TOLERANCE OF CITRUS ROOTSTOCKS TO ROOT PATHOGENS

by

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CHAPTER 1

GENERAL INTRODUCTION

Citrus, known for the fine flavour and quality of its fruit, is grown in nearly 50 countries throughout the world as a highly prized and economically important fruit crop (Castle, 1987). In 1995, southern Africa (including South Africa, Botswana, Zimbabwe, Namibia and Mozambique) was the 14th largest citrus producing region in the world with a total production of 1.02 million tonnes of citrus fruit. Current production is 1.33 million tonnes, which already exceeds the production of 1.3 million tonnes predicted for 2005 (Steve Burdette, Capespan Group Holdings (Pty) Ltd, Bellville, personal communication). After Spain and the United States of America, southern Africa is the third largest exporter of fresh citrus. From the approximately 16 million citrus trees planted on 58 000 ha in southern Africa in 1996, 64% of the total production was exported, 23% processed and 13% consumed locally (Barry, 1996). This ratio has not changed significantly (Steve Burdette, Capespan Group Holdings (Pty) Ltd, Bellville, personal communication). Outspan International Ltd.(now incorporated into Capespan Group Holdings), as one of the export-marketing agents for southern African citrus, exported 45.5 million cartons of citrus fruit in 1996 and 44.5 million cartons in 1997.

World-wide, diseases caused by fungi, bacteria, phytoplasms, viruses and nematodes are the most serious limitation to profitable production of citrus in otherwise suitable environments (Davies & Albrigo, 1994). *Phytophthora* de Bary species cause severe diseases of citrus world-wide (Timmer & Menge, 1988; Graham, 1990). *Phytophthora nicotianae* Breda de Haan (syn. *P. parasitica* Dastur) and *P. citrophthora* (R.E. Sm. & E.H. Sm.) Leonian are the most devastating fungal pathogens involved in citrus root rot (Klotz *et al.*, 1958). More recently, Zitko *et al.*

(1991) found *P. palmivora* var. *palmivora* (E. Butler) E. Butler to be a highly aggressive and damaging pathogen on certain citrus varieties in Florida, USA.

Under conditions conducive to disease, *P. nicotianae* causes tree decline and yield losses in many citrus cultivars (Graham, 1995a). Root rot occurs when the pathogen infects the root cortex and causes decay of fibrous roots (Graham, 1990; 1995a, b; Agostini *et al.*, 1991). The cortex turns soft, appears water-soaked and becomes somewhat discoloured. The fibrous roots slough their cortex leaving only the white, thread-like stele, giving the root system a stringy appearance (Graham, 1995a). Feeder roots are frequently affected in nurseries and the problem is often transferred from there into the field (Grech & Rijkenberg, 1992). *P. nicotianae* can also cause foot rot, which is recognised as an injury of the bark on the trunk or roots near the soil level. These lesions on the trunk or crown roots can girdle and kill the tree (Timmer & Menge, 1988; Graham, 1990).

Fusarium solani (Mart.) Appel & Wollenw., despite often being regarded as a "mild pathogen", also forms an important part of the citrus root rot complex (Labuschagne, 1994). It causes dry rot of citrus, characterised by death of the bark and brown staining of the scaffold roots and crown below the bud-union (Bender & Menge, 1984). It is also associated with feeder root rot which is characterised by cortical sloughing exposing the light-coloured stele in the centre of the root (Nemec & Zablotowich, 1981). *F. solani* furthermore is implicated in the "sudden death" syndrome. The leaves on an otherwise healthy tree suddenly wilt and turn yellow and the tree dies rapidly, often with the leaves and fruit still on the tree (Labuschagne, 1994).

Most researchers regard *F. solani* as a secondary parasite, because the fungus is also isolated from healthy citrus roots (Nemec, 1978; Graham *et al.*, 1983). Labuschagne & Kotzé (1988) demonstrated that waterlogging, girdling and high rates of inorganic nitrogen fertiliser, predispose citrus plants to root rot caused by *F. solani*. Carbohydrate depletion prior to inoculation also increasesthe susceptibility of citrus to infection by *F. solani* (Labuschagne *et al.*, 1992). Reduction in growth was more pronounced in the presence of both *F. solani* and *Tylenchulus semipenetrans* Cobb than each pathogen on its own (Van Gundy & Tsao, 1963; Nemec, 1978).

The citrus nematode, *T. semipenetrans*, is distributed throughout citrus growing regions of the world and causes citrus slow decline. By causing significant reductions in tree growth and yield over time, it is an important limiting factor in fruit production (McCarthy *et al.*, 1979; Kaplan & O'Bannon, 1981). Citrus slow decline is characterised by a reduction in fibrous root biomass, root disfunction, and undersized fruit (Duncan *et al.*, 1993). In susceptible rootstocks, juvenile stages of the female nematode will penetrate through the epidermal and hypodermal layers of the fibrous roots deep into the root cortex and parenchyma cells. Permanent feeding sites will be formed and the juveniles then mature into adults (Ferguson *et al.*, 1996). Hypersensitive reactions and the formation of wound periderm protect rootstocks against nematode penetration (Van Gundy & Kirkpatrick, 1964; Kaplan & O'Bannon, 1981).

Rootstocks are of primary importance to the citrus industry because of their long-term horticultural benefits (Castle, 1987; Niles *et al.*, 1995), such as tolerance to soil salinity, flooding, drought and cold injury, pronounced effects on scion vigour and size, fruit yield and size, juice quality and disease tolerance (Castle, 1987).

Although some root pathogens can be controlled by means of chemicals (Sandler *et al.*, 1989) and preventative phytosanitary methods such as soil fumigation and treated irrigation water (Grech & Rijkenberg, 1992), control through rootstock tolerance is a more desirable solution (Castle, 1987) because it is less expensive and more effective in the long run (Furr & Carpenter, 1961).

Tolerant rootstocks offer an excellent means of reducing losses from *Phytophthora* root (Carpenter & Furr, 1962) and foot (Afek *et al.*, 1990) rot. Over the years many workers have reported on rootstock resistance to *Phytophthora* spp. (Carpenter & Furr, 1962; Klotz *et al.*, 1967, 1968; Cameron *et al.*, 1972; Hutchison & Grimm, 1972; Whiteside, 1974; Carpenter *et al.*, 1975, 1981; Tuzcu, 1984; Smith *et al.*, 1987; Graham, 1990, 1995a, b; Agostini *et al.*, 1991; Hough, 1992; Broadbent & Gollnow, 1993). Rootstocks vary in their tolerance to *Phytophthora* spp. (Agostini *et al.*, 1991). *Phytophthora*-resistance is found in rootstock varieties and selections of most citrus, including trifoliate oranges, citranges, rough lemon, tangelos, Macrophylla and Volkamer lemon (Carpenter *et al.*, 1975; Wutscher, 1979; Hough, 1992; Kosola *et al.*, 1995). Menge & Nemec (1997) pointed out that in general, sweet oranges are very susceptible to *Phytophthora*, and according to Graham (1995b), may support high population densities of the fungus in soil within the root zone. Trifoliate orange, from which most of the *Phytophthora*-resistance is derived, may support lower soil populations of *Phytophthora*. Resistant citrus rootstocks have been recognised as important in the management of the citrus nematode (Kaplan & O'Bannon, 1981).

Very little research has been done on tolerance of rootstocks against *F. solani*. Citranges, Citrumelo, Macrophylla, Rough lemon and Cleopatra mandarin appear to be more susceptible to *Fusarium* spp. in the field than sour and sweet orange rootstocks (Menge, 1988).

Currently, Carrizo and Troyer citrange (*Citrus sinenses* (L.) Osb. x *Poncirus trifoliata* (L.) Raf. x *C. paradisi* Macf.) are the group of rootstocks most frequently used (37% of seed supplied by Capespan) in southern Africa. They have been ranked as tolerant to *Phytophthora* root rot and the citrus nematode, but somewhat susceptible to *Fusarium* dry rot (Rabe & Von Broembsen, 1991). Other rootstocks used in southern Africa include Rough lemon (*C. jambhiri* Lush.) (31%), Swingle citrumelo (*P. trifoliata* x *C. paradisi*) (17%), Volkamer (*C. volckameriana* Ten. & Pasq.) (7%), X639 hybrid [*P. trifoliata* x *C. reticulata* Blanco cv. Cleopatra mandarin (*C. reshni* Hort. ex Tan.)] (6%) and Minneola x Trifoliate hybrid [(*C. paradisi* x *C. reticulata*) x *P. trifoliata*)](1%) (Steve Burdette, Capespan Group Holdings (Pty) Ltd, Bellville, personal communication).

The objective of this study was to evaluate citrus rootstocks for resistance or tolerance against *P. nicotianae* and *F. solani* on their own and in combination, as well as against *T. semipenetrans*. The most reliable parameters to be used during screening for tolerance will also be assessed.

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CHAPTER 2

TOLERANCE OF CITRUS ROOTSTOCKS TO *PHYTOPHTHORA NICOTIANAE* AND *FUSARIUM SOLANI* UNDER GREENHOUSE CONDITIONS

ABSTRACT

Thirty different citrus rootstocks were evaluated for their tolerance to *Phytophthora nicotianae, Fusarium solani* and a combination of these two fungi in the greenhouse. Seedlings were grown in artificially infested media for three months and compared to Swingle citrumelo and Rough lemon rootstocks. Plant height, disease severity, feeder root length and root and shoot dry mass were used as parameters to determine the degree of tolerance or susceptibility of the rootstocks to the different pathogen treatments. Plant height and shoot mass were the most reliable parameters, whereas feeder root length was the most unreliable. Australian trifoliate and Shekwasha mandarin were highly tolerant of *P. nicotianae,* while Natsudaidai, Terra Bella citrange, Jacobsen trifoliate, Rusk citrange, Shekwasha mandarin and Pomeroy trifoliate were the most tolerant of *F. solani*. Swingle citrumelo nevertheless was more tolerant of both fungi then any of the above rootstocks.

INTRODUCTION

Throughout the world *Phytophthora* and *Fusarium* root rots are major soilborne diseases of citrus (Menge & Nemec, 1997).

Phytophthora nicotianae Breda de Haan (syn. *P. parasitica* Dastur) is the main cause of root rot in citrus (Timmer & Menge, 1988). Infection is usually characterised by decay of the small fibrous roots, which results in general decline and die-back of the leaf canopy (Blaker & MacDonald, 1986). Sixty-five percent of all citrus plantings in southern Africa is affected by this pathogen (Schutte, 1994).

Fusarium solani (Mart.) Appel & Wollenw., a common soilborne fungus (Menge & Nemec, 1997) and inhabitant on citrus roots (Nemec & Zablotowicz, 1981), is also associated with root rot and of major importance in terms of productivity of citrus (Labuschagne, 1994). However, there have been some conflicting reports about the pathogenicity of *F. solani*. Because of failures to infect citrus with *F. solani* in pot studies, Sherbakoff (1953), Martin *et al.* (1956), Graham *et al.* (1983) and Dandurand & Menge (1993) considered the fungus to be a saprophyte or weak pathogen colonising only rotted or injured roots. Sherbakoff (1953) considered *F. solani* to be playing a secondary role in the decline of citrus. Van Gundy & Tsao (1963), Nemec *et al.* (1980), Nemec & Zablotowicz (1981) and Labuschagne & Kotzé (1988) have demonstrated the pathogenicity of the fungus to citrus and showed that it is influenced by inoculation techniques, cultural practices and the environment. According to Labuschagne *et al.* (1987), *F. solani* can establish a symptomless infection in citrus roots and cause severe root rot under stress conditions.

Rootstocks are important in citrus production because of their potential resistance to soilborne pathogens (Kupper *et al.,* 1994). Tolerant rootstocks can offer an effective means of reducing losses from *Phytophthora* and *Fusarium* root rot (Carpenter & Furr, 1962; Labuschagne, 1994). Graham (1995a) defined root rot tolerance as the condition where plants are infected, but show little or no net root loss because root mass is maintained by root regeneration. Shurtleff & Averre (1997) described tolerance as the ability of a plant to endure an infectious disease, physiological disorder, adverse conditions or chemical injury without serious damage or yield loss. They defined resistance, on the other hand, as the inherent ability of an organism (host plant) to overcome or retard, completely or to some degree, the activity of a pathogen or other damaging factor. Lastly, susceptibility was defined as the inability of a plant to resist the effect of a pathogen or other damaging factor. Kosola *et al.* (1995) found the fine roots of rootstocks, for example those susceptible to *P. nicotianae*, to have a shorter lifespan and to support larger populations of *P. nicotianae* than the fine roots of a more tolerant rootstock.

Root pathogens of citrus can be chemically controlled, but it is very expensive (Sandler *et al.*, 1989) and certain pathogens have a tendency to become resistant towards some of the chemicals (Fisher, 1993). Tolerant rootstocks, on the other hand, can render protection, which is effective over a longer period of time (Furr & Carpenter, 1961).

The objective of this study was to evaluate various citrus rootstocks available in South Africa for tolerance to *P. nicotianae*, *F. solani* and the combined effect of these two pathogens under greenhouse conditions.

MATERIALS AND METHODS

Citrus rootstocks. Three-month-old seedlings of thirty different types of rootstocks (Table 1), were supplied by H F le Roux of Capespan Citrus Centre in Nelspruit. For ease of reading, genera and species names are not referred to in the text, but only the cultivar names. All seedlings of each cultivar were maintained in 44 x 190 x 20 cm asbestos pots, in a steam-pasteurised sand / peat / soil (1:1:1 v/v) mixture at 15 ± 2°C during night and 27 ± 2°C during day in a greenhouse. This was done because the rootstocks had to be evaluated in groups / batches because of limited greenhouse space. Seedlings were watered three times a week with distilled water. Red spider mite was controlled with alternate applications every six weeks of the following insecticides: 5 ml Dursban™, Efekto (a.i. chlorpyriphos 240 g^{-1}) 5 l^{-1} water, 200 ml Red Spidersprey™, Wonder (a.i. amitraz 200 g^{-1}) 100 l^{-1} water. The plants were fertilised every six weeks with 10 g Chemicult™, Kompel (macro-elements: 6.5% N, 2.7% P, 13.0% K, 7.0% Ca, 2.2% Mg, 7.5% S; micro-elements: 0.15% Fe, 0.024% Mn, 0.024% B, 0.005% Zn, 0.002% Cu, 0.001% Mo) 5 l^{-1} water. The remaining rootstocks (those that were planted in the second year) were transplanted every six months to fresh sand / peat / soil mixture. All rootstocks were maintained under these conditions until used.

Disease status of rootstocks at commencement of each trial. To confirm the absence of pathogens in plants at the start of each experiment, roots were randomly selected from each of six randomly selected seedlings of each cultivar and processed as follows: roots were washed, cut into segments ca. 5 mm in length, surface disinfested for 1 minute in 1.5% sodium hypochlorite solution and rinsed in sterile distilled water. Root segments (ten per medium) were plated on potato-dextrose agar (PDA) and a selective medium for *Fusarium,* namely rose-bengal-glycerine-urea agar (10 g glycerine, 1 g urea, 0.5 g L-alanine, 1 g quintozene (Terrachlor™, 75% a. i.), 0.5 g rose-bengal, 15 g Biolab™ PDA l^{-1} distilled water supplemented with 50 mg^{-1} streptomycin) (RbGU) (Van Wyk *et al.*, 1986).

Some root segments were only washed and then rinsed in sterile distilled water. These segments were plated (10 per medium) on a selective medium for *Phytophthora* spp., namely pimaracin-ampicillin-rifampicin-pentachloronitrobenzene-hymexazol (PARPH) [half-strength PDA (20 g Biolab™ PDA 800 ml^{-1} distilled water), supplemented after autoclaving with a solution containing: 100 mg quintozene, 250 mg ampicillin, 10 mg rifampicin, 10 mg pimaricin and 0.15 ml (103 ppm) hymexazol (Tachigaren™, 70% a. i.) added to 200 ml sterile distilled water (Jeffers & Martin, 1986)].

Plates were incubated at room temperature (25$\pm$2°C) in the dark and observed regularly over a two-week period for fungal growth. Sub-cultures from all fungal colonies were made and the fungi identified (Nelson *et al.*, 1983; Erwin & Ribeiro, 1996).

Pathogen isolates. A fresh isolate of *P. nicotianae* (obtained from infested soil in an orchard in the Sundags River Valley, Eastern Cape Province, South Africa) and *F. solani* isolate JF10 [isolated by Strauss (1992) from Rough lemon rootstock (scion Delta Valencia sweet orange), Letaba Estates, Northern Province, South Africa] were used. Fungal isolates were multiplied on PDA (39 g Biolab™ PDA l^{-1} distilled water). To maintain cultures between trials, *F. solani* was stored as a spore suspension (500 µl / vial) in autoclaved silica sand in sealed glass vials. *P. nicotianae* was stored as mycelial discs in sterile distilled water. Both pathogens were kept in darkness at room temperature (25±2°C).

Preparation of inoculum. Millet seed was used as carrier for fungal inoculum. 200 g millet seed in high-density polyethylene bags (17 cm x 25 cm) was moistured with 100 ml sterile distilled water and autoclaved for 20 minutes on two successive days. Each bag was inoculated with ten mycelial discs (6 mm in diameter) of either a seven-day-old *F. solani* or a 14-day-old *P. nicotianae* culture (Strauss, 1992). Inoculated bags were incubated for four weeks at 25±2°C in the dark and were regularly kneaded to ensure even spread of the fungus. Uninoculated millet seed was used as control.

Treatments. Rootstocks were tested against *P. nicotianae*, *F. solani* and a combination of these two fungi in groups of approximately ten at a time. An uninoculated treatment served as control. Six replicate plants per rootstock were used per treatment. The trials were conducted in 1996 and repeated 12 months later (1997) with the same rootstocks, but without the *P. nicotianae* / *F. solani* combination. In each experiment Swingle citrumelo and Rough lemon were included as reference rootstocks.

Inoculation. Millet seed inoculum of each fungus was added at a rate of 3 % v/v to a steamed sand / peat / soil (1:1:1 v/v) mixture and uniformly mixed. Seedlings were transplanted into the inoculated medium in 15 cm diameter plastic pots and grown for three months at 15±2°C during night and 27±2°C during day in a greenhouse. Pots were arranged in a block design, the cultivar replicates completely randomised within blocks to avoid cross-contamination. Seedlings were watered three times a week with distilled water.

Evaluation. At the beginning and end of each trial, seedling heights were measured from the soil-line to the top of the foliage. Percentage increase in plant height was calculated by subtracting the original height from the height at termination of the trial. Increase in plant height was expressed in relation to the plant height of the uninoculated control.

Three months after planting, the rootstocks were carefully removed from the pots, their root systems washed free from soil in tap water and processed as described below.

Disease severity was determined by rating each root system for visual root rot on a scale of 1 - 4 (1 = 0-25% of root system rotted, 2 = 26-50% of root system rotted, 3 = 51-75% of root system rotted and 4 = 76-100% of root system rotted). Increase in disease severity was expressed in relation to disease severity index of the uninoculated control and calculated as follows: inoculated (rating) - uninoculated (rating). After rating of disease severity, root samples were taken at random from the various treatments, and prepared and plated out as described above.

Feeder root length was determined after isolations were done by measuring the length of 0.1g of roots per rootstock with a Geotron™ (model nr. WLM1) root length meter (Geotron Systems (Pty) Ltd., P.O Box 2656, Potchefstroom, 2520). Results were expressed as the percentage increase in feeder root length and calculated as follows: ([uninoculated - inoculated] / uninoculated) x 100 (Gabor & Coffey, 1990). It was also expressed in relation to the feeder root length of the uninoculated control.

Root and foliage mass was determined after drying for 48 h at 80°C. Percentage reduction in root and shoot dry mass was calculated according to the following formula: ([uninoculated – inoculated]/uninoculated) x 100 (Gabor & Coffey, 1990). Reduction in root and shoot dry mass was expressed in relation to root and shoot dry mass of the uninoculated control.

Analysis of variance (ANOVA) was performed by means of GENSTAT 5 (GENSTAT Committee, 1993). Treatment means and interactions between the treatments were compared by means of Fisher's Protected Least Significant Difference (*LSD*) *t*-test procedure (Snedecor & Cochran, 1967). At 5% level of significance, the best group of rootstocks was designated tolerant (best group, T) and the poorest group susceptible (poorest group, S) based on the Multiple *t*-distribution test procedure of Gupta & Panchapakesan (1979).

RESULTS

Before planting no fungal pathogens could be isolated from the roots of the various rootstocks. At termination of the trails, *F. solani* and *P. nicotianae* infection was confirmed by isolations from the root systems in the respective treatments. Both pathogens were isolated from the roots of plants in the combination treatment, whereas no pathogens were isolated from the uninoculated control treatments.

Data of the 1996 and 1997 trials are presented in Tables 2-6 and 7-11 respectively.

According to feeder root length, the *Fusarium, Phytophthora* and *Fusarium - Phytophthora* combination treatments differed significantly ($P=0.05$) from each other (Tables 2 and 7), except with the third group of rootstocks tested in 1996. Treatment means of *F. solani* for the first group of rootstocks tested in 1996, and the second and third group of rootstocks tested in 1997 were all significantly lower than those of the other treatments. Treatment means for *Phytophthora* were significant lower than the other treatments for the second group of rootstocks tested in 1996 and the first group of rootstocks tested in 1997. Rootstocks also differed from one another, except for the third group of rootstocks tested in 1997. Rootstock x treatment interaction did not show significant differences between the rootstocks in all the trials except for the first group of rootstocks tested in 1996. Swingle citrumelo and Rough lemon varied in their response between the different groups of rootstocks.

With plant height as parameter, significant differences were evident between the *Fusarium* and *Phytophthora* treatments (Tables 3 and 8), but not between the *Fusarium* and *Fusarium-Phytophthora* combination treatments. In the third group of rootstocks tested during 1997, the treatment mean for *P. nicotianae* was higher (2.5%) than the treatment mean for *F. solani* (-2.0%) but otherwise, the rootstocks were more severely affected by *Phytophthora* than by *Fusarium* and the combination treatment. Rootstocks differed from one another and there were also differences in treatment x rootstock interaction. Growth of Rough lemon was severely to intermediately affected by the two pathogens. Although Swingle citrumelo was affected less, the results varied between experiments and actually indicated susceptibility in the second group of rootstocks. There was therefore poor consistency in the response of the reference rootstocks to the different pathogens. Plant height of Terra Bella citrange was only slightly affected by *F. solani* in both years. The same tendency occurred in the combination treatment, and to a lesser extent with *P. nicotianae*. Terra Bella citrange proved to be a more vigorous grower than the other rootstocks. Plant height of Australian trifoliate was only slightly affected by *P. nicotianae* in both years (5.9 and 14.8% respectively). Natsudaidai was slightly affected by *F. solani* (20.6 and 12.7%) but was severely impeded by *P. nicotianae* (2.1 and -8.9%) and combination (-4.6%) treatments. Jacobsen trifoliate was intermediate in its response to the pathogens in the first year, but was not much affected in the second year. Changsha mandarin increased only slightly in height in pathogen-infested soil in both years, but is in any case not a vigorous grower. Sunchusha mandarin and Rusk citrange were significantly affected by *P. nicotianae*. Obovoideae showed susceptibility to *F. solani* in both years. F80-8, Konejime, 1113 and Cleopatra mandarin were only slightly affected by both *Fusarium* and *Phytophthora*.

In terms of disease severity, the treatments differed significantly from one another in 1996, and in the first and third groups of rootstocks tested in 1997 (Tables 4 and 9). The *Fusarium* and *Fusarium-Phytophthora* combination treatments again did not differ from each other. The treatment mean of *Phytophthora* was consistently higher than that of the *Fusarium* and combination treatments. Rootstocks and treatment x rootstock interactions also differed significantly. Swingle citrumelo was not significantly affected by *P. nicotianae, F. solani* or the combination treatment. Rough lemon was severely affected by *P. nicotianae* in the first year, but appeared to be more intermediate in the second year. It also showed an intermediate reaction to the other two treatments in both years. Pomeroy trifoliate (0.7 and 0) and Rusk citrange (0.5 and 0.5) were tolerant to *F. solani* in both years. Both rootstocks also showed tolerance to *P. nicotianae* in the second year but were susceptible in the first year. Calamandrin (3 and 2.3) and Natsudaidai (2 and 2.3) showed high susceptibility to *P. nicotianae* in both years. Amblycarpa (1.7), Australian trifoliate (1), and Rubidoux trifoliate (0.8) were all intermediate in response to *Fusarium* in the first year, but tolerant in the second year (all 0.3). The latter rootstocks also showed greater vigour (growth) in the second year.

In terms of root mass (Tables 5 and 10) significant differences were evident between treatments, except for the first group in the second year. The tendency was for the *Phytophthora* treatment mean to be higher than that of the *Fusarium* and combination treatments. Rootstocks also showed significant differences, as did the treatment x rootstock interactions, except for the first group of rootstocks in both years. Swingle citrumelo showed a low percentage of root loss in all the treatments, except for the *P. nicotianae* treatment in the second group of rootstocks tested in 1996, where it was actually rated as susceptible. Rough lemon had an intermediate to high reduction in root mass in the *Fusarium* and *Phytophthora* treatments but was, however, tolerant to the combination treatment in the third group of rootstocks 1996. The root mass of Rusk citrange was affected little by *F. solani* in both years (13.4 and 2.4%). Natsudaidai (76.9 and 140%), Australian trifoliate (67.2 and 57.3%) and Japanese citron (93.2 and 77.5%) were all severely affected by *Phytophthora* in 1996 and 1997. Sunchusha mandarin responded with a substantial reduction in root mass (73 and 74.4%) to *F. solani* in both years. The tendency was for the root systems of Changsha mandarin and Shekwasha mandarin to be more severely affected by the *Fusarium* and *Phytophthora* treatments in 1996 than in 1997. However, these two mandarins were not severely affected by the combination treatment.

Fusarium and *Phytophthora* significantly reduced shoot mass of rootstocks (Tables 6 and 11). Again, the tendency was for treatment means of *Phytophthora* to be higher than that of the *Fusarium* and combination treatments. This is probably due to the high inoculum of *F. solani* that could have contained metabolites toxic to *P. nicotianae*. No differences occurred between the treatment means of the *Fusarium* (23.2%) and combination (25.3%) treatments in the first group of rootstocks in 1996. With the third group of rootstocks in 1996, the treatment means of the *Phytophthora* (48.3%) and combination (51.7%) treatment did not differ. No differences were evident between rootstocks in the first group in the second year. There was also no difference in terms of the rootstock x treatment interaction. For the rest, significant differences occurred between the rootstocks and the rootstocks x treatment interactions. Swingle citrumelo showed a low percentage reduction in shoot mass with all the pathogens, while Rough lemon showed a susceptible to tolerant response. Jacobsen trifoliate (2.1 and 32.1%), Rusk citrange (6.1 and 5.1%) and Shekwasha mandarin (26.5 and 13.2%) all showed relatively little reduction in shoot mass with *Fusarium* in both years. Changsha mandarin, on the other hand, showed a great reduction in shoot mass with both *Fusarium* and *Phytophthora* at first, but was rated as tolerant in the second year. It also appeared to be tolerant to the combination treatment (2.4%). Cleopatra mandarin and Natsudaidai were negatively affected by both *F. solani* and *P. nicotianae* in 1996 and 1997. Jacobsen trifoliate (76.2 and 53.7%), 1113 (67.1 and 72.2%), Rusk citrange (42.5 and 58.3%) and Japanese citron (77 and 59.4%) were all susceptible to *P. nicotianae*, while Sunchusha mandarin (64.1 and 69.7%) and Sunki mandarin (63.7 and 61.6%) were susceptible to *F. solani*. Rootstocks with a low percentage root loss generally showed a low percentage reduction in shoot mass.

DISCUSSION

The rootstocks were classified as tolerant, intermediate or susceptible for all three pathogen treatments in all three groups of rootstocks for both years. The term tolerant, rather than resistant, was used since even the most resistant rootstock can show root rot under conditions conducive to disease development (Menge & Nemec, 1997).

Overall, the pathogen treatments differed significantly from each other. The tendency was for the rootstocks to be more severely affected by *Phytophthora* than by *Fusarium* and the combination treatment. Generally, the *Phytophthora* / *Fusarium* combination showed less root rot, indicating a possible protective effect against *P. nicotianae* by *F. solani* (Strauss & Labuschagne, 1994). Where the treatments did not differ significantly from each other, the relevant parameter was not considered to be a reliable indicator of tolerance or susceptibility.

With root length as criterion, differences in rootstock response to the treatments were mostly non-significant. This could be attributed to feeder root loss occurring during the process of harvesting and washing of the root systems. This parameter is therefore not a reliable indicator of tolerance. A similar tendency was evident with root mass as parameter in the first group of rootstocks. Some correlation did exist between the reduction in root and shoot dry mass. A reduction in root mass usually correlated with reduction in shoot mass. This tendency was also reported by Matheron *et al.*, (1998).

Where increase in plant height and reduction in shoot mass were determined, significant differences consistently occurred between the treatments and treatment x rootstock interactions. The rootstocks also differed significantly within a parameter.

These two parameters are, therefore, considered to be the most reliable. Root and shoot mass showed some correlation, and reduction in root mass should therefore also be a reliable parameter. Increase in disease severity provided a relatively good indication of tolerance or susceptibility although not as good as plant mass.

In this study, rootstocks clearly showed differences in vigour. It is known that rootstocks impart vigour to the scion (Rabe & Von Broembsen, 1991) and that physiological status of the plant (e.g. age, nutrition, succulence, vigour, maturity of the growth flush etc.) is important in the evaluation of tolerance (Broadbent & Gollnow, 1993). Seedlings used during 1997 were 12 months older than those used in 1996. The older seedlings grew more vigorously, and hence showed greater tolerance to the pathogens. This observation corresponds with that of Graham (1990) who found three-month-old seedlings to be less tolerant than older seedlings. According to Zentmyer (1961) the phenomenon can be ascribed to the fact that younger seedlings may have higher rates of root exudation than older plants, which renders them more susceptible to root infection. However, the tolerance of older seedlings could also be due to phytoalexins accumulating in their roots (Afek & Sztejnberg, 1988).

The reference rootstock Swingle citrumelo was found to be tolerant to intermediate in most of the treatments. This rootstock is widely regarded as tolerant to *Phytophthora* spp. in particular (Castle *et al.*, 1988; Graham, 1990; Rabe & Von Broembsen, 1991) and is the fourth most commonly used rootstock in South Africa (Steve Burdette, Capespan Group Holdings (Pty) Ltd, Bellville, personal communication). In some instances, however, Swingle citrumelo appeared susceptible to *P. nicotianae*. This can possibly be attributed to the young seedlings being more susceptible.

The other reference rootstock, Rough lemon, was susceptible to *Phytophthora* and intermediate to *Fusarium*. It appeared to be intermediate to tolerant to the combination treatment, which can possibly be ascribed to the protective effect of *F. solani* (Strauss & Labuschagne, 1994). Rough lemon is known to be highly susceptible to *Phytophthora* infection (Klotz *et al.*, 1967; Broadbent, 1969; Grimm & Hutchison, 1973; Whiteside, 1974; Tuzcu *et al.*, 1984; Afek *et al.*, 1990; Agostini *et al.*, 1991; Broadbent & Gollnow, 1992; Feichtenberger *et al.*, 1992; Graham, 1995a; Kosola *et al.*, 1995). According to Hough (1992) the various clones of Rough lemon used in South Africa vary considerably in susceptibility to root rot. Labuschagne *et al.* (1996) found that Rough lemon had an intermediate response to *F. solani*, which agrees with the findings of the present study.

According to plant height, which proved to be a reliable parameter, Australian trifoliate was tolerant to *P. nicotianae*. However, according to the reduction in root mass, which is a less reliable parameter, it rated as susceptible to *P. nicotianae*. Hough (1992) found Australian trifoliate to be highly resistant to *Phytophthora* root rot according to a rating based on root rot and leaf yellowing. With the other parameters used, Jacobsen trifoliate and Pomeroy trifoliate were both tolerant to *F. solani*. Jacobsen trifoliate, however, appeared to be susceptible to *Phytophthora*, while Pomeroy trifoliate was intermediate. Pomeroy trifoliate has previously been reported to be highly tolerant to *P. citrophthora* and *P. parasitica* (= *P. nicotianae*) (Klotz *et al.*, 1967; Rabe & Von Broembsen, 1991). Trifoliate oranges are mostly tolerant to *Phytophthora*, but the tolerance can be variable (Castle, 1987). Trifoliate rootstocks have a slow growth rate with a medium tree size (Rabe & Von Broembsen, 1991). Yield and fruit size are usually good. Trifoliates are tolerant to the citrus nematode and resistant to tristeza, but sensitive to exocortis. They are also tolerant of poor drainage and high clay content. They are, however, sensitive to high sand, high salt, high pH and drought conditions.

Terra Bella citrange was tolerant to *F. solani* and tolerant to intermediate to *P. nicotianae* according to increase in plant height. Hough (1992) found Terra Bella citrange to be tolerant to *Phytophthora*. For the rest of the parameters it ranged from intermediate to susceptible, which is in contrast to the above. Rusk citrange was found to be fairly consistent in its tolerance to *F. solani*, but it was more susceptible to *P. nicotianae*. Horticultural trials conducted in Florida indicate that tree size is small with this rootstock (Gardner & Horanic, 1961, 1968). This is usually combined with a limited root system and drought intolerance (Castle, 1987). However, trees usually yield well and produce high quality fruit (Rabe & Von Broembsen, 1991). The rootstock is also exocortis and cold tolerant. C32-citrange appeared to be intermediate to tolerant to both *F. solani* and *P. nicotianae*. It was especially tolerant in its reaction to the different treatments according to shoot mass in 1996. Copeland (1988) also reported C32-citrange to be tolerant to *Phytophthora* root rot.

Shekwasha mandarin mostly appeared tolerant to *Fusarium*, but also showed tolerance to *Phytophthora* for different parameters, which is in agreement with findings by Klotz *et al.* (1967). Sunchucha mandarin and Sunki mandarin were susceptible to *F.*

solani and Sunchusha mandarin susceptible to *P. nicotianae* for different parameters. Both these mandarins have previously been reported to be susceptible to *Phytophthora* (Castle, 1987; Feichtenberger *et al.*, 1992; Graham, 1995a). Changsha mandarin and Cleopatra mandarin were both susceptible to *F. solani* and *P. nicotianae* in the present study. Cleopatra mandarin is one of the most widely studied rootstocks. It varied in response to *Phytophthora* infection and has previously been reported as tolerant (Tuzco *et al.*, 1984; Copeland, 1988), intermediate (Klotz *et al.*, 1967; Smith *et al.,* 1987; Agostini *et al.*, 1991) as well as susceptible (Graham, 1990; Broadbent & Gollnow, 1992; Feichtenberger *et al.,* 1992; Graham, 1995b). Bender (1995) also found Cleopatra mandarin to be susceptible to *F. solani*. This rootstock, despite several favourable attributes, remains of minor importance. It has two major flaws as a rootstock, namely, scions budded on it are often slow to bear and fruit size is small (Castle, 1987).

In the present study, according to plant height and shoot mass, Calamandrin, 1113, and Japanese citron were all susceptible to *P. nicotianae,* while Obovoideae was susceptible to *Fusarium* infection. Carpenter & Furr (1962) and Graham (1995a) also found Calamandrin to be susceptible to *P. nicotianae*.

Overall, the rootstocks that proved to be the most tolerant were the following: Australian trifoliate and Shekwasha mandarin (all *Phytophthora* tolerant) and Natsudaidai, Terra Bella citrange, Jacobsen trifoliate, Rusk citrange, Shekwasha mandarin and Pomeroy trifoliate (all *Fusarium* tolerant). Swingle citrumelo was tolerant to both *F. solani* and *P. nicotianae.* Rootstocks that proved to be the most susceptible were the following: Calamandrin, Jacobsen trifoliate, Natsudaidai and Japanese citron (all *Phytophthora* susceptible) and Changsha mandarin, Cleopatra mandarin, Rough lemon and Sunchusha mandarin (*Fusarium* and *Phytophthora* susceptible). These rootstocks were the most consistent between the two years according to selection based on the most reliable parameters.

It is important to remember that no commercially available rootstock possesses all the traits required by the citrus industry to overcome incompatibility, diseases and soil problems. The major problems must be identified and rootstocks that contain the qualities to suppress or overcome the problem must then be selected. No rootstock with complete resistance against root pathogens exists. An integrated control strategy is still the best way to provide control. For instance, the use of a tolerant rootstock together with a registered chemical and correct cultural practices can give optimum control. In the case of *F. solani*, there is currently no chemical or rootstock available to control the pathogen. The most effective control measure is to eliminate stress in trees by optimising growth conditions (Labuschagne *et al.*, 1996). It is of utmost importance to maintain soil conditions favourable for root development and to eliminate conditions such as soil compaction (Joubert, 1993). The ideal for controlling this pathogen would be to combine the above measures with a tolerant rootstock.

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Table 1: Citrus rootstocks evaluated in the greenhouse against *Phytophthora nicotianae*, *Fusarium solani* and the two pathogens in combination

GENUS AND SPECIES	CULTIVAR
Citrus aurantium L.	Gou Tou SO
C. depressa Hayata	Shekwasha mandarin
C. jambhiri Lush.	Rough lemon
C. limon (L.) Burm.f.	Milan lemon
C. limonia Osbeck	Japanese citron
C. macrophylla Wester	Macrophylla
C. natsudaidai Hayata	Natsudaidai
C. obovoidea Hort. ex Takahashi	Obovoideae
C. paradisi Macfady. x *C. reticulata* Blanco	Orlando tangelo
C. paradisi x *C. reticulata*	Sampson tangelo
C. reticulata	Changsha mandarin
C. reticulata	Cleopatra mandarin
C. reticulata	Sunchusha mandarin
C. sunki Hort. ex Tan	Sunki mandarin
Poncirus trifoliata (L.) Raf. var. *monstrosa* (T. Ito.) Swing x *C. sunki*	1112
P. trifoliata	Pomeroy trifoliate
P. trifoliata	Australian trifoliate
P. trifoliata	Jacobsen trifoliate
P. trifoliata	Rubidoux trifoliate
P. trifoliata var. *monstrosa* x *C. sunki*	1116
P. trifoliata var. *monstrosa* x *C. sunki*	1113
P. trifoliata x *C. paradisi*	F80-8 citrumelo
P. trifoliata. x *C. paradisi*.	Swingle citrumelo
P. trifoliata x *C. sinensis*	C32-citrange
P. trifoliata x *C. sinensis*	Rusk citrange
P. trifoliata x *C. sinensis*	Terra Bella citrange
Unknown - perhaps a *C. aurantium* hybrid	Smooth Flat Seville
Unknown parentage	Amblycarpa
Of unknown parentage, probably from the Philippines	Calamandrin
Unknown parentage	Konejime

Table 2: Increase in feeder root length of citrus rootstocks 3 months after inoculation with *Phytophthora nicotianae, Fusarium solani* and *P. nicotianae - F. solani* combination (1996)

Rootstock	% Increase in feeder root length[a]			
	Phytophthora	Combination	*Fusarium*	Rootstock mean
Swingle citrumelo	-85.0 S[b]	-38.1	-10.0	-44.4
Amblycarpa	73.6 T[c]	29.0	26.4	43.0 x[f]
C32-citrange	29.9 [d]	-48.0	-30.5	-16.2
Calamandrin	39.3 T	6.2	6.2	17.3
F80-8 citrumelo	24.7	27.5	-3.3	16.3
Gou Tou SO	28.0	24.2	-6.6	15.2
Konejime	-40.3	-17.7	-15.3	-24.4
Macrophylla	54.5 T	30.3	-2.0	27.6 x
Milan lemon	-78.3 S	-81.6 S	-104.4 S	-88.1 z
Orlando tangelo	53.5 T	54.6 T	40.9 T	49.7 x
Pomeroy trifoliate	44.6 T	22.5	28.0	31.7 x
Rough lemon	28.2	27.9	28.3	28.1 x
Treatment x Rootstock LSD	*{40.1}*	*{40.1}*	*{40.1}*	
Treatment mean	14.4 X[e]	3.1 X	-3.5 Y	
Treatment LSD	*[11.6]*	*[11.6]*	*[11.6]*	*Rootstock LSD (23.2)*
Swingle citrumelo	-8.2 *[g]	16.7 *	35.7 *	14.7 z
1113	26.8	56.6	51.8	45.1 x
Changsha mandarin	-0.4	15.5	37.2	17.4 z
Cleopatra mandarin	-3.6	-3.1	31.8	8.4 z
Jacobsen trifoliate	1.3	31.6	33.7	22.2 z
Natsudaidai	5.6	39.3	46.1	30.3
Obovoideae	12.4	34.8	55.9	34.4 x
Rusk citrange	7.8	26.9	13.7	16.1 z
Shekwasha mandarin	24.6	43.2	45.4	37.7 x
Terra Bella citrange	-13.8	32.8	40.1	19.7 z
Rough lemon	30.7	39.4	33.5	34.5 x
Treatment x Rootstock LSD	*{25.6}* *	*{25.6}* *	*{25.6}* *	
Treatment mean	7.6 Z	30.4 Y	38.6 X	
Treatment LSD	*[7.7]*	*[7.7]*	*[7.7]*	*Rootstock LSD (14.8)*
Swingle citrumelo	-7.5 *	15.7 *	16.4 *	8.2
1112	-22.2	1.9	-19.4	-13.3 z
1116	11.2	7.9	14.6	11.2
Australian trifoliate	2.8	-4.5	11.2	3.2 z
Japanese citron	35.9	42.1	56.9	44.9 x
Rubidoux trifoliate	11.2	25.5	24.7	20.5
Sampson tangelo	-18.3	11.7	0.7	-2.0 z
Smooth Flat Seville	0.0	-1.5	-5.8	-2.4 z
Sunchusha mandarin	0.0	-9.8	-5.7	-5.1 z
Sunki mandarin	-17.1	-37.4	27.6	-8.9 z
Rough lemon	30.5	32.6	33.5	32.2 x
Treatment x Rootstock LSD	*{32.0}* *	*{32.0}* *	*{32.0}* *	
Treatment mean	2.4 *	7.7 *	14.1 *	
Treatment LSD	*[9.7]*	*[9.7]*	*[9.7]*	*Rootstock LSD (18.5)*

a Feeder root length was determined by measuring the length of 0.1g roots. Results were expressed as the percentage increase in feeder root lengths and calculated as follows: ([uninoculated - inoculated]/uninoculated) x 100 (Gabor & Coffey, 1990)

b most susceptible (S) cultivars selected according to the Multiple *t*-distribution test (P=0.05), means followed by the same letter, do not differ significantly according to Fisher's protected *LSD* (*t*-test)

c most tolerant (T) cultivars selected according to the Multiple *t*-distribution test (P=0.05), means followed by the same letter, do not differ significantly according to Fisher's protected *LSD* (*t*-test)

d values not followed by any letter can be classified as intermediate in its response to the pathogen treatment according to the Multiple *t*-distribution test (P=0.05), and do not differ significantly according to Fisher's protected *LSD* (*t*-test), this is only where significant differences did occur within a treatment

e means followed by the same letter (X,Y,Z between treatments) do not differ significantly (P=0.05) according to Fisher's protected *LSD* (*t*-test) best cultivars selected according to the Multiple *t*-distribution test

f means followed by the same letter(x,z within rootstock mean) do not differ significantly (P=0.05) according to Fisher's protected *LSD* (*t*-test) best cultivars selected according to the Multiple *t*-distribution test

g* not significant.

Table 3: Increase in plant height of citrus rootstocks 3 months after inoculation with *Phytophthora nicotianae, Fusarium solani* and *P. nicotianae - F. solani* combination (1996)

Rootstock	% Increase in plant height[a]			
	Phytophthora	Combination	*Fusarium*	Rootstock mean
Swingle citrumelo	-3.6 [b]	5.3	1.3	1.0
Amblycarpa	-20.6	-17.6	-7.9	-15.3 z[f]
C32-citrange	-8.1	27.7 T[c]	13.1	10.9 x
Calamandrin	-25.4 S[d]	-15.8	-3.2	-14.8 z
F80-8 citrumelo	-9.1	11.5	3.9	2.1
Gou Tou SO	-21.9	-7.6	0.8	-9.6
Konejime	-12.7	2.8	7.5	-0.8
Macrophylla	-36.0 S	1.9	-3.3	-12.5 z
Milan lemon	-29.6 S	-14.0	-13.4	-19.0 z
Orlando tangelo	-28.6 S	-10.8	-16.9	-18.8 z
Pomeroy trifoliate	1.8	13.0	-0.3	4.8 x
Rough lemon	-10.0	-8.4	6.2	-4.1
Treatment x Rootstock LSD	*{12.3}*	*{12.3}*	*{12.3}*	
Treatment mean	-17.0 Y[e]	-1.0 X	-1.0 X	
Treatment LSD	*[3.5]*	*[3.5]*	*[3.5]*	*Rootstock LSD (7.1)*
Swingle citrumelo	-0.1	9.3	4.8	4.6 z
1113	8.3	14.4 T	7.5	10.1
Changsha mandarin	-9.8 S	8.1	-3.9 S	-1.8 z
Cleopatra mandarin	3.2	6.0	1.9	3.7 z
Jacobsen trifoliate	11.4	6.8	4.0	7.4
Natsudaidai	2.1	-4.6 S	20.6 T	6.1 z
Obovoideae	-3.0 S	5.8	-2.4 S	0.1 z
Rusk citrange	-14.4 S	8.9	3.1	-0.8 z
Shekwasha mandarin	8.0	14.7 T	7.7	10.1
Terra Bella citrange	17.6 T	24.1 T	20.5 T	20.7 x
Rough lemon	-1.1	-6.8 S	12.9 T	1.7 z
Treatment x Rootstock LSD	*{12.4}*	*{12.4}*	*{12.4}*	
Treatment mean	2.0 Y	7.9 X	7.0 X	
Treatment LSD	*[3.7]*	*[3.7]*	*[3.7]*	*Rootstock LSD (7.1)*
Swingle citrumelo	-0.8	-0.3	13.6 T	4.2 x
1112	-6.8	-7.1	6.1 T	-2.6 x
1116	-15.7	16.6 T	2.7	1.2 x
Australian trifoliate	5.9 T	3.5 T	1.7	3.7 x
Japanese citron	-5.3	3.9 T	3.6 T	0.7 x
Rubidoux trifoliate	-5.8	10.4 T	3.6 T	2.7 x
Sampson tangelo	-9.7	-11.8	-11.7	-11.1
Smooth Flat Seville	-1.4	2.4	10.7 T	3.9 x
Sunchusha mandarin	-18.1 S	-17.9 S	5.1 T	-10.3
Sunki mandarin	-13.9	1.2	8.0 T	-1.5 x
Rough lemon	-18.8 S	-15.8	-30.8 S	-21.8 z
Treatment x Rootstock LSD	*{13.7}*	*{13.7}*	*{13.7}*	
Treatment mean	-8.2 Y	-1.37 X	1.1 X	
Treatment LSD	*[4.1]*	*[4.1]*	*[4.1]*	*Rootstock LSD (7.9)*

a Increase in plant height was calculated by subtracting the original plant height of the seedlings, from the plant height three months later at termination of the trial, and it was expressed as percentage in relation to the uninoculated control

b values not followed by any letter can be classified as intermediate in its response to the pathogen treatment according to the Multiple *t*-distribution test (*P*=0.05), and do not differ significantly according to Fisher's protected *LSD* (*t*-test), this is only where significant differences did occur within a treatment

c most tolerant (T) cultivars selected according to the Multiple *t*-distribution test (*P*=0.05), means followed by the same letter do not differ significantly according to Fisher's protected *LSD* (*t*-test)

d most susceptible (S) cultivars selected according to the Multiple *t*-distribution test (*P*=0.05) means followed by the same letter do not differ significantly according to Fisher's protected *LSD* (*t*-test)

e means followed by the same letter (X,Y,Z between treatments) do not differ significantly (*P*=0.05) according to Fisher's protected *LSD* (*t*-test) best cultivars selected according to the Multiple *t*-distribution test

f means followed by the same letter(x,z within rootstock mean) do not differ significantly (*P*=0.05) according to Fisher's protected *LSD* (*t*-test) best cultivars selected according to the Multiple *t*-distribution test.

Table 4: Increase in disease severity in citrus rootstocks 3 months after inoculation with *Phytophthora nicotianae, Fusarium solani* and *P. nicotianae - F. solani* combination (1996)

Rootstock	Increase in Disease severity[a]			
	Phytophthora	**Combination**	***Fusarium***	**Rootstock mean**
Swingle citrumelo	0.8 T[b]	0.7 T	1.2	0.9 x[f]
Amblycarpa	3.0 S[c]	1.2	1.7	1.9
C32-citrange	1.8 [d]	1.2	1.7	1.6
Calamandrin	3.0 S	1.5	1.8	2.1 z
F80-8 citrumelo	1.8	1.3	1.2	1.4
Gou Tou SO	2.7 S	1.0	0.2 T	1.3 x
Konejime	2.7 S	1.3	0.7 T	1.6
Macrophylla	2.3 S	0.3 T	0.3 T	1.0 x
Milan lemon	2.7 S	1.3	1.7	1.9 z
Orlando tangelo	2.8 S	1.5	0.7 T	1.7
Pomeroy trifoliate	2.8 S	0.8 T	0.7 T	1.4
Rough lemon	2.2 S	0.7 T	1.2	1.3
Treatment x Rootstock LSD	*{0.7}*	*{0.7}*	*{0.7}*	
Treatment mean	2.4 Y[e]	1.1 X	1.1 X	
Treatment LSD	*[0.2]*	*[0.2]*	*[0.2]*	*Rootstock LSD (0.4)*
Swingle citrumelo	0.5 T	0.7 T	1.3	0.8 x
1113	1.0 T	1.0 T	0.7 T	0.9 x
Changsha mandarin	1.3	1.7	2.3 S	1.8 z
Cleopatra mandarin	1.4	2.0	2.0	1.8 z
Jacobsen trifoliate	1.5	1.3	1.2 T	1.3
Natsudaidai	2.0	1.7	1.5	1.7 z
Obovoideae	2.2 S	1.3	1.2 T	1.6
Rusk citrange	2.2 S	1.2 T	0.5 T	1.3
Shekwasha mandarin	1.0 T	0.5 T	1.2 T	0.9 x
Terra Bella citrange	1.5	1.7	1.5	1.6
Rough lemon	2.8 S	1.7	1.7	2.1 z
Treatment x Rootstock LSD	*{0.7}*	*{0.7}*	*{0.7}*	
Treatment mean	1.6 Y	1.3 X	1.4 X	
Treatment LSD	*[0.2]*	*[0.2]*	*[0.2]*	*Rootstock LSD (0.4)*
Swingle citrumelo	0.2 T	0.3	0.3	0.3 x
1112	0.8	0.5	0.5 T	0.6
1116	1.5 S	0.3 T	0.3	0.7 z
Australian trifoliate	0.8	0.8	1.0	0.9 z
Japanese citron	1.8 S	0.3	0.3	0.8 z
Rubidoux trifoliate	1.0	0.5	0.8	0.8 z
Sampson tangelo	0.4	1.3 S	1.5 S	1.1 z
Smooth Flat Seville	0.7	-0.3 T	-0.4 T	0.0 x
Sunchusha mandarin	1.2	0.5	0.5	0.7 z
Sunki mandarin	1.5 S	0.3	0.5	0.8 z
Rough lemon	2.0 S	0.7	0.7	1.1 z
Treatment x Rootstock LSD	*{0.7}*	*{0.7}*	*{0.7}*	
Treatment mean	1.1 Y	0.482 X	0.5 X	
Treatment LSD	*[0.2]*	*[0.2]*	*[0.2]*	*Rootstock LSD (0.4)*

a = inoculated - uninoculated, Root rot was scored on a scale of 1 - 4 where 1 = 0-25% of root system rotted; 2 = 26-50% of root system rotted; 3 = 51-75% of root system rotted and 4 = 76-100% of root system rotted, disease severity index was expressed in relation to disease severity index to the uninoculated control

b most tolerant (T) cultivars selected according to the Multiple *t*-distribution test (P =0.05), means followed by the same letter do not differ significantly according to Fisher's protected *LSD* (*t*-test)

c most susceptible (S) cultivars selected according to the Multiple *t*-distribution test (P=0.05) means followed by the same letter do not differ significantly according to Fisher's protected *LSD* (*t*-test)

d values not followed by any letter can be classified as intermediate in its response to the pathogen treatment according to the Multiple *t*-distribution test (P=0.05), and do not differ significantly according to Fisher's protected *LSD* (*t*-test), this is only where significant differences did occur within a treatment

e means followed by the same letter (X,Y,Z between treatments) do not differ significantly (P=0.05) according to Fisher's protected *LSD* (*t*-test), best cultivars selected according to the Multiple *t*-distribution test

f means followed by the same letter (x,z within rootstock mean) do not differ significantly (P=0.05) according to Fisher's protected *LSD* (*t*-test) best cultivars selected according to the Multiple *t*-distribution test.

Table 5: Reduction in root mass of citrus rootstocks 3 months after inoculation with *Phytophthora nicotianae, Fusarium solani* and *P. nicotianae - F. solani* combination (1996)

Rootstock	% Reduction in root dry mass[a]				
	Phytophthora	Combination	*Fusarium*		Rootstock mean
Swingle citrumelo	50.2 *[b]	0.4 *	39.4 *		30.0 x[d]
Amblycarpa	94.2 [c]	21.1	47.4		54.2 z
C32-citrange	70.1	61.1	70.1		67.1 z
Calamandrin	84.8	39.4	34.3		52.9 z
F80-8 citrumelo	67.3	24.8	65.7		52.6 z
Gou Tou SO	75.9	3.8	45.0		41.6 x
Konejime	63.7	46.6	38.0		49.4
Macrophylla	65.6	33.9	7.4		35.6 x
Milan lemon	71.0	36.0	37.2		48.1
Orlando tangelo	88.9	22.2	22.2		44.4 x
Pomeroy trifoliate	77.8	48.1	29.6		51.9 z
Rough lemon	72.0	10.0	30.0		37.3 x
Treatment x Rootstock LSD	*{35.8}* *	*{35.8}* *	*{35.8}* *		
Treatment mean	73.5 Y[e]	28.9 X	38.9 X		
Treatment LSD	*[10.3]*	*[10.3]*	*[10.3]*	*Rootstock LSD*	*(20.7)*
Swingle citrumelo	63.0 S[f]	20.4 T[g]	48.1		43.8 x
1113	79.9 S	6.6 T	17.6 T		34.7 x
Changsha mandarin	74.4 S	20.5 T	82.1 S		59.0
Cleopatra mandarin	63.2 S	16.7 T	68.4 S		49.4
Jacobsen trifoliate	70.3 S	15.5 T	-2.7 T		27.7 x
Natsudaidai	76.9 S	20.5 T	76.9 S		58.1
Obovoideae	29.1	40.6	36.8		35.5 x
Rusk citrange	50.2	69.7 S	13.4 T		44.4 x
Shekwasha mandarin	74.1 S	0.0 T	51.9 S		42.0 x
Terra Bella citrange	74.7 S	50.9	61.3 S		62.3
Rough lemon	81.1 S	50.8	65.9 S		65.9
Treatment x Rootstock LSD	*{30.6}*	*{30.6}*	*{30.6}*		
Treatment mean	67.0 Z	28.4 X	47.2 Y		
Treatment LSD	*[9.2]*	*[9.2]*	*[9.2]*	*Rootstock LSD*	*(17.6)*
Swingle citrumelo	12.6 T	15.9 T	11.0 T		13.2 x
1112	61.5	70.1 S	48.1		59.9
1116	54.4	33.3 T	31.6 T		39.8
Australian trifoliate	67.2 S	88.0 S	80.1 S		78.4 z
Japanese citron	93.2 S	80.5 S	30.2 T		68.0 z
Rubidoux trifoliate	28.0 T	51.7	23.3 T		34.3
Sampson tangelo	53.8	63.0	51.6		56.1
Smooth Flat Seville	63.9	55.3	52.6		57.3
Sunchusha mandarin	57.2	65.1	73.0 S		65.1 z
Sunki mandarin	62.7	62.7	58.8		61.4
Rough lemon	75.9 S	22.4 T	42.5		46.9
Treatment x Rootstock LSD	*{26.6}*	*{26.6}*	*{26.6}*		
Treatment mean	57.3 Y	55.3 Y	45.7 X		
Treatment LSD	*[8.0]*	*[8.0]*	*[8.0]*	*Rootstock LSD*	*(15.4)*

a ((uninoculated - inoculated)/uninoculated) x 100 (Gabor & Coffey, 1990)

b * not significant

c values not followed by any letter can be classified as intermediate in its response to the pathogen treatment according to the Multiple *t*-distribution test ($P=0.05$), and do not differ significantly according to Fisher's protected *LSD* (*t*-test), this is only where significant differences did occur within a treatment

d means followed by the same letter(x,z within rootstock mean) do not differ significantly ($P=0.05$) according to Fisher's protected *LSD* (*t*-test), best cultivars selected according to the Multiple *t*-distribution test

e means followed by the same letter (X,Y,Z between treatments) do not differ significantly ($P=0.05$) according to Fisher's protected *LSD* (*t*-test) best cultivars selected according to the Multiple *t*-distribution test

f most susceptible (S) cultivars selected according to the Multiple *t*-distribution test ($P=0.05$), means followed by the same letter differ significantly according to Fisher's protected *LSD* (*t*-test)

g most tolerant (T) cultivars selected according to the Multiple *t*-distribution test ($P=0.05$), means followed by the same letter differ significantly according to Fisher's protected *LSD* (*t*-test).

Table 6: Reduction in shoot mass of citrus rootstocks 3 months after inoculation with *Phytophthora nicotianae, Fusarium solani* and *P. nicotianae - F. solani* combination (1996)

Rootstock	% Reduction in shoot dry mass[a]			
	Phytophthora	**Combination**	***Fusarium***	**Rootstock mean**
Swingle citrumelo	31.3 T[b]	14.1 T	12.4 T	19.2 x[f]
Amblycarpa	75.8 S[c]	39.4 T	57.6 S	57.6 z
C32-citrange	23.4 T	15.7 T	10.0 T	16.3 x
Calamandrin	72.2 S	5.6 S	44.4	40.7 z
F80-8 citrumelo	52.9 S	27.5	47.1	42.5 z
Gou Tou SO	44.9 [d]	68.8 T	4.8 T	39.5 z
Konejime	39.2	8.2 T	11.8 T	19.7 x
Macrophylla	57.0 S	22.5 T	-2.2 T	25.8 x
Milan lemon	11.7 T	9.6	6.0 T	9.1 x
Orlando tangelo	86.4 S	37.9	37.9	54.0 z
Pomeroy trifoliate	72.7 S	33.3 T	15.2 T	40.4 z
Rough lemon	50.5	21.1	33.7	35.1
Treatment x Rootstock LSD	*{34.5}*	*{34.5}*	*{34.5}*	
Treatment mean	51.5 Y[e]	25.3 X	23.2 X	
Treatment LSD	*[10.0]*	*[10.0]*	*[10.0]*	*Rootstock LSD (19.9)*
Swingle citrumelo	45.3 S	10.7 T	34.7	30.2 x
1113	67.1 S	11.9 T	3.4 T	27.5 x
Changsha mandarin	49.9 S	2.4 T	74.3 S	42.2
Cleopatra mandarin	56.8 S	33.7	56.8 S	49.1 z
Jacobsen trifoliate	76.2 S	-5.8 T	2.1 T	24.2 x
Natsudaidai	72.9 S	16.0 T	70.1 S	53.0 z
Obovoideae	29.9	37.7	23.7 T	30.4 x
Rusk citrange	42.5	59.8 S	6.1 T	36.1
Shekwasha mandarin	60.6 S	6.8 T	26.5 T	31.3 x
Terra Bella citrange	62.5 S	41.7	50.0 S	51.4 z
Rough lemon	47.3 S	31.6	67.2 S	48.7 z
Treatment x Rootstock LSD	*{33.6}*	*{33.6}*	*{33.6}*	
Treatment mean	55.6 Z	22.4 X	37.7 Y	
Treatment LSD	*[10.1]*	*[10.1]*	*[10.1]*	*Rootstock LSD (19.4)*
Swingle citrumelo	17.3 T	10.0 T	25.8 T	17.7 x
1112	36.9	63.0 S	9.1 T	36.3
1116	57.5 S	34.2 T	34.2 T	42.0
Australian trifoliate	64.7 S	81.4 S	71.8 S	72.6 z
Japanese citron	77.0 S	74.5 S	9.1 T	53.5
Rubidoux trifoliate	25.5 T	49.3	19.9 T	31.6 x
Sampson tangelo	35.2 T	56.3 S	49.4	47.0
Smooth Flat Seville	52.4	55.4 S	46.4	51.4
Sunchusha mandarin	51.9	68.4 S	64.1 S	61.4 z
Sunki mandarin	53.6	53.6	63.7 S	57.0 z
Rough lemon	59.0 S	23.1 T	47.3	43.1
Treatment x Rootstock LSD	*{27.0}*	*{27.0}*	*{27.0}*	
Treatment mean	48.3 Y	51.7 Y	40.1 X	
Treatment LSD	*[8.1]*	*[8.1]*	*[8.1]*	*Rootstock LSD (15.6)*

a ((uninoculated - inoculated)/uninoculated) x 100 (Gabor & Coffey, 1990)

b most tolerant (T) cultivars selected according to the Multiple *t*-distribution test (P=0.05), means followed by the same letter do not differ significantly according to Fisher's protected *LSD* (*t*-test)

c most susceptible (S) cultivars selected according to the Multiple *t*-distribution test (P=0.05), means followed by the same letter do not differ significantly according to Fisher's protected *LSD* (*t*-test)

d values not followed by any letter can be classified as intermediate in its response to the pathogen treatment according to the Multiple *t*-distribution test (P=0.05), and do not differ significantly according to Fisher's protected *LSD* (*t*-test), this is only where significant differences did occur within a treatment

e means followed by the same letter (X,Y,Z between treatments) do not differ significantly (P=0.05) according to Fisher's protected *LSD* (*t*-test), best cultivars selected according to the Multiple *t*-distribution test

f means followed by the same letter(x,z within rootstock mean) do not differ significantly (P=0.05) according to Fisher's protected *LSD* (*t*-test),), best cultivars selected according to the Multiple *t*-distribution test

Table 7: Increase in feeder root length of citrus rootstocks 3 months after inoculation with *Phytophthora nicotianae* and *Fusarium solani* (1997)

Rootstock	% Increase in feeder root length[a]			
	Phytophthora	*Fusarium*		Rootstock mean
Swingle citrumelo	-8.0 *[b]	15.9 *		4.0
Amblycarpa	-31.4	44.0		6.3 x[d]
C32-citrange	-18.2	-9.4		-13.8 z
Calamandrin	-18.2	9.9		-4.1 z
F80-8 citrumelo	-36.0	-0.8		-18.4 z
Gou Tou SO	31.8	17.4		24.6 x
Konejime	-42.4	-6.8		-24.6 z
Macrophylla	14.3	45.0		29.7 x
Milan lemon	-17.5	2.6		-7.5 z
Pomeroy trifoliate	-28.4	25.1		-1.6 z
Rough lemon	-12.5	19.1		3.3
Treatment x Rootstock LSD	*{36.2}* *	*{36.2}* *		
Treatment mean	-15.1 Y[c]	14.7 X		
Treatment LSD	*[10.9]*	*[10.9]*	*Rootstock LSD*	*(25.6)*
Swingle citrumelo	20.7 *	-3.3 *		8.7 z
1113	19.9	1.4		10.6
Changsha mandarin	6.0	12.0		9.0 z
Cleopatra mandarin	30.9	38.3		34.6 x
Jacobsen trifoliate	-7.0	-11.0		-9.0 z
Natsudaidai	35.3	16.8		26.0 x
Obovoideae	18.8	9.4		14.1 x
Rusk citrange	-13.4	-13.4		-13.4 z
Shekwasha mandarin	32.6	14.1		23.3 x
Terra Bella citrange	8.6	-32.4		-11.9 x
Rough lemon	46.7	19.8		33.2 x
Treatment x Rootstock LSD	*{34.2}* *	*{34.2}* *		
Treatment mean	18.1 X	4.7 Y		
Treatment LSD	*[10.3]*	*[10.3]*	*Rootstock LSD*	*(24.2)*
Swingle citrumelo	10.7 *	-7.0 *		1.9 *
1112	24.9	-16.5		4.2
1116	9.6	-8.1		0.7
Australian trifoliate	19.9	-22.8		-1.5
Japanese citron	-1.5	-4.6		-3.0
Rubidoux trifoliate	14.7	19.5		17.1
Sampson tangelo	1.0	-34.4		-16.7
Smooth Flat Seville	4.0	-28.5		-12.3
Sunchusha mandarin	5.4	1.4		3.4
Sunki mandarin	-6.7	25.2		9.2
Rough lemon	25.0	18.6		21.8
Treatment x Rootstock LSD	*{37.9}* *	*{37.9}* *		
Treatment mean	9.7 X	-5.2 Y		
Treatment LSD	*[11.4]*	*[11.4]*	*Rootstock LSD*	*(26.8)*

a Feeder root length was determined by measuring the length of 0.1g roots. Results were expressed as the percentage increase in feeder root lengths and calculated as follows: ([uninoculated - inoculated]/uninoculated) x 100 (Gabor & Coffey, 1990)

b * not significant

c means followed by the same letter (X,Y,Z between treatments) do not differ significantly (P=0.05) according to Fisher's protected *LSD*(*t*-test), best cultivars were selected according to the Multiple *t*-distribution test

d means followed by the same letter(x,z within rootstock mean) do not differ significantly (P=0.05) according to Fisher's protected *LSD* (*t*-test) best cultivars were selected according to the Multiple *t*-distribution test.

Table 8: Increase in plant height of citrus rootstocks 3 months after inoculation with *Phytophthora nicotianae* and *Fusarium solani* and (1997)

Rootstock	% Increase in plant height[a]			
	Phytophthora	*Fusarium*	Rootstock mean	
Swingle citrumelo	3.8 [b]	7.2		5.5 x[f]
Amblycarpa	1.8	4.5		3.2 x
C32-citrange	-6.7 S[c]	20.6 T[d]		6.9 x
Calamandrin	-1.1	15.0 T		7.0 x
F80-8 citrumelo	-13.0 S	-7.8 S		-10.4 z
Gou Tou SO	1.4	0.1		0.8
Konejime	-6.7 S	-6.3 S		-6.5 z
Macrophylla	3.4	0.7		2.0 x
Milan lemon	2.5	6.9		4.7 x
Pomeroy trifoliate	-3.1	0.7		-1.2
Rough lemon	0.1	0.2		0.2
Treatment x Rootstock LSD	*{7.3}*	*{7.3}*		
Treatment mean	-1.6 Y[e]	3.8 X		
Treatment LSD	*[2.2]*	*[2.2]*	*Rootstock LSD*	*(5.1)*
Swingle citrumelo	-8.3 S	2.5 T		-2.9
1113	-14.8 S	-10.1 S		-12.4 z
Changsha mandarin	-9.9 S	-8.8 S		-9.3 z
Cleopatra mandarin	-7.7 S	-8.6 S		-8.1 z
Jacobsen trifoliate	7.3 T	7.4 T		7.7 x
Natsudaidai	-8.9 S	12.7 T		1.9 x
Obovoideae	-3.5	-17.8 S		-10.7 z
Rusk citrange	-8.9 S	-1.2		-5.1
Shekwasha mandarin	-1.1	0.5		-0.3
Terra Bella citrange	-6.3	8.3 T		1.0 x
Rough lemon	-15.3 S	-8.4 S		-11.8 z
Treatment x Rootstock LSD	*{10.3}*	*{10.3}*		
Treatment mean	-7.0 Y	-2.1 X		
Treatment LSD	*[3.1]*	*[3.1]*	*Rootstock LSD*	*(7.3)*
Swingle citrumelo	21.9 T	7.7		14.8 x
1112	-2.2	-0.5		-1.4
1116	9.7	-10.4 S		-0.3
Australian trifoliate	14.8 T	1.7		8.3 x
Japanese citron	5.9	1.5		3.7
Rubidoux trifoliate	16.1 T	1.6		8.9 x
Sampson tangelo	-3.7	-2.9		-3.3
Smooth Flat Seville	-5.8 S	-8.6 S		-7.2 z
Sunchusha mandarin	-16.0 S	0.1		-8.0 z
Sunki mandarin	-9.6 S	-15.3 S		-12.4 z
Rough lemon	-4.3	3.6		-0.4
Treatment x Rootstock LSD	*{11.1}*	*{11.1}*		
Treatment mean	2.5 X	-2.0 Y		
Treatment LSD	*[3.4]*	*[3.4]*	*Rootstock LSD*	*(7.9)*

a Increase in plant height was calculated by subtracting the original plant height of the seedlings, from the plant height three months later at termination of the trial, and it was expressed as percentage

b values not followed by any letter can be classified as intermediate in its response to the pathogen treatment according to the Multiple *t*-distribution test (P=0.05), and do not differ significantly according to Fisher's protected *LSD* (*t*-test)

c most susceptible (S) cultivars selected according to the Multiple *t*-distribution test (P=0.05), means followed by the same letter do not differ significantly according to Fisher's protected *LSD* (*t*-test)

d most tolerant (T) cultivars selected according to the Multiple *t*-distribution test (P=0.05), means followed by the same letter do not differ significantly according to Fisher's protected *LSD* (*t*-test)

e means followed by the same letter (X,Y,Z between treatments) do not differ significantly (P=0.05) according to Fisher's protected *LSD* (*t*-test), best cultivars were selected according to the Multiple t-distribution test

f means followed by the same letter (x,z within rootstock mean) do not differ significantly (P=0.05) according to Fisher's protected *LSD* (*t*-test), best cultivars were selected according to the Multiple *t*-distribution test

Table 9: Increase in disease severity in citrus rootstocks 3 months after inoculation with *Phytophthora nicotianae* and *Fusarium solani* (1997)

Rootstock	**Increase in Disease severity**[a]			
	Phytophthora	***Fusarium***		**Rootstock mean**
Swingle citrumelo	0.2 T[b]	0.7		0.4 x[f]
Amblycarpa	1.7 [c]	0.3 T		1.0
C32-citrange	0.8	0.8		0.8
Calamandrin	2.3 S[d]	1.0		1.7 z
F80-8 citrumelo	1.8 S	0.8		1.3 z
Gou Tou SO	1.0	0.8		0.9
Konejime	1.3	1.5		1.4 z
Macrophylla	1.5	1.3		1.4 z
Milan lemon	1.5	0.7		1.1
Pomeroy trifoliate	0.2 T	0.0 T		0.1 x
Rough lemon	1.3	0.7		1.0
Treatment x Rootstock LSD	*{0.7}*	*{0.7}*		
Treatment mean	1.2 Y[e]	0.8 X		
Treatment LSD	*[0.2]*	*[0.2]*	*Rootstock LSD*	*(0.5)*
Swingle citrumelo	0.5 T	0.2 T		0.3 x
1113	1.0	1.3		1.2
Changsha mandarin	1.3	2.0 S		1.7 z
Cleopatra mandarin	1.5	1.5		1.5 z
Jacobsen trifoliate	1.3	1.2		1.3
Natsudaidai	2.3 S	1.0		1.7 z
Obovoideae	0.8	2.2 S		1.5 z
Rusk citrange	0.5 T	0.5 T		0.5 x
Shekwasha mandarin	0.0 T	0.8		0.4 x
Terra Bella citrange	2.3 S	1.7 S		2.0 z
Rough lemon	1.3	1.5		1.4
Treatment x Rootstock LSD	*{0.7}*	*{0.7}*		
Treatment mean	1.2 *[g]	1.3 *		
Treatment LSD	*[0.2] **	*[0.2] **	*Rootstock LSD*	*(0.5)*
Swingle citrumelo	0.3 T	0.3 T		0.3 x
1112	1.5	1.7 S		1.6 z
1116	1.5	0.3 T		0.9
Australian trifoliate	1.0	0.3 T		0.7 x
Japanese citron	1.6 S	0.3 T		1.0
Rubidoux trifoliate	1.3	0.3 T		0.8
Sampson tangelo	2.2 S	0.8 T		1.5 z
Smooth Flat Seville	1.3	1.6 S		1.5 z
Sunchusha mandarin	1.2	0.7 T		0.9
Sunki mandarin	1.3	0.5 T		0.9
Rough lemon	1.3	1.2		1.3
Treatment x Rootstock LSD	*{0.6}*	*{0.6}*		
Treatment mean	1.3 Y	0.7 X		
Treatment LSD	*[0.2]*	*[0.2]*	*Rootstock LSD*	*(0.4)*

a = inoculated - uninoculated. Root rot were scored on a scale of 1 - 4 where 1 = no disease (0-25% of root system rotted); 2 = slight root rot (26-50% of root system rotted); 3 = moderate root rot (51-75% of root system rotted) and 4 = severe root rot (76-100% of root system rotted), disease severity index was expressed in relation to disease severity index to the uninoculated control

b most tolerant (T) cultivars selected according to the Multiple *t*-distribution test (P=0.05), means followed by the same letter do not differ significantly according to Fisher's protected *LSD* (*t*-test)

c values not followed by any letter can be classified as intermediate in its response to the pathogen treatment according to the Multiple *t*-distribution test (P=0.05), and do not differ significantly according to Fisher's protected *LSD* (*t*-test)

d most susceptible (S) cultivars selected according to the Multiple *t*-distribution test (P=0.05), means followed by the same letter do not differ significantly according to Fisher's protected *LSD* (*t*-test)

e means followed by the same letter (X,Y,Z between treatments) do not differ significantly (P=0.05) according to Fisher's protected *LSD* (*t*-test), best cultivars were selected according to the Multiple *t*-distribution test

f means followed by the same letter (x,z within rootstock mean) do not differ significantly (P=0.05) according to Fisher's protected *LSD* (*t*-test), best cultivars were selected according to the Multiple *t*-distribution test

g * not significant

Table 10: Reduction in root mass of citrus rootstocks 3 months after inoculation with *Phytophthora nicotianae* and *Fusarium solani* (1997)

Rootstock	**% Reduction in root dry mass**[a]			
	Phytophthora	***Fusarium***		**Rootstock mean**
Swingle citrumelo	9.6 *[b]	9.9 *		9.8 x[c]
Amblycarpa	41.0	34.0		37.5 z
C32-citrange	7.1	3.6		5.3 x
Calamandrin	55.6	64.2		59.9 z
F80-8 citrumelo	3.8	13.5		8.7 x
Gou Tou SO	9.3	14.2		11.8 x
Konejime	11.3	7.8		9.6 x
Macrophylla	25.0	27.1		26.0 x
Milan lemon	49.9	10.6		30.2 x
Pomeroy trifoliate	14.2	11.8		13.0 x
Rough lemon	51.1	29.1		40.1 z
Treatment x Rootstock LSD	*{33.9}* *	*{33.9}* *		
Treatment mean	25.3 *	20.5 *		
Treatment LSD	*[10.2]* *	*[10.2]* *	*Rootstock LSD*	*(24.0)*
Swingle citrumelo	1.0 T[d]	3.3 T		2.2 x
1113	75.1 [e]	63.6		69.3
Changsha mandarin	21.2 T	21.2 T		21.2 x
Cleopatra mandarin	54.9	67.4		61.2
Jacobsen trifoliate	53.2	35.0		44.1
Natsudaidai	140.0 S[f]	80.0		110.0 z
Obovoideae	58.3	72.7		65.5
Rusk citrange	57.1	2.4 T		29.8
Shekwasha mandarin	27.8 T	5.6 T		16.7 x
Terra Bella citrange	59.7	30.1 T		44.9
Rough lemon	63.3	35.4		49.3
Treatment x Rootstock LSD	*{33.4}*	*{33.4}*		
Treatment mean	55.6 Y[g]	37.9 X		
Treatment LSD	*[10.1]*	*[10.1]*	*Rootstock LSD*	*(23.6)*
Swingle citrumelo	-18.8 T	1.5 T		-8.7 x
1112	73.6 S	73.6 S		73.6 z
1116	55.2 S	12.2		33.7
Australian trifoliate	57.3 S	41.6		49.4
Japanese citron	77.5 S	70.2 S		73.8
Rubidoux trifoliate	70.4 S	32.6		51.5
Sampson tangelo	74.7 S	49.3		62.0 z
Smooth Flat Seville	61.1 S	33.3		47.2
Sunchusha mandarin	21.1	74.4 S		47.8
Sunki mandarin	56.1 S	21.1		38.6
Rough lemon	68.5 S	9.3		38.9
Treatment x Rootstock LSD	*{24.8}*	*{24.8}*		
Treatment mean	54.2 Y	38.1 X		
Treatment LSD	*[7.5]*	*[7.5]*	*Rootstock LSD*	*(17.6)*

a ([uninoculated - inoculated]/uninoculated) x 100(Gabor & Coffey, 1990)

b * not significant

c means followed by the same letter (x,z within rootstock mean) do not differ significantly (P=0.05) according to Fisher's protected *LSD* (*t*-test), best cultivars were selected according to the Multiple *t*-distrubution test

d most tolerant (T) cultivars selected according to the Multiple *t*-distribution test (P=0.05), means followed by the same letter do not differ significantly according to Fisher's protected *LSD* (*t*-test)

e values not followed by any letter can be classified as intermediate in its response to the pathogen treatment according to the Multiple *t*-distribution test (P=0.05), and do not differ significantly according to Fisher's protected *LSD* (*t*-test)

f most susceptible (S) of cultivars selected according to the Multiple *t*-distribution test (P=0.05), means followed by the same letter do not differ significantly according to Fisher's protected *LSD* (*t*-test)

g means followed by the same letter (X,Y,Z between treatments) do not differ significantly (P=0.05) according to Fisher's protected *LSD* (*t*-test), best cultivars were selected according to the Multiple *t*-distrubution test

Table 11 : Reduction in shoot mass of citrus rootstocks 3 months after inoculation with *Phytophthora nicotianae* and *Fusarium solani* (1997)

Rootstock	% Reduction in shoot dry mass[a]			
	Phytophthora	*Fusarium*		Rootstock mean
Swingle citrumelo	13.1 *[b]	24.8 *		19.0 *
Amblycarpa	17.4	24.8		21.1
C32-citrange	8.0	1.5		4.8
Calamandrin	35.0	54.1		44.6
F80-8 citrumelo	9.3	26.5		19.9
Gou Tou SO	12.2	26.5		19.7
Konejime	20.7	45.1		32.9
Macrophylla	16.7	14.6		15.6
Milan lemon	20.2	36.9		28.6
Pomeroy trifoliate	14.8	33.3		24.1
Rough lemon	26.2	37.2		31.7
Treatment x Rootstock LSD	*{33.2}*	*{33.2}*		
Treatment mean	17.6 X[c]	29.6 Y		
Treatment LSD	*[10.0]*	*[10.0]*	*Rootstock LSD*	*(23.5)* *
Swingle citrumelo	12.5 T[d]	13.1 T		12.8 x[f]
1113	72.2 S[e]	59.2		65.7 z
Changsha mandarin	33.8 T	21.6 T		27.7 x
Cleopatra mandarin	53.7 S	59.3 S		56.5 z
Jacobsen trifoliate	53.7 S	32.1 T		42.9
Natsudaidai	74.6 S	35.7 S		55.2 z
Obovoideae	50.4 [g]	75.7 S		63.0 z
Rusk citrange	58.3 S	5.1 T		31.7 x
Shekwasha mandarin	41.0	13.2 T		27.1 x
Terra Bella citrange	47.1	47.1		47.1 z
Rough lemon	50.2	29.1 T		39.7
Treatment x Rootstock LSD	*{29.3}*	*{29.3}*		
Treatment mean	49.8 Y	35.6 X		
Treatment LSD	*[8.8]*	*[8.8]*	*Rootstock LSD*	*(20.7)*
Swingle citrumelo	-14.4 T	-27.0 T		-20.7 x
1112	68.2 S	61.2 S		64.7 z
1116	46.1	30.8		38.4
Australian trifoliate	51.6	47.5		49.5
Japanese citron	59.4 S	64.5 S		62.0 z
Rubidoux trifoliate	76.5 S	57.5 S		67.0 z
Sampson tangelo	68.0 S	42.2		55.1 z
Smooth Flat Seville	69.2 S	42.9		56.1 z
Sunchusha mandarin	24.7	69.7 S		47.2
Sunki mandarin	71.5 S	61.6 S		66.6 z
Rough lemon	20.1	-2.0		9.0
Treatment x Rootstock LSD	*{19.1}*	*{19.1}*		
Treatment mean	49.2 Y	40.8 X		
Treatment LSD	*[5.8]*	*[5.8]*	*Rootstock LSD*	*(13.5)*

a ((uninoculated - inoculated)/uninoculated) x 100 (Gabor & Coffey, 1990)

b * not significant

c means followed by the same letter (X,Y,Z between treatments) do not differ significantly (P=0.05) according to Fisher's protected *LSD* (t-test), best cultivars were selected according to the Multiple t-distribution test

d most tolerant (T) cultivars selected according to the Multiple t-distribution test (P=0.05), means followed by the same letter do not differ significantly according to Fisher's protected *LSD* (t-test)

e most susceptible (S) cultivars selected according to the Multiple t-distribution test (P = 0.05), means followed by the same letter do not differ significantly according to Fisher's protected *LSD* (t-test)

f means followed by the same letter (x,z within rootstock mean) do not differ significantly (P=0.05) according to Fisher's protected *LSD* (t-test), best group of cultivars were selected according to the Multiple t-distribution test.

g values not followed by any letter can be classified as intermediate in its response to the pathogen treatment according to the Multiple t-distribution test (P=0.05), and do not differ significantly according to Fisher's protected *LSD* (t-test).

CHAPTER 3

RESPONSE OF CITRUS ROOTSTOCKS TO *PHYTOPHTHORA NICOTIANAE, FUSARIUM SOLANI* AND *TYLENCHULUS SEMIPENETRANS* INFECTION UNDER FIELD CONDITIONS

ABSTRACT

Eight citrus rootstocks were evaluated in two years for tolerance or susceptibility to *Phytophthora nicotianae, Fusarium solani* and *Tylenchulus semipenetrans* under simulated field conditions. The rootstocks tested were Swingle citrumelo, Carrizo citrange, X639 hybrid, Minneola x Trifoliate hybrid, C32-citrange, C35-citrange, Benton citrange and Rough lemon. The plants were grown in artificially infested field plots and evaluated after seven months. Plant height, disease severity index, feeder root length, nematode counts and percentage reduction in root and shoot dry mass were used as parameters to measure the tolerance or susceptibility of the rootstocks to the different pathogens. Swingle citrumelo and Rough lemon were included in each experiment as references representing a tolerant and susceptible rootstock, respectively. In the first year, significant differences occurred between the treatments, but not between the rootstocks within a treatment. In the second year, no differences occurred between the treatments nor between the rootstocks within a treatment. It was not possible to determine the best parameter for tolerance or susceptibility. The following rootstocks were found to be tolerant: Swingle citrumelo and C35-citrange (*Phytophthora* tolerant), Minneola x Trifoliate hybrid and C35 citrange (*Fusarium*

tolerant) and Swingle citrumelo (nematode tolerant). C32-citrange was tolerant to all three pathogens. Rootstocks that were susceptible were as follows: X639 hybrid, Minneola x Trifoliate hybrid (susceptible to *Phytophthora*), Carrizo citrange and Benton citrange (susceptible to *Fusarium*). Rough lemon showed tolerance to *Fusarium* in 1996, but was susceptible to all the pathogens in 1997.

INTRODUCTION

Rootstocks are of great importance in the intensive cultivation of citrus (Wutscher, 1979), particularly regarding resistance to pests and diseases (Castle, 1987).

Phytophthora spp., such as *Phytophthora nicotianae* Breda de Haan (syn. *P. parasitica* Dastur.) cause some of the most serious diseases of citrus (Timmer & Menge, 1988). Citrus rootstocks differ in their tolerance to root and foot rot (Castle, 1987) and can be either tolerant, susceptible or intermediate (Agostini *et al.*, 1991) towards *Phytophtora* spp. or other root pathogens. Tolerant rootstocks can offer excellent means of disease control against *Phytophthora* root and foot rot (Carpenter & Furr, 1962; Afek *et al.*, 1990). Tolerant rootstocks are also considered an important factor in the control of *Fusarium solani* (Mart.) Appel & Wollenw. (Labuschagne, 1994). *F. solani* is a pathogen that can cause feeder root rot of citrus by attacking the roots and trunks (Labuschagne *et al.*, 1987; Nemec *et al.*, 1980) as well as dry rot of the lower trunk and scaffold roots (Bender & Menge, 1984).

Most populations of the citrus nematode, *Tylenchulus semipenetrans* Cobb, reproduce on citrus rootstocks and cause trees to decline in vigour and yield (Niles *et al.*, 1995; Ferguson *et al.*, 1996). The use of a rootstock resistant to the citrus nematode could be an effective, economic and safe method of lowering nematode population densities (Verdejo-Lucas *et al.*, 1997). A substantial degree of resistance to the citrus nematode has been demonstrated in trifoliate orange (*Poncirus trifoliata* (L.) Raf.) and its hybrids (Baines *et al.*, 1960; Hearn *et al.*, 1974; McCarthy *et al.*, 1979).

The objective of this study was to evaluate citrus rootstocks that had previously been tested in other trials in the greenhouse for their tolerance to *P. nicotianae, F. solani* and *T. semipenetrans* under field conditions.

MATERIALS AND METHODS

Design and preparation of field plots. Fifteen field plots were established at the experimental farm of the University of Pretoria. The plots covered a total area of 17 m x 12.5 m. Each field plot was prepared by excavating a volume of soil (1.5 x 1 x 1 m deep), and fitting a rectangular fibreglass sidewall unit into the space. Plots were spaced 2 m apart. Each plot was filled with 170 l sandy loam soil. The soil was fumigated with methyl bromide (98 % methyl bromide + 2% chloropicrin) at a rate of 150 g 170 l^{-1} soil under clear high density polyethylene sheeting (150 μm thick) for 48 h and subsequently aerated as described by McLean & Kotzé (1992).

One day before planting, 90 g limestone-ammonium-nitrate (LAN) was distributed over each plot and mixed into the soil to a depth of 15 cm. The fertiliser was re-applied at the same rate every six weeks after planting.

Treatments. Treatments consisted of each rootstock inoculated with either *P. nicotianae, F. solani* or *T. semipenetrans* or left uninoculated (control). For the first group of rootstocks, four replicate plots were included for each treatment (except for the uninoculated control where only three were used). Each plot was planted with three plants of each rootstock (five rootstocks per plot), spaced 30 cm apart. For the second group of rootstocks, two replicate plots were used.

Fungal pathogens. A fresh isolate of *P. nicotianae* (obtained from infested soil from an orchard in the Sundags River Valley, Eastern Cape Province, South Africa) and *F. solani* isolate JF10 [isolated by Strauss (1992) from Rough lemon rootstock (scion Delta Valencia sweet orange), Letaba Estates, Northern Province, South Africa] were used. Fungal isolates were multiplied on potato-dextrose agar (39 g Biolab™ potato-dextrose agar (PDA) l^{-1} distilled water. *F. solani* was maintained as a spore suspension (500 μl / vial) in autoclaved silica sand in sealed glass vials. *P. nicotianae* was stored as mycelial discs in sterile distilled water. Both were kept in darkness at room temperature (25$\pm$2°C).

Inoculum was prepared as follows: 200 g millet seed in high-density autoclavable polyethylene bags (17 cm X 25 cm) was moistured with 100 ml distilled water and autoclaved for 20 minutes on two successive days. Ten mycelial discs (6 mm in diameter) from seven-day-old *F. solani* and 14-day-old *P. nicotianae* cultures were used to inoculate the millet seed pouches (Strauss, 1992). Inoculated bags were incubated for four weeks at 25°C in the dark. Pouches were regularly kneaded to ensure even spread of the fungus. For the *Fusarium* and *Phytophthora* treatments, each field plot was infested with 600 g of millet seed inoculum, mixed into the soil to a depth of 30 cm. Control plots received sterile millet seed at the same rate.

Tylenchulus semipenetrans. Prior to commencement of the experiments, nematode populations in the soil of the four *T. semipenetrans* plots were increased over a period of six months, by artificially infesting the soil with larvae and eggs and planting the infested soil to Rough lemon seedlings. Soil populations of *T. semipenetrans* were supplemented prior to planting by additional inoculum obtained from infected roots of 18-month-old

Rough lemon rootstocks, which were previously grown in infested nematode micro-plots, as well as from mature trees from the Mooinooi area in the North West Province. Eggs of *T. semipenetrans* were extracted from feeder roots using the egg extraction method of Coolen & d'Herde (1972). Egg suspensions were kept for three days at 3°C before inoculation. An egg concentration of 2 x 10^5 eggs 35 ml^{-1} water per root system was poured around each root system before covering it with soil.

At the start of each trial the concentration of larvae present in the soil was determined by means of the Baermann tray method (Whitehead & Hemming, 1965).

Citrus rootstocks. Five different rootstocks were evaluated in 1996 namely Carrizo citrange (*Poncirus trifoliata* (L.) Raf. x *Citrus sinensis* (L.) Osbeck.), Minneola x Trifoliate hybrid [(*Citrus paradisi* Macf. x *Citrus reticulata* Blanco) x *P. trifoliata*], X639 hybrid (*P. trifoliata* x *C. reticulata* cv. Cleopatra mandarin), Swingle citrumelo (*P. trifoliata* x *C. paradisi*) and Rough lemon (*C. jambhiri* Lush.). The one-year-old seedlings (not budded) were obtained from H F le Roux of Capespan Citrus Centre in Nelspruit.

In 1997, the following rootstocks were evaluated: C32-citrange (*P. trifoliata* x *C. sinensis*), C35-citrange (*P. trifoliata* x *C. sinensis*) and Benton citrange (*P. trifoliata* x *C. sinensis*). Swingle citrumelo and Rough lemon were again included as reference rootstocks. Seedlings were one year old, budded with Delta Valencia (*C. sinensis*) scion, and were obtained from Komati nursery near Nelspruit.

The plots (different treatments) were completely randomised. The cultivars within a plot were planted in rows that were randomised for each plot.

Plants were micro-irrigated once a week with borehole water and sprayed every six weeks with 5 ml Dursban™, Efekto (a.i. chlorpyriphos 240 g l^{-1}) 5 l^{-1} water to control insect pests.

Prior to each trial, isolations were made from the roots of randomly selected plants (six from each cultivar) to determine the presence of any root pathogens. Root segments, 5 mm in length, were washed in tap water, surface-disinfested for 1 minute in 1.5% sodium hypochlorite and rinsed with sterile distilled water. For the *Phytophthora* isolations, roots were only rinsed in sterile water. The root segments were plated on PDA and media selective for *Fusarium* and *Phytophthora*, respectively, using ten segments per medium per plant. The medium for *Fusarium* (rose-bengal-glycerine-urea agar) (RbGU)) contained 10 g glycerine, 1 g urea, 0.5 g L-alanine, 1 g quintozene (Terrachlor, 75% a. i.), 0.5 g rose-bengal, 15 g PDA 1 l^{-1} distilled water supplemented with 50 mg l^{-1} streptomycin (Van Wyk *et al.*, 1986). The medium selective for *Phytophthora* spp., pimaracin - ampicillin - rifampicin - pentachloronitrobenzene - hymexazol (PARPH), contained half-strength PDA (20 g 800 ml^{-1} distilled water) which was supplemented after autoclaving with 100 mg quintozene, 250 mg ampicillin, 10 mg rifampicin, 10 mg pimaricin and 0.15 ml (103 ppm) hymexazol (Tachigaren, 70% a. i.) added to 200 ml sterile distilled water, dissolved, and added to the half-strength PDA) (Jeffers & Martin, 1986).

Plates were incubated at 25 ± 2°C in the dark and observed every day for two weeks for fungal growth. Fungi were isolated into pure culture and identified (Nelson *et al.*, 1983; Erwin & Ribeiro, 1996).

Evaluation of resistance / tolerance. Both groups of rootstocks were evaluated seven months after planting. Plants were carefully removed from the soil and their root systems washed under running tap water.

Plant height was measured from the soil-line to the top of the foliage. In the first year plant height that was measured only at termination of the experiment, but in the second year increase in plant height was determined. Plant height and increase in plant height were expressed in relation to the height of the uninoculated control.

Root systems were evaluated for visual root rot on a scale of 1 to 4 (1= 0 to 25 % of root system showing rot symptoms, 2 = 26 to 50 % of root system showing rot symptoms, 3 = 51 to 75 % of root system showing rot symptoms and 4 = 76 to 100 % of root system showing rot symptoms). Increase in disease severity was also expressed in relation to disease severity index of the uninoculated control.

Root systems and foliage of plants were weighed after drying for 48 h at 80°C. Percentage reduction in root and shoot dry mass was calculated using the following formula: ([uninoculated – inoculated]/uninoculated) x 100 (Gabor & Coffey, 1990).

In the second trial, feeder root length was also determined. Fresh feeder roots weighing 0.1 g per rootstock were measured with a Geotron™ (Model nr. WLM1) (Geotron Systems (Pty) Ltd., P.O. Box 2656, Potchefstroom, 2520) root length meter. Results were expressed as the percentage increase in feeder root length and calculated according to the formula of Gabor & Coffey (1990). It was also expressed in relation to the increase in feeder root length of the uninoculated control.

Randomly-selected root segments from rootstock and treatment (six from each cultivar) were used for determination of fungal infection as described earlier.

Mature female nematodes were counted according to the modified Blender-sieve-colour technique of Van der Vegte (1973) to determine the degree of tolerance of the different rootstocks towards *T. semipenetrans*. As much soil as possible was removed from the root systems by lightly shaking the roots. Tertiary roots were cut into 2 cm long segments. Ten grams of roots per seedling were gently rinsed with tap water on a coarse sieve to remove most of the remaining soil particles. Rinsed roots were placed in a blender containing 250 ml tap water and homogenised, at a low speed for 60 seconds. The resultant suspension was poured through three sieves in series, namely 1 mm mesh (top), 212 μm (middle) and 25 μm (bottom). The residue on the top sieve was washed thoroughly with tap water.

The top sieve was removed and the washing process repeated on the middle sieve. The residue on the bottom sieve was transferred to a 40 mm diameter 25 μm sieve and stained for 60 minutes in 0.5 % acid fuchsin/lactophenol solution. After staining, the sieve was lifted from the staining solution, drained and the contents rinsed under a running tap until the filtrate was nearly colourless. The residue was washed into a 250 ml beaker, and filled up to the 200 ml mark with water. A few drops of 5 % acetic acid were added and the suspension was thoroughly mixed with a stream of air bubbles. The stained females (10 ml suspension / De Grisse counting dish) were counted under a stereo-microscope provided with illumination from below.

Analysis of variance (ANOVA) was performed by using the statistical program GENSTAT 5 (GENSTAT Committee, 1993). Treatment means and interactions between the treatments were compared by means of Fisher's Protected Least Significant Difference (*LSD*) *t*-test procedure (Snedecor & Cochran, 1967). At 5% level of significance, the best group of rootstocks was designated tolerant (best group, T) and the poorest group designated susceptible (poorest group, S) based on the Multiple *t*-distribution test procedure of Gupta & Panchapakesan (1979) .

RESULTS

Before planting no pathogenic fungi could be isolated from the roots of the different rootstocks. At termination of the first trial, *F. solani* and *P. nicotianae* were isolated at a mean rate of 64% and 77% from the respective treatments, and at the end of the second trail, at 64% and 59% respectively. A low incidence of *Fusarium oxysporum* Schlecht. em. W.C. Snyd. & H.N. Hansen. infection (7% and 10%) was detected.

Trial 1. At the time of planting, the nematode plots on average contained 150 *T. semipenetrans* larvae 5 g^{-1} soil. The mean numbers of females present in 10g roots at the end of the trial were as follows: Rough lemon 325; Swingle citrumelo 31; Minneola x Trifoliate hybrid 279; X639 hybrid 74 and Carrizo citrange 69.

Plant height was affected more severely by *F. solani* and *P. nicotianae* than by *T. semipenetrans* (Table 1). However, no differences were evident in the treatment x rootstock interaction or between rootstocks. Visually, the rootstocks were significantly more diseased in the nematode treatment than in the *Phytophthora* or *Fusarium* treatment. *T. semipenetrans* resulted in the greatest loss in root mass, followed by *P. nicotianae*. Differences in the treatment x rootstock interaction and rootstock means were non-significant. *T. semipenetrans* also had the greatest effect on shoot mass. Carrizo citrange and Rough lemon overall showed the greatest reduction in shoot mass.

Rootstocks that sustained a low percentage of root loss generally also showed a low percentage shoot loss and vice versa. The reference rootstock Swingle citrumelo was the least affected by *Phytophthora* and *T. semipenetrans* according to all the parameters. Rough lemon varied in its response to the different treatments.

Trial 2. At the time of planting, nematode plots contained an average of 180 *T. semipenetrans* larvae 5 g^{-1} soil. The mean number of females present in 10g roots at the end of the trial were as follows: Rough lemon 292; Swingle citrumelo 56; Benton citrange 63; C32-citrange 56 and C35-citrange 56.

With feeder root length as parameter, differences existed between rootstocks but not between rootstocks within a treatment (Table 2). Benton citrange showed the smallest increase in feeder root length for all the treatments. C32-citrange was the least affected by both *Phytophthora* and *Fusarium,* while Swingle was the least affected by the nematode. With plant height, there were no differences between treatment means. However, differences did occur between rootstock means and treatment x rootstock interaction means. Swingle citrumelo was the least affected by *Phytophthora* and *T. semipenetrans* (15.8 and 13.2% respectively), but it did not differ significantly from the other rootstocks according to the rootstock treatment interaction. Rough lemon was the least affected by *Fusarium* (18.6%). Overall, Swingle citrumelo and Rough lemon showed the greatest increase in height. C35-citrange (0.3%) was most susceptible to *Phytophthora*, Benton citrange (-3.2%) most susceptible to *Fusarium* and Rough lemon (-0.4%) most susceptible to *T. semipenetrans*. Data on root and shoot mass reductions only indicated differences in the rootstock means, with Benton citrange and Rough lemon showing the greatest reduction. Some correlation was evident between reduction in root and shoot mass in this trial.

DISCUSSION

There are several factors that can influence rootstock resistance, particularly under field conditions. According to Menge & Nemec (1997), Hartmann & Nienhaus (1974) showed that the resistance meganism of rootstocks could be affected by temperature. The time of year, when studies are conducted may have an important effect on the results. For example, during the colder months, rootstocks may undergo dormancy, and it could have an effect on their resistance, resulting in some becoming more and others less resistant than in the warmer months (Tuzcu *et al.*, 1984). Duncan *et al.* (1993) found that during the summer months, *T. semipenetrans* was less attracted to the roots, mainly because of changes in the carbohydrate concentration and extracts in the roots and soil moisture, making it less desirable for the migrating nematodes. Susceptibility of the rootstock can also be influenced by the scion variety. Some scion-rootstock combinations are not compatible, which greatly affects the nutrient status of the roots (Klotz *et al.*, 1968). The age of the rootstock also has a significant effect on resistance. The younger the rootstock, the more susceptible it is. For example, citrus rootstocks are the most sensitive to *Phytophthora* root rot and gummosis during the first two years in the field (Klotz *et al.*, 1958). The usefulness of some rootstocks resistant to *T. semipenetrans* may also be limited, because of the existence of physiological races or biotypes of the nematode (Verdejo-Lucas *et al.*, 1997).

The concentration of female nematodes on the fibrous roots of the different rootstocks was very low, and therefore not a clear indication of the possible tolerance of the rootstocks to the nematode. A possible explanation could be the age of the rootstocks. In both trials the rootstocks were very young, and the duration of the experiment perhaps to short for the nematode population to increase sufficiently.

A combination of the above factors could have influenced the tolerance of the rootstocks, and it is perhaps one of the reasons why so few significant differences occurred. Also, each rootstock needs its own ideal environmental conditions to perform at its peak, and it was impossible to suit the need of each one in the field.

Responses of the reference rootstocks to the different pathogens were not consistent between the two trails. This could be because the rootstocks in the second year were grafted with Delta Valencia scion, which can influence the degree of tolerance. Nevertheless, root and shoot mass reduction was generally related, which is in accordance with the finding of Matheron *et al.* (1998) that rootstocks that maintain a low percentage of root mass reduction, generally experience a low percentage of shoot mass reduction.

In conclusion, the rootstocks that appeared to be the most tolerant were the following: Swingle citrumelo and C35-citrange (*Phytophthora* tolerant), Minneola x Trifoliate hybrid and C35-citrange (*Fusarium* tolerant) and Swingle citrumelo (nematode tolerant). C32-citrange showed tolerance to all three pathogens. Rootstocks that were susceptible included the following: X639 hybrid, Minneola x Trifoliate hybrid (susceptible to *Phytophthora*), Carrizo citrange and Benton citrange (susceptible to *Fusarium*). Rough lemon was found to be tolerant to *Fusarium* in 1996, but was susceptible to all the pathogens in 1997.

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Table 1: Effect of *Phytophthora nicotianae*, *Fusarium solani* and *Tylenchulus semipenetrans* on citrus rootstocks in artificially infested soil[a] in microplots (1996)

Rootstock	*Phytophthora*	*Fusarium*	*T. semipenetrans*		Rootstock mean
	% Plant height[b]				
Swingle citrumelo	15.1 *[c]	6.9 *	51.4 *		24.4 *
Carrizo citrange	5.6	-0.4	45.3		16.8
Minneola x Trifoliate hybrid	3.0	1.2	25.2		9.8
X639 hybrid	1.8	4.2	37.7		14.6
Rough lemon	1.8	16.8	31.6		16.7
Treatment x Rootstock LSD	*{12.8}*	*{12.8}*	*{12.8}*		
Treatment mean	5.5 Y[d]	5.7 Y	38.2 X		
Treatment LSD	*[9.7]*	*[9.7]*	*[9.7]*	*Rootstock LSD*	*(12.8)*
	Increase in disease severity[e]				
Swingle citrumelo	0.1 *	0.7 *	1.6 *		0.8 x[f]
Carrizo citrange	0.8	0.5	1.9		1.1 x
Minneola x Trifoliate hybrid	0.5	0.9	2.2		1.2
X639 hybrid	0.9	1.2	2.7		1.6 z
Rough lemon	0.7	0.4	1.4		0.8 x
Treatment x Rootstock LSD	*{0.6}*	*{0.6}*	*{0.6}*		
Treatment mean	0.6 X	0.7 X	2.0 Y	*Rootstock LSD*	*(0.3)*
Treatment LSD	*[0.3]*	*[0.3]*	*[0.3]*		
	% Reduction in root dry mass[g]				
Swingle citrumelo	20.9 *	16.3 *	67.7 *		35.0 *
Carrizo citrange	44.5	19.9	89.9		51.4
Minneola x Trifoliate hybrid	23.1	0.7	80.6		34.8
X639 hybrid	35.2	17.8	83.8		45.6
Rough lemon	36.7	10.7	90.2		45.9
Treatment x Rootstock LSD	*{26.2}*	*{26.2}*	*{26.2}*		
Treatment mean	32.1 Y	13.1 X	82.4 Z		
Treatment LSD	*[11.7]*	*[11.7]*	*[11.7]*	*Rootstock LSD*	*(15.1)*
	% Reduction in shoot dry mass[h]				
Swingle citrumelo	-6.9 *	15.3 *	49.3 *		19.2 x
Carrizo citrange	44.8	24.3	84.5		51.2 z
Minneola x Trifoliate hybrid	8.3	-6.9	68.8		23.4 x
X639 hybrid	20.6	15.1	73.0		36.2 x
Rough lemon	30.5	12.5	84.8		42.6 z
Treatment x Rootstock LSD	*{33.1}*	*{33.1}*	*{33.1}*		
Treatment mean	19.4 X	12.1 X	72.1 Y		
Treatment LSD	*[14.8]*	*[14.8]*	*[14.8]*	*Rootstock LSD*	*(19.1)*

a Micro field plots were prepared as described under materials and methods

b Plant height was calculated by measuring the length at the end of the trial and was expressed as a percentage. It was expressed in relation to the plant length of the uninoculated control

c * not significant

d means followed by the same letter (X,Y,Z between treatments) do not differ significantly (P=0.05) according to Fisher's protected *LSD* (*t*-test)), the best rootstocks were selected according to the Multiple *t*-distribution test

e = inoculated - uninoculated, root rot were scored on a scale of 1 - 4 where 1 = no disease (0-25% of root system rotted); 2 = slight root rot (26-50% of root system rotted); 3 = moderate root rot (51-75% of root system rotted) and 4 = severe root rot (76-100% of root system rotted)

f means followed by the same letter (x,z within rootstock mean) do not differ significantly (P=0.05) according to Fisher's protected*LSD* (*t*-test)), the best rootstocks were selected according to the Multiple *t*-distribution test

g ([uninoculated - inoculated]/uninoculated) x 100 (Gabor & Coffey, 1990)

h ([uninoculated - inoculated]/uninoculated) x 100

Table 2: Effect of *Phytophthora nicotianae*, *Fusarium solani* and *Tylenchulus semipenetrans* on citrus rootstocks in artificially infested soil[a] in microplots (1997)

Rootstock	*Phytophthora*	*Fusarium*	*T. semipenetrans*		Rootstock[b] mean
	% Increase in feeder root length[c]				
Swingle citrumelo	24.4 *[d]	9.6 *	47.2 *		27.1 x[e]
C32-citrange	44.1	12.7	15.9		24.2 x
C35-citrange	8.2	7.8	30.6		15.5 x
Benton citrange	-22.7	0.0	-13.9		-12.2 z
Rough lemon	10.4	6.9	30.7		16.0 x
Treatment x Rootstock LSD	*{32.0}*	*{32.0}*	*{32.0}*		
Treatment mean	12.9 *	7.4 *	22.1 *		
Treatment LSD	*[14.3]*	*[14.3]*	*[14.3]*	*Rootstock LSD*	*(18.5)*
	% Increase in plant height[f]				
Swingle citrumelo	15.8 T[g]	9.4 T	13.2 T		12.8 x
C32-citrange	0.0 S[h]	4.8 S	5.1 S		5.5 z
C35-citrange	0.3 S	8.8 T	9.0 T		6.0 z
Benton citrange	6.1 S	-3.2 S	4.0 S		2.3 z
Rough lemon	8.2 T	18.6 T	-0.4 S		8.8 x
Treatment x Rootstock LSD	*{11.4}*	*{11.4}*	*{11.4}*		
Treatment mean	7.4 *	7.7 *	6.2 *		
Treatment LSD	*[5.1]*	*[5.1]*	*[5.1]*	*Rootstock LSD*	*(6.6)*
	% Reduction in root dry mass[i]				
Swingle citrumelo	-0.7 *	21.8 *	14.5		11.9 x
C32-citrange	-4.2	14.1	8.3 *		6.1 x
C35-citrange	20.3	6.0	24.4		16.9 x
Benton citrange	16.3	22.4	26.4		21.7 z
Rough lemon	41.2	27.4	46.3		38.3 x
Treatment x Rootstock LSD	*{31.8}*	*{31.8}*	*{31.8}*		
Treatment mean	14.6 *	18.4 *	24.0 *		
Treatment LSD	*[14.2]*	*[14.2]*	*[14.2]*	*Rootstock LSD*	*(18.4)*
	% Reduction in shoot dry mass[j]				
Swingle citrumelo	18.5 *	39.9 *	16.1 *		24.8 x
C32-citrange	17.7	17.1	13.4		16.1 x
C35-citrange	16.9	23.8	22.7		21.1 x
Benton citrange	38.8	26.0	39.5		34.8 z
Rough lemon	59.7	35.7	50.9		48.8 z
Treatment x Rootstock LSD	*{31.0}*	*{31.0}*	*{31.0}*		
Treatment mean	30.3 *	28.5 *	28.5 *		
Treatment LSD	*[13.9]*	*[13.9]*	*[13.9]*	*Rootstock LSD*	*(17.9)*

a Micro field plots were prepared as described under materials and methods

b rootstocks in this trail were grafted with Delta Valencia scion

c feeder root length was determined by measuring the length of 0.1 g roots. Results were expressed as the percentage increase in feeder root lengths and calculated as follows: ([uninoculated - inoculated]/uninoculated) x 100 (Gabor & Coffey, 1990)

d * not significant

e means followed by the same letter(x,z within rootstock mean) do not differ significantly (P=0.05) according to Fisher's protected *LSD* (*t*-test), the best rootstocks were selected according to the Multiple *t*-distribution test

f increase in plant height was calculated by subtracting the original plant length of the rootstocks, from the plant lengths seven months later at termination of the trial, and it was expressed in percentage. It was expressed in relation to the plant length of the uninoculated control

g most tolerant (T) cultivars selected according to the Multiple *t*-distribution test (P = 0.05), means followed by the same letter do not differ significantly according to Fisher's protected *LSD* (*t*-test)

h most susceptible (S) cultivars selected according to the Multiple *t*-distribution test (P=0.05) means followed by the same letter do not differ significantly according to Fisher's protected *LSD* (*t*-test)

i ([uninoculated - inoculated]/uninoculated) x 100

j ([uninoculated - inoculated]/uninoculated) x 100.

CHAPTER 4

ROOT REGENERATION RATE OF CITRUS ROOTSTOCKS UNDER GREENHOUSE CONDITIONS

ABSTRACT

Thirty different citrus rootstocks, varying in tolerance to fibrous root rot were evaluated for their ability to regenerate roots. One half of each rootstock's root system was removed longitudinally and the pruned rootstock planted in steam-pasteurised soil. Control plants were not subjected to root pruning. The plants were maintained for four weeks (first year) and eight weeks (second year), respectively, in the greenhouse. Root mass was recorded and the root growth potential of each rootstock was calculated. Rough lemon and Swingle citrumelo were used as reference rootstocks. The rootstocks were classified as either vigorous or non-vigorous according to their root growth potential. Pomeroy trifoliate, Swingle citrumelo, Terra Bella citrange, Cleopatra mandarin, Rusk citrange and 1113 were all vigorous in their growth. Macrophylla, C32-citrange, Obovoideae, Shekwasha mandarin, Australian trifoliate, Japanese citron and Rubidoux trifoliate were all non-vigorous. No consistent correlation was evident between root regeneration capacity and resistance to root pathogens. Root regeneration rate is therefore not a reliable parameter for screening tolerance in citrus rootstocks.

INTRODUCTION

Citrus root rot is a complex disease that affects all aspects of citrus production throughout the world (Menge & Nemec, 1997). Different fungi and nematodes contribute to the disease complex.

Phytophthora nicotianae Breda de Haan (syn. *P. parasitica* Dastur) causes losses due to rotting of roots and crown tissue in nurseries. It also causes foot rot (injury to the bark on the trunk or roots near the ground level) and fibrous root rot in orchards (Timmer & Menge, 1988). With fibrous root rot, the fleshy, non-woody feeder roots are attacked, rotted and, in the advanced stages, virtually destroyed. This eventually results in a slow decline in tree growth (Menge & Nemec, 1997) during which fruit size and yield may be dramatically reduced (Timmer & Menge, 1988).

Fusarium solani (Mart.) Appel & Wollenw. is the predominant fungus isolated from roots and rhizospere soil of citrus (Nemec & Zablotowicz, 1981; Graham *et al.*, 1985; Labuschagne *et al.*, 1987; Dandurand & Menge, 1993). This fungus can cause feeder root rot of citrus (Nemec & Zablotowicz, 1981; Strauss & Labuschagne, 1995), as well as dry rot of crowns and scaffold roots (Bender & Menge, 1984) and is a major limiting factor in the productivity of citrus (Labuschagne, 1994). *F. solani* varies in pathogenicity from non-pathogenic to severely pathogenic. The disease is aggravated by conditions that cause stress in the plant, for example waterlogging due to over-irrigation or poor drainage (Labuschagne, 1994).

The use of tolerant rootstocks is one of the best options for control of root pathogens (Agostini *et al.*, 1991). Several methods to evaluate resistance of citrus rootstocks to root pathogens have previously been described (Afek *et al.*, 1990). Resistance was assessed by means of the percentage seedlings surviving after being dipped in a *Phytophthora* zoospore suspension and planted in artificially infested media (Klotz *et* *al.*, 1958; Carpenter & Furr, 1962). Grimm & Hutchison (1973) and Tsao & Garber (1960) determined the percentage visual root rot of rootstocks grown for five to six weeks in artificially infested media. Klotz & De Wolfe (1965) used a colourant (2,3,5-triphenyl-2H-tetrazolium chloride (TTC)) which stains live tissue thereby indicating the percentage live roots. Grimm & Hutchison (1973) inoculated one-year-old seedlings with *Phytophthora* at the bark just above the soil line, and evaluated them after 6-8 weeks for resistance to *Phytophthora* infection.

Root growth potential or the ability to regenerate roots may also be a useful parameter in assessing differences in tolerance between rootstocks (Graham, 1995). The objective of this study was to determine the root regeneration rate of citrus rootstocks with the aim of correlating this capacity with their tolerance to *F. solani* and *P. nicotianae*.

MATERIALS AND METHODS

Citrus rootstocks. Thirty different rootstocks (Table 1), three months of age, were obtained from H F le Roux of Capespan Citrus Centre in Nelspruit. The seedlings were transplanted into 44 x 20 x 190 cm asbestos pots containing a steam pasteurised mixture of sand/peat/soil (1:1:1 v/v). Plants were maintained at 15±2°C during night and 27±2°C during day in a greenhouse and were watered three times a week with distilled water. Every six weeks the plants were alternately sprayed with 5 ml Dursban™, Efekto (a.i. chlorpyriphos 240 g^{-1}) 5 l^{-1} water and 200 ml Red Spidersprey™, Wonder (a.i. amitraz 200 g^{-1}), 100 l^{-1} water and fertilised with 10 g Chemicult™, Kompel (macro-elements: 6.5% N, 2.7% P, 13.0% K, 7.0% Ca, 2.2% Mg, 7.5% S; micro-elements: 0.15% Fe, 0.024% Mn, 0.024% B, 0.005% Zn, 0.002% Cu, 0.001% Mo) 5 l^{-1} water.

The rootstocks were evaluated in three groups of approximately ten rootstocks each, at monthly intervals in 1996. The experiment was repeated in 1997 with rootstocks that were between 15 and 17 months old. Orlando tangelo and Sampson tangelo were unavailable the second year. Swingle citrumelo was included as tolerant control and Rough lemon as susceptible control in all groups.

Disease status of rootstocks at the start of each trial. Roots were randomly selected from rootstocks to determine the presence of any root pathogens before each trial was planted. Root segments (5 mm in length) of six replicate plants per cultivar were washed and surface-disinfested for 1 minute in 1.5% sodium hypochlorite solution and subsequently rinsed with sterile distilled water. The root segments (ten per medium) were plated onto potato-dextrose agar (PDA) and rose-bengal-glycerine-urea agar selective for *Fusarium*, (10 g glycerine, 1 g urea, 0.5 g L-alanine, 1 g quintozene (Terrachlor™, 75% a. i.), 0.5 g rose-bengal, 15 g Biolab™ PDA l^{-1} distilled water supplemented with 50 mg l^{-1} streptomycin) (RbGU) (Van Wyk *et al.*, 1986).

Some root segments were only washed and then rinsed in sterile distilled water. These segments were plated (10 per medium) onto media selective for *Phytophthora* spp. [pimaracin-ampicillin-rifampicin-pentachloronitrobenzene-hymexazol (PARPH) (half-strength PDA (20 g Biolab™ PDA 800 ml^{-1} distilled water), supplemented after autoclaving with a solution consisting of 100 mg quintozene, 250 mg ampicillin, 10 mg rifampicin, 10 mg pimaricin and 0,15 ml (103 ppm) hymexazol (Tachigaren™, 70% a. i.) added to 200 ml sterile distilled water (Jeffers & Martin, 1986)].

Plates were incubated at room temperature (25 ± 2°C) in the dark and observed regularly over a two-week period for any fungal growth. Sub-cultures of all fungal colonies were made and the fungi identified (Nelson *et al.*, 1983; Erwin & Ribeiro, 1996).

Comparison of root regeneration potential. Plants were removed from the pots with the least root / soil disturbance as described by Kellam & Coffey (1985). One half of the root-soil mass of each seedling was bisected longitudinally with pruning shears and the half not attached to the stem was removed. The pruned seedlings were then transplanted into 1.5 l pots filled with steam-pasteurised sand/peat/soil (1:1:1 v/v) mixture. Control plants were not subjected to root pruning. Each rootstock treatment consisted of seven replicate plants, and were completely randomised in blocks. Plants were maintained at 15±2°C during night and 27±2°C during day in a greenhouse. The plants were watered three times a week with distilled water.

After four weeks (1996) and eight weeks (1997) the plants were carefully removed from the soil and their root systems washed under running tap water. The root mass of each seedling was determined after drying for 48 h at 80°C. Percentage reduction in root dry mass was calculated using the formula described by Gabor & Coffey (1990): ([control root system – pruned root system]/control root system) x 100.

Analysis of variance (ANOVA) was performed using the statistical program GENSTAT 5 (GENSTAT Committee, 1993). Treatment means and interactions between the treatments were compared by Fisher's Protected Least Significant Difference (*LSD*) *t*-test procedure (Snedecor & Cochran, 1967). The best group of rootstocks at the 5% level was assigned to be vigorous (best group, V) and the poorest group to be non-vigorous (poorest group, N) according to the Multiple *t*-distribution test procedure of Gupta & Panchapakesan (1979).

RESULTS

Before planting, no potentially pathogenic fungi could be isolated from the roots of the different rootstocks.

Reduction in root dry mass of the three-, four- and five-month-old rootstocks ranged from 0 to 72.9%, 22.2 to 71.9% and 16.7 to 71.0% respectively (Table 2). In the following year, reduction in root dry mass of the fifteen-, sixteen,-and seventeen-month-old rootstocks ranged from -5.8 to 72.6%, 1.2 to 59% and 36.2 to 88% respectively. No significant differences were evident in the 3- and 16- month-old rootstocks. Swingle citrumelo had a high root growth potential in all the trails. Rough lemon on the other hand, varied from a poor root growth potential in the first year to a far better potential in the second year. Most of the rootstocks showed a greater root regeneration rate in the older seedlings. Rootstocks that had a good root regeneration potential in both years were Pomeroy trifoliate, Swingle citrumelo, Terra Bella citrange, Cleopatra mandarin, Rusk citrange and 1113. Rootstocks with poor root regeneration abilities in both years were Macrophylla, C32-citrange, Obovoideae, Shekwasha mandarin, Australian trifoliate, Japanese citron and Rubidoux trifoliate.

DISCUSSION

Root regeneration commonly begins within a few days after infection of the original root system by a pathogen. New roots are usually formed from the taproot and live tissue of other roots at varying rates in different species and varieties. Within two weeks after infection, some new roots can be observed (Carpenter & Furr, 1962). According to Graham (1995), tolerance of rootstocks can be identified as the capability to regenerate fibrous roots in greenhouse and field soils infested with a particular root pathogen. In other words, a rootstock would be identified as tolerant when it exhibits a greater ability to regenerate roots than a more susceptible rootstock.

Swingle citrumelo, one of the reference rootstocks, had a high root growth potential in all the trials. It is known as highly tolerant to *Tylenchulus semipenetrans* Cobb. and *Phytophthora* spp. (Castle, 1987) Swingle citrumelo is not immune to infection as its roots support populations of *Phytophthora* (Graham, 1990). It does, however, have the ability to develop new roots subtending infected root tips (Graham, 1990). The resistance factors that limit infection to the root tip are not known, but it may relate to phytoalexins found in woody tissue of citrus infected with, for instance, *P. citrophthora* (R.E. Sm. & E.H. Sm.) Leonian (Afek & Sztejnberg, 1988). Root regeneration ability of Rough lemon, the other reference rootstock, varied from high and low. Rough lemon is known to be susceptible to root infection by *Phytophthora, Fusarium* and nematodes (Castle, 1987; Menge, 1988; Rabe & Von Broembsen, 1991).

For some rootstocks, a correlation existed between root regeneration ability and degree of tolerance to the different pathogens. Pomeroy trifoliate, Swingle citrumelo, Terra Bella citrange and Rusk citrange all had a high root growth potential, and were also found to be tolerant to *Fusarium* and *Phytophthora* in Chapter 2. Japanese citron had poor root regeneration ability and also showed susceptibility to *Phytophthora*. For the remaining rootstocks there was no correlation between root regeneration and tolerance to root pathogens.

Some authors consider root growth potential as an indication of tolerance to root pathogens (Kellam & Coffey, 1985; Blaker & McDonald, 1986; Graham, 1990; Graham, 1995). It was, however, noted by Graham (1995) that the capacity of a rootstock to regenerate roots in the presence of a pathogen is not necessarily related to the potential rate of root growth in the absence of the pathogen. For example, trifoliate orange had a low inherent root regeneration potential, but in the presence of a pathogen exhibited a greater ability to regenerate roots. Tolerance could therefore be more related to biochemical resistance (Afek & Stejnberg, 1988; Graham, 1995).

Most of the rootstocks showed a greater ability to regenerate roots in the second year, possibly because the rootstocks used in the second year were older and grown for a longer period of time before being evaluated.

Several factors can also influence the root regeneration potential of a rootstock. Environmental variables such as temperature can have a significant effect on root growth potential (Graham, 1995). Certain rootstocks loose the ability to regenerate roots in colder conditions, but regain it again when temperature increases (Graham, 1995). A low oxygen supply and high water content in soils will also inhibit growth and regeneration of roots (Stolzy *et al.*, 1965).

Root regeneration ability cannot be used as a reliable parameter for tolerance of a rootstock, because there is no direct correlation between root regeneration and resistance to certain root pathogens. Some rootstocks may have a great ability to regenerate roots after fibrous root damage, but can still be affected by the pathogen, particularly when environmental conditions favour disease.

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Table 1: Citrus rootstocks evaluated for their root regeneration ability in the greenhouse

GENUS AND SPECIES	CULTIVAR
C. aurantium L.	Gou Tou SO
C. depressa Hayata	Shekwasha mandarin
C. jambhiri Lush.	Rough lemon
C. limon (L.) Burm.f.	Milan lemon
C. limonia Osbeck	Japanese citron
C. macrophylla Wester	Macrophylla
C. natsudaidai Hayata	Natsudaidai
C. obovoidea Hort. ex Takahashi	Obovoideae
C. paradisi Macfady. x *C. reticulata* Blanco	Orlando tangelo
C. paradisi x *C. reticulata*	Sampson tangelo
C. reticulata	Changsha mandarin
C. reticulata	Cleopatra mandarin
C. reticulata	Sunchusha mandarin
C. sunki Hort. ex Tan	Sunki mandarin
Poncirus trifoliata (L.) Raf. var. *monstrosa* (T. Ito.) Swing x *C. sunki*	1112
P. trifoliata	Pomeroy trifoliate
P. trifoliata	Australian trifoliate
P. trifoliata	Jacobsen trifoliate
P. trifoliata	Rubidoux trifoliate
P. trifoliata var. *monstrosa* Swing x *C. sunki*	1116
P. trifoliata var. *monstrosa* Swing x *C. sunki*	1113
Poncirus trifoliata x *Citrus paradisi*	F80-8 citrumelo
P. trifoliata. x *C. paradisi*.	Swingle citrumelo
P. trifoliata x *C. sinensis*	C32-citrange
P. trifoliata x *C. sinensis*	Rusk citrange
P. trifoliata x *C. sinensis*	Terra Bella citrange
Unknown - perhaps a *C. aurantium* hybrid	Smooth Flat Seville
Unknown parentage	Amblycarpa
Unknown parentage, probably from the Philippines	Calamandrin
Unknown parentage	Konejime

Table 2: Root regeneration ability of citrus rootstocks four (1996) and eight (1997) weeks after 50% of their roots have been pruned off

Rootstock	% Reduction in root dry mass[a] 1996	1997
	Three- month old rootstocks	**Fifteen-month old rootstocks**
Swingle citrumelo	33.8 *[b]	-5.8 V[c]
Rough lemon	54.5	3.2 V
Pomeroy trifoliate	20.0	3.4 V
F80-8 citrumelo	41.7	5.5 V
Calamandrin	44.4	10.9 V
Amblycarpa	10.3	34.1 N[d]
Milan lemon	28.3	42.6 N
Gou Tou SO	29.2	50.2 N
Konejime	0.0	67.2 N
Macrophylla	43.3	69.9 N
C32-citrange	52.4	72.6 N
Orlando tangelo	72.9	.
Treatment x Rootstock LSD	*(37.9)*	*(39.3)*
	Four-month old rootstocks	**Sixteen-month old rootstocks**
1113	22.2 V	29.1 *
Natsudaidai	27.7 V	44.0
Swingle citrumelo	33.8 V	1.2
Terra Bella citrange	38.3 V	23.9
Cleopatra mandarin	41.7 V	32.9
Rusk citrange	45.0 V	33.2
Jacobsen trifoliate	45.8 V	47.0
Rough lemon	54.5 N	25.6
Obovoideae	59.5 N	33.8
Changsha mandarin	64.0 N	5.8
Shekwasha mandarin	71.9 N	59.1
Treatment x Rootstock LSD	*(24.4)*	*(51.5)*
	Five-month old rootstocks	**Seventeen-month old rootstocks**
Swingle citrumelo	33.8 V	38.7 V
Sampson tangelo	38.0 V	.
1116	16.7 V	83.2 N
Smooth Flat Seville	26.4 V	72.8 N
1112	42.0 [e]	35.0 V
Sunki mandarin	45.7	60.2
Australian trifoliate	64.6 N	60.3
Rough lemon	54.5 N	36.2 V
Sunchusha mandarin	71.0 N	46.3 V
Japanese citron	55.6 N	88.0 N
Rubidoux trifoliate	58.8 N	72.8 N
Treatment x Rootstock LSD	*(24.5)*	*(20.0)*

a ((unpruned - pruned)/unpruned) x 100

b * not significant

c most vigorous (V) cultivars selected according to the Multiple *t*-distribution test *(P*=0.05), means followed by the same letter do not differ significantly according to Fisher's protected *LSD* (*t*-test)

d most non-vigorous (N) cultivars selected according to the Multiple *t*-distribution test *(P*=0.05), means followed by the same letter do not differ significantly according to Fisher's protected *LSD* (*t*-test)

e values not followed by any letter can be classified as intermediate in its response to the treatment according to the Multiple t-distribution test (P=0.05) and do not differ significantly according to Fisher's protected *LSD* (*t*-test), this is only where significant differences did occur within a treatment.

CHAPTER 5

GENERAL DISCUSSION

Rootstock selection is a major consideration in every citrus growing operation (Davies & Albrigo, 1994) because it contributes to sustainability by providing disease resistance and long-term horticultural benefits for citrus production (Niles *et al.*, 1995).

The orange subfamily, Aurantioideae, in the Rutaceae, comprises 33 genera of which only three, *Citrus, Poncirus* (trifoliate orange) and *Fortunella* (kumquat), have been significant sources of citrus rootstocks or scions (Castle, 1987). *Phytophthora* foot rot and tristeza were the main stimuli to put more focus on rootstocks (Castle, 1987). Rootstock research in its infant stages involved only a few rootstocks namely Rough lemon (*Citrus jambhiri* Lush.), sour orange (*C. aurantium* L.), trifoliate orange (*P. trifoliata* [L.] Raf.), sweet orange (*C. sinensis* [L.] Osb.) and occasionally grapefruit (*C. paradisi* Macf.) or Cleopatra mandarin (*C. reticulata* Blanco), but because of tristeza, germplasm collections were established and breeding efforts intensified (Castle, 1987).

More than 20 horticultural characteristics can influence rootstock behaviour, e.g. tree vigour and size, depth of rooting, freeze tolerance, adaptation to soil conditions such as high salinity and pH or excess water, resistance or tolerance to nematodes and diseases like *Phytophthora* root and foot rot and citrus blight, fruit yield, size, texture, internal quality and maturity date (Castle, 1987; Davies & Albrigo, 1994). Although citrus producers often consider rootstocks only in terms of their yield, horticultural performance in the nursery remains the first, most important hurdle a potential rootstock must overcome to be successful (Matheron *et al.*, 1998). The rootstock should be vigorous, exhibit minimal branching and be adaptable to the prevalent soil type (Matheron *et al.*, 1998).

Profitable citriculture is highly dependent upon rootstock resistance to pests and diseases (Castle, 1987). Resistance can play a critical role in integrated disease management approaches (Matheron *et al.*, 1998) and can offer excellent means of reducing damage caused by root pathogens such as *Phytophthora nicotianae* Breda de Haan (syn. *P. parasitica* Dastur) (Graham, 1995), *Tylenchulus semipenetrans* Cobb. (Niles *et al.*, 1995) and *Fusarium solani* (Mart.) Appel & Wollenw. (Menge, 1988).

In the present study various citrus rootstocks were firstly evaluated in the greenhouse against the above root pathogens (Table 1). Different parameters were used to determine the degree of tolerance or susceptibility of the rootstocks. Subsequently, some of the rootstocks that were found to be tolerant in the greenhouse were evaluated in the field. Many of the newer rootstocks, which showed tolerance to the different pathogens, must still undergo intensive field evaluation. Rootstock breeding and field testing are time-consuming processes, often requiring 20 years or more before new rootstocks can be released (Davies & Albrigo, 1994).

Rootstocks evaluated in the present study were more severely affected by *P. nicotianae* than by *F. solani* or a combination of the two pathogens. The relatively low severity of the combination treatment could possibly be ascribed to the protective effect of *F. solani* against *P. nicotianae* (Strauss & Labuschagne, 1994).

Some rootstocks were found to be more vigorous in their growth than others. The physiological status of a plant (e.g. age, nutrition, succulence, vigour, maturity of the growth flush, etc.) is important in the evaluation of tolerance (Broadbent & Gollnow, 1993). It was also noted that plants stressed by adverse soil conditions such as drought (Duniway, 1977), waterlogging (Stolzy *et al.*, 1965) or salinity (Blaker & McDonald, 1986) are more susceptible to *Phytophthora* root rot.

Differences occurred between the rootstock x treatment interaction and it was possible to rate the rootstocks as tolerant or susceptible to the different treatments. Rootstocks that were the most tolerant overall were Australian trifoliate and Shekwasha mandarin (both *Phytophthora* tolerant) and Natsudaidai, Terra Bella citrange, Jacobsen trifoliate, Rusk citrange, Shekwasha mandarin and Pomeroy trifoliate (all *Fusarium* tolerant). Swingle citrumelo was tolerant to both *F. solani* and *P. nicotianae*. The above rootstocks were the most consistent over the two years according to the most reliable parameters.

Increase in plant height and reduction in shoot mass appeared to be the most reliable parameters for indicating tolerance or susceptibility. Some correlation also existed between reduction in root and shoot mass, which agrees with the findings of Matheron *et al.* (1998). Increase in feeder root length was not a reliable parameter. This could be attributed to root loss occurring during the process of harvesting and washing of the root system.

In the field experiments (Table 1), significant differences between treatments occurred in 1996 but not in 1997. The greatest effect was observed with *T. semipenetrans* for all the parameters, except plant height. Rootstocks that proved to be the most tolerant were the following: Swingle citrumelo and C35-citrange (*Phytophthora* tolerant), Minneola x Trifoliate hybrid and C35-citrange (*Fusarium* tolerant) and Swingle citrumelo (nematode tolerant). C32-citrange was tolerant to all three pathogens. Rootstocks that were found to be susceptible were the following: X639 hybrid, Minneola x Trifoliate hybrid (susceptible to *Phytophthora*), Carrizo citrange and Benton citrange (susceptible to *Fusarium*). Rough lemon was tolerant to *Fusarium* in 1996, but showed susceptibility to all the pathogens in 1997. Again, differences in vigour of growth of the different rootstocks were noted, and this could play a definite role in the tolerance of the rootstocks to the pathogens (Matheron *et al.*, 1998). Other factors that can also strongly influence rootstock resistance, especially in the field, are temperature (Menge & Nemec, 1997), scion variety (Klotz *et al.*, 1968), age of rootstock (Klotz *et al.*, 1958), the existence of physiological races or biotypes of *T. semipenetrans* (Verdejo-Lucas, 1997), nutrient status and succulence of the infected rootstock, soil characteristics and moisture levels (Matheron *et al.*, 1998).

Blaker & MacDonald (1986) and Graham (1990, 1995a) demonstrated that tolerant rootstocks produce new roots more rapidly than less tolerant rootstocks. Graham (1995b) defined tolerance as the capability of rootstocks to regenerate fibrous roots in the presence of root pathogens. In the present study some correlation existed between the root growth potential of rootstocks although this was not consistent throughout the study. Most of the rootstocks had a greater ability to regenerate roots in the second year, probably because these seedlings were 12 months older. Graham (1990) also noted that younger citrus seedlings are more susceptible to infections than older seedlings. Kellam & Coffey (1985) found in avocado (*Persea americana* Mill.) rootstocks that root growth potential, in the absence of a root pathogen, can be a useful parameter for the quantitative comparison of resistance to *Phytophthora* infections. However, Graham (1995b) indicated that the capacity of citrus rootstocks to regenerate roots in the presence of a pathogen is not necessarily related to their root growth rates in the absence of a pathogen. There was a correlation between the root regeneration ability and tolerance to pathogens for Pomeroy trifoliate, Swingle citrumelo, Terra Bella citrange, Rusk citrange and Japanese citron in the present study, but not for the other rootstocks.

The response of the different citrus rootstocks was compared with findings in the literature (Tables 2, 3 and 4). For most of the rootstocks, the findings in this study and from the literature were consistent, but for some, it was contradictory. There are several possible explanations. Firstly, susceptibility or resistance to gummosis (bark infection) is usually assumed to be applied to root rot as well. The rootstocks must be evaluated for root rot and gummosis, because it has been demonstrated that resistance to root rot is generally less than to bark infection (Furr & Carpenter, 1961; Grimm & Hutchison, 1973; Matheron *et al.*, 1998). Secondly, susceptibility or resitance to different *Phytophthora* spp. can vary (Matheron *et al.*, 1998).

Thirdly, the development of root rot can be influenced by several variables (as discussed above) in addition to the innate resistance of the citrus host (Matheron *et al.*, 1998). Finally, different selections of several *Citrus* spp. and citrus hybrids could display markedly different levels of resistance to colonisation by the pathogens. Significant variation in resistance among selections of sour orange and trifoliate orange to colonisation by *P. citrophthora* (R.E. Sm. & E.H. Sm.) Leonian has been reported (Matheron *et al.*, 1998).

Citriculture is a dynamic industry. New diseases such as blight appear. The need for new rootstocks can arise quickly. Urbanisation often shifts agriculture to poorer land with less water and of poorer quality. Industrial expansion in areas throughout the world has resulted in citrus being established in soils less suitable for available rootstocks. These events accentuate deficiencies in existing rootstocks (Castle, 1987). The ideal rootstock would be one that has a pest and disease resistance system, is compatible with scions producing an abundance of high-quality fruit, and shows adaptability to a multitude of soil and climatic conditions (Rabe & Von Broembsen, 1991). Such rootstocks do not presently exist. Rootstock evaluation is a slow and tedious process, and therefore a continuous activity.

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Table 1 : A summery of all the rootstocks used in all the experiments and their reaction to the different pathogens according to the different parameters used

Rootstocks	Feeder root length[a]								Plant height[b]								Disease severity[c]								Root dry mass[d]								Shoot dry mass[e]								Root regeneration[f]	
	Phytophthora		Combination		Fusarium		Nematode		Phytophthora		Combination		Fusarium		Nematode		Phytophthora		Combination		Fusarium		Nematode		Phytophthora		Combination		Fusarium		Nematode		Phytophthora		Combination		Fusarium		Nematode			
	A[g]	B[h]	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
Greenhouse experiments																																										
Swingle citrumelo	S[i]	*[l]	I		I	*			I	I	I		I	I			T	T	T		I	I			*	*	*		*	*			T	*	T		T	*			*	V[m]
Amblycarpa	T[j]		I		I				I	I	I		I	I			S	I	I		I	T											S		T		S					N[n]
C32-citrange	I[k]		I		I				I	S	T		I	T			I	I	I		I	I											T		T		T					N
Calamandrin	T		I		I				S	I	I		I	T			S	S	I		I	I											S		S		I					V
F80-8 citrumelo	I		I		I				I	S	I		I	S			I	S	I		I	I											S		I		I					V
Gou Tou SO	I		I		I				I	I	I		I	I			S	I	I		T	I											I		T		T					N
Konejime	I		I		I				I	S	I		I	S			S	I	I		T	I											I		T		T					N
Macrophylla	T		I		I				S	I	I		I	I			S	I	T		T	I											S		T		T					N
Milan Lemon	S		S		S				S	I	I		I	I			S	I	I		I	I											T		I		T					N
Orlando tangelo	T		T		T				S		I		I				S		I		T												S		I		I					N
Pomeroy trifoliate	T		I		I				I	I	I		I	I			S	T	T		T	T											S		T		T					V
Rough lemon	I		I		I				I	I	I		I	I			S	I	T		I	I											I		I		I					V
Swingle citrumelo	*	*	*		*	*			I	S	I		I	T			T	T	T		I	T			S	T	T		I	T			S	I	T		I	T			N	*
1113									I	S	T		I	S			T	T	T		T	I			S	T	T		T	I			S	S	T		T	I			V	
Changsha mandarin									S	S	I		S	S			I	I	I		S	S			S	T	T		S	T			S	T	T		S	T			N	
Cleopatra mandarin									I	S	I		I	S			I	I	I		I	I			S	I	T		S	I			S	S	I		S	S			V	
Jacobsen trifoliate									I	T	I		I	T			I	I	I		T	I			S	I	T		T	I			S	S	T		T	T			V	
Natsudaidai									I	S	S		T	T			I	S	I		I	I			S	S	T		S	I			S	S	T		S	S			V	
Obovoideae									S	I	I		S	S			S	I	I		T	S			I	I	I		I	I			I	I	I		T	S			N	
Rusk citrange									S	S	I		I	I			S	T	T		T	T			I	I	S		T	T			I	S	S		T	T			V	
Shekwasha mandarin									I	I	T		I	I			T	T	T		T	I			S	T	T		S	T			S	I	T		T	T			N	
Terra Bella citrange									T	I	T		T	T			I	S	I		I	S			S	I	I		S	T			S	I	I		S	I			V	
Rough lemon									I	S	S		T	S			S	I	I		I	I			S	I	I		S	I			S	I	I		S	T			N	
Swingle citrumelo	*	*	*		*	*			I	T	I		T	I			T	T	I		I	T			T	T	T		T	T			T	T	T		T	T			V	V
1112									I	I	I		T	I			I	I	I		T	S			I	S	S		I	S			I	S	S		T	S			I	V
1116									I	I	T		I	S			S	I	T		I	T			I	S	T		T	I			S	I	T		T	I			V	N
Australian trifoliate									T	T	T		I	I			I	I	I		I	T			S	S	S		S	I			S	I	S		S	I			N	I
Japanese citron									I	I	T		T	I			S	S	I		I	T			S	S	S		T	S			S	S	S		T	S			N	N
Rubidoux trifoliate									I	T	T		T	I			I	I	I		I	T			T	S	I		T	I			T	S	I		T	S			N	N
Sampson tangelo									I	I	I		I	I			I	S	S		S	T			I	S	I		I	I			T	S	S		I	I			V	
Smooth Flat Seville									I	S	I		T	S			I	I	T		T	S			I	S	I		I	I			I	S	S		I	I			V	N
Sunchusha mandarin									S	S	S		T	I			I	I	I		I	T			I	I	I		S	S			I	I	S		S	S			N	V
Sunki mandarin									I	S	I		T	S			S	I	I		I	T			I	S	I		I	I			I	S	I		S	S			I	I
Rough lemon									S	I	I		S	I			S	I	I		I	I			S	S	T		I	I			S	I	T		I	I			N	V
Field experiments																																										
Benton citrange		*				*		*		S				S		S									*	*			*	*	*	*	*	*			*	*	*	*		
C32-citrange		*				*		*		T				T		T									*	*			*	*	*	*	*	*			*	*	*	*		
C35-citrange		*				*		*		S				T		T									*	*			*	*	*	*	*	*			*	*	*	*		
Carrizo citrange									*				*		*		*				*		*		*	*			*	*	*	*	*	*			*	*	*	*		
Minneola X Trifoliate hybrid									*				*		*		*				*		*		*	*			*	*	*	*	*	*			*	*	*	*		
Rough lemon									*				*		*		*				*		*		*	*			*	*	*	*	*	*			*	*	*	*		
Swingle citrumelo									*				*		*		*				*		*		*	*			*	*	*	*	*	*			*	*	*	*		
X639 hybrid									*				*		*		*				*		*		*	*			*	*	*	*	*	*			*	*	*	*		

a feeder root length was determined by measuring the length of 0.1g roots (cm), and it was expressed as percentage increase in feeder root lengths
b plant height was determined by measuring the plant length (from soil-line to the top of foliage) at the beginning and end of the experiments, subtracting the end plant length from the plant length in the beginning and it was expressed as percentage increase in plant length
c disease severity was determined by rating each root system for visual root rot on a scale of 1-4 (1 = 0-25% of root system rotted, 2 = 25-50% of root system rotted, 3 = 50-75% of root system rotted and 4 = 75-100% of root system rotted, increase in disease severity was expressed in relation to disease severity of
d root mass was determined after drying for 48h at 80°C, and calculated as follows: ([uninoculated - inoculated]/uninoculated) x 100
e shoot mass was determined after drying for 48h at 80°C, and calculated as follows: ([uninoculated - inoculated]/uninoculated) x 100
f 50% of root system was pruned off and root regeneration ability determined
g 1996 (first year in which experiments were conducted)
h 1997 (second year in which experiments were conducted)
i cultivars susceptible (S) in their response to the different pathogens
j cultivars tolerant (T) in their response to the different pathogens
k cultivars intermediate (I) in their response to the different pathogens
l no significant differences (*) between cultivars
m vigorous (V) root growth potential
n non-vigorous (N) root growth potential

Table 2: A summery of the response (this study and from the literature) of different citrus cultivars to *Phytophthora* spp. and the parameter(s) used

REFERENCE	ROOTSTOCK	PARAMETER	Present study
		Lesion length	
Afek *et al.*, 1990	Macrophylla	R[a]	I to S
	Trifoliate orange	R	Varied[d]
	Sour orange	I[b]	I
	Rough lemon	S[c]	S
		Disease rating	
Klotz *et al.*, 1967	Pomeroy trifoliate	R	I
	Carrizo citrange	I	I
	Cleopatra mandarin	I	S
	Shekwasha mandarin	T	T[e]
	Macrophylla	I	I to S
	Rough lemon	S	S
Tuzco *et al.*, 1984	Jacobsen trifoliate	R	S
	Rubidoux trifoliate	R	Varied
	Carrizo citrange	R	I
	Macrophylla	S	I to S
	Natsudaidai	S	S
Smith *et al.*, 1987	Carrizo citrange	S	I
	Cleopatra mandarin	S	S
	Rough lemon	S	S
	Sour orange	S	I
Graham, 1990	Sour orange	S	I
	Cleopatra mandarin	S	S
	Carrizo citrange	S	I
	Trifoliate orange	T	Varied
	Swingle citrumelo	T	T
Graham, 1995a	Cleopatra mandarin	S	S
	Sunchusha mandarin	S	S
	Calamandrin	S	S
	Changsha mandarin	S	S
	X639 hybrid	I	S
	Carrizo citrange	I	I
	C35-citrange	T	S
	F80-8 citrumelo	I	I to S
	Sour orange	S	I
	Obovoideae	S	I to S
	Gou Tou Sour orange	S	I
	Smooth Flat Seville	S	I to S

REFERENCE	ROOTSTOCK	PARAMETER	Present study
		Lesion length and disease rating	
Broadbent & Gollnow,	Carrizo citrange	R	I
	Swingle citrumelo	R	T
	C35 citrange	R	S
	Smooth Flat Seville	T	I to S
	Rough lemon	S	S
		Plant height	
Carpenter & Furr, 1962	Rubidoux trifoliate	T	Varied
	Swingle citrumelo	T	T
	Carrizo citrange	S	I
	Rough lemon	S	S
		Root regeneration	
Graham, 1995b	Swingle citrumelo	V[f]	V
	Trifoliate orange	V	Varied
	Carrizo citrange	N[g]	I
	Sour orange	N	N
	Cleopatra mandarin	N	V
		Plant height, trunk diameter, colour of foliage and disease rating	
Hough, 1992	Carrizo citrange	S	I
	X639 hybrid	I	S
	Minneola x Trifoliate hybrid	I	S
	Terra Bella citrange	R	Varied
	Rough lemon	Varied	S
	Sampson tangelo	I	Varied
	Australian trifoliate	R	Varied
		Root and shoot mass, lesion lengths, plant survival	
Matheron *et al.*, 1998	C35-citrange	T	S
	Benton citrange	T	S
	Gou Tou SO	T	I
	Shekwasha mandarin	T	T
	Sunchusha mandarin	T	S
	Macrophylla	T	I to S

a resistant (R) in reaction to *Phytophthora* infection
b intermediate (I)
c susceptible (S)
d was not possible to classify it as either tolerant, intermediate or susceptible
e tolerant (T)
f vigorous (V) in root growth potential
g non-vigorous (N) in root growth potential

Table 3: A summery of the response (this study and from the literature) of different citrus cultivars to *Fusarium solani* and *Tylenchulus semipenetrans* and the parameter(s) used

REFERENCE	ROOTSTOCK	PARAMETER	Present study
Fusarium solani			
		Disease rating	
Nemec *et al*., 1980	Macrophylla	T[a]	T to I
Nemec, 1994	Trifoliate orange	T	Varied[d]
	Carrizo citrange	I to S	S
	Rough lemon	I to S	I
	Cleopatra mandarin	S[b]	S
	Orlando tangelo	S	T to I
		Root and shoot mass, disease rating, plant height	
Labuschagne *et al*., 1996	Carrizo citrange	T	S
	X639 hybrid	T	I
	Swingle citrumelo	I[c]	T to I
	Rough lemon	I	S

REFERENCE	ROOTSTOCK	PARAMETER	Present study
	Tylenchulus semipenetrans		
		Females / g roots	
Hutchison & O' Bannon, 1972	Rubidoux trifoliate	R[e]	Not evaluated against nematode
McCarthy *et al*., 1979	Rubidoux trifoliate	T	Not evaluated against nematode
	Pomeroy trifoliate	T	T
	Swingle citrumelo	T	T
	C35-citrange	T	I
Duncan *et al*., 1994	Trifoliate orange	R	Not evaluated against nematode
	Swingle citrumelo	R	T
	Rough lemon	S	S
Niles *et al*., 1995	Rubidoux trifoliate	R	Not evaluated against nematode
	Pomeroy trifoliate	R	T
	Swingle citrumelo	R	T
	Carrizo citrange	S	I

a tolerant (T) in reaction to *F. solani* or *T. semipenetrans* infection
b susceptible (S)
c intermediate (I)
d was not possible to classify it as either tolerant, intermediate or susceptible
e resistant (R)

Table 4: A summery of the response (this study and from the literature) of different citrus cultivars to Phytophthora spp. and Tylenchulus semipenetrans

ROOTSTOCK	REFERENCE	RESPONSE			
		Phytophthora spp.	Present study	*Tylenculus semipenetrans*	Present study
Rough lemon	Davies & Albrigo, 1994	S[a]	I to S	S	S
Milan lemon		S	Varied[d]	S	
Macrophylla		R[b]	Varied	S	
Sour orange		T[c]	I[e]	S	
Smooth Flat Seville		T	I to S	S	
Cleopatra mandarin		T	S	S	
Trifoliate orange		R	Varied	R	
Carrizo citrange		T	I	T	I
Swingle citrumelo		T	T	T	T
Carrizo citrange	Copeland, 1988	T	I	T	I
Trifoliate orange		T	Varied	T	
Rough lemon		S	I to S	S	S
Milan lemon		T	Varied		
Cleopatra mandarin		T	S	S	
Amblycarpa		T	Varied	S	
Sour orange		T	I to S	S	
Macrophylla		R	I to S	S	
C32-citrange		T	T to I	T	T
C35-citrange		T	T to I	T	I
Rough lemon	Rabe & Von Broembsen, 1991	S	I to S	S	S
Cleopatra mandarin		I	S	S	
Carrizo citrange		T	I	T	I
Trifoliate orange		T	Varied	T	
X639 hybrid		T	S	T	I
Swingle citrumelo		T	T	T	T

a susceptible (S) reaction to *Phytophthora* spp. or *T. semipenetrans* infection
b resistant (R)
c tolerant (T)
d was not possible to classify it as either tolerant, intermediate or susceptible
e intermediate (I)

TOLERANCE OF CITRUS ROOTSTOCKS TO ROOT PATHOGENS

by

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RESUMè

Thirty-five citrus rootstocks were evaluated for tolerance to *Phytophthora nicotianae* (syn. *P. parasitica*) and *Fusarium solani* on their own and in combination, as well as against the citrus nematode, *Tylenchulus semipenetrans*. The most reliable parameters for screening of tolerance were also established. The following emerged from the investigation:

The rootstocks were more severely affected by *P. nicotianae* than by *F. solani* or a combination of the two fungi. Plant height and reduction in shoot mass were the best parameters of tolerance, whereas increase in feeder root length was not reliable. Australian trifoliate and Shekwasha mandarin were highly tolerant to *P. nicotianae*, while Natsudaidai, Terra Bella citrange, Jacobsen trifoliate, Rusk citrange, Shekwasha mandarin and Pomeroy trifoliate were the most tolerant to *F. solani*. Swingle citrumelo, C32- and C35-citrange were the most tolerant to both fungi. C32-citrange

and Swingle citrumelo were tolerant to *T. semipenetrans*. Rootstocks that consistently rated as susceptible were Calamandrin, Jacobsen trifoliate, Natsudaidai, Japanese citron, X639-hybrid and Minneola x Trifoliate hybrid (all *Phytophthora* susceptible). Carrizo citrange and Benton citrange were susceptible to *F. solani*, whereas Changsha mandarin, Rough lemon, and Sunchusha mandarin showed susceptibility to both *Fusarium* and *Phytophthora*. Rough lemon was the most susceptible to *T. semipenetrans*. Sampson tangelo, Smooth Flat Seville and 1112 were all intermediate to *P. nicotianae* while Calamandrin and F80-8 citrumelo were intermediate to *F. solani*.

With the exception Swingle citrumelo, Pomeroy trifoliate, Terra Bella citrange, Rusk citrange and Japanese citron, no consistent correlation was found between root regeneration and disease resistance to root pathogens.

VERDRAAGSAAMHEID VAN SITRUSONDERSTAMME TEENOOR WORTELPATOGENE

deur

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SAMEVATTING

Vyf-en-dertig sitrusonderstamme is geëvalueer vir verdraagsaamheid teenoor *Phytophthora nicotianae* (sinoniem *P. parasitica*) en *Fusarium solani*, afsonderlik en in kombinasie, asook teen die sitrusaalwurm, *Tylenchulus semipenetrans*. Die betroubaarste maatstawwe vir die siftingsproses is ook daargestel. Die volgende is gevind:

Onderstamme is tot 'n groter mate beinvloed deur *P. nicotianae* as deur *F. solani* of 'n kombinasie van die twee swamme. Planthoogte en vermindering in loofmassa was die betroubaarste maatstawwe, terwyl voedingswortellengte verdraagsaamheid die swakste weerspieël het. Australiese trifoliaat en Shekwasha mandaryn was verdraagsaam teenoor *P. nicotianae*, terwyl Natsudaidai, Terra Bella sitrange, Jacobsen trifoliaat, Rusk sitrange, Shekwasha mandaryn en Pomeroy trifoliaat die verdraagsaamste was teen *F. solani*. Swingle sitrumelo, C32- en C35- sitrange was die mees verdraagsaam

teen beide die patogene. C32-sitrange en Swingle sitrumelo was verdraagsaam teen *T. semipenetrans*. Calamandrin, Jacobsen trifoliaat, Natsudaidai, Japanese sitron, X639 hibried en Minneola x Trifoliaat hibried was konsekwent vatbaar vir *Phytophthora*. Carrizo sitrange en Benton sitrange was vatbaar vir *F. solani*, terwyl Changsha mandaryn, growweskilsuurlemoen en Sunchusha mandaryn vatbaarheid getoon het teen beide *Fusarium* en *Phytophthora*. Growweskilsuurlemoen was die vatbaarste vir *T. semipenetrans*. Sampson tangelo, "Smooth Flat Seville" en 1112 was intermediêr teen *P. nicotianae*, terwyl Calamandrin en F80-8 sitrumelo intermediêr was teen *F. solani*.

Behalwe vir Swingle sitrumelo, Pomeroy trifoliaat, Terra Bella sitrange, Rusk sitrange and Japanese sitron kon geen konsekwente korrelasie gevind word tussen wortelregenerasievermoë en verdraagsaamheid teenoor wortelpatogene nie.