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# **AVOCADO SUNBLOTCH INDEXING**



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### **OPSOMMING**

Pcgings om blomknoppe van avokados te vries om sonvleksiekte mbv PAGE te indekseer was nie suksesvol nie. Buitendien het dit ook voorgekom asofdie eerste en laaste blomknoppe wat verskyn nie bevredigend vir PAGE-indeksering is nie.

#### SUMMARY

Attempts to freeze flower buds for avocado sunblotch PAGE indexing were not successful. In addition, it appears that the first and last flower buds to emerge are not satisfactory for PAGE indexing.

#### INTRODUCTION

The use of PAGE indexing for avocado sunblotch using flower buds was shown to be 100% successful with all 52 samples tested giving positive results (da Graca and Mason, 1983). Since flower buds are only available for a limited time, am investigation was undertaken to determine whether samples could be frozen and kept at -20 °C until the indexing could be performed. This would eliminate the need to index all the selected trees in a limited period of time.

During collaborative work with the Citrus and Subtropical Fruit Research Institute (CSFRI) to establish the use of the technique in the laboratories there, an interesting observation with regard to the chronological timing of flower bud sampling was made and is reported on.

## MATERIALS AND METHODS

Flower buds from a sunblotch infected Fuerte tree in the Eastern Transvaal were collected and placed in a deep freeze (-20 °C). After 1 month five grammes of this sample were analyzed for ASV-RNA by PAGE (da Graca and Mason, 1983). At the end of the second month a further fivegramme sample was analyzed

## **RESULTS AND DISCUSSION**

In the freezing experiment ASV-RNA was readily detectable after one month, but not after two months freezing, although the host 4S and 5S were present. After two months freezing the flower buds had begun to turn brown. More satisfactory results could possibly be obtained by freezing the samples in liquid nitrogen.

The observation made with the three samples collected at different times was that while the September sample gave a very clear picture result, the early and late samples, i.e. August and October, were negative.

These results have introduced some limitations to the PAGE technique, although freezing in liquid nitrogen may overcome some of these. The limitation of the availability of flower buds will not necessarily be overcome by using leaf tissue since Baksh, Lee and Garnsey (in press) found that citrus exocortis viroid could only be detected in field samples of citrus leaves collected during summer.

The results obtained during field trials in South Africa in 1982 clearly demonstrate that the technique is satisfactory (da Graca and Mason, 1983), and a very similar method is used in this country for routinely indexing chrysanthemums for viroids (G Thompson, pers comm.). Problems encountered at the CSFRI appear to be due to laboratory practice rather than to any faults with the technique itself.

Although 100% detection was obtained here, lower success rates have been obtained using similar, but not identical, methods in the USA (Utermohlen and Ohr, 1981) and Australia (Palukaitis *et al* 1981). This latter paper showed that the use of cDNA probe techniques is more sensitive. Another technique is dot-spot self-hybridization with self-hybridization with <sup>32</sup>P-labelled RNA (M Bar-Joseph, pers comm.). However, it is now believed that no method is able to identify sunblotch infection with absolute certainty (Allen, 1983). These techniques have not yet been developed in South Africa, but plans are now being made to test them here.

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