PROBLEMS WITH PAGE INDEXING FOR AVOCADO SUNBLOTCH

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OPSOMMING

'n Her-ondersoek na die gebruik van blomknop-ekstrakte by die PAGE indeksering van avokadosonvlek het getoon dat die variabiliteit in viroid-v/akke te hoog is vir die gebruik van hierdie metode as 'n roetine indekseringstegniek.

SUMMARY

A re-examination of the use of PAGE indexing for avocado sunblotch using flower bud extracts showed that the variability in viroids levels is too great for its use as a routine indexing technique.

INTRODUCTION

There are at present four methods for detecting avocado sunblotch, viz. i) biological indexing, ii) PAGE analysis, iii) ³²P-labelled complementary DNA and iv) dot blot hybridization. The cDNA technique has been found to be the most sensitive (Spiegel, Alper & Allen, 1984), but since it also failed to detect all infected plants the continued parallel use of biochemical and biological indexing is recommended in Israel.

Not all laboratories are equipped to handle cDNA techniques, and the encouraging results obtained by PAGE analysis of flower bud extracts in our laboratory (da Graca & Mason, 1983) gave rise to the hope that it could be used for routine indexing. However, in the following year it failed to live up to expectations when tested at the CSFRI (Moll, Hussey & van Vuuren, 1984). The following paper re-examines this technique and confirms it variability.

MATERIALS AND METHODS

The method used throughout was one described earlier (da Graca & Mason, 1983). Flower buds were collected at various intervals from May through to September 1984 from sunblotch-infected Fuerte trees at Baynesfield, Natal. On two occasions (June and August) buds were collected from infected Edranol and Fuerte trees from the Eastern Transvaal lowveld.

As a standard control, leaves from symptomless carrier trees in our phytotron were used.

RESULTS

Table 1 shows the results of the PAGE indexing trials. Clear positive results were obtained only once from each of the two areas under test, in June (E. Transvaal) and July (Natal) but in neither case did all the trees give positive reactions. The symptomless carrier control gave positive results throughout the trial period.

DISCUSSION

The discrepancy between these results and those reported earlier cannot be fully explained at this stage. The technique itself appears satisfactory since the symptomless carrier control gave positive results on each occasion, and it has worked satisfactorily in another laboratory with Northern Transvaal samples (C. Rey, pers. comm. 1984).

It is known that the level of viroid can vary greatly even in one branch (Allen & Dale, 1981; Palukaitis et al., 1981) and that these levels can sometimes be too low to detect by PAGE. Our results confirm an earlier observation that the time of sampling is important (da Graca, 1984). The effects of environmental conditions such as the recent drought on viroid levels are not known, but may well have a detrimental effect.

The recent development of synthetic oligonucleotides probes (M. Bar-Joseph, pers. comm. 1984) may provide a more reliable rapid indexing technique, but the retention of biological indexing using high temperatures (da Graca & van Vuuren, 1982) is advised.

TABLE 1. Detection of sunblotch	by PAGE of infected flower bud
extracts	

Area	Cultivar	Sampling date	No. positive/ No. tested
Natal	Fuerte	23/ 5/84 5/ 6/84 10/ 7/84 24/ 7/84 6/8/84	2/ 7 0/ 7 5/10 1/ 8 0/10
E. Transvaal	Edranol Fuerte	5/ 9/84 27/ 6/84 15/ 8/84 27/ 6/84 15/ 8/84	0/ 7 3/ 5 0/ 7 0/ 1 0/ 4

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REFERENCES

ALLEN, RN & DALE, JL 1981. Application of rapid biochemical methods for detecting avocado sunblotch disease. *Ann. Appl. Biol.* 98: 451 - 461

- DA GRACA, JV 1984. Avocado sunblotch indexing. S. Afr. Avocado Growers' Assoc. Yrbk. 7: 72
- DA GRACA, JV & MASON, TE 1983. Detection of avocado sunblotch viroid in flower buds by polyacrylamide gel electrophoresis. *Phytopathol. Z.* 108: 262 266
- DA GRACA, JV & VAN VUUREN, SP 1981. Use of high temperature to increase the rate of avocado sunblotch symptom development in indicator seedlings. *Pl. Dis.* 65: 46 47
- MOLL, JN, HUSSEY, KM & VAN VUUREN, SP 1984. Sunblotch indexing for the plant improvement scheme. S. Afr. Avocado Growers' Assoc. Yrbk. 7: 24.
- PALUKAITIS, P, RAKOWSKI, AG, ALEXANDER, PM & SYMONS, RH 1981. Rapid indexing of the sunblotch disease of avocados using a complementary DNA probe to avocado sunblotch viroid. *Ann. Appl. Biol.* 98: 439 449.
- SPIEGEL, S, ALPER, M& ALLEN, RN 1984. Evaluation of biochemical methods for the diagnosis of the avocado sunblotch viroid in Israel. *Phytoparasitica* 12: 37 43.