. Congr. Pl. Prot. 71st Ann Meet. Am. Phytopath. Soc.* No. 220.

10. SEMANCIK, J.S. & P.R. DESJARDINS. 1980. Multiple small RNA species and the viroid hypothesis for the sunblotch disease of avocado. *Virology* 104: 117 - 121.
11. THOMAS, W. & N.A. MOHAMED. 1979. Avocado sunblotch — a viroid disease? *Australasian Plant Pathol.* 8: 1 - 3.