

# **Developing and optimizing techniques to facilitate the development of avocado rootstocks resistant to *Phytophthora cinnamomi***

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## **ABSTRACT**

The release for semi-commercial evaluation of the first new rootstock from the ITSC rootstock breeding programme is discussed. Building of the *Phytophthora* unit was completed during July 1998 and 5000 seedlings have been screened but clonal propagation of selections will only commence in the new unit during 1999. Results and techniques, used by the ITSC and Californian breeding programmes up to the present, are discussed. New techniques and exciting developments for 1999 are highlighted.

## **INTRODUCTION:**

Since the introduction of *Phytophthora* tolerant clonal rootstocks as a counter to avocado root rot, the South African industry had to largely rely on Duke 7. New imported rootstocks proved to be unsatisfactory and in some cases disastrous. For this reason as well as the large financial impact that avocado root rot, caused by *Phytophthora cinnamomi*, has on the South African avocado industry, the avocado rootstock programme of the ARC-Institute for Tropical and Subtropical crops was started in 1992. The major objective was to develop a range of avocado rootstocks which are tolerant to *Phytophthora* root rot. Progress was reported by Koekemoer, Breedts, Manicom and Bijzet, (1994); Breedts, Koekemoer and Bijzet (1995); Bijzet, Breedts, Koekemoer and Cilliers (1996); Bijzet, Van Vuuren & Schroeder (1997) and Bijzet (1998).

Six seasons have elapsed since the breeding programme was initiated, during which approximately 41,000 seedlings have been screened and 55 selections have been made for further testing. During this time various techniques for breeding and screening have been tried and tested to culminate in the development of an Avocado *Phytophthora* Unit (Bijzet, 1998).

## **OVERVIEW OF MATERIAL AND METHODS:**

Various stages in the rootstock breeding programme were described by Bijzet, Sippel & Koekemoer (1993). These were subsequently altered to achieve greater efficiency (Bijzet, 1998).

- **Rootstock breeding**

Since 1992, open pollinated seeds from avocado rootstocks and from other cultivars in close proximity to the avocado rootstock material were germinated for screening. As was emphasized before, pollen derived from non-resistant sources detracts from the efficiency of the current procedure for producing seedlings. Breeding efforts are diverted more and more from the use of open pollinated sources towards controlled pollination. Future plans include creating polyploidy, embryo rescue and optimizing the controlled pollination efforts.

- **Phase 1 screening**

The protocol has been finalized to screen Phase I seedlings. It is done by planting seed directly into bins filled with *Phytophthora cinnamomi* soil. The seeds are left to germinate and subsequently to die of *Phytophthora* root rot. Indicator plants show whether the disease pressure is correct and if not, a mycelium suspension is applied approximately 120 days after germination. Surviving seedlings are selected. If the percentage surviving selections are too high, further elimination is done after an inspection of the root systems. The surviving seedlings are then treated as described by Koekemoer *et al.* (1994) and transplanted to black 50 litre rubber dustbins that are filled with sterilized soil. This allows for proper root expansion and subsequent top growth which is required for further multiplication of the selections.

- **Clonal propagation**

Clonal multiplication in addition to *Phytophthora* tolerance/resistance has been included as a selection criterion, as easy and effective clonal multiplication of rootstocks is an important nursery practice. As each new selection comprises only one plant, clonal multiplication is required to provide a large number of plants to be used for additional *Phytophthora* tests as well as horticultural evaluations. A detailed account of the process was given by Bijzet (1998).

- **Statistical screening**

The second phase, a statistical screening of the surviving selections, then follows. For this, 20 to 30 cuttings of each selection and of Duke 7 as control are made. These are grafted with Hass and then planted in bins filled with sterilized soil to which a known concentration of *Phytophthora mycelium* is added. Valid comparisons can be made and selections better than Duke 7 are included in field trials. The field trial will be carried out at Burgershall and Westfalia in an orchard known to have a high incidence of *Phytophthora*.

- **Escape trees from the industry**

Orchard trees being sole survivors or showing exceptional signs of vigour under apparent root rot pressure have been termed escape trees. Any such tree found, is salvaged and multiplied for testing along with the selections made from the first screening.

## RESULTS AND DISCUSSION

- **Rootstock breeding**

Open pollinated seedlings are still abundantly available and the major part of the 5000 seedlings screened during 1998 still comprises open seedlings. Hand pollinations were hampered by large numbers of overcast days during the pollination period. On overcast days all the flowers observed on the trees were in the male stage all day and could not be pollinated with the pollen that was collected. During the coming season, storage of pollen will be investigated and implemented. Again seeds from the polycross nursery were taken.

- **Phase 1 screening of seedlings**

The 5000 seedlings screened during 1998 bring the total of seedlings screened since the inception of the programme in 1992 to 41,000. Only five seedlings were selected.

- **Clonal propagation**

Commencement of clonal propagation of selections was contingent on the completion of the new *Phytophthora* unit during the 1998 season. Unfortunately the contractor did not finish building the unit in time to exploit the past season. However, all preparations have been completed for the coming season and clonal propagation will go ahead according to plan.

- **Statistical screening of cuttings**

The 1992-1995 selections have been multiplied and are currently being statistically tested by the pathology department. With regard to true resistance the results are disappointing: However, two of the selections have proven promising by having substantially larger root masses by the end of the trial period than Duke 7. These selections will be promoted to a field trial.

- **Escape trees**

The escape tree designated SARS/97-01 (PvT) that was recovered in the Hazyview area and found to be tolerant to waterlogged conditions and *Phytophthora* (Bijzet, 1998) was multiplied and planted in a field trial in co-operation with Dr. Anton Hough. The rootstock is currently being tested with Pinkerton, Hass, Fuerte, Ryan and Lamb Hass as scions. Dr. Hough was impressed with the performance of this particular rootstock under the very wet conditions during the past season. The ITSC will motivate for the semi-commercial release of this rootstock selection during the coming season. This will mean that farmers who are interested can order between 200 and a 1,000 trees of this rootstock grafted with a scion of their choice. Farmers taking part in this semi commercial release will have to be prepared to sign a non-propagation agreement and to disclose data with regard to these trees on request. Farmers should also realize that these materials are still under investigation and that there are certain risks involved.

## RESEARCH PLANNED FOR THE 1999 SEASON

Optimization of the inoculum used in the breeding programme is of the utmost importance. A study with regard to strains and difference in virulence will be undertaken during the 1999 season. This will entail collecting samples from all avocado producing areas and isolating and testing for *Phytophthora cinnamomi* from these samples. The first tetraploids will be induced by use of colchicine. These polyploids will then be tested for *Phytophthora* resistance. Pollen collection and storage will be investigated and optimized for future use. Although the screening protocol has been finalized, the best screening medium is still not known and will consequently be tested during the 1999 season. The results will dictate the medium used in future screenings. There is also a need for a wider base of breeding material. At this stage the only cultivar with true resistance to *Phytophthora cinnamomi* seems to be G755 otherwise known as the Martin Grande. This cultivar rarely flowers or bears any fruit in South Africa and is thus of little use in a breeding programme. Importation of wild avocado material and other *Persea* species is thus important. This will take place during October 1999.

According to a communication (2<sup>nd</sup> February 1999) with Dr. J. Menge who is in charge of the California rootstock breeding programme, they have abandoned the use of the Zentmyer-tank as they are afraid of losing certain types of resistance with this hydroponic method. They now use soil mixed with vermiculite.

According to Dr. Menge their biggest constraints are also with regard to breeding. They also use largely open pollinated seeds supplemented with seeds from small isolation blocks. They are currently investigating the need for controlled pollination.

## CONCLUSION

Completion of the *Phytophthora* unit and the possible semi-commercial release of SA-RS/97-01 are both signs that the breeding programme will have outgrown its developmental stage by the end of 1999 and when entering the new millennium the ITSC rootstock breeding programme will have become an automated breeding programme able to deliver new *Phytophthora* resistant/tolerant rootstocks on a regular basis.

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