



Final report

FUSARIUM DRY ROT OF POTATOES IN SOUTH AFRICA

2016

A report written by Cornel Millard on work done by D.J Theron and S.L. Venter during the 1980' and 1990s.



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EXECUTIVE SUMMARY

A total of 20 *Fusaria* taxa have been associated with the *Fusarium* dry-rot complex in South Africa. A total of nine *Fusarium* spp. have been identified as dry-rot pathogens in South Africa. A more diverse *Fusarium* population was isolated from tubers with dry-rot lesions than from those with stem-end-rot lesions. This might be ascribed to *Fusarium* dry-rot infection occurring mainly through wounds, while stem-end-rot infection is more complex.

Fusarium spp. were isolated either singly or in combination from diseased tubers, obscuring designation of the primary causal pathogen. *Fusarium solani* and *F. oxysporum* were the predominant species recovered from the diseased tubers with the latter in much higher densities, especially from dry-rot lesions.

Fourteen *Fusarium* spp. were isolated from dry rot and stem-end rot lesions of potato tubers collected from 51 farms in two major dryland production regions of South Africa during the summer of 1985/86. *Fusarium oxysporum* and *F. solani* were the predominant species isolated. Eight species (*F. oxysporum*, *F. crookwellense*, *F. solani*, *F. sambucinum*, *F. acuminatum*, *F. graminearum*, *F. scirpi* and *F. equiseti*) caused typical dry rot lesions on inoculated potato tubers. *Fusarium oxysporum* was the most pathogenic, followed by *F. crookwellense*, *F. solani* and *F. sambucinum*.

Fusarium proliferatum, *F. polyphialidicum*, *F. merismoides*, *F. culmorum* and *F. acuminatum* subsp. *armeniicum* were isolated for the first time in South Africa from potato tubers from potatoes on 64 farms in eight irrigated regions of South Africa. *Fusarium subglutinans* and *F. graminearum* Gr. II, which were isolated from tubers originating from dry land regions was not isolated during the survey in irrigated regions.

It could also be concluded after the extensive surveys conducted in the potato producing areas of South Africa (that isolates resembling *F. coeruleum* and *F. solani* var. *coeruleum*) were probably not associated with dry rot of potatoes in South Africa.

The optimum temperature for infection by fusaria causing dry rot was reported to be 15°C, however, in this study the maximum decay of stored potato tubers occurred on tubers incubated at 25°C. The level of decay was higher than that reported in other countries. Local isolates of pathogenic fusaria are possibly adapted to the high temperatures which prevail in the production regions.

A potato tuber disc baiting technique was developed to provide a rapid, effective quantitative and qualitative means of assessing the absolute inoculum potential of *Fusarium* dry rot pathogens in soil adhering to tubers after lifting. This inexpensive and simple technique could be used commercially to predict the risk of storage rot.

Most of the *Fusarium* spp. isolates evaluated, differed significantly in sensitivity against the fungicides evaluated e.g. all five *F. oxysporum* isolates which differed significantly in sensitivity from one another irrespective of the fungicide used.

Prochloraz had the highest overall inhibitory effect on mycelial growth of the *Fusarium* spp. followed by carbendazim, imazalil and captab. Mancozeb, one of two fungicides registered for the control of *Fusarium* dry rot in South Africa, was not effective in controlling the colony growth of *F. solani* and *F. oxysporum*, the predominant dry-rot pathogens in South Africa, by more than 42% relative to the control. When compared with prochloraz, mancozeb resulted in only 10% control of the colony growth of these two species.

Sensitivity between the five representative isolates of each species to the different fungicides differ significantly, probably due to diversity within the species rather than to a build-up of resistance inherited to exposure to the different fungicides. The differences in sensitivity to fungicides between the *Fusarium* spp., as well as between isolates of the same species, emphasized the need to test fungicides against all the target *Fusarium* spp. causing dry rot of potatoes, as well as to more than one isolate of each species. *In vitro* screening of fungicides only gives an indication of which fungicides could possibly control a disease.

This study showed that thiabendazole resistance appeared to be absent from isolates of *F. solani*, *F. oxysporum* and *F. sambucinum* in South Africa since mycelial growth of the South African isolates was inhibited on agar medium containing ≥ 10 mg a.i./l thiabendazole.

Theron found that fungicides affected the quality of treated seed pieces. Treatment of seed pieces with imazalil, prochloraz, propiconazole and penconazole caused serious damage to sprouts of seed pieces. Although some fungicides or the inert ingredients are phytotoxic, damaging the, not all the damage to the sprouts, could be attributed to fungicide treatments. Some damage was caused mechanically during the cutting process and fungicide treatments because even sprouts of the non-fungicide-treated control pieces were damaged.

Throughout all the autumn plantings the uncut, untreated seed tubers resulted in significantly better plant stands and yields than the treated seed pieces, irrespective of the time of treatment prior to planting. During the spring plantings the uncut, untreated seed tubers did not perform significantly better than most of the seed pieces treated the day prior to planting. The uncut untreated seed tubers performed significantly better than the other seed treatments 14 days prior to planting, except for the seed tubers cut and treated 14 days prior to planting with carbendazim.

A reliable laboratory method for screening potato clones for resistance to *Fusarium solani* at an early stage in a breeding programme was developed. The high correlations (0.87 – 0.99) between resistance to dry rot of different potato cultivars and lines found during four successive seasons demonstrated the reliability of the inoculation technique used. The technique is suitable to early stages of a breeding programme when large numbers of selections have to be tested. Evaluation should be done at 25°C, and *F. oxysporum* and *F. solani* should be included in future screening tests.

This study showed that Fusaric production by *F. oxysporum* isolates seemed to play a major role in the development of dry rot in potato tubers of susceptible cultivars. A correlation was found between virulence of isolates and fusaric acid production. The cultivars differed in their tolerance against *F. oxysporum* and the ranking order of cultivars corresponded with ranking orders previously reported.

In vitro culture combined with somaclonal variation for the selection of fusaric acid resistant potato phenotypes has potential, since somaclonal variation can already be present in the explant or can be induced by the technique. Both these methods proved useful for the induction of resistance to fusaric acid in callus and cell suspension cultures. More resistant calli were obtained when the toxin was added to the medium. Fusaric acid can be used as a selective agent and results indicate that calli of the more tolerant potato genotypes could be selected between concentrations of 0.2 and 0.3 mM fusaric acid. To reach its full potential the selection system requires high regeneration capacity in the *in vitro* explant material. Regenerated plants displaying *in vitro* resistance against the culture filtrate of *F. oxysporum* should be tested in field trials for resistance against the pathogen.

Chapter 1

FUSARIUM DRY ROT OF POTATOES

INTRODUCTION

According to Booth (1971), Von Martius (1842) first reported a fungus, *Fusisporium solani* Mart., associated with potato (*Solanum tuberosum* L.) tuber rot, which Saccardo (1881) reassigned to *Fusarium solani* (Mart.) Sacc. Based on the taxonomic system of Nelson *et al.* (1983) it is today known as *Fusarium solani* (Mart.) Appel & Wollenw. emend. Snyder & Hans. Although Von Martius (1842) did not consider this fungus as the causal organism of the disease, artificial infections showed conclusively that it caused tuber rot (Pethybridge & Bowers, 1908; Wehmer, 1987), and numerous other soil-borne *Fusarium* spp. have since been reported to cause dry rot (Booth, 1971; Boyd, 1972; Seppänen, 1989).

Fusarium dry rot manifests in three ways, namely storage rot (post-harvest decay), seed piece decay and stem-end rot. Storage rot is the most common form of *Fusarium* dry rot and is commonly referred to as dry rot. Seed piece decay occurs after planting, while in the case of stem-end rot, infection occurs through the stolon of the tuber, and symptoms are evident at harvest (Boyd, 1972; Carpenter, 1915; Foister, 1940; Jones & Woltz, 1981; Nielsen, 1981; Pethybridge & Lafferty, 1917; Powelson *et al.*, 1993).

Fusarium dry rot became problematic when potato production was mechanised, because *Fusarium* pathogens are wound pathogens (Seppänen, 1989). Although comprehensive figures were lacking, and *Fusarium* dry rot seldom reached epidemic proportions (Booth, 1971), Powelson *et al.* (1993) concluded that it probably causes greater losses in the storage and transit of both seed and commercial potatoes than any other post-harvest disease. Average crop losses attributed to *Fusarium* dry rot have been estimated at 6 to 25% (Chelkowski, 1989; Foister, 1940) and reports of more than 60% of tubers being affected during long-term storage were no exception (Carnegie *et al.*, 1990). *Fusarium* dry rot is considered to be mainly a problem in the seed industry, because seed tubers are stored for prolonged periods, while potatoes destined for consumption are normally utilised soon after harvest. It is also a major cause of seed piece decay after planting, resulting in reduced plant stands (Boyd, 1972; Nielsen, 1981).

DISEASE SYMPTOMS

Fusarium dry rot is characterised by a dry, powdery rot of infected tubers. A moist rot may occur if secondary infection with soft rot bacteria (mainly *Erwinia* spp. – now known as *Pectobacterium* spp.) are involved, resulting in a more rapid decay of the infected tubers (Moore, 1945; Nielsen, 1981; Powelson *et al.*, 1993). *Fusarium* dry rot symptoms can be confused with symptoms of gangrene caused by *Phoma exigua* var. *foveata* and *P. exigua* var. *exigua* (Boyd, 1972) and symptoms caused by *Botrytis cinerea* (Nielsen, 1981) and *Gliocladium roseum* (Langerfeld, 1977; Theron & Holz, 1991a). Symptoms of infection with different *Fusarium* spp. differ to a certain extent from one another (Boyd, 1972; Carpenter, 1915; Moore, 1945; Nielsen, 1981); however, these differences are not distinct and tubers are often colonised by more than one *Fusarium* species. The only certain way to determine which species is the causal organism is to isolate and identify the associated organisms (Theron & Holz, 1989).

Storage rot

Tuber lesions at wounds are visible as small brown areas around one month of storage. Infection spreads slowly, and the periderm covering lesions sinks and wrinkles, sometimes in concentric rings round the site of infection as the dead tissue becomes dry and powdery. Sporodochial pustules are frequently formed on the dead periderm, possibly pink or white when exposed to light, and blue – or at least with a blue base – when light is excluded. Rotten tubers shrivel and become mummified (Boyd, 1972; Foister, 1940; Nielsen, 1981). Affected tissues are usually fawn or light brown in colour, and stain to a darker brown when exposed to air. The advancing margin of the rot integrates into the healthy tissue. Due to the loss of moisture from the tissue, cavities often lined with white, yellow or pink mycelium are formed below the site of infection. In storage, blue, black, purple, grey, white, yellow or pink spore masses may form in these cavities. Older dead tissue assumes a variety of colours and is dry and spongy in texture (Boyd, 1972; Foister, 1940; Langerfeld, 1978; Nielsen, 1981). At low temperatures, internally infected tissue often becomes firm and dry or even powdery (Powelson *et al.*, 1993).

Seed piece decay

Fusarium spp. are considered the main cause of seed piece decay (Boyd, 1972). Although whole seed tubers can become infected through wounds sustained during storage or preparation for planting, the primary cause for concern is decay of seed tubers cut into seed pieces. As the cost of potato seed constitutes the greater part (35-40%) of production costs of potatoes, some farmers cut seed tubers into seed pieces. The cut surfaces expose tuber tissue to desiccation and are major infection sites for bacterial and/or fungal pathogens causing seed piece decay (Nelson *et al.*, 1993; Nolte *et al.*, 1987). Bacterial decay (e.g. soft rot), mainly caused by *Pectobacterium* spp., and fungal decay (e.g. dry rot), caused by *Fusarium* spp., alone or in combination, are the primary causes of potato seed piece decay (Davis *et al.*, 1983; Misca & Nelson, 1975; Nelson *et al.*, 1993; Nielsen, 1981; Nolte *et al.*, 1987).

Brown-to-black flecks appear on the cut surfaces of stored seed pieces about one week after infection by *Fusarium*, and depressions or pits appear within two weeks. Mycelium often grows on the depressed surfaces, which may become slimy and black from bacterial growth under humid conditions, accelerating seed piece decay. Numerous cut-surface infections cause lesions to merge, and the seed piece rots from the surface inwards, destroying the buds as the decay progresses (Eddins, 1940; Nielsen, 1981).

In the field, shrivelling of infected seed tubers and pitting of infected seed pieces may not be evident. The surface covering the lesions is brown, and the underlying necrotic tissues have fewer cavities. Necrotic tissues may attract soil insects and larvae such as the seed-corn maggot (*Delia tlatura* Meig.), which serves as vector of *Pectobacterium* spp. in wet soils, which often enter as secondary pathogens (Eddins, 1940; Nielsen, 1981). Symptoms caused by *Fusarium* spp. alone, or in combination with *Pectobacterium* spp., include total or partial decay of seed pieces, reduction of plant vigour and poor stands (Eddins, 1940; Escende & Echandi, 1988). Often, single sprouts emerge from partly decayed seed pieces, giving rise to small, slow-growing plants that are more susceptible to other diseases and which produce low yields (Eddins, 1940; Nielsen, 1981).

Stem-end rot

Although stem-end rot symptoms are very similar to those of storage rot, these symptoms are usually visible at harvest, localised on the stem-end of the tuber. Some of the diseased tubers may show a sunken, wrinkled, dry

stem-end rot of varying diameters, while others may only show a slight sign of rot around the point of the stolon attachment. When the latter tubers are cut across near the base, vascular browning, extending to various depths in the tuber, is visible. Vascular browning may affect the whole vascular ring, but more often involves only separate portions of it. The tissues surrounding the affected cells in the vascular ring usually appear brown, yellowish or water-soaked (Carpenter, 1915; Goss, 1936; Haskell, 1916; Schippers, 1962).

CAUSAL ORGANISMS

Fusarium spp. are distributed worldwide as saprophytes, soil inhabitants, and pathogens of many plants. At the time of the study, the identification of some species was difficult and still not clear. The fact that different species were reported under the same name and a single species under different names was also confusing (Seppänen, 1989). *Fusarium* spp. associated with diseases of potatoes were no exception. Many *Fusarium* spp. have been associated with potato tuber rots (Boyd, 1972; Chambers, 1973; Hanson *et al.*, 1996; Manici & Cerato, 1992; McKee, 1952; Savor & Maček, 1994; Seppänen, 1981a; Theron & Holz, 1989; Tivoli & Jouan, 1981; Wollenweber & Reinking, 1935) and stem-end rot (Carpenter, 1915; Goss, 1936; Haskell, 1916; Schippers, 1962; Wollenweber & Reinking, 1935) of the potato.

The *Fusarium* spp. associated with dry rot of potatoes have been referred to in the literature under at least 32 names and these were classified in 16 species according to the taxonomic system of Nelson *et al.* (1983). Amongst these 16 species, *F. solani* [= *F. coeruleum* (Libert) Sacc; *F. solani* (Mart.) Sacc.var. *coeruleum* (Libert) Bilai], *F. sambucinum* (Fuckel) [= *F. trichothecoides* Wollenw.; *F. sulphureum* Schlecht.] and *F. avenaceum* (Fr.) Sacc. [= *F. arthrosporoides* Sherb.] were the dominant species associated with dry rot of potatoes in the northern hemisphere (Boyd, 1972; Seppänen, 1989). In the southern hemisphere the most prevalent species were *F. oxysporum* Schlecht. emend. Snyd. & Hans. and *F. solani* (Stubbs, 1971; Chambers, 1973; Turkensteen, 1987; Tivoli *et al.*, 1988; Theron & Holz, 1989). All further references to *Fusarium* spp. causing dry rot of potatoes were of the 16 species accepted by Nelson *et al.* (1983).

The pathogenicity of *Fusarium* spp. associated with dry rot differs from one species to the other (Moore, 1945; Seppänen, 1983a; Theron & Holz, 1990), as well as within species. Furthermore, results obtained with a given fungus at different temperatures were not similar, implying that the virulence of the fungi may change with the environmental conditions (Seppänen, 1982b; 1983a).

Amongst dry-rot pathogens, *F. solani* has been most intensively investigated. Wollenweber and Reinking (1953) reported growth at temperatures between 3 and 30°C, with the optimum growth temperature 15- 28°C, at relative humidity (RH) of 50-80%, while several authors reported the optimum growth temperature to be between 15 and 20°C (Moore, 1945; McKee, 1954; Langerfeld, 1978; Tivoli & Jouan, 1981; Seppänen, 1981b; 1982b). However, Theron and Holz (1990) found that South African isolates grew optimally between 25-35°C. High RH (90-98%) was found to be more favourable for growth than low RH (70-80%) (Weiss *et al.*, 1928; Moore, 1945).

Fusarium avenaceum is pathogenic over a wide range of temperatures, but is favoured by high temperatures, 20-25°C (McKee, 1954; Langerfeld, 1978), the optimum being near 30°C (Tivoli & Jouan, 1981; Seppänen, 1981b; 1982b). A high RH, especially at higher temperatures, is necessary for infection and growth (Moore, 1945; Langerfeld, 1978; Seppänen, 1981b).

Fusarium sambucinum can infect potatoes over a wide range of temperatures (2-30°C) and grows optimally at temperatures of 15-25°C (Weiss *et al.*, 1928), although it penetrates potato tubers more rapidly at 10-12°C under dry rather than moist conditions (Weiss *et al.*, 1928; Seppänen, 1981b). A temperature of 25°C and a high RH are reported to favour dry rot, but the fungus could also cause dry rot at lower temperatures (Goss, 1921).

Theron and Holz (1990) could not determine a distinct optimum temperature for *F. sambucinum* at temperatures ranging between 5 and 35°C, although 25°C seemed to be optimum for the cv. BP-1, consistent with the results of Goss (1921).

Fusarium oxysporum is pathogenic over a wide range of temperatures, but is favoured by high temperatures, 16-30°C, the optimum being 25°C (Weiss *et al.*, 1928; Goss, 1921; Theron & Holz, 1990). High RH is also necessary for its growth (Weiss *et al.*, 1928; Goss, 1921).

It seems if *Fusarium* dry rot develops most rapidly under conditions of high RH and that the optimum temperature lies between 15-25°C. Relative humidities of at least 50% do not alter *Fusarium* dry rot development, but lower humidities retard disease development. Disease development will even continue at the coldest temperatures safe for potato storage (Langerfeld, 1978; Seppänen, 1989).

DISEASE MANAGEMENT

The control of *Fusarium* dry rot should begin long before the crop is harvested. Therefore, cultural control (e.g. seed selection, timely planting and choice of suitable cultivars) are necessary to keep *Fusarium* dry rot to a minimum. It is important to eliminate damage to tubers during and after harvesting at all costs because dry-rot pathogens primarily infect tubers through wounds. A holistic approach is the only effective way of managing *Fusarium* dry rot of potatoes (Powelson *et al.*, 1993).

In 1891, Bolley recommended Mercuric chloride, as a treatment of seed tubers prior to planting, for the control of tuber-borne diseases (Boyd, 1960), making Organomercury compounds the first chemicals used with various degrees of control (Small, 1945). Later, Diphenyl was found to reduce infection under laboratory conditions, but proved both ineffective and phytotoxic to tubers in the field (McKee & Boyd, 1962). Since the banning of the use of mercury compounds due to health risks, various other alternative chemicals have been tested (Boyd, 1960).

With the introduction of systemic fungicides in the 1960s, several researchers study their efficacy against potato tuber diseases. Since the late 1970s potato growers have relied heavily on the various formulations of the benzimidazole fungicides, benomyl and thiabendazole (TBZ). These fungicides, especially TBZ, were effective for the control of *Fusarium* dry rot in seed and stored potatoes (Cayley *et al.*, 1979; Carnegie *et al.*, 1990) and applied to the tubers either in a dust, dip spray (high volume or as ultra-low volume) or fumigation treatment after harvest, prior to storage or as a seed tuber treatment prior to planting (Leach, 1970; Murdock & Wood, 1972; Copeland & Logan; 1975; Leach, 1975; Logan, 1975; Cayley *et al.*, 1979; Hide & Cayley, 1980).

The efficacy of these treatments was highly dependent on the concentration of the product used (Leach, 1975), the effectiveness of covering the tuber surfaces, the amount of soil present on the tuber surfaces (Logan, 1975; Hide, 1986a), the time of application (Murdock & Wood, 1972; Hide & Cayley, 1980; 1985; Hide, 1986a) and the sensitivity of the *Fusarium* spp. present (Hanson *et al.*, 1996). The importance of avoiding a delay in treating tubers with fungicides after injury and possible infestation by dry-rot pathogens was clearly showed by Hide and Cayley (1980; 1985). However, during the 1990s, resistance to TBZ, the only registered post-harvest fungicide in most potato producing countries, has been reported (Langerfeld, 1986; Tivoli *et al.*, 1986; Carnegie *et al.*, 1990; Langerfeld, 1990; Hide *et al.*, 1992; Von Stachewicz *et al.*, 1992; Desjardins *et al.*, 1993; Kawchuk *et al.*, 1994; Hanson *et al.*, 1996), likely altering potato growers' practises for *Fusarium* dry rot control (Desjardins *et al.*, 1993).

Chapter 2

FUSARIUM SPECIES ASSOCIATED WITH DRY AND STEM-END ROT OF POTATO TUBERS

BACKGROUND TO STUDY

This report summarises the studies conducted by D.J. Theron in partial fulfilment of the requirements for the degree of Master of Science in Agriculture at the University of Stellenbosch, under the guidance of Prof. Gustav Holz, and the degree of Philosophiae Doctor at the University of the Free State, under the guidance of Prof. W.F.O. Marasas and Prof. M.J. Wingfield. Research was conducted at the Agricultural Research Council's Vegetable and Ornamental Plant Institute near Roodeplaat (Roodeplaat). Five peer-reviewed articles were published from the MSc dissertation (see References), but none from the PhD thesis. This report summarises the experiments, results, discussions and recommendations. The techniques applied are described and significant results are presented.

INTRODUCTION

At the start of this study in 1985, *Fusarium* dry and stem-end rot occurred commonly in South Africa, but the identity of the *Fusarium* spp. involved remained unclear. Dippenaar (1934) isolated *Fusarium oxysporum*, *F. coeruleum* (*F. solani*), *F. solani* and *F. sambucinum* from potatoes with dry rot, while Jandrell, Van Reenen and Hill (unpublished) isolated *F. equiseti*, *F. avenaceum* and *F. merismoides*. However, the pathogenicity of these species was not tested. Visser (1975) identified *F. solani* as the main causal organism of potato dry rot in Mpumalanga, then one of South Africa's main dryland production areas. These contrasting reports might have been because of the different classification systems followed and the variability of certain species (Nelson *et al.*, 1983). The objective of this study was to identify the *Fusarium* spp. responsible for dry and stem-end rot produced in 10 production areas of South Africa, and their role in the dry rot disease complex. This study was done in two phases: The first in the two major dryland regions of South Africa, and the second in regions where potato is irrigated.

MATERIALS AND METHODS

Collection of samples

This study was carried out in two phases. In the summer of 1985/86, samples were collected from two dryland regions namely the Eastern Free State and Mpumalanga. Potato tubers with dry and stem-end rot lesions were sampled at sorting tables of 24 farms in the Free State and 27 farms in Mpumalanga. Samples from irrigated fields, were collected in the late eighties from 8 farms in Limpopo, North West and Northern Cape each, 9 farms in Kwazulu-Natal and Eastern Cape each, 10 farms in Western Free State and Sandveld each and 12 farms in the Western Cape. Thus tubers were collected from 125 farms in 10 regions throughout South Africa. The popular cultivars at the time when the research was done were BP1, Up-to-date and Vanderplank. These have, however, been replaced largely by modern cultivars. Tubers were kept in paper bags at 5°C and 50–70% relative humidity (RH) until isolations were made.

Isolation and identification of micro-organisms

Twenty tubers (10 with dry and stem-end rot respectively) from each locality were washed, surface-disinfested (3% NaOCl, 15 min) and air-dried. Tubers were cut in half and five discs (2 mm³) dissected from the periphery of discoloured tissue of each tuber. The discs were placed on potato dextrose agar (PDA) plates and incubated at 25±2°C for 4-5 days under intermittent light (fluorescent plus black light; 12 h cycles) (Nelson *et al.*, 1983). Developing colonies were examined microscopically (10x) and organisms other than *Fusarium* identified directly. Single-spore isolates of each *Fusarium* sp. were obtained by a modified technique of Tousson and Nelson (1976). Spores were harvested directly from colonies with a wire loop and streaked onto water agar (2%) plates. After 24 h incubation at 25±2°C, single spores were transferred to carnation leaf agar (CLA) (Fisher *et al.*, 1982) section of divided Petri dishes. These cultures were incubated at 25±2°C for 2 days where after agar plugs (2-3 mm³) with mycelium were cut aseptically from the leading edge of developing colonies and transferred to the PDA section of the divided dishes. After 10-14 days *Fusarium* spp. were identified according to the system of Nelson *et al.*, (1983), but *F. compactum* and *F. nygamai* were identified according to Gordon (1952) and Burgess and Trimboli (1986), respectively. Spores from two different isolates of each species per sample were mass-transferred to CLA slants, incubated for 1 week as described previously and lyophilized. . The frequency and relative density of the *Fusarium* spp. were calculated according to McMullen and Stack (1984) as follows:

$$\text{Frequency (\%)} = \frac{\text{Number of tuber samples of occurrence of a species or combination of species}}{\text{Total number of tuber samples}} \times 100$$

$$\text{Relative density (\%)} = \frac{\text{Number of isolates of a species or combination of species}}{\text{Total number of isolates}} \times 100$$

Statistical analysis

Data was statistically analysed using analysis of variance. The least significant difference (LSD) function of SAS/STAT program for personal computers (Statistical Analysis Systems Inc., 1985) was used, to test for significant differences between means of the organisms isolated from the tubers according to Tukey's tests.

Pathogenicity tests

Single-isolate inoculations: During the study on samples from dryland regions, fifteen isolates of each *Fusarium* sp. from different localities in the production areas, were randomly selected. Lyophilized isolates was transferred to CLA plates (Fisher *et al.*, 1982). After incubation at 25±2°C for 2 weeks under intermittent light (fluorescent plus black light; 12 h cycles), spores were washed from cultures with sterile distilled water and the suspension diluted to 1x10⁴ CFU/ml (macro- and microconidia). Sound, unblemished tubers (cultivar Up-to-Date), selected at harvest from plants grown under normal commercial conditions in field plots at Roodeplaat, (ARC VOP) were disinfested for 15 min in 3% sodium chlorite (NaOCl) and air-dried. Twenty tubers (75–150 g) were inoculated with each isolate halfway between rose and heel ends by injecting 0.2 ml of the spore suspension 8 mm into the tissue with a Socorex 2-187 self-refilling type syringe (Theron & Holz, 1987). Control tubers received sterile distilled water. Tubers were wrapped in paper bags and kept a 25±2°C and 50-70% RH to support dry rot development (Visser, 1975).

Single-species vs. mixed-species inoculations: Inoculum of the most aggressive isolate of each *Fusarium* sp. was prepared as described above. Disinfested potato tubers (cultivar Up-to-Date) were injected with 0.2 ml of either single-species inoculum (1×10^4 CFU/ml), or mixed-species inoculum (1×10^4 CFU/ml; equal number of spores per species). Tubers were incubated in paper bags as described above.

During the study on samples from regions where potato is irrigated, the procedure was as follow: Five isolates of each *Fusarium* sp., obtained from different farms from each of the eight production regions, were selected at random. Five pathogenic *Fusarium* isolates of each species from the survey in Free State and Mpumalanga (Theron & Holz, 1989) were also included for comparative purposes. Inoculum was prepared by transferring lyophilized isolates to carnation leaf agar (Fisher *et al.*, 1982). After incubation at $25 \pm 2^\circ\text{C}$ for 2 weeks under intermittent light (Nelson *et al.*, 1983), conidia were washed from cultures with sterile distilled water and the suspension diluted to 1×10^4 propagules/ml (macro- and microconidia). Sound, unblemished potato tubers (cv. Up-to-Date), selected at harvest from plants grown under normal commercial conditions in field plots at Roodeplaat were surface-disinfested and allowed to dry. Twenty tubers (75–150 g) were inoculated with each isolate approximately halfway between rose and heel ends by injecting 0.2 ml of the conidial suspension 8 mm into the tissue with a Socorex 2-187 self-refilling type syringe (Theron & Holz, 1987). Control tubers received sterile distilled water. Tubers were wrapped in paper bags and kept a $25 \pm 2^\circ\text{C}$ and 50-70% relative humidity to promote dry rot development (Theron & Holz, 1987). Representative pathogenic isolates were deposited in the culture collection of the Programme on Mycotoxins and Experimental Carcinogenesis (PROMEC), Medical Research Council (MRC), P.O. Box 19070, Tygerberg 7505, South Africa.

Disease assessment: After 3 weeks, tubers were cut in half at the inoculation site and the extent of dry rot development determined according to a modified disease index scale of Wiersema (1977) (Figure 1). Results were showed as percentage decay based on the method of Kremer and Unterstenhöfer (1967).

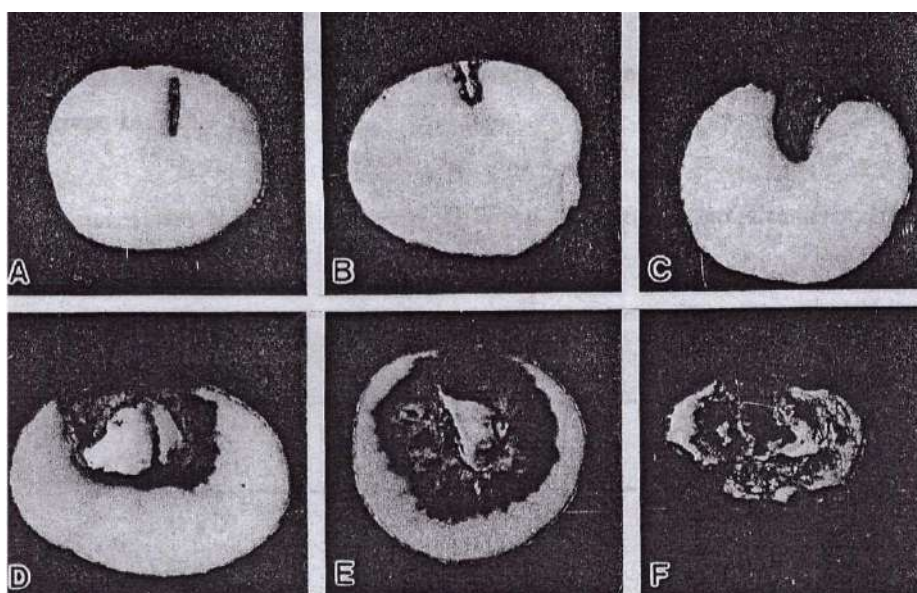


Figure 1. Disease index of potato tubers 3 weeks after inoculation with *F. solani*.

- A. Restricted discolouration with no dry rot development at inoculation site (rating = 0). B. Restricted discolouration with limited dry rot at the inoculation site (rating = 1). C. Advanced discolouration with increased dry rot development at the inoculation site (rating = 2). D. Advanced discolouration with extensive dry rot development at the inoculation site (rating = 3). E. Extensive discolouration and dry rot but tubers not completely decayed (rating = 4). F. Tubers completely decayed (rating = 5)

RESULTS AND DISCUSSION

Samples from the dryland regions (Eastern Free State and Mpumalanga)

Identification of organisms in dry and stem-end rot lesions

Fusarium spp. predominated from tubers with dry and stem-end rot. They were identified as *F. oxysporum* Schlecht. emend. Snyder & Hans., *F. solani* (Mart.) Appel & Wollenw. emend. Snyder & Hans., *F. crookwellense* Burgess, Nelson & Tousson, *F. sambucinum* Funckel, *F. acuminatum* Ell. & Ev., *F. graminearum* Schwabe gr. 1 & 2, *F. scirpi* Lambotte & Fautr., *F. equiseti* (Corda) Sacc., *F. moniliforme* Sheldon, *F. subglutinans* (Wollenw. & Reinking) Nelson, Tousson & Marasas comb. nov., *F. reticulatum* Mart., *F. nygamai* sp. nov., *F. compactum* (Wollenw.) Gordon and *F. chlamydosporum* Wollenw. & Reinking. The species were isolated singly or in combination, with *F. oxysporum* and *F. solani* most frequently isolated as single species. When more than one *Fusarium* sp. was isolated from a single tuber, dry or stem-end rot was ascribed to the organism with the highest incidence. *Fusarium oxysporum* and *F. solani* were the major fusaria associated with dry and stem-end rot lesions. Predominant *Fusarium* spp. recovered from dry and stem-end rot lesions of tubers collected from different localities in the two production areas are presented in Table 1.

Table 1. Predominant *Fusarium* spp., based on frequency of isolation, recovered from dry or stem-end rot lesions of potato tubers collected from different localities in two dryland production regions

<i>Fusarium</i> spp. ^a	Relative frequency (% of total)			
	Eastern Free State		Mpumalanga	
	Dry rot	Stem-end rot	Dry rot	Stem-end rot
<i>F. oxysporum</i> (MRC 4640)	39.7	52.6	43.2	28.9
<i>F. solani</i> (MRC 4768)	24.3	29.1	34.9	67.8
<i>F. equiseti</i> (MRC 4675)	16.6	5.8	1.3	0.1
<i>F. sambucinum</i> (MRC 4664)	9.4	8.8	12.2	2.1
<i>F. crookwellense</i> (MRC 4639)	6.6	1.8	3.5	0
<i>F. scirpi</i> (MRC 4676)	1.9	0.3	3.2	0.4
<i>F. acuminatum</i> (MRC 4793)	1.0	1.5	0	0
<i>F. subglutinans</i> (MRC 4685)	0	0	0.9	0.3
<i>F. graminearum</i> gr. 1 (MRC 4673)	0.1	0	0.6	0
<i>F. moniliforme</i> (MRC 4679)	0	0	0.1	0.4
<i>F. graminearum</i> gr. 2 (MRC)	0	0	0.1	0
<i>F. chlamydosporum</i> (MRC 4642)	0.1	0	0	0
<i>F. reticulatum</i> (MRC 4681)	0.1	0.1	0	0
<i>F. compactum</i> (MRC 4641)	0.1	0	0	0
<i>F. nygamai</i> (MRC 4683)	0.1	0	0	0

^aNumbers of representative isolates deposited in the culture collection of the Medical Research Council (MRC), P.O. Box 70, Tygerberg 7505, South Africa.

Pathogenicity

Single-isolate inoculations: Eight of the 14 *Fusarium* spp. caused typical dry rot symptoms on artificially-inoculated tubers. Percentage decay established by different isolates of a specific *Fusarium* sp. differed considerably, but the levels of rot caused by *F. oxysporum* and *F. scirpi* were much more uniform. The mean percentage of decay caused by the individual species is indicated in Figure 2.

Single-species vs. mixed-species inoculations: The *Fusarium* spp. used to prepare mixed inocula were consistently isolated from inoculated tubers, however, *F. oxysporum* and *F. solani* predominated in lesions where either had been present in mixed inocula. Furthermore, *F. oxysporum* and *F. solani* enhanced decay when present in these mixed inocula. The mean percentage of decay caused by the mixed species inocula is indicated in Figure 2.

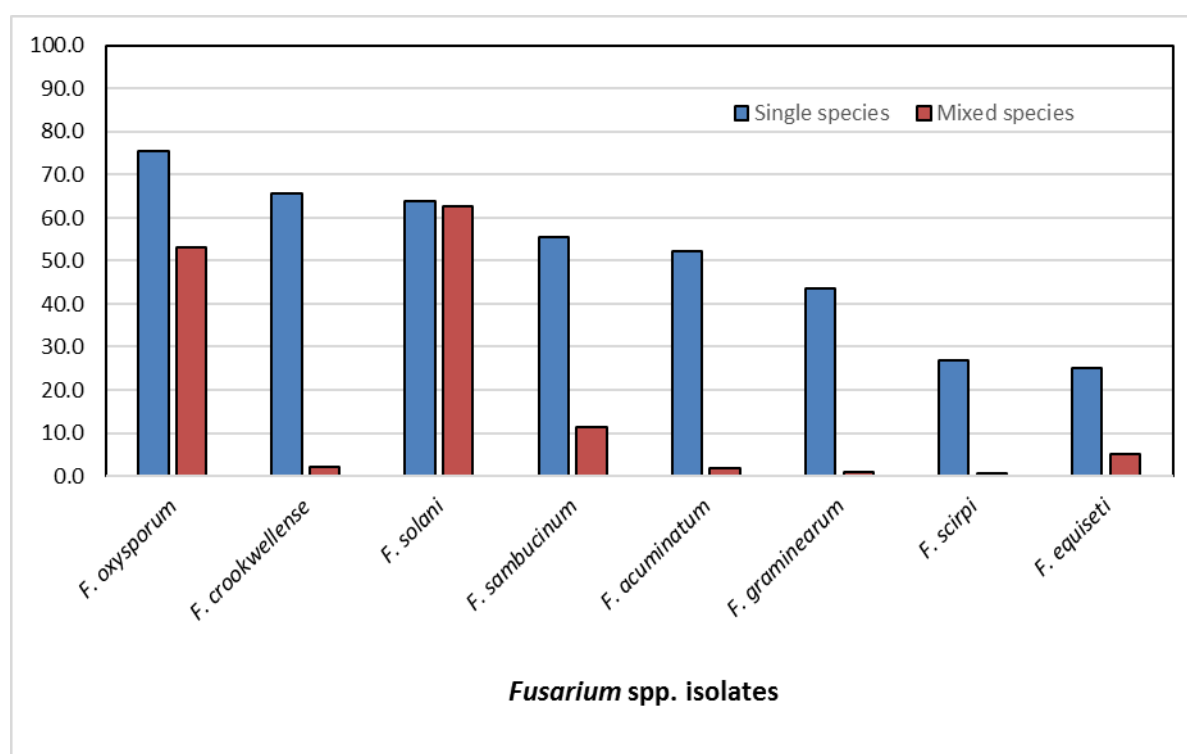


Figure 2. Mean percentage of decay (Y-axis) of potato tubers from two dryland regions, caused by *Fusarium* spp. three weeks after inoculation with single-species inocula.

At the time of the study *F. solani*, *F. sambucinum*, *F. trichothecioides* and *F. avenaceum* were known to be pathogens of potato tubers throughout the world (Wollenweber & Reinking, 1935; Boyd, 1972), while *F. oxysporum* and *F. culmorum* were the dominant fusaria causing dry rot in Australia (Chambers, 1973). While *F. equiseti*, *F. avenaceum* and *F. merismoides* (Jandrell *et al.*, unpublished) and *F. solani* (Visser, 1975), had been considered the main pathogens causing dry rot in Mpumalanga. In this study, D.J. Theron showed that a different *Fusarium*-dry rot situation occurred in the two main dryland production areas of South Africa. *Fusarium oxysporum* and *F. solani* were the major species recovered from diseased tubers. Additionally, *F. acuminatum* and *F. graminearum* were identified for the first time as pathogens of potatoes in South Africa. Some *F. graminearum* isolates obtained from tubers collected from farms in Mpumalanga were assigned to group 2. Members of this group, then prevalent in the eastern USA and Europe, were considered primarily as pathogens of foliage of maize and wheat (Burgess & Liddell, 1983; Nelson *et al.*, 1983). Furthermore, *F. scirpi* and *F. crookwellense* were for the first time positively associated with dry and stem-end rot of potatoes, and their pathogenicity verified. Although *F. crookwellense* had previously been

isolated from potato tubers, it was confined to sunken or dimple-like necrotic lesions (Burgess *et al.*, 1982), which are not typical symptoms of dry or stem-end rot.

Except for *F. equiseti* and *F. crookwellense*, which were isolated less frequently from tubers collected in Mpumalanga, no meaningful differences were found in the occurrence of fusaria from the two local production areas. Potatoes were usually rotated with maize and wheat in Mpumalanga and the Eastern Free State, respectively. *Fusarium equiseti* (Maas & Kotzé, 1985) and *F. crookwellense* (Van Wyk *et al.*, 1986) are pathogens of wheat rather than maize. The crop rotation system followed in Eastern Free State might have contributed to differences in occurrence of these pathogens between the two regions.

The same fusaria were isolated from tubers with dry and stem-end rot lesions, although stem-end rot was more prevalent in the Eastern Free State than in Mpumalanga. In the Eastern Free State, tubers are left for 3-4 months in the cool soils before being lifted. This practise might enable pathogens to enter tubers via the senescent stolons (Pethybridge & Lafferty, 1917).

When more than one *Fusarium* sp. was isolated from a single tuber, designation of the main casual pathogen was obscured. Seppänen (1983) gave each *Fusarium* sp. isolated an equal share in disease development, while Theron considered the *Fusarium* sp. most frequently isolated from discoloured tissue of a tuber the major pathogen. Differences in pathogenicity between the different *Fusarium* spp., the differences in aggressiveness between isolates of a given *Fusarium* sp., and the occurrence of the different fusaria in dry and stem-end rot lesions following mixed-species inoculations supported Theron's decision. Although each *Fusarium* sp. included in the mixed-species inocula was consistently recovered from discoloured tissue, the least pathogenic *Fusarium* spp. were usually isolated at low frequencies, possibly acting as saprophytes. However, it was unknown why *F. oxysporum* and *F. solani* predominate in lesions.

Conclusion

Fourteen *Fusarium* spp. were isolated from dry rot and stem-end rot lesions of potato tubers collected from 51 farms in two major dryland production regions of South Africa during the summer of 1985/86. *Fusarium oxysporum* and *F. solani* were the predominant species isolated. Eight species (*F. oxysporum*, *F. crookwellense*, *F. solani*, *F. sambucinum*, *F. acuminatum*, *F. graminearum*, *F. scirpi* and *F. equiseti*) caused typical dry rot lesions on inoculated potato tubers. *Fusarium oxysporum* was the most pathogenic, followed by *F. crookwellense*, *F. solani* and *F. sambucinum*.

Samples from the irrigated regions (Limpopo, North West, Western Free State, Northern Cape, Sandveld, Western Cape, Kwazulu-Natal, Eastern Cape)

Theron obtained potato tuber samples with dry-rot lesions surprisingly easy from all eight production regions, as samples were taken directly after harvest at the sorting tables. *Fusarium* dry rot is a post-harvest problem which usually develops during storage (Boyd, 1972). The results of this study emphasized the importance of this disease in South Africa, as an increase in *Fusarium* dry rot is expected when tubers are stored for prolonged periods. In South Africa, however, there is a constant supply of fresh potatoes throughout the year because potatoes are produced in 16 geographically different production regions. As a result of this, there is no need to store potatoes, except for seed potatoes.

The survey indicated that tuber samples with stem-end-rot lesions were not as easily obtainable as those with dry-rot lesions in the eight regions, especially in the Eastern Cape and the Sandveld regions where stem-end rot appeared to be almost absent. In both these regions, samples were taken from winter plantings suggesting that the incidence of stem-end rot is less prevalent during the cooler seasons. According to the survey conducted by Theron and Holz (1989) in Mpumalanga and the Eastern Free State, two dryland production regions, tuber samples with stem-end-rot lesions were easily obtainable in these regions. Tubers are usually left for 3-4 months in the soil in these two dryland production regions before being lifted, especially in the Eastern Free State. In irrigated regions, where the present survey was conducted, tubers are lifted soon after senescence which may explain why tuber samples with stem-end-rot lesions were not easily obtained from these regions. These results confirmed the results of Pethybridge and Lafferty (1917) that showed that the longer tubers are left in the soil after senescence, the greater the probability that pathogens will enter via the senescent stolons.

The predominant fungi isolated from dry-rot and stem-end rot lesions of potato tubers were members of the genus *Fusarium*. Eighteen *Fusarium* species (*F. solani*, *F. oxysporum*, *F. sambucinum*, *F. equiseti*, *F. scirpi*, *F. acuminatum*, *F. crookwellense*, *F. graminearum* Gr. I, *F. nygamai*, *F. compactum*, *F. culmorum*, *F. chlamydosporum*, *F. proliferatum*, *F. reticulatum*, *F. polyphialidicum*, *F. moniliforme*, *F. merismoides*, and *F. acuminatum* subsp. *armeniicum*) were isolated, either singly or in combination. *Fusarium oxysporum* was most frequently isolated as a single species from tubers with dry-rot and stem-end rot lesions, followed by *F. solani* (Table 2.1). These species also occurred as the predominant combination pair followed by the combination of *F. oxysporum* and *F. equiseti*.

Nine of the 18 *Fusarium* spp. isolated from the periphery of discoloured tissue of potato tubers with either dry rot or stem-end rot symptoms, caused typical dry-rot symptoms on artificially inoculated tubers (Table 2.2). Pathogenicity of eight of the nine species has been reported previously (Theron & Holz, 1989). *Fusarium culmorum* was identified for the first time as a dry-rot pathogen of potatoes in South Africa. Virulence varied from 15.9 to 87.8% (mean 50.1 ±19.1%) for the nine pathogenic species.

Table 2.1. Frequency and relative density of *Fusarium* spp. in lesions of potato tubers with dry and stem-end rot, collected in eight irrigated regions of South Africa^a.

<i>Fusarium</i> spp.	Frequency (%) ^b		Relative density(%) ^c	
	Dry rot	Stem-end rot	Dry rot	Stem-end rot
<i>F. solani</i>	23.5	36.4	24.1	39.1
<i>F. oxysporum</i>	56.5	47.7	58.1	46.4
<i>F. sambucinum</i>	2.8	1.4	2.5	1.1
<i>F. equiseti</i>	5.1	3.5	6.0	3.4
<i>F. scirpi</i>	0.6	1.1	0.7	1.0
<i>F. acuminatum</i>	4.0	0.9	2.6	1.0
<i>F. crookwellense</i>	2.4	1.0	1.9	0.8
<i>F. graminearum</i> Gr. 1	0.3	-	0.2	-
<i>F. nygamai</i>	0.7	5.1	0.5	4.6
<i>F. compactum</i>	0.9	0.9	0.8	0.6
<i>F. culmorum</i>	1.0	1.7	0.9	1.7
<i>F. chlamydosporum</i>	0.5	-	0.3	-
<i>F. proliferatum</i>	0.1	-	0.1	-
<i>F. reticulatum</i>	0.5	0.2	0.7	0.2
<i>F. polyphialidicum</i>	0.4	-	0.2	-
<i>F. moniliforme</i>	0.4	-	0.2	-
<i>F. a. subsp. armeniacum</i>	0.3	-	0.2	-
n	824	302	2746	1112
F-value ($p \leq 0.05$)	192.0	60.0	206.8	55.2
LSD _T 0.05	2.8	5.9	2.8	6.2

^aSurface-disinfested tubers collected at sorting tables, were cut in half and five discs randomly dissected from the periphery of discoloured tissue and plated on PDA.

^bFrequency (%) = [Number of tuber samples of occurrence of a certain organism/total number (n) of samples] x 100

^cRelative density = [Total number of isolates of a certain organism/total number (n) of isolates] x 100

Table 2.2. Mean percentage dry rot of potato tubers 3 weeks after inoculation with different isolates of 18 *Fusarium* species isolated from dry rot and stem-end rot lesions^a from eight irrigated regions in South Africa.

<i>Fusarium</i> spp.	Number of isolates	% Decay ^b		
		Min.	Max.	Mean \pm SD
<i>F. solani</i>	45	46.4	86.6	76.8 ^c \pm 13.4
<i>F. oxysporum</i>	45	56.1	85.4	70.3 \pm 7.7
<i>F. crookwellense</i>	25	47.6	86.6	66.7 \pm 13.2
<i>F. acuminatum</i>	25	30.5	87.8	51.4 \pm 16.5
<i>F. sambucinum</i>	30	42.7	73.2	49.2 \pm 8.4
<i>F. culmorum</i>	15	28.7	71.7	47.1 \pm 9.7
<i>F. graminearum</i> Gr. 1	15	24.4	64.7	42.0 \pm 10.2
<i>F. scirpi</i>	30	15.9	36.6	26.8 \pm 6.4
<i>F. equiseti</i>	45	15.9	51.2	20.2 \pm 8.5
<i>F. nygamai</i>	20	0	0	0
<i>F. compactum</i>	20	0	0	0
<i>F. chlamyosporum</i>	15	0	0	0
<i>F. proliferatum</i>	10	0	0	0
<i>F. reticulatum</i>	15	0	0	0
<i>F. polyphialidicum</i>	10	0	0	0
<i>F. moniliforme</i>	15	0	0	0
<i>F. merismoides</i>	5	0	0	0
<i>F. a. subsp. armeniacum</i>	5	0	0	0
Control	-	0	0	0

^aTwenty, sound, unblemished, surface-disinfested tubers, cultivar Up-to-Date, were injected with 2ml of a spore suspension (1×10^4 CFU/ml) and kept at $25 \pm 2^\circ\text{C}$ and 50-70%RH.

^bDry rot determined based on a disease index, where 0 = no lesion development and 5 = tuber completely decayed. Results presented as percentage dry rot calculated based on the method of Kremer and Unterstenhöfer (1967).

^cEach mean value represented 20 observations for each isolate.

In this survey as well as the previous one (Theron & Holz, 1989), Theron showed that the *Fusarium* dry-rot complex in South Africa's potato production regions differs from that in other potato producing countries of the world, especially those in the northern hemisphere. *Fusarium* spp. were isolated either singly or in combination from diseased tubers, obscuring designation of the primary causal pathogen. The *Fusarium* sp. most frequently isolated from the discoloured tissue of a tuber was considered to be the major pathogen. This decision was supported by the results obtained from mixed-species inoculations and the recovery of the more virulent *Fusarium* spp. in higher densities by Theron and Holz (1989). *Fusarium solani* and *F. oxysporum* were the predominant species recovered from the diseased tubers with the latter in much higher densities, especially from dry-rot lesions. This finding was also supported by the pathogenicity tests.

Fusarium proliferatum, *F. polyphialidicum*, *F. merismoides*, *F. culmorum* and *F. acuminatum* subsp. *armeniaceum* were isolated for the first time in South Africa from potato tubers during this survey. *Fusarium subglutinans* and *F. graminearum* Gr. II, which were previously isolated by Theron and Holz (1989), was not isolated during this survey.

Thus a total of 20 *Fusaria* taxa have been associated with the *Fusarium* dry-rot complex in South Africa. *Fusarium culmorum* was the only additional species identified for the first time as a pathogen of potatoes in South Africa. Thus a total of nine *Fusarium* spp. have been identified as dry-rot pathogens in South Africa. Jandrell, Van Reenen and Hill (ARC-VOP Roodeplaat, unpublished data) previously isolated *F. merismoides* and *F. avenaceum* from potato tubers.

In this survey, Theron isolated *F. merismoides* mainly in combination with *F. oxysporum*, but the pathogenicity of this species could not be confirmed, contrary to Førsund (1980) and Seppänen (1981a) whom reported *F. merismoides* as a dry-rot pathogen. Although *F. avenaceum* was not isolated during this and the previous survey, it has been reported to be an important dry-rot pathogen elsewhere (Boyd, 1972; Seppänen, 1981a).

A more diverse *Fusarium* population was isolated from tubers with dry-rot lesions than from those with stem-end-rot lesions. This might be ascribed to *Fusarium* dry-rot infection occurring mainly through wounds, while stem-end-rot infection is more complex. Therefore, mainly specialised species that are able to infect the intact tuber (Boyd, 1972), cause stem-end rot infection. *Fusarium solani*, *F. oxysporum* and *F. equiseti* were the only species consistently isolated from potato tubers with dry-rot lesions from all the production regions.

Leach (1985) reported that rotation crops or cropping sequences had no direct relationship to *Fusarium* populations in the soil or on dry rot, and that contaminated seed tubers are the primary source of infection. However, in this study Theron indicated that there may be a relationship between the occurrence of some of the *Fusarium* spp. in certain production regions that may be ascribed to type of crop rotation and climatic conditions. For example, *F. crookwellense* and *F. graminearum* mainly occurred in the regions where either maize or wheat are rotated with potatoes. Van Wyk *et al.* (1987) isolated *F. culmorum* mainly from diseased crowns of wheat grown in the Western Free State and not from wheat grown in the Eastern Free State. Similarly, Theron isolated *F. culmorum* only from potato tubers samples from the Western Free State and the climatically similar Northern Cape and Sandveld. In these regions, potatoes are planted predominantly in rotation with wheat and maize. The occurrence of *F. proliferatum* in Ceres and Eastern Cape may be related to the rotation with onions in those regions as it is known that this species can cause post-harvest rotting of onions (Naudé & Jooste, 1989).

CONCLUSIONS AND RECOMMENDATIONS

Theron showed that the *Fusarium* dry-rot complex in South Africa's potato production regions differs from that in other potato producing countries of the world, especially those in the northern hemisphere. *Fusarium* spp. were isolated either singly or in combination from diseased tubers, obscuring designation of the primary causal pathogen. *Fusarium solani* and *F. oxysporum* were the predominant species recovered from the diseased tubers with the latter in much higher densities, especially from dry-rot lesions.

Fusarium proliferatum, *F. polyphialidicum*, *F. merismoides*, *F. culmorum* and *F. acuminatum* subsp. *armeniicum* were isolated for the first time in South Africa from potato tubers during this survey. *Fusarium subglutinans* and *F. graminearum* Gr. II, which were isolated from tubers originating from dry land regions was not isolated during this survey in irrigated regions.

Thus a total of 20 *Fusaria* taxa have been associated with the *Fusarium* dry-rot complex in South Africa. *Fusarium culmorum* was the only additional species identified for the first time as a pathogen of potatoes in South Africa. Thus a total of nine *Fusarium* spp. have been identified as dry-rot pathogens in South Africa.

A more diverse *Fusarium* population was isolated from tubers with dry-rot lesions than from those with stem-end-rot lesions. This might be ascribed to *Fusarium* dry-rot infection occurring mainly through wounds, while stem-end-rot infection is more complex.

The knowledge gained by this study on the *Fusarium* dry-rot complex and the associated *Fusarium* spp. in the different production regions should form the basis for the development of an effective *Fusarium* dry-rot control strategy for South Africa. A valuable outcome of this study was that all potato breeding lines were screened against *F. solani* and *F. oxysporum* for dry-rot resistance, whereas in the past only *F. solani* was included.

Chapter 3

DIFFERENTIATION BETWEEN SOUTH AFRICAN AND FOREIGN ISOLATES OF *FUSARIUM SOLANI*, *FUSARIUM SOLANI* VAR. *COERULEUM* AND *F. COERULEUM* FROM POTATO TUBERS WITH DRY ROT

INTRODUCTION

At the time of this study many *Fusarium* spp. have been associated with tuber rots of potato, of which *F. solani*, *F. sambucinum*, and *F. avenaceum* were the dominant species associated with Fusarium dry rot in the northern hemisphere (Boyd, 1972; Jones & Woltz, 1981; Seppänen, 1989), and *F. oxysporum* and *F. solani* were the most prevalent in the southern hemisphere (Stubbs, 1971; Chambers, 1973; Turkensteen, 1987; Tivoli *et al.*, 1988; Theron & Holz, 1989). In published research, *F. solani* [= *F. solani* var. *coeruleum* and *F. coeruleum*] and *F. sambucinum* [= *F. sulphureum*] had received the most attention, as dry rot pathogens of potatoes.

Due to the fact that plant pathologists use different taxonomic systems to identify and name the *Fusarium* strains, confusion could arise when results were compared. Besides *F. oxysporum*, *F. solani* was the predominant species associated with dry rot and stem-end rot of potatoes in South Africa (Theron & Holz, 1989). These two species were identified according to the taxonomic system of Nelson *et al.* (1983). In order to compare the results in this study with published reports, Theron had to determine whether South African isolates identified as *F. solani* were conspecific with foreign isolates referred to in the literature as *F. solani*, *F. coeruleum* and/or *F. solani* var. *coeruleum*.

MATERIALS AND METHODS

Isolates

Theron included twenty-four *F. solani* isolates from potato dry-rot and stem-end rot tubers in South Africa as well as five *F. solani*, seven *F. solani* var. *coeruleum* and twenty-one *F. coeruleum* isolates obtained from various foreign countries, in this study. South African isolates were identified using the taxonomic system of Nelson *et al.* (1983) and representative cultures were deposited in the culture collection of the Programme on Mycotoxins and Experimental Carcinogenesis (PROMEC), Medical Research Council (MRC), South Africa. All cultures were single spored using a modified technique of Nelson *et al.* (1983) as described by Theron and Holz (1989). Cultures were incubated at 25±2°C under intermittent light (Nelson *et al.*, 1983) on CLA-slants (Fischer *et al.*, 1982) until sporulation occurred, and then kept at 5°C in the dark.

Cultural characteristics

Carnation leaf pieces were transferred aseptically from the CLA-slants to the CLA section of divided Petri dishes and incubated at 25±2°C for two days. Agar plugs (3 mm in diameter) with mycelium were cut aseptically from the margins of developing colonies and transferred to the PDA section of the divided dishes (Nelson *et al.*, 1983).

After 10-15 days incubation at $25\pm 2^{\circ}\text{C}$ under intermittent light, the identity of cultures was confirmed using the taxonomic system of Nelson *et al.* (1983).

Photography

Photomicrographs of sporodochia formed on carnation leaves were made 10 days after incubation at $25\pm 2^{\circ}\text{C}$ under intermittent light using a Wild stereo microscope equipped with a Nikon camera. Photographs of macroconidia suspended in distilled water were taken using a Nikon microscope at x400 (x40 objective and a x10 eyepiece).

Growth on potato-dextrose agar

Carnation leaf pieces were transferred from the CLA-slants to PDA plates and incubated for 10 days at $25\pm 2^{\circ}\text{C}$ under intermittent light. Mycelia plugs (3 mm in diameter) were cut aseptically with a cork borer from the edge of a colony of each isolate and transferred to the centre of 90-mm plastic PDA plate. Plates (3 per treatment) supporting mycelia plugs of each isolate were incubated in a randomized block design at 5, 10, 15, 25, 30, or 35°C in the dark. Colony diameters were measured after 3 and 6 days incubation, and averages were calculated.

Pathogenicity

Inoculum was prepared by transferring carnation leaf pieces from the CLA-slants to CLA-plates. After incubation at $25\pm 2^{\circ}\text{C}$ for 2 weeks under intermittent light (Nelson *et al.*, 1983), spores were washed from cultures with sterile distilled water and the suspension diluted to 1×10^4 propagules/ml (macro- and microconidia). Sound unblemished tubers, cv. Up-to-date, selected after harvest from plants grown under normal commercial conditions in field plots at Roodeplaat (ARC VOP) were disinfected for 15 min in 3% NaOCl and allowed to dry. Fifteen tubers (75-150 g each) were inoculated approximately halfway between the rose and heel-ends, on either side of the tubers, by injecting 0.2 ml of the conidial suspension of each isolate 8 mm into the tissue with a Socorex 2-187 self-refilling syringe (Theron & Holz, 1987). Tubers (five per treatment, three replicates) were wrapped in paper bags and incubated in a randomized block design at $25\pm 2^{\circ}\text{C}$ and 50-70% RH.

After 3 weeks incubation, tubers were cut in half at the inoculation sites and the extent of dry-tot development determined on a disease index scale of 0 to 5, with 0 = restricted discolouration with no dry-rot development at the inoculation site and 5 = tubers completely decayed (Theron & Holz, 1987). The results were expressed as percentage decay according to the method of Kremer and Unterstenhöfer (1967).

Inoculum was prepared from the four most virulent isolates of each group of isolates (*F. solani* South African, *F. solani* foreign, *F. solani* var. *coeruleum* and *F. coeruleum*) and inoculated into tubers as described above. Tubers (five per treatment, three replicates) were wrapped in paper bags and incubated in a randomized block design at 5, 15 and 25°C for three weeks, after which the percentage of dry rot was determined as described above.

Statistical analysis

The data were statistically analysed using analysis of variance. The least significant difference (LSD) function of the SAS/STAT program for personal computers (Statistical Analysis System Inc., 1989) was used, to test for significant differences according to Tukey's test ($p = 0.05$).

RESULTS AND DISCUSSION

Cultural characteristics

The five foreign *F. solani* isolates obtained from P.E Nelson and D. Rodriguez had similar characteristics than the *F. solani* isolates from dry-rot lesions of potatoes in South Africa, but the *F. coeruleum* and *F. solani* var. *coeruleum* isolates differed from the *F. solani* isolates. Cultural and morphological descriptions are presented in the following section with the emphasis placed on the differences between the *F. solani* isolates and *F. coeruleum* and *F. solani* var. *coeruleum* isolates.

F. solani

Colonies: Colonies rapidly reached an average colony diameter of 33.9 ± 3.1 and 38.5 ± 6.4 mm at 25 and 30°C, respectively, in three days. Aerial mycelium was usually rather sparse, floccose, felt-like, sometimes zonate and whitish to cream in colour (Figure 3.1). Pigmentation of colonies did not vary, and the under surfaces of the colonies were predominantly cream-coloured (Figure 3.1). Some of the isolates had light greenish to bluish flecks, especially old cultures. Sporulation usually commenced in the aerial mycelium as microconidia cohering in false heads after 2 to 3 days. Most of the isolates formed an abundance of macroconidia in white to cream-white sporodochia (Figure 3.2) which became visible in the area of the point of inoculation after 4 to 7 days resulting in these cultures having a pionnotal appearance (Figure 3.1).

Conidiophores: Primary conidiophores arised laterally from hyphae in the aerial mycelium, at first unbranched, later sparsely branched. Secondary, initially simple but later short multibranched, conidiophores occurred which soon merged, forming sporodochial or pionnotal layers. Microconidia were produced on long and slender monophialides, mostly with distinct colarettes. Macroconidia were produced on shorter, subcylindric obclavate or doliiform monophialides.

Conidia: Micro- and macroconidia were abundantly produced by all the isolates. Microconidia were single- or two-celled and oval, ellipsoid to subcylindrical and macroconidia were long, slightly curved, relatively wide, thick walled and predominantly 3- to 4- septate and occasionally 5-septate. For most of their length the dorsal and ventral surfaces were parallel. The apical cells were short, slightly narrow, more or less pointed and hooked, especially for the foreign isolates, and the basal cells were distinctly notched or sometime foot-shaped (Figure 3.3).

Chlamydospores: Chlamydospores formed abundantly after ± 14 days terminally or intercalary in hyphae or conidia either single or in pairs but rarely in chains or clusters. They were globose to subglobose and their walls usually smooth, but some of the isolates occasionally produced rough-walled chlamydospores.

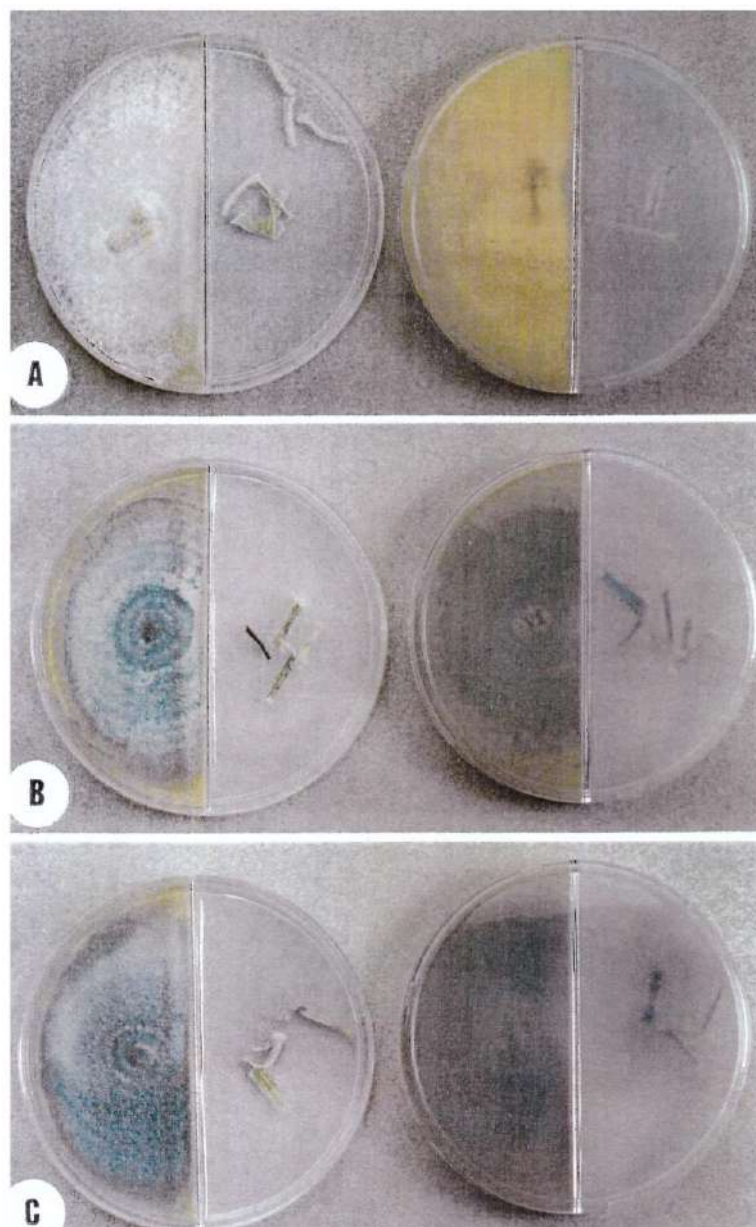


Figure 3.1. Colony morphology of *Fusarium solani* (A), *F. solani* var. *coeruleum* (B) and *F. coeruleum* (C) on PDA after 14 days incubation at 25°C under intermittent light.

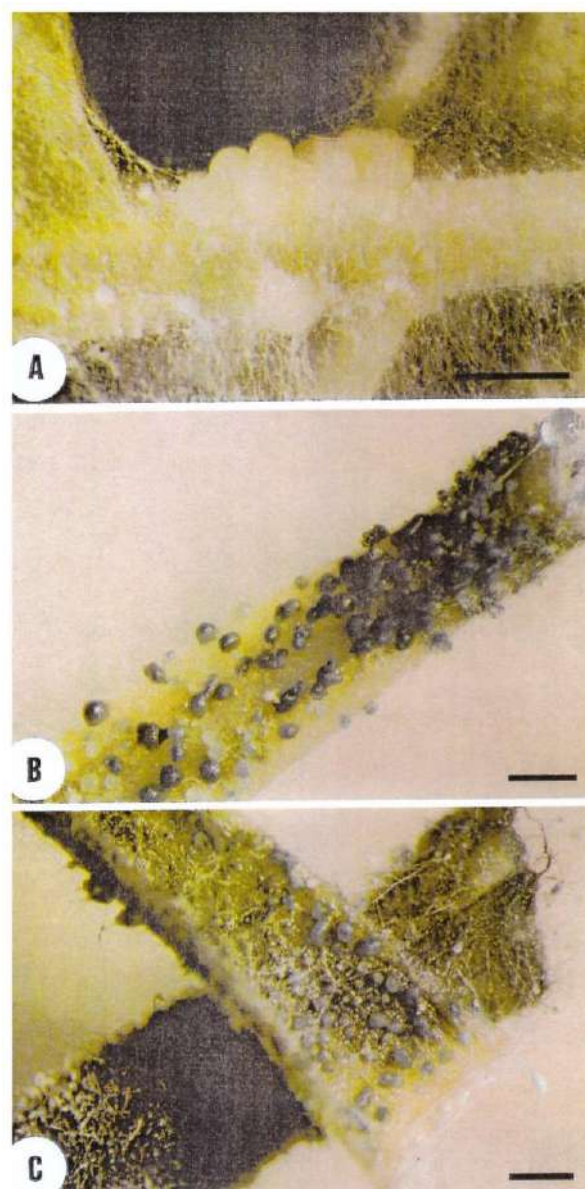


Figure 3.2. Sporodochia produced on carnation leaves by *Fusarium solani* (A), *F. solani* var. *coeruleum* (B) and *F. coeruleum* (C) after 14 days incubation on CLA at 25°C under intermittent light. Bars = 1 mm.

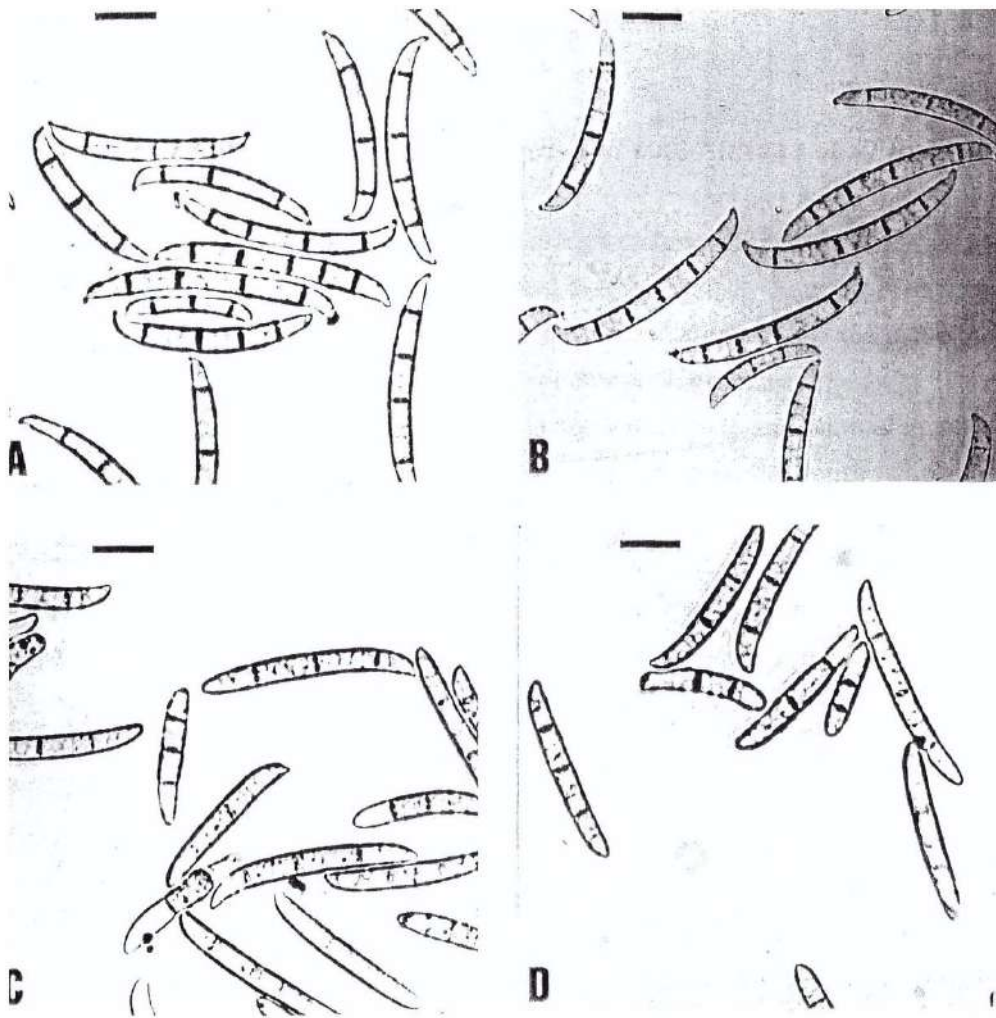


Figure 3.3. Macroconidia produced in sporodochia, formed on CLA by *Fusarium solani* isolates, South African (A) and foreign (B), *F. solani* var. *coeruleum* (C) and *F. coeruleum* (D) after 14 days incubation at 25°C under intermittent light. Bars = 10 µm.

The isolates of the *F. solani* group (South African and foreign *F. solani* isolates), matched the description of *F. solani* (Nelson *et al.*, 1983). The most notable cultural and morphological characteristics were the homogeneous whitish to cream colour of these isolates, the occurrence of long monophialides bearing microconidia in false heads and the production of macroconidia in cream coloured sporodochia. The macroconidia were mostly 3- to 4-septate, with short apical cells, more or less pointed and hooked and the basal cells distinctly notched. These characteristics matched the description of almost all the *Fusarium* spp. except *F. coeruleum*, which are associated with dry rot of potatoes in the sections Martiella and Ventricosum described by Gerlach and Nirenberg (1982). The *F. solani* isolates (South African and foreign) could not be assigned with confidence to any one of these species, but they fit the descriptions of *F. javanicum* and *F. solani* best.

F. coeruleum and *F. solani* var. *coeruleum*

Colonies: Compared to the *F. solani* isolates, colony growth was slow, reaching 25.7 ± 4.1 and 9.2 ± 1.5 mm at 25 and 30°C, respectively, in three days. Aerial mycelium was usually rather sparse or even absent and when present it was floccose, felt-like with a dense mycelial-mat, grey-violet to bluish in colour (Figure 3.1). Pigmentation of the colonies was rather variable and the under surfaces of the colonies were dark purple, almost black, but predominantly bluish to deep blue-purple (Figure 3.1). Sporulation in freshly isolated cultures was abundant and commenced rapidly in the aerial mycelium forming masses of conidia in sporodochia or pionnotes. Newly formed sporodochia were usually cream to buff coloured turning grey-violet to blue-purple with age (Figure 3.2). Most of the isolates had a slimy appearance with sporodochia becoming confluent, covering large parts of the colony, usually in zones, resulting in cultures with a pionnotal appearance (Figure 3.1).

Conidiophores: Conidiophores arised initially as single lateral phialides in the aerial mycelium or loosely branched, however, when formed in sporodochia or pionnotal layers, branching was very dense. Phialides were short, monopialidic, with distinct colarettes almost cylindrical and rather slender. Long monopialides bearing microconidia in false heads were not produced by these isolates.

Conidia: Microconidia were sparsely produced by some of the isolates, but macroconidia were abundantly produced in all isolates. Microconidia were poorly developed, either single- or double-celled, oval, ellipsoid to sub-cylindrical. Microconidia were produced sparsely by some isolates. None of the microconidia, when present, were formed on distinct conidiogenous cells.

Macroconidia were long, sub-cylindrical, only slightly curved, less than those of *F. solani*, relatively wide, thick walled and predominantly 3-septate, occasionally 4-septate. Most often the septa were indistinct. For most of the length of the macroconidia, the dorsal and ventral surfaces were parallel. The apical cells were blunt, but never pointed or hooked as those produced by the *F. solani* isolates. The basal cells were notched but distinctly foot-shaped cells never occurred (Figure 3.3).

Chlamydospores: Terminal or intercalary chlamydospores formed abundantly in hyphae or conidia, either single, in pairs, in chains or in clusters. They were usually globose to subglobose and smooth-walled.

Characteristics of the *F. coeruleum* and *F. solani* var. *coeruleum* isolates were consistent with those published for *F. coeruleum* (Gerlach & Nirenberg, 1982) and partly to *F. solani* var. *coeruleum* (Booth, 1971), but differed from the description of *F. solani* (Burgess & Lidell, 1983; Nelson *et al.*, 1983). The most pronounced difference was the absence of long monopialides bearing microconidia in false heads. Although some of the isolates of *F. coeruleum* and *F. solani* var. *coeruleum* did produce microconidia sparsely, the total absence thereof is not typical of *F. solani* (Nelson *et al.*, 1983). Macroconidia were produced by all the isolates in grey-violet to blue-purple sporodochia whereas the sporodochia of the *F. solani* isolates were always cream coloured. The *F. coeruleum* and *F. solani* var. *coeruleum* isolates produced macroconidia with distinctly notched basal cells, but foot-shaped basal cells were absent. The apical cells were blunt and never pointed or hooked such as those produced by the *F. solani* isolates. The macroconidia were predominantly 3-septate with indistinct septa. These characteristics, as well as the predominantly bluish to deep blue-purple colour of the *F. coeruleum* and *F. solani* var. *coeruleum* isolates matched the description of *F. coeruleum* (Gerlach & Nirenberg, 1982) better than the descriptions of Burgess and Lidell (1983) and Nelson *et al.* (1983) of *F. solani*.

Growth on potato-dextrose agar

Except for a few South African and foreign *F. solani* isolates, colony diameters of the isolates, irrespective of the incubation temperature, did not differ significantly ($p \leq 0.05$) from each other within a taxon. The colony diameters of the *F. solani*-group (South African and foreign *F. solani* isolates), did not differ significantly ($p \leq 0.05$) from each other. The same was found between the isolates of the *F. coeruleum*-group (*F. solani* var. *coeruleum* and *F. coeruleum* isolates) (Table 3.1). The isolates of the *F. coeruleum*-group grew significantly better ($p \leq 0.05$) than those of the *F. solani*-group after incubation at 15°C. The optimum temperature for the *F. solani*-group was 30°C followed by 25°C and for the *F. coeruleum*-group 25°C followed by 20°C.

Colony diameters reached after three days incubation at 25 and 30°C is considered an important characteristic for the identification of *Fusarium* spp. and colony diameters of 21-29 mm and 26-36 mm for *F. solani* at 25 and 30°C, respectively, were reported by Burgess and Lidell (1983). In this study Theron compared the colony diameters of the *F. solani*-group and the *F. coeruleum*-group at 25 and 30°C. Mean colony diameter of the *F. solani*-group (33.9 and 38.6 mm), although larger, were comparable with the mean colony diameters published by Burgess and Lidell (1983), while those of the *F. coeruleum*-group (25.7 and 9.2 mm) were not comparable, especially at 30°C. *Fusarium solani* have been included as the only species in the section Martiella by Burgess and Liddell (1983) and Nelson *et al.* (1983), while Nelson *et al.* (1983) considered species such as *F. javanicum*, *F. coeruleum*, *F. solani* var. *coeruleum*, *F. solani*, *F. eumartii* and *F. ventricosum*, associated with dry rot of potatoes, as synonyms.

Table 3.1. Mean colony diameters (mm) of South African and foreign isolates of *Fusarium solani*, *F. solani* var. *coeruleum* and *F. coeruleum*, 3 days after incubation at different temperatures on PDA plates.

Fusarium isolates	Temperature °C							
	5	10	15	20	25	30	35	Mean
<i>F. solani</i> : South Africa	5.0	5.0	8.9	27.6	33.7	37.8	22.4	20.1
<i>F. solani</i> : Foreign	5.0	5.0	8.5	29.0	35.1	42.5	24.3	21.3
<i>F. solani</i> var. <i>coeruleum</i> : Foreign	5.0	7.2	12.5	25.4	25.6	8.9	7.0	13.1
<i>F. coeruleum</i> : Foreign	5.0	6.0	11.9	25.5	25.7	9.3	7.1	12.9
Mean	5.0	5.7	10.4	26.7	29.9	24.2	15.0	
LSD _(T) 5%								
Taxa (A)	3.8							
Temperature (B)	2.7							
A x B	3.1							

Gerlach and Nirenberg (1982) reported that the growth rates of *F. coeruleum* and *F. ventricosum* were rather slow, with colony diameters of 56-62 mm and 38-42 mm, respectively, after 10 days incubation at 25°C compared to 73-80 mm for *F. solani*.

In a study conducted by Burgess and Lidell (1983), the colony diameters of the *F. coeruleum* or *F. solani* var. *coeruleum* isolates (25.7 mm and 9.2 mm) did not correspond with the colony diameters of *F. solani* (21-29 mm and 26-36 mm) at 25 and 30°C, respectively, after three days incubation on PDA. The optimal growth

temperature for these isolates was between 20 and 25°C, which corresponds to previous reports (Moore, 1945; Langerfeld, 1978; Seppänen, 1981b; Seppänen, 1982; Tivoli *et al.*, 1983). This is different to those for the *F. solani* isolates, where the optimum temperatures appear to be between 25 and 35°C for the South African isolates (Theron & Holz, 1990).

Pathogenicity

At 25°C the South African *F. solani* isolates were significantly more ($p \leq 0.05$) virulent than all the foreign isolates, resulting in significantly more ($p \leq 0.05$) dry rot in the tubers inoculated with the South African *F. solani* isolates and kept at 25°C for three weeks than the foreign isolates (Table 3.2).

Significantly less ($p \leq 0.05$) dry rot developed in inoculated tubers which were kept at 5°C, especially in the tubers inoculated with isolates of the *F. solani* group, than the tubers kept at either 15 or 25°C. The foreign isolates, irrespective of the taxon, resulted in significantly more ($p \leq 0.05$) dry rot in inoculated tubers which were kept at 15°C than the tubers kept at either 5 or 25°C. The optimum temperature for dry rot development caused by the South African *F. solani* isolates and the foreign isolates were 25 and 15°C, respectively.

All the isolates were pathogenic to potato tubers. Tubers inoculated with the South African *F. solani* isolates developed the highest percentage of dry rot when kept at 25°C, while those inoculated with the foreign isolates developed the highest percentage of dry rot when kept at 15°C. This study confirmed that the South African *F. solani* isolates are better adjusted to the higher prevailing temperatures in South Africa (Theron & Holz, 1990). Various reports indicated that dry rot caused by *F. solani*, *F. coeruleum* and *F. solani* var. *coeruleum* develops faster at temperatures ranging between 15 and 20°C (Moore, 1945; Boyd, 1972; Langerfeld, 1978; Seppänen, 1981b; Seppänen, 1982; Tivoli *et al.*, 1983). However, the findings of this study agreed with the report of Seppänen (1981b) that dry rot caused by *F. solani* var. *coeruleum* develops faster at temperatures between 12-24°C and *F. solani* at temperatures of 24°C and higher. At 5°C a substantial percentage of dry rot developed in tubers inoculated with *F. coeruleum* and *F. solani* var. *coeruleum*, however, virtually no dry rot developed in tubers inoculated with *F. solani* isolates, irrespective of their origin.

Although Snyder and Hansen (1941), Nelson *et al.* (1983) and Burgess and Liddell (1983) considered *F. coeruleum* and *F. solani* var. *coeruleum* as synonyms of *F. solani*, considerable cultural and morphological differences occur between isolates of these taxa and the *F. solani* (South African and foreign isolates). Theron recommended that caution should be exercised when comparing results with reports published on these taxa and that the phylogenetic classification of *Fusarium* proposed by O'Donnell (1996) might distinguish between species more accurately.

In this study Theron established that the South African *F. solani* isolates associated with potato dry rot are unique because they require higher temperatures for dry-rot development than the foreign dry-rot isolates. It could also be concluded after the extensive surveys conducted in the potato producing areas of South Africa (Theron & Holz, 1989; Section 2.2) that isolates resembling *F. coeruleum* and *F. solani* var. *coeruleum* were probably not associated with dry rot of potatoes in South Africa.

Table 3.2. Dry rot of potato tubers, cv. Up-to-Date, 3 weeks after inoculation with selected South African and foreign isolates of *F. solani*, *F. solani* var. *coeruleum* and *F. coeruleum* from potato tubers with dry rot and incubated at different temperatures.

<i>Fusarium</i> isolates ^a	Percentage dry rot ^b			
	Temperature °C			
	5	15	25	Mean
<i>F. solani</i>: South Africa				
Fs 2	9.2	38.6	75.8	41.2
Fs 5	7.2	36.1	76.4	39.9
Fs 13	6.3	30.3	74.6	37.1
Fs 23	5.8	29.4	76.8	37.3
Mean	7.1	33.6	75.9	38.9
<i>F. solani</i>: Foreign				
Fs 71	7.0	55.0	43.7	35.2
Fs 72	3.9	47.9	38.3	30.0
Fs 75	7.8	58.3	43.6	36.6
Fs 78	4.0	45.7	40.8	30.2
Mean	5.7	51.7	41.6	33.0
<i>F. solani</i> var. <i>coeruleum</i>: Foreign				
Fsc 58	18.3	71.2	59.1	49.5
Fsc 61	18.1	72.9	57.5	49.5
Fsc 63	20.3	71.3	57.2	49.6
Fsc 64	18.0	74.3	58.7	50.3
Mean	18.7	72.4	58.1	49.7
<i>F. coeruleum</i>: Foreign				
Fc 33	21.3	77.7	62.9	54.0
Fc 38	22.7	76.7	62.9	54.1
Fc 51	24.7	77.3	62.3	54.8
Fc 54	17.8	71.7	61.9	50.5
Mean	21.6	75.9	62.5	53.4
Overall mean	13.3	58.4	59.5	
LSD _(T) 5%				
Isolates	10.0			
Taxa (A)	5.0			
Temperature (B)	4.3			
A x B	4.7			

^aThe four most virulent isolates of each taxon.

^bDry rot determined according to a disease index, where 0 = no lesion development and 5 = tuber completely decayed. Results presented as percentage of decay calculated according to the method of Kremer and Unterstenhöfer (1967). Each value represents 30 observations.

When inoculated tubers were incubated at 5 and 15°C, the *F. solani* var. *coeruleum* and *F. coeruleum* isolates were significantly ($p \leq 0.05$) more virulent than the South African and foreign *F. solani* isolates (Table 2.3).

Chapter 4

EFFECT OF TEMPERATURE ON DRY ROT DEVELOPMENT OF POTATO TUBERS INOCULATED WITH DIFFERENT *FUSARIUM* SPECIES

INTRODUCTION

Dry rot of stored potatoes is caused by several *Fusarium* spp. of which *Fusarium coeruleum*., *F. sambucinum*, *F. trichothecioides* and *F. avenaceum* dominated globally as pathogens causing dry rot of stored potatoes (Wollenweber & Reinking, 1935; Boyd, 1972). However, a different situation occurred in the in the dryland production areas of South Africa: *F. oxysporum* and *F. solani* were the major and *F. sambucinum*, *F. equiseti*, *F. acuminatum*, *F. graminearum*, *F. crookwellense* and *F. scirpi* were the minor causal agents of *Fusarium* dry rot of stored potatoes. Since nothing was known locally about the epidemiology of these fusaria this study investigated the effect of temperature on dry rot development of potato tubers inoculated with local isolates of some fusaria. Tubers of the three most commonly cultivated potato cultivars in South Africa were used. More than one species may occur in a single lesion, therefore interactions between *Fusarium* spp., temperature and cultivar were determined as well.

MATERIALS AND METHODS

Inoculation and incubation

Inoculum was prepared by transferring lyophilized isolates of the most aggressive isolate of *F. oxysporum*, *F. solani*, *F. crookwellense*, *F. sambucinum*, *F. acuminatum*, *F. scirpi* and *F. equiseti* CLA plates (Fisher *et al.*, 1982). After incubation at $25\pm 2^{\circ}\text{C}$ for 2 weeks under intermittent light (fluorescent plus black light; 12 h cycles), spores were washed from cultures with sterile distilled water and the suspension diluted to 1×10^4 CFU/ml (macro- and microconidia). Sound, unblemished tubers of potato cultivars Up-to-Date, BP1 and Vanderplank, selected at harvest from plants grown under normal commercial conditions in field plots at Roodeplaat, were disinfected (3% NaOCl, 15 minutes) and air-dried. Twenty-four tubers (75–150 g) of each cultivar were inoculated with each isolate halfway between rose and heel ends by injecting 0.2 ml of the spore suspension 8 mm into the tissue with a Socorex 2-187 self-refilling type syringe (Theron & Holz, 1987). Tubers (8 tubers per treatment, three replicates) were wrapped in paper bags and incubated in a randomized block design at 5, 15, 25 and 35°C and $65\pm 5\%$ RH.

Disease assessment

After 3 weeks, tubers were cut in half at the inoculation site and the extent of dry rot development determined with a disease index scale used by Theron and Holz (1987). Results were showed as percentage decay based on the method of Kremer and Unterstenhöfer (1967). The data were analysed using analysis of variance and the LSD were calculated with the Tukey-test using the statistical programme Genstat.

Growth on potato-dextrose agar

A lyophilized isolate of each *Fusarium* sp. was transferred to PDA plates and incubated for 10 days at 25±2° under intermittent light (fluorescent plus black light, 12 h cycles). Mycelial plugs (3 mm diameter) were cut aseptically with a cork borer from the edge of a colony of each *Fusarium* sp. and transferred to the centre of PDA plates. Separate plates (10 per treatment) with mycelial plugs of each *Fusarium* sp. were incubated in a randomized block design at 5, 10, 15, 20, 25, 30 and 35°C. Colony diameters were measured after 3 days incubation.

RESULTS AND DISCUSSION

Fusaria, temperature and dry rot development

The level of decay caused by each *Fusarium* sp., irrespective of cultivar, is presented in Table 4.1. Incubation temperature influenced the dry rot development caused by the seven fusaria significantly ($p \leq 0.01$). At 5°C, *F. oxysporum*, *F. equiseti*, *F. scirpi* and *F. solani* were the least active pathogens, causing virtually no decay, and no significant difference occurred between the most active pathogens, *F. sambucinum* and *F. crookwellense*. Decay increased significantly ($p \leq 0.01$) with a rise in temperature above 5°C, except for *F. sambucinum*. *Fusarium crookwellense* was significantly more active at 15°C than the other fusaria while *F. scirpi* was the least active. The activity level of *F. scirpi* and *F. equiseti* plateaued at 15°C. The optimum temperature for decay by *F. sambucinum*, *F. acuminatum*, *F. oxysporum* and *F. crookwellense* was 25°C, and the percentage decay caused by the three most active fusaria (*F. acuminatum*, *F. oxysporum* and *F. crookwellense*) did not differ significantly. *F. equiseti* and *F. solani* had an optimum of 15-25°C and 25-35°, respectively, but *F. scirpi* had no distinct optimum. Contrary to the other isolates, decay caused by *F. solani* peak at 35°C while *F. equiseti* was the least active. The average percentage of decay caused by isolates of each species indicated that their pathogenicity differed. *F. acuminatum* was the most pathogenic, followed by *F. crookwellense*, *F. oxysporum* and *F. solani*, *F. sambucinum*, and *F. scirpi* and *F. equiseti*.

Table 4.1. Decay caused by seven *Fusarium* spp. on potato tubers^a kept for 3 weeks at four incubation temperatures and uniform (65±5%) relative humidity.

<i>Fusarium</i> spp.	Decay ^b (%) at different incubation temperatures (°C)				
	5	15	25	35	Mean per <i>Fusarium</i> sp.
<i>F. crookwellense</i>	24.20 efg	35.60 d	58.96 a	16.13 hij	33.72 B
<i>F. sambucinum</i>	19.24 ghi	23.38 fg	29.24 e	21.73 fg	23.40 D
<i>F. acuminatum</i>	14.49 ij	26.16 ef	60.04 a	47.00 b	36.92 A
<i>F. oxysporum</i>	3.38 k	22.84 fg	57.84 a	23.67 fg	26.93 C
<i>F. solani</i>	2.24 k	20.60 gh	40.31 cd	43.11 bc	26.57 C
<i>F. equiseti</i>	2.24 k	20.56 gh	20.58 gh	11.98 j	13.84 E
<i>F. scirpi</i>	0.87 k	14.78 ij	15.02 ij	14.20 ij	11.22 F
Mean per temperature	9.52 A	23.42 B	40.28 D	25.40 C	
Source of variation	F	Probability (P)			LSD
<i>Fusarium</i> spp. (A)	351.76	0.001			2.25
Temperature (B)	994.14	0.001			1.48
Interaction (AxB)	165.29	0.001			5.39

^aSurface-disinfested tubers of three potato cultivars (Vanderplank, Up-to-Date and BP1) were injected with 0.2 ml of a spore suspension (1×10^4 CFU/ml).

^bDry rot determined according to disease index where 0 = no lesion development and 5 = tuber completely decayed. Results showed as percentage decay based on method of Kremer and Unterstenhöfer. Values in columns followed by the same letter do not differ significantly according to Tukey's multiple range test ($p \leq 0.01$).

Fusaria, cultivar and dry rot development

The level of decay caused by different fusaria, irrespective of temperature, is given in Table 4.2. Vanderplank was significantly more ($p \leq 0.01$) resistant to *F. sambucinum*, *F. oxysporum*, *F. solani* and *F. crookwellense* than Up-to-Date or BP1. However, the three cultivars were equally susceptible to *F. equiseti* and *F. scirpi*. Up-to-Date was significantly more ($p \leq 0.01$) susceptible than BP1 to *F. solani*. Averaging the data for each cultivar showed that BP1 and Up-to-Date were equally susceptible to dry rot and that Vanderplank was significantly more ($p \leq 0.01$) resistant.

Table 4.2. Decay caused by seven *Fusarium* spp. on potato tubers^a of three potato cultivars kept for 3 weeks at different incubation temperatures and uniform (65±5%) relative humidity.

<i>Fusarium</i> spp.	Decay ^b (%) of cultivars		
	Vanderplank	BP1	Up-to-Date
<i>F. acuminatum</i>	33.98 bc	40.03 a	36.75 ab
<i>F. crookwellense</i>	27.30 de	37.33 ab	36.53 ab
<i>F. solani</i>	19.76 f	25.64 e	34.32 b
<i>F. oxysporum</i>	19.63 f	31.72 cd	29.45 cde
<i>F. sambucinum</i>	18.38 fg	26.52 e	25.30 e
<i>F. equiseti</i>	11.28 hi	14.58 gh	15.65 fgh
<i>F. scirpi</i>	8.77 i	13.82 gh	11.07 hi
Mean per cultivar	19.87 A	27.09 B	27.01 B
Source of variation	F	Probability (P)	LSD (r)5%
<i>Fusarium</i> spp. (A)	351.76	0.001	2.25
Cultivars (B)	994.14	0.001	1.17
Interaction (AXB)	165.29	0.001	4.67

^aSurface-disinfested tubers of three potato cultivars (Vanderplank, Up-to-Date and BP1) were injected with 0.2 ml of a spore suspension (1×10^4 CFU/ml).

^bDry rot determined according to disease index where 0 = no lesion development and 5 = tuber completely decayed. Results showed as percentage decay based on method of Kremer and Unterstenhöfer. Values in columns followed by the same letter do not differ significantly according to Tukey's multiple range test ($p \leq 0.01$).

Interaction between *Fusarium* spp., temperature and cultivar

With the exception of *F. equiseti* significant differences occurred in cultivar reaction to fusaria at some incubation temperatures (results not shown). At 5°C, Vanderplank inoculated with *F. oxysporum* was significantly more resistant than Up-to Date. At 15°C, Vanderplank was significantly more resistant than BP1 to *F. oxysporum* or to *F. crookwellense*. At 25°C, Vanderplank was significantly more resistant to *F. scirpi*, *F. sambucinum* or *F. crookwellense* than BP1, than both BP1 and Up-to-Date to *F. oxysporum*, and Up-to-Date to *F. solani*.

Growth on potato dextrose agar

A rise in temperature increased the growth of each pathogen to a different extent (Figure 4.1). At 5°C, colony diameter of the different fusaria was similar. Growth of *F. oxysporum* and *F. crookwellense* was minimal at 10°C while *F. solani* showed slow growth at 10 and 15°C. *Fusarium equiseti* followed by *F. crookwellense*, *F. sambucinum*, *F. scirpi*, *F. oxysporum* and *F. acuminatum* showed maximum growth at 25°C. Only *F. solani* which showed maximum growth at 35°C, had a higher growth rate at 35°C than at 5°C.

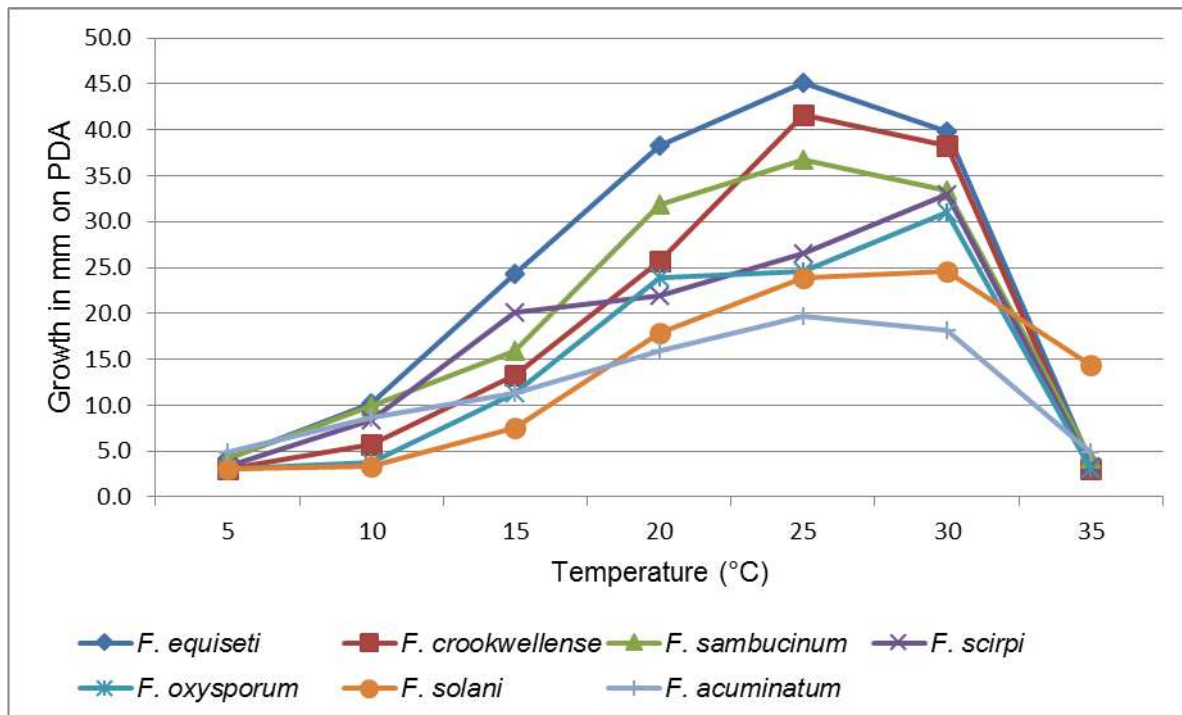


Figure 4.1. Growth of *Fusarium* spp. on potato-dextrose agar incubated for 3 days at different incubation temperatures.

The optimum temperature for infection by fusaria causing dry rot is reported to be approximately 15°C (Weiss *et al.*, 1928; Moore, 1945; Seppänen, 1981a, 1981b, 1982b; Tivoli *et al.*, 1983). Theron found that fusaria generally associated with dry rot in the main dryland production areas of South Africa caused most rot at 25°C. The percentage of decay caused by the two major dry rot pathogens *F. oxysporum* and *F. solani*, as well as by *F. sambucinum*, was significantly higher at 25°C than at 15°C. The optimum temperature for dry rot development by *F. sambucinum* and *F. solani*, the predominant pathogens world-wide, was significantly higher at 25°C and at 35°C, respectively, compared with values of 10-15°C (Weiss *et al.*, 1928; Seppänen, 1981a, 1981b, 1982) and 15 - 20°C (Weiss *et al.*, 1928; Moore, 1945; McKee, 1954; Boyd, 1972; Seppänen, 1981a, 1981b, 1982b; Tivoli *et al.*, 1983) reported from elsewhere. Local isolates of these pathogens appear to be adapted to the high temperatures which prevail in the production areas of Mpumalanga and the eastern Orange Free State.

The finding that *F. acuminatum* was the most pathogenic *Fusarium* sp. was surprising because this organism was considered to be a minor pathogen in the disease complex, and occurred at very low frequencies in lesions caused by mixed-species inoculations. Its insignificance in the potato-dry rot complex might be due to a poor competitive ability, as indicated by mixed-species inoculations, and its poor saprophytic growth on PDA.

The different cultivars did not react uniformly to a given pathogen at different temperatures, emphasizing the need to perform tests at a standardized temperature when evaluating cultivars for disease resistance or for effectiveness of disease control measures. Since different cultivars showed significant differences toward most fusaria at 25°C, evaluation should be done at this temperature.

Locally, dry rot was predominantly caused by *F. oxysporum* and *F. solani* but these pathogens caused virtually no decay at 5°C, therefore dry rot can be retarded by cold storage. In areas with a known history of dry rot, cold storage should be integrated with the cultivation of a resistant cultivar such as Vanderplank.

CONCLUSIONS AND RECOMMENDATIONS

The optimum temperature for infection by fusaria causing dry rot was reported to be 15°C, however, in this study the maximum decay of stored potato tubers occurred on tubers incubated at 25°C. The level of decay was higher than that reported in other countries. Local isolates of pathogenic fusaria are possibly adapted to the high temperatures which prevail in the production regions. Locally, dry rot is usually caused by *F. oxysporum* and *F. solani*. However, these pathogens caused virtually no decay at 5°C, and dry rot can therefore be quite effectively retarded by cold storage. In areas with a known history of dry rot, cold storage should be integrated with the cultivation of a resistant cultivar such as Vanderplank.

Chapter 5

BIOASSAY FOR QUANTIFICATION AND QUALIFICATION OF *FUSARIUM* SPECIES CAUSING DRY ROT OF POTATO TUBERS

INTRODUCTION

At the time of this study dry rot caused by eight *Fusarium* spp. (Theron & Holz, 1989) was the most important storage disease of potato tubers in South Africa (Visser, 1975). The incidence of *Fusarium* dry rot varies among production areas and fields within a production area (McKee & Boyd, 1952; Lapwood *et al.*, 1979), and Du Plooy and Van der Plank (1972) had reported annual losses as high as 60%. These fusaria occur in field soil adhering to tubers (Small, 1944; Foister *et al.*, 1945; Boyd, 1952b; Folsom, 1959; Schippers, 1962; Leach & Nielsen, 1975; Leach, 1985) and usually invade tubers through wounds sustained during lifting or grading (Small, 1944; Foister *et al.*, 1952; Schippers, 1962; Tivoli *et al.*, 1983), resulting in dry rot during storage.

A reliable system for predicting the dry rot potential of a potato crop before harvest was needed to determine the suitability, nature, and duration of storage and the feasibility of post-harvest fungicide applications (Lapwood *et al.*, 1979; Adams *et al.*, 1985). Plant bioassays or tuber baiting techniques based on assessment of inoculum levels and tuber susceptibility (McKee & Boyd, 1952; Tivoli *et al.*, 1986), have been developed for predicting the *Fusarium* dry rot potential of field soils (Foister *et al.*, 1945, 1952; McKee & Boyd, 1952b; Schippers, 1962; Chambers, 1973; Leach & Nielsen, 1975; Lapwood *et al.*, 1979; Seppänen, 1983; Tivoli *et al.*, 1983; Jeffries & Boyd, 1984; Adams *et al.*, 1985; Tivoli *et al.*, 1986) but they could not discriminate between soilborne pathogens causing similar symptoms (Jeffries & Boyd, 1984). Furthermore, these bioassays were time-consuming and results were not available before decisions on control and storage strategies had to be made.

A potato baiting technique that provided a quantitative assessment of the absolute potential of *Pythium aphanidermatum* in naturally-infested sugar beet field soils was developed shortly before the start of this study (Stanghellini *et al.*, 1983; Stanghellini & Kronland, 1985). The objective of this study was to evaluate a similar technique for assessing the absolute inoculum potential of *Fusarium* spp. associated with potato dry rot, in field soils.

MATERIALS AND METHODS

Soil samples

Soil samples were obtained from potato fields from 27 farms in Mpumalanga, 24 farms in the Eastern Free State, and from field plots at Roodeplaat. The cropping history in these production areas was mostly unknown, but potatoes are usually rotated with maize or wheat in a 3 – 4 year rotation cycle. Samples from Roodeplaat were collected from two plots with a one-year potato and three-year rotation with *Eragrostis curvula*, a plot planted previously with vegetables and a plot of potatoes planted in a virgin soil. One kilogram samples of soil adhering to tubers when they were lifted were collected from operating grading machines, air-dried for 24 h and kept in paper bags at 5°C until required.

Preliminary studies on tuber discs

Three soil samples from potato fields were randomly selected, air-dried and sieved through 1.0 mm mesh sieves. The tuber disc technique described by Stanghellini and Kronland (1985) was adapted to bait soilborne *Fusarium* spp. and colonization of the discs by these fungi was also determined.

Experiment 1: Three petri dishes (9 cm diameter) were filled with 24 g prepared soil per sample. Sound unblemished tubers (cultivar Vanderplank), selected at harvest from potato plants grown under commercial conditions in field plots at VOPRI, were washed under running tap water to remove adhering soil, surface-disinfested (3% NaOCl, 15 min) and air-dried. Ten discs (5 mm diameter, 10 mm thick) were cut from tubers with a cork borer halfway between the rose and heel ends, placed on the soil surface in each petri dish and incubated at 25±2°C.

After 24 h discs were removed and the adhering soil and mycelium were washed away in running tap water. Discs were surface-disinfested (70% ethanol, 2 min), rinsed for 3 min in sterile distilled water and air-dried on sterile paper towels in a laminar airflow cabinet. Each disc was sliced in five sequential sections (2 mm thick), starting from the surface that had touched the soil. The sections were plated on a selective rose bengal-glycerin-urea (RbGU) medium for *Fusarium* (Van Wyk *et al.*, 1986) and incubated under intermittent light (fluorescent plus black light; 12 h cycle) at 25±2°C.

After 7 days developing colonies were examined with a light microscope under low magnification (10x). Colonies of single *Fusarium* spp. were transferred directly to the CLA section of divided petri dishes (Fischer *et al.*, 1982). Single-spore isolates of each species were obtained directly from colonies of mixed cultures and transferred to the CLA section of divided petri dishes and incubated at 25±2°C under intermittent light (fluorescent plus black light; 12 h cycle) for 48 h. Agar plugs (2-3 mm³) cut aseptically from the leading edge of developing colonies, were transferred to the PDA section of the divided plates and incubated at 25±2°C under intermittent light for 10-14 days. *Fusaria* that developed from each section after 24 h were identified according to Nelson *et al.* (1983). Colonization of different sections of the tuber discs by the *Fusarium* spp. was also determined.

Experiment 2: Ten petri dishes per sample were filled with 24 g of soil from two potato fields and tuber discs (5 mm diameter, 5 mm thick) prepared from freshly lifted potato tubers (cultivar Up-to-Date) were placed in the petri dishes as described before. A water agar plug (5 mm diameter, 3 mm thick) was placed on top of each potato disc. The dishes were divided in two groups and incubated at 25±2°C for 24 and 48 h, respectively. The tuber discs were then removed, separated, disinfested and dried as described before. A 2 mm section was sliced from the portion of the dried discs that had touched the soil and discarded. The remainder of each disc and the water agar plugs were plated on RbGU medium and incubated at 25±2°C as described before. The percentages of tuber discs and water plugs colonized by *Fusarium* spp. were determined after 7 days.

Experiment 3: Twenty petri dishes per sample were filled with 24 g of soil from two potato fields, and tuber discs (5 mm diameter, 5 mm thick) were prepared from freshly-lifted or physiologically old (kept after harvest in paper bags for 90 days at 5°C, 50-70% RH) potato tubers (cultivars Up-to-Date and Vanderplank) as described before. Ten discs were placed on top of the soil in each petri dish, incubated at 25±2°C for 24 h, and then disinfested, dried and cut as before. The remaining portions of the dried discs were plated on RbGU medium and incubated at 25±2°C. The percentage of tuber discs colonized by *Fusarium* spp. was determined after 7 days.

Determination of population density and inoculum potential

Five different techniques were used to estimate the population density and the absolute inoculum potential of all soils collected.

Soil-dilution plate technique: Air-dried, sieved (1.0 mm mesh) soil from each sample was ground in a mortar and pestle. Subsamples of 0.25 g were added to 100 ml of 0.1% water agar. Suspensions were shaken on a wrist action shaker for 30 min and 1 ml volumes were then spread on the surface of RbGU medium. Plates were incubated at $25\pm 2^{\circ}\text{C}$ for 7-10 days under intermittent light. The number of *Fusarium* colonies per subsample was determined microscopically (10x) and converted to CFU per gram of soil.

Debris technique: Soils were treated for isolation of *Fusarium* spp. from plant debris as described by Nelson *et al.* (1983). Debris, mainly potato plant tissue, was air-dried overnight on sterile paper towels and stored in paper envelopes in a desiccator until tested. Two hundred pieces (2-5 mm length, 0.3-1 mm diameter) of debris from each sample were plated on RbGU medium (10 pieces per plate). Plates were incubated at $25\pm 2^{\circ}\text{C}$ for 6-10 days under intermittent light. Fusaria that developed from each piece of debris were identified as described before and the incidence of each species was expressed as a percentage of the total number of *Fusarium* isolates.

Natural infections: One hundred tubers damaged mechanically by grading machines during harvesting were collected from each of the four Roodeplaat plots. The tubers were kept in crates at $25\pm 2^{\circ}\text{C}$ and 50-70% RH for six weeks to assist natural development of dry rot (Section 1.2). Tubers were then cut in half at the infection site, and five discs (2mm^3) were randomly dissected from the periphery of discoloured tissue of each tuber, plated on PDA plates and incubated at $25\pm 2^{\circ}\text{C}$ for 4-5 days under intermittent light as before. Developing colonies were examined microscopically (10x) and organisms other than fusaria identified directly. Single spores of *Fusarium* spp. were transferred to CLA and PDA and identified according to Nelson *et al.* (1983). The percentage of infections caused by dry rot *Fusarium* spp. (singly or in combinations) was regarded as an estimate of the absolute inoculum potential.

Whole tuber technique: Sound, unblemished tubers from potato plants (cultivar Vanderplank), grown under commercial conditions in field plots at VOPRI, were selected at harvest, surface-disinfested (3% NaOCl, 15 min) and allowed to dry. Tubers (75-150 g) were pressed against a sterilized rigid peg (5 mm diameter, 10 mm length) to wound them on both sides halfway between the rose and heel ends. Fifty tubers were inoculated by placing 0.5 g air-dried, sieved soil (1.0 mm mesh) into each wound with a sterile spatula. Control tubers were wounded but not uninoculated. Tubers were wrapped in paper bags and incubated at $25\pm 2^{\circ}\text{C}$ and 50-70% RH for 6 weeks to allow development of dry rot. The occurrence of *Fusarium* spp. in discoloured tissue was determined as described for natural infections. Absolute inoculum potential was estimated as the percentage of infections caused by dry rot *Fusarium* spp. (singly or in combination).

Tuber disc method: The absolute inoculum potential of the different soils was assessed by a standardized tuber disc method (see Results). Petri dishes (10 per sample) were filled with soil and tuber discs (5 mm diameter, 5 mm thick) prepared from freshly lifted potato tubers (cultivar Up-to-Date) as described in experiment 3. Ten tubers discs were placed on the soil in each petri dish and incubated at $25\pm 2^{\circ}\text{C}$. After 24 h discs were removed, disinfested and dried as described before. A 2-mm section was cut from the portion of the dried discs that had touched the soil and discarded. The remainder of each disc was plated on RbGU medium and incubated at $25\pm 2^{\circ}\text{C}$ for 7 days as described before. The percentage of tuber discs colonized by dry rot *Fusarium* spp. (singly or in combination) was regarded as the absolute inoculum potential in soils.

RESULTS AND DISCUSSION

Colonization of tuber discs by soilborne fusaria

During the initial 24 h, soilborne fusaria penetrated the first 2 mm of 94.4% of the tuber discs. Colonization decreased significantly ($p \leq 0.01$) to 60, 56.7, 16.7 and 4.4% of the tuber discs of the sections 2-4, 4-6, 6-8 and 8-10 mm from the soil contact zone, respectively.

The incidence of fusaria isolated from sequential sections cut from tuber discs is shown in Table 5.1. A high incidence of several *Fusarium* spp. occurred in the first 2 mm of the tuber discs, although some of them, like *F. compactum*, *F. moniliforme*, and *F. subglutinans* were not dry rot pathogens (Section 1.1). Fusaria causing dry rot predominated in the top 6 mm sections of the discs and common saprophytes were rarely found. *Fusarium oxysporum* penetrated farther than other fusaria and was isolated singly from nearly 75% of sections cut from the distal 2 mm of colonized discs (Table 5.1).

Table 5.1. Incidence of *Fusarium* spp. isolated from five sequential sections^b cut from potato tuber discs^a incubated for 24 hours at 25±2 °C on soil from three potato fields^c.

<i>Fusarium</i> spp.	Fusaria (% of total)				
	Section 1	Section 2	Section 3	Section 4	Section 5
<i>F. oxysporum</i>	22.87	51.03	58.23	43.97	83.33
<i>F. solani</i>	13.70	27.90	38.07	45.7	16.67
<i>F. equiseti</i>	48.07	11.50	3.70	10.33	0.00
<i>F. scirpi</i>	7.93	7.30	0.00	0.00	0.00
<i>F. compactum</i>	1.63	2.17	0.00	0.00	0.00
<i>F. moniliforme</i>	1.67	0.00	0.00	0.00	0.00
<i>F. sambucinum</i>	0.97	0.00	0.00	0.00	0.00
<i>F. graminearum</i>	2.17	0.10	0.00	0.00	0.00
<i>F. subglutinans</i>	1.00	0.00	0.00	0.00	0.00

^aTuber discs were plated on the soil surface in petri dishes (10 discs per dish, three replicates per treatment) and incubated at 25±2°C.

^bFive sequential sections, starting from the portion that had been in contact with the soil (section 1), were cut from surface-disinfested (70% ethanol, 2 min) tuber discs. The sections were incubated at 25±2°C on RbGU medium.

^cSamples obtained from three farms in Mpumalanga.

Incidence of fusaria isolated from baits incubated for two different incubation periods, is shown in Table 5.2. *Fusarium* spp. colonized significantly more ($p \leq 0.01$) tuber discs than water agar plugs. Furthermore, significantly more ($p \leq 0.01$) baits were colonized after 48 h of incubation than after 24 h. After 48 h the discs had dried out and some were covered by a mycelial mat which hampered isolation. *Fusarium scirpi*, *F. acuminatum* and *F. crookwellense* were isolated from tuber discs but not from water agar plugs, while *F. oxysporum* was isolated significantly more ($p \leq 0.01$) from water agar plugs than from tuber discs (Table 5). *Fusarium* spp. colonised

significantly more discs ($p \leq 0.01$) of cultivar Up-to-Date than of Vanderplank, and more of physiologically old than freshly lifted tubers (results not shown).

Table 5.2. Incidence of *Fusarium* spp. isolated from baits^a incubated for two different incubation periods at $25 \pm 2^\circ\text{C}$ on soil^b from potato fields.

<i>Fusarium</i> spp.	Fusaria (% of total)			
	Tuber disc		Water agar	
	24 h	48 h	24 h	48 h
<i>F. oxysporum</i>	47.20	63.70	72.60	59.05
<i>F. solani</i>	29.85	17.95	23.10	27.40
<i>F. equiseti</i>	13.75	9.80	2.15	11.45
<i>F. sambucinum</i>	5.55	3.20	0.00	0.00
<i>F. scirpi</i>	1.80	3.35	0.00	0.00
<i>F. acuminatum</i>	1.85	1.00	0.00	0.00
<i>F. crookwellense</i>	0.00	1.00	0.00	1.05

^aTuber discs, each layered on the top surface with a water agar plug, were plated on the soil surface in petri dishes (10 discs per dish, five replicates per treatment) and incubated at $25 \pm 2^\circ\text{C}$.

^bSamples obtained from two farms in the then eastern Orange Free State.

Inoculum density and inoculum potential of potato field soils

Table 5.3 shows the incidence of *Fusarium* spp. in plant debris recovered from 51 potato field soils, and in baits used for assessing the absolute potential of these soils. *Fusarium oxysporum* was the predominant *Fusarium* sp. recovered from tuber baits and plant debris. It was isolated more frequently from plant debris than from the tuber baits. However, *F. solani*, one of the major colonizers of tuber baits, was isolated infrequently from plant debris. *Fusarium crookwellense*, *F. scirpi*, *F. acuminatum* and *F. graminearum* occurred infrequently in plant debris, and were seldom isolated from tuber discs.

Inoculum densities of the 51 potato field soils, as determined by soil dilution, ranged between 560 and 10 360 *Fusarium* propagules per gram of soil, with an average value of 3880 for soils of the eastern Orange Free State, and 3792 for Mpumalanga. The absolute inoculum potential of these soils, determined by the two tuber baiting techniques, ranged between 2 and 94%.

Regression analysis of data of the different assessments indicated highly significant ($p \leq 0.01$) correlation ($r = 0.96$) between results of the whole tuber and tuber discs techniques but a low correlation between inoculum density determined by soil dilution plate technique and the absolute inoculum potential determined by the whole tuber or tuber disk technique. The data of the plant debris assay did not correlate positively with data from either of the tuber baiting methods (results not shown).

Table 5.3. Occurrence of *Fusarium* spp. in plant debris recovered from soils from different potato fields and in baits used for assessment of the absolute inoculum potential of these soils.

<i>Fusarium</i> spp.	Incidence (% of total)					
	Eastern Free State			Mpumalanga		
	Plant debris	Whole tuber	Tuber disc	Plant debris	Whole tuber	Tuber disc
<i>F. oxysporum</i>	57.5	49.2	51.7	64.6	51.7	44.6
<i>F. solani</i>	16.3	43.8	46.2	12.6	42.3	49.2
<i>F. equiseti</i>	12.6	2.7	1.2	8.5	2.5	4.4
<i>F. scirpi</i>	4.3	0.5	0	5.3	1.3	0.1
<i>F. sambucinum</i>	2.1	1.6	0.4	1.0	1.0	1.2
<i>F. subglutinans</i>	1.4	0	0	4.4	0.2	0
<i>F. reticulatum</i>	2.5	0.1	0	0.3	0.1	0
<i>F. moniliforme</i>	0.5	0	0	1.4	0.2	0.1
<i>F. chlamyosporum</i>	0.2	0	0	0.6	0.1	0
<i>F. nygamai</i>	0.8	0	0.2	0	0.2	0.1
<i>F. crookwellense</i>	0.7	1.0	0.2	0	0	0
<i>F. compactum</i>	0.2	0.2	0.1	0.5	0.1	0.3
<i>F. dimerum</i>	0.2	0	0	0.3	0	0
<i>F. acuminatum</i>	0.4	0.5	0	0	0	0
<i>F. graminearum</i> gr.1	0.1	0.4	0	0.4	0.3	0
<i>F. merismoides</i>	0.2	0	0	0	0	0
<i>F. graminearum</i> gr. 2	0	0	0	0.1	0	0

Inoculum density and inoculum potential of the Roodeplaats soils

Inoculum densities, expressed as *Fusarium* propagules per gram of soil, of the four Roodeplaats field plots ranged between 500 and 7880. The absolute inoculum potential of each soil, as determined by the tuber disk and whole tuber bioassays, was considerably higher than indicated by the incidence of naturally-infected tubers (Table 5.4). However, the relative value of the four soils, as shown by the different bioassays, was essentially similar.

Table 5.4. The absolute inoculum potential of dry rot causing fusaria in soil from four Roodeplaat field plots^a assessed by different bioassays.

Soil sample	Absolute inoculum potential (%)		
	Tuber discs ^b	Inoculated tubers ^c	Naturally infected tubers ^d
VOPRI 1	44	39	26
VOPRI 2	28	15	17
VOPRI 3	46	39	23
VOPRI 4	8	6	3

^aVOPRI 1 and 2 = two plots with a one-year potato and three-year *Eragrostis curvula* rotation; VOPRI 3 = a plot planted previously with vegetables; VOPRI 4 = a plot of potatoes planted in virgin soil.

^bThe percentage tuber discs colonized by a dry rotting *Fusarium* (species singly or in combination) was regarded as the absolute inoculum potential.

^{c,d}The percentage successful infections considered as the absolute inoculum potential.

The relative frequencies of isolation of *Fusarium* spp. from debris from the VOPRI soils and from the tuber baits are given in Table 5.5. *Fusarium oxysporum* was predominantly isolated from soil debris, the different baits and from discoloured tissue of naturally-infected tubers. It was also the sole organism associated with dry rot of naturally-infected tubers attained from two of the field plots. Contrary to soils from the two dryland production areas, *F. oxysporum* occurred at noticeably lower levels in debris than in tuber baits, whereas *F. solani* occurred at much lower frequencies in the Roodeplaat soils than in the potato field soils. Regression analysis indicated significant correlation between the *Fusarium* population density as determined by soil dilutions and the absolute inoculum potential as determined by the various tuber bioassays (results not shown).

Table 5.5. Relative frequency of isolation of *Fusarium* spp. from debris of four Roodeplaat field soils^a and from baits used for determination of the absolute inoculum potential of the soils.

<i>Fusarium</i> spp.	Fusaria (% of total)			
	Soil Debris	Tuber discs	Inoculated tubers	Naturally infected tubers
Roodeplaat 1				
<i>F. oxysporum</i>	66.9	90.4	80.0	97.9
<i>F. solani</i>	4.5	4.8	20.0	2.1
<i>F. equiseti</i>	6.5	4.8	0	0
<i>F. nygamai</i>	21.4	0	0	0
<i>F. compactum</i>	0.7	0	0	0
Roodeplaat 2				
<i>F. oxysporum</i>	53.8	75.0	70.0	100.0
<i>F. solani</i>	13.1	16.7	30.0	0
<i>F. equiseti</i>	7.6	8.3	0	0
<i>F. nygamai</i>	24.3	0	0	0
<i>F. moniliforme</i>	0.7	0	0	0
<i>F. compactum</i>	0.5	0	0	0
Roodeplaat 3				
<i>F. oxysporum</i>	33.9	50.0	85.7	91.6
<i>F. solani</i>	41.2	32.1	14.3	8.4
<i>F. equiseti</i>	20.6	17.9	0	0
<i>F. nygamai</i>	4.3	0	0	0
Roodeplaat 4				
<i>F. oxysporum</i>	75.5	75.0	65.8	100.0
<i>F. solani</i>	13.3	25.0	34.2	0
<i>F. equiseti</i>	3.1	0	0	0
<i>F. nygamai</i>	5.2	0	0	0
<i>F. compactum</i>	2.1	0	0	0
<i>F. scirpi</i>	0.8	0	0	0

^aRoodeplaat 1 and 2 = two plots with a one-year potato and three-year *Eragrostis curvula* rotation; Roodeplaat 3 = a plot planted previously with vegetables; Roodeplaat 4 = a plot of potatoes planted in virgin soil.

Knowledge of the inoculum density or the absolute inoculum potential of a soilborne pathogen is required to accurately predict the impact of a soilborne disease (Baker, 1971; Mitchell, 1972; James, 1974; Bouhout, 1979; Gilligan, 1983; Lockwood, 1985; Leath & Carroll, 1985; Stanghellini & Kronland, 1985). Different methods for measurement of inoculum densities of soilborne fusaria should be compared carefully as certain isolation methods might favour selective isolation of certain species (McMullen & Stack, 1983) and these methods fail to distinguish between pathogenic and non-pathogenic forms of a pathogen, or between more and less aggressive forms of a specific *Fusarium* sp. (Small, 1945; Bouhout, 1979; Stanghellini & Kronland, 1985).

Assessment of the absolute potential, which involves collection of soils from fields and a host bioassay, estimates the maximum capacity of a pathogen population to infect a population of fully susceptible host plants under conditions optimum for infection (Mitchell, 1979). Theron showed in this study that the potato tuber disc technique provided an effective quantitative estimate of the absolute inoculum potential of *Fusarium* dry rot pathogens in soil adhering to tubers after lifting. The technique proved to be reliable because the slopes of the regression lines calculated from data of bioassays of soils obtained from diverse regions or soil with different histories of potato cultivation, did not differ significantly from each other.

This simple, inexpensive technique could be applied to predict the risk of storage rot. Unlike other tuber bioassays (McKee & Boyd, 1952; Lapwood *et al.*, 1979; Tivoli *et al.*, 1983), few tubers are needed, soil samples are easily collected and handled, and results are available within 8-10 days. Most soilborne fusaria causing dry rot should be isolated from discs 5 mm in diameter, 5 mm thick, incubated for 24 h on soil and a 2 mm section of the part that had touched the soil should be discarded. The fusaria that cause dry rot are primarily regarded as tuberborne fungi (Foister *et al.*, 1945; Schippers, 1962; Tickle & Boyd, 1974; Leach & Nielsen, 1975; Leach, 1985) and propagules in soil adhering to tubers are known to cause tuber rot (Small, 1945; Boyd, 1952; McKee & Boyd, 1952; Folsom, 1959; Nielsen & Johnson, 1972). Therefore, soils for the tuber disc bioassay should not be sampled by conventional techniques (Schippers, 1962), but collected from tubers during lifting or grading. Tubers should be sampled and soil collected from them approximately 14 days before harvesting, in order to predict the risk of *Fusarium* dry rot of a particular crop. The same tubers should be used for preparation of the baits to exclude differences in susceptibility between cultivars, or between tubers of the same cultivar (Schippers, 1962; Tivoli *et al.*, 1983; Jeffries & Boyd, 1984; Tivoli *et al.*, 1986).

A survey of dry rot of the same tuber lots from which the soil samples had been obtained showed that *F. oxysporum* and *F. solani* were the major *Fusarium* spp. associated with the dry rot disease complex in the two dryland production areas, while *F. equiseti*, *F. sambucinum*, *F. scirpi*, *F. crookwellense*, *F. acuminatum* and *F. graminearum* were minor pathogens (Section 1.1). The same *Fusarium* spp. were also isolated from tuber discs incubated on these soils, although at higher frequencies than from naturally-infected tubers. However, the different fusaria in naturally-infected tubers and colonized tuber discs occurred at a similar order of magnitude. Only *F. sambucinum* occurred less commonly in colonized discs than in naturally-infected tubers. Theron mentioned that pathogen populations or pathogenicity of *F. sambucinum* may have decreased during the 3-5 month that the potato field soils were stored at 5°C before being assessed, because *F. sulphureum* (*F. sambucinum*) populations have been reported to decline in soil after 6 month storage at 4°C (Jeffries & Boyd, 1984; Leath & Carroll, 1985).

Fusarium oxysporum was the major pathogen isolated from the tuber discs incubated on the four VOPRI soils, and almost solely responsible for dry rot of naturally-infected tubers. Therefore, the tuber disc technique provides an effective and reliable way to assess the relative frequencies at which different fusaria causing dry rot occur in soil adhering to tubers. Theron stated that this method could be of great value when planning control strategies because on certain farms, some of the minor fusaria, like *F. equiseti* and *F. crookwellense*, were recovered at relatively high frequencies from both tubers and soils. Furthermore, dry rot fusaria differ substantially in their temperature requirements, and at low temperatures, minor fusaria, like *F. acuminatum* and *F. crookwellense*, are distinctly more pathogenic than *F. oxysporum* or *F. solani*.

The rate at which the different sections of tuber discs were colonized showed that *F. oxysporum*, and *F. solani* are the fastest growers and most competitive *Fusarium* spp. explaining their predominance in lesions that develop following mixed-inoculum inoculations. It might also be an important reason why these two *Fusarium* spp. predominate in soils adhering to tubers, ensuring subsequent development of dry rot in the two main dryland production areas.

CONCLUSIONS AND RECOMMENDATIONS

A potato tuber disc baiting technique was developed to provide a rapid, effective quantitative and qualitative means of assessing the absolute inoculum potential of *Fusarium* dry rot pathogens in soil adhering to tubers after lifting. This inexpensive and simple technique could be used commercially to predict the risk of storage rot. However, soils for the tuber disc bioassay should not be sampled by conventional techniques, but collected from tubers 14 days before harvesting at lifting or during grading. The same tubers should be used for preparation of the baits to eliminate differences in susceptibility between cultivars, or between tubers of the same cultivar.

Chapter 6

CHEMICAL CONTROL OF FUSARIUM DRY ROT OF POTATOES

BACKGROUND

In South Africa, *Fusarium* dry rot is considered the most important post-harvest disease of potatoes (Theron & Holz, 1989), and losses due to *Fusarium* dry rot are mainly considered a problem in the seed industry where potatoes are stored for prolonged periods. Although other *Fusarium* spp. have been isolated locally from dry-rot lesions, *F. solani* and *F. oxysporum* were considered the main causal organisms of dry rot of potatoes (Theron & Holz, 1989).

During the 15-20 years prior to the study, fungicides had increasingly been used for the control of *Fusarium* dry rot (Carnegie *et al.*, 1990) which develops at wounds made either at harvest, grading or later during storage when tubers become more susceptible (Boyd, 1967; Nielsen, 1981; Seppänen, 1981a). Various studies achieved good control by applying fungicides to tubers after harvest, controlling *Fusarium* dry rot during storage, or to seed that has been cut prior to planting, controlling seed piece decay (McKee & Boyd, 1962; Leach, 1978; Hide, 1986). At the time of this study, thiabendazole, as a dip, spray or mist treatment, and mancozeb, as a dip or seed dressing, were the only fungicides registered in South Africa for the control of *Fusarium* dry rot (Krause *et al.*, 1996) and had been used extensively in local potato production since the early 1970's. However, the incidence of *Fusarium* dry rot had increased over the years, irrespective of the use of these two registered fungicides.

PART 1 IN VITRO SCREENING OF FUNGICIDES

INTRODUCTION

The objective of this study was to evaluate different fungicides *in vitro* against *Fusarium* spp. associated with dry rot of potatoes in south Africa because the registered fungicides were tested only against *F. solani* and other fungicides have been reported to be more effective (Hide & Cayley, 1985; Maughan *et al.*, 1991).

MATERIAL AND METHODS

Screening of fungicides against dry-rot pathogens

Twenty-six fungicides were screened *in vitro* for their efficiency of control of mycelial growth of *F. solani*, *F. oxysporum*, *F. sambucinum*, *F. equiseti*, *F. scirpi*, *F. acuminatum*, *F. crookwellense* and *F. graminearum* Gr. 1 associated with dry rot and stem-end rot of potatoes in South Africa. Lyophilized conidia of the most virulent isolates of each species were transferred to CLA plates (Fisher *et al.*, 1982) and incubated at 25°C under intermittent light (Nelson *et al.*, 1983). Fungicide suspensions were prepared in sterile distilled water (pH 7.2).

Paper-disc plate technique

A paper-disc plate technique, as described by Thornberry (1950), was used. Conidia were washed from 14-day-old cultures on CLA plates with sterile distilled water, and the suspension diluted to 2×10^5 conidia/ml. Potato dextrose agar plates, prepared 4 days prior to the experiment, were covered with 0.5 ml of the spore suspension and placed for 3 hr in a laminar airflow cabinet to dry. Sterile filter paper discs (5 mm diameter), cut from Whatman no. 4 filter paper, were dipped in a fungicide suspension (2000 mg a.i./L) and placed on the PDA plates, three per plate. Filter paper discs, dipped in sterile distilled water, served as controls. Five replicates were used for each treatment combination. Four days after incubation at 25°C in plastic bags in the dark, the inhibition zones were measured and 5 mm were subtracted to correct for the size of the filter paper discs.

Poisoned medium technique

A poisoned medium technique, as described by Jones and Ehret (1976), was used. Mycelial plugs (3 mm diameter) were cut aseptically with a cork borer from the leading margin of one-week-old developing colonies on the CLA plates, transferred to PDA plates and incubated as before. After seven days mycelial plugs (5 mm diameter) were cut aseptically with a cork borer from the edge of the colony of each *Fusarium* sp. and placed on 2% PDA plates, three per plate, containing 500 or 2000 mg a.i./L of a fungicide, added after the autoclaved agar had cooled to 60°C. PDA plates without fungicides added, served as controls. For each treatment combination, five replicates were used. The radial growth of the isolates was measured, 7 days after incubation at 25°C in the dark and 5 mm was subtracted to correct for the size of the mycelial plugs.

Efficiency against different isolates

Eleven fungicides, including those most effective for the inhibition of mycelial growth, and mancozeb which is registered in South Africa, were evaluated, using the paper-disc plate technique as described before, for their efficiency against five representative pathogenic isolates of each species including the most virulent isolates.

Statistical analysis

The data were statistically analysed using analysis of variance. The least significant difference (LSD) function of SAS/STAT program for personal computers (Statistical Analysis Systems Inc., 1989) was used, to test for significant differences according to Tukey's test ($p=0.05$).

RESULTS AND DISCUSSION

Paper-disc plate technique

The results of the mycelium inhibition zones of the most virulent isolates of each dry rot pathogen, surrounding the filter paper discs soaked with fungicides, after 4 days of incubation on PDA plates are showed in Table 6.1. Mycelium growth, irrespective of the *Fusarium* spp., was significantly ($p \leq 0.05$) inhibited by filter paper discs soaked with prochloraz, both formulations, followed by captafol, captab, imazalil and carbendazim (SC 51%).

Chloroneb, pencycuron, procymidone, propamocarb, thiophanate methyl and vinclosolin had no effect on the growth of *Fusarium* spp., whereas dichlorophen and iprodione did not differ significantly ($p \geq 0.05$) from them.

The two fungicides registered for the control of *Fusarium* dry rot in South Africa, thiabendazole and mancozeb, did not feature amongst the nine best fungicides for the inhibition of mycelium growth. The sensitivity of *Fusarium* spp. to the fungicides differed from one another: for example, fenpropimorf inhibited the mycelial growth of *F. solani* significantly more ($p \leq 0.05$) than the other species, while chlorothalonil, had no effect on *F. solani* but inhibited all the other dry-rot pathogens significantly ($p \leq 0.05$) more. Propiconazole and triadimenol had a significantly higher ($p \leq 0.05$) inhibitory effect on mycelium growth of *F. equiseti* and *F. scirpi* than on the other *Fusarium* spp., with the latter fungicide being effective only against these two species.

Table 6.1 Mycelial inhibition zones (mm), of the most virulent isolate of each *Fusarium* spp., surrounding the filter paper discs, soaked with fungicides (2000 mg a.i./L), measured after 4 days of incubation on PDA plates at 25°C^a.

Fungicides	<i>Fusarium</i> species ^b								Mean	LSD _T 5%	% control ^c
	<i>F. sol</i>	<i>F. oxy</i>	<i>F. sam</i>	<i>F. equi</i>	<i>F. scir</i>	<i>F. acu</i>	<i>F. croo</i>	<i>F. gra</i>			
Benodanil	7.4	6.3	8.1	1.6	6.6	6.9	1.6	11.6	6.4	1.8	15.2
Benomyl	27.3	14.3	14.1	3.9	11.7	7.2	11.0	12.3	13.4	4.7	31.9
Captab	18.3	26.7	25.4	17.9	19.8	25.7	22.2	30.8	24.6	5.9	58.6
Captafol	24.9	22.9	30.4	29.7	23.3	28.8	30.6	43.1	29.1	4.4	69.3
Carbendazim ^d	38.8	23.9	19.1	19.1	19.4	14.8	18.9	25.0	23.8	1.9	56.7
Carbendazim ^e	37.1	22.3	17.3	17.3	13.6	13.7	20.7	21.9	21.8	1.5	51.9
Chloroneb	0	0	0	0	0	0	0	0	0	- ^f	0
Chlorothalonil	0	5.1	6.2	5.2	7.1	8.0	6.1	5.7	5.6	1.8	13.3
Dichlorophen	0	0	5.5	0	0	5.0	0	8.7	2.8	0.9	6.7
Fenpropimorf	40.4	2.8	1.6	0	2.4	2.0	1.2	0	7.6	2.4	18.1
Folpet	10.9	14.9	9.2	7.1	7	17.7	7.5	13.7	12.3	2.5	29.3
Imazalil	13.8	26.7	27.3	29.5	39.3	10.3	29.4	38.0	25.3	3.8	60.2
Iprodione	0	5	1.6	0	0	0	0	7.5	1.6	0.9	3.8
Mancozeb	7.1	3.8	6.0	2.4	6.5	9.5	2.8	7.8	5.9	1.5	14.0
Metiram	10.3	8.6	6.9	0	1.6	6.3	5.0	4.8	5.7	1.8	13.6
Penconazole	12.0	13.3	17.9	37.8	34.2	3.3	7.7	8.2	15.7	1.4	37.4
Pencycuron	0	0	0	0	0	0	0	0	0	-	0
Prochloraz	40.3	43.1	40.9	27.9	31.9	48.1	36.7	52.7	42.0	2.6	100.0
Prochloraz Mg.Cl	29.1	31.0	28.8	17.1	27.8	34.3	27.9	38.1	31.1	5.0	74.0
Propiconazole	6.7	6.5	29.3	35.2	34.3	7.1	20.2	9.6	20.3	1.9	48.3
Procymidone	0	0	0	0	0	0	0	0	0	-	0
Propamocarb	0	0	0	0	0	0	0	0	0	-	0
Thiabendazole	34.4	10.6	15.2	15.9	17.1	8.9	23.3	15.1	15.6	2.5	37.1
Thiophanate methyl	0	0	0	0	0	0	0	0	0	-	0
Triadimenol	0	0	0	14.0	18.2	0	0	0	3.6	1.3	8.6
Vinclosolin	0	0	0	0	0	0	0	0	0	-	0
Control	0	0	0	0	0	0	0	0	0	-	0
LSD _T 5%	1.7	2.7	3.1	3.1	1.6	2.6	2.1	2.5	2.1	2.8	

^aThree measurements were taken for each of five replicates (n = 15)

^b*F. Solani*; *F. oxysporum*; *F. sambucinum*; *F. equiseti*; *F. scirpi*; *F. acuminatum*; *F. crookwellense*; *F. graminearum* Gr. 1

^cPercentage mycelium inhibition of that of the best fungicide treatment (prochloraz).

^dCarbendazim, SC 51%, Derosal

^eCarbendazim, BP 65%, Bavistin

^fNot estimable

Mycelium growth of *F. solani* and *F. oxysporum*, the predominant dry-rot pathogens in South Africa, was significantly ($p \leq 0.05$) inhibited by both formulations of prochloraz and carbendazim, followed by captafol, thiabendazole and captab than the other fungicides.

Poisoned medium technique

Twenty-two of the 26 fungicides reduced the growth of the *Fusarium* spp. colonies with more than 50% relative to the control when grown on PDA, amended with 2000 mg a.i./L of the fungicides (results not shown). The seven

fungicides which reduced the colony growth most (>94.7%), did not differ significantly ($p \geq 0.05$) from each other, therefore the trial was repeated with the fungicides applied to the medium at a concentration of 500 mg a.i./L. Seventeen of the 26 fungicides still reduced the growth of the dry-rot pathogen colonies with more than 50% relative to the control when grown on PDA, amended with 500 mg a.i./L of these fungicides (Table 6.2). However, the efficiency of control between these two concentrations of the fungicides benodanil, benomyl, dichlorophen, folpet, mancozeb, metiram and penconazole, differed more than 10%.

PDA amended with 500 mg a.i./L of imazalil, prochloraz (EC 45%) or thiabendazole reduced the colony growth of the *Fusaria* with more than 95% relative to the control, significantly ($p \leq 0.05$) more than the other fungicides (Table 6.2). However, all the fungicides tested reduced the colony growth of the *Fusaria* significantly ($p \leq 0.05$) more than the control. The colony growth of both *F. solani* and *F. oxysporum*, the predominant dry-rot pathogens in South Africa, was totally reduced by imazalil, prochloraz (both formulations) and thiabendazole. Benodanil and fenpropimorf, however, reduced the colony growth of only *F. solani* by 100% and benomyl and carbendazim (both formulations) only that of *F. oxysporum* (Table 6.2).

Efficiency against different isolates

Most of the *Fusarium* spp. isolates evaluated, differed significantly ($p \leq 0.05$) in sensitivity against the fungicides evaluated e.g. all five *F. oxysporum* isolates which differed significantly ($p \leq 0.05$) in sensitivity from one another irrespective of the fungicide used (results not shown).

Prochloraz reduced the mycelium growth of all the *Fusarium* spp. significantly ($p \leq 0.05$) more than the other fungicides, except for *F. solani*, where it did not differ from fenpropimorf and for *F. equiseti* and *F. scirpi* where it did not differ from penconazole (Table 6.3). In comparison to the other species, fenpropimorf had no inhibitory effect on mycelial growth ($p \leq 0.05$), except for *F. solani* and *F. sambucinum*.

Table 6.2 Percentage control of the colony growth of the most virulent isolate of each *Fusarium* spp., measured after 7 days of incubation at 25°C on PDA plates containing fungicides (500 mg a.i./L) which were added to the media after the autoclaved agar had cooled to 60°C^a.

Fungicides	<i>Fusarium</i> species ^b								Mean	LSD _T 5%
	<i>F. sol.</i>	<i>F. oxy.</i>	<i>F. sam</i>	<i>F. equi</i>	<i>F. scir.</i>	<i>F. acum</i>	<i>F. crook</i>	<i>F. gram</i>		
Benodanil	100.0	57.1	78.5	79.6	65.9	76.1	75.3	87.8	77.0	3.3
Benomyl	86.5	100.0	59.7	58.8	100.0	100.0	100.0	100.0	86.4	1.6
Captab	80.0	76.4	78.3	37.0	37.5	47.2	46.7	85.6	61.9	4.1
Captafol	78.3	77.2	54.5	48.2	40.5	58.5	62.6	81.5	64.2	4.8
Carbendazim ^c	71.4	100.0	100.0	86.8	93.3	100.0	100.0	100.0	91.6	3.4
Carbendazim ^d	69.2	100.0	100.0	87.3	82.0	100.0	96.1	100.0	89.7	3.8
Chloroneb	0.1	20.2	18.0	36.1	39.9	1.1	43.6	54.3	29.5	5.8
Chlorothalonil	67.0	73.5	55.9	70.9	67.4	61.9	70.2	75.0	67.3	2.8
Dichlorophen	33.2	57.5	57.7	77.6	51.5	33.5	59.1	74.7	57.3	3.2
Fenpropimorf	100.0	50.7	73.6	77.0	75.3	68.8	82.7	80.4	78.7	2.8
Folpet	72.7	74.3	39.4	30.7	34.5	45.5	45.7	81.8	54.7	6.4
Imazalil	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	- ^e
Iprodione	46.2	52.3	36.8	33.5	28.0	31.3	55.2	68.8	47.8	4.6
Mancozeb	37.1	43.1	40.2	41.6	40.2	60.8	57.3	82.6	45.5	3.1
Metiram	37.5	53.1	59.7	53.7	37.2	42.0	49.2	71.7	49.8	4.4
Penconazole	66.2	83.6	82.0	100.0	100.0	59.1	87.1	88.3	82.3	2.7
Pencycuron	0.1	27.5	10.1	10.8	5.5	0.6	28.4	38.0	14.0	2.5
Prochloraz	100.0	100.0	100.0	97.7	100.0	100.0	100.0	100.0	99.7	0.7
Prochloraz Mg.Cl	100.0	100.0	96.5	87.6	89.0	100.0	88.9	100.0	94.4	3.3
Propiconazole	74.4	83.1	79.4	100.0	100.0	74.4	100.0	90.5	89.1	1.5
Procymidone	40.1	55.1	10.1	23.2	24.2	21.0	28.4	48.9	34.1	5.3
Propamocarb	0.2	30.3	11.3	47.9	5.5	4.0	28.4	64.9	24.0	5.7
Thiabendazole	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	96.4	0.7
Thiophanate methyl	67.0	39.1	28.7	43.9	30.8	27.8	73.2	38.0	48.2	7.8
Triadimenol	42.3	58.3	66.1	100.0	88.7	44.9	65.8	79.3	65.5	4.4
Vinclosolin	46.2	63.1	30.7	40.7	39.6	26.1	41.6	55.4	46.2	4.5
LSD _T 5%	3.6	3.9	4.9	3.8	4.3	3.8	3.5	3.1	6.1	

^aPercentage inhibition of colony diameter relative to the control. Three measurements were taken for each of five replicates (n = 15)

^b*F. Solani*; *F. oxysporum*; *F. sambucinum*; *F. equiseti*; *F. scirpi*; *F. acuminatum*; *F. crookwellense*; *F. graminearum* Gr. 1

^cCarbendazim, SC 51%, Derosal

^dCarbendazim, BP 65%, Bavistin

^eNot estimable

Table 6.3 Average mycelial inhibition zones (mm) of five pathogenic isolates of eight *Fusarium* spp. surrounding the filter paper discs, soaked with fungicides (2000 mg. a.i./L), measured after 4 days of incubation on PDA plates at 25°C^a.

Fungicides	<i>Fusarium</i> species ^b							
	<i>F. sol</i>	<i>F. oxy</i>	<i>F. sam</i>	<i>F. equi</i>	<i>F. scir</i>	<i>F. acum</i>	<i>F. crook</i>	<i>F. gram</i>
Benodanil; BP 50%	6.5	0.4	4.3	2.6	3.7	0.8	0.0	8.8
Benomyl; BP 50%	22.7	7.1	14.7	0.6	7.6	2.6	9.9	7.3
Captab; BP 50%	13.5	15.2	14.7	14.7	9.0	8.2	9.1	11.7
Carbendazim; SC 51%	31.4	16.4	14.2	17.4	15.6	6.1	17.0	20.6
Fenpropimorf; EC 75%	44.2	0.4	2.9	0.0	0.0	0.4	0.0	0.0
Imazalil; BP 75%	10.3	14.2	28.7	38.3	35.0	7.7	25.3	30.0
Mancozeb; BP 80%	4.5	0.8	6.1	0.0	0.4	2.9	4.0	1.9
Penconazole; EC 10%	8.4	10.3	11.5	48.3	37.4	5.1	7.3	14.8
Prochloraz; EC 45%	42.7	43.5	53.8	46.7	37.0	43.9	43.3	48.5
Propiconazole; EC 25%	4.3	3.2	25.1	39.2	33.4	4.5	10.7	13.7
Thiabendazole; EC 45%	23.5	4.0	13.8	10.5	11.8	3.5	6.5	15.6
Control	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
LSD _(T) 5%	1.5	0.9	1.3	2.2	1.0	1.3	1.1	1.2

^aThree measurements were taken for each of five replicates (n = 15)

^b*F. Solani*; *F. oxysporum*; *F. sambucinum*; *F. equiseti*; *F. scirpi*; *F. acuminatum*; *F. crookwellense*; *F. graminearum* Gr. 1

Penconazole had a significantly higher ($p \leq 0.05$) inhibitory effect on mycelial growth of *F. equiseti* and *F. scirpi* than the other fungicides, except for prochloraz (Table 6.3).

Prochloraz had the highest overall inhibitory effect on mycelial growth of the *Fusarium* spp. followed by carbendazim, imazalil and captab (Table 6.3). Mancozeb, one of two fungicides registered for the control of Fusarium dry rot in South Africa, was not effective in controlling the colony growth of *F. solani* and *F. oxysporum*, the predominant dry-rot pathogens in South Africa, by more than 42% relative to the control (Table 6.2). When compared with prochloraz, mancozeb resulted in only 10% control of the colony growth of these two species (Table 6.3).

Efficiency against different isolates

Most of the *Fusarium* spp. isolates evaluated, differed significantly ($p \leq 0.05$) in sensitivity against the fungicides evaluated e.g. all five *F. oxysporum* isolates which differed significantly ($p \leq 0.05$) in sensitivity from one another irrespective of the fungicide used (results not shown).

Prochloraz reduced the mycelium growth of all the *Fusarium* spp. significantly ($p \leq 0.05$) more than the other fungicides, except for *F. solani*, where it did not differ from fenpropimorf and for *F. equiseti* and *F. scirpi* where it did not differ from penconazole (Table 6.3). In comparison to the other species, fenpropimorf had no inhibitory effect on mycelial growth ($p \leq 0.05$), except for *F. solani* and *F. sambucinum*. Penconazole had a significantly higher ($p \leq 0.05$) inhibitory effect on mycelial growth of *F. equiseti* and *F. scirpi* than the other fungicides, except

for prochloraz (Table 6.3). Prochloraz had the highest overall inhibitory effect on mycelial growth of the *Fusarium* spp. followed by carbendazim, imazalil and captab (Table 6.3).

Mancozeb, one of two fungicides registered for the control of *Fusarium* dry rot in South Africa, was not effective in controlling the colony growth of *F. solani* and *F. oxysporum*, the predominant dry-rot pathogens in South Africa, by more than 42% relative to the control (Table 6.2). When compared with prochloraz, mancozeb resulted in only 10% control of the colony growth of these two species (Table 6.3).

Theron stated that the fungicides formulated as wettable powders were less effective than those formulated as either an emulsified or as a suspendible concentration when evaluated by using a paper-disc technique, possibly due to the poorer solubility of the fungicides formulated as wettable powders and their ineffective diffusibility into the media (Torgenson, 1967). Therefore, Theron stressed the importance of using more than one screening method to evaluate the fungicides.

This study showed that irrespective of the *in vitro* screening technique used, prochloraz was the most effective fungicide to inhibit mycelial growth of the *Fusarium* species, followed by imazalil, carbendazim, propiconazole, thiabendazole, captafol, captab, penconazole, benomyl and benodanil. These fungicides, except for propiconazole, penconazole and benodanil, were also the most effective against *F. solani* and *F. oxysporum*, the predominant dry-rot pathogens in South Africa. The *Fusarium* spp. reacted differently on treatments with the different fungicides.

Sensitivity between the five representative isolates of each species to the different fungicides differ significantly, probably due to diversity within the species rather than to a build-up of resistance inherit to exposure to the different fungicides. The differences in sensitivity to fungicides between the *Fusarium* spp., as well as between isolates of the same species, emphasized the need to test fungicides against all the target *Fusarium* spp. causing dry rot of potatoes, as well as to more than one isolate of each species (Shephard, 1987).

Thiabendazole and mancozeb were the only two fungicides registered in South Africa for the control of *Fusarium* dry rot of potatoes at the time of this study (Krause *et al.*, 1996). Although Thiabendazole was rated fifth in these trials, it was not as effective against *F. oxysporum* as against *F. solani*, the predominant dry-rot pathogens found in South Africa (Theron & Holz, 1989). Mancozeb was unable to control the colony growth of either of these two pathogens more than 42% relative to the control. This could explain the poor control and the increase of the incidence of *Fusarium* dry rot locally, irrespectively of the use of these two fungicides.

In vitro screening of fungicides only gives an indication of which fungicides could possibly control a disease. Therefore, Theron recommended further tests where these selected fungicides to determine their efficiency on a commercial scale before recommendations can be made to potato producers. These trials should include different times of application, such as harvesting and before planting as well as other external factors which may have an influence on the efficiency of the fungicides or on *Fusarium* dry rot development.

PART 2. CONTROL OF STORAGE ROT

INTRODUCTION

In South Africa, *F. solani* and *F. oxysporum* are the predominant, of nine *Fusarium* spp., found to be dry-rot pathogens (Theron & Holz, 1989). *Fusarium* dry rot develops at wounds created either at harvest, grading or later during storage when tubers become more susceptible (Boyd, 1967; Nielsen, 1981; Powelson *et al.*, 1993) and can be controlled by applying fungicides to tubers after harvest (McKee & Boyd, 1962; Leach, 1971; Leach, 1978; Hide, 1986).

In South Africa, losses due to *Fusarium* dry rot are mainly problematic in the seed industry where potatoes are stored for prolonged periods. Although tuber inspections are conducted after harvesting, grading and packing, 15.1% of the 18.3 million bags (25 kg) of potato seed presented for certification, during 1995 to 1998, still contained more than 5% tubers with dry-rot symptoms per bag (Database, South African Potato Seed Certification Service, Potatoes South Africa). The percentage of *Fusarium* dry rot found during tuber inspections can be expected to increase during storage.

As stated above, the incidence of *Fusarium* dry rot has increased over the years despite the use of thiabendazole and mancozeb. According to Allan and Moolman (1987) this could be ascribed to the increase in the use of mechanical harvesters, grading and packing equipment, increasing mechanical damage of tubers, therefore providing more infection ports for dry-rot pathogens (Boyd, 1972; Nielsen, 1981; Powelson *et al.*, 1993). Other possible reasons are the time of application, incorrect fungicide dosage, poor coverage and differences in susceptibility between *Fusarium* spp. or even the possibility of fungicide resistance (Murdock & Wood, 1972; Leach, 1975; Logan, 1975; Hide & Cayley, 1985; Tivoli *et al.*, 1986; Carnegie *et al.*, 1990; Hide *et al.*, 1992). Thiabendazole resistance has widely been reported in *F. sambucinum* isolates in France (Tivoli *et al.*, 1986), the Netherlands (Meijers, 1986), Germany (Langerfeld, 1986), the United Kingdom (Hide *et al.*, 1992), United States of America (Desjardins *et al.*, 1993; Hanson *et al.*, 1996) and Canada (Kawchuk *et al.*, 1994). Resistance has also been found in isolates of *F. solani* in Germany (Langerfeld, 1990) and North America (Hanson *et al.*, 1996), *F. culmorum* in the United Kingdom (Hide *et al.*, 1992) and North America (Hanson *et al.*, 1996), *F. acuminatum* and *F. oxysporum* in North America (Hanson *et al.*, 1996) although in low numbers. Von Stachewicz *et al.* (1992) reported *F. sambucinum* also to be resistant to carbendazim.

The objective of this study was to determine the efficiency of thiabendazole and mancozeb as well as other more effective fungicides selected during *in vitro* screening trials, against the spectrum of *Fusarium* spp. associated with dry rot of potatoes in South Africa (Theron & Holz, 1989). In most farming operations the time interval between harvesting and fungicide treatment of the tubers is 6 hr or longer, even 48 to 72 hours. Therefore, Theron also addressed the effect of delaying fungicide treatments and investigated the possibility of resistance in South African isolates of *F. solani*, *F. oxysporum* and *F. sambucinum* to thiabendazole.

MATERIALS AND METHODS

Time required for infection

Inoculum was prepared by transferring lyophilized conidia from the most virulent dry-rot isolates of *F. solani* (MRC 6360) and *F. oxysporum* (MRC 6379) obtained during the survey (Section 2.2) to carnation-leaf-agar (CLA) (Fischer *et al.*, 1982). After incubation at 25°C for 2 weeks under intermittent light (Nelson *et al.*, 1983),

conidia were washed from cultures with sterile distilled water, and the suspension diluted to 2×10^5 conidia/ml. Sound, unblemished potato tubers of the cultivars Up-to-Date, BP1 and Vanderplank, selected at harvest from plants grown under commercial conditions in field plots at the ARC-Roodeplaat (VOPI), Pretoria, South Africa were washed under running tap water to remove adhering soil, surface-disinfested and allowed to dry.

***In vitro* trial**

Ten tuber discs (5 mm in diameter, 5 mm thick), cut from tubers with a cork borer about half-way between the rose- and heel-ends, were placed in a petri dish lined with filter paper and moistened with sterile distilled water. Discs were inoculated by placing a drop (20 μ l) of a spore suspension of either *F. solani* or *F. oxysporum* (2×10^5 conidia/ml) on top of each. Petri dishes were sealed with parafilm and incubated at 25°C. After incubation for different time intervals (0, 1, 4, 8, 24, 30, 48, 64 and 72 hr), the discs were surface-disinfested (70% ethanol, 2 min), rinsed for 3 min in sterile distilled water and dried on sterile paper towels in a laminar airflow cabinet. Dried discs were plated (5 discs/plate) on a selective rose bengal-glycerin-urea (RbGU) medium for *Fusarium* (van Wyk *et al.*, 1986) and incubated at 25°C under intermittent light as described previously. Colonies were examined after 7 days with a light microscope under low magnification (x10) and the percentage of discs colonised with *Fusarium* determined. Two sets of controls (non-inoculated and inoculated tuber discs) were included in these trials. Neither of the control sets were surface-disinfested. A 3-level factorial experimental design, with four replicates and 30 tubers discs for each treatment combination, was used.

***In vivo* trial**

Two holes (5 mm in diameter, 10 mm deep) were cut from the same tubers, used in the *in vitro* trial (75 – 150 g) with a cork borer on either side about half-way between the rose- and heel-ends. Twenty tubers were inoculated with 100 μ l of the inoculum per hole. The tubers were wrapped in papers bags (7 per bag) and incubated at $25 \pm 2^\circ\text{C}$ and 65–75% RH. After incubation for different time intervals (0, 1, 4, 8, 24, 30, 48, 64 and 72 hr), the tubers were surface-disinfested (70% ethanol, 2 min), rinsed for 3 min in sterile distilled water, air-dried on a bench inside the laboratory and then incubated for a further 3 weeks at $25 \pm 2^\circ\text{C}$ and 65–75% RH. The tubers were cut in half at the inoculation sites and the percentage of tuber wounds in which dry-rot symptoms had developed, determined. Two sets of controls were included in these trials, i.e. non-inoculated and inoculated tubers. Neither of the control sets were surface-disinfested. A 3-level factorial experimental design, with two replicates and 14 tubers for each treatment combination, was used.

Fungicide evaluations

Lyophilized conidia of the same *F. solani* and *F. oxysporum* isolates were transferred to CLA and incubated at 25°C under intermittent light. After 10 days, mycelial plugs (3 mm in diameter) were cut aseptically with a cork borer from the edge of the colony of each *Fusarium* culture and transferred to Erlenmeyer flasks (500 ml) containing 250 ml of a 2% malt extract solution and incubated for 10 weeks at 25°C on an orbital shaker. The inoculum suspension was diluted with sterile distilled water to 1.4×10^5 conidia/ml.

Eight fungicides selected during *in vitro* screening trials (Section 2.3.1) were evaluated in these *in vivo* studies. Sound, unblemished potato tubers (cv. Up-to-Date; 75–150 g) selected at harvest from plants grown under commercial conditions in field plots at ARC- Vegetable and Ornamental Plants (Roodeplaat), were washed,

surface-disinfested and dried as described before. Tubers were wounded on both sides about half-way between the rose- and heel-ends with a sterilised rigid peg (5 mm in diameter, 10 mm long) (Theron & Holz, 1991) after which they were inoculated by dipping for 5 min in a spore suspension of either *F. solani* or *F. oxysporum* or a mixture of the two species. Inoculated tubers were air-dried, incubated at 25±2°C and 65–75% RH until fungicide-treated by dipping them for 5 min in 2000 mg a.i./L of a fungicide at different time intervals (4, 12, 24, 48 and 72 hr) after inoculation. Tubers dipped in distilled water served as controls. The treated tubers were wrapped in paper bags (5 per bag), incubated at either 25 or 5 °C for 3 and 8 weeks, respectively, and then cut in half at the inoculation sites and the percentage of tuber wounds in which dry-rot symptoms had developed, determined. A 3-level factorial experimental design, with three replicates and 21 tubers for each treatment combination, was used.

Re-isolations were made from dry-rot lesions (15 per treatment) of potato tubers inoculated with a mixture of *F. solani* and *F. oxysporum* and kept at 25°C for 3 weeks, by dissecting five discs (2 mm³) randomly from the periphery of discoloured tissue of each lesion. The discs were placed on PDA plates and incubated at 25±2°C for 4–5 days under intermittent light (Nelson *et al.*, 1983). Developing colonies were examined with a light microscope under low magnification (10x). The frequency and relative density at which *F. solani*, *F. oxysporum* or a mixture of the two species were reisolated, were calculated according to McMullen and Stack (1984).

$$\text{Frequency (\%)} = \frac{\text{Number of tuber lesions of occurrence of a } \textit{Fusarium} \text{ sp. or a mixture thereof}}{\text{Total number of tuber lesions}} \times 100$$

$$\text{Relative density (\%)} = \frac{\text{Number of isolates of a } \textit{Fusarium} \text{ sp. or a mixture thereof}}{\text{Total number of isolates}} \times 100$$

Statistical analysis

All data sets were statistically analysed using analysis of variance. The least significant difference (LSD) function of the SAS/STAT program for personal computers (Statistical Analysis Systems Inc., 1989) was used, to test for significant differences according to Tukey's test ($p = 0.05$).

Thiabendazole sensitivity trials

Lyophilized conidia of 25 isolates each of *F. solani*, *F. oxysporum* and *F. sambucinum* isolated from potato tubers with dry-rot symptoms were randomly selected and transferred to malt extract agar (MEA) and incubated at 25°C in the dark. After 7 days mycelial plugs (5 mm in diameter) were cut aseptically from the edge of the colony of each *Fusarium* culture with a cork borer and placed centrally onto 2% MEA plates containing 0, 1, 10 or 20 mg a.i./L thiabendazole, respectively, added after the autoclaved agar had cooled to 60°C. The trial consisted of three replicates (5 petri dishes per replicate). The radial growth of the isolates was measured 4 days after incubation at 25°C in the dark and the sensitivity to thiabendazole determined. Thiabendazole resistant isolates were included as controls, one *F. coeruleum* isolate (Nr. 7 – 1990) obtained from Dr. E. Langerfeld, Federal Biological Research Centre for Agriculture and Forestry, Braunschweig, Germany and two *F. sambucinum* isolates (F130 & F140) obtained from Dr. D.L. Corsini, USDA, Agricultural Research Service, Idaho, USA. Methods for assessing resistance to the benzimidazole fungicides are not standardised (Desjardins *et al.*, 1993; Hide *et al.*, 1992; Tivoli *et al.*, 1986). Isolates with radial growth at 10 mg a.i./L thiabendazole were considered as resistant in this study.

RESULTS AND DISCUSSION

Time required for infection

In vitro and in vivo trials: Tuber discs and tuber wounds, irrespective of the potato cultivar, inoculated with *F. oxysporum* were significantly ($p \leq 0.05$) more colonised and infected, respectively, than those inoculated with *F. solani*. The cultivar Up-to-Date was significantly ($p \leq 0.05$) more colonised or infected by both *F. solani* and *F. oxysporum* than BP1 or Vanderplank, while Vanderplank was also significantly ($p \leq 0.05$) less colonised or infected than BP1. None of the tuber discs, but 7.1% of the tuber wounds, surface-disinfested immediately after inoculation with either *F. solani* or *F. oxysporum*, were colonised or showed dry-rot symptoms respectively. The percentage of tuber discs and tuber wounds colonised and infected increased significantly ($p \leq 0.05$) with an increased delay in the time of surface-disinfestation after inoculation (Tables 6.4 and 6.5).

While tuber discs inoculated with *F. oxysporum*, irrespective of the potato cultivar, were 100% colonised 24-30 hr after surface-disinfestation, discs inoculated with *F. solani* were 100% colonised 48-72 hr after surface-disinfestation, depending on the potato cultivar (Table 6.4). Surface-disinfestation of inoculated tubers after 24 hr resulted in an average of 55% tuber wounds expressing dry-rot symptoms (Table 6.5). Significantly more ($p \leq 0.05$) tubers, depending on the cultivar, expressed dry-rot symptoms after inoculation with *F. oxysporum* and surface-disinfested 24 hr later (52.5 - 70%), than those inoculated with *F. solani* (40 and 60%) (Table 6.5).

None of the non-inoculated control tuber discs or tuber wounds were colonised or expressed dry-rot symptoms respectively (results not shown). The inoculated, non-surface-disinfested control tuber discs were 100% colonised (Table 6.4), while between 55 and 100% of the non-surface-disinfested tuber wounds expressed dry-rot symptoms depending on the cultivar and *Fusarium* sp. used as inoculum (Table 6.5).

Table 6.4 Percentage potato tuber discs colonised after artificial inoculation with a spore suspension of either *F. solani* or *F. oxysporum*, surface-disinfested after different time intervals, and incubated at 25°C for 7 days on RbGU medium.

<i>Fusarium</i> spp.	Percentage discs colonised									
	Time intervals (hr)									
	0	1	4	6	24	30	48	54	72	Mean
Cultivar Up-to-Date										
<i>F. solani</i>	0 ^a	20.0	45.0	45.0	70.0	90.0	100	95.0	100	62.8
Control ^b	-	-	-	-	-	-	-	-	100	-
<i>F. oxysporum</i>	0	30.0	65.0	70.0	100	100	100	100	100	73.8
Control	-	-	-	-	-	-	-	-	100	-
Mean	0	25.0	55.0	57.5	85.0	95.0	100	97.5	100	68.3
Cultivar BP1										
<i>F. solani</i>	0	30.0	45.0	40.0	70.0	75.0	85.0	95.0	100	60.8
Control	-	-	-	-	-	-	-	-	100	-
<i>F. oxysporum</i>	0	25.0	40.0	55.0	95.0	100	100	100	100	68.3
Control	-	-	-	-	-	-	-	-	100	-
Mean	0	27.5	42.5	47.5	82.5	87.5	92.5	97.5	100	64.2
Cultivar Vanderplank										
<i>F. solani</i>	0	20.0	25.0	35.0	65.0	75.0	85.0	100	100	56.1
Control	-	-	-	-	-	-	-	-	100	-
<i>F. oxysporum</i>	0	25.0	45.0	55.0	100	100	100	100	100	69.4
Control	-	-	-	-45.0	-	-	-	-	100	-
Mean	0	22.0	35.5		82.5	87.5	92.5	100	100	62.8
Overall mean	0	25.0	44.2	50.0	83.3	90.0	98.3	100	100	
Source of variation									LSD(τ)5%	
<i>Fusarium</i> spp. (A) ^c									0.65	
Cultivars (B)									0.79	
Time of surface-disinfestation (C)									1.37	
A x B									1.12	
A x C									1.94	
B x C									2.37	
A x B x C									4.46	

^aFive observations for each of four replicates (n = 20)

^bInoculated tuber discs, either with *F. solani* or *F. oxysporum*, but not surface-disinfested. Data not included in the statistical analysis.

^cOverall mean of the percentage tuber discs colonised by either *F. solani* (59.6%) or *F. oxysporum* (70.5%) irrespective of the potato cultivar or the time interval of surface-disinfestation after inoculation.

Table 6.5 Percentage tuber wounds expressing dry-rot symptoms after artificial inoculation with a spore suspension of either *F. solani* or *F. oxysporum*, surface-disinfested after different time intervals, and incubated at 25±2° C and 65 – 75% RH for 3 weeks

<i>Fusarium</i> spp.	Percentage tubers expressing dry-rot symptoms									
	Time intervals (hr)									
	0	1	4	6	24	30	48	54	72	Mean
Cultivar Up-to-Date										
<i>F. solani</i>	10.0 ^a	10.0	30.0	40.0	60.0	70.0	65.0	85.0	85.0	50.6
Control ^b	-	-	-	-	-	-	-	-	85.0	-
<i>F. oxysporum</i>	10.0	15.0	30.0	40.0	67.5	75.0	90.0	100	100	57.5
Control	-	-	-	-	-	-	-	-	100	-
Mean	10.0	12.5	30.0	40.0	63.8	72.5	77.5	87.5	92.5	54.0
Cultivar BP1										
<i>F. solani</i>	7.5	17.5	30.0	40.0	40.0	42.5	52.5	55.0	67.5	39.2
Control	-	-	-	-	-	-	-	-	72.5	-
<i>F. oxysporum</i>	0	20.0	35.0	35.0	52.5	62.5	77.5	82.5	90.0	50.6
Control	-	-	-	-	-	-	-	-	92.5	-
Mean	3.8	18.8	32.5	37.5	46.3	52.5	65.0	68.8	78.8	44.9
Cultivar Vanderplank										
<i>F. solani</i>	7.5	10.0	20.0	25.0	40.0	40.0	45.0	45	47.5	30.1
Control	-	-	-	-	-	-	-	-	55.0	-
<i>F. oxysporum</i>	7.5	10.0	40.0	52.5	70.0	70.0	70.0	67.5	70.0	50.8
Control	-	-	-	-	-	-	-	-	70	-
Mean	7.5	10.0	30.0	38.8	55.0	55.0	57.5	56.3	58.8	41.0
Overall mean	7.1	13.8	30.8	38.8	55.0	60.0	66.7	70.9	76.7	
Source of variation									LSD(τ)5%	
<i>Fusarium</i> spp. (A) ^c									0.80	
Cultivars (B)									0.98	
Time of surface-disinfestation (C)									1.70	
A x B									1.39	
A x C									2.40	
B x C									2.94	
A x B x C									4.16	

^aTwenty observations for each of two replicates (n = 40)

^bInoculated tubers, either with *F. solani* or *F. oxysporum*, but not surface-disinfested. Data not included in the statistical analysis.

^cOverall mean of the percentage tuber wound expressing dry-rot symptoms caused by either *F. solani* (40.3%) or *F. oxysporum* (53.0%) irrespective of the potato cultivar or the time interval of surface-disinfestation after inoculation.

The study showed that the longer surface-disinfestation or fungicide treatment of inoculated tuber discs or tubers was delayed, the more they became colonised and infected by *Fusarium* spp. *In vitro* and *in vivo* trials showed that surface-disinfestation 1 hr after inoculation was not fully effective in halting colonisation and infestation, especially when tuber discs and tubers were inoculated with *F. oxysporum*, therefore infestation can take place within 1 hr, under optimal conditions. Surface-disinfestation 72 hr after inoculation was ineffective in halting the tuber discs or tubers from becoming totally colonised and infected.

Hide and Cayley (1980; 1985) demonstrated the importance of avoiding delay in treating tubers with fungicides after injury and possible infestation by dry-rot pathogens. This study showed that none of the fungicides evaluated could control dry-rot development fully when tubers were treated 4 hr after inoculation and the amount of *Fusarium* dry rot increased with further delays in treatment. In South Africa it is common for some potato growers to leave their harvested potatoes for up to 18 hr on trailers before grading and fungicide treatment. Theron reasoned that this practice, and the fact that mechanisation has increased locally over the years, leading to more mechanically damaged tubers especially during the harvesting process (Allen & Moolman, 1987), could have contributed to the increase in *Fusarium* dry rot and the failure of fungicide treatments.

Fungicide evaluations

This study showed that delaying fungicide treatments after tubers had been inoculated with either *F. solani* or *F. oxysporum* or a mixture thereof, resulted in a significantly higher ($p \leq 0.05$) percentage of the tuber wounds expressing dry-rot symptoms (results not shown). Very few tubers inoculated with *F. solani* or *F. oxysporum*, fungicide-treated 4 hr after inoculation and kept at 5°C, developed dry-rot symptoms, especially tubers treated with either prochloraz, thiabendazole or imazalil. When tubers were inoculated with a mixture of these two species, only prochloraz was reasonably effective when applied 4 hr after inoculation, and the tubers kept at 5°C.

Wounds on tubers kept at 5°C developed significantly less ($p \leq 0.05$) *Fusarium* dry-rot symptoms than those kept at 25°C (Table 6.6). This results confirmed the report of Theron and Holz (1990) that lower temperatures will decrease the amount of *Fusarium* dry rot. Wounds of tubers inoculated with either *F. solani* or *F. oxysporum*, and kept at 5°C, expressed approximately 32% less dry-rot symptoms than those kept at 25°C, while tubers inoculated with a mixture of *F. solani* and *F. oxysporum* expressed only 15% less dry-rot symptoms when kept at 5°C. Significantly more ($p \leq 0.05$) wounds of the untreated control tubers developed dry-rot symptoms, irrespective of the *Fusarium* sp. with which the tubers were inoculated or the temperature at which they were kept, than the fungicide-treated tubers (Table 6.6). However, the untreated control tubers kept at 5°C developed less *Fusarium* dry rot than those kept at 25°C, proving the value of cold storage as a control practice.

Prochloraz followed by thiabendazole and imazalil gave significantly ($p \leq 0.05$) better control of dry rot in tubers inoculated with *F. solani* or *F. oxysporum* or a mixture thereof than any of the other fungicides (Table 6.6). Prochloraz, thiabendazole and imazalil gave the best control of *Fusarium* dry rot irrespective of the *Fusarium* spp. inoculated into the tubers, confirming reports that these fungicides gave the best control of dry rot caused by *F. solani* [= *F. solani* var. *coeruleum*] or *F. sambucinum* [= *F. sulphureum*] (Hide & Cayley, 1985; Maughan *et al.*, 1991). Thiabendazole and mancozeb were the only fungicides registered in South Africa for the control of *Fusarium* dry rot (Krause *et al.*, 1996). Although mancozeb did not differ significantly ($p \leq 0.05$) from captab for the control of *Fusarium* dry rot caused either by *F. solani* or *F. oxysporum* (Table 6.6), it was the least effective fungicide tested of the two fungicides registered in South Africa for the control of *Fusarium* dry rot. Thiabendazole was one of the more effective fungicides, but less effective than various other fungicides controlling dry rot caused by a mixture of *F. solani* and *F. oxysporum* than with either of the two *Fusarium* spp. on their own (Table 6.6). Although thiabendazole was not as effective as prochloraz, it was more effective in controlling dry rot caused by *F. solani* than by *F. oxysporum* whereas mancozeb was the least effective of all the fungicides tested. These results confirmed the findings of the *in vitro* fungicide screening trials, where mancozeb was also found to be less effective in inhibiting the mycelial growth of the dry-rot pathogens.

Table 6.6 Percentage tuber wounds expressing dry-rot symptoms after dip treated in fungicides (2000 mg a.i./L) at different time intervals after inoculation with a spore suspension of either *F. solani*, *F. oxysporum* or a mixture thereof, and kept at 5°C for 8 weeks or at 25°C for 3 weeks^a.

Fungicides	Percentage dry rot								
	<i>F. solani</i>			<i>F. oxysporum</i>			<i>F. solani</i> + <i>F. oxysporum</i>		
	Incubation temperature		Mean	Incubation temperature		Mean	Incubation temperature		Mean
	5°	25°C		5°	25°C		5°	25°C	
Control	70.5	99.5	84.9	43.4	74.3	58.9	56.2	87.1	71.7
Mancozeb	42.9	81.9	62.4	27.6	59.1	43.4	41.4	57.6	49.5
Captab	42.8	82.4	62.6	22.4	62.9	42.7	36.2	52.3	44.3
Captafol	37.6	79.5	58.6	24.8	59.1	42.0	33.8	52.8	43.3
Benomyl	42.4	77.2	59.8	24.9	57.6	41.3	35.7	53.3	44.5
Carbendazim	36.7	73.8	55.8	20.0	55.2	37.6	31.9	41.9	36.9
Imazalil	27.6	56.7	42.2	20.0	45.2	32.6	36.6	40.1	38.4
Thiabendazole	23.4	42.4	32.9	18.1	47.1	32.6	33.8	43.3	38.6
Prochloraz	20.0	38.1	29.1	13.3	38.6	26.2	22.8	34.3	28.6
Mean	38.2	70.2		23.8	55.5		36.5	51.5	
LSD _(T) 5%									
Temperature (A)	0.46			0.33			0.29		
Fungicide (B)	0.97			0.69			0.62		
A x B	1.37			0.36			0.87		

^aTubers (Up-to-Date) were wounded on both sides about half-way between the rose- and heel-ends.

^bMean of the percentage tuber wounds expressing dry-rot symptoms irrespective of the time interval of fungicide-treatments after inoculation or the temperature at which these tubers were kept.

Theron mentioned that the fact that Fusarium dry rot in South Africa is mainly caused by *F. solani* or *F. oxysporum* or a combination of these species could also explain the failure to control this disease with these registered fungicides.

Fusarium oxysporum was re-isolated significantly more ($p \leq 0.05$) frequently and in higher density than *F. solani* from fungicide-treated tubers inoculated with a combination of *F. solani* and *F. oxysporum* (results not shown), but in approximately a 1:1 ratio from the dry-rot lesions of the untreated control tubers. This study indicated that the part played by each species causing dry rot was equal, however fungicide treatments were not as effective against *F. oxysporum* as they are against *F. solani*. This was also found when the fungicides were screened *in vitro*.

Theron stated that the reasons for the increase in Fusarium dry rot in South Africa, in spite of the use of the two registered fungicides, appear to be the increase in the incidence of mechanically damaged tubers owing to mechanisation. If these damaged tubers are not treated timeously and effectively with fungicides effective for the control of all the *Fusarium* spp. associated with dry rot, especially *F. oxysporum* an increase of dry rot symptoms will occur. Tubers are less susceptible to infection when kept under conditions favourable to wound healing prior to infection (Escande & Ehandi, 1988; Hide & Cayley, 1985; Nolte *et al.*, 1987), however, tubers are already contaminated with dry-rot pathogens when harvested. If tubers are injured during harvesting or subsequent handling, inoculum is introduced into wounds and is present before the wound healing process has begun (Hide & Cayley, 1985; Powelson *et al.*, 1993). Tuber wounds cannot heal quick enough to prevent Fusarium dry rot when kept under conditions favourable to wound healing (20°C and a relatively high humidity) which is also

favourable to *Fusarium* infection. At least 7 days of wound healing are required to prevent infection of the wounds (Nolte *et al.*, 1987). When tubers are kept at low temperatures, wound healing, will be slower, but the fungal growth and the infection process will also be suppressed by the lower temperature, thus the pathogen will remain more accessible to the surface-applied fungicides. Theron recommended that losses due to *Fusarium* dry rot will be reduced by limiting mechanical damage to tubers, applying a fungicide treatment (preferable prochloraz) as soon as possible after harvest and keeping the tubers in cold storage.

Thiabendazole sensitivity trials

No mycelial growth occurred in cultures of the 25 South African isolates of *F. solani*, *F. sambucinum* and *F. oxysporum* after 4 days incubation at 25°C on 2% malt extract agar containing ≥ 10 mg a.i./L thiabendazole, while the resistant control isolates grew well on plates amended with 20 mg a.i./L thiabendazole. At 1 mg a.i./L thiabendazole the mycelial growth of South African isolates of *F. solani*, *F. sambucinum* and *F. oxysporum* was significantly less ($p \leq 0.05$), i.e. 79.0, 27.3% and 27.2% respectively, than at 0 mg a.i./L thiabendazole, contrary to the mycelial growth of the resistant isolates, *F. solani* [= *F. coeruleum*] and *F. sambucinum* which did not differ significantly ($p \leq 0.05$) at 10 mg a.i./L thiabendazole and 0 mg a.i./L thiabendazole.

This study showed that thiabendazole resistance appeared to be absent from isolates of *F. solani*, *F. oxysporum* and *F. sambucinum* in South Africa since mycelial growth of the South African isolates was inhibited on agar medium containing ≥ 10 mg a.i./L thiabendazole. Theron could consequently have dismissed thiabendazole resistance as a contributing factor to the increase of *Fusarium* dry rot in South Africa, but emphasized the importance to follow strategies limiting the chances of the South African *Fusarium* population developing resistance. Fungicides, especially fungicides belonging to the benzimidazole group, should be applied timeously at the recommended dosage and only when absolutely necessary. Alternating fungicides belonging to the benzimidazole group (thiabendazole, benomyl and carbendazim) with those in the imidazole group (prochloraz and imazalil) were recommended, because no cross resistance occurs between these groups and this practice will lower the selection pressure on the fungal population for resistant strains to develop. However, care should be taken not to import *Fusarium* infected potato seed tubers from abroad which could contain *Fusarium* spp., resistant to thiabendazole.

PART 3. CONTROL OF SEED-PIECE DECAY

Introduction

As the cost of potato seed tubers represents the greater part (35 to 40%) of the production costs of potatoes, the use of tubers, cut into seed pieces is a customary planting practice of some farmers. This cutting process could lead to the spread of tuber-borne pathogens throughout the seed lot and to the soil causing considerable losses during the growing season or thereafter and exposes tuber tissue to desiccation and bacterial and/or fungal seed-piece decay (Nelson *et al.*, 1993; Nolte *et al.*, 1987). Bacterial decay, e.g. soft rot, caused mainly by *Pectobacterium* spp. and fungal decay, e.g. dry rot, caused by *Fusarium* spp. and their combinations are the primary cause of potato seed-piece decay (Davis *et al.*, 1983; Misca & Nelson, 1975; Nelson *et al.*, 1993; Nielsen, 1981; Nolte *et al.*, 1987). Total or partial decay of seed pieces, reduction of plant vigour and poor stands are symptomatic of seed-piece decay (Escande & Echandi, 1988). Often single sprouts emerge from partly decayed seed pieces, developing in small, slow growing plants more susceptible to other diseases, resulting in

low yields (Nielsen, 1981). Cut seed tubers contaminated with *Fusarium* spp. will increase soil contamination and is an important source of contamination of progeny tubers, resulting in high levels of Fusarium dry rot in storage (Leach, 1985).

Various fungicide treatments in the form of dusts or mists are often applied to cut seed tubers as a preventative measure against seed-piece decay. Seed-piece treatments can be applied to planted cut seed or to pre-cut seed before healing begins. Adverse weather during the planting season are related to seed-piece decay. Seed-piece decay is usually minimal when high-quality seed is used and the cutting surfaces are properly healed after cutting, assuming that soil conditions at planting and in the first few weeks after planting favour rapid emergence. Seed-piece treatments under optimal soil conditions at planting until emergence may be of little benefit, however, if unfavourable environmental conditions develop soon after planting, treated seed pieces usually produce a better plant stand. Seed-piece treatments are a valuable preventative measure because environmental conditions are not always optimal at planting, cannot be predicted and may change afterwards (Secor & Gudmestad, 1993).

In South Africa seed-piece decay is an important problem due to the hot and dry climate. Plants developing from decaying seed pieces are usually small and under stress, making them more susceptible to other diseases, resulting in yield losses. During the time of this study, emergence and final stands of potatoes which were often reduced due to seed-piece decay mainly caused by *Fusarium* spp., was a major concern in South Africa. Long-term protection of seed pieces is best provided by the natural barriers produced by the tubers in response to wounding, however, both *Pectobacterium* spp. and *Fusarium* spp. could become established more quickly than these barriers can be formed. Chemical seed-piece treatment could be employed to slow or even stop the infection process (Nolte *et al.*, 1987; Nelson *et al.*, 1993).

The planting of wound-healed, fungicide-treated potato seed pieces was recommended for the control of seed-piece decay (Nelson *et al.*, 1993; Powelson *et al.*, 1993; Schultz, 1981). In South Africa mancozeb was the only fungicide registered for treating potato seed pieces (Krause *et al.*, 1996). However, mancozeb was not effective in controlling either *Fusarium* growth *in vitro* or dry rot of *Fusarium* inoculated tubers. The aim of this study was to evaluate different protective chemicals, time of application, wound-healing and seasonal influences on seed-piece decay, because these practices are not always effective in South Africa.

MATERIALS AND METHODS

Planting material

Sprouted certified seed tubers of the cultivar Up-to-date ($\pm 100 - 150$ g) were cut lengthwise into two seed pieces ($\pm 50 - 75$ g each) either 14 days or the day before planting and immediately dip-treated for 5 min. in 10 fungicides (2000 mg a.i./L) selected during *in vitro* screening trials (Section 2.3.1). Seed pieces dip-treated in water for 5 min. and uncut ($\pm 50 - 75$ g), untreated seed tubers, served as controls. Treated seed pieces were placed in plastic crates and dried in a well-ventilated shady location before being placed in a dark room at 25°C and 65 - 75% RH until planting. The seed pieces cut and fungicide-treated 14 days prior to planting, were evaluated the day before planting to determine the effect of the fungicide treatments on the sprouts and on the quality of the seed tubers.

Field preparation

All the trials were conducted under irrigation on fields subjected to a four year crop rotation with *Eragrostis curvula* at ARC-Vegetable and Ornamental Plants (Roodeplaat), Pretoria, South Africa. To allow the natural breakdown of plant debris, cultivation of the fields commenced one season prior to planting. A fine seedbed was prepared prior to planting and irrigated till water field capacity was reached. Fertiliser 3:2:1 (25) was applied in the furrows, according to a recommendation based on the soil analysis of the fields.

Determining the soil inoculum potential

Eighteen soil samples (± 100 g each) were randomly taken 25 cm deep from the field plots during planting, thoroughly mixed and the inoculum potential determined according to the technique described by Theron and Holz (1991) using the same source of seed tubers planted.

Plantings

Trials were carried out during the autumn (February) and spring (August) of three consecutive years (1990 - 1992). Seed tubers were hand planted in 9 m rows, 30 tubers each. Spacing between and within rows was 75 cm and 30 cm respectively. Border rows were planted between each treatment row using untreated seed tubers, cv. Up-to-date. A randomized block design with three replicates (3 x 9 m rows) for each treatment combination was used. Seed pieces treated 14 days prior to planting were planted in a separate block than those treated one day before planting. Irrigation of the trials was scheduled twice weekly to maintain soil moisture at field water capacity, based on data obtained from a class-A evaporation pan. The maximum and minimum temperatures were recorded daily for the duration of the growing season and the mean temperature calculated.

Evaluation

The mean plant stands were calculated three and six weeks after planting and the yield (kg / 9m row) determined after harvest. The data were statistically analysed using the Genstat 5 PC Program (Genstat 5 Committee, 1987) and the means compared using Tukey's test ($p \leq 0.05$).

RESULTS AND DISCUSSION

Quality of fungicide treated seed pieces

The sprouts of those seed pieces treated with either carbendazim, captab, thiabendazole, or untreated control, were significantly less ($p \leq 0.05$) damaged ($\leq 21\%$) than those treated with one of the other fungicides, while those treated with propiconazole, penconazole or prochloraz were either seriously damaged or dead (Table 6.7).

The quality of seed pieces, treated with carbendazim was significantly better ($p \leq 0.05$) than the quality of those treated with captafol, imazalil, mancozeb, prochloraz and captab, as more than 81% of the seed pieces were not dehydrated or rotten at the time of planting. Although the untreated control seed pieces were significantly less ($p \leq 0.05$) dehydrated than those treated with imazalil, prochloraz, captafol and mancozeb, it resulted in significantly ($p \leq 0.05$) more rotten seed pieces than the fungicide-treated seed pieces (Table 6.7).

Results obtained with propiconazole and penconazole were not included in the statistical analysis because seed pieces treated with these fungicides showed serious damage to the sprouts, were dehydrated or even rotten, and wound healing on the cut surface of seed pieces was almost absent. A relatively high percentage (4.1%) of seed pieces treated with propiconazole, rotted during the 14 days prior to planting (Table 6.7).

Although exceptionally good quality seed tubers were used in these trials, low plant stands and yields experienced during 1991 could partly be attributed to the poor quality of seed tubers, especially those used during the autumn planting of 1991 (data not shown).

Theron found that fungicides affected the quality of treated seed pieces. Treatment of seed pieces with imazalil, prochloraz, propiconazole and penconazole caused serious damage to sprouts of seed pieces. Although some fungicides or the inert ingredients are phytotoxic, damaging the sprouts (Secor & Gudmestad, 1993), not all the damage to the sprouts, could be attributed to fungicide treatments. Some damage was caused mechanically during the cutting process and fungicide treatments because even sprouts of the non-fungicide-treated control pieces were damaged.

Some fungicides treatments interfered with the wound healing process as well, confirming reports of these detrimental effects on the natural healing of freshly cut seed tubers by Nolte *et al.* (1987) and Sanford (1951).

Table 6.7 Effect of fungicide treatments on the sprout and tuber quality of seed pieces (cv. Up-to-Date, 50 – 75 g) planted 13 days after cutting, fungicide-treated (2000 mg a.i./L, 5 min. dip) and stored at 25°C and 65 – 75% RH.

Fungicides	Quality of sprouts			Quality of sprouts		
	% Normal	% Damage	% Dead	% Firm	% Dehydrated	% Decay
Benomyl	77.5	17.8	4.7	71.8	26.0	2.2
Carbendazim	91.0	7.7	1.3	81.8	17.2	1.0
Thiabendazole	79.0	17.5	3.5	70.8	26.0	3.2
Imazalil	33.8	34.5	33.3	50.0	46.5	3.5
Prochloraz	44.5	51.2	6.0	62.8	34.4	2.8
Captafol	48.5	47.5	4.0	45.3	51.2	3.5
Captab	85.5	10.8	3.7	65.8	30.8	3.4
Mancozeb	77.3	19.8	2.8	57.8	41.2	1.0
Propiconazole	0	90.5	9.5	37.1	59.1	3.8
Penconazole	0	32.4	67.6	1.9	97.1	1.0
Control	88.8	7.2	4.0	76.7	15.7	7.6
LSD(T)5%	12.1	14.1	7.0	15.3	14.8	3.8

Treatments which delay wound healing or cause malformation of the wound barriers may contribute to the penetration of organisms which cause seed-piece decay (Nolte *et al.*, 1987). The same fungicides which were responsible for damage to the sprouts, including captafol and mancozeb, interrupted the wound healing process.

Freshly cut seed pieces treated with captab and thiabendazole, in a combination, have a detrimental effect of the deposition of suberin and wound periderm formation (Escande & Echandi, 1988; Stevenson *et al.*, 1986), however, Captab did not interfere considerably with the wound healing process (Nolte *et al.*, 1987). Both fungicides, although not as a combination treatment, were evaluated during these trials. Wound healing was only affected when captab was used, since the seed pieces treated with captab were significantly ($p \leq 0.05$) more dehydrated than the untreated control seed pieces, indicating that wound healing was not adequate. Seed pieces treated with thiabendazole were not significantly ($p \leq 0.05$) more dehydrated than the untreated control seed pieces.

Soil inoculum potential

Theron reported in previous studies that fungicides differ in their effectiveness for the control of mycelial growth of *Fusarium* spp. or Fusarium dry rot. The inoculum potentials of the soils were almost the same for the three consecutive (1990, 1991 & 1992) autumn (36, 43 and 35%) and spring (13, 15 and 10%) plantings. The predominant species isolated from infected tuber disks were *Fusarium solani* and *F. oxysporum*, with *F. solani* responsible for almost 60% of the rotting. Therefore, Theron expected that those fungicides, most effective for the control of *F. solani*, would also be most effective for the control of seed-piece decay.

Since the difference in the mean inoculum potential of the field soils measured during the autumn plantings (38%) and during the spring plantings (12.7%) correlated with the results obtained, it can be concluded that the inoculum present in the soils contributed to the occurrence of seed-piece decay.

Planting

Although the mean temperature during the growing period was 3.8°C higher for the three consecutive spring plantings than for the autumn plantings, the mean temperature during the first 7 days after planting was 9°C lower for the spring plantings (data not shown). During the autumn plantings significantly poorer ($p \leq 0.05$) plant stands (Table 6.8) and yields (Table 6.9) were obtained than during the spring plantings, possibly caused by the temperatures during the growing period, especially the temperatures during the first 7 days after planting, which could enhance seed-piece decay.

During the autumn plantings, plant stands derived from seed pieces treated 14 days prior to planting did not differ significantly ($p \geq 0.05$) from those treated the day prior to planting (Table 2.10). However, seed pieces treated one day prior to planting resulted in significantly ($p \leq 0.05$) higher yields than those treated 14 days prior to planting (Table 6.9). During the spring plantings, significantly better ($p \leq 0.05$) plant stands (Table 6.8) and yields (Table 6.9) were derived from those seed pieces treated the day prior to planting than from those treated 14 days prior to planting allowing for wound healing.

Throughout all the autumn plantings the uncut, untreated seed tubers resulted in significantly better ($p \leq 0.05$) plant stands and yields than the treated seed pieces, irrespective of the time of treatment prior to planting. During the spring plantings the uncut, untreated seed tubers did not perform significantly ($p \geq 0.05$) better than most of the seed pieces treated the day prior to planting (Tables 6.8 and 6.9). The uncut untreated seed tubers performed significantly ($p \leq 0.05$) better than the other seed treatments 14 days prior to planting, except for the seed tubers cut and treated 14 days prior to planting with carbendazim (Tables 6.8 and 6.9).

Table 6.8 Plant stands 6 weeks after planting fungicide-dip-treated seed pieces (cv. Up-to-Date, 50 – 75 g) stored at 25°C and 65 – 75% RH until planting.

Fungicides 2000 mg a.i./L	Seed tubers cut and treated 14 days before planting					Overall mean ^d
	Autumn plantings (1990 – 1992)		Spring plantings (1990 – 1992)		Mean ^c	
	Mean ^a	Overall mean ^b	Mean ^a	Overall mean ^b		
Benomyl	18.0	18.6	20.7	23.8	19.4	21.2
Carbendazim	22.8	21.9	27.3	27.6	25.1	24.8
Thiabendazole	21.3	22.0	22.7	24.5	22.0	23.3
Imazalil	16.6	16.9	13.3	19.1	15.0	18.0
Prochloraz	16.6	16.7	11.8	19.5	14.2	18.1
Captafol	18.6	17.6	14.4	20.8	16.5	19.2
Captab	20.2	19.5	17.4	23.2	18.8	21.4
Mancozeb	19.0	19.5	18.0	23.4	18.5	21.5
Seed pieces dipped in water for 5 min	16.0	15.1	18.5	23.8	17.3	19.5
Untreated whole seed tubers	28.1	28.0	29.4	29.7	28.8	28.9
Mean	19.7	19.7	19.4	23.5	19.6	
	Seed tubers cut and treated one day before planting					
Benomyl	19.1		26.8		23.4	
Carbendazim	21.0		27.8		24.4	
Thiabendazole	22.7		26.2		24.5	
Imazalil	17.2		24.9		21.1	
Prochloraz	16.7		27.1		21.9	
Captafol	16.6		27.2		21.9	
Captab	18.7		29.0		23.9	
Mancozeb	20.0		28.7		24.4	
Seed pieces dipped in water for 5 min	14.1		29.1		21.6	
Untreated whole seed tubers	27.8		29.9		28.9	
Mean	19.4		27.7		23.6	
LSD _(T) 5%						
Planting season						1.2
Planting time (A)	1.5		1.1			1.9
Seed treatment (B)	5.4		3.8			3.8
Interaction (A x B)	7.6		5.4			7.8

^aPlant stands, number of plants out of 30 seed pieces planted in 9 m rows, for three consecutive seasons. Thirty observations for each of three replicates for each season (n = 90). ^bOverall mean irrespective of the time the seed tubers were cut and fungicide-treated prior to planting (n = 180). ^cMean irrespective of the season (n = 180).

^dOverall mean irrespective of the season and time the seed tubers were cut and fungicide-treated prior to planting (n = 360). ^eSeed pieces dipped in water for 5 minutes. ^fUntreated whole seed tubers (50 – 75 g).

Table 6.9 Yield 15 weeks after planting fungicide-dip-treated seed pieces (cv. Up-to-Date) stored at 25°C and 65 – 75% RH until planting in field plots under irrigation.

Fungicides 2000 mg a.i./L	Seed tubers cut and treated 14 days before planting					Overall mean ^d
	Autumn plantings (1990 – 1992)		Spring plantings (1990 – 1992)		Mean ^c	
	Mean ^a	Overall mean ^b	Mean ^a	Overall mean ^b		
Benomyl	16.1	19.2	39.7	48.3	27.9	33.8
Carbendazim	19.4	20.9	54.3	56.4	36.9	38.7
Thiabendazole	17.2	19.0	42.4	47.9	29.8	33.5
Imazalil	11.4	13.9	22.3	31.9	16.9	22.9
Prochloraz	12.7	15.3	25.2	39.5	19.0	27.4
Captafol	16.3	16.5	30.1	41.6	23.2	29.1
Captab	16.2	17.2	37.0	47.8	26.6	32.5
Mancozeb	16.2	18.3	37.4	47.5	26.8	32.9
Seed pieces dipped in water for 5 min	15.2	15.1	44.5	50.9	29.9	33.0
Untreated whole seed tubers	27.7	28.3	60.5	60.9	44.1	44.6
Mean	16.8		39.4		28.1	
	Seed tubers cut and treated one day before planting					
Benomyl	22.2		56.9		39.6	
Carbendazim	22.4		58.5		40.5	
Thiabendazole	20.8		53.4		37.1	
Imazalil	16.4		41.5		29.0	
Prochloraz	17.9		53.8		35.9	
Captafol	16.6		53.0		34.8	
Captab	18.2		58.6		38.4	
Mancozeb	20.3		57.6		39.0	
Seed pieces dipped in water for 5 min	15.0		57.2		36.1	
Untreated whole seed tubers	28.8		61.2		45.0	
Mean	19.9		55.2		37.6	
LSD _(T) 5%						
Planting season					1.2	
Planting time (A)	1.3		3.3		4.8	
Seed treatment (B)	4.9		11.9		6.2	
Interaction (A x B)	6.9		16.9		7.4	

^aYield, kg for each 9 m row planted with 30 treated seed pieces during three consecutive seasons. One observation for each of three replicates for each season (n = 3). ^bOverall mean irrespective of the time the seed tubers were cut and fungicide-treated prior to planting (n = 6). ^cMean irrespective of the season (n = 6). ^dOverall mean irrespective of the season and time the seed tubers were cut and fungicide-treated prior to planting (n = 12).

After the first autumn planting the use of propiconazole and penconazole as seed-piece treatments was discontinued because almost no plants emerged from seed pieces treated with these fungicides, possibly due to phytotoxicity (data not shown). During the autumn plantings seed pieces treated with thiabendazole gave the best control of seed-piece decay, followed by the carbendazim, mancozeb, captab and benomyl treatments, while carbendazim followed by the untreated control seed pieces, thiabendazole, benomyl, captab and

mancozeb gave the best control of seed-piece decay during the spring plantings (Tables 6.8 and 6.9). During the autumn plantings the imazalil-, prochloraz-treated and untreated control seed pieces resulted in significantly lower ($p \leq 0.05$) plant stands and yields than the thiabendazole-treated seed pieces, while the imazalil-, prochloraz- and captafol-treated seed pieces resulted in significantly lower plant stand and yields than the carbendazim-treated seed pieces during the spring plantings (Tables 6.8 and 6.9).

When seed pieces, cut from physiologically old seed tubers, originating from seed lots with a high percentage *Fusarium* dry rot infected tubers, were planted during the 1991 autumn and spring plantings, plant stands and yields were greatly reduced. As *Fusarium* propagules found in soil adhering to tubers (Boyd, 1952; Folsom, 1959; Jeffries, 1978; Nielsen & Johnson, 1972; Small, 1944) have been shown to cause dry rot, post-harvest decay and seed-piece decay (McKee & Boyd, 1952; Small, 1945; Theron & Holz, 1991), Theron believed that the *Fusarium* propagules were randomly spread when cutting the seed and that the fungicide treatments were not sufficient to control the high inoculum present. Furthermore, the unusually high temperatures experienced the first 7 days after planting during the 1991 autumn planting, contributed to more seed piece decay, because seed pieces are more inclined to seed-piece decay when soil temperatures exceed 20°C (Escande & Echandi, 1988). Poor results were obtained with seed pieces planted during autumn when temperatures were high. The low yields experienced, irrespective of the use of fungicide-treated seed pieces or whole seed tubers, is common for autumn plantings in South Africa.

Cutting of seed tubers disrupt apical dominance and plants from pre-cut seed pieces emerges sooner than those from whole seed tubers or seed pieces planted the same day when cut (Chase *et al.*, 1988). However, Theron observed no significant difference ($p \geq 0.05$) in the time of plant emergence when already sprouted seed tubers were cut, and planted either directly or 14 days later. Although the planting of wound-healed potato seed pieces is recommended for the control of seed-piece decay (Lapwood *et al.*, 1984), Nolte *et al.* (1987) reported that both *Pectobacterium* spp. and *Fusarium* spp. can become established more quickly than wound healing can take place. Theron found that the planting of wound-healed seed pieces did not result in higher plant stands or yields except during the autumn plantings when higher plant stands were found. However, these differences did not differ significantly ($p \geq 0.05$) from those cut and treated one day prior to planting. The best results were achieved during the spring plantings using seed pieces cut and treated one day prior to planting. Soil temperatures during the spring plantings, especially early in the growing season, encouraged plants to emerge sooner than during the autumn plantings and suppressed the growth of seed-piece decay organisms. The optimal temperature for growth and dry rot development caused by *F. solani* and *F. oxysporum* the predominant species associated with this disease in South Africa, was reported to be $\geq 25^\circ\text{C}$ (Theron & Holz, 1991).

Carbendazim, thiabendazole, mancozeb, captab and benomyl seed-piece treatments resulted in the best plant stands and yields, confirming a report of De and Mahasin (1993) that good plant stands and yields were achieved when carbendazim- and mancozeb-treated seed pieces were planted although not always better than the untreated control seed pieces. Mancozeb was reported to be an effective seed-piece treatment especially when seed-piece decay is caused by *F. solani* (Hahm *et al.*, 1993). These treatments did not differ significantly ($p \geq 0.05$) from those achieved by planting whole seed tubers. The untreated control seed pieces resulted, second to the carbendazim-treated seed pieces, in the highest plant stands and yields during the spring plantings. However, the use of untreated seed pieces cannot be recommended, because untreated seed can serve as a source of inoculum (Leach, 1985). Data obtained in this study suggested that although plant stands and yields did not improve every year, treatment of seed pieces with fungicides can effectively improve the plant stands and yields.

The EC formulation of prochloraz was phytotoxic when applied to seed pieces. Denner *et al.* (1998) reported that treatment of uncut seed tubers with prochloraz, either as an EC or DP formulation did not significantly affect plant

stands or yields. Although the EC formulation of prochloraz seemed to be phytotoxic to seed pieces, and resulted in low plant stands and yields, it was found to be the best fungicide for the control of *Fusarium* dry rot, post-harvest decay, when freshly harvested tubers were artificially inoculated and dip-treated (Section 2.3.2). Theron stated that prochloraz could possibly be a good seed-piece treatment when using the DP formulation, as this formulation was shown not to be phytotoxic to uncut seed tubers (Denner *et al.*, 1998).

In this study, seed pieces were dip-treated, because it is relatively easy to use, the best coverage is accomplished (Hide, 1986) and it was the most common application method used by potato growers in South Africa. However, a dip-treatment of seed tubers prior to planting is not a highly recommended method of treating seed tubers (Hide, 1986; Secor & Gudmestad, 1993) because tubers can easily be contaminated with other tuber-borne pathogens, especially pathogens responsible for tuber rotting e.g. soft-rot bacteria. Spraying seed tubers, especially using ultra-low volume spraying equipment, or even dusting, are more recommended methods to applying fungicides (Logan, 1975; Hide, 1986; Nolte *et al.*, 1987).

In this study the use of uncut tubers resulted in significantly higher plant stands and yields, except in the case of the spring plantings when seed tubers, cut and treated one day prior to planting, were used. Theron stated that the use of seed pieces can reduce production costs, but care should be taken not to increase the risks of seed piece decay while trying to save on seed costs, by only using healthy, not physiologically old, seed tubers for cutting. It was also recommended that seed pieces should be treated with one of the above-mentioned fungicides to eliminate the spread of *Fusarium* propagules from the soil and should be planted the day after cutting in cool, moist soil. Theron advised potato growers to carefully consider the economic impact of the use of seed pieces in addition to the extra labour cost of cutting and fungicide-treatment of seed tubers as well as the risk of seed-piece decay, versus uncut seed tubers, before deciding to use cut seed tubers.

Chapter 7

LABORATORY ASSESSMENT OF POTATO TUBER RESISTANCE TO DRY ROT CAUSED BY *FUSARIUM SOLANI*

INTRODUCTION

As variation in susceptibility of potato cultivars to *Fusarium* dry rot is well-known (Moore, 1945; Boyd, 1952a; Boyd, 1952c; Boyd, 1972; Seppänen, 1981c), breeding for resistance is an important approach in the control of this disease (Leach & Webb, 1981). Although a number of inoculation and evaluation techniques have been used in breeding programmes (Boyd, 1952a; Boyd, 1952c; Ayers & Robinson, 1954; Jellis, 1975; Wiersema, 1977; Leach & Webb, 1981; Jellis & Starling, 1983) a lack of a reliable tuber inoculation technique was one of the major obstacles in screening potato clones for resistance to dry rot, especially in the early stages of the breeding programme when many selections have to be tested (Jellis, 1975; Leach & Webb, 1981). The objective of this study was to develop a reliable inoculation and evaluation technique to determine the resistance of potato tubers to *F. solani*.

MATERIALS AND METHODS

Sound, unblemished potato tubers of eight cultivars and nine breeding lines, produced under commercial conditions in field plots at VOPRI, were selected at harvest, disinfested (3% NaOCl, 15 min) and air-dried. The tubers were kept in crates for 3 months at 5°C to allow breakdown of resistance observed at harvest in most cultivars (Boyd 1952b; Boyd, 1952d; McKee, 1954). Two days before inoculation the tubers were removed from cold storage and kept at room temperature (25±2°C).

Inoculum of 1×10^4 CFU/ml (macro- and microconidia) was prepared by harvesting spores from a two-week-old culture of a single-spored, lyophilized isolate of *F. solani* (PREM 48287) obtained from a naturally-infected potato tuber, grown at 25°C on CLA (Fisher *et al.*, 1982). Twenty tubers (75 – 150 g) of each cultivar or breeding line were inoculated halfway between the rose and heel ends by injecting 0.2 ml of inoculum 8 mm into the tissue with a Socorex 2-187 self-refilling type syringe. The 12G, 38-mm needle was shortened to 10 mm, the tip sealed and an aperture of 0.5 mm diameter made 2 mm from the tip to prevent clogging by tuber tissue. Control tubers were treated with sterile distilled water. The tubers were placed in paper bags and kept at 25±2°C and 50-70% RH to promote dry rot development (Visser, 1975). After 3 weeks, tubers were cut in half at the inoculation site and the extent of dry rot development determined according to a modified disease index scale (Figure 1) used by Wiersema (1977).

RESULTS

The disease index of tubers of different cultivars and breeding lines inoculated with *F. solani* is given in Table 7.1.

Table 7.1. Disease index^a of potato tubers^b of different cultivars and breeding lines 3 wk. after inoculation^c with *Fusarium solani*.

Cultivar or breeding line	Harvest date				
	10/01/85	18/07/85	07/12/85	10/07/86	Mean
Elsa	3.40 s	3.60 s	4.60 s	4.00 s	3.90 S
73-75-74	3.00 st	2.80 t	4.25 st	2.80 t	3.21 T
Kimberley Choice	2.95 st	2.50 tu	3.95 t	2.65 tu	3.01 TU
74-75-49	2.65 tu	2.60 t	3.95 t	2.60 tuv	2.95 TU
74-45-228	2.70 tu	2.70 t	3.25 u	2.70 tu	2.84 TUV
Sackfiller	2.65 tu	2.50 tu	3.15 u	2.60 tuv	2.73 TUV
74-45-44	2.65 tu	2.50 tu	2.85 uv	2.60 tuv	2.65 TUV
73-75-9	2.55 tu	2.50 tu	2.75 uvw	2.50 tuv	2.58 TUV
74-45-238	2.60 tu	2.40 tuv	2.25 wx	2.45 tuvwx	2.43 UVW
138/65	2.40 tuv	2.35 tuv	2.40 vwx	2.40 tuvwx	2.39 UVW
Buffelspoort	2.45 tuv	2.00 uvwx	2.20 wx	2.10 uvwxy	2.19 VWX
73-75-71	2.35 tuv	1.80 wxy	2.40 vwx	1.85 xyz	2.10 VWXY
Up-to-Date	2.40 tuv	1.90 vwxy	2.10 xy	1.95 wxy	2.09 VWXY
Vanderplank	2.05 uvw	1.90 vwxy	1.30 z	2.00 vwy	1.81 WXYZ
BP1	1.75 vwx	1.50 xy	1.65 yz	1.55 yz	1.61 XYZ
75-97-9	1.60 wx	1.50 xy	1.55 z	1.60 yz	1.57 YZ
Late Harvest	1.30 x	1.40 y	1.35 z	1.35 z	1.37 Z

^aDisease index: 0 = no lesion development; 5 = tuber completely decayed. Values in columns followed by the same letter do not differ significantly according to Duncan's multiple range test ($P=0.05$).

^bBefore inoculation sound, unblemished, surface-disinfested (3% NaOCl, 15 min) tubers were kept in crates for 3 month at 5°C to allow natural breakdown of resistance observed at harvest.

^cInoculated tubers were kept at 25±2°C at 50-70% RH to promote dry rot development.

The high correlations (0.87 – 0.99) between resistance to dry rot of different potato cultivars and lines found during four successive seasons indicated the reliability of the inoculation technique used. This can be attributed to standardization of tuber size (75 – 150 g), inoculation site (halfway between rose and heel ends), inoculum (dose and spore density) and depth of inoculum placement (8 mm) because these variables can affect the extent of dry rot development (Boyd, 1952a). Lyophilisation preserved the high pathogenicity of the pathogen, while incubation on

CLA ensured high and uniform spore production (Fisher *et al.*, 1982; Nelson *et al.*, 1983). The simple screening technique is suitable to early stages of a breeding programme when large numbers of selections have to be tested.

Although BP1 and Vanderplank were previously reported to be resistant to this pathogen (Visser & Kotzé, 1979), the locally available cultivars (BP1, Vanderplank and Up-to-Date) and a breeding line (75-97-9) were moderately resistant to *F. solani*. Other *Fusarium* spp. also cause dry rot of potato tubers (Carpenter, 1915; Moore, 1945; Ayers, 1956; Boyd, 1972; Seppänen, 1981a). The survey of the *Fusarium* spp. associated with dry and stem-end rot of potato tubers (Section 1.1) indicated that some of these species might be of greater importance than *F. solani* in the main dryland production areas of South Africa. Therefore, these fusaria should be included in future screening tests.

CONCLUSIONS AND RECOMMENDATIONS

Breeding for resistance is an important approach in the control of *Fusarium* dry rot of potatoes. A reliable laboratory method for screening potato clones for resistance to *Fusarium solani* at an early stage in a breeding programme was developed during 1985 to 1986. The high correlations (0.87 – 0.99) between resistance to dry rot of different potato cultivars and lines found during four successive seasons demonstrated the reliability of the inoculation technique used. The simple screening technique is suitable to early stages of a breeding programme when large numbers of selections have to be tested. However, this study indicated that cultivars did not react uniformly to a given pathogen at different temperatures. Therefore, when evaluating cultivars for disease resistance or for effectiveness of disease control measures, test should be performed at standardized temperature. Different cultivars showed significant different reactions toward most fusaria at 25°C, therefore, evaluation should be done at this temperature. Since *F. oxysporum* and *F. solani* were the major *Fusarium* spp. associated with the disease complex in the two dryland production areas, *F. oxysporum* should be included in future screening tests.

Chapter 8

DEVELOPMENT OF AN *IN VITRO* SELECTION TECHNIQUE OF POTATO CULTIVARS FOR IMPROVED RESISTANCE TO *FUSARIUM OXYSPORUM*

BACKGROUND TO THE STUDY

The primary objective of the study conducted by Venter at ARC-Vegetable and Ornamental Plants (ARC-VOP) at Roodeplaat, in fulfilment of the requirements for the degree of Doctor of Philosophy Botany in the Faculty of Biological and Agricultural Sciences, Department of Botany of the University of the Pretoria, under the guidance of Prof. A. Eicker and dr D.I Ferreira in 1997, was to develop an optimal *in vitro* system where selective agents could be added to select potato cultivars with improved resistance to *Fusarium oxysporum*. This report summarises the literature review, experiments, results, discussions and recommendations. The techniques applied are described and significant results are presented.

INTRODUCTION

Venter explained that at the time of the study, despite the existence of substantial breeding programmes, the most widely grown potato varieties in South Africa were still the old, established ones. They had very good qualities, but lacked adaptation to some environmental factors and resistance to disease, resulting in a decline in yield, quality and market value. An alternative solution could have been to improve a popular variety rather than creating a new one. At that stage plant tissue culture had drawn attention from plant pathologists and breeders aiming for disease resistance, since it could meet the requirements for screening a large number of individuals in a small space (Chawla & Wenzel, 1987a). Callus and plant cell cultures had provided an appealing alternative for increased genetic variability, relatively fast and without sophisticated technology.

Meyer (1967) found that *F. oxysporum* represents 80 – 90% of the total mycobiota of the rhizosphere of several agricultural crops. *Fusarium oxysporum* causes dry rot, stem-end rot and wilt of potatoes (Nelson *et al.*, 1981). Due to the high frequency and economic importance of *Fusarium*, it was important to have potato cultivars and breeding lines with improved *Fusarium* resistance, which do not differ agronomically from their breeding parents. Conventional breeding strategies are time-consuming, but the process could be shortened by the development of a model, using cell biological techniques, by which improved resistance of potato cultivars and breeding lines to *F. oxysporum* f. sp. *tuberosi* could be obtained. Hence, an effective *in vitro* screening and selection technique had to be developed for South African potato cultivars. This was meant to be the first model of its kind in South Africa.

Phytotoxins are useful tools for the induction and selection of disease resistant plants by using tissue culture (Branchard, 1984; Daub, 1984; Wenzel, 1985) and was first used in resistance breeding by Wheeler & Luke in 1955. It is imperative to determine the correlation of resistance to a pathogen and resistance to its toxins for such use of phytotoxins (Kuo *et al.*, 1970; Byther & Steiner, 1972; Matern *et al.*, 1978). It was known that fungi of the genus *Fusarium* produce biologically active secondary metabolites showing phytotoxicity in bioassays on plants, but the level of knowledge on their role in pathogenesis and interaction of *Fusarium* with plants was limited (Hartman-Mitchell *et al.*, 1983; Scheffer, 1993). Fusaric acid (5-butylpyridine-2-carboxylic acid), a non-specific toxin, is produced by many *Fusarium oxysporum* forma speciales (Davies, 1969; Prasad & Chaudhary, 1974; Mutert *et al.*,

1981). Toyoda *et al.*, (1984) selected tomato calli resistant to fusaric acid and Wenzel *et al.*, (1984) have used fusaric acid as a selective agent on barley.

Many potato cultivars are susceptible hosts to the pathogen *F. oxysporum* which may produce substantial quantities of fusaric acid in still cultures or in infected plants. Venter explained that for the production of resistant plants utilizing cell culture one of two methods may be used namely: (i) the selection of cell lines resistant to toxins produced by the pathogen *in vitro* followed by regeneration of plants from resistant cell lines and (ii) screening regenerated plants from unselected cell cultures and identifying resistant somaclonal variants. "Somaclonal variation" is defined as the variation seen in plants obtained from any form of cell culture (Larken & Scowcroft, 1981). The first method was successful in producing potato plants resistant to *F. oxysporum* (Behnke, 1980a) and *Phytophthora infestans* (Behnke, 1979; 1980b) as well as alfalfa plants resistant to *F. oxysporum* f.sp. *medicaginis* (Hartman *et al.*, 1984a, b) and the second method, where potential somaclonal variants are screened, proved to be successful in identifying potato plants resistant to *Alternaria solani* (Matern *et al.*, 1978) and to *P. infestans* (Sheppard *et al.*, 1980).

In a study on tomatoes, Shahin & Spivey (1986) found that the degree to which *in vitro* selection can be utilized depend on whether the recovered undesirable heritable traits could restrict the breeding process. They found that many undesirable traits were recovered besides the targeted one (*Fusarium* wilt resistance). Therefore, unless there is a method to sort out those undesirable changes, the use of *in vitro* selection will be cumbersome to the plant breeder. Still, *in vitro* selection has its advantage as a potential source of novel breeding material for traits otherwise unobtainable by conventional breeding methods.

Although the first reports of disease resistant mutants produced by cell culture techniques had dated back to the 1970's, only a few of these plants had found practical value in crop improvement. The lack of reliable resistance tests at the whole plant level and frequent sterility or other agronomically undesirable traits in the regenerants could have been the most important reasons (Sacristan, 1986). The most important limitation in the use of cell culture systems for induction and selection of disease resistant mutants was the difficulty in finding indisputable indicators for plant resistance at cellular level. The general technique used at that moment was based on the application of behavioural criteria, i.e. survival and growth under the influence of a selective agent.

Venter stated that *in vitro* approaches hold considerable potential for increasing the genetic diversity available to the breeder and hence would be of use in disease resistance breeding. *In vitro* selection for improved resistance of potato cultivars to *F. oxysporum* causing dry-rot implied the induction of variation in tissue cultures of the crop (i.e. callus cultures, cell suspension cultures, protoplast cultures) followed by selecting cells with desired traits at cellular level using a selective agent, preferably a fungal toxin. This method had been applied successfully for the first time by Gengenbach & Green (1975) for the selection of *Helminthosporium* resistant corn plants. Since then, that approach had been used for the selection of *Fusarium* resistance in different crops (Behnke, 1980a, b; Hartman *et al.*, 1984a; Arcioni *et al.*, 1987), but only a few performed genetic analysis of the acquired resistant material. Shahin & Spivey (1986) performed genetic analysis on selected monogenic *Fusarium* resistance in protoplast cultures of tomato using fusaric acid as selective agent. Venter mentioned that close examination of their results raised the question whether cells selected for insensitivity for fusaric acid produced more *Fusarium* resistant plants than the non-selected controls. Therefore, a further examination of the potential of *in vitro* selection for *Fusarium* resistance seemed necessary.

According to Venter three factors determine the success of *in vitro* selection. Firstly, the tissue culture techniques must be sufficiently developed. Secondly, the variation in the tissue cultures must be broad enough to contain cell with the desired characteristic. Thirdly, the selective agent applied must play an important role in the pathogenesis. Although success with *in vitro* selection had been obtained in a few crops, an effective model for screening and

selection against *Fusarium oxysporum* forma speciales for any specific crop had not yet been developed. To develop an effective model, the three factors discussed above should have been in place for every crop.

The objective of this study was therefore to develop an effective *in vitro* selection technique to select potato somaclones or breeding lines with improved resistance to *Fusarium oxysporum*. The work was done in four phases:

1. The role which the toxin played in disease development (dry rot) in the potato cultivars was determined (Part 1).
2. The toxicity of *F. oxysporum* culture filtrate and fusaric acid on cell and callus cultures of different resistant and susceptible genotype was described to determine the relationship between susceptibility of potatoes to *F. oxysporum*, fusaric acid and culture filtrates of *F. oxysporum* (Part 2).
3. The regenerated potato plants was tested for improved resistance to *F. oxysporum*. This programme was dependant on linkage to the conventional potato breeding programme. This linkage would ensure that the tissue culture material developed had the agronomic performance that is required (Part 3)

The potato cultivars used during the study were recommended by the researchers of the Fusarium-resistance breeding programme at ARC-VOP (Roodeplaat). The potato cultivars Late Harvest, BP1, Vanderplank, Kimberley Choice and Up-to-Date were used in this study. Late Harvest was included since it was the cultivar with the best existing resistance against Fusarium dry rot in South Africa (Steyn *et al.*, 1991). BP1, Up-to-Date and Vanderplank were considered to be moderately resistant and Kimberley Choice was the most susceptible cultivar. These cultivars were the more popular cultivars and were included as standard cultivars in the potato breeding programme in South Africa. Of all the commercial seed potato cultivars grown in South Africa, 46% was BP1, 19% Up-to-Date and 7% Vanderplank (Nortje, 1997).

PART 1. ROLE OF FUSARIC ACID IN FUSARIUM DRY ROT DEVELOPMENT

PRODUCTION OF FUSARIC ACID BY *FUSARIUM OXYSPORUM*

Introduction

Plant pathogens produce various toxic compounds to culture media and plant tissues (Drysdale, 1982) that cause morphological and biochemical changes in plant tissues and contribute to the pathogenicity or virulence of the organisms producing the toxin. *Fusarium* sp. produce several toxins including fusaric acid (Drysdale, 1982; Hardborne, 1983). This study was the first on the production of fusaric acid by *Fusarium oxysporum*, which causes dry rot of potato tubers.

Material and Methods

Culture filtrate

Erlenmeyer flasks containing 30 ml culture medium were inoculated with single agar plugs (1mm³) of three *Fusarium oxysporum* isolates, isolated from potato tubers with dry rot symptoms. Their virulence had been verified by Theron & Holz (1989). The flasks were incubated at 22±1°C on an orbital shaker in the dark (50 rpm) for the duration of the experiment. Mycelial growth was determined every 24 h for 30 days. The mycelium and culture medium from five

flasks were individually separated by a Buchner funnel, the mycelium weighed and the mean fresh mass (g) recorded.

Toxin preparation and extraction

Crude filtrates and a culture medium only control treatment, were acidified with 1N HCl and extracted with an equal volume of ethyl acetate. The organic phase was removed and vacuum-dried, the residues dissolved with 5 mL methanol and stored at -20°C. The fusaric acid peak (HPLC) of the samples was identified by co-elution with pure fusaric acid added to the sample. Extraction efficiency was determined by spiking half of the sample with 0.015 mg/mL pure fusaric acid (5-butyl picolinic acid) prior to acidification. Eight replicates were extracted.

Analysis of fusaric acid

Culture filtrates were collected every 24 h for 32 days and tested for the presence of fusaric acid using reversed phase High Performance Liquid Chromatography (HPLC) on a Bondclone 10 C18 column (10 µm, 300 x 3.9 mm). A 50 µL aliquot of culture filtrate, culture medium or pure fusaric acid was injected and eluted isocratically with eluting buffer (Julien, 1988). The absorption was recorded with a UV detector at 254 nm, using a Beckman System Gold Model injector, pump and UV detector Model 168. Standard curves were established with pure fusaric acid. The column was stored in 100% methanol and equilibrated with eluting buffer 1 h before use. Samples were injected as methanol solutions.

Results and discussion

Extraction efficiency

Fusaric acid eluted in a reproducible manner as a sharp peak with an elution time of 5.56 min and a temperature of 20±1°C. Pure fusaric acid co-injected with the sample increased the height of the peak, and the product eluted at this peak had the characteristic UV spectrum of fusaric acid. Extraction efficiency (% fusaric acid recovered) was determined from day 0 to 4 for all three isolates by spiking half of the sample with 0.015 mg/mL pure fusaric acid. Fusaric acid (85.46%) was recovered with a coefficient of variance of 8.1%.

Mycelial growth and fusaric acid production

Isolate F.o 1 showed the least mycelium growth and produced the most fusaric acid, while isolate F.o 6 showed the most mycelium growth and produced the least fusaric acid. Both the differences in growth and fusaric acid between isolates F.o 1 and F.o 6 were significant ($P < 0.001$). The growth curves for all three isolates showed a logarithmic growth phase from days 2 - 18, after which a short stationary phase followed. Mycelium growth decreased after 20 days and at day 20 the fresh weight reached 1.46 g for isolate F.o 6, 1.26 g for isolate F.o 4 and 1.14 g for isolate F.o 1. The fusaric acid production curves showed a lag phase from days 0 - 5 and an exponential growth phase from days 6-18. After day 18 a stationary phase was reached and the fusaric acid concentration remained constant from then until day 32, when the experiment ended. The concentration of fusaric acid differed significantly between the different isolates. On day 20 the difference was 0.042 mg/mL for isolate F.o 1, 0.040 mg/mL for F.o 4 and 0.038 mg/mL for isolate F.o 6. It is important to determine the concentration of fusaric acid after the stationary phase has been reached.

CORRELATION BETWEEN FUSARIC ACID PRODUCTION AND VIRULENCE OF ISOLATES OF *FUSARIUM OXYSPORUM* THAT CAUSES POTATO DRY ROT IN SOUTH AFRICA.

Introduction

Fusarium dry rot is mostly a post-harvest disease and can become a serious problem when infected potatoes are stored. Breeding for potato cultivars resistant to *Fusarium* species causing dry rot is an important control strategy world-wide. Genetic modification of plants for disease resistance requires that resistant individual plants must be distinguished from large populations of susceptible plants, by inoculating them with the pathogen (Yoder, 1983). Toxins produced by pathogens can be used for the identification of disease resistant plants *in vitro* or *in vivo* (Gracen *et al.*, 1971; Galston, 1974; Earle, 1978; Wenzel, 1985; Daub, 1986; Branchard, 1984; Brettel & Ingram, 1979), however, the toxins must play a role in disease development and if the toxin is pathologically important, the role in disease development should be defined.

Phytotoxins may either be a pathogenicity- or a virulence factor (Yoder, 1980; 1983; Toyoda *et al.*, 1991). A pathogenicity factor is needed by the pathogen to cause disease, whereas a virulence factor is not needed to initiate disease but, changes the degree of disease development (Yoder, 1980). Fusaric acid is produced by many *F. oxysporum* formae sp. which cause diseases of many important crops (Mégnégneau & Branchard, 1988). The objective of this study was to determine the role of fusaric acid in the development of potato dry rot caused by *F. oxysporum*.

Material and Methods

Virulence tests

Conidial suspensions of twelve isolates of *F. oxysporum* isolated from potato tubers with dry rot symptoms (D.J Theron), their virulence verified by Theron & Holz (1989), were prepared as described by Venter *et al.*, (1992). Twenty unblemished, disinfected potato tubers of cultivars BP1, Kimberley Choice (KC) and Late Harvest (LH) were inoculated approximately halfway between rose and heel ends by injecting 0.2 ml of the spore suspension into the tissue with a Socorex 2-187 self refilling type syringe. Tubers were incubated in paper bags and kept at 25±2°C and 50-70 % RH to promote dry rot development. After a 3 week incubation period, tubers were cut in half at the inoculation site and the degree of dry rot development was determined according to a disease index scale used by Theron & Holz (1989). A randomized block design with three replicates was used.

Production of fusaric acid in culture filtrates

Erlenmeyer flasks containing 30 ml culture medium were inoculated with single agar plugs (1mm³) from the same mycelium as used in the virulence tests. The flasks were incubated at 22±1°C in the dark on an orbital shaker (50 rpm) for 20 days after which fusaric acid was extracted.

Fusaric acid preparation and extraction

Crude filtrates and a culture medium control were acidified with 1N HCl and extracted with an equal volume of ethyl acetate. Extraction efficiency was determined by spiking half of the sample with 0.05 mg/ml pure fusaric acid (5-butyl picolinic acid) prior to acidification. The organic phase was removed and vacuum-dried, the residues dissolved in 5 ml methanol and stored at -20°C. Twelve replicates of each isolate were extracted.

Analysis of fusaric acid in the culture filtrates

The culture filtrate was analysed by High Performance Liquid Chromatography (HPLC). A 50 μl sample of culture filtrate was eluted isocratically from a reversed phase Bondclone 10 C18 column (10 μm , 300 x 3.9 mm) with 40% methanol and 60% of a aqueous solution of 0.62 mM Na_2EDTA and 2% H_3PO_4 (Julien, 1988) at a flow rate of 1 mL/min. The eluate was monitored at 254 nm, using a Beckman System Gold Model 166 UV detector. The flow rate was 1 mL/min. Standard curves were established with pure fusaric acid. The column was stored in 100% methanol and equilibrated with eluting buffer 1 h before use. Samples were injected as methanol solutions.

Results and Discussion

Virulence of isolates

The isolates differed significantly in virulence and the cultivars differed in their tolerance against *Fusarium* dry rot ($P < 0.001$) (Table 8.1). Late Harvest (0.43) was the most tolerant with Kimberley Choice (1.14) mildly susceptible and BP1 (1.55) the most susceptible cultivar ($P < 0.001$). Values in brackets are the mean dry rot index value of each cultivar obtained after inoculation with the *F. oxysporum* isolates and corresponded with results obtained by Steyn *et al.*, (1991). The ranking order of the *F. oxysporum* isolates with the different cultivars corresponded to a high degree with the mean value of the ranking order (Table 8.1) which supports the virulence ranking order granted to the isolates by D.J Theron.

Table 8.1 Average dry rot development in tubers of BP1, Kimberley Choice (KC) and Late Harvest (LH), inoculated with *F. oxysporum* isolates as well as concentration of fusaric acid production in culture filtrates of 12 different isolates in vitro after twenty days. Values indicated with the same symbol are not statistically different.

Isolates	Mean dry rot disease index ¹				Fusaric acid concentrate ² (mg/ml)
	BP1	LH	KC	Average	
H ₂ O	0.069 a	0.000 a	0.000 a	0.022 a	0.000 a
N6D10/2(3)	0.067 a	0.000 a	0.069 a	0.044 a	0.019 b
N7P5/2(5)	0.267 ab	0.034 a	0.000 a	0.090 a	0.029 e
WNNR 1174	0.481 ab	0.000 a	0.107 a	0.222 a	0.024 d
N6P7/2(3)	0.680 ab	0.033 a	0.103 a	0.273 a	0.019 b
N6P16/2(5)	0.759 b	0.067 ab	0.233 a	0.356 a	0.022 c
F. o 7	1.885 c	0.741 cde	1.423 b	1.352 b	0.033 g
F. o 1	2.536 d	0.138 abc	1.846 bc	1.508 bc	0.053 k
F. o 4	2.609 d	0.423 abcd	2.500 c	1.812 bcd	0.048 i
F. o 2	2.500 cd	0.828 de	2.115 bc	1.825 bcd	0.032 f
F. o 5	2.923 d	0.696 bcde	2.286 c	1.848 bcd	0.038 h
F. o 6	2.385 cd	1.217 e	2.000 bc	1.889 cd	0.033 g
F. o 8	2.864 d	1.333 e	2.577 c	2.261 d	0.051 j

¹Disease index: 0 = no lesion development; 5 = tuber completely decayed

²Fusaric acid concentration (mg/ml) twenty days after inoculation with *F. oxysporum* isolates.

Efficiency of fusaric acid extraction

Fusaric acid eluted in a reproducible manner as a sharp peak with an elution time of 5.6 min and a temperature of 20±1°C. Pure fusaric acid co-injected with the sample increased height of the peak and the product eluted at this peak had the characteristic UV spectrum of fusaric acid. Extraction efficiency (% fusaric acid recovered) was determined by spiking half of the sample with 0.05 mg/ml pure fusaric acid. Ninety-six percent fusaric acid was recovered with a coefficient of variance (CV) of 2.0%. All measurements were adjusted accordingly.

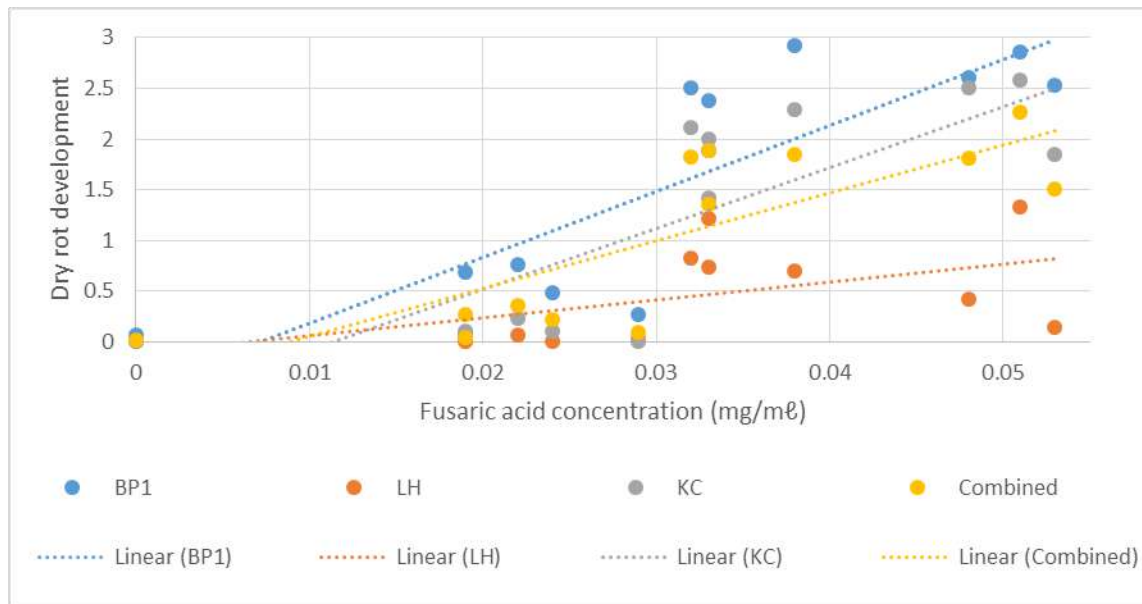


Figure 8.1 Correlation between fusaric acid production (20 days incubation, in vitro) of different *Fusarium oxysporum* isolates and dry rot development in potato tubers of three cultivars, namely BP1, Late Harvest (LH), Kimberley Choice (KC) and the combined cultivars.

Concentration of fusaric acid in culture filtrates of *F. oxysporum* isolates

Differences in fusaric acid production between most isolates was statistically significant ($P < 0.001$) (Table 8.1). The five non-virulent isolates had the lowest ranking order, which corresponded with the virulence obtained in the pathogenicity test.

To determine whether the fusaric acid concentration obtained in the culture filtrates of the *F. oxysporum* isolates influenced dry rot expression, the correlation between dry rot expression in the three different cultivars respectively and the mean dry rot expression of the three cultivars (Table 8.1), was determined. The linear regression (fusaric acid concentration against dry rot disease index value) of the mean for all three cultivars, or for the cultivars BP1 and Kimberley Choice fitted significantly ($P = 0.01$) with R^2 60.2, 69.0 and 66.0% respectively (Figure 8.1). The linear regression of Late Harvest could not be fitted significantly and the correlation was weak ($R^2 = 28.15\%$) due to the fact that Late Harvest is a tolerant cultivar and the respective *F. oxysporum* isolates had little effect on this cultivar.

PART 2. RELATIONSHIP BETWEEN FUSARIC ACID, CULTURE FILTRATES OF *F. OXYSPORUM* AND *F. OXYSPORUM* RESISTANCE

IN VITRO SELECTION FOR FUSARIC ACID RESISTANT POTATO PLANTS

Introduction

Plant tissue culture had attracted growing interest from plant pathologists and breeders aiming for disease resistance, since it could meet the requirements for screening a large number of individuals in a small space. *In vitro*

selection of *Fusarium* resistance was used in various crops (Behnke, 1980a; Hartman *et al.*, 1984a; Arcioni *et al.*, 1987; Chawla & Wenzel, 1987a,b), but in spite of the many efforts, incidental successes have been achieved (Löffler & Mouris, 1992). Resistant plants have been obtained in two ways: by selecting cell lines resistant to purified or crude toxin(s) produced by the pathogen followed by regeneration of plants (Hartman *et al.*, 1984a; Rines & Luke, 1985; Shahin & Spivey, 1986) or by screening regenerated plants obtained from unselected cell cultures with toxin(s) to identify resistant somaclonal variants (Shepard *et al.*, 1980).

One of the requirements for *in vitro* selection is a suitable selective agent, preferably a toxin produced by the pathogen (Löffler & Mouris, 1992). Phytotoxins are useful tools for the induction and selection of disease-resistant plants by using tissue culture (Branchard, 1984; Daub, 1984; Wenzel, 1985), since some phytotoxins are well characterized as single molecules that are easier to use in a selection programme opposed to fungal or bacterial pathogens (Mégnégneau & Branchard, 1988). Although the phytotoxin fusaric acid was studied widely for its potential as selective agent for *in vitro* selection (Julien, 1988; Matsui & Watanabe, 1988; Mégnégneau & Branchard, 1988), its role during pathogenesis was still not clear (Löffler & Mouris, 1992). Venter *et al.*, (1996) and Venter & Steyn (1997) found a correlation between fusaric acid production and virulence of isolates of *F. oxysporum* that causes potato dry rot in South Africa.

In this study Venter determined the toxicity of fusaric acid on callus cultures and leaf discs of five potato cultivars with different resistance levels to *F. oxysporum* in order to determine the relationship between susceptibility to *F. oxysporum* and to fusaric acid.

Material and Methods

Callus cultures of potato cultivars Late Harvest (LH), Vanderplank (VdP), BP1, Up-to-Date (UTD) and Kimberley Choice (KC) were established by culturing leaves produced from four week old *in vitro* plantlets, approximately 1 – 1.5 cm in length, with the apical and basal 3 mm removed, were placed with the abaxial side in contact with callus inducing medium. In addition stem segments produced from four week old *in vitro* plantlets, 10 mm in length, containing nodes but not leaves, were placed flat on nutrient media, consisting of modified MS salts and vitamins (Murashige & Skoog, 1962), 2 g/l casein hydrolysate, 30 g/l sucrose 2 mg/l 2,4-dichloro-phenoxyacetic acid (2,4-D) and 0.25 mg/l kinetin (KIN). In order to test the lethality of the toxin on callus, the callus were divided into pieces 0.5 cm in diameter and put into petri dishes (9 cm diameter) containing 0, 0.05, 0.1, 0.15, 0.25, 0.3, 0.35, 0.4 and 0.5 mM fusaric acid in callus-initiating media. The fusaric acid solution was filter-sterilized (0.45 µm Millipore filter) and added to luke warm autoclaved callus-initiating medium. For each fusaric acid concentration the pH was adjusted before the autoclaving (20 min at 121°C) to obtain a pH of 5.7 after fusaric acid addition.

Fresh weight of callus of each treatment was determined after 4 weeks and the relative fresh mass increase was determined according to the formula: $(X_n - X_0 / X_c - X_0) \times 100$ where X_n = fresh mass for concentration n, X_c = fresh mass of the control and X_0 = fresh mass of initial inoculum. A dose reaction curve was obtained to determine the lethal concentration of fusaric acid on callus growth and where 90% of the callus died.

Callus which survived the concentration where 90% calli died were selected and to test the resistance of the selected callus lines, callus were maintained on non-toxic media for 3 months (Chawla & Wenzel, 1987a, b) and then placed back on the same concentration of toxic medium and non-toxic medium, respectively. Increase in fresh weight was measured after 4 weeks as described before. After this step, callus was transferred to shoot induction medium (Lam, 1977) to regenerate plants.

A callus bioassay was used to determine the effect of fusaric on callus colour. Discolouration was scored using a modified index of Löffler & Mouris (1992) ranging from 0 (healthy, light yellow callus) to 3 (strong discolouration). Calli were incubated on callus-initiating medium containing 0, 0.05, 0.1, 0.15, 0.25, 0.3, 0.35, 0.4 and 0.5 mM fusaric acid in the dark at $24\pm 1^\circ\text{C}$. After 4 weeks (30 days) calli were evaluated for growth and colour.

Leaves of selected plants from each progeny were cut and placed on regeneration medium (MS medium supplemented with 20 g/l sucrose, 2 mg/l zeatin (added after autoclaving), 0.02 mg/l 1-naphtyl acetic acid (NAA), 0.02 mg/l gibberellic acid (GA_3), 1.5 mg/l STS and 7.5 mg/l agar with pH 5.8), containing 0.2 mM fusaric acid for testing the resistance of regenerated plants. The leaves with the apical and basal 1-3 mm removed were placed with the adaxial side facing upwards on plates containing the toxic regeneration medium. Leaf discs were subcultured onto fresh toxic medium once a week and when callus production could be visualised on the cut surfaces of the leaves (5-8 weeks, depending on the cultivar), the leaves were transferred to regeneration medium without auxin (but with toxin) to stimulate shoot production. Emerged shoots (1 cm in length) were rooted (modified MS salts and vitamins, containing 0.01 mg/l NAA, 0.1 mg/l GA_3 , 25 g/l sucrose and 7g/l agar with the pH adjusted to 5.5 in 250 ml plastic tubs), without toxin.

Another method for testing the resistance of regenerated plants was used. After the shoots had reached a certain size, leaflets were removed and tested for resistance to fusaric acid by immersing leaflets from sterile cultures into a liquid medium containing MS-medium with 0.2 mM fusaric acid.

Results and Discussion

It was well-known that fusaric acid is toxic to many plants and this study confirmed the toxicity of fusaric acid to potatoes. Fusaric acid decreased the regeneration of plantlets at concentrations of 0.01mM and higher. The non-differentiated callus tissue of potatoes was sensitive to fusaric acid. Callus pieces was killed at a concentration of 0.3 to 0.5 mM fusaric acid in the callus-inducing medium within 30 days. Both the callus and leaflet assays of potato can be used to demonstrate the toxic activity of fusaric acid to potatoes. From these assays it was evident that sensitivity is expressed in non-differentiated tissue, a very important criteria for *in vitro* selection. A distinctive effect of fusaric acid on callus is greyish discolouration rather than browning of the callus associated with the inhibition of polyphenol oxidases and peroxidases (Drysdale, 1982). Discolouration of calli was evaluated after 30 days. At the higher fusaric acid concentrations (0.25 and 0.3 mM fusaric acid), calli became bleached and greyish rather than brown.

Fusaric acid at concentrations of 0.05 and 0.01 mM had little effect on the relative growth of callus cultures, while addition of fusaric acid at a concentration of 0.15 mM gave growth rates of 41.2 to 48.6% for the different cultivars. Fusaric acid added at concentrations above 0.15 mM to the callus inducing medium significantly affected the growth of the callus ($P \leq 0.05$). There was very little relative growth at concentrations of 0.25 and 0.3 mM and at a concentration of 0.35 mM fusaric acid all cultivars except for Late Harvest, had no or even negative relative growth, possibly because Late Harvest is more tolerant to *F. oxysporum* than the other cultivars (Steyn *et al.*, 1991). At 0.4 and 0.5 mM fusaric acid all cultivars had negative relative growth. Relative growth of callus was inversely related to the toxin concentration. A concentration of 0.2 mM fusaric acid (where 90% of callus died) was selected to identify resistant callus. All calli of the cultivar Kimberley Choice have been inhibited at a concentration of 0.3 mM fusaric acid, possibly because Kimberley Choice is the most susceptible cultivar to potato dry rot (Steyn *et al.*, 1991).

Five thousand pieces of callus of each cultivar were transferred to fusaric acid selection medium (containing 0.2 mM fusaric acid) and resistant calli were transferred to fresh medium every 4 weeks to 4 months. After the first week of selection about 90% of the calli died, with 11.26% of Late Harvest calli and 6.58% of Kimberley Choice calli still viable (Table 8.2). When the resistant calli were transferred to fresh medium the survival rate was between 93.83%

and 86.01% for the different cultivars and kept increasing with successive transfers (Table 8.2). Therefore it could be assumed that these calli exhibited resistance to the toxicity of fusaric acid.

Table 8.2 Response of callus cultures of 5 potato cultivars to fusaric acid (0.2 mM). A = 5000 (no. of callus cultures initially inoculated). Values indicated with the same symbol are not statistically different.

Cultivar	First selection phase		Second selection phase		Third selection phase		Fourth selection phase	
	No. of calli (I)	% I/A x 100	No. of calli (II)	% II/I x 100	No. of calli (III)	% III/II x 100	No. of calli (IV)	% IV/III x 100
LH	563 a	11.26	518 a	92.01	513 a	99.03	509 a	99.22
KC	329 d	6.58	283 d	86.01	273 d	96.48	268 d	98.2
BP1	489 c	9.78	434 c	88.75	427 c	98.39	423 c	99.06
VdP	523 b	10.46	482 b	92.16	480 b	99.59	475 b	99.0
UTD	486 c	9.72	456 c	93.83	443 c	97.15	440 c	99.32
LSD _T (5%)	32		27		24		19	

Resistant calli obtained after selection on toxic medium were maintained on non-toxic callus initiation medium. Calli kept continuously on toxic medium became more friable, but regained compactness if kept on non-toxic medium in between (Chawla & Wenzel, 1987a). Calli were screened again on toxic medium after three months to ascertain resistance. Of all the calli tested 98.67% still exhibited resistance to toxicity of fusaric acid, although there was much less relative fresh weight increase on toxic medium (8.7 – 13.8%) than on the non-toxic medium (controls) (156 – 192%) for various cultivars. However, more growth occurred than in the unselected material which showed a decrease in relative fresh weight. When parts of actively growing calli were placed on shoot regeneration medium, resistant Late Harvest calli produced 137 green plants, Vanderplank 92, BP1 118, Kimberley Choice 63, and Up-to-Date 110. Selection on toxic media had a significant inhibitory effect on the regeneration potential of calli.

EFFECT OF FUNGAL CULTURE FILTRATE OF *FUSARIUM OXYSPORUM* ON CELL SUSPENSION AND CALLUS CULTURES OF POTATO IN SOUTH AFRICA

Introduction

At the stage of the study there was no potato cultivar in South Africa which was resistant to *F. oxysporum* causing dry rot. Plant tissue cultures could be used to either select disease resistant genotypes by *in vitro* selection or to evaluate disease resistance. Callus grown in the presence of a culture filtrate or toxins has been widely used for the selection of disease resistant lines (Mégignéau & Branchard, 1991). Although this method does not guarantee that plants regenerated from resistant calli will also be resistant to the pathogen, it has been effectively applied to several plant-pathogen systems (Behnke, 1979, 1980a; Sacristan, 1982; Thanutong *et al.*, 1983; Hartman *et al.*, 1984a;

Toyoda *et al.*, 1984; Arcioni *et al.*, 1987; Chawla & Wenzel, 1987a). Venter stated that a positive correlation between *in vitro* culture filtrate resistance and whole plant resistance is important for the success of the use of tissue culture for the evaluation of disease resistance or to obtain plants with improved resistance. A tissue culture method to differentiate between calli derived from cultivars which are resistant or susceptible to the pathogen, by adding fungal culture filtrate containing phytotoxic metabolites to a callus growth medium was developed by Gray *et al.*, (1986), Wilmot *et al.*, (1989) and Connel *et al.*, (1990), where the extent of inhibition of callus growth caused by culture filtrate treatment was related to the level of resistance of the cultivar. Since *Fusarium oxysporum* has a high capacity for synthesizing toxins *in vitro*, it's an appropriate pathogen to use in such a study.

Venter *et al.*, (1996) found that a correlation exists between fusaric acid production of *F. oxysporum* and the development of dry rot in potatoes in South Africa. The first *in vitro* selection was performed using callus from leaf discs, where fusaric acid, a non-host specific toxin, was used as selective agent. In this study Venter aimed to describe the effect of culture filtrates of *F. oxysporum* on cell suspension and callus culture of potato cultivars and thereby develop an *in vitro* evaluation method by which potato cultivars with improved resistance to *F. oxysporum* could be selected.

Material and Methods

Plant material

Virus-free potato material of five cultivars, Late Harvest (LH), BP1, Up-to-Date (UTD), Kimberley Choice (KC) and Vanderplank (VdP) was obtained from the genebank at the ARC-VOP.

Culture filtrate

A highly aggressive isolate of *F. oxysporum* isolated from potatoes with dry-rot symptoms (supplied by D.J Theron) and the virulence ascertained by Venter *et al.*, (1996), were cultured on potato dextrose agar (PDA) and maintained as described by Venter *et al.*, (1992). Two agar blocks (1mm³) of 10 day old cultures were placed in 100 ml of *Fusarium* culture medium and the liquid cultures were incubated (Venter & Steyn, 1998). Filtrates were collected by vacuum filtration (Whatman #41), filter sterilized with a 0.22 µm Millipore filter unit and stored at -20°C to -70°C until use. The pH of the crude filtrate was adjusted to pH 5.7 with 1N HCl. Fungal culture filtrate was added to luke-warm autoclaved medium. Preliminary tests of culture filtrate toxicity involved dilution series of 4, 6, 8 and 10% (v/v).

Cell suspension cultures

Well established cell suspension cultures were obtained by transferring 2 g (fresh mass) of friable callus to 100 ml Erlenmeyer flasks containing 15 ml liquid medium to initiate a cell suspension culture. The flasks were agitated continuously on a rotary shaker at 80 rpm under continuous dim light (10 µmol/m²/s) at 22±1°C. The medium was replaced every seven days to establish a fine, fast growing suspension culture. On subculturing during the first three weeks in culture, cells were allowed to settle to the bottom of the flask and the supernatant removed with a sterile pipette. Fresh medium was added and after the third week the suspension produced enough cells to be subcultured. With the first subculture into fresh medium large clumps were removed, but smaller clumps still occurred. As the cell suspensions developed, they were transferred to 250 ml Erlenmeyer flasks later to 500 ml Erlenmeyer flasks. The amount of media with cells was always 20% of the volume of the flask, while 25% of the suspension volume consisted of cells. Cell viabilities were determined by staining with FDA (Fluorescein diacetate) (Widholm, 1972; Larkin, 1976). The stained cells were inspected under a microscope with UV illumination to check for viability.

About 7 days before plating the cells, the *Fusarium* culture filtrates were added to the cell suspension cultures. The cell suspension cultures were plated on callus initiating medium containing the culture filtrates at the same concentrations as in the cell suspension medium. Fifty petri dishes (9 cm diameter) were plated per treatment. At the

same time cell suspension cultures which had not been inoculated with culture filtrate were also plated on the callus initiation medium containing different concentrations of culture filtrate. Microcalli that were formed were placed on shoot induction medium and the regenerated shoots were rooted and the regenerated plants were screened for resistance to the culture filtrate. Dose response curves of the cells to the culture filtrate of *F. oxysporum* were determined to find the concentration where 90% of the cells were killed. Relative fresh mass increase was determined according to the following equation: $(X_n - X_0) / (X_c - X_0) \times 100$ where X_n = fresh mass for concentration n , X_c = fresh mass of the control and X_0 = initial fresh mass.

Analysis of fusaric acid in culture filtrate

Culture filtrates from 30-day old cultures of *F. oxysporum* were tested for the presence of fusaric acid using Reversed Phase HPLC as described previously (Venter & Steyn, 1998). The effect of 6% culture filtrate on the callus cultures was determined over a period of time and correlated with increase of fusaric acid in culture filtrates determined by HPLC.

Results and Discussion

Eight percent of the culture filtrate was sufficient to kill all the original cells of all the cultivars except for Late Harvest which were killed at a 10% culture filtrate concentration (Figure 8.2). At a concentration of 8% culture filtrate 90% of the potato cells died. At all concentrations Late Harvest showed a significant better growth rate than that of Vanderplank, Up-to-Date and BP1, whereas Kimberley Choice had a significant poorer growth rate than all the other cultivars ($P < 0.001$). This results supported the ranking order of Late Harvest as the most tolerant cultivar to *F. oxysporum* causing dry rot, BP1, Up-to-Date and Vanderplank as moderately resistant and Kimberley Choice as the most susceptible cultivar granted by Steyn *et al.*, (1991).

Resistant plants were recovered by three routes, namely (i) without culture filtrate, (ii) with selection against *Fusarium* culture filtrate only during the callus phase and (iii) selection against *Fusarium* culture filtrate during cell suspension as well as callus phases. All the cultivars produced less micro-calli, shoots, plantlets and resistant plants on media where only the callus initiating medium contained the culture filtrate compared to where both the cell suspension culture and the callus initiating medium contained culture filtrate. Cell suspension and callus cultures with no culture filtrate produced far less resistant individuals than the other two treatments, although some resistant individuals were produced. Late Harvest produced more resistant individuals than any other cultivar and Kimberley Choice less than all the other, while little variation occurred between the other three cultivars. This supported the ranking order Steyn *et al.*, (1991) granted these cultivars.

In greenhouse tests 74% of the regenerated plants were phenotypically similar to the original cultivars, but it was still too early to draw conclusions about their usefulness. The other 26% plants varied slightly in some morphological characteristics, such as height, leaf shape, tuber shape and flower characteristics. A total of 436 Late Harvest, 219 Vanderplank, 259 BP1, 264 Up-to-Date and 132 Kimberley Choice individuals exhibiting resistance to *F. oxysporum* culture filtrate were obtained.

The concentration of fusaric acid in the culture filtrates determined by HPLC (Venter *et al.*, 1996; Venter & Steyn, 1998) equalled approximately 2.8 mM after 28 days. Time influenced the effect of the culture filtrate on the callus cultures (Figure 8.3) since the accumulation of fusaric acid in the medium corresponds with the increase of toxicity. Fusaric acid was found in the culture filtrates after 6 days and the concentration increased over time (Venter & Steyn, 1998).

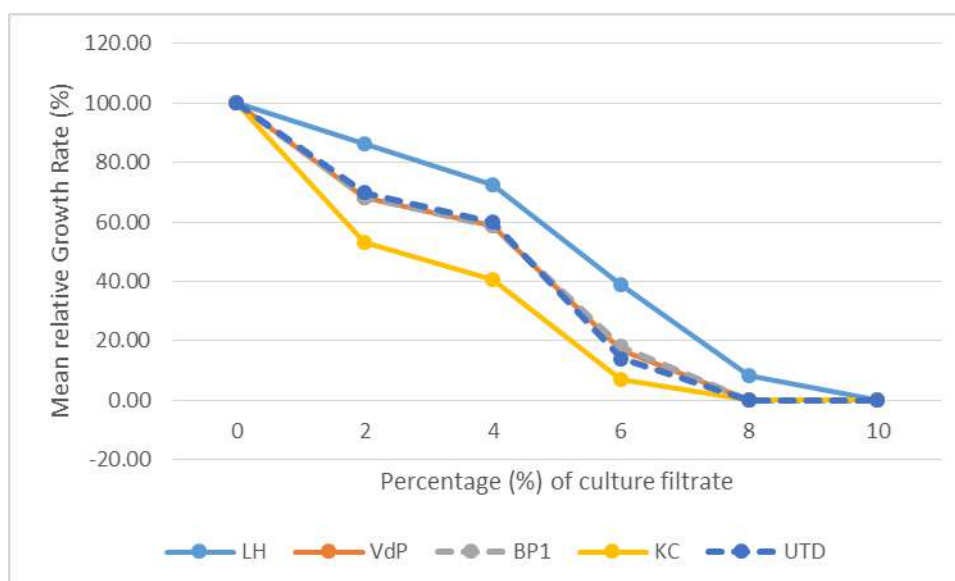


Figure 8.2. Dose response curves of cell suspension cultures of five potato cultivars to a culture filtrate of *Fusarium oxysporum*. ($P \leq 0.05$; $LSD_T = 11.76$)

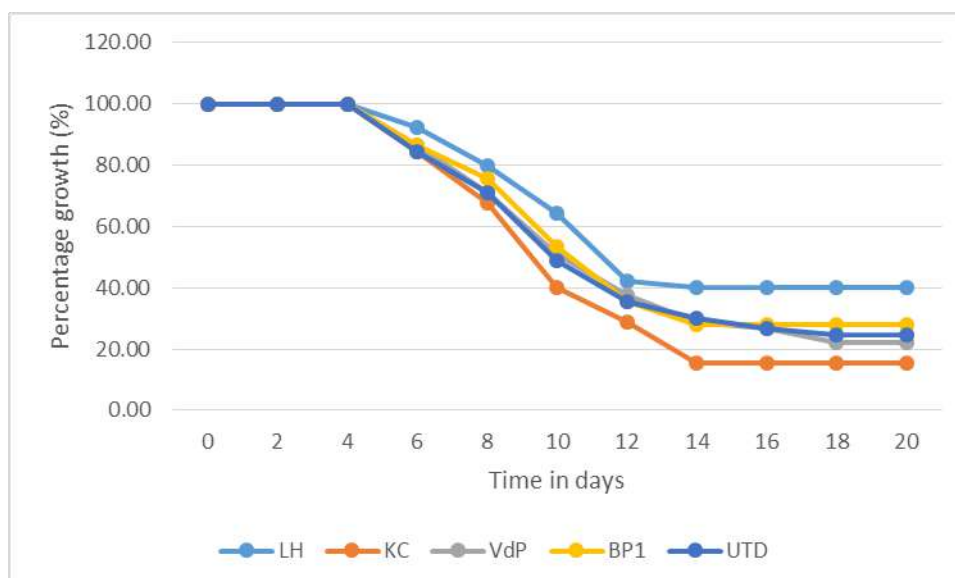


Figure 8.3 Influence of time on the effect of *Fusarium oxysporum* culture filtrate on callus cultures of five potato cultivars (% Growth = $x / 50 \times 100$ calli is alive), ($P \leq 0.05$; $LSD_T = 4.8$).

Based on the colour bioassay, toxic activity started at day 8 and at day 18 almost all the calli of all the cultivars were completely greyish, a characteristic symptom for the presence of fusaric acid.

More than one component could be responsible for the toxic activity of the culture filtrate, however, the increase of toxic activity in the culture filtrate corresponded with the production of fusaric acid in culture filtrates (Venter & Steyn, 1998; Venter *et al.*, 1996). Furthermore, the symptoms noticed after exposure of the callus to culture filtrate were typically caused by fusaric acid (Drysdale, 1982; Löffler & Mouris, 1992), indicating that symptoms were at least partially due to fusaric acid. This study as well as a previous study (Venter *et al.*, 1996) showed that fusaric acid is produced by *F. oxysporum* causing dry rot of potatoes. Fusaric acid is toxic to both differentiated and non-differentiated tissues of potato and plays an important role in potato dry rot development of various cultivars (Venter *et al.*, 1996). However, regenerated plants should be tested in field trials for resistance against the pathogen *F. oxysporum*. It could be that the approaches described in this study could have an impact on the production of novel

disease-resistant potato plants, particularly when conventional breeding methods have failed to answer a specific disease problem, but the degree to which it could be utilized would depend on whether the recovered undesirable heritable traits could hamper the breeding process. In this study undesirable traits were also recovered besides the targeted ones, indicating that unless there is a method to resolve those undesirable changes, the use of *in vitro* selection will be cumbersome to the plant breeder.

PART 2. A RAPID VIABILITY ASSAY FOR GROWTH RESPONSE OF POTATO CELL SUSPENSION AND CALLUS CULTURES TO FUSARIC ACID AND CULTURE FILTRATE OF *FUSARIUM OXYSPORUM*

Introduction

The exploitation of cell cultures for the selection of superior genotypes could be a valuable tool for the breeder, but it was still handicapped by technical problems (Wenzel, 1985). Selection for resistance using toxins required exact definition of the parameters the cell culture was subjected to. Furthermore, the factors used for selection had to be tested over a wide range of concentrations to determine the growth response of the cell populations. Cell sensitivity could be determined by several methods such as cell counting, fresh and dry mass, packed cell volume, mitotic index and viability staining, but some of these methods were laborious and cumbersome, since they required large quantities of cells, several repetitions and tedious microscopic examinations (Tepper *et al.*, 1991). The measurements were often very variable and may have required sophisticated and expensive equipment and in many cases the sampling and measuring were not frequent enough and may have resulted in incorrect conclusions (Gonzales & Widholm, 1985). Smith, (1951) reported that viable cells of a variety of organisms were able to reduce various tetrazolium salts. Tetrazolium salts are reduced to red formazan (Nachlas *et al.*, 1960, Towill & Mazur, 1975). Steponkus & Lanphear (1967) reported an assay in which viable cells reduced 2,3,5-triphenyltetrazolium chloride (TTC) to red formazan which was extracted with ethanol and its absorbance was measured (Steponkus & Lanphear, 1967), while Towill & Mazur (1975) reported further studies on the reduction of TTC as a viability assay for plant tissue cultures.

TTC reduction has been used as a viability assay for many years (Bennet & Loomis, 1949) and is a simple rapid means of assessing viability of a large number of samples. There is a correlation between the amount of formazan formed by a sample and the percentage viable cells within the sample as measured by cell division as criteria (Towill & Mazur, 1975). TTC reduction gives a reasonably accurate estimate of viability for leaf discs, stem sections and tissue cultures (Steponkus, 1971). Correspondence between regrowth and TTC reduction is not always absolute (Steponkus & Lanphear, 1967), therefore, initial studies using two or more methods of determining viability were advisable. The objective of this study was to evaluate a simple, rapid growth measuring method developed to determine the *in vitro* sensitivity of *F. oxysporum* and established a selection system for cell lines of potato resistant to the toxins produced by *F. oxysporum*.

Material and Methods

Culture filtrate

A highly aggressive isolate of *F. oxysporum* isolated from potatoes with dry-rot symptoms (supplied by D.J Theron) and the virulence ascertained by Venter *et al.*, (1996), were prepared as described in 3.4.1 and 3.4.2. Media contained 4, 5, 6, 7, 8, 10 and 12% (v/v) culture filtrates of *F. oxysporum* causing dry rot on potatoes.

Fusaric acid

Fusaric acid containing media were prepared as described in 3.4.1 at concentrations of 0.0, 0.05, 0.1, 0.15, 0.2, 0.25, 0.3, 0.35, 0.4, 0.45, 0.5, 1.0 and 1.5 mM fusaric acid.

Cell suspension cultures

Cell suspension cultures of the potato cultivars Late Harvest (LH), BP1, Up-to-Date (UTD), Kimberley Choice (KC) and Vanderplank (VdP) were prepared as described.

Cell viability assay

The TTC (0.2%) was dissolved in the reaction mixture, prepared by mixing (v/v) (2:1) 0.05 M sodium phosphate buffer pH 7.5 and liquid MS medium (Murashige & Skoog, 1962). Two ml cells were subcultured to sterile centrifuge tubes and cells were centrifuged at 200 x g (1 000 rpm) for 7 min. Packed cell volume was recorded and supernatant discarded. The TTC solution (5 ml) was added to the remaining cells and incubated at 26±1°C in the dark for 18 – 22 h. After incubation, the cells were pelleted by centrifugation at 1 000 rpm for 7 min and the supernatant discarded.

Cells were washed once with sterile distilled water, centrifuged again and the supernatant discarded. Red formazan was extracted from the pelleted cells with 5 ml of 96% ethanol for 30 min (of which 15 min at 60°C in the beginning). This brief heating aided in extracting the formazan from large clumps. The absorbance of the extract was read at 485 nm with a Beckman spectrophotometer.

Resistance of leaf tissue of regenerated plantlets to the culture filtrate and fusaric acid was tested by immersing leaf segments of plantlets *in vitro* in the liquid subculture medium with the culture filtrate or the toxin (0.2 mM fusaric acid).

Growth rate and viability of cell suspension cultures and calli on toxic medium were measured to determine toxicity of the culture filtrate and fusaric acid. Growth rate was calculated and the TTC assay as described above was used as a viability assay for the cell suspension and callus cultures exposed to the toxic media.

Results and Discussion

Cell suspension cultures and callus cultures were not affected on subculture media containing fungal culture filtrate up to 4% and fusaric acid up to 0.1 mM. However, cell suspension cultures became bleached and greyish on subculture media containing 8 – 12% fungal culture filtrate and 0.35 to 1.5 mM fusaric acid. Late Harvest cell suspension cultures were more tolerant to both the fusaric acid and culture filtrate containing media than any of the other cultivars, whereas Kimberley Choice was more susceptible ($P \leq 0.5$). These results correlated with results obtained in previous studies as well as with the ranking order Steyn *et al.*, (1991) granted to the potato cultivars.

A correlation existed between the three methods evaluated. A lag phase of ±two days and a fast growth rate between the second and fifth days for the various potato cultivars was noticed. At low fusaric acid (0.01 and 0.02 mM) and culture filtrate concentrations cells continued to grow until around the 20th day. Viability assays corresponded to the percentage growth and packed cell volume curves. A definite difference in absorbance (TTC assay) between lethal and non-lethal fusaric acid and culture filtrate concentrations existed, although 0.03 mM fusaric acid and 5% culture filtrate showed an intermediate response. The TTC method allow the continuous following of the response of cultured cells to fusaric acid and culture filtrates of *F. oxysporum*. The advantages of this approach are that small quantities of plant cells in suspension are sufficient for several replications and treatment, frequent measurements can be taken since the time required for each determination is short and simple, the same system can be used to measure the effect of various other factors on cell growth and development *in vitro* and the

type of growth and texture of aggregates do not influence results which is often a problem when using other methods e.g. the photometric system described by Tepper *et al.*, (1991).

All cell cultures of Kimberley Choice died on subculture medium containing 0.3 mM fusaric acid and 7% culture filtrate, whereas cell cultures of Vanderplank, Up-to-Date and BP1 died on subculture medium containing 0.4 mM fusaric acid and 10% culture filtrate. Cell cultures of Late Harvest died at 0.5 mM fusaric acid and 12% culture filtrate. Cell suspension cultures were plated on toxic media containing the same concentrations of fusaric acid and culture filtrate than that of the cell suspension media. All callus pieces of Kimberley Choice died on media containing 0.3 mM fusaric acid and 7% culture filtrate as was the case with the cell suspension cultures, but depending on the cultivar, 10 – 20% of the callus pieces of the other cultivars survived and grew well on this medium. Callus of Late Harvest was less affected by 0.4 mM fusaric acid and 8% culture filtrate than the other cultivars, but 40-60% of the callus pieces died on this medium. One mM fusaric acid killed pieces of all the cultivars. These results correlated with the ranking order granted to the cultivars by Steyn *et al.*, (1991) and the resistance of the potato cell suspension cultures and calli correlated with the resistance of the original plants to *Fusarium* dry rot caused by the fungus. Therefore, bioassays of potato calli and cell suspension cultures with culture filtrates and fusaric acid are good assay systems for evaluating resistance of potato plants to the disease, because the influence of any other pathogen is eliminated.

This system could therefore be used for *in vitro* selection of resistant cell lines of potatoes to *Fusarium* dry rot as reported for other plant species (Gengenbach & Green, 1975; Behnke, 1980a, b; Hartman *et al.*, 1984a; Arai & Takeuchi, 1993). The effect of culture filtrate and fusaric acid on leaves also agreed with pathogenicity of the pathogen to original plants, implying that culture filtrate of the pathogen could be used for the evaluation of *Fusarium* resistance. Fusaric acid, a non-specific toxin produced by *Fusarium*, has almost the same effect than that of the culture filtrate on the growth and viability of cells and calli – especially at low concentrations, while at concentrations higher than 0.45 mM the difference in response of the calli between the cultivars is not clear. In this study a system for evaluating the resistance of potato cultivars to *Fusarium* dry rot was established, allowing the production of new cultivars or more tolerant cultivars from a selected mutant cell.

PART 3. FIELD EVALUATION OF LATE HARVEST CLONES WITH IMPROVED *IN VITRO* RESISTANCE AGAINST FUSARIC ACID AND/OR CULTURE FILTRATE OF *FUSARIUM OXYSPORUM*

Introduction

Cultivars, clones or breeding lines with improved *Fusarium* resistance, which do not differ agronomically from their breeding parents, were needed. Conventional breeding strategies are time-consuming and the *Fusarium* resistance breeding programme was based on conventional breeding methods. Therefore, alternative methods for the development and induction of new characteristics in cultivars and clones should have been investigated. Plant tissue culture had drawn attention from plant pathologists and breeders aiming for disease resistance, since it could meet the requirements for screening a large number of individuals in a small space (Chawla & Wenzel, 1987). Hence, an effective *in vitro* screening and selection model needed to be developed for South African potato cultivars, and several studies were conducted with the objective to develop such a model. The objective of this study was field evaluation of clones of the potato cultivar Late Harvest with improved *in vitro* resistance to fusaric acid and *F. oxysporum* culture filtrates which was induced using *in vitro* screening and selection techniques described in 3.4. Field evaluation was done to determine whether clones developed from callus and/or cell lines with improved *in vitro* resistance have improved field resistance, but at the same time have agronomic characteristics similar to the

original Late Harvest cultivar. This would be to the advantage of the potato industry and similar methods could be developed for other diseases and resistance breeding programmes.

Material and Methods

Thirty-five clones of the potato cultivar Late Harvest were selected from all the selected clones developed from cell and/or callus vines with *in vitro* resistance against *F. oxysporum* culture filtrates and/or fusaric acid as described above. The selection of the 35 clones was based on tuber shape, absence of malformation and leaf growth viability.

The 35 lines and a control (Late Harvest) were multiplied in the field during 1993 and 1994 and were planted in a randomized block design with three replications in October 1995. Thirty tubers were planted in 9 m rows. The inter-row spacing was 1 m and intra-row spacing, 30 cm. All the tubers were of the same physiological age and the same source. Fertilizer applications were based on regular soil analysis, while weeds, diseases and insect pests were controlled by chemicals registered for use on potatoes. Irrigation was scheduled according to a class A evaporation pan.

After harvesting all the lines were evaluated for their agronomic characteristics. They were evaluated according to size (small, medium and large), malformation of the tubers, secondary growth, eye depth, growth crack, tuber shape and stolon indentation. Total marketable and unmarketable yields were also determined.

After the agronomic characteristics were evaluated 20 unblemished, medium (100 – 250 g) tubers were selected from each of the three replications and kept in brown paper bags at 4 - 5°C for 12 weeks. The laboratory assessment of potato tuber resistance to dry rot was conducted according to the method described by Theron & Holz (1987). After the tubers were kept at room temperature for two days, inoculations were done by injecting a 0.2 ml spore suspension (1×10^4 propagules/ml) into each tuber. Tubers were placed in paper bags and kept at 25°C and 50 - 70% RH for 21 days to promote dry rot development. After incubation the tubers were cut in half and the extent of dry rot development determined according to a dry rot disease index scale of Theron & Holz, (1987).

Analysis of variance for marketable and unmarketable yields were done according to Genstat 5 release 3.2 (1995). The mean values were tested for differences against the control cultivar, Late Harvest (clone 7) by means of Dunnett's multiple comparative test using SAS (SAS Institute INC. 1989). Index values were given according to specific agronomic characteristic of the potato, the higher the value, the stronger the characteristic. These values were used to determine whether the clones differed agronomically from the control Late Harvest by using the chi-square test using Genstat 5 release 3.2 (1995). Only clones which differed significantly from the control cultivar, Late Harvest (clone 7) were indicated, since only clones which performed better than the control would be acceptable for using in the potato breeding programme.

Results and Discussion

Clone 166 had a significantly higher marketable yield than the control cultivar Late Harvest and the other clones ($P < 0.005$). Except for clone 166 no statistical differences existed between the clones and the control cultivar for all the agronomical characteristics which indicated that the genotypes of the clones were not altered. Although no statistical differences existed for agronomical characteristics, it was important to determine whether the clones had improved resistance against *Fusarium oxysporum* since only clones which performed the same or better than the control cultivar would have been included in the potato breeding programme. Six of the 35 clones showed significantly better resistance against *F. oxysporum* than the control cultivar Late Harvest.

The clones with the highest average level of resistance against *F. oxysporum* were clone 45 with a disease index of 1.46, clones 86, 141 and 209 with an index of 1.39 and clones 166 and 228 with an index of 1.37, while the disease index for the Late Harvest control was 1.28. Steyn *et al.*, (1991) and Theron & Holz (1987) reported disease index values of 1.23 and 1.37 for Late Harvest, respectively. No significant differences existed between the other clones and the control Late Harvest. However, this evaluation must be repeated in the *Fusarium* resistance breeding programme to determine the stability of the resistance, before the clones could be included in the potato breeding programme.

Although six of the 35 selected clones had improved field resistance (14.2%), the success rate was relatively low, considering only 35 from all the lines or clones with *in vitro* resistance could be evaluated, as all the other clones were disqualified based on irregular tuber shape, malformation or poor leaf growth viability, prior to this evaluation. The cost and time implication of both conventional breeding methods and *in vitro* selection methods should be considered when deciding whether the success rate is acceptable. Not all cell lines stably resistant to *F. oxysporum* filtrate, regenerated plants with increased resistance to *Fusarium in vivo*, could explain the low success rate. Venter stated that not all cell lines showing stable resistance to *Fusarium* spp. filtrate, regenerated plants with increased resistance to *Fusarium in vivo*, because the same phenotype, i.e. resistance to toxic filtrates, may be due to different genetic or possibly epigenetic changes in different lines. Daub (1986) found that in selection for resistance against a crude pathogen filtrate, as many resistant cell lines as possible must be isolated, since many of them may have been selected for resistance to components other than the putative toxin, but if enough are generated, some may carry the desired resistance. Thus, the variation may be due to the strong selection pressure resulting in different types of mutation, acting at different stages of the reaction to a particular toxin or pathogen. This implies that genetic rather than epigenetic changes at the cellular level have taken place, that has to be checked by segregation experiments and the reaction of lines insensitive to the toxin has to be compared with their reaction to the pathogen (Chawla & Wenzel, 1987). Venter stated that the results of this study supported the concept of using cell culture technology to increase resistance to *Fusarium* species, but revealed the need to learn more about the biochemical and physiological bases of the interaction of pathogen versus plant *in vivo* and in cell culture *in vitro*.

CONCLUSION AND RECOMMENDATION

Fusaric acid is produced by *F. oxysporum* causing dry rot of potatoes. A correlation was found between virulence of isolates and fusaric acid production. Growth curves from specific isolates could be used to determine the concentration of fusaric acid in culture filtrate after specific lengths of time. The cultivars differed in their tolerance against *F. oxysporum* and the ranking order of cultivars corresponded with ranking orders previously reported. From the results it was evident that Fusaric production by *F. oxysporum* isolates seemed to play a major role in the development of dry rot in potato tubers of susceptible cultivars.

Both the callus and leaflet assays of potato confirmed the toxic activity of fusaric acid to potatoes. *In vitro* culture combined with somaclonal variation for the selection of fusaric acid resistant potato phenotypes has potential, since somaclonal variation can already be present in the explant or can be induced by the technique. Both these methods proved useful for the induction of resistance to fusaric acid in callus and cell suspension cultures. More resistant calli were obtained when the toxin were added to the medium. Fusaric acid can be used as a selective agent and results indicate that calli of the more tolerant potato genotypes could be selected between concentrations of 0.2 and 0.3 mM fusaric acid. To reach its full potential the selection system requires high regeneration capacity in the *in vitro* explant material.

Regenerated plants displaying *in vitro* resistance against the culture filtrate of *F. oxysporum* should be tested in field trials for resistance against the pathogen *F. oxysporum*. Screened lines with improved resistance in field trials, should become part of the potato breeding programme. Results of this study indicated that the application of *in vitro* selection for the improved resistance of potato cultivars to *F. oxysporum*, by adding only one characteristic without altering the remaining genotype should be researched further. The system developed could be a handy tool for breeding of potato cultivars resistant to *Fusarium* dry rot. Although *in vitro* improvement cannot replace conventional breeding, it can be valuable in supporting breeding programmes. The value and advantage of the developed *in vitro* technique is that a large number of breeding lines can be screened in a relatively short period of time and in a limited space, right through the year, which is not possible in conventional methods. However, this system is dependent on linkage to the conventional potato breeding programme to ensure that the tissue culture material developed has the agronomic performance that is required.

REFERENCES

- ABDEL-MONIEM, M.F., 1977. Fungi causing tuber rots of potatoes and their effect on stand and yield. *Agricultural Research Review* 55: 107 - 111.
- ABDEL-MONIEM, M.F., 1977. Fungi causing tuber rots of potatoes and their effect on stand and yield. *Agricultural Research Review* 55: 107 - 111.
- ALLAN, R.S. & MOOLMAN, G.F.J., 1987. Technical report: Mechanical damage to potatoes. Directorate: Agricultural Engineering and Water Supply, Department of Agriculture and Water Supply, Private Bag X515, Silverton, 0127, South Africa.
- ALTOMARE, C., LOGRIECO, A., BOTTALICO, A., MULÉ, G., MORETTI, A. & EVIDENTE, A., 1995. Production of type A trichothecenes and enniatin B by *Fusarium sambucinum* Fuckel sensu lato. *Mycopathologia* 129: 177 - 181.
- ARAI, M. & TAKEUCHI, M. 1993. Influence of Fusarium wilt toxin(s) on carnation cells. *Plant Cell Tissue and Organ Culture* 34: 287 – 293.
- ARCIONI, S., PEZZOTTI, M. & DAMIANI, F. 1987. *In vitro* selection of alfalfa plants resistant to *Fusarium oxysporum* f.sp. *medicaginis*. *Theoretical and Applied Genetics* 74: 700 – 705.
- AYERS, G.W., 1956. The resistance of potato varieties to storage decay caused by *Fusarium sambucinum* F6 and *Fusarium coeruleum*. *American Potato Journal* 33:249 - 254.
- AYERS, G.W., 1972. *Fusarium* decay in potatoes. *Canadian Agricultural* 17: 38 - 39.
- AYERS, G.W. & ROBINSON, D.B., 1954. An inoculation technique for the study of dry rot of potatoes. *American Potato Journal* 31:278 - 281.
- BAKER, R., 1971. Analyses involving inoculum density of soil-borne plant pathogens in epidemiology. *Phytopathology* 61: 1280 - 1292.
- BEHNKE, M. 1979. Selection of potato callus for resistance to culture filtrates of *Phytophthora infestans* and regeneration of resistant plants. *Theoretical and Applied Genetics* 55: 69 – 71.
- BEHNKE, M. 1980a. Selection of dihaploid potato callus for resistance to the culture filtrate of *Fusarium oxysporum*. *Pflanzenzücht* 85: 254 – 258.
- BEHNKE, M. 1980b. General resistance to late blight of *Solanum tuberosum* plants regenerated from callus resistant to culture filtrates of *Phytophthora infestans*. *Theoretical and Applied Genetics* 56: 151 – 152.
- BENNETT, N. & LOOMIS, W.E. 1949. Tetrazolium chloride as a test reagent for freezing injury of seed corn. *Plant Physiology* 24: 162 – 174.
- BOOTH, C., 1971. The Genus *Fusarium*. Commonwealth Mycological Institute, Kew, Surrey, England.
- BOUHOUT, K., 1979. Estimation of inoculum density and inoculum potential: Techniques and their value for disease prediction. Pages 21 - 34 in: B. Schippers and W. Gams, eds. Soil borne plant pathogens. Academic Press, New York.

- BOYD, A.E.W., 1952a. Dry-rot disease of the potato. IV. Laboratory methods used in assessing variations in tuber susceptibility. *Annals of Applied Biology* 39: 322 - 329.
- BOYD, A.E.W., 1952b. Dry-rot disease of the potato. V. Seasonal and local variations in tuber susceptibility. *Annals of Applied Biology* 39: 330 - 338.
- BOYD, A.E.W., 1952c. Dry-rot disease of the potato. VI. Varietal differences in tuber susceptibility obtained by injection and riddle-abrasion methods. *Annals of Applied Biology* 39: 339 - 350.
- BOYD, A.E.W., 1952d. Dry-rot disease of the potato. VII. The effect of storage temperature upon subsequent susceptibility of tubers. *Annals of Applied Biology* 39: 351 - 357.
- BOYD, A.E.W., 1960. Fungicidal dipping and other treatments of seed potatoes in Scotland. *European Potato Journal* 3: 137 - 154.
- BOYD, A.E.W., 1967. The effects of length of the growing period and of nutrition upon potato tuber susceptibility to dry rot (*Fusarium coeruleum*). *Annals of Applied Biology* 60: 231 - 240.
- BOYD, A.E.W., 1972. Potato storage diseases. *Review of Plant Pathology* 51: 297 - 321.
- BURGESS, L.W. & LIDDELL, G.M., 1983. Laboratory manual for *Fusarium* research. Department of Plant pathology and Entomology, University of Sydney, Sydney, Australia.
- BURGESS, L.W. & TRIMBOLI, D., 1986. Characterization and distribution of *Fusarium nygamai* sp. nov. *Mycologia* 78: 223 - 229.
- BURGESS, L.W., NELSON, P.E. & TOUSSOUN, T.A., 1982. Characterization, geographic distribution and ecology of *Fusarium crookwellense* sp. nov. *Transactions of the British Mycological Society* 79: 497 - 505.
- BURGESS, L.W., FORBES, G.A., WINDELS, C., NELSON, P.E., MARASAS, W.F.O. & GOTT, K.P., 1993. Characterization and distribution of *Fusarium acuminatum* subsp. *armeniicum* subsp. nov. *Mycologia* 85: 119 - 124.
- BYTHER, R.S. & STEINER, G.W. 1972. Use of helminthosporoside to select sugarcane seedlings resistant to eye spot disease. *Phytopathology* 62: 466 - 476.
- CARDI, T., IANNAMICO, V., AMBROSIO, F.D., FILLIPONE, E. & LURQUIN, P.F. 1993. *In vitro* regeneration and cytological characterization of shoots from leaf explants of three accessions of *Solanum commersonni*. *Plant Cell, Tissue and Organ Culture* 34: 107 - 114.
- CARNEGIE, S.F., RUTHVEN, A.D., LINDSAY, D.A. & HALL, T.D., 1990. Effects of fungicides applied to seed potato tubers at harvest or after grading on fungal storage diseases and plant development. *Annals of Applied Biology* 116: 61 - 72.
- CARPENTER, C.W., 1915. Some potato tuber-rots caused by species of *Fusarium*. *Journal of Agricultural Research* 5: 183 - 225.
- CASSELS, A.C., COLEMAN, M., FARREL, G., LONG, R., GOETH, E.M. & BOYTON, N. 1986. Screening for virus resistance in tissue culture adventitious regenerants and their progeny. In: Horn, W., Jensen, C.J., Odenbach, W. & Schieder, O. (Eds). *Genetic manipulation in plant breeding*. Walter de Gruyter Publ., Berlin. p535 - 545.
- CASSELS, A.C., FARREL, G. & COLEMAN, M.C. 1987. Somaclonal variation as a source of novel virus resistance in potato character improvement. Xth Triennial conference of the European Association of Potato Research. Aalborg, Denmark. p. 104

- CAYLEY, G.R., HIDE, G.A., LORD, K.A., AUSTIN, D.J. & DAVIES, A.R., 1979. Control of potato storage diseases with formulations of thiabendazole. *Potato Research* 22: 177 - 190.
- CHAMBERS, S.C., 1973. Studies on *Fusarium* species associated with "pathogen-tested" seed potatoes in Victoria. *Australian Journal of Experimental Agriculture and Animal Husbandry* 13: 718 - 723.
- CHAMBERS, S.C. & MILLINGTON, J.R., 1974. Studies on *Fusarium* species associated with a field planting "pathogen-tested" potatoes. *Australian Journal of Agricultural Research* 25: 293 - 297.
- CHASE, R.W., SILVA, G.H. & KITCHEN, R.B., 1988. Effects of pre-cutting and fungicide treatment of potato seed. *American Potato Journal* 65:473.
- CHAWLA, H.S. & WENZEL, G. 1987a. *In vitro* selection for fusaric acid resistant barley plants. *Plant Breeding* 99: 159 – 163.
- CHAWLA, H.S. & WENZEL, G. 1987b. *In vitro* selection of barley and wheat for resistance against *Helminthosporium sativum*. *Theoretical and Applied Genetics* 74: 841 – 845.
- CHELKOWSKI, J., 1989. Toxigenicity of *Fusarium* species causing dry-rot of potato tubers. Pages 435-440 in: JK. Chelkowski, ed. *Fusarium Mycotoxins, Taxonomy and Pathology*. Elsevier Publishing Co., New York.
- CHONA, B.L., 1932. The occurrence in England of a potato wilt disease due to *Fusarium oxysporum* Schlecht. *Transactions of the British Mycological Society* 17: 229 - 235.
- COLEMAN, M., WAUGH, R. & POWELL, W. 1990. Genetical analysis of *in vitro* cell and tissue culture response in potato. *Plant Cell Tissue and Organ Culture* 23: 181 – 186.
- CONNEL, S.A., LEGG, T. & HEALE, J.B. 1990. Sensitivity of cells and protoplasts of Hop cultivars to cytotoxic components of culture filtrates of *Verticillium albo-atrum* isolates from Hop. *Plant Pathology* 39: 92 - 101
- COPELAND, R.B. & LOGAN, C., 1975. Control of tuber diseases especially gangrene, with benomyl, thiabendazole and other fungicides. *Potato Research* 18: 179 - 188.
- DAUB, M.E. 1984. A cell culture approach for the development of disease resistance: studies on the phytotoxin cercosporin. *Horticultural Science* 19: 18 – 23.
- DAUB, M.E. 1986. Tissue culture and the selection to pathogens. *Annual review of Phytopathology* 24: 159 – 186.
- DAVIES, D. 1969. Fusaric acid in selective pathogenicity of *Fusarium oxysporum*. *Phytopathology* 59: 1391 – 1395.
- DAVIS, J.R., SORENSEN, L.H. & CORSINI, G.S., 1983. Interaction of *Erwinia* spp. and *Fusarium roseum* 'Sambucinum' on the Russet Burbank potato. *American Potato Journal* 60: 409 - 421.
- DE, B.K. & MAHASIN, M., 1993. Evaluation of fungicides to control seed piece decay of potatoes in the plains of West Bengal. *Environment and Ecology* 11: 324 - 326.
- DENNER, F.D.N., MILLARD, C.P., GELDENHUYS, A. & WEHNER, F.C., 1998. Treatment of seed potatoes with prochloraz for simultaneous control of silver scurf and black dot on progeny tubers. *Potato Research* 40: 221 – 227.
- DESJARDINS, A.E., 1989. Rotting potatoes harbour harmful toxins. *Science News* 135: 238.
- DESJARDINS, A.E. & BEREMAND, M., 1987. A genetic system for trichothecene toxin production in *Gibberella pulicaris* (*Fusarium sambucinum*). *Phytopathology* 77: 678 - 683.

- DESJARDINS, A.E., CHRIST-HARNED, E.A., McCORMIC, S.P. & SECOR, G.A., 1993. Population structure and genetic analysis of field resistance to thiabendazole in *Gibberella pulicaris* from potato tubers. *Phytopathology* 83: 164 - 170.
- DESJARDINS, A.E. & PLATTNER, R.D., 1989. Trichothecene toxin production by strains of *Gibberella pulicaris* (*Fusarium sambucinum*) in liquid culture and in potato tubers. *Journal of Agricultural and Food Chemistry* 37: 388 - 392.
- DIPPENAAR, B.J., 1934. *Fusarium*-rots in potatoes. *Farming in South Africa* 9:58.
- DIXON, R.A. 1985. Isolation and maintenance of callus and cell suspension cultures. In: Dixon, A.R. (Ed.). *Plant Cell Culture: A Practical Approach*. 1 – 20.
- DODDS, J.A. & ROBERTS, L.W. 1985. Experiments in Plant Tissue Culture. Second Edition. Cambridge University Press. Cambridge. P 1 – 81, 94 – 103.
- DRYSDALE, R.B. 1982. The production and significance in phytopathology of toxins produced by species of *Fusarium*. In: Moss, M.O. & Smith, J.E. (Eds). *The applied mycology of Fusarium* pp. 95 – 105.
- DU PLOOY, F.I & VAN DER PLANK, J.E., 1972. Potato developing programme since 1964. Transvaal Region and Institute for Plant Protection.
- DUNCAN, D.R., WILLIAMS, M.E., ZEHR, B.E. & WILHOLM, J.M. 1985. The production of callus capable of plant regeneration from immature embryos of numerous *Zea mays* genotypes. *Planta* 165: 322 – 332.
- EDDINS, A.H., 1940. Potato seed-piece rot caused by *Fusarium oxysporum*. *Phytopathology* 30: 181 - 183.
- EARLE, E.D. 1978. Phytotoxin studies with plant cells and protoplasts. In: Thorpe, T.A. (Ed.) *Frontiers of Plant tissue cultures, Proceedings of the 4th International Congress. Plant tissue and cell culture*. Calgary. 363 – 372.
- EL-BANNA, A.A., SCOTT, P.M., LAU, P-Y., SAKUMA, T., PLATT, H. & CAMPBELL, V., 1984. Formation of trichothecenes by *Fusarium solani* var. *coeruleum* and *Fusarium sambucinum* in potatoes. *Applied and Environmental Microbiology* 47: 1169 - 1171.
- ESCANDE, A.R. & ECHANDI, E., 1988. Wound-healing and the effect of soil temperature, cultivars and protective chemicals on wound-healed potato seed pieces inoculated with seed piece decay fungi and bacteria. *American Potato Journal* 65: 741 - 752.
- ESTRADA, R., TOUVAR, P. & DODDS, J.H. 1986. Induction of *in vitro* tubers in a broad range of potato genotypes. *Plant Cell, Tissue and Organ Culture* 7: 3 – 10.
- EWING, E.E. 1985. Cuttings as a simplified model of the potato plant. In: Li, P.H. (Ed.) *Potato Physiology*. Academic Press, New York. 153 – 207.
- EWING, E.E. 1990. Induction of tuberization in potato. In: Vayda, M.E. & Park, W.D. (Eds). *The Molecular and Cellular Biology of the Potato*. C.A.B. International, Wallingford. 25 – 41.
- FISH, N. & JONES, M.G.K. 1988. A comparison of tissue culture response between related tetraploid and dihaploid *S. tuberosum* genotypes. *Plant Cell Tissue and Organ Culture* 15: 201 – 210.
- FISHER, N.L., BURGESS, L.W., TOUSSOUN, T.A. & NELSON, P.E.M., 1982. Carnation leaves as a substrate for preserving cultures of *Fusarium* species. *Phytopathology* 72: 151 - 153.
- FOISTER, C.E., 1940. Dry rot disease of potatoes. *Scottish Journal of Agriculture* 23: 63 - 67.

- FOISTER, C.E., WILSON, A.R. & BOYD, A.E.W., 1945. Potato dry rot and gangrene as soil-borne diseases. *Nature* 155: 793 - 794.
- FOISTER, C.E., WILSON, A.R. & BOYD, A.E.W., 1952. Dry-rot disease of the potato. I. Effect of commercial handling methods on the incidence of the disease. *Annals of Applied Biology* 39: 29 - 37.
- FOLSOM, D., 1959. Potato tuber bruise rots in relation to crop rotation in Maine 1945-1956. *American Potato Journal* 36: 154 - 161.
- FORTI, E., MANDOLINO, G. & RANALLI, P. 1991. *In vitro* tuber induction: influence of the variety and of the media. *Acta Horticulturae* 300: 127 - 131.
- FORSLINE, P.L. & LANGILLE, A.R. 1976. An assessment of the modifying effect of kinetin on *in vitro* tuberization of induced and noninduced tissues of *Solanum tuberosum*. *Canadian Journal Botany* 54: 2513 - 2516.
- FORSUND, E., 1980. Tuber dry rot caused by *Fusarium merismoides* Cda. *Potato Research* 23: 478.
- FOULGER, D. & JONES, M.G.K. 1986. Improved efficiency of genotype-dependent regeneration from protoplasts of important potato cultivars. *Plant Cell Reports* 5: 72 - 76.
- GAVINLERTVATANA, P. & LI, P.I. 1980. The influence of 2,4-D and kinetin on leaf callus formation in different potato species. *Potato Research* 23: 115 - 120.
- GALSTON, A.W. 1974. Molding new plants. *Natural History* 83: 94 - 96.
- GENGENBACH, B.G. & GREEN, C.E. 1975. Selection of T-cytoplasm maize callus cultures resistant to *Helminthosporium maydis* race T pathotoxin. *Crop Science* 15: 645 - 649.
- GERLACH, W. & NIRENBERG, H., 1982. The genus *Fusarium* - A pictorial Atlas: Mitteilungen aus der Biologischen Bundesanstalt für Land- and Forstwirtschaft. Berlin-Dahlem 209: 1 - 406.
- GILLIGAN, C.A., 1983. Modelling of soilborne pathogens. *Annual Review of Phytopathology* 21: 45 - 64.
- GINDRAT, D. & PILLOUD, R., 1985. La pourriture fongiques des tubercules de pomme de terre en atmosphère contrôlée. *Potato Research* 28: 153 - 160.
- GONZALES, R.A.A & WIDHOLM, J.M. 1985. In: Dixon, R.A. (Ed.). *Plant Cell Culture: A practical approach*. IRL Press, Oxford. 67 - 78.
- GOLIŃSKI, P., VESONDER, R.F., LATUS-ZIĘTKIEWICZ, D. & PERKOWSKI, J., 1988. Formation of fusarenome X, nivalenol, zearalenone, α -trans-zearalenol, β -trans-zearalenol and Fusarin C by *Fusarium crookwellense*. *Applied and Environmental Microbiology* 54: 2147 - 2148.
- GORDON, W.L., 1952. The occurrence of *Fusarium* species in Canada. II. Prevalence and taxonomy of *Fusarium* species in cereal seed. *Canadian Journal of Botany* 30: 209 - 251.
- GOSS, R.W., 1940. A dry rot of potato stems caused by *Fusarium solani*. *Phytopathology* 30: 160 - 165.
- GOSS, R.W., 1921. Temperature and humidity studies of potatoes of some Fusaria rots of the Irish potato. *Journal of Agricultural Research* 22: 65 - 83.

- GOSS, R.W., 1936. *Fusarium* wilts of potato, their differentiation, and the effect of environment upon their occurrence. *American Potato Journal* 13: 171 - 180.
- GRACEN, V.E., FORSTER, M.J., SAYRE, K.D. & GROGAN, C.O. 1971. Rapid method for selecting resistant plants for control of southern corn leaf blight. *Plant Disease Reporter* 55: 469 – 470.
- GRAY, L.E., GUAN, Y.Q. & WIDHOLM, J.M. 1986. Reaction of soybean callus to culture filtrates of *Phialophora gregata*. *Plant Science* 47: 45 – 55.
- HAHM, Y.I. PARK, C.S., AHN, J. & CHOI, K.S., 1993. Studies on the cause of seed-piece decay and the effect of seed-piece treatments on emergence and yield in potatoes “Superior”. *RDA Journal of Agricultural Science, Horticulture* 35: 530 - 533.
- HANSON, L. E., SCHWAGER, S.J. & LORIA, R., 1996. Sensitivity to thiabendazole in *Fusarium* species associated with dry rot of potatoes. *Phytopathology* 86: 378 - 384.
- HANZEL, J.J., MILLER, J.P., BRINKMAN, M.A. & FENDOZ, E. 1985. Genotype and media effects on callus formation and regeneration in barley. *Crop Science* 25: 27 – 31.
- HARDBORNE, J.B., 1983. Toxin of plant-fungal interactions. In: Keeler, R.F. & Tu, A.T. (Eds). *Plant and Fungal toxins*. Marcel Dekker, New York. p743 – 782.
- HARMEY, M.A. CROWLEY, M.P. & CLINCH, P.E.M. 1966. The effect of growth regulators on tuberization of cultured stem pieces of *Solanum tuberosum*. *European Potato Journal* 9: 146 – 151.
- HARTMAN, C.L., McCOY, T.J. & KNOUS, T.R. 1984a. Selection of alfalfa (*Medicago sativa*) cell lines and regeneration of plants resistant to the toxin(s) produced by *Fusarium oxysporum* f. sp. *medicaginis*. *Plant Science Letters* 34: 183 – 194.
- HARTMAN, C.L., McCOY, T.J. & KNOUS, T.R. 1984b. Field testing and preliminary progeny evaluation of alfalfa regenerated from cell lines resistant to the toxins produced by *Fusarium oxysporum* f. sp. *medicaginis*. *Phytopathology* 74: 818.
- HARTMAN-MITCHELL, C.L., KNOUS, T.R. & McCOY, T.J. 1983. Toxic components produced by *Fusarium oxysporum* f.sp. *medicaginis* and their use in alfalfa cell culture selection techniques. *Phytopathology* 73: 829.
- HASKELL, R.J., 1916. Potato wilt and tuber rot caused by *Fusarium eumartii*. *Phytopathology* 6: 321 - 327.
- HIDE, G.A., 1986a. *Phoma* and *Fusarium* rots of important “new” potatoes. *Plant Pathology* 35: 126 - 127.
- HIDE, G.A., 1986b. Some problems in the chemical control of potato tuber diseases. Crop protection of sugar beet and crop protection and quality of potatoes: part II. *Aspects of Applied Biology* 13: 263 - 272.
- HIDE, G.A. & CAYLEY, G.R., 1980. Test of fungicides for controlling gangrene (*Phoma exigua* var. *foveata*) and dry rot (*Fusarium solani* var. *coeruleum* and *F. sulphureum*) on potatoes during storage. *Potato Research* 23: 395 - 403.
- HIDE, G.A. & CAYLEY, G.R., 1985. Effects of delaying fungicide treatment of wounded potatoes on the incidence of *Fusarium* dry rot in store. *Annals of Applied Biology* 107: 429 - 438.
- HIDE, G.A., READ, P.J. & HALL, S.M., 1992. Resistance to thiabendazole in *Fusarium* species isolated from potato tubers affected by dry rot. *Plant Pathology* 41: 745 - 748.
- HORNOK, L., 1982. Dry rot of potato tubers caused by *Fusarium trichothecoides* Wollenweber, a new fungus newly recorded in Hungary. *Acta Phytopathologica Academiae Scientiarum Hungaricae* 17: 81 - 83.

- HULME, J.S., HIGGINS, E.S & SHIELDS, R. 1992. An efficient genotype-independent method for regeneration of potato plants from leaf tissue. *Plant Cell tissue and Organ Culture* 31: 161 – 167.
- HUSSEY, G., STACEY, N.J. 1984. Factors affecting the formation of *in vitro* tubers of potato (*Solanum tuberosum*). *Annals of Botany* 53: 565 – 578.
- JACOBSON, E. 1981. Polyploidisation in leaf callus tissue and in regenerated plants of dihaploid potato. *Plant Cell Tissue and Organ Culture* 1: 77 – 84.
- JACOBSON, E. 1987. Genetic diversity in protoplast- and cell-derived plants of potato. In: Bajaj, Y.P.S. (Ed.). *Biotechnology in Agriculture and Forestry* Vol 3: Potato 358 – 374.
- JAMES. W.C., 1974. Assessment of plant diseases and losses. *Annual Review of Phytopathology* 12: 27 - 48.
- JAMIESON, C.O. & WOLLENWEBER, H.W., 1912. Phytopathology: An external dry rot of potato tubers caused by *Fusarium trichothecioides* Wollenw. *Journal of the Washington Academy of Sciences* 2: 146 - 152.
- JEFFRIES, C.J., 1978. Transmission studies on the potato pathogens *Fusarium solani* var. *coeruleum* and *Fusarium sulphureum*. Ph.D Thesis, University of Edinburgh. Edinburgh, Scotland.
- JEFFRIES, C.J., BOYD, A.E.W. & PATTERSON, L.J., 1984. Evaluation of selective media for the isolation of *Fusarium solani* var. *coeruleum* and *Fusarium sulphureum* from soil and potato tuber tissue. *Annals of Applied Biology* 105: 471 - 481.
- JELLIS, G.J., 1975. Screening potato clones for resistance to dry rot (*Fusarium solani* var. *coeruleum*). *Annals of Applied Biology* 81: 417 - 418.
- JELLIS, G.J. & STARLING, N.C., 1983. Resistance to powdery dry rot (*Fusarium sulphureum*) in potato tubers. *Potato Research* 26: 295 - 301.
- JONES, A.L. & EHERET, G.R., 1976. Isolation and characterization of benomyl-tolerant strains of *Monilia fructicola*. *Plant Disease* 60: 765 - 769.
- JONES, J.P. & WOLTZ, S.S., 1981. *Fusarium*-incited diseases of tomato and potatoes and their control. Pages 157-168 in: P.E. Nelson, T.A. Toussoun & Cook, eds. *Fusarium: Diseases, Biology and Taxonomy*. The Pennsylvania State University Press, University Park, PA.
- JULIEN, M. 1988. Effects of the *Fusarium* sp. toxins and selections of crude toxin resistant strains in mesophyll cell cultures of *Asparagus officinalis*. *Plant Physiology & Biochemistry* 26: 713 – 721.
- KAWCHUK, L.M., HOLLEY, J.D., LYNCH, D.R. & CLEAR, R.M., 1994. Resistance to thiabendazole and thiophenate-methyl in Canadian isolates of *Fusarium sambucinum* and *Helminthosporium solani*. *American Potato Journal* 71: 185 - 192.
- KEIL, M., SANCHEZ-SERRANO, J.J. & WILLMITZER, L. 1989. Both wound-inducible and tuber specific expression are mediated by the promotor of a single member of the protease inhibitor II gene family. *EMBO Journal* 8: 1323 – 1330.
- KIM, J. & LEE, Y., 1994. Sambutoxin, a new mycotoxin produced by toxic *Fusarium* isolates obtained from rotted potato tubers. *Applied and Environmental Microbiology* 60: 4380 - 4386.
- KIM, J., LEE, Y. & YU, S., 1995. Sambutoxin-producing isolates of *Fusarium* species and occurrence of sambutoxin in rotten potato tubers. *Applied and Environmental Microbiology* 61: 3750 - 3751.

- KRAUSE, M., NEL, A. & VAN ZYL, K., 1996. A guide to the use of pesticides and fungicides in the Republic of South Africa. Department of Agriculture. Directorate of Livestock Improvement and Agriculture Production Resources, Technical Advice to the Registrar (Act No. 36/1947). Directorate of Agriculture Information, Private Bag X144, Pretoria, 0001, South Africa.
- KREMER, FR. W. & UNTERSTENHÖFER, G., 1967. Computation of results of crop protection experiments by the method of Townsend and Hueberger. *Pflanzenschutz-Nachrichten "Bayer"* 20: 625 - 628.
- KREMER, F.R.W. & UNTERSTENHÖFER, G., 1967. Computation of results of crop protection experiments by the method of Townsend and Hueberger. *Pflanzenschutz-Nachrichten "Bayer"* 20: 625 - 628.
- KRIEK, N.P.J., MARASAS, W.F.O., STEYN, M.P.S., VAN RENSBURG, S.J. & STEYN, M., 1977. Toxicity of a moniliformin-producing strain of *Fusarium moniliforme* var. *subglutinans* isolated from maize. *Food and Cosmetics Toxicology* 15: 579 - 587.
- KUBEK, D.J. & SHULER, M.L. 1978. On the generality of methods to obtain single-cell plant suspension cultures. *Canadian Journal of Botany* 56: 2521 – 2527.
- KUO, M.S., YODER, O.C. & SCHEFFER, R.P. 1970. Comparative specificity of the toxin of *Helminthosporium carbonum* and *Helminthosporium victoriae*. *Phytopathology* 60: 365 – 368.
- LAFONT, P., GIRARD, T., PAYEN, J., SARFATI, J. & GAILLARDIN, M., 1983. Contamination de pommes de terre de consommation par des fusariotrichothecenes. *Microbiologie Aliments Nutrition* 1: 147 - 152.
- LAM, S. 1975. Shoot formation in potato tuber discs in tissue cultures. *American Potato Journal* 52: 103 – 106.
- LAM, S. 1977. Regeneration of plantlets from single cells in potatoes. *American Potato Journal* 54: 575 – 580.
- LANGERFELD, E., 1977. *Gliocladeum roseum* Bainier als Ursache von Schäden an Pflanzkartoffelknollen. Mitteilungen aus der Biologischen Bundesanstalt für Land- und Forstwirtschaft. Berlin-Dahlem 184: 1 – 81.
- LANGERFELD, E., 1978. *Fusarium coeruleum* (Lib.) Sacc. Als Ursache von Lagerfäulen an Kartoffelknollen. Mitteilungen aus der Biologischen Bundesanstalt für Land- und Forstwirtschaft. Berlin-Dahlem 184: 1 - 81.
- LANGERFELD, E., 1986. Thiabendazole resistance in *Fusarium sulphureum*. *Nachrichtenblatt des Deutschen Pflanzenschutzdienstes (Braunschweig)* 38: 165 - 168.
- LANGERFELD, E., 1990. Thiabendazole resistance in *Fusarium coeruleum*. *Nachrichtenblatt des Deutschen Pflanzenschutzdienstes (Braunschweig)* 42: 79.
- LAPWOOD, D.H., READ, P.J. & SPOKES, J., 1984. Methods for assessing the susceptibility of potato tubers of different cultivars to rotting by *Erwinia carotovora* subspecies *atroseptica* and *carotovora*. *Plant Pathology* 33:13 - 20.
- LAPWOOD, D.H., BELL, F., HARRIS, R.I., HIDE, G.A. & ADAMS, M.J., 1979. Possibilities of forecasting potato storage diseases from tests on seed tuber and crop samples. *Plant Pathology* 28: 181 - 190.
- LARKIN, P.J. 1976. Purification and viability determinations of plant protoplasts. *Planta (Berl.)* 128: 213 – 216.
- LARKIN, O.J. & SCOWCROFT, W.R. 1981. Somaclonal variation – a novel source of variability from cell cultures for plant improvement. *Theoretical and Applied Genetics* 60: 197 – 214.

- LATUS-ZIĘTKIEWICZ, D., PERKOWSKI, J. & CHELOWSKI, J., 1988. *Fusarium* species as pathogens of potato tubers during storage and their ability to produce mycotoxins. Pages 99-104 in: *Mycotoxin Research*, Special Edition: European Seminar "Fusarium- Mycotoxins, Taxonomy and Pathogenicity". Warsaw, Poland, September 8 - 10.
- LATUS-ZIĘTKIEWICZ, D., PERKOWSKI, J., TANAKA, T., GAMAMOTO, S., KAWAMURA, O., SUGIURA, Y. & UENO, Y., 1990. Formation of trichothecenes and zearalone by *Fusarium* isolates from potato tubers. *Microbiologie Aliments Nutrition* 8: 143 - 147.
- LEACH, S.S., 1970. Evaluation of post-harvest-prestorage fungicidal treatment for the control of *Fusarium* tuber rot of potatoes. *Phytopathology* 60: 1299.
- LEACH, S.S., 1971. Postharvest treatment for the control of *Fusarium* dry rot development in potatoes. *Plant Disease Reporter* 55: 723 - 726.
- LEACH, S.S., 1975. Control of postharvest *Fusarium* tuber dry rot of white potatoes. U.S. Department of Agriculture. Agricultural Research Services – N.E. 55: 1 - 19.
- LEACH, S.S., 1978. Quality of stored potatoes improved by chemical treatment. *American Potato Journal* 55: 155 - 159.
- LEACH, S.S., 1985. Contamination of soil and transmission of seedborne potato dry rot fungi (*Fusarium* spp.) to progeny tubers. *American Potato Journal* 62: 129 - 136.
- LEACH, S.S. & NIELSEN, L.W., 1975. Elimination of fusarial contamination on seed potatoes. *American Potato Journal* 52: 211 - 218.
- LEACH, S.S. & WEBB, R.E., 1981. Resistance of selected potato cultivars and clones to *Fusarium* dry rot. *Phytopathology* 71: 623 - 629.
- LEATH, S. & CARROLL, R.B., 1985. Use of ridge regression to predict yield reduction by *Fusarium* sp. in selected soybean cultivars. *Canadian Journal of Plant Pathology* 7: 58 - 66.
- LECLERC, Y., DONNELLY, D.J. & SEABROOK, J.E.A. 1994. Microtuberization of layered shoots and nodal cuttings of potato: The influence of growth regulators and incubation periods. *Plant Cell, Tissue and Organ Culture* 37: 113 – 120.
- LOCKWOOD, J.L., 1985. Soilborne plant pathogens: Concepts and connections. *Phytopathology* 76: 20 - 27.
- LÖFFLER, H.J.M. & MORRIS, J.R. 1992. Fusaric acid: phytotoxicity and *in vitro* production by *Fusarium oxysporum* f.sp. *lilii*, the causal agent of basal rot in lilies. *Netherland Journal of Plant Pathology* 98: 107 – 115.
- LOGAN, C., 1975. Potato tuber disinfection by thiabendazole mist application. *Agriculture in Northern Ireland* 48:438 - 440.
- LOGRIECO, A., FRISULLO, S. & BOTTALICO, A., 1987. Specie di *Fusarium* associate a marciumi di Patate da "seme" in Italia meridionale e relativi saggi di patogenicit  e tossicit . *Informatare Fitopatologico* 37: 33 - 36.
- LOGRIECO, A., BOTTALICO, A. & SOLFRIZZO, M., 1988. Toxigenic *Fusarium* species isolated from rotted potato tubers. Pages 105-110 in: *Mycotoxin Research*, Special Edition: European Seminar "Fusarium- Mycotoxins, Taxonomy and Pathogenicity" Warsaw, Poland, September 8 - 10.
- MAAS, ERNA M.C. & KOTZ , J.M., 1985. *Fusarium equiseti* crown rot of wheat in South Africa. *Phytophylactica* 17: 169 - 170.
- MANICI, L.M. & CERATO, C., 1992. Studio su alcuni funghi agenti di marciume dei tuberi di patata. *Informatore Fitopatologico* 42: 41 - 46.

- MATERN, U., STROBEL, G. & SHEPARD, J. 1978. Reaction to phytotoxin in a potato population derived from mesophyll protoplasts. *Proceedings of the National Academy of Sciences United States of America* 75: 4939 – 4953.
- MATSUI, Y. & WATANABE, M. 1988. Quantitative analysis of fusaric acid in the culture filtrate and soybean plants inoculated with *Fusarium oxysporum* var. *redolens*. *Journal of Rakuno, Gakuen University* 13: 159 – 167.
- MAUGHAN, J.P., SHANMUGANATHAN, N. & HEPWORTH, G., 1991. Fungicide treatments for the control of storage rots of seed potatoes. *Australasian Plant Pathology* 20: 142 - 145.
- McKEE, R.K., 1952. Dry-rot disease of the potato II. Fungi causing dry rot of seed potatoes in Britain. *Annals of Applied Biology* 39: 38 - 43.
- McKEE, R.K., 1954. Dry rot disease of potato. VIII. A study of the pathogenicity of *Fusarium coeruleum* (Lib.) Sacc. and *Fusarium avenaceum* (Fr.) Sacc. *Annals of Applied Biology* 41: 417 - 434.
- McKEE, R.K. & BOYD, A.E.W., 1952. Dry-rot disease of the potato. III. A biological method of assessing soil infectivity. *Annals of Applied Biology* 39: 44 - 53
- McKEE, R.K. & BOYD, A.E.W., 1962. Dry-rot disease of the potato. IX. The effect of diphenyl vapour on dry rot infection of potato tubers. *Annals of Applied Biology* 50: 89 - 94.
- McKLEAN, J.G. & WALKER, J.C., 1941. A comparison of *Fusarium avenaceum*, *F. oxysporum* and *F. solani* var. *eumartii* in relation to potato wilt in Wisconsin. *Journal of Agricultural Research* 63: 495 - 525.
- McMULLEN, M.P. & STACK, R.W., 1983. Effects of isolation techniques and media on the differential isolation of *Fusarium* species. *Phytopathology* 73: 458 - 462.
- McMULLEN, M.P. & STACK, R.W., 1984. The effect of surface mining and reclamation on *Fusarium* populations of grassland soils. *Reclamation and Revegetation Research* 2: 253 - 266.
- MéGNÉGNEAU, B. & BRANCHARD, M. 1988. Toxicity of fusaric acid observed on callus cultures of various *Cucumis melo* genotypes. *Plant Physiology and Biochemistry* 26: 585 – 588.
- MEIJERS, C.P., 1986. What can we do this year on the storage diseases of seed potatoes? *Aardappelwereld* Juli: 13 - 15.
- MEREDITH, C. 1979. Shoot development in established callus cultures of cultivated tomato (*Lycopersicon esculentum* MILL.) *Zeitschrift für Pflanzenphysiologie* 95: 405 – 411.
- MEYER, I.A. 1967. Reserches sur les fusarioses. II. Ecologie et pathogenie du *Fusarium oxysporum*. *Annals Epiphyties* 18: 241 – 247.
- MISKA, J.P. & NELSON, G.A., 1975. Potato seed-piece decay: a bibliography, 1930-1975. *Canadian Plant Disease Survey* 55: 126 - 146.
- MITCHELL, J.E., 1979. The dynamics of the inoculum potential of populations of soilborne plant pathogens in the soil ecosystem. Pages 3 - 20 in: B. Schippers and W. Gams, eds. *Soil borne plant pathogens*. Academic Press, New York.
- MOORE, F.J., 1945. A comparison of *Fusarium avenaceum* and *Fusarium coeruleum* as causes of wastage in stored potato tubers. *Annals of Applied Biology* 32: 304 - 309.
- MOTTLEY, J. & KEEN, B. 1987. Indirect assessment of callus fresh weight by non-destructive methods. *Plant Cell Reports* 6: 389 – 392.

- M'RIBU, H.K. & VEILLEUX, R.E. 1990. Effect of genotype, explant, subculture interval and environmental conditions on regeneration of shoots from *in vitro* monoloids of a dihaploid potato species, *Solanum phureja* Juz. & Buk. *Plant Cell Tissue and Organ Culture* 23: 171 – 179.
- MULÉ, G., LOGRIECO, A., STEA, G. & BOTTALICO, A., 1997. Clustering of trichothecene-producing *Fusarium* strains determined from 28S ribosomal DNA sequences. *Applied and Environmental Microbiology* 63: 1843 - 1846.
- MURASHIGE, T. 1973. Nutrition of plant cells and organs *in vitro*. *In vitro* 9 (2): 81 - 85
- MURASHIGE, T. & SKOOG, F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiologia Plantarum* 15: 473 – 497.
- MURDOCK, A.W. & WOOD, R.K.S., 1972. Control of *Fusarium solani* rot of potato tubers with fungicides. *Annals of Applied Biology*. 72: 53 - 62.
- MUTERT, W.U., LUETFRING, H., BARU, W. & STRACK, D. 1981. Formation of fusaric acid by fungi of the genus *Fusarium*. *Zeitschrift für Naturforschung Section C* 36c: 338 - 339
- NACHLAS, M.M., MARGULIES, S.I. & SELIGMAN, A.M. 1960. Sites of electron transfer to tetrazolium salts in the succinoxidase system. *The Journal of Biological Chemistry* 235: 2739 – 2743.
- NAUDÉ, S.P. & JOOSTE, W.J., 1989. Five fungal pathogens of storage onions. *Phytophylactica* 21: 110.
- NELSON, P.E., TOUSSOUN, T.A. & COOK, R.J. 1981. *Fusarium: Diseases, Biology and Taxonomy*. Pennsylvania State University Press. University Park, Pennsylvania.
- NELSON, P.E. DESJARDINS, A.E. & PLATTNER, R.D., 1993. Fumonisin, mycotoxins produced by *Fusarium* species: Biology, chemistry and significance. *Annual Review of Phytopathology* 31: 233 - 252.
- NELSON, P.E., TOUSSOUN, T.A. & MARASAS, W.F.O., 1983. *Fusarium* species. An illustrated manual for identification. The Pennsylvania State University Press. University Park. London.
- NIELSEN, L.W., 1981. *Fusarium* dry rots. Pages 58-60 in: Hooker, ed. Compendium of Potato Diseases, APS Press, St Paul, Minnesota.
- NIELSEN, L.W. & JOHNSON, J.T., 1972. Seed potato contamination with fusarial propagules and their removal by washing. *American Potato Journal* 49: 391 - 396.
- NIRENBERG, H.I., 1995a. The European *Fusarium sambucinum* Project. *Mycopathologia* 129: 127.
- NIRENBERG, H.I., 1995b. Morphological differentiation of *Fusarium sambucinum* Fuckel sensu stricto, *F. torulosum* (Berk. & Curt.) Nirenberg comb. Nov. and *F. venenatum* Nirenberg sp. nov. *Mycopathologia* 129: 131 - 141.
- NOLTE, P., SECOR, G.A. & GUDMESTAD, N.C., 1987. Wound healing, decay and chemical treatment of cut potato tuber tissue. *American Potato Journal* 64: 1 - 9.
- NORTJE, P.F. 1997. Potato Seed Production in South Africa. Proceedings of the African Potato Association Congress. Pretoria. South Africa.
- OCHATT, S.J. & CASO, O.H. 1986. Differential requirements among tissue source in *Solanum tuberosum* L. spp. *andigena* callus cultures. *Turrialba* 36: 363 – 368.
- PALMER, C.E. & SMITH, O.E. 1969. Cytokinins and tuber initiation in the potato *Solanum tuberosum* L. *Nature* 221: 279 – 280.

- PALMER, C.E. & SMITH, O.E. 1970. Effect of kinetin on tuber formation on isolated stolons of *Solanum tuberosum* L. cultured *in vitro*. *Plant Cell Physiology* 11: 303 – 314.
- PERL, A., AVIV, D. & GALUN, E. 1988. Ethylene and *in vitro* culture of potato: Suppression of ethylene generation vastly improves protoplast yield, plating efficiency and transient expression of an alien gene. *Plant Cell Reports* 7: 403 – 406.
- PETHYBRIDGE, G.A & BOWERS, E.H., 1908. Dry rot of the potato tuber. *Economical Proceedings of the Dublin Society* 1: 547 - 588.
- PETHYBRIDGE, G.A & LAFFERTY, H.A., 1917. Further observations on the cause of the common dry-rot of the potato tuber in the British Isles. *Scientific Proceedings of the Royal Dublin Society Series* 15: 193 - 224.
- POWELSON, M.L., JOHNSON, K.B. & ROWE, R.C., 1993. Management of diseases caused by soil-borne pathogens. Pages 149 - 158 in: R.C. Rowe, ed. *Potato Health Management*. APS Press, St Paul, MN.
- PRASAD, M. & CHAUDHARY, S.K. 1974. Amino acids and fusaric acid production in tissues of *Cajanus cajan* infected with *Fusarium oxysporum* f. sp. *udum*. *Phytopathology Zeitschrift* 81: 339 – 345.
- RAI, R.P., 1979. *Fusarium equiseti* (Corda) Sacc. causing dry rot of potato tubers – A new report. *Indian Phytopathology* 48: 1043 - 1045.
- RANALLI, P., BIZARRI, M., BORGHI, L. & MARI, M. 1994. Genotypic influence on *in vitro* induction, dormancy, length, advancing age and agronomical performance of potato microtubers (*Solanum tuberosum* L.). *Annals of Applied Biology* 125: 161 – 173.
- RANCHARD, M., 1984. Application des vitro méthodes à la mise en oeuvre de programmes de sélection de plantes résistantes à des maladies. *Agronomie* 4: 905 – 911.
- RINES, H.W. & LUKE, H.H. 1985. Selection and regeneration of toxin-insensitive plants from tissue cultures of oats (*Avena sativa*) susceptible to *Helminthosporium victoriae*. *Theoretical and Applied Genetics* 71: 16 – 21.
- ROEST, S. & BOKELMANN, G.S. 1976. Vegetative propagation of *Solanum tuberosum* L. *in vitro*. *Potato Research* 19: 173 – 178.
- SACRISTAN, M.D. 1982. Resistance responses to *Phoma lingam* of plants regenerated from selected cell and embryogenic cultures of haploid *Brassica napus*. *Theoretical and Applied Genetics* 61: 193 – 200.
- SACRISTAN, M.D. 1986. Isolation and characterization of mutant cell lines and plants: Disease resistance. In: Vasil, I.K. (Ed). *Cell culture and somatic cell genetics of plants*. Vol 3. Academic Press. Florida. 57 – 63.
- SAVOR, J. & MAČEK, J., 1994. Studies on the causal agents (*Fusarium* spp.) of dry rot of potatoes (*Solanum tuberosum* L.) in Slovenia. *Mededeling en – Faculteit Landbouwkundige en Toegepaste Biologische Wetenschappen, Universiteit Gent*. 59: 885 - 894.
- SCHEFFER, R.P. 1983. Toxins as chemical determinants of plant disease. In: Daly, M & Deverall, B.J. (Eds). Academic Press, New York. 1 – 40.
- SCHIPPERS, P.A., 1962. Dry rot of the potato; preliminary publication. *European Potato Journal* 5: 132 - 146.
- SECOR, G. & GUDMESTAD, N.C., 1993. Handling and planting seed tubers. Pages 27 - 34 in: R.C. Rowe, ed. *Potato Health Management*. APS Press, St Paul, MN.

- SCHMIDT, R., ZAJKOWSKI, P. & WINK, J. 1995. Toxicity of *Fusarium sambucinum* Fuckel *sensu lato* to brine shrimp. *Mycopathologia* 129: 173 - 175.
- SCHULTZ, O., 1981. Tuber seed treatment. Page 67 in: W.J. Hooker, ed. Compendium of Potato Diseases. American Phytopathology Society, St Paul, MN.
- SEPPÄNEN, E., 1980. On the growth of some *Fusarium* species in cultivar Bintje under different environmental conditions. *Potato Research* 23: 478.
- SEPPÄNEN, E., 1981a. *Fusariums* of the potato in Finland. I. On the *Fusarium* species causing dry rot in potatoes. *Annales Agriculturae Fenniae* 20: 156 - 160.
- SEPPÄNEN, E., 1981b. *Fusariums* of the potato in Finland. II. On the growth optima of *Fusarium* species in tubers of cultivar Bintje. *Annales Agriculturae Fenniae* 20: 161 - 176.
- SEPPÄNEN, E., 1981c. *Fusariums* of the potato in Finland. III. Varietal resistance of potatoes to some *Fusarium* species. *Annals of Applied Biology* 81: 177 - 183.
- SEPPÄNEN, E., 1982. *Fusariums* of the potato in Finland. V. Further investigations into the growth optima of *Fusarium* species in potato tubers. *Annales Agriculturae Fenniae* 21: 162 - 168.
- SEPPÄNEN, E., 1983a. *Fusariums* of the potato in Finland. VI. Varietal tuber resistance to *Fusarium* species. *Annales Agriculturae Fenniae* 22: 8 - 17.
- SEPPÄNEN, E., 1983b. *Fusariums* of the potato in Finland. VIII. Occurrence of the pathogens causing dry rot and gangrene. *Annales Agriculturae Fenniae* 22: 115 - 119.
- SEPPÄNEN, E., 1989. *Fusariums* as pathogens of potato tubers and their pathogenicity. Pages 421 - 433 in: J Chelkowski, ed. *Fusarium Mycotoxins, Taxonomy and Pathology*. Elsevier Publishing Co., New York.
- SHAHIN, E. & SPIVEY, R. 1986. A single dominant gene for *Fusarium* wilt resistance in protoplast derived tomato plants. *Theoretical and Applied Genetics* 73: 64 - 169.
- SHARP, W.R. & LARSEN, P.O. 1979. Plant Cell and Tissue Culture: Current applications and potential. *Plant Cell and Tissue Culture: Principles and Applications*. Sharp, W.R., Larsen, P.O., Paddock, E.F. & Raghavan, V. (Eds). p15 - 122.
- SHEPPARD, M.C., 1987. Screening for fungicides. *Annual Review of Phytopathology* 25: 189 - 206.
- SHEPPARD, J., BIDNEY, D. & SHAHIN, E. 1980. Potato protoplasts in crop improvements. *Science* 208: 17 - 24.
- SHERBAKOFF, C.D., 1915. *Fusarium* of potatoes. *Memoires of the Cornell Agricultural Experimental Station* 6: 97 - 270.
- SIEGFRIED, R. & LANGERFELD, E., 1978. Vorläufige Untersuchungen über die Production von Toxinen durch Fäuleerreger bei Kartoffeln. *Potato Research* 21: 335 - 339.
- SMALL, T., 1944. Dry rot of potato (*Fusarium coeruleum* [Lib.] Sacc.). Investigation on the sources and time of infection. *Annals of Applied Biology* 31: 290 - 295.
- SMALL, T., 1945. The effect of disinfecting and bruising on the incidence of dry rot (*Fusarium coeruleum* [Lib.] Sacc.). *Annals of Applied Biology* 33: 211 - 221.
- SMITH, F.E. 1951. Tetrazolium salt. *Science* 113: 751.

- SNYDER, W.C. & HANSEN, H.N., 1941. The species concept in *Fusarium*. *American Journal of Botany* 27: 64 - 67.
- STANGHELLINI, M.E., STOWELL, L.J., KRONLAND, W.C. & BRETZEL, P., 1983. Distribution of *Pythium aphanidermatum* in rhizosphere soil and factors affecting expression of the absolute inoculum potential. *Phytopathology* 73: 1463 - 1466.
- STANGHELLINI, M.E. & KRONLAND, W.C., 1985. Bioassay for quantification of *Pythium aphanidermatum* in soil. *Phytopathology* 75: 1242 - 1245.
- STEPONKUS, P.L. 1971. Effect of freezing on dehydrogenase activity and reduction of triphenyl tetrazolium chloride. *Cryobiology* 8: 570 - 573.
- STEPONKUS, P.L. & LANPHEAR, F.O. 1967. Refinement in the triphenyl tetrazolium chloride method of determining cold injury. *Plant Physiology* 42: 1423 - 1426.
- STEVENSON, W., STEWART, J. & SANDERSON, P., 1986. The effect of thiabendazole seed piece treatment on Monona potatoes in Wisconsin. *American Potato Journal* 63: 191 - 205.
- STEYN, P.J. MCKENZIE, M. & VISSER, A.F. 1991. Resistance in potato tubers to dry rot caused by *Fusarium solani*. *Journal of the South African Society for Horticultural Sciences* 1: 19 - 21.
- STEYN, P.S., VLEGGAR, R., RABIE, C.J., KRIEK, N.P.J. & HARRINGTON, J.S., 1978. Trichothecene mycotoxins from *Fusarium sulphureum*. *Biochemistry* 17: 949 - 951.
- STUBBS, L.L., 1971. Plant Pathology in Australia. *Review of Plant Pathology* 50: 461 - 478.
- SYDENHAM, E.W., MARASAS, W.F.O., THIEL, P.G., SHEPHARD, G.S. & NIEUWENHUIS, J.J., 1991. Production of mycotoxins by selected *Fusarium graminearum* and *F. crookwellense* isolates. *Food Additives and Contaminants* 8: 31 - 41.
- TAVAZZA, R., TAVAZZA, M., ORDAS, R.J., ANCORA, G. & BENVENUTO, E. 1988. Genetic transformation of potato (*Solanum tuberosum*): an efficient method to obtain transgenic plants. *Plant science* 59: 175 - 181.
- TEPPER, T., ZIV, M. & ASHRI, A. 1991. A simple rapid photometric estimation of the growth response of immobilized cells to fusaric acid and gamma radiation. *Plant Cell reports* 10; 481 - 484.
- THANUTONG, P., FURUSAWA, I. & YAMAMOTO, M. 1983. Resistant tobacco plants from protoplast-derived calluses selected for their resistance to *Pseudomonas* and *Alternaria* toxins. *Theoretical and Applied Genetics* 66: 209 - 215.
- THERON, D.J. & HOLZ, G., 1987. Laboratory assessment of potato tuber resistance to dry rot caused by *Fusarium solani*. *Phytophylactica* 17: 521 - 523.
- THERON, D.J. & HOLZ, G., 1989. *Fusarium* species associated with dry and stem-end rot of potatoes in South Africa. *Phytophylactica* 21: 175 - 181
- THERON, D.J. & HOLZ, G., 1990. Effect of temperature on dry rot development of potato tubers inoculated with different *Fusarium* species. *Potato Research* 33: 109 - 117.
- THERON, D.J. & HOLZ, G., 1991a. Dry rot of potatoes caused by *Gliocladium roseum*. *Plant Pathology* 40: 302 - 305.
- THERON, D.J. & HOLZ, G., 1991b. Prediction of potato dry rot based on the presence of *Fusarium* in soil adhering to tubers at harvest. *Plant Disease* 75: 126 - 130.

- THRANE, U. & HANSEN, U., 1995. Chemical and physiological characterization of taxa in the *Fusarium sambucinum* complex. *Mycopathologica* 129: 183 - 190.
- TICKLE J.H. & BOYD, A.E.W., 1974. The influence of the seed tuber in transmission of potato dry rot with a selective medium for the detection of propagules of *Fusarium solani* var. *coeruleum* in field soils. *Potato research* 17: 353.
- TIVOLI, B. & JOUAN, B., 1981. Inventaire, fréquence et agressivité des différentes espèces ou variétés de *Fusarium* responsables de la pourriture sèche des tubercules de pomme de terre. *Agronomy* 1: 787 - 794.
- TIVOLI, B., CORBIÉRE, R. & JOUAN, B., 1983. Influence de la température et de l'humidité sur le comportement dans le sol de 3 espèces ou variétés de *Fusarium* responsables de la pourriture sèche des tubercules de pomme de terre. *Agronomy* 3: 1001 - 1009.
- TIVOLI, B., JOUAN, B. & LEMARCHAND, E., 1983. Etude de facteurs modifiant la mesure du potentiel infectieux des sols infestés par les *Fusarium* responsables de la pourriture sèche des pomme de terre. *Potato Research* 16: 203 - 218.
- TIVOLI, B., ABDUL RAZZAQ, K., JOUAN, B & LEMARCHAND, E., 1986. Etude comparée des capacités infectieuses des différentes espèces ou variétés de *Fusarium* responsables de la pourriture sèche des tubercules de pomme de terre. *Potato Research* 29: 13 - 32.
- TIVOLI, B., DELTOUR, A., MOLET, D., BEDIN, P. & JOUAN, B., 1986. Isolation of thiabendazole-resistant strains of *F. roseum* var. *sambucinum* from potato tubers. *Agronomie* 6: 219 - 224.
- TIVOLI, B., TORRES, H. & FRENCH, E.R., 1988. Inventaire, distribution et agressivité des espèces ou variétés de *Fusarium* rencontrées sur la pomme de terre ou dans son environnement dans différentes zones agroécologiques du Pérou. *Potato Research* 31: 681 - 690.
- TORGENSON, D.C., 1967. Determination and measurement of fungitoxicity. Pages 93-123 in: D.C. Torgenson, ed. *Fungicides: An advanced treatise*. Volume 1. Academic Press, New York.
- TOWILL, L.E. & MAZUR, P. 1975. Studies on the reduction of 2,3,5-triphenyltetrazolium chloride as a viability assay for plant tissue cultures. *Canadian Journal of Botany* 53: 1097 - 1102.
- TOYODA, H., HAYASHI, H., YAMAMOTO, K. & HIRAI, T. 1984. Selection of resistant tomato calli to fusaric acid. *Annals Phytopathological Society of Japan* 50: 538 - 540.
- TOYODA, H., KATSURAGI, K., TAMAI, T. & OUCHI, S. 1991. DNA Sequence of genes for detoxification of fusaric acid, a wilt-inducing agent produced by *Fusarium* species. *Journal of Phytopathology* 133: 265 - 277.
- TURKENSTEEN, L.J., 1987. Survey of diseases and pests in Africa. Fungal and bacterial pathogens. *Acta Horticulturae* 213: 151 - 159.
- UPSTONE, M.E., 1970a. A corky rot of Jersey Royal potato tubers caused by *Fusarium oxysporum*. *Plant Pathology* 19:165 - 167.
- UPSTONE, M.E., 1970b. A potato tuber rot caused by *Fusarium sporotrichioides* Sherb. *Plant Pathology* 19: 150.
- VAN WYK, P.S., LOS, O. & KLOPPERS, F.J., 1986. Crown rot of wheat caused by *Fusarium crookwellense*. *Phytophylactica* 18: 91 - 92.
- VAN WYK, P.S., SCHOLTZ, D.J. & LOS, O., 1986. A selective medium for the isolation of *Fusarium* spp. from soil debris. *Phytophylactica* 18: 67 - 69.

- VAN WYK, P.S., LOS, O., PAUER, G.D.C. & MARASAS, W.F.O., 1987. Geographic distribution and pathogenicity of *Fusarium* species associated with crown rot of wheat in the Orange Free State, South Africa. *Phytophylactica* 19: 271 - 274.
- VAN WYK, P.S., SCHOLTZ, D.J. & LOS, O., 1986. A selective medium for the isolation of *Fusarium* spp. from soil debris. *Phytophylactica* 18: 67 - 69.
- VENTER, S.L., THERON, D.J., STEYN, P.J., FERREIRA, D.I. & EICKER, A. 1992. Relationship between vegetative compatibility and pathogenicity of isolates of *Fusarium oxysporum* f. sp. *tuberosi* from potato. *Phytopathology* 82: 858 - 862.
- VENTER, S.L., STEYN, P.J. & STEYN, H.S.F. 1996. Production of fusaric acid by *Fusarium oxysporum*. *Potato Research* 39: 79 - 84.
- VENTER, S.L. & STEYN, P.J. 1998. Correlation between fusaric acid production and virulence of isolates of *Fusarium oxysporum* that causes potato dry rot in South Africa. *Potato Research* 41: 289 - 294.
- VESONDER, R.F., GOLIŃSKI, P., PLATTNER, R. & ZIETKIEWICZ, D.L., 1991. Mycotoxin formation by different geographic isolates of *Fusarium crookwellense*. *Mycopathologia* 113: 11 - 14.
- VISSER, C.P.N., 1975. Droëvrot van aartappels op die Transvaalse Hoëveld. M.Sc. (Agric.) Thesis. University of Pretoria.
- VISSER, C.P.N. & KOTZÉ, J.M., 1979. Die vatbaarheid van drie aartappelcultivars vir *Fusarium*-droëvrot en die ekonomiese belang van die siektes op die Transvaalse Hoëveld. Tegnieese mededeling No. 158. Departement van Landbou Tegnieese Dienste. Republiek van Suid-Afrika.
- VISSER, R.G.F. 1991. Regeneration and transformation of potato by *Agrobacterium tumefaciens*. *Plant Tissue Culture Manual* B5: 1 - 9.
- VON MARTIUS, C.F.P., 1842. Die kartoffelepidemie der letzten Jahre oder die Stochfäule und Räude der Kartoffel geschildert und in ihren ursächlichen erörtert. *Akademie der Wissenschaften, München*. 20: 1 - 70.
- VON STACHEWICZ, H., BURTH, U. & RATHKE, S., 1992. Fungizidresistenz bei *Fusarium*-Trockenfäuleerregern der Kartoffel in den neuen Bundesländern. *Nachrichtenblatt des Deutschen Pflanzenschutzdienstes (Stuttgart)* 44: 97 - 100.
- WEHMER, C., 1897. Untersuchungen über Kartoffelkrankheiten. II. Ansteckungsversuche mit *Fusarium solani* (die Fusariumfäule). *Centralblatt für Bakteriologie* 25/26: 727 - 742.
- WEISS, F., LAURITZEN, J.I. & BRIERELY, P., 1928. Factors in the inception and development of *Fusarium* rot in stored potatoes. United States Department of Agriculture, Technical Bulletin No 62: 1 - 36. United States Department of Agriculture, Washington, D.C.
- WENZEL, G. 1985. Strategies in unconventional breeding for disease resistance. *Annual Review of Phytopathology* 23: 149 - 172.
- WENZEL, G., KÖHLER, F., SHCUCHEMAN, R., FRIEDT, W. & FEROUGHI-WEHR, B. 1984. *In vitro* selection for disease resistance. In: Anonymous (Eds). British Crop Protection Conference - Pests and diseases. BCPC publications. Croydon.
- WENZLER, H., MIGNERY, G., MAY, G. & PARK, W. 1989. A rapid and efficient transformation method for the production of large numbers of transgenic potato plants. *Plant Science* 63: 79 - 85.
- WHEELER, V.A., EVANS, N.E., FOULGER, D., WEBB, K.J., KARP, A., FRANKLIN, J. & BRIGHT, S.W.J. 1985. Shoot formation from explant cultures of fourteen potato cultivars and studies of the cytology and morphology of regenerated plants. *Annals of Botany* 55: 309 - 320.

- WHEELER, H.E & LUKE, H.H. 1955. Mass screening for disease resistant mutants in oats. *Science* 122: 1229.
- WIERSEMA, H.T., 1977. Laboratory testing for the resistance of potato tubers to dry rot. *Potato Research* 20: 268 - 269.
- WIDHOLM, J.M. 1972. The use of fluorescein diacetate and phenosafranin for determining viability of cultured plant cell. *Stain Technology* 47(4): 189 - 194.
- WILLMOT, D.B., NICKELL, C.D., WIDHOLM, J.M. & GRAY, L.E. 1989. Evaluation of soybean resistance to *Phialophora gregata* culture filtrate in tissue culture. *Theoretical and Applied Genetics* 77: 227 – 232.
- WOLLENWEBER, H.W., 1913. Studies on the *Fusarium* problem. *Journal of Phytopathology* 3: 24 - 50.
- WOLLENWEBER, H.W. & REINKING, O.A., 1935. Die Fusarien, ihre Beschreibung, Schadwirkung und Bekämpfung. Paul Parey, Berlin.
- WU, W., NELSON, P.E., COOK, M.E. & SMALLEY, E.B., 1990. Fusarochromanone production by *Fusarium* isolates. *Applied and Environmental Microbiology* 56: 2989 - 2993.
- YODER, O.C. 1980. Toxins in pathogenesis. *Annual Review of Phytopathology* 18: 103 – 129.
- YODER, O.C. 1983. Use of pathogen produced toxins in genetic engineering of plants and pathogens. In: Kosuge, T., Meredith, C.P. & Hollaender, H. (Eds.) *Genetic Engineering of plants*. Plenum Press, New York. pp. 335 – 353.

OUTPUTS

PEER REVIEWED ARTICLES

STEYN, P.J. MCKENZIE, M. & VISSER, A.F. 1991. Resistance in potato tubers to dry rot caused by *Fusarium solani*. *Journal of the South African Society for Horticultural Sciences* 1: 19 – 21.

THERON, D.J. & HOLZ, G., 1987. Laboratory assessment of potato tuber resistance to dry rot caused by *Fusarium solani*. *Phytophylactica* 17: 521-523.

THERON, D.J. & HOLZ, G., 1989. *Fusarium* species associated with dry and stem-end rot of potatoes in South Africa. *Phytophylactica* 21: 175-181

THERON, D.J. & HOLZ, G., 1990. Effect of temperature on dry rot development of potato tubers inoculated with different *Fusarium* species. *Potato Research* 33: 109-117.

THERON, D.J & HOLZ, G., 1991a. Dry rot of potatoes caused by *Gliocladium roseum*. *Plant Pathology* 40: 302-305.

THERON, D.J. & HOLZ, G., 1991b. Prediction of potato dry rot based on the presence of *Fusarium* in soil adhering to tubers at harvest. *Plant Disease* 75: 126-130.

VENTER, S.L., THERON, D.J., STEYN, P.J., FERREIRA, D.I. & EICKER, A. 1992. Relationship between vegetative compatibility and pathogenicity of isolates of *Fusarium oxysporum* f. sp. *tuberosi* from potato. *Phytopathology* 82: 858 - 862.

VENTER, S.L., STEYN, P.J. & STEYN, H.S.F. 1996. Production of fusaric acid by *Fusarium oxysporum*. *Potato Research* 39: 79 – 84.

VENTER, S.L. & STEYN, P.J. 1998. Correlation between fusaric acid production and virulence of isolates of *Fusarium oxysporum* that causes potato dry rot in South Africa. *Potato Research* 41: 289 – 294.

PRESENTATIONS AT SCIENTIFIC MEETINGS

DU PLOOY, S.L. Onderskeiding van formae speciales in *Fusarium oxysporum*. Fusarium Workshop. October 1988.

VENTER, S.L. & THERON, D.J. Differentiation of isolates of *Fusarium oxysporum* f.sp. *tuberosi* on the basis of vegetative compatibility. Nasionale aartappel simposium. August 1989.

VENTER, S.L. & THERON, D.J. Differentiation of isolates of *Fusarium oxysporum* f.sp. *tuberosi* on the basis of vegetative compatibility and virulence. Southern African Society for Plant Pathology Congress. January 1990.

VENTER, S.L. & THERON, D.J. Vegetative Compatibility and Pathogenicity of *Fusarium oxysporum* f.sp. *tuberosi* and *lycopersici*. SASPP Congress. Pietermaritzburg. January 1991.

- VENTER, S.L. *In vitro* selektering van aartappel cultivars vir bestandheid teen *Fusarium oxysporum* f.sp. *tuberosi*. First annual symposium PBRC. November 1991.
- KRITZINGER, E.M., OELOFSE, D. & VENTER, S.L. Invloed van 'n fitotoksien en Kultuurfiltrate van *Phytophthora nicotianae* var. *nicotianae* op tabaksel selsuspensie kulture. Southern African Society for Plant Pathology Congress. January 1992.
- VENTER, S.L. & STEYN, P.J. Correlation between fusaric acid production and virulence of isolates of *Fusarium oxysporum* that causes potato dry rot. 1993. Southern African Society for Plant Pathology Congress. 1993
- VENTER, S.L. & THERON, D.J. Differentiation of isolates of *Fusarium oxysporum* on the basis of vegetative compatibility and pathogenicity. SASPP Congress. 1992.
- VENTER, S.L., STEYN, P.J. & STEYN, H.S.F. Production of Fusaric acid by *Fusarium oxysporum*. The Southern African Society for Plant Pathology Congress. 1993.
- THERON, D.J., VENTER, S.L., MILLARD, C.P., BURGER, J., GREYLING, M. MARASSAS W.F.O. & WINGFIELD, M.J. Comparison of local and foreign *Fusarium sambucinum* isolates using RAPD's, VCGI's and virulence tests. The Southern African Society for Plant Pathology. 33rd Congress. 1995.
- VENTER, S.L. GERNTHOLTZ, U.J. & UYS, M.D.R. 1996. Vegetative Compatibility Groups of *Fusarium oxysporum* isolates of onion, tomato and potato. The Southern African Society for Plant Pathology.
- VENTER, S.L. & STEYN, P.J. Correlation between Fusaric acid production and virulence of isolates of *Fusarium oxysporum* that causes potato dry rot. 13th triennial conference of the European Association for Potato Research. Veldhoven, The Netherlands. 1996.
- VENTER, S.L. & STEYN, P.J. Correlation between Fusaric acid production and virulence of isolates of *Fusarium oxysporum* that causes potato dry rot. 1997. African Potato Association. Pretoria.
- VENTER, S.L. & STEYN, P.J., 1998. Field evaluation of Late Harvest clones with improved *in vitro* resistance against fusaric acid and/or culture filtrate of *Fusarium oxysporum*. XXV International Horticultural Congress (IHC) Benelux.
- VENTER, S.L. Onderskeiding van isolate van *Fusarium oxysporum* f.sp. *tuberosi* op grond van vegetatiewe verenigbaarheid. Roodeplaat Bulletin. September 1989.

CONTRIBUTIONS AT SHORT COURSES

- D.J. Theron. 1993. Belang van *Fusarium* siektes by aartappelproduksie. Aartappelkursus, Citrusdal.
- D.J. Theron. 1996. Chemiese beheer van *Fusarium* droeëvrot. Aartappelkorkursus, Roodeplaat.
- D.J. Theron. 1996. Chemiese beheer van *Fusarium*saadstukverrotting by gesnyde moere. Aartappelkorkursus, Roodeplaat.