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PRELIMINARY REPORT ON AVOCADO SUNBLOTCH FIELD INDEXING

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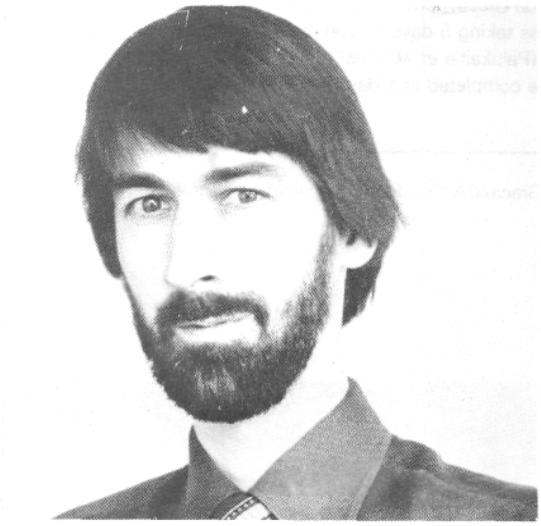


Dr J da Graca

In April 1981 John da Graça received his Ph.D. at the University of Natal, Pietermaritzburg, for his research on avocado sunblotch disease.

Dr Da Graça, who was born in Zimbabwe, obtained his B.Sc.(Agric.) in 1971 from Pietermaritzburg, and went on to obtain his M.Sc.(Agric.) in plant virology at the same university.

In 1974 he joined the Plant Pathology section of the Citrus and Subtropical Fruit Research Institute, and was involved in research on various viral diseases of citrus and subtropical fruits, including avocado sunblotch.

In 1979 he returned to the University of Pietermaritzburg to take up an appointment as lecturer in the Department of Microbiology and Plant Pathology.

His main concern with sunblotch has been the development of improved indexing techniques. The standard indexing technique with indicator seedlings, was speeded up by using higher glasshouse temperatures. Meanwhile, efforts to develop a rapid laboratory technique were continuing. The demonstration in Australia and New Zealand that a viroid-like RNA is associated with sunblotch led Dr Da Graça to develop a rapid laboratory test for the disease, reported in the 1981 Yearbook, which is now being tested on field trees.

OPSOMMING

Peile van sonvlek-verwante RNA in blare van volwasse boordbome is dikwels te laag om betroubaar deur PAGE waargeneem te word. Voorlopige bevindings dui aan dat

RNA peile in blomknoppies aansienlik hoër is en dit mag 'n geskikte weefsel wees vir vinnige indekseringtegniek.

SUMMARY

Levels of sunblotch-associated RNA in mature leaves of field trees are often too low to reliably detect by PAGE. However, preliminary findings indicate that RNA levels in flower buds are considerably higher and this may prove to be suitable tissue for the rapid indexing technique.

INTRODUCTION

Following the development of a rapid polyacrylamide gel electrophoresis (PAGE) technique for testing for avocado sunblotch disease in glasshouse seedlings (da Graca, 1981) a study was initiated to test this technique on field trees, and to determine which tissue was most suitable for sampling. Preliminary results are presented ¡n this paper. The urgent need for a reliable diagnostic test for sunblotch has recently been highlighted in Natal where between 5 and 10% of the trees in two plantings of 3—4 yr. old trees from a reputable nursery developed symptoms of sunblotch (B.N. Wolstenholme, pers. comm. 1981).

MATERIALS AND METHODS

The method used for extracting the RNA's was as described by da Graca (1981), and based on that of Pfannenstiel *et al* (1980), except that 1,4 g polyvinylpyrrolidone (PVP) was included ¡n the maceration step.

Mature leaf samples from sunblotch-infected trees of five cultivars were obtained from one Natal and two Transvaal sources. The extracts were analyzed by slab gel electrophoresis (Winter *et al,* 1977) and the gel was stained in ethidium bromide.

A comparison was then made between slab and column gel electrophoresis. Five-Gram 'recovery' growth mature leaf samples from three sunblotch-infected Fuerte trees and a three-gram combined flower samples from two of these trees were extracted, 10 µl (the maximum possible with the available apparatus) of each was then analyzed on a slab gel, and 100 µl analyzed by column gel electrophoresis.

This experiment was followed by a comparison of 'recovery' growth leaf and flower bud extracts (5 g per sample) from two Fuerte trees by column gel electrophoresis.

RESULTS

The results of the indexing of known sunblotch-infected trees by slab PAGE are presented in Table 1.

Of 25 infected samples tested only three gave a positive result. In one case a sample from the 'recovery' growth of an Edranol tree tested positive whilst the sample from that tree's rootstock shoot tested negative, and in another case the opposite occurred.

Figs. 1 and 2 show the results of the experiment comparing the slab and column gel analyses. In the slab gel the glasshouse symptomless carrier Edranol seedling (control) tested positive but none of the field Fuerte leaf or flower samples gave a positive result (Fig. 1). However, on the column gels where ten times the volume of extract was analyzed all were positive, the field samples containing much less sunblotch-associated RNA than the glasshouse control.

Fig. 3 shows the comparison of extracts from mature leaves and flower buds from Fuerte 'recovery' growth. The flower buds gave clear positive reactions whilst no sunblotch RNA bands can be seen in the leaf samples. The extraction and analysis of these samples was repeated twice. In one faint bands were observed in the leaf samples whilst the flower bud samples were in all cases clearly positive.

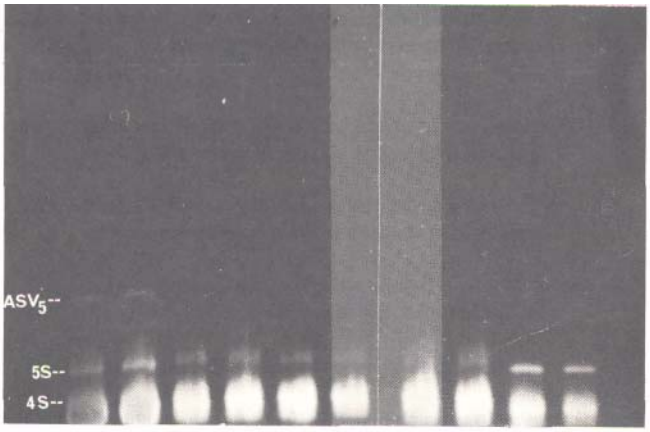


FIG. 1: Slab PAGE of avocado leaf RNA's stained with ethidium bromide. From left to right in pairs: symptomless carrier Edranol glasshouse seedling, three Fuerte trees ('recovery' growth), combined flower sample from two Fuerte trees

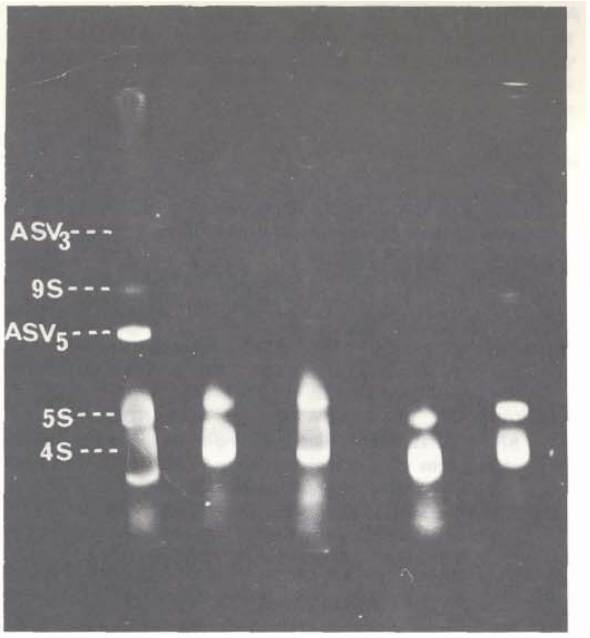
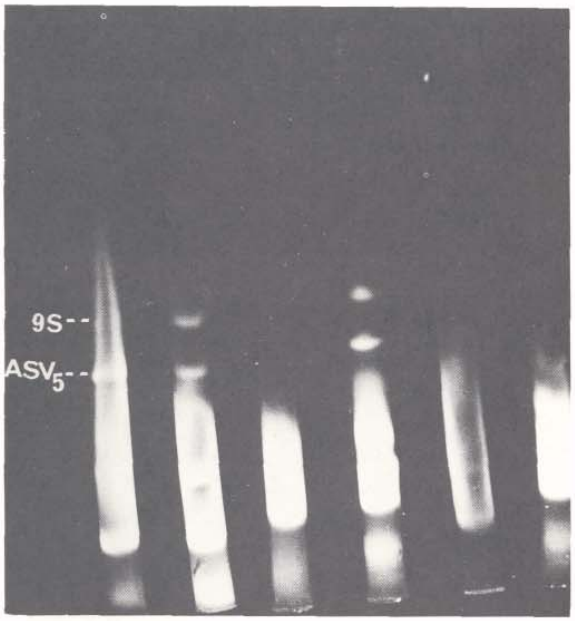


FIG. 2: Column PAGE of avocado leaf RNA's stained with ethidium bromide. From left to right in pairs: symptomless carrier Edranol glasshouse seedling, three Fuerte trees ('recovery' growth), combined flower sample from two Fuerte trees



'FIG. 3: Column PAGE of avocado RNA's stained with ethidium bromide. From left to right: symptomless carrier Edranol glasshouse seedling leaves, 'recovery' growth flower buds (Fuerte tree no. 1), 'recovery' growth leaves (Fuerte tree no. 1), 'recovery' growth flower buds (Fuerte tree no. 2), 'recovery' growth leaves (Fuerte tree no. 2), healthy flower buds

TABLE 1: Indexing of known sunblotch-infected avocado trees by slab PAGE using 10 µl per sample

Sources	Cultivar & Status	No. tested	No. positive
N.Transvaal	Fuerte with symptoms	2	0
	Fuerte 'recovery' growth	2	1
	Edranol with symptoms	2	0
	Ryan with symptoms	2	0
E.Transvaal	Edranol with symptoms	5	0
	Edranol 'recovery' growth	5	1
	Mexican rootstock (symptom-less)	4	1
Natal	Fuerte 'recovery' growth	3	0

DISCUSSION

Palukaitis et *al* (1981) have reported that the level of the viroid-like RNA in sunblotch-infected avocados can vary 10 000-fold from 0,2 to 2 x 10^{-5}% by weight. This would account for the very low, rate of detection by slab gel PAGE where only 10µl of the extract could be applied. Use of larger volumes, as was the case with the column gels, allowed detection in three samples which were negative on the slab gel.

Allen and Dale (1981) found very little difference in the level of RNA in mature leaves and young flowers. The one flower sample tested in this study (Fig. 2) supports this, but the results shown in Fig. 3 clearly indicate that flower buds have considerably higher levels of the disease associated RNA.

It is now planned to test a number of infected trees of different cultivars using flower buds. One problem is that such material is available for only a short period. A preliminary test on glasshouse material was therefore conducted to determine whether very young leaves might also contain high levels of the sunblotch RNA. However, the levels were found to be no higher than in mature leaves. Mohamed and Thomas (1980) found higher levels in mature leaves than in young leaves.

Palukaitis et *al* (1981) compared two rapid indexing methods, PAGE and complementary DNA probe, and found the latter to be more sensitive. However they used toluidine blue to stain the gels, and research in both California and South Africa has shown this to be far less sensitive than ethidium bromide (Semancik and Desjardins, 1980; da Graca, 1981). The complementary DNA probe is also a longer process taking 5 days to complete, excluding the preparation of the DNA (Palukaitis *et al.* (1981). The PAGE method on the other hand can be completed in 1 day (da Graca, 1981).

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