CFPA	DFPT	DFTS	Winetech
Canning Fruit Producers' Assoc. Submit to: Wiehahn Victor PO Box 426 Paarl, 7620 Tel: +27 (0)21 872 1501 **inmaak@mweb.co.za**	Deciduous Fruit Producers' Trust Submit to: Louise Liebenberg PO Box 12789 Die Boord, 7613 Tel: +27 (0)21 882 8470/1 **louise@dfptresearch.co.za**	Dried Fruit Technical Services Submit to: Dappie Smit PO Box 163 Paarl, 7622 Tel: +27 (0)21 870 2900 **dappies@dtd.co.za**	Submit to: Jan Booysen PO Box 825 Paarl, 7624 Tel: +27 (0)21 807 3324 **booysenj@kwv.co.za**
			X

Indicate (X) client(s) to whom this final report is submitted.
Replace any of these with other relevant clients if required.

FINAL REPORT
FOR 2007

PROGRAMME & PROJECT LEADER INFORMATION

	Programme leader	Project leader
Title, initials, surname	Dr PH Fourie	Dr PH Fourie
Present position	Snr Researcher	Snr Researcher
Address	Dept Plant Pathology University of Stellenbosch Private Bag X1 Matieland 7602	Dept Plant Pathology University of Stellenbosch Private Bag X1 Matieland 7602
Tel. / Cell no.	021-8083721	021-8083721
Fax	021-8084956	021-8084956
E-mail	phf@cri.co.za	phf@cri.co.za

PROJECT INFORMATION

Project number	USPP07/2006

Project title	Fungal pathogens associated with rootstock cane necrosis

Industry programme	**CFPA**	
	DFPT	
	DFTS	
	Winetech	Plant protection
	Other	

Fruit kind(s)	Grapevine

Start date (dd/mm/yyyy)	January 2006	**End date** (dd/mm/yyyy)	December 2007*

***Project not funded in 2007/8 and Final Report is therefore submitted.**

FINAL SUMMARY OF RESEARCH PROJECT

PROGRAMME & PROJECT LEADER INFORMATION

	Programme leader	Project leader
Title, initials, surname	Dr PH Fourie	Dr PH Fourie
Institution	Department of Plant Pathology University of Stellenbosch	Department of Plant Pathology University of Stellenbosch
Tel. / Cell no.	021-8083721	021-8083721
E-mail	phf@cri.co.za	phf@cri.co.za

PROJECT INFORMATION

Project number	USPP07/2006

Project title	Fungal pathogens associated with rootstock cane necrosis

Fruit kind(s)	Grapevines

Start date (dd/mm/yyyy)	January 2006	**End date** (dd/mm/yyyy)	December 2007

During 2005 and 2006 samples of rootstock cuttings were collected during the period of harvesting and preparation of rootstock cuttings. These samples were examined for any external and internal symptoms. Isolations, from observed internal symptoms, were made onto PDA. Fungi isolated at the highest frequency from these symptoms were *Botryosphaeriaceae* and *Phomopsis* spp., with *Phaeoacremonium* spp. and *Phaeomoniella chlamydospora* isolated at much lower frequencies. These genera have all been shown to be important grapevine trunk pathogens, infecting pruning wounds and other mechanical wounds, ultimately causing decline and dieback of nursery and mature grapevines. Some isolates, representing *Phoma* and *Acremonium* spp., were also isolated, although these are not recognised as grapevine pathogens. Management strategies aimed at preventing wound infection of rootstock canes by abovementioned pathogens are recommended. These include treatment of pruning wounds on mother vines with pruning wound protecting agents, trellising of mother vines to prevent injury of the rootstock canes and careful inspection and selection of canes prior to grafting.

FINAL REPORT

1. Problem identification and objectives

State the problem being addressed and the ultimate aim of the project.

Rootstock cane necrosis (i.e. dead zones in rootstock canes or cuttings) has been identified in several Vine Improvement Association reports as a major cause of graftling failure in grapevine nurseries. Moreover, if these necrotic lesions are not detected during certification, it would most definitely lead to premature dieback of grapevines in vineyards. The aim of this project was to determine whether rootstock cane necrosis is caused by one or more fungal pathogens. If this rootstock cane necrosis is revealed to be caused by a fungal pathogen or group of pathogens, epidemiological and management studies will be proposed.

2. Workplan (materials & methods)

List trial sites, treatments, experimental layout and statistical detail, sampling detail, cold storage and examination stages and parameters.

Phase 1: Determine biotic nature of rootstock cane necrosis (2006)

In 2005, a small number of samples were collected and examined and preliminary results reported in the 2006 progress report. However, during 2006, more extensive surveys were conducted during the harvesting and preparation of rootstock cuttings in co-operation with KWV-Vititec. Seven batches of samples were obtained from 6 different rootstock producers in 4 different areas (Table 1). Samples consisted of rootstock cuttings, representing different rootstock cultivars and clones (Table 1) that exhibited necrotic lesions. Atotal of 532 cuttings were examined externally and observed symptoms photographed. Subsequent to external examination, rootstock cuttings were dissected into 1–2 cm sections. These sections were again examined for any internal wood necrosis symptoms. Any internal symptoms were again photographed. Subsequent to examination, at least one rootstock section, representing each type of internal symptom, were selected for pathogen isolation. One asymptomatic section was also included to determine the possible presence of latent pathogen infection.

The selected rootstock sections were triple sterilised by immersion into 70% ethanol for 30 s, 1 min in 0.35% NaOCl and again for 30 s in 70% ethanol. After strelisation, sections were split longitudinally and 4 pieces of tissue were removed aseptically from the edge of each symptom type and plated out onto potato dextrose agar (PDA) amended with streptomycin sulphate (40 mg/L) to reduce bacterial growth. All isolations were done under sterile conditions in a laminar flow. After isolation, dishes were incubated at ± 25 ˚C for 4 weeks. Fungal growth on the dishes were carefully monitored, with fungi growing from the tissue pieces sub cultured through hyphal tipping onto fresh PDA dishes. Pure fungal cultures obtained after hyphal tipping were identified to genus, and where possible, to species level based on morphological characteristics. Fungi belonging to potentially pathogenic genera were stored in sterile water and on PDA slants for future identification and pathogenicity testing.

Table 1. Areas and rootstock cultivars and clones surveyed during 2006.

Batch	Cultivar	Clone	Area
A	R 99	RY 13 C	Piketberg
B	R 99	RY 179 B	Piketberg
C	R 110	RQ 28 C	Rawsonville
D	R 110	RQ 28 C	Rawsonville
E	101-14	AA 219 A	Vanrhynsdorp
F	US 8-7	UC 1	Rawsonvile
G	R 99	RY 13 E	Nieuwoudtville

Phase 2: Determine the causal pathogens of rootstock cane necrosis (2007, 2008)

This phase of the project was not completed due to the project being terminated.

In this phase, all representative isolates, not already identified to species level, within potentially pathogenic genera would have been identified to species level based on molecular and morphological characteristics. Following species identification, infection studies would have been conducted on selected rootstock cultivars to determine the pathogenicity and virulence of the identified fungal species. Representative isolates, with relevant information, are maintained at the Department of Plant Pathology, University of Stellenbosch.

3. Results and discussion

State results obtained and list any benefits to the industry. Include a short discussion if applicable to your results.
This final discussion must cover ALL accumulated results from the start of the project, but please limit it to *essential* information.

Results

Milestone	Achievement
1. Determine biotic nature of rootstock cane necrosis	Milestone completed.
2. Species-identification of fungi associated with rootstock cane necrosis	Milestone not completed due to project termination.
3. Determine pathogenicity and virulence of identified fungi	Milestone not completed due to project termination.
4. Determine pathogenesis of rootstock cane necrosis	Milestone not completed due to project termination.

Phase 1: Determine biotic nature of rootstock cane necrosis

During 2005, a preliminary assay was conducted and two batches of rootstock cuttings exhibiting necrotic symptoms were sampled during rootstock cutting preparation. Symptoms that were observed included mild to severe cankers, watery necrosis and general dried-out samples. When examining these canes internally, the external necrotic lesions manifested itself as light to dark brown discolouration of the underlying vascular tissue. Isolations from all observed symptoms was made onto PDA medium and yielded a great variety of fungi that included saprophytes as well known grapevine trunk pathogens. Pathogens isolated with the highest frequency were *Phomopsis* and *Botryosphaeriaceae* spp., while *Phaeomoniella chlamydospora* and *Phaeoacremonium* spp. were isolated at a much lower frequency. These preliminary results indicated that trunk disease pathogens are involved in rootstock necrosis, which could be an important source of infection that manifests itself in older, more established vineyards.

In 2006, 7 batches of rootstock cuttings, representing 4 different areas, 4 cultivars and 6 clones were sampled (Table 1). External symptoms observed on these cuttings included mild to severe cankers (Figure 1) and necrosis (Figure 2) as well as surface wounds (Figure 3) and cracks (Figure 4). Upon internal observation, the necrosis observed externally manifested itself as brown (Figure 5) to black (Figure 6) vascular streaking and watery (Figure 7) and brown necrosis (Figure 8). Isolations made from these observed symptoms onto PDA medium again yielded a great variety of fungi, which included saprophytes as well as several isolates of known grapevine trunk pathogens. A total of 57 isolates representing unknown and possible grapevine pathogens were obtained. Pathogens isolated in the highest percentage during the 2006 survey were *Fusarium* spp. (33.3%), *Botryosphaeriaceae* spp. (31.6%) and *Phomopsis* spp. (19.3%), while *Pilidiella* (8.8%), and *Phaeoacremonium* (1.8%) spp. was isolated at a much lower percentage. *Phoma* and *Acremonium* spp. were also isolated at low percentages (3.5 and 1.8%, respectively) from brown streaking and brown necrosis (Table 2). However, the status of these fungi as grapevine pathogens is currently unknown. *Botryosphaeriaceae* and *Fusarium* spp. were isolated, at varying percentages from all observed symptom types, except asymptomatic tissue, while *Phomopsis* spp. were isolated from all symptom types, including asymptomatic tissue (Table 2). *Phaeoacremonium* spp. was isolated only from brown necrosis, similar to *Pilidiella* spp., which was also isolated from black streaking (Table 2).

Table 2. Total percentage of various fungi obtained in survey as well as percentage of isolates obtained from different internal symptom types observed.

Fungus	Total[1]	Internal symptom types				
		Brown streaking	Black streaking	Brown necrosis	Watery necrosis	Asymptomatic tissue
Acremonium spp.	1.8	1.8[2]	-	-	-	-
Botryosphaeriaceae spp.	31.6	1.7	15.8	12.3	1.8	-
Fusarium spp.	33.3	3.5	5.3	21.0	3.5	-
Phaeoacremonium spp.	1.8	-	-	1.8	-	-
Phoma spp.	3.5	-	-	3.5	-	-
Phomopsis spp.	19.3	5.3	3.4	5.3	3.5	1.8
Pilidiella spp.	8.8	-	7.0	1.8	-	-

[1] Percentage of isolates of specific fungus calculated from a total of 57 isolates obtained.
[2] Percentage isolates of fungus obtained from specific symptom type.

Figure 1. Canker observed on surface of a rootstock cane.

Figure 2. Necrosis and fruiting bodies observed on the surface of a rootstock cane.

Figure 3. Wound observed on surface of rootstock cane.

Figure 4. Cracks observed in the bark of a rootstock cane.

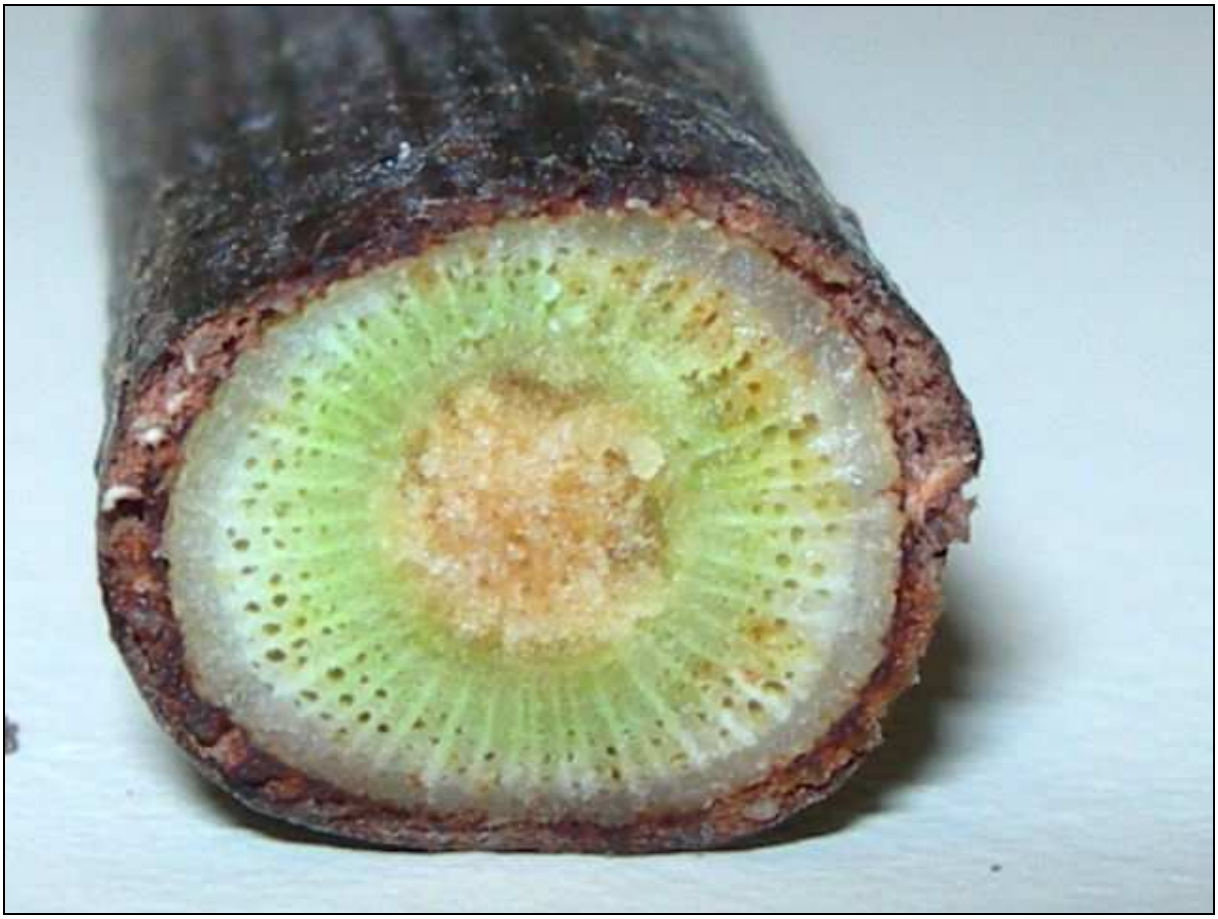

Figure 5. Brown spots, visible as brown vascular streaking, when rootstock cane is split longitudinally.

Figure 6. Arch-shaped lesions, visible as black vascular streaking, when rootstock canes are split longitudinally.

Figure 7. Watery necrosis observed internally in a rootstock cane.

Figure 8. Brown necrosis observed internally in a rootstock cane.

Phase 2: Determine the causal pathogens of rootstock cane necrosis (2007, 2008)

The molecular identification of unknown isolates, as well as the proposed infection studies was not conducted due to the termination of the research project.

Discussion

In previous studies into the etiology of grapevine trunk diseases, several *Botryosphaeriaceae*, *Phomopsis* and *Phaeoacremonium* spp. as well as *Phaeomoniella chlamydospora* have been identified associated with grapevines in South Africa and other grape producing countries of the world (Mugnai *et al*., 1999; van Niekerk *et al*., 2004a, 2005; Mostert *et al*., 2006). These pathogens cause premature decline and dieback of grapevines, consequently causing great economic loss due to decline in yield and grape quality of infected grapevines (Mugnai *et al*., 1999; van Niekerk *et al*., 2004a, 2005; Mostert *et al*., 2006). Pruning wounds and wounds caused by mechanical injuries are furthermore know to be important infection portals for all of these pathogens (Larignon and Dubos, 2000; van Niekerk *et al*., 2004a, 2005).

The presence of these pathogens in the rootstock samples examined in this trial supports earlier findings that indicated that *Botryosphaeriaceae*, *Phomopsis* and *Phaeoacremonium* spp. as well as *Phaeomoniella chlamydospora* are often present in propagation material and the young grapevine plants propagated from this material (Halleen *et al*., 2003; Fourie and Halleen, 2002; Fourie and Halleen, 2004a). Consequently, these infected nursery plants are used in the establishment of new commercial vineyards. Several *Fusarium* spp. have also previously been shown to be associated with grapevines, causing root necrosis and ultimately decline and dieback of the infected vines (Marais, 1979, 1980; Ferreira *et al*., 1989). In a recent study, van Coller (2004) furthermore found that *F. oxysporum, F. proliferatum* and *F. solani* all induced root rot as well as reduced dry weight production in artificially inoculated nursery vines. *Pilidiella* spp. has also previously been reported to be associated with white rot of mature grapevines in several countries (van Niekerk *et al*., 2004b). However, it is unknown if *Pilidiella*, as well as the *Phoma* and *Acremonium* spp.

isolated from the root stock canes in this study, should be regarded as potential important pathogens of nursery or even mature grapevines. A further study, employing artificial inoculation studies, is therefore required to fully elucidate the pathogenic nature of these last three fungal genera.

The large number of especially *Botryosphaeriaceae* and *Phomopsis* spp. associated with the root stock canes examined in this study is, however, of great concern. It is therefore recommended that pruning wounds made on rootstock and scion mother vines should be treated with wound protecting agents to prevent infection by these and other trunk pathogens as infected mother plants could give rise to infected propagation material (Fourie and Halleen, 2004a). A further preventative management strategy that could be employed is the use of trellising systems in the cultivation of rootstock mother vines. Trellising will prevent the rootstock shoots being exposed to possible infection propagules of pathogens such as *Pa. chlamydospora* and *Fusarium* spp, which have been found to be present in vineyard soils or in puddles of standing water (Marais, 1979; Rooney *et al*., 2001; Retief *et al.*, 2006). Trellising will also prevent possible mechanical injuries of shoots that could act as infection points for the various pathogens. Pre-grafting inspection of rootstock canes and other propagation material for visible symptoms of pathogen infection and injury is also suggested as this will ensure only high quality material being used in the propagation process. Subsequent treatment of propagation material in the nursery could also be employed and here the recommendations, as outlined by Fourie and Halleen (2004b; 2006), can be followed.

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4. Accumulated outputs

List ALL the outputs from the start of the project.
The year of each output must also be indicated.

Technology developed

- The fungal genera possibly involved in rootstock cane necrosis were determined.
- Selected cultures, of the various genera isolated, were stored and added to the culture collection for possible future research.
- Symptoms associated with each stored culture were also recorded for possible future use.

Human resources developed/trained

- The symptomology, isolation and generic identification will be conducted by the 4th year Plant Pathology students under supervision of USPP Disease Clinic personnel.

Patents

None

Publications (popular, press releases, semi-scientific, scientific)

- Possible publication of results in a relevant scientific journal.

Presentations/papers delivered

None

4. Total cost summary of project

	Year	CFPA	DFPT	DFTS	Winetech	THRIP	Other	TOTAL
Total cost in real terms for year 1	2006				**30 240**	**30 240**		**60 480**
Total cost in real terms for year 2								
Total cost in real terms for year 3								
Total cost in real terms for year 4								
Total cost in real terms for year 5								
TOTAL					**30 240**	**30 240**		**60 480**