

Development of Avocado Rootstocks with Improved Resistance/Tolerance to Phytophthora Root rot

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ABSTRACT

During the 1996 season 3323 seedlings yielded the high number of 190 selections. During 1997 these were reduced to 10 selections and have been transplanted to be multiplied for the statistical screening phase. Another 3646 seedlings have been screened during 1997, and 20 were selected. The polycross unit that was finalized during 1996/97 produced its first seeds as predicted. However, larger numbers are needed from this facility. Due to the genetic make-up of each seedling being different, difficulty was experienced in multiplying these seedlings and little results were obtained. It was thus decided that the ease with which a seedling can be multiplied should also be a selection criterion. With this in mind the facilities were further upgraded with the building of a *Phytophthora* unit which will house both the screening phases as well as the multiplication of selections. While awaiting completion of this unit, the facility of the pathology department was utilised as an interim measure to complete the first multiplication cycle. However, results on the statistical screening are still expected from the pathology department.

UITTREKSEL

Die 1996 seisoen het 190 seleksies uit 'n totaal van 3323 saailmge opgelewer. Die seleksies is gedurende 1997 vermmder tot slegs tien. Uit die, 3646 saailmge van 1997 is slegs 20 geselekteer. Saad is vir die eerste keer vanuit die polikruiseenheid, wat gedurende die 1996/97 finansiele jaar voltooi is, geoes. Daar word egter aansienlik meer saad benodig vanuit die fasiliteit. As gevolg van die saailing seleksies wat geneties van mekaar verskil, is daar probleme ondervind tydens die vermeerderingsfase van die seleksies. Daar is gevoldik besluit dat die gemak waarmee 'n saailingseleksie vermeerder kan word, voortaan ook 'n seleksiekriterium behoort te wees. Met die oog hierop is 'n *Phytooththora*-eenhied beplan en gebou ten einde die probleem aan te spreek. Die eenheid sal beide siflingsfases sowel as die vermeerderingsfase kan hanteer. As interim maatreef is daar van die patologie-afdeling se fasiliteite gebruik gemaak vir vermeerdering van die eerste seleksies maar resultate ten opsigte van die statistiese evaluasie was nog nie beskikbaar vir publikasie nie.

INTRODUCTION

At the ARC-institute for tropical and subtropical crops the major objective in the avocado rootstock breeding programme is the development of a range of avocado rootstocks which are tolerant to *Phytophthora* root rot. The reason for this is the large financial impact that avocado root rot, caused by *Phytophthora cinnamomi*, has on the South African avocado industry. The ITSC's avocado rootstock programme was started in 1992. Progress was reported by Koekemoer, Breedts, Manicom and Bijzet (1994); Breedts, Koekemoer and Bijzet (1995); Bijzet, Breedts, Koekemoer and Cilliers (1996) and Bijzet, Van Vuuren & Schroeder (1997).

Easy and effective clonal multiplication of rootstocks is an important nursery practice. Clonal multiplication is therefore, in addition to *Phytophthora* tolerance/resistance, a selection criterion in the breeding programme. Another 3646 seedlings were screened for *Phytophthora* susceptibility in 1997. Three percent of these seedlings survived the initial screening and since the initiation of the programme in 1992, a total of 36 679 seedlings were screened and 81 selections were made.

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 (figure 1).

Rootstock breeding

Since 1992, open pollinated seeds from avocado rootstocks and from other cultivars in close proximity of the avocado rootstock material were germinated for screening.

Pollen derived from non-resistant sources detracts from the efficiency of the current procedure of producing seedlings. An isolated orchard consisting only of rootstock material is needed. An orchard of this kind will be very costly to maintain in view of the distance that it would have to be removed from other avocado orchards. This problem was solved by renovating an old shade cloth structure of approximately 1000 m². The structure consists of 6 terraces, each 3 metres wide, 50 metres long and with 15 well-drained plant pots 1.25 m in diameter and spaced 3 metres apart, giving 90 pots in total. This area is covered with shade cloth which is supported by treated poles. The result is an area of approximately 1000 m² that can be isolated from other avocado plantings, enclosing pollinators and only rootstock material with potential resistance to *Phytophthora* root rot (figure 2).

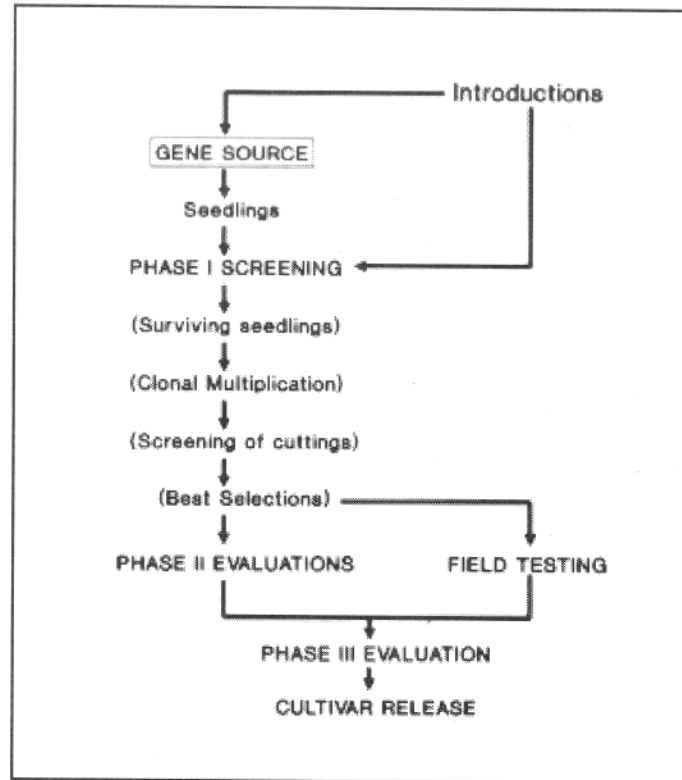


Figure 1. ITSC - rootstock breeding strategy

Phase I screening

The protocol has been finalized to screen Phase I seedlings. This is done by planting seed directly in bins filled with *Phytophthora cinnamomi* soil. The seeds are left to germinate and subsequently 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 proper root expansion and subsequent top growth which is required for further multiplication of the selections.



Figure 2. The polycross unit showing the six terraces with plant pots in the shadenet enclosure

Clonal propagation

The main methods of propagating avocado clonally were described by Frolich & Platt (1972); Ernst (1978) and Moll & Wood (1980). The method of Moll & Wood (1980) was chosen to multiply selected *Phytophthora* tolerant seedlings since limited material is available for grafting and a large number of plants are required for additional *Phytophthora* tests as well as horticultural evaluations.

Seed of a sunblotch-free avocado tree, cv. Edranol, planted in small plastic bags (70 mm diameter, 150 mm high) containing a well-drained, sterile medium. After germination, the nurse seedlings are grafted to the required selections to be multiplied. At budburst, the nurse plant is transferred to darkness for etiolation.

Two methods follow from this step, depending on the growth habit of the selection:

- When leaves develop during the etiolation process, cuttings are taken while still in the dark when the shoot stops growing actively (approximately 300-400 mm). Cuttings are taken at internode length and placed in a mist bed with bottom heating at 26°C and a mist blow of two seconds every minute.
- Selections, which do not develop leaves in the dark, are removed from the etiolation chamber when the etiolated shoots are approximately 20-30 mm long. The shoots are painted black with a butimenbased tree sealing compound and the plants are left in daylight to develop normal green leaves. When one or two leaves have developed, the shoot is cut and placed in a mist bed as described above.

Roots develop approximately 4-8 weeks later. When the roots have developed, the cuttings are transplanted to the same size plastic bags with the same medium as the nurse seedlings and are left under the mist spray for a week where after they are hardened off.

The statistical screening

The second phase, a statistical screening of the surviving selections then follows. For this, 20 to 30 cuttings of each selection, as well as Duke 7 as control, are made. These are grafted with Hass and are 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 and in a Phase II programme. The field trial will be carried out at Burgershall 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. Thirty-four escape trees were identified by SAAGA on the farms of producers over the past few years. These were tested in the laboratory of the University of Pretoria by means of the detached root technique for tolerance/resistance (Van der Merwe, Maas & Kotzé, 1990). Although not significantly so, nine of the 34 trees tested, showed a higher degree of resistance than G755 and six trees were found to be significantly more resistant than Duke 7. Three of the trees were significantly more susceptible than Edranol and probably survived in the field only due to the fact that the roots did not come into contact with *Phytophthora* inoculum.

The tasks of obtaining rooted cuttings from each of the identified trees were delegated to the Avocado Cultivar Evaluation Committee of SAAGA. This seems to be a tedious task as all the trees are grafted with a scion and the producers are not eager to sacrifice a good bearing tree for the sake of further testing of the rootstock material. Enticing roots to make water shoots and various other methods, have been suggested. The search for and testing of escape trees is an ongoing task of the breeding programme.

New ideas and developments

When clonal propagation was included as part of the research programme, facilities were needed to handle the workload. The *Phytophthora* unit (figure 3) was consequently designed and consists of a facility housing two *Phytophthora* screening bins of 10m long and 1m wide. One bin will be used for screening seedlings and the other for screening cuttings. The unit also includes a 3m x 1m mistbed and an etiolation room. Ample floor space is left for the placing of nurse seedlings as well as transplanted selections.

Explanation of events taking place in the *Phytophthora* unit (figure 3)

Seeds are harvested from the polycross unit (figure 2) and then planted in bin 1 and screened. The surviving selections are transplanted to the 50 litre bins situated in area 2. Budwood is then taken from these plants and grafted on nurse seedlings on standby in area 3. The grafted nurse seedlings are transferred to the etiolation room (4) from where etiolated cuttings are taken to the mistbed (5). Rooted cuttings are transplanted

to bags ready in area 6. The rooted cuttings in area 6 are then grafted with Hass and then transplanted in bin 7 for statistical screening. Selections statistically excelling in bin 7 are then promoted to a field trial and are simultaneously included into the Phase II rootstock programme for horticultural evaluation.

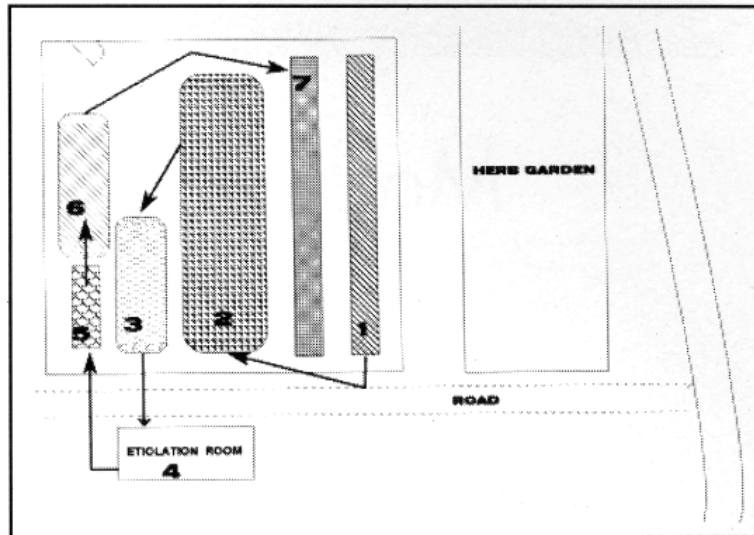


Figure 3. Flow of events through the *Phytophthora* unit

Table 1. Summary of breeding parents used, that yielded selections from 1992 to 1997.

FEMALE	PLANTED	SELECTED	FEMALE	PLANTED	SELECTED	FEMALE	PLANTED	SELECTED
BARR DUKE	2148	11	HILCOA 16#7	206	1	NUMLICH 70	114	1
BRUWER	60	1	HILCOA 5	208	2	PIETOU	43	1
D9	237	2	I399	245	1	PT37	505	1
DUKE 7	4385	14	I413	208	1	REED	466	2
DUKE SDL 1	2407	4	JOVO	368	2	TEAGUE	610	2
DUKE SDL 2	702	7	LOHNHEISS HASS	344	3	THOMAS	178	1
G6	3447	2	NA565	467	2	TORO CANYON	70	1
H222	508	1	NN63	108	1	ZUTANO	122	1
H709	116	1	NO NAME	1349	9	SLEIGH	32	1
HASS 4TH GEN 271		5						

Table 2. Summary of breeding parents that did not yield selections from 1992 to 1997.

FEMALE	PLANTED	FEMALE	PLANTED	FEMALE	PLANTED	FEMALE	PLANTED	FEMALE	PLANTED
*1-17-1	11	*DU TOIT	41	LATA - S(x)	10	H670	342	NN10	66
*1-17-2	13	*DU TOIT	17	ZUTANO -S(x)	20	HASS	548	NUMLICH 111	296
*1-25-1.7	20	*FOURIE	5	#86	173	HAYES	154	OA 184	229
*1-25-8	17	*GOUBRECTS	70	ADDO Selection	20	HAZZARD	177	P-PARENT	239
*1-30-1	12	*GRAVES	131	ALLBOYCE	12	HORSHIM	1036	P3	987
*1-30-2	3	*KOEN	5	BACON	280	HX204	57	P6	25
*1-30-3	4	*KOTZE	18	BALBOA	147	I388	19	PARIDA (P1)	267
*2-68-1	2	*MARAIS	6	BORCHARD	125	I392	189	PINKERTON	265
*2-69-1	6	*MOSTERT	26	CANADA	123	I414	100	REGAL	5
*2-70-1	2	*RIETSPRUIT	2	COLIN V33	4	IRVINE	29	RINTON	1082
*4-5-1	90	*VAN WYK	6	EDRANOL	1191	J241	195	RYAN	171
*ALBERTS	30	*VAN ZYL	33	ESTER	934	KARIKA	38	SCOTLAND	47
*BOSSINGER	10	*VISSER	27	ETTINGER	366	LATA	144	SHARWILLE	260
*BOTHIA	58	COLIN V33 - P(x)	5	FUERTE	54	LULA	20	SHEPPARD	25
*BURGER	10	DUKE 7 - P(x)	15	G22	2374	MOAZ	14	T142	42
*COETZEE	22	DUKE 7 (escape)	1	G755C	15	NA526	63	TOPA-TOPA	1295
*COLBY	28	DUKE 7 (escape)	1	GA13	34	NA66	30	TX531	558
*CRONJE	24	DUKE SDL - S(x)	5	GWEN	361	NABAL	132	WHITSEL	401
*CUTHBERTS	77	G6 - S(x)	22	H287	61	NDLC	8	WI SDL	11

*Seeds collected from trees in home gardens in the Western Cape.
S(x) - breeding parent that was self-pollinated.

P(x) -A breeding parent in the polycross unit.

RESULTS AND DISCUSSION

Rootstock breeding

Detailed accounts of the results were given by Bijzet *et al.* (1993), Koekemoer *et al.* (1994); Breedts *et al.* (1995); Bijzet *et al.* (1996) and Bijzet *et al.* (1997.) The polycross nursery yielded its first seeds. Only a few seeds were available from the polycross unit and none of these were selected during the final screening. The aim is to plant 6000 seeds for screening during 1998 when seed from the polycross unit is again available. A summary of breeding parents used, the number of seeds planted and selections made is given in table 1. Breeding parents used but not yielding any selections are listed in table 2.

Phase I screening of seedlings

From 1992 to 1997 a total of 36 679 seedlings screened for resistance/ tolerance to *Phytophthora*. A summary of seeds harvested and seedlings selected is given in table 3.

The period 1992 till 1997 yielded 81 selections which were included in the clonal propagation process. During the multiplication period of selections made between 1992 and 1995, 33 of the weaker selections died. Twelve of these seedlings originally showed strong root development and few signs of *Phytophthora* root rot when selected, but died after removal of the seed lobes and subsequent transplantation. The 30 selections made during 1996 and 1997 are still being propagated and a percentage of these might succumb to the same fate during the process of seed lobe removal and transplantation. The seed lobes being a great source of nutrients seem to keep the

seedling alive and upon natural or manual removal of the seed lobes, the seedling does not have the resources to withstand the root rot anymore.

The screening of the seedlings in the 1994 season was done differently than the other seasons. The 18,757 seeds were first germinated and then transplanted to the *Phytophthora* infested soil whereas during the other seasons the seeds were directly germinated in bins containing the *Phytophthora* infested soil. The shorter period of time that the seedlings, of this particular season, were in contact with *Phytophthora cinnamomi* thus accounts for the high number of seedlings which were noted under Screen 0 (table 3), as surviving after 12 weeks.

Table 3: Screening of open pollinated seedlings

Year	Planted	Screen 0	Screen 1	Screen 2	Selection	Multiplication phase	Survived
1992	5717	2894	107	68	12	9	7
1993	2799	1648	108	15	15	3	2
1994	18757	16381	26	20	20	9	9
1995	2437	278	7	4	4	4	0
1996	3323	1508	190	66	10	10	?
1997	3646	831	308	93	20	20	?
Totals	36679	23540	746	266	81	55	18

Screen 0 accounts for the surviving seedlings \pm 12 weeks after planting. Screen 2 accounts for the surviving seedlings following a further 4 weeks after Screen 1 is a manual selection at this time.

Selection is the final manual selection at this time

Of the 3,323 seeds that were harvested and screened in 1996, more than 50% survived the initial screening although, judging from the indicator plants in the bin, a high *Phytophthora* pressure was maintained. The surviving seedlings were mainly from the new gene source. On close inspection of the roots, 190 seedlings were selected which accounted for 5% of the total seedlings screened. As 5% is still a high selection rate, these selections were not transplanted immediately but were subjected to a further *Phytophthora* pressure for a period of four weeks after which 66 survived. The weaker seedlings were discarded and only ten seedlings were kept. These are now in the multiplication phase.

During 1997 a total of 3,646 seeds were planted and again three quarters of the seed were taken from the gene source at Levubu research station. This time only 2.55% survived the initial screening of which only 20 were kept after close inspection of the roots. These 20 seedlings are currently being prepared for the multiplication phase. The first available cuttings from these seedlings are in the process of being top worked in orchard J9 at Nelspruit in order to obtain efficient numbers of budwood material for further multiplication.

Clonal propagation

Requests for the clonal propagation of the selections were put to the ITSC nursery. Due to the genetic make-up of each seedling being different, difficulty was experienced in

multiplying seedlings and little results were obtained. It was then decided that the ease with which a seedling can be multiplied should also be a selection criterion. As this is almost a research project on its own, this task was diverted from the nursery. The first results were reported during 1996/1997 (Bijzet, Van Vuuren & Schroeder).

Statistical screening of cuttings

The 1992-1995 selections have been multiplied and are currently being statistically tested by the pathology department. Results were unfortunately not available for this report but will be reported on next season.

The statistical screening method will be finalized pending the outcome of this experiment.

Escape trees

Dr. Anton Hough succeeded in obtaining two trees in the Hazy view area to make shoots and subsequently supplied the institute with 24 and 60 rooted cuttings of two selections named TR and PvT respectively. More cuttings will be made available for statistical screening of the selections. The owner of PvT has indicated that he would like to obtain breeding rights to this specific material. A non propagating agreement between the ITSC and the owner was signed and the owner has agreed that the ITSC share ownership of the breeding rights in exchange of evaluating the selection for *Phytophthora* resistance as well as horticultural soundness.

New ideas and developments

The *Phytophthora* unit was designed in 1996 and building commenced in February 1998, but will, however be used during the 1998 season for screening and multiplication of seedling material as described under material and methods.

CONCLUSION

Seed, awaited with great anticipation from the polycross unit, was harvested and screened. No selections resulted from this particular consignment of seed but larger numbers of seed are needed from this facility. However, the prospects are good as the trees are yet another year older and a permanent source of pollinators will be used from this year on. A further 3,646 seeds were germinated and screened for resistance and 0.55 percent of the seedlings were selected. Upgrading of the facilities, with the building of the *Phytophthora* screening unit to accommodate clonal multiplication is a milestone reached in this programme. The *Phytophthora* unit solves the bottle neck in the process with a swifter second phase screening of the selections envisioned. Exciting results are expected from the statistical screening of cuttings. Success with the PvT Selection emphasizes the importance of identifying and recovering material from escape trees.

REFERENCES

- BIJZET, Z., SIPPEL, A.D. & KOEKEMOER, P.J.J. 1993. Avocado breeding: a progress report. *South African Avocado Growers' Association Yearbook* 16:86 - 89.
- BIJZET, Z., BREEDT, HJ. KOEKEMOER, P.J.J. & CILLJERS, B. 1996. Avocado rootstock breeding new developments and intricacies. *South African Avocado Growers' Association Yearbook* 19:14 - 15.
- BIJZET, Z., BREEDT, HJ. KOEKEMOER, P.J.J. & CILLIERS, B. 1997. Progress with local selections in the quest towards a more tolerant/resistant avocado rootstock than Duke 7. *South African Avocado Growers' Association Yearbook* 20:17 - 23.
- BIJZET, Z., VAN VUUREN, S.P. & SCHROEDER L., 1997. Progress with local selections in the quest towards a more *Phytophthora* tolerant/ resistant avocado rootstock than Duke 7. *South African Avocado Growers' Association Yearbook* 20:17 - 23
- BREEDT, HJ. KOEKEMOER, P.J.J. & BIJZET, Z., 1995. Vorderingsverslag: Seleksies van *Phytophthora* bestände Avokado onderstamme 1992 tot 1994. *Suid Afrikaanse Avokadokwekers Vereniging Jaarboek*. 18:4 - 6
- ERNST, A.A. 1978. New promising technique for rooting difficult-to-root avocado (*Persea americana* Mill) cuttings. *Citrus and Subtrop. Fr. J* 532:6 - 10
- FROLICH, E.F. & PLATT, R.G. 1972. Use of the etiolation technique in rooting avocado cuttings. *Calif. Avocado Soc. Yrb.* 55:97 - 109
- KOEKEMOER, P.J.J., BREEDT, HJ. MANICOM, B.Q. & BIJZET, Z, 1994. Selection of *Phytophthora* tolerant avocado rootstocks. *South African Avocado Growers' Association Yearbook* 17:72 - 74
- MOLL, J.N. & WOOD, R. 1980. An efficient method for producing rooted avocado cuttings, *Subtropica* 1(11): 9-12
- VAN DER MERWE, M de V., MAAS, E.M.C. & KOTZÉ, J.M.1990. Evaluation of field trees for resistance to *Phytophthora cinnamomi* by means of the detached root technique. *South African Avocado Growers' Association Yearbook* 13: 63 - 64