



# Screening of avocado rootstock material for tolerance to *Phytophthora cinnamomi*

by

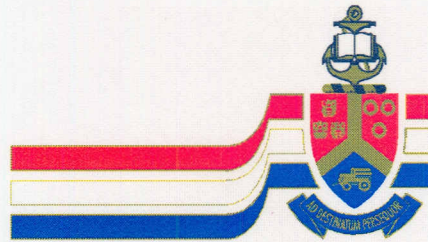
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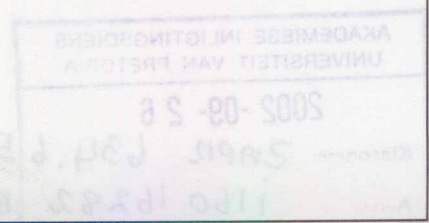
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**March 2002**





**There is a time in the life of every problem when it is big  
enough to see, yet small enough to solve.**

**Mike Leavitt**

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## DECLARATION

I declare that this dissertation, for the degree of MSc(Agric), has never been submitted for any degree at any university. The research work reported is the result of my own original investigation, except where acknowledged.

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Zelda Bijzet

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Date



# **CHAPTER 1**

## **General introduction**

## 1.1. GENERAL INTRODUCTION

Root rot of avocados (*Persea americana* Mill.) is a devastating disease caused by *Phytophthora cinnamomi* Rands, and from observations and information obtained, Zentmyer (1979) concluded that root rot in South Africa was a problem of much greater importance than in California. The avocado was introduced into South Africa in the last decade of the nineteenth century and is now mainly grown in Letaba/Tzaneen, Hazyview/Nelspruit, Louis Trichardt/ Levubu and KwaZulu-Natal (Garbers, 1987; Ben Ya'acov & Michelson, 1995). For many years, the South African industry was established on Mexican rootstocks and soon 80% of the avocado trees were infected with *P. cinnamomi*, necessitating the implementation of control measures (Kotzé, 1986).

According to Kotzé (1985) the four principles on which management of avocado root rot depends are disease-free nursery plants, sound orchard practices, judicious use of fungicides and resistant rootstocks. The Avocado Plant Improvement Scheme (APIS) was initiated in 1993 with the founding of the South African Avocado Nurserymen Association (ANA) and is jointly managed by the South African Avocado Growers' Association (SAAGA) and ANA. (Anon, 1993) APIS ensures that certified nurseries produce and supply disease-free nursery plants to prospective buyers. Darvas *et al.* (1983) first reported effective chemical control of avocado root rot with the use of fosetyl-Al as a trunk injection.

The effect of *P. cinnamomi* in South African orchards has greatly diminished with the use of fosetyl-Al trunk injections, clean nursery material and sound orchard practices, including among other good soil preparation prior to planting (Broekman, 1993). It should, however, be remembered that fungicides have a tendency to become less effective after continuous usage (Kotzé, 1985). Breeding and selection of tolerant rootstocks such as Duke 7 is therefore the ultimate solution (Wolstenholme, 1987).

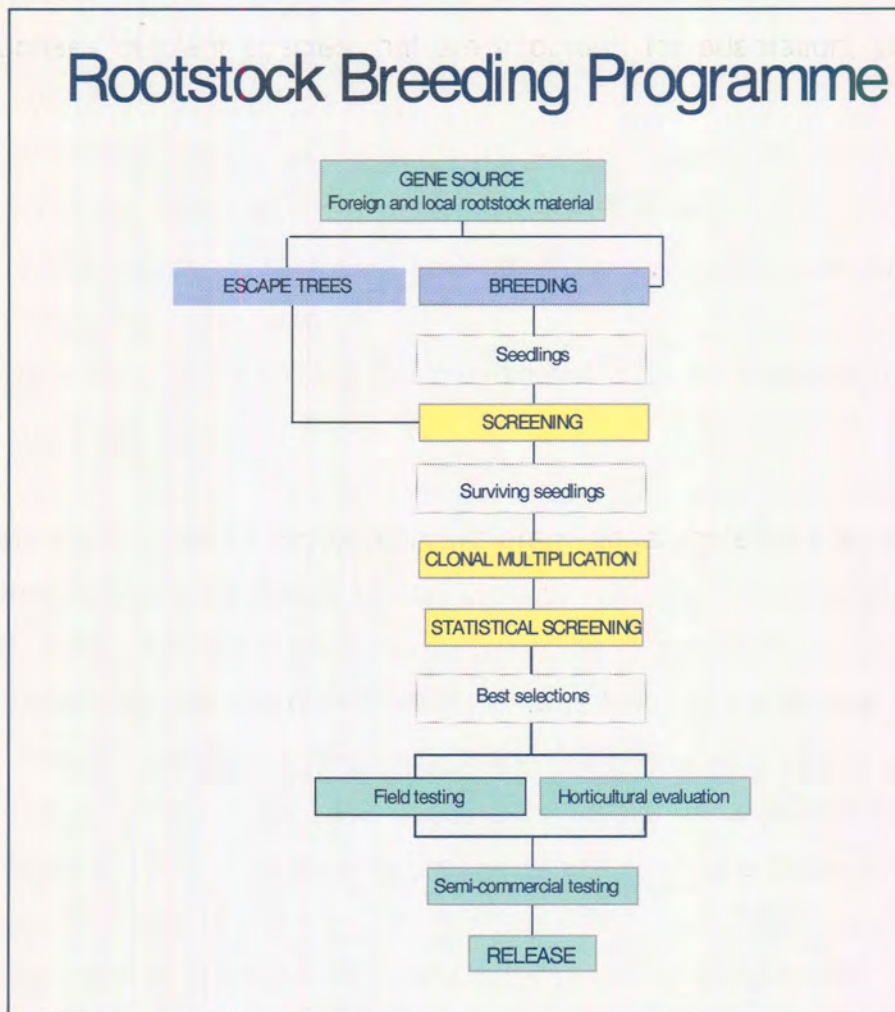
A breeding strategy for the development of avocado rootstocks with resistance to, or an increased tolerance to, *P. cinnamomi* was proposed by Bijzet *et al.* (1993). According to Poehlman (1987) the basic elements of such a plant breeding strategy are to:

1. recognise the morphological traits and the physiological and pathological responses of plant species that are important for adaptation, yield, and quality of the particular species;
2. design techniques that will evaluate the genetic potential for these traits in strains of the particular species, in this case *P. americana*
3. search for sources of genes with the desired traits that may be utilised in a breeding programme; and
4. devise means of combining the genetic potential for these traits into an improved cultivar.

Since the introduction of *Phytophthora*-tolerant clonal rootstocks as a counter to avocado root rot, the South African avocado industry had to largely rely on Duke 7. New imported rootstocks proved to be unsatisfactory and in some cases disastrous. For this reason, and the large financial impact that avocado root rot has on the South African avocado industry, the avocado rootstock breeding programme of the ARC-Institute for Tropical and Subtropical Crops was initiated in 1992. The major objective was to develop a range of avocado rootstocks that are tolerant to *Phytophthora* root rot. Bijzet *et al.* (1993) described various stages in the rootstock breeding programme, which is outlined in Figure 1.1 and summarised below:

Rootstock material of local and foreign origin is maintained in a gene source block at Nelspruit. This plant material is utilised in a breeding programme and the resulting seedlings undergo an initial screening regarding resistance/tolerance to *P. cinnamomi* to eliminate inferior genotypes. Material recovered from apparently resistant trees found in the field also undergoes an initial screening. Selections, comprising single seedlings from the initial screening, are multiplied and incorporated in a statistical screening of clonal material. The objective is to determine their performance relative to the clonal Duke 7,

which is regarded as a standard or control rootstock in South Africa as it is tolerant to *P. cinnamomi* and the dominant rootstock in the industry. Selections with a performance better than Duke 7 are promoted to a field evaluation and are incorporated into horticultural trials to determine their production potential. Only selections passing both these field tests are released as new rootstocks.



**Figure 1.1** Different stages in the avocado rootstock breeding programme

In the period 1992 to 1997, 38 984 seedlings have been screened and 91 selections were made for further testing. During this time various techniques for breeding and screening have been used. These methods were largely unscientific, resulting in a crude selection process. In Chapter 3 constraints were identified with regard to each of the four elements in the breeding strategy as outlined previously, the most profound being the specific

constraints with regard to combining the genetic potential of the various traits into an improved cultivar and the crude screening process.

It is interesting to note the sequence in which Poehlman (1987) mentions these four elements. Combining the genetic potential into an improved cultivar is only fourth on his list. This is because although hybridisation is the heart of developing rootstocks with improved tolerance or resistance, it is futile if there is no way of detecting the beneficial genotypes. In order to design techniques for the detection of the beneficial genotypes, the physiological and pathological responses of the plant to the pathogen, i.e. the host/pathogen interaction, must be known to a certain extent.

The practical research aspect of this dissertation was thus aimed at optimising the techniques for the detection of beneficial genotypes, taking into account the interaction between *P. americana* and *P. cinnamomi*. Questions that are addressed here include (i) the correct medium for screening, (ii) fast and effective cloning of single selections, and (iii) evaluation of clonal material with regard to *P. cinnamomi* tolerance.



## **CHAPTER 2**

### **Literature review**

## 2.1. INTRODUCTION

In the selection of avocado (*Persea americana* Mill.) rootstocks with an enhanced tolerance/resistance to *Phytophthora cinnamomi* Rands, there are three aspects involved: the host, the pathogen and the interaction of the host with the pathogen. The most important issues pertaining to these three aspects are discussed below.

## 2.2. THE HOST

### 2.2.1. AVOCADO ROOTSTOCKS

In South Africa, the predominant desired trait for avocado rootstocks is adequate tolerance to *P. cinnamomi* root rot. Only limited resistance to the fungus is known in certain lines of avocado and *Persea schiedeana* Nees. Rootstocks with dwarfing characteristics, as well as rootstocks with drought-, waterlogging- and freeze tolerance, could be an added benefit to the avocado industry. Tolerance to salinity is not such an issue in South Africa as it is in Israel, for instance.

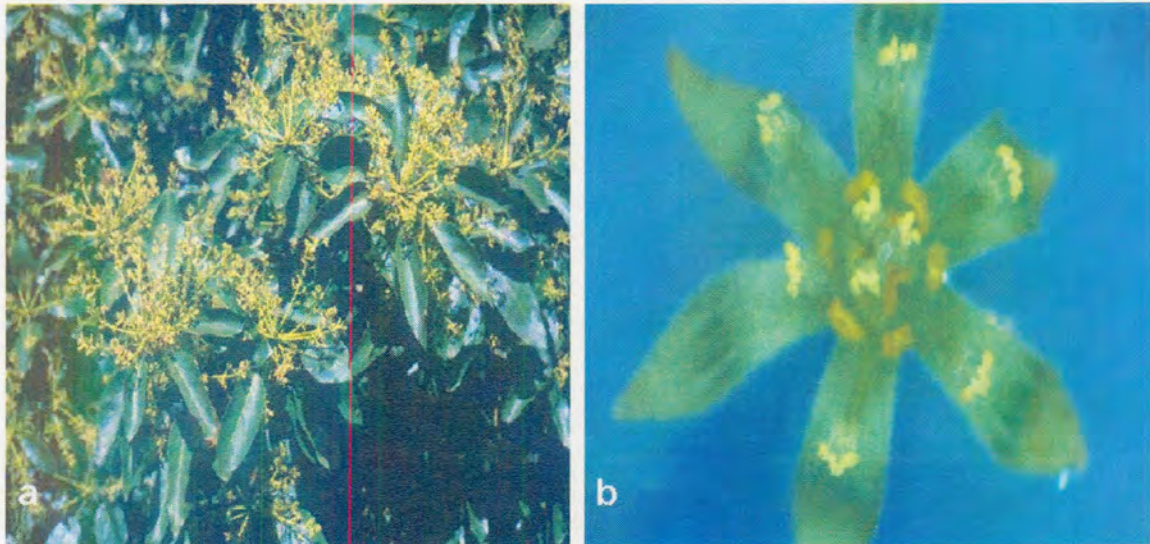
An important and often overlooked characteristic, due to the importance of *P. cinnamomi*, is the influence of the rootstock on production and fruit quality. Rootstocks play a vital role in the productivity of an avocado orchard and their selection should thus be as important as selection of the scion. An orchard can still be top-worked to replace an unproductive or redundant cultivar, but replacement of the rootstock is a very expensive operation. The host (i.e. avocado) and characteristics of the host are discussed in as far as it bears direct relevance to breeding and selection of an avocado rootstock.

### 2.2.2. BOTANY

The avocado tree is variable in shape, can either be upright or spreading, and can reach a height of up to 15 to 18 m. Grafted trees are mostly 10 to 12 m high if not pruned (Robbertse, 2001). Successful artificial crossing in a breeding programme depends on the flower morphology and behaviour. In South Africa, depending on the cultivar, inflorescence bud development starts from March to June. Bud bursting and opening of the first flowers commence

towards July and August and trees may continue to flower up to the end of September, beginning of October (Robbertse, 2001).

Avocado flowers are small and thus difficult to emasculate or pollinate (Figure 2.1). The flowers are pale yellow-green and consist of six (three outer and three inner) perianth members, nine stamens and a pistil.



**Figure 2.1** a Illustration of the size and number of avocado inflorescences, b Avocado flower showing the pollen sac valves with pollen (courtesy P.J. Robbertse)

The anther of each stamen consists of four pollen sacks, which open with valves. The pollen from each sac usually sticks in a clump to the opened valve until it is removed by insects or drops with the flower. Methods of pollen collection successfully applied in other fruit crops such as vacuum devices have not been effective with avocado (Bergh, 1975).

All avocado cultivars and seedlings, irrespective of race, exhibit a mechanism called synchronous, protogynous dichogamy, and fall in one of two complementary groups, designated A and B (Robbertse, 2001). Flowers of the A-group of cultivars open in the morning of the first day and function for three to four hours as female flowers. These flowers close to open again the next day in the afternoon, but this time functioning as male flowers for a few hours before closing for the second time. During the female phase a white papillate, receptive stigma is exposed while the stamens are prostrate with



closed anthers. During the male stage, the stamens are upright, with open anther locules, presenting the pollen.

In the B-group of cultivars, the flowers open for the first time in the afternoon as functional female flowers with receptive stigmas and closed anthers. They close in the evening and re-open the next morning as functional male flowers. Flowers of the A and B groups of cultivars are therefore synchronised (Figure 2.2). If any A-group cultivar has open flowers with functional female parts, any B-group cultivar will have open flowers with functional male parts for cross-pollination. Flowers always open in the female phase first (protogynous) (Robbertse, 2001).

	Day 1		Day 2	
Type A	♀			♂
Type B		♀	♂	
	Morning	Afternoon	Morning	Afternoon

**Figure 2.2** Synchronous, protogynous dichogamy of avocado flowers in the A and B groups

The diurnal opening and closing of the flowers is affected by low temperatures and to some extent by day length. Under favourable weather conditions with average day temperatures above 20 °C, there is very little overlap of female and male phases on the same tree. Unfavourable conditions can interfere with the opening and closing of the flowers, causing a greater overlap of the male and female phases on the same tree which will promote self-pollination. In case of not being pollinated, the unpollinated flower is abscised after the second opening.

Grafted seedling trees have a tap root system while clonal rootstock trees start with adventitious roots. Root growth in the tropics is more or less continuous, but in cooler areas it is more seasonal, alternating with the shoot flushes (Robbertse, 2001). Avocado roots have no, or at very best a few, root hairs and require high soil oxygen levels to function optimally.

Andosols are soils derived from volcanic ash and have a low bulk density of 0.4 to 0.8 g cm<sup>-3</sup>, which allows for a high air-filled porosity, and are thus ideal for avocado roots (Wolstenholme, 2001). These andosols are found in the mountain rainforests of Mexico and Central America, where the avocado evolved. The litter layer, breaking down to humus, that is present in these rainforests aids feeder root proliferation and increases the water holding capacity (WHC) of the soil (Wolstenholme, 2001). This is important, as water uptake by avocado roots is relatively inefficient. Plant media should therefore have a high air-filled porosity but at the same time support a high WHC.

The juvenile period of avocados can last for 5 to 15 years. Girdling of seedling trees can help to reduce the duration of the juvenile stage, although grafting juvenile scions onto mature rootstocks do not seem to have any effect. This is important in view of certain breeding strategies such as back-cross programmes. Fruit are also needed for the descriptive process in order to register plant breeder's rights, although the new selection is exclusively intended as a rootstock.

### 2.2.3. TAXONOMY OF THE AVOCADO

The commercial avocado belongs, like cinnamon and camphor, to the aromatic Lauraceae family. This family contains some 45 genera and over 1000 species (Bergh, 1969). The genus *Persea* is divided into two subgenera, *Eriodaphne* and *Persea*. These two genera have so far proven totally incompatible, which is unfortunate as the *Eriodaphne* subgenus, containing most of the species, have some species with resistance to root rot caused by *P. cinnamomi*.

The subgenus *Persea* includes the avocado, which Popenoe (1927) divided into three distinguishable horticultural races with distinctive characteristics. These three types were also referred to as ecological races according to their origin: Mexican, Guatemalan and West Indian. Isozyme data indicated that these races are too different to be merely separate forms, but not different enough to be separate species. They were thus classified as subspecies of *P. americana* and are respectively *P. americana* var. *drymifolia*, *P. americana*

var. *guatemalensis* and *P. americana* var. *americana* (Bergh & Ellstrand, 1986).

Originally, Popenoe (1927) used ripening time to distinguish the three races. Since then many other characteristics have been identified as being distinctive (Table 2.1). With regard to increasing tropical adaptation the racial order is Mexican, Guatemalan and West Indian, with the latter race being the only one that is well adapted to truly tropical climates (Bergh, 1992).

**TABLE 2.1:** Comparison of the three horticultural races of *Persea americana* with regard to characteristics relevant to rootstocks (Bergh, 1969, 1975, 1992; Bergh & Ellstrand, 1986)

Character	MEXICAN	GUATEMALAN	WEST INDIAN
Native region	Mexican highlands	Guatemalan highlands	Tropical lowlands
Climatic adaptation	Semi-tropical	Sub-tropical	Tropical
Cold tolerance	Most	Intermediate	Least
Heat tolerance	High	Low	High
Low humidity tolerance	High	Intermediate	Low
Salinity tolerance	Least	Intermediate	Most
Iron chlorosis tolerance	Intermediate	Least	Most

The **Mexican race** is indigenous to the cool, subtropical highland forests of Mexico, altitude 1500 to 3000 m and situated between 16 and 25° N (Bergh, 1992). It appears as though this race has the least useful genes. It is, however, of importance to the Californian industry due to its high level of resistance to cold (Bergh, 1975; Bergh & Ellstrand, 1986).

The **Guatemalan race** is indigenous to the tropical highlands of Guatemala with an altitude of up to 2900 m, but is best adapted to altitudes between 1000 and 2000 m, ±15° N. The Guatemalan race is not tolerant to extremes such as high temperatures and low humidity, but has horticultural characteristics that are much needed in a breeding programme (Bergh, 1975).

It was originally believed that the **West Indian race** was indigenous to the West Indian Islands in the Atlantic Ocean. It became evident that it rather originated in the hot and humid Pacific lowlands of the tropical Central or northern regions of South America (between 82° and 92° W and 10° N), most probably Columbia (Storey *et al.*, 1986). It is thus the most tropical of the three races and occurs from sea level to an altitude of 1000 m. The need for a hot climate with a high humidity imply that, in South Africa, West Indian avocado trees are mainly grown in the hot, humid KwaZulu-Natal coastal belt which is unsuitable for other avocado races.

There are no known sterility barriers among the three races, or among members of the *P. americana* complex. Where trees grow in close proximity, hybridisation occurs readily and a cultivar can consequently be a mixture of two or more races. Examples of such hybrids include the cultivar Fuerte, apparently a natural Mexican x Guatemalan hybrid and Hass, which is believed to be 85 % Guatemalan and 15 % Mexican (Bergh & Ellstrand, 1986; Bergh, 1992).

According to Bergh (1975) the Mexican race is genetically more resistant to *Verticillium* en *Dothiorella*, but very susceptible to anthracnose.

#### **2.2.4. ORIGIN OF VARIOUS ROOTSTOCKS**

Rootstock research received high priority at the University of California since the establishment of an avocado industry in California (Reuther, 1961). During the 1940's rootstock research in California concentrated on productivity as influenced by Mexican and Guatemalan rootstocks (Halma, 1954).

*P. cinnamomi* as the causal agent of avocado tree decline was identified in 1942, with the result that rootstock research henceforth was mostly aimed at discovering rootstocks resistant or tolerant to this disease. The search for a root rot resistant rootstock was well underway in California when the cultivar Duke was discovered in 1951 (Zentmyer, 1978). It was found to be not as tolerant to *P. cinnamomi* as *Persea borbonia* (L.) K. Spreng and *Persea*

*skutchii* Mez., which are species from the *Persea* subgenus *Eriodaphne*, but was selected due to its cold tolerance, a trait inherited from its Mexican parentage (Zentmyer *et al.*, 1963) and a very necessary characteristic in California.

The first selection made from **Duke** seedlings that were screened in *P. cinnamomi* infested soil was named **Duke 6** merely because of its position in the bed (Zentmyer & Thorn, 1956). The following year another Duke seedling was selected and named **Duke 7**. This selection seemed to perform better than **Duke 6** which often appeared chlorotic (Zentmyer, 1978). **Barr Duke** was a third generation seedling derived from **Duke 6** and was found to have an outstanding performance in a severe root rot situation as well as having dwarfing characteristics (Coffey, 1987).

The selection **D9** was the result of **Duke** budwood being subjected to gamma radiation. Although a high level of root rot resistance exists in this selection, it does not propagate as easily as the other Duke rootstocks (Zentmyer & Schieber, 1982; Menge *et al.*, 1992).

The **G6** clone is the result of budwood collected in 1971 from a tree in Guatemala. Seed from this tree was also planted and a selection **G6#1** was made. However, the parent selection **G6** performed better with regard to *P. cinnamomi* resistance. Although **G6** originated in Guatemala, it belongs to the Mexican race (Du Plooy, 1991).

Another series of rootstocks, consisting of the **G755A**, **G755B** and **G755C** cultivars, originated from fruit collected at a native market in Coban, Guatemala, in September 1975. The fruit were said to be coyou that came from a village north-east of Coban (Zentmyer *et al.*, 1988). “Coyou” or “Chucte” are names given by the Guatemalan natives to fruit of *P. schiedeana*. This rootstock series was found to be more tolerant to *P. cinnamomi* than **Duke 7** (Kotzé, 1987). **G755** have been named Martin Grande after Martin Cumes who died in 1981 (Zentmyer *et al.*, 1988).

**Martin Grande** establishes easily in severe root rot situations but does not perform well in California due to the cold winter temperatures (Coffey, 1987), and has a history of chlorosis where not expected (Kotzé, 1987). Isozyme analysis confirmed in 1986 that the G755 series is a hybrid between *P. americana* and *P. schiedeana* (Elstrand *et al.*, 1986). No significant differences in tolerance between the three selections with regard to *P. cinnamomi* could be detected (Coffey & Guillemet, 1987).

**Thomas** was recovered in 1979 from a rootstock of a Fuerte tree growing in Escondido (Du Plooy, 1991). This tree survived in a root rot area where other trees had died, and is thus designated an escape tree. **Thomas** is a Mexican type and according to Coffey & Guillemet (1987) has resistance comparable to **Martin Grande**.

Another Mexican type escape tree is **Toro Canyon**. Although little is known about this rootstock, Gabor & Coffey (1990) described it as having intermediate tolerance to *P. cinnamomi*, approximately the same as **Duke 7**. Other escape trees include **G1008**, which belongs to a different *Persea* species, *Persea steyermarkii* Allen, and the **Parida**-series. Little is known about these selections (Du Plooy, 1991).

**Borchard** is a specialty rootstock from the Brokaw nursery in California suited for alkaline soils and has no resistance to *Phytophthora* root rot.

### 2.2.5. ROOTSTOCK–SCION INTERACTIONS

The ideal aim with regard to choosing a rootstock, would be to select it for a certain soil type, climate and scion. To be able to do this, different combinations of scions and rootstocks should be tested in various soil types and climatic zones. This has been attempted but proved to be of a long-term and expensive nature.

From trials done in South Africa some trends were evident. However, these trends were not significant to such an extent as to make conclusive

recommendations. The following interesting facts nevertheless emerged from various trials conducted around the world:

- **Grafting compatibility**

Generally, there are no compatibility problems within *P. americana* between rootstock and scion as no visible graft incompatibility could be detected. Among trees of different *Persea* species, avocado is only compatible with species belonging to the subgenus *Persea* and not with those of the subgenus *Eriodaphne*. Visual ranking in California confirmed that the scion-rootstock unions of **G755A**, **G755B** and **Borchard** were significantly different from the other rootstocks and an experimental rootstock, **G1033**, which had a noticeable bulge at the bud union (Du Plooy, 1991). In comparison to the other two **G755** selections, trees on **G755C** have very smooth bud unions (Du Plooy, 1991).

- **Precocity and yield**

Rootstock type does have an effect on productivity in all cultivars investigated (Ben-Ya'acov *et al.*, 1993). The ranking of productivity of rootstocks on the basis of yield per tree can change when they are evaluated according to yield per unit of occupied area (Ben-Ya'acov *et al.*, 1993). The productivity as influenced by rootstock-scion combination has been very consistent over years (Ben-Ya'acov *et al.*, 1993).

Dwarfing and productive rootstocks as well as vigorous and non-productive rootstocks can be found in each of the three horticultural races of avocado. Some rootstocks reported with a dwarfing effect such as **D9** only had a dwarfing effect for the first three years (Du Plooy, 1991).

Rootstock-scion combination is important in itself and in some cases, a certain combination is non-productive while the rootstock or scion of this combination is productive with another partner. The **G755** rootstock is not agreeable with the scion **Hass** as very low yields were obtained from **Hass** on **Martin Grande** as opposed to **Hass** on **Duke 7** (Sippel *et al.*, 1994).

- **Rootstock effect on quality**

It is known that quality characteristics of fruit can be influenced by the rootstock in citrus (Bitters, 1961; Gardener, 1969; Monteverde *et al.*, 1988; Recupero *et al.*, 1992.). Little, however, has been done to evaluate horticultural attributes such as tree productivity, vegetative vigour and fruit quality of avocado rootstocks (Arpaia *et al.*, 1992). **Hass** fruit size was the same on both productive and unproductive rootstocks (Arpaia *et al.*, 1992). Köhne (1992) found that **Hass** fruit produced on **Duke 7** were shorter and rounder with larger seeds than on two other rootstocks. It was postulated by Smith (1993), that post-harvest quality of **Fuerte** fruit was influenced by the rootstock.

According to Arpaia (1993), fruit dry weight was little affected by any of the five rootstocks used in a trial. Kadman & Ben-Ya'acov (1976) found that fruit from scions on Guatemalan rootstocks had a higher oil percentage than those on Mexican rootstocks. In Australia, Mexican rootstocks induced maturity earlier in the season than other rootstocks (Young, 1992).

In Israel it was observed that the harvest period was influenced by different rootstocks (Ben-Ya'acov & Michelson, 1995). The rootstock can also affect the fruit quality by its influence on the uptake of nutrients (Bingham & Nelson, 1971).

- **Rootstock effect on climatic adaptation**

It was found that a frost resistant rootstock could not confer frost resistance to a sensitive scion (Krezdorn, 1973).

- **General rootstock aspects**

Bergh (1969) made the following observation because seedling rootstocks were still the norm at the time:

The Mexican race is well adapted to California. The other adapted race, the Guatemalan, is more susceptible to cold, high pH chlorosis, *Verticillium* wilt

and *Dothiorella* canker. Mexican rootstocks are also generally used in South Africa and Israel. This is largely because of availability. Guatemalan rootstocks in Israel had a chlorosis problem and West Indian rootstocks gave poor results in South Africa. West Indian seedlings are preferred in Central America and the Philippines as rootstocks. Mexican rootstocks have been reported as unsatisfactory in Australia.

The West Indian race as either rootstock or scion has proven most tolerant to high salt injury in Texas and Israel, whereas the Mexican race is most susceptible in this regard.

#### **2.2.6. CURRENT ROOTSTOCKS IN USE**

Rootstocks have primarily been used in avocado to produce true-to-type scions. Selecting the right rootstock has become important, as it is evident that rootstocks have an influence on the scion with regard to characteristics such as fruit quality and productivity, and thus ultimately on the income produced by an orchard.

To date **Duke 7** proved to be the tried and tested rootstock for most situations in South Africa. However, due to market sophistication and competition, a wider selection of rootstocks is needed to reduce input costs as well as the risk of building an industry on one rootstock.

The Mexican race has provided nearly all of the rootstocks used in California as can be seen from Table 2.2 (Gabor *et al.*, 1990).

Rootstocks currently being investigated in South Africa include a few local selections. Five of these selections were included in trials done at the University of California (Roe & Morudu, 1999). The results are summarised in Table 2.3.

**Table 2.2** Californian rootstocks and their relevance to South Africa

Rootstock/ Selection	Horticultural race and geographic origin	Relevance in South Africa
Duke 7	Mexican Riverside, California Duke Seedling	Duke 7 is currently the preferred rootstock for optimal soil conditions. This rootstock is the best suited to Pinkerton and Hass with regard to yield. Both G6 and Thomas were found to be more suited to Fuerte as a rootstock than Duke 7.
Martin Grande (G755 series)	Hybrid <i>P. americana</i> x <i>P. schiedeana</i> Coban, Guatemala Market collection.	Available in South Africa. It is highly <i>Pc</i> tolerant and suitable to marginal soil conditions as it is a better forager. It is cold sensitive and thus only suitable for warmer areas. It is a vigorous grower and not suitable for Hass due to low productivity.
Thomas	Mexican Escondido, California. Field collection, root rot area.	Equal in <i>Pc</i> tolerance to G755. Under optimal conditions, productivity of Hass on Thomas is equal to that of Hass on Duke 7, but it is significantly lower under stress conditions. Thomas is a good rootstock for Fuerte and Ryan, especially with regard to yield determined on a tree volume basis.
D9	Mexican	This rootstock was tested in South Africa, but was not accepted as it proved unsuccessful as a substitute for Duke 7.
Barr Duke	Mexican	This rootstock was tested in South Africa, but was not accepted as it proved unsuccessful as a substitute for Duke 7.
Toro canyon	Mexican Toro Canyon, California. Field collection, root rot area	Not currently used in South Africa
G6	Mexican Acatengo volcano, Guatemala.	Being phased out because it is inferior to Duke 7. It is also suspected that an earlier source of G6 was infected by the sun blotch viroid. Not suitable for Hass, but seems to produce good yields with Fuerte especially with regard to yield determined on a volume basis.
Borchard	Mexican	Not <i>Pc</i> tolerant but good for high salt conditions Not currently used in South Africa

*Pc* = *Phytophthora cinnamomi*

**Table 2.3** Summarised results of rootstock evaluations done in California

Site	Comparison
7 year trial at South Coast Field station (SCFS) (low <i>Pc</i> pressure)	Hass yield on Duke 7 > <b>Merensky 2</b> > Thomas > Borchard > D9 (Merensky is a vigorous rootstock. Other South African selections not included)
8 year trial at SCFS (low <i>Pc</i> )	Hass yield on <b>Jovo</b> > Thomas > Toro Canyon > Parida
2 year trial at Camarillo (Heavy <i>Pc</i> )	Health of trees on Spencer > Vc256 > Thomas > <b>Merensky 4</b> > <b>Merensky 3</b>
From greenhouse screening trials	In terms of <i>Pc</i> tolerance: G755A = Thomas > Pp4= <b>Merensky 3</b> > <b>Merensky 2</b> > <b>Merensky</b> > Duke 7 > Velvick

*Pc* = *Phytophthora cinnamomi*

The rootstocks Merensky, Merensky 2, Merensky 3, Merensky 4 and Jovo are local selections made by Westfalia during the 1980's. An escape tree in a Fuerte orchard with seedling rootstocks gave rise to a selection designated SA-RS97/1. This rootstock was found tolerant to waterlogging as well as to *P. cinnamomi*.

Release of these new rootstocks, with appropriate knowledge regarding the rootstock influence, is anticipated in the near future.

## **2.3. THE PATHOGEN**

### **2.3.1. PHYTOPHTHORA CINNAMOMI**

The genus *Phytophthora* is closely related to the genus *Pythium* and both genera are classified in the family *Pythiaceae*, so named because the genus *Pythium* was described first (Erwin & Ribeiro, 1996). In the genus *Phytophthora*, 67 species have been described. Most of these are important plant pathogens that cause significant production losses in a wide range of agricultural and forestry based industries in the tropical and temperate zones of the world (Zentmyer, 1980).

*P. cinnamomi* is a soilborne plant pathogen that was first described by Rands (1922) as the causal agent of stripe canker on cinnamon trees (*Cinnamomum burmanii* Blume) in Sumatra. Since the discovery of *P. cinnamomi* it has spread to, or had been found in, 70 countries all over the world as a pathogen of over a 1000 plant species (Zentmyer, 1985). *P. cinnamomi* on avocado was first described by Tucker (1927) in Puerto Rico.

In South Africa, *P. cinnamomi* was first described on avocado in 1931 (Doidge & Bottomley; 1931; Wager, 1931). Von Broembsen & Kruger (1985) presented evidence that *P. cinnamomi* may be indigenous to the South Western Cape Province of South Africa, as the pathogen was isolated from many native plants in undisturbed locations and also from rivers flowing from remote mountain areas.

From the host list of *P. cinnamomi* it is notable that the pathogen prefers woody plants and hosts in the herbaceous and vegetable groups are relatively scarce (Zentmyer, 1980). Host species are included in the families, Proteaceae, Fabaceae, Myrtaceae, Lauraceae, Epacridaceae, Pinaceae, Ericaceae, Fagaceae, Rutaceae and Cupressaceae. Important non-hosts include most grains, banana, coffee, cotton, sugarcane and many vegetables. Monocotyledonous plants are rarely hosts of *P. cinnamomi* (Zentmyer, 1980).

### 2.3.2. TAXONOMIC POSITION OF *PHYTOPHTHORA CINNAMOMI*

The genus *Phytophthora* was first so named by Anton de Bary in 1876. The name *Phytophthora* is derived from Greek that literally means *phyto* (plant) and *phthora* (destroyer) (Erwin & Ribeiro, 1996).

*Phytophthora* is commonly referred to as a fungus and has along with other oomycetous microorganisms been part of the Kingdom *Fungi*. Erwin & Ribeiro (1996), however, discussed the concept proposed by Dick (1995a, b) that *Phytophthora*, based on its evolutionary phylogeny, belongs in another kingdom, *Chromista*. This kingdom has only recently been acknowledged (Cavalier-Smith, 1986, 1987; Barr, 1992; Dick 1995a, b).

### 2.3.3. UNIQUE FEATURES OF *PHYTOPHTHORA*

Unique features of *Phytophthora*, *Pythium* and other oomycetes distinguishing them from most other fungi (Erwin & Ribeiro, 1996) are:

- The major part of the life cycle is primarily diploid whereas the higher fungi are haploid
- Cell walls are composed of cellulose and  $\beta$ -glucans and not chitin
- Mycolaminarin, a  $\beta$ -glucan, is the characteristic storage carbohydrate
- Zoospores are biflagellate, one whiplash and the other a tinsel flagellum (the flagellar rootlet morphology is similar to that of the heterokont algae)
- Exogenous  $\beta$ -hydroxy sterols are needed for sporulation as *Phytophthora* does not synthesise sterols
- *Phytophthora* and *Pythium* are uniquely resistant to polyene antibiotics such as pimaricin.

### 2.3.4. LIFE CYCLE OF *PHYTOPHTHORA CINNAMOMI*

As has already been mentioned, *Oomycetes* and more specifically *Phytophthora* are different from higher fungi. With regard to the life cycle, the main difference is that the major part of the life cycle is primarily diploid whereas the higher fungi are haploid (Zentmyer, 1983; Griffith *et al.*, 1992)

As most other species in the genus, *P. cinnamomi* produces four spore stages: sporangia, zoospores and chlamydozoospores in the asexual phase and oospores in the sexual phase (Zentmyer, 1980). These two phases are depicted in Figure 2.3.

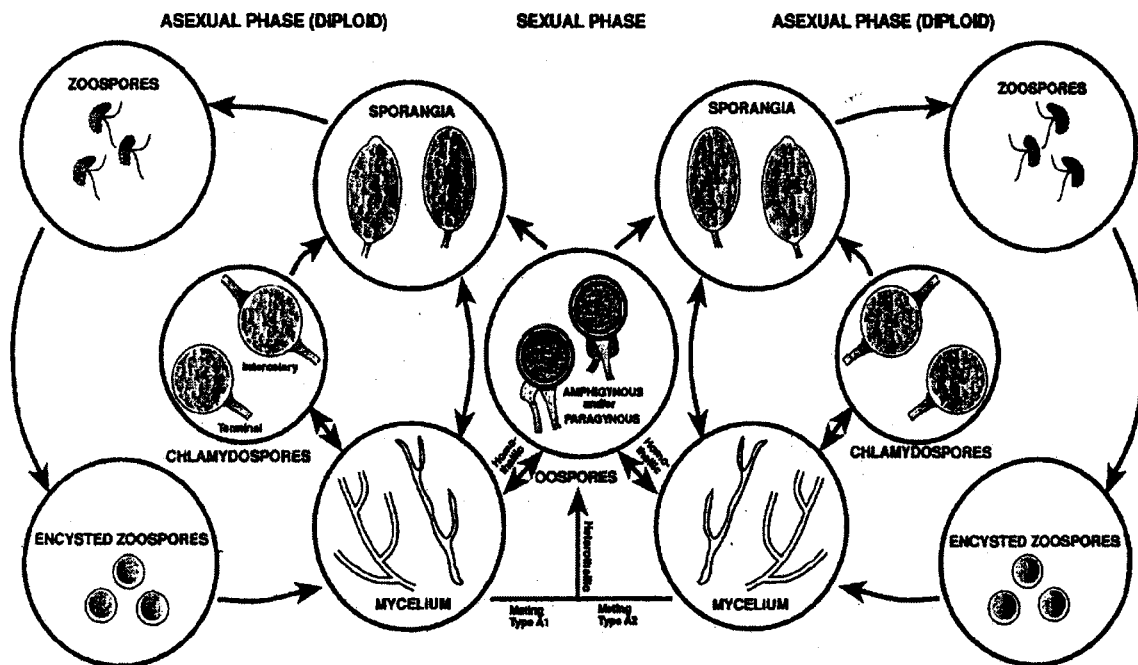


Figure 2.3 Life cycle of *Phytophthora cinnamomi* (Erwin & Ribeiro, 1996).

#### 2.3.4.1. Asexual life cycle

*Phytophthora* spp. produce asexual spores under suitable environmental conditions (optimum temperature, moisture and nutrient status.)

- **Sporangia (Gr. *spora*, a seed: *angeion*, a vessel)**

Asexual reproduction in *Phytophthora* spp. takes place by means of sporangia or more precise zoosporangia that can either germinate directly or

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differentiate into zoospores (Erwin & Ribeiro, 1996). Sporangia are produced on sporangiophores which differ slightly from vegetative hyphae but are often similar in diameter than the hyphae (Ribeiro, 1978). Sporangia vary in size and shape of which the latter can be spherical, subspherical, ovoid, obovoid, ellipsoid, limoniform, pyriform, obpyriform, turbinate and obturbinate. By transmitted light microscopy, the sporangia appear hyaline to light yellow. The sporangia can either be papillate, semi-papillate or non-papillate (the *papilla* (nipple) refers to a 'plug' or mucilaginous area of the inner sporangium wall that can absorb water and has a different refractive index than the rest of the sporangium).

- **Zoospores**

In *Phytophthora*, unlike in *Pythium*, complete differentiation of zoospores occurs inside the sporangia before it is released through the apex of the sporangium or expelled into a vesicle (Zentmyer, 1983). Zoospores emerge in an amoeboid fashion through an exit pore that is smaller than the diameter of the zoospore (Ribeiro, 1978; Gizi, 1983). The zoospores are reniform in shape and with two, heterokont flagella attached near the middle of the concave side. These two morphologically different flagella facilitate the swimming of zoospores towards host tissue for infection. Zoospores can swim for hours but as soon as they cease to swim, they encyst within minutes. Encysted zoospores can either germinate directly to produce additional zoospores (repeated emergence) or to form vegetative hyphae.

- **Chlamydospores**

Chlamydospores are spherical to oval, hyaline to deep brown and can have either a thick or a thin wall. They are non-papillate and borne either terminally or intercallary. Although these structures closely resemble hyphal swellings they can be distinguished because they are delimited from the mycelium by septa (Blackwell 1949; Hemmes 1983). Their strong birefringence and the lack of a separate (oogonial) wall differentiate them from oospores. Chlamydospores are usually formed under unfavourable conditions. Weste & Vinthage (1979) observed the *in vitro* production of chlamydospores under dry



conditions in soil, gravel and in host tissue. Depending on the environmental conditions, chlamydospores will germinate to form one of the following: sporangia, vegetative hyphae or additional chlamydospores. *Phytophthora* spp. can survive as chlamydospores for at least up to six years in soil (Zentmyer & Mircetich, 1966).

#### **2.3.4.2. Sexual life cycle**

*P. cinnamomi* is heterothallic. Sexual reproduction comprises an antheridial and oogonial interaction resulting in the formation of an oospore, presumably by fusion of gametangial nuclei (Ribeiro, 1978). The oogonium can be pyriform, smooth, hyaline to yellowish and delimited from the hyphae by a septum. The antheridium is usually produced as a multinucleate swollen hyphal tip cut off by a septum. The antheridium attaches firmly to the oogonium at an early stage of development and the attachment can either be paragynous (attached to one side of the oogonium) or amphigynous (the oogonium grows through the antheridium) (Ribeiro, 1978).

After fertilisation a single, smooth, spherical, hyaline to yellowish oospore develops, that nearly fills the interior of the oogonium. These oospores have thick walls that enable them to survive outside the host. Oospores are the most resistant structures produced and can survive for many years in soil (Mckay, 1957; Duncan & Cowan, 1980).

Oospores germinate by forming a germ tube, which can either initiate mycelial growth directly, or terminate into a sporangium that produces zoospores.

#### **2.3.5. STRAINS OR RACES OF *PHYTOPHTHORA CINNAMOMI***

Pathogenicity refers to the ability of the pathogen to infect a specific host (Shaner *et al.*, 1992). Although Torgeson (1954) coined the term "physiological race", there is no evidence for physiological races of *P. cinnamomi* but varying degrees of virulence of isolates on different host were reported (Crandall *et al.*, 1945; Torgeson, 1954; Manning & Crossan, 1966a, b).

Virulence refers to the ability of a pathogen to overcome specific resistance genes present in a particular host-plant species (Shaner *et al.*, 1992).

Zentmyer & Guillemet (1981) found that an A2 isolate from avocado was highly pathogenic to avocado but not to camellia, whereas an A1 isolate from *Camellia japonica* L. caused severe reaction on both avocado and camellia. Rands (1922) also mentioned two strains, the one more virulent on cinnamon than the other. White (1937) noted that *P. cinnamomi* isolates from rhododendron and cinnamon were more virulent on avocado than an avocado isolate of the fungus. Manning & Crossan (1966a) found variation among 13 isolates of *P. cinnamomi* with regard to the degree of pathogenicity to six cultivars of *Taxus*. Manning & Crossan (1966b) also reported differential pathogenic effects on the same and different host plants, thus further proving the existence of different races of *P. cinnamomi*. Weste (1975) worked with *Nothofagus cunninghamii* (Hook.f.) Oerst. and found that both A1 and A2 mating types from Australia were pathogenic to this host but the A1 was more virulent.

Not only were significant differences found between isolates in pathogenicity to different hosts and virulence on the same host (Zentmyer, 1980) but Shepherd & Forrester (1977) also found significant differences in the growth rate of the isolates they observed. The isolates derived from baiting grew faster than those from direct plating. They speculated that the isolates from direct planting were probably heterokaryotic for growth rate determinants, whereas those obtained by baiting represented the homokaryotic effect of zoospores that have a single nucleus and thus vary less than the mycelium.

### **2.3.6. POPULATION STRUCTURE OF *PHYTOPHTHORA CINNAMOMI* IN SOUTH AFRICA**

Population genetic studies of *P. cinnamomi* are limited to two isozyme studies (Old *et al.*, 1984, 1988) and a RAPD study (Chang *et al.*, 1996). The two studies revealed a relatively uniform population structure with two A2 and two A1 multilocus isozyme genotypes of *P. cinnamomi* in Australia. A small *P. cinnamomi* population from Papua, New Guinea, showed higher levels of

genetic diversity in the A1 mating type population (seven A1 multilocus isozyme genotypes), while the A2 mating type population was resolved in only two multilocus isozyme genotypes. According to Goodwin (1997), the overall levels of genetic diversity in populations of *P. cinnamomi* from Australia and Papua were lower than expected from a heterothallic, outbreeding oomycete.

Linde *et al.* (1997) determined the population structure of *P. cinnamomi* in South Africa and found the isozyme gene diversity ( $H_{exp} = 0.115$ ) to be similar to that of the Australian *P. cinnamomi* population. The low level of isozyme diversity was also reflected in low levels of genotypic diversity in the South African *P. cinnamomi* population. A higher level of genotypic diversity was observed in the Mpumalanga Province than in the Western Cape Province but this could have been due to a smaller sample size from the latter region (Linde *et al.*, 1997).

### **2.3.7. VARIATION IN PATHOGENICITY AMONG SOUTH AFRICAN ISOLATES OF *PHYTOPHTHORA CINNAMOMI*.**

The long-term success of breeding and selection programmes partly depends on the variation in pathogenicity of the pathogen population (Linde, 1998). Linde (1998) evaluated *P. cinnamomi* isolates from South Africa for differences in growth rate *in vitro* and levels of pathogenicity towards *Eucalyptus smithii* (Donn ex Smith) in the field. The isolates differed significantly with regard to growth rate *in vitro*, as well as in levels of pathogenicity to *E. smithii* in the field. A positive correlation was found between growth rate *in vitro* and levels of pathogenicity in the field. Culture age, geographic origin and genetic background had a significant effect on the growth rate *in vitro*. Season of inoculation and average minimum temperatures at trial sites influenced the levels of pathogenicity in the field. Contrary to the *in vitro* work, however, geographic origin had no significant effect on the levels of pathogenicity in the field.

### **2.3.8. ISOLATION AND DETECTION OF *PHYTOPHTHORA CINNAMOMI***

Conventional means of isolating pathogenic fungi are unsuccessful for most species of *Phytophthora* (Zentmyer, 1980). Erwin & Ribeiro (1996) state that

the isolation of *Phytophthora* species involves the interaction of selecting freshly diseased plant tissue, placing it on the proper selective agar medium, and looking for the emergence of non-septate hyphae on the agar medium. Apart from plant tissue, isolation of *Phytophthora* can also take place from water and from soil by means of baiting with a selective host.

#### 2.3.8.1. Isolation from tissue

*P. cinnamomi* can easily be isolated from diseased avocado roots (Zentmyer, 1980). The affected tissue should ideally be in an active stage of infection since *Phytophthora* is particularly difficult to isolate from necrotic tissue (Erwin & Ribeiro, 1996). Isolation of *P. cinnamomi* from some plants, e.g. shortleaf pine and eucalyptus, is more difficult than from avocado. Small pieces of tissue can be placed on the surface of a selective medium in a Petri dish (Erwin & Ribeiro, 1996). All utensils such as the scalpel should be dipped frequently in fresh ethanol and burned off during the plating of the root pieces.

In the absence of a suitable selective medium a weak medium such as cornmeal agar or diluted V8 juice agar at about a fifth of the recommended concentration can be used. A selective medium, however, assures a higher percentage of success. Surface disinfestation of infected material is generally not required when a selective medium is used (Mitchell & Kannwischer-Mitchell, 1992). Zentmyer (1980) reported that dipping root tissue in 70% alcohol for 15-30 seconds, followed by blotting on a paper towel, is effective with regard to avocado roots.

The two most effective selective media are P<sub>10</sub>VP and P<sub>10</sub>ARP. The PVP medium was developed by Tsao & Ocana (1969) and modifications of the formula have been widely used. The selective medium P<sub>10</sub>VP contains low dosages of pimarin, vancomycin and pentachloronitrobenzene (PCNB). Pimarin is a polyene antibiotic that is active against most fungi except the *Pythiaceae* and is also not active against some species of *Mortierella*. Vancomycin is an antibiotic that is active against most Gram-negative and Gram-positive bacteria. PCNB is a fungicide that is active against most fungi except the *Oomycetes*. The selective medium P<sub>10</sub>ARP (Mitchell &

Kannwischer-Mitchell, 1992) also contains pimaricin and PCNB to inhibit non-pythiaceous fungi and includes ampicillin and rifampicin to inhibit bacteria.

The presence of *Pythium* makes isolation of *Phytophthora* from roots and soil difficult (Ribeiro, 1978). One of the methods that have been found to provide a degree of success against *Pythium* is the use of a selective medium containing hymexazol (Ribeiro, 1978). Hymexazol is a fungicide, 3-hydroxy-5-methyl isoxazole. Reducing the pimaricin dosage to 5 mg and adding 50 mg hymexazol and 100 mg PCNB l<sup>-1</sup> medium, modify the PARP (Kannwischer & Mitchell, 1978) medium to PARPH (Jeffers & Martin, 1986).

### 2.3.8.2. Isolation from soil

Isolations of *Phytophthora* spp. directly from soil were seldom successful prior to the development of selective media (Tsao, 1983). Since *Phytophthora* spp. typically are found at low densities in soil, the development of selective media that inhibit faster-growing and more numerous microorganisms has made possible the detection and isolation as well as quantification of propagules of *Phytophthora* (Mitchell & Kannwischer-Mitchell, 1992).

According to Mitchell & Kannwischer-Mitchell (1992) the method of isolation for quantitative estimates depends on the spore structure that is present and is also influenced by the species targeted. Chlamydospores of *P. cinnamomi* are easy to collect and germinate on selective media, but not so readily from other *Phytophthora* spp. This is probably because *P. cinnamomi* survives in soil as chlamydospores (Mitchell & Kannwischer-Mitchell, 1992). The method of collecting soil samples is also important with regard to quantitative estimates (Mitchell & Kannwischer-Mitchell, 1992) as *Phytophthora* propagules are more sensitive to heat, cold and drying than many members of the *Deuteromycota*, *Ascomycota* and *Basidiomycota* (Tsao, 1983).

Zentmyer (1980) found that by simply sprinkling soil crumbs on the surface of a P<sub>10</sub>VP selective agar plate, *Phytophthora* could be qualitatively detected. Zentmyer (1980) regarded this method to be highly suitable for *P. cinnamomi*. Quantitative estimates of populations of *Phytophthora* spp. from soil are

generally based on a serial dilution end point (SDEP) and a soil-dilution plating method which are fully described by Tsao (1983) and Mitchell & Kannwischer-Mitchell (1992).

### **2.3.8.3. Isolation by baiting with selective hosts.**

Since most *Phytophthora* spp. are difficult to isolate from decayed tissue or from soil, the bait method has been used for nearly a century to aid isolation (Mitchell & Kannwischer-Mitchell, 1992). Selective media were only developed during the 1960s and until then baiting with specific hosts was the only method that could be used. Various techniques of baiting, also called trapping or host-infection, have been reported (Tsao, 1983). These methods could be categorised into three groups:

1. Inserting soil or infested tissue into a wound or hole made on a fleshy fruit such as an apple
2. Planting of seedlings, rooted cuttings, etc., into soil in pots or in the field, followed by thorough wetting
3. Floating or partially immersing various kinds of bait in a soil and water mixture where the water to soil ratio is high.

For the purpose of this dissertation, only the methods applicable to *P. cinnamomi* will be discussed:

With regard to group one, Campbell (1949) reported the use of apple fruit as bait material. Holes were made in the fruit and these were filled with soil and incubated at 15-27 °C for five to ten days. The disadvantage was that other *Phytophthora* spp, *Pythium* and other soil fungi were also isolated.

With regard to group two, Zentmyer & Ohr (1978) found that *Persea indica* (L.) K. Spreng seedlings, their roots submerged in a soil suspension (5 g of soil per 250 ml of water), gave results within two to three days. For the isolation from pine soils, newly germinated *Lupinus angustifolius* L. seedlings were placed bare root in a suspension of the test soil (Chee & Newhook, 1965). This method is also successful for four other *Phytophthora* spp. Other seedlings used for the isolation of *P. cinnamomi* include *Chamaecyparis* (Roth

& Kuhlman, 1963), *Eucalyptus* and *Banksia* (Podger *et al.*, 1965), *Jacaranda* (Brodrick *et al.*, 1976), avocado (Broadbent & Baker, 1974), pine (Campbell & Copeland, 1954) and rooted pineapple crowns (Anderson, 1951; Klemmer & Nakano, 1962).

With regard to group three, Gerrettson-Cornell (1974) buried apple slices in wet soil, as well as lupin radicles immersed in 200 ml water over 25 g of soil. The percentage recovery of *P. cinnamomi* was lower with apple slices than with lupin radicles. Dance *et al.* (1975) also used lupin radicles but in addition floated pine and cedar needles in a soil suspension and found more *Phytophthora* spp. to be isolated than just *P. cinnamomi*. *Eucalyptus* cotyledons, floated on water added to soil, were used by Marks & Kassaby (1974) and Greenlagh (1978), while Linderman & Zeitoun (1977) used *Eucalyptus* leaf discs. Anderson (1951) immersed the bases of young pineapple leaves in a soil suspension.

Whole avocado fruit has been partly imbedded in flooded soil by Zentmyer *et al.* (1960) and Zentmyer & Ohr (1978), while Brodrick *et al.* (1976) used avocado 'cukes' to isolate *P. cinnamomi*. Pegg (1977) used citrus leaf pieces floated on the surface of a soil suspension. Of these the avocado fruit was found to be a very selective trap for *P. cinnamomi* (Zentmyer *et al.*, 1967; Zentmyer & Ohr, 1978). Isolating *P. cinnamomi* using whole avocado fruit depended on temperature. The most effective and rapid isolation occurred with mature fruit with green rinds at 24 to 27 °C.

#### **2.3.8.4. Isolation of *Phytophthora* from bodies of water**

Various species of *Phytophthora* have been isolated from bodies of water that are used for irrigation (Klotz *et al.*, 1959). For experimental detection of *Phytophthora*, water samples can be passed through filters with a porosity that will retain the *Phytophthora* propagules (Erwin & Ribeiro, 1996). Klotz *et al.* (1959) used bait to isolate *Phytophthora* from reservoirs in California. Lemon fruit were placed in perforated plastic bags and incubated in the canal or reservoir for two weeks. *Phytophthora* was then isolated from the diseased tissue of the lemon fruit. Thompson & Allen (1974) used whole leaves from

*Citrus jambhiri* Lush (rough lemon) as bait and found it to be more effective than the fruit. Thompson & Allen (1974) also passed water through a series of filters and then incubated the filters on P<sub>10</sub> VP agar. They found the most propagules to be retained on the 8 µm filters and thus considered zoospores to be the main type of propagule. Filter paper discs that are used to isolate *Pythium* were also suspended in reservoirs for nine days and then plated on P<sub>10</sub> ARP (Robertson, 1975). Various other seedlings and leaf baits were reported by McIntosh (1966) and Pittis & Colholm (1984).

*P. cinnamomi* was detected by Von Broemsen (1984a, b) in all the rivers of the Southwestern Cape region of South Africa. Water was passed through Nucleopore filters (45 mm diameter) after which the filters were transferred to Petri dishes and covered with P<sub>10</sub> VPH agar.

### 2.3.9. MAINTENANCE OF CULTURE

Liquid nitrogen and freeze-drying as applied to other fungi have not been equally successful with *Oomycetes* (Zentmyer, 1980).

Isolates of *Phytophthora* spp. have traditionally been maintained on common media such as cornmeal, V8 juice, lima bean and potato-dextrose agar. They have been preserved by serial subculturing or by storage for one year or more on agar slants covered with mineral oil at 6-12°C (Mitchell & Kannwischer-Mitchell, 1992). According to Erwin & Ribeiro (1996), Zentmyer and Erwin have maintained their collection at 15 °C on slants of V8 juice agar covered with mineral oil. Zentmyer (1980) found that a high percentage of cultures could be maintained under mineral oil for up to four years.

Cultures can also be stored successfully in sterile distilled water (Boesewinkel, 1976; Ann & Ko, 1990). Small blocks of agar, with actively growing *Phytophthora*, are placed successfully in autoclaved screw-capped bottles filled with sterile distilled water.

Isolates generally retain virulence in storage, but loss of virulence and genetic degeneration or attenuation of strains maintained in culture have been reported (Shaw, 1988)

### **2.3.10. MORPHOLOGY AND IDENTIFICATION OF *PHYTOPHTHORA CINNAMOMI***

Identification of many *Phytophthora* species is relatively easy. However, the morphological differences among some of the species are so small and some characteristics are so variable that the genus is considered, even by experts, to be difficult (Erwin & Ribeiro, 1996).

**For the purpose of this dissertation, isolates for identification was submitted to a *Phytophthora* expert at the ARC Plant Protection Research Institute at Roodeplaat.** The subject has, however, been studied and the illustrations in Ho *et al.* (1995) was found to acceptably facilitate an understanding of the differences amongst *Phytophthora* spp. Keys for the classification of *Phytophthora* spp. were also compiled amongst others, by Waterhouse (1963) and Newhook *et al.* (1978) and discussed by Zentmyer (1980).

Waterhouse (1963) grouped the *Phytophthora* spp. into six groups, based on a series of morphological and physiological parameters. *P. cinnamomi* is heterothallic, has non-papillate sporangia and resides in Group VI of the Waterhouse key. The most prominent morphological characteristics of *P. cinnamomi* are summarised in Table 2.4.

**Table 2.4:** Characteristics of *Phytophthora cinnamomi* (Ribeiro, 1978; Zentmyer, 1980; Ho *et al.*, 1995)

Colonies	Profuse tough aerial mycelium, sometimes appressed on cornmeal agar, uniform and radiate on V8, and rosette-like on potato-dextrose agar.
Main hyphae	5-8 $\mu\text{m}$ wide, irregular to coralloid, free branching or clustered.
Hyphal swellings	Spherical, clustered or single, mostly terminal, 20-52 $\mu\text{m}$ diameter. Hyphal swellings form abundantly in water.
Sporangiophores	Simple (unbranched) or sympodially branched and typically proliferate through an empty sporangium.
Sporangia	Ovoid, obpyriform, or ellipsoid to elongated ellipsoid and non-papillate. Sporangia are tapered or rounded at the base, noncaducous, proliferate internally and are borne terminally. Not readily produced in axenic cultures.
Zoospores	Motile by two flagella (a whiplash and a tinsel), usually uninucleate, mainly ovoid, bluntly pointed at the anterior end, with a longitudinal groove running along the zoospore body. Encysted zoospores germinate by a germ tube but occasionally the germ tube terminates in a miniature sporangium.
Chlamydospores	Globose, terminal, thin-walled (1 $\mu\text{m}$ ) and formed singly or in clusters either on parent hyphae or on new hyphal branches.
Antheridia	Predominantly amphigynous, bicellular, 12-20 $\mu\text{m}$ .
Oogonia	Spherical, 35 – 50 $\mu\text{m}$ , surface smooth, Thickness of oogonial wall 1-2 $\mu\text{m}$ or less.
Oospores	Plerotic, i.e. nearly fills the oogonium and the wall of the oospore is 1–3 $\mu\text{m}$ thick.
General	Sex organs not produced in single culture. Heterothallic.

## 2.4. THE HOST-PATHOGEN INTERACTION

### 2.4.1. SYMPTOMS ON AVOCADO TREES

The host range of *P. cinnamomi* is extensive and different types of lesions are caused on different hosts. On avocado the primary invasion is of the small absorbing (feeder) roots (1-3 mm in diameter) producing a brownish-black firm rot with little progression into larger roots of about pencil thickness (Zentmyer, 1980). After a tree has been infected, the leaves on the tree become smaller than normal and turn pale green to yellow green (Zentmyer *et al.*, 1976). As the disease progresses wilting occurs followed by a heavy leaf drop that gives the tree a sparse appearance. In advanced stages of the disease, branches die, new growth is often absent and fruit are small. It frequently happens that a diseased tree will set a heavy fruit crop as the loss of many roots has a girdling effect (Zentmyer *et al.*, 1976). Feeder roots are difficult to find in advanced stages of the disease, causing the tree to take up less nutrients and moisture (Zentmyer *et al.*, 1976). Infested container-grown avocado plants appear yellow and stunted followed by defoliation and dieback of branches from the top of the plant.

*P. cinnamomi* can also invade the trunks of avocado trees and cause cankers. The white exudation from the bleeding cankers consists of a unique seven-carbon sugar, ketoheptose, also known as D-mannoketoheptose (La Forge, 1917). Cankers on avocado are, however, more commonly caused by *Phytophthora citricola* Sawada than by *P. cinnamomi* (Zentmyer *et al.*, 1974).

### 2.4.2. MECHANISMS OF PATHOGENESIS

Biflagellate zoospores are the major infecting agents of *P. cinnamomi* (Hardham, 1995). The interaction between host and pathogen involves zoospore taxis, encystment, cyst adhesion, germination and germ tube tropism (Deacon & Donaldson, 1993).

Zoospores were observed to germinate on the surface of excised roots after attraction of zoospores by, and encystment on, susceptible avocado roots. Invasion taking place through unwounded tissue is followed within 24–36

hours by the development of a brown lesion several millimetres in length (Zentmyer, 1961). Lesions then spread rapidly in the feeder roots and mycelium of *P. cinnamomi* can be found throughout the root within 72 hours.

#### 2.4.2.1. Chemotaxis

Zentmyer (1961) studied the effect of various chemicals and weak electrical currents on motile zoospores and found that infection of avocado by *P. cinnamomi* takes place via zoospores that are chemically attracted by root exudates (positive chemotaxis) to the region of elongation of the avocado root. Zoospores are more actively attracted to the root tip than to the region of differentiation. In his work with excised avocado root tips, Zentmyer (1961) also found that zoospores encysted at different distances from excised roots. This could be in response to a concentration gradient of a stimulatory exudate from the root (Zentmyer, 1980). Germ tubes of germinating zoospores were attracted towards the avocado roots. The attraction of zoospores was specific to live avocado roots, as roots that were killed did not attract any zoospores.

Ho & Zentmyer (1977) investigated the infection of avocado and other *Persea* spp. with both the A1 and A2 mating types of *P. cinnamomi*. They concluded that:

- The root tips of susceptible as well as resistant *Persea* spp. equally attracted both mating types.
- Germ tubes of both mating types penetrated the root epidermis directly and colonised the cortical tissue of both susceptible and resistant *Persea* spp.
- Brown lesions appeared within 24 hours on *P. americana* as well as *P. indica* and involved the whole feeder root.
- Brown lesions on the resistant species (*Persea pachypoda* Nees and *P. borbonia*) were smaller and confined to the root tip
- The A2 type isolate from avocado was more virulent to avocado than the A1 type isolated from camellia.
- Exudates from *P. americana* and *P. indica* consisted of eight different amino acids.

Zentmyer (1980) found that the rate of root attack was reduced if the leaves of avocado seedlings were removed partially or completely or by girdling the larger roots. This was because zoospores were less attracted by the feeder roots of the manipulated plants than by the roots of undisturbed plants. According to Zentmyer (1980) the implication of this is that products formed by the leaves move to the roots and are involved in chemotaxis as stimulatory exudates e.g. amino acids, sugars and possibly growth hormones. It is also plausible, however, that leaves produce growth hormones (auxins) that in turn stimulate root growth and spores are attracted to actively growing roots.

Davis & Menge (1977) found that endotrophic mycorrhizal roots of avocado seedlings were infected more severely than nonmycorrhizal roots.

#### **2.4.2.2. Electrotaxis**

Khew & Zentmyer (1974) noted three basic types of electrotaxis, namely attraction, repulsion and immobilisation. Zoospores were found to be negatively charged as they were readily stained by positive stains such as fast green, neutral red, safranin and crystal violet. No staining could be achieved with negative stains. Micro-electrophoresis further showed the zoospores to move towards the anode in an electrical field.

#### **2.4.3. INVASION**

Scanning electron microscope studies showed that, within one hour from the accumulation of zoospores on the root surface of avocado roots, germ tubes had already penetrated the epidermis (Ho & Zentmyer, 1977). Within 48 hours intercellular as well as intracellular mycelium was found in the cortex of the roots and rapid collapse of the parenchyma cells occurred (Ho & Zentmyer, 1977). Four to six days following invasion hyphal swelling and chlamydospores were formed. After six to eleven days oospores were detected in roots invaded by the A2 mating type (Zentmyer, 1952).

No differences were found in the mode of penetration and early post-penetration development of the pathogen between resistant and susceptible

species of *Persea*, Disease development was, however, slower in the resistant species.

Mycelium of *P. cinnamomi* is also able to invade avocado roots (Zentmyer, 1980). This infection was observed when excised roots of young avocado seedlings were placed on the surface of a *P. cinnamomi* culture on PDA. No sporangia or other spores were present and invasion took place in the region of root elongation as with zoospore infection.

#### **2.4.4. TYPES OF RESISTANCE AND TERMINOLOGY**

##### **2.4.4.1. Variance in pathogenicity**

Pathogenicity reflects the ability of a fungal pathogen to infect a specific host (Shaner *et al.*, 1992). Variation in pathogenicity among isolates within a species has long been recognised. Loss of virulence with continued culturing is not an uncommon phenomenon (Erwin, 1966). Recognition of this type of variation is of paramount importance to plant pathologists and plant breeders.

Aggressiveness, on the other hand, is a term applied to the pathogen to account for different degrees of ability of the pathogen to parasitise the host and has a quantitative connotation.

##### **2.4.4.2. Pathogenicity: The race concept**

Black (1952) identified four dominant genes for resistance to *Phytophthora infestans* (Mont.) de Bary in *Solanum demissum* Lindl. and, based on his genetic analysis, postulated 16 different races of *P. infestans*. Since then all 16 races have been isolated in nature (Ribeiro, 1978). Toxopeus (1956) then postulated a gene-for-gene relationship for the *Phytophthora:Solanum* system. The gene-for-gene relationship states that for each gene determining resistance in the host plant, there is a specific gene determining virulence in the pathogen (Flor, 1956). Van der Plank (1963) coined the term “vertical pathogenicity” which was based on certain populations of *P. infestans* being pathogenic to one or more potato host cultivars except for the cultivars with specific inherited resistant (R) genes. Robinson (1969) avoided the term ‘race’ and rather preferred “vertical pathodemes” or “vertical pathotypes”

Van der Plank (1963) described two types of resistance in host plants:

**Vertical resistance** (Monogenic, major gene, hypersensitivity) is resistance due to a single dominant gene and is effective against certain races and ineffective against other races of the pathogen.

**Horizontal resistance** (Polygenic, minor gene, field resistance, non-specific resistance, multigenic resistance) reduces the amount of disease development by slowing down the rate of increase of the disease and is related to various components of the pathogenic process. The resistance is not race-specific, i.e. it does not show a differential cultivar x race interaction.

## **2.4.5. RESISTANCE MECHANISMS**

### **2.4.5.1. External defence mechanisms**

The external surfaces of the host plant are specific with regard to composition, texture and function that can contribute to one or another defence mechanism such as (Erwin & Ribeiro, 1996):

- The release of chemicals that keep the pathogen at a distance
- A recognising mechanism of the host that prevents the pathogen from entering the plant
- Physical barriers such as wound tissue and callus that form.

### **2.4.5.2. Internal defence mechanisms**

Internal defence mechanisms can include (Erwin & Ribeiro, 1996):

- An impenetrable cork layer close to the root tip that can not be broken down by the enzymes of the pathogen. The disease becomes localised.
- Toxins that are produced and stored and which are released if an irritation, caused by the pathogen, occurs.

### **2.4.5.3. Induced resistance**

Various mechanisms can be involved when a defence mechanism is established following a previous infection. These defence mechanisms represent the specific resistance (genetic resistance) with regard to a specific pathogen (Erwin & Ribeiro, 1996).

#### 2.4.5.4. Escape mechanisms

Escape is defined as the situations where an inherently susceptible host appears to be resistant in a pathogen-infested area. This can be due to conditions not favouring infection or due to a mutation of the host in the case of clonal plants or genetic recombination in the case of a seedling (Erwin & Ribeiro, 1996).

#### 2.4.6. MECHANISMS OF RESISTANCE IN AVOCADO

It is more difficult to find host resistance to pathogens with a wide host range than with narrow host ranges and Zentmyer (1980) has presented evidence that this is particularly true for *P. cinnamomi*. Zentmyer (1952) undertook various expeditions to Central America as part of a concerted effort to find an avocado rootstock resistant to *P. cinnamomi*. These expeditions resulted in the location and importation of types of avocado and species related to the avocado. These species were screened for their resistance to *P.cinnamomi* and the results are summarised in Table 2.5.

**Table 2.5** Degree of resistance of various *Persea* spp. and related genera in the Lauraceae to *Phytophthora cinnamomi*. (Zentmyer & Schroeder, 1958; Zentmyer, 1980).

PERSEA SPP.	SOURCE	RESISTANCE
<i>P. alba</i> Nees	Brazil	High
<i>P. caerulea</i> (Ruiz & Pavon) Mez	Venezuela, Costa Rica	High
<i>P. chrysophylla</i> Kopp	Colombia	High
<i>P. donnell-smithii</i> Mez	Guatemala, Honduras	High, variable
<i>P. borbonia</i> (L.) K. Spreng	South east United States	Usually high some variability
<i>P. durifolia</i> Mez	Peru	Moderate
<i>P. haenkeana</i> Mez	Peru	Moderate
<i>Umbellularia californica</i> Hook & Arnold.) Nult.	California	Moderate
Various spp. of: <i>Nectandra</i> , <i>Ocotea</i> & <i>Phoebe</i>	Latin America	Usually moderately high
<i>P. floccosa</i> Mez	Mexico	Low
<i>P. lingue</i> (Ruiz & Pavon) Nees	Chile	Low
<i>P. longipes</i> (Schlecht.) Meissn.	Mexico	Low
<i>P. portoricensis</i> Britt. & P. Wills.	Guatemala, El Salvador	Low
<i>P. schiedeana</i>	Costa Rica, Guatemala	Usually low, some moderate
<i>P. nubigena</i> L.O. Willm. ( = <i>P. gigantea</i> L.O. Willm)	Guatemala	Generally low, some Exceptions
<i>P. americana</i>	Mexico, Central and South America	Generally low, some exceptions
<i>P. indica</i>	Canary islands	Very low

The results in Table 2.5 indicate the possibility of finding a rootstock, resistant to *P. cinnamomi*, for the avocado. This can be achieved either by grafting onto these species or by using them in a breeding programme. Frolich *et al.* (1958) tested the compatibility of species within the *Persea* genus and the results (Table 2.6) indicate that the species were either compatible with *P. americana* or with *P. borbonia* but not with both. Incompatibility was defined as the inability for a shoot to grow and survive for longer than a year .

**Table 2.6** Compatibility (graft and cross) of species within the *Persea* genus

	<i>americana</i>	Aguacate de Mico	<i>floccosa</i>	<i>gigantea</i>	<i>longipes</i>	<i>nubigena</i>	<i>schiedeana</i>	<i>borbonia</i>	<i>caerulea</i>	<i>chrysophylla</i>	<i>donell-smithii</i>	<i>durifolia</i>	<i>indica</i>	<i>lingue</i>	<i>portorcensis</i>	<i>skutchii</i>
<i>Americana</i>	+	+	+	+	+	+	+	○	○	○	○	○	○	○	○	○
Aguacate de Mico	+	+	-	-	-	-	-	○	○	○	○	○	○	○	○	○
<i>Floccosa</i>	+	-	+	-	-	-	-	○	-	-	○	-	-	-	-	○
<i>Gigantea</i>	+	-	-	+	-	-	-	○	-	-	○	-	-	-	-	○
<i>Longipes</i>	+	-	-	-	+	-	-	○	-	-	○	-	-	-	-	○
<i>Nubigena</i>	+	-	-	-	-	+	-	○	-	-	○	-	-	-	-	○
<i>Schiedeana</i>	+	-	-	-	-	-	+	○	-	-	○	-	-	-	-	○
<i>Borbonia</i>	○	○	○	○	○	○	○	+	+	+	+	+	+	+	+	+
<i>Caerulea</i>	○	-	-	-	-	-	-	+	+	-	-	+	-	-	-	-
<i>Chrysophylla</i>	○	-	-	-	-	-	-	+	-	+	-	+	-	-	-	-
<i>donell-smithii</i>	○	○	○	○	○	○	○	+	+	-	+	-	+	-	-	+
<i>Durifolia</i>	○	-	-	-	-	-	-	+	+	+	+	+	-	-	-	-
<i>Indica</i>	○	○	-	-	-	-	-	+	-	-	+	-	+	-	-	-
<i>Lingue</i>	○	-	-	-	-	-	-	+	-	-	-	-	-	+	-	-
<i>Portorcensis</i>	○	-	○	-	-	-	-	+	-	-	-	-	-	-	+	-
<i>Skutchii</i>	○	○	○	○	○	○	○	+	-	-	-	+	-	-	-	+

○ = INCOMPATIBLE

+ = COMPATIBLE

- = NOT TESTED

The basis of resistance in avocado to *P. cinnamomi* has not been clearly determined yet. Eradicating *P. cinnamomi* as a problem will be a step closer to reality once the genetic basis of root rot resistance is understood (Elstrand *et al.*, 1986). The resistance mechanism can either be chemical (i.e. phytoalexins, elicitors and inhibitors) or anatomical.

A chemical isolated from several resistant *Persea* spp. was found to be anti-fungal in nature (Schönbeck & Schlösser, 1976). As it has first been isolated from *P. borbonia*, the compound was given the name "borbonol". It has been found in tolerant *Persea* spp. as well as in some of the susceptible *Persea* spp. However, concentrations in the susceptible species were much lower than in the resistant and tolerant species (Zentmyer, 1980). *In vitro* experiments indicated that borbonol inhibits the growth of *P. cinnamomi* at  $1 \mu\text{g ml}^{-1}$  in solution culture (Ribeiro, 1978). Zaki *et al.* (1980) determined that borbonol primarily affects hyphal growth of *P. cinnamomi* as it induced abnormal hyphal swellings and excessive hyphal branching. They postulated that borbonol may inhibit some aspect of fungal development such as cell wall biosynthesis.

Another chemical response is that of root electrolyte leakage. Zoospores of *P. cinnamomi* were found to be less attracted to roots of cultivars tolerant to the pathogen than to roots of susceptible species. Zentmyer (1961) found that electrolyte leakage from roots inoculated with *P. cinnamomi* was correlated with their susceptibility to the pathogen. The composition of these electrolytes interested Botha & Kotzé (1989a) and their investigation showed that a combination of amino acids in root exudates resulted in the attraction of zoospores but that individual amino acids had little or no chemotactic effect on zoospores.

Khew & Zentmyer (1973) concluded that arginine, aspartic acid and glutamic acid attracted more zoospores than other amino acids and that different amino acids in different concentrations could explain the variance in tolerance or susceptibility of avocado cultivars to *P. cinnamomi*. This was confirmed by Botha & Kotzé (1989b) who stated that sugar content of the root exudate did

not attract zoospores but that it was the amino acid glutamic acid that attracted the most zoospores.

Phillips *et al.* (1987) were of the opinion that resistance in avocado could be explained by something more than borbonol. After inoculating Duke 7 avocado roots 4 mm behind the root tip, the histology of the diseased root revealed three zones, namely heavily necrotic, lesion end point and healthy root tissue that was uninfected. The heavily infected zone (zone 1) extended from the point of inoculation into differentiated tissue with secondary wall thickening and stellar tissue containing the vascular cambium. At the lesion end point (zone two) hyphal development became restricted to a smaller region of the cortex. Cells neighbouring the necrotic region had undergone rapid peri- and anticlinal cell division, with the possible presence of cork tissue. These cells had thickened, with intercellular spaces extending from the epidermis to the endodermis, and was identified as necrophyllactic periderm (Mullick, 1975).

Pathogen invasion is terminated in zone three and is marked by two major anatomical responses (Phillips *et al.*, 1987):

- The production of necrophyllactic periderm in the cortex, and
- The isolation of phloem bundles by the increase in cell numbers through periclinal cell division and the accumulation of suberin between central cell walls and intercellular spaces.

## **2.4.7. SCREENING FOR RESISTANCE**

### **2.4.7.1. Methods of screening**

The prerequisite for success, with regard to a rootstock breeding programme, is mass screening in excess of 10 000 seedlings per annum. The screening should be fast, reliable, stern, cost-effective and consistent.

Screening has widely been discussed in so far as laboratory techniques are concerned and include colonisation of excised root tips (Kellam & Coffey, 1985), lesion development on etiolated shoots (Kellam & Coffey, 1985; Dolan

& Coffey, 1986), electrolyte leakage (Zilberstein & Pinkas, 1987) and the detached root inoculation method (Botha *et al.*, 1989).

Other methods include the screening of candidate rootstocks in naturally infested soil (Zentmyer & Richards, 1952), infesting soil or sand by adding ground mycelium (Tsao & Garber, 1952), dipping intact roots in a spore or mycelium suspension (Klotz & DeWolfe, 1960) and growing infected rootstock seedlings in a nutrient solution. The nutrient solution test is very severe and gives results in a short time; in susceptible plants 90 – 95 % of the roots are rotted in the 12-day incubation period at 24° C (Zentmyer & Mirchetich, 1965; Zentmyer, 1982).

#### **2.4.7.2. Favourable conditions for plants and *Phytophthora cinnamomi***

According to Wilkinson *et al.* (1981), zoospores do not move very far by themselves in soil but swimming zoospores moved about twice the distance of cysts. Column experiments showed that zoospores in sand moved 35 cm behind the wetting front (free water) in sand, 44 cm in sandy clay and 48 cm in loam. They failed to move at all in silt.

Drainage of soil in pots is much less efficient than in the field, especially if plants grow slower and transpiration is limited. Adding peat moss, perlite, vermiculite, sand or redwood sawdust increased the porosity of the soil and thus improves drainage (Baker, 1957).

According to Duniway (1983) the most decisive factor that governs the severity of root disease is the length of time that the soil remains saturated or near saturation. Tippet *et al.* (1985, 1987) concluded that the progress of lesions in woody plants were influenced by the phloem-moisture ratio. In their work with Jarrah, Tippet *et al.* (1987) found that *P. cinnamomi* was much more sensitive to low water potentials in the bark than it was to those in the soil. When the relative water content in the phloem was reduced to 70 %, growth of *P. cinnamomi* ceased.

According to Sterne *et al.* (1977) *P. cinnamomi* caused very little root disease in *P. indica* plants when the soil matric potential was maintained above -250 millibar (-25 kPa) in a sandy loam soil. The percentage of diseased roots, however, increased to 100 % at 0, -50, and -100 millibar (0, -5 and -10 kPa). Gisi *et al.* (1981) and Gisi (1983) showed that maximum numbers of sporangia were formed under flooded to saturated soil conditions when the inoculum was on the soil surface. Maximum sporangia were formed at -160 millibar (-16 kPa) if the inoculum was buried 5-20 mm deep.

One of the dangers of continuous flooding to induce *Phytophthora* root rot is the development of an anaerobic condition. Some root diseases that are caused by anoxia could be confused with *Phytophthora* root rot.

## 2.5. CONCLUSION

It is evident from the literature that complex host-pathogen interactions with regard to avocado and *P. cinnamomi* exist. The currently available avocado rootstock germplasm include individuals with different tolerant mechanisms and it appears as though vertical resistance with regard to *P. cinnamomi* does not exist in *P. americana*. This factor complicates a breeding programme for resistance towards *Phytophthora* root rot as an unknown number of multiple genes have to be recombined to find a beneficial phenotype to aid tolerance with regard to the fungus.

Another complicating aspect is the detection of beneficial phenotypes. This is due to the possible variation in host-pathogen interactions. These interactions must thus be taken into account when screening of newly-created phenotypes is considered.

Knowledge of the pathogen is of utmost importance in order to ensure that a representative and pure isolate of *P. cinnamomi* is used in the screening of potential new avocado rootstocks. The literature has shown that screening results can be influenced by various physical and physiological factors as well as by other pathogens.



Development of new genotypes with regard to avocado rootstocks is difficult due to various physical constraints such as flower morphology and behaviour and, in addition to this, field trials are expensive and time-consuming. The literature should thus be utilised to optimise the screening process in order to ensure that beneficial genotypes are not overlooked or that unnecessary material does not make the breeding programme unwieldy and costly.



## **CHAPTER 3**

**Overview of the Avocado Rootstock Breeding  
and selection programme at the ARC-Institute  
for Tropical and Subtropical Crops**

**1991 - 1997**

### 3.1. INTRODUCTION

Since the introduction of *Phytophthora* tolerant clonal rootstocks as a counter to avocado (*Persea americana* Mill) 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 and the large financial impact that root rot, caused by *Phytophthora cinnamomi* Rands, has on the South African avocado industry, the avocado rootstock programme of the ARC-Institute for Tropical and Subtropical crops commenced in 1991. The major objective was to develop a range of avocado rootstocks that are tolerant to *Phytophthora* root rot. Progress was reported by Koekemoer *et al.* (1994), Breedts *et al.* (1995), Bijzet *et al.* (1996, 1997) and Bijzet (1998).

Between 1991 (when the breeding programme was initiated) and 1997, seven seasons elapsed during which 38 984 seedlings have been screened and 91 selections were made for further testing. During this time various techniques for breeding and screening have been tried and tested. These are reviewed and discussed in this chapter.

### 3.2. ROOTSTOCK BREEDING

#### 3.2.1. INTRODUCTION

With the increased importance of *Phytophthora* root rot (PRR) in the avocado orchards of California in the 1950's, a concerted effort was made to find a rootstock resistant to *P. cinnamomi* (Zentmyer & Schroeder, 1958). The *Persea* genus is divided into two subgenera; the subgenus *Persea* of which the avocado is a member and the much larger *Eriodaphne* subgenus (Kopp, 1966). A few members of the *Eriodaphne* subgenus have absolute resistance to *P. cinnamomi*, but unfortunately these two subgenera have proved to be totally (hybridisation and graft) incompatible (Frolich *et al.*, 1958; Bergh, 1969, 1992).

The avocado generally has a low resistance towards *P. cinnamomi* and the tolerance seems to be of quantitative nature. The discovery of the cultivar Duke, with some tolerance to PRR, indicated the possibility of selecting even more tolerant types, followed by vegetative propagation (Zentmyer & Thorn, 1956).

The incompatibility with resistant members of the *Eriodaphne* subgenus compels the breeder to utilise the quantitative nature of resistance to PRR found in *P. americana*. With regard to quantitative inherited characteristics, a cultivar is said to have a good general combining ability when its progeny is generally of a good quality irrespective of its mating partner. The general combining ability of a plant parent is determined by additive genetic effects of its genotype and determines whether a given strain should repeatedly be used in a breeding programme (Poehlman, 1987). Specific combining ability is shown by a particularly favourable cross, between two parents, each otherwise displaying low general combining abilities. Favourable offspring is often registered as new cultivars but usually fail as breeding parents.

The opposite of this is the cumulative effect of favourable genes. Exploitation of this property of quantitative genes is based on the use of two cultivars that are both outstanding with regard to the same quantitative characteristic. It is desirable to use two avocado rootstock cultivars that are not closely related to each other in order to minimise the number of common favourable genes in their genotypes. The progeny (F1) is planted and screened. It is often wise to intercross the F1 progeny followed by selection in the F2 generation (Poehlman, 1987; Falconer, 1989).

Since 1992, fruits from avocado rootstock plants in South Africa and from other cultivars planted in close proximity to the avocado rootstock material were harvested and the seed (Figure 3.1) germinated for screening. These seeds were thus the result of open pollination.

As was emphasised before, pollen derived from non-resistant plants detracts from the efficiency of the current procedure for producing seedlings. Breeding efforts were to be diverted more and more from the use of open pollinated sources towards controlled pollination. Avocado seedlings can be produced with varying degrees of parental certainty, and with varying advantages and disadvantages:

- In open pollination there is no certainty with regard to the male parent. Depending on the degree of isolation a high proportion of out-crossing

can be expected. The advantages is low labour and other costs permitting the rapid analysis of large seedling numbers, depending on orchard space. Disadvantages are that little is learnt of the inheritance of commercial traits and seedlings may be inferior due to uncontrolled pollination by inferior pollen.

- Semi-controlled pollination (poly-cross nurseries) where a population is isolated from pollen originating from non-tolerant or non-resistant plants. The male parent is unknown but part of a demarcated group. The disadvantage is that isolating material can be costly.
- The most can be learnt from controlled pollination where both parents are known. The cost implication to facilitate controlled pollination in the case of *P. americana* is, however, very high.



**Figure 3.1** Avocado seed after extraction ready to be germinated

### **3.2.2. MATERIALS AND METHODS:**

#### **3.2.2.1. Open pollination**

Since 1992, open pollinated seeds from avocado rootstocks and from other cultivars in the close proximity of the avocado rootstock material were germinated for screening. Bijzet *et al.* (1993), Koekemoer *et al.* (1994), Breedt *et al.* (1995) and Bijzet *et al.* 1996,1997 gave detailed accounts of the methods and is summarised as follows:

Seed was collected from selected open-pollinated trees, which may produce a high proportion of out-crossing.

A total of 122 breeding parents, from five different groups, were used:

- Seed from known *Phytophthora* tolerant cultivars = 85 breeding parents
- Seed from local rootstock and scion selections = 37 breeding parents
- Seed from imported rootstock and scion selections = 34 breeding parents
- Seed derived from other cultivars = 24 breeding parents
- Seed derived from unknown origins

#### **3.2.2.2. Poly-cross nurseries (Semi-controlled pollination):**

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 (Berg, 1969). 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.

Renovating an old shade cloth structure (Figure 3.2), of approximately 1000 m<sup>2</sup>, solved this problem. The structure consisted of six terraces, each 3 m wide, 50 m long and with 15 well-drained plant pots 1.25 m in diameter and spaced 3 m apart, giving 90 pots in total. This area was covered with shade cloth suspended on treated poles. The result is an area of approximately 1000 m<sup>2</sup> that can be isolated from other avocado plantings, enclosing pollinators and only rootstock material with potential resistance to *Phytophthora* root rot (Figure 3.3).



**Figure 3.2** Old shade cloth structure during the process of renovation.

<b>MOTHER TREES</b>		G755A	4601	Wurtz	Duke 7	Lancefield	Colin V33
G6	Lata	G755B	4603	Wilg	Barr Duke	Topa-Topa	Teague
Jovo	Zutano	G755C	4604	G22	D9	Toro Canyon	Thomas



**Figure 3.3** Poly-cross nursery: Renovated shade cloth structure with some mother trees already planted.

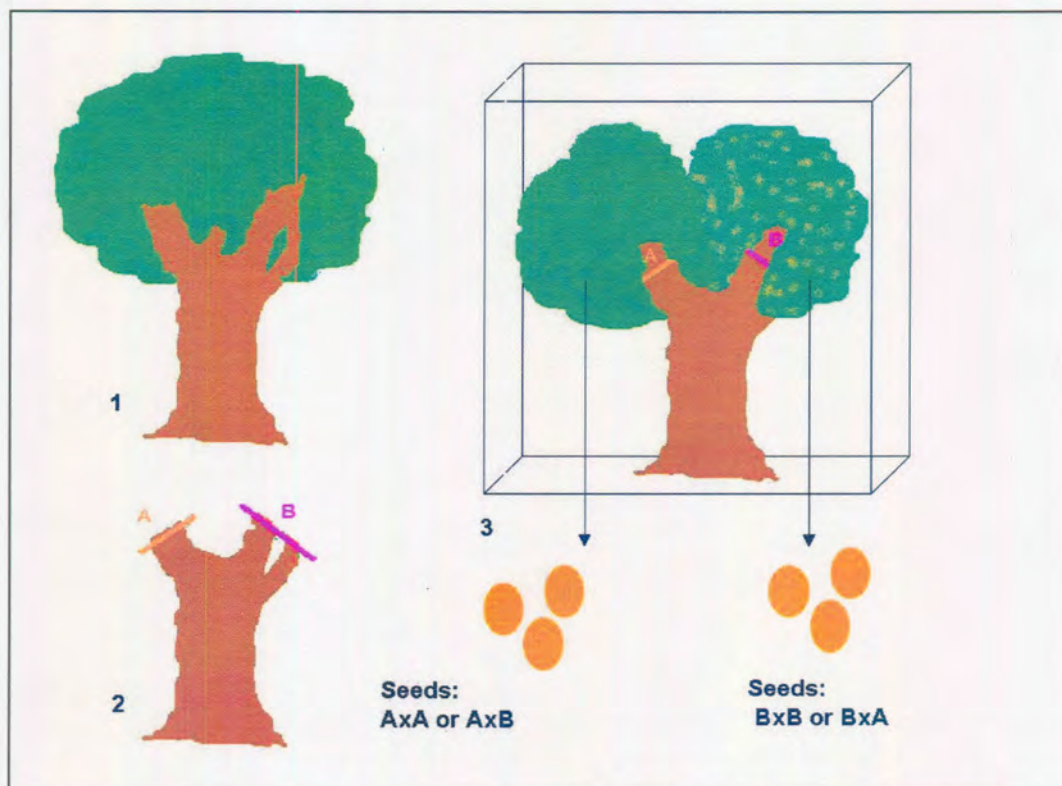
### 3.2.2.3. Controlled pollination:

With this scenario both female and male parents are known and both have a degree of tolerance or resistance to *P. cinnamomi*. This can be achieved by:

- Hand-pollination, in which case pollen is collected from flowers just after the first female stage. The pollen is then mixed with talc and applied with a small brush to the stigma of the female parent. Using whole flowers directly (like

a brush) can also convey pollen. Hand pollination was done during the first opening when flowers of the maternal parent were in the female stage and never during the second opening. Only a few flowers per inflorescence were pollinated in the mid-afternoon and only during optimal weather conditions as prescribed by Berg (1969).

- Top working the male parent into the female tree. In the absence of an ungrafted rootstock tree for a specific cross, two rootstock parents are top worked onto a decapitated stem (Figure 3.4) of an elected healthy tree, followed by caging with pollinators as soon as the new branches are flowering again. The male parent of the seed derived from any one of these two controlled situations is still unknown but the options are now limited to two possibilities as the seeds can either be the product of a self-pollination or the product of a cross between A and B.



**Figure 3.4** 1. Elected tree to be used for top working.  
 2. Elected tree after decapitation.  
 3. Elected tree enclosed in a cage, illustrating the new growth of top work A and B.  
 4. Seed extracted from fruit, harvested from each branch.

- Selfing as a result of effective isolation where fruit is harvested from a tree in the middle of an orchard consisting of a single cultivar. This is based on the assumption that most flowers will be subjected to self-pollination if there is no other cultivar within 100 m from the particular tree (Berg, 1969).
- Selfing can also be achieved by enclosing a single tree with pollinators in a cage. An illustration of such a cage is given in Figure 3.5.



**Figure 3.5** Type of cage that was used to encage top-worked and /or single trees for selfing.

Top-working and selfing have been restricted to a group consisting of *Phytophthora*-tolerant cultivars.

### 3.2.3. RESULTS AND DISCUSSION:

The general combining ability of 122 breeding parents could be determined by utilising mainly seed derived from open pollination from these breeding parents. The breeding parents can be classified into five different groups (Table 3.1).

As expected, the largest percentage of selections originated from the group of breeding parents that are known to be tolerant to *P. cinnamomi*. This group yielded 60 percent of the total selections. It was, however, also the group of which the most seed was planted, namely 18535 seeds. Another group that showed potential is that of imported selections.

**Table 3.1 Account of seed planted and selections made from the five different breeding parent groups.**

Group	Number of parents	Seeds planted	Selections made	% selections per seeds planted	% of total selections
<i>Phytophthora</i> -tolerant cultivars	85	18535	55	0.30	60.44
Local rootstock and scion selections	37	1193	4	0.34	4.40
Imported rootstock and scion selections	34	7779	23	0.30	25.27
Other cultivars	24	10037	2	0.02	2.20
Unknown origin	-	1440	7	0.49	7.69
<b>Total</b>	<b>180</b>	<b>38984</b>	<b>91</b>		

Individual parent performance and the genotypes that did not yield any selections are given in Tables 3.2 and 3.3, respectively.

**Table 3.2 Individual parent performance**

Breeding parent	Group	Seed planted	Selections	Percentage
No Name	Unknown	1440	7	0.49
Reed	Scion	484	2	0.41
Toro Canyon	Rootstock	70	1	1.43
D9	Rootstock	302	4	1.32
Zutano	Rootstock	143	1	0.70
Teague	Rootstock	705	4	0.57
Thomas	Rootstock	178	1	0.56
Jovo	Rootstock	571	3	0.53
Barr Duke	Rootstock	2961	13	0.44
Duke sdl	Rootstock	3207	11	0.34
Lata	Rootstock	293	1	0.34
Duke 7	Rootstock	4924	16	0.32
G6	Rootstock	3604	2	0.06
Western Cape mix	Local	961	4	0.42
H222	Imported	708	1	0.14
PT37	Imported	706	1	0.14
H670	Imported	342	1	0.29
Na565	Imported	530	2	0.38
I413	Imported	248	1	0.40
I399	Imported	245	1	0.41
Lohnheiss Hass	Imported	363	3	0.83
Numlioh 70	Imported	114	1	0.88
NN63	Imported	108	1	0.93
Hilcoa 5	Imported	208	2	0.96
Hass 4th generation	Imported	271	5	1.85
NN10	Imported	66	2	3.03

**Table 3.3 Summary of breeding parents that did not yield selections from 1992 to 1997.**

FEMALE	PLANTED	FEMALE	PLANTED	FEMALE	PLANTED	FEMALE	PLANTED
COLIN V33	5	G22	2374	IRVINE	29	PARIDA (P1)	267
#86	173	G755C	15	J241	195	PINKERTON	265
ADDO sel.	20	GA13	34	KARIKA	38	REGAL	5
ALLBOYCE	12	GWEN	361	LULA	20	RINTON	1082
BACON	280	H287	61	MOAZ	14	RYAN	171
BALBOA	147	HASS	548	NA66	30	SCOTLAND	47
BORCHARD	125	HAYES	154	NABAL	132	SHARWILLE	260
CANADA	123	HAZZARD	177	NDLC	8	SHEPPARD	25
COLIN V33	4	HORSHIM	1036	NUMLICH 111	296	T142	42
EDRANOL	1191	HX204	57	OA 184	229	TOPA-TOPA	1295
ESTER	934	I388	19	P-PARENT	239	TX531	558
ETTINGER	366	I392	189	P3	987	WHITSEL	401
FUERTE	54	I414	100	P6	25	WI SDL	11

No selections were made from controlled pollination situations, as the number of seeds available per season was relatively low. Specific combining abilities could thus not be determined. The hidden potential of the current rootstock population, however, is evident, even in an open pollination situation. It is also evident that large numbers of seedlings have to be screened to find suitable combinations.

The implementation and maintenance of a poly-cross nursery and controlled pollination make thus even more sense. In order to make full use of the additive effect and the exploitation of cumulative effects of favourable genes, careful consideration should go into selecting parents for a poly-cross nursery. New material arising from the breeding programme must be incorporated regularly in this poly-cross nursery. This would include material (F1) not promoted to cultivar status due to it not being substantially better than the standard Duke 7.

Other methods of controlled pollination have proved to be unpractical. Hand pollination failed not only because pollen is sparse, sticky and difficult to collect, but only a few hundred of the approximately one million flowers that are borne on a single tree, persists to maturity. Top working two rootstock cultivars onto one tree followed by encaging also failed. In Table 3.4 a list is given of combinations tested.

**Table 3.4 Combinations top-worked for controlled cross-pollination**

Combination	Parent A	Parent B	Combination	Parent A	Parent B
1	Thomas	G6	8	Colin V33	G6
2	Thomas	Toro canyon	9	Colin V33	Toro canyon
3	Thomas	Wurtz	10	Colin V33	Wurtz
4	Thomas	Duke 7	11	Colin V33	Duke 7
5	Thomas	Lancefield	12	Colin V33	Lancefield
6	Thomas	Duke 9	13	Colin V33	Duke 9
7	Thomas	Barr Duke	14	Colin V33	Barr Duke

The reason for this failure was that some of the material, such as Thomas, dominated the other top-worked branches on the same tree, as it is a very fast and strong grower. Flowering times of the cultivars also did not always coincide. New chemicals that recently became available should help to surmount these problems. The other constraint is the pollination agent that has to be implemented such as bees. Difficulty was experienced in obtaining colonies to engage with the trees. These colonies had to be very small to be able to survive on one tree. In most instances of engaging the colonies were lost. No apiarist would be prepared to place beehives with these risks involved. Some success was achieved by using blowflies, but the number of seeds obtained from these cages remained too low (15 to 30 fruit per cage) to be able to determine the specific combining ability of two cultivars. This low number of progeny did not justify the costs and effort that had been incurred by these procedures.

### **3.3. SEEDLING SCREENING FOR *PHYTOPHTHORA* RESISTANCE/ TOLERANCE**

#### **3.3.1. INTRODUCTION**

The aim of this facet was to screen as many seedlings as possible whilst keeping it cost- and time-effective.

Although hybridisation is the heart of developing rootstocks with improved tolerance/resistance it is futile if there is no way of detecting the beneficial genotypes. It is of the utmost importance that this screening process be optimised in order to make sure that right genotypes are selected. The process should neither be too strict nor too lenient. If it is too strict, genotypes could be discarded that can contribute to the gene pool. If the process is too lenient the succeeding phases will be voluminous and unwieldy. If all the interactions and contaminants are not taken into account, screening is not optimised and the process can become lenient if genotypes escape the pathogen or too strict if selections loose roots and are dying due to something other than PRR, for example sub-optimal feeding, *Fusarium*, etc.

### 3.3.2. MATERIALS AND METHODS

The first screening trial was done in 1992 and it was decided to screen seedlings by germinating seed directly into *Phytophthora*-infested soil. (Figure 3.6).



**Figure 3.6** Seeds planted directly into *Phytophthora*-infested soil.

Seedlings were inoculated once, between the plants, with pea (*Pisum sativum* L.) seeds colonised by a local isolate of *P. cinnamomi*. Sixteen weeks after inoculation, seedlings were selected by visual assessment of root lesions. An

example of the plants and roots that were observed during this assessment can be seen in Figure 3.7.



**Figure 3.7** Visual assessment of roots 16 weeks after inoculation (left) and an example of selections made (right).

Selected seedlings were then dipped in a  $2 \text{ g l}^{-1}$  captan solution for approximately one minute, before being transplanted into sterilised pine bark in 12 l plastic bags. Seven days after transplanting, seedlings were treated with fosetyl-AI. A balanced nutrient solution (Chemicult) was applied at weekly intervals at  $2 \text{ g l}^{-1}$ . The fosetyl-AI treatment was a precautionary measure as the selections were now promoted to a multiplication phase in the nursery, whilst the purpose of the nutrient solution was to boost growth of the selections for further multiplication.

In 1993 seeds were germinated in vermiculite in the greenhouse and then transplanted into vermiculite in a concrete bin with dimensions 12 x 0.9 x 0.4 m (length x width x depth). The concrete bin was lined with thick black plastic for waterproofing. Seedlings were inoculated twice with pea seeds colonised by *P. cinnamomi*, between the plants and on the vermiculite surface, followed by light irrigation. Visual assessment was done after 16 weeks following the last inoculation and selections that were made were treated as in 1992.

In 1994 the seeds were germinated in vermiculite in the greenhouse and then transplanted into a 12 x 0.9 x 0.4 m concrete bin with bottom heating and filled

with naturally infested soil. A minimum of 120 days was allowed before additional inoculum in the form of mycelium was applied. Visual assessment of roots was done within 45 days after additional inoculation. Seedlings with actively growing roots were selected and replanted in the bin and left for another 21 days after which a final selection was done. The selections were treated similarly to those in 1992.

The procedure from 1995 to 1999 was as follows: Seed was planted directly in concrete bins filled with *P. cinnamomi*-infested soil. The seeds were left to germinate and subsequently to die of *Phytophthora* root rot. Indicator plants (cv. Edranol) showed whether the disease pressure was sufficient or not. If not, a mycelium suspension was applied approximately 120 days after germination. Surviving seedlings were selected after another 45 days. If the percentage surviving selections were too high, further elimination was done after an inspection of the root systems.



**Figure 3.8** Black 50 l bins used for transplantation.

The surviving seedlings were treated as in 1992, except for the 12 l bags being substituted by black 50 l rubber dustbins that were filled with sterilised top soil (Figure 3.8). Transplanting to these bins allowed proper root expansion and

subsequent top growth, which is required for further multiplication of the selections.

### 3.3.3. RESULT AND DISCUSSION

From 1992 to 1998 a total of 38982 seeds were harvested, planted and screened for resistance/tolerance to *P. cinnamomi*. The screening process in 1992 started with a marginal infrastructure and only *Phytophthora*-tolerant parents were utilised. In 1994 additional space was negotiated and the number of seeds collected were increased by 187% from 5717 to 16381. The results of the screening process over the seven years are summarised in Table 3.5.

**TABLE 3.5 Screening of open pollinated seedlings.**

Year	Seed planted	Screen-120	Screen - 45	Screen-45m	Screen-21	Selection	Clonal Phase
1992	5717	2894	564	107	68	12	9
1993	2799	2689	1648	108	27	15	3
1994	16381	15391	5430	26	20	20	9
1995	2437	278	60	7	4	4	4
1996	3323	1508	280	190	66	10	7
1997	3646	831	308	100	93	20	9
1998	4679	1895	502	200	98	10	5
<b>Totals</b>	<b>38982</b>	<b>25486</b>	<b>8792</b>	<b>738</b>	<b>364</b>	<b>91</b>	<b>46</b>

**Screen -120** accounts for the germination and surviving (just before inoculation)  $\pm$ 120 days after planting. **Screen - 45** is the surviving seedlings 45 days after inoculation.

**Screen - 45m** is a manual selection at this time.

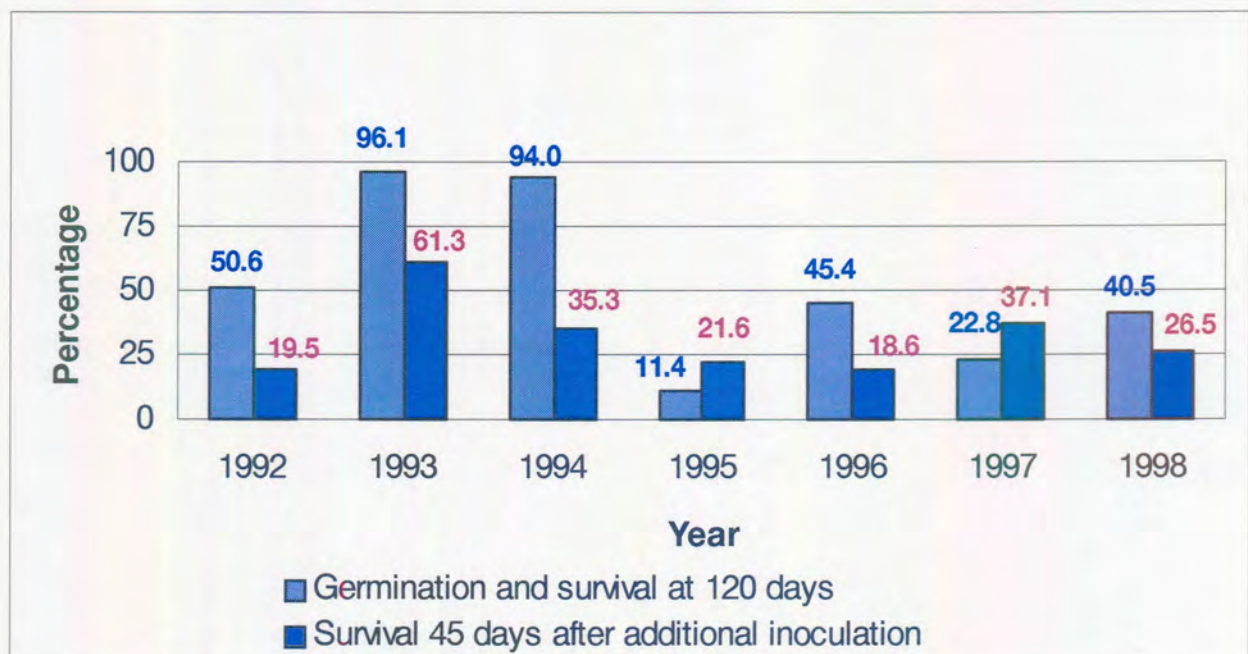
**Screen - 21** accounts for the surviving seedlings following a further 21 days after Screen 45.

**Selection** is the final manual selection at this time.

Of the 38982 seeds planted only 66 % germinated. This indicated a problem with the method of screening that was used. A total of 91 selections were made from 24486 seedlings and only 46 were promoted to the clonal phase. All of these selections were the result of a subjective visual screening process.

Various laboratory methods had been contemplated but none was considered to be sufficiently time- and cost-effective to cope with the large numbers of seed that were envisaged to be screened in a year.

In Figure 3.9 the germination and survival rate 120 days after planting and the survival rate 45 days after inoculation are compared from year to year. Germination in 1993 and 1994 was significantly higher than in the other years. These two years differed from the rest in so far as that the seeds were germinated in vermiculite and then transplanted into the screening bins, which optimised germination.



**Figure 3.9** Germination and survival from planting to 45 days after inoculation.

The low germination and survival rates in 1992 and during the period 1995 to 1998 could be attributed to the fact that germination was done directly in soil. The use of unsterilised soil from a *P. cinnamomi* diseased orchard is a complicating factor as interference by any soilborne pathogen could have inhibited germination. Edranol clonal plants included in the bins indicated that disease pressure with regard to established roots systems was not very high during the first 120 days. It is thus difficult to scientifically postulate the reason for the low germination as it could be ascribed to soil pathogens and/or soil physical properties, therefore indicating vermiculite as a better rooting medium.

In all instances, except 1993, survival 45 days after inoculation was low. Slow development of disease symptoms and low seedling mortality were evident in 1993 where the seedlings were grown and inoculated in vermiculite (Figure 3.10). However after a second inoculation and flooding of the bin, 15 *Phytophthora*-tolerant selections were made.



**Figure 3.10** Germination and screening in vermiculite showing a high germination and survival rate 45 days after inoculation.

The problem encountered with vermiculite as screening medium was at that stage reflected by the mean percentage of seedlings selected (12 selections in 1992 = 0.21 % and 15 in 1993 = 0.53%). The percentage seedlings selected in 1993 with the use of vermiculite was perceived to be too high and the procedure reverted to the use of soil. Comparing data of seven seasons illustrated the high inhibition with regard to germination due to the use of naturally *P. cinnamomi*-infested soil prior to germination. If the number of “seeds planted” (Table 3.5) and thus germination is disregarded, and the percentage of selections is calculated from the number of seedlings that were inoculated (Screen-120), the results do not differ that much (0.42 % selections in 1992 and 0.56 % selections in 1993).

It can be seen in Figure 3.11 that, due to the use of naturally-infested soils prior to germination, a large number of seed was discarded without having germinated.



**Figure 3.11** Visual assessment of seedlings and discarding of seeds that did not germinate in the naturally-infested soil.

The seedlings in Figure 3.10 that were germinated and screened in vermiculite, for *P. cinnamomi*-tolerance can again be seen in Figure 3.12 during the visual root assessment of the surviving seedlings in 1993. Compared to the result in Figure 3.11, the contrast between germination and screening in vermiculite and germination and screening in naturally-infested soil is evident.



**Figure 3.12** Visual assessment of surviving seedlings screened in vermiculite: 1993.

In the case of naturally-infested soil even the taproot was in some cases destroyed (Figure 3.13) which is contradictory to the belief that *P. cinnamomi* only attacks feeder roots.

For future breeding, the screening processes and in particular the physical condition of the growth medium and also the dispersion of the inoculum, will have to be addressed and optimised in order to create an effective and scientific screening protocol. The breeding programme is worthless if beneficial genotypes are being lost or overlooked due to a subjective screening process.



**Figure 3.13** An avocado seedling with a rotten taproot and no feeder roots.

### **3.4. CLONAL MULTIPLICATION AND STATISTICAL SCREENING**

A total of 46 selections entering the clonal phase was still a large number of selections to test in the field. The inherent resistance/tolerance as well as the degree of tolerance with regard to the standard Duke 7 had not yet been determined. A statistical and scientific method was thus needed. However, statistical comparison of selections amongst each other as well as with the standard Duke 7 necessitated clonal multiplication of the selections.

This task was left to the ITSC nursery, but it did not tie in with the normal nursery activities and it was soon reported that some of the selections were difficult to multiply. The process took too long and clonal multiplication in addition to *Phytophthora* tolerance/resistance was included as a selection criterion as easy and effective cloning of rootstocks is the basis of fruit crop production. As each new selection comprises only one plant, clonal multiplication was required to provide at least 40 plants to be used for additional *Phytophthora* tests, and this does not include plants needed for horticultural evaluations.

A suitable *modus operandi* to optimise this selection criterion is therefore needed to supply the plants required for additional *Phytophthora* tests.

### **3.5. CONCLUSION**

Each of the steps outlined in Figure 1.1 needs to be optimised. The most crucial element of these is the screening of seedlings whereas the biggest bottleneck is at the clonal multiplication process. Once these problems have been solved the breeding aspect could be attended to.



## **CHAPTER 4**

**Optimisation of the screening programme:**

**1998 - 2000**

## **4.1. SUITABILITY OF VARIOUS MEDIA FOR THE SCREENING OF AVOCADO BREEDING MATERIAL WITH REGARD TO *PHYTOPHTHORA CINNAMOMI* TOLERANCE**

### **4.1.1. INTRODUCTION**

The ultimate solution to the negative financial impact that avocado (*Persea Americana* Mill.) root rot has on the South African avocado industry, is tolerant rootstocks. The development of tolerant rootstocks is usually done by screening seedlings resulting from controlled pollination (Bijzet *et al.*, 1993; Koekemoer *et al.*, 1994; Breedts *et al.*, 1995.)

The prerequisite for success, with regard to a rootstock breeding programme, is mass screening in excess of 10 000 seedlings per annum (Bergh, 1969; Poehlman, 1987). The screening should be fast, reliable, stern, cost-effective and consistent. Screening has widely been discussed as far as laboratory techniques are concerned and include colonisation of excised root tips (Kellam & Coffey, 1985), lesion development on etiolated shoots (Kellam & Coffey, 1985; Dolan & Coffey, 1986), electrolyte leakage (Zilberstein & Pinkas, 1987) and the detached root inoculation method (Botha *et al.*, 1989). Other methods include the screening of candidate rootstocks in naturally-infested soil (Zentmyer & Richards, 1952), infesting soil or sand by adding ground mycelium (Tsao & Garber, 1960), dipping intact roots in a spore or mycelium suspension (Klotz & DeWolfe, 1960) and growing infected rootstock seedlings in a nutrient solution. The nutrient solution test is very severe and provides results in a short time; in susceptible plants 90 – 95 % of the roots are rotted within 12-days at 24 °C (Zentmyer, 1982).

Due to the bulkiness of avocado seed and seedlings, *in vitro* screening methods have proved impractical and too costly for the mass screening of 10000+ seedlings. In the period 1991 to 1997, the rootstock breeding programme of the ARC-ITSC thus reverted to screening in naturally-infested soil (Zentmyer & Richards, 1952, Bijzet *et al.*, 1993) with the exception of one season when the soil was substituted with sterile vermiculite. It was found

that germination was low in naturally-infested soil relative to germination in sterile vermiculite (Chapter 3). These methods lacked scientific merit and a suitable screening medium had to be identified.

The present trial has its origin in the insufficient comparison of the suitability of different growth media with regard to both the host and the pathogen. The objective was to identify a medium which would be conducive to the development of optimal *Phytophthora cinnamomi* Rands inoculum whilst at the same time would not impair the development of the plant. The physical requirements for both *P. cinnamomi* and the avocado host are given in Table 4.1.

**Table 4.1** Physical requirements for *Phytophthora cinnamomi* and the avocado host (Zentmyer, 1980; Wolstenholme, 2001).

Characteristic	<i>P. cinnamomi</i>	Avocado
Temperature	24	24-30
PH	6.5	5
Aeration	6.8 – 7.2 ppm O <sub>2</sub>	60-80% Air filled porosity
Moisture	Saturated to flooded	Field (container) capacity
Light	Dark	Light
Substrate-texture	Moderate to heavy	Light to moderate

Media can be divided into two different groups, namely aggregate cultures and water cultures (Mason,1990). In water culture, nutrients are dissolved in water, which is brought in contact with the roots. The water is either aerated or roots are allowed to be in contact with air as well as with the nutrient solution. Aggregates consist of small particles of chemically inert substances providing a suitable environment for the plant roots to grow. A suitable aggregate holds sufficient moisture but drains off the excess, allowing adequate aeration (Mason, 1990). Aggregate media include vermiculite, perlite, sand, gravel, scoria, pumice, diatomite, rockwool, expanded clay and expanded plastics. Choice of media is based on suitability for the pathogen as well as the host, availability, consistency and cost.

## 4.1.2. MATERIALS AND METHODS

### 4.1.2.1. Media

Five media (Figures 4.1, 4.2) were selected, namely 100 % silica sand (used in swimming pool filters), 100 % vermiculite, soil, a water culture based on the Zentmyer tank (Zentmyer, 1960) and a mist spray based on the Schwalbach system (Mason, 1990). The soil used in this study was never fertilised or cultivated before, had a clay content of 35 %, and was collected from virgin land in the Nelspruit area.



**Figure 4.1** Different growth media for the screening of *Phytophthora cinnamomi*. From left to right: soil, sand and vermiculite



**Figure 4.2** Different water cultures for the screening of *Phytophthora cinnamomi*. Left an aerated water tank and right a mist system.

Media temperatures were maintained at  $24 \pm 2$  °C. Containers used for the trial were plastic baths 485 x 350 mm with a capacity of 15 l and were filled

with medium to a depth 120 mm. Air-filled porosity (AFP) and water-holding capacity (WHC) of the soil, sand and vermiculite are given in Table 4.2

**Table 4.2** Air-filled porosity and water-holding capacity of the media

Medium	Air-filled porosity (Volume %)	Water-holding capacity (Volume %)
Sand	38.10	28.12
Soil	10.41	42.32
Vermiculite	15.20	37.48

The water culture consisted of water-filled containers in which the roots were also in contact with air provided by aerating the water in the containers with a 50 Hz pump. Water for the mist spray system was delivered by a mono-phase 0.74 kW Electro pump through a 20 mm PVC pipe (Figure 4.3). Water was fed to the mist chamber (container) via spaghetti tubing connected to a DAN mist spreader with nozzle size: violet 35 l hr<sup>-1</sup> (200 kPa). Mist was applied for two minutes with a seven-minute interval. Seedling trays were modified to support the plants for the water culture as well as the Schwalbach system (Figure 4.3). All the equipment and media were steam sterilised (Baker & Roistacher, 1957) prior to commencement of the trial.



**Figure 4.3** An improvised Schwalbach system: Water was fed from the bottom by means of spaghetti tubes (left) and the plants were supported by a modified seedling tray (right)

#### 4.1.2.2. Plants

Commercially produced, 18-month-old rooted Duke 7 plants were used. Plants were received in wedge-shaped micro-holders from Allesbeste nursery, Tzaneen. Plant height (root tip to growth point) ranged from 190 to 320 mm and the mass from 5.1 to 8.75 g. The root systems consisted of primary roots and structural and feeder roots could thus not be distinguished at this early stage.

Plants were transplanted into the sterile media and were left to stabilise and grow in the media for four weeks. During the stabilising period, the media were kept at container capacity ( $\pm 1$  kPa) that was determined for the soil, silica sand and vermiculite as described by Handreck & Black (1984). After stabilising in the media and before inoculation the plants were carefully removed from the media, washed, weighed and replanted.

#### 4.1.2.3. Inoculation

A life culture of a *P. cinnamomi* isolate (PREM 50801) was provided by J.A. Duvenhage of Merensky Technological Services, Tzaneen. A mycelium inoculum was prepared according to the method of Duvenhage & Maas (1990).

The inoculum was added to the planting media and water culture at 100 ml l<sup>-1</sup> (Duvenhage & Maas, 1990). A medium similar to that of Duvenhage & Maas (1990), but without *P. cinnamomi*, was added to the control treatments at 100 ml l<sup>-1</sup> planting medium. The sand, soil and vermiculite were flooded for 48 hours after inoculation whilst the water culture was aerated as usual. The roots of the plants grown in the Schwalbach system were dipped for 48 hours in an inoculum suspension equal to the concentration that was added to the other media and then placed back in the mist system. After 48 hours of flooding the soil, sand and vermiculite, the water was drained to container capacity. Flooding and draining were repeated twice with a 48-hour interval, after which the media were maintained at container capacity. The culture of *P. cinnamomi* that was used in this study was identified by W.J Botha of the Plant Protection Research Institute, Pretoria.

#### 4.1.2.4. Trial layout and statistics

The trial layout was a randomised block design in a greenhouse with natural light and a temperature range of 18 to 30 °C. Each of the three aggregate planting media consisted of an uninfected control (-P-F), an uninfected flooded control (-P;+F) and an infected, flooded treatment (+P;+F). The water culture and Schwalbach system consisted of an uninfected control (-P) and an infected treatment (+P), thus 13 treatment combinations in total. These 13 treatment combinations were replicated five times and each treatment combination consisted of 10 observation points (plants), thus 50 plants per treatment (Figure 4.4).



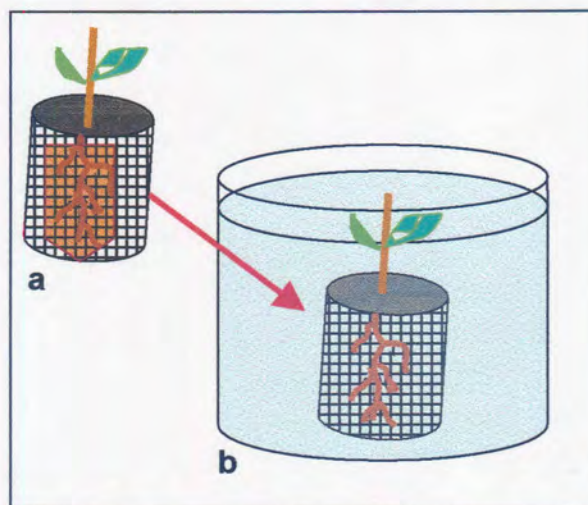
**Figure 4.4** A randomised block design with 13 treatments, replicated five times, each replicate consisting of 10 plants, thus totalling 650 plants.

Data were tested for normality with a Proc Univariate and analysis of variance was done with the GLM procedure of SAS. Means were also compared with a protected Fisher test at 95 % level of significance.

#### 4.1.2.5. Assessing the effect of flooding and *Phytophthora cinnamomi* on the plants in the various media

##### 4.1.2.5.1. Intact root system screening

Four weeks after inoculation the area of each container was divided into 10 blocks with each block containing a plant. Each block containing a plant was then carefully removed, placed in a wire screen cylinder (mesh size = 0.5 mm<sup>2</sup>) and immersed in sterile water (Figure 4.5) until the medium was separated from the roots (Bloodworth *et al.*, 1958). The plants were removed from the cylinder and the fresh mass of each plant was determined.



**Figure 4.5** a) Roots enclosed in a wire screen cylinder b) Cylinder immersed in water to remove the medium

The above-ground part of the plants were then separated from the roots. The roots were separated into structural roots and feeder roots according to the method of Van Vuuren (1997). The fresh mass of the structural roots, feeder roots and leaves (including the stem) was determined after which these components were dried at 60 °C for 48 hours to determine the physical parameters.

##### 4.1.2.5.2. Disease assessments

Prior to drying, the root systems were assessed for necrosis and root infection. The percentage of the total root system of a given plant that was necrotic was estimated visually according to the key of Duvenhage *et al.*

(1992). Brittle and excessively discoloured roots were considered to be necrotic and white to light brown fleshy roots to be alive and healthy.

Necrosis was also determined with the aid of a Geotron root measurement apparatus (WLM1). The technique involved measuring the total root length of each plant, followed by a second measurement after removal of the necrotic roots. The percentage of necrotic roots was calculated from these two measurements.

Root infection was determined by plating 10 randomly selected 1-cm-long feeder root segments of each plant on a *Phytophthora*-selective medium (PARPH) (Kelham & Coffey, 1985). *Phytophthora* recovery was recorded after three days incubation at 23 °C in the dark. Root infection was expressed as the percentage of root segments yielding *P. cinnamomi*.

#### 4.1.2.5.3. Physical parameters

The percentage increase in fresh mass for each treatment was calculated for the post –inoculation period. The dry mass of the total root component as well as the different root components (i.e. structural and feeder roots) were used to calculate the percentage of feeder roots present at the end of the trial. According to Zentmyer (1980), *P. cinnamomi* only invades the small feeder roots or absorbing roots, which have no root hairs. The fungus does not progress from the small feeder roots to the more mature secondary or structural roots and only rarely invades larger roots. The percentage feeder roots were therefore used as an indication of the plant's ability to cope with stress situations under various conditions.

The viable root to shoot ratio (VRSR) was calculated according to the formula of Ploetz & Schaffer (1989):

$$\text{VRSR} = [\text{RT} - (\text{RT} \times \text{RN})] / \text{ST}$$

where RT is root dry mass, RN is necrosis as the portion of the total root system that was necrotic, and ST is shoot dry mass.

#### 4.1.2.6. Re-isolation of *Phytophthora cinnamomi* and estimating root infection

Isolations from the media as well as the roots were done for confirmation of the presence or absence of *P. cinnamomi*.

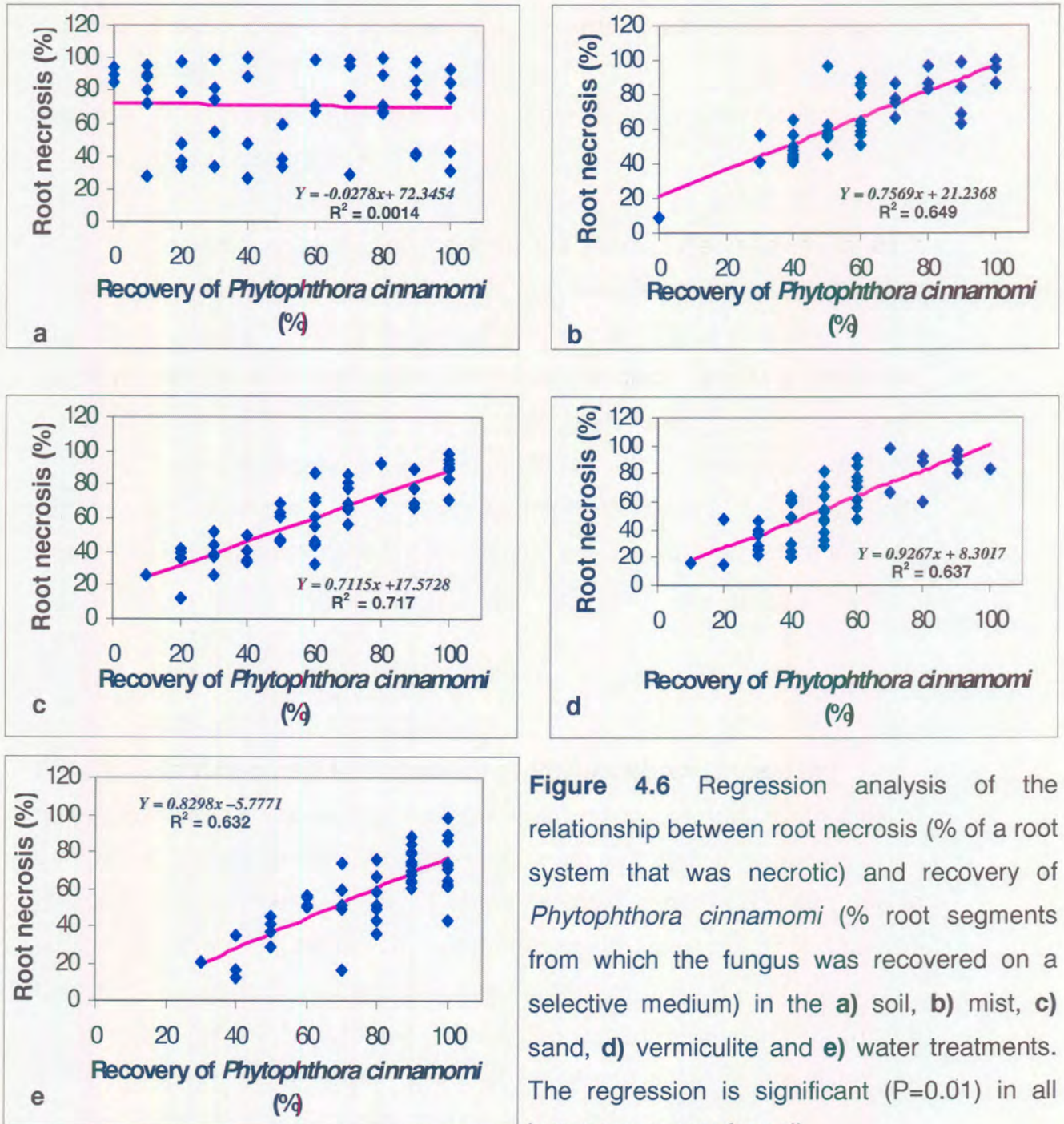
Prior to the removal of the plants from the media, the medium in each container (except for the Schwalbach system) was baited with whole avocado fruit (Zentmyer *et al.*, 1960). In addition to this, a sample of each medium per container was collected and baited with citrus leaf discs (Grimm & Alexander, 1973). Five citrus leaf discs and five avocado discs (taken from the surface of avocado fruit) per container were transferred to a hymexazol selective medium for the isolation of *Phytophthora* spp. (Tsao & Guy, 1977). Fifty healthy looking root tips per treatment were collected to ensure that infection was still in an active stage. The root tips were incubated on the medium of Tsao & Guy (1977).

#### 4.1.3. RESULTS AND DISCUSSION:

##### 4.1.3.1. Root necrosis and recovery of *Phytophthora cinnamomi*

No *P. cinnamomi* could be isolated from roots of the noninoculated treatments, although some necrosis was evident. This indicates that the necrosis found in the inoculated treatments could partly have been due to factors other than *P. cinnamomi*. Nevertheless, recovery of *P. cinnamomi* from necrotic roots in the inoculated treatments was positively correlated with the extent of root necrosis, the only exception being the soil medium (Figure 4.6). Sand, vermiculite, water, and mist are therefore suitable as screening media to test avocado plants for tolerance to *P. cinnamomi*. Soil, on the other hand, displayed a lot of necrosis that could not be attributed to *P. cinnamomi* and should rather be avoided.

Working in a peat-perlite potting medium and a Rockdale fine sandy loam soil, Ploetz & Schaffer (1989, 1992) also found a percentage of root necrosis in noninoculated, flooded and nonflooded treatments that could thus not be attributed to *P. cinnamomi*. They did, however, not explain the phenomenon.

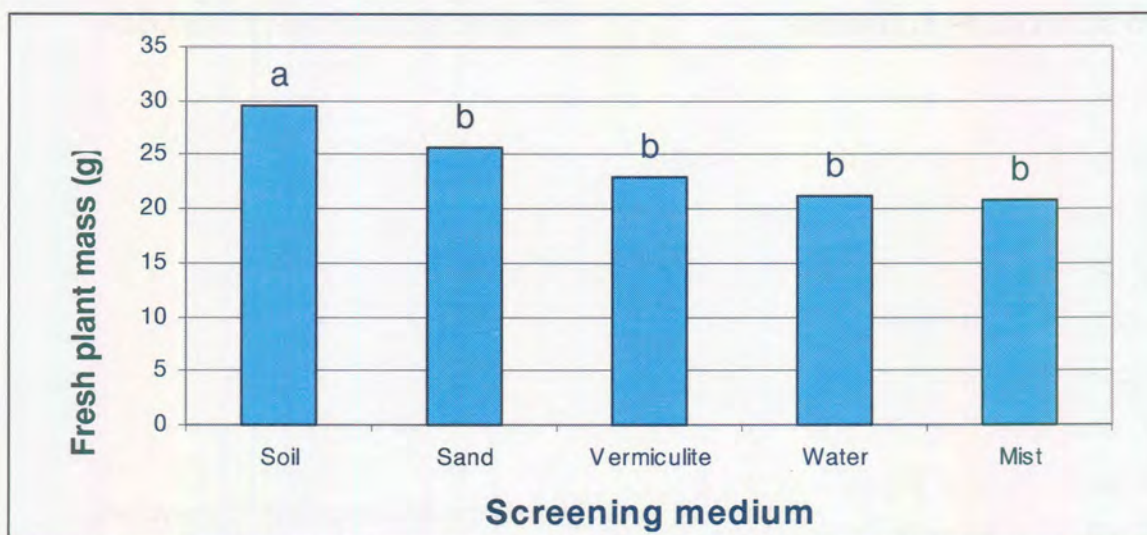


**Figure 4.6** Regression analysis of the relationship between root necrosis (% of a root system that was necrotic) and recovery of *Phytophthora cinnamomi* (% root segments from which the fungus was recovered on a selective medium) in the **a)** soil, **b)** mist, **c)** sand, **d)** vermiculite and **e)** water treatments. The regression is significant ( $P=0.01$ ) in all instances except for soil.

Since it is often difficult to recover *P. cinnamomi* from parasitised tissue (Ploetz & Schaffer, 1987), *P. cinnamomi* may have been responsible for even a larger portion of root necrosis in this study than data on the recovery of the pathogen might suggest.

#### 4.1.3.2. Plant response

Growth measured as fresh mass during the stabilising period, prior to inoculation was significantly better in soil than in sand, vermiculite, water and mist (Schwalbach system) (Figure 4.7). This was expected as soil is not an inert medium and contains minerals that are available to the plants. Growth of plants in the sand, vermiculite, water and mist did not differ significantly from each other. This indicates that moisture stress was not a factor in any of the media at this stage of the trial as irrigation was maintained at container capacity and no *P. cinnamomi* inoculum had been applied.



**Figure 4.7** Growth of clonal Duke 7 avocado plants in different media during a four week stabilising period (pre-inoculation period) (Treatments followed by the same letter do not differ significantly at  $P=0.05$ ).

After flooding and application of *P. cinnamomi* mycelium, the results of the control treatments (-P-F) indicated that overall growth of the plants had increased in the sand and vermiculite and that it had decreased in the soil, mist and water treatments (Table 4.3).

**Table 4.3** Influence of *Phytophthora cinnamomi* root rot and flooding on the growth of clonal Duke 7 avocado plants in different media

Treatment	Increase in fresh mass (%) <sup>w</sup>	Feeder roots (%) <sup>x</sup>	Necrosis (%) <sup>y</sup>	Viable root to shoot ratio	
Vermiculite	-P-F <sup>v</sup>	19.19 c <sup>z</sup>	67.85 a	9.58 fg	0.31 abc
	-P+F	23.27 bc	62.38 ab	10.94 fg	0.30 abc
	+P+F	-4.76 d	46.55 cde	56.75 cd	0.21 bcd
Sand	-P-F	36.94 a	64.67 ab	9.37 fg	0.26 bcd
	-P+F	32.97 ab	57.27 abc	12.06 f	0.34 ab
	+P+F	18.21 c	35.43 e	60.26 bc	0.17 cd
Soil	-P-F	20.95 bc	58.81 abc	26.22 e	0.23 bcd
	-P+F	-17.36 de	48.89 cd	46.86 d	0.23 bcd
	+P+F	-23.41 e	38.11 de	70.93 a	0.12 d
Water	-P	-16.93 de	56.35 abc	17.58 f	0.45 a
	+P	-23.17 e	47.19 cde	58.12 c	0.17 cd
Mist	-P	-40.08 f	69.10 a	3.20 g	0.46 a
	+P	-27.47 ef	54.05 bc	67.41 ab	0.13 d

<sup>v</sup> -P and +P = noninoculated and inoculated with *P. cinnamomi*, and -F and +F = nonflooded and flooded, respectively.

<sup>w</sup> Percentage increase in fresh mass = fresh mass in the pre-inoculation period – fresh mass in the post-inoculation period

<sup>x</sup> %Feeder roots = feeder root dry mass divided by total root mass x 100

<sup>y</sup> Percent necrosis was measured for each root system at the end of the trial.

<sup>z</sup> Means within columns followed by the same letter are not significantly different at P=0.05

The decrease of growth in non-inoculated water and mist treatments indicates that these media could not sustain growth for the given period without additional nutrients. Soil was the only medium where flooding significantly reduced overall growth. This could be attributed to the low air-filled porosity and slow draining between flooding periods.

Fresh mass was significantly reduced by *P. cinnamomi* in vermiculite and sand.

The percentage increase in fresh mass could not be used to distinguish between the uninoculated (-P) and inoculated (+P) water and mist treatments but did differ significantly with regard to the soil, sand and vermiculite. Although increase in fresh mass was significantly influenced by *P. cinnamomi*

in the soil medium it did not differ significantly from the noninoculated but flooded (-P+F) treatment.

Contrary to the belief of Kellam & Coffey (1985) no detrimental effect was experienced by the removal of the plants from the media and the subsequent baring of the roots during the weighing process prior to flooding and inoculation. This was consistent with previous observations (not documented).

Flooding had no influence on the feeder root percentage in the sand, vermiculite and soil treatments. Percentage necrosis was not influenced by flooding except in the soil treatment.

Feeder root percentages were significantly reduced by *P. cinnamomi* in all the media except soil and water. A similar tendency was evident with root necrosis. Percentage necrosis was a good indication of *P. cinnamomi* infection except in the soil treatment. Viable root to shoot ratio (VRSR) significantly distinguished between the inoculated and uninoculated sand, water and mist treatments.

#### 4.1.4. CONCLUSION

All the media tested except soil are suitable as screening media to test avocado plants for tolerance to *P. cinnamomi*. It is, however, evident from Table 4.3 that the evaluation criterion depends on the medium that is used. Feeder root percentage was a good criterion for clonal material in all the media except soil and water, whereas necrosis was a good criterion in all the media except soil where the high level of necrosis could have been due to factors other than *P. cinnamomi*.

Sand and vermiculite emerged as the best media for screening based on growth. Both these media had no adverse effect on growth of the plants after flooding. The matrix potentials of sand and vermiculite apparently were conducive to the development of *P. cinnamomi* zoospores and consequent infection of the feeder roots. The silica sand that was used in this trial is the



more expensive of the two media but can be steam-sterilised with ease whereas vermiculite tends to collapse during sterilising and would have to be replenished or replaced every screening season, making it more expensive in the long run.

Soil was an unreliable screening medium as a high incidence of necrosis, attributed to the slow drainage following flooding, was evident. Different soils would probably also give different results, indicating the risks involved in using soils.

As shoot growth in sand and vermiculite did not readily reflect the root situation, screening of seedlings would still depend on a subjective evaluation of the roots since determining feeder root percentages of 10 000 individual plants is not viable. Percentage necrosis could prove valuable as it can be done with a root measuring apparatus. Sand and vermiculite are, however, effective for the screening of genetic material that relies on root regeneration as a mechanism of resistance.

The Schwalbach system (mist) reflects stress in the root region readily in the shoots. Root regeneration is not relevant, as the effect of *P. cinnamomi* is intense and rapid in the absence of additional fertiliser or foliar feeds.

A further trial, incorporating vermiculite, sand and mist, to evaluate the effect of the medium and *P. cinnamomi* over time is recommended. This would entail assessing samples throughout the screening period for stress symptoms. Various methods with regard to stress evaluation have been disregarded due to financial constraints and it would be beneficial if these could also be included.

## **4.2. SCREENING OF CLONAL AVOCADO SELECTIONS FOR TOLERANCE TO *PHYTOPHTHORA CINNAMOMI***

### **4.2.1. INTRODUCTION**

Rootstock material, consisting of foreign and local rootstock selections, is maintained in a gene source block at Nelspruit, where it is utilised in a breeding programme (Bijzet *et al.*, 1993; Koekemoer *et al.*, 1994; Breedts *et al.*, 1995; Bijzet, 1998.). The resulting seedlings undergo an initial screening with regard to *P. cinnamomi* to eliminate inferior genotypes. Material recovered from apparently resistant trees found in the field also undergoes an initial screening. Selections from the initial screening are multiplied and incorporated in a statistical screening to determine their performance relative to Duke 7, the major rootstock in South Africa and regarded as the standard or control. Selections with a performance better than Duke 7 are promoted to a *P. cinnamomi* field test and are incorporated into horticultural trials to determine their production potential.

Each of the selections from the initial screening comprises one seedling plant with a taproot. The degree of inherent resistance/tolerance compared to the standard clonal Duke 7 has thus not yet been determined. Horticultural field trials are costly and require space. A screening of clonal material was thus required to ensure that the best candidates are promoted to field-testing.

The 91 selections, remaining after the initial screening, have been tested with the detached root inoculation method (Van der Merwe, 1995) and selections that were not significantly better than the standard cultivar Duke 7, were discarded. None of the selections was, however, significantly better than Duke 7 and 46 selections equal to Duke 7 remained. This is still a high number of selections to be promoted to a field trial. The purpose of this study was therefore to determine if any of the selections had a better root regeneration ability than Duke 7 and hence a better chance of survival. Only 14 selections could be etiolated successfully and thus be multiplied for the trial.

## 4.2.2. MATERIALS AND METHODS

### 4.2.2.1. Plant material, inoculation and evaluation

Fourteen potential rootstock selections (Table 4.4), including Duke 7 and Edranol, were multiplied to have 40 clonal plants of each selection (Bijzet *et al.*, 1997).

**Table 4.4** Short codes and descriptions of selections

Code	Selection	Description
A	92-4-3	Seedling of Duke seedling
B	2-64-1	Seedling from an unknown seedling tree in Paarl
C	92-2-2	Barr Duke seedling
D	94-2-4	Barr Duke Seedling
E	94-9-1-2	Teague seedling
F	2-62-1	Seedling from an unknown seedling tree in Paarl
G	92-1-2/5	Duke 7 seedling
H	92-1-1/1	Duke 7 seedling
I	94-1-12	Duke 7 seedling
J	Duke 7	Duke 7 clonal
K	SA-RS97/1	Escape tree (unknown seedling rootstock)
L	92-1-2/1	Duke 7 seedling
M	92-5-1	Seedling of a Duke seedling
N	92-1-2/2	Duke 7 seedling
O	94-9-1	Teague seedling
P	Edranol	Edranol clonal

Each plant was planted in a separate container in 100 % silica sand (used in swimming pool filters). The trial layout was a randomised block design in a greenhouse with natural light and a temperature regime of 18 to 30 °C. All the equipment and media were steam-sterilised (Baker & Roistacher, 1957) prior to commencement of the trial. Each treatment consisted of five replicates and four plants per replicate. As soon as the plants have acclimatised, 20 plants of each selection were inoculated with *P. cinnamomi* according to the method of Duvenhage & Maas (1990). The remaining 20 plants served as controls. The sand was flooded for 48 hours after inoculation and the water was drained to container capacity. Flooding and draining were repeated twice with a 48-hour interval, after which the sand was maintained at container capacity. Three months after inoculation, the root system of each plant was harvested



and divided into structural and feeder roots (Van Vuuren, 1997). The roots were dried and the feeder root mass calculated as a percentage of the total root mass.

#### **4.2.2.2. Re-isolation of *Phytophthora cinnamomi***

After removal of the plants, the sand in each container was baited with whole avocado fruit (Zentmyer *et al.*, 1960). In addition a sample of sand was also taken from each container and baited with citrus leaf discs (Grimm & Alexander, 1973). Five citrus leaf discs and five avocado discs (taken from the surface of whole avocado fruit) per container were plated on a hymexazol medium selective for *Phytophthora* spp. (Tsao & Guy, 1977). Ten healthy looking root tips per plant were collected from all the treatments to ensure that infection was still in an active stage. The root tips were incubated on the medium of Tsao & Guy (1977).

#### **4.2.2.3. Data analysis**

The data were tested for normality with the Univariate procedure of SAS and analysis of variance was done with the GLM procedure of SAS. The means were also compared with a protected Fisher test at 95 % level of significance.

### **4.2.3. RESULTS AND DISCUSSION**

#### **4.2.3.1. Overall influence of *Phytophthora cinnamomi* on the root components**

*P. cinnamomi* had a significant effect on root development (Table 4.5). The mean total and structural root component were significantly higher in the inoculated treatments than in the control treatments. Feeder roots, however, had a lower dry mass in the inoculated treatments than in the control treatments.

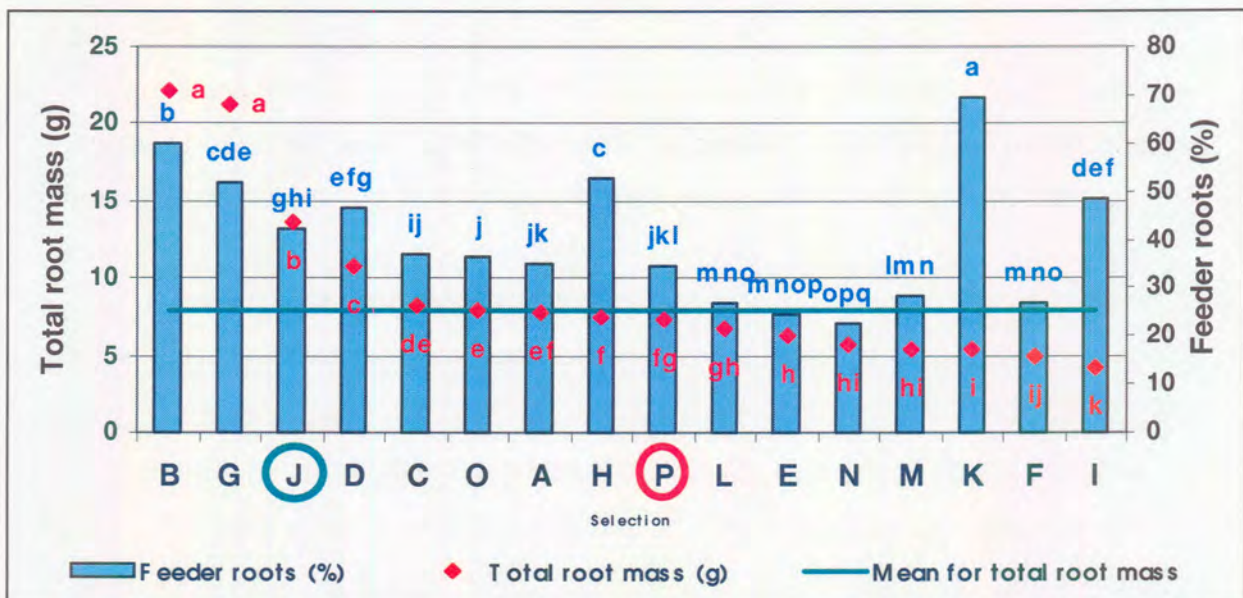
**Table 4.5** Overall effect of *Phytophthora cinnamomi* on root development of clonal avocado selections.

Root Component	Mean dry mass (g)	
	Control	Inoculated
Structural	4.58b*	5.48a
Feeder	2.96a	2.53b
Total	8.00a	8.59b

\* Values followed by the same letter within a row do not differ significantly at P=0.05

**4.2.3.2. Variation amongst selections irrespective of *Phytophthora cinnamomi* inoculation**

Feeder root percentages relative to actual dry mass of the total root component for the control treatments of the 14 selections are given in Figure 4.8. The influence of *P. cinnamomi* is thus excluded.



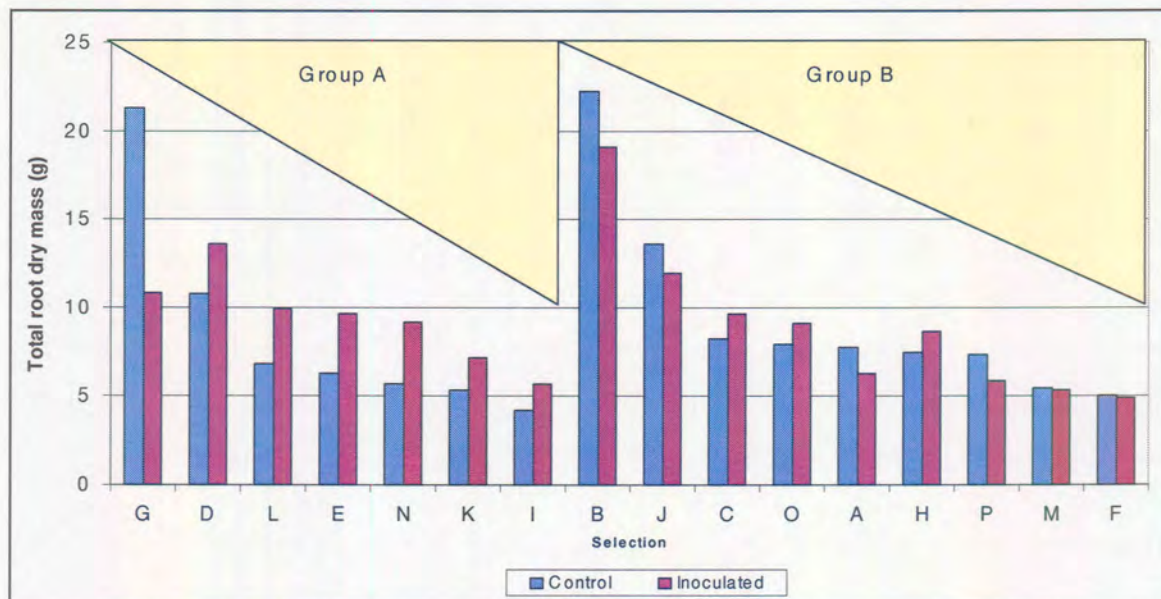
**Figure 4.8** Feeder root percentages relative to actual dry mass of the total root component for the control treatments of 14 clonal avocado selections including the standard cultivar Duke 7 (J) and a susceptible control Edranol (P). Values within parameters followed by the same letter do not differ significantly at P=0.05.

Figure 4.8 indicates that the feeder root percentages of selections K and B were significantly higher than the feeder root percentages of the other

selections, selection K having the highest feeder root percentage overall. Selection B had the highest root mass (although not higher than G) as well as the second-highest feeder root percentage. By contrast, selection K, with the highest feeder root percentage, had a total root mass significantly lower than that of the standard rootstock Duke 7. The root mass of selection K was even lower than the mean for the 14 selections included in the trial. This indicates that, although feeder root percentage is a good criterion for clonal material, it is not a representative parameter for comparing tolerance to *P. cinnamomi* amongst genetically different selections.

#### 4.2.3.3. Total root component before and after inoculation

Table 4.5 indicated that the total root mass was overall significantly higher in the inoculated treatment than in the control treatment. If this is viewed in detail, two groups can be distinguished (Figure 4.9). Group A represents the selections of which the total dry root mass differed significantly between the control and inoculated treatments, whereas group B includes the selections that did not display significant differences.

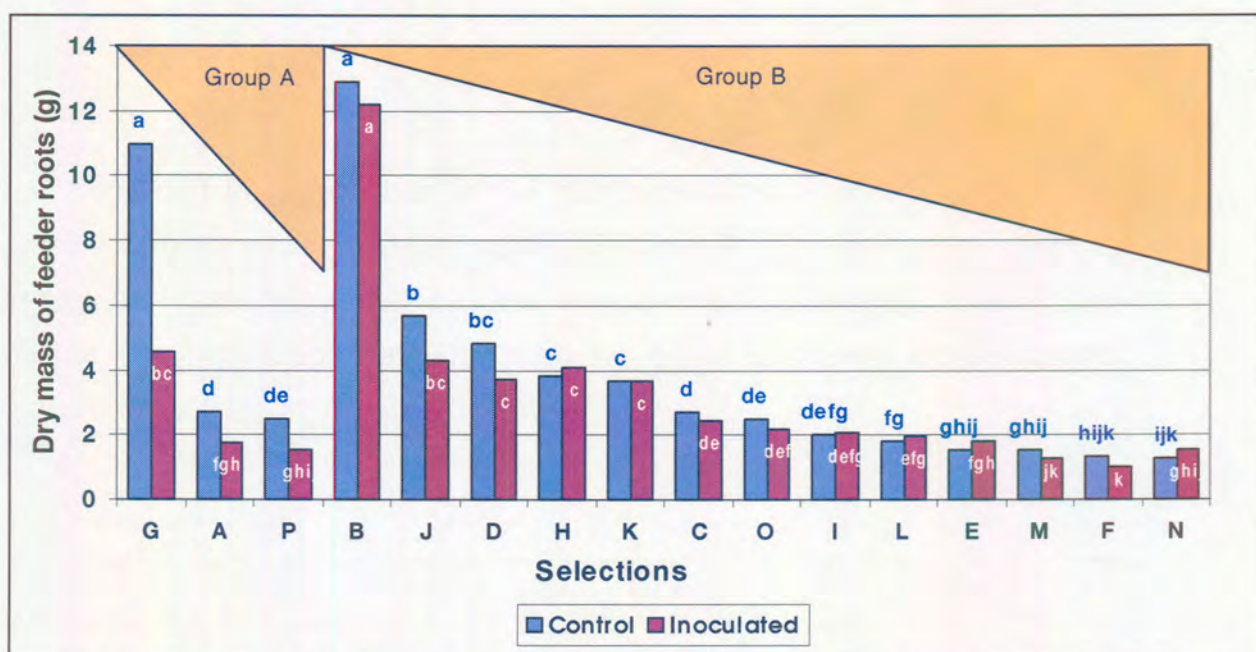


**Figure 4.9** Comparison of the dry mass of the total root component in the control and the inoculated treatments with regard to significant (group A) and a non-significant (group B) differences.

In group A, only selection G showed a decrease in root mass whereas the other selections (D, L, E, N, K and I ) had an increase in root mass following inoculation.

#### 4.2.3.4. Effect of *Phytophthora cinnamomi* on the feeder root component

In order to identify the best selection with regard to tolerance or resistance the influence of *P. cinnamomi* on the feeder root component was studied (Figure 4.10).



**Figure 4.10** Comparison of the dry mass of the feeder root component in the control and the inoculated treatments with regard to significant (group A) and non-significant (group B) difference.

The data in Figure 4.10 indicate that, with the exception of selections G, A and P, none showed a significant reduction in feeder root mass and would thus seem to be equally tolerant to *P. cinnamomi*. Selections that could definitely be discarded at this stage would thus be G, A and P. This, however, still leaves twelve selections that are apparently equal to Duke 7 in feeder root regeneration. Although Figure 4.10 showed that the feeder root component of selection D, L, E, N, K and I (Group B) did not increase significantly, Figure 4.9 indicated an increase in dry mass of the total root component of these

selections, thus suggesting an increase in the structural root component. According to Schaffer *et al.* (1991) the formation of adventitious (fleshy, white) roots is a reaction to severe stress situations and is supposedly aimed at increasing the area for oxygen absorption.

In order to determine the most probable selections for further evaluation, a summary of the root characteristics of the fourteen selections and two control cultivars is given in Table 4.6.

**Table 4.6** Comparison of clonal avocado selections with regard to their root components as affected by *Phytophthora cinnamomi*.

(Values = difference between control and inoculation values)

Total (g)	Structural (g)	Feeder (g)	Feeder %	Difference between Control and Inoculation values
B	A	B	B	
19.11	9.16	12.19	64.61	
J	J	J	H	No significant difference
11.95	7.26	4.29	47.84	
C	P	H	G	Significant Increase
9.63	4.05	4.10	42.74	
O	M	D	J	Significant Increase
9.12	4.04	3.70	37.69	
A	F	K	A	Significant Decrease
6.26	3.83	3.68	30.25	
H	D	C	P	Significant Increase
8.66	9.64	2.46	28.73	
P	L	O	M	Significant Increase
5.84	7.99	2.20	24.15	
M	E	I	F	Significant Increase
5.33	7.84	2.06	21.59	
F	N	L	N	Significant Increase
4.89	7.60	1.95	17.12	
D	C	E	K	Significant Decrease
13.58	6.73	1.78	57.13	
L	O	N	I	Significant Decrease
9.94	6.48	1.56	38.35	
E	H	M	D	Significant Decrease
9.64	4.48	1.27	28.54	
N	I	F	C	Significant Decrease
9.18	3.40	1.01	28.64	
K	K	G	O	Significant Decrease
7.18	2.84	4.55	27.31	
I	B	A	L	Significant Decrease
5.66	6.49	1.74	19.65	
G	G	P	E	Significant Decrease
10.84	6.14	1.51	18.63	

Selection G in Table 4.6 indicates the danger of using feeder root percentage instead of mass when comparing different genotypes with each other. The feeder root percentage of G was the third highest of all the selections but the actual feeder root as well as the total root mass have been reduced drastically as is evident from Figures 4.9 and 4.10.

Avocado rootstocks need to be more than just tolerant to *P. cinnamomi*. Factors such as compatibility, precocity, yield and effect on quality should be taken into account as well (Bijzet & Sippel, 2001) and can only be determined

in a field trial. It can, however, be hypothesised that a larger root system would enhance the foraging ability of the avocado tree.

A large number of physiological processes in plants depend on leaf water potential and the avocado is no exception (Sterne *et al.*, 1978). A genetically larger root system would thus not be so easily prone to water stress as a smaller root system. The feeder root mass is also important, as the development of new increments of growth by continued elongation of existing roots and the initiation of new feeder roots are considered to be important features with regard to absorption by roots (Esau, 1977). This growth creates new absorbing surfaces, and it brings these surfaces in contact with new areas of soil.

The selections thus destined for further evaluation are B, H, D and K. Selections C, O, I, L, E, M, F and N are temporarily disregarded due to their total root mass (Figure 4.8) being significantly lower than that of Duke 7.

### **4.3. CONCLUSION**

The detached root inoculation method (unpublished data) were only able to eliminate 45 of the original 91 selections. An alternative method was needed as the remaining number of selections was still too high to be promoted to the field. Comparison of feeder root percentage in non-inoculated and inoculated treatments were also not sufficient to facilitate the final selection of candidate rootstocks from a large number of potential selections with initial characteristics similar to that of Duke 7.

Four selections could be made based on the hypothesis that a larger root system will be a better forager and thus enhances the horticultural aspects of the rootstock scion combination. This hypothesis has, however, not yet been proven with regard to different selections and should be tested. It is also worthwhile to repeat the trial grafting the selections with a scion such as Hass, which is known to exert a further drain on the rootstock.

A revised method of Kelham & Coffey (1985) can be suggested for enhanced differentiation of the apparently similar selections, where the influence of *P. cinnamomi* is assessed over time. This method will identify the selections with an ability to maintain root regeneration, facing *P. cinnamomi* pressure, over time.



## **CHAPTER 5**

**Clonal propagation as a selection criterion of  
potential new avocado rootstocks**

## 5.1. INTRODUCTION

A comparison of avocado (*Persea americana* Mill) rootstock selections amongst each other and with the standard Duke 7 necessitated clonal multiplication of the selections. This task was left to the ITSC nursery, but it did not tie in with the normal nursery activities and it was soon reported that some of the selections were difficult to multiply. Clonal multiplication is an important nursery practice and was included, in addition to *Phytophthora* tolerance, as selection criterion for avocado rootstocks. As each new selection comprises only one plant, clonal multiplication was required to provide at least 40 plants to be used for additional *Phytophthora* tests. This number excluded plants needed for horticultural evaluations.

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 preferred to multiply selected *Phytophthora* root rot tolerant seedlings since limited material was available for grafting and a large number of plants are required for additional *Phytophthora* tests as well as for horticultural evaluations that might follow at a later stage. This process represents only one of many methods available for multiplying avocado rootstocks. Thus, if a rootstock is found to be difficult to multiply by the method selected for the purpose of this project but has an excellent tolerance/resistance to *Phytophthora*, it could still be recommended for further investigation. This will, however, only be possible if sufficient material can be produced, by means of a different method, for these tests. If commercial release of such a rootstock is not viable due to the difficulty, and subsequent high costs involved in multiplication, the rootstock might still be useful as a breeding parent.

## 5.2. MATERIALS AND METHODS

The trial was performed with the fourteen selections listed in Chapter 4, Table 4.4.

Seeds of a sun-blotch-free Edranol avocado tree were planted, as nurse seeds, in small plastic bags (70 mm diameter, 150 mm high) containing a well-drained sterile medium. Care was taken to ensure that the nurse seeds were fairly uniform in size and mass. After germination, the nurse seedlings were grafted with the selections to be multiplied.

At bud burst the nurse plants were transferred to a dark-room for etiolation. Two methods were followed from this step, depending on the growth habit of the selection:

1. When leaves developed during the etiolation process, cuttings were taken while in the dark, when the shoot had stopped growing actively (approximately 300-400 mm). (Figure 5.1) Cuttings were 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.

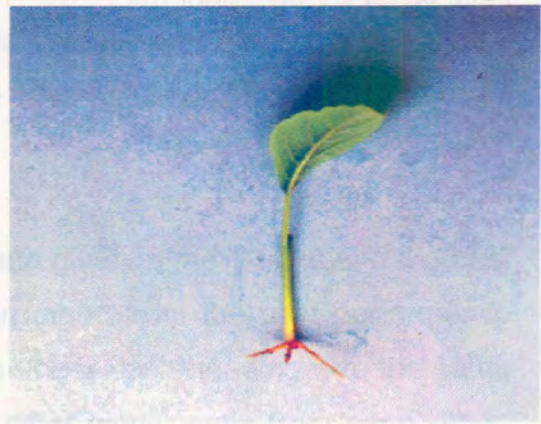


**Figure 5.1** Etiolated avocado selection with leaves that had developed.

2. Selections that did not develop leaves in the dark were removed from the etiolation chamber when etiolated shoots were approximately 200-300 mm long. The shoots were painted black with a bitumen-based tree-sealing compound (Figure 5.2) and the plants were left in daylight to develop normal green leaves. When one or two leaves had developed, the shoot was cut and placed in a mist bed as described above.



**Figure 5.2** Etiolated avocado selection without leaf development



**Figure 5.3** Rooted internode-length cutting

Roots developed approximately 4-8 weeks later (Figure 5.3). When the roots had developed, the cuttings were transplanted to the same size bag with medium as the nurse seedling. The transplanted cuttings were left under the mist spray for a week whereafter they were hardened off.

L Duke 7 N	D F O	Duke 7 H O	O Duke 7 N	O L D
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Selections that consistently excelled are marked in green and ones that consistently failed are marked in red. The standard Duke 7 is marked in blue. K, a local escape tree, was the best overall performer. Some selection like Duke 7 and C were variable with regard to their performance. The overall performance of selection O was disappointing.

Scions from the selections were grafted on nurse seedlings during November and transferred to the dark as previously described. The material was inspected every fourth day. Cuttings were taken and placed in a mistbed as described above. The dates of each action were recorded. In each instance Duke 7 was regarded as the standard for the sake of comparison. Easy and effective clonal multiplication of avocado rootstocks was quantified by the following measures:

- Number of days from grafting to first cut
- Number of cuttings per nurse seed
- Percentage of cuttings rooted
- Number of days to rooting

Data were statistically analysed as a randomised block design with SAS. A selection refers to a single seedling plant that was selected during the initial screening. Limited material was thus available for multiplication purposes. Four bud sticks were taken from each selection and grafted on four different nurse seeds. These were considered as the replicates. The data were tested for normality with a UNIVARIATE procedure and an analysis of variance was done with the GLM procedure of SAS. The means were also compared with a protected Fisher test at 99 % level of significance.

### **5.3. RESULTS**

The coefficient of variance ranged from 18 % to 28 % for the various measurements. The distribution of the data was fairly normal for each measurement except for the number of cuttings that were taken per graft, in which case a transformation was done. This resulted in the coefficient of variance for this specific analysis being lowered from 18 % to 7 %. The replicates did not differ significantly ( $P=0.01$ ) for any of the analyses that were done.

### 5.3.1. NUMBER OF DAYS FROM GRAFTING TO FIRST CUT AND BETWEEN CUTS

The prolific nature of each selection regarding the production of new shoots was determined by noting the number of days from grafting the selection until the first cut could be made. Complementary to this the time from cut-to-cut, i.e the number of days that elapsed until another internode length cutting/cuttings was available, was also recorded.

**Table 5.1** Time from grafting of clonal avocado selections on nurse seedlings until the first cutting could be taken.

Selection	Mean number of days*
N	80.0 a
Duke 7	72.5 a
E	70.5 ab
O	63.3 abc
A	45.0 bcd
B	45.0 bcd
D	44.8 bcd
I	44.8 bcd
K	43.0 cd
G	41.3 cd
C	39.0 cd
H	38.8 cd
M	38.3 cd
L	38.0 cd
F	34.3 d

\* Values not followed by the same letter differ significantly according to a protected Fisher test (P=0.01)

The time from grafting to first cut varied between 34.3 and 80 days (Table 5.1). In this case the selections with the shortest time between grafting to first cutting made are the most efficient.

The time that elapsed until another internode length cutting or sometimes more than one cutting was available from each graft per selection is given in Table 5.2. There is a need for a prolific rootstock that produces new cuttings at shorter intervals in order to produce larger numbers of rooted cuttings in a shorter period of time.

**Table 5.2** Time that elapsed until another internode cutting or cuttings per nurse seedling was available

Selection	Mean number of days between cuts*	
O	39.8	a
F	31.5	ab
B	31.3	ab
G	29.7	abc
E	29.6	abc
Duke 7	26.0	bcd
H	25.6	bcd
D	23.2	bcde
L	23.0	bcde
M	22.7	bcde
A	21.3	bcde
I	18.9	cde
N	16.8	de
K	13.9	e
C	13.3	e

\* Values not followed by the same letter differ significantly according to a protected Fisher test (P=0.01)

The number of days between cuts varied from 13.3 to 39.8. Only two selections were significantly better than Duke 7 namely K (Sa-RS97/1) and C (92-2-2).

### 5.3.2. NUMBER OF CUTTINGS PER NURSE SEED

The data did not fit a normal distribution very well so a transformation was done. The data in Table 5.3 represent a transformation back to normal values. Ten of the selections were significantly more prolific than Duke 7. K, G and C, however, performed significantly better than any of the other selections, including Duke 7.

**Table 5.3** Mean number of cuts that were produced per nurse seedling per selection.

Selection	*Mean number of cuttings per graft	
K	50.0	a
G	30.7	b
C	29.0	b
D	14.0	c
M	13.7	cd
L	12.6	cde
I	12.0	cde
N	11.1	cde
E	11.0	cde
A	11.0	cde
F	9.9	def
B	9.2	ef
Duke 7	7.2	fg
H	6.3	g
O	5.7	g

\* Values not followed by the same letter differ significantly according to a protected Fisher test (P=0.01)

The number of cuts per nurse seed is also important as it enhances the final number of cuttings that will be available for rooting. It furthermore has implications with regard to space requirements for etiolation, as fewer bags with

grafted nurse seeds are needed for a selection that produces a large number of cuts per nurse seed.

### 5.3.3. PERCENTAGE OF CUTTINGS THAT ROOTED

The percentage of cuttings that can be successfully rooted is the most important selection criterion with regard to clonal ability. It is at this stage that many selections fail to produce results. The rooting ability of the selections that were evaluated is depicted in Table 5.4.

**Table 5.4** Rooting ability of selections as determined by the percentage of cuttings that rooted.

<b>Selection</b>	<b>Mean percentage of cuts that rooted*</b>
K	82.5 a
E	77.3 ab
B	74.1 abc
F	69.4 abcd
G	65.9 abcd
L	64.5 abcd
H	62.5 bcd
I	55.8 Cde
C	53.2 De
A	52.0 De
D	39.7 ef
M	38.2 ef
O	26.3 fg
<b>Duke 7</b>	<b>21.7 fg</b>
N	9.4 g

\* Values not followed by the same letter differ significantly according to a protected Fisher test (P=0.01).

The best selection was K but it did not differ significantly from E, B, F, G and L. It could be argued that a rooting percentage of less than 70 % is not economically feasible, which excludes most of the selections, including Duke 7.

### 5.3.4. NUMBER OF DAYS TO ROOTING

Another factor that has an influence on the clonal ability of a selection is the period of time that elapses from the day that the cutting is put into the mist bed until it has formed roots and can be transplanted. Saving time is always important but in this instance the primary need for a short period from cutting to rooting is the constant possibility of contamination with pathogens and the consequent loss of material. Table 5.5 shows the time that elapsed for each selection from entering the mistbed to being transplanted as a rooted cutting.

**Table 5.5** Time from entering the mistbed to being rooted.

Selection	Mean number of days*	
D	114.5	a
L	92.4	b
O	83.0	bc
I	73.5	cd
B	71.8	cd
A	66.3	d
H	64.2	d
E	62.2	d
M	45.6	e
C	45.4	e
F	44.3	e
G	39.4	e
K	31.1	ef
N	23.3	f
Duke 7	19.0	f

\* Values not followed by the same letter differ significantly according to a protected Fisher test (P=0.01).

Duke 7 performed very well with regard to this characteristic, but not significantly better than selections N and K. The variation amongst selections ranged from 19 days to 114.5 days.

In Table 5.6 a summary is presented of the data given in the graphs and the best selections are highlighted. Selections are arranged from the best performer to the worst performer per characteristic. Areas under each heading with the same colour represent the selections that did not differ significantly from each other. Selections under the same heading shaded in a specific colour differed significantly from those shaded differently.

**Table 5.6** Summary of cloning ability of avocado selections

# days to 1 <sup>st</sup> cut	#days : cut-cut	# of cuttings per nurse seed	% of cuts that rooted	# of days from cut to rooting
F	<b>C</b>	<b>K</b>	<b>K</b>	<b>Duke 7</b>
L	<b>K</b>	G	E	N
M	N	<b>C</b>	B	<b>K</b>
H	I	D	F	G
<b>C</b>	A	M	G	F
G	M	L	L	<b>C</b>
<b>K</b>	L	I	H	M
I	D	N	I	E
D	H	E	<b>C</b>	H
B	<b>Duke 7</b>	A	A	A
A	E	F	D	B
<b>O</b>	G	B	M	I
E	B	<b>Duke 7</b>	<b>O</b>	<b>O</b>
<b>Duke 7</b>	F	H	<b>Duke 7</b>	L
N	<b>O</b>	<b>O</b>	N	D

Selections that consistently excelled are marked in green and ones that consistently failed are marked in red. The standard Duke 7 is marked in blue. K, a local escape tree, was the best overall performer. Some selection like Duke 7 and C were variable with regard to their performance. The overall performance of selection O was disappointing.

## 5.4. CONCLUSION

The cloning ability of the different selections varied considerably even though they shared the same parent. One of the mechanisms with regard to tolerance in Duke 7 (one of the parents of these selections) is its considerable root growth potential (Coffey, 1991). It would thus be expected that the percentage cuttings that rooted as well as the number of days from cut to root should give an indication of the potential of the selections with regard to one of the mechanisms for tolerance. This will be taken into account during further experiments with these seedling. The 91 selections, remaining after the initial screening, have been tested with the detached root inoculation method (Van der Merwe, 1995) and selections that were not significantly better than the standard cultivar Duke 7, were discarded.

Most of the selections performed better than the standard cultivar Duke 7 except for time from cut to root. This would be expected to some extent as the Duke 7 material was taken from a mature tree whilst the single plant selections were never older than two years, when the material for cloning was taken. Kadman (1976) concluded that getting material from mature trees to root was more difficulty than to root material still in a juvenile state. Any selection thus not performing to the same standard as Duke 7 is not acceptable.

The largest constraint experienced during this trial was the inability to etiolate some of the selections, which could thus not be rooted or undergo further testing. These selections might possess beneficial genes and will not be discarded but will be incorporated as mother trees in the breeding programme.

## **CHAPTER 6**

### **General conclusion**

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## 6. GENERAL DISCUSSION

*Phytophthora* root rot caused *Phytophthora cinnamomi* Rands is a devastating disease of avocados (*Persea americana* Mill.), with immense financial implications to the producer. A review of the literature indicated the existence of complex host-pathogen interactions with regard to avocado and *P. cinnamomi*. It is evident that vertical resistance to *P. cinnamomi* does not exist in *P. americana*. Avocado rootstock germplasm that is currently available does, however, include individuals with different mechanisms of tolerance that can be utilised in a breeding programme. The incompatibility between the group of *Persea* spp. that exhibit resistance and the group that *P. americana* belongs to is a complicating factor in a breeding programme aimed at establishing *Phytophthora* root rot resistant rootstocks for avocado production. In order to find a beneficial phenotype to aid increased tolerance with regard to *P. cinnamomi*, an unknown number of multiple genes have to be recombined.

Detection of a beneficial genotype is the most crucial element in a breeding programme. Possible variation in host-pathogen interactions could thus be a further complication and these interactions must be taken into account when screening of newly created phenotypes is considered. Various physical and physiological factors as well as other pathogens can influence screening results. Ignorance with regard to these factors will induce high costs and render the breeding programme ineffective.

Various physical constraints such as flower morphology and behaviour as well as expensive and time-consuming field trials are further complicating factors. The literature should thus be utilised to optimise the screening process in order to ensure that beneficial genotypes are not overlooked or that unnecessary material does not make the breeding programme unwieldy and costly.

An overview of the breeding programme between 1991 and 1997 has shown that the methods previously used by the breeding team at the ARC-ITSC, were not scientifically based. The following problems were foremost:

- Inoculum was either derived from local isolates of the pathogen or from old storage cultures. Local isolates were not positively identified as *P. cinnamomi* by a mycologist whilst loss of virulence and various genetic degeneration or attenuation of strains maintained in culture could have occurred.
- Soil from an avocado orchard with *P. cinnamomi* symptoms was used without determining the pathogen complex or even determining whether *P. cinnamomi* was present or not.
- Methods were not consistent from season to season

The material that was initially selected between 1991 and 1997 nevertheless proved to be equal to the standard cultivar Duke 7. Only three of these selections were discarded, at the end of the trials done for this dissertation, due to their inability to match the characteristics of Duke 7.

With regard to choice of screening media it was indicated that soil is not an effective medium for the initial screening of breeding products. A high incidence of necrosis, attributed to the slow drainage following the flooding of the soil, was evident. Soil is not an inert standardised medium and repeatability from season to season will be difficult. The inherent properties, for instance the mineral status and clay content, could differ between seasons.

All the other media tested, namely sand, vermiculite, water and the Schwalbach system, were equal in performance. The medium that will be used in future will depend on the preference of the breeder as each medium has its own pro's and con's. The initial capital outlay of both the water and Schwalbach systems is high. These two systems also need intensive care, as temporary clogging of nozzles in the Schwalbach system and interrupted



delivery of oxygen to the water system, could cause the results to be inaccurate or even unattainable.

Vermiculite is prone to weathering and it is expected that continuous use would not be an option. Steam sterilising of vermiculite could contribute to a loss of volume of this medium. Personally, sand was preferred with the precept that only silica sand should be used due to its unique characteristics. Optimal germination of avocado seeds has previously been achieved in vermiculite. It is thus recommended that seeds be first germinated in vermiculite after which the seedlings can be transplanted to sterilised silica sand for the screening process as was described in this dissertation.

Sand and vermiculite are effective for the screening of genetic material that relies on root regeneration as a mechanism of resistance. Shoot growth in the sand and vermiculite does not reflect the root situation and screening of seedlings will still be dependent on a subjective evaluation of the roots as determining feeder root percentage of 10 000 individual plants is not viable. Percentage necrosis could prove valuable as this can be done mechanically. Although the Schwalbach system (mist) reflects stress in the root region readily in the shoot growth, as indicated in Table 4.3, root regeneration is not facilitated as the effect of *P. cinnamomi* is intense and rapid in the absence of additional fertiliser or leaf feeds.

It was also evident from Table 4.3 that the evaluation criterion to be used depends on the medium that is used. Feeder root percentage was a good criterion for clonal material in all the media except for soil and water. Necrosis was a good criterion for clonal material in all the media except for soil.

There was a definite difference with regard to the cloning ability of the different selections even where they shared the same parent. An inability to be etiolated was displayed by some of the selections and these could thus not be vegetatively propagated and were not tested further.

One of the mechanisms involved in the tolerance of Duke 7 (one of the parents of these selections) is its considerable growth potential. It would thus be expected that the percentage cuttings that rooted as well as the number of days from cut to root should give an indication of the potential of the selections with regard to this mechanism of tolerance. This, however, does not reflect clearly when comparing Tables 4.6 and 5.6.

Most of the selections performed better than the standard cultivar Duke 7. This would be expected to some extent as the Duke 7 material was taken from a mature tree whilst the single plant selections were never older than two years when the material for cloning was collected. Any selection not performing to the same standard as Duke 7 is not acceptable, as rooting cuttings taken from mature trees are more difficult than rooting material still in a juvenile state.

Valuable information was obtained with regard to various media and the criteria to be used during screening. This knowledge must be taken into account in the planning of future breeding projects. Knowledge of the clonal ability of a potential new rootstock is important for both the nursery and the producer from a financial point of view.



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# SUMMARY



## SUMMARY

### **Screening of avocado rootstock material for tolerance to *Phytophthora cinnamomi***

by

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During the initiation and execution of a rootstock breeding programme to overcome the financially crippling disease, *Phytophthora* root rot of avocado, various constraints have been identified for both the breeding as well as the screening aspect of the programme. A review of the literature revealed a complex host-pathogen interaction that should be taken into account in the recombination and screening of genetic material.

With the detection of beneficial genotypes being the crux of a breeding programme, this dissertation was focused on the screening of rootstock material for tolerance to *Phytophthora cinnamomi*. Screening should be scientific but at the same time also be time and cost effective. Specific attention was given to (i) the correct medium for screening mass numbers of seedlings, (ii) fast and effective cloning of single selections, and (iii) evaluation of clonal material for tolerance to *P. cinnamomi*.

Soil as a screening medium was compared with three inert hydroponic media as well as one aeroponic system. Only soil was found to be ineffective due to its properties. The other media tested, namely, sand, vermiculite, water and the aeroponic system were equal in performance. The medium to be used will depend on the preference of the breeder as each medium has its own pro's



and con's. It was, however, found that the evaluation criterion to be applied depends on the medium that is used.

With regard to cloning of single selections, a definite difference with regard to the cloning ability of the different selections was found. An inability to be etiolated was displayed by some of the selections and these could thus not be vegetatively propagated and were not further tested.

One of the tolerance mechanisms in the standard cultivar Duke 7, is root regeneration. It was thus expected that this characteristic cloning would give an indication of the rootstock's ability to tolerate *P. cinnamomi*. This could not be confirmed, but most of the selections did, however, perform better than Duke 7.

Comparison of feeder root percentage in non-inoculated and inoculated treatments was not sufficient for facilitating the final selection of candidate rootstocks from a large number of potential clonal selections. Four selections were made, based on the hypothesis that a larger root system will be a better forager and thus enhance the horticultural aspects of the rootstock-scion combination.

Valuable information was obtained with regard to various mediums and criteria to be used during mass screening and final screening of clonal selections. This knowledge must be taken into account in the planning of future breeding projects. During this project a total of 38 984 seedlings were screened and four selections were made. For both the nursery and the producer, knowledge of the clonal ability of a potential new rootstock is important from a financial point of view.



## Samevatting

### Onderzoek van avokado onderstam materiaal vir verdraagsaamheid teenoor *Phytophthora cinnamomi* .

deur

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Tydens die implementering van 'n onderstamteelprogram met die oog op die teëwerking van die finansiële impak wat *Phytophthora* wortelvrot of die avokado bedryf is verskillende tekortkominge aangedui. Struikelblokke in die kruisingsprogram sowel as in die sifting van die nageslag is geïdentifiseer. Literatuur het aangedui dat rekombinasie en seleksie van genetiese materiaal onderhewig is aan komplekse gasheer-patogeen interaksies.

Die mees kritieke aspek van 'n teelprogram is die identifisering van voordelige nuwe genotipes en daarom is gefokus op die seleksie van onderstammateriaal. Alhoewel finansiële beperkinge bepaal dat sifting van potensiële nuwe onderstamme so koste- en tydseffektief as moontlik gedoen moet word, moet dit steeds wetenskaplik uitgevoer word. Aandag is veral aan die volgende aspekte gegee: (i) identifisering van geskikte media vir massasifting, (ii) evaluering van *Phytophthora cinnamomi* verdraagsaamheid van vegetatiewe onderstammateriaal, en (iii) klonering van enkel seleksies.

Vir 'n geskikte siftingsmedium is grond vergelyk met drie inerte hirdoponiese mediums, naamlik sand, vermikuliet en water, asook met 'n aeroponiese sisteem. Slegs grond is as ongeskik gevind as gevolg van sekere fisiese eienskappe. Die ander media wat getoets is, naamlik sand, vermikuliet, water

en die aeroponiese sisteem blyk almal ewe bruikbaar te wees. Die medium wat gebruik gaan word hang grootliks af van die navorser se voorkeur en begroting aangesien elkeen sy eie voor- en nadele inhou. Verskillende evalueringskriteria is egter vir elke medium geïdentifiseer.

Definitiewe verskille ten opsigte van die vinnige en effektiewe vermeerdering van enkel seleksies is gevind, selfs vir die met 'n gemeenskaplike ouer. Van die seleksies het swak gereageer op etiolering en kon dus nie verder vermeerder of getoets word nie.

Wortelregenerasie is by Duke 7 een van die oorlewingsmeganismes teen *P. cinnamomi*. Daar is dus verwag dat hierdie kenmerk 'n aanduiding sou kon gee van 'n seleksies se verdraagsaamheid teenoor *P. cinnamomi* te wees. Die hipotese kon egter nie vanuit die data bevestig word nie.

Die verskil tussen persentasie voedingswortels van geïnfekteerde en nie-geïnfekteerde plante was nie voldoende om die finale selektering van potensiële onderstamme vanuit 'n groep klonaal vermeerderde onderstamme te bewerkstellig nie. Behalwe in drie van die seleksies kon geen statistiese verantwoordbare verlies aan voedingswortels gevind word nie en het meeste van die seleksies soos Duke 7 vertoon. Vier seleksies is gemaak op grond van die hipotese dat onderstamme met 'n groter wortelmasse beter voeders is en dus tuinboukundige eienskappe van die onderstam–bostam kombinasie verbeter.

Waardevolle inligting is ingesamel ten opsigte van die onderskeie media en die metingskriteria wat gebruik kan word vir die sifting van groot aantalle saailinge en die finale sifting van klonale seleksies. Die kennis moet gebruik word in die beplanning van toekomstige onderstamteelprogramme. Tydens hierdie projek is 38 984 saailinge getoets en vier seleksies gemaak. Vanuit 'n finansiële oogpunt is die vinnige en ekonomiese vermeerdering van dié potensiële onderstamme 'n belangrike aspek.