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.

Rootstock Breeding Programme

GENE SOURCE
Foreign and local rootstock material

ESCAPE TREES   BREEDING

Seedlings

SCREENING

Surviving seedlings

CLONAL MULTIPLICATION

STATISTICAL SCREENING

Best selections

Field testing   Horticultural evaluation

Semi-commercial testing

RELEASE
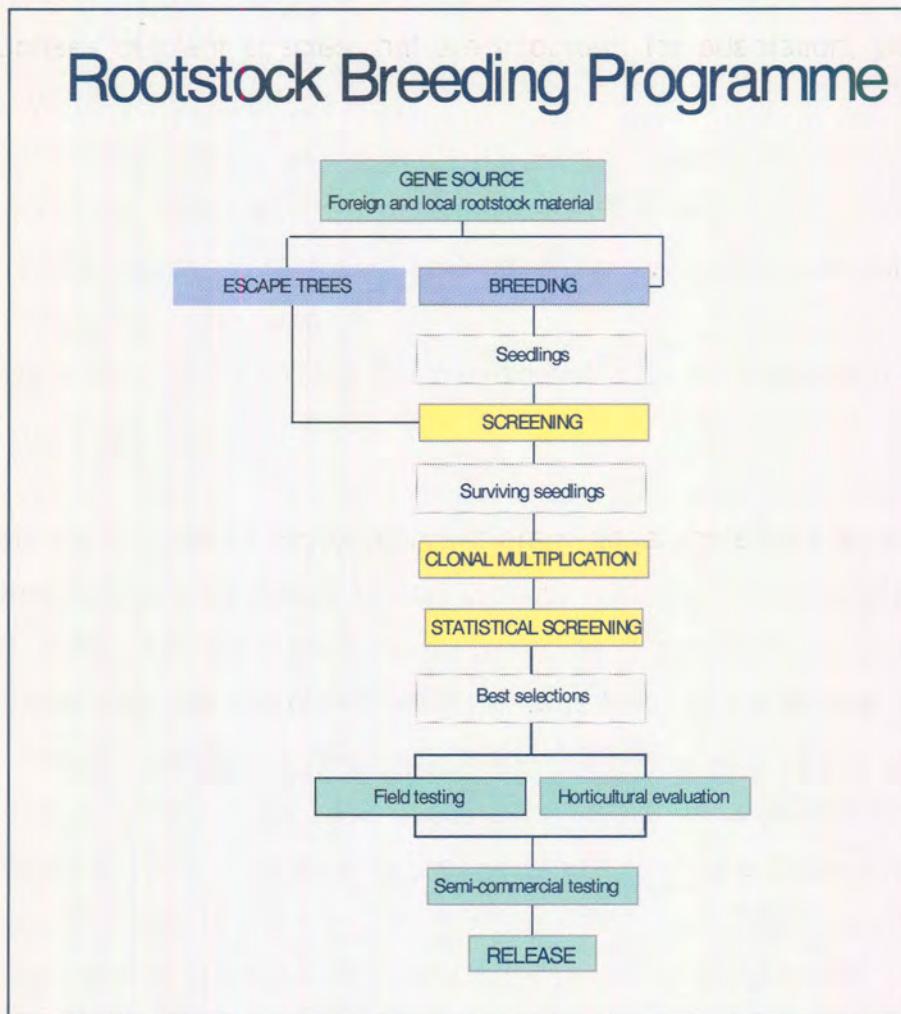
Figure 1.1   Different stages in the avocado rootstock breeding programme

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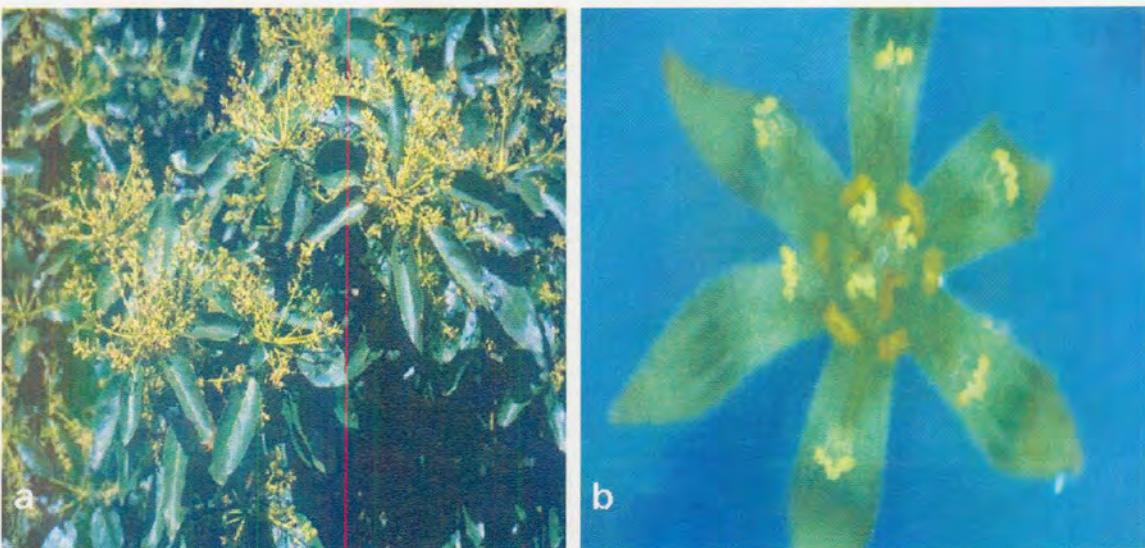
Figure 2.1   **a**   Illustration of the size and number of avocado inflorences, **b**   Avocado flower showing the pollen sac valves with pollen (courtesy P.J. Robbertse)

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^{-3}, 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, Guatamalan 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	Guatamalan 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 Guatamalan hybrid and Hass, which is believed to be 85 % Guatamalan 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, V*erticillium* 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 *P. americana x P. schiedeana* Coban, Guatamala Market collection.	Available in South Africa. It is highly *Pc* 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 *Pc* 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, Guatamala.	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 *Pc* 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 *Pc* pressure)	Hass yield on Duke 7 > **Merensky 2** > Thomas > Borchard > D9 (Merensky is a vigorous rootstock. Other South African selections not included)
8 year trial at SCFS (low *Pc*)	Hass yield on **Jovo** > Thomas > Toro Canyon > Parida
2 year trial at Camarillo (Heavy *Pc*)	Health of trees on Spencer > Vc256 > Thomas > **Merensky 4 > Merensky 3**
From greenhouse screening trials	In terms of *Pc* tolerance: G755A = Thomas > Pp4=**Merensky 3 > Merensky 2** > **Merensky** > 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 β-glucans and not chitin
- Mycolaminarin, a β-glucan, is the characteristic storage carbohydrate
- Zoospores are biflagelate, one whiplash and the other a tinsel flagellum (the flagellar rootlet morphology is similar to that of the heterokont algae)
- Exogenous β-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 chlamydospores in the asexual phase and oospores in the sexual phase (Zentmyer, 1980). These two phases are depicted in Figure 2.3.
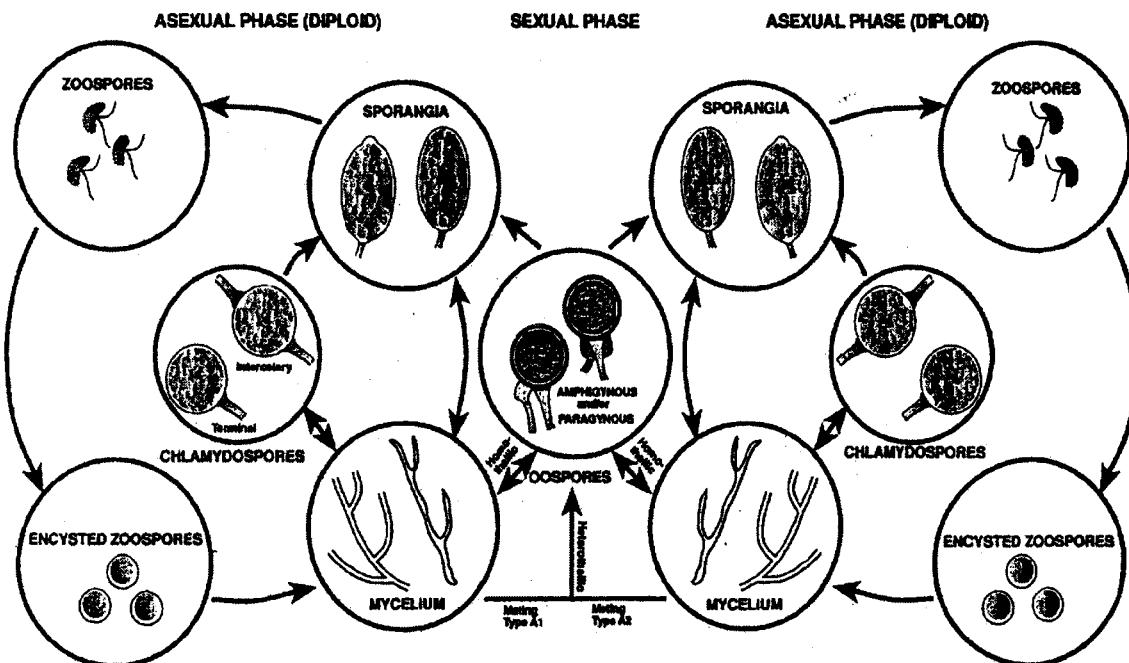

Figure 2.3   Life cycle of *Phytophthora cinnamomi* (Erwin & Ribeiro, 1996).

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

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 sperical, 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 growths 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 isoyme genotypes), while the A2 mating type population was resolved in only two multilocus iozyme 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 form 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 smithiii* (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. smithiii* 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 $P_{10}VP$ and $P_{10}ARP$. The PVP medium was developed by Tsao & Ocana (1969) and modifications of the formula have been widely used. The selective medium $P_{10}VP$ contains low dosages of pimaricin, vancomycin and pentachloronitrobenzene (PCNB). Pimaricin 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 $P_{10}ARP$ (Mitchell &

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

The precence 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 isoxyazole. Reducing the pimaricin dosage to 5 mg and adding 50 mg hymexazol and 100 mg PCNB l^{-1} 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 $P_{10}VP$ 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), *Euacalyptus* 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_{10} 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_{10} 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_{10} 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 µm wide, irregular to coralloid, free branching or clustered.
Hyphal swellings	Spherical, clustered or single, mostly terminal, 20-52 µm 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 longitudal 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 (1µm) and formed singly or in clusters either on parent hyphae or on new hyphal branches.
Antheridia	Predominantly amphigynous, bicellular, 12-20 µm.
Oogonia	Spherical, 35 – 50 µm, surface smooth, Thickness of oogonial wall 1-2 µm or less.
Oospores	Plerotic, i.e. nearly fills the oogonium and the wall of the oospore is 1–3 µm 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

Biflaggelate 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).

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

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

O = 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 form *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 μg 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 necrophylactic 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 necrophylactic 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), Breedt *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 m², 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 m² 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.

MOTHER TREES		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 inflorecence 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.
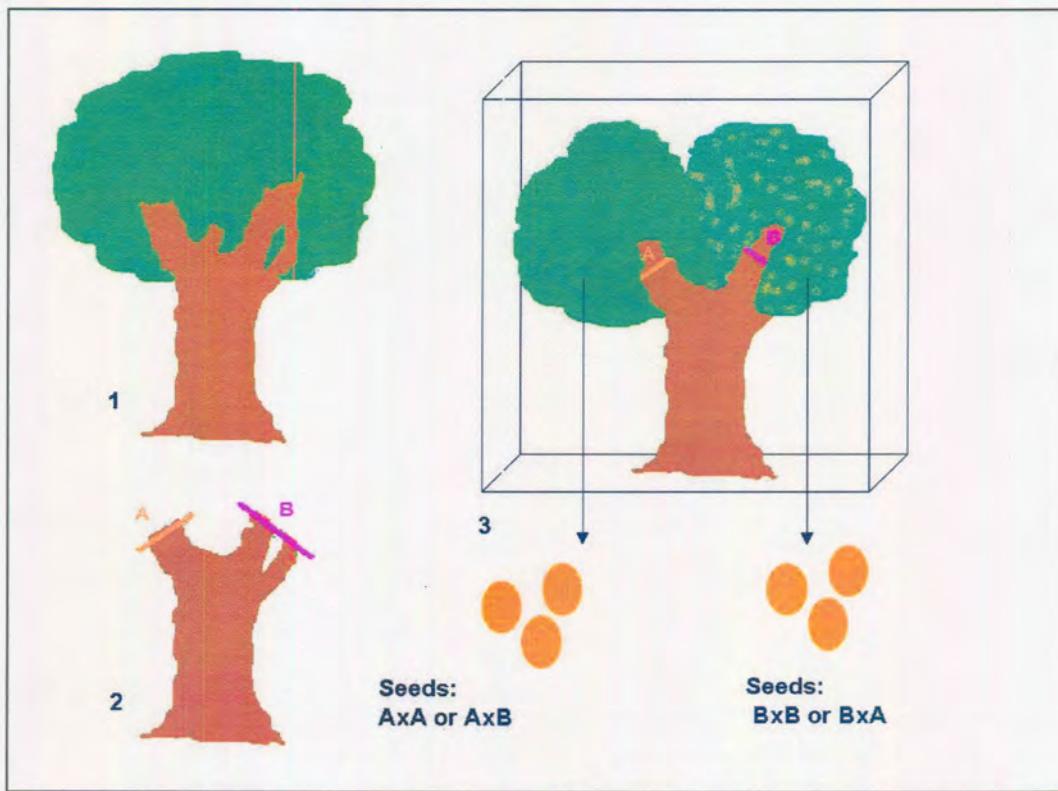
Figure 3.4   1. Elected tree to be used for top working.

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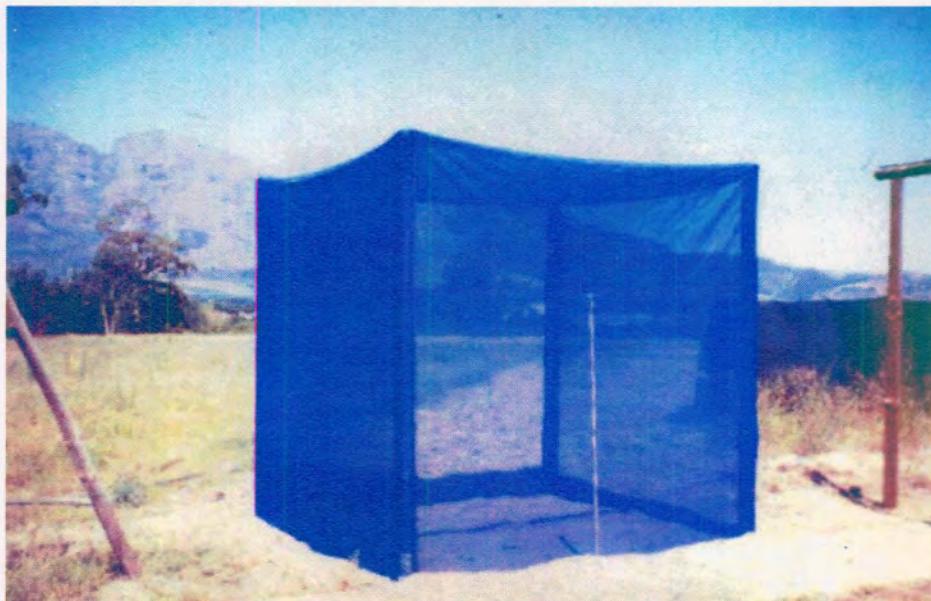
Figure 3.5   Type of cage that was used to encage top-worked and /or single trees for selfing.

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
Phytophthora-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
Total	180	38984	91		

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 geneartion	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 encage with the trees. These colonies had to be very small to be able to survive on one tree. In most instances of encaging 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).
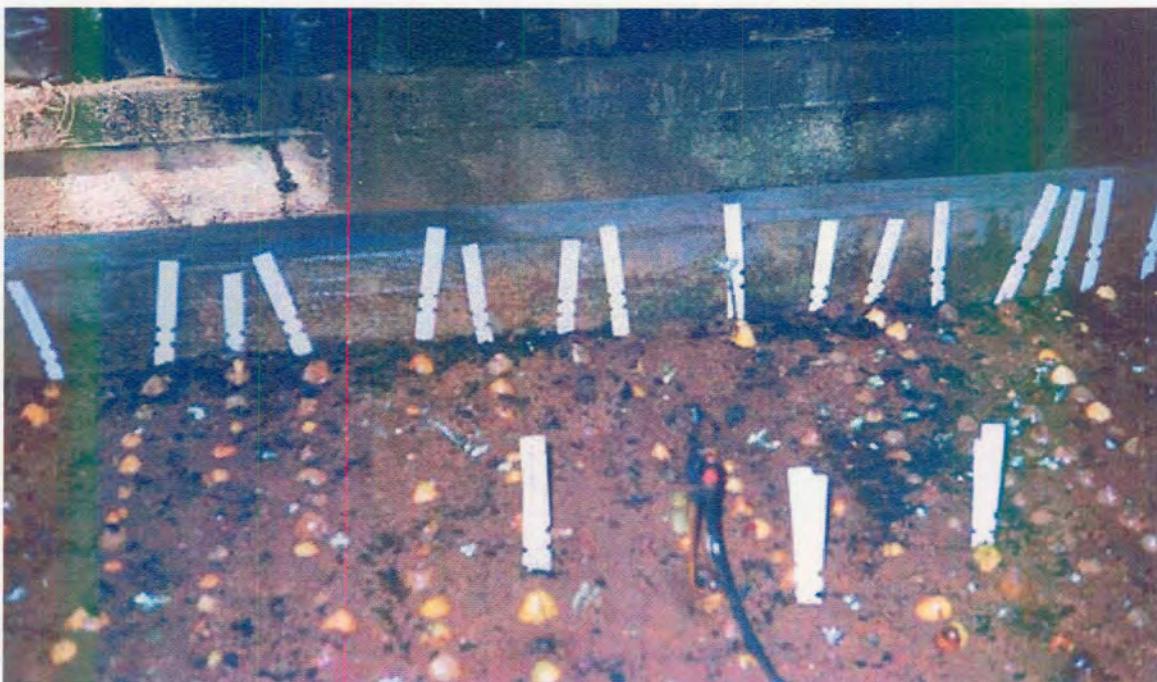
Figure 3.6   Seeds planted directly into *Phytophthora*-infested soil.

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 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-Al. A balanced nutrient solution (Chemicult) was applied at weekly intervals at 2 g l^{-1}. The fosetyl-Al 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
Totals	38982	25486	8792	738	364	91	46

Screen –120 accounts for the germination and surviving (just before inoculation) ±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 in1993 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.
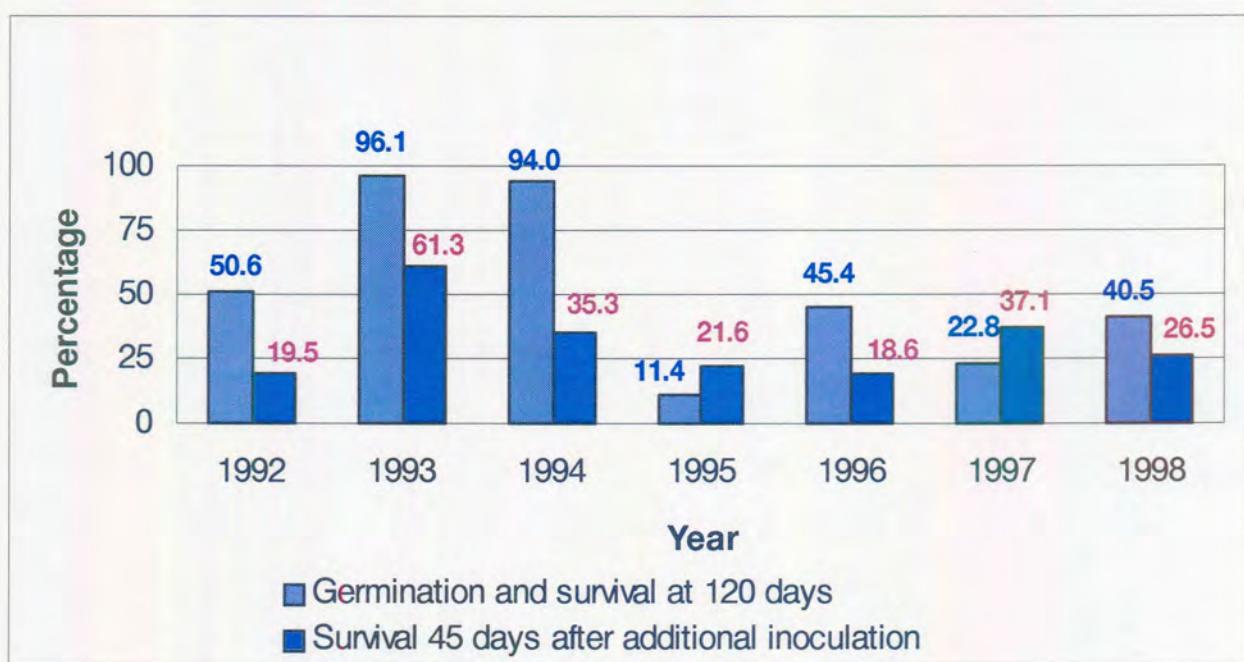
Figure 3.9   Germination and survival from planting to 45 days after inoculation.

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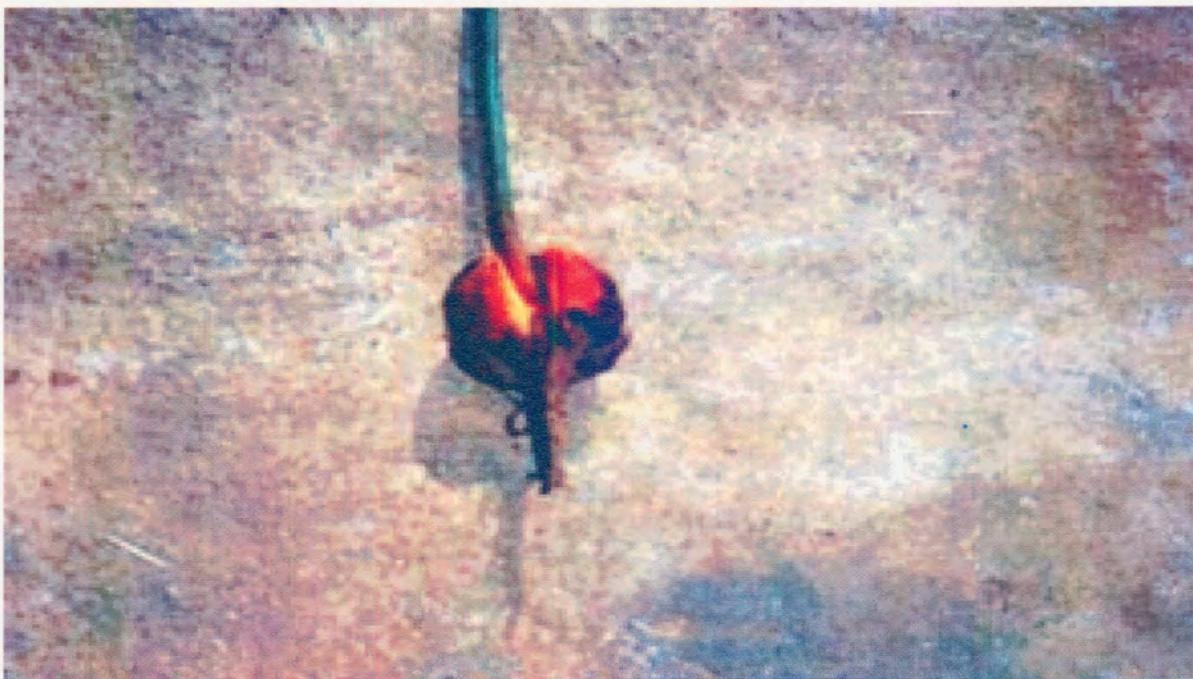
Figure 3.13 An avocado seedling with a rotten taproot and no feeder roots.

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.