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Development of a nursery good agricultural practices framework and protocol for the South African citrus nursery industry

by

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LIST OF ABBREVIATIONS

APHIS	Animal and Plant Health Inspection Services
APIS	Agricultural Product Inspection Service
ARC	Agricultural Research Council
bp	base pair
CBS	Citrus Black Spot
CGA	Citrus Grower Association
CGIAR	Consultative Group on International Agricultural Research
CHRP	Citrus Health Response Plan
CIP	Citrus Improvement Programme
CRI	Citrus Research International
CSFRI	Citrus and Subtropical Fruit Research Institute
DNA	Deoxyribonucleic Acid
dNTP	Deoxynucleotriphosphate
DoA	Department of Agriculture
DTBIA	Direct Tissue Blot Immunoassay
ELISA	Enzyme-Linked Immunosorbent Assay
EU	European Union
FAO	Food Agricultural Organization
GAP	Good Agricultural Practices
g	gram
IBPGR	International Board for Plant Genetics Resources
IOCV	International Organization of Citrus Virologists
ISCN	International Society of Citrus Nurserymen
ITSC	Institute for Tropical and Subtropical Crops
mg	milligram
ml	millilitre
mm	millimetre
NSW	New South Wales
PCA	Polyclonal Antiserum

LIST OF ABBREVIATIONS

PCR	Polymerase Chain Reaction
PHC	Packhouse Codes

PPECB	Perishable Products Export Control Board
PUC	Production Units Codes
RISA	Radio Immunosorbent Assay
RNA	Ribonucleic Acid
RT	Reverse Transcription
SACIP	South Africa Citrus Improvement Programme
SACNA	South Africa Citrus Nursery Association
SAGAP	South African Good Agricultural Practices
SANAS	South African National Accreditation System
SPS	Sanitary and Phytosanitary Measures
SSEM	Serologically Specific Electron Microscopy
µl	microlitre
µM	micro Molar
USA	United States of America
USDA	United State Department of Agriculture
WTO	World Trade Organization

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CHAPTER 1

GENERAL INTRODUCTION

Citrus is one of the most important fruit crops in world trade. Citrus originated in South East Asia and was introduced to the rest of the world during the middle ages (Spiegel-Roy & Goldschmidt, 1996). Citrus fruits have been cultivated and enjoyed for over 400 years and is currently grown in nearly every country of the world particularly within 40º north-south latitude (Gmitter & Hu, 1990). Within this region, north eastern India and northern Burma are believed to be the centre of origin. Alternative evidence, however, suggests that the Yunnan Province in South-Central China might also be as important due to the diversity of species found in this area and the system of rivers that could have provided dispersal to the south (Gmitter & Hu, 1990). Citrus plants were introduced to the European traders around 310 B.C. and only arrived in the southern parts of Africa around 1650 A.D. (Reuther *et al*., 1967). In South Africa, citrus plants were imported during 1654 from the island of St. Helena (Oberholzer, 1969).

The South African Citrus Industry was established in the early 1800s and the South African Co-operative Citrus Exchange in 1926 (Mabiletsa, 2003). The first fruit export attempts to England were undertaken in 1886 but only in 1907, 3000 cartons of South African oranges were sold in Britain (Paterson *et al.,* 2003). From then onwards the South African Citrus Industry became a major force on the export markets (Davies & Albrigo, 1994), played a vital role in the economy and social welfare of the country and became a global quality trendsetter in fresh fruit trade.

In terms of world citrus production, Brazil is the largest producer, followed by China, the United States of America (USA), and Spain (Table 2.2) (http://www.cga.co.za). In the southern hemisphere, South Africa is one of the top five countries exporting citrus fruits. South Africa is mainly competing with countries such as Argentina, Australia and Chile during the peak season, which is April to October and with Egypt, Israel, Spain and the USA towards the end of the marketing season. In Africa, the largest citrus producing countries are Algeria, Egypt, Morocco and South Africa. South Africa is the 12th largest citrus producing region in the world and the 2nd largest exporter of fresh citrus fruits after Spain (Table 2.3) (http://www.cga.co.za). In South Africa, the citrus industry is the second largest earner of foreign exchange through agricultural exports (Mabiletsa, 2003). Citrus is grown in eight out of the nine provinces in South Africa and the main citrus season runs from March towards the beginning of October (Mabiletsa, 2003).

Citrus belongs to the genus *Citrus L.,* subtribe *Citrinae*, family *Rutaceae* and Subfamily *Aurantioideae* (Mukhopadhyay, 2004). Citrus fruit is rich in Vitamin C and has been used to prevent scurvy since the 17th century (FAO, 1998). Like any other crop, citrus trees can be attacked by many pathogens that can affect the roots, leaves and fruits. Citrus black spot (CBS) is the most important disease of citrus caused by *Guignardia citricarpa* Kiely, anamorph *Phyllosticta citricarpa* (McAlpine) van de Aa, Synonyms *Phoma citricarpa* (McAlpine) or *Phyllosticta citricarpa* (McAlpine) Petrak (Kotzé, 1981; Herbert & Grech, 1985). Black spot symptoms on the fruit rind disqualify fruit for export purposes to CBS sensitive markets (Kotzé, 1981; Snowdon, 1990).

This study will focus on citrus black spot for the development of a nursery good agricultural practices framework and protocol in order to produce certified disease free nursery stock. Therefore, the history, method of spread and detection method of CBS will be discussed together with preventative measures.

Citrus black spot was first noticed and reported in Australia in 1895 from diseased fruit originating from citrus-growing areas within New South Wales (NSW) and Queensland (Benson, 1895). Since then, the disease has spread globally and now affects all types and cultivars of citrus in most of the major citrus producing countries (Timmer, 1999; Timmer *et al.,* 2000). The first record of CBS in South Africa was in 1929 from the Pietermaritzburg region (Doidge, 1929). The disease has since been reported from several new sites in South Africa and has mainly been distributed by infected nursery trees with latent infections (Wager, 1952).

Other countries in which CBS is a major problem include Argentina (Garran, 1996), Bhutan (European Union, 1998), Brazil (European Union, 2000a; 2000b), China, Hong Kong, Indonesia (European Union, 1998), India (Brodrick, 1969), Kenya, Mozambique (European Union, 1998), Nigeria (Baayen *et al*., 2002), Peru, Philippines and Coastal regions of Australia, Swaziland, Uruguay, Taiwan, Venezuela (Timmer *et al.,* 2000), West Indies (Calavan, 1960), Zambia and Zimbabwe (European Union, 1998). The disease is absent in Mediterranean regions subject to winter rainfall, e.g. Israel, Italy, Portugal, Spain, Greece, Cyprus (European Union, 1988; Baayen *et al.,* 2002), Chile as well as in California and Texas in the USA (Cook, 1975; Kotzé, 1981; Baayen *et al.,* 2002). The chances of future occurrence of this important fungal disease in major citrus producing countries largely depend on effective application of quarantine measures and the initial eradication steps taken when the first disease outbreaks occur.

In South Africa, the disease is prominent in many parts of the Northern Province (Wager, 1952; Kotzé, 1981) but not in the Northern Cape or Western Cape (Paul *et al.,* 2005). In terms of the Agricultural Pest Act, 1983 (Act No. 36 of 1983) the main objective is to prevent agricultural pests from spreading to new areas (http://www.nda.agric.za). Within the act it is prohibited and illegal to move citrus propagation material from areas where CBS occur (Mpumalanga, Limpopo and KwaZulu-Natal) to areas where it does not occur (Western Cape and Northern Cape) (http://www.nda.agric.za). The Western Cape and parts of the Northern Cape are the only regions in South Africa officially recognized as citrus black spot free, allowing growers to export to sensitive markets such as the USA, Japan, Korea and European Union Countries (European Union, 1998; USDA/APHIS, 2002; Mabiletsa, 2003; Le Roux, 2004; Paul *et al.,* 2005).

Two types of *G. citricarpa* spores cause infection of citrus namely windborne ascospores contained in pseudo perithecia and waterborne conidia contained in pycnidia (Kiely, 1949a; Kotzé, 1963; 1996). In wet weather, conidia ooze from the pycnidia and require running water for dissemination and specific conditions for germination. Ascospores are considered the most efficient means of dissemination of CBS in areas where the disease is already established (Kiely, 1949a; Kotzé, 1963; 1996).
Citrus black spot can cause major economic losses annually if not controlled effectively, particularly fruits destined for export (Kotzé, 1981). The pathogen mainly causes cosmetic symptoms on the fruits that affect only the rind and not the internal quality of the fruit (Kotzé, 1981). Citrus black spot was originally not considered a disease of major importance but has since become a major concern in terms of phytosanitary requirements and is now regarded as a barrier to trade. Citrus consignments for export can be rejected based on the presence of a single black spot on some of the fruit in a pallet. This will inevitably result in huge financial losses for the producers or exporters. In South Africa for instance, Kotzé (1963), estimated losses between 1960 and 1961 at Letaba Estates to be around 10.1% of the total crop and 9.5% of possible total income despite a CBS control programme.

Where the disease is not controlled, losses of more than 80% of unprotected fruit are not uncommon (McOnie, 1964). Brodrick (1969) estimated over R1.5 million spent by the South African Citrus Industry to control CBS. The cost of control measures of CBS in South Africa during the 1995 season amounted to between R11 million and R16.5 million. During the 1997 season, South Africa spent in the region of R30 to R50 million on fungicides alone (mainly mancozeb and benomyl) for pre-harvest control of citrus black spot (http://www.up.ac.za/academic/fabi/citrus/blackspot.html).

The greatest risk of introducing CBS to other production regions is by means of latently infected bud wood or foliage of nursery trees, provided that environmental conditions are favourable (Wager,

1949; 1951). Therefore nurseries play an important role in the spread and manifestation of CBS disease in areas previously known to be disease-free. The main objective of this study is therefore to design a nursery protocol that can be used by citrus producers to monitor and detect CBS infections in the nurseries. By screening latent nursery material and providing CBS certified free planting material the industry can be protected from further spreading of the disease. In order to integrate current nursery practices and internationally recognised standards, a nursery best practices programme was developed.

In this mini dissertation, a brief literature review is provided as a general overview of global citrus trade, the history of CBS, its spread and how it affects trade. It also covers general background information about citrus nursery production practices. Chapter three provides basic information regarding nursery practices, legislation and regulations involved in citrus nurseries and nursery management practices and monitoring and detection of diseases and pests. Chapter four provides information about the development and implementation of a South African citrus nurserygap for the purpose of establishing a regulatory system to prevent CBS distribution through nurseries. By establishing a framework to certify citrus nursery trees CBS free producers can procure trees for sensitive citrus production areas currently free of this phytosanitary disease. In addition the standard was benchmarked against Globalgap and the national regulation for export producers to ensure compliance with international and local requirements. Chapter 5 is a general discussion about the value of developing a nursery good agricultural practices framework for the South African Citrus Industry.

Finally this study provides a protocol that can be used by the citrus industry to monitor and detect diseases in citrus nurseries within a citrus nursery improvement programme using a good agricultural practices model.

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CHAPTER 2

LITERATURE REVIEW

2.1 INTRODUCTION

Citrus is primarily valued for the fruits, which is consumed fresh (sweet orange, tangerine, grapefruit etc.) processed into juices or jams or added to dishes and beverages (Davies & Albrigo, 1994). Fresh citrus fruits are rich in Vitamin C, which play a vital role in the prevention of scurvy (Gorinstein *et al.,* 2001). Citrus has many other uses including animal fodder, craft and fuel wood (Timmer *et al.,* 1999). In the Arab world, citrus have been used as a condiment for fish (Roistacher & Civerolo, 1989). Due to its excellent taste, nutritional value and ornamental features, citrus was rapidly introduced to most parts of the world. In South Africa, citrus plants arrived during 1654 from the island of St. Helena and were planted on Jan van Riebeeck's farm, Borsheuvel, situated above what is today called the Cape Town Suburb of Clairmont (Oberholzer, 1969).

South Africa is the 2nd largest exporter of fresh citrus fruits in the world and approximately 60% of the total citrus production is exported to other countries (http://www.cga.co.za). Citrus, like many other crops, is facing major threats due to pests and pathogens that affect the roots, stem and fruits. Citrus black spot (CBS) is one of the most important fungal diseases of citrus worldwide. The fruit symptoms represent necrotic lesions on fruit that make them unacceptable for marketing. When the disease is severe, black spot may cause extensive premature fruit drop that reduces yields (Kotzé, 1981). Therefore, CBS must be controlled to achieve profitable production and ensure market access. Citrus black spot has been a significant production problem in a number of countries in Southeast Asia, Africa, South America and also in Australia. The disease can further be introduced into CBS free countries via movement of infected fruit or illegal introduction of vegetative plant material.

In many parts of the world, citrus is produced as an export commodity and markets are demanding high standards (Mabiletsa, 2003). In South Africa 90% of citrus fruits from unprotected trees were claimed to be unfit for export (McOnie, 1964b) and losses of more than 80% of unprotected fruits were reported to be common (McOnie, 1964d). In South Africa, summer rain is the most important factor in the establishment of the disease and then reading epidemic proportions. The disease has not been known to disappear or decline (Kotzé, 1981). Therefore, control of CBS is of utmost importance

as it is difficult to eliminate once it has established and it causes unsightly external lesions on the fruit and renders it unacceptable for export (Timmer *et al.*, 2000).

Due to phytosanitary risks, fruits with CBS symptoms are unacceptable for export to the European Union (EU) due to quarantine regulations (European Union, 2000a; Bonants *et al.,* 2003,). Similar phytosanitary restrictions also affect exports of citrus fruits from Argentina (European Union, 2000a) and Brazil (European Union, 2000b) into the EU. New phytosanitary requirements are aimed at preventing the introduction of CBS and its subsequent establishment in the EU and the USA. Imports of citrus from CBS affected regions are not entirely prohibited, but fruit may only be imported if evidence can be provided that effective pre-harvest spray programmes exist, infected fruit are culled and fruit were inspected at the packhouse or at the port. In order to gain access to lucrative markets such as the EU and USA, a region must be officially declared CBS free and citrus orchards must be inspected to assess the absence of CBS. In addition, the effectiveness of the chemical spray programmes must be determined and fruit must be inspected at the packhouse and port and considered free of CBS symptoms (Bonants *et al.,* 2003).

The Sanitary and Phytosanitary (SPS) agreement represents measures taken to protect human, animal, plant life or health from risks associated with imported agricultural commodities (WTO, 1995). To prevent the use of SPS standards as trade obstacles, the agreement stipulates that countries should base their SPS standards on international guidelines and recommendations and that it must be scientifically based. It also permits countries to establish its own SPS standards, above the international level, on a non-discriminatory basis as long as it can provide a scientific justifiable reason supported by a risk assessment study.

In this literature review, aspect related to citrus trade and the history of CBS is discussed. The impact of the disease on trade of fresh citrus fruits has resulted in the development of a protocol to be used for detecting the pathogen in citrus nurseries. This protective measure has been established to ensure that the disease is not introduced into disease free areas.

2.2 GLOBAL CITRUS INDUSTRY

Citrus originates from the *Rutacea* and exists in various types cultivated around the world (Table 2.1). Brazil is the largest producer of citrus in the world and its industry is oriented towards production of oranges for processing purpose (Table 2.2). China is the second largest producer of citrus followed by the USA (http://www.cga.co.za). Although South Africa is one of the smallest producers of citrus it is

one of the major export countries of fresh fruit (Table 2.3). Today, the edible types of citrus include citron, sour orange, lime, lemon, sweet orange, shaddock, grapefruit, mandarin and kumquats (Saunt, 2000).

Table 2.1: Types of citrus cultivars and their place of origin (Saunt, 2000)

Type of citrus	**Botanical names**	**Year of reported origin**	**Place of origin**
Citron	*Citrus medica* L.	330 B.C.	China and India
Sour orange	*Citrus aurantium* L.	700 B.C.	South-east Asia
Limes	*Citrus aurantifolia* L.	-	East India
Lemon	*Citrus limon* L.	-	-
Sweet orange	*Citrus sinensis* L.	-	South-east Asia
Shaddock	*Citrus granidsi* L.	-	Malaysia and India
Grapefruit	*Citrus paradisi* L	-	West Indies
Mandarin	*Citrus reticulata* L.	-	Indo-China
Kumquats	*Fortunella margarita L.*	-	South-eastern China

Table 2.2: World Fresh Citrus Production

	Years of Citrus Production					
Country	**2001/02**	**2002/03**	**2003/04**	**2004/05**	**2005/06**	**Rank**
Brazil	20,674	17,735	21,392	18903	18238	1
China	12,027	12,466	13,884	15228	14985	2
USA	14,800	13,731	14,784	10499	10451	3
Mexico	6,357	6,080	6,583	6910	6030	4
Spain	5,751	5,944	6,232	6181	5378	5
India	4,700	4,632	4,662	4662	4662	6
Italy	3,016	2,763	2,757	3321	3525	7
Iran	2,956	2,977	3,037	3037	3037	8
Egypt	2,900	2,481	2,318	2706	3050	9
Argentina	2,566	2,465	2,550	2670	2430	10
Turkey	2,390	1,955	2,093	2317	2499	11
South Africa	1,816	1,586	1,560	1543	1743	12
Japan	1,633	1,433	1,425	1341	1378	13
Morocco	1,154	1,293	1,108	1321	1245	14
Indonesia	968	1,530	2,071	1312	1312	15

Source: Food Agriculture Organization (Citrus Growers Association: Key Industry Statistics, 2007). Volume expressed in metric tonnes.

2.3 SOUTH AFRICA CITRUS INDUSTRY

South Africa is an important player in the global citrus industry and generates considerable income and employment in the country (Mabiletsa, 2003). The Citrus industry is the 2nd largest earner of foreign exchange through agricultural exports (Mabiletsa, 2003). Citrus is grown in eight out of the nine provinces in South Africa and the season extends from late February to early October. South Africa is also the 2nd largest exporter of citrus in the world and amongst the leading exporters of fresh quality fruit from the Southern Hemisphere (http://www.cga.co.za). In terms of exports, South Africa is competing with countries such as Argentina, Chile and Australia during the peak season, which is April to October and with Israel, Spain, Egypt and the United States of America (USA) towards the end of the marketing season. There are important environmental differences between production regions in South Africa based on rainfall seasons and other climatic factors. The Eastern and Western Cape dominate in the production of Navels while the Limpopo Province dominates in the production of Valencias (Veldman *et al.*, 1996).

Table 2.3: World Fresh Citrus Export volumes between 2001 and 2006

	Years of Citrus Production					
Country	**2001/02**	**2002/03**	**2003/04**	**2004/05**	**2005/06**	**Rank**
Spain	3,142	3,313	3,564	3,117	3,238	1
South Africa	981	1,043	1,049	1,080	1,213	2
USA	1,081	1,117	1,136	919	930	3
Turkey	826	740	607	877	948	4
Argentina	421	486	548	642	580	5
Egypt	303	414	491	605	650	6
Morocco	430	484	438	490	543	7
Mexico	225	347	399	413	399	8
China	184	241	344	386	380	9
Greece	317	398	303	235	337	10

Source: Food Agriculture Organization (Citrus Growers Association: Key Industry Statistics, 2007). Volumes expressed in metric tonnes.

Large volumes of grapefruit are grown in Mpumalanga and Limpopo Provinces, while soft citrus grows better in a more Mediterranean climate. Therefore, almost half of all soft citrus produced in South Africa originates from the Western Cape (Appendix C). The Eastern Cape produces the highest volume of lemons (49%) (PPECB Export Directory, 2007/2008). Citrus export earnings were R2.5 billion a year in 2003 and 2007, and R4.3 billion in terms of citrus export earnings

(http://www.nda.agric.za; (Directorate: Agriculture Statistics). The South African Citrus Industry exports its fruits to more than 60 countries. Europe has traditionally been the most important market for South African citrus exports. While the United Kingdom remains South Africa's biggest market, new markets were established in the Middle East, Asia and the Far East (PPECB Export Directory, 2007/2008). About 60 million cartons of fresh citrus were exported during the 2001 citrus season. Six years later approximately 80 million cartons of fresh citrus were exported during the 2006/2007 season (PPECB Export Directory, 2007/2008). The Middle East and Mediterranean countries received the highest volumes of lemons, limes and oranges whereas central Europe received the biggest share of kumquats (Table 2.4). The Far East also received the highest quantity of grapefruits as compared to other markets. The bulk of soft citrus was exported to the United Kingdom. The least fruit was exported to African countries.

The citrus industry employ approximately 100 000 people at nurseries, on farms and in packhouses with an unknown number of seasonal workers employed during the season to harvest and pack fruit (Mabitsela, 2003). The citrus industry has for the past ten years shown tremendous growth with the planting of new citrus trees (Appendix A & B). In 2007, the South Africa Citrus Industry had 1746 Production Units registered with the Citrus Growers Association (CGA) (http://www.cga.co.za). In South Africa, citrus is produced as an export commodity and is therefore a highly competitive industry. Maintaining a good cost competitive position requires high fruit quality standards and keeping abreast in new world market trends.

Table 2.4: Export markets for South African Citrus between 2002-2007 Citrus Seasons (PPECB Export Directory, 2007/2008)

Major Export	**Percentage of citrus volumes exported per group**					
Destination	**Grapefruit**	**Kumquats**	**Lemons**	**Limes**	**Oranges**	**Soft Citrus**
Africa	0.31	-	0.86	-	0.71	0.61
America	2.01	-	0.55	-	6.33	13.82
Central Europe	31.32	67.23	18.70	-	28.92	19.73
Far East & Asia	48.05	21.47	22.31	12.00	22.82	11.38
Middle East & Mediterranean	8.40	5.65	45.65	83.40	31.23	7.44
United Kingdom	9.91	5.65	11.93	4.60	9.98	47.02
Total Percent =100						

2.4 TRADE POLICY OF AGRICULTURAL PRODUCTS INSPECTIONS IN SOUTH AFRICA

During the 2001 season, The National Department of Agriculture (DoA), through the Citrus Growers Association (CGA), instituted a product tracking system for citrus. The system provides that growers exporting under the "Special Export Markets Programme" (USA, Japan, Korea and EU) must register with the DoA and obtain Production Unit Codes (PUC's) per orchard, per farm or number of farms and packhouse codes (PHC's) (http://www.nda.agric.za). The DoA is also responsible for phytosanitary inspections, monitoring of all orchards, packhouses, inspection depots and implementation of Good Agricultural Practices (GAP).

The purpose of the registration is to be able to identify and withdraw all fruit produced from areas that are considered not to be free of CBS. The hit list of all suspended citrus packhouses is circulated every year by the DoA under the Directorate of Plant Health, Sub-directorate Import/Export Programmes before the start of the citrus season. This is communicated to The National Programme Manager of Standards and Protocols at the Perishable Product Export Control Board (PPECB). This board is the official inspection body responsible for the selection and monitoring of the biometric sampling, quality inspection and initiation of the cold chain. This allows for inspectors to be well informed about the packhouses, which are allowed to pack for special markets, and those who are prohibited due to the presence of CBS. If CBS is detected during inspection, the pallets are flagged as a measure to indicate their CBS status at the pack house level. A rejected consignment of citrus is considered a "Strike" and if three consignments are rejected for the presence of CBS the production unit code (PUC) is suspended from the special markets programme for the duration of the season. The rejected consignment is then diverted to a non CBS sensitive market. It is difficult to get the exact statistics of CBS rejections due to divergence of fruits to other markets. Suspended producers to be reinstated for the next export season, have to provide documentation to the DoA of the corrective actions taken. The DoA and USDA-Agricultural Plant Health Inspection Service (APHIS) will conduct orchard and packhouse inspections at the suspended operator during the previous season to assure compliance with GAP. Once cleared, suspended units will be reinstated based upon compliance with programme requirements.

The following procedures should be followed for a product destined to special markets programme e.g. fruits exported to the USA (USDA Citrus Export Programme, 2006). Citrus fruits destined for special markets are subjected to cold treatment in transit with cold treatment at 31ºC or below for 24

days (USDA Citrus Export Programme, 2006). All pallets must be accompanied by USDA official passed labels on all four sides. These labels are issued and controlled by USDA-APHIS. Vessels and containers to be used will be USDA-APHIS approved and have valid USDA-APHIS certificates of approval. A list of USDA-APHIS approved vessels and containers can be found on the following website: http://www.aphis.usda.gov/ppq/manuals/vessellist-external.html. At the port of entry, USDA-APHIS will examine and verify the cold treatment log.

2.5 SOUTH AFRICAN LOGISTICAL CHALLENGES

Citrus is exported through four ports in Southern Africa, which are Cape Town, Durban, Maputo and Port Elizabeth and shipped overseas in refrigerated containers or conventional reefers (Appendix E) (PPECB Export Directory, 2006/2007). Durban and Port Elizabeth receives the biggest volume of fruits followed by Cape Town harbour, whilst small volumes pass through Maputo into Mozambique. The biggest logistical challenge facing the industry is the lack of capacity at these ports. Citrus exporters are not only competing for space with other exporters in the citrus industry but also with other industry sectors. Systems are overloaded giving rise to inadequate stacking, space and shortage of electrical plugs in points for containers. In Cape Town harbour, a fruit terminal that is exposed to the South Easter wind increases the problem. The South Easter wind can reach speeds of up to 80km/h therefore making it difficult for fruit to be loaded into ships, causing further delays in the port. All fruit are loaded into the reefer under the supervision of the PPECB (official export certification agency for perishable products destined for export as mandated by the South Africa Government, via the DoA).

2.6 CITRUS BLACK SPOT

2.6.1 Detection and identification of citrus black spot symptoms

Citrus black spot fruit symptoms vary to the extent that confusion exists regarding their status and description (Schüepp, 1961; Kotzé, 2000). Symptoms are known as hard spot, shot hole spot, freckle spot and spreading or virulent spot (Fig 2.1) (Kiely, 1949a). Hard spot and shot hole spot lesions are circular and brown with slight depressions, which gradually sink in the centre developing into crater-like depressions, with a grey-white centre and black margins encircled by green rind tissue (Kotzé, 1981). The smaller orange freckle spots usually appear on the half of the fruit exposed to the sun, up to several hundred spots per fruit. When conditions are favourable for spotting, hard spot development is replaced by freckle spot, which drastically reduces the quality of

fruits. Virulent spot are common, developing 2-3 weeks after freckle spot appearance with the onset of warmer conditions. Freckle spot may develop into virulent lesions and virulent spots may engulf freckle and hard lesions (Kiely, 1960; McOnie, 1964e).

2.6.2 Disease symptoms on leaves and twigs

Symptoms first appear on mature leaves and petioles as tiny circular red to red-brown spots, visible on both sides of the leaf surface (Fig 2.2) (Timmer *et al.,* 2000). With time, the border of the spots darken with an even brown to black ring. Spots remain small, not exceeding 3mm. Leaf lesions often have yellow halos. Citrus trees introduced to new sites can be tested for latent infections by sampling green leaves (Kiely, 1949a).

2.6.3 Means of movement and dispersal

2.6.3.1 Dissemination of citrus black spot in fruits

Citrus black spot can primarily be introduced into a new area through infected budwood or affected trees. Up to date, no scientific evidence exists that symptomatic fruit can be a source of inoculum. This fact is currently debated in mitigation arguments between South Africa and the European Union (EU). Pycnidiospores form on fruit and these are not airborne, therefore the risk of spread from fruit is relatively low (Whiteside, 1965).

a. Hard spots. b. Virulent spots. c. Speckled spots.

Fig 2.1: Examples of classic (a) hard spots with black pycnidia caused by *Guignardia citricarpa*; (b) virulent spots caused by *Guignardia citricarpa* and (c) speckled spots caused by *Guignardia citricarpa (Source: http://edis.ifas.ufl.edu/PP135).*

Figure 2.2: Leaf spots caused by *Guignardia citricarpa* *(Source: http://edis.ifas.ufl.edu/PP135).*

In 2001, Korf and colleagues indicated that the CBS pathogen cannot survive on postharvest spots and will not pose a threat to CBS sensitive markets. The fruit leaf pathway was questioned by the EU in a response on the risk assessment document prepared by South Africa. It was reported that mycelium can be isolated postharvest from CBS spots from infected fruit and that it could result in the introduction of the pathogen in CBS free markets (Agostini *et al.,* 2006). Subsequently, it was proven that infected fruit do not pose a phytosanitary risk, therefore cannot lead to infection and colonisation of freshly detached leaves or natural leaf litter or represent a source of inoculum in citrus orchard for these leaves (Truter *et al.,* 2006).

2.6.3.2 Global dissemination of citrus black spot

Citrus black spot was first described by Kiely (1929), in New South Wales, Australia on fruits in the citrus growing areas around Sydney. Today CBS is found in Africa (Mozambique, South Africa, Swaziland and Zimbabwe), Asia (China, Indonesia, Taiwan, Hon-Kong, Philippians and Japan) and in the Americas (Argentina, Brazil and Peru) (Kotzé, 2000; Peres & Timmer, 2003). Citrus black spot was initially introduced into most countries through the importation of infected budwood or trees.

2.6.3.3 Introduction of citrus black spot into South Africa

Jan van Riebeeck first introduced citrus into South Africa in 1654 from St. Helena (Oberholzer, 1969). Subsequently, introductions of citrus planting material came from the Dutch merchants trading with the Orient (Oberholzer, 1969). The citrus industry grew rapidly during the 1800s, with the discovery of gold and diamonds. This resulted in an increased demand for agricultural produce including citrus (Oberholzer, 1969). Citrus black spot was introduced into South Africa through infected trees around 1929 from Australia (Kotzé, 1981). The disease was first reported in 1929 from Pietermaritzburg where it was described as being introduced through nursery trees with latent

infections (Doidge, 1929). It was considered that the disease then spread into Zimbabwe from South Africa on budwood or nursery trees early in the 1960's, before restrictions were placed on the movement of plant material out of South Africa (Whiteside, 1965).

2.6.3.4 Spread of citrus black spot through planting materials

Grafting is the joining of parts of plants together in such a way that they unite and continue to grow as a single unit (Hall, 2003). The part of the plant that becomes the upper portion or the top of the new plant is called the scion and the part, which becomes the lower portion that includes the root, is called the stock or rootstock. Grafting can transform low quality fruit trees to become the best commercial varieties. Like any nursery practices if proper care is not followed in the selection of budwood or during the grafting process it can lead to infection of the stem and leaves on healthy trees (Schüepp, 1961). Citrus black spot has been reported to spread through grafting and special care should therefore be taken.

Grafting is the beginning of the actual plant propagation process and to be successful certain particulars need to be addressed (Mukhopadhyay, 2004): (i) the stock and scion must be compatible otherwise it will not unite; (ii) cambial regions of scions and stock must be in intimate contact. The cut should be held tightly for proper healing and to ensure the flow of water and nutrients; (iii) grafting should be done at the right time, age and are considered ideal if the stock and scion are at the right size and growth stage; (iv) after grafting, all cut surfaces must be protected from desiccation or drying out. This can be done by covering the graft area with wax and tape; (v) proper care must be given to the graft until it unites. Shoots from the stock must be removed as they can choke out the scion, and (vi) the grafting equipments such as a bud knife should be kept razor sharp and sanitized during grafting to avoid spread of diseases (Mukhopadhyay, 2004). Citrus black spot has been reported to spread through grafting and special care should therefore be taken. Grafting of citrus trees can also lead to transmission of other pathogens (Appendix D).

2.6.3.5 Citrus black spot spread within an orchard

Two types of spores can spread CBS in an orchard, the asexual (conidia), which develops in fruits and leaves on the plant tree and the asexual (ascospores), which develops in leaves and litter at various stage of decomposition (Kotzé, 1996). Macroconidia are waterborne and requires droplets of water for their emergence and dispersal (Wager, 1952). Pycnidia have no special release mechanism for expelling conidia into the atmosphere (Kotzé, 1981). Ascospores are airborne, can

spread over short and long distances and by wind it can spread for kilometers. Although conidia may cause infection of citrus, the ascospores are seen to be the primary source of infection where citrus is cultivated (Kotzé, 1963; 1996).

Germination and infection of ascospores requires the presence of free surface water or wetting of leaves (Kotzé, 1964; 1981). Even a small amount of rain enhances release of ascospores and some ascospores continue to be released as long as the leaves are moist. Ascospores are dispersed by air currents and deposited on leaf and fruit surface where they germinate and form dormant infections (McOnie, 1967; Huang & Chang, 1972). Ascospores, once germinated, produce appressoria with infection pegs that penetrate the cuticle. The infection pegs produce at their tips, between the cuticle and the upper epidermal cells, knots of fungal tissue which are considered to establish the latent infection (McOnie, 1967; Kotzé, 1981).

2.7 HOW DOES CITRUS BLACK SPOT AFFECT INTERNATIONAL TRADE?

Export procedures have changed considerably since the first detailed description of CBS in 1899. Today, fruit infected with CBS are not allowed into the EU due to quarantine regulations. Similar, phytosanitary restrictions affect the export of citrus from Southern Hemisphere countries such as Argentina (European Union, 2001a) and Brazil (European Union, 2000b) into the EU. Since citrus is a high-value fruit crop technical barriers to trade for certain countries exist. The greatest risk of introducing CBS to other countries is by means of latently infected budwood or foliage of nursery trees, provided that environmental conditions are favourable (Wager, 1949; 1951).

In South Africa, the Western Cape (the magisterial districts of Clanwilliam, Piketburg, Paarl, Stellenbosch, Somerset West, Wellington, Robertson, Montagu, Swellendam, Heidelberg, Ladismith, Hermanus, Caledon, Bredasdorp and Strand) and parts of the Northern Cape (Hartswater and Warrenton) are currently free of CBS, and represent areas from were citrus can be exported to sensitive market such as the EU, USA, Japan and Korea (Paul *et al.,* 2005). The USDA has a phytosanitary pre-clearance and inspection programme in the Western Cape for South African citrus destined for the US markets (USA Citrus Export Special Markets Programme, 2006). Several other countries such as Japan, Korea, China, Iran and the EU also have pre-clearance programmes for citrus exports to their markets (http://www.nda.agric.za/docs/NPPOZA/page_g.htm). If CBS were found during inspection, the Executive Officer (PPECB Inspectors) has to declare that the consignment was passed but not given clearances for the European market. There are three conditions that qualify citrus for export to the EU and other sensitive markets. Firstly, the fruit must

originate in the CBS-free areas of the Western Cape and parts of the Northern Cape; secondly, fruit must originate in orchards free from CBS and lastly fruit must originate in orchards that were subjected to appropriate treatments against the disease and show no symptoms of the diseases during quality or phytosanitary inspection (Agricultural News, 2002). In Fig 2.3 CBS inspection requirements in the supply chain is indicated. If the citrus production programme complies with all the stages and requirements indicated in Fig 2.3 then the fruits can be exported to any sensitive market without experiencing any problem for rejection of CBS.

2.8 ECONOMIC IMPACT OF CITRUS BLACK SPOT

Citrus black spot is a serious disease of citrus cultivars in Australia (Kiely, 1949b; 1969), Guangdong province in China (Fawcett, 1936) and South Africa (McOnie, 1964b). Before the adoption of control measures, heavy losses in oranges cultivar Valencia had been reported in the coastal orchards in New South Wales (Kiely, 1949a; 1949b). In South Africa 90% of fruits from unprotected trees were unfit for export (McOnie, 1964b) and losses of more than 80% of unprotected fruits were reported to be common (McOnie, 1964d). In South Africa, summer rains is the most important factor in the establishment of an epidemic and so far CBS has not been known to disappear or decline once it has reached the epidemic stage (Kotzé, 1981). Although CBS is mainly a fruit disease with pre-harvest lesions, some postharvest spots may develop rendering the fruit unmarketable (Korsten Communication, 2004).

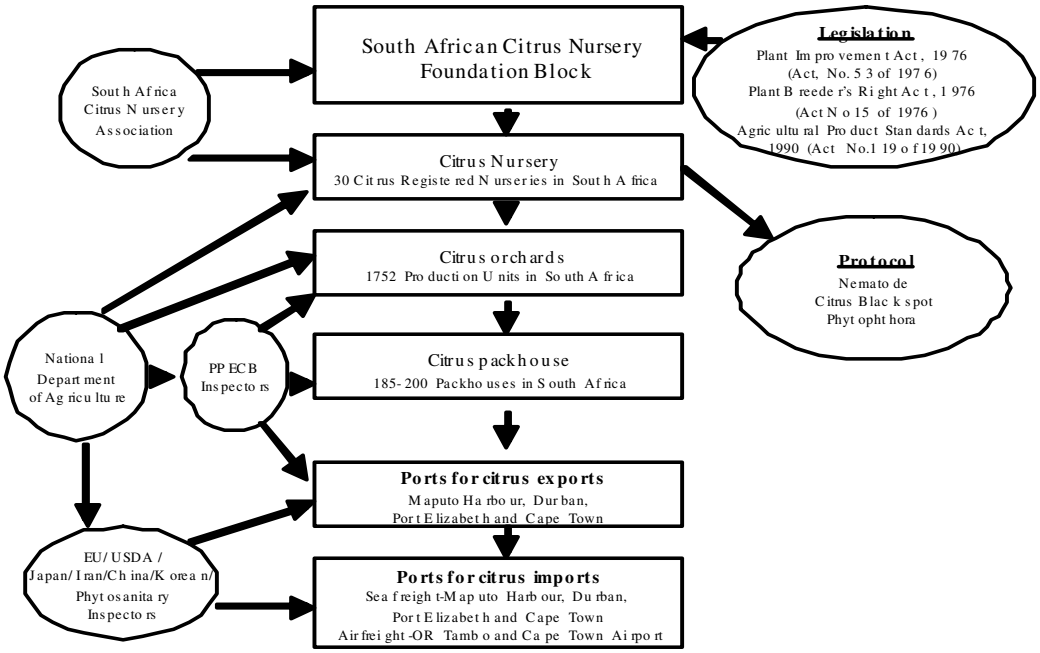

Fig. 2.3 Summary of citrus black spot inspection points within the citrus supply chain. Information extracted from, PPECB Export Directory, 2007/2008 http://www.cri.co.za; http://www.cga.co.za; http://www.nda.agric.za.

2.9 REGISTERED CITRUS BLACK SPOT SPRAY PROGRAMMES

Due to the presence of CBS, most countries face fruit trade restrictions (Baayen *et al.,* 2002; Bonants *et al*., 2003). Commercial control of CBS can only be achieved through the use of plant protection products. Therefore, control of CBS is of utmost importance as it causes unsightly external lesions on the fruit and renders it unacceptable for export (Timmer *et al.,* 2000). Various fungicides treatments have been explored to reduce the risk of introduction of CBS to the countries that are considered to be CBS free (Appendix F & H).

Since most commercial grown citrus species are susceptible except *C. aurantium* (sour oranges), a range of fungicides have been tested and recommended for protection and control (Kotzé, 1981). The use of preventive sprays such as Bordeaux + white oil, Bordeaux + zineb/mezineb (Kiely, 1949a; 1969; Wager, 1952), mancozeb (Kellerman & Kotzé, 1973; 1979), benomyl + mineral oil (Kotzé, 1981) have been reported to give adequate control. Other control measures include orchard sanitation together with the removal of mature fruits before the new crop set to prevent pycnidial inoculum getting washed down. Stripping nursery trees of leaves before being sold has also been recommended (Wager, 1952).

Currently, a recommended spray programme exists that is required in CBS infected areas (Appendix F & G) (Nel *et al*., 2003). When applying contact fungicide, spray application have to be carefully timed. Recommended intervals between sprays must not be exceeded. The maximum period that the different fungicides can protect fruit at registered concentration is as follows: Mancozeb (28 days), Zineb (21 days), Copper oxychloride/copper hydroxide (35 days) and Maneb/Zinc oxide (28 days) (Schutte, 2003).

Infection seldom occurs before mid-October, therefore, the protection period stretches from mid-October until end of January. Systemic fungicides are capable of penetrating the epidermis and the cuticle, and killing the mycelium present. Existing lesions can also heal once the infection is eliminated. Except for Fosetyl-Al/mancozeb, all the systemic fungicides belong to the benzimadazole group of fungicides. Addition of oil to the systemic fungicides is essential to ensure smooth penetration (Schutte, 2003).

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CHAPTER 3

CITRUS NURSERY MANAGEMENT PRACTICES

3.1 INTRODUCTION

A citrus nursery is defined as a "geographically distinct location where citrus nursery stock is produced for wholesale or retail" (CHRP, 2006-2007). It is essential that citrus production start with planting of clean nursery material and growing the seedlings according to optimum nursery practice to ensure ultimate plant health. Factors associated with tree health are rooted in the selection of the correct and best scion cultivars which are vegetatively compatible. It is also affected by scion and rootstock forming a healthy union and the susceptibility of scion cultivars to endemic pathogens in specific ecological niches to be limited due to a non host relationship (Timmer & Duncan, 1999). Key elements that will determine ultimate nursery planting material selection will include variety of fruit, final destination of the intended product i.e. as fresh or for processing (i.e. juice, jams etc), location of the production unit or orchard, history of disease in the unit and surrounding area (Whiteside, 1967; Kotzé, 1981).

It is a well known fact that nursery planting material can represent a source of inoculum and contribute to the spread of a disease and its ultimate manifestation in disease free areas such as CBS (Appendix I) (Kotzé, 1993; Van Broekhuizen & Swart, 2003; Swart, 2004). If climatic conditions support disease development and the pathogen is introduced into a production area via nursery planting material it can result in the establishment of the disease in the area (Timmer & Duncan, 1999). Careful selection of healthy high quality nursery stock is therefore the single most important decision any grower or producer will make that will ultimately affect the financial success of new plantings. In this chapter the national nursery framework of the country, classification of nurseries, nursery applicable legislation, regulations and codes of practice in particular citrus nursery management practices will be discussed. Also information and guidelines on how to start a nursery and the steps involved in growing certified disease free nursery stock will be provided.

3.2 HISTORY OF CITRUS NURSERIES

Citrus nurseries gradually opened as the demand for new trees increased (Corrado, 1787). The first centre dedicated to the production of citrus trees was built near Lecce in the Italian Pouilles. The centre was developed by Corrado, a religious man of the Celestian order, who published his remarkable treatise on "The physiology of the citrus and how to control and multiply them" (Corrado,

1787). Ten years later in 1797, Cavanilles stressed the importance of effective nursery practices for the establishment of citrus plantations in Valencia, Spain according to Naples in 1797. The technology developed in these European countries proved to be crucial for the reinforcement of good citrus nursery practices and the introduction of citrus cultivation techniques in the new world. The global phytophthora epidemics resulted in the development of tolerant rootstock such as the sour orange (Bonavia, 1888).

The citrus industry slowly developed at the beginning of the 20th century due to the economic difficulties caused by the two World Wars (Corrado, 1787). Accidental introduction of tristeza in Argentina and Brazil was attributed to the use of contaminated budwood coming from South Africa (Zeman, 1931; Bitancourt, 1943). In no less than 15 years the disease affected millions of trees (Costa, 1956). The establishment of the International Organization of Citrus Virologists (IOCV) in 1957 benefited the citrus producing countries worldwide. In 1981, the International Society of Citrus Nurserymen (ISCN) was created and aimed at promoting safe and efficient multiplication techniques for quality planting materials (Roistacher, 1991; Bernard, *et al.,* 1998).

South Africa's Citrus Improvement Programme (SACIP) was established in 1973 as a joint project between the South African Co-operative Citrus Exchange and the Citrus and Subtropical Fruit Research Institute (CSFRI) (http://www.cri.co.za/cip/index.htm). Dr. E. C. Calavan of the University of California, Riverside drafted the basic guidelines of the SACIP, after consulting visit to South Africa in 1972 (von Broembsen, 1978 & 1984). The programme was to consist of various phases to provide nurserymen disease-free, horticulturally superior plant material. Significant progress during the past years in shoot-tip grafting, virus indexing and tristeza cross protection has enable Dr. Calavan recommendation to be fully exploited. Applications forms for participation in the programme were sent to all registered citrus nurserymen in South Africa in 1973. This effort leads to the formation of the South African Citrus Nurserymen's Association (SACNA) in 1974. An annual conference has been held by SACNA ever since and served the important function of providing a forum for personal contact between citrus nurserymen and relevant research and extension workers. The first trees were certified under the programme in 1976 from budwood cut in 1975 (Lee, 1986).

In 1977, Mr Chester Roistacher visited South Africa as a guest of the citrus exchange and contributed significantly to the adoption of sound indexing techniques (von Broembsen, 1978 & 1984). All nurserymen were given a list of the approved parents and these were the only sources which were subsequently recognised for the production of certified trees. In 1977, Dr. H. de Lange of the CSFRI visited California and his visit lead to the introduction of shoot-tip grafting of citrus to South Africa

after he learned the technique from Mr. C. Roistacher (von Broembsen, 1978 & 1984). This technique has been used to remove pathogens from a wide range of citrus cultivars; including individual trees which had repeatedly performed outstandingly in the annual horticultural evaluation of the interim programmes budwood sources trees.

The Qutspan Foundation Block was then established in 1980, releasing the first virus-free certified budwood in 1980 and the first certified seed in 1986. In 2007, the South African Citrus Nursery Industry had 19 accredited nurseries. Certification schemes provide a basic platform for all integrated pest management practices. It is therefore important for the management of insect and fungal pests and the programme ensures that the grower is planting healthy germplasm of the highest horticultural quality (OEPP/EPPO, 1991). The components of a certification programme include a quarantine programme, a clean stock programme and a certification programme to provide for distribution of the high quality, virus tests propagating material into the citrus industry for the benefit of the citrus industry as a whole (http://www.cri.co.za/cip/index.htm).

Locally, as part of the South Africa Citrus Improvement Programme, container grown trees are produced in the nursery according to strict guidelines and nursery had to provide evidence that they comply with certain production requirements prior to registration (Lee, 1988). Following application, nurseries will be inspected by DoA on the regulations and requirements. Nurseries that meet the criteria will be registered as “Citrus black spot –secure”. Compliance inspections will be conducted at least once a year and failure to adhere to these guidelines may result in nursery quarantines. These inspections include:

- Inspection of structures, standard operating procedures, nursery sanitation and records i.e. assess training, water analysis and pesticides records
- Inspection following permit application to allow movement of batches of trees to areas of low pest prevalence. These inspections would entail visual inspection of trees in the relevant batch for CBS symptoms, as well as a sample of mature leaves to be submitted for laboratory detection of *Guignardia citricarpa* according to a DoA protocol http://www.nda.agric.za; Plant Health, policies, norms and standards)

3.3 CLASSIFICATION OF NURSERIES

A plant nursery is a place where plants, trees, shrubs, ground covers, vines and herbaceous plants are propagated and grown (Mason, 1994). Like any nursery, citrus nurseries are classified and described

according to four major criteria: ownership, function, production system and type of plant material to be produced (Table 3.1).

3.3.1 Quarantine nursery

Quarantine nurseries are nurseries where plants are held during a period of quarantine (Davidson *et al.,* 1988). They are operated by national government or representative industry bodies mandated with the responsibility of administering national or regional laws or regulations, related to the importation of plants (Davidson *et al.,* 1988).

Table 3.1 Classifications of Nurseries

I. Ownership:

A. Private industry
    1. Proprietorship
    2. Partnership
    3. Corporation
B. Government agencies
    1. Country
    2. City
    3. State
    4. National
C. Educational institutions
    1. Botanical gardens
    2. Universities

II. Function:

A. Production
    1. Wholesale
    2. Retail
    3. Non-profit
B. Mail-order
C. Landscape
D. Wholesale plant distribution
E. Quarantine
F. Research

III. Production system:

A. Field production
    1. Bare-root
    2. Soil-balled
B. Container production

IV. Product:

A. General. Many types of hardy plant
B. Speciality
    1. Fruit
        a. General
        b. Tree fruits, including nuts
        c. Small fruits
    2. Ornamental
        a. General
        b. Shade and flowering trees
        c. Evergreens (narrow or broad leaves)
        d. Shrubs
        e. Roses
        f. Ground covers and vines
        g. Herbaceous perennials
        h. Indoor plants (house plants)
    3. Forest and conservation
        a. Reforestation
        b. Wildlife habitat
        c. Erosion control
    4. Propagation and liner materials
        a. Budded plants
        b. Grafted plants
        c. Rooted cuttings
        d. Seedlings
        e. Rootstocks

Source: (Davidson *et al.,* 1988).

3.3.2 Foundation block nursery

Foundation block nurseries usually belong to privately owned institutions or may be maintained by public agencies ((Davidson *et al.,* 1988). Foundation block type of nurseries are nurseries that are producing certified citrus trees that are indexed for pathogens on a regular basis so that they should meet horticultural quality required in the certification regulation. The citrus foundation block type of nurseries also supply citrus seed to nurseries around the world (http://www.cri.co.za/cip/index.htm).

3.3.3 Production nursery

Production nurseries are nurseries that propagate and produce plants for a variety of purposes, including fruit production, environmental quality improvement programmes, conservation projects and research and educational programmes (Davidson *et al.,* 1988).

3.3.4 Landscape nursery

Landscape nurseries design and construct attractive and functional landscapes, which utilize the resources within the organization. This type of nursery produces woody ornamental plants that are larger than those available from production nurseries (Davidson *et al.*, 1988).

3.3.5 Plant material distribution centres

The primary function of a plant distribution center or retail nursery is to relocate or purchase and assemble a large variety and volume of plants in a convenient location for landscape or garden plants, which in turn are resold to landscape nurserymen or at retail level to the public in large volumes. The plant material distribution center relocates plant material from a central stock production unit and holds it in the distribution center or grows it further in an adjacent or nearby nursery to enhance final quality prior to sale (Davidson *et al.,* 1988).

3.3.6 Home garden nursery

Home garden nurseries are small garden nurseries that sell garden plants directly to the public on a small scale (Mason, 1994).

3.4 SOUTH AFRICAN CITRUS NURSERIES

Citrus nurseries need to be accredited in order to produce budwood, which are free from pests and diseases. The Citrus Improvement Programme was established in 1973 as a joint project between the South African Co-operative Citrus Exchange and the Citrus and Subtropical Fruit Research Institute (CSFRI) in order to obtain certified trees of various cultivars for commercial orchards (http://www.cri.co.za/cip/index.htm). In 1997, shoot tip grafting was introduced at the Citrus and Subtropical Fruit Research Institute (now called the Institute for Tropical and Subtropical Crops (ITSC) in order to provide a virus-free source of budwood material. The ITSC and Citrus Research International (CRI) are responsible for shoot-tip grafting and pre-immunisation of material with a mild strain of citrus tristeza virus. The Citrus Foundation Block is responsible for increase and release of propagation material to the citrus industry national and international. The Outspan Foundation Block was established in 1980, releasing the first virus-free certified budwood in 1984 and the first certified seed in 1986.

Nurseries producing certified citrus trees must be accredited in accordance with a quality management system and have to be audited twice a year. A certified citrus tree must be made from certified propagation material, tested for pathogens for about four times per annum, and found to be free of harmful pathogens before being sold or moved to a new region. In South Africa, Du Roi nursery is one of the largest citrus nurseries and fully accredited with the South Africa Citrus Improvement Programme (SACIP), which is regulated by the Citrus Growers Association of Southern Africa http://www.duroinursery.co.za. The number of citrus nurseries participating in the Citrus Improvement Programme has decreased from 46 in 1999 to 23 in 2005; of which only 19 are fully accredited (Table 3.2 and Table 3.3).

Table 3.2 Citrus Nurseries Certified to conform to the standards required for the South African Citrus Improvement Scheme-November 2007

No	Nursery name	Town	Area

1	Apapanzi Nursery	Kirkwood	Eastern Cape
2	B.F. Joubert Nursery	Kirkwood	Eastern Cape
3	Baddaford Nursery	Fort Beaufort	Eastern Cape
4	Casmar Nursery	Mooinooi	North West Province
5	Du Roi Nursery	Letsitele	Limpopo
6	Esselen Nursery	Malelane	Mpumalanga
7	H.J. Joubert Nursery	Montague	Western Cape
8	Letsitele Nursery	Letsitele	Limpopo
9	Mistkraal Nursery	Kirkwood	Eastern Cape
10	Ngwenya Nursery	Malelane	Mpumalanga
11	Nucellar Nursery	Simondium	Western Cape
12	Paksaam Nursery	Patensie	Eastern Cape
13	Sondagsrivier Nursery	Kirkwood	Eastern Cape
14	Stargrow Nursery	Die Boord	Western Cape
15	Tweeling Nursery	Kirkwood	Eastern Cape
16	Vaalharts Nursery	Hartswater	Northern Cape
17	Waterfall Nursery	Adelaide	Eastern Cape
18	Westfalia Nursery	Duiwelskloof	Limpopo
19	Witkrans Nursery	Boshoek	North West Province

Source: Citrus Grower Association, 2007.

Table 3.3 South African Citrus Nurseries (S.A. Citrus Improvement Scheme) (http://www.cri.co.za/cip/index.htm & http://www.cga.co.za/site/files/5438/Accredited_nurseries_oct2004(1).xls).

No	South African Citrus Nursery Association	Nursery name	Accredited	Town	Area
1	-	Allandale Farms	-	Addo	Eastern Cape
2	Member	Apapanzi Nursery	November 2007	Kirkwood	Eastern Cape
3	Member	Baddaford Nursery	Farm Nursery	Fort Beaufort	Eastern Cape
4	Member	B.F. Joubert Nursery	November 2007	Kirkwood	Eastern Cape
5	-	Bonny Lea Nursery	Farm Nursery	Kirkwood	Eastern Cape
6	Member	Casmar Nursery	November 2007	Mooinooi	North West Province
7	-	Crocodile Valley Citrus Co Pty Ltd	Farm Nursery	Nelspruit	Mpumalanga
8	-	Comrie Nursery-Mooinooi	-	Pretoria West	Gauteng
9	Member	Du Roi Nursery	November 2007	Letsitele	Limpopo
10	-	Eendracht Nursery	Farm Nursery	Sunland	Eastern Cape
	Member	Esselen Nursery	November 2007	Malelane	Mpumalanga
11	Member	Esselen Nursery-Noordkaap	Stop	Jan Kempdorp	Northern Cape
12	-	Gamtoos Nursery	-	Patensie	Eastern Cape
13	Member	H.J. Joubert	November 2007	Montagu	Western Cape
14	-	Kleinbegin Nursery	-	Sunland	Eastern Cape
15	-	La Rhyn Sitrus Nursery	November 2007	Citrusdal	Western Cape
16	Member	Letsitele Nursery	November 2007	Letsitele	Limpopo

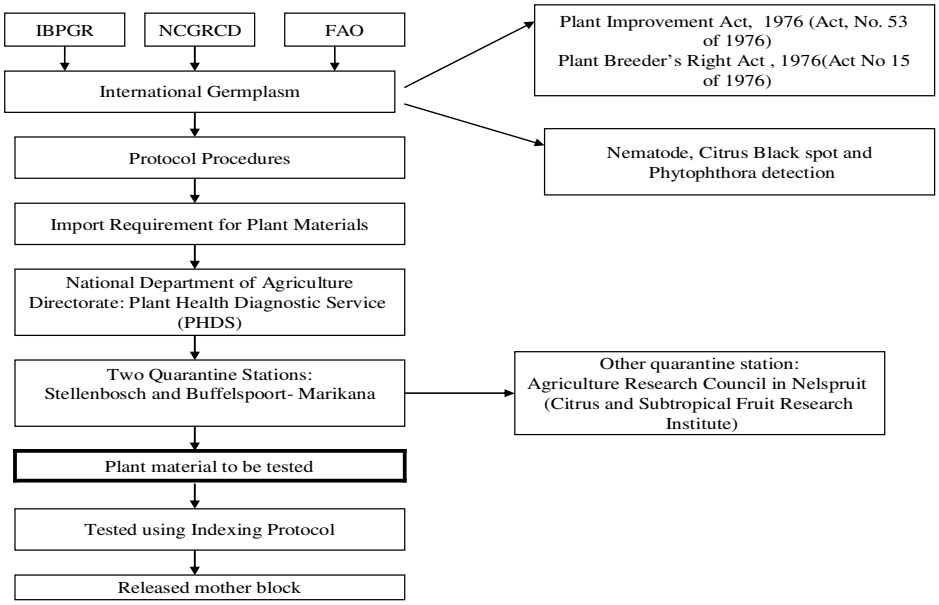
Table 3.3 **Continued:** South African Citrus Nurseries (S.A. Citrus Improvement Scheme) (http://www.cri.co.za/cip/index.htm & http://www.cga.co.za/site/files/5438/Accredited_nurseries_oct2004(1).xls).

No	South African Citrus Nursery Association	Nursery name	Accredited	Town	Area
17	Member	Mistkraal Nursery	November 2007	Kirkwood	Eastern Cape
18	Member	Ngwenya Nursery	November 2007	Malelane	Mpumalanga
19	Member	Nucellar Nurseries Cedarberg Citrusdal	November 2007	Simondium	Western Cape
20	Member	Paksaam Nursery	November 2007	Patensie	Eastern Cape
21	-	Porterville Nursery	Wholesale selling	Porterville	Western Cape
22	-	Sondagsrivier Nursery	November 2007	Kirkwood	Eastern Cape
23	Member	Stargrow Nursery	November 2007	Citrusdal	Western Cape
24	-	Tulbagh Bosbou Nursery	Wholesale selling	Tulbagh	Western Cape
25	Member	Tweeling Nursery	November 2007	Kirkwood	Eastern Cape
26	Member	Vaalharts Nursery	November 2007	Hartswater	Northern Cape
27	Member	Waterfall Nursery	November 2007	Adelaide	Eastern Cape
28	Member	Witkrans Nursery	November 2007	Boshoek	North West Province
29	-	Zululand Nursery	Wholesale selling	Eshowe	Kwazulu Natal
30	Member	Westfalia Citrus Nursery	November 2007	Duiwelskloof	Limpopo

3.5 INTERNATIONAL GERM COLLECTION

The growth, vitality and competitiveness of the South African Citrus Industry depend on safe access to national and worldwide germplasm resources. The ever-increasing movement of germplasm internationally involves a risk of accidentally introducing plant quarantine pests along with the host plant material (Roistacher, 1991). In order to minimize the risk, effective testing procedures are required to ensure that distributed citrus plant material is free of pests that are of quarantine concern (Frison & Taher, 1991). The Consultative Group on International Agricultural Research (CGIAR) established the International Board for Plant Genetic Resources (IBPGR) in 1974 in order to minimize the global risk of introducing pests that are of quarantine concern. The National Clonal Germplasm Repository for Citrus and Dates (NCGRCD) in Riverside, California, is the world repository for virus tested citrus budwood and is charged with serving the needs of users of citrus and date palm germplasm (Reuther, 1981; Gumpf *et al*., 1996; Bash, 1999; Krueger, 1999 & 2000b). In Fig 3.1 the guidelines for international germplasm movement using Republic of South Africa as an example is indicated.

Fig 3.1 Chain for international germplasm movement in the Republic of South Africa



The Food and Agricultural Organization (FAO) and International Board for Plant Genetic Resources (IBPGR) launched a collaborative programme for the safe and expeditious movement of germplasm. The FAO mandate is to assist its member's government to strengthen their plant quarantine service whilst the IBPGR mandate is to further improve the collecting, conservation and use of genetic diverse useful plants for the benefit of people throughout the world (Frison & Taher, 1991). The main aim for the joint of FAO and IBPGR programme is to generate a series of crop specific technical guidelines that will provide relevant information on disease indexing and other procedures that will aid in ensuring phytosanitary safety when germplasm is moved internationally.

3.6 QUARANTINE STATIONS FOR PHYTOSANITARY CLEARANCE

The DoA in South Africa under the Division Plant Health Diagnostic Services have two official quarantine stations, Stellenbosch (Cape Town) and Buffelspoort-Marikana (North West Province) (http://www.nda.agric.za/doc/NPPOZA/Citrus.htm). The two quarantine stations provide diagnostic services to detect and identify harmful plants and pathogens (nematodes, insects, mites, viruses, virus-like disease, bacteria, fungi and phytoplasmas) on imported plant propagating materials and plant products in terms of the Agricultural Pest Act, 1983 (Act No. 36 of 1983). Application to import vegetative propagation materials must be submitted to the Pretoria office of the DoA.

The quarantine imported citrus plant materials must be received and inspected by the DoA officials at the plant quarantine station prior to forwarding the parcel to the Citrus and Subtropical Fruit Research Institute (CSFRI) in Nelspruit. The ARC institute manage the quarantine evaluation of citrus propagation material on behalf of the DoA. Diagnostic methods employed include the Enzyme-Linked Immunosorbent Assay (ELISA), biological indexing (herbaceous and woody indicators) and Reverse Transcription- Polymerase Chain Reaction (RT-PCR) for the identification of virus, viroids and phytoplasmas. The BIOLOG system is used for the identification of bacteria and DNA based method is used for the identification of nematodes. DNA barcording project for the

identifying of mealybugs (http://www.nda.agric.za/doc/NPPOZA/Citrus.htm) and scales is currently underway.

The quarantine detention of imported propagation materials consists of an inspection of the plant materials and the phytosanitary documentation and an appropriate decontamination treatment before the material is grafted onto virus free rootstock. After completion of indexing material is released to the citrus foundation block whilst screening continues for another year (http://www.nda.agric.za/doc/NPPOZA/Citrus.htm).

3.7 LAWS AND REGULATIONS

The nursery industry, like any other major industry must comply with certain international or national laws and regulations. The national laws and regulations are promulgated by the Government of South Africa primarily for the protection of people and spread of pests and diseases to other countries, which are considered to be free from various pests and diseases (http://www.nda.agric.za/docs/plant_improvement/REGULATIONS.htm). National laws and regulations that are of major concern to the nursery industry include: (i) Plant Improvement Act, 1976 (Act No. 53 of 1976), (ii) Agricultural Pest Act, 1983(Act No. 36 of 1983) and (iii) Plant Breeder's Right Act, 1976 (Act No. 15 of 1976). Table 3.3 also includes the lists of all national laws and regulations that are of concern to citrus nursery.

(i) Plant Improvement Act, 1976 (Act No. 53 of 1976)

The main objective with the Plant Improvement Act, 1976 (Act No. 53 of 1976) is to provide a regulatory framework for the registration of premises from which the sale of certain plants or the cleansing, packing and sale of certain propagating material may be undertaken, to prescribe the conditions subject to which such plants or propagating material may be sold for the purposes of cultivation. The other main function is to provide for the recognition of certain varieties of plants; for a system of certification of plants and propagating material with the objective of maintaining the quality of certain plant and propagating material and ensuring the usefulness of the product thereof for agricultural and

industrial purposes; and for the control of the import and export of certain plants and propagating material and to provide for incidental matters (http://www.nda.agric.za/docs/plant_improvement/REGULATIONS.htm).

(ii) Agricultural Pest Act, 1983 (Act No. 36 of 1983)

The main purpose of the Agricultural Pest Act, 1983 (Act No. 36 of 1983) is to provide for measures by which agricultural pests may be prevented and combated (http://www.nda.agric.za/docs/plant_improvement/REGULATIONS.htm). The Act also mandates the Directorate Plant Health to regulate plants, plants products and other regulated articles when imported into South Africa. Plants, plant products and related materials are capable of harbouring quarantine pests, which if they enter South Africa with imported commodities and establish, may endanger the South African agricultural, horticultural or forestry sectors. Similar, pests that occur in South Africa may endanger countries to which we export and as a result South Africa may loose its exports markets.

(iii) Plant Breeder's Act, 1983 (Act No. 15 of 1976)

The main purpose of the Plant Breeder's Act, 1983 (Act No. 15 of 1976) is to provide for a system where plant breeders rights relating to varieties of certain kinds of plants may be granted and registered, for the requirements which have to be compiled with for the grant of such rights; for the protection of such rights and the grant of licences in respect of the exercise thereof; and to provide for incidental matters such as preventing introducing alien plants into the Republic of South Africa or plants that are not registered with plant breeders rights or contravene it (http://www.nda.agric.za/docs/plant_improvement/REGULATIONS.htm).

3.8 NURSERY PRACTICES

The term certification programme is often wrongly referred to as a clean stock programme, which is used as propagating sources that would enable multiplication of clean nursery

plants (Lee, *et al.,* 1999). A true certification programme is a marriage of three separate, but well integrated programmes, which ensures the production of healthy and high quality nursery trees, and provides protection against unknowing importation of pests. The three programmes which comprise a certification programme are:

- Quarantine programme for the safe introduction of select horticultural germplasm
- Clean stock programme for the testing and therapy treatment to identify domestic sources and to produce pathogen free material for commercial use
- Certification programme to provide a method of maintaining the pathogen free materials and making them available to nurseries and growers to benefit the industry. Healthy plants from the quarantine programme and the clean stock programme provide the necessary foundation material for the certification programme

3.8.1 Quarantine programmes

Citrus growers worldwide are always looking for new citrus germplasm which may offer a competitive advantage against the older cultivars e.g. an earlier or later maturity variety which may extend an already recognized market or better fruit pigmentation, unique fruit shape, seedless fruit etc (Lee *et al*., 1999). These are the common reasons why citrus growers are interested in importing citrus germplasm. Uncontrolled importation of budwood to another country or regions has the risk of introducing new pests and pathogens, which in certain cases could introduce a foreign pest or pathogen and subsequently cause important economic losses (Davidson *et al.,* 1988). The risk of introducing new pest and pathogens to a country or region can be minimized by carefully controlling and regulating introduction through permits and quarantine stations to allow for the importation of foreign plant varieties. In these stations plant material is tested for the presence of new pests and pathogens.

Quarantine programmes are usually operated by plant protection service, under the jurisdiction of the Ministry of agriculture of a country (Lee *et al.,* 1999). In South Africa the Directorate of Plant Health and Agricultural Product Inspection Service (APIS) who

issues the import permit according to international standard procedures and Agricultural Pests and Regulation (1013) handles the importation of plant material. Imported plant material is collected at the ports of entry by plant health inspectors from the DoA and send to one of the quarantine stations at Buffelspoort (North West Province) or Plant Quarantine Station in Stellenbosch (Cape Town) depending on the nearest position to the import point (http://www.nda.agric.za/docs/plant_improvement/REGULATIONS.htm).

Quarantine procedure which can be used for safe importation of citrus plant material to minimize entrance of new plant disease and pests are propagating imported budwood maintained in quarantine greenhouses located far away from the respective production region. Another alternative procedure is the incubation of budwood in tubes (Navaroo *et al*., 1984). This procedure was developed in Spain for safe introduction of citrus varieties and has proven to be effective. Imported citrus seed should be imported from countries, which have recognized certified seed sources trees. Bud sticks received from other countries are cleaned thoroughly and sterilized to remove pests or pathogens and are immediately placed in test tubes and incubated in a closed growth chamber.

Imported budwood is tested for the presence of pathogens and either thermotherapy or shoot tip grafting methods are applied to free introduced germplasm from the pathogens present in the original germplasm (Vives *et al.,* 2003). It is important to note that germplasm is only free of the pathogens for which it has been tested. For example, the decline of Roble orange on Swingle citrage rootstock in Florida appears to be due to the presence of citrus leaf blotch virus, a pathogen not recognized until recently (Galipienso *et al.,* 2001; Vives *et al*., 2003). There are now reports of citrus tristeza virus and huanglong bin being seed transmitted (Hartung *et al.,* 2003). Uncontrolled importation of budwood to another country or regions has the risk of introducing new pests and pathogens, which in some instances would cause important economic losses (Davidson *et al.,* 1988).

3.8.2 Clean stock programmes

Several steps are involved in the establishment of a clean stock programme, this includes

the selection of mother trees of local cultivars, indexing of the selected mother trees; recovery of pathogen free plants by shoot tip grafting *in vitro* and thermal therapy; indexing of the plant recovery; horticultural evaluation of healthy plants and maintenance of healthy plants (Timmer & Duncan, 1999). Selection of mother trees should be done based on higher fruit colour, early or late ripening. Pruning and grafting tools should be adequately disinfected with the use of sodium hypochlorite (Roistacher *et al.,* 1969 & Roistacher *et al.,* 1980).

3.8.3 Certification programmes

The main purpose of certification programmes is to guarantee the sanitary status and true character of propagating material in commercial propagation in nurseries (Davidson *et al.,* 1988). Certification programmes impose legal regulations governing nursery operations and inspection of trees used for nursery propagation (Timmer & Duncan, 1999). Certified nursery trees gives guarantee that they meet the requirements for horticultural quality in the certification regulations, and nurseries must also keep records to show that they have complied with the regulations (Davidson *et al.,* 1988). The most important components of a certification programme is:

- Well-trained and dedicated personnel with experience
- Plants should be grown with the best available cultural practices, pest control, labeling and planting location
- Special precautions should be taken to control pests and fungal diseases
- Pruning and grafting tools should be adequately disinfected prior to any fruit picking, grafting of any nursery plant, to prevent the spread of mechanically transmitted diseases (Rom & Carlson, 1987)
- Careful record keeping and labelling of plants during the whole process of propagation
- Nursery operations should be continuously under inspection to minimize pest or diseases introductions at early stage

3.8.4 Nursery site selection

Nursery site selection should be done with considerable care and thought, since location can play a major effect on the overall success of the nursery (Timmer & Duncan, 1999). Establishing a nursery sites requires a large investment in land and facilities, familiarity with the laws and restrictions on all levels and units of government. Before selecting the site for nursery, all factors should be considered and evaluated. If possible advice should be given from specialists in the area of real estate, soil science, horticulture, entomology, extension services and plant pathology (Davidson *et al.,* 1988). The factors listed in Table 3.4 should be considered when selecting a site.

3.8.5 Organisation and development of the nursery

Organisation and development of a nursery site should be done with considerable care and planning to maximize efficiency of production and to minimize loss of time (Timmer & Duncan, 1999). Plans to develop the nursery site include the location of production areas, irrigation facilities, administrative headquarters, parking, windbreaks, employee facilities and storage facilities. The best sites should be reserved for plant production. Nursery production areas of nurseries include propagation greenhouses, seedbeds, liner beds and the container yards. While developing the overall plan for the nursery, consideration should be given to construction of storage areas for equipment, tools, fertilizers, pesticides and other materials (Davidson *et al.,* 1988).

Nurseries will need storage areas for the basic ingredients of their growing media, a holding area for containers and special storage tanks for fertilizer concentrate (Mason, 1994). An effective irrigation system is an important requirement in the development of container production nursery areas (Timmer & Duncan, 1999). Visitor facilities such as parking areas and toilet facilities should be considered as well as employee facilities such as canteen or lunch areas with washing and toilet facilities and locker rooms a foreman's office should be considered and carefully planned, properly constructed and maintained (Davidson *et al.,* 1988). Clean and neat facilities for employees are important for

maintaining high morale, which will have a direct effect upon productivity. Public access areas should also be considered linked with display and selling areas particularly in landscape nurseries. Product flow should be considered with a receivable area for incoming and raw material, a preparation area, plant multiplication area, growing areas, display areas and sales as well as off loading areas for bulk consignment and sales.

3.9 NURSERY DISEASES AND PESTS

Citrus nursery plants are subject to numerous diseases which affects growth and its commercial value. The most important of these diseases are Phytophthora which affects the root system and ultimate growth and yield (Spiegel-Roy & Goldschmidt, 1996). Citrus nursery diseases and major registered control measures are listed in Appendix I and Appendix J.

Table 3.4 Factors to consider when selecting a nursery site (Source: Davidson *et al.,* 1988)

Ecological	**Sociological**	**Economic**	**Biological**	**Political**
Regional climate: particularly wind, hail or storms that can affect stability off green house structures etc.	Increased population	Cost of land, annual taxes and property assessments	Diseases: known history of presence of pathogens	Land reform consideration. Land re-institution
Topography: access to main roads. Steep slopes, rock and large stones areas should be avoided.	Wealth and leisure time	Cost of labour	Pests: presence of insect species	Black Economic Empowerment issues
Soil: access to quality soil free from pathogens. Gravel and dust roads, air pollution which will affect plant health should be avoided.	Access to quality schools, churches, hospitals, civic association and cultural centres	Distance from industrial areas to purchase equipment, stock etc.	Weeds	Job creation
Water: access to adequate quality water and free from pathogens	Lifestyle and ethnic background of the people	Access to funding, loans etc.	Free from waterborne diseases	Capacity building incentives

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CHAPTER 4

SOUTH AFRICAN CITRUS NURSERYGAP

PROTOCOL AND COMPLIANCE CRITERIA

VERSION 1.0

JUNE, 2009

INTRODUCTION

Citrus is one of the most important horticultural crops cultivated in South Africa. It is not only of economic importance for the country but also important in terms of trade, foreign exchange and

employment opportunities. Citrus, like any other crop which are commercially cultivated, is plagued by numerous pests and diseases. Since nurseries can potentially play an important role in the spread and manifestation of pests and diseases it is imperative to ensure that all planting material used are certified healthy and that production practices are managed effectively. Implementation of Good Agricultural Practices (GAP) may help to improve sound nursery production practices and the risk of non-compliance and spread of pest and diseases into new areas.

Good Agricultural Practices refer to any collection of specific procedures or methods, which when applied to agriculture, produce results that are in harmony with the values of the proponents of those practices. When applied to nurseries, GAP provide the opportunity to assess and decide which best nursery practices to follow at each step in the production process. In this Chapter, the following GAP principles will be discussed: geographical location, building and greenhouse physical structures, soil and substrate management, source of mother material, fertilizer use, storage, irrigation/fertigation, chemical disease and pest control programme, waste and pollution management, nursery hygiene, worker hygiene, strategy and welfare and protocols for detecting pathogens.

Each nursery practice protocol is aimed at allowing a comprehensive management strategy, providing for the capability for tactical adjustments in response to changes. Good Agricultural Practices in the nursery requires implementation of a comprehensive management strategy which involves knowing, understanding, planning, measuring, monitoring and record keeping. Adoption of GAP by the nursery industry may result in higher production and also help to reduce the risk of non-compliance with national and international regulations, standards and guidelines (GlobalGAP and national regulations).

The use of certified disease free planting material will contribute substantially towards improving the productivity and competitiveness of citrus production in South Africa. Starting with a healthy, disease and pest free citrus plant is an essential first step in any successful disease management strategy. Careful selection of healthy, high quality stock is the single most important decision affecting financial success of new plantings and has the greatest cost benefit ratio of any step in the nursery management strategy.

The main objective with GAP is to ensure that the best nursery management practices are developed and integrated into a single manual that can prevent the spread of pests and diseases into new areas particularly citrus black spot.

SOUTH AFRICAN CITRUS GOOD NURSERY PRACTICES

CONTENTS **PAGE**

1. DEFINITIONS

For the purpose of this standard the following definitions apply:

"Accredited Certification Body"	Is an organisation accredited by an authorising body such as South African National Accreditation System (SANAS) to undertake third party assessment of quality management systems such as GlobalGaP, Hazard Analysis Critical Control Point (HACCP) and to award and withdraw certificates of registration to theses standards.
"Approved citrus nursery site"	A defined area which meets the certification requirements as prescribed by the Department of Agriculture.
"*Approved soil pit"*	A soil source used for filling purpose, highway or road construction, or as an ingredient in plant growing or potting media which meets the Department of Agriculture requirements as to the absence of nematodes attacking citrus.
"*Approved Laboratory"*	Laboratory which complies to certain minimum performance criteria set by an accrediting agency and has received *a* certificate to verify their status.
"A sample bag"	As strong plastic bag with a minimum thickness of 30 microns into which a sample will be placed.
"*Best Management Practices"*	Are all practices which are designed to minimize the risk of introduction or survival of pathogens in a nursery.
"*Budwood"*	A portion of a stem or branch with a vegetative bud(s) used in propagation by means of budding or grafting.
"*Certified budwood*"	Budwood which are obtained from the Citrus Foundation Block.
"*Certified citrus*	A nursery which has been certified by the Department of Agriculture as meeting the requirements for

nursery"	production of citrus nursery stock free of pests and diseases.
"*Citrus*"	All species of the genera Citrus, Poncirus and Fortunella including any hybrids thereof.
"*Compliance criteria*"	To take all necessary actions to ensure and maintain compliance with criteria established in the protocol.
"*Control point"*	A point, step or procedure at which control can be applied and is essential to prevent or eliminate a disease or reduce it to an acceptable level.
"*Delimitation survey"*	A survey done to determine the extent of the infestation within a nursery site.
"Disinfection"	Is the killing, inhibition or removal of microorganisms that cause disease.
"*Enzyme Linked Immuno Sorbent Assay"*	A serological laboratory test which uses antibodies coupled with indicators to detect the presence of viruses.
"Germplasm"	Plants intended for use in breeding or conservation programs.
"*Good Agricultural Practices"*	Are those minimum control measures, actions and procedures that have to be performed to produce a product that meets these standards such as employee hygiene and training, field sanitation and critter control for pests and diseases.
"*Growing medium*"	Any medium used to grow plants in (in which the plants roots are established).
"Horticulturally true-to-type"	A plant which conforms to the description of a particular cultivar and which is from the same genetic line of descent as that cultivar.
"*Media"*	Refers to any soil or substrate in which citrus plants are grown.
"*Nuclear stock"*	A plant or plants which were originally tested and found to be free of pests and pathogens and maintained in such a way as to retain their pest free status.
"*Nursery"*	Any location where nursery stock is grown, propagated, stored or sold.
"*Nurseryman"*	Any person engaged in the production or collection of nursery stock for sale or distribution.

"*Nursery Certificate*"	A document issued by the State Crop Pest Commission, or an equivalent agency of another state, declaring that the plants grown by the person named on the document have been inspected and found to be apparently free from injurious plant pests.
"*Nursery block"*	A contiguous grouping of plants similar in size/age and rootstock that can be sampled together which are at least two meters from other contiguous groupings of plants in the same area.
"*Nursery site*"	A geographically separated location of a nursery that has a distinct physical address and have appropriate biosecurity measures to prevent the movement of diseases from one site to another site.
"*Nursery site quarantine*"	It's a site in a nursery which hosts plants and associated plants that shall not be moved within or out of the quarantine block.
"*Nursery stock*"	Any plants for planting, including houseplants, propagative material that are grown in a nursery and tree seedlings for reforestation.
"*Pasteurization"*	Is a process of heating products for the purpose of destroying viruses and harmful organisms such as bacteria, protozoa, molds and yeasts.
"*Pathogen"*	Any bacteria, fungi, nematodes and virus or virus like organism that is harmful to citrus.
"*Polymerase* Chain *Reaction PCR)"*	A highly sensitive laboratory test that can detect small amounts of DNA or RNA in a plant tissue sample by amplification of a specific DNA or RNA segment.
"Pest "	Refers to any insect that is harmful to citrus.
"*Propagative material"*	Any live plant material used to produce nursery stock, including cuttings, budwood, seeds, seedlings, air layering and tissue culture.
"*Rootstock"*	A plant used as a recipient understock in budding or grafting.
"*Scion tree"*	A citrus tree grown in accordance with rule from budwood taken from a registered foundation tree and

	registered with the department as a source of budwood
"*Seed source*"	A tree that supplies seed for propagation which has been determined by the nurserymen as being apparently horticulturally true-to-type and found free from diseases.
"Shall"	Describes a feature or behaviour that is mandatory for an implementation that conforms to this document.
"Should"	For an implementation that conforms to this document, describes a feature or behaviour that is recommended but not mandatory.
"*Source tree*"	A citrus tree that has met all of the requirements as a source of budwood or propagative material, e.g. scion tree, seed source.
"*Sterilization*"	Refers to the process in which all living cells, spores and viruses are completely destroyed or removed from an object or environment.

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2. AIMS OF THE DOCUMENT

The aim of this document is to prevent, minimize or eliminate diseases and pests within the nursery production system and also to ensure that disease free certified propagation material are produced in the nursery. Equipment to be used in the nursery for production practices must be sterilized in order to prevent contamination. All workers must be trained in personal hygiene and best nursery practices. An effective nursery management system should be established and nurseries must be registered with the South African Nursery Association, National Department of Agriculture and annually inspected by Plant Health Inspectors. This manual represents a guideline to ensure compliance and contains all the current nursery practices and requirements as set out by the South African citrus nursery industry. The document has also been developed in line with the GlobalGAP nursery document and references the most important applicable national regulations and legislation of importance to the citrus nursery industry.

3. PURPOSE

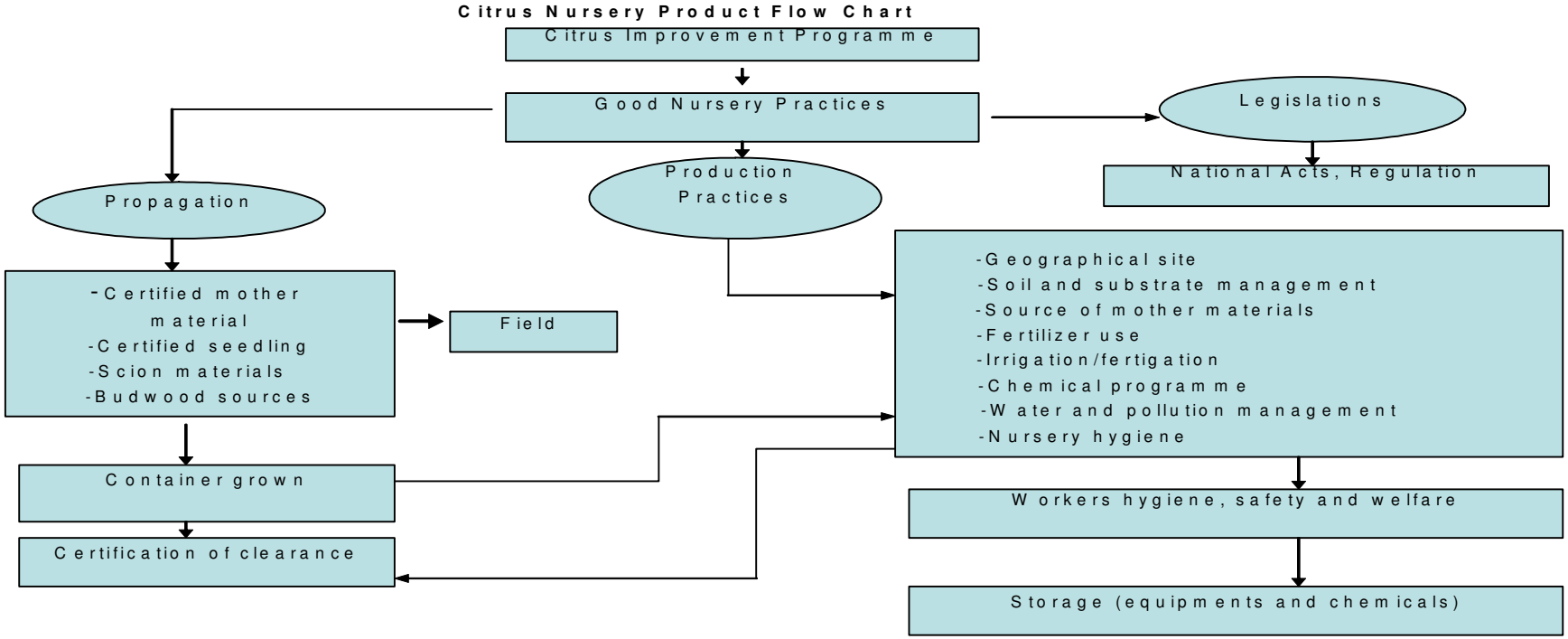
For the long-term profitability and growth of the South African Citrus Industry it is important that propagation material is pathogen free and obtained from a certified grower.

4. HOW TO USE THE STANDARD AND PROTOCOLS

The standard and protocol is primarily designed to help citrus nurserymen follow GAP in the nursery (Fig 4.1). The citrus nursery product flow chart is discussed in more detail under criteria for citrus nursery production practices section. The standards must ensure a responsible approach to worker's health and safety when it comes to tasks such as handling of chemicals. The standard and protocol should be implemented by the South Africa Citrus Industry and can be used as a compliance guideline for inspectors. Plant samples must be taken to test for the absence of certain pathogens. Samples can only be analysed by an approved laboratory.

This document and standard were prepared by: Mr. Mbulaheni Thomas Mutengwe and Prof Lise Korsten from the Department of Microbiology and Plant Pathology, University of Pretoria for the South African Citrus Nursery Industry in collaboration with the National Department of Agriculture. Special standards technical input was obtained from Mr. Frans Beneke (Lead Auditor) from the South African National Accreditation System.

Fig 4.1 Summary of inspection and certification procedures and flow lines in the citrus nursery scheme (Davidson *et al.*, 1988)



5. In the GAP Criteria for Citrus Nursery Production Practices (Page 77-104) and Protocol for Detecting Pathogens (Page 104-114) Footnotes (Non-highlighted points are from these references)

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5.1 The following control points and compliance criteria have been extracted from the following documents using a colour coding system to differentiate between the various documents:

Blue highlighted points are from Kotzé, J. M., 2005b. Draft recommendations to monitor for CBS in citrus nurseries, CRI Annual Report 2005:293-294. Swart, S. H., 2004. Developing a protocol for detecting CBS in citrus nurseries. CRI Annual Report 2004: 324-327. Agricultural Pests Act (Act No.36 of 1983) Notice R. 461 amended on the 25 April 2008.

Tan highlighted points are from National Department of Agriculture websites http://www.nda.agric.za. South Africa Good Agriculture Practices (SAGAP) accessed on 15 January 2008.

Dark gray highlighted points are from Davies, F. S. & Albrigo, L. G., 1994. Citrus. Crop Production Science in Horticulture. CAB International, 18-110.

Dark Yellow highlighted points are from Futch, S. H., 2005. Maximizing Weed Control in Florida Citrus. Horticultural Science Department Document HS 1007, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida, Gainesville, FL 32611.

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Gold highlighted points are from the book Safety and Health in Agricultural Work. 1965. International Labour Office Geneva, 91-92.

Gray highlighted points are from EurepGAP (General Regulations Guideline for Propagation Material) Version 1.0-May 2006.

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Bright green highlighted points are from EurepGAP (Control Points and Compliance Criteria Integrated Farm Assurance) fruit and vegetables version 3.0-March 2007.

Red highlighted points are from South Africa Department of Labour. http://www.labour.gov.za accessed on 20 October 2007.

Yellow highlighted points are from Citrus Research International (CRI) (Pty) Ltd. Citrus Improvement Programme. Published documents are: (i) South African Citrus Nursery Accreditation Scheme, Document No. A_NRY, effective date: 2003 and revision no. 2.0. Compiled by C. Keith Roxburgh and authorised by H. le Roux, F. van Vuuren, M. du Toit & P. Kingston.

(ii) Working Instruction-Nursery Sanitation, Document No. W1004, effective date: 07 June 1997 and revision no. 0.0. Compiled by C. Keith Roxburgh and authorised by S. Burdette.

(iii) Working Instruction-Water Treatment and Testing, Document No. W1002, effective date: 19 June 1997 and revision no. 0.0. Compiled by C. Keith Roxburgh and authorised by S. Burdette.

(iv) Working Instruction-Nursery sampling method for pathogen analysis, Document No. W1001, effective date: 09 May1997 and revision no. 0.0. Compiled by C. Keith Roxburgh and authorised by S. Burdette.

Green highlighted points are from Nursery Management Administration and culture. Second Edition by Harold Davidson, Roy Mecklenburg & Curtis Peterson (1988) Prentice Hall, Eaglewood Cliffs, New Jersey 07632, 16-361.

6. CRITERIA FOR CITRUS NURSERY PRODUCTION PRACTICES:

NO	CONTROL POINT	COMPLIANCE CRITERIA	LEVEL
6.1	**GEOGRAPHICAL LOCATION:**		
6.1.1	**Selection and registration of a nursery site:**		
6.1.1.1	Have all factors been considered for selection of a nursery site?	Soil, water and environment are the major factors to be considered in selecting a site for nursery production. Other factors that should also be considered are: ecological, economic, sociological, biological and historical factors.	Major
6.1.1.2	Does a designated official inspect the site?	The site has to be inspected by the National Department of Agriculture, Directorate: Agriculture Products Inspection Service (APIS) http://www.nda.agric.za or appointed agents.	Major
6.1.1.3	Has the official site inspection included all required specifications?	Site inspection shall include inspection of structures, standards operating procedures, nursery sanitation and records.	Major
6.1.1.4	Has the official site inspection included an inspection of the mother block and nearby orchards?	The site inspection should include visual inspections of the health status of the mother trees and nearby orchards specifically paying attention to phytosanitary diseases such as CBS.	Major
6.1.1.5	Has an official certificate of approval been issued for citrus production on the site?	Certificates have to be issued and signed by a competent authority that the sites have been approved for citrus production.	Major
6.1.2	**Site history:**		
6.1.2.1	Is there evidence that a Risk Assessment has been done prior to selecting a new nursery site?	Evidence that a risk assessment has been done should be available and include the following: type of soil, erosion, quality and level of groundwater, availability of sustainable water sources, and impact on and of the adjacent area. When the assessment identifies a non-controllable risk that is critical to health and/or the environment, the site must not be used for agricultural activities.	Major
6.1.2.2	Is validation of the Risk Assessment Study conducted?	Validation of the risk assessment study should be conducted. Having on record evidence that the risk assessment study was	Major

6. CRITERIA FOR CITRUS NURSERY PRODUCTION PRACTICES:

NO	CONTROL POINT	COMPLIANCE CRITERIA	LEVEL
		done by a registered professional environmentalist qualified in this field (Conservation of Agricultural Resources Act, 1983 (Act No. 43 of 1983) which state that all impacts on soil, soil potential, pollution of soil and bad soil management practices that lead to degradation of the soil.	
6.1.2.3	Provide evidence of a corrective action plan that specify strategies to minimize all identified risks on new nursery sites?	Corrective action plans should identify the risk, its potential severity and probability as well as the list of measures taken to prevent or to control the risk.	Minor
6.1.2.4	Does the selected area have a history of CBS or is it close to an area with a history of CBS?	If yes, nurseries in CBS infected areas must be on a CBS control programme. The programme should preferably consist of registered contact fungicides e.g. copper oxychloride, copper hydroxide or mancozeb, preferably in tank mixture with sporekill and not Benzimidazoles or the Strobilurines to prevent the development of pathogen resistance.	Major
6.1.2.5	How is it confirmed that the area is free from CBS in the case of an existing nursery?	Proof of CBS absence through comprehensive surveys and Production Unit Code (PUC), which are registered with the National Department of Agriculture, should be available.	Major
6.1.3	**Site management:**		
6.1.3.1	Is the nursery registered with the Citrus Growers Association and National Department of Agriculture?	The nursery should be registered with the Citrus Growers Association, Citrus Improvement Programme and the National Department of Agriculture (Roxburgh, 2003).	Must
6.1.3.2	What is the Citrus Black Spot (CBS) status of the nursery?	Determine if the nursery is categorized as a Citrus Black Spot secure designation or not free from CBS (DoA, CRI & SACNA, 2007).	Major
6.1.3.3	Has a CBS monitoring system been established for the mother trees or block used for propagative material in the nursery?	Documented records that reference each production area from where propagation material has been collected should be available.	Major

6. CRITERIA FOR CITRUS NURSERY PRODUCTION PRACTICES:

NO	CONTROL POINT	COMPLIANCE CRITERIA	LEVEL
6.1.3.4	Has the citrus orchards from where budwood/scion material is collected been certified to a Good Agricultural Practices standard?	Evidence should be provided that budwood or scion material obtained is compliant to a Good Agricultural Practices standard such as GlobalGAP.	Major
6.1.3.5	Has a visual identification or reference system for mother trees, blocks and the nursery been established?	Every mother block, nursery or greenhouse is physically identifiable e.g. using landmarks, a unique code, name, number or colour that can be linked to records that reference the area.	Minor
6.1.3.6	Has the nursery positioned for drainage rainwater control been planned to ensure effective runoff?	The nursery should be well positioned so that no rain water from adjacent land may enter the nursery. A good drainage system is therefore required.	Major
6.2	**BUILDING AND GREENHOUSE PHYSICAL STRUCTURES:**		
6.2.1	**Greenhouse**		
6.2.1.1	What are the advantages of having greenhouse structures in the nursery?	Greenhouse structures are necessary for the production of index plants and for indexing.	Minor
6.2.1.2	Which materials are used to construct the greenhouse?	Glasshouses with brick, polycarbonate walls and polycarbonate roofing are used to construct greenhouse in the nursery. Corrugated fiberglass rather than glass is recommended where hail is a problem. The structure should be enclosed to avoid foliar wetting from rain.	Minor
6.2.1.3	Has the size of the greenhouse been calculated on sound nursery management practices?	The size of the greenhouse will depend upon the amount of indexing and research to be carried out.	Minor
6.2.1.4	Has greenhouse access been restricted?	Greenhouse access should be restricted in order to reduce the spread of pest and diseases.	Major
6.2.1.5	How is the floor for greenhouse structure?	Greenhouse flooring can be of concrete with provisions for drainage. Gravel flooring with concrete walkways is highly recommended.	Minor

6. CRITERIA FOR CITRUS NURSERY PRODUCTION PRACTICES:

NO	CONTROL POINT	COMPLIANCE CRITERIA	LEVEL
6.2.1.6	Has the optimal growing compartments been established to ensure best cultivation conditions?	Optimal growing compartments should be established which include at least three compartments: a cool room for indexing citrus graft-transmissible pathogens which are best expressed in plants grown under relatively cool temperature; a relatively warm temperature room primarily used for growing plants and hot room which may be used for pre-conditioning budwood prior to thermotherapy.	Minor
6.2.1.7	Does the facility have adequate environmental control infrastructure?	Greenhouse should have gas heaters, cooling fans, evaporator coolers, supplements lighting and temperature control.	Minor
6.2.1.8	Are hand wash facilities with soap and drying cloth paper etc available at the entrance of the Greenhouse?	For hygienic purpose hand wash facilities with soap and drying cloth should be available at the entrance of greenhouse.	Major
6.2.2	**Toilets facilities:**		
6.2.2.1	Are toilet facilities available within the nursery yard?	Workers should be provided with toilets facilities separate for each sex (male and female).	Major
6.2.2.2	Are sign boards erected or positioned to effectively indicate the direction of toilets?	Sign boards should indicate the nearest toilet facility.	Minor
6.2.2.3	Are adequate hands washing facilities present at the toilets?	Adequate hand washing facilities should be available at the toilets.	Major
6.2.2.4	Does an effective toilet cleaning program exist and is it implemented and maintained?	Toilet cleaning program should be implemented as part of hazard critical control point (HACCP).	Major
6.2.2.5	Are the toilets conforming to the requirements of the national healthy authority?	Toilets facilities should conform to the requirements of the competent healthy authority. For personal cleansing supply toilets with toilets paper. Water facilities with soap and towels should be provided.	Major
6.3	**SOIL AND SUBSTRATE MANAGEMENT:**		
6.3.1	**Site impact environment:**		

6. CRITERIA FOR CITRUS NURSERY PRODUCTION PRACTICES:

NO	CONTROL POINT	COMPLIANCE CRITERIA	LEVEL
6.3.1.1	How is the soil in areas around the nursery conserved?	Soil in areas around the nursery should be conserved through application of mulches such as bark husks to the soil helps to protect the soil surface from splash or wind erosion. The success of soil conservation practices depended upon good design, construction, proper use and maintenance of the soil.	Major
6.3.1.2	Is a disinfected footpath present at the entrance of the nursery and is it maintained and managed?	To prevent the spread of disease such as *Phytophthora,* disinfected footpath such as copper should be present at the entrance of the nursery. Footpath should be maintained and managed all the time.	Major
6.3.2	**Substrates management:**		
6.3.2.1	Does the nurseryman participate in substrate recycling programmes?	The nurseryman must keep records with quantities recycled and dates. Invoices and loading dockets are acceptable. If there is no participation in a recycling program available, it should be justified.	Recom.
6.3.2.2	If chemicals are used to sterilize substrates for re-use, has the location of sterilization been recorded?	When substrates are sterilized at the nursery, the name or reference of the field, orchard or greenhouse should be recorded. If sterilized off-site the name and location of the company which sterilizes the substrates should be provided as well as evidence of effective adequate treatment.	Major
6.3.2.3	If chemicals are used to sterilize substrates for re-use, has the date of sterilization, type of chemicals, method of sterilization and name of the operator been recorded?	The following should be recorded: the dates of sterilization (day/month/year); the name and active ingredient of the chemical; the machinery used (e.g.1000 l-tank); the method (e.g. drenching, fogging); the operator's name (the person who applied the chemicals and did the sterilization) and verification of the activity.	Minor
6.3.2.4	If steaming is used to sterilized substrates for re-use, has records been kept?	Documented evidence that shows that steaming is used should be available.	Recom.

6. CRITERIA FOR CITRUS NURSERY PRODUCTION PRACTICES:

NO	CONTROL POINT	COMPLIANCE CRITERIA	LEVEL
6.3.2.5	Are substrates traceable to the source of origin and that it does not come from designated conservation areas?	Records should be available to prove origin of the substrates being used. These records demonstrate that the substrates do not come from designated conservation areas.	Recom.
6.4	**SOURCE OF MOTHER MATERIAL:**	The choice of propagation material plays an important role in the production process and by using the correct varieties can help reduce the number of fertilizer and plant protection product applications.	
6.4.1	**Legislation:**		
6.4.1.1	Which legislations is the nursery industry must comply too?	Legislations which are of concern to the nursery industry includes: Agricultural Pest Act, 1983 (Act No. 36 of 1983), Plant Improvement Act, 1976 (Act No. 53 of 1976), Plant Breeders Right Act, 1976 (Act No. 15 of 1976), Agricultural Product Standards Act, 1990 (Act No.119 of 1990), Conservation of Agricultural Resources Act, 1983 (Act No. 43 of 1983), National Water Act, 1998 (Act No. 36 of 1998), National Environment Management Ac, 1998 (Act No.107 of 1998), Occupational Health and Safety Act, 1993 (Act No. 85 of 1993), Designated Areas Development Act, 1979 (Act No. 87 of 1979), Fertilizers, Farm Feeds, Agricultural Remedies and Stock Remedies Act, 1947 (Act No. 36 of 1947) and Labour Relations Act, 1995 (Act No. 66 of 1995). But not limited to other Acts or national legislations which are relevant to the nursery industry.	Major
6.4.1.2	Why is legislation important in the nursery industry?	Legislation is important to the nursery as it is promulgated by government primarily for the protection of people, environment, fauna and flora.	Major
6.4.1.3	Which aspects of the law does the nursery comply too?	The nursery should comply with environmental pesticides, labour standards, licenses, occupational safety, pest control, including disease, insects and pests and lastly sales taxes.	Major

6. CRITERIA FOR CITRUS NURSERY PRODUCTION PRACTICES:

NO	CONTROL POINT	COMPLIANCE CRITERIA	LEVEL
6.4.1.4	What role does Agricultural Product Standard Act, 1990 (Act No. 119 of 1991) play in the nursery?	Agricultural Product Standard Act, 1990 (Act No. 119 of 1991) provides control over the sale and export of certain agricultural products, control over sale of certain imported products and controls over other related products.	Major
6.4.1.5	What role does Plant Breeders Right Act, 1976 (Act No. 15 of 1976) related to the nursery industry?	Plant Breeder Rights Act, 1976 (Act No. 15 of 1976) provide a system where under plant breeders rights relating to varieties of certain kinds of plants may be granted and registered for the protection of such rights and grant of licenses in respect of the exercise.	Major
6.4.1.6	What role does Plant Improvement Act, 1976 (Act No. 53 of 1976) play in the nursery?	Plant Improvement Act, 1976 (Act No. 53 of 1976) provide a regulatory framework for the registration of premises from which the sale of certain plants, the cleansing, packing and sale of certain propagating material may be undertaken, to prescribe the conditions subject to which such plants or propagating material may be sold for the purposes of cultivation.	Major
6.4.1.7	What role does Agricultural Pest Act, 1983 (Act No. 36 of 1983) play in the nursery?	Agricultural Pest Act, 1983 (Act No. 36 of 1983) provides for measures by which agricultural pests may be prevented and combated from spreading from one area to another area especially imported products.	Major
6.4.1.8	What role does National Water Act, 1998 (Act No. 36 of 1998) play in the nursery?	National Water Act, 1998 (Act No. 36 of 1998) provide for fundamental reform of the law relating to water resources, to repeal certain laws and to provide for matters connected therewith.	Major
6.4.1.9	What role does Conservation of Agriculture Resources Act, 1983 (Act No. 43 of 1983) play in the nursery?	Conservation of Agriculture Resources Act, 1983 (Act No. 43 of 1983) provides for the conservation of the natural agricultural resources by the maintenance of the production potential of land, by combating and prevention of erosion and weakening or	Major

6. CRITERIA FOR CITRUS NURSERY PRODUCTION PRACTICES:

NO	CONTROL POINT	COMPLIANCE CRITERIA	LEVEL
		destruction of water resources and by the protection of the vegetation and the combating of weeds and invader plants.	
6.4.1.10	What role does Designated Areas Development Act, 1979 (Act No. 87 of 1979) play in the nursery?	Designated Areas Development Act, 1979 (Act No. 87 of 1979) provides for measures for the promotion of the density of population and farming activities in certain areas.	Major
6.4.1.11	What role does Labour Relations Act, 1995 (Act No. 66 of 1995) play in the nursery?	Labour Relations Act, 1995 (Act No. 66 of 1995) promote economic development, social justice, labour peace and democracy in the workplace. Applicable to workers in the citrus nursery industry.	Major
6.4.1.12	What role National Environment Management Act, 1998 (Act No.107 of 1998) play in the nursery?	National Environment Management Act, 1998 (Act No.107 of 1998) provide for co-operative, environmental governance by establishing principles for decision-making on matters affecting the environment, institutions that will promote co-operative governance and procedures for coordinating environmental factors exercised by organs of state and to provide for matters connected therewith.	Major
6.4.1.13	What role Occupational Health and Safety Act, 1993 (Act No. 85 of 1993) play in the nursery?	Occupational Health and Safety Act, 1993(Act No. 85 of 1993) provide for the health and safety of persons at work and for the health and safety of persons in connection with the use of plant and machinery, the protection of persons other than persons at work against hazards to health and safety arising out of or in connection with the activities of persons at work, to establish an advisory council for occupational health and safety and to provide for matters connected therewith.	Major
6.4.1.14	What role does Fertilizers, Farm Feeds, Agricultural Remedies and Stock Remedies Act, 1947 (Act No. 36 of 1947) play in the nursery?	Fertilizers, Farm Feeds, Agricultural Remedies and Stock Remedies Act, 1947 (Act No. 36 of 1947) provide for the appointment of a Registrar of Fertilizers, Farm Feeds and Agricultural Remedies; for the registration of fertilizers, farm	Major

6. CRITERIA FOR CITRUS NURSERY PRODUCTION PRACTICES:

NO	CONTROL POINT	COMPLIANCE CRITERIA	LEVEL
		feeds, agricultural remedies, stock remedies, sterilizing plants and pest control operators; to regulate or prohibit the importation, sale, acquisition, disposal or use of fertilizers, farm feeds, agricultural remedies and stock remedies; to provide for designation of technical advisers and analysts.	
6.4.1.15	If using imported citrus material, are they compliant to South African legislation?	Imported rootstock, seed or budwood must be compliant to South African legislation (Plant Breeders Right Act, 1976 (Act No.15 of 1976). Obtain evidence that imported rootstock have a valid Phytosanitary Certificates form the DoA compliant with Plant Improvement Act, 1976 (Act No. 53 of 1976) and indexing strategies or therapy procedures have been followed.	Major
6.4.2	**Choice of variety or rootstock:**		
6.4.2.1	In case of own citrus breeding programmes have adequate plant breeding rights been considered?	Adequate plant breeding rights should be considered. Plant breeder's rights relating to varieties of certain kinds of plants should comply with the right of such plant breeding rights, for the protection of such rights and the grant of licenses in respect of the exercise according to Plant Breeders Right Act, 1976 (Act No. 15 of 1976).	Major
6.4.2.2	Is the nurseryman aware of the importance of effective crop husbandry in relation to the "mother plants" of the registered propagation material?	Cropping techniques and measures are adopted in the "mother plants" which can minimize inputs such as crop protection products and fertilizers in the registered propagation material.	Recom.
6.4.2.3	Does the variety or rootstock grown meet the International Union guidelines for the protection of new varieties of plants guidelines?	Records should be available that proves that the varieties grown have been obtained in accordance to local legislation and incompliance with intellectual property rights.	Minor
6.4.3	**Seed/rootstock quality:**		
6.4.3.1	Are the evidence that certified seed were used in the nursery?	A seed certificate of quality origin, pathogen free status and viability should be maintained.	Minor

6. CRITERIA FOR CITRUS NURSERY PRODUCTION PRACTICES:

NO	CONTROL POINT	COMPLIANCE CRITERIA	LEVEL
6.4.4	**Pest and disease resistance:**		
6.4.4.1	Are the varieties grown resistance/tolerance to commercially important pests and diseases?	The nurserymen have to make sure that varieties used for planting are resistance or tolerance to pests and diseases.	Recom.
6.4.5	**Seed treatments and dressings:**		
6.4.5.1	How are the seeds prepared in the nursery?	Rootstock seed are extracted from fruit normally harvested in the fall. The simplest means of extraction is to cut a horizontal ring into the fruit; just deeply enough to avoid cutting the seeds, then twist the two halves apart. An electric juicer can be used to collect the seeds, after which they should be separated from the accompanying pulp by repeated washing. Commercial seed operations commonly crush the fruit in an ice crusher, followed by treatment with pectinase enzymes at controlled temperatures, with constant stirring.	Minor
6.4.5.2	In case of own seed treatment, is evidence available of the product/s used, application method, dosage, date, applicant and validated?	When seed or rootstock has been chemically treated, there are records available with the name of the products, dosage application method, applicant signed off and the date of application and the range of pests and or diseases they control specified.	Recom.
6.4.5.3	How is citrus seed stored?	Citrus seed should be dipped in one percent 8-hydroxyquinoline sulphate, air dried and packaged for storage or planting. Seed should be stored in sealed plastic bag.	Major
6.4.5.4	How long is citrus seed stored?	Properly treated and packaged in sealed plastic bags, seed can be stored in a refrigerator for several months with little loss in viability.	Major
6.4.5.5	At what temperature is the citrus seed stored?	Seed are stored at 4-5°C for 6 months to 2 years.	Major
6.4.6	**Propagation material:**		
6.4.6.1	Is imported purchased propagation material free of	A Plant Health Certificate should be available complying with	Minor

6. CRITERIA FOR CITRUS NURSERY PRODUCTION PRACTICES:

NO	CONTROL POINT	COMPLIANCE CRITERIA	LEVEL
	visible signs of pests and diseases?	National Legislation Plant Improvement Act, 1979 (Act No. 53 of 1976).	Minor
6.4.6.2	Is locally purchased material free of visible signs of pests and diseases?	Locally purchased material should be free of visible signs of pests and diseases. When plants have visible signs of pests and disease damage, a justification should be available in terms of threshold treatment.	Major
6.4.6.3	Are plant health quality control systems operational for in-house nursery propagation?	A quality control monitoring system on visible signs of pests and disease should be in place and records are available for the monitoring system, which are monitored and signed off the product.	Minor
6.4.6.4	Are crop protection product treatments on in-house nursery propagation material applied during the plant propagation period recorded?	Records of crop protection product treatments applied during the plant propagation material should be available and include product name, application dates, applicant, application method, target pests and doses.	Minor
6.4.7	**Sowing/Planting:**		
6.4.7.1	Are containers used for planting clean?	Containers used for planting must be clean, free of pests, weed seeds, diseases, insects, nematodes, herbicide residues and sterilized without the production of toxic substances such as soluble salts, ammonia.	Major
6.4.7.2	Are records kept on sowing/planting methods, seed/planting rate, and sowing/planting date?	Records of sowing/planting method, planting rate/date and responsible team must be kept as a sign of evidence.	Minor
6.4.8	**Management of the mother block:**		
6.4.8.1	Has the protection of mother trees against pests, disease and weeds been achieved with the appropriate minimum propagation material protection product input?	All crop protection should be documented and include written justifications, target and intervention thresholds that mother trees have been protected against pests, diseases and weeds.	Minor
6.4.8.2	Are recognized Integrated Pest Management (IPM)	Evidence should be available to prove implementation of IPM	Recom.

6. CRITERIA FOR CITRUS NURSERY PRODUCTION PRACTICES:

NO	CONTROL POINT	COMPLIANCE CRITERIA	LEVEL
	techniques applied?	techniques.	
6.4.8.3	Has assistance with implementation of IPM systems been obtained through training?	Responsible person should receive formal training in IPM implementation.	Minor
6.5	**FERTILIZER USE:**		
6.5.1	**Quantity, quality and type of fertilizer:**		
6.5.1.1	Can the technically responsible person demonstrate competence to determine quantity and type of fertilizer (organic and inorganic) to be used?	Documented evidence must be available that demonstrates training and competence of the technically responsible person to determine quantity to apply and type of fertilizer to use.	Minor
6.5.2	**Method of application:**		
6.5.2.1	Can evidence be provided of type of fertilizer application method used and why it was selected?	Evidence should be provided of type of fertilizer application i.e. surface, subsurface injection, foliage and bark application.	Minor
6.5.3	**Time of application:**		
6.5.3.1	When is the best time to apply fertilizer?	The best time to apply fertilizer, other than nitrogenous materials, is prior to planting, since these materials should be incorporated into the soil.	Major
6.5.4	**Records of application:**		
6.5.4.1	Have all application dates of soil and foliar fertilizers both organic and inorganic, been recorded including field, orchard, nursery block?	Records should be kept of all fertilizer applications, detailing the geographical area, the name or reference of the field, nursery block where the registered product is located.	Minor
6.5.4.2	Have all application dates, method of application and operator of soil and foliar fertilizers both organic and inorganic, been recorded?	Detailed in the records of all fertilizer application are the exact dates (day/month/year) of application, machinery type used and the method including the name of the operator and that it has been signed off and verified.	Minor
6.5.5	**Application machinery maintenance and operation:**		
6.5.5.1	Is fertilizer application machinery kept in good condition and a general maintenance plan followed?	Maintenance records or invoices of spare parts of both organic and inorganic fertilizer application machinery should be available on request.	Minor

6. CRITERIA FOR CITRUS NURSERY PRODUCTION PRACTICES:

NO	CONTROL POINT	COMPLIANCE CRITERIA	LEVEL
6.5.5.2	Is inorganic fertilizer application machinery verified annually to ensure accurate fertilizer delivery?	Inorganic fertilizer application machinery should be verified annually. Prove of calibration certificate should be provided and filed for auditing purpose.	Recom.
6.5.6	**Organic fertilizer:**		
6.5.6.1	Is human sewage sludge not used in the nursery?	Assess that no human sewage sludge is used in the nursery.	Major
6.5.6.2	Has a risk assessment been carried out before application of organic fertilizer, considering its source and characteristics?	Documented evidence should be available to demonstrate that potential risks have been considered: disease transmission and method of compost inorganic fertilizer prior to use.	Minor
6.5.6.3	Has account been taken of the nutrient contribution of organic fertilizer?	An analysis is carried out, which takes into account the contents of N.P.K nutrients in organic fertilizers applied.	Recom.
6.5.6.4	Is organic fertilizer analysis done by a component laboratory and results where adequately interpreted?	Assess evidence that the correct analysis has been done in a recognized lab and that interpretation was done by a suitable qualified person.	Major
6.5.7	**Inorganic fertilizer:**		
6.5.7.1	Are purchased inorganic fertilizers accompanied by evidence of chemical content and purity?	Documented evidence detailing chemical content and purity should be available for all inorganic fertilizers used on propagation materials.	Recom.
6.6	**STORAGE:**		
6.6.1	**Building structure:**		
6.6.1.1	Does the storage area comply with local regulations in terms of being secure, well ventilated and fire resistant?	Storage area should comply with all the appropriate current national, regional and local legislations and regulations. The storage area should also be built of materials that are sound and robust, fire resistant, secure under lock and well ventilated.	Must
6.6.1.2	Is the storage area built of materials that are appropriate to the temperature conditions?	Storage area should be built of materials that protect against temperature extremes.	Must

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NO	CONTROL POINT	COMPLIANCE CRITERIA	LEVEL
6.6.1.3	Is there sufficient light in the storage room?	Storage facilities should be located in an area with sufficient natural and artificial lighting, to ensure that all product labels can be read easily on the shelves.	Must
6.6.1.4	Is the storage area in a location away from other materials?	Storage facilities should be located in a separate air space independent from any other materials.	Must
6.6.1.5	Is the storage shelving made of non-absorbent materials?	Storage area should be equipped with shelving non-absorbent in case of spillage, e.g. rigid plastic or metal.	Recom.
6.6.2	**Storage of fertilizer:**		
6.6.2.1	Is organic fertilizer stored in an appropriate manner, which reduces the risk of contamination of the environment?	If stored at the nursery, organic fertilizer should be stored in a designated area; at least 25 meters from direct water sources in order to reduce the risk of contamination.	Recom.
6.6.2.2	Are inorganic and organic fertilizers stored separate from plant propagation material?	Fertilizers should not be stored with plant propagation material.	Major
6.6.2.3	Are inorganic fertilizers stored in a clean and dry area?	Fertilizers should be stored in a clean well ventilated area free from waste and rainwater or condensation.	Minor
6.6.2.4	Is there an up to date inorganic fertilizer stock inventory available at the nursery?	A stock inventory, which indicates the contents of the store (type and amount), should be available and regularly updated	Minor
6.6.2.5	Are inorganic fertilizers stored separately from chemicals?	Inorganic fertilizer should be stored separately to prevent cross contamination between fertilizers and chemicals.	Major
6.6.2.6	Are inorganic fertilizers stored in a covered area?	Inorganic fertilizer should be stored in a covered area to protect them from atmospheric influences like sunlight, frost and rain.	Minor
6.6.2.7	Are fertilizers stored off the ground?	Obtain visual evidence that all fertilizer products are stored off the ground to prevent contamination.	Major
6.6.3	**Storage of pesticides and biocides:**		
6.6.3.1	Are chemicals stored in accordance with local regulations?	Chemical storage facilities should comply with all the appropriate current national, regional and local regulations.	Major
6.6.3.2	Are chemical stored in a secure location away from	Chemicals storage facilities should be located in a separate air	Minor

6. CRITERIA FOR CITRUS NURSERY PRODUCTION PRACTICES:

NO	CONTROL POINT	COMPLIANCE CRITERIA	LEVEL
	other chemicals?	space independent from any other materials. Storage facilities should be securely locked at all the times.	
6.6.3.3	Are chemicals stored in a location that is fire resistant?	Chemical storage facilities should be built of materials that are fire resistant.	Minor
6.6.3.4	Are chemicals stored in a location that is well lit?	Chemicals storage facilities should have sufficient natural an artificial light, to ensure that all products labels can be read easily on the shelves.	Minor
6.6.3.5	Are chemicals stored in a location that is well ventilated?	Chemicals storage facilities should have sufficient and constant ventilation of fresh air to avoid build up of harmful vapours.	Minor
6.6.3.6	Is the chemical product store able to retain spillage?	Storage facilities should have retaining tanks to ensure that there cannot be any leakage, seepage and contamination to the exterior of the store.	Minor
6.6.3.7	Are there facilities for measuring chemicals?	Storage facilities should have a filling/mixing area or have measuring equipment, which has been calibrated by a accredited certification body.	Major
6.6.3.8	Are there facilities to deal with spillage?	Storage facilities should be equipped with floor, brush, dustbin and plastic bags and a container of absorbent inert material such as sand.	Major
6.6.3.9	Are keys and access to the chemicals store limited to workers with formal training in the handling of chemicals?	Chemicals storage facilities should be kept locked and access only granted in the presence of persons who can demonstrate formal training in the safe handling and use of chemicals.	Must
6.6.3.10	Is the product inventory documented and readily available?	A stock inventory which indicates the contents (type and quantity) of the store should be available and updated.	Minor
6.6.3.11	Are all chemicals stored in their original package?	All chemicals that are stored should be kept in the original containers and packs, in the case of breakage.	Major
6.6.3.12	Are the chemicals stored approved for use in citrus?	All chemicals stored in the storage area are officially approved and registered for application in citrus.	Must

6. CRITERIA FOR CITRUS NURSERY PRODUCTION PRACTICES:

NO	CONTROL POINT	COMPLIANCE CRITERIA	LEVEL
6.6.3.13	Are liquids not stored on shelves above powders?	Liquids formulations should not be stored on shelves above powder or granular formulations to avoid spillage.	Minor
6.6.4	**Packing and storage areas:**		
6.6.4.1	Are produce handling and storage facilities and equipment cleaned and maintained so as to prevent contamination?	To prevent contamination, produce handling and storage facilities and equipment must be cleaned and maintained according to the cleaning schedule.	Must
6.6.4.2	Are cleaning agents stored to prevent chemical contamination of produce?	Cleaning agents, lubricants should be kept in a designated area, away from where produce is packed, to avoid chemicals contamination of produce.	Minor.
6.6.4.3	Are all forklifts and other driven transport trolleys clean and well maintained to avoid contamination through emissions?	Internal transport should be maintained to avoid product contamination with special attention to fume emissions. Forklifts and other driven transport trolleys should be electric or gas-driven.	Recom.
6.6.4.4	Are packing materials clean and stored in clean and hygienic conditions?	Packing materials including re-useable crates should be stored in a clean and hygienic area, to prevent product contamination until used.	Must
6.7	**IRRIGATION/FERTIGATION:**		
6.7.1	**Water treatment and testing:**		
6.7.1.1	Assess the water quality management plan and determine which steps is in place for ensuing that the water in the nursery is pathogen free?	Assess the total water management plan including steps such as ozone, UV light and heat. The most common treatment method is chlorination.	Major
6.7.2	**Predicting irrigation requirements:**		
6.7.2.1	Is there assess efficacy of irrigation schedule of nursery plants, seedlings etc?	Crop should be irrigated when the soil moisture in the root zone drops to a predetermined level. Soil moisture level can be determined by feeling the soil or soil moisture meters.	Minor
6.7.2.2	Have systematic methods of prediction been used to determine the water requirements of the propagation	Calculations should be available and supported by data records e.g. rain gauges, drainage trays for substrate, water tension	Recom.

6. CRITERIA FOR CITRUS NURSERY PRODUCTION PRACTICES:

NO	CONTROL POINT	COMPLIANCE CRITERIA	LEVEL
	material?	meters (%moisture in the soil) and soil maps.	
6.7.2.3	Is predicted rainfall taken into account when calculating irrigation application?	Documented records should be available of predicted and actual rainfall (rain gauges).	Recom.
6.7.2.4	Is evaporation taken into account when calculating irrigation applications?	The grower is able to demonstrate via documentation which data is used to calculate the evaporation rate and how.	Recom.
6.7.3	**Irrigation/fertigation methods:**		
6.7.3.1	Has the most efficient and commercially practical water delivery system been used to ensure the best utilization of water resources?	The irrigation system used is the most efficient available for the propagation material and accepted as such within good agricultural practices. Provided that irrigation systems should prevent foliar wetting of seedling and trees.	Recom.
6.7.3.2	Is there a water management plan to optimize water usage and reduce waste?	Documented plan should be available which outline the steps and actions to be taken to implement the management plan.	Recom.
6.7.3.3	Are records of irrigation/fertigation water usage maintained?	Records should be kept which indicate the date and volume per water or per irrigation unit. If the grower works with irrigation programmes, the calculated and actual irrigated water volume should be written down in the records, signed off and dated.	Recom.
6.7.4	**Quantity of irrigation water:**		
6.7.4.1	Has an annual risk assessment for irrigation/fertigation water pollution been completed?	The risk assessment must consider potential microbial, chemical or physical pollution of all sources of irrigation/fertigation water. Microbial limits must not exceed the following potable water (SABS 241) parameters: *E Coli*- not detectable. *Thermo tolerant faecal Coliforming* bacteria-10/110ml.	Recom.
6.7.4.2	Is irrigation water analysed at least once a year?	Irrigation water quality assessment should be done more frequently than annual.	Recom.
6.7.4.3	Does a suitable laboratory carry out microbial and chemical analysis?	It should be analysed by an accredited laboratory complying with ISO 17025.	Recom.
6.7.4.4	Is or has untreated sewage water not been used for	Untreated sewage water should not be used for irrigation/	Major

6. CRITERIA FOR CITRUS NURSERY PRODUCTION PRACTICES:

NO	CONTROL POINT	COMPLIANCE CRITERIA	LEVEL
	irrigation/fertigation?	fertigation. Where treated sewage is used, water should comply with the World Health Organization, standards.	Major
6.7.4.5	If contaminated water has been used has a Risk Assessment been done addressing chemical and heavy metal pollutants?	Risk assessment should be done if contaminated water is used. The risk analysis should documented record of any chemical residues and heavy metal contaminants.	Recom.
6.7.4.6	Does the analysis consider the microbial contaminate?	The risk should documented record of the relevant microbial contaminants.	Recom.
6.7.4.7	Have any adverse results been acted upon?	Records should be kept for what actions have been taken and what the results are so far.	Recom.
6.7.5	**Supply of irrigation/fertigation water:**		
6.7.5.1	Has irrigation water been obtained from sustainable sources?	Irrigation water should be obtained from sustainable sources that can supply water under normal conditions e.g. Dam	Recom.
6.7.5.2	Has advice on abstraction been from water authorities?	Documented records should be available e.g. letter, license. Reference: National Water Act, 1998 (Act No. 36 of 1998).	Recom.
6.8	**CHEMICAL PROGRAMME:**		
6.8.1	**Choice of pesticides and biocides:**		
6.8.1.1	Do you follow a recognized disease control program for pest and disease control in general?	Disease control program should be followed in order to control diseases and pests (DoA, CRI & SACNA, 2007).	Major
6.8.1.2	Is the type of plant protection product applied appropriate for the target as recommended on the product label?	All the plant protection products applied to the crop should be suitable and can be justified (according to recommendations label or official registration body).	Major
6.8.1.3	Do nurseries only use plant protection products that are registered in the country of use for the target crop?	All the plant protection products applied should be officially registered by the National Department of Agriculture. Directorate: Food Safety and Quality Assurance (Fertilizers, Farm Feeds, Agricultural Remedies and Stock Remedies Act, 1947 (Act No. 36 of 1947).	Major
6.8.1.4	Are invoices of registered plant protection products	Invoices of the registered chemical products used, must be kept	Minor

6. CRITERIA FOR CITRUS NURSERY PRODUCTION PRACTICES:

NO	CONTROL POINT	COMPLIANCE CRITERIA	LEVEL
	kept for record keeping?	for record keeping and available at the time of the inspection.	
6.8.1.5	Is a current list of plant protection products that are used and approved for use on crops being grown in the nursery kept for recording keeping?	An up to date documented list that takes into account any changes in national plant protection product legislation should be available for the commercial brand names of plant protection products.	Minor
6.8.1.6	Is the choice of plant protection products made by a qualified adviser, can they demonstrate competence?	Where the plant protection product records show that the technically responsible person making the choice of the plant protection products is a qualified adviser, technical competence can be demonstrated via official qualifications or specific training course attendance certificates.	Major
6.8.1.7	If the choice of plant protection products is made by the nursery manager, can competence and knowledge be demonstrated?	If the decision in terms of which chemical to use is made by the nursery manager must show proves of technical knowledge and certified training and course attendance etc.	Major
6.8.2	**Records of chemicals applications:**		
6.8.2.1	Have all the chemicals applications been recorded including the crop name or variety?	All protection product application records should specify the crop or variety treated (Hickey, 1986 & EurepGAP, Version 1.0, 2006).	Major
6.8.2.2	Have all the plant protection product applications been recorded including the location?	All plant protection product application records should specify the geographical area, the name or reference of the nursery, and the field, where the crop is located.	Major
6.8.2.3	Have all the plant protection product applications been recorded including application date?	All plant protection product application records should specify the exact dates (day/month/year) of the application. Record the actual date (end date, if applied more than one day) of application (Hickey, 1986 & EurepGAP, Version 1.0, 2006).	Major
6.8.2.4	Have all the plant protection product applications been recorded including the product trade name?	All plant protection product application records should specify the exact dates (including formulation). It must be possible to connect the trade name information to the active ingredient.	Major

6. CRITERIA FOR CITRUS NURSERY PRODUCTION PRACTICES:

NO	CONTROL POINT	COMPLIANCE CRITERIA	LEVEL
6.8.2.5	Has the operator been identified for plant protection product applications?	The operator for plant protection products should be identified and trained for correct applications of chemicals.	Minor
6.8.2.6	Have all the chemicals applications been recorded including justification for application?	All plant protection applications should be recorded including justification for application.	Minor
6.8.2.7	Have all the chemicals applications been recorded including appropriate information to identify the product quantity applied?	All plant protection application records should specify the amount of product to be applied in weight or volumes or the total quantity of water (or other carrier medium) and dosage in g/l or internationally recognized measures for chemicals.	Minor
6.8.2.8	Have all the chemicals applications been recorded including the technical authorization for application?	Responsible person making the chemicals application recommendation should be identified in the records.	Minor
6.8.2.9	Have all the chemicals application been recorded including the application machinery used?	The application machinery type, for all chemicals applied and the method used e.g. knapsack, high volume, U.L.V., dusting, via the irrigation system, fogger, aerial or other methods) all detailed chemical applied should be recorded.	Minor
6.8.3	**Application equipment management and operational procedures:**		
6.8.3.1	Is plant protection product application machinery kept in good condition and verified annually to ensure accurate application?	The plant protection product application should be kept in good state of repair with documented evidence of up to date maintenance sheets for all repairs, oil changes etc. It should also be verified and correct operation within the last 12 months and prove of verification date and also certification for verification.	Major
6.8.3.2	Is the producer involved in an independent calibration certification scheme, where available?	The producer's involvement in an independent calibration certification scheme should be documented.	Recom.
6.8.3.3	When mixing plant protection products, are the correct handling and filling procedures, followed as stated on the label?	Facilities, including appropriate measuring equipment, must be adequate for mixing plant protection products, so that the correct handling and filling procedures, as stated on the label can be followed (Hickey, 1986 & EurepGAP, Version 1.0, 2006).	Minor

6. CRITERIA FOR CITRUS NURSERY PRODUCTION PRACTICES:

NO	CONTROL POINT	COMPLIANCE CRITERIA	LEVEL
6.8.4	**Disposal of empty chemicals containers:**		
6.8.4.1	Is re-use of empty chemicals containers for purposes other than containing and transporting of the identical product avoided?	Plant protection products containers should not be re-used and documented.	Minor
6.8.4.2	Does disposal of empty plant protection product containers occur in a manner that avoids exposure to humans?	System should be in place to dispose empty chemicals containers and ensures that persons cannot come into physical contact with the empty containers by having secure storage point, safe handling system prior to the disposal.	Minor
6.8.4.3	Does disposal of empty chemicals occur in a manner that avoids contamination of the environment?	Disposal of empty chemicals containers should minimize the risk of contamination of the environment, water courses, flora and fauna.	Major
6.8.4.4	Are official collection and disposal systems used when available?	Where official and disposal exist, there should be documented records of participation by the producer.	Minor
6.8.4.5	If there is a collection system. Are the empty containers adequately stored, labelled and handed to the rules of collection?	All the empty chemicals, once emptied, are not re-used and have been adequately stored. Labelled and handled according to the requirements of official collection and disposal schemes where applicable.	Minor
6.8.4.6	Are empty containers kept secure until disposal is possible?	There should be secure store point for all empty plant protection products containers prior to disposal that is isolated from the crop and packaging materials e.g. permanently signed and with physically restricted access for persons and fauna.	Minor
6.8.4.7	Are all local regulations disposal or destruction of containers observed?	All the relevant national, regional and local regulations and legislation if exists, has been complied with regarding the disposal of empty chemicals containers.	Major
6.9	**WASTE AND POLLUTION MANAGEMENT**		
6.9.1	**Identification of waste and pollutants:**		
6.9.1.1	Have all possible waste products been identified in all	Waste products by the production process have to be identified,	Recom.

6. CRITERIA FOR CITRUS NURSERY PRODUCTION PRACTICES:

NO	CONTROL POINT	COMPLIANCE CRITERIA	LEVEL
	areas surrounding the nursery?	documented and catalogued.	Recom.
6.9.1.2	Have potential sources of pollution been identified?	Potential sources of pollution e.g. fertilizer excess, exhaust smoke for heating units has to be catalogued and documented for all the process.	Recom.
6.9.2	**Waste and pollution management action plan:**		
6.9.2.1	Are the nursery and premises clear of litter and waste?	Insignificant litter and waste on the designated areas are acceptable as well as the waste from current day's work. Other litter has to be cleared up. Areas where propagation materials are handled indoors have to be cleaned at least once a day.	Recom.
6.9.2.2	Is there a documented plan to avoid or reduce wastage and pollution and avoid the use of landfill or burning, by waste recycling?	A comprehensive, current documented plan that covers wastage reduction, pollution and waste recycling is supposed to be available.	Recom.
6.9.2.3	Has waste management plan been implemented?	Visible actions and measures at the nursery that confirm that the objectives of the waste and pollution action plan are being carried out.	Recom.
6.9.2.4	Do the premises have adequate provisions for waste disposal?	Designated areas to store litter and waste should be available. Different types of waste are identified and stored separately. Empty chemicals containers are rinsed with water crushed and stored in a secure area or room until disposal unless they are returnable to the distributor.	Recom.
6.10	**NURSERY HYGIENE:**		
6.10.1	**Risk Assessments:**		
6.10.1.1	Has a risk assessments for safe and healthy working conditions been carried out?	Risk assessment should be documented and based in national, regional and local legislation and sectorial agreements.	Recom.

6. CRITERIA FOR CITRUS NURSERY PRODUCTION PRACTICES:

NO	CONTROL POINT	COMPLIANCE CRITERIA	LEVEL
6.10.1.2	Has this risk assessment been used to develop an action plan to promote safe and healthy working conditions?	Action plan should be documented and refers to the non-compliance, the action to be taken with a timetable and the person responsible.	Recom.
6.10.2	**Facilities, equipment and accident procedures:**		
6.10.2.1	Are First Aid boxes present in the vicinity of the work?	Complete First Aid boxes according to national regulation and requirements must be available and accessible in the vicinity of the work.	Recom.
6.10.2.2	Are hazards clearly identified by warning signs?	Permanent and legible signs must indicate potential hazards e.g. waste pit, fuel tanks etc.	Recom.
6.10.2.3	Do accident and emergency procedures exist?	Written procedures must describe how to act in the event of an accident or emergency.	Minor
6.10.2.4	Are signs warning of potential dangers placed on access doors?	Permanent and clear hazard warning signs must be placed on or next to the access doors of the crop protection product and fertilizer storage facilities.	Minor
6.10.3	**Nursery sanitation:**		
6.10.3.1	How is the nursery fenced?	The nursery needs to be fenced to control any traffic through the nursery. The number of entrances into the nursery must be limited.	Major
6.10.3.2	Are footbaths installed in the nursery and how are they maintained?	Footbaths need to be installed at the entrances into the nursery. Where vehicles enter a vehicle dip needs to be constructed. Both the footbaths and vehicle dips need to contain copper oxychloride powder or copper solution. Footbaths must be constructed so that people who enter the nursery must go through them.	Major
6.10.3.3	What sanitation rules are implemented in the nursery?	Trees in the nursery can easily be infected with pathogens introduced by personnel. The following guidelines should be	Major

6. CRITERIA FOR CITRUS NURSERY PRODUCTION PRACTICES:

NO	CONTROL POINT	COMPLIANCE CRITERIA	LEVEL
		noted: All equipment entering or leaving the nursery must be clean of all plant material and decontaminated in accordance with Department of Agriculture procedures using approved decontamination products, Budding knives, clippers and other cutting implements shall be sterilized, all persons entering an approved structure shall walk through a sanitizing foot bath containing a decontaminant that is approved by Department of Agriculture such as copper sulphate, plant parts or sawdust, manure, soil entering the approved site for the production of commercial citrus nursery stock must be accompanied by Nematode Certificate, Clean clothes need to be worn in the nursery. Hands are to be washed before the commencement of work with soap and water. No feet, shoes, dirty implants must be placed on a tree or surface on which a trees stands. All surfaces on which trees are to stand must be swept and the sprayed with a 300g. 100 L copper oxychloride mixture. Nursery area and parameter shall remain weed free. The tips or heads of hose pipes used in the nursery must not touch the ground. Dust must be controlled in and around the nursery. Planting of suitable windbreaks and even lawn around the nursery is encouraged. No trees, once removed from the nursery are allowed back in the nursery.	
6.10.3.4	What steps are taken to ensure good sanitation in the seed germination area?	The equipment used in any germination room or area must be sterilized regularly. Hands must be washed before handling any seeds or plants. Ideally any personnel working in the room should put on a clean dust jacket before going into the room or area.	Major
6.10.4	**Weed management:**		

6. CRITERIA FOR CITRUS NURSERY PRODUCTION PRACTICES:

NO	CONTROL POINT	COMPLIANCE CRITERIA	LEVEL
6.10.4.1	What are the factors to be considered when developing a weed management program?	The following need to be considered: The application site which involves tree age, soil type, types of weeds present, the stage of weed growth, herbicide selection, spray nozzle, spray volumes and amounts of herbicides used. All these factors will directly affect cost and the success of the weed management program.	Major
6.10.4.2	How do you control weed management in citrus nursery?	There are many approaches one can use to suppress and control weeds within the grove. Preventative programmes which entails the use of practices such as sanitation, spot spraying or hand labour to prevent the source of weed infestation from spreading through the area. Mechanical programme which involves tillage or cultivation which are effective method of controlling annuals weeds. Chemical weed control which vary from location to location depending on the soil type, variety, method of herbicides application and specific weed species. Two groups of herbicides, soil-applied preemergence herbicides that should be applied to fairly clean soil surfaces prior to weed emergence and foliar applied post emergence herbicides that are applied after germination of weed seed. Chemical mowing to suppress the weed seed.	Major
6.10.4.3	How do you make sure that correct application of herbicides is in place?	Successful herbicides programs start with selecting the right herbicides or herbicides mixtures. All herbicides have label that state the requirements, application rates; weed controlled and personal protective equipments required during mixing or application. Equipments used must be calibrated and one have to remember the label is the law and must be followed in order to avoid environmental contaminations.	Major
6.10.4.4	How to avoid herbicides resistance?	Through avoiding repeated use of the same pesticides in the same area in order to reduce the risk of herbicides resistance.	Major

6. CRITERIA FOR CITRUS NURSERY PRODUCTION PRACTICES:

NO	CONTROL POINT	COMPLIANCE CRITERIA	LEVEL
6.11	**WORKERS HYGIENE, SAFETY AND WELFARE:**		
6.11.1	**Type of training and records:**		
6.11.1.1	Has formal training or instruction been given to all workers operating dangerous or complex equipment?	Records indicating training program are in place and that there is a copy of the attendances certificates or signed lists of workers who attend a training course. Records should also indicate subcontract service providers.	Minor
6.11.1.2	Is a record of training kept for each worker?	A record for each worker should be kept which contains the required training programmes and a copy of certificates.	Recom.
6.11.1.3	Is there a person trained in First Aid at the nursery?	At least one person should receive training in First Aid. Applicable Legislation in First Aid training must be in place for referral purposes.	Recom.
6.11.1.4	Has all workers received basic hygiene training for the handling of propagation material regarding hand cleaning, skin cuts, smoking, eating and drinking in permitted areas?	All employees both new and old should receive training in hygienic and all training should be documented.	Recom.
6.11.1.5	Are all subcontractors and visitors aware of the relevant demands personal hygiene?	Evidence for visitor personal hygiene procedures and requirements should be officially communicated to visitors and subcontractors.	Recom.
6.11.2	**Personal Hygiene:**		
6.11.2.1	Have workers received basic instructions in hygiene before handling produce?	Evidence that workers received hygiene training should be recorded. This includes personal cleanliness and also personal behaviour e.g. smoking and eating.	Major
6.11.2.2	Do the workers implement the hygiene instructions for handling produce?	Evidence should be shown to the auditor that workers are complying with the hygiene instructions.	Minor
6.11.2.3	Are all workers wearing outer garments that are clean and fit for purpose for the operation and able to protect products from contamination?	All workers in the nursery should wear outer garments that are clean and fit for operation according to the risk analysis.	Recom.

6. CRITERIA FOR CITRUS NURSERY PRODUCTION PRACTICES:

NO	CONTROL POINT	COMPLIANCE CRITERIA	LEVEL
6.11.2.4	Are smoking, eating, chewing and drinking confined to designated areas separated from plant materials?	Smoking, eating, chewing and drinking should confine to designated areas and are never allowed in the plant material or storage areas.	Minor
6.11.2.5	Are signs clearly displayed in the picking facilities with the main hygiene instructions for workers and visitors?	Signs with the main hygiene instructions must be visible displayed in the nursery.	Minor
6.11.2.6	Do workers in the nursery have access to clean toilets and hand washing facilities?	Toilets in good state should be available and hand washing facilities containing non-performed soap and dry facilities.	Major
6.11.3	**Crop Protection product handling:**		
6.11.3.1	Are the workers who handle and apply crop protection products trained?	Workers have to be trained on handling and applying crop protection via official qualifications or specific training course attendance certificates.	Minor
6.11.3.2	Are all staff has contact with crop protection products submitted voluntary to annual health checks in line with guidelines laid down in local codes of practice?	If applicable, health checks to which all staff which has contact with crop protection products are voluntary submitted comply with national, regional or local codes of practice.	Recom.
6.11.4	**Protective clothing/equipment maintenance and handling:**		
6.11.4.1	Are workers equipped with suitable protective clothing in accordance with label instructions?	Workers should be equipped with a complete sets of protective clothing (rubber boots, waterproof clothing, overalls, rubber gloves, face marks etc).	Major
6.11.4.2	Is protective clothing cleaned after use?	Protective clothes should be cleaned after use to avoid spread of diseases.	Minor
6.11.4.3	Is protective clothing and equipment stored separately from crop protection products?	All protective clothing and equipment including replacements filters etc., should be stored apart and physically separate from the crop protection products in a well-ventilated area.	Major
6.11.5	**Workers health, safety and welfare:**		
6.11.5.1	Is a member of management clearly identifiable as	Documentation is supposed to be available that demonstrates	Minor

6. CRITERIA FOR CITRUS NURSERY PRODUCTION PRACTICES:

NO	CONTROL POINT	COMPLIANCE CRITERIA	LEVEL
	responsible for workers health, safety and welfare issues?	that a clearly identified, named member of management for ensuring compliance with existing, current and relevant national and local regulations on workers health, safety and welfare issues.	
6.11.5.2	Do regular two-way communication meetings take place between management and employees? Are there records from such meetings?	Records that concerns about workers health, safety and welfare should be recorded in meetings planned. The auditor is not required to make judgments about the content, accuracy or outcome of such records.	Recom.
6.11.5.3	Are your employees aware and comply with Occupational Health and Safety Act, 1993 (Act No. 85 of 1993?	Employee should be aware and familiarized themselves with the Occupational Health and Safety Act, 1993 (Act No. 85 of 1993, which provide the health, and safety of persons at work and for the health and safety with the use of plant and machinery.	Regulatory requirement
6.11.6	**Visitor safety:**		
6.11.6.1	Are all visitors aware of the relevant demands on personal safety?	The nursery manager shall show evidence that personal safety procedures have been followed and are officially communicated to the visitors.	Recom.
6.11.6.2	Are records available of visitors and is it signed?	Record of visitor's attendance shall be available and signed.	Recom.

7. PROTOCOL FOR DETECTING PATHOGENS:

NO	CONTROL POINT	COMPLIANCE CRITERIA	LEVEL
7	**PROTOCOL FOR DETECTING PATHOGENS:**		
7.1	**Monitoring for the presence of *Phytophthora:***		
7.1.1	When is the right time to test soil for the presence of *Phytophthora?*	Being a soil borne pathogens, soil should be sampled for analysis prior to planting to determine the status of the soil.	Major
7.1.2	Has a *Phytophthora* detection system been established?	Through visual signs such as chlorotic symptoms, stunted in	Minor

7. PROTOCOL FOR DETECTING PATHOGENS:

NO	CONTROL POINT	COMPLIANCE CRITERIA	LEVEL
		plant growth and poorly developed root system. The use of leaf baiting in soil or water samples is useful for detecting contamination of irrigation water in nurseries and for checking nursery crops for infestation. *Phytophthora* can also be detected through the use of ELISA, whereby fibrous roots are cut into 0.5 inch lengths and mixed thoroughly (Maseko & Coutinho, 2002).	
7.1.3	Which precaution measures are in place to minimize the impact of *Phytophthora* disease?	One have to make sure that disease free planting material should be selected for planting, avoidance of low budding, use of susceptible rootstock, flood irrigation and flat bed system. Tree trunk should not be allowed to come into contact with irrigation water, regular monitoring for the disease symptoms should be done to control the disease at its initial stages. In doubt of the disease soil and infected samples should be checked in laboratory for *Phytophthora* http://edis.ifas.ufl.edu.	Major
7.1.4	Has an effective *Phytophthora* preventative programme been established in the nursery?	*Phytophthora* control is an integrated approach, which includes use of resistant rootstocks, appropriate cultural practices and use of chemicals such as Fosetyl-Al, Mefenoxam and application of Bordeaux paste http://edis.ifas.ufl.edu.	Major
7.2	**Monitoring for the presence of Nematodes:**		
7.2.1	When is the right time to test for the presence of nematode?	Before you plant anything in the area, soil sample should be drawn for laboratory analysis to determine the status of nematode (Kleynhans *et al.,* 1996).	Major
7.2.2	What can be done to determine potential nematode problems in a field before it is planted?	Samples from several areas in the same field where nematodes disease is suspected and placed in the bag for laboratory analysis (Kleynhans *et al.,* 1996).	Major
7.2.3	What are the symptoms of citrus nematodes damage on citrus plants?	Citrus nematode are not like gall-forming nematode, their symptoms are often non-descript and difficult to diagnose.	Major

7. PROTOCOL FOR DETECTING PATHOGENS:

NO	CONTROL POINT	COMPLIANCE CRITERIA	LEVEL
		Citrus nematode causes gradual deterioration of older trees, which leads to loss of vigour and yield. Leaves turn dull green and smaller than normal. Small twigs die back, resulting in a sparse canopy. Symptoms can be confused with nutrient deficiency symptoms (Timmer *et al*., 2000; Mukhopadhyay, 2004).	
7.2.4	Has a citrus nematode detection system been established?	Citrus nematode damage is difficult to detect with the naked eye and is mainly detected through a sparse feeder root system and the clinging of sand particles to the roots when the roots are washed in slow flowing water (Timmer *et al.,* 2000).	Major
7.2.5	Has an effective nematode preventative programme been established?	Dissemination of nematodes is often due to infested nursery stock obtained from infested nurseries where nematodes are spread from trees to trees by flood irrigation. Therefore, use of certified nematode-free planting stock approved by the Citrus Improvement Programme is the simplest method of avoiding the introduction of nematodes into the nursery. Disinfest equipment prior use in non-infested nursery by removing soil particles with high pressure water pipe. Decontamination of irrigation water through the use of filtration system (Timmer *et al*., 2000; Mukhopadhyay, 2004).	Major
7.2.6	How are nematodes managed or control in the nursery?	Nematodes management in citrus nursery relies heavily on exclusionary measures, use of resistant rootstocks, cultural practices, and preplant and post plant nematicide treatment such as Temik 15G and Nemacur 3 (Kleynhans *et al.,* 1996).	Major
7.2.7	What is considered to be a proper sample for citrus nematode detection?	A proper sample for citrus nematode detection is a minimum of 1 quart of the combination of roots and soil from each of the	Minor

7. PROTOCOL FOR DETECTING PATHOGENS:

NO	CONTROL POINT	COMPLIANCE CRITERIA	LEVEL
		diseased and healthy areas. The bag should be sealed immediately and kept in a cool area (Kleynhans *et al.,* 1996).	
7.2.8	How is the sample bags marked?	Label each sample bag properly with the grower's name, address, sample location, date, crop history, existing crop and the next crop proposed for the field in this case will be citrus trees (Kleynhans *et al.,* 1996).	Minor
7.2.9	Which method is used to transport samples?	Carrier, any vehicle or post provided that samples taken are in a container, which will not allow overheating and desiccation.	Minor
7.2.10	Which methods are used to diagnose nematodes in the laboratory?	The following methods are used for the detection of nematodes, Baermann-tray method, sieving centrifugal-flotation and sieving sedimentation (Kleynhans *et al.,* 1996). The choice of filtering after sieving depends primarily on whether the species can pass through the filter. Root samples can be processed by incubation or maceration. Incubation takes several days and is less expensive and maceration takes only several minutes.	Major
7.3	**Monitoring for the Presence of Tristeza virus:**		
7.3.1	What are the typical symptoms caused by Citrus Tristeza Virus (CTV)?	CTV severity vary markedly and may cause vein clearing, vein corking, leaf cupping and stem pitting in some citrus varieties regardless the rootstock used. Twigs are brittle and the bark is abnormally thick and growth is markedly reduced. Leaves are often chlorotic and dieback may occur (Garnsey *et al.,* 1998).	Major
7.3.2	How is CTV transmitted into the nursery?	CTV is usually transmitted via the movement of the infected budwood or nursery stock. CTV is also transmitted by several species of aphids. The brown citrus aphid (*Toxoptera citricida*), melon aphid (*Aphis gossypii*) and the spirea aphid (*Aphis spiraecola*). CTV can also be transmitted on contaminated tools in the field (Rocha-Peña *et al.,* 1995).	Major

7. PROTOCOL FOR DETECTING PATHOGENS:

NO	CONTROL POINT	COMPLIANCE CRITERIA	LEVEL
7.3.4	Has a CTV be detection system been established?	CTV can be readily identified by looking for pits in the trunk and branches in the field. For conformation of the CTV infection, biological indexing, serological tests and molecular tests must be performed (Roistacher & Moreno, 1991).	Major
7.3.5	Has an effective CTV preventative programme been established?	CTV is a vector-borne disease. Control of CTV is difficult if inoculum sources are widespread and aphid vectors are well established. The first line of defence in areas where CTV is not yet widespread are quarantine and budwood certification programme. The best method to check these diseases is to control the aphid population by application of insecticides and by the use of non-virulent cross protective Tristeza strains in the nursery and also plantations (Garnsey *et al.,* 1998).	Major
7.4	**Monitoring for the presence of insect and pest:**		
7.4.1	Which pest and insect causes economic damage in the citrus nursery?	Major pests of citrus are thrips, aphids, psyllids, whiteflies, scales, mealybugs, leaf miners, butterfly, fruit fly mites, snails, ants, leafhoppers and midges (Browning *et al.,* 1996).	Major
7.4.2	Has pest and insects detection system been established in the nursery?	**Thrips** (*Heliothrips haemorroides* Bouche, *Scirtothrips citri* Moulton and *Scirtothrips aurantii* Faure: Symptoms appearance depends on the degree of attack and the growth stage of the plants. Young leaves become thickened, distorted; shoot apex turns black, dies and falls off. Injury to flush foliage lead to misshapen and curled leaves. **Aphids** (*Aphis citricola, Aphis gossypii, Myzus persicae, Toxoptera aurantii, Toxoptera citricidus and Sinomeougoura citricola*: Aphids colonize the leaves whereby it expand and covering veins and petioles. Severe infestation leaves become deformed; curled, pale yellow and young shoots become twisted	Major

7. PROTOCOL FOR DETECTING PATHOGENS:

NO	CONTROL POINT	COMPLIANCE CRITERIA	LEVEL
		and wither. **Psyllids** (*Diaphorina citri and Trioza erytreae*): *D. citri* cause twisting of the leaves and *T. erytreae* lead to formation of characteristic bumps on the under surface of leaves. **Whiteflies** (*Aleurocanthus woglumi, Aleurothrixus floccosus, Dialeurodes citri and Dialeurodes citrifolii): Aleurocanthus woglumi* produce honeydew that contributes to the sever accumulation of sooty mold fungus. **Scale insects**: scale insects are sessile, small and often inconspicuous (Spiegel-Roy & Goldschmidt, 1996).	Major
7.4.3	Has an effective pest and insects preventative programme been established?	**Thrips:** monitoring and trapping of thrips is done by trapping the adults at the stage of their emergence from the pupae. No cultural control technique utilized for citrus thrips. A number of natural enemies attack citrus thrips including mites, spiders, lacewings and bugs. Chemical used for the control of thrips are abamectin, temephos, dimethoate, formetanate, pyrethroids and acephate. **Aphids:** natural enemy such as *T. citricidus* is used to control aphid's population in the nursery. **Scale insects:** Chemical treatment remained the reliable means of controlling scales. Chemicals such as Chloropyrifos, Pyriproxyfen, malathion and azimpos methyl. **Mealbugs:** use of selective insecticides such as chloropyrifos used	Major

7. PROTOCOL FOR DETECTING PATHOGENS:

NO	CONTROL POINT	COMPLIANCE CRITERIA	LEVEL
		for citrus thrips control will often prevent problems with mealbugs. **Mites:** chemicals such as Dicofol, abamectin wettable sulphur are used for the control of mites (Browning *et al.,* 1996).	
7.5	**Monitoring for the presence of citrus greening:**		
7.5.1	What are the symptoms of citrus greening disease?	Foliage symptoms of citrus greening are the blotchy mottling of leaves and leaf yellowing that may appear on a single shoot or branch. Other symptoms include twig dieback, poor flowering and stunted growth (Garnier & Bove, 2000).	Major
7.5.2	How is citrus greening transmitted?	Citrus greening is transmitted by two species of psyllids. One species, *Trioza erytreae*, occur in Africa and *Diaphorina citri* (De Graca, 1991; 2000).	Major
7.5.3	How to detect citrus greening in the nursery?	Citrus greening can be detected by the yellow shoot symptom. It can also be detected by grafting onto citrus indicators but may require multiple tests and use of Electron microscopy to confirm the presence of citrus greening disease and PCA-based assays have been developed for identification of greening disease (Garnier & Bove, 1993; 2000).	Major
7.5.4	How to control citrus greening diseases?	The general control strategy is to eradicate all existing sources of citrus greening disease and replant with citrus greening-free trees grown from clean budwood. Psyllid populations must also be reduced as much as possible through biological control (Garnier & Bove, 2000; Hung *et al.,* 2000).	Major

8. DETERMINATION OF CITRUS BLACK SPOT (CBS) STATUS OF NURSERY TREES:

NO	CONTROL POINT	COMPLIANCE CRITERIA	LEVEL
8.1	**Adjacent CBS infected orchards:**		
8.1.1	How do you prevent infection from adjacent CBS infected orchards?	Planting of windbreaker help in the prevention of CBS from adjacent infected orchards and effective chemical spray programmes, removal of litter (Swart, 2004).	Major
8.1.2	How effective is the windbreaker in preventing CBS infection to your nursery?	Windbreaker helps in reducing dehydration, eroding and abusive effects of strong winds. Also harbor insects and diseases such as CBS that could be injurious to the nursery crops (Davidson *et al.,* 1988).	Minor
8.2	**Inspection (detection and testing) of CBS:**		
8.2.1	Is there a system in place to assess the presence of CBS in the adjacent and nursery?	An effective CBS assessment protocol is required to ensure that trees are inspected for the presence of the disease (Swart, 2004).	Recom.
8.2.2	Is there a corrective action plan to minimize the impact of CBS?	A comprehensive, documented plan that covers preventative measures to minimize the impact of CBS, through following a spray programme and sanitation measures such as removal of dead leaves lying around or hidden between bricks and plastic containers (DoA, CRI & SACNA, 2007).	Major
8.3	**Sampling for CBS:**		
8.3.1	Has a formal training been given to all workers with regards to CBS identification of leaf samples?	Records indicate that the required instructions and training program are in place and that there is a copy of the attendance at the training or a signed list of workers who attended a training course. Records to include sub contracted service providers.	Minor
8.3.2	Which procedure do you follow before sampling?	Before leaves are sampled, nursery should be inspected. Note the distance from infected area, wind direction and irrigation system. Wetting of leaves in the nursery should be avoided.	Major
8.3.3	Which equipment is used to sample leaves for detecting CBS?	A set of knives and secateurs must be kept for use provided that all cutting tools are sterilized correctly to effectively kill pathogens present.	Major

8. DETERMINATION OF CITRUS BLACK SPOT (CBS) STATUS OF NURSERY TREES:

NO	CONTROL POINT	COMPLIANCE CRITERIA	LEVEL
8.3.4	How many leaves must be sampled for detecting CBS?	Samples of 2000 leaves should be collected randomly from the sample units for analysis of Citrus Black Spot. The size and number of samples also depend on the feasibility of the sampling procedure and costs or select 5-8 leaves with visibly developed pynicida or perithecia from the bulk sample. The experience of the sampler is paramount.	Major
8.3.5	Which sampling methods are used for detecting CBS?	Methods such as ascospores trapping culture from suspected lesions and PCR tests may be used to detect presence of CBS in the nursery.	Regulatory requirement
8.3.6	How were the sample block identified in the nursery?	The unique sample code, the block position in the nursery, the details of the rootstock and age, the number of trees in the block, the number of sub-samples taken and result of the analysis.	Major
8.3.7	How were the samples packed and dispatched to the diagnostic center?	Sampled leaves must be placed in clean and new brown paper bag and kept cool at room temperature until processing in the laboratory. Sample bags must be placed in a suitable container for dispatching by post or courier to an approved accredited Diagnostic Center. Dispatching forms must be included in the container before sending to the diagnostic center.	Major
8.3.8	How is the sample bags marked?	Each sample should be clearly labelled with an adhesive white label, on which the following details is recorded in pencil: Permit application reference number, Date of picking, nursery name, cultivar/rootstock combination, number of trees in batch and nursery's batch number (DoA Regulations).	Major
8.3.9	How is the information for tracebility purpose written in the sample for CBS analysis?	All the relevant information should be written on a plastic with pencil and placed inside the bag with the leaves. The samples must reach the laboratory as soon as possible (preferable within	Minor

8. DETERMINATION OF CITRUS BLACK SPOT (CBS) STATUS OF NURSERY TREES:

NO	CONTROL POINT	COMPLIANCE CRITERIA	LEVEL
		24 hrs) for the wet drying process. It is not advisable to place the samples under refrigeration because it delays the process.	Minor
8.3.10	Which method is used to transport samples to the laboratory for analysis?	Courier or post and also closed or tarpaulin vehicles. Not an open bakkie where the samples will be exposed to wind circulation and contamination of further spores. If fast transport service is used the nurseryman must specify the transport conditions and obtain evidence that it has been adhered too. Also make an appointment with the accredited laboratory to receive the samples according to their samples receivable procedures.	Major
8.4	**Laboratory analysis:**		
8.4.1	Which laboratory is the nursery using to analyze sampled leaves?	An accredited laboratory will be used to detect the presence of CBS.	Major
8.4.2	How is the leaf sample drawn prepared for laboratory analysis?	1) Wash leaves in running water to remove dirt and drain to remove excess water. 2) Air dry leaves for 12 hours out of direct sunlight or air dry leaves for 2 to 4 hours in direct sunlight. 3) Soak air-dried leaves in tap water for 30 minutes, drain to remove excess water and place in a 20 micron clear plastic bag. 4) Closed bag should be placed with leaves in an incubator at 42°C for 6 hours. 5) After 6 hours remove the bag from the incubator and mix leaves by shaking the bag. 6) Open the bag to allow leaves to air-dry and incubate under fluorescent and near UV light for 18 hours.7) Repeat steps 3 to 6 for at least 21 days or until ample fructification of Guignardia citricarpa is visible on the leaf surface. 8) It is important to monitor the moisture within the bag closely, since no fruiting bodies will develop if the leaves are too dry and the leaves will rot if it is wet. Correct moisture levels are only known through	Major

8. DETERMINATION OF CITRUS BLACK SPOT (CBS) STATUS OF NURSERY TREES:

NO	CONTROL POINT	COMPLIANCE CRITERIA	LEVEL
		experience.	
8.4.3	How do you process a leaf sample for pathogens analysis?	Care should be taken to prevent cross-contamination of samples and steps should be conducted in a laminar flow cabinet, one batch processed at a time, followed by sterilization of all equipment and flow cabinet work surface with 95% ethanol.	Major
8.4.4	Which equipment is used to select leaves samples?	Using the Unicore 1.2 mm diameter punch, which remove 20-25 maximum leaf pieces from the selected leaves; 1.5 ml Eppendorf tube which grind leaf sections to a very fine powder in the Eppendorf tube; using the plant mini DNA extraction kit (Qiagen).	Major
8.4.5	How do you detect the presence of CBS after testing the received sample?	Observation of symptoms.	Major
8.4.6	Are the results of the analysis recorded?	Results of all samples received and processed should be recorded. Aspects that should be noted include label details, dates received and processed, conditions of samples, results, in order to take corrective and preventive actions if the results show CBS infection.	Minor

9. NORMATIVE DOCUMENT

9.1 NURSERY PROTOCOLS:

The goal to having a nursery protocol is to establish a set of procedures that are used to find and eradicate the pathogens quickly and effectively if present in the nurseries. A detailed and thorough inspection should take place at the nursery to identify the presence of pathogens. Areas of consideration include: nursery stock, plant materials, soil and water usage and hygienic conditions within the nursery. The nursery management must ensure that work instructions are clearly displayed at all stations in the nursery and are available for quick referencing. The nursery management must make sure that no samples are taken from any tree that has been treated with a fungicide (Roxburgh, 1997).

Protocol 1: Nursery Sanitation Rules

1.1 Purpose of nursery sanitation rules: Citrus nursery stock in the nursery can easily be infected with pathogens introduced by animals or personnel and care must be taken that this does not happen (Roxburgh, 1997). Therefore, development of nursery hygiene practices is of paramount importance in reducing the spread of pests and disease; improving the efficacy of pest and disease control strategies and in particular, to educate nursery employees regarding the importance of good hygiene practices. The following guidelines, as suggested by Roxburgh (1997) should be considered:

(i) Clean clothes need to be worn in the nursery. It is adviserable to have a set of nursery dust jackets or overalls that the personnel only wear in the nursery which is regularly cleaned.

(ii) Hands must be washed before the commencement of work with soap and water. Through the day hands need to be washed regularly especially when moving from one task to another and when returning from tea or lunch breaks.

(iii) Feet, shoes or dirty implements should never come into contact with any surface where nursery stock is placed.

(iv) All implements to be used in the nursery are exclusively for use in the nursery. All implements must be washed thoroughly on a daily basis before being stored in a clean storage area.

(v) All surfaces on which nursery stock are to stand must be swept clean and then sprayed with a 300g/100 L copper oxychloride mixture or with any other effective and registered surface sterilant.

(vi) All dying or dead plant material must be removed from the nursery when observed. Weeds must be controlled in the nursery.

(vii) The tips or heads of hose pipes use in the nursery must never touch the ground or dirty surfaces.

(viii) Dust must be controlled in and around the nursery. Planting of suitable windbreaks around the nursery is encouraged.

(ix) No trees, once removed from the nursery, are allowed back in. If a tree falls off the structure on which it is standing, it should be destroyed and not put back onto the structure.

(x) Equipment such as germination frames which is used in a germination room or area must be sterilized regularly. Any effective registered sterilant can be used. Hands must be washed before handling any seeds or plants. Ideally all personnel working in the room should put on a clean jacket or overalls before going into the germination room or area.

Protocol 2: Protocol for Water Treatment and Testing

2.1 Purpose of water treatment and testing: The quality of irrigation water is very important in the production of citrus in the nursery. Contaminated or poor quality water can lead to further spread of pathogens. Therefore, the main aim of this protocol is to ensure that all water used in any part of the nursery must be tested for pathogens prior to use. Clean water is an important criteria for nursery accreditation (Roxburgh, 2003).

2.2 Method of sampling and analysis of water treatment: Various treatment methods are available to ensure that the water used in the nursery is considered to be pathogen free. Treatment methods such as ozone (Moens *et al.,* 1991; Runia & Amsing, 1996), ultra violet (Grech *et al.,* 1989; Moens & Hendrickx, 1989; Runia, 1994; Amsing & Runia, 1995; Hallman *et al.,* 2005), light and heat treatment (Runia & Amsing, 2001a; b; Hallman *et al*., 2005) and chlorination (Grech & Rijkenberg, 1992; Runia, 1995; Roxburgh, 1997) may be considered. The later treatment is the most common. The following steps need to be taken into consideration for effective chlorination of water. Flocculate if the water is dirty; filter the water before pumping into the dam or tank in which the chlorination will be done; the pH must be adjusted to 7.2 using any suitable registered chemical; add chlorine to the water and mix well; allow the water and chlorine to stand for 30min and then test the free chlorine with a DPD-FAS free Chlorine test kit. The level of free chlorine must be 3-6ppm and test the chlorinated water for a minimum of five times a year with a spore trap.

2.2.1 Flocculating water: Measure out the correct flocculant e.g. Alum for the size of the reservoir. Fill the flocculating reservoir until it is 90% full (Roxburgh, 1997). Then sprinkle the flocculant evenly over the water while the last 10% of the water is being pumped in. The water entering the flocculating reservoir must be pumped through a screen filter (1mm) to remove large dirt particles (Moens & Hendrickx, 1992). The water should stand for a minimum of 24 hrs to settle out. After 24 hrs check to see if the water is clear enough e.g. an object ±500mm below the surface must be visible. If not clear enough allow standing for a further 12-24 hrs.

2.2.2 Chlorination of water for *Pythium* and *Phytophthora*: Chlorination is the treatment of irrigation water for control of plant pathogens such as *Pythium* and *Phytophthora* (Cook & Devine, 1979, Grech *et al.,* 1989) as well as many other diseases (Brown *et al.,* 1986; Datnoff *et al.,* 1987). Water is pumped from the flocculating reservoir or water source via a slow sand filtration to the chlorinating reservoir as part of the purification process of irrigation water treatments (Ellis, 1985). Sand filtration can, however, serve as the first step in a combination filter system to remove most organic contamination products from the water. Ensure that the inlet of the reservoir is positioned in such a way that it will stir the water or have a system that can circulate the water in the reservoir. It is important that the reservoir should be covered. Measure out the correct amount of chlorine (Calcium hypochlorite or Sodium hypochlorite) for the size of the reservoir (Roxburgh, 1997). Fill the chlorinating reservoir until it is 90% full. High chlorine levels may be phytotoxic to certain crops or can restrict root development (Runia, 1995). Sprinkle or add the chlorine evenly into the water while the last 10% of the water is being pumped in. After the reservoir is filled to capacity, allow the water to circulate freely until the chlorine is well mixed. Let the water stand for 30min before measuring the free chlorine level with a DPD-FAS free Chlorine test kit (Ford, 1980). It must be between 3-6ppm. If the chlorine level is not 3ppm or higher more chlorine should be added, the water mixed and tested again after 30min.

2.2.3 Water test for *Phytophthora* and *Pythium*: South Africa is heavily dependent on surface water for irrigation and as agriculture intensifies the problem of contaminated irrigation water is on the increase (Hugo & Malan, 2006). It is important that nursery water must be tested for a minimum of five times a year with a *Phytophthora* spore trap. A spore trap consists of a round screw top plastic container with 6-10, 4mm holes drilled into the container 15mm from the top (Roxburgh, 1997). A hole is drilled in the centre of the lid through which a length of spaghetti tube is pushed and a 2 L/h dripper is attached to its end. The dripper will allow water to drip slowly into the container and run out of the 4 mm holes.

2.2.3.1 Installation of Phytophthora spore trap (Roxburgh, 1997)**:** Install the spore trap so that it will receive water every time the nursery is irrigated. The pressure in the line must not exceed 150 KPA. Place the spore trap where it will not be contaminated by soil or any other sources of pathogens e.g. run off water, next to trees. Ideally the spore trap should be inside the pump room in a plastic crate lifted off the ground with blocks or an open section of raised bed in the nursery. Protect the spore trap from the sun by wrapping it in aluminium foil.

2.2.3.2 Obtaining and storing the spore trap agar plates (Roxburgh, 1997)**:** A spore trap needs to be ordered from the local extension officer, nursery advisor or from the Citrus Research International. Two different types of agar plates with selective media must be ordered from CRI. One spore trap is used for the detection of the presence of *Phytophthora* and the other one used for the presence of *Pythium*. It is advisable that both plates must be ordered and used to test the water. The spore trap should be stored in the fridge at a temperature of between 5-10°C. The selective agar medium contains an antibiotic, which is sensitive and can only be stored for three weeks. If the plates are older than three weeks or if the media in the plates have shrunk or dried out, they must be discarded and new plates ordered.

2.2.3.3 Running the spore trap (Roxburgh, 1997): Clean the spore trap with soap and clean water and fill with irrigation water until it runs out. Cut 20 citrus leaf discs, approximately 4mm in diameter, with a paper punch or scissors and float on top of the water. The leaves used must come from a citrus tree that has not been sprayed with a fungicide or copper in the past 12 weeks. Close the lid of the spore trap.

2.2.3.4 Plating out the leaf discs (Roxburgh, 1997): Use the two different spore trap plates. After 48 hrs remove 14 floating leaf discs from the spore trap using tweezers, dry on a clean paper towel, and place seven leaf discs onto the agar of each plate. Seal the plates with tape and stick a completed label on each lid. Post the plates within two days of using them to Citrus Research International, Diagnostic Centre, P O Box 28, Nelspruit, Mpumalanga, 1200, South Africa. If the plates cannot be posted immediately store in the fridge for a maximum of five days until they can be posted.

2.2.3.5 Posting the Petri dish (Roxburgh, 1997): Make sure a complete label is applied on each lid. The spore trap plates are easily broken in the post and must be packed securely. Pack the spore trap plates between two sheets of cardboard and place them in a padded envelope. Address it to Citrus Research International, Diagnostic Centre, P O Box 28, Nelspruit, Mpumalanga, 1200 South Africa.

The results will be sent directly to sender with a copy to the nursery advisor or extension officer serving your nursery.

2.2.3.6 Water handling after treatment (Roxburgh, 1997): The reservoir in which the treated water is stored must be covered. A separate pump must be used to pump the treated water into the nursery. The pump used to fill the reservoir cannot be used for the nursery, as there will always be non-treated water in the pump, which will then contaminate the treated water.

Protocol 3: Checklist for Pesticides, Fungicides, Fertilizer Application and Handling (Marer *et al.*, 1988)

Checklist for planning pesticide, fungicide and fertilizer application and handling is aimed at improving efficacy of control strategies and in particular, to educate nursery managers and employees on effective application methods, timing and correct use of fungicides, pesticides and fertilizer. Its purpose is to give guidance both in correct use and effective application. The following procedure could reduce the associated risks (Marer *et al.,* 1988):

(i) Personal: Person should undergo a medical examination and be properly trained for this type of application.

(ii) Pesticide, fungicide and fertilizer application: Applicant should read and thoroughly understand the label information and instructions. Read material safety data sheet for information on hazards and know proper rate for application of pesticide, fungicide and fertilizer.

(iii) Equipment: Persons applying pesticides, fungicides and fertilizers must wear protective clothing (boots, gloves, respiratory equipment, eye protection and head wear). Select suitable application equipment for spraying (tank capacity, pressure range, volume of output, nozzle size, and pump compatible with formulation type). Lastly, equipment to be used should be properly calibrated prior to use.

(iv) Mixing: Clean water must be used for mixing pesticides or fungicides and water pH must be tested and the correct adjuvant obtained for correcting pH, preventing foaming and improving deposition. Check compatibility of pesticide tank mixes or fertilizer-pesticide combinations.

(v) Treatment site: Make sure that nursery stocks in the treatment area are in proper condition for pesticide application (correct growth stage, not under moisture stress and also complying with other requirements specified on pesticides labels).

(vi) Weather conditions: It is important to check if weather conditions are suitable for application (low wind, suitable temperature and no rain forecasted).

(vii) Application: Application profile should be established that are suitable for each treatment area and prevailing weather conditions. Equipment used should be checked frequently during application to assure that everything is working properly and is providing a uniform application.

(viii) Clean up: Equipment used should be properly cleaned and decontaminated after application. Personal protective clothing and equipment should be cleaned and stored according to approved methods. Disposable materials should be burned or disposed of in an approved way.

(ix) Disposable: Pesticides, fungicides and fertilizers should be burned or disposed of according to National Regulations (The Registrar (Act No. 36 of 1947) (Nel *et al.,* 2003).

(x) Storage: Unused pesticides, fungicides and fertilizers should be stored in a locked facility with limited access for later use and a signboard should also be provided for safety purposes.

(xi) Reports: The necessary reports must be filed.

(xii) Follow up: A treated area in the nursery is inspected after application to assure that the pesticides have controlled target pests without causing damage to non-target organisms.

(xiii) Damage: Damage, if it occurred, should be promptly reported.

Protocol 4: Protocol for Nematode Detection

4.1 Purpose of detecting nematode: Nematodes are worm-like microscopic organisms that live in the soil. The citrus nematode *Tylenchulus semipenetrans* causes slow decline of citrus in all citrus producing regions around the world (Kaplan, 1988). The aim of this protocol is to provide information and guidance on how to test samples for nematodes in the nursery.

4.2 Extraction and detection of nematode from soil samples

Currently, the CRI's, Diagnostic Centre in Nelspruit is using a procedure described by Van der Vegte (1973) to extract citrus nematodes from soil samples (Laura Huisman, Personal Communication, 2008). The procedure is based on the Baermann-tray method (van der Vegte, 1973). The procedure is as follows:

(i) Prepare a plastic tray, plastic gauze and tissue paper. Mix the soil samples thoroughly and place a 250cc of soil on the tissue paper. Dry soil should be soaked for a few hours. The mixture is stirred to free nematodes from the soil and suspend them in the water.

Flocculating agents, such as Separan NP10 (12.5µg/ml) might be used to help to break up soil aggregates in heavy clay soil.

(ii) Pour water into a plastic tray until the soil is saturated and let it stand at room temperature for 48 hrs.

(iii) Lift plastic gauze with tissue and soil carefully from the tray.

(iv) Pour the remaining water in the tray through a 38µM sieve. Rinse tray thoroughly to ensure that no nematodes stay behind in the tray.

(v) Rinse the nematodes from the sieve as there is a greater chance of nematode recovery into a 250cc beaker. Fill the beaker up to 100ml and mix suspension thoroughly with a stream of air bubbles.

(vi) Draw sub-samples into a Hawksley nematode counting slide and count the *Tylenchulus semipenetrans* juveniles. Repeat the test three times and use the average result from the test conducted in order to get the readings.

(vii) Counts are given per 250cc soil.

Protocol 5: Protocol for *Phytophthora* detection in nursery trees and plant growth medium

5.1 Purpose of the protocol: *Phytophthora* spp. cause the most serious and economically important soil borne disease of citrus (Timmer, 1988). In citrus nurseries, *Phytophthora* diseases may appear at any time during plant growth. The disease is spread through contaminated water, soil and even through indirect means such as nursery worker's contaminated hands and boots and implements. The aim of this protocol is to provide information and guidance on how to test samples for the presence of *Phytophthora* in citrus nurseries. *Phytophthora nicotianae* (Dastur) and *Phytophthora citrophthora* (Leonian) are the two most important species causing citrus diseases (Benson & Jones, 1980; Grech & Frean, 1987; Timmer, 1988).

5.2 Detection of *Phytophthora*

Currently, the CRI's, Diagnostic Centre in Nelspruit is using the leaf piece baiting technique (Grimm & Alexander, 1973) to detect *Phytophthora* in the nursery (Laura Huisman, Personal Communication, 2008). The following materials are used for the detection of *Phytophthora* in the nursery: One ice tray with 14 cubicles for each sample, clean untreated citrus leaves, clean punch to make leaf pieces, distilled water, pincette, paper towelling, Petri dishes with *Phytophthora* selective media and an incubator at 25ºC.

The method for detecting *Phytophthora* in the nursery is as follows:

(i) Make sure that the soil samples are thoroughly mixed before testing for *Phytopthora* spp.

(ii) Place 5ml soil in each cubicle of the ice tray, add distilled water to cubicles taking care not to overflow the cubicles.

(iii) Mix soil and water.

(iv) Float 1-2 citrus leaf pieces in each cubicle (diameter of leaf pieces 5mm x 5mm), cover ice trays with cardboard and let it stand at room temperature for 48hrs. If *Phytophthora* is present, zoospores will infect the citrus leaf pieces.

(v) Using the pincette, blot leaf pieces lightly on a paper towel and place seven pieces on a petri dish with *Phytophthora* selective media. The following selective media may be used for *Phytophthora* detection: prepare 500ml Potato Dextrose Agar and allow it to cool to 50ºC. Add 0.17g Ampicillin (250mg ampicillin capsules); 0.05g Hymexazole crystals (Sankyo Agro Co. Ltd); 0.01g Rifampicin (Rifcin 150mg capsules); 0.075g PCNB (Pentachloronitrobenzene) and 0.01g Pimaricin (Delvocid). Take note that two petri dishes are used for one ice tray. Media are poured into 90mm Petri dishes and stored in the fridge for no longer than 14 days.

(vi) Place Petri dishes in a dark incubator at 25ºC for 48hrs and lastly identify *Phytophthora* spp. *Phytophthora* can be distinguished from *Pythium* by its comparatively slow growing, stout and stiff branched hyphae, often with distinct and obvious hyphal swellings. *Pythium* cultures are generally fast growing, with finer and flexuous hyphae. Hymexazole is not added to selective media for *Pythium*.

Protocol 6: Protocol for sampling Citrus Black Spot

6.1 Purpose of the protocol: Citrus black spot (CBS) is caused by a fungus of which the asexual stage is called *Phyllostica citricarpa* (McAlpine) and sexual stage is called *Guignardia citricarpa* (Kiely) (Kotzé, 1981). Citrus black spot is one of the most important fungal disease of citrus in South Africa. Disseminating nursery trees can serve as a means of introducing *Guignardia citricarpa* into areas previously certified as CBS free areas (Kotzé, 1993; Van Broekhuizen & Swart, 2003; Swart, 2004). Fruit affected by CBS are not acceptable for export due to strict quarantine regulations (European Union, 2000a; Bonants *et al.,* 2003).

The purpose of sampling for CBS is to ensure that if CBS is present in the nursery, it must be detected early. What happens after CBS detection will depend on several factors and must be dealt

with separately once the protocol is established. The protocol is of special interest not only to nursery workers and nursery supervisors but also to the South Africa Citrus Industry.

6.2 Method of sampling and isolation for CBS: The size and the number of samples depend on the feasibility of the sampling procedure and costs. The experience of the sampler and processor is of paramount importance of the fungus. Care should be taken to prevent cross-contamination of samples and these steps should be conducted in a laminar flow cabinet, one batch processed at a time, followed by sterilisation of all equipment and flow cabinet work surfaces with 100% ethanol (Kotzé, 2005a). The sampling method is as follows:

(i) Leaves: Select 5-8 leaves with visibly developed pycnidia or perithecia from the bulk sample. Fruit: Select 5-8 fruit with lesions resembling black spot.

(ii) Leaves: Using the Unicore 1.2mm diameter punch, remove 20-25 (maximum) leaf pieces from the selected leaves. Fruit: Remove lesions from affected fruit by means of a sterilised scalpel.

(iii) When no perithecia or fungal structures are visible, select 10-12 leaves and remove not more than two punches from each. Preference is given to typical black spot lesions if at all present.

(iv) Add leaf or fruit sections to a 1.5ml Eppendorf tube and grind leaf sections to a very fine powder in the Eppendorf tube.

(v) Using the DNeasy Plant Mini DNA extraction kit (Qiagen), complete the DNA extraction process.

(vi) PCR amplification: Prepare 24µl PCR mix by adding optimised volumes of the following:

- dH_2O (adjusted volume to a final volume of 25µl total product)
- 5µl 10x buffer (or according to manufacturers specifications)
- 200µM of each dNTP or dNTP mix (according to manufacturers specifications)
- (10 pmol) CITRIC1 primer (5'-GAA AGG TGA TGG AAG GGA G-3'); (Meyer *et al*., 2006)
- (15 pmol) ITS4 primer
- 5U Taq polymerase (according to manufacturer's specifications)
- Add 1µl DNA to each eppendorf tube
- Positive controls: DNA from *Guignardia citricarpa*
- Negative controls: DNA from *Colletotrichum gloeosporioides* and H_2O

(vi) PCR cycle:

- Initial denaturation: 95˚C for 2min
- 35 cycles of: 93˚C for 30s, 56˚C for 45s and 72˚C for 90s
- Final extension: 72˚C for 7min

(vii) Visualisation:

- 1.25% agarose gel in 10% TBE buffer
- Band sizes to be observed: 620 bp for *Guignardia citricarpa.* No bands should be served in the negative controls

(viii) Identification of putative *Guignardia citricarpa* PCR products:

- Sequencing analyses will be used to determine whether all 620bp amplicons amplified from DNA samples were indeed *G. citricarpa*
- 620bp amplicons will be cut from agarose gels and purified using a QIAquick Gel Extraction kit (Qiagen, Valencia, CA, and USA)
- The resulting product will be sequenced using the ITS4 and citric 1 primers according to the sequencing reaction and cycle conditions as recommended by the manufacturer
- The identity of the sequences will be determined by BLAST analyses

Protocol 7: Method of Citrus Tristeza Virus Detection

Citrus tristeza virus (CTV) is a long, flexuous, rod-shaped virus which causes a decline of trees, stem-pitting and seedling yellows (van Vuuren, 2003). Control of CTV is through eradication of severe strains, quarantine certification and mild strain cross protection. The main purpose of the protocol is to differentiate methods that can be used to test CTV strains.

7.1 Sample preparation:

7.1.1 Preparation of tissue prints for testing: Appropriate sampling is critical for serological or molecular CTV detection. The standard sampling for nursery plants involves two young shoots or four leaves (Cambra *et al*., 2002). Clean cuts must be made on tender shoots and leave petioles, carefully press the fresh cut sections against a nitrocellulose membrane (0.45mm) and weight approximately 1g of plant material. Table 4.1 showing citrus cultivars to be used for virus indexing. Let the trace or the print dry for a few minutes. For routine testing, perform at least two printings per selected shoot and one per leaf petiole.

7.1.2 Preparation of plant extracts for testing: Weigh approximately 1g of plant material, cut it into small pieces and place in a suitable tube or plastic bag for processing (Cambra *et al.*, 2002). A flow diagram for virus diagnosis is shown in Fig 4.1. Approximately 20 volumes of extraction buffer is added to the sample where after the sample is homogenized in tubes using a Polytron, Homex 6 machine or any manual roller or hammer.

Table 4.1 Citrus Cultivars used for virus indexing (van Vuuren, 2003).

Citrus cultivar	**Virus indexing**
Troyer/Carrizo Citrange	For virus free rootstocks, STG and tatter leaf indexing
West Indian Lime	For CTV bio-indexing
Etrog Citron on Ruff Lemon (RL)	For Viroid indexing
Duncan or Marsh Grapefruit	For Impietratura indexing
Ponkan Mandarin on Ruff Lemon	For HLB indexing
Rough Lemon	For STG of Trifoliates, virus free rootstock for lemons and etrog
Parsons Special Mandarin on RL	For Cachexia indexing
Madame Vinous on RL	For Psorosis and HLB bio-indexing
Nagami kumquat on Nules Clementine on RL	For possible citrus leaf blotch bio-indexing

7.1.3 Extraction buffer: The extraction buffer consist of Phosphate buffer saline (PBS) pH 7.2-7.4 supplemented with 0.2% sodium diethyl dithiocarbamante or 0.2% mercaptoethanol. Samples for serological testing can be prepared in tubes or in plastic bags. Samples for molecular testing must be prepared in appropriate individual plastic bags.

7.2 Screening Tests

7.2.1 Biological Indexing: The traditional method to detect CTV is to graft-inoculate *Citrus aurantifolia* (Key lime or Mexican lime) plants (Mukhopadhyay, 2004). The indicator plant, Mexican Lime (*Citrus aurantifolia*) is used and is also referred to as the seedling lime index (Huang *et al.,* 2004). This host reacts to most isolates by developing cleanings or flecks in the veins of new leaves formed after inoculation. In some cases the severity of symptoms is indicative of the isolate's virulence in other hosts, but the correlation is not exact.

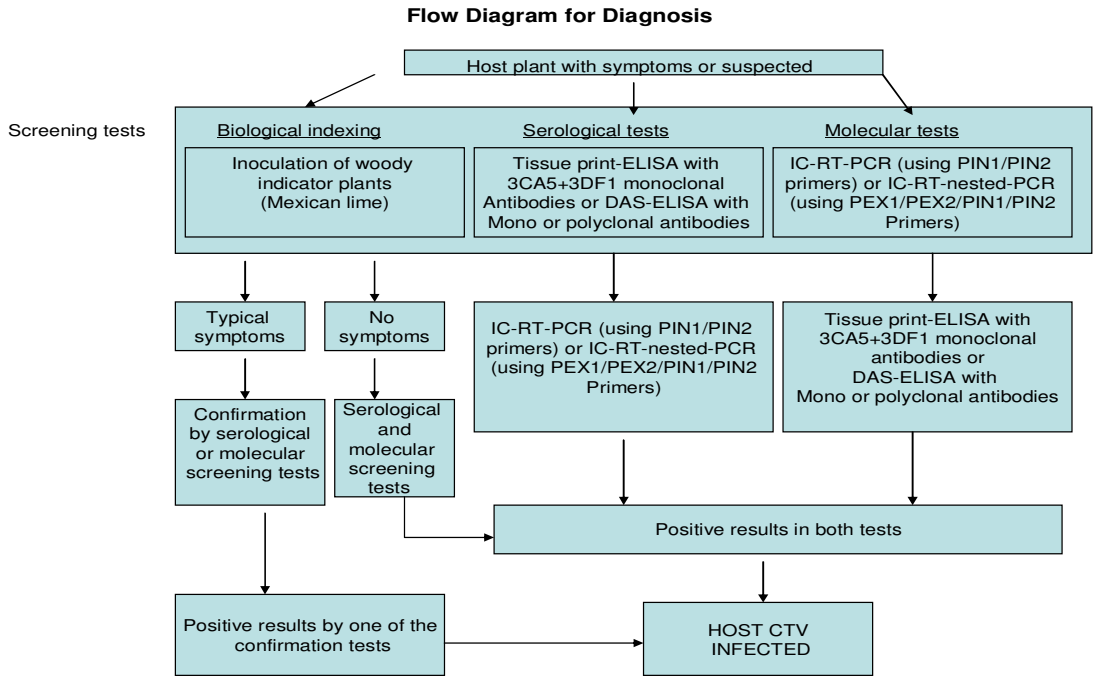

Fig 4.2 Flow diagram for virus detection (Cambra *et al.,* 2002).

To determine CTV decline effects sweet orange plants grafted on sour orange rootstock are inoculated and monitored for symptoms of dwarfing and chlorosis over a 6-12 month period. However, some isolates that cause decline of field trees grafted on sour orange are symptomless in the same condition, under glasshouse or screenhouse conditions (Ballester-Olmos *et al.,* 1993). Stem pitting is determined by inoculating seedlings of an appropriate or susceptible cultivar, usually sweet orange or grapefruit, and monitoring growth over 12-15 months. The main stem of the indicator plants is striped of bark and examined for stem pitting. To test an isolate for seedling yellows, the isolates is grafted to a seedling of sour orange, lemon, or grapefruit and observed for 6-12 months in the greenhouse for stunting and chlorosis symptoms. This often is considered a presumptive indication for a severe isolate of CTV, but some virulent stem-pitting isolates do not cause seedling yellows and vice versa. Some isolates induce seedling yellows, but do not cause stem pitting in grapefruit or sweet orange. The sensitivity of biological indexing is very low as mild symptoms may contain severe strains that are expressed only in specific conditions (Cambra *et al.,* 1993).

7.2.2 Serological Test Methods: Several serological detection procedures have been developed from polyclonal and monoclonal antibodies valuable in detection of CTV. These include SDS-immunodiffusion (Brlansky *et al.,* 1984), direct tissue blot immunoassay (DTBIA) (Garnsey *et al.,* 1993), enzyme-linked immunosorbent assay (ELISA), dot-immunoblotting assay (Rocha-Peña *et*

al., 1991), radio-immunosorbent assay (RISA) (Rocha-Peña *et al.,* 1991), using 25I serologically specific electron microscopy (Brlansky *et al.,* 1984), in situ immunoassay (Stewart, 2006), in situ immunofluorescence (ISIF) (Brlansky *et al.,* 1984), and a Western blot assay (Rocha-Peña *et al.,* 1991). CTV monoclonal antibodies (MCAs-13) method is commonly used as a first step to differentiate decline and non-decline inducing isolates of CTV (Huang *et al.,* 2004) and they recognise all CTV strains.

Other methods that are used for Serological tests to detect CTV are Tissue print-ELISA and DAS-ELISA techniques (Garnsey & Cambra, 1991). Citrus tristeza virus strains are inoculated to all plants prior to planting in the orchard to help prevent trees from getting the more severe strains. This is called Mild Strain Cross-Protection. Therefore, in order to confirm that every plant has been pre-immunized serology test are implemented.

7.2.3 Seedling indexing for citrus tristeza virus: Seedling indexing to Mexican lime is still a very powerful tool for detection of tristeza virus (Roistacher, 1991). The procedure for CTV seedling indexing is as follows:

(i) Collect budsticks from a minimum of four quadrants of each tree. For routine re-indexing of important foundation or mother-block trees where there may be some danger of possible infection by vector transmission, collect from eight sectors of each tree.

(ii) Buds (buds with eyes, blind buds or chip buds), leaf discs or leaf pieces can be used for seedling indexing. Graft two inoculum buds or leaf pieces or a minimum of five or six leaf discs per plant.

(iii) Place inoculum buds or leaf pieces in the lower part of the test seedling, removing as few leaves as possible from the lower stems. The seedling can be cut back to 20-25cm from the soil surface at the time of inoculation or at two or three weeks after inoculation when wrapping tapes are cut and the inoculum is observed for survival. The time to cut back is decided upon according to the specific environmental conditions in each plant laboratory.

(iv) Mexican lime is the recommended general indicator for identification of all types of tristeza.

(v) Allow all shoots to develop for the first three growth flushes, then prune and train as single shoot.

(vi) Cool temperatures are necessary for maximum tristeza symptom expression in plants. Warm temperatures above 35ºC may suppress development of vein clearing and stem pitting symptoms in Mexican lime seedlings (Roistacher *et al.,* 1974). The preferred greenhouse temperatures for all tristeza and seedling-yellows indexing are 24-28ºC maximum during the

day and 17-21ºC minimum at night. Temperature control is especially important when checking for mild isolates.

(vii) Symptoms: vein clearing, vein darkening, leaf cupping and stem pitting. Vein corking for severe isolates.

Future methods which can be used by the South African Citrus Industry are PCR systems aimed at differentiating between different strains by targeting the conserved p23 gene and the variable 5′ half of the citrus tristeza virus genome (Stewart, 2006). An oligonucleotide microarray was recently developed to differentiate T30 and T36 strains of CTV and may be adapted for the simultaneous detection of citrus tristeza virus (Stewart, 2006).

Protocol 8: Detection of Citrus Greening

8.1 Purpose of the protocol: Citrus greening is one of the most serious and devastating diseases affecting production, yield and the quality and economic value of the fruit (Garnier & Bové, 2000). Presently three species of the causal organism are recognized: Candidatus Liberibacter species (*Africanus, Asiaticus and Americanus*) (Catling, 1970; Bove′, 2006; Le Roux *et al.,* 2006). For indexing citrus greening disease, the South Africa Citrus Industry is using biological and molecular indexing methods. The PCR indexing is currently done at the University of Pretoria by Prof. Gerhard Pietersen. The aim of the protocol is to provide information and guidance on how to test samples for citrus greening disease in the nursery.

8.2 Method of sampling and isolation for citrus greening disease

Visual symptoms and biological indexing have been the historical means of diagnosis of citrus greening (Miyakawa, 1980 & Roistacher, 1991). Later, detection systems were developed using electron microscopy (Lafleche *et al.,* 1970), HLB-specific fluorescent substance (Schwarz, 1968), and enzyme-linked immunosorbent assay (ELISA) with monoclonal antibodies (Garnier *et al*., 1987). PCR based detection methods were developed based on the 16s ribosomal DNA and other regions of the bacterial genome (Jagoueix *et al.,* 1996; Tian *et al.,* 1996 & Hocquellet *et al.,* 1999). Sensitive detection methods for confirmation of symptoms developed include real time-quantitative PCR (qPCR) (Li, *et al.,* 2006) and loop-mediated isothermal amplifications (Okuda *et al.,* 2005). TagMan based real-time quantitative polymerase chain reaction methodology was developed for detection of *Candidatus asiaticus* in *Diaphorina citri* (Manjunath *et al.,* 2007).

8.2.1 Biological indexing: Use of indicator plants is the most widely used method to detect greening at field level (Mukhopadhyay, 2004). Seedlings of sweet orange are mostly used as the indicator plants. Grapefruit seedlings can also be used in the absence of severe tristeza strains. The suspected plant is used as the scion and the sweet orange as the rootstock to conduct graft transmission and characteristic symptom development. Seedlings should be grown one per container as a single shoot to approximately 1m, with a thickness of 5 to 7mm. A minimum of five plants should be inoculated to index a given source tree. Each plant can be inoculated with two side grafts, two to three leaf-piece grafts or a side graft and two leaf-piece grafts. Positive and negative control plants are essential in any indexing procedure.

Side grafts are performed between 10 to 14 days after inoculation. The bottom ends of the polyethylene sleeves are opened to permit partial drying around the sides. After three weeks the polyethylene is removed in order to observe grafts for survival. Plants are then cut back to approximately 25cm from the soil surface. One terminal shoot must be staked to grow as a single stick. The wrapping tape surrounding the leaf graft is cut two to three weeks after inoculation and inoculation survival should be recorded. A temperature requirement for holding indicator plants is 20-25ºC for African greening and 25ºC for Asian greening.

In 1883, Garnier and Bove′ used a technique for direct observation of the organisms. The technique is as follows (Roistacher, 1991):

(i) Pieces of leaf midrib tissue from leaves showing typical mottle symptoms of greening are cut or diced into 1mm pieces, using a razor blade.

(ii) They are fixed in 4% glutaraldehyde in a O.1M cacodylate (phosphate) buffer Ph 7.5 for 6 hours.

(iii) rinsed three times in the same buffer and post-fixed with 1% osmium tetroxide in the same buffer.

(vi) Dehydrated in alcohol and embedded in Epon 812 and thin sectioned with an ultramicrotome.

(v) Stained with lead citrate and observed in the electron microscope.

Protocol to extract DNA from citrus plants (Doyle, 1990): Chop fine petioles and midribs with scalpel blades; weight chopped fine petioles and midribs into grams; macerate in 3ml CTAB (plus 2-ME) in maceration bags in Homex 6. Transfer 2ml of macerate to 2ml Eppendorf tubes. Then shake in 60ºC incubator for 30min; centrifuge 3000rpm for 5min, recover 900µl of supernatant and place in new tube. Add 900µl volume of chloroform, isoamyl alcohol (24:1) and invert to mix.

Centrifuge 14000rpm for 5min at 10ºC; place 800µl upper aqueous phase in a new tube; add 2/3 volume (480µl) of cold iso-propanol and mix gently. Incubate for 20min at -80ºC; centrifuge 14000rpm for 20min; carefully remove supernatant (pellet may be loose). Add 1ml cold wash buffer to pellet and swirl; centrifuge 14000rpm for 10min (4ºC). Carefully remove supernatant (pellet may be loose); add 1ml cold wash buffer to pellet and swirl; centrifuge 14000rpm for 10min (4ºC). Then dry in speedy-vac for 3min, resuspend in 100µl TE buffer and store until used (use 1µl of DNA in 40µl Greening (PCR reaction).

Detection of the greening organism by microscopic examination may be difficult and requires expertise and experience. It is important to look at healthy controls and helpful to examine known positive sources. If the organism is found it is good confirmation of symptom diagnosis. However, if not found, this does not mean it is not present and its' absence cannot be taken as negative in certification programme diagnosis.

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CHAPTER 5

GENERAL DISCUSSION

Citrus belongs to the genus *Citrus L.,* family *Rutaceae*, and subfamily *Aurantioideae* (Mukhopadhyay, 2004). Citrus is one of the world's most important fruit crops and although it originated from South East Asia it is currently cultivated throughout the world (Spiegel-Roy & Goldschmidt, 1996).

The South African Citrus Industry was established in the 1800s and the South African Co-operative Citrus Exchange in 1926 once the potential for exports of fresh fruit was realised (Mabiletsa, 2003). The South African citrus industry is regarded as one of the most important agricultural industries in the world in terms of export volumes of fresh fruit and is ranked 2nd in world trade figures (Citrus Growers Association, 2007). In 1995, the citrus industry exported 43 million cartons of citrus to 60 countries with a gross value of R1.6 billion. In 2006/2007 the industries growth was reflected in the 90 million cartons of fresh citrus fruits exported to more than 60 countries (PPECB Export Directory, 2007/2008). Fresh citrus fruits are also sold locally to 19 fresh produce markets nationwide (Bloemfontein, Cape Town, Durban, East London, Johannesburg, Klerksdorp, Kimberley, Mpumalanga, Nelspruit, Polokwane, Pretoria, Port Elizabeth, Pietermaritzburg, Springs, Umtata, Uitenhange, Witbank, Welkom and Vereeniging) (Mabiletsa, 2005).

Agriculture not only plays a major role in the economic growth and development of the country, but also has a distinct role in the broadening of the economy and to provide social options for rural people. The South Africa Citrus Industry employs approximately 100 000 workers and is a significant contributor in the creation of job opportunities and economic growth and trade (Mabiletsa, 2003).

As with any commercial crop, citrus is subjected to various pests and diseases which has a negative impact on yield, causes varying degrees of damage to the fruit and impact on market access (Davies & Albrigo, 1994). It is evident from this study that in order to prevent short commercial life of citrus trees with low yields and poor quality fruits, nurseryman should only select healthy nursery planting material certified free from pest and diseases and implement good nursery practices. The production of citrus trees, propagated from registered pathogen-free sources has been proven to lower the incidence of diseases and pests in the citrus industries of the world (Timmer *et al.,* 2000).

Globalisation has resulted in increased international trade and tourism, which poses a threat to plant health in the form of alien pest and diseases introductions (Serial No. PH 0001). Many of the major citrus production countries including South Africa have stringent nursery regulations and has established budwood registration programmes. This is essential for the long-term success of a developing industry (Davies & Albrigo, 1994). Illegal movement of citrus plant material is difficult to control since the public is not always aware of the risks involved and the potential threat to the local industry. However, using proper legal import channels, the risk of introducing foreign pest can be reduced (Appendix E).

GlobalGAP is a private sector body that sets voluntary standards for the certification of agricultural products around the globe http://www.globalgap.org. Although adopted globally, GlobalGAP often do not specifically fulfill industry demands such as addressing in country legislation, compliance with local regulation and including industry specific protocols. The GlobalGAP standard is primarily designed to reassure consumers on how citrus propagation material is produced in the nursery by minimising detrimental environmental impact of nursery operations, reducing the use of chemical inputs and ensuring a responsible approach to workers' health and safety. GlobalGAP serves as a practical manual for good agricultural practices and operates like a satellite navigation system. It equips members with a reliable tool kit, which allows each partner in the supply chain to position themselves in a global market with respect to consumer requirements.

The South African citrus industry is dedicated to continue with improvements of horticultural and ethical practices within the citrus nursery industry. Citrus Research International (CRI) has taken the initiative to create standards and protocols for the citrus nursery industry to ensure that certified healthy rootstock are used by the South African citrus industry. With the formation of the Citrus Improvement Programme (CIP) in 1973 as a joint project between the South Africa Co-operative Citrus Exchange and the Citrus and Subtropical Fruit Research Institute (CSFRI) (http://www.cri.co.za/cip/index.htm), the industry established a sound basis for good practices. The main objective of the CIP is to increase profitability of the Southern African citrus industry, by ensuring that citrus growers are supplied with nursery trees of the highest quality made from the best genetic citrus material and free from any harmful pathogens.

In order to maintain a successful nursery industry, good nursery practises should be adopted and implemented to ensure compliance to national standards, industry protocols and regulatory requirements. This mini dissertation provides a base for the establishment of such a South Africa citrus nursery good nursery practices programme.

REFERENCES

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APPENDIX A

NEW CITRUS PLANTING PER REGION (BUDS)

	Year of new plantings										
Region	**1996**	**1997**	**1998**	**1999**	**2000**	**2001**	**2002**	**2003**	**2004**	**2005**	**2006**
Eastern Cape	885,180	975,658	976,098	833,139	590,269	473,369	441,902	546,660	427,080	478,079	469,452
Kwazulu Natal	180,150	228,200	134,540	111,550	55,100	38,250	20,600	36,030	16,500	15,500	31,550
Limpopo Province	1,014,455	1,237,556	931,980	562,480	575,980	712,026	804,120	930,650	1,070,735	1,252,503	777,085
Mozambique	8,600	1,400	11,000	30,980	38,100	8,500	0	5,400	0	12,250	3,000
Mpumalanga	424,155	488,110	349,705	399,166	376,440	192,190	376,205	244,590	214,450	312,552	312,240
North West Province	25,560	313,100	243,380	126,920	137,600	109,665	121,350	101,100	86,990	82,780	135,090
Northern Cape	74,790	124,940	80,065	81,960	85,615	152,215	156,905	116,990	68,720	46,850	34,940
Swaziland	70,624	74,905	48,418	59,940	41,830	43,550	49,400	10,600	0	42,225	2,000
Western Cape	1,061,090	1,265,797	843,454	441,548	380,675	181,534	272,850	365,665	368,255	383,089	311,49
Zimbabwe	338,635	669,415	550,197	222,524	217,615	12,290	39,250	21,700	0	1,700	
Other African States							1,400		28,000		
Total	4,083,239	5,379,081	4,168,837	2,870,207	2,499,224	1,923,589	2,283,982	2,379,730	2,280,730	2,627,528	2,076,851

Source: Citrus Growers Association, South Africa (Key Industry Statistics, 2007).

REFERENCE

(1) Citrus Grower Association (CGA), South Africa. http://www.cga.co.za. Key Industry Statistics 2007: 1-48.

APPENDIX B

NEW CITRUS PLANTING PER VARIETY (BUDS)

	Year of new plantings										
Region	**1996**	**1997**	**1998**	**1999**	**2000**	**2001**	**2002**	**2003**	**2004**	**2005**	**2006**
Clementine	182,774	409,345	768,098	385,928	67,070	20,410	51,200	39,460	95,855	38,100	31,950
Ellendale	5,935	26,420	36,450	68,707	8,650	4,020	0	2,760	1,270	2,700	500
Grapefruit	540,909	512,566	104,868	234,580	156,280	149,932	224,180	237,800	318,700	542,053	375,130
Grapefruit Hybrid	40,575	12,800	777	6,285	5,500	10,139	5,630	2,250	1,700	7,100	5,300
Kumquat	30,910	29,410	23,550	12,500	4,700	5,925	3,400	5,450	9,406	9,450	12,130
Lemon	607,742	974,031	536,630	113,650	259,530	183,105	138,120	260,140	244,322	120,980	57,880
Lime	2,410	11,750	12,440	19,830	11,150	29,090	36,835	37,700	9,000	6,630	29,670
Mandarin Hybrid	192,872	150,680	205,046	316,586	322,980	140,834	176,720	265,430	138,880	139,412	201,651
Mid-Season Oranges	28,629	15,420	11,375	16,810	16,930	13,350	10,730	4,720	3,140	1,830	9,970
Navel	722,502	1,095,778	831,511	628,240	742,004	622,712	857,885	862,090	708,650	884,720	581,377
Satsuma	101,451	142,167	128,469	161,855	72,550	61,800	94,200	134,675	67,798	60,964	52,835
Seville	0	650	3,928	100	0	200	1,330	20	0	0	0
Valencia	1,626,530	1,998,064	1,505,695	905,136	831,880	679,322	683,572	526,790	682,015	813,859	718,458
Total	3,135,822	3,135,822	3,135,822	3,135,822	3,135,822	1,921,089	2,283,802	2,379,385	2,280,730	2,627,528	2,076,851

Source: Citrus Growers Association, South Africa (Key Industry Statistics, 2007).

REFERENCE

(1) Citrus Grower Association (CGA), South Africa. http://www.cga.co.za. Key Industry Statistics 2007: 1-48.

APPENDIX C

Export volumes for the past five seasons as from 2001-2006 Citrus Seasons (Statistic by Mr. Cyril Julius (National Programme Manager: Citrus) PPECB, 2007)

Production Region	Product Group	Product	2002	2003	2004	2005	2006
Berg River	Citrus fruit	Grapefruit	26,838	14,570	20,230	22,950	31,821
		Lemons	674,043	740,360	849,634	853,889	584,868
		Oranges	6.712.781	7,372,320	7,698,869	6,384,913	7,130,115
		Soft Citrus	2, 594,588	3,827,086	4,820,139	3,782,597	3,899,688
Berg River total			**10,008,250**	**11,954,336**	**13,388,872**	**11,044,349**	**11,646,492**
Ceres	Citrus Fruit	Oranges	55,738	24,625	28,573	9,549	17,448
		Soft Citrus	151,805	194,629	194,546	187,156	262,497
Ceres total			**207,543**	**219,254**	**223,119**	**196,705**	**279,945**
Eastern Cape	Citrus Fruit	Grapefruit	213,250	282,810	298,560	312,204	382,872
		Lemons	2,929,677	3,517,896	4,307,738	3,760,206	4,957,551
		Oranges	11,063,833	12,319,714	13,196,626	11,690,496	15,309,457
		Soft Citrus	1,388,520	1,602,690	2,005,963	1,956,302	2,643,998
Eastern Cape total			**15,595,280**	**17,723,110**	**19,808,887**	**17,719,208**	**23,293,878**
Elgin Grabouw Villiersdrop	Citrus Fruit	Grapefruit	-	13,401	14,621	-	-
		Lemons	5,680	32,580	18,678	25,730	-
		Oranges	64,701	89,021	71,901	4,039	-
		Soft Citrus	739,848	724,088	693,839	165,106	414
Elgin Grabouw Villiersdrop total			**810,229**	**859,090**	**799,039**	**194,875**	**414**
Free State	Citrus Fruit	Lemons	5,3680	36,785	730	28,598	20,920
		Oranges	64,701	338,380	10,950	328,711	321,340
		Soft Citrus	739,848	12,745	-	12,705	27,208
Free State total			**179,859**	**387,910**	**11,680**	**370,014**	**369,468**
Klein Karoo	Citrus Fruit	Lemons	253,964	322,816	467,768	273,367	236,398
		Oranges	322,370	339,128	376,817	319,895	311,805
		Soft Citrus	1,400,166	1,698,083	2,375,686	2,704,163	3,096,467
Klein Karoo total			**1,976,500**	**2,360,027**	**3,220,301**	**3,297,425**	**3,644,670**
Kwazulu Natal	Citrus Fruit	Grapefruit	2,111,318	2,670,078	2,392,392	2,788,028	2,381,428
		Lemons	301,372	380,834	380,200	368,688	323,370
		Limes	16,728	-	2,280	34,116	1,800
		Oranges	2,134,783	4,932,209	1,593,828	1,519,577	1,615,240
		Soft Citrus	45,255	77,210	39,775	64,212	35,524
Kwazulu Natal total			**4,609,456**	**8,060,331**	**4,408,475**	**4,774,621**	**4,357,362**
Langkloof	Citrus Fruit	Oranges	27,380	13,980	8,750	3,920	-
		Soft Citrus	49,400	51,189	29,600	27,450	18,575
Langkloof total			**76,780**	**65,169**	**38,350**	**31,370**	**18,575**
Limpopo Area	Citrus Fruit	Grapefruit	3,300,590	3,674,204	3,447,525	4,686,522	2,909,610
		Lemons	408,457	403,013	413,001	505,772	359,418
		Limes	-	-	1,40	2,400	-
		Oranges	12,745,604	15,474,660	15,720,384	16,701,617	14,267,828
		Soft Citrus	152,253	281,709	110,526	431,359	457,819
Limpopo Area Total			**16,606,904**	**19,833,586**	**19,692,843**	**22,327,670**	**17,994,675**

APPENDIX C (Cont.)

Export volumes for the past five seasons as from 2001-2006 Citrus Seasons (Statistic by Mr. Cyril Julius (National Programme Manager: Citrus) PPECB, 2007)

Production Region	Product Group	Product	2002	2003	2004	2005	2006
Mpumalanga Area	Citrus Fruit	Grapefruit	4,293,730	3,83,174	4,452,140	6,278,999	2,988,365
		Lemons	120,060	175,860	240,068	184,993	102,605
		Limes	12,960	34,368	19,132	-	-
		Oranges	4,232,202	4,818,214	4,733,374	5,327,507	4,605,028
		Soft Citrus	23,070	76,796	15,765	46,059	41,278
Mpumalanga Area total			**8,682,022**	**8,943,412**	**9,460,479**	**11,837,558**	**7,737,276**
Mozambique	Citrus Fruit	Grapefruit	9,650	12,670	23,655	57,140	7,480
		Lemons	-	1,260	280	-	-
		Oranges	199,968	593,350	16,330	10,500	-
Mozambique total			**209,618**	**607,280**	**40,265**	**67,640**	**7,480**
North West Area	Citrus Fruit	Grapefruit	5,094	23,118	10,254	10,660	6,406
		Lemons	342,414	615,495	738,320	828,756	647,839
		Limes	1,265	1,533	1,384	589	1,811
		Oranges	6,057,478	8,401,764	4,569,043	6,838,616	6,556,274
		Soft Citrus	170,477	225,942	187,516	212,992	127,492
North West Area total			**6,576,727**	**9,267,852**	**5,506,517**	**7,891,613**	**7,339,822**
Orange River	Citrus Fruit	Grapefruit	17,210	4,262	17,230	64,490	133,815
		Lemons	18,490	6,840	96,355	148,282	100,260
		Oranges	6,230	13,060	70,335	86,008	134,410
		Soft Citrus	9,600	2,680	4,662	6,594	12,189
Orange River total			**51,530**	**26,842**	**188,582**	**305,374**	**380,674**
Other Africa Countries	Citrus Fruit	Grapefruit	953,815	712,625	836,703	868,115	640,484
		Lemons	48,580	34,790	41,930	44,596	41,820
		Limes	2,640	-	-	-	-
		Oranges	1,076,204	1,291,569	683,060	556,905	525,015
		Soft Citrus	18,480	14,770	39,320	17,560	26,520
Other Africa Countries total			**2,099,719**	**2,053,754**	**1,601,013**	**1,487,176**	**1,233,839**
Other Western Cape Area	Citrus Fruit	Grapefruit	6,379	3,184	9,441	5,738	5,103
		Lemons	76,744	88,144	55,083	81,639	55,796
		Limes	-	5	1,122	23	-
		Oranges	206,629	504,365	178,692	68,694	54,715
		Soft Citrus	47,815	46,346	45,348	37,369	66,537
Other Western Cape total			**337,567**	**642,044**	**289,686**	**193,463**	**182,151**
Stellenbosch	Citrus Fruit	Grapefruit	155,711	19,912	73,025	-	-
		Lemons	84,107	175,293	207,542	180,483	227,862
		Oranges	977,091	443,364	384,803	237,506	339,622
		Soft Citrus	326,647	471,790	633,331	585,861	567,248
Stellenbosch total			1,543,556	1,110,359	1,298,701	1,003,850	1,134,732
Grand Total			**69,571,540**	**84,114,356**	**79,976,809**	**82,742,911**	**79,621,453**

APPENDIX D

Graft transmissible pathogens of citrus having vectors or other means of natural spread (Roistacher, 1991).

Graft transmissible disease	**Causal agent**	**Vector means of spread**
Citrus blight	Unknown	Unknown
Citrus chlorotic dwarf	Unknown virus-like	*Parabemisia myricae*
Citrus variegated chlorosis	Strain of *Xylella Fastidiosa* Wells	Several species of sharpshooters (Hemiptera: Cicadellidae)
Citrus greening	*Candidates liberobacter* Asiaticum *& L. Africanum* (Asian & African greening)	*Diaphonna citri* kuwayama *trioza ertreae*
Leprosis	Citrus leprosis rhabdovirus	*Brevipalpus spp.* mites
Natural spread psorosis	Citrus psorosis virus	Unknown
Satsuma dwarf	Satsuma dwarf Nepovirus	Unidentified soil borne agent
Stubborn	*Spiroplasma citri* Saglio	Leafhoppers
Tristeza	Citrus tristeza	Several aphids species Such as *Toxoptera citricida*
Witches broom	Candidates phytoplasma Aurantifolia	Hishimonus phylitis
Woody gall	Citrus vein enation virus	Aphids, *T. citricida* and *Aphis*

REFERENCE

(1) Roistacher, C. N., 1991. Graft-transmissible diseases of citrus, handbook for detection and diagnosis. International Organization of Citrus Virologists, Riverside, Food and Agriculture Organization of the United Nations, Rome, 286.

APPENDIX E

PORTS OF ENRTY AS PUBLISHED IN THE REPUBLIC OF SOUTH AFRICAN GOVERNMENT NOTICE R1013

Border control posts	Railway station within the municipal area	Main post offices	Container Depots	Airports	Harbours
Vioolsdrift, Nakop, Groblers Bridge, Kopfontein Gate, Schilpad's Gate, Ramatlabana, Beit Bridge, Lebombo, Border Gate (Mananga), Oshoek, Nerston, Mahamba, Golela, Fickburg Bridge, Maseru Bridge	Cape Town Durban East London Johannesburg Port Elisabeth Pretoria	Cape Town Durban East London Johannesburg Port Elisabeth Pretoria	Cape Town Port Elisabeth Durban City Deep	Cape Town Port Elisabeth Johannesburg Durban	Cape Town Port Elisabeth East London Durban

APPENDIX F

Preventative citrus black spot control programmes (Department of Agriculture and Citrus Research International (Nel *et al.,* 2003 and CRI, 2003).

Weather conditions	Fungicide (Active Ingredient/s)	Dosage/100litres water	Application date/interval
Wet season	Mancozeb * 200g*		First application after 100% petal falls in early October. Follow up with a further four applications at 25 day intervals.
	Copper oxycloride or Copper hydroxide	200g	Four applications at 30-35 day intervals commencing in early October.
	Zineb	200g	6-7 applications at 19-21 day intervals commencing in early October.
	Maneb/Zinc Oxide	200ml	Five applications at 28 day intervals commencing during the 3rd week in October.
Normal season	Mancozeb*	200g*	Four applications at 25 day intervals commencing during the 3rd week in October.
	Copper oxycloride or Copper hydroxide	200g	Three applications at 30-35 day intervals commencing during the 3rd week in October.
	Zineb	200g	Five applications at 19-21 day intervals, commencing during the 3rd week in October.
	Maneb/Zinc Oxide	200ml	Four applications at 28 day intervals commencing in early October.
Normal and wet season	Azoxystrobin + Mancozeb + Mineral oil	20-30ml + 150g + 0.5%	15-30 November and 1-7 January. Wet season: 30ml and normal season : 20ml.
	Trifloxystrobin + Mancozeb + Mineral oil	10g + 150g + 0.5%	15-30 November and 1-7 January. An additional application of Mancozeb early in October and again in February will improve control under conditions conducive to heavy infections pressure.
	Pyraclostrobin + Mancozeb + Mineral oil	10g + 150g + 0.5%	Five applications at 19-21 day intervals, commencing during the 3rd week in October.
	Kresoxim-methyl + Mancozeb + Mineral oil	20 + 150g + 0.5%	Four applications at 28 day intervals commencing in early October. Valencia Only. Apply from 15-30 November and 1-7 January

- If mixed with 0.5% (or less) narrow range mineral oil the mancozeb dosage may be reduced to 150g per 100 litres water.
- The spray program used/recommended by Citrus Research International is similar to that recommended by the Department of Agriculture.

REFERENCES

(1) Nel, A., Krause, M. & Khelawanlall, N., 2003. A Guide for the Control of Plant Disease compiled by Department of Agriculture 2nd Edition. Republic of South Africa, 31-34.

(2) Citrus Research International Integrated Production Guidelines for Export Citrus Volume III Integrated Pest and Disease Management. Grout, T. G. (Editor), 2003.

APPENDIX G

Corrective citrus black spot control programme (Department of Agriculture and Citrus Research International (Nel *at al.,* 2003 and CRI, 2003).

Tree condition/age	Fungicide (Active Ingredients)	Dosage/100litres water	Application date/interval
Late cultivars; trees younger than 20 years which are growing vigorously	Benomyl + mancozeb + mineral oil*	50g + 200g + 0.5%	1-20 December
	Split application are also registered**	25g + 200g + 0.5%	15-30 November and 1-7 January
	Carbendazim + mancozeb + mineral oil*	55ml + 200g + 0.5%	1-20 December
	Split applications are also registered**	27.5ml + 200g + 0.5%	15-30 November and 1-7 January
Old neglected trees with a history of high Susceptibility	Benomyl + mancozeb + mineral oil*	75g + 200g + 0.5%	1-20 December
	Carbendazim + mancozeb + mineral oil*	82.5ml + 200g + 0.5%	1-20 December
Lemons	Benomyl + mancozeb + mineral oil*	50g + 200g + 0.5%	1-20 December and 1-15 January
Early cultivars	Benomyl + mancozeb + mineral oil*	50g + 200g + 0.5%	1-20 December
	Split applications are also registered**	25g + 200g + 0.5%	1-14 November and 1-7 January
Valencias under 20 years, grapefruit, navels and mid-season oranges	Thiophanate-methyl + mancozeb + mineral oil*	50g + 200g + 0.5%	15-30 November and 14-21 January
Valencias over 20 years	Thiophanate-methyl + mancozeb + mineral oil*	70g + 200g + 0.5%	15-30 November and 14-21 January
All cultivars irrespective of tree condition	Fosetyl-Al/mancozeb	450g	The first application to be sprayed just after the first flush has hardened followed by a second spray 50 days later.

- The spray program used/recommended by Citrus Research International is similar to that recommended by the Department of Agriculture.

REFERENCES

(1) Nel, A., Krause, M. & Khelawanlall, N., 2003. A Guide for the Control of Plant Disease compiled by Department of Agriculture 2nd Edition. Republic of South Africa, 31-34.

(2) Citrus Research International Integrated Production Guidelines for Export Citrus Volume III Integrated Pest and Disease Management. Grout, T. G. (Editor), 2003.

APPENDIX H:

SOME OF THE LAWS AND REGULATIONS THAT ARE RELAVENT TO THE SOUTH AFRICAN CITRUS NURSERY INDUSTRY

Laws and regulations:	**Aims:**
National Environment Management Act, 1998 (Act No. 107 of 1998)	-To provide for co-operative, environmental governance by establishing principles for decision-making on matters affecting the environment, institutions that will promote co-operative governance and procedures for coordinating environmental functions exercised by organs of the state and to provide for matters connected therewith.
Agricultural Product Standards Act, 1990 (Act No. 119 of 1990)	-To provide for control over the sale and export of certain agricultural products, control over the sale of certain imported agricultural products and control over other related products and for matters connected therewith.
Agricultural Pests Act, 1983 (Act No. 36 of 1983)	-To provide for measures by which agricultural pests may be prevented and combated.
Plant Improvement Act, 1976 (Act No. 53 of 1976)	-To provide a regulatory framework for the registration of premises from which the sale of certain plants or the cleansing, packing and sale of propagating material may be undertaken, to prescribe the conditions subject to which such plants or propagating material may be sold for the purposes of cultivation.
Conservation of Agricultural Resources Act, 1983 (Act No. 43 of 1983)	-To provide for the conservation of the natural agricultural resources by the maintenance of the production potential of land, by the combating and prevention of erosion and weakening or destruction of water resources and by the protection of the vegetation and the combating of weeds and invader plants.
Occupational Health and Safety Act, 1993 (Act No. 85 of 1993)	-To provide for the health and safety of persons at work and for the health and safety of persons in connection with the use of plant and machinery; the protection of persons other than persons at work against hazards to health and safety arising out of or in connection with the activities of persons at work, to establish an advisory council for occupational health and safety and to provide for matters connected therewith.
Designated Areas Development Act, 1979 (Act No. 87 of 1979)	-To provide for measures for the promotion of the density of population and of certain activities in certain areas designated by the Minister for the purpose.
Labour Relations Act, 1995 (Act No. 66 of 1995)	-To promote economic development, social justice, labour peace and democracy in the workplace. Applicable to workers in the citrus nursery industry.
National Water Act, 1998 (Act No. 36 of 1998)	-To provide for fundamental reform of the law relating to water resources, to repeal certain laws and to provide for matters connected therewith.

APPENDIX H:

SOME OF THE LAWS AND REGULATIONS THAT ARE RELAVENT TO THE SOUTH AFRICAN CITRUS NURSERY INDUSTRY (CONTINUED)

REFERENCES

(i) http://www.info.gov.za/gazette/acts/1998/a107-98.pdf

(ii) http://www.labour.gov.za & http://www.info.gov/gazette/acts/1998/a36-98.pdf

(iii) http://www.agriculure,kzntl.gov.za

(iv) http://www.acts.co.za/occupational_health_and_safety_act_1993.htm

(v) http://www.nda.agric.za/docs/agriculutral product stnadards/REGULATIONS.htm

APPENDIX I: Summary of global citrus nursery diseases.

Disease name	Symptoms locations	Inoculums/causes	Means of spread/ causal conditions	Control /remediation	References
	Soilborne diseases				
Damping off	-recently germinated seedlings collapse and die rapidly.	-decaying organic matter in soil.	-use of infested nursery stock. Equipments moves from infested area to non infested groves. Irrigation water when using furrow or flood irrigation.	-purchase certified citrus plants from nurseries that are registered with Citrus Improvement Scheme. -equipments should be cleaned before moved from one area to another area. - use systemic fungicides e.g. furalaxyl.	-Nel *et al.,* 2003. -Timmer *et al.,* 2000. -Department of Agriculture, 2007. -Kotzé, 2000.
Fertilizer burn	-recently germinating seedlings collapse and die rapidly.	-misuse of fertilizer.	- over application of fertilizer in the nursery.	-follow prescribed application on the labels. -consult chemicals company or consultant for help.	-Timmer *et al.,* 2000. -Browning *et al.,*1995.
Fumigation-induced stunting, nutritional deficiency and soil pH	-plants stunted and may be chlorotic.	-improper application of fumigation.	-poor spray distribution leading to excessive overlap. -improper nozzle placement -inadequate distribution of herbicides in the tank.	-follow prescribed application on the labels. -consult chemicals company or consultant for help.	-Timmer *et al.,* 2000. -Mukhopadhyay, 2004.
Nematodes injury and water damage	-fibrous root thickened and dead.	-soil and water - movement of infested nursery trees from one area to another.	-movement of soil, water and plant material. - wind and animals may also move nematodes pests. -rainfall.	-carefully cleaning of farm machinery. -use certified seed and other propagation materials. -hot water treatment of diseased propagative materials.	-Nel *et al.,* 2003. -Timmer *et al.,* 2000. -Department of Agriculture, 2007. -Mukhopadhyay, 2004. -Bedford, 1971.

APPENDIX I: Summary of global citrus nursery diseases.

Disease name	Symptoms locations	Inoculums/causes	Means of spread/ causal conditions	Control /remediation	References
				-applying nematicides: terbufos fosthiazate, fenamiphos.	
Phytophthora root rot	-fibrous root thickened and dead.	-zoospores carried by irrigation or rainwater to the roots. -prolonged contact of water with trees trunk exacerbate the diseases. -heavy and poorly drained soil.	-use of infested nursery stock, irrigation water when using furrow or flood irrigation. -use of susceptible rootstock. -low budding. -retention of water for longer period in beds. -flood irrigation and flat bed system. -repeated use of same land for nursery raising	-irrigation management (use of tensiometers to determine whether the trees are receiving too much water or too little water). -use of resistant rootstocks such as sour orange. -avoid flood irrigation. -seeds should be treated with fungicides before sowing e.g. Furalaxyl. -seeds should be sown in trays or in nursery beds where water cannot stand for longer periods and nursery should be fumigated before sowing. - nursery operation equipment should be separated from the orchard equipment to avoid contamination.	-Nel *et al.,* 2003. -Timmer *et al.,* 2000. -Department of Agriculture, 2007. -Erwin & Ribeiro, 1996.
	Seedling diseases				
Phytophthora foot rot, herbicide injury and	- trees suddenly turn yellow and frequently die back or die completely.	-zoospores carried by irrigation or rainwater to the	-use of infested nursery stock, irrigation water when using furrow or	-irrigation management (use of tensiometers to determine whether the trees is receiving	-Timmer *et al.,* 2000. -Department of Agriculture, 2007.

APPENDIX I: Summary of global citrus nursery diseases **(Continued).**

Disease name	Symptoms locations	Inoculums/causes	Means of spread/ causal conditions	Control/remediation	References
fertilization problem	-chlorosis most intense in midrib and blotchy pattern present.	roots. -prolonged contact of water with trees trunk exacerbate the diseases. -heavy and poor drained soil.	flood irrigation. -use of susceptible rootstock and low budding. -retention of water for longer period in beds. -flood irrigation and flat bed system. -repeated use of same land for nursery raising.	too much water or too little water. -use of resistant rootstock such as sour orange. -avoid flood irrigation.	-Erwin & Ribeiro, 1999. -Nel *et al.,* 2003.
	Leaf diseases				
Citrus canker	-necrotic lesions, often with chlorotic margins	-soil, crop residues and roots. -perennial plants. -weeds. -soil, crop residues and roots. -perennial plants. -weeds	-wind driven rain, overhead irrigation and contaminated equipment. -vegetative propagating material. -insects, seeds, water and animals. -plant's stomata, wounds on leaves or damage caused by citrus leaf miner larvae (*Phyllocnistis citrella).*	-if disease already established itself, eradication of all nursery trees is the only solution. -pruning, copper sprays, use of resistant cultivars, soil treatments, diseases forecasting, removal of infected leaves and stems, windbreaks or netting. -copper used to control canker: copper sulfate, copper hydroxide and copper carbonate. -applying quarantine measures.	-Cavalloro & Martino, 1986. -Whiteside, 1985. -Hardy, 1985a & b. -Graham & Gottwald, 1991. -Stall & Seymour, 1983. -Graham *et al,* 1989. -Swing & Civerolo, 1993. -Stall *et al*., 1980.
Citrus black spot	-small, round, sunken necrotic spots with gray centres, each	-spores (conidia and ascospores)	-grafting infected twigs on healthy trees.	-orchard sanitation, include removal of dead leaves in the	-McOnie, 1964(a, b, c, d and e).

APPENDIX I: Summary of global citrus nursery diseases **(Continued).**

Disease name	Symptoms locations	Inoculums/causes	Means of spread/ causal conditions	Control/remediation	References
	surrounded by a dark brown ring and yellow halo.	produced on dead leaves on the ground. -wet conditions.	-rain, water and wind. -nursery trees/budwood with latent infection	nursery. -purchase certified citrus plants from nursery registered with Citrus Improvement Scheme. -use of fungicides for control: azoxystrobin, benomyl+ mancozeb, copper hydroxide, carbendazim+mancozeb, zineb, copper oxychloride, mancozeb, fosetyl-Al/ mancozeb, maneb/zinc oxide, trifloxystrobin, pyraclostrobin-	-Kotzé, 1981. -Whiteside, 1967. -Wager, 1952. -Schüepp, 1961. -Nel *et al.,* 2003. -Timmer *et al.,* 2000
Scab diseases	-protuberances with a warty appearance and leaf distortion.	-dead twigs.	- dispersed by rainfall, irrigation and wind.	-copper oxychloride.	-Bushong & Timmer, 2000. -Nel *et al.,* 2003. -Timmer *et al.,* 2000.
Nutritional deficiency and spray toxicities	-leaves have chlorotic spot patterns in young leaves. -lower leaves mostly affected.	-misuse of fungicides.	-over application of fungicides. -lack of nutrients, soil should have 13 nutrients elements essential to all plant growth.	-follow prescribed application on the labels. -consult chemicals company or consultant for help.	-Timmer *et al.,* 2000.
Sooty mold	-leaves are covered with a brown or black film that can be removed by rubbing with moist cloth.	-sooty mold do not actually infect the plant, instead they grow on the sugar	-honey dew excreted by aphids, brown soft scale, citrus black flies and citrus whitefly.	-reduction of insects causing honeydew e.g. whiteflies.	-Mukhopadhyay, 2004. -Timmer *et al.*, 2000.

APPENDIX I: Summary of global citrus nursery diseases **(Continued).**

Disease name	Symptoms locations	Inoculums/causes	Means of spread/ causal conditions	Control/remediation	References
		exudates of insects such as aphids, brown soft scale, black flies and whiteflies.			
	(iv) Trees diseases				
Tristeza	-trees are budded on sour orange, leaves turn yellow in cool weather and chlorosis most intense in midrib and blotchy pattern present.	-prolonged contact of water with trees trunk exacerbate the diseases. -heavy and poor drained soil.	-several aphids species such as *Toxoptera citricida.* -movement of the infected budwood or nursery stock with citrus tristeza virus (CTV).	-quarantine and budwood certification programs. -aphids control may be useful in nurseries , but regarded as a practical long-term strategy for commercial plantings.	-Bar-Joseph *et al.,* 1989. -Timmer *et al.,* 2000. -Garnsey *et al.,* 1998. -Rocha-Peña, 1995.
Tristeza stem Pitting	-step pitting in twigs or branches		-CTV can be transmitted by budding and grafting.	-use of virus-free budwood should be used.	-Mukhopadhyay, 2004.
Nematodes	-fibrous roots alive but stubby or distorted	-soil and water. -some isolates of citrus tristeza virus.	-movement of soil, water and plant material. - wind and animals may also move nematodes pests.	-sanitation of equipment to remove soil and root debris. -use certified seed and other propagation materials. -hot water treatment of diseased propagative materials. -applying nematicides: terbufos, fosthiazate, fenamiphos.	-Timmer *et al.,* 2000. -Department of Agriculture, 2007.

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APPENDIX J: Major groups of arthropod citrus pests and registered pesticides for control.

Class/Order/Family	Representative species/ common name	Nature of injury	Control	References
Acari: **Prostigmata**			**Registered Pesticides**	
Eriophyidae	*Eriophyes sheldoni* (Ewing) Citrus bud mites	-Mites feed in buds, stunted trees and causing grotesque deformations of fruit and leaves.	-Aldicarb, amitraz, etoxazole, bromopropylate, chinomethionat, endosulfan,fenpropathrin+phenthoate, methamidophos, propargite, polysulphide sulfur, triazophos.	-Nel *et al.,* 2003. -Department of Agriculture, 2007. -Spiegel-Roy & Goldschmidt, 1996. -Browning *et al.,* 1995. -Mukhopadhyay, 2004. -Bedford, 1971.
	Phyllocoptruta oleivora (Ashmead) Citrus rust mite	-Mites feed on epidermal cells, causing silvering of yellow fruit and russeting of oranges.	-Aldicarb, bromopropylate, cyhexatin, chinomethionat, etoxazole,fenbutatin oxide, mancozeb, sulfur, profenofos, propargite, methamidophos,triazophos, polysulphide sulfur.	
Tarsonemidae	*Polypahgotarsonemus latus* (Banks) Broad mite	-Silvering of lemons, leaf curl.	-Bromopropylate, mancozeb, sulfur, propargite.	
Tenuipalpidae	*Brevipalpus phoenicis*(Geijskes) Citrus flat mite	-Chlorosis of fruit and leaves, galls on stems of seedlings.	-Amitraz, bromopropylate, dicofol, chinomethionat, etoxazole, fenbutatin oxide, propargite, spiradiclofen, polysulphide sulfur, sulfur, propargite.	
Tetranychidae	*Panonychus citri*(McGregor) Citrus red mite	-Defoliation, fruit drop, discoloration, reduction of fruit size	-Amitraz, bromopropylate, dicofol, chinomethionat, etoxazole, fenbutatin oxide, propargite, spiradiclofen, mineral oil, tetradifon, triazophos, triazophos+mineral oil.	

APPENDIX J: Major groups of arthropod citrus pests and registered pesticides for control **(Continued).**

Class/Order/Family	Representative species/ common name	Nature of injury	Control	References
Insecta: Homptera			**Registered Pesticides:**	
Aleyrodidae	*Aleurocanthus woglumi* (Ashby)Citrus blackfly *Dialeurodes citri* (Ashmead) Citrus whitefly	-Whiteflies and blackflies suck the sap from leaves and shoots, draining the plant and producing large amounts of honeydew, on which sooty mold fungi develop, blocking photosynthesis and contaminating fruit.	-Controlled by pesticides applied primarily for control of scale insects.	-Nel *et al.,* 2003. -Department of Agriculture, 2007. -Spiegel-Roy & Goldschmidt, 1996. -Browning *et al.,* 1995. -Mukhopadhyay, 2004. -Bedford, 1971.
Aphididae	*Aphis spiraecola* (Patch) Spirea aphid *Toxoptera aurantii* (Boyer de Fonscolombe) Black citrus aphid	-Aphids suck sap from young growth, causing leaf curl, blossom drop and sooty mold. Some species are vectors of tristeza and other diseases.	-Acetamiprid, aldicarb, chlorpyrifos, canola oil, azinphos-methyl, dimethoate,demeton-S-methyl, chlorpyrifos+cypermethrin, parathion, endosulfan, imidacloprid, methomyl, mercaptothion, methamidophos, mevinphos, oxydemeton-methyl, pirimicarb, profenofos, prothiofos, thiacloprid, triazophos.	
Coccidae	*Ceroplastes destructor* (Newstead) White wax scale *Coccus hesperidum* L. brown soft scale	-Soft and wax scales suck sap from leaves and branches, producing copious amounts of honeydew and sooty mold. Defoliation, fruit drop and dead wood may result and blemished fruit may be culled.	-Methidathion, methomyl, triazophos, azinphos-methyl, dimethoate, mercaptothion, methamidophos, mineral oil-light/medium.	
Diaspididae	*Lepidosaphes beckii* (Newman) Purple scale	-Soft and wax scales suck sap from leaves and branches, producing copious amounts of honeydew and sooty mold. Defoliation, fruit drop and dead wood may result and blemished fruit may be culled.	-Acetamiprid, aldicarb, chlorpyrifos, dimethoate, endosulfan, triazophos, methamidophos, mevinphos, profenofos, oxydemeton methyl,	

APPENDIX J: Major groups of arthropod citrus pests and registered pesticides for control **(Continued).**

Class/Order/Family	Representative species/ common name	Nature of injury	Control	References

Insecta: Homptera			**Registered Pesticides:**	
Diaspididae	*Unaspis yanonensis (*Kuwana) Arrowhead scale *Chrysomphalus aonidum* L. Red scale	-Armored scale insects suck the contents of cells on all parts of the tree, causing defoliation, fruit drop, dying back of twigs and branches, distortion and discoloration of fruit and culling of infested fruit. Severe attack may result in death of trees.	amitraz, bromopropylate, dicofol, chinomethionat, etoxazole, fenbutatin oxide, fenpropathrin+ mineral oil, propargite, tetradifon, triazophos+ mineral oil, buprofezin+mineral oil, imidacloprid, mercaptothion, mineral oil: light, paraffin white oil, parathion, pyriproxyfen+mineral oil, mineral oil-chlorpyrifos, thiamethoxam, dimethoate+methidathion, dimethoate+ mineral oil.	-Nel *et al.,* 2003. -Department of Agriculture, 2007. -Spiegel-Roy & Goldschmidt, 1996. -Browning *et al.,* 1995. -Mukhopadhyay, 2004. -Bedford, 1971.
Margarodidae	*Icerya purchase* (Maskell) Cottony cushion scale	-Sucking of sap from leaves and twigs may cause defoliation, fruit drop and heavy sooty mold.		
Pseudococcidae	*Planococcus calceolariae* (Risso) Citrus mealbug *Pseudococcus citriculus* (Green) Citriculus mealbug	-Sucking of sap from leaves and twigs may cause defoliation, fruit drop and heavy sooty mold. -Mealbugs suck sap, producing honeydew and sooty mold, sometimes attracting lepidopterous fruit pests. Feeding at fruit stems may cause fruit drop.	-Acetamiprid, buprofezin, buprofezin+ chlorpyrifos, mercaptothion, dimethoate, chlorpyrifos/cypermethrin, imidacloprid, methidathion, methomyl, mineral oil, omethoate, parathion, profenofos, prothiofos.	
Psyllidae	*Trioza erytreae* (Del Guercio) Citrus psylla	-The principal vector of greening disease, nymphs cause leaf galls.	-Aldicarb, chlorpyrifos, acetamiprid, dimethoate, endsulfan, methamidophos ,mevinphos, oxydemeton methyl, profenofos, triazophos, profenofos (premium grade).	

APPENDIX J: Major groups of arthropod citrus pests and registered pesticides for control **(Continued).**

Class/Order/Family	Representative species/ common name	Nature of injury	Control	References

			Registered Pesticides:	
Cicadellidae	*Empoasca citrusa* (Theron) Green citrus leafhopper	-Leafhoppers suck fruit, causing dark or chlorotic spotting, even fruit drop.	-Carbaryl, thiamethoxam, pymetrozine, diafenthiuron, aldicarb, acetamiprid.	-Nel *et al.,* 2003. -Department of Agriculture, 2007.
Insecta:Heteroptera				
Coreidae	*Leptoglossus phyllopus* (L.) leaf-footed bug	-Sucking of sap causes wilting of growth tips, and sometimes fruit loss.	-Mercaptothion, parathion, omethoate+ mineral oil, meracptothion+mineral oil, pyrethrum.	-Spiegel-Roy & Goldschmidt, 1996.
Pentatomidae	*Phynchocoris humeralis* (Thunberg) Citrus green stinkbug	-Stinkbugs such sap, causing serious crop loss.		-Browning *et al.,* 1995.
Thysanoptera:				-Mukhopadhyay, 2004.
Thripidae	*Frankliniella occidentalis* (Pergande) Western flower thrips *Scirtostocerca Americana* (Drury) Citrus thrips	-Thrips feed on epidermal scarring fruit, destroying buds, dwarfing and distorting leaves.	-Abamectin, acetamiprid, fipronil, formetanate, azdirachtin+pyrethrum, beta-cypermethrin, cypermethrin, fenpropathrin+phenthoate, sulfur, mercaptothion, imidacloprid, temephos, methamidophos, methiocarb, parathion profenofos, prothiophos, spinosad, tartar emetic, tau-fluvalinate, thiacloprid, triazophos.	-Bedford, 1971.
Diptera				
Tephritidae	*Anastrepha ludens* (Loew) Mexican fruit fly *Bactrocera tyromi* (Froggatt) Queensland fruit fly *Bactrocera dorsalis* (Hendel) Oriental fruit fly *Ceratitis capitata* (Wiedenmann) Mediterranean fruit fly.	-Fruit flies oviposit in ripening fruit, the punctures and developing larvae causing decay and fruit drop. Serious crop loss may result. Many species are on quarantine lists, prevent export of citrus and other fruit in South Africa.	-Mercaptothion+fish emulsion (attractant), mercaptothion, permetrhin/ fruit fly attractant, spinosad, protein hydrolysate, trichlorfon, trimedlure.	

APPENDIX J: Major groups of arthropod citrus pests and registered pesticides for control **(Continued).**

Class/Order/Family	**Representative species/ common name**	**Nature of injury**	**Control**	**References**

Hymenoptera:			**Registered Pesticides**	
Formicidae	*Atta sexdens* (L.) Leaf-cutting ant. *Iridomyrmex humilis* (Mayr) Argentine ant	-Many ants feed on honeydew, fostering homopterous pests and decimating their natural enemies. Leaf cutting ants feed on fungi grown on cut leaves and may cause serious damage to foliage.	**-** Alpha-cypermethrin, chlorpyrifos**,** hydramethylnon, isazofos.	-Nel *et al.,* 2003. -Department of Agriculture, 2007 -Spiegel-Roy & Goldschmidt, 1996. -Browning *et al.,* 1995. -Mukhopadhyay, 2004. -Bedford, 1971.
Lepidoptera:				
Geometridae	*Ascotis selanaria reciprocaria* (Walker) Citrus looper	-Caterpillars feed on fruit, leaves and blossoms.	-Endosulfan, esfenvalerate,profenofos, fenvalerate, profenofos (premium grade), zeta-cypermentrhin.	
Lynetiidae	*Phyllocnistis citrella* (Stainton) Citrus leaf miner	-Caterpillars mine leaves, may kill young Trees.	**-**Disulfoton, disulfoton/triadimenol, trichlorfon.	
Noctuidae	*Helicoverpa armigera* (Hübner) American bollworm	-Pierce fruits and causes blemishes mark.	-*Bacillus thuringiensis* var. Kurstaki, chlorpyrifos, cypermethrin, endosulfan, fenpropathrin+phenthoate, methomyl, mevinphos, profenofos, spinosad, profenofos (premium grade).	
Olethreutidae	*Cryptophlebia leucotreta* (Meyrick) False codling moth	-Caterpillars bore into ripening fruit, causing decay and fruit drop.	-Cypermenthrin, E-8 dodecenyl acetate/Z-8 dodecenyl acetate/E/Z-8 dodecenol, fenpropathrin, parathion-methyl, pheromone/permethrin, teflunezuron, triflumuron.	

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APPENDIX K: CHECKLIST FOR CITRUS NURSERY PRODUCTION PRACTICES:

NO	CONTROL POINT	LEVEL	COMPLIANCE CRITERIA
6.1	**GEOGRAPHICAL LOCATION:**		
6.1.1	**Selection and registration of a nursery site:**		
6.1.1.1	Have all factors been considered for selection of a nursery site?	Major	
6.1.1.2	Does a designated official inspect the site?	Major	
6.1.1.3	Has the official site inspection included all required specifications?	Major	
6.1.1.4	Has the official site inspection included an inspection of the mother block and nearby orchards?	Major	
6.1.1.5	Has an official certificate of approval been issued for citrus production on the site?	Major	
6.1.2	**Site history:**		
6.1.2.1	Is there evidence that a Risk Assessment has been done prior to selecting a new nursery site?	Major	
6.1.2.2	Is validation of the Risk Assessment Study conducted?	Major	
6.1.2.3	Provide evidence of a corrective action plan that specify strategies to minimize all identified risks on new nursery sites?	Minor	
6.1.2.4	Does the selected area have a history of CBS or is it close to an area with a history of CBS?	Major	
6.1.2.5	How is it confirmed that the area is free from CBS in the case of an existing nursery?	Major	
6.1.3	**Site management:**		
6.1.3.1	Is the nursery registered with the Citrus Growers Association and National Department of Agriculture?	Must	
6.1.3.2	What is the Citrus Black Spot (CBS) status of the nursery?	Major	
6.1.3.3	Has a CBS monitoring system been established for the mother trees or block used for propagative material in the nursery?	Major	

CHECKLIST FOR CITRUS NURSERY PRODUCTION PRACTICES:

NO	CONTROL POINT	LEVEL	COMPLIANCE CRITERIA

6.1.3.4	Has the citrus orchards from where budwood/scion material is collected been certified to a Good Agricultural Practices standard?	Major	
6.1.3.5	Has a visual identification or reference system for mother trees, blocks and the nursery been established?	Minor	
6.1.3.6	Has the nursery positioned for drainage rainwater control been planned to ensure effective runoff?	Major	
6.2	**BUILDING AND GREENHOUSE PHYSICAL STRUCTURES:**		
6.2.1	**Greenhouse**		
6.2.1.1	What are the advantages of having greenhouse structures in the nursery?	Minor	
6.2.1.2	Which materials are used to construct the greenhouse?	Minor	
6.2.1.3	Has the size of the greenhouse been calculated on sound nursery management practices?	Minor	
6.2.1.4	Has greenhouse access been restricted?	Major	
6.2.1.5	How is the floor for greenhouse structure?	Minor	
6.2.1.6	Has the optimal growing compartments been established to ensure best cultivation conditions?	Minor	
6.2.1.7	Does the facility have adequate environmental control infrastructure?	Minor	
6.2.1.8	Are hand wash facilities with soap and drying cloth paper etc available at the entrance of the Greenhouse?	Major	
6.2.2	**Toilets facilities:**		
6.2.2.1	Are toilet facilities available within the nursery yard?	Major	
6.2.2.2	Are sign boards erected or positioned to effectively indicate the direction of toilets?	Minor	

CHECKLIST FOR CITRUS NURSERY PRODUCTION PRACTICES:

NO	CONTROL POINT	LEVEL	COMPLIANCE CRITERIA
6.2.2.3	Are adequate hands washing facilities present at the toilets?	Major	
6.2.2.4	Does an effective toilet cleaning program exist and is it implemented and maintained?	Major	
6.2.2.5	Are the toilets conforming to the requirements of the national	Major	

	healthy authority?		
6.3	**SOIL AND SUBSTRATE MANAGEMENT:**		
6.3.1	**Site impact environment:**		
6.3.1.1	How is the soil in areas around the nursery conserved?	Major	
6.3.1.2	Is a disinfected footpath present at the entrance of the nursery and is it maintained and managed?	Major	
6.3.2	**Substrates management:**		
6.3.2.1	Does the nurseryman participate in substrate recycling programmes?	Recom.	
6.3.2.2	If chemicals are used to sterilize substrates for reuse, has the location of sterilization been recorded?	Major	
6.3.2.3	If chemicals are used to sterilize substrates for re-use, has the date of sterilization, type of chemicals, method of sterilization and name of the operator been recorded?	Minor	
6.3.2.4	If steaming is used to sterilized substrates for re-use, has records been kept?	Recom.	
6.3.2.5	Are substrates traceable to the source of origin and that it does not come from designated conservation areas?	Recom.	
6.4	**SOURCE OF MOTHER MATERIAL:**		
6.4.1	**Legislation:**		
6.4.1.1	Which legislations is the nursery industry must comply too?	Major	
6.4.1.2	Why is legislation important in the nursery industry?	Major	
6.4.1.3	Which aspects of the law does the nursery comply too?	Major	

CHECKLIST FOR CITRUS NURSERY PRODUCTION PRACTICES:

NO	CONTROL POINT	LEVEL	COMPLIANCE CRITERIA
6.4.1.4	What role does Agricultural Product Standard Act, 1990 (Act No. 119 of 1991) play in the nursery?	Major	
6.4.1.5	What role does Plant Breeders Right Act, 1976 (Act No. 15 of 1976) related to the nursery industry?	Major	
6.4.1.6	What role does Plant Improvement Act, 1976 (Act No. 53 of 1976) play in the nursery?	Major	
6.4.1.7	What role does Agricultural Pest Act, 1983 (Act No. 36 of 1983)	Major	

	play in the nursery?		
6.4.1.8	What role does National Water Act, 1998 (Act No. 36 of 1998) play in the nursery?	Major	
6.4.1.9	What role does Conservation of Agriculture Resources Act, 1983 (Act No. 43 of 1983) play in the nursery?	Major	
6.4.1.10	What role does Designated Areas Development Act, 1979 (Act No. 87 of 1979) play in the nursery?	Major	
6.4.1.11	What role does Labour Relations Act, 1995 (Act No. 66 of 1995) play in the nursery?	Major	
6.4.1.12	What role National Environment Management Act, 1998 (Act No.107 of 1998) play in the nursery?	Major	
6.4.1.13	What role Occupational Health and Safety Act, 1993 (Act No. 85 of 1993) play in the nursery?	Major	
6.4.1.14	What role does Fertilizers, Farm Feeds, Agricultural Remedies and Stock Remedies Act, 1947 (Act No. 36 of 1947) play in the nursery?	Major	
6.4.1.15	If using imported citrus material, are they compliant to South African legislation?	Major	
6.4.2	**Choice of variety or rootstock:**		
6.4.2.1	In case of own citrus breeding programmes have adequate plant	Major	

CHECKLIST FOR CITRUS NURSERY PRODUCTION PRACTICES:

NO	CONTROL POINT	LEVEL	COMPLIANCE CRITERIA
	breeding rights been considered?		
6.4.2.2	Is the nurseryman aware of the importance of effective crop husbandry in relation to the “mother plants” of the registered propagation material?	Recom.	
6.4.2.3	Does the variety or rootstock grown meet the International Union guidelines for the protection of new varieties of plants guidelines?	Minor	
6.4.3	**Seed/rootstock quality:**		
6.4.3.1	Are the evidence that certified seed were used in the nursery?	Minor	
6.4.4	**Pest and disease resistance:**		
6.4.4.1	Are the varieties grown resistance/tolerance to commercially	Recom.	

	important pests and diseases?		
6.4.5	**Seed treatments and dressings:**		
6.4.5.1	How are the seeds prepared in the nursery?	Minor	
6.4.5.2	In case of own seed treatment, is evidence available of the product/s used, application method, dosage, date, applicant and validated?	Recom.	
6.4.5.3	How is citrus seed stored?	Major	
6.4.5.4	How long is citrus seed stored?	Major	
6.4.5.5	At what temperature is the citrus seed stored?	Major	
6.4.6	**Propagation material:**		
6.4.6.1	Is imported purchased propagation material free of visible signs of pests and diseases?	Minor	
6.4.6.2	Is locally purchased material free of visible signs of pests and diseases?	Major	
6.4.6.3	Are plant health quality control systems operational for in-house nursery propagation?	Minor	

CHECKLIST FOR CITRUS NURSERY PRODUCTION PRACTICES:

NO	CONTROL POINT	LEVEL	COMPLIANCE CRITERIA
6.4.6.4	Are crop protection product treatments on in-house nursery propagation material applied during the plant propagation period recorded?	Minor	
6.4.7	**Sowing/Planting:**		
6.4.7.1	Are containers used for planting clean?	Major	
6.4.7.2	Are records kept on sowing/planting methods, seed/planting rate, and sowing/planting date?	Minor	
6.4.8	**Management of the mother block:**		
6.4.8.1	Has the protection of mother trees against pests, disease and weeds been achieved with the appropriate minimum propagation material protection product input?	Minor	
6.4.8.2	Are recognized Integrated Pest Management (IPM) techniques applied?	Recom.	
6.4.8.3	Has assistance with implementation of IPM systems been obtained through training?	Minor	

6.5	**FERTILIZER USE:**		
6.5.1	**Quantity, quality and type of fertilizer:**		
6.5.1.1	Can the technically responsible person demonstrate competence to determine quantity and type of fertilizer (organic and inorganic) to be used?	Minor	
6.5.2	**Method of application:**		
6.5.2.1	Can evidence be provided of type of fertilizer application method used and why it was selected?	Minor	
6.5.3	**Time of application:**		
6.5.3.1	When is the best time to apply fertilizer?	Major	
6.5.4	**Records of application:**		
6.5.4.1	Have all application dates of soil and foliar fertilizers both	Minor	

CHECKLIST FOR CITRUS NURSERY PRODUCTION PRACTICES:

NO	CONTROL POINT	LEVEL	COMPLIANCE CRITERIA
	organic and inorganic, been recorded including field, orchard, nursery block?		
6.5.4.2	Have all application dates, method of application and operator of soil and foliar fertilizers both organic and inorganic, been recorded?	Minor	
6.5.5	**Application machinery maintenance and operation:**		
6.5.5.1	Is fertilizer application machinery kept in good condition and a general maintenance plan followed?	Minor	
6.5.5.2	Is inorganic fertilizer application machinery verified annually to ensure accurate fertilizer delivery?	Recom.	
6.5.6	**Organic fertilizer:**		
6.5.6.1	Is human sewage sludge not used in the nursery?	Major	
6.5.6.2	Has a risk assessment been carried out before application of organic fertilizer, considering its source and characteristics?	Minor	
6.5.6.3	Has account been taken of the nutrient contribution of organic fertilizer?	Recom.	
6.5.6.4	Is organic fertilizer analysis done by a component laboratory and results where adequately interpreted?	Major	
6.5.7	**Inorganic fertilizer:**		

6.5.7.1	Are purchased inorganic fertilizers accompanied by evidence of chemical content and purity?	Recom.	
6.6	**STORAGE:**		
6.6.1	**Building structure:**		
6.6.1.1	Does the storage area comply with local regulations in terms of being secure, well ventilated and fire resistant?	Must	
6.6.1.2	Is the storage area built of materials that are appropriate to the temperature conditions?	Must	

CHECKLIST FOR CITRUS NURSERY PRODUCTION PRACTICES:

NO	CONTROL POINT	LEVEL	COMPLIANCE CRITERIA
6.6.1.3	Is there sufficient light in the storage room?	Must	
6.6.1.4	Is the storage area in a location away from other materials?	Must	
6.6.1.5	Is the storage shelving made of non-absorbent materials?	Recom.	
6.6.2	**Storage of fertilizer:**		
6.6.2.1	Is organic fertilizer stored in an appropriate manner, which reduces the risk of contamination of the environment?	Recom.	
6.6.2.2	Are inorganic and organic fertilizers stored separate from plant propagation material?	Major	
6.6.2.3	Are inorganic fertilizers stored in a clean and dry area?	Minor	
6.6.2.4	Is there an up to date inorganic fertilizer stock inventory available at the nursery?	Minor	
6.6.2.5	Are inorganic fertilizers stored separately from chemicals?	Major	
6.6.2.6	Are inorganic fertilizers stored in a covered area?	Minor	
6.6.2.7	Are fertilizers stored off the ground?	Major	
6.6.3	**Storage of pesticides and biocides:**		
6.6.3.1	Are chemicals stored in accordance with local regulations?	Major	
6.6.3.2	Are chemical stored in a secure location away from other chemicals?	Minor	
6.6.3.3	Are chemicals stored in a location that is fire resistant?	Minor	
6.6.3.4	Are chemicals stored in a location that is well lit?	Minor	
6.6.3.5	Are chemicals stored in a location that is well ventilated?	Minor	
6.6.3.6	Is the chemical product store able to retain spillage?	Minor	

6.6.3.7	Are there facilities for measuring chemicals?	Major	
6.6.3.8	Are there facilities to deal with spillage?	Major	
6.6.3.9	Are keys and access to the chemicals store limited to workers with formal training in the handling of chemicals?	Must	
6.6.3.10	Is the product inventory documented and readily available?	Minor	

CHECKLIST FOR CITRUS NURSERY PRODUCTION PRACTICES:

NO	CONTROL POINT	LEVEL	COMPLIANCE CRITERIA
6.6.3.11	Are all chemicals stored in their original package?	Major	
6.6.3.12	Are the chemicals stored approved for use in citrus?	Must	
6.6.3.13	Are liquids not stored on shelves above powders?	Minor	
6.6.4	**Packing and storage areas:**		
6.6.4.1	Are produce handling and storage facilities and equipment cleaned and maintained so as to prevent contamination?	Must	
6.6.4.2	Are cleaning agents stored to prevent chemical contamination of produce?	Minor.	
6.6.4.3	Are all forklifts and other driven transport trolleys clean and well maintained to avoid contamination through emissions?	Recom.	
6.6.4.4	Are packing materials clean and stored in clean and hygienic conditions?	Must	
6.7	**IRRIGATION/FERTIGATION:**		
6.7.1	**Water treatment and testing:**		
6.7.1.1	Assess the water quality management plan and determine which steps is in place for ensuing that the water in the nursery is pathogen free?	Major	
6.7.2	**Predicting irrigation requirements:**		
6.7.2.1	Is there assess efficacy of irrigation schedule of nursery plants, seedlings etc?	Minor	
6.7.2.2	Have systematic methods of prediction been used to determine the water requirements of the propagation material?	Recom.	
6.7.2.3	Is predicted rainfall taken into account when calculating irrigation application?	Recom.	
6.7.2.4	Is evaporation taken into account when calculating irrigation	Recom.	

	applications?		
6.7.3	**Irrigation/fertigation methods:**		

CHECKLIST FOR CITRUS NURSERY PRODUCTION PRACTICES:

NO	CONTROL POINT	LEVEL	COMPLIANCE CRITERIA
6.7.3.1	Has the most efficient and commercially practical water delivery system been used to ensure the best utilization of water resources?	Recom.	
6.7.3.2	Is there a water management plan to optimize water usage and reduce waste?	Recom.	
6.7.3.3	Are records of irrigation/fertigation water usage maintained?	Recom.	
6.7.4	**Quantity of irrigation water:**		
6.7.4.1	Has an annual risk assessment for irrigation/fertigation water pollution been completed?	Recom.	
6.7.4.2	Is irrigation water analysed at least once a year?	Recom.	
6.7.4.3	Does a suitable laboratory carry out microbial and chemical analysis?	Recom.	
6.7.4.4	Is or has untreated sewage water not been used for irrigation/fertigation?	Major	
6.7.4.5	If contaminated water has been used has a Risk Assessment been done addressing chemical and heavy metal pollutants?	Recom.	
6.7.4.6	Does the analysis consider the microbial contaminate?	Recom.	
6.7.4.7	Have any adverse results been acted upon?	Recom.	
6.7.5	**Supply of irrigation/fertigation water:**		
6.7.5.1	Has irrigation water been obtained from sustainable sources?	Recom.	
6.7.5.2	Has advice on abstraction been from water authorities?	Recom.	
6.8	**CHEMICAL PROGRAMME:**		
6.8.1	**Choice of pesticides and biocides:**		
6.8.1.1	Do you follow a recognized disease control program for pest and disease control in general?	Major	
6.8.1.2	Is the type of plant protection product applied appropriate for the target as recommended on the product label?	Major	

CHECKLIST FOR CITRUS NURSERY PRODUCTION PRACTICES:

NO	CONTROL POINT	LEVEL	COMPLIANCE CRITERIA
6.8.1.3	Do nurseries only use plant protection products that are registered in the country of use for the target crop?	Major	
6.8.1.4	Are invoices of registered plant protection products kept for record keeping?	Minor	
6.8.1.5	Is a current list of plant protection products that are used and approved for use on crops being grown in the nursery kept for recording keeping?	Minor	
6.8.1.6	Is the choice of plant protection products made by a qualified adviser, can they demonstrate competence?	Major	
6.8.1.7	If the choice of plant protection products is made by the nursery manager, can competence and knowledge be demonstrated?	Major	
6.8.2	**Records of chemicals applications:**		
6.8.2.1	Have all the chemicals applications been recorded including the crop name or variety?	Major	
6.8.2.2	Have all the plant protection product applications been recorded including the location?	Major	
6.8.2.3	Have all the plant protection product applications been recorded including application date?	Major	
6.8.2.4	Have all the plant protection product applications been recorded including the product trade name?	Major	
6.8.2.5	Has the operator been identified for plant protection product applications?	Minor	
6.8.2.6	Have all the chemicals applications been recorded including justification for application?	Minor	
6.8.2.7	Have all the chemicals applications been recorded including appropriate information to identify the product quantity applied?	Minor	
6.8.2.8	Have all the chemicals applications been recorded including the	Minor	

CHECKLIST FOR CITRUS NURSERY PRODUCTION PRACTICES:

NO	CONTROL POINT	LEVEL	COMPLIANCE CRITERIA
	technical authorization for application?		
6.8.2.9	Have all the chemicals application been recorded including the application machinery used?	Minor	

6.8.3	**Application equipment management and operational procedures:**		
6.8.3.1	Is plant protection product application machinery kept in good condition and verified annually to ensure accurate application?	Major	
6.8.3.2	Is the producer involved in an independent calibration certification scheme, where available?	Recom.	
6.8.3.3	When mixing plant protection products, are the correct handling and filling procedures, followed as stated on the label?	Minor	
6.8.4	**Disposal of empty chemicals containers:**		
6.8.4.1	Is re-use of empty chemicals containers for purposes other than containing and transporting of the identical product avoided?	Minor	
6.8.4.2	Does disposal of empty plant protection product containers occur in a manner that avoids exposure to humans?	Minor	
6.8.4.3	Does disposal of empty chemicals occur in a manner that avoids contamination of the environment?	Major	
6.8.4.4	Are official collection and disposal systems used when available?	Minor	
6.8.4.5	If there is a collection system. Are the empty containers adequately stored, labelled and handed to the rules of collection?	Minor	
6.8.4.6	Are empty containers kept secure until disposal is possible?	Minor	
6.8.4.7	Are all local regulations disposal or destruction of containers observed?	Major	
6.9	**WASTE AND POLLUTION MANAGEMENT**		
6.9.1	**Identification of waste and pollutants:**		

CHECKLIST FOR CITRUS NURSERY PRODUCTION PRACTICES:

NO	CONTROL POINT	LEVEL	COMPLIANCE CRITERIA
6.9.1.1	Have all possible waste products been identified in all areas surrounding the nursery?	Recom.	
6.9.1.2	Have potential sources of pollution been identified?	Recom.	
6.9.2	**Waste and pollution management action plan:**		
6.9.2.1	Are the nursery and premises clear of litter and waste?	Recom.	
6.9.2.2	Is there a documented plan to avoid or reduce wastage and pollution and avoid the use of landfill or burning, by waste	Recom.	

	recycling?		
6.9.2.3	Has waste management plan been implemented?	Recom.	
6.9.2.4	Do the premises have adequate provisions for waste disposal?	Recom.	
6.10	**NURSERY HYGIENE:**		
6.10.1	**Risk Assessments:**		
6.10.1.1	Has a risk assessments for safe and healthy working conditions been carried out?	Recom.	
6.10.1.2	Has this risk assessment been used to develop an action plan to promote safe and healthy working conditions?	Recom.	
6.10.2	**Facilities, equipment and accident procedures:**		
6.10.2.1	Are First Aid boxes present in the vicinity of the work?	Recom.	
6.10.2.2	Are hazards clearly identified by warning signs?	Recom.	
6.10.2.3	Do accident and emergency procedures exist?	Minor	
6.10.2.4	Are signs warning of potential dangers placed on access doors?	Minor	
6.10.3	**Nursery sanitation:**		
6.10.3.1	How is the nursery fenced?	Major	
6.10.3.2	Are footbaths installed in the nursery and how are they maintained?	Major	
6.10.3.3	What sanitation rules are implemented in the nursery?	Major	

CHECKLIST FOR CITRUS NURSERY PRODUCTION PRACTICES:

NO	**CONTROL POINT**	**LEVEL**	**COMPLIANCE CRITERIA**
6.10.3.4	What steps are taken to ensure good sanitation in the seed germination area?	Major	
6.10.4	**Weed management:**		
6.10.4.1	What are the factors to be considered when developing a weed management program?	Major	
6.10.4.2	How do you control weed management in citrus nursery?	Major	
6.10.4.3	How do you make sure that correct application of herbicides is in place?	Major	
6.10.4.4	How to avoid herbicides resistance?	Major	
6.11	**WORKERS HYGIENE, SAFETY AND WELFARE:**		

6.11.1	**Type of training and records:**		
6.11.1.1	Has formal training or instruction been given to all workers operating dangerous or complex equipment?	Minor	
6.11.1.2	Is a record of training kept for each worker?	Recom.	
6.11.1.3	Is there a person trained in First Aid at the nursery?	Recom.	
6.11.1.4	Has all workers received basic hygiene training for the handling of propagation material regarding hand cleaning, skin cuts, smoking, eating and drinking in permitted areas?	Recom.	
6.11.1.5	Are all subcontractors and visitors aware of the relevant demands personal hygiene?	Recom.	
6.11.2	**Personal Hygiene:**		
6.11.2.1	Have workers received basic instructions in hygiene before handling produce?	Major	
6.11.2.2	Do the workers implement the hygiene instructions for handling produce?	Minor	
6.11.2.3	Are all workers wearing outer garments that are clean and fit for purpose for the operation and able to protect products from	Recom.	

CHECKLIST FOR CITRUS NURSERY PRODUCTION PRACTICES:

NO	CONTROL POINT	LEVEL	COMPLIANCE CRITERIA
	contamination?		
6.11.2.4	Are smoking, eating, chewing and drinking confined to designated areas separated from plant materials?	Minor	
6.11.2.5	Are signs clearly displayed in the picking facilities with the main hygiene instructions for workers and visitors?	Minor	
6.11.2.6	Do workers in the nursery have access to clean toilets and hand washing facilities?	Major	
6.11.3	**Crop Protection product handling:**		
6.11.3.1	Are the workers who handle and apply crop protection products trained?	Minor	
6.11.3.2	Are all staff has contact with crop protection products submitted voluntary to annual health checks in line with guidelines laid down in local codes of practice?	Recom.	
6.11.4	**Protective clothing/equipment maintenance and handling:**		

6.11.4.1	Are workers equipped with suitable protective clothing in accordance with label instructions?	Major	
6.11.4.2	Is protective clothing cleaned after use?	Minor	
6.11.4.3	Is protective clothing and equipment stored separately from crop protection products?	Major	
6.11.5	**Workers health, safety and welfare:**		
6.11.5.1	Is a member of management clearly identifiable as responsible for workers health, safety and welfare issues?	Minor	
6.11.5.2	Do regular two-way communication meetings take place between management and employees? Are there records from such meetings?	Recom.	
6.11.5.3	Are your employees aware and comply with Occupational Health and Safety Act, 1993 (Act No. 85 of 1993?	Regulatory requirement	

CHECKLIST FOR CITRUS NURSERY PRODUCTION PRACTICES:

NO	CONTROL POINT	LEVEL	COMPLIANCE CRITERIA
6.11.6	**Visitor safety:**		
6.11.6.1	Are all visitors aware of the relevant demands on personal safety?	Recom.	
6.11.6.2	Are records available of visitors and is it signed?	Recom.	
7	**PROTOCOL FOR DETECTING PATHOGENS:**		
7.1	**Monitoring for the presence of *Phytophthora:***		
7.1.1	When is the right time to test soil for the presence of *Phytophthora?*	Major	
7.1.2	Has *Phytophthora* detection system been established?	Minor	
7.1.3	Which precaution measures are in place to minimize the impact of *Phytophthora* disease?	Major	
7.1.4	Has an effective *Phytophthora* preventative programme been established in the nursery?	Major	
7.2	**Monitoring for the presence of Nematodes:**		
7.2.1	When is the right time to test for the presence of nematode?	Major	
7.2.2	What can be done to determine potential nematode problems in a field before it is planted?	Major	
7.2.3	What are the symptoms of citrus nematodes damage on citrus	Major	

	plants?		
7.2.4	Has a citrus nematode detection system been established?	Major	
7.2.5	Has an effective nematode preventative programme been established?	Major	
7.2.6	How are nematodes managed or control in the nursery?	Major	
7.2.7	What is considered to be a proper sample for citrus nematode detection?	Minor	
7.2.8	How is the sample bags marked?	Minor	
7.2.9	Which method is used to transport samples?	Minor	

CHECKLIST FOR CITRUS NURSERY PRODUCTION PRACTICES:

NO	CONTROL POINT	LEVEL	COMPLIANCE CRITERIA
7.2.10	Which methods are used to diagnose nematodes in the laboratory?	Major	
7.3	**Monitoring for the Presence of Tristeza virus:**		
7.3.1	What are the typical symptoms caused by Citrus Tristeza Virus (CTV)?	Major	
7.3.2	How is CTV transmitted into the nursery?	Major	
7.3.4	Has a CTV be detection system been established?	Major	
7.3.5	Has an effective CTV preventative programme been established?	Major	
7.4	**Monitoring for the presence of insect and pest:**		
7.4.1	Which pest and insect causes economic damage in the citrus nursery?	Major	
7.4.2	Has pest and insects detection system been established in the nursery?	Major	
7.4.3	Has an effective pest and insects preventative programme been established?	Major	
7.5	**Monitoring for the presence of citrus greening:**		
7.5.1	What are the symptoms of citrus greening disease?	Major	
7.5.2	How is citrus greening transmitted?	Major	
7.5.3	How to detect citrus greening in the nursery?	Major	
7.5.4	How to control citrus greening diseases?	Major	
8.	**DETERMINATION OF CITRUS BLACK SPOT (CBS)**		

	STATUS OF NURSERY TREES:		
8.1	**Adjacent CBS infected orchards:**		
8.1.1	How do you prevent infection from adjacent CBS infected orchards?	Major	
8.1.2	How effective is the windbreaker in preventing CBS infection to your nursery?	Minor	

CHECKLIST FOR CITRUS NURSERY PRODUCTION PRACTICES:

NO	CONTROL POINT	LEVEL	COMPLIANCE CRITERIA
8.2	**Inspection (detection and testing) of CBS:**		
8.2.1	Is there a system in place to assess the presence of CBS in the adjacent and nursery?	Recom.	
8.2.2	Is there a corrective action plan to minimize the impact of CBS?	Major	
8.3	**Sampling for CBS:**		
8.3.1	Has a formal training been given to all workers with regards to CBS identification of leaf samples?	Minor	
8.3.2	Which procedure do you follow before sampling?	Major	
8.3.3	Which equipment is used to sample leaves for detecting CBS?	Major	
8.3.4	How many leaves must be sampled for detecting CBS?	Major	
8.3.5	Which sampling methods are used for detecting CBS?	Regulatory requirement	
8.3.6	How were the sample block identified in the nursery?	Major	
8.3.5	Which sampling methods are used for detecting CBS?	Regulatory requirement	
8.3.6	How were the sample block identified in the nursery?	Major	
8.3.7	How were the samples packed and dispatched to the diagnostic center?	Major	
8.3.8	How is the sample bags marked?	Major	
8.3.9	How is the information for tracebility purpose written in the sample for CBS analysis?	Minor	
8.3.10	Which method is used to transport samples to the laboratory for analysis?	Major	
8.4	**Laboratory analysis:**		
8.4.1	Which laboratory is the nursery using to analyze sampled	Major	

	leaves?		
8.4.2	How is the leaf sample drawn prepared for laboratory analysis?	Major	

CHECKLIST FOR CITRUS NURSERY PRODUCTION PRACTICES:

NO	CONTROL POINT	LEVEL	COMPLIANCE CRITERIA
8.4.3	How do you process a leaf sample for pathogens analysis?	Major	
8.4.4	Which equipment is used to select leaves samples?	Major	
8.4.5	How do you detect the presence of CBS after testing the received sample?	Major	
8.4.6	Are the results of the analysis recorded?	Minor	