FUSARIUM DRY ROT OF POTATOES: ETIOLOGY, EPIDEMIOLOGY, TOXICITY AND CONTROL

A dissertation submitted in fulfilment of requirements for the degree of Philosophiae Doctor in the Faculty of Agriculture, Department of Plant Pathology of the University of the Orange Free State, Bloemfontein

By

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DECLARATION

I the undersigned hereby declare that the dissertation submitted herewith for the degree Doctor of Philosophy to the University of the Orange Free State, contains my own independent work and has hitherto not been submitted for any degree at any other university.

Daniël Jacobus Theron

May 1999

This dissertation is dedicated to my parents, Helena and our children.

"The most beautiful thing we can experience is the mysterious; it is the source of all true art and science. He to whom this emotion is a stranger, who can no longer pause to wonder and stand rapt in awe, is as good as dead: his eyes are closed."

Albert Einstein, 1930.

"As science advances its store of knowledge, more and more efforts are required of man's imagination in order to breath life into statistics and give direction to the new technology"

The editors, 1977. The World's Last Mysteries.

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PREFACE

Various *Fusarium* species have been associated with dry rot of potato tubers. There are three manifestations of Fusarium dry rot, *viz* storage rot, seed piece decay and stem-end rot. This dissertation presents a study of the occurrence, importance, control, toxicity and population structure of two of the important *Fusarium* species. The dissertation is presented as a compilation of eight manuscripts; each as an independent unit represented in five separate chapters. Some lack of continuity between chapters has been unavoidable due to the fact that the projects have developed over a period of approximately 10 years.

Chapter one represents a literature review on the current status of Fusarium dry rot in the world. This is a summary of earlier reviews, scientific papers and research reports, and also addresses recent developments in Fusarium dry rot research internationally.

Fusarium dry rot became a serious problem when potato production was mechanized. This is due to the characteristic nature of the *Fusarium* pathogens as wound parasites. In South Africa, losses due to Fusarium dry rot are mainly considered to be a problem in the seed industry where potatoes are stored for prolonged periods. The commercial crops are usually consumed within one month after harvesting. Annual losses in the seed industry are between 5 and 15%, and individual cases of as high as 60% have been recorded. Various complaints have been received from local potato producers. Their main concern has been the inefficiency of the two fungicides registered for the control of Fusarium dry rot.

During a survey in 1989 in two major dryland potato production regions of South Africa 14 *Fusarium* species were isolated, either singly or in combination, from potato tubers with dry rot. Eight species were associated with tuber rots of which *F. solani* and *F. oxysporum* were the major species responsible for the dry-rot and stem-end-rot problems in these regions. Chapter two reports on the *Fusarium* species isolated during an extensive survey in the eight remaining potato production regions of South Africa and their pathogenicity.

Two fungicides are registered in South Africa for the control of Fusarium dry rot. The efficiency of these two fungicides with regard to the control of Fusarium dry rot is under suspicion. Chapter three represents a study on the *in vitro* and *in vivo* screening of various fungicides. Part one reports on the efficiency of 24 fungicides inhibiting the mycelial growth *in vitro* of the *Fusarium* species associated with dry rot in South Africa. Parts two and three report

on the results of chemical and agronomical control measures evaluated during *in vivo* trials.

Fusarium solani and *F. sambucinum* are the most common species associated with Fusarium dry rot of potatoes in the northern hemisphere. In chapter four, parts one and two, isolates of *F. solani* and *F. sambucinum* from potatoes with dry rot in South Africa are characterized and compared with various foreign *F. solani, F. solani* var. *coeruleum, F.coeruleum, F. sambucinum, F. sulphureum* and *F. roseum* var. *sambucinum* isolates. Cultural, morphological, pathogenic, molecular and genetic relationships and similarities are also compared.

Fusarium species have long been known to produce important secondary metabolites, which cause different physiological and pharmacological responses in plants and animals. The interest in toxigenic *Fusarium* species is increasing world-wide owing to the discovery of a growing number of naturally occurring *Fusarium* mycotoxins which threaten human and animal health. Since the late 1970's, isolates of *Fusarium* species associated with dry rot of potatoes have been associated with mycotoxin production. Chapter five reports on a preliminary study to determine the toxicity of the 18 *Fusarium* species, pathogenic as well as non-pathogenic, associated with potato dry rot and stem-end rot in South Africa.

This dissertation attempts to represent a balance between practical and applied scientific research. It is hoped that this study will contribute to a better understanding of Fusarium dry rot of potatoes by the scientific community and also reduce the occurrence of this disease.

CHAPTER 1

FUSARIUM DRY ROT OF POTATOES

INTRODUCTION

Von Martius (1842) was the first person to report a fungus, *Fusisporium solani* Mart., associated with a potato (*Solanum tuberosum* L.) tuber rot which Saccardo (1881), according to Booth (1971), transferred to *Fusarium solani* (Mart.) Sacc. According to the taxonomic system of Nelson, Toussoun & Marasas (1983) it is today known as *Fusarium solani* (Mart.) Appel & Wollenw. emend. Snyd. & Hans. Von Martius (1842) did not consider this fungus as the causal organism of the disease, but artificial infections showed conclusively that it can cause a tuber rot (Wehmer, 1897; Pethybridge & Bowers, 1908). Numerous other soil-borne *Fusarium* spp. have also since been reported to cause dry rot (Booth, 1971; Boyd, 1972; Seppänen, 1989).

There are three manifestations of Fusarium dry rot, *viz* 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, and is also an important form of Fusarium dry rot. In the case of stem-end rot, infection apparently occurs through the stolon of the tuber and symptoms are evident at harvest (Carpenter, 1915; Pethybridge & Lafferty, 1917; Foister, 1940; Boyd, 1972; Jones & Woltz, 1981; Nielsen, 1981; Powelson, Johnson & Rowe, 1993).

Fusarium dry rot first became a serious problem when potato production was mechanized. This was due to the characteristic nature of the *Fusarium* pathogens as wound parasites (Seppänen, 1989). Although comprehensive figures are lacking, and Fusarium dry rot seldom reaches epidemic proportions (Booth, 1971), it probably causes greater losses in storage and transit of both seed and commercial potatoes, than any other post-harvest disease (Powelson *et al.*, 1993). Average annual crop losses attributed to Fusarium dry rot have been estimated at 6 to 25% (Foister, 1940; Chełkowski, 1989). Reports of more than 60% of the tubers being affected during long-term storage are no exception (Carnegie, Ruthven, Lindsay & Hall, 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).

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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.) are also involved, resulting in a more rapid decay of the infected tubers (Moore, 1945; Nielsen, 1981; Powelson *et al.*, 1993). Fusarium dry-rot symptoms are in some respects similar to those of gangrene caused by *Phoma exigua* var. *foveata* (Foister) Boerema and *P. exigua* var. *exigua* (Foister) Malcolmson (Boyd, 1972). Gangrene commences as a small depression and the tissue remains reasonably firm with a well-defined margin between the rotten and healthy tissue, which is unlike Fusarium dry rot (Foister, 1940). Fusarium dry-rot symptoms can sometimes also be confused with symptoms caused by *Botrytis cinerea* Pers. (Nielsen, 1981) and *Gliocladium roseum* Bain (Langerfeld, 1977; Theron & Holz, 1991a).

Symptoms of infection with different *Fusarium* spp. differ to a certain extent from one another (Carpenter, 1915; Moore, 1945; Boyd, 1972; Nielsen, 1981). These differences are, however, not distinct and tubers are often colonized by more than one *Fusarium* sp. (Theron & Holz, 1989). The only certain way of determining which species is the causal organism, is to isolate and identify the associated organisms (Theron & Holz, 1989).

Storage rot

After about one month of storage, tuber lesions at the wounds are visible as small brown areas. Infection spreads slowly and periderm covering the lesions sinks and wrinkles, sometimes in concentric rings round the site of infection, as the dead tissues became dry and powdery. Sporodochial pustules are frequently formed on the dead periderm, and these may be 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 (Foister, 1940; Boyd, 1972; Nielsen, 1981).

The affected tissues are usually fawn or light brown in colour, and stain to a darker brown within a few minutes when exposed to air. The advancing margin of the rot diffuses and merges into the healthy tissue. Because of the loss of moisture from the tissue, cavities are formed below the site of infection. These cavities are often lined with white, yellow or pink mycelium which is visible when the tubers are cut. In storage, blue, black, purple, gray, 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 (Foister, 1940; Boyd, 1972; 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 as the main cause of seed piece decay (Boyd, 1972). Although whole seed tubers can also become infected through wounds sustained during storage or preparation for planting, the primary cause of concern is the decay of seed tubers cut into seed pieces. The cost of potato seed constitutes the greater part (35-40%) of the production costs of potatoes. It is, therefore, customary for some farmers to cut seed tubers into seed pieces. The cut surfaces expose tuber tissue to desiccation and are also major infection courts for bacterial and/or fungal pathogens causing seed piece decay (Nolte, Secor & Gudmestad, 1987; Nelson, Secor, Gudmestad & Preston, 1993). Bacterial decay e.g. soft rot, mainly caused by *Erwinia* spp. and fungal decay, e.g. dry rot, caused by *Fusarium* spp. and their combinations, are the primary causes of potato seed piece decay (Davis, Sorensen & Corsini, 1983; Misca, & Nelson, 1975; Nelson *et al.*, 1993; Nielsen, 1981; Nolte *et al.*, 1987).

When seed pieces are stored, brown to black flecks appear on the cut surfaces about one week after infection by *Fusarium* and depressions or pits appear within 2 wk. Mycelium often grows on the depressed surfaces, and under humid conditions, depressions may become slimy and black from bacterial growth. Soft rot bacteria may also invade through the *Fusarium* lesions and accelerate seed piece decay. Numerous cut-surface infections cause lesions to coalesce and the seed piece rots from the surface inwards, destroying the buds as the decay progresses (Eddins, 1940; Nielsen, 1981).

In the field, the 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 *Erwinia* spp. In wet soils, *Erwinia* spp. often enter as secondary pathogens (Eddins, 1940; Nielsen, 1981). Symptoms caused by *Fusarium* spp., alone or in combination with *Erwinia* spp., include total or partial decay of seed pieces, reduction of plant vigour and poor stands (Eddins, 1940; Escande & 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 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, localized on the stem-end of the tuber. Some of the diseased tubers may express a sunken, wrinkled, dry stem-end rot of varying diameters, while others may only express a slight indication of rot around the point of the stolon attachment. When the latter tubers are cut across near the base, browning is evident in the region of the ring of the vascular bundles. This discolouration may extend to various depths in the tuber, sometimes affecting the whole vascular ring, but more often involving only separate portions of it. The tissues surrounding the affected cells in the vascular ring usually appear brown, yellowish or water-soaked (Carpenter, 1915; Haskell, 1916; Goss, 1936; Schippers, 1962).

CAUSAL ORGANISMS

Fusarium spp. are distributed worldwide as saprophytes, soil inhabitants, and pathogens of many plants. The identification of some species is difficult and is still not clear. The fact that different species are reported under the same name and a single species under different names is also confusing (Seppänen, 1989). *Fusarium* spp. associated with diseases of potatoes are no exception. Many *Fusarium* spp. have been associated with potato tuber rots (Wollenweber & Reinking, 1935; McKee, 1952; Boyd, 1972; Chambers, 1973; Seppänen, 1981a; Tivoli & Jouan, 1981; Theron & Holz, 1989; Manici & Cerato, 1992; Savor & Maček, 1994; Hanson, Schwager & Loria, 1996) and stem-end rot (Carpenter, 1915; Haskell, 1916; Wollenweber & Reinking, 1935; Goss, 1936; Schippers, 1962) 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 are classified in 16 species according to the taxonomic system of Nelson *et al.* (1983) in Table 1. Amongst these 16 species, *F. solani* [= *F. coeruleum* (Libert) Sacc; *F. solani* (Mart.) Sacc. var. *coeruleum* (Libert) Bilai], *F. sambucinum* (Fuckel) [= *F. trichothecioides* Wollenw.; *F. sulphureum* Schlecht.] and *F. avenaceum* (Fr.) Sacc. [= *F. arthrosporioides* Sherb.] are 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 are *F. oxysporum* Schlecht. emend. Snyd. & Hans. and *F. solani* (Stubbs, 1971; Chambers, 1973; Turkensteen, 1987; Tivoli, Torres & French, 1988; Theron & Holz, 1989).

Most of the research on Fusarium dry rot concerns strains of *F. solani* and *F.*

sambucinum. However, strains of these species are often referred to under one or more of the synonyms by which they are also known. This makes it difficult for the non-specialist to draw conclusions from previous findings. Often strains of these species have been incorrectly named or even wrongly identified resulting in confusion amongst researchers. New initiatives in the traditional area of morphological research, such as computer-aided identification, and the modern techniques used by molecular biologists, such as analysis of ribosomal RNA sequences and fingerprinting with polymerase chain reaction have provided new insights to species relations (Hall, 1995). For example, a European *F. sambucinum* project was initiated in 1989 amongst scientists world-wide using different methods and approaches. A joint conclusion was drawn on the taxonomic position of *F. sambucinum sensu lato* (Nirenberg, 1995a). Results led to a conclusion that strains of *F. sambucinum* and its synonyms should be divided into three species. These include *F. sambucinum* Fuckel *senso stricto*, *F. torulosum* (Berk. & Curt.) Nirenberg comb. nov. and *F. venenatum* Nirenberg sp. nov. All three species included strains isolated from potatoes (Nirenberg, 1995b).

For the purpose of this review all further references to *Fusarium* spp. causing dry rot of potatoes will be to the 16 species accepted by Nelson *et al.* (1983) (Table 1). *Fusarium sambucinum s. str.*, *F. torulosum* and *F. venenatum* were not included in Table 1, because it is not clear whether the strains of these three species, although isolated from potatoes (Nirenberg, 1995b), were in fact isolated from dry-rot lesions. *Fusarium oxysporum* f. sp. *tuberosi* Snyder & Hansen was wrongly cited as a dry-rot pathogen of potatoes by Venter, Theron, Steyn, Ferreira & Eicker (1992), and is therefore also not included in Table 1.

HISTOPATHOLOGY

Fusarium spp. cannot infect intact tuber periderm (Nielsen, 1981) because they are primarily wound pathogens, and rotting is seldom observed before lifting (Foister, Wilson & Boyd, 1952; Chambers & Millington, 1974). Cuts and wounds breaking the periderm incited either during harvest, grading, transportation and storage, when tubers become more susceptible, or when seed tubers are cut, are the major infection courts (Small, 1945 & 1946; Foister *et al.*, 1952; Boyd, 1967; Nielsen, 1981; Powelson *et al.*, 1993). Scarified wounds were reported to be more susceptible to infection than clear cuts (Boyd, 1972). Pethybridge & Lafferty, (1917) could

artificially infect potato tubers through lenticels, eyes, sprouts and through scab pustules caused by *Streptomyces scabies* (Thaxt.) Waksman & Henrici. McIntosh (1944) also maintained that infections could occur through lenticels. Foister *et al.*, (1952), however, obtained no infections by this route and there were no further reports thereof (Nielsen, 1981). Natural infections associated with powdery scab, caused by *Spongospora subterranea* (Wallr.) Langerh. are often found (Foister *et al.*, 1952). Surface lesions caused by late blight [*Phytophthora infestans* (Mont.) De Bary], mop top virus and possibly other tuber diseases, can also become infected. Wounds caused by the feeding of insects and frost are sometimes infected (Weiss, Lauritzen & Brierely, 1928; Foister *et al.*, 1952; Nielsen, 1981). Although Boyd (1972) never observed tuber infection prior to lifting, Carpenter (1915), Haskell (1916), Goss (1936) and Schippers (1962) considered it possible for infection to occur through stolons, resulting in stem-end rot. Mooi (1950), also showed experimentally that tuber infection was possible through the stolon-end of the tuber when haulms were damaged or removed. In South Africa the occurrence of tubers expressing stem-end-rot symptoms is common (Theron & Holz, 1989).

Hyphae of *F. solani* are at first intercellular, becoming intracellular in dead cells, whereas *F. avenaceum* (McKee, 1954), *F. culmorum* (W. G. Smith) Sacc. and *F. sambucinum* (Langerfeld, 1978) immediately kill, penetrate and colonize the cells surrounding the point of infection. In order to infect and colonize plant tissue, a pathogen must penetrate two defensive barriers of the plant, i.e. the cuticle and the cell wall. To overcome these barriers, the production of cell wall degrading enzymes by the pathogen is necessary. The dry rot causing *Fusarium* spp. do not seem to be able to overcome the first barrier, owing to the fact that they infect primarily through wounds. *Fusarium* spp. are, however, able to produce degrading enzymes to overcome the cell wall (Urbanck, 1989).

The breakdown of the tuber tissue through enzymatic activity seems to progress in a stepwise fashion. First pectinmethylesterase (PME) of host origin is activated or PME is released from the pathogen. This enzyme group catalyses the demethylation of methyl esters of polygalacturonic acid, i.e. the pectic material of the middle lamellae and cell walls. The pectin chains are then available to depolymerizing enzymes, e.g. polygalacturonase (PG), which are more effective on substrates with a low degree of esterification. PG catalyses them, splitting the glycosidic bonds in the pectin chains. The degradation of the structural integrity results in cell separation which makes penetration of fungal hyphae in the tuber tissue possible (Olsson, 1989).

Langerfeld (1978) reported that *F. solani* has pectolytic, proteolytic and cellulytic activities. In contrast to *F. sambucinum*, *F. solani* synthesises its polygalacturonase constitutively, whereas constitutive synthesis of pectinmethylesterase and cellulase occurs in both fungi. These activities, as well as the penetration mode of *F. solani*, are advantageous due to the maceration of the pectin- and cellulose containing cell walls and middle lamellae. Degradation of the pectic substances in the middle lamellae and cell walls can also render other wall polymers susceptible to attack by hemicellulose, cellulose and protein-degrading enzymes. At this stage of pathogenesis, the pectin degrading enzymes disappear (Olsson, 1989).

Langerfeld (1978), stated that some researchers believe that the main factor responsible for pathogenicity of *Fusarium* spp., causing dry rot, lies in their ability to halt phenol production in the tuber tissue. Desjardins, Gardner & Weltring (1992) thus showed that *F. sambucinum* can detoxify the potato phyto-alexins, rishitin and lubimin, and that this detoxification is necessary for a high level of virulence in potato tubers.

In developing lesions, hyphae may be sparse in intercellular spaces, with host cells showing little reaction to the pathogen. Towards the centre of the lesion, less starch is present and the abundant mycelium may be confined to the intercellular spaces by suberin deposited in host cell walls and intercellular spaces. In susceptible tissue, starch hydrolysis and suberin deposits are lacking. Small lesions restricted to the site of infection may be underlaid by a continuous layer of wound meristem cells, with suberin deposition. Characteristics of the reaction depend on the pathogen, the resistance of the tuber and the part of the lesion examined (Nielsen, 1981).

DISEASE CYCLE

Fusarium spp. associated with dry rot are soil-borne and are common in most soils where potatoes are grown. They can survive as chlamydospores or mycelium in decaying plant debris in field soils for several years, but the primary inoculum is generally carried on the tuber surfaces (Boyd, 1952a; Folsom, 1959; Nielsen & Johnson, 1972; Jeffries, 1978; Jones & Woltz, 1981; Adams & Lapwood, 1983; Leach, 1985; Seppänen, 1989; Theron & Holz, 1991b). Some infections develop on tubers prior to harvesting, causing a stem-end decay (Carpenter, 1915; Haskell, 1916; Schippers, 1962; Powelson *et al.*, 1993), but most occur during or after harvest. Surface-borne propagules contaminate containers and equipment used for the handling or storage

of potatoes, and enter wounds incited by the handling of the tubers (Boyd, 1972; Jeffries, 1978; Langerfeld, 1978; Seppänen, 1989). *Fusarium* spores may also be present in the air in potato stores (Pethybridge & Lafferty, 1917; Small, 1944) or in dust (Small, 1946) or in contaminated boxes, pre-germination containers and sacks (Pethybridge & Lafferty, 1917). However, these sources present a negligible risk unless the tubers have been freshly wounded (Pethybridge & Lafferty, 1917; Small, 1946; Foister *et al.*, 1952). Infected tubers will decay during storage (Nielsen, 1981), but the spreading of infection from diseased to healthy tubers in the warehouse does not normally occur (Small, 1946).

Seed tubers infected during the cutting and planting process or thereafter in the soil, will most likely rot in the soil after planting (Boyd, 1972; Langerfeld, 1978; Seppänen, 1989). The planting of infected seed tubers is also a major source of infection of the soil and the progeny. This results in considerable losses during and after the growing season. Infected seed tubers are also exposed to desiccation and bacterial seed piece decay (Tickle & Boyd, 1974; Jeffries, 1978; Adams & Lapwood, 1983; Leach, 1985; Nolte *et al.*, 1987; Nelson *et al.*, 1993).

EPIDEMIOLOGY

The host and its resistance to Fusarium dry rot

Variation in resistance of potato cultivars to Fusarium dry rot has long been known, but none of the available cultivars are immune (Moore, 1945; Boyd, 1952a; Leach & Webb, 1981; Seppänen, 1981c; Theron & Holz, 1987). Differences in varietal reactions to different *Fusarium* spp. have also been reported (Boyd, 1972; Wastie, Stewart & Brown, 1989; Theron & Holz, 1990; Platt, 1992). The resistance of the cultivars depends on two major determinants: the genetic composition (genotype) of the cultivar and the surrounding environment, which strongly influences the expression of the genetic information (phenotype) (Falconer, 1981).

The resistance of the tubers can be divided into physiological and mechanical mechanisms (Boyd, 1952b; Leach & Webb, 1981). The components involved in the physiological mechanisms, however, are complex and poorly understood (Boyd, 1972; Clarke, 1972). Among those biochemical components that may be involved, both phenolics and glycoalkaloids exhibit fungicidal characteristics (Corsini & Pavek, 1980; Olsson, 1988). Mechanical mechanisms of possible importance are the cell wall and middle lamella, which have a high content of pectic

substances (Olsson, 1988). An improved structural integrity due to cross-linkages between the carboxylic groups of the pectin chains via divalent cations will make the tissue of the host plant less susceptible to degradation (Olsson, 1988). Cultivars directly more resistant to mechanical damage, owing to stronger cell walls and thicker skins, will indirectly also be less susceptible to Fusarium dry rot (Small, 1946; Foister *et al.*, 1952; Boyd, 1967; Langerfeld, 1978; Nielsen, 1981; Powelson *et al.*, 1993).

Wound healing which is greatly affected by temperature, oxygen and carbon dioxide levels and humidity, can reduce infection. Deposition of suberin in the cell walls does not prevent infection, but the formation of wound periderm does (O'Brien & Leach, 1983; Nolte *et al.*, 1987; Plissey, 1993). Nolte *et al.* (1987) reported that both *Erwinia* spp. and *Fusarium* spp. can, however, become established more quickly than wound-healing can take place.

Tubers of the same cultivar also differ in their resistance to Fusarium dry rot. It is also well known that different parts of a tuber vary in composition. For example, it has been found that the central zone of a tuber is less resistant than the cortex. The heel-end normally also contains higher percentages of dry matter and starch than the rose-end, and is usually more susceptible to Fusarium dry rot (Boyd, 1952b).

Environmental effects on the susceptibility of tubers

As has already been mentioned, the susceptibility of the potato tubers to Fusarium dry rot is greatly influenced by the environmental conditions under which potatoes are planted, grown, harvested and stored.

Soil and growing conditions: The planting of seed pieces in dry, hot soil (especially sandy soil) immediately after cutting results in poor wound healing. Excessive seed piece decay may, therefore, follow (Secor & Gudmestad, 1993). If the soil temperature and soil moisture are suitable for rapid sprout growth and emergence (13-16°C, 70-80% of available soil water), seed tuber or seed piece decay after planting may be of little consequence (Nielsen, 1981; Secor & Gudmestad, 1993). Fusarium wilts and stem-end infections, leading to stem-end rot, will most likely occur when plants are grown under stress due to hot, dry conditions (Haskell, 1916; Goss, 1936; Schippers, 1962).

Fusarium dry rot is more severe after dry and hot growing seasons. Mooi (1950) considered this to be related to the effect of higher temperature, advancing the rate of maturity

which will result in tubers being more susceptible. Ayers & Ramsay (1961) concluded that warm dry soil favoured the propagation of the pathogen, while Lansade (1950), suggested that the operative factor was the higher dry matter content of the tuber associated with such conditions. In many respects, susceptibility appears to be associated with a higher dry matter content, e.g. the stolon-end of a tuber is more susceptible than the rose-end (Boyd, 1952a). Susceptibility, and the dry matter content are also reduced if the growing period is shortened either by late planting or early haulm removal (Boyd, 1967). There is a great variation in tuber susceptibility, depending on the age of the tuber. Immature tubers are quite susceptible to Fusarium dry rot, but their susceptibility decreases towards the end of the growing season and is lowest at harvest (Pethybridge & Laverty, 1917; Small, 1945; Boyd, 1952a).

Fertilization: Schippers (1962) reported that the type and quantity of fertilizers applied has an influence on tuber susceptibility. The application of nitrogen alone, which lowers dry matter content, tended to increase susceptibility (Schippers, 1962; Boyd, 1967). The association between dry matter content and the susceptibility of the tubers seems to be indirect (Boyd, 1972; Langerfeld, 1978). No potash effect was observed (Schippers , 1962; Boyd, 1967). However, an interaction between nitrogen and potash dressing was found to occur (Schippers 1962). Langerfeld (1973) found that additional applications of Mg or Ca to an ordinary N-P-K supply reduced the incidence of dry rot caused by *F. solani*. This was also reported by Olsson (1988) in the case of extra Mg, and occasionally extra Ca, applications. This effect can be explained by the pectin-bond Mg, which gives a firmer cell wall and middle lamella than the pectin-bond Ca.

Harvesting: At the end of the growing period, vines slowly senesce and photosynthesis decreases. Tuber growth slows, and the skins begins to set. When the vines have died or are killed artificially, tuber growth stops and the skins are finally set. Apart from the fact that immature tubers are more susceptible to Fusarium dry rot (Boyd, 1967), tubers in which the skins have not set properly, will also be more susceptible to mechanical damage when harvested. This will increase Fusarium dry-rot development (Boyd, 1972; Plissey, 1993). Potatoes harvested from cold, wet soils or dry, cloddy soil are more susceptible to bruising and thus more prone to Fusarium dry-rot infection (Plissey, 1993). Specht (1968) found that with each 1°C drop in temperature below 15°C, a 10% increase in the rate of damage occurs.

Storage: Despite precautions taken during harvesting and handling, some damage to the tuber surfaces will inevitably occur. Rapid wound healing minimizes *Fusarium* infection (Nielsen,

1981; Plissey, 1993). Wound healing is greatly affected by storage conditions, e.g. temperature, humidity and ventilation, and occurs most rapidly at ±21°C and RH of 95-99%. These conditions are, however, also favourable to Fusarium dry-rot development (Plissey, 1993).

Pre-storage conditions are of considerable importance affecting the susceptibility of tubers to Fusarium dry rot (Seppänen, 1982a & 1989). Lansade (1950) found the growth rate of *F. solani* in tubers to be higher when kept at lower pre-storage temperatures. When tubers were pre-stored at a high humidity and high temperature, the growth rate was, however, reduced. This means that the physiological defence of tubers, i.e. the ability to localise an infection to the wound area, is temperature and humidity dependant. This may be explained by the fact that high humidity and high temperatures enhance wound-healing, whereas at low humidity and low temperatures, wound-healing is slow, allowing the pathogen access (Nielsen, 1981; Plissey, 1993). Weiss *et al.*, (1928) found that below 10°C, even with favourable humidity, tubers cannot form a protective layer which will exclude the more virulent dry-rot organisms. Boyd (1952c) showed that pre-storage at 3-4°C did not decrease tuber resistance, but that pre-storage at -1°C or 22°C did. The resistance of tubers pre-stored for 2 wk at 6 or 24°C and a low humidity (RH 45%) decreased rapidly (Seppänen, 1982a & 1989). It is, therefore, necessary to distinguish between the infection phase and colonization of the pathogen.

In the case of already manifested rots, increasing the storage temperature will result in more Fusarium dry rot (Langerfeld, 1978). During storage, tubers will become more susceptible to Fusarium dry rot (Pethybridge & Lafferty, 1917; Boyd. 1952c). Cultivars differ greatly in relation to their susceptibility to Fusarium dry rot during storage and will also react differently under different storage conditions (Boyd. 1952c).

Variation in the pathogenicity of dry-rot pathogens

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

Amongst dry-rot pathogens, *F. solani* has been most intensively investigated. Wollenweber & Reinking (1935) reported growth at temperatures between 3 and 30°C, with the

optima being between 15 and 28°C, especially at relative humidity (RH) of 50-80%. Subsequently 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 & Holz (1990) found that South African isolates grew optimally between 25 and 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 best 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). Two optimum temperatures (12 and 24°C) on cv. Bintje were, however, reported for *F. sambucinum*, but on cvs. Pito and Sabina only one optimum, between 24-30°C, was found (Tivoli & Jouan, 1981). Theron & 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 the optimum for the cv. BP-1. This was 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 would appear that 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).

TOXIGENICITY

The genus *Fusarium* has long been known to produce important secondary metabolites which cause different physiological and pharmacological responses in plants and animals. They are best known for the production of the trichothecene mycotoxins, but they also produce a variety of other compounds such as other mycotoxins, pigments, antibiotics and phytotoxins (Nelson, Desjardins & Plattner, 1993).

The interest in toxigenic *Fusarium* spp. is increasing world-wide owing to the discovery of a growing number of naturally occurring *Fusarium* mycotoxins which are of practical importance as threats to human and animal health (Marasas, Nelson & Toussoun, 1984). Most of the research concerning the toxigenicity of *Fusarium* spp. has been conducted on cereals and the *Fusarium* pathogens thereof. In the past, however, most of the *Fusarium* spp. which have been associated with dry rot, and even some isolates isolated from rotten potato tubers, were reported to produce various mycotoxins (Marasas *et al.*, 1984). However, no research was conducted to determine if these *Fusarium* isolates could produce toxins in infected tubers and their possible threat to the consumers of potatoes.

It is only since the late 1970's that the *Fusarium* isolates associated with dry rot have received serious attention from researchers interested in their toxigenicity (Chełkowski, 1989). Strains of *F. sambucinum*, a major cause of dry rot in many countries, have been associated with trichothecene production. These isolates were obtained from rotten potato tubers collected in Germany (Siegfried & Langerfeld, 1978), France (Lafont, Girard, Payen, Sarfati & Gaillardin, 1983), Poland (Latus-Ziętkiewicz, Perkowski & Chełkowski, 1988) and Iran, with a high-incidence area of human esophageal cancer (Steyn, Vleggaar, Rabie, Kriek & Harington, 1978) as well as several other locations (Desjardins & Plattner, 1989). As part of the European *F. sambucinum* Project (Nirenberg, 1995a), strains of *F. sambucinum s. l.*, divided into three species (*F. sambucinum s. str.*, *F. torulosum* and *F. venenatum*) (Nirenberg, 1995b), were screened for the production of secondary metabolites *in vitro*. Thirteen strains, isolated from potato tubers, were tested and they all produced some secondary metabolites (Altomare, Logrieco, Bottalico, Mulé, Moretti & Evidente, 1995; Thrane & Hansen, 1995) and were also highly toxic in the biological assays (Altomare *et al.*, 1995). Type A trichothecenes, in particular diacetoxyscirpenol (DAS), were produced by all the *F. sambucinum s. str.* and *F. venenatum* strains, but not by the

F. torulosum strains. It also appears as if these stains were more toxic than the strains isolated from crops other than potatoes. All but one *F. sambucinum s. str.* strain, produced DAS as well as other trichothecenes, whereas the *F. venenatum* strains produced only DAS (Altomare *et al.*, 1995; Thrane & Hansen, 1995). In contrast to these findings, Kim & Lee (1994) reported that none of the *F. sambucinum*, *F. oxysporum* and *F. solani* strains that they isolated from rotted potato tubers in Korea were able to produce trichothecenes. However, a new toxin, sambutoxin, was produced *in vitro* by strains of *F. sambucinum* and *F. oxysporum* but not *F. solani* (Kim & Lee, 1994).

In spite of the widespread occurrence of *F. sambucinum* causing dry rot of potatoes, and the documented toxigenicity thereof (Marasas *et al.*, 1984; Desjardins & Beremand, 1987), only a limited study has been done on the ability of this species to produce trichothecenes in potato tubers (Desjardins & Beremand, 1987). The same is true for other *Fusarium* spp. that might also produce known or even unknown mycotoxins in colonized potato tubers. In France, Lafont *et al.*, (1983), found several trichothecene toxins in potato tubers naturally and artificially infected with *F. sambucinum*. In Canada, El-Banna, Scott, Lau, Sakuma, Platt & Campbell (1984) found low concentrations of trichothecenes in potato tubers artificially infected with a strain of *F. sambucinum,* which had originally been isolated from potato tubers. In Korea, Kim, Lee & Yu (1995) reported the occurrence of sambutoxin in 42.9% of tubers, naturally infected with *F. sambucinum* and *F. oxysporum,* at levels of 15.8 to 78.1 ng of toxin/g rotten tissue. Recently Desjardins & Plattner (1989) reported that strains of *F. sambucinum* can produce trichothecenes, including 15-monoacetoxyscirpenol and 4-15-diacetoxyscirpenol, as well as other minor trichothecenes, in artificially infected potato tubers. Even apparently disease-free parts of infected potatoes contained the mycotoxins at levels of about 10% of those in the diseased portion. Therefore, the removal of rotten parts will not completely eliminate the toxin. Since trichothecenes are heat stable, it seems unlikely that these mycotoxins would be destroyed by the usual procedures of preparing potatoes for human consumption (Desjardins, 1989).

The ability to produce mycotoxins has also been found in *Fusarium* spp., other than *F. sambucinum*, isolated from potato tubers with dry-rot symptoms (El-Banna *et al.*, 1984; Latus-Ziętkiewicz *et al.*, 1988; Latus-Ziętkiewicz, Perkowski, Tanaka, Gamamoto, Kawamura, Sugiura & Ueno, 1990). These results suggest that potato tubers are a suitable medium for mycotoxin production, although not as suitable as cereal grains (wheat, maize, rice) (Chełkowski, 1989).

DISEASE MANAGEMENT

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

Management through agronomic practices

Planting: Although *Fusarium* spp. are soil-borne, the primary source of infection of the progeny is infected or contaminated seed tubers (Boyd, 1952a; Folsom, 1959; Jeffries, 1978; Nielsen & Johnson, 1972; Jones & Woltz, 1981; Seppänen, 1989; Theron & Holz, 1991b). Therefore, the planting of clean non-infected or fungicide-treated seed is essential. Seed pieces should be planted immediately after cutting and fungicide treatment. Although *Fusarium* propagules can survive in the soil for several years (Boyd, 1972; Langerfeld, 1978; Secor & Gudmestad, 1993), and tuber rotting *Fusarium* spp. "generally" do not infect other plants or plant organs (Nielsen, 1981), a crop rotation system will lower the soil inoculum (Jones & Woltz, 1981; Nielsen, 1981). Clearly, more information is required about the cross-pathogenicity of *Fusarium* spp. causing dry rot of potato tubers and other plants. Potato cultivars differ in their susceptibility to Fusarium dry rot, but none of the available cultivars are immune (Moore, 1945; Boyd, 1952a; Seppänen, 1981c). Therefore, the planting of more tolerant cultivars, especially when Fusarium dry rot is a known limitation, is recommended (Nielsen, 1981).

Careful selection of the planting date, and thereby avoiding excessive dry and hot spells, may also alleviate stem-end-rot infections (Carpenter, 1915; Haskell, 1916; Goss, 1936; Schippers, 1962). Planting should be conducted when the soil temperature and soil moisture are suitable for rapid sprout growth and emergence. This will also decrease the extent of seed tuber decay, especially that of seed pieces (Nielsen, 1981; Secor & Gudmestad, 1993).

Care should be taken when applying fertilizers. An excessive application of nitrogen, which may reduce the tubers's dry matter content, tends to increase tuber susceptibility (Scippers, 1962; Boyd, 1967). Additional applications of Mg or Ca to an ordinary N-P-K supply, may reduce

the incidence of Fusarium dry rot (Langerfeld, 1973; Olsson , 1989).

Harvesting and grading: This is by far the most critical stage with regard to Fusarium dry-rot development because during these practices tubers are most likely to be damaged. It is, therefore, essential not to harvest immature tubers of which the skins are not properly set and which will be more susceptible to bruising and thus more prone to Fusarium dry-rot infection. Harvesting, especially from cold, wet soils or dry, cloddy soils should also be avoided (Boyd, 1972; Plissey, 1993). Tubers harvested when temperatures are low will also result in more bruising (Plissey. 1993). Excess soil should be removed from the tubers. This will reduce the presence of high concentrations of *Fusarium* propagules which are very likely to occur in these soils (Nielsen, 1981; Plissey, 1993).

Care should be taken during harvesting and handling of the tubers to prevent mechanical damage (Nielsen, 1981; Plissey, 1993). Harvested tubers should not be allowed to lie in direct sunlight or be subjected to cold weather for prologed periods, but should be transported to warehouses as soon as possible (Plissey, 1993). Sanitation in the warehouse and in respect of the equipment used for the grading and storage of the tubers is recommended, as these could also harbour *Fusarium* propagules (Pethybridge & Lafferty, 1917; Small, 1946; Foister *et al.,* 1952). During grading, diseased tubers should be removed and discarded. Seed tubers may be treated with an effective fungicide, dust or liquid spray, before storage (Plissey, 1993).

Despite precautions taken during harvesting and handling, some injury to the tubers will occur. A curing or sweat period must be provided to promote proper wound healing prior to long-term storage. Wound healing is greatly affected by temperature, oxygen and carbon dioxide levels and humidity. Although the process occurs most rapidly at about 21°C, compromises must be made to prevent tuber decay (Small, 1946; Foister *et al.*, 1952, Boyd, 1967, Nielsen 1981; Powelson *et al.*, 1993). Most pathogens decline in activity at lower temperatures. If the crop is generally healthy, the curing temperature can safely be maintained at 14- 16°C. However, if any significant amount of tuber diseases is present, a curing temperature nearer to 10°C is preferable. (Plissey, 1993).

Storage: Potatoes stored in a well-ventilated room at a temperature of 4 ±1°C will slow Fusarium dry-rot development (Plissey, 1993). Although Fusarium dry rot may even continue at the coldest temperatures safe for potato storage (Langerfeld, 1978; Seppänen, 1989). Infections

are not likely to take place under these conditions (Plissey, 1993).

Cold-stored potatoes should not be handled until they are ready for planting because they are more susceptible to bruising. They should, however, be warmed slowly for a week to temperatures of 20-25°C before planting or cutting into seed pieces (Nielsen, 1981).

Chemical control

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

The introduction of systemic fungicides in the 1960's prompted several researchers to study their efficacy against potato tuber diseases. During the past 20 years potato growers have relied heavily on the benzimidazole fungicides, benomyl and thiabendazole (TBZ), which are available in various formulations. Of these, especially TBZ was found to be effective for the control of Fusarium dry rot in seed and stored potatoes (Cayley, Hide, Lord, Austin & Davies, 1979; Carnegie *et al.*, 1990). These fungicides are 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 is 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). Hide & Cayley (1980 & 1985) clearly demonstrated the importance of avoiding a delay in treating tubers with fungicides after injury and possible infestation by dry-rot pathogens. Recently resistance to TBZ, the only registered post-harvest fungicide in most potato producing countries, has been reported (Langerfeld, 1986; Tivoli, Deltour, Molet, Bedin & Jouan, 1986; Carnegie *et al.*, 1990; Langerfeld, 1990; Hide, Read & Hall, 1992; Von Stachewicz, Burth & Rathke, 1992; Desjardins, Christ-Harned, McCormick & Secor, 1993; Kawchuk, Holley, Lynch

& Clear, 1994; Hanson *et al.*, 1996). This is likely to alter potato growers' practices for Fusarium dry rot control (Desjardins *et al.*, 1993).

Biological control

Breeding for enhanced disease-resistant potato cultivars, including resistance to Fusarium dry rot, has become important and receives attention throughout the world. However, none of the commonly grown potato cultivars are immune to Fusarium dry rot (Moore, 1945; Boyd, 1952a; Leach & Webb, 1981; Seppänen, 1981c). Because of this and the general public concern about pesticide residues in food products and in the environment, research on alternative control strategies for fungal diseases of potatoes has been stimulated (Desjardins *et al.*, 1993).

Biological control of post-harvest diseases of fruits and vegetables has proven to be feasible in numerous studies (Wisniewski & Wilson, 1992). Thus far, biological control measures have not been used against Fusarium dry rot even though promising results have been reported, using microbial antagonists (Schisler & Slininger, 1994). Burr, Schroth & Suslow (1978) reported an increase in yield after seed pieces were treated with specific strains of *Pseudomonas fluorescens* and *P. putida*, but during their study, the effect of the bacterial antagonists against *Fusarium* pathogens was not tested. Schisler & Slininger (1994), however, showed that strains belonging to the genera *Pseudomonas, Enterobacter* and *Pantoea* could suppress dry rot incited by *F. sambucinum*. Promising results were found when *P. fluorescens* or *E. cloacae* were tested on potatoes under commercial storage conditions (Schisler, Kleinkopf, Slininger, Bothast & Ostrowski, 1997). Effective biological control methods are needed, not to replace management practices already in place, but to fill the gaps in a holistic management strategy (Powelson *et al.*, 1993).

CONCLUSIONS

The increase in Fusarium dry rot since the beginning of this century is a classical example of how a pathogen can develop into an economic problem by changed production methods. The increasing mechanisation of potato production and storage, resulted in greater numbers of damaged potato tubers and the subsequent increase in Fusarium dry rot. It is, therefore, important to approach the Fusarium dry-rot problem time and again, because production practices are changing, in spite of the fact that the "older knowledge" is still correct and relevant. The ability

to technically and commercially realize the phytopathological knowledge in the potato production usually poses a problem.

Fusarium dry rot of potatoes probably causes greater losses in storage and transit than any other post-harvest disease. The numerous reports on this disease show that this problem is far greater in the seed potato industry than in the commercial sector. The way potatoes are treated prior to marketing already reflects the cause of the problem. Potatoes for commercial use, are stored for much shorter periods than seed potatoes. Furthermore, commercial potatoes are normally graded once, whereas seed potatoes are graded for size and diseased tubers after harvest, and again after storing before planting, for fungicide treatments and/or cutting into seed pieces.

Knowledge of the production practices from harvest to planting is of great importance in understanding the infection cycle of the *Fusarium* spp. With each renewed tuber damage, the potential for infection increases. The planting of infected seed tubers also serves as a source of infection of the soil, as well as the progeny. Assuming that potato production without tuber damage will not be possible within the foreseeable future, the first alternative would be to control the pathogens directly, using chemicals.

For more than half a century, such attempts have given varying results. Relatively good control was achieved, using mercury-compounds. Since banning the use of these compounds, new fungicides have become available. Of these, TBZ has proven very effective for the control of Fusarium dry rot in seed and stored potatoes. Recently, however, field resistance to this compound was observed in some of the *Fusarium* spp. associated with dry rot. This, as well as the general public concern about pesticide residues in food products and in the environment, pose new challenges to researchers to find new methods of controlling Fusarium dry rot.

Apart from chemical control strategies, there are agronomic measures that are more important than the "easy" way of combatting a disease chemically. The breeding of skin-fast cultivars is just as important as breeding for physiological resistance. The breeding of cultivars that are more resistant to mechanical damage should be given priority, as this factor is equally effective against all fungal and bacterial wound pathogens.

Although a great deal of research has been done on Fusarium dry rot, there is still a great deal of controversy, especially on the nomenclature of the *Fusarium* spp. associated with the disease. More research is also needed on the mechanisms of infestation, as well as the physiologically resistant effects of tubers. The fact that *Fusarium* spp. can produce mycotoxins

in infected potato tubers should be clarified in order to eliminate any possible health risk to the consumer.

Unfortunately there is no short cut for the control of Fusarium dry rot and the only effective approach will be a holistic approach. To be able to follow a holistic approach, it is of the utmost importance that one should understand the disease, which may differ between countries, and also take into account the specific agronomic practices followed in and the specific needs for any particular country.

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Table 1. *Fusarium* species reported as dry rot pathogens of potato tubers in the potato producing countries of the world

Fusarium species[a]	References[b]	Synonyms	References[b]
F. acuminatum Ell. & Ev.	13; 28; 32	*F. scirpi* Lambotte & Fautr. var. *acuminatum* (Ell. & Ev.) Wollenw.	27; 28
F. avenaceum (Fr.) Sacc.	6; 7; 13; 18; 20; 21; 22; 29	*F. avenaceum* (Corda) Fr. Sacc. *F. arthrosporioides* Sherb. *F. roseum* Lk. emend. Snyd. & Hans. var. *avenaceum* (Sacc.) Snyd. Hans. *F. subulatum* Appel & Wollenw.	3; 4; 11; 28; 31 4; 6; 7; 20; 22; 24; 28; 29 23 41
F. culmorum (W.G. Smith) Sacc.	5; 6; 7; 11; 13; 28; 29	*F. roseum* Lk. emend. Snyd. & Hans. var. *culmorum* (Schwabe) (sic.) Snyd. & Hans. *F. rubiginosum* Appel & Wollenw.	3; 27; 31; 33; 34 41
F. crookwellense Burgess, Nelson, Toussoun	13; 18; 32		
F. equiseti (Corda) Sacc.	3; 6; 13; 19; 25; 28; 32		
F. flocciferum Corda	3; 27; 28; 31		
F. lateritium Nees	26		
F. graminearum Schwabe	27; 28; 29; 32	*F. roseum* Lk. emend. Snyd. & Hans. var. *graminearum* (Schwabe) Snyd. & Hans.	27; 33; 34
F. merismoides Corda	10; 28		
F. oxysporum Schlecht. emend. Snyd. & Hans.	6; 7; 13; 19; 28; 29; 30; 32; 36; 37; 40	*F. redolens* Wollenw. *F. orthoceras* Appel & Wollenw. *F. oxysporum* Schlecht.	27; 28; 31 41 4; 5; 8; 14; 20; 21; 24; 38; 39
F. sambucinum Fuckel	4; 11; 13; 17; 19; 28; 29; 32	*F. discolor* var. *sulphureum* (Schlecht.) Appel & Wollenw. *F. roseum* Lk. emend. Snyd. & Hans. var. *sambucinum* (Fuckel) Snyd. & Hans. *F. sulphureum* Schlecht. *F. trichothecoides* Wollenw.	5; 42 23; 33; 34; 35 3; 4; 17; 27; 28; 39 4; 5; 15; 16; 17; 18; 28; 29; 40; 41
F. scirpi Lambotte & Fautr.	32	*F. gibosum* Appel & Wollenw.	41; 42

F. semitectum Berk. & Rav.	2; 19	*F. roseum* Lk. emend. Snyd. & Hans. var. *arthrosporioides* (Sherb.) Messiaen & Cassini pro parte	33; 34; 35
F. solani (Mart.) Appel & Wollenw. emend. Snyd. & Hans.	6; 7; 13; 19; 32; 36; 37	*F. coeruleum* (Libert) Sacc. *F. eumartii* Carpenter *F. javanicum* Koord. var. *radicicola* Wr. *F. solani* (Mart.) Sacc. var. *coeruleum* (Libert) Bilai *F. solani* (Mart.) Sacc. var. *coeruleum* (Sacc.) Booth comb. nov. *F. solani* (Mart.) Sacc. *F. solani* (Mart.) App. & Wr. var. *eumartii* (Carp.) Wr. *F. ventricosum* Appel & Wollenw.	4; 5; 9; 16; 20; 22; 24; 29; 30; 40; 41; 42 5; 23; 24; 40 42 7; 11; 18; 28; 29; 33; 34; 35 3 4; 5; 6; 17; 24; 28 12; 20 5; 41; 42
F. sporotrichioides Sherb.	1; 4; 19; 27; 28; 29; 39		
F. tricinctum (Corda) Sacc.	4; 20; 27; 28; 29		

[a] Classified according to the taxonomic system of Nelson *et al.*, (1983).

[b]Authors: 1 = Abdel-Moniem (1977); 2 = Ayers (1972); 3 = Booth (1971); 4 = Boyd (1972); 5 = Carpenter (1915); 6 = Chambers (1973); 7 = Chambers & Millington (1974); 8 = Chona (1932); 9 = Foister (1940); 10 = Førsund (1980); 11 = Gindrat & Pilloud (1985); 12 = Goss (1940); 13 = Hanson *et al.*, 1996; 14 = Hide (1986b); 15 = Hornok (1982); 16 = Jamieson & Wollenweber (1912); 17 = Jeffries, Boyd & Patterson (1984); 18 = Logrieco, Frisulla & Bottalico (1987); 19 = Manici & Cerato (1992); 20 = McKee (1952); 21 = McKlean & Walker (1941); 22 = Moore (1945); 23 = Nielsen (1981); 24 = Pethybridge & Lafferty (1917); 25 = Rai (1979); 26 = Savor (1986); 27 = Seppänen (1980); 28 = Seppänen (1981a); 29 = Seppänen (1983b); 30 = Sherbakoff (1915); 31 = Stubbs (1971); 32 = Theron & Holz (1989); 33 = Tivoli, Conbière & Jouan (1983a); 34 = Tivoli & Jouan (1981); 35 = Tivoli, Jouan & Lemarchand (1983b); 36 = Tivoli, Torres & French (1988); 37 = Turkensteen (1987); 38 = Upstone (1970a); 39 = Upstone (1970b); 40 = Weiss *et al.*, (1928); 41 = Wollenweber (1913); 42 = Wollenweber & Reinking (1935).

CHAPTER 2

FUSARIUM SPECIES ASSOCIATED WITH DRY ROT AND STEM-END ROT OF POTATOES IN SOUTH AFRICA

ABSTRACT

The predominant fungi isolated from dry-rot and stem-end-rot lesions of potato tubers, collected from 74 farms in eight major potato production regions of South Africa, were members of the genus *Fusarium*. Eighteen *Fusarium* species were isolated, either singly or in combination. In some cases, as many as four species were isolated from a single lesion. *Fusarium oxysporum* and *F. solani* were the predominant species isolated from both dry-rot and stem-end-rot lesions. Nine species (*F. oxysporum, F. solani, F. sambucinum, F. acuminatum, F. crookwellense, F. graminearum* Gr. I, *F. culmorum, F. scirpi* and *F. equiseti*) caused typical dry-rot lesions on artificially inoculated potato tubers. 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.

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INTRODUCTION

Fusarium dry rot is mostly associated with a post-harvest disease of potato (*Solanum tuberosum* L.) tubers (Nielsen, 1981). Fusarium dry rot seldom reaches epidemic proportions (Booth, 1971). Although comprehensive figures are lacking, dry rot probably causes greater losses in storage and transit of both seed and commercial potatoes, than any other post-harvest disease (Powelson, Johnson & Rowe, 1993). Average annual crop losses attributed to Fusarium dry rot have been estimated at between 6 and 25% (Foister, 1940; Chełkowski, 1989). However, reports that more than 60% of tubers in storage can be affected, are not uncommon (Carnegie, Ruthven, Lindsay & Hall, 1990).

Many *Fusarium* spp. have been associated with tuber rots of the potato (McKee, 1952; Boyd, 1972; Chambers, 1973; Seppänen, 1981; Tivoli & Jouan, 1981; Manici & Cerato, 1992; Savor & Maček, 1994; Hanson, Schwager & Loria, 1996). Fusarium dry rot and stem-end rot occur commonly in South Africa. During a survey carried out by Theron & Holz (1989) in the two major dryland potato production regions of South Africa, *Fusarium solani* (Mart.) Appel & Wollenw. emend. Snyd. & Hans. and *Fusarium oxysporum* Schlecht. emend. Snyd. & Hans. were found to be the major species responsible for the dry-rot and stem-end-rot problems experienced in these regions. Various other *Fusarium* species were also associated with these diseases and the Fusarium dry-rot complex in these regions differed from those in other parts of the world. Therefore, it is essential to extend the survey to the other important potato production regions, mainly those under irrigation. A clear picture of the Fusarium dry-rot and stem-end-rot situation in South Africa is essential to develop a strategy to control the problem. This paper reports on the *Fusarium* spp. isolated during a survey and their relative importance in the dry-rot disease complex in South Africa.

MATERIALS AND METHODS

Collection of samples

Potato tubers, with dry-rot lesions (Fig. 1) and stem-end-rot lesions (Fig. 2) were sampled at sorting tables in the Northern Province (8 farms), North West (8 farms), Free State (10 farms in the Western Free State), KwaZulu-Natal (9 farms), Eastern Cape (9 farms), Western Cape (12

farms in the Ceres and 10 farms in the Sandveld) and Northern Cape (8 farms) (Fig. 3). Tubers were kept in paper bags at 5°C and 50 - 70% relative humidity until isolations were made. Samples consisted mainly of two cultivars, Bp-1 and Up-to-Date, which are the main cultivars produced in South Africa.

Isolation and identification of micro-organisms

Tuber samples were washed, surface-disinfested (3% sodium hypochlorite for 15 min) and allowed to dry. Tubers were cut in half, and five discs (2 mm^3) were randomly 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 lights: 12 h cycles) (Nelson, Toussoun & Marasas, 1983). Developing colonies were examined with a light microscope (10X) and organisms other than *Fusarium* identified directly. Single-conidial isolates of each separate *Fusarium* species were obtained by using a modification of the technique of Nelson *et al.* (1983) as previously described (Theron & Holz, 1989). After 10 - 14 days incubation, on divided Petri dishes containing PDA in the one half and carnation-leaf agar (CLA) (Fisher, Burgess, Toussoun & Nelson, 1982) in the other, the *Fusarium* spp. were identified. All the species were identified according to the system of Nelson *et al.* (1983) with the exception of *F. compactum* (Wollenw.) Gordon, *F. nygamai* Burgess & Trimboli, *F. acuminatum* subsp. *armeniacum* Forbes, Windels & Burgess, and *F. polyphialidicum* Marasas, Nelson, Toussoun & Van Wyk which were identified according to Gordon (1952), Burgess & Trimboli (1986), Burgess, Forbes, Windels, Nelson, Marasas & Gott (1993) and Marasas, Nelson, Toussoun & Van Wyk (1986), respectively. Conidia from two representative isolates of each species per sample were mass-transferred to CLA slants, incubated as described previously until sporulation occurred, and lyophilized.

The frequency and relative density of the *Fusarium* spp. were calculated according to McMullen & 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 were statistically analysed by 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*:** Five isolates of each *Fusarium* species, obtained from different farms, were selected at random from each of the eight production regions. Five pathogenic *Fusarium* isolates of each species from the previous survey (Theron & Holz, 1989) were also included for comparative purposes. Inoculum was prepared by transferring lyophilized conidia to CLA. After incubation at 25 ±2°C for 2 wk under intermittent light (Nelson *et al.*, 1983), conidia were washed from cultures with sterile distilled water, counted in a haemacytometer, and the suspension diluted to 1 x 10^4 conidia/ml. Sound, unblemished potato tubers, cv. Up-to-Date, selected at harvest from plants grown under commercial conditions at the Roodeplaat Vegetable and Ornamental Plant Institute were disinfested for 15 min in 3% sodium hypochlorite and allowed to dry. Twenty tubers (75-150 g each) were inoculated approximately halfway between the rose and heel ends, 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). Control tubers were injected with sterile distilled water. Tubers were wrapped in paper bags and kept at 25 ±2°C and 50-70% relative humidity to promote dry-rot development (Theron & Holz, 1987).

Disease assessment

After 3 wk of incubation, tubers were cut in half through the inoculation site and the extent of Fusarium dry-rot development determined with the disease index scale used by Theron & Holz (1987). Results were expressed as percentage decay according to the method of Kremer & Unterstenhöfer (1967). 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.

RESULTS

Collection of samples

Potato tuber samples with Fusarium dry-rot symptoms (mean 11.1 tubers/farm) were obtained from all 74 farms in the eight potato production regions sampled. Samples with stem-end-rot symptoms were less abundant (mean 4.5 tubers/farm) and could be obtained only from 41 of the farms visited. In the Eastern Cape and the Sandveld regions, stem-end rot was virtually absent. Only eight tubers were obtained from two farms and three tubers from one farm, in these two production regions, respectively.

Isolation and identification of micro-organisms

Tuber samples with dry-rot and those with stem-end-rot symptoms, collected at 74 farms in the eight production regions, were significantly ($P \leq 0.05$) more frequently and also at significantly ($P \leq 0.05$) higher densities colonised by *Fusarium* spp. than the other organisms (Tables 1; 2). In the Northern Cape, however, *Fusarium* spp. were not isolated at significantly ($P \leq 0.05$) higher frequencies or densities than *Alternaria* spp., common saprophytes, *Gliocladium roseum* Bainer and bacteria from dry-rotted potato tubers (Table 1). The frequencies and relative densities at which *Fusarium* spp. were isolated from tubers with stem-end-rot symptoms, sampled in the KwaZulu Natal, Sandveld and Northern Cape production regions, were also not significantly ($P \leq 0.05$) higher than those at which some of the other organisms were isolated (Table 2).

G. roseum was isolated from tubers in all eight production regions, but was not equally abundant in every region. The highest incidence of *G. roseum* was found in the Northern Cape followed by the Northern Province. *Phoma* spp., were seldom isolated. *Alternaria* spp. were frequently isolated at relatively high densities, especially from tuber samples obtained from farms in Ceres and the Northern Cape. Except for the *Fusarium* spp., *Alternaria* spp. were isolated at significantly ($P \leq 0.05$) higher frequencies and relative densities than most of the other organisms, irrespective of isolation from tubers with dry-rot or stem-end-rot lesions (Tables 1; 2).

The *Fusarium* spp. isolated represented the following 18 species: *F. solani*, *F. oxysporum*, *F. sambucinum* Fuckel, *F. equiseti* (Corda) Sacc., *F. scirpi* Lambotte & Fautr., *F. acuminatum* Ell. & Ev., *F. crookwellense* Burgess, Nelson & Toussoun, *F. graminearum* Schwabe Gr. I, *F.*

nygamai, *F. compactum*, *F. culmorum* (W. G. Smith), *F. chlamydosporum* Wollenw. & Reinking, *F. proliferatum* (Matsushima) Nirenberg, *F. reticulatum* Mont., *F. polyphialidicum*, *F. moniliforme* Sheldon, *F. merismoides* Corda, and *F. acuminatum* subsp. *armeniacum*. These species were isolated either singly or in combination. In some cases as many as four *Fusarium* spp. were isolated from a single lesion (results not shown). *Fusarium oxysporum* was most frequently isolated as a single species from tubers with dry-rot lesions (26.6%) and stem-end-rot lesions (28.5%), followed by *F. solani* (10.7 and 22.8%), respectively. These species also occurred as the predominant combination pair followed by the combination of *F. oxysporum* and *F. equiseti*, (20 and 18.9%) from dry-rot lesions and (18.9 and 8.1%) from stem-end-rot lesions, respectively.

Where more than one *Fusarium* sp. was isolated from a single tuber, dry rot or stem-end rot were attributed to the species with the highest density (Tables 3; 4). Using these criteria, *F. oxysporum* and *F. solani* were isolated at significantly ($P \leq 0.05$) higher frequencies (±52 and 30%) and relative densities (± 51 and 31%), respectively, than the other *Fusarium* spp. In cases where *F. merismoides* were isolated, neither dry rot or stem-end rot could be attributed to this species according to the criteria used. These two species were followed by *F. equiseti*, *F. acuminatum*, *F. nygamai*, *F. crookwellense*, *F. scirpi*, *F. culmorum* and *F. sambucinum*, irrespective of the production region.

Pathogenicity tests

***Single-isolate inoculations*:** Nine of the 18 *Fusarium* spp. recovered from the periphery of discoloured tissue of potato tubers with either dry rot or stem-end rot caused typical dry-rot symptoms on artificially inoculated tubers (Table 5). Except for *F. culmorum*, which was identified for the first time as a dry-rot pathogen of potatoes in South Africa, pathogenicity of the other eight species has been reported previously (Theron & Holz, 1989). Virulence varied from 15.9 to 87.8% (mean 50.1 ±19.1%) for the nine pathogenic species. The virulence of the *F. acuminatum* isolates was the most variable, i.e. 30.5 to 87.8% (mean 51.4 ±16.5%), whereas the isolates of *F. oxysporum* and *F. scirpi* were less variable, i.e. 56.1 to 85.4% (mean 70.3 ±7.7%) and 15.9 to 36.6% (mean 26.8 ±6.4%), respectively, of all the pathogenic species (Table 5).

DISCUSSION

The ease with which potato tuber samples with dry-rot lesions were obtained from all eight production regions, was unexpected in view of the fact that 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). This finding highlights the importance of this disease in South Africa, as one would expect an increase in Fusarium dry rot when tubers are stored for prolonged periods. In South Africa, however, potatoes are produced throughout the year, albeit in the different production regions. Owing to this, there is no need to store potatoes, except for seed potatoes. No figures are available for losses due to Fusarium dry rot in respect of potatoes sold to consumers. One can only predict that these losses would be high if the potatoes have been stored.

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. These findings could not be ascribed to cultivar differences. The samples, irrespective of the region, consisted either of the cultivar Bp-1 or Up-to-Date. In both these instances, samples were taken from winter plantings and it would appear as if the incidence of stem-end rot is less prevalent during the cooler seasons. According to Theron & Holz (1989), who conducted their survey 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 the irrigation regions, where the present survey was conducted, tubers are lifted soon after senescence which may also explain the fact that tuber samples with stem-end-rot lesions were not commonly obtained from these regions. These results would support the view of Pethybridge & Lafferty (1917) who showed that the longer tubers are left in the soil after senescence, the greater the probability that pathogens will enter via the senescent stolons.

G. roseum, which is also known to be associated with dry rot of potatoes (Theron & Holz, 1991), was isolated from all eight production regions, but was not equally abundant in every region. The highest incidence of *G. roseum* was found in the Northern Cape. The reason for the high incidence of *G. roseum* is not known. *Phoma* spp., including *P. exigua* Desm. var. *foveata*

(Foister) Boerema and *P. exigua* Desm. var. *exigua* Malcomson & Gray causing gangrene of potato tubers (Boyd, 1972; Seppänen, 1983) which can easily be confused with Fusarium dry rot, were seldom isolated. Gangrene usually develops during storage, especially during cold storage (Boyd, 1972). In South Africa a serious outbreak of gangrene occurred in 1973 on cold stored potatoes, but since then very few cases of gangrene on potatoes have been reported (Young, personal communication). One might, therefore, conclude that gangrene is not a problem in the production of potatoes in South Africa at present.

Several *Fusarium* spp. have been associated with dry rot of potatoes and *F. solani, F. sambucinum* (*F. sulphureum* Schlecht.), *F. trichothecioides* Wollenw. (*F. sambucinum*) and *F. avenaceum* (Fr.) Sacc. are known to be pathogens of potato tubers throughout the world (Wollenweber & Reinking, 1935; Boyd, 1972; Seppänen, 1981; Tivoli & Jouan, 1981). *Fusarium oxysporum* and *F. culmorum* are the dominant *Fusarium* spp. causing dry rot in Australia (Chambers, 1973). Recently *F. oxysporum* was also found to be an important *Fusarium* sp. associated with dry rot of potatoes in the northeastern United States (Hanson *et al.*, 1996) and Italy (Manici & Cerato, 1992). The present survey as well as the previous one (Theron & Holz, 1989), indicates that a different Fusarium dry-rot situation occurs in the potato production regions of South Africa. In South Africa some of the major *Fusarium* spp., associated with dry rot of potatoes, are of minor importance elsewhere and *vice versa*. Theron & Holz (1990) also reported that the South African isolates in general, require higher temperatures for optimal dry-rot development.

Fusarium spp. were isolated either singly or in combination from diseased tubers. When more than one *Fusarium* sp. was isolated from a single tuber, designation of the primary causal pathogen was obscured. Seppänen (1983) gave each *Fusarium* sp. isolated an equal share in disease development. In our survey it was assumed that the *Fusarium* sp. most frequently isolated from the discoloured tissue of a tuber was the major pathogen. This assumption was supported by the results of the mixed-species inoculations and the recovery of the more virulent *Fusarium* spp. in higher densities (Theron & Holz, 1989). Using these criteria *F. 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 is also supported by the pathogenicity tests. The fact that a higher percentage of Fusarium dry rot occurred when *F. solani* and *F. oxysporum* were inoculated as a mixture than when they were inoculated singly (Theron & Holz, 1989),

indicates that these two species may act synergistically to cause dry rot. Alternatively, that their mechanisms of colonising tuber tissue might differ. Hyphae of *F. solani* are first intercellular, becoming intracellular in dead cells, whereas *F. avenaceum* (McKee, 1954), *F. culmorum* and *F. sambucinum* (Langerfeld, 1978) immediately kill, penetrate and colonize the cells surrounding the point of infection. If *F. oxysporum* does function in the same manner as *F. avenaceum*, *F. culmorum* and *F. sambucinum*, it might explain the possible synergistic action with *F. solani*.

The following *Fusarium* spp. were isolated from potato tubers for the first time in South Africa during this survey: *F. proliferatum*, *F. polyphialidicum*, *F. merismoides*, *F. culmorum* and *F. acuminatum* subsp. *armeniacum*. Thus a total of 20 *Fusarium* taxa have been associated with the Fusarium dry-rot complex in South Africa. *Fusarium subglutinans* (Wollenw. & Reinking) Nelson, Toussoun & Marasas and *F. graminearum* Gr. II, which were previously isolated by Theron & Holz (1989), was not isolated during this survey. *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 & Hill (Vegetable and Ornamental Plant Institute, Roodeplaat, unpublished data) previously isolated *F. merismoides* and *F. avenaceum* from potato tubers. During this survey *F. merismoides* was mainly isolated in combination with *F. oxysporum*, but the pathogenicity of this species could not be confirmed. In contrast, Førsund (1980) and Seppänen (1981) reported *F. merismoides* as a dry-rot pathogen. Although *F. avenaceum* was not isolated during this and the previous survey (Theron & Holz, 1989), it has been reported to be an important dry-rot pathogen elsewhere (Boyd, 1972; Sëppanen, 1981).

A more diverse *Fusarium* population was isolated from tubers with dry-rot lesions than from those with stem-end-rot lesions. This might be attributed to Fusarium dry-rot infection occurring mainly through wounds, while stem-end-rot infection is more complex. It is, therefore, mainly specialised species that are able to infect the intact tuber (Boyd, 1972). *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 in general had no direct relationship to the *Fusarium* populations in the soil or on dry rot, and that contaminated seed tubers are considered as the primary source of infection. However, there appears to be a relationship between the differences in the occurrence of some of the *Fusarium* spp. in the

production regions, found in the present study, that may be ascribed to the type of crop rotation and climatic conditions. For instance, *F. crookwellense* and *F. graminearum* mainly occurred in the regions where either maize or wheat are rotated with potatoes. In a survey carried out by Van Wyk, Los, Pauer & Marasas (1987), *F. culmorum* was isolated mainly from diseased crowns of wheat grown in the Western Free State and not from wheat grown in the Eastern Free State. Similarly, *F. culmorum* was isolated only from potato tubers originating 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 because, it is known that this species can cause post-harvest rotting of onions (Naudé & Jooste, 1989).

The knowledge gained by this study about 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. For example, all potato breeding lines are currently screened against *F. solani* and *F. oxysporum* for dry-rot resistance, whereas in the past only *F. solani* was included. Fungicides should also be evaluated for their efficiency in controlling dry rot caused by all the predominant *Fusarium* spp.

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Table 1. Frequency and relative density of organisms in lesions of potato tubers with dry rot, collected in eight potato producing regions of South Africa[a]

Organism	Frequency (%)[b]									Relative density (%)[c]								
	Regions[d]								Mean	Regions[d]								Mean
	NP	NW	WFS	KZN	EC	CE	SV	NC	%	NP	NW	WFS	KZN	EC	CE	SV	NC	%
Fusarium spp.	88.6	90.3	78.8	95.1	97.7	75.7	97.9	33.4	82.6	75.7	67.7	53.4	75.1	82.6	46.0	82.8	24.7	63.2
Gliocladium roseum	14.0	7.2	5.8	8.9	3.3	9.6	2.0	24.9	9.1	4.3	1.9	3.5	3.4	0.5	3.2	0.9	14.1	3.8
Phoma spp.	0	2.5	2.6	1.6	0	2.8	0	10.4	2.4	0	0.6	0.5	0.7	0	1.0	0	3.1	0.7
Alternaria spp.	3.7	15.1	12.6	4.6	1.1	58.5	0	42.6	18.5	0.9	3.9	4.1	1.1	0.6	27.3	0	20.3	7.9
Rhizoctonia spp.	14.4	11.3	15.4	1.6	0.9	3.7	0.8	4.8	6.4	7.0	6.3	7.2	0.6	0.3	2.1	0.1	1.9	3.1
Colletotrichum spp.	2.1	0	2.6	4.4	2.2	14.5	2.1	8.0	4.9	1.1	0	0.8	0.8	0.8	4.7	0.6	1.8	1.4
Common saprophytes	14.6	12.6	24.7	8.0	6.7	16.4	30.1	35.4	18.6	5.9	7.3	10.5	3.6	1.8	6.3	10.6	17.9	7.9
Bacteria	9.2	22.6	20.6	36.7	28.4	27.4	16.5	21.4	23.1	5.1	9.7	6.2	14.7	13.5	8.9	4.9	14.9	9.5
No growth	0	11.0	27.2	0	0	1.2	0	6.1	5.7	0	4.3	13.8	0	0	0.5	0	1.4	2.5
n	89	65	101	88	86	189	120	86	824	519	360	549	480	581	1117	784	465	4855
F-value ($P \leq 0.05$)	66.6	36.7	21.2	68.6	143.7	31.9	53.6	3.9	229.6	138.9	40.5	27.7	243.1	272.9	28.2	112.1	3.8	378.2
$LSD_{(T)}$ 0.05	9.6	12.8	14.4	10.6	7.5	13.1	12.4	20.3	4.6	5.8	9.5	8.8	4.4	4.6	8.2	7.2	13.2	2.8

[a]Surface-disinfested tubers (3% sodium hypochlorite, 15 min), collected at sorting tables, were cut in half and five discs (2 mm^3) randomly dissected from the periphery of discoloured tissue and plated on potato dextrose agar.

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

[c]Relative density = [Total number of isolates of a certain organism/total number (n) of isolates] x 100.

[d]Regions: NP = Northern Province (8 farms); NW = North West (8 farms); WFS = Western Free State (10 farms); KZN = KwaZulu-Natal (9 farms); EC = Eastern Cape (9 farms); CE = Ceres (12 farms); SV = Sandveld (10 farms) and NC = Northern Cape (8 farms).

Table 2. Frequency and relative density of organisms in lesions of potato tubers with stem-end rot, collected in eight potato producing regions of South Africa[a]

Organism	Frequency (%)[b]									Relative density (%)[c]								
	Regions[d]								Mean %	Regions[d]								Mean %
	NP	NW	WFS	KZN	EC	CE	SV	NC		NP	NW	WFS	KZN	EC	CE	SV	NC	
Fusarium spp.	92.9	80.0	78.2	68.3	100.0	93.7	100.0	58.7	81.8	76.6	64.8	57.6	51.7	76.5	63.7	61.2	31.8	58.1
Gliocladium roseum	6.5	16.7	27.8	1.5	0	1.7	0	21.0	10.4	1.2	1.2	4.5	0.3	0	0.7	0	15.7	4.1
Phoma spp.	0	0	0	1.5	0	2.8	0	0	0.9	0	0	0	0.3	0	1.9	0	0	0.5
Alternaria spp.	1.1	10.3	15.7	2.8	0	36.6	0	31.4	19.1	0.5	3.9	4.1	0.5	0	13.6	0	18.6	8.2
Rhizoctonia spp.	11.7	6.7	13.3	3.3	0	4.0	0	1.4	5.8	3.6	3.3	6.1	0.9	0	2.0	0	0.3	2.3
Colletotrichum spp.	7.0	0	2.0	0	0	18.5	8.4	7.3	8.2	0.7	0	0.8	0	0	5.7	3.0	1.8	2.2
Common saprophytes	26.5	20.0	35.1	24.4	0	9.7	50.0	28.3	23.2	6.6	8.2	10.0	8.6	0	3.5	30.0	13.3	8.8
Bacteria	21.8	23.3	14.9	51.0	66.7	17.7	25.0	21.5	24.0	9.4	7.5	6.0	36.3	23.5	8.2	5.9	13.8	12.2
No growth	0	13.3	28.0	5.4	0	3.6	0	16.7	9.1	0	11.0	10.8	1.6	0	0.6	0	6.1	3.6
n	45	30	38	40	3	120	8	18	302	264	155	208	262	17	705	49	183	1843
F-value ($P \leq 0.05$)	37.7	17.3	4.8	15.6	-[e]	56.5	3.3	3.4	45.7	84.4	30.6	23.6	17.3	-	99.1	2.9	2.8	75.4
$LSD_{(T)}$ 0.05	13.6	17.4	30.7	18.6	-	11.0	60.3	27.6	8.2	7.7	11.0	10.5	13.4	-	5.7	39.8	17.6	4.7

[a]Surface-disinfested tubers (3% sodium hypochlorite, 15 min), collected at sorting tables, were cut in half and five discs (2 mm^3) randomly dissected from the periphery of discoloured tissue and plated on potato dextrose agar.

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

[c]Relative density = [Total number of isolates of a certain organism/total number (n) of isolates] x 100.

[d]Regions: NP = Northern Province (7 farms); NW = North West (3 farms); WFS = Western Free State (5 farms); KZN = KwaZulu-Natal (4 farms); EC = Eastern Cape (1 farm); CE = Ceres (11 farms); SV = Sandveld (2 farms) and NC = Northern Cape (8 farms).

[e]Not estimable

Table 3. Frequency and relative density of *Fusarium* spp. in lesions of potato tubers with dry rot, collected in eight potato producing regions of South Africa[a]

Fusarium spp	Frequency (%)[b]									Relative density (%)[c]								
	Regions[d]								Mean %	Regions[d]								Mean %
	NP	NW	WFS	KZN	EC	CE	SV	NC		NP	NW	WFS	KZN	EC	CE	SV	NC	
F. solani	40.5	22.3	31.5	25.8	37.1	16.8	7.6	5.2	23.5	42.4	27.4	31.2	24.6	35.1	17.2	7.7	7.6	24.1
F. oxysporum	52.5	63.0	41.0	65.3	57.1	37.8	82.6	60.5	56.5	50.2	61.9	44.9	68.8	59.6	41.2	81.8	61.9	58.1
F. sambucinum	0	1.1	2.0	0	1.1	12.2	1.4	0	2.8	0	0.6	0.7	0	1.3	12.0	1.5	0	2.5
F. equiseti	4.4	4.3	4.1	2.4	2.7	5.6	4.1	16.8	5.1	4.4	4.4	4.6	2.1	3.0	8.8	6.8	15.6	6.0
F. scirpi	0.9	1.3	0.6	0	0	1.0	0.8	0	0.6	0.9	1.3	0.9	0	0	1.1	0.8	0	0.7
F. acuminatum	0	2.2	3.3	0	0	17.4	0	4.9	4.0	0	1.9	1.1	0	0	11.3	0	4.0	2.6
F. crookwellense	0	0	2.7	6.5	1.1	6.6	0	0	2.4	0	0	4.2	4.6	0.8	3.8	0	0	1.9
F. graminearum Gr. I	0	1.2	1.1	0	0	0	0	0	0.3	0	0.7	1.2	0	0	0	0	0	0.2
F. nygamai	1.7	0	1.1	0	0	0	2.0	1.0	0.7	2.2	0	1.2	0	0	0	1.0	0.7	0.5
F. compactum	0	0	1.1	0	0	0.4	0	8.3	0.9	0	0	0.4	0	0	0.3	0	8.3	0.8
F. culmorum	0	1.0	5.7	0	0	0	0.7	0	1.0	0	0.3	5.9	0	0	0	0.2	0	0.9
F. chlamydosporum	0	1.3	0.6	0	0	0	0	3.3	0.5	0	0.7	0.2	0	0	0	0	2.0	0.3
F. proliferatum	0	0	0	0	0	0.4	0	0	0.1	0	0	0	0	0	0.6	0	0	0.1
F. reticulatum	0	0	1.0	0	0	2.0	0	0	0.5	0	0	0.3	0	0	3.8	0	0	0.7
F. polyphialidicum	0	0	2.0	0	0	0	0.7	0	0.4	0	0	1.4	0	0	0	0.2	0	0.2
F. moniliforme	0	1.4	1.0	0	0.9	0	0	0	0.4	0	0.4	1.1	0	0.2	0	0	0	0.2
F. a. subsp. *armeniacum*	0	1.0	1.1	0	0	0	0	0	0.3	0	0.6	1.0	0	0	0	0	0	0.2
n	89	65	101	88	86	189	120	86	824	326	215	345	347	362	550	476	125	2746
F-value ($P \leq 0.05$)	40.3	15.5	28.2	60.3	27.7	12.0	121.2	8.9	192.0	44.0	16.1	34.8	115.5	28.2	13.1	81.8	9.5	206.8
$LSD_{(T)}$ 0.05	6.8	11.1	6.1	6.0	8.5	8.2	5.0	13.9	2.8	6.5	11.1	5.9	4.5	8.5	8.2	6.1	13.8	2.8

[a]Surface-disinfested tubers (3% sodium hypochlorite, 15 min), collected at sorting tables, were cut in half and five discs (2 mm^3) randomly dissected from the periphery of discoloured tissue and plated on potato dextrose agar.

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

[c]Relative density = [Total number of isolates of a certain organism/total number (n) of isolates] x 100.

[d]Regions: NP = Northern Province (8 farms); NW = North West (8 farms); WFS = Western Free State (10 farms); KZN = KwaZulu-Natal (9 farms); EC = Eastern Cape (9 farms); CE = Ceres (12 farms); SV = Sandveld (10 farms) and NC = Northern Cape (8 farms).

Table 4. Frequency and relative density of *Fusarium* spp. in lesions of potato tubers with stem-end rot, collected in eight potato producing regions of South Africa[a]

Fusarium spp.	Frequency (%)[b]									Relative density (%)[c]								
	Regions[d]								Mean %	Regions[d]								Mean %
	NP	NW	WFS	KZN	EC	CE	SV	NC		NP	NW	WFS	KZN	EC	CE	SV	NC	
F. solani	28.8	36.7	33.6	39.3	100.0	36.5	33.4	36.0	36.4	21.9	35.5	40.1	40.2	100.0	39.9	47.1	34.6	39.1
F. oxysporum	42.3	56.7	46.3	52.1	0	42.7	66.7	60.7	47.7	19.2	55.0	43.4	52.3	0	39.4	52.9	64.3	46.4
F. sambucinum	0	0	0	0	0	3.3	0	0	1.4	0	0	0	0	0	3.4	0	0	1.1
F. equiseti	4.7	6.7	7.0	0	0	4.2	0	3.3	3.5	10.7	9.5	3.8	0	0	4.4	0	1.1	3.4
F. scirpi	0	0	0	0	0	3.7	0	0	1.1	0	0	0	0	0	3.5	0	0	1.0
F. acuminatum	0	0	0	0	0	3.2	0	0	0.9	0	0	0	0	0	3.7	0	0	1.0
F. crookwellense	0	0	0	8.6	0	0.5	0	0	1.0	0	0	0	7.6	0	0.1	0	0	0.8
F. nygamai	20.7	0	0	0	0	4.5	0	0	5.1	14.7	0	0	0	0	4.5	0	0	4.6
F. compactum	3.6	0	0	0	0	0.7	0	0	0.9	7.6	0	0	0	0	0.2	0	0	0.6
F. culmorum	0	0	13.1	0	0	0	0	0	1.7	0	0	12.6	0	0	0	0	0	1.7
F. reticulatum	0	0	0	0	0	0.8	0	0	0.2	0	0	0	0	0	0.8	0	0	0.2
n	45	30	38	40	3	120	8	18	302	181	103	125	147	11	450	30	65	1112
F-value ($P \leq 0.05$)	9.4	6.4	13.0	5.7	-[e]	13.0	9.2	12.1	60.0	10.0	8.7	10.8	5.9	-	10.6	2.9	11.0	55.2
$LSD_{(T)}$ 0.05	13.0	22.3	12.7	22.2	-	11.8	22.1	16.6	5.9	12.8	18.6	14.4	22.2	-	13.1	37.0	18.1	6.2

[a]Surface-disinfested tubers (3% sodium hypochlorite, 15 min), collected at sorting tables, were cut in half and five discs (2 mm^3) randomly dissected from the periphery of discoloured tissue and plated on potato dextrose agar.

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

[c]Relative density = [Total number of isolates of a certain organism/total number (n) of isolates] x 100.

[d]Regions: NP = Northern Province (7 farms); NW = North West (3 farms); WFS = Western Free State (5 farms); KZN = KwaZulu-Natal (4 farms); EC = Eastern Cape (1 farm); CE = Ceres (11 farms); SV = Sandveld (2 farms) and NC = Northern Cape (8 farms).

[e]Not estimable.

Table 5. Mean percentage dry rot of potato tubers 3 wk after inoculation with different isolates of 18 *Fusarium* species isolated from dry-rot and stem-end-rot lesions[a]

Fusarium species	Number of isolates	MRC numbers[b]	% Decay[c]		
			Min.	Max.	Mean ± SD
F. solani	45	6358-6362	46.4	86.6	76.8[d] ±13.4
F. oxysporum	45	6379-6383	56.1	85.4	70.3 ±7.7
F. crookwellense	25	6444-6448	47.6	86.6	66.7 ±13.2
F. acuminatum	25	6449-6453	30.5	87.8	51.4 ±16.5
F. sambucinum	30	6454-6458	42.7	73.2	49.2 ±8.4
F. culmorum	15	6833-6837	28.7	71.7	47.1 ±9.7
F. graminearum Gr. I	15	4672-4673	24.4	64.7	42.0 ±10.2
F. scirpi	30	6838-6842	15.9	36.6	26.8 ±6.4
F. equiseti	45	6828-6832	15.9	51.2	20.2 ±8.5
F. nygamai	20	-	0	0	0
F. compactum	20	-	0	0	0
F. chlamydosporum	15	-	0	0	0
F. proliferatum	10	-	0	0	0
F. reticulatum	15	-	0	0	0
F. polyphialidicum	10	-	0	0	0
F. moniliforme	15	-	0	0	0
F. merismoides	5	-	0	0	0
F. a. subsp. *armeniacum*	5	-	0	0	0
Control	-	-	0	0	0

[a]Twenty, sound, unblemished, surface-disinfested (3% sodium hypochlorite, 15 min) tubers, cv. Up-to-Date, were injected with 2 ml of a spore suspension (1 x 10^4 conidia/ml) and kept at 25 ±2°C and 50-70% relative humidity.

[b]Accession numbers of representative isolates of each species 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.

[c]Dry rot determined according to a disease index, where 0 = no lesion development and 5 = tuber completely decayed. Results presented as percentage dry rot calculated according to the method of Kremer & Unterstenhöfer (1967).

[d]Each mean value represent 20 observations for each isolate.

Fig. 1. Potato production regions in South Africa where potato tubers with dry-rot and stem-end-rot lesions were sampled during grading.

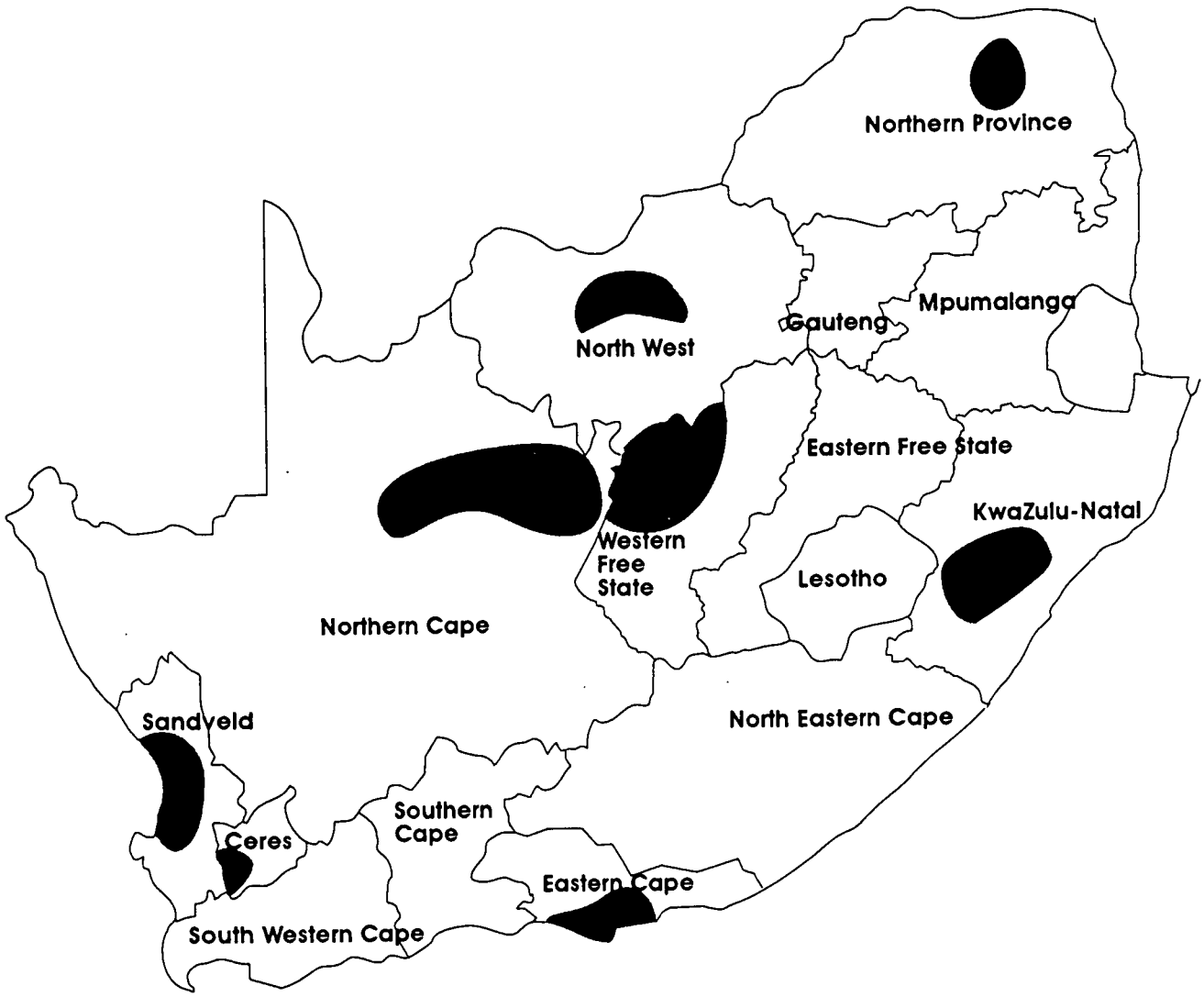
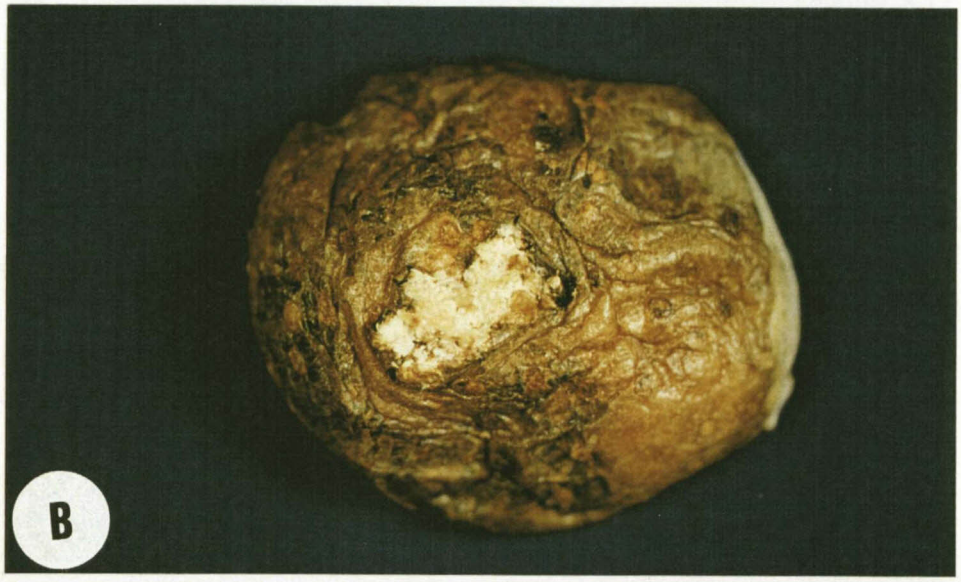

Northern Province
Mpumalanga
Gauteng
North West
Eastern Free State
KwaZulu-Natal
Western
Free
State
Lesotho
Northern Cape
Sandveld
North Eastern Cape
Southern
Cape
Ceres
Eastern Cape
South Western Cape

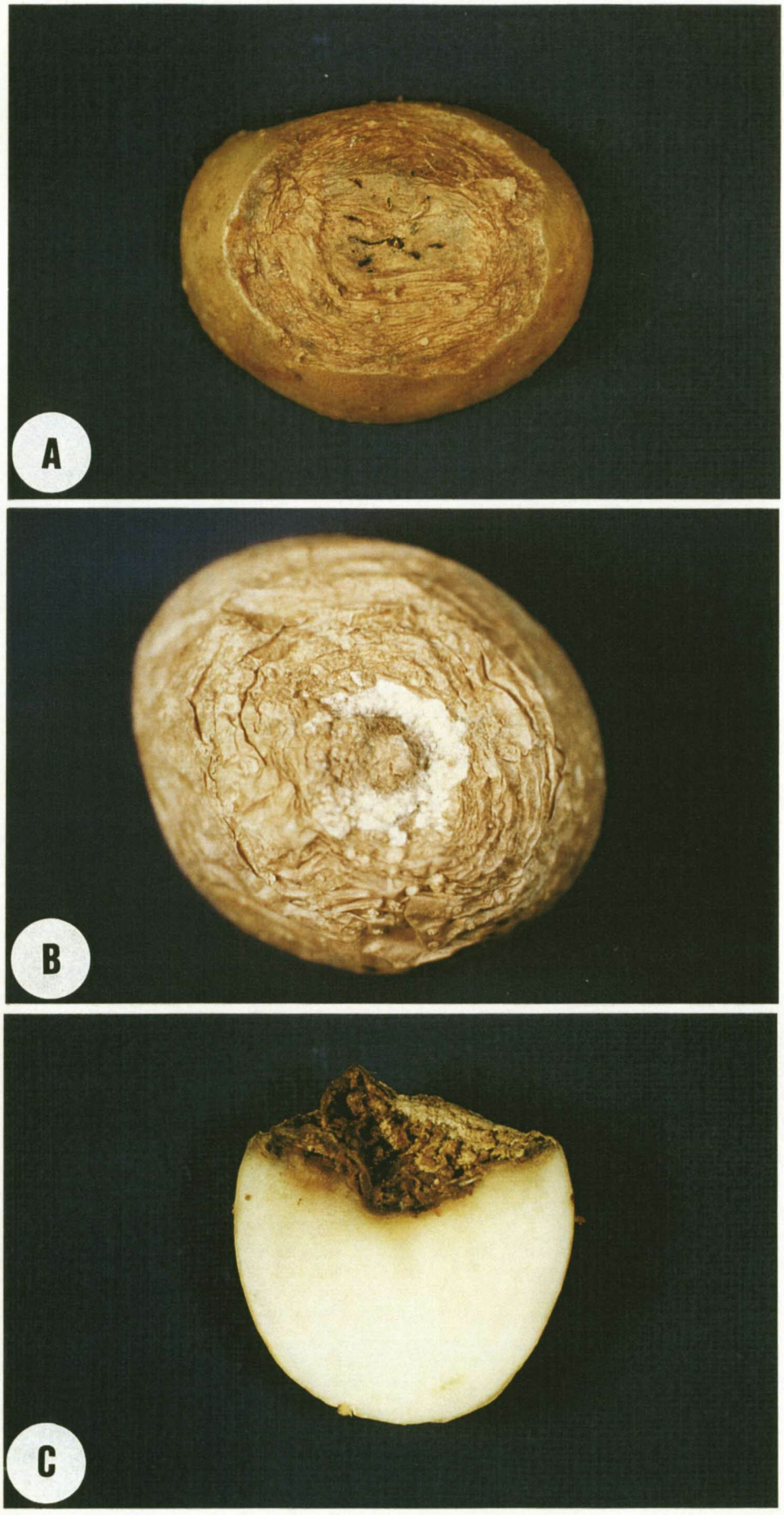
Fig. 2. Fusarium dry-rot, (A & B) external and (C) internal, symptoms.


A


B


C

Fig. 3. Fusarium stem-end-rot, (A & B) external and (C) internal, symptoms.


A
B
C

CHAPTER 3

CHEMICAL CONTROL OF FUSARIUM DRY ROT OF POTATOES: I. *IN VITRO* SCREENING OF FUNGICIDES

ABSTRACT

Twenty six fungicides were evaluated *in vitro* against *Fusarium* spp. and *Gliocladium roseum*, associated with dry rot of potatoes in South Africa. A paper-disc plate, as well as a poisoned medium technique, were used. The fungicides were evaluated at concentrations of 2000 and 500 mg a.i./*l*. The sensitivity of five isolates of each of the pathogens to eleven of the most effective fungicides was also evaluated.

Irrespectively of the *in vitro* screening technique used, prochloraz was the most effective fungicide as far as the inhibition of the mycelium growth of the dry-rot pathogens was concerned, 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 sensitivity between the isolates of each species to the different fungicides was also found to differ significantly, except for the *G. roseum*. The appearance of differences in sensitivity to fungicides between the pathogens, as well as isolates of the same pathogen, stresses the need to test fungicides against all the target pathogens associated with dry rot of potatoes, as well as to more than one isolate of each species.

Thiabendazole and mancozeb are the only two fungicides registered in South Africa for the control of Fusarium dry rot of potatoes. Thiabendazole was rated fifth in these trials, but was not as effective against *F. oxysporum* as against *F. solani*, which are the predominant dry-rot pathogens found in South Africa. Mancozeb was unable to control the colony growth of either of these two pathogens by more than 42% of that of the control.

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INTRODUCTION

Fusarium dry rot of potatoes, caused by several *Fusarium* spp., is considered as an important disease of potatoes world-wide, wherever potatoes are produced (Boyd, 1972; Seppänen, 1981). In South Africa, Fusarium dry rot is also considered as the most important post-harvest disease of potatoes (Theron & Holz, 1989). Losses due to Fusarium dry rot are mainly considered a problem in the seed industry where potatoes are stored for prolonged periods. Annual losses in the South African seed industry, due to Fusarium dry rot, are between 5 and 15%, with individual cases as high as 60% having been recorded (Du Plooy & Van der Plank, 1972). Although other *Fusarium* spp. have been isolated locally from dry-rot lesions, *F. solani* (Mart.) Appel & Wollenw. emend. Snyd. & Hans. and *F. oxysporum* Schlecht. emend. Snyd. & Hans. are considered as the main causal organisms of dry rot of potatoes (Theron & Holz, 1989; Chapter 2). *Gliocladium roseum* Bainier has also been reported as a dry-rot causing pathogen locally (Theron & Holz, 1991).

During the past 15 - 20 years, fungicides have increasingly been used for the control of storage diseases of potatoes, including Fusarium dry rot (Carnegie, Ruthven, Lindsay & Hall, 1990). Fusarium dry rot develops at wounds made either at harvest, grading or later during storage when tubers become more susceptible (Boyd, 1967; Nielsen, 1981; Seppänen, 1981). Good control has been achieved 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, 1971; Leach, 1978; Hide, 1986). Thiabendazole, as a dip, spray or mist treatment, and mancozeb, as a dip or seed dressing, are the only fungicides registered in South Africa for the control of Fusarium dry rot (Krause, Nel & Van Zyl, 1995) and have been used extensively in potato production locally since the early 1970's. The incidence of Fusarium dry rot has, however, increased over the years, irrespective of the use of these two registered fungicides.

Owing to the fact that these two fungicides were tested only against *F. solani* and because other fungicides have been reported more effective (Hide & Cayley, 1985; Maughan, Shanmuganathan & Hepworth, 1991), a study was undertaken to evaluate different fungicides *in vitro* against *Fusarium* spp. (Theron & Holz, 1989), and *G. roseum* (Theron & Holz, 1991), associated with dry rot of potatoes in South Africa.

MATERIAL AND METHODS

Screening of fungicides against dry-rot pathogens

Twenty six fungicides (Table 1) were screened *in vitro* for their efficiency with regard to the control of mycelial growth of *F. solani*, *F. oxysporum*, *F. sambucinum* Fuckel, *F. equiseti* (Corda) Sacc., *F. scirpi* Lambotte & Fautr., *F. acuminatum* Ell. & Ev., *F. crookwellense* Burgess, Nelson & Toussoun, *F. graminearum* Schwabe Gr. I (Theron & Holz, 1989) and *G. roseum* (Theron & Holz, 1991) associated with dry rot and stem-end rot of potatoes in South Africa. Lyophilized conidia of the most virulent isolates of each species (Chapter 2) were transferred to carnation-leaf-agar (CLA) (Fisher, Burgess, Toussoun & Nelson, 1982) and incubated at 25 °C under intermittent light (fluorescent plus black lights: 12 hr cycles) (Nelson, Toussoun & Marasas, 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 the 14-day old cultures on the CLA plates with sterile distilled water, counted in a haemacytometer, and the suspension diluted to 20 x 10^4 conidia/ml. Potato-dextrose agar (PDA) 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 (5mm 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. For each treatment combination five replicates were used. 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 & 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 7 days mycelial plugs (5 mm diameter) were cut aseptically with a cork borer from the edge of the colony of each *Fusarium* sp. and *G. roseum* and placed on to 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 found to be the most effective for the inhibition of mycelial growth, as well as mancozeb which is registered in South Africa, were evaluated, using the paper-disc plate technique as was described previously, for their efficiency against five representative pathogenic isolates of each species including the most virulent isolates.

Statistical analysis

The data were statistically analysed by 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

Paper-disc plate technique

Mycelium growth, irrespective of the dry-rot pathogen, was significantly ($P \leq 0.05$) inhibited by filter paper discs impregnated 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 mycelium growth of the dry-rot pathogens, whereas dichlorophen and iprodione did not differ significantly ($P \geq 0.05$) from them. Thiabendazole and mancozeb, the only two fungicides registered for the control of Fusarium dry rot in South Africa, did not feature amongst the nine best fungicides for the inhibition of mycelium growth. The sensitivity of the dry-rot pathogens to the fungicides differed from one another. For instance fenpropimorf did inhibit the mycelial growth of *F. solani* significantly ($P \leq 0.05$) more than the other species. Chlorothalonil, however, 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 ($P \leq 0.05$) higher inhibitory effect on the 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 2). Mycelium growth of *F. solani* and *F. oxysporum*, the predominant dry-rot pathogens in South Africa, was significantly ($P \leq 0.05$) inhibited by

prochloraz and carbendazim, both formulations, followed by captafol, thiabendazole and captab than the other fungicides (Table 2).

It appeared that the fungicides formulated as wettable powders were less effective, using a paper-disc pate screening technique, than those formulated as either an emulsified or a suspendible concentration. Prochloraz as an emulsified concentration (EC 45%) could for example inhibit the mycelial growth of the dry-rot pathogens significantly ($P \leq 0.05$) more than the suspendible concentration (WP 50%). The same was found for carbendazim, although the differences were not significant ($P \geq 0.05$) (Table 2). It was therefore decided to evaluate the same series of fungicides using the poisoned medium technique.

Poisoned medium technique

Twenty-two of the 26 fungicides restricted the growth of the dry-rot pathogen colonies to more than 50% of that of the control when grown on PDA, amended with 2000 mg a.i./l of the fungicides (Table 3). The seven fungicides which restricted the colony growth most (> 94.7%), did not differ significantly ($P \geq 0.05$) from one another and the trial was therefore repeated with these fungicides applied to the medium at a concentration of 500 mg a.i./l.

Seventeen of the 26 fungicides still restricted the growth of the dry-rot pathogen colonies to more than 50% of that of the control when grown on PDA, amended with 500 mg a.i./l of these fungicides (Table 4). The efficiency of control between these two concentrations, differs more than 10% for benodanil, benomyl, dichlorophen, folpet, mancozeb, metiram and penconazole (Tables 3; 4).

PDA amended with 500 mg a.i./l of imazalil, prochloraz (EC 45%) or thiabendazole restricted the colony growth of the dry-rot pathogens to more than 95% of that of the control which was also significantly ($P \leq 0.05$) more than the other fungicides (Table 4). All the fungicides evaluated, however, restricted the colony growth of the dry-rot pathogens 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 restricted by imazalil, prochloraz (both formulations) and thiabendazole. Benodanil and fenpropimorf, however, restricted the colony growth of only *F. solani* by 100% and benomyl and carbendazim (both formulations) only that of *F. oxysporum* (Tables 3; 4).

Efficiency against different isolates

Except for the *G. roseum* isolates, most of the *Fusarium* spp. isolates evaluated, differed significantly ($P \leq 0.05$) in sensitivity against the fungicides evaluated (Tables 5 - 13). For instance, all five *F. oxysporum* isolates differed significantly ($P \leq 0.05$) in sensitivity from one another irrespective of the fungicide used (Table 6).

Prochloraz inhibited the mycelium growth of all the species significantly ($P \leq 0.05$) more than the other fungicides, except for those of *F. solani* (Table 5), in which case it did not differ from fenpropimorf, and for *F. equiseti* (Table 8) and *F. scirpi* (Table 9) where it did not differ significantly ($P \geq 0.05$) from penconazole. Fenpropimorf had a significantly ($P \leq 0.05$) higher inhibitory effect on the mycelial growth of all five isolates of *F. solani* (Table 5), but did not differ from prochloraz. In comparison to the other species, fenpropimorf had no inhibitory effect and did not differ significantly ($P \geq 0.05$) from the control, except for *F. sambucinum* and *G. roseum* (Tables 6 - 13). Penconazole had a significantly ($P \leq 0.05$) higher inhibitory effect on the mycelium growth of *F. equiseti* (Table 8) and *F. scirpi* (Table 9) than the other fungicides, except for prochloraz.

Prochloraz had the highest overall inhibitory effect on the mycelial growth of the dry-rot pathogens, followed by carbendazim, imazalil and captab. The results obtained with mancozeb, one of two fungicides registered for the control of Fusarium dry rot in South Africa, were very disappointing. It 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% of that of the control (Tables 3; 4). When compared with prochloraz, mancozeb resulted in only 10% control of the colony growth of these two species (Tables 2; 5; 6).

DISCUSSION

In this study, it appeared that the fungicides formulated as wettable powders were less effective than those formulated as either an emulsified or a suspendible concentration when evaluated by using a paper-disc technique. These findings could be attributed to the poorer solubility of the fungicides formulated as wettable powders and also the possibility that their diffusibility into the media is not effective (Torgenson, 1967). It is, therefore, important that more than one screening method be used to evaluate the fungicides.

Irrespective of the *in vitro* screening technique used, prochloraz was the most effective fungicide inhibiting the mycelial growth of the dry-rot pathogens. This was followed by imazalil, carbendazim, propiconazole, thiabendazole, captafol, captab, penconazole, benomyl and benodanil. Prochloraz, carbendazim, imazalil, thiabendazole and benomyl have also previously been reported to be effective for the control of Fusarium dry rot (Leach, 1971; Murdoch & Wood, 1972; Hide & Cayley, 1980). The dry-rot pathogens reacted differently on treatments with the different fungicides. Differences in efficiency of fungicides for the control of Fusarium dry rot, caused by different *Fusarium* spp., have also been reported previously (Hide & Cayley, 1980; 1985; Maughan *et al.*, 1991).

Sensitivity between the five representative isolates of each species to the different fungicides was also found to differ significantly, except for the *G. roseum* isolates. These differences can probably be attributed to diversity within the species rather than to a build-up of resistance inherit to exposure to the different fungicides. The appearance of differences in sensitivity to fungicides between the pathogens, as well as between isolates of the same pathogen, stresses the need to test fungicides against all the target pathogens associated with dry rot of potatoes, as well as to use more than one isolate of each species (Shephard, 1987).

Thiabendazole and mancozeb are the only two fungicides registered in South Africa for the control of Fusarium dry rot of potatoes (Krause *et al.*, 1995). Thiabendazole was rated fifth in these trials, but was not as effective against *F. oxysporum* as against *F. solani*, the predominant dry-rot pathogens found in South Africa (Theron & Holz, 1989, Chapter 2). Mancozeb could not control the colony growth of either of these two pathogens more than 26%. This could be a reason for 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 or a selection of fungicides which could possibly control a disease. Therefore, it is necessary to conduct further tests with these selected fungicides to determine their efficiency on a commercial scale before recommendations can be made to potato producers. These tests should include trials on different times of application, such as after harvesting and before planting. Other external factors which may also have an influence on the efficiency of the fungicides or on Fusarium dry-rot development should also be investigated in these trials.

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Table 1. Fungicides screened *in vitro* for their efficiency to inhibit mycelial growth of *Fusarium* spp. and *Gliocladium roseum* associated with dry rot and stem-end rot of potatoes in South Africa

Active ingredient	Trade name	Formulation	Supplier
benodanil	Calirus	WP 50%	BASF
benomyl	Benlate	WP 50%	Agricura
captab	Orthocide	WP 50%	AECI
captafol	Difolatan	SC 48%	Agricura
carbendazim	Derosal	SC 51%	BASF
carbendazim	Bavistin	WP 50%	BASF
chloroneb	Demosan	SDR 65%	Agricura
chlorothalonil	Bravo	WP 75%	Agricura
dichlorophen	Xanbac D	EC 20%	Plaaskem
fenpropimorf	Corbell	EC 75%	BASF
folpet	Folpan	WP 50%	Zeneca
imazalil	Fungazil	WP 75%	Jansen Pharmaceutica
iprodione	Rovral	SC 25%	Rhône-Poulenc
mancozeb	Dithane M45	WP 80%	Zeneca
metiram	Polyram Combi	WP 80%	BASF
penconazole	Topaz	EC 10%	Ciba-Geigy
pencycuron	Monceren	SC 25%	Bayer
prochloraz	Sportak	EC 45%	AgrEvo
prochloraz Mg. chloride	Octave	WP 50%	Schering
propiconazole	Tilt	EC 25%	Ciba-Geigy
procymidone	Sumisclex	SC 25%	Agricura
propamocarb	Previcur N	EC 68.6%	AgrEvo
thiabendazole	Tecto	SC 45%	MSD Agvet
thiophanate-methyl	Topsin-M	WP 65%	AECI
triadimenol	Bayfidan	EC 25%	Bayer
vinclosolin	Ronilan	WP 50%	BASF

Table 2. Mycelial inhibition zones (mm), of the most virulent isolate of each dry-rot pathogen, surrounding the filter paper discs, impregnated with fungicides (2000 mg a.i./l), measured after 4 days of incubation on potato-dextrose agar plates at 25 °C[a]

Fungicides	Dry rot pathogens[b]									**Mean**	F -value (P ≤0.05)	$LSD_{(T)}$ 5%	% Control[c]
	F. sol.	*F. oxy.*	*F. sam.*	*F. equi.*	*F. scir.*	*F. acum.*	*F. crook.*	*F. gram.*	*G. ros.*				
benodanil	7.4	6.3	8.1	1.6	6.6	6.9	1.6	11.6	7.3	**6.4**	25.2	1.8	15.2
benomyl	27.3	14.3	14.1	3.9	11.7	7.2	11.0	12.3	18.9	**13.4**	16.7	4.7	31.9
captab	18.3	26.7	25.4	17.9	19.8	25.7	22.2	30.8	34.3	**24.6**	7.4	5.9	58.6
captafol	24.9	22.9	30.4	29.7	23.3	28.8	30.6	43.1	28.3	**29.1**	15.7	4.4	69.3
carbendazim[d]	38.8	23.9	19.1	19.1	19.4	14.8	18.9	25.0	35.0	**23.8**	114.3	1.9	56.7
carbendazim[e]	37.1	22.3	17.3	17.3	13.6	13.7	20.7	21.9	31.9	**21.8**	239.8	1.5	51.9
chloroneb	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	**0.0**	-[f]	-	0.0
chlorothalonil	0.0	5.1	6.2	5.2	7.1	8.0	6.1	5.7	7.2	**5.6**	13.7	1.8	13.3
dichlorophen	0.0	0.0	5.5	0.0	0.0	5.0	0.0	8.7	6.3	**2.8**	117.7	0.9	6.7
fenpropimorf	40.4	2.8	1.6	0.0	2.4	2.0	1.2	0.0	18.3	**7.6**	270.1	2.4	18.1
folpet	10.9	14.9	9.2	7.1	7.0	17.7	7.5	13.7	22.5	**12.3**	37.5	2.5	29.3
imazalil	13.8	26.7	27.3	29.5	39.3	10.3	29.4	38.0	13.5	**25.3**	62.7	3.8	60.2
iprodione	0.0	5.0	1.6	0.0	0.0	0.0	0.0	7.5	0.0	**1.6**	74.0	0.9	3.8
mancozeb	7.1	3.8	6.0	2.4	6.5	9.5	2.8	7.8	7.5	**5.9**	21.7	1.5	14.0
metiram	10.3	8.6	6.9	0.0	1.6	6.3	5.0	4.8	7.8	**5.7**	25.9	1.8	13.6
penconazole	12.0	13.3	17.9	37.8	34.2	3.3	7.7	8.2	7.1	**15.7**	658.1	1.4	37.4
pencycuron	0.0	0.0	0.0	0.0	0.0	0.0	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	56.5	**42.0**	110.0	2.6	100.0
prochloraz Mg.Cl	29.1	31.0	28.8	17.1	27.8	34.3	27.9	38.1	45.4	**31.1**	20.5	5.0	74.0
propiconazole	6.7	6.5	29.3	35.2	34.3	7.1	20.2	9.6	33.8	**20.3**	388.5	1.9	48.3
procymidone	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	**0.0**	-	-	0.0
propamocarb	0.0	0.0	0.0	0.0	0.0	0.0	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	0.0	**15.6**	116.1	2.5	37.1
thiophanate methyl	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	**0.0**	-	-	0.0
triadimenol	0.0	0.0	0.0	14.0	18.2	0.0	0.0	0.0	0.0	**3.6**	236.1	1.3	8.6
vinclosolin	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	**0.0**	-	-	0.0
control	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	**0.0**	-	-	0.0
F - value (P ≤ 0.05)	615.6	156.4	122.3	127.2	303.7	175.5	260.6	294.5	456.5	142.6			
$LSD_{(T)}$ 5%	1.7	2.7	3.1	3.1	1.6	2.6	2.1	2.5	2.1	2.8			

[a]Three measurements were taken for each of five replicates (n = 15).

[b]*F. solani* (MRC 6358); F. oxysporum (MRC 6379); F. sambucinum (MRC 6454); F. equiseti (MRC 6828); F. scirpi (MRC 6838); F. acuminatum (MRC 6449); F. crookwellense (MRC 6444); F. graminearum Gr. I (MRC 4672) and *Gliocladium roseum* (PREM 48918). (MRC and PREM) = Accession numbers of representative isolates deposited in the culture collection of the Programme on Mycotoxins and Experimental Carcinogenesis (PROMEC), Medical Research Council (MRC), P. O. Box 19070, Tygerberg 7505 and in the National Collection of Fungi, (PREM), Private Bag X 134, Pretoria 0001, South Africa.

[c]Percentage mycelium inhibition of that of the best fungicide treatment (prochloraz).

[d]Carbendazim, SC 51%, Derosal.

[e]Carbendazim, BP 65%, Bavistin.

[f]Not estimable.

Table 3. Percentage control of the colony growth of the most virulent isolate of each dry-rot pathogen, measured after 7 days of incubation at 25°C on potato-dextrose agar plates containing fungicides (2000 mg a.i./l) which were added to the media after the autoclaved agar had cooled to 60 °C[a]

Fungicides	Dry rot pathogens[b]									Mean	F -value (P ≤0.05)	$LSD_{(T)}$ 5%
	F. sol.	*F. oxy.*	*F. sam.*	*F. equi.*	*F. scir.*	*F. acum.*	*F. crook.*	*F. gram.*	*G. ros.*			
benodanil	100.0	83.6	84.6	85.0	86.6	78.4	91.5	95.9	100.0	**89.5**	35.5	3.7
benomyl	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	85.8	**98.4**	1290.6	0.4
captab	81.3	80.0	79.7	37.6	38.7	50.6	60.7	86.7	78.7	**66.0**	36.4	9.2
captafol	79.6	80.8	55.4	49.6	41.5	63.6	71.6	83.2	79.8	**67.2**	49.9	6.2
carbendazim[c]	80.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	79.8	**95.5**	171.6	1.9
carbendazim[d]	71.8	100.0	100.0	91.9	92.7	100.0	100.0	100.0	76.7	**92.6**	87.3	8.0
chloroneb	0.1	24.3	20.9	39.0	43.0	10.2	46.0	57.1	68.4	**34.3**	166.5	4.9
chlorothalonil	70.1	73.9	58.5	73.8	69.2	64.8	75.1	79.3	75.9	**71.2**	26.6	3.5
dichlorophen	42.7	59.1	76.2	81.0	68.0	40.3	77.4	82.9	79.8	**67.5**	57.1	6.2
fenpropimorf	100.0	57.5	74.5	78.4	82.6	70.5	89.1	81.5	100.0	**81.6**	74.1	4.6
folpet	76.6	77.6	69.0	47.3	59.1	50.0	70.2	84.5	81.8	**68.5**	45.3	5.8
imazalil	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	**100.0**	-[e]	-
iprodione	56.2	58.3	51.6	35.5	31.7	38.1	56.4	74.5	87.7	**54.4**	129.4	4.6
mancozeb	37.1	49.1	40.6	48.8	34.6	63.6	62.4	87.2	78.3	**56.7**	26.3	9.7
metiram	82.6	58.7	68.4	61.7	70.2	75.6	66.5	81.8	83.4	**72.1**	19.2	6.0
penconazole	77.0	85.2	90.4	100.0	100.0	100.0	100.0	100.0	100.0	**94.7**	322.6	1.4
pencycuron	0.0	31.5	13.0	13.7	8.5	5.1	30.5	40.8	8.7	**16.9**	41.5	6.2
prochloraz	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	**100.0**	-	-
prochloraz Mg.Cl	100.0	100.0	100.0	100.0	100.0	100.0	90.1	90.8	100.0	**97.9**	11.9	3.5
propiconazole	82.2	86.0	100.0	100.0	100.0	79.0	100.0	100.0	100.0	**94.1**	634.7	1.0
procymidone	44.5	59.5	13.0	26.3	28.0	26.7	30.7	51.9	60.1	**37.9**	63.8	6.0
propamocarb	0.2	36.7	13.0	50.8	8.5	9.7	30.7	67.7	27.3	**27.2**	133.5	5.5
thiabendazole	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	76.7	**97.4**	3469.1	0.4
thiophanate methyl	69.2	48.7	40.6	46.2	37.2	39.2	78.3	44.8	87.7	**54.7**	144.8	4.5
triadimenol	44.9	62.3	77.7	100.0	100.0	56.8	71.8	81.0	63.2	**73.1**	192.7	3.9
vinclosolin	55.3	64.3	38.3	44.2	41.8	31.8	46.0	59.5	79.1	**51.1**	93.5	4.4
F - value (P ≤ 0.05)	281.2	485.3	260.5	196.1	130.7	330.6	375.9	213.3	269.3	146.3		
$LSD_{(T)}$ 5%	5.3	3.0	5.3	5.7	7.7	4.9	3.4	3.5	3.7	5.6		

[a]Percentage inhibition of colony diameter of that of the control. Three measurements were taken for each of five replicates (n = 15).

[b]*F. solani* (MRC 6358); F. oxysporum (MRC 6379); F. sambucinum (MRC 6454); F. equiseti (MRC 6828); F. scirpi (MRC 6838); F. acuminatum (MRC 6449); F. crookwellense (MRC 6444); F. graminearum Gr. I (MRC 4672) and *Gliocladium roseum* (PREM 48918). (MRC and PREM) = Accession numbers of representative isolates deposited in the culture collection of the Programme on Mycotoxins and Experimental Carcinogenesis (PROMEC), Medical Research Council (MRC), P. O. Box 19070, Tygerberg 7505 and in the National Collection of Fungi, (PREM), Private Bag X 134, Pretoria 0001, South Africa.

[c]Carbendazim, SC 51%, Derosal.

[d]Carbendazim, BP 65%, Bavistin.

[e]Not estimable.

Table 4. Percentage control of the colony growth of the most virulent isolate of each dry-rot pathogen, measured after 7 days of incubation at 25°C on potato-dextrose agar 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	Dry rot pathogens[b]									Mean	F -value (P ≤0.05)	$LSD_{(T)}$ 5%
	F. sol.	*F. oxy.*	*F. sam.*	*F. equi.*	*F. scir.*	*F. acum.*	*F. crook.*	*F. gram.*	*G. ros.*			
benodanil	100.0	57.1	78.5	79.6	65.9	76.1	75.3	87.8	72.3	**77.0**	113.4	3.3
benomyl	86.5	100.0	59.7	58.8	100.0	100.0	100.0	100.0	72.3	**86.4**	1019.0	1.6
captab	80.0	76.4	78.3	37.0	37.5	47.2	46.7	85.6	68.8	**61.9**	190.8	4.1
captafol	78.3	77.2	54.5	48.2	40.5	58.5	62.6	81.5	76.7	**64.2**	128.9	4.8
carbendazim[c]	71.4	100.0	100.0	86.8	93.3	100.0	100.0	100.0	72.7	**91.6**	104.6	3.4
carbendazim[d]	69.2	100.0	100.0	87.3	82.0	100.0	96.1	100.0	73.1	**89.7**	15.4	3.8
chloroneb	0.1	20.2	18.0	36.1	39.9	1.1	43.6	54.3	52.2	**29.5**	101.8	5.8
chlorothalonil	67.0	73.5	55.9	70.9	67.4	61.9	70.2	75.0	63.6	**67.3**	40.0	2.8
dichlorophen	33.2	57.5	57.7	77.6	51.5	33.5	59.1	74.7	71.1	**57.3**	211.0	3.2
fenpropimorf	100.0	50.7	73.6	77.0	75.3	68.8	82.7	80.4	100.0	**78.7**	235.4	2.8
folpet	72.7	74.3	39.4	30.7	34.5	45.5	45.7	81.8	68.0	**54.7**	83.2	6.4
imazalil	100.0	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	77.9	**47.8**	118.8	4.6
mancozeb	37.1	43.1	40.2	41.6	40.2	60.8	57.3	82.6	36.4	**45.5**	340.6	3.1
metiram	37.5	53.1	59.7	53.7	37.2	42.0	49.2	71.7	43.5	**49.8**	53.2	4.4
penconazole	66.2	83.6	82.0	100.0	100.0	59.1	87.1	88.3	74.3	**82.3**	219.2	2.7
pencycuron	0.1	27.5	10.1	10.8	5.5	0.6	28.4	38.0	4.7	**14.0**	250.0	2.5
prochloraz	100.0	100.0	100.0	97.7	100.0	100.0	100.0	100.0	100.0	**99.7**	9.9	0.7
prochloraz Mg.Cl	100.0	100.0	96.5	87.6	89.0	100.0	88.9	100.0	100.0	**94.4**	26.3	3.3
propiconazole	74.4	83.1	79.4	100.0	100.0	74.4	100.0	90.5	100.0	**89.1**	474.5	1.5
procymidone	40.1	55.1	10.1	23.2	24.2	21.0	28.4	48.9	55.3	**34.1**	78.8	5.3
propamocarb	0.2	30.3	11.3	47.9	5.5	4.0	28.4	64.9	23.3	**24.0**	119.2	5.7
thiabendazole	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	68.0	**96.4**	1867.6	0.7
thiophanate methyl	67.0	39.1	28.7	43.9	30.8	27.8	73.2	38.0	85.0	**48.2**	62.0	7.8
triadimenol	42.3	58.3	66.1	100.0	88.7	44.9	65.8	79.3	44.3	**65.5**	179.7	4.4
vinclosolin	46.2	63.1	30.7	40.7	39.6	26.1	41.6	55.4	71.9	**46.2**	20.1	4.5
F - value (P ≤ 0.05)	607.0	334.4	335.9	440.6	431.3	599.6	400.8	282.5	358.5	127.5		
$LSD_{(T)}$ 5%	3.6	3.9	4.9	3.8	4.3	3.8	3.5	3.1	3.5	6.1		

[a]Percentage inhibition of colony diameter of that of the control. Three measurements were taken for each of five replicates (n = 15).

[b]*F. solani* (MRC 6358); F. oxysporum (MRC 6379); F. sambucinum (MRC 6454); F. equiseti (MRC 6828); F. scirpi (MRC 6838); F. acuminatum (MRC 6449); F. crookwellense (MRC 6444); F. graminearum Gr. I (MRC 4672) and *Gliocladium roseum* (PREM 48918). (MRC and PREM) = Accession numbers of representative isolates deposited in the culture collection of the Programme on Mycotoxins and Experimental Carcinogenesis (PROMEC), Medical Research Council (MRC), P. O. Box 19070, Tygerberg 7505 and in the National Collection of Fungi, (PREM), Private Bag X 134, Pretoria 0001, South Africa.

[c]Carbendazim, SC 51%, Derosal.

[d]Carbendazim, BP 65%, Bavistin.

[e]Not estimable.

Table 5. Mycelial inhibition zones (mm) of five pathogenic *F. solani* isolates surrounding the filter paper discs, impregnated with fungicides (2000 mg a.i./l), measured after 4 days of incubation on potato-dextrose agar plates at 25 °C[a]

Fungicides	Isolates[b]					Mean
	MRC6358	MRC6359	MRC6360	MRC6361	MRC6362	
benodanil; BP 50%	6.8	6.3	6.7	5.6	6.9	**6.5**
benomyl; BP 50%	26.1	21.6	23.4	22.2	20.3	**22.7**
captab; BP 50%	14.8	14.0	14.4	11.6	12.8	**13.5**
carbendazim; SC 51%	33.4	31.1	29.2	30.9	32.3	**31.4**
fenpropimorf; EC 75%	41.0	46.0	42.2	48.1	44.0	**44.2**
imazalil; BP 75%	10.4	9.9	10.4	10.7	10.1	**10.3**
mancozeb; BP 80%	4.8	4.3	4.8	4.3	4.1	**4.5**
penconazole; EC 10%	9.8	9.3	8.0	8.1	6.7	**8.4**
prochloraz; EC 45%	42.4	41.3	43.1	44.9	42.0	**42.7**
propiconazole; EC 25%	4.6	4.2	4.1	4.3	4.1	**4.3**
thiabendazole; EC 45%	31.8	16.6	18.0	19.8	31.3	**23.5**
Control	0.0	0.0	0.0	0.0	0.0	**0.0**
Mean	**18.8**	**17.1**	**17.0**	**17.5**	**17.9**	

Source of variation	F-value ($P \leq 0.05$)	$LSD_{(T)}$ 5%
Isolates (A)	18.97	0.8
Fungicides (B)	1000	1.5
A x B	11.00	2.9

[a]Three measurements were taken for each of five replicates (n = 15).

[b]Accession numbers of representative isolates of *F. solani* 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.

Table 6. Mycelial inhibition zones (mm) of five pathogenic *F. oxysporum* isolates surrounding the filter paper discs, impregnated with fungicides (2000 mg a.i./l), measured after 4 days of incubation on potato-dextrose agar plates at 25 °C[a]

Fungicides	Isolates[b]					**Mean**
	MRC6379	MRC6380	MRC6381	MRC6382	MRC6383	
benodanil; BP 50%	2.0	0.0	0.0	0.0	0.0	**0.4**
benomyl; BP 50%	7.4	7.1	6.7	6.9	7.2	**7.1**
captab; BP 50%	17.9	13.8	10.7	17.1	16.3	**15.2**
carbendazim; SC 51%	17.6	15.0	16.1	17.2	16.3	**16.4**
fenpropimorf; EC 75%	2.0	0.0	0.0	0.0	2.0	**0.4**
imazalil; BP 75%	18.8	8.1	9.0	18.2	17.0	**14.2**
mancozeb; BP 80%	2.0	0.0	0.0	2.0	0.0	**0.8**
penconazole; EC 10%	12.1	9.8	7.8	10.0	12.0	**10.3**
prochloraz; EC 45%	45.0	41.8	43.8	44.4	42.6	**43.5**
propiconazole; EC 25%	6.0	0.0	0.0	0.0	10.0	**3.2**
thiabendazole; EC 45%	10.0	10.0	0.0	0.0	0.0	**4.0**
Control	0.0	0.0	0.0	0.0	0.0	**0.0**
Mean	**11.7**	**8.8**	**7.8**	**9.7**	**10.1**	

Source of variation	F-value ($P \leq 0.05$)	$LSD_{(T)}$ 5%
Isolates (A)	96.43	0.4
Fungicides (B)	1000	0.9
A x B	38.91	1.7

[a]Three measurements were taken for each of five replicates (n = 15).

[b]Accession numbers of representative isolates of *F. oxysporum* 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.

Table 7. Mycelial inhibition zones (mm) of five pathogenic *F. sambucinum* isolates surrounding the filter paper discs, impregnated with fungicides (2000 mg a.i./l), measured after 4 days of incubation on potato-dextrose agar plates at 25 °C[a]

Fungicides	Isolates[b]					**Mean**
	MRC6454	MRC6455	MRC6456	MRC6457	MRC6458	
benodanil; BP 50%	6.6	0	5.3	3.3	6.3	**4.3**
benomyl; BP 50%	14.1	16.5	12.5	14.3	16.0	**14.7**
captab; BP 50%	18.3	15.8	12.7	13.5	13.0	**14.7**
carbendazim; SC 51%	16.3	13.9	14.5	12.6	13.4	**14.2**
fenpropimorf; EC 75%	0.0	7.1	0.0	3.6	4.0	**2.9**
imazalil; BP 75%	27.8	25.0	30.7	26.6	33.4	**28.7**
mancozeb; BP 80%	6.7	6.2	6.2	6.5	4.8	**6.1**
penconazole; EC 10%	14.7	12.5	10.2	10.1	10.2	**11.5**
prochloraz; EC 45%	52.6	58.5	52.6	51.7	53.8	**53.8**
propiconazole; EC 25%	26.8	24.7	24.3	24.3	25.3	**25.1**
thiabendazole; EC 45%	15.2	17.0	14.4	7.3	15.0	**13.8**
Control	0.0	0.0	0.0	0.0	0.0	**0.0**
Mean	**16.6**	**16.4**	**15.3**	**14.5**	**16.3**	

Source of variation	F-value ($P \leq 0.05$)	$LSD_{(T)}$ 5%
Isolates (A)	33.26	0.7
Fungicides (B)	1000	1.3
A x B	13.01	2.4

[a]Three measurements were taken for each of five replicates (n = 15).

[b]Accession numbers of representative isolates of *F. sambucinum* 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.

Table 8. Mycelial inhibition zones (mm) of five pathogenic *F. equiseti* isolates surrounding the filter paper discs, impregnated with fungicides (2000 mg a.i./l), measured after 4 days of incubation on potato-dextrose agar plates at 25 °C[a]

Fungicides	Isolates[b]					**Mean**
	MRC6828	MRC6829	MRC6830	MRC6831	MRC6832	
benodanil; BP 50%	0.0	0.0	6.8	6.2	0.0	**2.6**
benomyl; BP 50%	3.0	0.0	0.0	0.0	0.0	**0.6**
captab; BP 50%	15.3	13.3	12.3	16.1	16.3	**14.7**
carbendazim; SC 51%	18.9	21.4	15.6	17.7	13.4	**17.4**
fenpropimorf; EC 75%	0.0	0.0	0.0	0.0	0.0	**0.0**
imazalil; BP 75%	37.9	35.9	38.6	43.5	35.6	**38.3**
mancozeb; BP 80%	0.0	0.0	0.0	0.0	0.0	**0.0**
penconazole; EC 10%	46.6	45.4	53.7	52.4	43.4	**48.3**
prochloraz; EC 45%	46.1	52.3	46.0	47.1	41.9	**46.7**
propiconazole; EC 25%	38.3	38.6	38.2	42.7	38.1	**39.2**
thiabendazole; EC 45%	21.7	24.7	0.0	6.2	0.0	**10.5**
Control	0.0	0.0	0.0	0.0	0.0	**0.0**
Mean	**18.9**	**19.3**	**17.6**	**19.3**	**15.7**	

Source of variation	F-value ($P \leq 0.05$)	$LSD_{(T)}$ 5%
Isolates (A)	53.73	0.8
Fungicides (B)	1000	2.2
A x B	33.59	2.8

[a]Three measurements were taken for each of five replicates (n = 15).

[b]Accession numbers of representative isolates of *F. equiseti* 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.

Table 9. Mycelial inhibition zones (mm) of five pathogenic *F. scirpi* isolates surrounding the filter paper discs, impregnated with fungicides (2000 mg a.i./l), measured after 4 days of incubation on potato-dextrose agar plates at 25 °C[a]

Fungicides	Isolates[b]					**Mean**
	MRC6838	MRC6839	MRC6840	MRC6841	MRC6842	
benodanil; BP 50%	5.3	2.7	4.7	0.0	6.0	**3.7**
benomyl; BP 50%	8.1	7.6	7.8	7.2	7.2	**7.6**
captab; BP 50%	9.7	10.1	8.3	7.2	9.5	**9.0**
carbendazim; SC 51%	16.6	16.1	14.8	15.8	14.8	**15.6**
fenpropimorf; EC 75%	0.0	0.0	0.0	0.0	0.0	**0.0**
imazalil; BP 75%	37.6	34.4	34.6	34.4	34.0	**35.0**
mancozeb; BP 80%	2.0	0.0	0.0	0.0	0.0	**0.4**
penconazole; EC 10%	41.6	35.8	34.8	38.4	36.3	**37.4**
prochloraz; EC 45%	35.1	37.0	36.2	37.1	39.5	**37.0**
propiconazole; EC 25%	35.0	32.6	34.1	34.0	31.5	**33.4**
thiabendazole; EC 45%	13.0	11.3	11.0	11.6	12.3	**11.8**
Control	0.0	0.0	0.0	0.0	0.0	**0.0**
Mean	**17.0**	**15.6**	**15.5**	**15.5**	**16.0**	

Source of variation	F-value ($P \leq 0.05$)	$LSD_{(T)}$ 5%
Isolates (A)	24.30	0.5
Fungicides (B)	1000	1.0
A x B	7.81	1.8

[a]Three measurements were taken for each of five replicates (n = 15).

[b]Accession numbers of representative isolates of *F. scirpi* 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.

Table 10. Mycelial inhibition zones (mm) of five pathogenic *F. acuminatum* isolates surrounding the filter paper discs, impregnated with fungicides (2000 mg a.i./l), measured after 4 days of incubation on potato-dextrose agar plates at 25 °C[a]

Fungicides	Isolates[b]					**Mean**
	MRC6449	MRC6450	MRC6451	MRC6452	MRC6453	
benodanil; BP 50%	4.2	0.0	0.0	0.0	0.0	**0.8**
benomyl; BP 50%	6.1	0.0	0.0	6.9	0.0	**2.6**
captab; BP 50%	7.4	8.7	7.8	8.8	8.3	**8.2**
carbendazim; SC 51%	7.4	8.3	7.5	7.2	0.0	**6.1**
fenpropimorf; EC 75%	0.0	0.0	0.0	2.0	0.0	**0.4**
imazalil; BP 75%	7.4	7.4	7.4	8.7	7.4	**7.7**
mancozeb; BP 80%	2.0	4.0	2.0	2.2	4.1	**2.9**
penconazole; EC 10%	4.2	4.9	6.7	7.1	2.9	**5.1**
prochloraz; EC 45%	38.2	49.7	54.0	35.7	42.0	**43.9**
propiconazole; EC 25%	2.7	6.6	6.7	3.3	3.4	**4.5**
thiabendazole; EC 45%	7.7	0.0	10.0	0.0	0.0	**3.5**
Control	0.0	0.0	0.0	0.0	0.0	**0.0**
Mean	**7.3**	**7.5**	**8.5**	**6.9**	**5.7**	

Source of variation	F-value ($P \leq 0.05$)	$LSD_{(T)}$ 5%
Isolates (A)	31.47	0.7
Fungicides (B)	1000	1.3
A x B	25.02	2.5

[a]Three measurements were taken for each of five replicates (n = 15).

[b]Accession numbers of representative isolates of *F. acuminatum* 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.

Table 11. Mycelial inhibition zones (mm) of five pathogenic *F. crookwellense* isolates surrounding the filter paper discs, impregnated with fungicides (2000 mg a.i./l), measured after 4 days of incubation on potato-dextrose agar plates at 25 °C[a]

Fungicides	Isolates[b]					**Mean**
	MRC6444	MRC6445	MRC6446	MRC6447	MRC6448	
benodanil; BP 50%	0.0	0.0	0.0	0.0	0.0	**0.0**
benomyl; BP 50%	9.2	10.0	10.2	9.4	10.4	**9.9**
captab; BP 50%	15.4	6.2	6.3	11.5	6.3	**9.1**
carbendazim; SC 51%	18.2	16.9	17.8	16.7	15.5	**17.0**
fenpropimorf; EC 75%	0.0	0.0	0.0	0.0	0.0	**0.0**
imazalil; BP 75%	22.9	26.3	27.4	24.3	25.8	**25.3**
mancozeb; BP 80%	3.5	5.3	0.0	4.8	6.2	**4.0**
penconazole; EC 10%	7.1	7.3	7.3	8.0	6.7	**7.3**
prochloraz; EC 45%	41.5	41.2	44.6	41.0	48.0	**43.3**
propiconazole; EC 25%	10.8	9.1	10.9	11.8	11.0	**10.7**
thiabendazole; EC 45%	21.0	0.0	0.0	6.3	5.1	**6.5**
Control	0.0	0.0	0.0	0.0	0.0	**0.0**
Mean	**12.5**	**10.2**	**10.4**	**11.1**	**11.2**	

Source of variation	F-value ($P \leq 0.05$)	$LSD_{(T)}$ 5%
Isolates (A)	33.28	0.6
Fungicides (B)	1000	1.1
A x B	34.11	2.1

[a]Three measurements were taken for each of five replicates (n = 15).

[b]Accession numbers of representative isolates of *F. crookwellense* 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.

Table 12. Mycelial inhibition zones (mm) of five pathogenic *F. graminearum* Gr. I isolates surrounding the filter paper discs, impregnated with fungicides (2000 mg a.i./l), measured after 4 days of incubation on potato-dextrose agar plates at 25 °C[a]

Fungicides	Isolates[b]					**Mean**
	MRC4672	MRC4673	3	4	5	
benodanil; BP 50%	11.1	7.8	10.2	7.9	7.1	**8.8**
benomyl; BP 50%	7.2	8.1	7.6	6.7	7.1	**7.3**
captab; BP 50%	13.0	10.9	11.6	11.1	12.1	**11.7**
carbendazim; SC 51%	20.1	20.1	20.1	18.2	24.4	**20.6**
fenpropimorf; EC 75%	0.0	0.0	0.0	0.0	0.0	**0.0**
imazalil; BP 75%	29.9	27.9	30.1	30.3	31.7	**30.0**
mancozeb; BP 80%	4.0	0.0	0.0	0.0	5.3	**1.9**
penconazole; EC 10%	16.1	16.8	13.5	12.1	15.2	**14.8**
prochloraz; EC 45%	49.5	53.5	35.1	52.7	51.9	**48.5**
propiconazole; EC 25%	11.3	16.1	15.2	14.2	11.8	**13.7**
thiabendazole; EC 45%	13.1	12.6	12.0	10.9	29.4	**15.6**
Control	0.0	0.0	0.0	0.0	0.0	**0.0**
Mean	**14.6**	**14.5**	**13.0**	**13.7**	**16.4**	

Source of variation	F-value ($P \leq 0.05$)	$LSD_{(T)}$ 5%
Isolates (A)	76.96	0.6
Fungicides (B)	1000	1.2
A x B	38.71	2.2

[a]Three measurements were taken for each of five replicates (n = 15).

[b]Accession numbers of representative isolates of *F. graminearum* Gr. I 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.

Table 13. Mycelial inhibition zones (mm) of five pathogenic *G. roseum* isolates surrounding the filter paper discs, impregnated with fungicides (2000 mg a.i./l), measured after 4 days of incubation on potato-dextrose agar plates at 25 °C[a]

Fungicides	Isolates[b]					**Mean**
	PREM 48918	PREM 48919	3	4	5	
benodanil; BP 50%	6.8	6.4	6.9	6.7	7.0	**6.8**
benomyl; BP 50%	12.9	12.3	16.0	14.7	14.2	**14.0**
captab; BP 50%	9.1	8.1	10.0	9.7	9.3	**9.2**
carbendazim; SC 51%	27.3	27.6	26.5	26.5	26.1	**26.8**
fenpropimorf; EC 75%	12.1	13.9	14.5	14.2	14.7	**13.9**
imazalil; BP 75%	8.1	7.2	6.4	6.4	6.1	**6.9**
mancozeb; BP 80%	6.0	6.3	6.3	6.6	6.5	**6.3**
penconazole; EC 10%	6.2	6.0	6.3	6.9	6.7	**6.4**
prochloraz; EC 45%	55.7	53.6	55.6	57.4	55.8	**55.6**
propiconazole; EC 25%	30.1	32.8	31.9	29.8	31.7	**31.2**
thiabendazole; EC 45%	0.0	0.0	0.0	0.0	0.0	**0.0**
Control	0.0	0.0	0.0	0.0	0.0	**0.0**
Mean	**14.5**	**14.5**	**15.0**	**14.9**	**14.9**	

Source of variation	F-value ($P \leq 0.05$)	$LSD_{(T)}$ 5%
Isolates (A)	NS[c]	0.9
Fungicides (B)	1000	1.0
A x B	3.55	1.8

[a]Three measurements were taken for each of five replicates (n = 15).

[b]Accession numbers of representative isolates of *G. roseum* deposited in the National Collection of Fungi, (PREM), Private Bag X134, Pretoria 0001, South Africa.

[c]Not significant.

CHAPTER 3

CHEMICAL CONTROL OF FUSARIUM DRY ROT OF POTATOES: II. CONTROL OF STORAGE ROT

ABSTRACT

The time required for *Fusarium solani* and *F. oxysporum* to infect potato tubers was determined. Six different fungicides were evaluated for their efficiency to control Fusarium dry rot of potatoes, caused by *F. solani* or *F. oxysporum* when applied at different time intervals after tuber inoculation and kept at different temperatures. The presence or absence of thiabendazole resistance was also determined in the South African *F. solani, F. oxysporum* and *F. sambucinum* dry-rot pathogen populations. Both *F. solani* and *F. oxysporum* could colonise and infect potato tuber disks within 1 hr after inoculation. Therefore the time of fungicide application is crucial. The incidence of disease increased with increased delays in fungicide treatments after tuber inoculation. Less Fusarium dry rot developed in tubers held at 5 °C than at 25 °C. Tubers treated with prochloraz 4 hr after inoculation with either *F. solani* or *F. oxysporum* or a mixture thereof, were almost completely free from dry rot, especially when kept at 5 °C rather than at 25 °C. Thiabendazole resistance appears to be absent in South African isolates of *F. solani, F. oxysporum* and *F. sambucinum.*

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INTRODUCTION

Fusarium dry rot of potatoes, a world-wide problem wherever potatoes are produced, is caused by several *Fusarium* spp. (Boyd, 1972; Seppänen, 1981). In South Africa, *F. solani* (Mart.) Appel & Wollenw. emend. Snyd. & Hans. and *F. oxysporum* Schlecht. emend. Snyd. & Hans. are the predominant, of nine *Fusarium* spp., found to be dry-rot pathogens (Theron & Holz, 1989; Chapter 2). The disease develops at wounds made either at harvest, grading or later during storage when tubers become more susceptible (Boyd, 1967; Nielsen, 1981; Powelson, Johnson & Rose, 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 considered to be a problem in the seed industry where potatoes are stored for prolonged periods. The commercial crops are usually consumed within one month after harvesting and, therefore, less Fusarium dry rot occurs in crops destined for local consumption. Annual losses in the seed industry due to Fusarium dry rot are between 5 and 15%, and individual cases of as high as 60% have been recorded (Du Plooy & Van der Plank, 1972). In South Africa, tuber inspections are conducted after harvesting, grading and packing. Even though most of the damaged and rotting tubers have already been discarded by the time of inspection, still 15.1% of the 18.3 million bags (25 kg) of potato seed presented for certification, during 1995 to 1998, 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.

Thiabendazole and mancozeb are the only fungicides registered in South Africa for the control of Fusarium dry rot (Krause, Nel & Van Zyl, 1996) and have been used extensively in potato production since the early 1970's. In spite of the use of these fungicides, the incidence of Fusarium dry rot has increased over the years. This could be attributed to the increase in the use of mechanical harvesters, grading and packing equipment, increasing mechanical damage of tubers (Allan & Moolman, 1987). This would provide more infection ports for dry-rot pathogens (Boyd, 1972; Nielsen, 1981; Powelson *et al.*, 1993). Other possible reasons for the inconsistent control achieved using these fungicides 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, Deltour, Molet, Bedin & Jouan, 1986; Carnegie, Ruthven, Lindsay & Hall, 1990; Hide, Read & Hall, 1992). Thiabendazole resistance has widely been reported in *F. sambucinum* (Fuckel) 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, Schwager & Loria, 1996) and Canada (Kawchuk, Holley, Lynch & Clear, 1994). Resistance has also been found in isolates of *F. solani* in Germany (Langerfeld, 1990) and North America (Hanson *et al.*, 1996), *F. culmorum* (W. G. Smith) Sacc. in the United Kingdom (Hide *et al.*, 1992) and North America (Hanson *et al.*, 1996), *F. acuminatum* Ell & Ev. and *F. oxysporum* in North America (Hanson *et al.*, 1996) although in low numbers. Von Stachewicz, Burth & Rathke (1992) reported *F. sambucinum* [= *F. sulphureum* Schlecht.] also to be resistant to carbendazim.

A study was undertaken to determine the efficiency of thiabendazole and mancozeb against the spectrum of *Fusarium* spp. associated with dry rot of potatoes in South Africa (Theron & Holz, 1989; Chapter 2). Other more effective fungicides than thiabendazole and mancozeb, selected during *in vitro* screening trials, were also included in this study. In most farming operations the time lapse between harvesting and eventual fungicide treatment of the tubers is 6 hr or longer, with 48 to 72 hr not uncommon. The effect of delaying fungicide-treatments was also addressed in this study and the possibility of resistance in South African isolates of *F. solani, F. oxysporum* and *F. sambucinum* to thiabendazole was investigated.

MATERIAL AND METHODS

Time required for infection

The most virulent dry-rot isolates of *F. solani* (MRC 6360) and *F. oxysporum* (MRC 6379), obtained from naturally-infected potato tubers during a survey (Chapter 2) were selected. Inoculum was prepared by transferring lyophilized conidia to carnation-leaf-agar (CLA) (Fisher, Burgess, Toussoun & Nelson, 1982). After incubation at 25 °C for 2 wk under intermittent light (fluorescent plus black lights: 12 hr cycles) (Nelson, Toussoun & Marasas, 1983), conidia were washed from cultures with sterile distilled water, counted in a haemacytometer, and the suspension diluted to 20 x 10^4 conidia/ml. Sound, unblemished potato tubers of the cultivars Up-

to-Date, BP-1 and Vanderplank, selected at harvest from plants grown under commercial conditions in field plots at the Roodeplaat Vegetable and Ornamental Plant Institute (VOPI), Pretoria, South Africa were washed under running tap water to remove adhering soil, surface-disinfested (3% sodium hypochlorite, 15 min) and allowed to dry.

***In vitro trial*:** Tuber disks (5 mm in diameter, 5 mm thick) were cut from tubers with a cork borer about half-way between the rose- and heel-ends. Ten disks were placed in each Petri dish, lined with filter paper and moistened with sterile distilled water. Disks were inoculated by placing a drop (20μl) of a spore suspension of either *F. solani* or *F. oxysporum* (20 x 10^4 conidia/ml) on top of each. The 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 disks were surface-disinfested (70% ethanol, 2 min), rinsed for 3 min in sterile distilled water and allowed to dry on sterile paper towels in a laminar airflow cabinet. Dried disks were plated (5 disks/plate) on a selective rose bengal-glycerin-urea (RbGU) medium for *Fusarium* (Van Wyk, Scholtz & Los, 1986) and incubated at 25 °C under intermittent light as described previously (Fig. 1).

Colonies were examined after 7 days with a light microscope under low magnification (x10) and the percentage of disks colonised with *Fusarium* determined (Fig. 1). Two sets of controls were included in these trials. The one control consisted of non-inoculated and the other of inoculated tuber disks. Neither of the control sets were surface-disinfested. A 3-level factorial experimental design, with four replicates and 30 tuber disks 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 by placing 100 μl of the inoculum into each hole. The tubers were wrapped in paper bags, 7 per bag, and incubated at 25 ±2 °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 and allowed to dry on a bench inside the laboratory and then incubated for a further 3 wk at 25 ±2 °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 (Fig. 2). 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 described above were selected, 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 wk at 25 °C on an orbital-shaker. Inoculum was prepared by diluting the suspensions with sterile distilled water to $1,4 \times 10^5$ conidia/ml. Conidia were counted using a haemacytometer.

Eight fungicides selected during previous *in vitro* screening trials (Chapter 2; Part 1) (Table 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 the VOPI were washed, surface-disinfested and dried as described before. Tubers were wounded on either side about half-way between the rose- and heel-ends by pressing them against 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 allowed to dry, 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. The fungicide suspensions were constantly stirred while the tubers were treated. Tubers dipped in distilled water served as controls. The treated tubers were wrapped in paper bags, 5 per bag, kept at either 25 or 5 °C for 3 and 8 wk, 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.

Reisolations 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 wk by dissecting five disks ($2\ mm^3$) randomly from the periphery of discoloured tissue of each lesion. The disks were placed on potato-dextrose agar 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 & 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 by 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 five 2% MEA plates, three replicates, containing 0, 1, 10 or 20 mg a.i./l thiabendazole, added after the autoclaved agar had cooled to 60 °C. 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, Christ-Harned, McCormick & Secor, 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

Time required for infection

***In vitro and in vivo trials*:** Tuber disks 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 BP-1 or Vanderplank. The latter cultivar was also significantly ($P \leq 0.05$) less colonised or infected than BP-1. None of the tuber disks, but 7.1% of the tuber wounds, surface-disinfested immediately after inoculation with either *F. solani* or *F. oxysporum*, were colonised or displayed dry-rot symptoms respectively. The percentage of tuber disks 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 2; 3).

Tuber disks inoculated with *F. oxysporum,* irrespective of the potato cultivar, were 100% colonised 24 - 30 hr after surface-disinfestation, whereas disks inoculated with *F. solani* were 100% colonised 48 - 72 hr after surface-disinfestation, depending on the potato cultivar (Table 2). Delaying surface-disinfestation of inoculated tubers to 24 hr, resulted an average of 55% tuber wounds expressing dry-rot symptoms (Table 3). Significantly ($P \leq 0.05$) more tuber wounds, between 52.5 and 70%, depending on the cultivar, expressed dry-rot symptoms after inoculation with *F. oxysporum* and surface-disinfested 24 hr later, than those inoculated with *F. solani*, in which case only between 40 and 60% expressed dry-rot symptoms (Table 3).

None of the non-inoculated control tuber disks or tuber wounds were colonised or expressed dry-rot symptoms respectively (results not shown). The inoculated, non-surface-disinfested control tuber disks were 100% colonised (Table 2), whereas 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 3).

Fungicide evaluations

Delaying fungicide treatments after tubers had been inoculated with either *F. solani* (Table 4) or *F. oxysporum* (Table 5) or a mixture thereof (Table 6), resulted in a significantly ($P \leq 0.05$) a higher percentage of the tuber wounds expressing dry-rot symptoms. Wounds on the tubers kept at 5 °C expressed significantly ($P \leq 0.05$) less dry-rot symptoms than those kept at 25 °C. Approximately 30% less of the tuber wounds expressed dry-rot symptoms after being inoculated with either *F. solani* or *F. oxysporum* (Tables 4; 5) and 15% less in those inoculated with a mixture of the two species (Table 6), and kept at 5 °C than in those tubers kept at 25 °C. Significantly ($P \leq 0.05$) more wounds of the non-fungicide-treated control tubers expressed 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 (Tables 4 - 6).

Prochloraz 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. This was followed by thiabendazole, imazalil and carbendazim (Tables 4 - 6). Of the two fungicides registered in South Africa for the control of Fusarium dry rot, mancozeb was in most instances the least effective fungicide tested, although it did not differ significantly ($P \geq 0.05$) from captab for the control of Fusarium dry rot caused either by *F. solani* (Table 4) or *F. oxysporum* (Table 5). Although thiabendazole was one of the more effective fungicides, it was less effective than various other fungicides controlling dry rot caused by a mixture of *F. solani* and *F. oxysporum* (Table 6) than when tubers were inoculated with either of the two *Fusarium* spp. on their own (Tables 4; 5).

Fusarium oxysporum was reisolated significantly ($P \leq 0.05$) more frequently and also in higher density than *F. solani* from tubers inoculated with a mixture of *F. solani* and *F. oxysporum* (Table 7). *Fusarium solani* and *F. oxysporum* were isolated in approximately a 1:1 ratio from the non-fungicide-treated control tubers. Comparing this to the ratio in which these two species were isolated from the fungicide-treated tubers, it would appear that the fungicides are not as effective against *F. oxysporum* as they are against *F. solani*.

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 (Fig. 3). In contrast 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 ($P \leq 0.05$), i.e. 79.0, 27.3% and 27.2% respectively, less than their mycelial growth at 0 mg a.i/l thiabendazole. In contrast, the mycelial growth of the resistant isolates, *F. solani* [= *F. coeruleum*] and *F. sambucinum* at 10 mg a.i/l thiabendazole did not differ significantly ($P \geq 0.05$) from their mycelial growth at 0 mg a.i/l thiabendazole (Fig. 3).

DISCUSSION

The longer the surface-disinfestation or fungicide treatment of the inoculated tuber disks or tubers was delayed, the more they became colonised and infected by *Fusarium* spp. During the *in vitro* and *in vivo* trials, surface-disinfestation 1 hr after inoculation was not fully effective in stopping colonisation and infestation, especially when tuber disks and tubers were inoculated with *F. oxysporum*. This is a clear indication that infestation can take place within 1 hr, under optimal conditions, making it difficult for the surface-disinfectant to come into contact with the pathogen. Surface-disinfestation 72 hr after inoculation had no effect on stopping the tuber disks or tubers from becoming totally colonised and infected. In laboratory experiments with tubers held at 16-18 °C, Bogucka & Lewosz (1978) according to Hide & Cayley (1985), detected a loss of fungicide efficacy when wounds were treated 2 hr after inoculation with *F. sambucinum*.

Hide & Cayley (1980; 1985) also demonstrated the importance of avoiding delay in treating tubers with fungicides after injury and possible infestation by dry-rot pathogens. None of the fungicides evaluated were able to control dry-rot development fully when tubers were treated 4 hr after inoculation. Treatment after 12 hr was even less effective and the amount of Fusarium dry rot increased with further delays in treatment. It is common for some potato growers in South Africa to leave their harvested potatoes for up to 18 hr on trailers before grading and fungicide treatment. 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.

Prochloraz, thiabendazole and imazalil gave the best control of Fusarium dry rot

irrespective of the *Fusarium* spp. inoculated into the tubers. These fungicides have also previously been reported to give the best control of dry rot caused by *F. solani* [= *F. solani* var. *coeruleum*] or *F. sambucinum* [= *F. sulphureum*] (Hide & Cayley, 1985; Maughan, Shanmuganathan & Hepworth, 1991). Thiabendazole and mancozeb are the only fungicides registered in South Africa for the control of Fusarium dry rot (Krause *et al.*, 1996). Thiabendazole, although not as effective as prochloraz, was more effective in controlling dry rot caused by *F. solani* than by *F. oxysporum*. Of all the fungicides tested, mancozeb was the least effective. During the *in vitro* fungicide screening trials, mancozeb was also found to be less effective in inhibiting the mycelial growth of the dry-rot pathogens (Chapter 3; Part 1). The fact that Fusarium dry rot in South Africa is mainly caused by *F. solani* or *F. oxysporum* or a combination of these species (Theron & Holz, 1989; Chapter 2) could also explain the failure to control this disease with these registered fungicides.

F. oxysporum was reisolated more frequently and in the highest density from tubers inoculated with a combination of *F. solani* and *F. oxysporum*. These two species were reisolated from the dry-rot lesions of the untreated control tubers in a ratio of approximately 1:1, indicating that the part played by each species causing dry rot was equal. However, when the tubers were fungicide-treated, *F. oxysporum* was reisolated significantly ($P \leq 0.05$) more frequently and also in higher densities than *F. solani*. It would appear that the fungicide treatments are not as effective against *F. oxysporum* as against *F. solani*. This was also found when the fungicides were screened *in vitro* (Chapter 3; Part 1).

Significantly ($P \leq 0.05$) less Fusarium dry rot developed in the tubers kept at 5 °C than those kept at 25 °C. Theron & Holz (1990) also reported that lower temperatures will decrease the amount of Fusarium dry rot. Approximately 32% less wounds expressed dry-rot symptoms in tubers inoculated with either *F. solani* or *F. oxysporum*, and kept at 5 °C than those kept at 25 °C. However, tubers inoculated with a mixture of *F. solani* and *F. oxysporum* expressed only 15% less dry-rot symptoms when kept at 5 °C. The reason for only a 15% difference in the percentage wounds expressing dry-rot symptoms at the two storage temperatures for the mixed-species-inoculations compared to the 32% difference for the single- species-inoculations is unclear. The fact that even the non-fungicide-treated control tubers kept at 5 °C developed less Fusarium dry rot than those kept at 25 °C, clearly demonstrated the value of cold storage as a control practice.

Very few *F.* solani or *F. oxysporum* inoculated tubers, fungicide-treated 4 hr after

inoculation and kept at 5 °C, developed dry-rot symptoms. This was especially prominent in tubers treated with either prochloraz, thiabendazole or imazalil. In the case of tubers 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.

It has been reported that tubers are less susceptible to infection when kept under conditions favourable to wound healing prior to infection (Escande & Echandi, 1988; Hide & Cayley, 1985; Nolte, Secor & Gudmestad, 1987). In practice, however, tubers are already contaminated with dry-rot pathogens when harvested. When such tubers are injured during harvesting or subsequent handling, inoculum is introduced into these wounds and is, therefore, present before the wound healing process has begun (Hide & Cayley, 1985; Powelson *et al.*, 1993). Tuber wounds cannot heal rapidly 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 necessary to prevent infection of the wounds (Nolte *et al.*, 1987). When tubers are kept at low temperatures, wound healing, which does not seem to be a main consideration preventing infection, will be slower. The fungal growth and the infection process will also be suppressed by the lower temperature and therefore the pathogen will remain more accessible to the surface-applied fungicides. Low-temperature storage, combined with a prochloraz treatment as soon as possible after harvest, grading and packing, are therefore recommended to keep dry-rot levels down, especially when long-term storage is required.

In South Africa, thiabendazole resistance appears to be absent from isolates of *F. solani*, *F. oxysporum* and *F. sambucinum* since mycelial growth of South African isolates was inhibited on agar medium containing ≥ 10 mg a.i./l thiabendazole. Resistance could consequently be ruled out as a contributing factor to the increase of Fusarium dry rot in South Africa. It is, therefore, import to follow strategies limiting the chances of the South African *Fusarium* population developing resistance. When fungicides, especially fungicides belonging to the benzimidazole group are used, it is import to apply them 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) can also be recommended. No cross resistance occurs between these groups and this practice will lower the selection pressure on the fungal population for resistant strains to develop. Care should,

however, also be taken not to import *Fusarium* infected potato seed tubers from abroad which could contain *Fusarium* spp., resistant to thiabendazole.

The reasons for the increase in Fusarium dry rot in South Africa, in spite of the use of the two registered fungicides, appears to be the increase in the incidence of mechanically damaged tubers owing to mechanisation. The fact that these tubers are not treated timeously and effectively with fungicides capable of controlling all the *Fusarium* spp. associated with dry rot, especially *F. oxysporum* also contributes to an increase of dry rot. It is recommended that losses due to Fusarium dry rot will be reduced by limiting mechanical damage to tubers, applying a fungicide treatment (preferably prochloraz) as soon as possible after harvest and keeping the tubers in cold storage.

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Table 1. Fungicides evaluated for their effectiveness to control Fusarium dry rot of potato tubers caused by *F. solani* and *F. oxysporum* or a combination thereof

Active ingredient	Trade name	Formulation	Supplier
benomyl	Benlate	WP 50%	Agricura
captab	Orthocide	WP 50%	AECI
captafol	Difolatan	SC 48%	Agricura
carbendazim	Derosal	SC 51%	BASF
imazalil	Fungazil	WP 75%	Jansen Pharmaceutica
mancozeb	Dithane M45	WP 80%	Zeneca
prochloraz	Omega	EC 45%	AgrEvo
thiabendazole	Tecto	EC 45%	MSD Agvet

Table 2. Percentage potato tuber disks 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

Fusarium spp.	Percentage disks colonised									
	Time intervals (hr)									
	0	1	4	6	24	30	48	54	72	**Mean**
Cultivar Up-to-Date										
F. solani	0[a]	20.0	45.0	45.0	70.0	90.0	100	95.0	100	**62.8**
Control[b]	-	-	-	-	-	-	-	-	100	-
F. oxysporum	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 BP-1										
F. solani	0	30.0	45.0	40.0	70.0	75.0	85.0	95.0	100	**60.0**
Control	-	-	-	-	-	-	-	-	100	-
F. oxysporum	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										
F. solani	0	20.0	25.0	35.0	65.0	75.0	85.0	100	100	**56.1**
Control	-	-	-	-	-	-	-	-	100	-
F. oxysporum	0	25.0	45.0	55.0	100	100	100	100	100	**69.4**
Control	-	-	-	-	-	-	-	-	100	-
Mean	**0**	**22.5**	**35.0**	**45.0**	**82.5**	**87.5**	**92.5**	**100**	**100**	**62.8**
Overall mean	**0**	**25.0**	**44.2**	**50.0**	**83.3**	**90.0**	**95.0**	**98.3**	**100**	

Source of variation	F-value (P≤ 0.05)	$LSD_{(T)}$ 5%
Fusarium spp. (A)[c]	1083.80	0.65
Cultivars (B)	94.32	0.79
Time of surface-disinfestation (C)	5763.82	1.37
A x B	20.89	1.12
A x C	130.22	1.94
B x C	21.86	2.37
A x B x C	13.15	4.46

[a]Five observations for each of four replicates (n=20).

[b]Inoculated tuber disks, either with *F. solani* or *F. oxysporum*, but not surface-disinfested. Data not included in the statistical analysis.

[c]Overall mean of the percentage tuber disks 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 3. 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 wk

Fusarium spp.	Percentage tubers expressing dry-rot symptoms									
	Time intervals (hr)									
	0	1	4	6	24	30	48	54	72	**Mean**
Cultivar Up-to-Date										
F. solani	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	-
F. oxysporum	10.0	15.0	30.0	40.0	67.5	75.0	90.0	90.0	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 BP-1										
F. solani	7.5	17.5	30.0	40.0	40.0	42.5	52.5	55.0	67.5	**39.2**
Control	-	-	-	-	-	-	-	-	72.5	-
F. oxysporum	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										
F. solani	7.5	10.0	20.0	25.0	40.0	40.0	45.0	45.0	47.5	**30.1**
Control	-	-	-	-	-	-	-	-	55.0	-
F. oxysporum	7.5	10.0	40.0	52.5	70.0	70.0	70.0	67.5	70.0	**50.8**
Control	-	-	-	-	-	-	-	-	70.0	-
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	F-value (P≤ 0.05)	$LSD_{(T)}$ 5%
Fusarium spp. (A)[c]	1077.14	0.80
Cultivars (B)	384.64	0.98
Time of surface-disinfestation (C)	1757.48	1.70
A x B	90.68	1.39
A x C	58.04	2.40
B x C	50.79	2.94
A x B x C	14.53	4.16

[a]Twenty observations for each of two replicates (n=40).

[b]Inoculated tubers, either with *F. solani* or *F. oxysporum*, but not surface-disinfested. Data not included in the statistical analysis.

[c]Overall mean of the percentage tuber wounds 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.

Table 4. Percentage tuber wounds expressing dry-rot symptoms after dip treated in fungicides at different time intervals after inoculation with a spore suspension *F. solani*, and kept at 5 °C for 8 wk or at 25 °C for 3 wk[a]

Fungicides (2000 mg a.i/l)	Percentage dry rot						
	Time of fungicide treatment after inoculation (hr)						
	4	12	24	48	72	**Mean**	**Overall mean[b]**
	Incubation temperature 5 °C						
control	50.0[c]	61.9	66.7	73.8	100.0	**70.5**	**84.9**
mancozeb	9.5	26.2	40.5	52.4	85.7	**42.9**	**62.4**
captab	7.1	26.2	40.5	45.2	95.2	**42.8**	**62.6**
captafol	9.5	21.4	31.0	42.9	83.3	**37.6**	**58.6**
benomyl	7.1	21.4	42.9	50.0	90.5	**42.4**	**59.8**
carbendazim	7.1	16.7	33.3	42.9	83.3	**36.7**	**55.8**
imazalil	2.4	11.9	28.6	38.1	57.1	**27.6**	**42.2**
thiabendazole	2.4	16.7	23.8	31.0	42.9	**23.4**	**32.9**
prochloraz	2.4	7.1	16.7	23.8	50.0	**20.0**	**29.1**
Mean	**10.8**	**23.2**	**35.9**	**44.5**	**76.5**	**38.2**	
	Incubation temperature 25 °C						
control	97.6	100.0	100.0	100	100.0	**99.5**	
mancozeb	73.8	85.7	81.0	83.3	85.7	**81.9**	
captab	71.4	78.6	78.6	88.1	95.2	**82.4**	
captafol	71.4	76.2	78.6	85.7	85.7	**79.5**	
benomyl	61.9	66.7	81.0	88.3	88.1	**77.2**	
carbendazim	47.6	66.7	83.3	85.7	85.7	**73.8**	
imazalil	38.1	50.0	85.7	52.4	57.1	**56.7**	
thiabendazole	23.8	42.9	47.6	54.8	42.9	**42.4**	
prochloraz	9.5	35.7	45.2	50.0	50.0	**38.1**	
Mean	**55.0**	**66.9**	**75.7**	**76.5**	**76.7**	**70.2**	
Overall mean	**32.9**	**45.1**	**54.8**	**60.5**	**76.6**		

Source of variation	F-value (P≤ 0.05)	$LSD_{(T)}$ 5%
Incubation temperature (A)	18649.17	0.46
Fungicide treatment (B)	2557.41	0.97
Time of treatment (C)	4045.44	0.72
A x B	202.27	1.37
A x C	1252.34	1.02
B x C	42.99	2.16
A x B x C	28.90	3.05

[a]Tubers (cv. Up-to-Date) were wounded on both sides about half-way between the rose- and heel-ends.

[b]Overall mean 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.

[c]Fourteen observations for each of three replicates (n=42).

Table 5. Percentage tuber wounds expressing dry-rot symptoms after dip treated in fungicides at different time intervals after inoculation with a spore suspension *F. oxysporum*, and kept at 5 °C for 8 wk or at 25 °C for 3 wk[a]

Fungicides (2000 mg a.i/l)	Percentage dry rot						
	Time of fungicide treatment after inoculation (hr)						
	4	12	24	48	72	**Mean**	**Overall mean[b]**
	Incubation temperature 5 °C						
control	31.1[c]	33.3	40.5	50.0	61.9	**43.4**	**58.9**
mancozeb	11.9	16.7	26.2	38.1	45.2	**27.6**	**43.4**
captab	7.1	11.9	23.8	31.0	38.1	**22.4**	**42.7**
captafol	7.1	16.7	23.8	35.7	40.5	**24.8**	**42.0**
benomyl	4.8	14.3	21.4	35.7	45.2	**24.9**	**41.3**
carbendazim	4.8	11.9	21.4	26.2	35.7	**20.0**	**37.6**
imazalil	2.4	7.1	21.4	31.0	38.1	**20.0**	**32.6**
thiabendazole	2.4	7.1	14.3	30.0	35.7	**18.1**	**32.6**
prochloraz	2.4	7.1	11.9	21.4	23.8	**13.3**	**26.2**
Mean	**8.6**	**14.0**	**22.7**	**33.3**	**40.5**	**23.8**	
	Incubation temperature 25 °C						
control	73.8	73.8	76.2	73.8	73.8	**74.3**	
mancozeb	52.4	54.8	61.9	61.9	64.3	**59.1**	
captab	59.5	61.9	61.9	64.3	66.7	**62.9**	
captafol	52.4	54.8	59.5	64.3	64.3	**59.1**	
benomyl	45.2	50.0	59.5	66.7	66.7	**57.6**	
carbendazim	45.2	50.0	59.5	59.5	61.9	**55.2**	
imazalil	35.7	40.5	45.2	50.0	54.8	**45.2**	
thiabendazole	42.9	45.2	50.0	47.6	50.0	**47.1**	
prochloraz	19.0	31.0	42.9	47.6	52.4	**38.6**	
Mean	**47.3**	**51.3**	**57.4**	**59.5**	**61.7**	**55.5**	
Overall mean	**28.0**	**32.7**	**40.0**	**46.4**	**51.1**		

Source of variation	F-value (P≤ 0.05)	$LSD_{(T)}$ 5%
Incubation temperature (A)	35614.42	0.33
Fungicide treatment (B)	1286.69	0.69
Time of treatment (C)	2604.61	0.52
A x B	90.14	0.36
A x C	439.51	0.73
B x C	13.70	1.56
A x B x C	21.70	2.19

[a]Tubers (cv. Up-to-Date) were wounded on both sides about half-way between the rose- and heel-ends.

[b]Overall mean 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.

[c]Fourteen observations for each of three replicates (n=42).

Table 6. Percentage tuber wounds expressing dry-rot symptoms after dip treated in fungicides at different time intervals after inoculation with a spore suspension of a mixture of *F. solani* and *F. oxysporum*, and kept at 5 °C for 8 wk or at 25 °C for 3 wk and kept at 5 °C for 8 wk or at 25 °C for 3 wk[a]

Fungicides (2000 mg a.i./l)	Percentage dry rot						
	Time of fungicide treatment after inoculation (hr)						
	4	12	24	48	72	**Mean**	**Overall mean[b]**
	Incubation temperature 5 °C						
control	45.2[c]	50.0	57.1	61.9	66.7	**56.2**	**71.7**
mancozeb	26.2	31.0	45.2	50.0	54.8	**41.4**	**49.5**
captab	21.4	26.2	35.7	50.0	47.6	**36.2**	**44.3**
captafol	16.7	26.2	38.1	42.9	45.2	**33.8**	**43.3**
benomyl	23.8	28.6	38.1	42.9	45.2	**35.7**	**44.5**
carbendazim	16.7	19.0	35.7	42.9	45.2	**31.9**	**36.9**
imazalil	19.0	30.6	38.1	45.2	50.0	**36.6**	**38.4**
thiabendazole	21.4	26.2	33.3	42.9	45.2	**33.8**	**38.6**
prochloraz	4.8	14.2	26.2	33.3	35.7	**22.8**	**28.6**
Mean	**21.7**	**28.0**	**38.6**	**45.8**	**48.4**	**36.5**	
	Incubation temperature 25 °C						
control	88.1	85.7	88.1	88.1	85.7	**87.1**	
mancozeb	50.0	57.1	57.1	59.5	64.3	**57.6**	
captab	42.6	45.2	52.4	57.1	64.3	**52.3**	
captafol	42.9	52.4	52.4	52.4	64.3	**52.8**	
benomyl	38.1	50.0	57.1	59.5	61.9	**53.3**	
carbendazim	28.6	38.1	45.2	47.6	50.0	**41.9**	
imazalil	26.2	38.1	42.9	45.2	52.4	**40.1**	
thiabendazole	26.2	40.5	47.6	50.0	52.4	**43.3**	
prochloraz	16.7	23.8	33.3	45.2	52.4	**34.3**	
Mean	**39.9**	**47.9**	**52.8**	**56.1**	**60.9**	**51.5**	
Overall mean	**30.8**	**37.9**	**45.8**	**50.9**	**54.6**		

Source of variation	F-value ($P \leq 0.05$)	$LSD_{(T)}$ 5%
Incubation temperature (A)	10325.04	0.29
Fungicide treatment (B)	2946.70	0.62
Time of treatment (C)	3552.52	0.46
A x B	293.47	0.87
A x C	143.72	0.65
B x C	34.25	1.38
A x B x C	18.99	1.95

[a]Tubers (cv. Up-to-Date) were wounded on both sides about half-way between the rose- and heel-ends.

[b]Overall mean 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.

[c]Fourteen observations for each of three replicates (n=42).

Table 7. Frequency and relative density of *Fusarium* spp. reisolated either singly or in combination from dry-rot lesions of potato tubers inoculated with a spore suspension of *F. solani* and *F. oxysporum*, fungicide-treated and kept at 25 °C for 3 wk[a]

Fungicides 2000 mg a.i./l	Frequency (%)[b] *Fusarium* spp. reisolated				Relative density (%)[c] *Fusarium* spp. reisolated		
	F. solani	*F. oxysporum*	Mixture	**Mean**	*F. solani*	*F. oxysporum*	Mixture
control	80.0	86.7	100.0	**86.9**	17.9	19.9	62.2
mancozeb	20.0	100.0	26.7	**48.9**	3.4	87.3	9.3
captan	40.0	80.0	66.7	**62.2**	15.4	53.3	31.3
captafol	0.0	100.0	20.0	**40.0**	0.7	94.7	4.6
benomyl	20.0	73.3	60.0	**52.2**	10.7	53.3	36.0
carbendazim	13.3	93.3	73.3	**60.0**	6.7	66.0	27.3
imazalil	13.3	86.7	53.3	**51.1**	6.0	66.7	27.3
thiabendazole	0.0	100.0	26.7	**42.2**	3.4	87.3	9.3
prochloraz	20.0	100.0	33.3	**51.1**	4.0	85.3	10.7
Mean (%)	**23.0**	**91.1**	**51.1**		**7.6**	**68.2**	**24.2**

Source of variation	F-value ($P \leq 0.05$)	$LSD_{(T)}$ 5%	F-value ($P \leq 0.05$)	$LSD_{(T)}$ 5%
Species (A)	16736.24	0.74	13866.60	0.75
Treatments (B)	960.02	1.28	-	NS[d]
A x B	614.74	2.21	730.20	2.26

[a]Surface-disinfested tubers (cv. Up-to-Date) were cut in half through the dry-rot lesion and five discs (2 mm^3) randomly dissected from the periphery of discoloured tissue and plated on potato dextrose agar at 25 °C.

[b]Frequency (%) = [Number of tuber lesions of occurrence of a *Fusarium* sp. or a mixture thereof / total number (n) of tuber lesions] x 100. (n = 15).

[c]Relative density (%) = [Number of isolates of a *Fusarium* sp. or a mixture thereof / total number (n) of isolates] x 100. (n = 75 maximum).

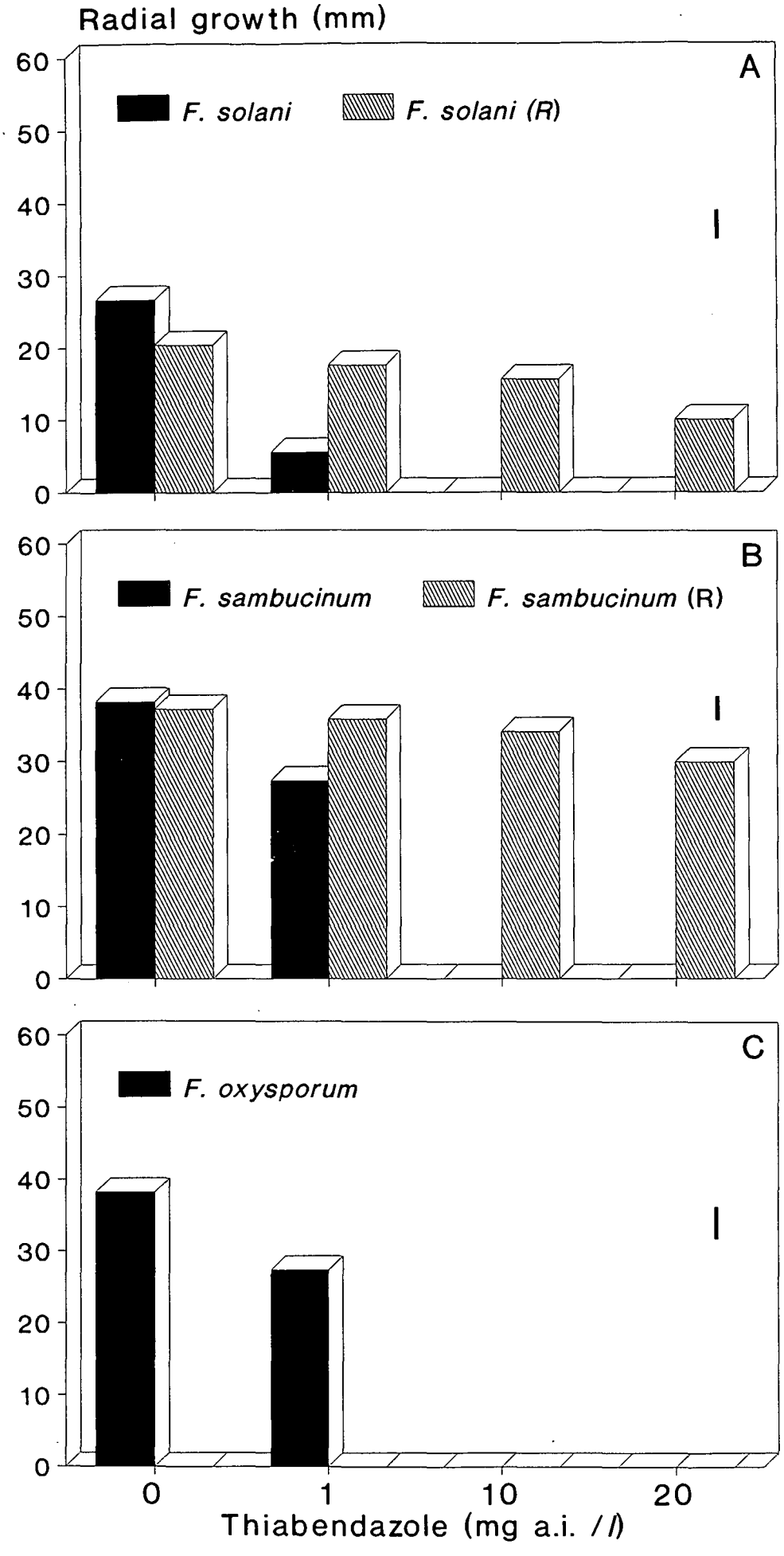
[d]Not significant.

Fig. 1. Potato tuber disks colonised (A) or not (B) after artificial inoculation with a spore suspension of either *F. solani* or *F. oxysporum*, surface-disinfested after different time intervals and incubated for 7 days at 25 °C under intermittent light on rose bengal-glycerin-urea medium medium.

Fig. 2. Potato tuber wounds expressing dry-rot symptoms (A) or not (B) after artificial inoculation with a spore suspension of either *F. solani* or *F. oxysporum*, surface-disinfested after different time intervals and incubated for 3 weeks at 25 ±2 °C and 65 - 75 % RH.

Fig. 3. Mean radial growth (mm) of 25 South African isolates each of *F. solani* (A), and *F. sambucinum* (B), and *F. oxysporum* (C) isolates after 4 days incubation in the dark at 25 °C on 2% malt extract agar containing different concentrations of thiabendazole compared to one *F. solani* [= *F. coeruleum*] and two *F. sambucinum* thiabendazole resistant (R) isolates. Five replicates, 9 cm petri dishes, for each treatment combination were used. Bar indcates $LSD_{(T)}$ 5% for all comparisons:

A = 4.8; B = 3.8 and C = 5.3.


Radial growth (mm)
A
F. solani
F. solani (R)
B
F. sambucinum
F. sambucinum (R)
C
F. oxysporum
0
10
20
30
40
50
60
0
1
10
20
Thiabendazole (mg a.i. / l)

CHAPTER 3

CHEMICAL CONTROL OF FUSARIUM DRY ROT OF POTATOES: III. CONTROL OF SEED-PIECE DECAY

ABSTRACT

The cost of potato seed tubers constitutes the greater part of the production costs of potatoes. Therefore the use of tubers cut into seed pieces is a customary planting practice of some farmers. In South Africa seed-piece decay is a significant problem, mainly because of the severe hot and dry climate. Seed-piece decay symptoms include total or partial decay of seed pieces, reduction of plant vigour and poor stands resulting in low yields. During six consecutive, three spring and three autumn, plantings, 10 fungicides, as well as the time of cutting and fungicide treatments of the potato seed tubers, were evaluated for their effectiveness in controlling seed-piece decay. Not only did some of the fungicides damage the sprouts, but they also interfered with the wound healing process. Fungicide-treated or non-treated potato seed pieces which were planted during the three spring plantings, resulted in significantly better plant stands and yields than those planted during autumn. Seed pieces treated the day before planting gave significantly better results than those treated 14 days before planting and wound healing allowed, especially during the spring plantings. The efficiency of the fungicide treatments were influenced by the quality of the seed tubers, as well as the temperatures during the growing season, especially those during the first few days after planting. Carbendazim-treated seed pieces, irrespective of the time of application, generally resulted in the best plant stands and yields followed by thiabendazole, benomyl, mancozeb, captab and the non treated control. Uncut, non-treated potato seed tubers gave significantly better results than the fungicide-treated or non-treated seed pieces, especially those planted during autumn, except for the seed pieces which were planted during spring the day after fungicide treatment.

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INTRODUCTION

The health and performance of a potato crop are influenced by many factors. Of those directly controlled by the producer, none are more important than the selection of good quality seed. Once a high-quality seedlot has been purchased, management procedures implemented before planting determine seed performance to a great degree. The way seed tubers are handled, prepared and planted is just as important as the quality of the seed tubers. Establishing a good plant stand of healthy, vigorous plants is a significant step towards high yields and a good quality crop (Secor & Gudmestad, 1993).

The cost of potato seed tubers constitutes the greater part (35 to 40%) of the production costs of potatoes. Therefore the use of tubers, cut into seed pieces is a customary planting practice of some farmers. This cutting process could, however, lead to two major problems if precautions are not taken. Firstly, it could lead to the possible spread of tuber-borne pathogens throughout the seedlot and to the soil which would cause considerable losses during the growing season or thereafter. Secondly, it exposes tuber tissue to desiccation and bacterial and/or fungal seed-piece decay (Nelson, Secor, Gudmestad & Preston, 1993; Nolte, Secor & Gudmestad, 1987). Bacterial decay, e.g. soft rot, caused mainly by *Erwinia* 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, Sorensen & Corsini, 1983; Misca, & Nelson, 1975; Nelson *et. al.*, 1993; Nielsen, 1981; Nolte *et al.*, 1987). Symptoms include total or partial decay of seed pieces, reduction of plant vigour and poor stands (Escande & Echandi, 1988). Often single sprouts emerge from partly decayed seed pieces, giving rise to small, slow growing plants which are also more susceptible to other diseases, resulting in low yields (Nielsen, 1981). Cut seed tubers contaminated with *Fusarium* spp. will also increase soil contamination and is an important source of contamination of the progeny tubers, which can result in a high levels of Fusarium dry rot in storage (Leach, 1985).

As a preventative measure against seed-piece decay, various fungicide treatments in the form of dusts or mists are often applied to cut seed tubers. Seed-piece treatments can be applied to seed which is cut and planted or to pre-cut seed before healing begins. Serious problems with seed-piece decay are often related to adverse weather during the planting season. When high-quality seed is used and the cutting surfaces properly healed after cutting, seed-piece decay is

usually minimal. This is assuming that soil conditions at planting and in the first few weeks after planting favour rapid emergence. There may be little benefit from seed-piece treatments if soil conditions are optimal at planting and until emergence. However, if unfavourable environmental conditions develop soon after planting, treated seed pieces usually produce a better plant stand. Because environmental conditions are not always optimal at planting, cannot be predicted and may also change afterwards, seed-piece treatments are a valuable precautionary measure. Seed-piece treatments may also be of some use in managing other seed-borne diseases including Rhizoctonia stem canker, Pythium leak and scab (Secor & Gudmestad, 1993).

In South Africa seed-piece decay is a significant problem, mainly because of the severe hot and dry climate. When plants develop from decaying seed pieces, these plants are usually small and under stress, making them more susceptible to other diseases, resulting in yield losses. Emergence and final stands of potatoes are often reduced owing to seed-piece decay, mainly caused by *Fusarium* spp., which is a major cause for 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, but both *Erwinia* spp. and *Fusarium* spp. can become established more quickly than these barriers can be formed. For this reason chemical seed-piece treatment can 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 is, recommended for the control of seed-piece decay (Nelson *et. al.*, 1993; Powelson, Johnson & Rowe, 1993; Schultz, 1981). In South Africa mancozeb is the only fungicide registered for treating potato seed pieces (Krause, Nel & Van Zyl, 1996). Mancozeb was, however, reported not to be effective in controlling either *Fusarium* growth *in vitro* or dry rot of *Fusarium* inoculated tubers (Chapter 3, Part 1; 2). Because these practices are not always effective in South Africa, a study to evaluate different protective chemicals, time of application, wound-healing and seasonal influences on seed-piece decay was undertaken.

MATERIALS AND METHODS

Planting material

Certified seed tubers of the cultivar Up-to-date were used in all the field trials. Sprouted seed tubers (±100 - 150 g) were cut by hand lengthwise into two seed pieces (±50 - 75 g each)

either 14 days or the day before planting and then immediately dip-treated for 5 min. in 10 fungicides (2000 mg a.i./l) selected during previous *in vitro* screening trials (Chapter 3, Part 1) (Table 1). Seed pieces dip-treated in water for 5 min., as well as uncut (±50 - 75 g), non-fungicide-treated seed tubers, served as controls. Treated seed pieces were placed in plastic crates and allowed to dry in a well-ventilated shady location before being placed in a dark room at 25 °C and 65 to 75% RH until planting. The day before planting the seed pieces, cut and fungicide-treated 14 days prior to planting, were evaluated for the effect the fungicide treatments had on the sprouts, as well as on the quality of the seed tubers.

Field preparation

All the trials were conducted under irrigation at the Roodeplaat Vegetable and Ornamental Plant Institute (VOPI), Pretoria, South Africa. A four year crop rotation with *Eragrostis curvula* (Schrad.) Nees was used on these fields. Cultivation of the fields commenced one season prior to planting, allowing the natural breakdown of plant debris. A fine seed bed 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. Soil samples were thoroughly mixed and the inoculum potential determined according to the technique described by Theron & 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, '91 and '92). Seed tubers were hand planted in 9 m rows, 30 tubers each. Spacing between and within the rows was 75 cm and 30 cm respectively. Border rows were planted between each treatment row using non-treated 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 to those treated one day before planting.

Irrigation of the trials was scheduled twice weekly, maintaining the soil moisture at field water capacity, calculating the amount of water needed from the data obtained from a class-A evaporation pan. The maximum and minimum temperatures were recorded daily for the duration of the growing seasons 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

Quality of fungicide treated seed pieces

The sprouts of those seed pieces treated with either carbendazim, captab, thiabendazole, or non-treated control, were significantly ($P \leq 0.05$) less ($\leq 21\%$) damaged than those treated with one of the other fungicides. The sprouts of those seed pieces treated with propiconazole, penconazole or prochloraz were either seriously damaged or dead (Table 2).

The quality of the seed pieces, treated with carbendazim was significantly ($P \leq 0.05$) better 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. The non-treated control seed pieces were significantly ($P \leq 0.05$) less dehydrated than those treated with imazalil, prochloraz, captafol and mancozeb, but resulted in significantly ($P \leq 0.05$) more rotten seed pieces than the fungicide-treated seed pieces (Table 2).

Results obtained with propiconazole and penconazole were not included in the statistical analysis. Seed pieces treated with these fungicides did not only have serious damage to the sprouts but were also dehydrated or even rotten. Wound healing on the cut surface of seed pieces treated with these two fungicides was almost absent. For instance, a relatively high (4.1%) percentage of seed pieces treated with propiconazole, rotted during the 14 days curing period prior to planting (Table 2).

Exceptionally good quality seed tubers were used in these trials, except those obtained during 1991, of which those used during the autumn planting of 1991 were the worst. The low

plant stands and yields experienced during that year could partly be attributed to the poor quality seed tubers used (data not shown).

Soil inoculum potential

Previously it was found that fungicides differ in their effectiveness for the control of mycelial growth of *Fusarium* spp. (Chapter 3, Part 1) or Fusarium dry rot (Chapter 3, Part 2). The inoculum potentials of the soils were more or less the same for the three consecutive (1990, '91 & '92) autumn (36, 43 & 35%) and spring (13, 15 & 10%) plantings. *Fusarium solani* (Mart.) Appel & Wollenw. emend. Snyd. & Hans. and *F. oxysporum* Schlecht. emend. Snyd. & Hans. were the predominant species isolated from infected tuber disks, with *F. solani* responsible for almost 60% of the rotting. Therefore, we expected that those fungicides, most effective for the control of *F. solani*, would also be most effective for the control 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 (Table 3). Significantly ($P \leq 0.05$) poorer plant stands (Tables 4; 5) and yields (Table 6) were obtained during the autumn plantings than during the spring plantings. This could be attributed to the temperatures during the growing period, especially the temperatures during the first 7 days after planting, which could enhance seed-piece decay.

Plant stands during the autumn plantings, obtained 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 (Tables 4; 5). 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). During the spring plantings, significantly ($P \leq 0.05$) better plant stands (Tables 4; 5) and yields (Table 6) were obtained with those seed pieces treated the day prior to planting than with those treated 14 days prior to planting allowing for wound healing.

Throughout all the autumn plantings the uncut, untreated seed tubers resulted in significantly ($P \leq 0.05$) 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 ($P \geq 0.05$) better than most of the seed pieces treated the day prior to planting (Tables 4 - 6). Except for the seed tubers cut and treated 14 days prior to planting with carbendazim, the uncut untreated seed tubers performed significantly ($P \geq 0.05$) better than the other seed treatments 14 days prior to planting (Tables 4 - 6).

The use of propiconazole and penconazole as seed-piece treatments was discontinued after the first autumn planting. Virtually no plants emerged from seed pieces treated with these fungicides, possibly owing to phytotoxicity (data not shown). During the autumn plantings the seed pieces treated with thiabendazole gave the best control of seed-piece decay, followed by the carbendazim, mancozeb, cabtab and benomyl treatments. The imazalil-, prochloraz-treated and non-treated control seed pieces resulted in significantly ($P \leq 0.05$) lower plant stands and yields than the thiabendazole-treated seed pieces (Tables 4 - 6). With the exception of the non-treated control seed pieces, which were second to carbendazim, the best seed-piece treatment, the same fungicide treatments as for the autumn plantings were found to give adequate control of seed-piece decay during the spring plantings. Imazalil-, prochloraz- and captafol-treated seed pieces also resulted in significantly lower plant stand and yields during the spring plantings than the carbendazim-treated seed pieces (Tables 4 - 6).

DISCUSSION

Fungicides were found to affect the quality of treated seed pieces. The sprouts of seed pieces treated with imazalil, prochloraz, propiconazole and penconazole were seriously damaged. Although some fungicides or the inert ingredients are known to be phytotoxic, damaging the sprouts (Secor & Gudmestad, 1993), not all the damage to the sprouts, which occurred during these trials, could be attributed to fungicide treatments. Some of the damage was also caused mechanically during the cutting process and fungicide treatments in view of the fact that even sprouts of the non-fungicide- treated control seed pieces were damaged.

Not only did some of the fungicides damage the sprouts, but some also interfered with the wound healing process. These detrimental effects on the natural healing of freshly cut seed tubers have been reported (Nolte *et al.*, 1987; Sanford, 1951). 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 wound healing process was interrupted

by the same fungicides responsible for damage to the sprouts, including captafol and mancozeb.

Treating freshly cut seed pieces with captab and thiabendazole, in a combination, was reported to have a detrimental effect of the deposition of suberin and wound periderm formation (Escande & Echandi, 1988; Stevenson, Stewart & Sanderson, 1986). Captab was, however, reported by Nolte *et al.* (1987) not to interfere appreciably with the wound healing process. Both fungicides, although not as a combination treatment, were also evaluated during these trials. Wound healing was apparently only affected when captab was used, because the seed pieces treated with captab were significantly ($P \leq 0.05$) more dehydrated than the non-treated 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 non-treated control seed pieces.

The mean inoculum potential of the field soils measured during the autumn plantings (38%) was almost three times higher than that measured (12.7%) during the spring plantings. These differences in the inoculum potential of the field soils during the two planting seasons correlated with the results obtained. Therefore, it can be concluded that the inoculum present in the soils contributed to the occurrence of seed-piece decay.

Plant stands and yields during the 1991 autumn and spring plantings were greatly reduced when seed pieces, cut from physiologically old seed tubers and originating from seed lots in which a high percentage Fusarium dry rot infected tubers were present, were planted. *Fusarium* propagules have been found in the soil adhering to tubers (Boyd, 1952; Folsom, 1959; Jeffries, 1978; Nielsen & Johnson, 1972; Small, 1944) and have been shown to be responsible for dry rot, post-harvest decay and seed-piece decay (McKee & Boyd, 1952; Small, 1945; Theron & Holz, 1991). Therefore, it is believed that the *Fusarium* propagules were randomly spread when cutting the seed and that the fungicide treatments were not adequate to control the high inoculum present. The exceptionally high temperatures experienced the first 7 days after planting during the 1991 autumn planting, also contributed to more seed-piece decay. Escande & Echandi, (1988) reported that when soil temperatures exceed 20 °C, seed pieces are more inclined to seed-piece decay. 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 has been found to disrupt apical dominance and plants from pre-cut

seed pieces emerge sooner than those from whole seed tubers or seed pieces planted the same day when cut (Chase, Silva & Kitchen, 1988). This might be true when non-sprouted seed tubers are cut. However, during these trials no significant ($P \geq 0.05$) difference was observed 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, Read & Spokes, 1984), Nolte *et al.* (1987) reported that both *Erwinia* spp. and *Fusarium* spp. can become established more quickly than wound healing can take place. During these trials 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, however, achieved during the spring plantings using seed pieces cut and treated one day prior to planting. It was established that the soil temperatures during the spring plantings, especially early in the growing season, encouraged plants to emerge sooner than during the autumn plantings and also 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 ≥25 °C. (Theron & Holz, 1990).

Carbendazim, thiabendazole, mancozeb, captab and benomyl seed-piece treatments resulted in the best plant stands and yields. De & Mahasin (1993) also reported that good plant stands and yields were achieved when carbendazim- and mancozeb-treated seed pieces were planted although not always better than the non-treated seed pieces. The non-treated control seed pieces resulted, second to the carbendazim-treated seed pieces, in the highest plant stands and yields during the spring plantings. Mancozeb was reported to be an effective seed-piece treatment especially when seed-piece decay is caused by *F. solani* (Hahm, Park, Ahn & Choi, 1993). These treatments also did not differ significantly ($P \geq 0.05$) from those achieved by planting whole seed tubers. Although non-treated seed pieces gave good results, the use of non-treated seed pieces cannot be recommended, because non-treated seed can also serve as a source of inoculum (Leach, 1985). Data presented suggests that although plant stands and yields do not improve every year, treating seed pieces with fungicides can effectively improve the plant stands and yields.

The EC formulation of prochloraz was found to be phytotoxic when applied to seed pieces. Denner, Millard, Geldenhuys and Wehner, (1998) reported that the treatment of uncut

seed tubers with prochloraz, either as an EC or DP formulation did not significantly affect plant stands or yields. Although these results were not significant, the treatment of seed tubers with the EC formulation resulted in lower plant stands and yields than the treatment with the DP formulation (Denner, personal communication). Although prochloraz as the EC formulation seems to be phytotoxic when treating seed pieces, resulting in low plant stands and yields during these trials, the EC formulation 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 (Chapter 3; Part 2). It would, therefore, appear 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 all instances in this study, seed pieces were dip-treated. This method of application was chosen because it is relatively easy to use, the best coverage is also accomplished (Hide, 1986) and it is also the most common application method used by potato growers in South Africa. A dip-treatment of seed tubers prior to planting is, however, 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. Erwinia soft rot is often a problem when tubers are dip-treated, especially when the seed tubers are not properly dried after treatment or black leg (*Erwinia carotovora* var. *atroseptica*) can be the result in subsequent crops. 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). Some potato growers use ultra-low volume spraying or dusting application practices for treating their seed tubers. However, the general view amongst growers is that given the dusting equipment available, dust-treating potato seed tubers is not feasible under their farming practices.

Throughout the trials, the use of uncut seed 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. The use of seed pieces can reduce production costs, but it is important that care is taken not to increase the risks of seed piece decay while trying to save on seed costs. Therefore, only healthy, not physiologically old, seed tubers should be used for cutting. Seed pieces must also 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. The planting of seed pieces in dry soils when the mean daily temperatures exceed 18 °C, especially during the first 7 - 14 days after planting, could lead to catastrophic results. In economic terms the use of seed pieces alongside the extra labour cost of cutting and fungicide-treating the seed tubers as well as the risk of seed-piece decay, versus uncut seed tubers should be considered carefully by potato growers before deciding to use cut seed tubers.

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Table 1. Fungicides evaluated for their effectiveness as seed-piece treatments

Active ingredient	Trade name	Formulation
benomyl	Benlate	W.P. 50%
carbendazim	Derosal	S.C. 51%
thiabendazole	Tecto	E.C. 45%
imazalil	Fungazil	W.P. 75%
prochloraz	Omega	E.C. 45%
captafol	Difolatan	S.C. 48%
captab	Orthocide	W.P. 50%
mancozeb	Dithane M45	W.P. 80%
propiconazole	Tilt	E.C. 25%
penconazole	Topaz	E.C. 10%

Table 2. 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[a]	Quality of sprouts			Quality of seed pieces		
	% 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
F-value ($P \leq 0.05$)	67.05	29.91	43.06	14.65	14.77	3.21
$LSD_{(T)}$ ($P= 0.05$)	12.1	14.1	7.0	15.3	14.8	3.8

[a]Only one set of data (105 tubers) is presented because the use of propiconazole and penconazole as seed-piece treatments was discontinued after the 1990 autumn planting. All other means are based on six mean values, three consecutive autumn (February) and spring (August) plantings, each calculated from between 105 and 130 tubers depending on the year and season.

Table 3. Temperature data for the duration of the growing periods of the three consecutive (1990, '91 and '92) autumn (February) spring (August) plantings

Month	Temperature °C								
	1990			1991			1992		
	Max	Min	Mean	Max	Min	Mean	Max	Min	Mean
Feb.	28.4	14.5	**21.5**	29.0	16.4	**22.7**	32.6	16.9	**24.8**
Mch.	27.6	14.3	**21.0**	26.2	15.1	**20.6**	29.8	13.4	**21.7**
April	25.5	11.6	**18.6**	25.8	4.7	**12.8**	28.3	10.7	**19.5**
May	22.3	5.5	**13.9**	23.6	5.2	**14.5**	24.7	2.8	**13.8**
June	15.5	1.2	**8.4**	20.0	2.8	**11.4**	21.6	0.9	**11.3**
Mean[a]	**27.9**	**16.1**	**22.0**	**33.4**	**18.7**	**26.1**	**31.2**	**11.6**	**21.4**
Mean	**23.9**	**9.4**	**16.6**	**24.9**	**8.8**	**16.9**	**27.4**	**8.9**	**18.2**
Aug.	23.2	3.7	**13.5**	23.8	3.0	**13.4**	21.6	3.3	**12.5**
Sept.	26.7	7.1	**16.9**	26.9	9.7	**18.4**	29.9	10.8	**20.4**
Oct.	28.2	12.8	**20.5**	29.2	12.6	**20.9**	30.2	13.7	**21.9**
Nov	30.6	14.9	**22.8**	28.6	14.0	**21.3**	28.0	14.5	**21.3**
Dec.	29.9	16.1	**23.0**	27.9	15.0	**21.5**	29.8	16.6	**23.2**
Mean[a]	**25.5**	**5.1**	**15.3**	**25.3**	**7.9**	**16.6**	**18.9**	**1.1**	**10.0**
Mean	**27.7**	**10.9**	**19.3**	**27.3**	**10.9**	**19.1**	**27.9**	**11.8**	**19.8**

[a]Mean temperature for 7 days after planting.

Table 4. Plant stands 3 wk 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				Mean[c]	Overall mean[d]
	Autumn plantings (1990, '91, '92)		Spring plantings (1990, '91, '92)			
	Mean[a]	Overall mean[b]	Mean[a]	Overall mean[b]		
benomyl	15.9	16.3	16.2	19.8	16.1	18.1
carbendazim	18.7	17.3	22.2	22.6	20.5	20.0
thiabendazole	19.2	18.3	16.8	19.0	18.0	18.7
imazalil	15.5	14.1	9.9	11.0	12.7	12.6
prochloraz	14.5	14.4	8.1	15.1	11.3	14.8
captafol	15.4	14.7	10.5	16.9	13.0	15.8
captab	16.9	16.4	14.0	19.9	15.5	18.2
mancozeb	16.8	17.0	15.1	19.6	16	18.3
control 1[e]	15.6	14.1	16.2	21.3	15.9	17.7
control 2[f]	26.2	25.9	25.7	25.7	26.0	25.8
Mean	**17.5**	**16.9**	**15.5**	**19.1**	**16.5**	
Seed tubers cut and treated one day before planting						
benomyl	16.7		23.4		20.1	
carbendazim	15.9		22.9		19.4	
thiabendazole	17.3		21.2		19.3	
imazalil	12.6		12.1		12.4	
prochloraz	14.3		22.0		18.2	
captafol	13.9		23.3		18.6	
captab	15.9		25.7		20.8	
mancozeb	17.2		24.1		20.7	
control 1[e]	12.6		26.3		19.5	
control 2[f]	25.5		25.6		25.6	
Mean	**16.1**		**22.8**		**19.5**	
	F-value ($P \leq 0.05$)	$LSD_{(T)}$ 5%	F-value ($P \leq 0.05$)	$LSD_{(T)}$ 5%	F-value ($P \leq 0.05$)	$LSD_{(T)}$ 5%
Planting season					17.45	1.7
Planting time (A)	NS[g]	1.4	105.35	1.3	48.36	2.1
Seed treatment (B)	9.54	5.0	13.67	4.6	11.42	4.8
Interaction (AxB)	4.38	7.1	7.59	6.5	5.23	6.7

[a]Plant 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).

[b]Overall mean irrespective of the time the seed tubers were cut and fungicide-treated prior to planting. (n = 180).

[c]Mean irrespective of the season (n = 180).

[d]Overall mean irrespective of the season and time the seed tubers were cut and fungicide-treated prior to planting. (n = 360).

[e]Seed pieces dipped in water for 5 min.

[f]Non-treated whole seed tubers (50 - 75 g).

[g]Not significant.

Table 5. Plant stands 6 wk 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				Mean[c]	Overall mean[d]
	Autumn plantings (1990, '91, '92)		Spring plantings (1990, '91, '92)			
	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
control 1[e]	16.0	15.1	18.5	23.8	17.3	19.5
control 2[f]	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	
control 1[e]	14.1		29.1		21.6	
control 2[f]	27.8		29.9		28.9	
Mean	**19.4**		**27.7**		**23.6**	
	F-value (P≤ 0.05)	$LSD_{(T)}$ 5%	F-value (P≤ 0.05)	$LSD_{(T)}$ 5%	F-value (P≤ 0.05)	$LSD_{(T)}$ 5%
Planting season					19.36	1.2
Planting time (A)	NS[e]	1.5	249.03	1.1	57.28	1.9
Seed treatment (B)	9.27	5.4	17.16	3.8	13.2	3.8
Interaction (AxB)	3.31	7.6	8.79	5.4	5.89	7.1

[a]Plant 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).

[b]Overall mean irrespective of the time the seed tubers were cut and fungicide-treated prior to planting. (n = 180

[c]Mean irrespective of the season (n = 180).

[d]Overall mean irrespective of the season and time the seed tubers were cut and fungicide-treated prior to planting. (n = 360).

[e]Seed pieces dipped in water for 5 min.

[f]Non-treated whole seed tubers (50 - 75 g).

[g]Not significant.

Table 6. Yield 15 wk after planting fungicide-dip- treated seed pieces (cv. Up-to-date, 50 - 75 g) 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				Mean[c]	Overall mean[d]
	Autumn plantings (1990, '91, '92)		Spring plantings (1990, '91, '92)			
	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
control 1[e]	15.2	15.1	44.5	50.9	29.9	33.0
control 2[f]	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	
control 1[e]	15.0		57.2		36.1	
control 2[f]	28.8		61.2		45.0	
Mean	**19.9**		**55.2**		**37.6**	
	F-value ($P \leq 0.05$)	$LSD_{(T)}$ 5%	F-value ($P \leq 0.05$)	$LSD_{(T)}$ 5%	F-value ($P \leq 0.05$)	$LSD_{(T)}$ 5%
Planting season					107.83	1.2
Planting time (A)	15.26	1.3	87.35	3.3	6.23	4.8
Seed treatment (B)	13.97	4.9	9.52	11.9	4.27	6.2
Interaction (AxB)	10.67	6.9	2.62	16.9	3.81	7.4

[a]Yield, 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).

[b]Overall mean irrespective of the time the seed tubers were cut and fungicide-treated prior to planting. (n = 6).

[c]Mean irrespective of the season (n = 6).

[d]Overall mean irrespective of the season and time the seed tubers were cut and fungicide-treated prior to planting. (n = 12).

[e]Seed pieces dipped in water for 5 min.

[f]Non-treated whole seed tubers (50 - 75 g).

CHAPTER 4

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

ABSTRACT

Twenty-four South African *Fusarium solani* isolates from potato tubers with dry-rot symptoms were compared to 33 foreign *Fusarium solani, F. solani* var. *coeruleum* and *F. coeruleum* dry-rot isolates. Morphologically all the South African and foreign *F. solani* isolates matched the description of *F. solani.* The foreign isolates referred to as *F. solani* var. *coeruleum* and *F. coeruleum* differed from the *F. solani* isolates. On potato-dextrose agar (PDA) the colonies of the *F. solani* isolates were always whitish to cream coloured whereas those of the *F. solani* var. *coeruleum* and *F. coeruleum* isolates were predominantly bluish to deep blue-purple. Long monophialides bearing microconidia in false heads, a key characteristic for the *F. solani* isolates, were absent in the *F. solani* var. *coeruleum* and *F. coeruleum* isolates, although microconidia were produced sparsely in these isolates. Macroconidia were produced abundantly in sporodochia by all the isolates examined. The sporodochia of the *F. solani* isolates were always cream coloured, whereas those of the *F. solani* var. *coeruleum* and *F. coeruleum* isolates were grey-violet to blue-purple. The macroconidia produced by the *F. solani* isolates were predominantly 3- to 4- septate with their basal cells notched, some times foot-shaped and their apical cells short, pointed and slightly hooked, whereas the macroconidia produced by the *F. solani* var. *coeruleum* and *F. coeruleum* isolates were predominantly 3- septate, their basal cells notched and their apical cells blunt. The linear colony growth of the *F. solani* var. *coeruleum* and *F. coeruleum* isolates (25.7 mm and 9.2 mm) did not agree with that reported for *F. solani* (21 - 29 mm and 26 - 36 mm) at 25 and 30 °C, respectively, after three days incubation on PDA. The optimum temperature for linear growth rate for the South African and foreign *F. solani* isolates were significantly ($P \leq 0.05$) higher (30 °C) than that of the foreign *F. solani* var. *coeruleum* and *F. coeruleum* isolates (25 °C). At 25 °C the South African *F. solani* isolates resulted in significantly ($P \leq 0.05$) more dry-rot than the foreign isolates, whereas at 15 °C the foreign isolates resulted in significantly ($P \leq 0.05$) more dry-rot than the South African isolates.

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INTRODUCTION

Many *Fusarium* spp. have been associated with tuber rots of potato *(Solanum tuberosum* L.). Of these *F. solani* (Mart.) Appel & Wollenw. emend. Snyd. & Hans., *F. sambucinum* (Fuckel) and *F. avenaceum* (Fr.) Sacc. are the dominant species associated with Fusarium dry rot in the northern hemisphere (Boyd, 1972; Jones & Wolltz, 1981; Seppänen, 1989). In contrast, *F. oxysporum* Schlecht. emend. Snyd. & Hans. and *F. solani* are most prevalent in the southern hemisphere (Stubbs, 1971; Chambers, 1973; Turkensteen, 1987; Tivoli, Torres & French, 1988; Theron & Holz, 1989). In published research, *F. solani* [= *F. solani* (Mart.) Sacc. var. *coeruleum* (Sacc.) Booth and *F. coeruleum* (Lib.) Sacc.] and *F. sambucinum* [= *F. sulphureum* (Schlecht.)] have received most attention, as dry rot pathogens of potatoes.

Von Martius (1842) was the first to report a fungus *Fusisporium solani* Mart., associated with a potato tuber rot which Saccardo (1881), according to Booth (1971), transferred to *Fusarium solani* (Mart.) Sacc. This species was later transferred to *F. solani* (Mart.) Appel & Wollenw. pro parte (Appel & Wollenweber, 1910). Von Martius (1842) did not consider this fungus as the casual agent of the disease. The first artificial infections which proved conclusively that the fungus can cause a tuber rot were made by Wehmer (1897) and Pethybridge & Bowers (1908). Wollenweber & Reinking (1935) considered *Fusisporium solani* Mart. as a synonym of either *F. solani* (Mart.) Appel & Wollenw., *F. solani* (Mart.) var. *martii* (Appel & Wollenw. sub specie) Wollenw. and *F. solani* (Mart.) Appel & Wollenw. var. *eumartii* (Carp.) Wollenw.

The section Martiella was first proposed by Wollenweber (1913) for three species i.e. *F. solani* (Mart.), *F. coeruleum* (Lib.) and *F. martii* Appel & Wollenw. Although both *F. solani* and *F. coeruleum* were associated with potato dry rot, only *F. coeruleum* was considered as a wound pathogen responsible for dry rot (Wollenweber, 1913). The section Ventricosum also proposed by Wollenweber (1913) included one species, *F. ventricosum* Appel & Wollenw., which was also associated with dry rot of potatoes.

Wollenweber & Reinking (1935) revised the section Martiella and three species, seven varieties and three forms were included. Amongst these, *F. javanicum* Koord. var. *radicicola* Wollenw. [= *F. radicicola* Wollenw.], *F. coeruleum* (Lib.) Sacc. and *F. solani* (Mart.) Appel & Wollenw. var. *striatum* (Sherb.) Wollenw. [= *F. striatum* Sherb.] and *F. solani* (Mart.) Appel & Wollenw. var. *eumartii* (Carp.) Wollenw. [= *Fusisporium solani* Mart. pro parte and *F. eumartii*

Carpenter] were considered as dry-rot or stem-end-rot pathogens of potato tubers (Wollenweber & Reinking, 1935). The section Ventricosum as proposed by Wollenweber (1913) was kept unchanged (Wollenweber & Reinking, 1935).

Snyder and Hansen (1941) regarded the system of Wollenweber & Reinking (1935) as unworkable and reduced all the species, varieties and forms in the section Martiella to *F. solani* (Mart.) Appel & Wollenw. emend. Snyd. & Hans. This species was subdivided into five *formae speciales* on the basis of pathogenicity (Snyder & Hansen, 1941). Their work with *F. solani*, which is also generally accepted as reliable, showed that the variations are heritable (Nelson, Toussoun & Marasas, 1983). According to Booth (1971), Snyder & Hansen (1940) also included *F. ventricosum*, section Ventricosum, as a synonym of *F. solani* in the section Martiella. No evidence for this could, however, be obtained from the work of Snyder & Hansen (1940; 1941).

Booth (1971) revised the section Martiella and also included the species in section Ventricosum into this section. Four species were included in the section Martiella, of which *F. solani* (Mart.) Sacc. emend. Snyd. & Hans. pro parte [= *Fusisporium solani* Martius and *Fusarium javanicum* Koord.] and *F. ventricosum* Appel & Wollenw. are associated with dry rot of potatoes. Nineteen *formae speciales* were proposed for *F. solani*, which included *F. solani* (Mart.) Sacc. f. sp. *eumartii* (Carp.) Snyd. & Hans, [= *F. eumartii* (Carp.) and *F. solani* var. *eumartii* (Carp.) Wollenw.], *F. solani* (Mart.) Sacc. f. sp. *radicicola* (Wollenw.) Snyd. & Hans. [= *F. javanicum* Koord. var. *radicicola* Wollenw.] and *F. solani* (Mart.) Sacc. var. *coeruleum* (Sacc.) Booth comb. nov. [= *F. coeruleum* (Lib.) Sacc.] which were associated with dry rot of potatoes (Booth, 1971).

Gerlach and Nirenberg (1982) chose to use the taxonomic concepts of Wollenweber and Reinking (1935) and published "The genus *Fusarium*- A Pictorial Atlas" in 1982. Gerlach and Nirenberg (1982) also revised the section Martiella and included six species in contrast to the three species, seven varieties and three forms of Wollenweber and Reinking (1935). Of these, the following were associated with potato dry rot: *F. javanicum* Koord. [= *F. javanicum* Koord. var. *radicicola* Wollenw. and *F. solani* (Mart.) Sacc. f. sp. *radicicola* (Wollenw.)], *F. solani* (Mart.) Sacc. [= *F. solani* (Mart.) Appel & Wollenw., *F. solani* (Mart.) Appel & Wollenw. var. *striatum*, *F. solani* (Mart.) Sacc. Snyd. & Hans. pro parte], *F. coeruleum* (Lib.) Sacc. [= *F. solani* (Mart.) Sacc. var. *coeruleum* (Sacc.) Booth] and *F. eumartii* (Carp.) [= *F. solani* (Mart.) Appel & Wollenw. var. *eumartii* (Carp.) Wollenw., *F. solani* (Mart.) Sacc. f. sp. *eumartii* (Carp.) Snyd.

& Hans.]. The section Ventricosum was retained by Gerlach and Nirenberg (1982) and included only the species *F. ventricosum* Appel & Wollenw.

Nelson *et al.* (1983) and Burgess & Liddell (1983) chose to follow the concept of Snyder and Hansen (1941) and accepting only one species, *F. solani* (Mart.) Appel & Wollenw. emend. Snyd. & Hans. Accordingly, *F. javanicum* Koord., *F. coeruleum* (Lib.) Sacc., *F. solani* (Mart.) Sacc. var. *coeruleum* (Sacc.) Booth, *F. solani* (Mart.) Sacc., *F. eumartii* (Carp.) and *F. ventricosum* Appel & Wollenw., associated with dry rot of potatoes, were considered as synonyms of *F. solani* (Nelson *et al.* 1983).

New initiatives in the traditional area of morphological research, such as computer-aided identification, and modern techniques used by molecular biologists, such as analysis of ribosomal RNA sequences and fingerprinting with polymerase chain reaction have provided new insights to species relationships (Hall, 1995). A DNA sequence database for all *Fusarium* spp. is being developed by O'Donnell (1996). This will be used to construct a phylogenetically-based classification system and will also develop molecular tools for the rapid and accurate identification of unknown isolates. According to O'Donnell (1996) "approximately 50 phylogenetically species within *F. solani.*" have been identified. It is not clear whether this approach will bring order in the classification of the genus *Fusarium*, or whether it will it lead to more confusion amongst taxonomists and pathologists.

Due to the fact that plant pathologists use different taxonomic systems in order to identify and name the *Fusarium* strains, confusion can arise when results are compared. Besides *F. oxysporum, F. solani* is the predominant species associated with dry rot and stem-end rot of potatoes in South Africa (Theron & Holz, 1989; Chapter 2). 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, it was essential to determine whether South African isolates identified as *F. solani* are conspecific with foreign isolates referred to in the literature as *F. solani*, *F. coeruleum* and/or *F. solani* var. *coeruleum*.

MATERIALS AND METHODS

Isolates

Fifty-seven isolates of *F. solani*, *F. solani* var. *coeruleum* and *F. coeruleum*, isolated from

potato tubers were included in this study (Table 1). These included the following: twenty-four *F. solani* isolates from potato dry-rot and stem-end-rot tubers in South Africa; five *F. solani*, seven *F. solani* var. *coeruleum* and twenty-one *F. coeruleum* isolates obtained from various foreign countries. 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), P. O. Box 19070, Tygerberg 7505, South Africa. All cultures were single spored using a modified technique of Nelson *et al.* (1983) as described by Theron & Holz (1989). Cultures were incubated at 25 ±2°C under intermittent light (fluorescent plus black lights; 12 hour cycles) (Nelson *et al.*, 1983) on carnation-leaf agar (CLA)- slants (Fisher, Burgess, Toussoun & Nelson, 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 section containing CLA of divided Petri dishes and incubated at 25 ±2 °C for two days. Agar plugs (3 mm in diameter) with mycelium were then cut aseptically from the margins of developing colonies and transferred to the potato-dextrose agar (PDA) (Nelson *et al.*, 1983) section of the divided dishes. After 10-15 day incubation at 25 ±2 °C under intermittent light (fluorescent plus black lights; 12 hour cycles), 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 ±2 °C under intermittent light (fluorescent plus black lights; 12 hour cycles) 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 (Oxoid) plates. After 10 days incubation as above, mycelial 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 Petri dishes, containing PDA (Oxoid). Plates (3 per treatment) supporting mycelial 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 computed.

Pathogenicity

Inoculum was prepared by transferring carnation leaf pieces from the CLA-slants to CLA-plates. After incubation at 25 ±2 °C for 2 weeks under intermittent light (fluorescent plus black lights; 12 hour cycles) (Nelson *et al.*, 1983), spores were washed from cultures with sterile distilled water and the suspension diluted to 1 x 10^4 propagules/ml (macro- and microconidia). Counts were made with a haemacytometer. Sound unblemished tubers, cv. Up-to-Date, selected after harvest from plants grown under normal commercial conditions in field plots at the Roodeplaat Vegetable and Ornamental Plant Institute, Pretoria, South Africa were disinfected for 15 min in 3% sodium hypochlorite 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 ±2 °C and 50-70% relative humidity.

After 3 weeks incubation, tubers were cut in half at the inoculation sites and the extent of dry-rot 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 & Unterstenhöfer (1967).

The four most virulent isolates of each group of isolates (*F. solani* South African, *F. solani* foreign, *F. solani* var. *coeruleum* and *F. coeruleum*) were selected. Inoculum was prepared from them 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 by 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

Cultural characteristics

The *F. solani* isolates from dry-rot lesions of potatoes in South Africa, as well as the five foreign *F. solani* isolates, obtained from P. E Nelson and D. Rodriques (Table 1) had similar cultural characteristics. The *F. coeruleum* and *F. solani* var. *coeruleum* isolates, however, 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: On PDA growth was rapid reaching 33.9 ±3.1 and 38.5 ±6.4 mm at 25 and 30°C, respectively, in three days (Table 2). Aerial mycelium usually rather sparse, floccose, felt-like, sometimes zonate and whitish to cream in colour (Fig. 1).

Pigmentation - isolates did not vary, and the under surfaces of the colonies were predominantly cream-coloured (Table 1 & Fig. 1). For some of the isolates, light greenish to bluish flecks occurred, especially in old cultures.

Sporulation - usually commenced rapidly in the aerial mycelium as microconidia cohering in false heads after 2 to 3 days. Macroconidia were abundantly formed by most of the isolates in white to cream-white sporodochia (Fig. 2) which became visible in the vicinity of the point of inoculation after 4 to 7 days resulting in these cultures having a pionnotal appearance (Fig. 1).

Conidiophores: Primary conidiophores arising 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.

Phialides - microconidia were produced on long and slender monophialides, mostly with distinct collarettes. Macroconidia were produced on shorter, subcylindric to slightly 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.

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 (Fig. 3).

Chlamydospores: More or less abundantly formed after ±14 days, terminal 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, however, rough-wall chlamydospores were occasionally produced by some of the isolates.

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

Colonies: On PDA growth was slow, compared with the *F. solani* isolates, reaching 25.7 ±4.1 and 9.2 ±1.5 mm at 25 and 30°C, respectively, in three days (Table 2). 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 (Fig. 1).

Pigmentation - rather variable and the under surfaces of the cultures were dark purple, almost black, but predominantly bluish to deep blue-purple (Table 1& Fig. 1).

Sporulation - in freshly isolated cultures abundant, 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 (Fig. 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 (Fig. 1).

Conidiophores: Initially arising as single lateral phialides in the aerial mycelium or branching loosely, however, when formed in sporodochia or pionnotal layers, branching was very dense.

Phialides - were short, monophialidic, with distinct collarettes almost cylindrical and rather

slender. Long monophialides bearing microconidia in false heads were not produced by these isolates.

Conidia: Microconidia were sparsely produced by some of the isolates, however, 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 (Table 1). 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 (Fig. 3).

Chlamydospores: More or less abundantly formed, terminal or intercalary in hyphae or conidia, occurring either single, in pairs, in chains or in clusters. Usually globose to subglobose and smooth-walled.

Growth on potato-dextrose agar

Colony diameters of the isolates, irrespective of the incubation temperature, did not differ significantly ($P \leq 0.05$) from each other within a taxon, except for a few South African and foreign *F. solani* isolates. The colony diameters of the *F. solani* isolates, South African and foreign [= *F. solani*- group], did not differ significantly ($P \leq 0.05$) from each other. The same was found between the *F. solani* var. *coeruleum* and *F. coeruleum* isolates [= *F. coeruleum*-group] (Tables 2; 3). The isolates of the *F. coeruleum*-group grew significantly ($P \leq 0.05$) better (12.1 mm) at 15 °C than those of the *F. solani*-group (8.8 mm). The optimum temperature for the *F. solani*-group was 30 °C, followed by 25 °C and for the *F. coeruleum*-group the optimum was 25 °C, followed by 20 °C (Tables 2 and 3).

Pathogenicity

At 25 °C the South African *F. solani* isolates were significantly ($P \leq 0.05$) more virulent than all the foreign isolates (Table 4). The most virulent isolate (Fs 2 = MRC 6358), a South

African *F. solani* isolate, resulted in 72.2% dry rot, whereas two foreign *F. solani* isolates (Fs 71 and Fs 72) resulted in the lowest (22.8%) dry rot (Table 4). When inoculated tubers were incubated at different temperatures, 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 5).

Significantly ($P \leq 0.05$) less dry rot developed in inoculated tubers which were kept at 5 °C, especially in the tubers inoculated with South African and foreign *F. solani* isolates, than the tubers kept at either 15 or 25 °C. Significantly ($P \leq 0.05$) more dry rot developed in the tubers inoculated with the South African *F. solani* isolates and kept at 25 °C for three weeks than the foreign isolates. The foreign isolates, irrespective of the taxon, resulted in significantly ($P \leq 0.05$) more 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 appears to be 25 and 15 °C, respectively (Table 5).

DISCUSSION

The *F. solani* isolates, from potato dry rot lesions in South Africa as well as the isolates obtained from P. E. Nelson and D. Rodriques, matched the description of *F. solani* (Nelson *et al.*, 1983). The most striking cultural and morphological characteristics were the homogeneous whitish to cream colour of these isolates, the presence 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 more or less the description of all the *Fusarium* spp., except *F. coeruleum*, which are associated with dry rot of potatoes in the sections Martiella and Ventricosum described by Gerlach & Nirenberg (1982). The *F. solani* isolates (South African and foreign) could not be assign with confidence to any one of these species, but they fit the descriptions of *F. javanicum* and *F. solani* best.

Colony diameters reached after three days incubation at 25 and 30 °C is considered an important characteristic for the identification of *Fusarium* spp. (Burgess & Liddell, 1983). For *F. solani*, colony diameters of 21-29 mm and 26-36 mm at 25 and 30 °C, respectively, were reported (Burgess & Liddell, 1983). The colony diameters of the *F. solani* isolates, South African and foreign [= *F.*

solani- group] and the foreign *F. solani* var. *coeruleum* and *F. coeruleum* isolates [= *F. coeruleum*-group] were compared under the set conditions at 25 and 30 °C. Those of the *F. solani*-group (33.9 and 38.6 mm), although larger, were comparable with the mean colony diameters published (Burgess & Liddell, 1983). Those of the *F. coeruleum*-group (25.7 and 9.2 mm) were not comparable, especially at 30 °C. Burgess & Liddell (1983) and Nelson *et al.* (1983) have included *F. solani* as the only species in the section Martiella. Species such as *F. javanicum*, *F. coeruleum*, *F. solani* var. *coeruleum*, *F. solani*, *F. eumartii* and *F. ventricosum*, associated with dry rot of potatoes, were considered as synonyms by Nelson *et al.* (1983). Of these synonyms, *F. coeruleum* and *F. ventricosum* growth rates 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* (Gerlach & Nirenberg, 1982).

Characteristics of the *F. coeruleum* and *F. solani* var. *coeruleum* isolates were consistent with those published for *F. coeruleum* (Gerlach & Nirenberg, 1982) and to a certain extent to *F. solani* var. *coeruleum* (Booth, 1971), but differed from the description of *F. solani* (Burgess & Liddell, 1983; Nelson *et al.*, 1983). The most notable difference was the absence of long monophialides bearing microconidia in false heads. Some of the isolates of *F. coeruleum* and *F. solani* var. *coeruleum* did, however, produce microconidia sparsely, but 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, however, foot-shaped basal cells were absent. The apical cells were blunt but were never pointed or hooked such as those produced by the *F. solani* isolates. The macroconidia were predominantly 3-septate and most often the septa were indistinct. 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 & Liddell (1983) and Nelson *et al.* (1983) of *F. solani*.

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, respective, after three days incubation on PDA (Burgess & Liddell, 1983). The optimal growth temperature for these isolates was between 20 and 25 °C, which corresponds to previous reports (Moore, 1945; Langerfeld, 1978; Seppänen, 1981; Seppänen, 1982; Tivoli, Corbiére & Jouan, 1983). This is different to those for the *F. solani* isolates, where the optimum temperature appears to be between 25 and 35 °C for the South African isolates (Theron & Holz, 1990).

All the isolates examined in this study 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, whereas those inoculated with the foreign isolates developed the highest percentage of dry rot when kept at 15 °C. Therefore, it appears 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* occurs most rapidly at temperatures ranging between 15 and 20 °C (Moore, 1945; Boyd, 1972; Langerfeld, 1978; Seppänen, 1981; Seppänen, 1982; Tivoli, *et al.*, 1983). Seppänen (1981), however, reported that dry rot caused by *F. solani* var. *coeruleum* occurs most rapidly at lower temperatures (12 - 24 °C) than *F. solani* (≥ 24 °C) which do agree with the findings in the present studies. Even at 5 °C a substantial percentage of dry rot developed in tubers inoculated with these taxa, except for the *F. solani* isolates, irrespective of their origin, in which case virtually no dry rot developed.

Although Snyder & Hansen (1941), Nelson *et al.* (1983) and Burgess & Liddell (1983) considered *F. coeruleum* and *F. solani* var. *coeruleum* as synonyms of *F. solani*, substantial cultural and morphological differences occur between isolates of these taxa and the *F. solani* (South African and foreign) isolates. Care should therefore be taken when comparing results with reports published on these taxa. It is in such cases that approaches, such as the phylogenetic classification of *Fusarium* proposed by O'Donnell (1996) might help to distinguish between species more precisely.

The South African *F. solani* isolates associated with potato dry rot, are unique in the sense that they require higher temperatures for dry-rot development than the foreign dry-rot isolates. It can also be concluded after the extensive surveys conducted in the potato producing areas of South Africa (Theron & Holz, 1989; Chapter 2) that isolates resembling *F. coeruleum* and *F. solani* var. *coeruleum* are probably not associated with dry rot of potatoes in South Africa.

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Table 1. Sources and cultural characteristics of isolates of *Fusarium solani*, *F. solani* var. *coeruleum* and *F. coeruleum* from potato tubers with dry rot

Accession number	Original number	Geo-graphical origin	Obtained from	Cultural characteristics		
				Pig-mentation[a]	Mono-phialides & falseheads[b]	Micro-conidia[b]
F. solani: South Africa						
Fs 1 MRC[c] 6360	N9/D4/1(3)	SA	D.J. Theron	Cream	+	+
Fs 2 MRC 6358	KB1/P3/1(5)	SA	D.J. Theron	Cream	+	+
Fs 3	AP91/60(3)	SA	D.J. Theron	Cream	+	+
Fs 4 MRC 6359	N9/P2/1(4)	SA	D.J. Theron	Cream	+	+
Fs 5 MRC 6361	NO. 9	SA	C.P.N. Visser	Cream	+	+
Fs 6 MRC 6362	D4/D5/5(3)	SA	D.J. Theron	Cream	+	+
Fs 7	KB9/D13/1(5)	SA	D.J. Theron	Cream	+	+
Fs 8	D3/D10/1(4)	SA	D.J. Theron	Cream	+	+
Fs 9	KB2/D9/5(4)	SA	D.J. Theron	Cream	+	+
Fs 10	KB12/D2/1(5)	SA	D.J. Theron	Cream	+	+
Fs 11	KB11/P5/5	SA	D.J. Theron	Cream	+	+
Fs 12	N7/D3/1(5)	SA	D.J. Theron	Cream	+	+
Fs 13 MRC 4797	O6/2/10/D18	SA	D.J. Theron	Cream	+	+
Fs 14	W14/P4/1(5)	SA	D.J. Theron	Cream	+	+
Fs 15	N9/P11(5)	SA	D.J. Theron	Cream	+	+
Fs 16	D4/D15/5(4)	SA	D.J. Theron	Cream	+	+
Fs 17	W17/P2/1(5)	SA	D.J. Theron	Cream	+	+
Fs 18	D8/P3/1(4)	SA	D.J. Theron	Cream	+	+
Fs 19	AP91/60(1)	SA	D.J. Theron	Cream	+	+
Fs 20	O8/1/2P5	SA	D.J. Theron	Cream	+	+
Fs 21	O6/2/10D18	SA	D.J. Theron	Cream	+	+
Fs 22	T3/1/1P17	SA	D.J. Theron	Cream	+	+
Fs 23 MRC 4678	T2/1/5D17	SA	D.J. Theron	Cream	+	+
Fs 24	T1/3/5D5	SA	D.J. Theron	Cream	+	+
F. solani: Foreign						
Fs 69	S-0661	Palestine	P.E. Nelson	Cream	+	+
Fs 71	S-1060	Canada	P.E. Nelson	Cream	+	+
Fs 72	S-1247	Peru	P.E. Nelson	Cream	+	+
Fs 75	S-1259	Peru	P.E. Nelson	Cream	+	+
Fs 78	FMI 139	USA	D. Rodriques	Cream	+	+

F. solani var *coeruleum*: Foreign						
Fsc 58	4-5a	UK	G.T. Jellis	Dark purple	-	+
Fsc 59	4-6	UK	G.T. Jellis	Dark purple	-	-
Fsc 61	87-29-186F	France	B. Tivoli	Dark purple	-	+
Fsc 62	87-A-134bF	France	B. Tivoli	Blue/Purple	-	-
Fsc 63	87-A-113aF	France	B. Tivoli	Blue/Purple	-	-
Fsc 64	89-35-5F	France	B. Tivoli	Dark purple	-	+
Fsc 65	86-29-59F	France	B. Tivoli	Blue/Purple	-	+
F. coeruleum: Foreign						
Fc 26	Fc 7	UK	R.L. Wastie	Bluish	-	+
Fc 30	F 37	USA	D.L. Corsini	Blue/Purple	-	+
Fc 31	F 124	USA	D.L. Corsini	Blue/Purple	-	-
Fc 33	F 128	USA	D.L. Corsini	Blue/Purple	-	-
Fc 34	F 40	USA	D.L. Corsini	Blue/Purple	-	-
Fc 35	Fc 7'90	Germany	E. Langerfeld	Bluish	-	-
Fc 36	Fc 8'72	Germany	E. Langerfeld	Bluish	-	-
Fc 37	Fc 9'72	Germany	E. Langerfeld	Blue/Purple	-	-
Fc 38	Fc 10'90	Germany	E. Langerfeld	Bluish	-	-
Fc 39	Fc 11'90	Poland	E. Langerfeld	Blue/Purple	-	-
Fc 45	6	UK	G.A Hide	Bluish	-	+
Fc 46	24	UK	G.A Hide	Bluish	-	+
Fc 47	25	UK	G.A Hide	Blue/Purple	-	+
Fc 48	73	UK	G.A Hide	Dark purple	-	+
Fc 50	76	UK	G.A Hide	Dark purple	-	+
Fc 51	158	UK	G.A Hide	Dark purple	-	+
Fc 52	211	UK	G.A Hide	Bluish	-	-
Fc 53	212	UK	G.A Hide	Bluish	-	+
Fc 54	213	UK	G.A Hide	Dark purple	-	+
Fc 56	236	UK	G.A Hide	Blue/ Purple	-	-
Fc 57	237	UK	G.A Hide	Dark purple	-	+

[a]Pigmentation of the under surfaces of the colonies on PDA.

[b]+ = present; - absent.

[c]Accession numbers of representative *F. solani* isolates from South Africa, 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.

Table 2. 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 potato-dextrose agar-plates

Fusarium isolates	Temperature °C							
	5	10	15	20	25	30	35	**Mean**
F. solani: South Africa								
Fs 1 MRC[a] 6360	5.0[b]	5.0	8.3	32.7	41.0	48.3	26.0	**23.8**
Fs 2 MRC 6358	5.0	5.0	10.0	28.0	32.3	34.3	17.3	**18.9**
Fs 3	5.0	5.0	9.7	26.3	34.0	34.0	22.0	**19.4**
Fs 4 MRC 6359	5.0	5.0	8.3	31.0	38.7	49.3	26.7	**23.4**
Fs 5 MRC 6361	5.0	5.0	9.0	27.0	32.7	33.3	16.0	**18.3**
Fs 6 MRC 6362	5.0	5.0	8.7	26.3	33.0	34.3	20.7	**19.0**
Fs 7	5.0	5.0	8.0	26.7	31.7	33.0	17.7	**18.4**
Fs 8	5.0	5.0	9.3	26.7	31.0	34.0	19.0	**18.6**
Fs 9	5.0	5.0	8.7	28.3	35.7	35.3	19.3	**19.6**
Fs 10	5.0	5.0	8.7	24.7	29.7	32.7	19.0	**17.8**
Fs 11	5.0	5.0	10.3	30.0	37.0	44.3	16.7	**22.6**
Fs 12	5.0	5.0	9.3	25.3	29.3	35.3	23.7	**19.0**
Fs 13 MRC 4797	5.0	5.0	8.3	29.7	33.0	34.0	17.3	**18.9**
Fs 14	5.0	5.0	7.3	29.7	33.0	35.0	23.7	**19.8**
Fs 15	5.0	5.0	9.7	30.0	37.7	46.0	34.0	**23.9**
Fs 16	5.0	5.0	10.0	27.0	31.3	35.0	24.7	**19.7**
Fs 17	5.0	5.0	7.0	27.0	36.0	47.6	24.0	**21.7**
Fs 18	5.0	5.0	8.0	27.0	35.3	47.3	24.7	**21.8**
Fs 19	5.0	5.0	8.0	25.0	29.7	34.0	21.3	**18.3**
Fs 20	5.0	5.0	7.3	27.0	33.0	36.7	18.7	**19.0**
Fs 21	5.0	5.0	10.0	24.7	32.0	32.0	22.0	**18.7**
Fs 22	5.0	5.0	10.0	26.0	33.0	32.7	23.3	**19.3**
Fs 23 MRC 4678	5.0	5.0	9.0	25.7	32.3	33.0	26.7	**19.5**
Fs 24	5.0	5.0	10.3	30.0	37.3	44.3	22.7	**22.1**
Mean	**5.0**	**5.0**	**8.9**	**27.6**	**33.7**	**37.8**	**22.4**	**20.1**
F. solani: Foreign								
Fs 69	5.0	5.0	9.0	27.0	30.0	36.0	13.0	**17.9**
Fs 71	5.0	5.0	9.3	29.0	34.0	33.0	13.7	**18.5**
Fs 72	5.0	5.0	6.3	27.3	34.3	48.0	32.0	**22.6**
Fs 75	5.0	5.0	7.0	32.3	40.0	49.0	32.0	**24.3**
Fs 78	5.0	5.0	10.7	29.3	37.3	46.0	31.0	**23.5**
Mean	**5.0**	**5.0**	**8.5**	**29.0**	**35.1**	**42.5**	**24.3**	**21.3**

F. solani var. *coeruleum*: Foreign								
Fsc 58	5.0	7.0	12.7	28.0	27.3	9.0	7.0	**13.7**
Fsc 59	5.0	8.0	14.0	28.0	28.3	10.3	7.0	**14.4**
Fsc 61	5.0	6.7	12.3	26.3	28.3	9.7	7.0	**13.6**
Fsc 62	5.0	6.0	12.0	27.0	21.0	8.0	7.0	**12.3**
Fsc 63	5.0	7.3	12.7	25.7	28.0	9.0	7.0	**13.5**
Fsc 64	5.0	8.0	12.0	24.3	25.0	8.0	7.0	**12.8**
Fsc 65	5.0	7.3	12.0	18.7	21.3	8.0	7.0	**11.3**
Mean	**5.0**	**7.2**	**12.5**	**25.4**	**25.6**	**8.9**	**7.0**	**13.1**
F. coeruleum: Foreign								
Fc 26	5.0	5.0	11.7	23.7	22.0	5.0	5.0	**11.0**
Fc 30	5.0	5.0	10.0	26.0	26.3	9.0	7.3	**12.7**
Fc 31	5.0	6.3	8.3	14.3	20.7	9.0	6.3	**10.0**
Fc 33	5.0	6.0	12.7	29.0	29.0	10.7	8.0	**14.3**
Fc 34	5.0	5.0	10.0	26.0	27.0	12.7	8.0	**13.4**
Fc 35	5.0	6.0	11.0	26.0	27.0	9.3	8.0	**13.2**
Fc 36	5.0	5.0	12.0	25.3	26.7	9.0	7.0	**12.9**
Fc 37	5.0	6.3	12.7	29.0	28.3	9.0	8.0	**14.0**
Fc 38	5.0	5.0	13.0	27.0	28.0	9.0	7.3	**13.5**
Fc 39	5.0	5.0	12.7	28.3	29.0	9.0	7.0	**13.7**
Fc 45	5.0	5.0	11.0	26.0	27.0	9.0	7.0	**12.9**
Fc 46	5.0	6.7	13.7	25.0	27.3	10.0	7.0	**13.5**
Fc 47	5.0	7.0	11.7	25.0	23.7	13.7	7.0	**13.3**
Fc 48	5.0	6.3	12.7	26.7	28.0	9.0	7.0	**13.5**
Fc 50	5.0	8.0	13.3	26.7	29.0	10.0	7.0	**14.1**
Fc 51	5.0	8.0	12.3	28.0	28.0	9.0	7.0	**13.9**
Fc 52	5.0	7.0	11.3	28.0	28.3	9.0	7.0	**13.7**
Fc 53	5.0	5.0	12.0	27.7	28.0	9.0	7.0	**13.4**
Fc 54	5.0	6.3	12.0	27.3	27.3	9.0	7.0	**13.4**
Fc 56	5.0	6.0	13.0	19.0	15.7	8.7	7.0	**10.6**
Fc 57	5.0	7.0	13.0	21.0	12.7	8.0	7.0	**10.5**
Mean	**5.0**	**6.0**	**11.9**	**25.5**	**25.7**	**9.3**	**7.1**	**12.9**
Overall mean	**5.0**	**5.7**	**10.4**	**26.7**	**29.9**	**24.2**	**15.0**	

Source of variation	F-value (P≤ 0.05)	$LSD_{(T)}$ 5%
Isolates	1.87	6.9
Taxa (A)	8.81	3.8
Temperature (B)	323.49	2.7
A x B	167.43	3.1

[a]Accession numbers of representative *F. solani* isolates from South Africa, 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.

[b]Each value represent three observations, each consisted of a 90-mm plastic Petri dish containing potato-dextrose agar (Oxoid).

Table 3. Colony diameters (mm) of South African and foreign isolates of *Fusarium solani*, *F. solani* var. *coeruleum* and *F. coeruleum*, 6 days after incubation at different temperatures on potato-dextrose agar-plates

Fusarium isolates	Temperature °C							
	5	10	15	20	25	30	35	**Mean**
F. solani: South Africa								
Fs 1 MRC[a] 6360	5.0[b]	7.0	19.0	43.3	51.0	58.0	23.3	**29.5**
Fs 2 MRC 6358	5.0	5.0	16.7	51.0	70.0	83.0	45.0	**39.4**
Fs 3	5.0	7.0	17.3	42.3	56.0	56.3	32.3	**30.9**
Fs 4 MRC 6359	5.0	5.0	16.0	50.7	69.7	81.7	44.0	**38.9**
Fs 5 MRC 6361	5.0	7.0	16.7	43.3	54.3	57.0	24.7	**29.7**
Fs 6 MRC 6362	5.0	6.7	15.0	44.0	56.7	58.0	36.0	**31.6**
Fs 7	5.0	5.7	15.0	43.0	53.3	55.3	26.0	**29.0**
Fs 8	5.0	6.0	17.0	43.0	51.3	56.3	24.3	**29.0**
Fs 9	5.0	6.3	17.3	45.0	59.0	60.3	39.7	**33.2**
Fs 10	5.0	5.7	16.3	37.7	47.0	54.3	32.3	**28.3**
Fs 11	5.0	6.7	19.3	49.0	63.7	79.3	47.7	**38.7**
Fs 12	5.0	6.0	17.3	37.7	49.0	54.3	35.0	**29.2**
Fs 13 MRC 4797	5.0	7.0	16.7	44.7	54.3	54.3	26.3	**29.8**
Fs 14	5.0	7.3	17.0	45.3	55.7	61.0	39.3	**33.0**
Fs 15	5.0	7.0	17.0	50.0	63.3	84.7	56.0	**40.4**
Fs 16	5.0	6.3	19.0	42.0	54.3	59.7	34.3	**31.5**
Fs 17	5.0	7.0	16.0	43.3	62.3	82.0	40.3	**36.6**
Fs 18	5.0	6.7	17.3	42.7	57.3	64.3	37.3	**33.0**
Fs 19	5.0	5.7	16.3	39.7	51.0	55.0	31.0	**29.1**
Fs 20	5.0	5.7	16.3	43.7	54.0	57.7	24.7	**29.6**
Fs 21	5.0	6.7	19.0	38.0	52.3	56.0	38.0	**30.7**
Fs 22	5.0	6.0	17.0	39.7	48.7	57.3	39.0	**30.4**
Fs 23 MRC 4678	5.0	6.0	15.7	42.7	52.0	54.7	49.3	**32.2**
Fs 24	5.0	7.3	19.0	48.3	65.7	79.7	23.0	**35.4**
Mean	**5.0**	**6.4**	**17.1**	**43.8**	**56.3**	**63.3**	**35.4**	**32.5**
F. solani: Foreign								
Fs 69	5.0	6.0	17.0	43.0	55.7	57.7	15.0	**28.5**
Fs 71	5.0	8.0	20.0	45.7	59.7	55.7	15.0	**29.9**
Fs 72	5.0	5.0	13.0	46.0	63.7	80.7	51.0	**37.8**
Fs 75	5.0	5.0	12.3	50.0	68.0	81.3	60.7	**40.3**
Fs 78	5.0	8.3	19.0	45.3	61.3	76.3	52.0	**38.2**
Mean	**5.0**	**6.5**	**16.3**	**46.0**	**61.7**	**70.3**	**38.7**	**34.9**

F. solani var. *coeruleum*: Foreign								
Fsc 58	5.0	13.0	27.0	44.3	40.0	9.0	5.0	**20.5**
Fsc 59	5.0	14.3	28.0	45.7	45.3	10.3	5.0	**22.0**
Fsc 61	5.0	13.0	26.0	43.0	46.0	9.7	5.0	**21.1**
Fsc 62	5.0	13.0	27.3	34.3	33.0	8.0	5.0	**18.0**
Fsc 63	5.0	14.3	27.7	43.7	45.7	9.0	5.0	**21.5**
Fsc 64	5.0	14.7	25.3	40.0	40.3	8.0	5.0	**19.8**
Fsc 65	5.0	13.0	22.3	30.7	35.0	8.0	5.0	**17.0**
Mean	**5.0**	**13.6**	**26.2**	**40.2**	**40.8**	**8.9**	**5.0**	**20.0**
F. coeruleum: Foreign								
Fc 26	5.0	5.0	22.7	37.7	34.3	5.0	5.0	**16.4**
Fc 30	5.0	9.3	22.0	41.7	41.7	9.0	5.0	**19.1**
Fc 31	5.0	12.0	16.7	23.0	34.0	9.0	5.0	**15.0**
Fc 33	5.0	12.6	25.0	47.3	45.7	10.7	5.0	**21.6**
Fc 34	5.0	12.0	22.3	42.7	41.3	9.3	5.0	**20.0**
Fc 35	5.0	12.3	24.7	42.0	43.7	9.3	5.0	**20.3**
Fc 36	5.0	10.0	24.0	42.3	42.3	9.3	5.0	**19.7**
Fc 37	5.0	12.3	26.3	45.0	42.3	9.0	5.0	**20.7**
Fc 38	5.0	13.0	27.0	43.3	45.7	9.0	5.0	**21.1**
Fc 39	5.0	13.7	27.0	45.3	47.3	9.0	5.0	**21.8**
Fc 45	5.0	12.0	22.3	41.7	45.0	9.0	5.0	**20.0**
Fc 46	5.0	13.3	25.7	39.7	43.7	10.0	5.0	**20.3**
Fc 47	5.0	15.0	23.0	40.7	34.3	13.7	5.0	**19.5**
Fc 48	5.0	14.7	27.3	44.0	46.3	9.0	5.0	**21.6**
Fc 50	5.0	15.0	27.3	44.7	48.3	10.0	5.0	**22.2**
Fc 51	5.0	14.0	24.3	45.3	46.3	9.0	5.0	**21.3**
Fc 52	5.0	14.0	26.0	46.3	47.7	9.0	5.0	**21.9**
Fc 53	5.0	11.7	25.0	44.7	45.0	9.0	5.0	**20.8**
Fc 54	5.0	12.0	26.3	46.0	46.7	9.0	5.0	**21.4**
Fc 56	5.0	11.0	26.0	29.3	24.0	8.7	5.0	**15.6**
Fc 57	5.0	13.0	28.3	37.0	26.3	8.0	5.0	**17.5**
Mean	**5.0**	**12.3**	**24.7**	**41.4**	**41.5**	**9.2**	**5.0**	**19.9**
Overall mean	**5.0**	**9.4**	**20.9**	**42.7**	**49.4**	**37.3**	**20.8**	

Source of variation	F-value (P≤ 0.05)	$LSD_{(T)}$ 5%
Isolates	1.52	7.2
Taxa (A)	7.32	3.5
Temperature (B)	342.87	2.5
A x B	183.27	2.9

[a]Accession numbers of representative *F. solani* isolates from South Africa, 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.

[b]Each value represent three observations, each consisted of a 90-mm plastic Petri dish containing potato-dextrose-agar (Oxoid).

Table 4. Mean percentage dry rot of potato tubers, cv. Up-to-Date, 3 weeks after inoculation with South African and foreign isolates of *F. solani*, *F. solani* var. *coeruleum* and *F. coeruleum* from potato tubers with dry rot and incubated at 25 °C

Fusarium taxa	Number of isolates	Percentage dry rot[a]		
		Minimum	Maximum	**Mean[b]**
F. solani - South African	24	40.6	72.2	**58.5**
F. solani - Foreign	5	22.8	27.8	**24.2**
F. solani var. *coeruleum* - Foreign	7	32.2	55.6	**46.7**
F. coeruleum - Foreign	21	25.6	56.9	**45.9**
Source of variation	F-value ($P \leq 0.05$)		$LSD_{(T)}$ 5%	
Taxa	74.96		2.3	

[a]Dry rot determined according to a disease index, were 0 = no lesion development and 5 = tuber completely decayed. Results presented as percentage of decay calculated according to the method of Kremer & Unterstenhöfer (1967).

[b]Each mean value represents 30 observations for each isolate.

Table 5. 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

Fusarium isolates[a]	Percentage dry rot[b]			
	Temperature °C			
	5	15	25	Mean
	F. solani: South Africa			
Fs 2 MRC 6358[c]	9.2	38.6	75.8	**41.2**
Fs 5 MRC 6361	7.2	36.1	76.4	**39.9**
Fs 13 MRC 4797	6.3	30.3	74.6	**37.1**
Fs 23 MRC 4678	5.8	29.4	76.8	**37.3**
Mean	**7.1**	**33.6**	**75.9**	**38.9**
	F. solani: 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**
	F. solani var. *coeruleum*: 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**
	F. coeruleum: 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**	

Source of variation	F-value ($P \leq 0.05$)	$LSD_{(T)}$ 5%
Isolates	5.91	10.0
Taxa (A)	53.48	5.0
Temperature (B)	292.03	4.3
A x B	166.72	4.7

[a]The four most virulent isolates of each taxon.

[b]Dry rot determined according to a disease index, were 0 = no lesion development and 5 = tuber completely decayed. Results presented as percentage of decay calculated according to the method of Kremer & Unterstenhöfer (1967). Each value represent 30 observations.

[c]Accession numbers of representative *F. solani* isolates from South Africa, 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.

Fig. 1 Colony morphology of *Fusarium solani* (A), *F. solani* var. *coeruleum* (B) and *F. coeruleum* (C) on potato-dextrose agar after 14 days incubation at 25 °C under intermittent light (fluorescent plus black lights; 12 hour cycles).

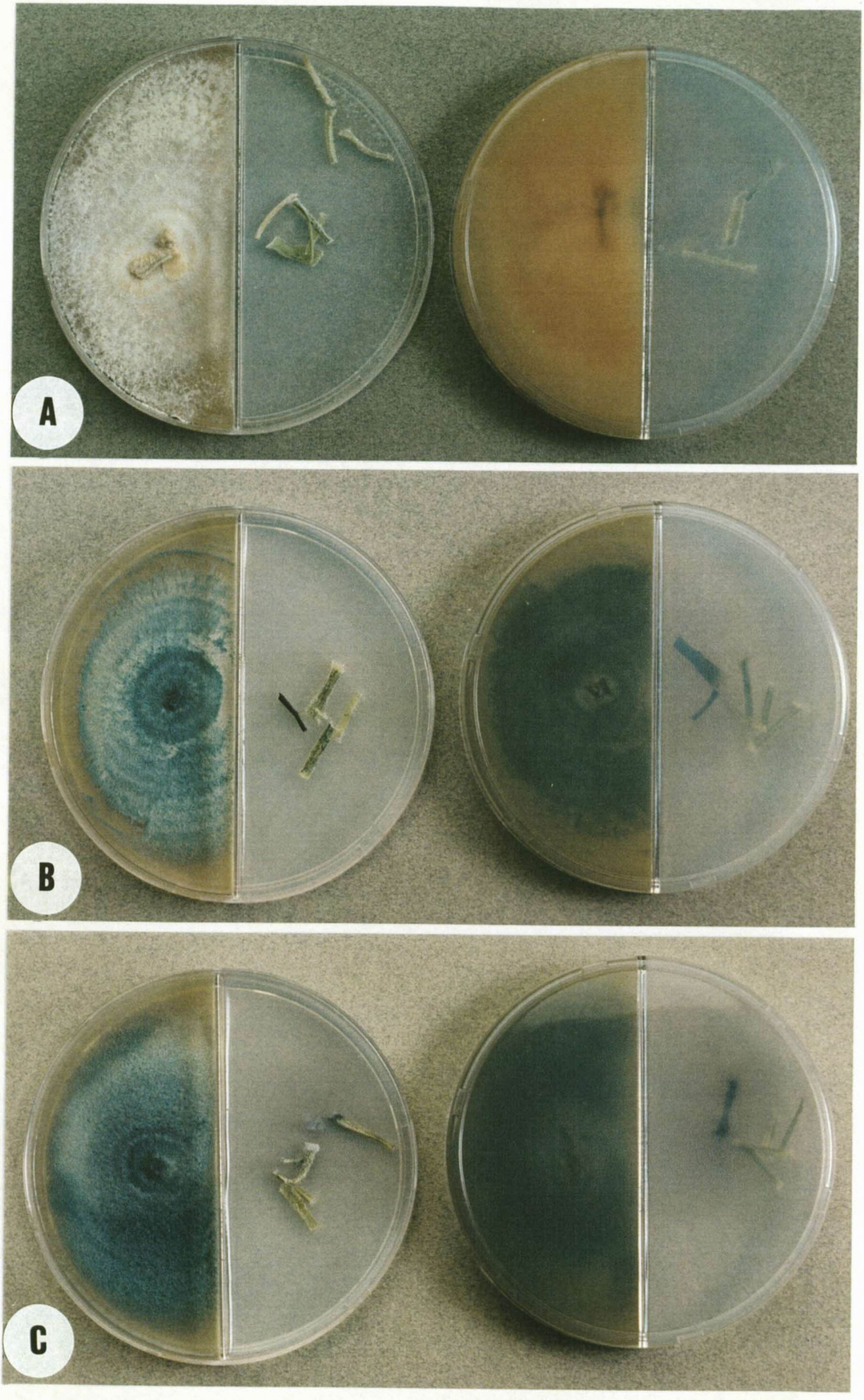

A
B
C

Fig. 2 Sporodochia produced on carnation leaves by *Fusarium solani* (A), *F. solani* var. *coeruleum* (B) and *F. coeruleum* (C) after 14 days incubation on carnation-leaf agar at 25 °C under intermittent light (fluorescent plus black lights; 12 hour cycles). Bars = 1 mm.

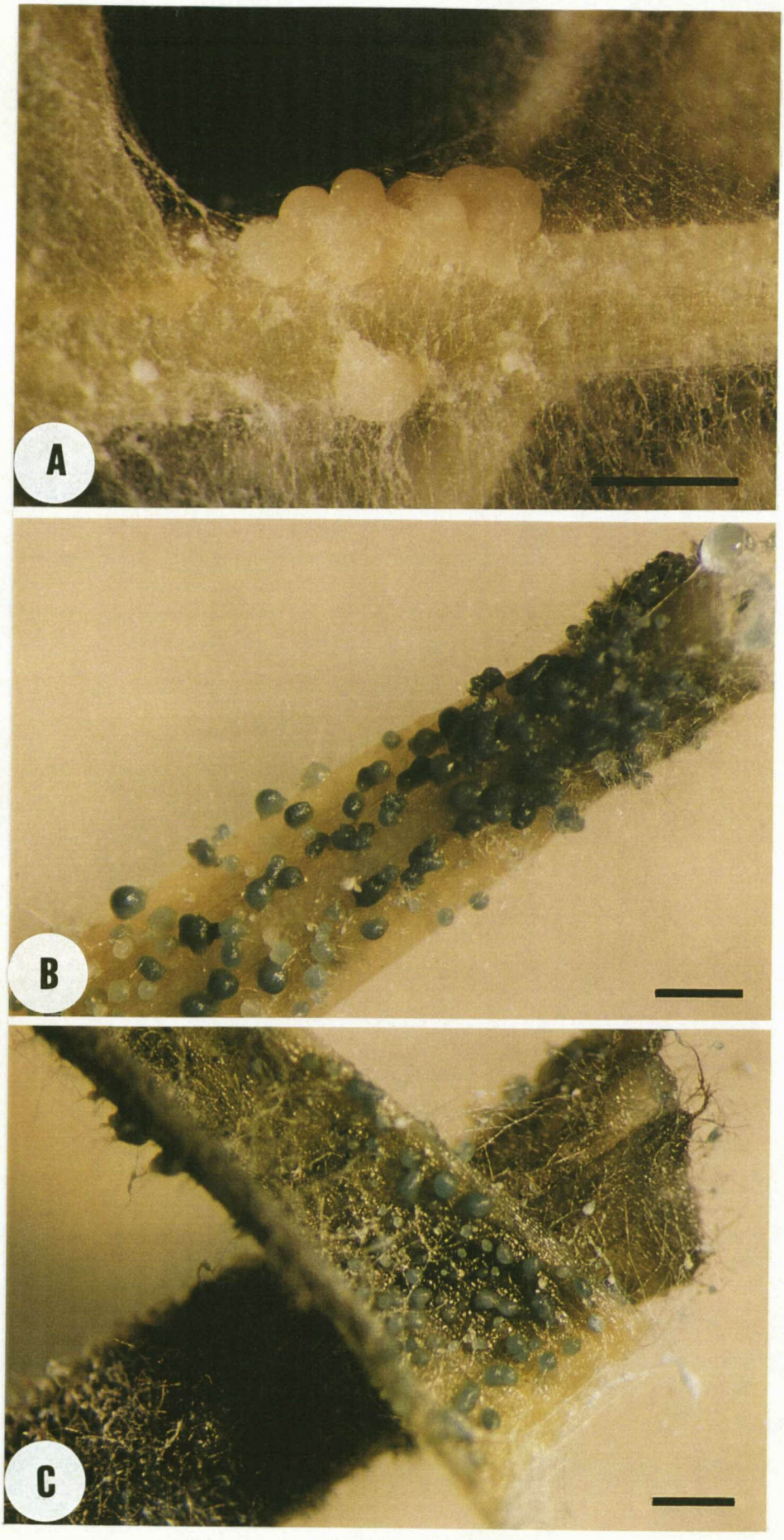
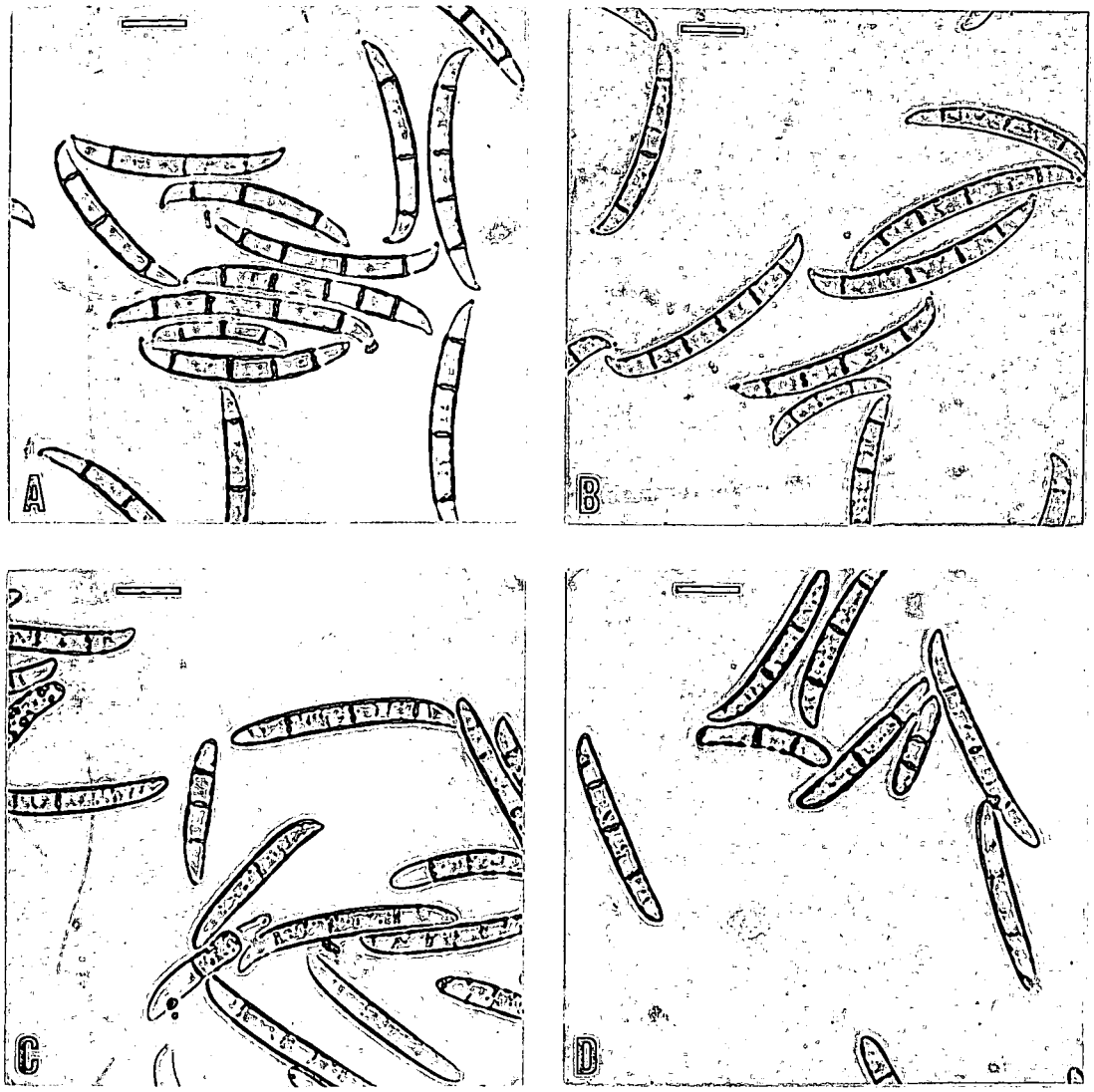

A
B
C

Fig. 3 Macroconidia produced in sporodochia, formed on carnation-leaf agar 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 (fluorescent plus black lights; 12 hour cycles). Bars = 10 μm.


A
B
C
D

CHAPTER 4

TAXONOMY: II. DIFFERENTIATION BETWEEN SOUTH AFRICAN AND FOREIGN ISOLATES OF *FUSARIUM SAMBUCINUM SENSU LATO* FROM POTATO TUBERS WITH DRY ROT

ABSTRACT

Seventy-two *Fusarium sambucinum* isolates, 25 South African and 47 foreign (19 *F. sambucinum*, 23 *F. sulphureum* and 5 *F. roseum* var. *sambucinum*), isolated from dry-rotted potato tubers, were compared with one another. Although cultures of the South African isolates appeared to be distinct, they could not be differentiated morphologically from the foreign isolates using the taxonomic system of Nelson *et al.* (1983). The differentiation of the foreign isolates into different taxa could also not be warranted based only on morphological characteristics. The linear growth of all the isolates increased with increasing temperature to an optimum of 25 °C, which was also the optimum temperature for dry-rot development. Although the South African isolates had more rapid linear growth rates than the foreign isolates, they were less virulent than the foreign isolates. No vegetative compatibility occurred between the South African and foreign isolates. The South African isolates were divided into six VCG's with the most and least virulent isolates occurring in distinct VCG's. Only three VCG's were obtained for the foreign isolates, with no correlation either to their origin or to the taxon into which these isolates were identified. None of the sexual crosses between the South African isolates and the *F. sambucinum* (*Gibberella pulicaris*) strains, (MAT1-1 or MAT1-2), were successful. Crossing some South African isolates with each other resulted in fertile crosses and back crosses with the parents and progeny were also successful. The ascospores produced from crosses between the South African isolates were significantly ($P \leq 0.05$) larger than those produced when the *G. pulicaris* strains were crossed with each other. Five South African *F. sambucinum* isolates generated PCR products of the expected size with *F. venenatum* primers. This finding indicates that some of the *F. sambucinum* isolates obtained from potato tubers in South Africa are conspecific with *F. venenatum*. Much more work, including detailed morphological, genetic and molecular comparisons between the South African isolates of *F. sambucinum* and authentic isolates of *F. venenatum*, is required to resolve the identity of these enigmatic South African isolates from potato tubers with dry rot.

Manuscript to be submitted to Mycological Research

INTRODUCTION

Many *Fusarium* spp. have been associated with tuber rots of potato *(Solanum tuberosum* L.). Of these *F. solani* (Mart.) Appel & Wollenw. emend. Snyd. & Hans.[= *F. solani* (Mart.) Sacc. var. *coeruleum* (Sacc.) Booth and *F. coeruleum* (Libert) Sacc.], *F. sambucinum* (Fuckel) [= *F. sulphureum* Schlecht.] and *F. avenaceum* (Fr.) Sacc. are the dominant species associated with Fusarium dry rot in the northern hemisphere (Boyd, 1972; Jones & Wolltz, 1981; Seppänen, 1989). In the southern hemisphere, however, *F. oxysporum* Schlecht. emend. Snyd. & Hans. and *F. solani* are most often associated with Fusarium dry rot (Stubbs, 1971; Chambers, 1973; Turkensteen, 1987; Tivoli, Torres & French, 1988; Theron & Holz, 1989). According to Booth (1971), *F. sambucinum* is also considered as a serious dry rot pathogen of potatoes in Australia. Of these species *F. solani* and *F. sambucinum* received the most attention by researchers.

F. sambucinum [= *F. trichothecioides* Wollenw.] was first reported as a cause of dry rot of potato tubers by Jamieson & Wollenweber (1912) and this was also the first description of a definite tuber rot conclusively demonstrated to be caused by a *Fusarium* sp. In contrast to this Wollenweber (1913) claimed that the first artificial infections which proved conclusively that *F. solani* [= *F. solani* (Mart.) Sacc.] caused a special tuber rot of potatoes were made by Wehmer (1897) and Pethybridge & Bowers (1908). Nevertheless, *F. sambucinum* has been shown also to be a serious dry-rot pathogen.

Various *Fusarium* spp. are considered to be synonyms of *F. sambucinum* (Nelson, Toussoun & Marasas, 1983; Chapter 1). These include *F. trichothecioides*, *F. bactridioides* Wollenw., *F. sulphureum* Schlecht., *F. sambucinum* Fuckel var. *trichothecioides* (Wollenw.) Bilai, *F. roseum* Lk. emend. Snyd. & Hans. 'Sambucinum' and *F. roseum* Lk. emend. Snyd. & Hans. var. *sambucinum* (Fuckel) Snyd. & Hans. Of these, *F. trichothecioides* [teleomorph unknown], *F. sulphureum*, [teleomorph = *Gibberella cyanogena* (Sollm.) Wollenw.], *F. sambucinum* [teleomorph = *G. pulicaris* (Fr.) Sacc.] (Booth, 1971) and *F. roseum* var. *sambucinum* [teleomorph = *G. pulicaris*] (Tivoli & Jouan, 1981) are true dry-rot pathogens of potatoes, causing substantial losses during storage (Boyd, 1972).

Apart from *F. oxysporum* and *F. solani*, the predominant species associated with dry rot and stem-end rot of potatoes in South Africa, *F. sambucinum* has also frequently been isolated in relatively high densities in three important potato producing regions (Theron & Holz, 1989;

Chapter 2). Although these *F. sambucinum* isolates in general corresponded morphologically to the taxonomic concept for *F. sambucinum* published by Nelson *et al.* (1983), they differed in several respects. Therefore they may represent a discrete taxon.

A fundamental problem inherent in *Fusarium* identification is that members of the genus vary widely in morphological and non-morphological characteristics, including virulence, and these criteria are used in taxonomic systems (Windels, 1991). Various "new" techniques, based on physiology (*formae speciales* and races) or genetics, have been described and put into practice in systematic and taxonomic studies within *Fusarium* species. These techniques are, however, not recognized by the International Code of Botanical Nomenclature, Article 4.3 (13) (Windels, 1991). In recent years various useful techniques, based on genetics, have been developed, including vegetative compatibility groupings (VCG) that allows population analysis (Leslie, 1990; Correll, 1991), mating populations (Leslie, 1991), restriction fragment length polymorphism (RFLP) (Michelmore & Hulbert, 1987), random amplification of polymorphic DNA (RAPD) (Williams, Kuberlik, Livak, Rafolski & Tingey, 1991) and primed polymerase chain reaction (PCR) (Welsh & McClelland, 1991). These techniques are mainly employed to distinguish between strains or groups within a species.

These techniques were recently employed during the European *F. sambucinum* Project launched in 1989 to draw a joint conclusion on the taxonomic position of *F. sambucinum* (Nirenberg, 1995a). From this project it was concluded that strains of *F. sambucinum sensu lato* should be divided into three species; *F. sambucinum* Fuckel *sensu stricto*, *F. torulosum* (Berk. & Curt.) Nirenberg comb. nov. and *F. venenatum* Nirenberg sp. nov. (Nirenberg, 1995b). This three-species concept was supported by computer-aided morphometrical analysis of the macroconidia (Hagen & Hagen, 1995), phylogenetic relationship determined from ribosomal RNA sequences (Logrieco, Peterson & Bottalico, 1995), random amplified polymorphic DNA polymerase chain reaction (RAPD PCR) (Hering & Nirenberg, 1995), esterase zymogram analysis (Szécsi, Turóczi & Bordás, 1995) and *in vitro* toxin production (Altomare, Logrieco, Bottalico, Mulé, Moretti & Evedente, 1995; Thrane & Hansen, 1995).

In this study, some of the above techniques were used to compare South African and foreign *F. sambucinum* isolates in order to relate results obtained in South Africa with those reported in the literature and to determine the taxonomic placement of the South African isolates.

MATERIALS AND METHODS

Isolates

Seventy-two isolates of *F. sambucinum sensu lato*, isolated from potato tubers, were included in this study (Table 1). These included the following: Twenty-five South African *F. sambucinum* isolates from potato tubers with dry rot and stem-end rot and 47 foreign isolates representing 19 *F. sambucinum*, 23 *F. sulphureum* and 5 *F. roseum* var. *sambucinum* isolates obtained from various countries. Of the foreign *F. sambucinum* isolates, three were originally received from Dr. Anne E. Desjardins (Northern Regional Research Centre, USDA-ARS, Peoria, IL 61604, USA) as *G. pulicaris* strains (Desjardins & Beremand, 1987). The South African isolates were identified according to 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), P. O. Box 19070, Tygerberg 7505, South Africa. All the cultures were single spored using a modified technique of Nelson *et al.* (1983) as described by Theron & Holz (1989). The cultures were incubated at 25 ±2 °C under intermittent light (fluorescent plus black lights; 12 hour cycles) (Nelson *et al.*, 1983) on carnation-leaf agar (CLA) slants (Fisher, Burgess, Toussoun & Nelson, 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 section of divided Petri dishes containing CLA and incubated at 25 ±2 °C for two days. Mycelial plugs (3 mm in diameter) were cut aseptically with a cork borer from the margins of the developing colonies and transferred to the potato-dextrose agar (PDA) section of the divided dishes. PDA was prepared from freshly harvested potato tubers, cv. Up-to-Date (Nelson *et al.*, 1983). After a period of incubation of 10 - 15 days at 25 ±2 °C under intermittent light (fluorescent plus black lights; 12 hour cycles), the identity of the cultures was confirmed according to the taxonomic system of Nelson *et al.* (1983).

Linear growth rates on PDA

Carnation leaf pieces were transferred aseptically from the CLA slants to PDA (Oxoid)

plates. After 10 days incubation as above, mycelial plugs (3 mm in diameter) were cut aseptically with a cork borer from the edges of colonies of each isolate and transferred to the centre of 90-mm plastic Petri dishes containing PDA (Oxoid). Separate plates (3 per treatment) supporting mycelial 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.

Linear growth rates on minimal medium (MM)

Mycelial plugs (4 mm^2) cut from the edge of seven-day old colonies of five South African isolates of *F. sambucinum*, Fsam 1, Fsam 3, Fsam 4, Fsam 6 and Fsam 7 (MRC 6454 - 6458), grown on MM, were placed onto the centres of fresh MM plates. These were incubated in Chex-All bags (Propper, NY) at 28 °C under intermittent light (fluorescent plus black lights; 12 hour cycles) (Nelson *et al.*, 1983) for up to 10 days. During this time positions of colony margins were marked on the undersides of the Petri dishes on four radii, at the same time everyday. The mean linear extent rates (mm/day) were calculated from these measurements for all the isolates and compared to those of *F. venenatum* (Yoder & Christianson, 1998).

Pathogenicity

Inoculum was prepared by transferring carnation leaf pieces from the CLA slants to CLA plates. After a period of incubation of 2 weeks at 25 ±2 °C under intermittent light (fluorescent plus black lights; 12 hour cycles) (Nelson *et al.*, 1983), spores were washed from the cultures with sterile distilled water and the suspension was diluted to 1 x 10^4 propagules/ml (macro- and microconidia). Counts were made with a haemacytometer. Sound, unblemished tubers (cv. Up-to-Date), selected after harvest from plants grown under normal commercial conditions in field plots at the Roodeplaat Vegetable and Ornamental Plant Institute, Pretoria, South Africa were disinfected for 15 min in 3% sodium hypochlorite and allowed to dry. Fifteen tubers (75 - 150 g each) were inoculated approximately halfway between the rose- and heel-ends, either side of the tuber, 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 ±2 °C and 50 - 70% relative humidity.

After incubation for three weeks, the tubers were cut in half at the inoculation sites and

the extent of dry-rot development was determined using 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 percentages of dry rot according to the method of Kremer & Unterstenhöfer (1967).

The four most virulent isolates of each group of isolates (South African *F. sambucinum* and foreign *F. sambucinum*, *F. sulphureum* and *F. roseum* var. *sambucinum*) were selected. Inoculum was prepared from them and tubers were inoculated as described previously. Tubers, cv. Up-to-Date, (five per treatment, three replicates) were wrapped in paper bags and incubated in a randomized block design at 5, 15 an 25 °C for three weeks after which the percentage dry rot was determined as described previously.

Statistical analysis

The data were statistically analysed by analysis of variance. The least significant difference (LSD) function of the SAS/STAT program for personal computers (SAS Institute Inc., 1989) was used for significant differences according to Tukey's test ($P \leq 0.05$).

Vegetative compatibility tests

Carnation leaf pieces from the 25 South African *F. sambucinum* isolates, as well as from the 47 foreign *F. sambucinum*, *F. sulphureum* and *F. roseum* var. *sambucinum* isolates (Table 1) were transferred from the CLA slants to water agar plates. After a period of incubation of 7 days at 25 ±2 °C under intermittent light (fluorescent plus black lights; 12 hour cycles) (Nelson *et al.*, 1983), mycelial 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 Petri dishes containing minimal media (MM), with only nitrate as a source of nitrogen (Puhalla, 1985) and incubated as above for 12 days.

***Generation of* nit-*mutants*:** Chlorate-resistant mutants were generated by plating mycelial plugs (3 mm diameter), cut from the edge of a colony of each isolate and transferred to the centre of 90-mm plastic Petri dishes, eight replicates for each chlorate containing medium. The following chlorate-containing media were used: potato-sucrose chlorate agar (KPS), minimal chlorate agar (KMM), potato-dextrose chlorate agar (PDC) (Correll, Klittich & Leslie, 1987), malt agar (CMA) and Czapek-Dox chlorate agar (CDA) (Venter, Theron, Steyn, Ferreira &

Eicker, 1992) all amended with potassium chlorate (15 g/l). These plates were incubated as above and examined after 7, 10 and 12 days for fast-growing, chlorate-resistant sectors (Fig. 1). Mycelial plugs (3 mm in diameter) were cut from the growing margins of each sector, transferred to plates containing (MM) and incubated at 25 °C for 10 days. *Nit*-mutants showing sparse mycelial growth were retained for further study, while the wild types with profuse aerial mycelium were discarded. To verify these *nit*-mutants, they were plated onto a complete medium (CM) (Correll *et al.*, 1987) and those which showed wild-type growth were considered as *nit*-mutants.

***Phenotyping of* nit-*mutants*:** The retained *nit*-mutants of each isolate were phenotyped for their ability to utilize nitrate, nitrite, hypoxanthine, ammonium and uric acid as sole source of nitrogen on the five phenotyping media. Small pieces of the *nit*-mutants were cut from the MM-plates and transferred to one plate each of MM (= nitrate), nitrite (= NO_2), hypoxanthine (= HX), ammonium tartrate (= NH_4) and uric acid (= UA) medium, and their colony morphology was evaluated after a period of incubation of four days as above (Correll *et al.*, 1987; Leslie, 1990). The growth of the *nit*-mutants was scored as follows: - for no or minimal growth and + for profuse, wild-type growth. All the *nit*-mutants should have been - on MM and + on NH_4. Mutants which complied with these requirements were phenotyped as follows:

Phenotype	MM	NO_2	HX	NH_4	UA
nit-1	-	+	+	+	+
nit-3	-	-	+	+	+
nit-M	-	+	-	+	+

Thus *nit*-1 utilizes nitrite, hypoxanthine, ammonium tartrate and uric acid, *nit*-3 utilizes hypoxanthine, ammonium tartrate and uric acid, but not nitrite and *nit*-M utilizes nitrite, ammonium tartrate and uric acid, but not hypoxanthine (Cove, 1976).

***Vegetative compatibility grouping*:** Pairings (*nit*-1 or *nit*-3 with *nit*-M) were made by placing mycelia plugs (3 mm in diameter) cut from the growing margins of each *nit*-mutant 1-3 cm apart on MM in all combinations and were incubated for 21 days as described above (Correll *et al.*, 1987). Where *nit*-1 mutants were not formed by the isolates, *nit*-3 mutants were used. Genetic complementation was indicated by the presence of vigorous growth of aerial mycelia on MM, where the mycelia of two *nit*-mutant colonies came in contact and formed a hetrokaryon

(Fig. 1). When *nit*-mutants of two different isolates formed a heterokaryon, their parent isolates were assigned to the same vegetative compatibility group (VCG). All other pairs in respect of which no heterokaryons were formed, were considered vegetative incompatible, and their parent isolates were assigned to different VCG's.

Sexual compatibility

***Source of isolates*:** Sexual compatibility was determined between the 25 South African isolates as well as with two *G. pulicaris* tester strains (Fsam 42 and Fsam 44) and female sterile strains (Fsam 43 and Fsul 67). Data on mating type and sex of these *G. pulicaris* strains were previously published; Fsam 42 = male$^+$ and female$^+$, Mat1-1 and Fsam 44 = male$^+$ and female$^+$, Mat1-2 (Desjardins & Beremand, 1987; Hohn, Desjardins & McCormick, 1993; Desjardins, 1995) and Fsam 43 = male$^+$ and female$^-$, Mat1-2 and Fsul 67 = male$^+$ and female$^-$, Mat1-2, (Hohn *et al*, 1993). Fsul 67 (= MRC 514 = R 5389) was originally isolated from potatoes in Iran and is known for its trichothecene mycotoxin production (Steyn, Vleggaar, Rabie, Kriek & Harington, 1978). The isolates used in this study were kept on CLA slants at 5 °C in the dark.

***Media*:** The media used for crosses were potato-dextrose agar (PDA) (Nelson *et al.*, 1983), carnation-leaf agar (CLA) (Fisher *et al.*, 1982), potato-carrot agar (PCA) (Lawrence, Nelson & Toussoun, 1985), V-8 juice agar and water agar with a 2 to 3-cm twig of mulberry (*Morus alba* L.) pre-sterilized by intermittent steaming for three days (MTA) (Desjardins & Beremand, 1987). For all the crosses carnation leaf pieces were transferred from the CLA slants onto complete medium (CM) (Correll *et al.*, 1987) and incubated at 25 ±2 °C under intermittent light (fluorescent plus black lights; 12 hour cycles) (Nelson *et al.*, 1983).

***Crosses*:** For the initial screening of the different media, mycelial plugs (3 mm in diameter) were cut aseptically with a cork borer from the edge of a colony on CM of each of the *G. pulicaris* strains which would serve as a recipient (female parent) and plated onto one of each of the test media and incubated inversely for 3 - 5 weeks at 20 °C in the dark until fertilization had taken place. Those isolates which served as the donor (male parent) were plated on 8-ml CM slants (Correll *et al.*, 1987) in test-tubes and incubated at 25 ±2 °C under intermittent light for 7 days. For subsequent crosses, the recipient isolates were grown on MTA. The following crosses were made: *G. pulicaris* strains with each other (Table 7), which also served as controls in all the subsequent crosses; all the South African isolates either as male or as female with the *G. pulicaris*

strains (Table 7); and 10 South African isolates (Fsam 1-10) with each other, either as the male or as the female parent (Table 8). Crosses which produced non-fertile perithecia (protoperithecia) were repeated. Progeny derived from ascospores produced in fertile crosses were also backcrossed with their original parents.

***Fertilization*:** A 3 ml of 2.5% Tween-60 was added to each CM slant of the donor isolates (male parents) by means of a sterile pipette and scratched lightly to dislodge the conidia. Isolates serving as the female parents were fertilized by adding 1 ml of the conidial suspension to the specific MTA plates (three plates/cross) and spreading it over the entire surface with a glass "hockey stick". MTA-plates were then incubated upright in a single layer at 15 °C in an incubator with low-level intermittent light (Desjardins & Beremand, 1987). All the crosses were examined weekly from the second to the eighth week for the presence of protoperithecia and mature perithecia with ascospore masses extruded from their ostioles. A cross producing protoperithecia was repeated and was scored as infertile if mature perithecia did not form during the second attempt. The progeny (single ascospore isolates), resulting from the crosses made between the South African isolates, were back-crossed with their parents. The single ascospore isolates were also crossed with each other in all possible combinations.

***Ascospore isolation and measurements*:** Ascospores were isolated according to the procedure described by Desjardins and Beremand (1987) from all the fertile crosses. At the same time that ascospore suspensions were poured onto a thin layer of water agar, macroscopic permanent slides were prepared. One hundred ascospores produced in each of four fertile crosses between the *G. pulicaris* strains, as well as in between four fertile crosses between South Africa isolates were used for determining the length, width, periphery and surface area of each ascospore using a Kontron Video Plan Image analyser program (Version 2.5, April, 1993, Kontron Electronic GMBH, Germany) connected to a Nikon Microphot -FXA microscope. The data for the ascospore measurements were statistically analysed using the least significant difference (LSD) function of the SAS/STAT program for personal computers (SAS Institute Inc., 1989).

Photography

Photographs of macroconidia and ascospores suspended in distilled water were taken using a Nikon microscope at x 400 (x 40 objective and a x 10 eyepiece). Photomicrographs of

perithecia, formed on mulberry twigs, were made using a Wild stereo microscope equipped with a Nikon camera. For scanning electron microscopy (SEM), ascospores were fixed in 2.5% glutaraldehyde in 0.075 M phosphate buffer (pH 7.4) for two hours. After rinsing in same buffer (3 x 15 min.), the material was dehydrated in a graded series of ethanol and critical point dried in a Hitachi HCP-2 (Ltd., Tokyo, Japan) critical point dryer, mounted, sputter-coated with gold and viewed in a JSM 840 (JEOL, Tokyo, Japan) electron microscope operated at 5 kV.

PCR analysis

***Source of isolates*:** The PCR analysis was performed by Dr. Wendy T. Yoder (Novo Nordish Biotech Inc., 1445 Drew Avenue, Davis CA 95616, USA) on the following five South African isolates: Fsam 1, Fsam 3, Fsam 4, Fsam 6 and Fsam 7 (MRC 6454 - 6458).

***DNA extractions*:** Each isolate was subcultured onto MM (Yoder & Christianson, 1998) and incubated in Chex All bags at 25 °C for five days after which four plugs (4 mm^2) were cut aseptically from the edge of a colony of each isolate and used to inoculate glass baffled shake flasks (125 ml) in duplicate, containing 25 ml YPG (Yoder & Christianson, 1998). These flasks were incubated at 28 °C on an orbital shaker and shaken at 150 rpm for 3 - 4 days. After incubation, mycelia were harvested and DNA extracted as described in Yoder & Christianson (1998). DNA was quantified by running 5 μl of each prep on a 1% agarose gel run in TAE buffer along with five standards of known concentration.

***PCR reactions*:** Species-specific primers used in this study were:
SAM-E CAGAAGCGGAGCAAGTTCACAATC and CAGAAGCGGATGGAGATGTAAAGT;
TOR-B CAAAGCGCTCCCTCAATCTCGTAC and CAAAGCGCTCATCAACTCCATATA;
VEN-B GGCGGATAAGGATAGTGGTAGAAG and GGCGGATAAGCAAATAAGATGCTT
(Yoder & Christianson, 1998).

All PCR reactions contained 50 mM KCl, 10 mM Tris HCl ph 8, 1.5 mM $MgCl_2$, 100 mM each dATP, dCTP, dGTP and dTTP (Pharacia Biotech), 25 pmol species-specific primer, 25 ng genomic DNA of each isolate, 2.5 U Taq DNA polymerase (Boeringer Mannheim) and sterile distilled water to a total volume of 50 μl. Two drops of mineral oil were added to overlay the mixture in each tube. PCR reactions were performed using a Stratagene Robocycler 40. The program was 1 cycle of 5 min at 94 °C followed by 45 cycles of 1 min at 94 °C, 1 min at 60 °C and 2 min at 72 °C. Thirteen μl of the PCR products from each reaction were run immediately on

RESULTS

Cultural characteristics

All the isolates matched the description of *F. sambucinum* by Nelson *et al.* (1983) and Burgess & Liddell (1983). However, the foreign *F. sambucinum, F. sulphureum* and *F. roseum* var. *sambucinum* isolates differed from each other, especially in respect of the pigment produced on PDA. The colour of the aerial mycelium varied from whitish to pink to carmine red, with some isolates tinged with red and with the undersurfaces of most of the cultures being yellow, peach to tan and some carmine red (Table 1; Fig. 2). Although a carmine red undersurface is characteristic of many isolates of *F. sambucinum* on PDA (Nelson *et al.*, 1983), none of the other 45 foreign isolates produced a red pigment on PDA with the exception of Fsam 43 which was carmine red and Fsul 54 which was tinged with red. Microconidia were absent in all the isolates. Macroconidia were produced sparsely in the mycelium by all the isolates, however, most of the isolates produced relatively short and stout, usually 4-5- septate, some 3-septate, macroconidia with thick walls and slightly curved ventral and dorsal surfaces (Fig. 3) in cream to orange sporodochia which matched the description of *F. sambucinum* (Nelson *et al.*, 1983). Insufficient cultural and morphological differences were therefore observed between these foreign isolates to justify their being considered as separate taxa. The majority of the cultures of the South African isolates were culturally and morphologically much more homogeneous than the foreign isolates.

On PDA growth of the South African isolates was rapid, 36.0 mm at 25 °C and 25.8 mm at 30 °C after three days incubation (Table 2). White, floccose aerial mycelium was formed which became pink to salmon with the under surfaces of the cultures peach in colour, but never carmine red or tan to brown. Only isolates of *F. sambucinum* obtained from potatoes grown in the Ceres area (KB = original number - Chapter 2), Fsam 2, 18, 19, 21, 22 and 23 expressed yellow to tan undersurfaces when cultured on PDA (Table 1). The colony diameters of these six isolates after 3 or 6 days growth on PDA at different temperatures were also significantly ($P \leq 0.05$) smaller than the other South African isolates (Tables 2; 3). Macroconidia were produced sparsely in the mycelium of most isolates. However, they were produced in orange sporodochia occurring either sparsely in the mycelium or on the carnation leaves. The macroconidia were relatively short and stout, usually 4 - 5-septate, some 3-septate, with thick walls and slightly curved ventral and dorsal surfaces. The basal cells were distinctly notched, but foot-shaped cells also occurred. The apical

cells were constricted into a snout, resembling a dolphin's nose, and strongly curved (Fig. 3). Microconidia were absent. Chlamydospores were sparsely produced in intercalary chains or clumps.

Linear growth rates on potato-dextrose agar

The optimum growth, 34.8 and 71.3 mm, occurred at 25 °C after 3 and 6 days, respectively, for all the South African and foreign isolates, which was significantly ($P \leq 0.05$) greater than at any of the other temperatures. At 30 °C the majority of the South African isolates of *F. sambucinum* grew more rapidly, however not significantly ($P \geq 0.05$), than at 20 °C. The foreign isolates grew more rapidly at 20 °C than at 30 °C, however the differences in growth were only significant ($P \leq 0.05$) for the *F. sambucinum* and *F. roseum* var. *sambucinum* isolates but not for the *F. sulphureum* isolates. The growth rates of the South African isolates were significantly ($P \leq 0.05$) greater than those of the foreign isolates irrespective of the incubation temperature and time of incubation. Differences also occurred between the growth rates of isolates within a taxa, e.g. Fsam 1, Fsam 18, Fsam 19, Fsam 21, Fsam 22 and Fsam 23, which grew significantly ($P \leq 0.05$) slower than the other South African isolates (Tables 2; 3). Colony diameters reached after 3 days incubation at 25 and 30 °C are considered an important characteristic for the identification of *Fusarium* spp. (Burgess & Liddell, 1983). Comparing the colony diameters of the groups of isolates under the set conditions for *F. sambucinum* (24-35 mm at 25 °C and 11-21 mm at 30 °C) (Burgess & Liddell, 1983), the colony diameters of the South African isolates where slightly larger at 36.0 ±10.1 and 25.8 ±5.5mm at 25 and 30 °C, respectively.

Linear growth rate on minimal medium (MM)

The mean growth rate of the five South African isolates (8.32 ±0.86 mm/day) was greater than that of *F. venenatum* (7.1 ±0.62 mm/day) reported by Yoder & Christianson (1998).

Pathogenicity

Most of the isolates within a taxon differed significantly ($P \leq 0.05$) from each other in their virulence (Table 1). The South African isolates were significantly ($P \leq 0.05$) less virulent (28.7% dry rot) than the foreign isolates, with the *F. sulphureum* isolates being the most virulent (61.4%), followed by the isolates of *F. roseum* var. *sambucinum* (61.2%) and *F. sambucinum* (52.8%)

(Tables 1; 4). The most virulent isolate Fsul 47, an isolate from Germany, resulted in 79.4% dry rot, whereas Fsam 18, a South African isolate, resulted in the lowest percentage (2%) dry rot (Table 1).

Significantly ($P \leq 0.05$) less (52.2%) dry rot developed in the inoculated tubers kept at 5 °C than those kept at 15 or 25 °C. This was especially noticeable in the tubers inoculated with the South African isolates. Only 17.8% dry rot developed in the tubers inoculated with these isolates and kept at 5 °C compared to 35.8% and 57.7% dry rot development in the tubers kept at 15 °C and 25 °C, respectively (Table 5). Inoculated tubers, irrespective of the taxa, kept at 25 °C resulted in a significantly ($P \leq 0.05$) higher percentage dry rot, 18.1% more, than those kept at 15 °C (Table 5). Thus 25 °C also appears to be the optimum temperature for dry rot development. Although significantly ($P \leq 0.05$) more dry rot developed in tubers kept at 15 °C than at 5 °C, irrespective of the taxa used as inoculum, these differences were not as extensive (5.5 ±2.25% difference) between the taxa of the foreign isolates compared to the 18% difference in the case of the South African isolates. Approximately 40% more dry rot occurred in tubers inoculated with the South African isolates and kept 25 °C than at 5 °C, whereas only 29.1, 17.2 and 20.6% more dry rot occurred in the tubers inoculated with the foreign *F. sambucinum, F. sulphureum* and *F. roseum* var. *sambucinum* isolates, respectively, and kept 25 °C than at 5 °C. When the four most virulent isolates of each group of isolates were compared, *F. sulphureum* was significantly ($P \leq 0.05$) more virulent than the most virulent isolates of the other groups. Some isolates in each group of isolates also differed significantly ($P \leq 0.05$) from each other in their virulence (Table 5).

Vegetative compatibility tests

***Selection, characterization and pairing of* nit-*mutants*:** Chlorate-resistant sectors (Fig. 1) were produced by all the isolates used in this study. The number of sectors produced, differed substantially amongst the isolates, as well as between the different chlorate-containing media used. Most of the chlorate-resistant sectors were produced on the PDC and KPS media. The majority (94%) of these sectors were discarded when wild-type growth was observed on MM. Isolates showing sparse growth could readily be characterized as *nit*-1, *nit*-3 or *nit*-M mutants, with the majority being *nit*-1. *Nit*-1 and *nit*-3 mutants were able to produce heterokaryons (Fig. 1) when paired with *nit*-M mutants of the same isolate, and no vegetative self-incompatibility groups were found between the South African isolates. However, seven of the foreign isolates were self-incompatible (Table 1).

***VCG diversity in the 25 South African and 47 foreign isolates*:** The 25 South African isolates of *F. sambucinum* could be assigned to six different VCG's, SA-1 to SA-6. The source, VCG's and virulence of these isolates are summarized in Table 1. Of these, VCG SA-1 was the largest VCG, containing members from all three potato producing regions from which *F. sambucinum* isolates were obtained and represented 32% of the isolates tested. This VCG also included some of the most virulent isolates. VCG SA-3 and VCG SA-4 were also geographically widespread and contained a further 12% and 16% of the isolates tested, respectively. Although all the isolates in VCG SA-1 showed no cross-complementation with isolates from any other VCG, weak cross-reactivity was occasionally observed upon pairing certain VCG SA-3 isolates with VCG SA-4 isolates. These data suggest that VCG SA-3 and VCG SA-4 may be very similar. VCG SA-2 contained only one stem-end rot isolate from the Eastern Free State and VCG SA-3 and VCG SA-6 three isolates each, one stem-end rot and two dry rot isolates from Mpumalanga, the Eastern Free State and Ceres, respectively. The less virulent isolates were clearly assigned to VCG SA-5 (Table 1) and included those isolates with the slowest growth (Tables 2; 3).

The foreign isolates were assigned to three VCG's, FI-1 to FI-3. No distinct vegetative compatibility occurred between the South African and the foreign isolates. However, weak cross-reactivity was observed upon pairing the VCG SA-5 isolates with certain VCG FI-1 isolates. This may suggest that these VCG's may overlap and that isolates in these groups may even be similar. VCG FI-1 was the largest VCG, containing members from all the potato producing countries from which *F. sambucinum* isolates were obtained and represented ±49% of the isolates tested. No correlation occurred between the VCG's and their origin, virulence or "species" to which these isolates were assigned. The *G. pulicaris* strains Fsam 42-44 and Fsul 67 which crossed with each other, however, were all assigned to VCG FI-2 (Table 1). Desjardins, Christ-Harned, McCormick & Secor, (1993) and Desjardins (1995) also tested some of these foreign isolates for vegetative compatibility, e.g. Fsam 32 - 37, Fsam 42, Fsul 58 - 61 and Fsul 64 - 67 (Table 1). According to their results it would appear that our VCG's, FI-1, FI-2 and FI-3 correspond to their VCG's 01 and 03 and/or 04, respectively with the exception of Fsam 33, Fsam 42 and Fsul 64 - 67 which were designated to 0X by them, which does not indicate a particular VCG but rather indicate an isolate that was incompatible with all isolates against which it was tested.

Sexual compatibility

Crosses between the *G. pulicaris* strains, conducted on CLA, PCA and V-8 agar, resulted in the production of protoperithecia, except for the cross between Fsam 42$^{\varphi}$ (MAT1-1) and Fsul 67$^{\sigma}$ (MAT1-2) which did not result in the production of protoperithecia on any of the three media. Mature perithecia extruding ascospores from their ostioles were produced in all the crosses between the *G. pulicaris* strains on MTA or, rarely, on CLA and V-8 agar (Table 6).

Several protoperithecia were produced when crosses were made between the 25 South African isolates, (Fsam 1-25) used as female and as male parents, with the *G. pulicaris* strains. These crosses included the following: South African isolates as female parent; Fsam 2, 18, 23 X Fsam 42 (MAT1-1), Fsam 18, 19, 22, 23 X Fsam 43 (MAT1-2), Fsam 18, 19, 22 X Fsam 44 (MAT1-2) and Fsam 19, 22 X Fsul 67 (MAT1-2). South African isolates as male parent; Fsam 11-21, 23-25 X Fsam 44 (MAT1-2). None of these crosses were, however, fertile even when repeated (Table 7).

Crosses made between the 10 South African isolates, (Fsam 1-10) resulted in three fertile crosses, Fsam 8 as female parent X Fsam 7, 9, 10 as male parents. Of these, the latter two crosses were only successful after the second attempt. Several protoperithecia were also produced in the following crosses, the first mentioned isolate was used as the female parent, Fsam 1 X Fsam 3, 6 , 9 and Fsam 10 X Fsam 8, but no fertile perithecia were produced after repeating these crosses (Table 8). Single ascospore isolates, 35 from each cross, were generated from the progeny (ascospores) of these fertile crosses. Fertile perithecia were also produced with 7% of these isolates (F1 population) when backcrossed with their parents. None of these four South African isolates that crossed with each other (Fsam 7,8,9 and 10), resulted in fertile crosses when crossed with the Mat1-1 and MAT1-2 *G. pulicaris* strains (Fsam 42, 43, 45 and Fsul 67) after various attempts. Thus it can be concluded that the South African isolates do not belong to the same mating population as the *G. pulicaris* strains.

Perithecia in fertile crosses were produced abundantly on the mulberry twigs but seldom directly on the agar-surfaces. The perithecia produced when compatible South African isolates were crossed with each other where most often formed singly or in pairs, but seldom in clumps as was found when the *G. pulicaris* strains were crossed with each other (Fig. 4).

Ascospores produced in crosses were all fusiform to ellipsoid, curved and slightly constricted at the three transverse septa. However, those produced in crosses between the South African isolates,

Fsam 8 ,7, 9, 10 and MRC 7554, a single ascospore isolate (progeny of the fertile cross between Fsam 8 and 7), were significantly ($P \leq 0.05$) longer and larger, but not wider, than those produced between the *G. pulicaris* strains, Fsam 42, 33, 44 and Fsul 67 (Table 9; Fig. 5; 6).

PCR analysis

Five of the South African isolates, Fsam 1, Fsam 3, Fsam 4, Fsam 6 and Fsam 7, generated negative results with the *F. torulosum* (TOR-B) and *F. sambucinum* (SAM-E) primers but were positive (i.e. generated bands of the expected size) with the *F. venenatum* (VEN-B) primers (Fig. 7).

DISCUSSION

According to Nelson *et al.* (1983), a wide variety of taxa are grouped together under *F. sambucinum*, making this a large heterogeneous, and from the point of view of cultural appearance on PDA, a rather varied group. All seventy-two *Fusarium sambucinum* isolates, 25 South African and 47 foreign (19 *F. sambucinum*, 23 *F. sulphureum* and 5 *F. roseum* var. *sambucinum*) not only matched the description of Nelson *et al.* (1983), but their colony diameters on PDA after three days incubation at 25 and 30 °C corresponded with those published for the species (Burgess & Liddell, 1983) except that the colony diameters of the South African isolates were slightly larger, particularly at 30 °C. The majority of the South African isolates had a faster growth rate than the foreign isolates which also appeared to be more closely related to *F. trichothecioides* as described by Booth (1971). Differences occurred between the taxa of foreign isolates and also occurred between isolates within a specific taxon, and therefore, based on the cultural characteristics, the division of these isolates into separate taxa is not warranted. According to the three-species concept for *F. sambucinum*, proposed by Nirenberg (1995b), the majority of the South African isolates did not fit in any of these species, especially not that of *F. torulosum*.

The linear growth of all the South African and foreign isolates increased with increasing temperature until an optimum temperature of 25 °C was reached. The linear growth rates of the majority of the South African isolates were greater than those of the foreign isolates, but not significantly ($P \geq 0.05$) different. The six isolates, Fsam 2, 18, 19, 21, 22 and 23, all obtained from the Ceres potato production area, had a significantly ($P \leq 0.05$) slower growth rate and were also

significantly ($P \leq 0.05$) less virulent than the other South African isolates. It appears that these six isolates may be more closely related to the foreign *F. sambucinum sensu lato* isolates.

The optimum temperature for dry-rot development appears to be 25 °C. Dry-rot development was much more inhibited in the case of tubers inoculated with the South African isolates and kept at 5 or 15 °C, than in the case of tubers inoculated with the foreign isolates. These results are in agreement with the presumption that the South African isolates are adapted to the higher prevailing temperatures of South Africa (Theron & Holz, 1990). Goss (1921) and Weiss, Lauritzen & Brierely (1928) reported that *F. trichothecioides* and *F. sulphureum* can infect potato tubers over a wide temperature range (2 - 30 °C), with 25 °C being the optimum for *F. trichothecioides*, especially at high relative humidity. The South African isolates were significantly ($P \leq 0.05$) less virulent than the foreign isolates. This, as well as the fact that only a few of these isolates could produce chlamydospores in culture, which could also contribute to their poor survival in field soils (Theron & Holz, 1989), may possibly be the reason why *F. sambucinum* is not as serious a problem in South Africa as in other potato producing countries. Moreover, Jeffries, Boyd & Patterson (1984) also reported that the rate of survival of *F. sambucinum* in soils tends to decline rapidly compared to those of *F. solani* and *F. oxysporum*, the predominant Fusarium dry-rot pathogens in South Africa (Theron & Holz, 1989; Chapter 2).

No vegetative compatibility occurred between the South African and foreign *F. sambucinum* isolates, although weak cross-reactivity was observed upon paring South African isolates belonging to VCG SA-5, i.e. Fsam 2, 18, 19, 21, 22 and 23, with certain foreign (VCG FI-1) isolates. This indicated that these two VCG's may overlap and that the isolates assigned to them are more closely related than the rest of the South African isolates. This was also demonstrated in their growth rates. These six isolates did not cross with each other or with Fsam 7, 8, 9 or 10 to produce a teleomorph. With the exception of Fsam 21, the other five isolates did however produce protoperithecia when crossed with the *G. pulicaris* strains. These facts support the view that these six isolates are more closely related to the foreign isolates than to the other South African isolates.

Despite the fact that the occurrence of *F. sambucinum* has thus far been restricted to only three potato producing regions in South Africa (Theron & Holz, 1989; Chapter 2), a high degree of genetic diversity, based on the six VCG's found, appears to be present in the South African isolates obtained from dry rotted potatoes, compared to the three VCG's found in the foreign isolates. Our results concerning the VCG's for the North American isolates, e.g. Fsam 32 and Fsam 34 - 37,

agree with the findings of Desjardins *et al.* (1993), but not for the European isolates, e.g. Fsul 58 and Fsul 60, which were designated in the present studies to two different VCG's, viz. FI-2 and FI-3 respectively, which Desjardins (1995) designated to the same VCG, viz.07. Fsul 61 which was designated to VCG FI-1 in the present studies was designated to VCG 06 by Desjardins (1995). The reason for this is not very clear. However, it is possible that the isolates assigned to VCG FI-2 and FI-3 can be divided into more VCG's, e.g. VCG FI-3 appears to include both VCG 03 and VCG 04 as was reported by Desjardins (1995). No correlation, either to the origin or the taxa into which the foreign isolates were divided, occurred between the three VCG's obtained for these foreign isolates. Five isolates, viz. Fsam 33, Fsam 42, Fsul 64, Fsul 65 and Fsul 67, which Desjardins *et al.* (1993) and Desjardins (1995) designated to VCG 0X, which does not indicate a particular VCG group, were designated to either VCG FI-1 or FI-2 in the present studies. Fsul 66 could not be designated to a particular VCG in the present studies which was also reported by Desjardins (1995) for this particular isolate.

VCG diversity is indicative of a well-established fungal population, the introduction of numerous VCG's into a new area and/or regular sexual reproduction (Brasier, 1987). Despite the widespread cultivation of potatoes and their long association with Fusarium dry rot, which would suggest the potential for substantial genetic diversity in the population of *F. sambucinum* (*G. pulicaris*) in North America, Desjardins *et al.* (1993) and Desjardins (1995) reported that the genetic diversity appears to be quite limited as the *F. sambucinum* population consists mainly of three VCG's. They attributed this to the complete absence of mating type MAT1-2 amongst a total of 121 isolates, isolated from potato tubers in 12 states in the USA over the course of 30 years and suggested that MAT1-2 has not yet been introduced into potato seed and tuber production to any significant degree, and that sexual reproduction does not contribute to the generation of the genetic diversity of the isolates in the United States.

Of all the media used in the sexual crossing techniques, MTA medium was the most consistent, supporting the best production of perithecia and resulting in the most fertile crosses when crossing the *G. pulicaris* strains, as also reported by Desjardins & Beremand (1987). The fact that Fsul 67 (= MRC 514 = R5389) crossed with the *G. pulicaris* strains confirms that *F. sulphureum* is conspecific with *F. sambucinum sensu stricto* (Desjardins & Beremand, 1987). This means that the teleomorph of *F. sulphureum* is *G. pulicaris*, and not *G. cyanogena* as stated by Booth (1971). Although protoperithecia were produced when some of the South African *F. sambucinum* isolates, either

used as males or females, were crossed with the *G. pulicaris* strains, they failed to yield fertile crosses, even after repetition. Researchers in the USA were also unable to obtained fertile crosses when crossing five South African isolates, MRC 4665 to MRC 4670, obtained during the survey conducted by Theron & Holz (1989) with the *G. pulicaris* strains (Dr. Anne E. Desjardins, Northern Regional Research Center, USDA-ARS, Peoria, IL 61604, USA, personal communication, 1992). The occurrence of protoperithecia in these specific crosses, however, could be attributed to the production of non-fertile perithecia by the *G. pulicaris* strains used (Desjardins & Beremand, 1987). As part of the European *Fusarium sambucinum* Project, a number of *F. sambucinum sensu lato* strains were crossed. Most of the *F. sambucinum sensu stricto* strains were fertile with the *G. pulicaris* strains, but no fertile crosses were obtained when crossed with *F. venenatum* strains (Desjardins & Nelson, 1995). The fact that the South African *F. sambucinum* isolates also did not result in fertile crosses when crossed with the *G. pulicaris* strains, means that they are not part of the *F. sambucinum sensu stricto* mating population of the *G. pulicaris* strains.

Fertile crosses were, however, obtained when South African isolates were crossed with each other. Isolates produced from single ascospores could also successfully be backcrossed with their parents. The ascospores produced from these fertile crosses between the South African isolates were significantly ($P \leq 0.05$) longer and larger, but not wider, compared with those produced by the crosses between the *G. pulicaris* strains. This is the first report of South African *F. sambucinum* isolates from potatoes, producing a teleomorph, which may also explain the high degree of genetic diversity found in these isolates. This raises the possibility that sexual reproduction of *F. sambucinum* occurs in the field or in storage in South Africa. Although direct evidence is lacking, the presence of a large number of VCG's is consistent with sexual reproduction and recombination of vegetative compatibility loci (Leslie, 1993).

The lack of fertile crosses between the *G. pulicaris* strains and the South African isolates suggest that these isolates are not *F. sambucinum sensu stricto* (teleomorph *G. pulicaris*). Although the ability to form a fully fertile cross with a *G. pulicaris* strains can unequivocally determine the species of a fertile isolate, it is more problematic to exclude an isolate only because it is infertile (Desjardins & Nelson, 1995). The fact, however, that the South African isolates did not cross with the *G. pulicaris* strains, but crossed with each other, producing distinctive ascospores as well as the vegetative non-compatibility between them and the foreign isolates implies that they belong in a new mating population and probably in a new taxon, distinct from *F. sambucinum sensu stricto*

(teleomorph *G. pulicaris*). The latter is more acceptable because no vegetative compatibility occurred between these and the foreign isolates. Glass & Kuldau (1992) demonstrated that vegetative compatibility, and therefore the formation of a heterokaryon, is regulated by allelic and non-allelic genetic systems which are different from the genetic system regulating the sexual functions, mating type recognition, and therefore strains that are vegetatively incompatible can mate and *vice versa*. The results of Desjardins *et al.* (1993) and Desjardins (1995) proved that *F. sambucinum* isolates, belonging to the same mating type, are vegetatively incompatible. According to Beremand, Desjardins, Hohn & Van Middelsworth (1991) only heterothallism was observed in the *F. sambucinum* isolates studied by them, but they found no evidence for additional mating populations although such groups have been reported for other *Fusarium* spp. (Booth, 1971; Matuo & Snyder, 1973; Hsieh, Smith & Snyder, 1977; Leslie. 1991).

On the basis of the PCR analysis, the five isolates representing the majority of the South African *F. sambucinum* isolates, are conspecific with *F. venenatum*. In contrast, the data generated by measurement of the linear extent rates indicate that these isolates are not conspecific with *F. venenatum*. These isolates also do not agree morphologically with the description of *F. venenatum* (Nirenberg, 1995; Yoder & Christianson, 1998) as none were red, chlamydospores were produced sparsely and their growth rates were faster. Therefore one should be extremely careful accepting results obtained from PCR-analysis as the alpha and omega to distinguish between species.

Unfortunately no South African isolates were included in the European *F. sambucinum* Project. It appears as if the majority of the South African isolates from potato tubers are members of a new *Fusarium* sp. of which the teleomorph has also been discovered in this study. Much more work, including detailed morphological, genetic and molecular comparisons between the South African isolates of *F. sambucinum* and authentic isolates of *F. venenatum*, is required to resolve the identity of these enigmatic South African isolates from potato tubers with dry rot.

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Table 1. Source, pathogenicity and vegetative compatibility groups of South African and foreign isolates of *F. sambucinum sensu lato* from potato tubers with dry rot

Accession number	Original number	Geographical origin	Obtained from	Pigmentation[a]	% Dry rot[b]	VCG[c]
F. sambucinum: South Africa						
Fsam 1 MRC 6454[d]	T1/1/4D12a	SA	D.J. Theron	Peach	38.0	SA-4
Fsam 2	KB4/P9/2(4)	SA	D.J. Theron	Yellow/Tan	22.0	SA-5
Fsam 3 MRC6455	T3/1/6D16	SA	D.J. Theron	Peach	31.3	SA-3
Fsam 4 MRC6456	O2/4/4D16	SA	D.J. Theron	Peach	27.3	SA-4
Fsam 5	O3/1/3P7	SA	D.J. Theron	Peach	47.3	SA-4
Fsam 6 MRC6457	O2/2/7P16	SA	D.J. Theron	Peach	22.0	SA-2
Fsam 7 MRC6458	KB1/D19/3(3)	SA	D.J. Theron	Peach	45.3	SA-4
Fsam 8 MRC7436	O3/1/3P5	SA	D.J. Theron	Peach	33.3	SA-3
Fsam 9 MRC7550	O2/2/7P10	SA	D.J. Theron	Peach	47.3	SA-1
Fsam 10 MRC7551	O3/4/7D7	SA	D.J. Theron	Peach	42.7	SA-1
Fsam 11	O1/1/5D8	SA	D.J. Theron	Peach	38.7	SA-1
Fsam 12	O4/4/7D7	SA	D.J. Theron	Peach/Tan	22.1	SA-6
Fsam 13	T1/1/4P59	SA	D.J. Theron	Peach	25.4	SA-6
Fsam 14	T1/1/4P11	SA	D.J. Theron	Peach	38.3	SA-1
Fsam 15	KB4/D8/5(3)	SA	D.J. Theron	Peach	22.1	SA-6
Fsam 16	KB1/D19/4(3)	SA	D.J. Theron	Peach	44.1	SA-1
Fsam 17	T1/1/4D11	SA	D.J. Theron	Peach	30.7	SA-1
Fsam 18	KB4/D6/5	SA	D.J. Theron	Yellow/Tan	2.0	SA-5
Fsam 19	KB2/D9/4(3)	SA	D.J. Theron	Yellow/Tan	6.7	SA-5
Fsam 20	O2/2/7P10	SA	D.J. Theron	Peach	39.3	SA-1
Fsam 21	KB2/D5/4(3)	SA	D.J. Theron	Yellow/Tan	6.0	SA-5
Fsam 22	KB4/D3/2(3)	SA	D.J. Theron	Peach	20.0	SA-5
Fsam 23	KB4/P1/5(2)	SA	D.J. Theron	Yellow/Tan	14.0	SA-5
Fsam 24	AP91/603	SA	D.J. Theron	Peach	30.0	SA-1
Fsam 25	KB1/D26/4	SA	D.J. Theron	Peach	20.7	SA-3
Mean					**28.7**	
F. sambucinum: Foreign						
Fsam 26	Fsa 504c	France	R.L. Wastie	Yellow	29.2	-
Fsam 27	Fsam F49	USA	D.L. Corsini	Yellow	61.0	FI-1
Fsam 28	Fsam F130	USA	D.L. Corsini	Yellow	68.3	FI-1
Fsam 29	Fsam F134	USA	D.L. Corsini	Yellow/Tan	64.4	FI-1
Fsam 30	Fsam F138	USA	D.L. Corsini	Yellow	62.3	FI-1
Fsam 31	Fsam F140	USA	D.L. Corsini	Yellow/Tan	59.4	FI-1
Fsam 32	R-9146	Canada	P.E. Nelson	Yellow/Tan	57.5	FI-2
Fsam 33	R-9149	USA	P.E. Nelson	Yellow	20.1	FI-2
Fsam 34	R-9262	USA	P.E. Nelson	Yellow	73.3	FI-1
Fsam 35	R-9256	USA	P.E. Nelson	Peach	75.0	FI-3
Fsam 36	R-9271	USA	P.E. Nelson	Yellow/Tan	70.4	FI-1
Fsam 37	R-9283	USA	P.E. Nelson	Yellow	77.3	FI-1
Fsam 38	Fsam 4/70	Netherlands	L.J. Turkensteen	Yellow	12.4	FI-3
Fsam 39	Fsam 3/70	Netherlands	L.J. Turkensteen	Peach	65.3	FI-1
Fsam 40	Fsam 2/70	Netherlands	L.J. Turkensteen	Peach	46.4	FI-1
Fsam 41	Fsam 1/70	Netherlands	L.J. Turkensteen	Yellow/Tan	70.4	-
Fsam 42 MRC6970[e]	R6380	Germany	A.E. Desjardins	Yellow	7.4	FI-2
Fsam 43 MRC6971[ef]	R8575	SA	A.E. Desjardins	Red	17.4	FI-2
Fsam 44 MRC6972[ef]	R9153=64995	Netherlands	A.E. Desjardins	Yellow	65.3	FI-2
Mean					**52.8**	

F. sulphureum: Foreign						
Fsul 45	Fsul X	UK	R.L. Wastie	Yellow	60.2	FI-1
Fsul 46	Fsul 6-4	UK	G.T. Jellis	Yellow	53.2	FI-2
Fsul 47	Fsul 6-1	Germany	G.T. Jellis	Yellow	79.4	-
Fsul 48	Fsul 2'72	Germany	E. Langerfeld	Yellow/Tan	68.4	FI-2
Fsul 49	Fsul 3'72	Germany	E. Langerfeld	Yellow	73.0	FI-2
Fsul 50	Fsul 4'72	Germany	E. Langerfeld	Yellow	70.9	FI-1
Fsul 51	Fsul 5'72	Germany	E. Langerfeld	Yellow	55.3	FI-1
Fsul 52	Fsul 6'72	Germany	E. Langerfeld	Yellow	51.4	FI-1
Fsul 53	Fsul 1790	Netherlands	L.J. Turkensteen	Yellow	62.3	FI-1
Fsul 54	Fsul 1800	Netherlands	L.J. Turkensteen	Yellow	58.1	FI-1
Fsul 55	Fsul 1799	Netherlands	L.J. Turkensteen	Yellow	65.4	FI-1
Fsul 56	Fsul 1408	Netherlands	L.J. Turkensteen	Yellow/Tan	46.3	-
Fsul 57	Fsul 1409	Netherlands	L.J. Turkensteen	Yellow	57.4	FI-1
Fsul 58	94	UK	G.A. Hide	Peach	56.4	FI-2
Fsul 59	144 (R9357)	Netherlands	G.A. Hide	Peach	63.4	FI-1
Fsul 60	229	UK	G.A. Hide	Yellow	59.4	FI-3
Fsul 61	230	UK	G.A. Hide	Yellow	67.3	FI-1
Fsul 62	231	UK	G.A. Hide	Yellow	57.4	FI-1
Fsul 63	232	UK	G.A. Hide	Yellow	64.4	-
Fsul 64	233	UK	G.A. Hide	Yellow	67.4	FI-1
Fsul 65	234	UK	G.A. Hide	Yellow	69.4	FI-1
Fsul 66	235	UK	G.A. Hide	Yellow	30.0	-
Fsul 67 MRC514[e]	R5389	Iran	W. F. O. Marasas	Yellow	76.0	FI-2
Mean					**61.4**	
F. roseum var. *sambucinum*: Foreign						
Frsam 68	89-29-48	France	B. Tivoli	Yellow	24.4	FI-2
Frsam 69	88-29-5	France	B. Tivoli	Yellow	67.4	-
Frsam 70	86-22-3	France	B. Tivoli	Yellow	73.4	FI-3
Frsam 71	90-02-1	France	B. Tivoli	Yellow/Tan	75.4	FI-3
Frsam 72	89-91-1	France	B. Tivoli	Yellow	65.4	FI-3
Mean					**61.2**	

Source of variation	F-value ($P \leq 0.05$)	$LSD_{(T)}$ 5%
Taxa	147.7	6.7
Isolates	798.8	1.6

[a]Pigmentation of the under surfaces of the colonies on PDA after incubation at 25 ±2 °C under intermittent light (fluorescent plus black lights; 12 hour cycles).

[b]Dry rot determined 3 weeks after inoculated tubers were kept at 25 ±2 °C an 50 - 70% relative humidity according to a disease index, were 0 = no lesion development and 5 = tuber completely decayed. Results presented as percentage of decay calculated according to the method of Kremer & Unterstenhöfer (1967). Each value represents the mean of 30 observations.

[c]VCG = vegetative compatibility grouping of isolates determined by the method of Correll *et al.* (1987). Weak cross-reactivity was observed upon pairing VCG SA-5 isolates with VCG FI-1.

[d]Accession numbers of representative *F. sambucinum* isolates 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.

[e]*G. pulicaris* strains (Desjardins & Beremand, 1987).

[f]Not from potato tubers with dry rot. R8575 from soil and R9153 from *Brassica oleracea*.

Table 2. Colony diameters (mm) of South African and foreign isolates of *Fusarium sambucinum*, *F. sulphureum* and *F. roseum* var. *sambucinum* 3 days after incubation at different temperatures on potato-dextrose-agar plates

Fusarium isolates	Temperature °C							
	5	10	15	20	25	30	35	Mean
F. sambucinum: South African								
Fsam 1 MRC 6454[a]	5.0[b]	15.7	20.0	29.5	38.0	28.7	7.0	**20.6**
Fsam 2	5.3	9.3	12.3	18.0	20.0	18.0	5.7	**12.7**
Fsam 3 MRC 6455	5.7	15.7	19.7	35.0	43.3	28.7	6.0	**22.0**
Fsam 4 MRC 6456	5.0	11.7	19.3	31.3	40.0	31.7	5.3	**20.6**
Fsam 5	5.3	18.8	17.3	29.2	44.3	29.3	8.3	**21.8**
Fsam 6 MRC 6457	6.0	17.0	18.7	29.0	42.7	30.7	6.0	**21.4**
Fsam 7 MRC 6458	5.3	14.3	20.0	25.3	44.7	30.0	7.0	**20.9**
Fsam 8 MRC 7436	5.7	15.0	18.8	26.3	40.7	33.3	6.7	**20.9**
Fsam 9 MRC 7550	5.0	13.0	17.3	24.3	42.0	29.3	7.3	**19.7**
Fsam 10 MRC 7551	5.0	19.0	22.0	34.0	47.7	32.7	5.7	**23.7**
Fsam 11	5.0	18.0	19.3	25.0	42.3	30.3	6.0	**20.8**
Fsam 12	5.0	15.3	17.0	26.0	43.0	28.6	5.7	**20.1**
Fsam 13	5.0	15.0	20.3	30.7	40.3	26.7	5.7	**20.5**
Fsam 14	8.0	14.7	15.3	27.0	41.0	26.0	5.7	**19.7**
Fsam 15	5.0	15.7	17.5	24.0	40.7	25.7	7.3	**19.4**
Fsam 16	5.0	15.7	19.5	27.7	35.7	27.3	8.0	**19.8**
Fsam 17	5.3	18.0	20.7	32.5	47.3	30.3	7.0	**23.0**
Fsam 18	6.0	10.0	13.0	15.0	19.3	18.7	8.0	**12.9**
Fsam 19	6.3	12.7	15.7	16.7	20.0	17.0	8.3	**13.8**
Fsam 20	5.0	12.7	20.7	26.0	38.7	22.0	7.7	**19.0**
Fsam 21	6.0	9.5	15.3	15.7	20.3	19.3	7.7	**13.4**
Fsam 22	6.0	9.7	13.3	13.3	17.3	16.3	8.3	**12.0**
Fsam 23	6.0	11.0	11.6	14.0	18.7	15.7	7.3	**12.0**
Fsam 24	5.0	16.3	15.3	22.0	32.3	24.0	8.7	**17.7**
Fsam 25	5.3	12.8	20.5	29.0	40.0	24.0	6.3	**19.7**
Mean	**5.5**	**14.3**	**17.6**	**25.1**	**36.0**	**25.8**	**6.9**	**18.7**
F. sambucinum: Foreign								
Fsam 26	5.0	17.2	17.7	35.2	43.0	14.7	5.0	**19.7**
Fsam 27	6.0	15.3	13.3	26.7	34.3	22.0	5.0	**17.5**
Fsam 28	5.0	14.2	14.3	23.7	27.3	17.7	5.0	**15.3**
Fsam 29	5.3	14.0	16.0	26.0	32.0	20.3	5.0	**16.9**
Fsam 30	5.0	10.0	12.3	23.8	35.3	19.7	5.0	**15.9**
Fsam 31	5.0	8.3	14.5	26.0	32.0	21.7	5.7	**16.2**
Fsam 32	5.0	9.0	8.7	20.3	22.6	14.7	5.0	**12.2**
Fsam 33	5.0	13.7	11.8	21.7	26.3	20.3	5.3	**14.9**
Fsam 34	6.0	12.3	14.7	24.5	26.3	13.7	5.7	**16.2**
Fsam 35	5.7	14.3	13.7	22.0	37.3	13.7	6.0	**16.1**
Fsam 36	5.0	15.2	15.7	23.7	26.7	18.0	5.7	**15.7**
Fsam 37	5.0	14.0	16.3	23.3	28.3	20.0	5.7	**16.1**
Fsam 38	6.0	18.3	19.7	29.3	39.0	16.3	5.3	**19.1**
Fsam 39	5.0	16.0	15.0	28.2	36.3	23.0	5.0	**18.4**
Fsam 40	5.0	10.7	14.7	27.8	48.3	22.7	5.3	**19.2**
Fsam 41	6.0	15.8	16.7	23.5	37.3	21.0	5.7	**18.0**
Fsam 42	5.7	15.3	19.0	31.3	46.0	17.7	5.0	**20.0**
Fsam 43	5.7	13.3	13.8	26.8	35.7	23.0	5.3	**17.7**
Fsam 44	5.0	13.0	16.7	22.3	29.3	18.0	5.0	**15.6**
Mean	**5.3**	**13.7**	**15.0**	**25.6**	**34.4**	**18.9**	**5.3**	**16.9**

F. sulphureum: Foreign								
Fsul 45	5.3	14.3	13.5	20.2	33.3	35.0	5.0	**18.1**
Fsul 46	5.0	14.3	13.3	20.3	34.3	21.0	5.0	**16.2**
Fsul 47	6.0	14.7	13.5	18.0	33.3	18.7	5.0	**15.6**
Fsul 48	5.0	15.0	16.8	19.0	45.3	22.7	5.0	**18.4**
Fsul 49	5.0	11.8	17.7	18.7	48.0	23.3	5.0	**18.5**
Fsul 50	5.0	9.7	12.7	18.7	23.3	20.0	5.3	**13.5**
Fsul 51	5.3	11.8	16.3	24.7	39.0	19.7	5.0	**17.4**
Fsul 52	5.3	14.7	14.0	21.0	34.3	20.0	6.0	**16.5**
Fsul 53	5.7	13.3	16.2	23.0	33.0	21.0	5.0	**16.7**
Fsul 54	6.0	13.3	15.0	22.7	30.7	20.0	6.0	**16.2**
Fsul 55	6.0	10.5	16.3	19.0	29.0	19.7	6.0	**15.2**
Fsul 56	5.7	10.3	15.3	20.7	31.3	22.0	5.3	**15.8**
Fsul 57	5.0	13.8	14.3	21.0	43.3	17.3	5.3	**17.1**
Fsul 58	5.0	13.7	16.3	21.7	32.0	19.3	5.0	**16.1**
Fsul 59	5.0	14.0	15.0	19.7	30.3	19.7	5.7	**15.6**
Fsul 60	5.0	10.3	14.0	18.7	46.0	17.7	5.7	**16.8**
Fsul 61	6.0	17.8	20.7	29.7	46.3	22.3	5.0	**21.1**
Fsul 62	5.3	13.3	20.0	21.3	37.0	15.3	5.3	**16.8**
Fsul 63	5.0	10.5	15.7	21.7	29.3	17.3	5.0	**14.9**
Fsul 64	5.7	9.0	12.3	23.0	30.0	17.0	5.3	**14.6**
Fsul 65	5.0	10.3	15.7	23.7	37.0	16.3	5.0	**16.1**
Fsul 66	5.0	12.7	10.3	15.0	21.7	14.3	5.0	**12.0**
Fsul 67	5.0	16.0	15.3	21.0	28.7	15.7	5.0	**15.2**
Mean	**5.3**	**12.8**	**15.2**	**21.0**	**34.6**	**19.8**	**5.3**	**16.3**
F. roseum var. *sambucinum*: Foreign								
Frsam 69	5.7	14.7	14.2	28.7	34.0	14.0	5.7	**16.7**
Frsam 70	5.7	13.5	15.0	26.2	24.3	19.7	6.0	**15.8**
Frsam 71	5.0	14.3	16.7	35.3	35.0	20.1	5.0	**18.8**
Frsam 72	5.0	13.7	11.7	31.5	30.7	17.3	5.0	**16.4**
Mean	**5.3**	**13.8**	**14.6**	**30.4**	**31.6**	**17.0**	**5.3**	**16.9**
Overall mean	**5.4**	**13.6**	**15.9**	**24.3**	**34.8**	**21.5**	**5.9**	

Source of variation	F-value (P≤ 0.05)	$LSD_{(T)}$ 5%
Isolates	12.71	3.6
Taxa (A)	426.9	1.7
Temperature (B)	2121.24	1.2
Interaction (A x B)	672.43	1.4

[a]Accession numbers of representative South African *F. sambucinum* isolates deposited in the culture collection of the Programme on Mycotoxins and Experimental Carcinogenesis (PROMEC), of the Medical Research Council (MRC), P. O. Box 19070, Tygerberg 7505, South Africa.

[b]Each value represents the mean of three measurements.

Table 3. Colony diameters (mm) of South African and foreign isolates of *Fusarium sambucinum*, *F. sulphureum* and *F. roseum* var. *sambucinum* 6 days after incubation at different temperatures on potato-dextrose-agar plates

Fusarium isolates	Temperature °C							
	5	10	15	20	25	30	35	Mean
F. sambucinum: South African								
Fsam 1 MRC 6454[a]	9.0[b]	37.0	45.7	57.0	78.7	57.7	7.0	**41.7**
Fsam 2	9.3	20.0	22.0	30.3	33.7	31.7	6.3	**21.9**
Fsam 3 MRC 6455	11.0	34.7	42.7	69.3	88.0	52.7	7.7	**43.7**
Fsam 4 MRC 6456	8.0	28.7	44.3	60.0	72.7	55.7	5.7	**39.3**
Fsam 5	10.3	38.3	44.0	64.0	78.0	52.3	9.3	**42.3**
Fsam 6 MRC 6457	12.3	36.0	39.3	57.0	83.0	54.0	7.0	**41.2**
Fsam 7 MRC 6458	10.3	32.7	42.3	52.7	87.0	68.0	7.3	**42.9**
Fsam 8 MRC 7436	9.7	35.0	42.0	52.0	83.0	61.3	7.0	**41.4**
Fsam 9 MRC 7550	9.0	26.0	38.3	48.3	90.0	62.3	7.7	**40.2**
Fsam 10 MRC 7551	13.3	42.0	54.0	68.7	90.0	55.0	6.0	**47.0**
Fsam 11	9.0	37.0	40.7	51.0	88.7	66.0	6.0	**42.6**
Fsam 12	9.7	33.0	46.7	57.0	84.7	52.3	6.7	**41.4**
Fsam 13	10.0	34.0	44.3	64.0	86.7	48.3	6.7	**42.0**
Fsam 14	12.3	33.3	45.0	59.7	90.0	50.7	5.7	**42.4**
Fsam 15	10.7	34.3	41.3	48.3	90.0	54.0	9.0	**41.1**
Fsam 16	9.0	36.0	40.7	64.0	86.7	68.0	8.7	**44.7**
Fsam 17	12.0	38.0	45.7	64.3	88.3	55.7	7.7	**44.5**
Fsam 18	10.0	18.0	23.0	27.7	36.3	35.0	16.7	**23.8**
Fsam 19	10.7	21.0	26.3	29.7	37.3	30.0	14.3	**24.2**
Fsam 20	8.3	32.0	41.7	53.3	87.3	62.0	7.7	**41.8**
Fsam 21	11.7	31.7	34.3	37.0	43.3	31.3	9.0	**28.3**
Fsam 22	9.7	15.0	21.3	21.0	30.3	32.7	11.7	**20.2**
Fsam 23	10.7	18.3	24.0	25.7	34.0	30.0	10.3	**21.9**
Fsam 24	9.3	36.0	36.3	43.7	87.3	59.3	10.3	**40.3**
Fsam 25	11.0	33.3	51.7	63.7	83.3	51.0	7.3	**43.1**
Mean	**10.2**	**31.3**	**39.1**	**50.8**	**73.5**	**51.1**	**8.3**	**37.8**
F. sambucinum: Foreign								
Fsam 26	8.0	34.0	42.7	67.0	84.3	44.0	5.0	**40.7**
Fsam 27	11.0	25.0	29.7	47.7	77.7	39.7	5.0	**33.7**
Fsam 28	8.7	23.0	30.0	44.7	54.3	37.7	5.0	**29.0**
Fsam 29	10.3	25.0	35.0	50.7	64.3	39.3	5.0	**32.8**
Fsam 30	8.0	18.3	24.0	43.7	71.0	40.7	5.0	**30.1**
Fsam 31	8.3	14.0	30.0	49.0	68.3	41.3	5.7	**31.0**
Fsam 32	7.0	16.0	24.0	36.0	40.7	24.0	5.0	**21.8**
Fsam 33	7.3	22.0	23.7	39.3	53.0	46.0	5.3	**28.1**
Fsam 34	9.0	21.3	31.0	46.3	63.7	23.0	5.7	**28.6**
Fsam 35	8.0	22.7	27.3	39.0	67.3	23.7	6.0	**27.7**
Fsam 36	7.7	27.3	30.7	45.3	58.3	39.3	5.7	**30.6**
Fsam 37	8.0	24.0	26.3	44.3	60.3	46.3	5.7	**30.7**
Fsam 38	10.7	36.3	45.3	59.7	86.3	27.7	5.3	**38.8**
Fsam 39	7.7	28.0	33.0	57.3	82.7	53.3	5.0	**38.1**
Fsam 40	7.7	22.3	33.7	58.3	90.0	50.0	6.0	**38.3**
Fsam 41	9.0	26.7	31.0	42.7	71.7	39.0	6.0	**32.3**
Fsam 42	11.3	35.0	40.7	63.3	90.0	39.7	5.0	**40.7**
Fsam 43	9.0	24.7	33.7	55.0	78.0	50.3	5.0	**36.5**
Fsam 44	7.0	23.0	30.7	41.7	66.0	45.0	5.0	**31.2**
Mean	**8.6**	**24.7**	**31.7**	**49.0**	**69.9**	**39.5**	**5.3**	**32.7**

F. sulphureum: Foreign								
Fsul 45	7.0	26.7	30.3	42.7	88.3.	54.0	5.0	**36.3**
Fsul 46	8.0	25.0	28.7	39.7	85.0	53.7	5.0	**35.0**
Fsul 47	8.0	25.0	29.3	35.3	86.7	45.3	5.0	**33.5**
Fsul 48	8.0	25.3	38.0	40.7	88.7	48.0	5.0	**36.2**
Fsul 49	7.3	23.7	33.7	40.0	90.0	59.3	5.0	**37.0**
Fsul 50	8.0	17.3	22.7	34.7	48.0	42.3	5.0	**25.4**
Fsul 51	8.3	24.3	33.7	48.7	48.0	37.0	5.0	**29.3**
Fsul 52	8.0	22.7	25.3	35.7	61.3	47.0	6.0	**29.4**
Fsul 53	9.0	24.7	31.3	44.0	60.0	43.0	5.7	**31.1**
Fsul 54	9.3	24.7	30.7	41.7	58.3	43.3	6.0	**30.6**
Fsul 55	10.3	21.3	34.3	38.0	55.3	38.7	6.0	**29.1**
Fsul 56	8.7	21.3	28.0	38.3	68.0	53.7	5.3	**31.9**
Fsul 57	8.0	23.3	32.0	42.3	86.7	30.3	5.7	**32.6**
Fsul 58	7.3	30.0	30.0	42.0	88.3	30.7	5.0	**33.3**
Fsul 59	8.0	25.0	29.0	39.7	54.7	40.3	5.0	**28.8**
Fsul 60	8.3	22.3	29.3	43.3	90.0	42.7	5.0	**34.4**
Fsul 61	11.0	35.3	45.3	59.7	90.0	52.7	5.3	**42.8**
Fsul 62	8.3	27.3	29.7	45.0	88.0	41.3	5.3	**35.0**
Fsul 63	7.7	19.0	29.7	51.3	58.7	38.7	5.0	**30.0**
Fsul 64	9.3	18.7	25.0	41.3	59.3	38.0	5.0	**28.1**
Fsul 65	7.0	21.0	31.7	46.3	72.7	47.3	5.0	**33.0**
Fsul 66	7.3	14.7	17.3	29.3	52.3	42.0	5.0	**24.0**
Fsul 67	7.3	24.3	27.7	38.7	56.3	35.3	5.0	**27.8**
Mean	**8.2**	**23.6**	**30.1**	**41.7**	**71.1**	**43.7**	**5.2**	**31.9**
F. roseum var. *sambucinum*: Foreign								
Frsam 68	8.0	23.0	33.7	59.3	65.7	29.3	5.0	**32.0**
Frsam 69	9.3	24.0	28.3	51.0	69.3	32.3	5.7	**31.4**
Frsam 70	11.7	23.7	28.0	48.0	51.7	36.3	6.0	**29.3**
Frsam 71	8.0	27.0	38.3	66.0	73.7	42.0	5.0	**37.1**
Frsam 72	8.7	24.0	26.0	65.0	74.7	39.3	5.0	**34.7**
Mean	**9.1**	**24.3**	**30.9**	**57.9**	**67.0**	**35.9**	**5.3**	**32.9**
Overall mean	**9.1**	**26.6**	**33.7**	**47.9**	**71.3**	**44.6**	**6.3**	

Source of variation	F-value (P≤ 0.05)	$LSD_{(T)}$ 5%
Isolates	14.11	4.6
Taxa (A)	246.8	4.3
Temperature (B)	1979.42	1.4
Interaction (A x B)	531.42	1.8

[a]Accession numbers of representative South African *F. sambucinum* isolates deposited in the culture collection of the Programme on Mycotoxins and Experimental Carcinogenesis (PROMEC), of the Medical Research Council (MRC), P. O. Box 19070, Tygerberg 7505, South Africa.

[b]Each value represents the mean of three measurements.

Table 4. Mean percentage dry rot of potato tubers, cv. Up-to-Date, 3 weeks after inoculation with South African and foreign isolates of *F. sambucinum*, *F. sulphureum* and *F. roseum* var. *sambucinum* from potato tubers with dry rot and incubated at 25 °C

Fusarium taxa	Number of isolates	Percentage of dry rot[a]		
		Minimum	Maximum	Mean[b]
F. sambucinum - South African	25	2.0	47.3	28.7
F. sambucinum - Foreign	19	7.4	77.3	52.8
F. sulphureum - Foreign	23	30.0	79.4	61.4
F. roseum var. *sambucinum* - Foreign	5	24.4	75.4	61.2
Source of variation	F-value ($P \leq 0.05$)		$LSD_{(T)}$ 5%	
Taxa	147.7		6.7	

[a]Dry rot determined according to a disease index, were 0 = no lesion development and 5 = tuber completely decayed. Results presented as percentages of decay calculated according to the method of Kremer & Unterstenhöfer (1967).

[b]Each mean value represents 30 observations for each isolate.

Table 5. Dry rot of potato tubers kept at different temperatures for 3 weeks after inoculation with selected South African and foreign isolates of *F. sambucinum*, *F. sulphureum* and *F. roseum* var. *sambucinum* from potato tubers with dry rot

Fusarium isolates[a]	Percentage dry rot[b]			
	Temperature °C			
	5	15	25	Mean
	F. sambucinum: South African			
Fsam 5	17.0	32.0	56.1	**35.0**
Fsam 9	18.0	30.0	55.8	**34.6**
Fsam 11	19.0	33.3	57.0	**36.6**
Fsam 20	17.3	47.7	61.7	**42.2**
Mean	**17.8**	**35.8**	**57.7**	**37.1**
	F. sambucinum: Foreign			
Fsam 34	56.7	56.0	80.3	**64.3**
Fsam 35	59.3	62.7	87.0	**69.7**
Fsam 37	52.7	63.0	78.0	**64.9**
Fsam 41	50.3	50.3	89.3	**63.3**
Mean	**54.8**	**58.0**	**83.9**	**65.6**
	F. sulphureum: Foreign			
Fsul 47	64.3	71.7	91.7	**75.9**
Fsul 49	68.7	81.0	81.3	**77.0**
Fsul 50	72.3	72.7	97.7	**80.9**
Fsul 67	81.3	83.3	85.0	**83.2**
Mean	**71.7**	**77.2**	**88.9**	**79.3**
	F. roseum var. *sambucinum*: Foreign			
Frsam 70	60.0	62.3	86.3	**69.6**
Frsam 71	64.7	69.0	84.3	**72.7**
Frsam 72	61.7	80.3	82.3	**74.7**
Mean	**64.4**	**72.1**	**85.0**	**73.8**
Overall mean	**52.2**	**60.8**	**78.9**	**64.0**

Source of variation	F-value (P≤ 0.05)	$LSD_{(T)}$ 5%
Isolates	2.65	5.8
Taxa (A)	182.65	2.9
Temperature (B)	231.48	2.5
Interaction (A x B)	173.52	2.7

[a]The four most virulent isolates of each group.

[b]Dry rot determined according to a disease index, were 0 = no lesion development and 5 = tuber completely decayed. Results presented as percentages of decay calculated according to the method of Kremer & Unterstenhöfer (1967). Each value represents the mean of 30 observations.

Table 6. Perithecia production of crosses between *Gibberella pulicaris* strains on different media[a]

Crosses	Media[b]				
	PDA	CLA	PCA	V-8 agar	MTA
Fsam 42♀ X Fsam 43♂	PNS[c]	PS[e]	PNS	PS	PS
Fsam 42♀ X Fsam 44♂	NP[d]	PNS	PNS	PS	PS
Fsam 42♀ X Fsul 67♂	NP	NP	NP	NP	PS
Fsam 44♀ X Fsam 42♂	PNS	PS	PNS	PS	PS

[a]Fsam 42 = male$^+$ and female$^+$, MAT1-1; Fsam 43 = male$^+$ and female$^-$, MAT1-2; Fsam 44 = male$^+$ and female$^+$, MAT1-2 and Fsul 67 = male$^+$ and female$^-$, MAT1-2.

[b]Media: PDA = potato-dextrose agar, CLA = carnation-leaf agar, PCA = potato-carrot agar, V-8 agar and MTA = mulberry-twig agar.

[c]PNS = protoperithecia, no ascospores extruded from their ostioles.

[d]NP = no perithecia.

[e]PS = perithecia with ascospores extruded from their ostioles.

Table 7. Results of crosses between *Gibberella pulicaris* strains and South African *Fusarium sambucinum* isolates from potato tubers with dry rot

Male parent	Female parent																										
G. pulicaris strains[a]	*F. sambucinum* isolates																										
	Fsam 42	Fsam 44	Fsam 1	Fsam 2	Fsam 3	Fsam 4	Fsam 5	Fsam 6	Fsam 7	Fsam 8	Fsam 9	Fsam 10	Fsam 11	Fsam 12	Fsam 13	Fsam 14	Fsam 15	Fsam 16	Fsam 17	Fsam 18	Fsam 19	Fsam 20	Fsam 21	Fsam 22	Fsam 23	Fsam 24	Fsam 25
Fsam 42	-	O	X	O	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	O	X	X	X	X	O	X	X
Fsam 43	O	O	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	O	O	X	X	O	O	X	X
Fsam 44	O	-	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	O	O	X	X	O	x	x	X
Fsul 67	O	O	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	O	X	X	O	X	X	X
Female parent	Male parent																										
G. pulicaris strains	*F. sambucinum* isolates																										
	Fsam 1	Fsam 2	Fsam 3	Fsam 4	Fsam 5	Fsam 6	Fsam 7	Fsam 8	Fsam 9	Fsam 10	Fsam 11	Fsam 12	Fsam 13	Fsam 14	Fsam 15	Fsam 16	Fsam 17	Fsam 18	Fsam 19	Fsam 20	Fsam 21	Fsam 22	Fsam 23	Fsam 24	Fsam 25		
Fsam 42	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		
Fsam 44	X	X	X	X	X	X	X	X	X	X	O	O	O	O	O	O	O	O	O	O	O	X	O	O	X		

[a]Fsam 42 = male$^+$ and female$^+$, Mat1-1; Fsam 43 = male$^+$ and female$^-$, Mat1-2; Fsam 44 = male$^+$ and female$^+$, Mat1-2; Fsul 67 = male$^+$ and female$^-$, Mat1-2.

- Isolates that did not produce perithecia when selfed are indicated by dash.

O Mature perithecia with extruded ascospores from their ostioles.

X No perithecia.

O Protoperithecia.

Table 8. Results of crosses between South African isolates of *Fusarium sambucinum* from potato tubers with dry rot

Male parent	Female parent									
	Fsam 1	Fsam 2	Fsam 3	Fsam 4	Fsam 5	Fsam 6	Fsam 7	Fsam 8	Fsam 9	Fsam 10
Fsam 1	-	X	X	X	X	X	X	X	X	X
Fsam 2	X	-	X	X	X	X	X	X	X	X
Fsam 3	○	X	-	X	X	X	X	X	X	X
Fsam 4	X	X	X	-	X	X	X	X	X	X
Fsam 5	X	X	X	X	-	X	X	X	X	X
Fsam 6	○	X	X	X	X	-	X	X	X	X
Fsam 7	X	X	X	X	X	X	-	○	X	X
Fsam 8	X	X	X	X	X	X	X	-	X	○
Fsam 9	○	X	X	X	X	X	X	○	-	X
Fsam 10	X	X	X	X	X	X	X	○	X	-

- Isolates that did not produce perithecia when selfed are indicated by dash.

X No perithecia.

○ Protoperithecia.

○ Mature perithecia with extruded ascospores from their ostioles.

Table 9. Measurements of ascospores produced in fertile crosses between *Gibberella pulicaris* strains and between South African isolates of *Fusarium sambucinum sensu lato* from potato tubers with dry rot

Crosses	Measurements[a]			
	Length (μm)	Width (μm)	Periphery (μm)	Surface area (μm^2)
G. pulicaris strains[b]				
Fsam 42$^{\varphi}$ x Fsam 43$^{\sigma}$	22.4	7.6	52.1	119.3
Fsam 42$^{\varphi}$ x Fsam 44$^{\sigma}$	22.0	8.0	52.0	120.4
Fsam 42$^{\varphi}$ x Fsul 67$^{\sigma}$	23.2	6.3	52.2	109.8
Fsam 44$^{\varphi}$ x Fsam 42$^{\sigma}$	21.9	7.9	51.8	118.1
Mean	**22.4**	**7.5**	**52.0**	**116.9**
South African isolates				
Fsam 8$^{\varphi}$ x Fsam 7$^{\sigma}$	30.8	7.4	67.8	154.3
Fsam 8$^{\varphi}$ x Fsam 9$^{\sigma}$	29.6	7.2	65.9	142.6
Fsam 8$^{\varphi}$ x Fsam 10$^{\sigma}$	27.7	7.1	61.8	135.5
Fsam 8$^{\varphi}$ x MRC 7554$^{\sigma}$ [c]	30.2	7.4	67.6	152.4
Mean	**29.6**	**7.3**	**65.8**	**146.2**
F-value ($P < 0.05$)				
Cross	9.33	13.13	5.96	6.37
Origin	281.79	NS[d]	127.98	158.93
LSD $_{(T)}$ 5%				
Cross	1.6	0.7	3.5	12.3
Origin	1.1	0.5	2.5	8.7

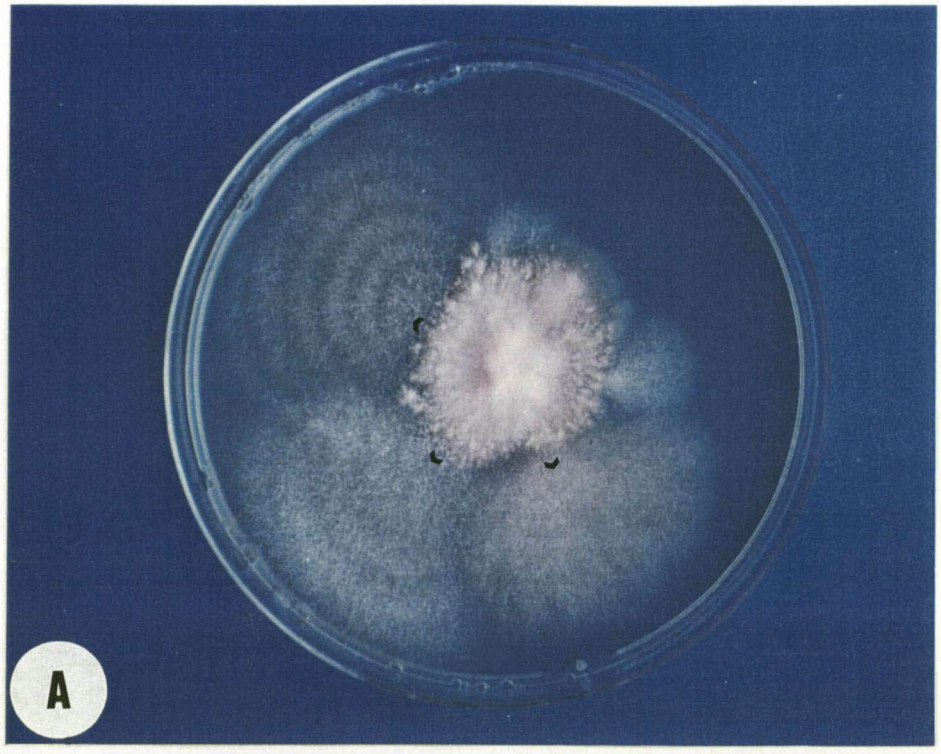
[a]One hundred ascospores produced in each of the fertile crosses, length, width, periphery and surface area determined on permanent macroscopic slides using a Kontron Video Plan Image analyser program (Version 2.5, April 1993, Kontron Electronic GMBH, Germany) connected to a Nikon Microphot-FXA microscope.

[b]Fsam 42 = male^{+} and female^{+}, Mat1-1; Fsam 43 = male^{+} and female^{-}, Mat1-2; Fsam 44 = male^{+} and female^{+}, Mat1-2; Fsul 67 = male^{+} and female^{-}, Mat1-2.

[c]Single-ascospore isolate, deposited in the culture collection of the Programme on Mycotoxins and Experimental Carcinogenesis (PROMEC), of the Medical Research Council (MRC), P. O. Box 19070, Tygerberg 7505, South Africa, progeny of a cross between Fsam 8 and Fsam 7.

[d]Not significant ($P \geq 0.05$).

Fig. 1 Chlorate resistant sectors of *F. sambucinum* on the chlorate containing medium KPS (potato-sucrose chlorate medium)(A) and vegetative compatibility reaction between *nit*-mutants of *F. sambucinum* (B).


A

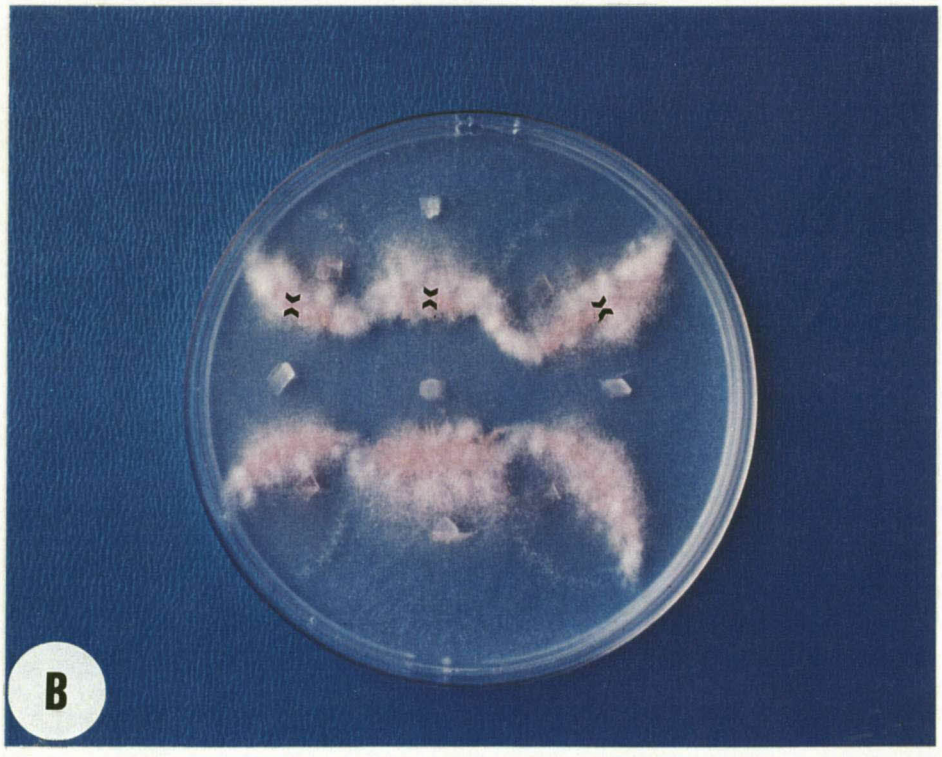
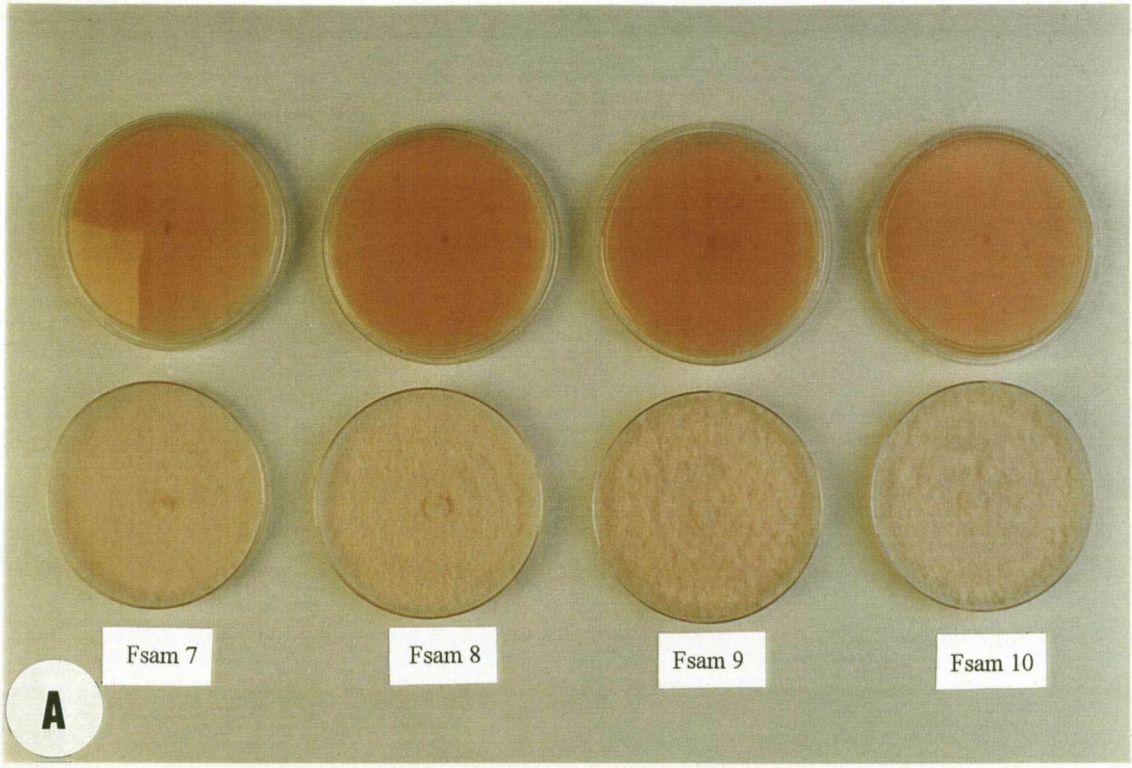

B

Fig. 2. Colony morphology of isolates of South African *Fusarium sambucinum* (A) from potato tubers with dry rot and foreign strains of *Gibberella pulicaris* (anamorph = *F. sambucinum sensu stricto*) (B) on potato-dextrose agar after 14 days incubation at 25 °C under intermittent light (fluorescent plus black lights; 12 hour cycles).


Fsam 7
Fsam 8
Fsam 9
Fsam 10
A

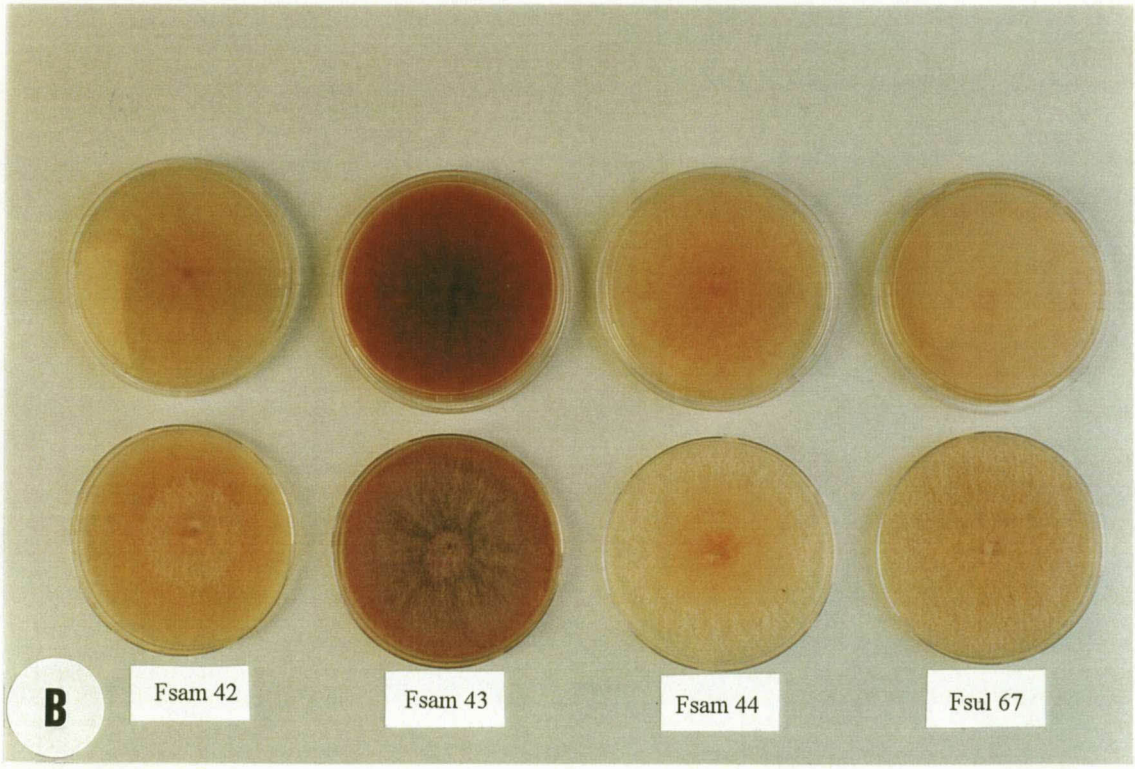
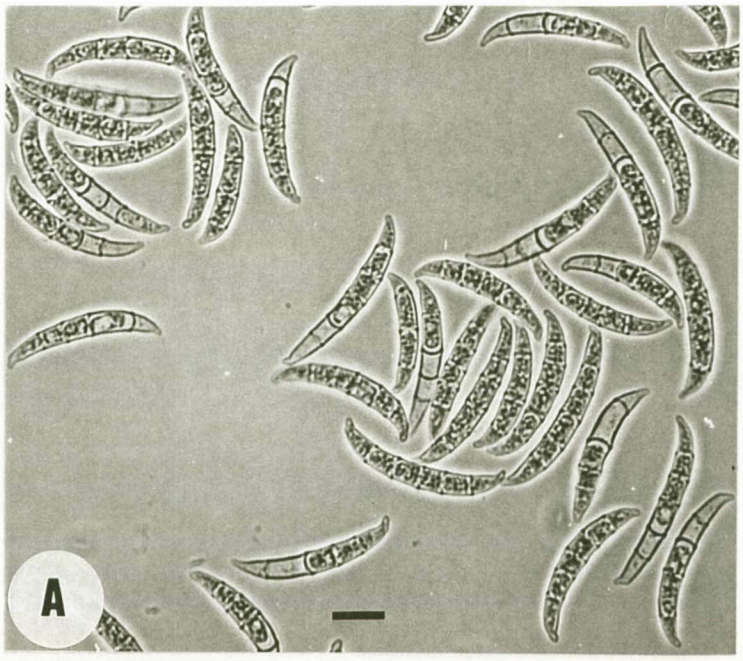

Fsam 42
Fsam 43
Fsam 44
Fsul 67
B

Fig. 3. Macroconidia produced in sporodochia, formed on carnation leaves by South African isolates of *Fusarium sambucinum* (Fsam 8) (A) from potato tubers with dry rot and foreign isolates of *F. sambucinum* (Fsam 42) (B) after 14 days incubation on carnation-leaf agar at 25 °C under intermittent light (fluorescent plus black lights; 12 hour cycles). Bars = 10 μm.


A

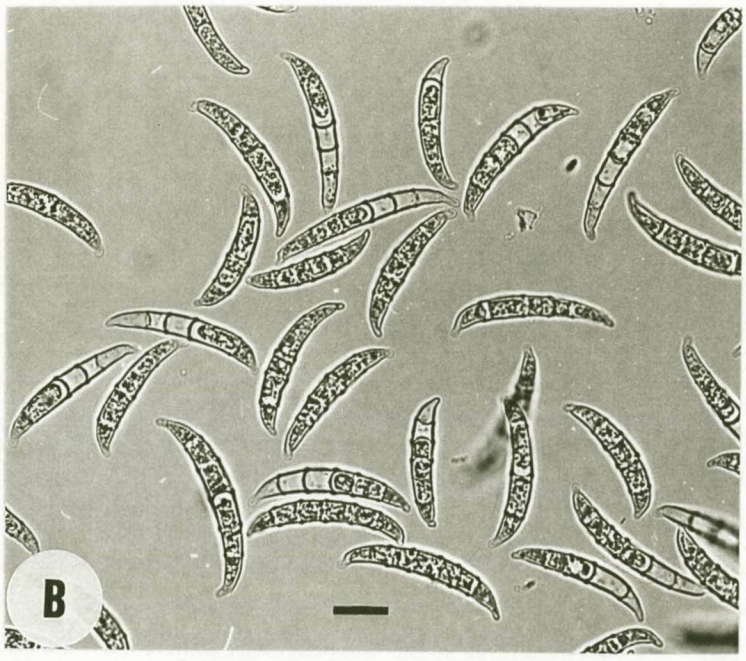
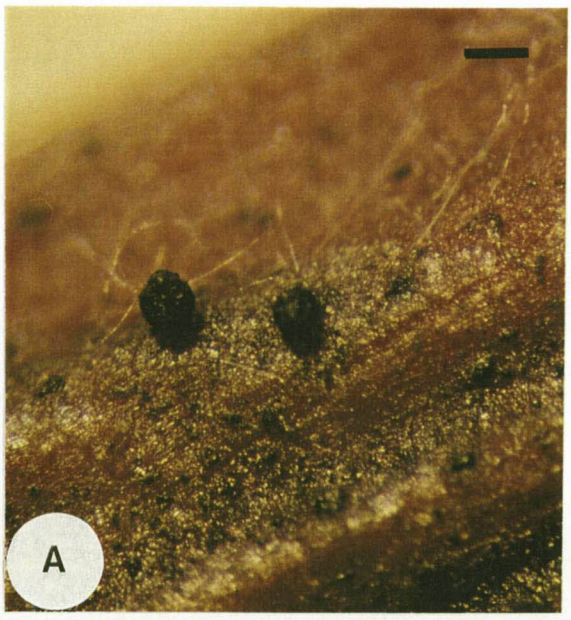

B

Fig. 4. Perithecia produced on mulberry (*Morus alba* L.) twigs in fertile crosses between South African isolates of *Fusarium sambucinum* (Fsam 8^{φ} X Fsam 7^{σ}), from potato tubers with dry rot, have been crossed with each other (A & B) and between strains of *Gibberella pulicaris* (anamorph = *F. sambucinum sensu stricto*) (Fsam 42^{φ} X Fsam 44^{σ}) have been crossed with each other (C &D). Note the ascospores extruded from the ostioles of fertile perithecia (B & D). Bars = 0.5 mm.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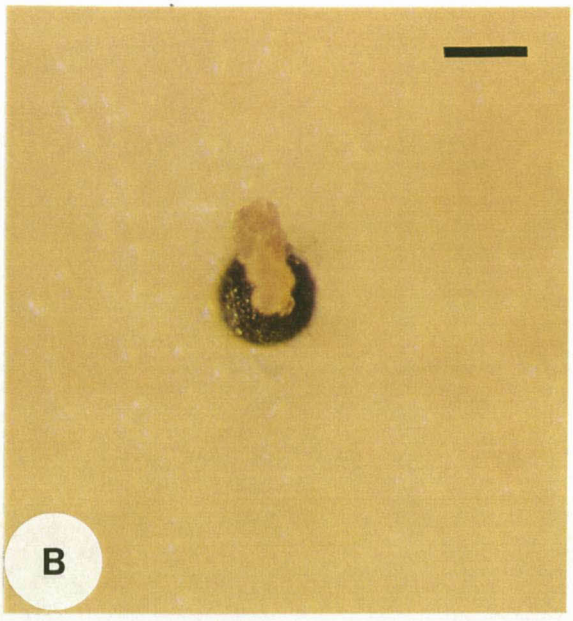
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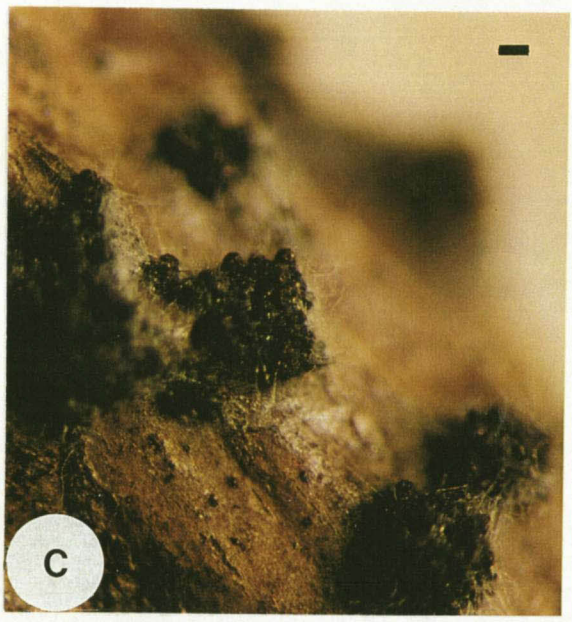

C

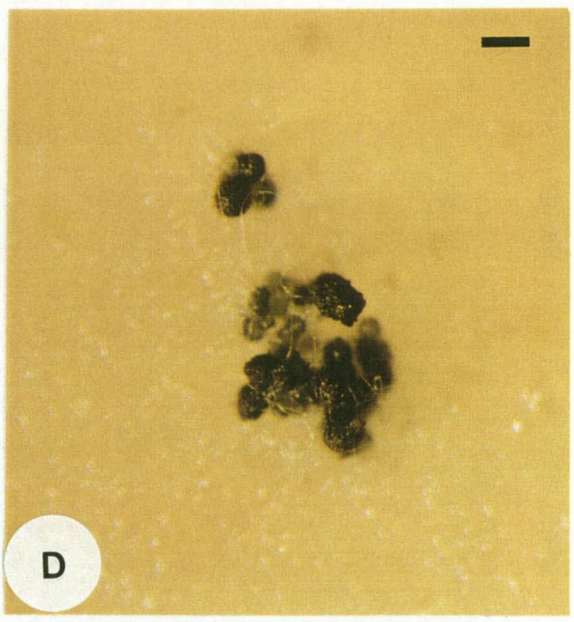
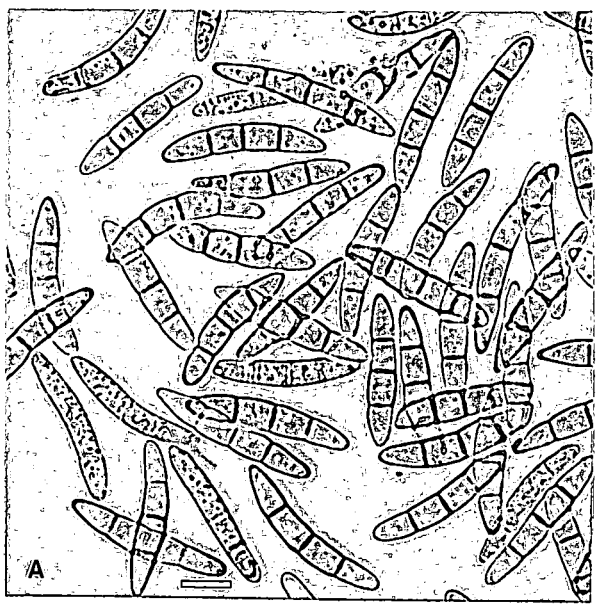

D

Fig. 5. Ascospores from fertile perithecia produced on mulberry (*Morus alba* L.) twigs in fertile crosses between South African isolates of *Fusarium sambucinum* (Fsam 8$^{\varphi}$ X Fsam 7$^{\sigma}$) from potato tubers with dry rot (A & B) and between strains of *Gibberella pulicaris* (anamorph = *F. sambucinum sensu stricto*) (Fsam 42$^{\varphi}$ X Fsam 44$^{\sigma}$) (C & D). Bars = 10 μm.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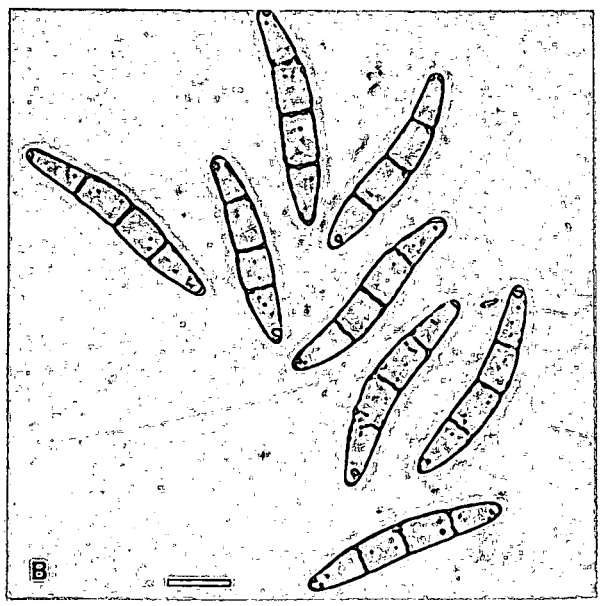
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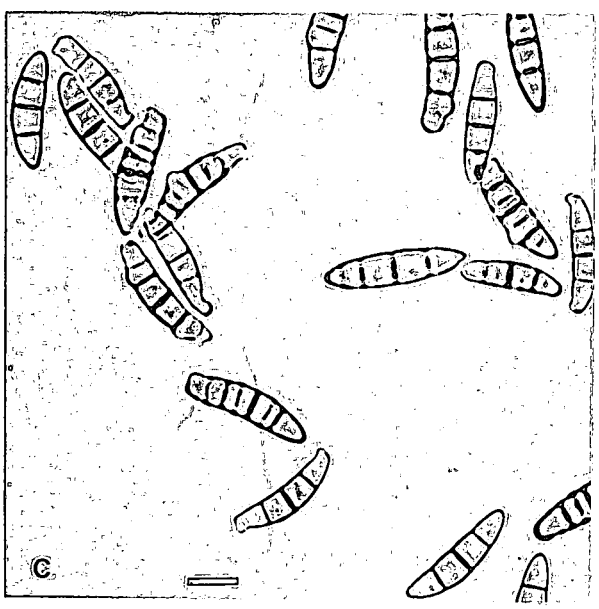
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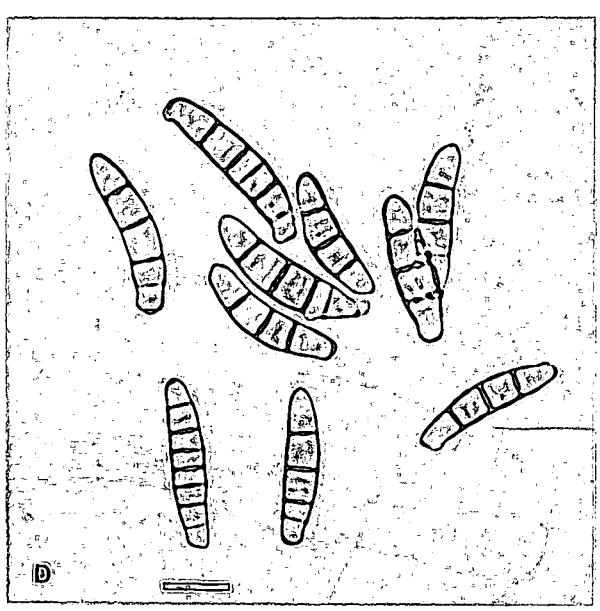
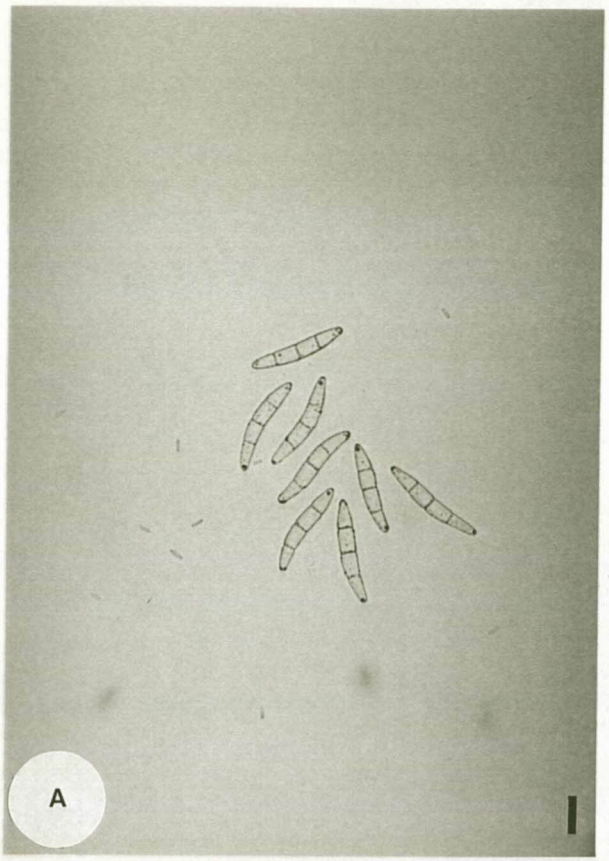

D

Fig. 6. Ascospores from fertile perithecia produced on mulberry (*Morus alba* L.) twigs in fertile crosses between South African isolates of *Fusarium sambucinum* (Fsam 8^{φ} X Fsam 7^{σ}) from potato tubers with dry rot (A, B & C) and between strains of *Gibberella pulicaris* (anamorph = *F. sambucinum sensu stricto*) (Fsam 42^{φ} X Fsam 44^{σ}) (D, E & F). Light micrography (A & D), bars = 10 μm. Scanning electronmicrography (B & C and E & F), bars = 10 μm.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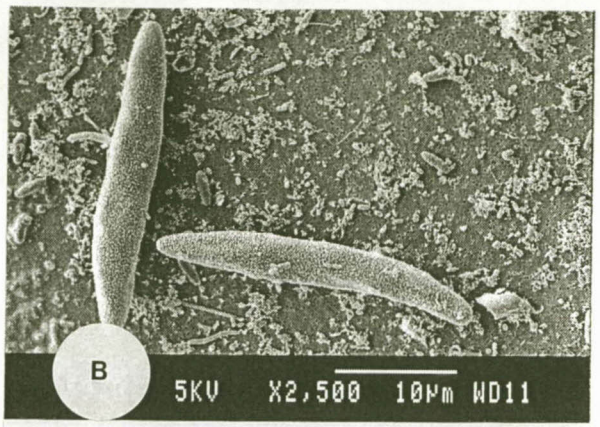
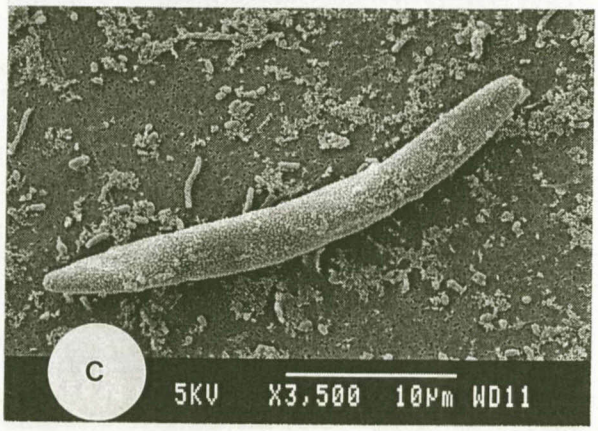

B
5KV X2,500 10µm WD11


C
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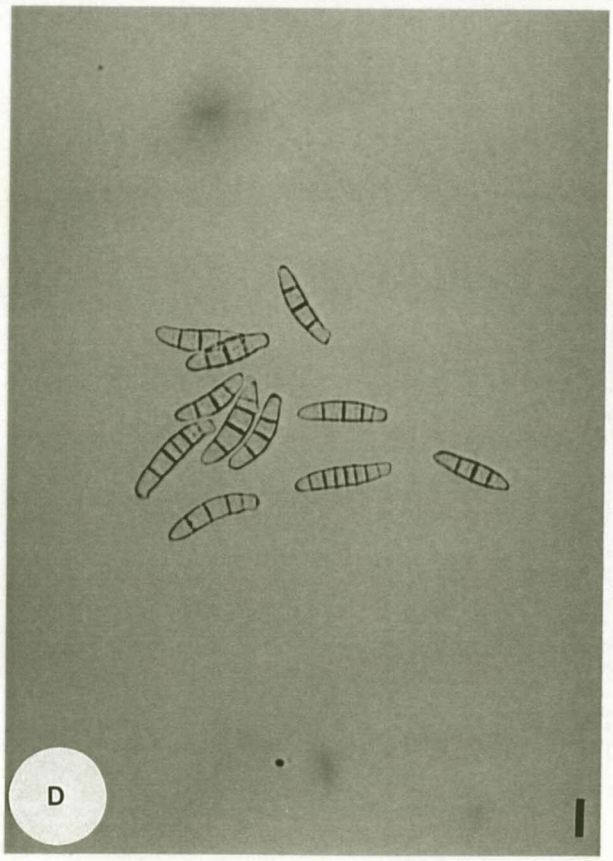
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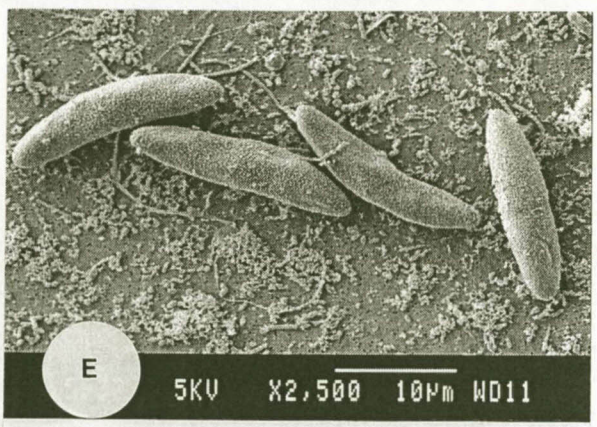
E
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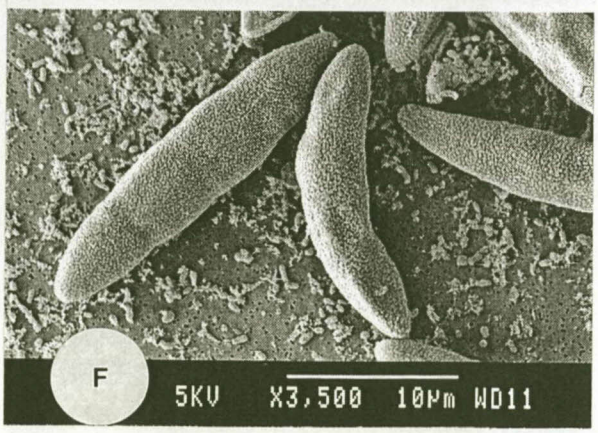

F
5KV X3,500 10µm WD11

Fig. 7. Agarose gel showing lack of PCR products when five South African isolates were PCR-ed with TOR-B primers (A) and SAM-E primers (B) but bands of the predicted size when VEN-B primers were used (B).

Key to the lanes:

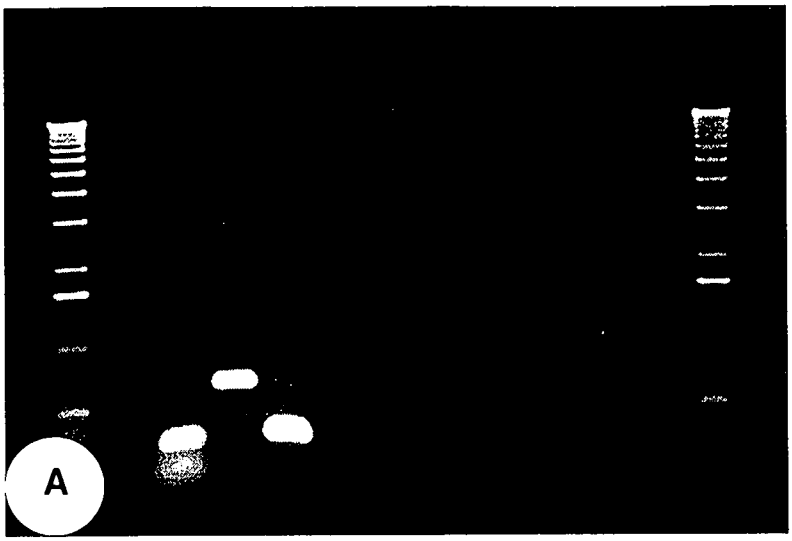
1 *F. venenatum* #93 PCR product using VEN-B primers
2 *F. torulosum* #90 PCR product using TOR-B primers
3 *F. sambucinum* #83 PCR product using SAM-E primers

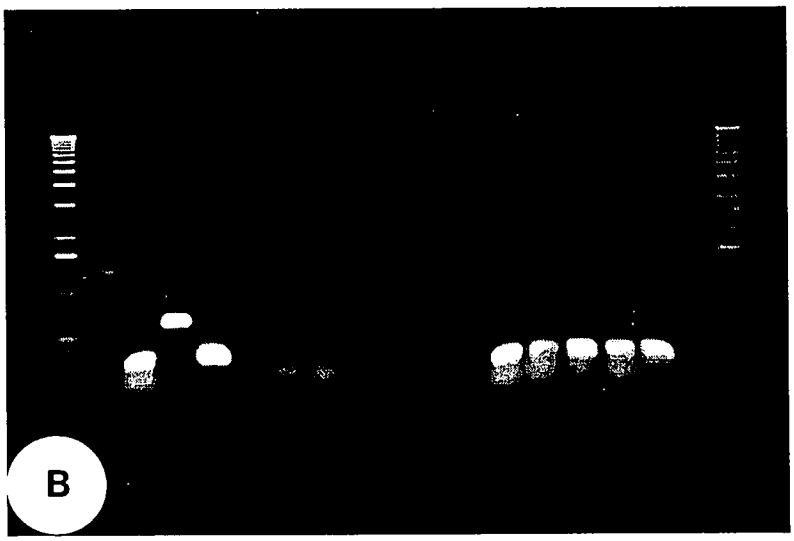
4 Fsam 1 using TOR-B primers
5 Fsam 3 using TOR-B primers
6 Fsam 4 using TOR-B primers
7 Fsam 6 using TOR-B primers
8 Fsam 7 using TOR-B primers

9 Fsam 1 using SAM-E primers
10 Fsam 3 using SAM-E primers
11 Fsam 4 using SAM-E primers
12 Fsam 6 using SAM-E primers
13 Fsam 7 using SAM-E primers

14 Fsam 1 using VEN-B primers
15 Fsam 3 using VEN-B primers
16 Fsam 4 using VEN-B primers
17 Fsam 6 using VEN-B primers
18 Fsam 7 using VEN-B primers

Y Boehringer Mannheim DNA Molecular Weight Markers X


A
Y 1 2 3 4 5 6 7 8 Y


B
Y 1 2 3 9 10 11 12 13 14 15 16 17 18 Y

CHAPTER 5

TOXICITY OF *FUSARIUM* ISOLATES FROM POTATO TUBERS WITH DRY ROT IN SOUTH AFRICA

ABSTRACT

The genus *Fusarium* has long been known to include important mycotoxin-producing species that have been implicated in human and animal diseases. The Fusarium dry-rot complex of potatoes found in South Africa differs from that in the rest of the world and the fact that diseased or damaged tubers are often fed to cattle stresses the urgency to determine the possible threat posed by these *Fusarium* spp. to the industry. A preliminary study was undertaken to determine the toxicity of the *Fusarium* spp., pathogenic as well as non-pathogenic, associated with potato dry rot and stem-end rot in South Africa. The acute toxicity of a diet, contaning autoclaved maize meal on which isolates of these *Fusarium* spp. were cultured, was determined in one-day-old Pekin ducklings. With the exception of *Fusarium graminearum*, all the other *Fusarium* spp. tested caused death of the ducklings, indicating the involvement of mycotoxins. Differences in the toxicity of the various *Fusarium* spp., as well as within isolates of the same species, were evident. Diets containing maize meal cultures of isolates of *F. nygamai*, *F. moniliforme*, *F. acuminatum*, *F. culmorum*, *F. compactum*, *F. subglutinans* and *F. reticulatum* caused 100% mortality of the ducklings between 3 and 7 days after feeding commenced and with a mean feed intake of 68.8 g. The most toxic *Fusarium* sp. was *F. nygamai*, because only 14 ±2 g of feed was sufficient to kill all the ducklings within three days.

Manuscript to be submitted to Mycopathologia

INTRODUCTION

Fusarium dry rot of potatoes (*Solanum tuberosum* L.) is a problem wherever potatoes are produced. This disease is caused by several *Fusarium* spp. (Boyd, 1972; Seppänen, 1989). *Fusarium solani* (Mart.) Appel & Wollenw. emend. Snyd. & Hans., *F. sambucinum* Fuckel and *F. avenaceum* (Fr.) Sacc. are the dominant species in the northern hemisphere (Boyd, 1972; Seppänen, 1989). In the southern hemisphere, *F. oxysporum* Schlecht. emend. Snyd. & Hans. and *F. solani* are the dominant species associated with the disease (Stubbs, 1971; Chambers, 1973; Turkensteen, 1987; Tivoli, Torres & French, 1988; Theron & Holz, 1989; Chapter 2). In South Africa, Fusarium dry rot of potatoes is considered as the most important post-harvest disease of potatoes (Theron & Holz, 1989; Chapter 2).

Fusarium spp. have long been known to produce important secondary metabolites, which cause different physiological and pharmacological responses in plants and animals. They are best known for the production of the trichothecene mycotoxins, but they may also produce a variety of other compounds such as other mycotoxins, pigments, antibiotics and phytotoxins (Nelson, Desjardins & Plattner, 1993).

The interest in toxigenic *Fusarium* spp. is increasing world-wide owing to the discovery of a growing number of naturally occurring *Fusarium* mycotoxins which threats human and animal health (Marasas, Nelson & Toussoun, 1984). Most of the research concerning the toxigenicity of *Fusarium* spp. has been conducted on cereals and the *Fusarium* pathogens of cereals. Previously, many of the *Fusarium* spp. that have been associated with potato dry rot, including some isolates from rotten potato tubers, were reported to produce various mycotoxins (Marasas *et al.*, 1984), but no research was conducted to determine their possible threat to the consumers of potatoes.

It is only since the late 1970's that isolates of *Fusarium* spp. associated with potato dry rot have received serious attention from researchers (Chełkowski, 1989). For instance, isolates of *F. sambucinum* (*F. sulphureum* Schlecht.), a major cause of potato dry rot in many countries, have been associated with trichothecene production. These isolates were obtained from rotten potato tubers collected in Germany (Siegfried & Langerfeld, 1978), in Iran, from a high-incidence area of human esophageal cancer (Steyn, Vleggaar, Rabie, Kriek & Harington, 1978), in France (Lafont, Girard, Payen, Sarfati & Gaillardin, 1983), in Poland (Latus-Ziętkiewicz, Perkowski & Chełkowski, 1988), and from several other geographically widely separated locations (Desjardins

& Plattner, 1989).

As part of the European *F. sambucinum* Project (Nirenberg, 1995a), isolates of *F. sambucinum*, divided into three species [*F. sambucinum* Fuckel *sensu stricto*, *F. torulosum* (Berk. & Curt.) Nirenberg comb. nov. and *F. venenatum* Nirenberg sp. nov.] (Nirenberg, 1995b), were screened *in vitro* for the production of secondary metabolites. Of the 29 isolates screened, 13 (9 = *F. sambucinum sensu stricto*, 2 = *F. torulosum* and 2 = *F. venenatum*) were isolated from rotten potato tubers, and all of them produced secondary metabolites (Altomare, Logrieco, Bottalico, Mulé, Moretti & Evidente, 1995; Thrane & Hansen, 1995). These isolates were also highly toxic in the biological assays (Altomare *et al.*, 1995; Schmidt, Zajkowski & Wink, 1995). Type A trichothecenes, in particular diacetoxyscirpenol (DAS), were produced by all 14 *F. sambucinum sensu stricto* and six *F. venenatum* isolates, but not by the nine *F. torulosum* isolates. The isolates from potato tubers were apparently more toxic than the isolates from other sources. All the *F. sambucinum sensu stricto* isolates, except for one, produced DAS as well as other trichothecenes, whereas the *F. venenatum* isolates produced DAS exclusively (Altomare *et al.*, 1995; Thrane & Hansen, 1995).

In contrast to these findings, Kim & Lee (1994) reported that none of the *F. sambucinum sensu lato*, *F. oxysporum* and *F. solani* isolates from rotten potato tubers in Korea were able to produce trichothecenes. However, a new toxin, sambutoxin, was produced *in vitro* by isolates of *F. sambucinum sensu lato* and *F. oxysporum*. According to Altomare *et al.* (1995) the high toxicity shown by *F. sambucinum sensu stricto* and *F. venenatum* isolates to *Artemia salina* L., may be associated with the amounts of trichothecenes produced by these isolates. However, they suggested the production of unknown toxic compounds by the *F. torulosum* isolates tested by them, because they could not find a correlation between the levels of known toxins detected and the toxicity observed in their biological assays. Despite of the widespread occurrence of *F. sambucinum sensu lato* in association with dry rot of potatoes and the documented toxigenicity of isolates from a wide variety of habitats (Marasas *et al.*, 1984; Desjardins & Beremand, 1987; Altomare *et al.*, 1995; Thrane & Hansen, 1995), only limited information is available on the ability of this species to produce trichothecenes in potato tubers.

The following *Fusarium* spp. isolated from potato tubers with dry rot have also been reported to produce mycotoxins: *F. avenaceum* (Logrieco, Frisullo & Bottalico, 1987; Logrieco, Bottalico & Solfrizzo, 1988; Latus-Ziętkiewicz *et al.*, 1988), *F. crookwellense* Burgess, Nelson

& Toussoun (Logrieco *et al.*, 1987; Goliński, Versonder, Latus-Ziętkiewicz & Perkowski, 1988; Logrieco *et al.*, 1988; Latus-Ziętkiewicz *et al.*, 1988; Latus-Ziętkiewicz, Perkowski, Tanaka, Gamamoto, Kawamura, Sugiura & Ueno, 1990; Mulè, Logrieco, Stea & Bottalico, 1997), *F. culmorum* (W. G. Smith) Sacc. (Lafont *et al.*, 1983, Latus-Ziętkiewicz *et al.*, 1988), *F. equiseti* (Corda) Sacc. (Latus-Ziętkiewicz *et al.*, 1990), *F. graminearum* Schwabe (Lafont *et al.*, 1983), *F. oxysporum* (Kim & Lee , 1994), *F. solani* (El-Banna, Scott, Lau, Sakuma, Platt & Campbell, 1984) and *F. subglutinans* (Latus-Ziętkiewicz *et al.*, 1988).

Because the Fusarium dry-rot complex of potatoes in South Africa differs from that in the northern hemisphere, it is essential to determine the possible toxicological threat that these *Fusarium* spp., associated with dry rot, could pose to the consumers of potatoes. In this study, the toxicity of the *Fusarium* spp., pathogenic and non-pathogenic, associated with dry rot and stem-end rot of potatoes in South Africa was determined.

MATERIALS AND METHODS

Isolates

During a survey conducted in ten potato producing regions of South Africa, *Fusarium* spp. were isolated from potato tubers with either dry-rot or stem-end rot lesions (Theron & Holz, 1989; Chapter 2). Seventy-four isolates were included in this study (Table 1). These included seven *F. acuminatum* Ell. & Ev., seven *F. crookwellense*, five *F. culmorum*, six *F. equiseti*, two *F. graminearum* Gr. I, seven *F. oxysporum*, fifteen *F. sambucinum*, seven *F. scirpi* Lambotte & Fautr. and six *F. solani* isolates, all pathogenic to potatoes. Two isolates of each of the following non-pathogenic species were also included: *F. chlamydosporum* Wollenw. & Reinking, *F. compactum* (Wollenw.) Gordon, *F. moniliforme* Sheldon, *F. nygamai* Burgess & Trimboli, *F. reticulatum* Mont. and *F. subglutinans* (Wollenw. & Reinking) Nelson, Toussoun & Marasas. Cultures of all these 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, lyophilised and identified according to the taxonomic system of Nelson, Toussoun & Marasas (1983), except for *F. compactum* and *F. nygamai* which were identified according to Gordon (1952) and Burgess & Trimboli (1986), respectively.

Preparation of bulk cultures of Fusarium spp.

A standard technique at PROMEC, described by Sydenham, Marasas, Thiel, Shephard & Nieuwenhuis (1991), was used for the preparation of bulk cultures of the 74 *Fusarium* isolates (Table 1). Yellow maize kernels in 2-litre glass fruit jars (400 g of maize and 400 ml of distilled water/jar) were autoclaved at 121 °C and 103 kPas for one hour on each of two consecutive days. The autoclaved maize was inoculated with lyophilized conidia of each *Fusarium* isolate and incubated in a growth room at 25 ±2 °C in the dark for 21 days. The contents of the jars were then dried at 45 °C for 24 hours, ground in a laboratory mill and the resulting meal stored at 4 °C until use.

Acute toxicity trials in ducklings

Diets were prepared by mixing the mouldy maize meal with an equal weight of commercial chicken mash. The control diets contained 50% maize meal prepared from autoclaved, non-inoculated yellow maize and 50% chicken mash. These diets were fed *ad libitum* to groups of 4 one-day-old Pekin ducklings for 14 days. The ducklings and their respective feeds were weighed at the beginning and end of each experiment and feed intake values were calculated on the basis of the amount of food remaining at the conclusion of the test (Kriek, Marasas, Steyn, van Rensburg & Steyn, 1977).

RESULTS

Except for *F. graminearum* Gr. I, all the *Fusarium* spp. tested were acutely toxic to the ducklings. The species differed in toxicity as reflected by the percentage duckling mortality: *F. equiseti* - 54.2%, *F. oxysporum*, *F. solani* and *F. chlamydosporum* - 75%, *F. crookwellense* - 89.3%, *F. scirpi* - 92.9%, *F. sambucinum* - 93.3% and the other species - 100% (Table 2). The most toxic *Fusarium* sp. was *F. nygamai*, because only 14 ±2 g of feed intake proved to be lethal to the ducklings which died within 2.7 - 3 days after feeding commenced, followed by *F. moniliforme*, *F. acuminatum*, *F. culmorum*, *F. compactum*, *F. subglutinans and F. reticulatum* (Table 2). Apart from one isolate each of *F. crookwellense* (MRC 6447), *F. sambucinum* (MRC 4663) and *F. scirpi* (MRC 6839), all the other isolates of these species tested resulted in all four ducklings dying after feeding on a diet containing maize meal colonized by these species (Table 2).

The mean day of death also varied between the *Fusarium* spp. as well as between isolates of the same species (Table 2). All the ducklings fed on a diet containing culture material of *F. nygamai*,

died within 2.7 - 3.0 days, whereas all those fed on a diet containing *F. reticulatum* died within 6.0 - 8.7 days. This rapid or retarded death syndrome was also in accordance with the amount of feed intake for most of the species. In the case of some species only a small amount of feed proved to be lethal, e.g. only 12 and 16 g of the diet containing *F. nygamai* culture material was needed to cause 100% mortality, whereas between 120 and 136 g of the diet was needed in the case of *F. subglutinans*. Differences in the toxicity of isolates of the same species were also evident. For example an intake of between 26 and 98 g of diets containing *F. culmorum* culture material of different isolates was necessary to be 100% lethal, in which case the ducklings died between 4.7 and 7.0 days of feeding (Table 2).

DISCUSSION

Pathogenic and non-pathogenic isolates of the fifteen *Fusarium* spp. isolated from potato tubers revealing dry-rot symptoms were included in these trials. All the species except *F. graminearum* were acutely toxic at varying degrees to one-day-old Pekin ducklings, which were fed *ad libitum* on a diet containing maize meal colonized with different isolates of each species. Of these, *F. nygamai* appeared to be the most toxic, followed by *F. acuminatum* and *F. moniliforme*.

The quantity of feed consumed and the time taken for the ducklings to die, did not correlate in all instances. Thus some ducklings took relatively longer to die while consuming less of their diets than others that consumed more of their diets and died more rapidly. These findings may be due to the presence of different types of mycotoxins produced by the *Fusarium* spp. or their ability or inability to produce large quantities of mycotoxins in the maize kernels. For instance, isolates of *F. crookwellense* have been reported to produce only zearalenone on maize kernels and not on rice, whereas *F. avenaceum* isolates produce moniliformin on both maize and rice, but in different quantities (533 mg/kg and 1600 mg/kg respectively) (Logrieco *et al.*, 1988).

Fusarium spp. associated with potatoes are known to be able to produce different mycotoxins (Seppänen, 1989). Isolates of *F. avenaceum, F. sambucinum, F. subglutinans, F. crookwellense, F. equiseti, F. graminearum* Gr. I, *F. culmorum, F. solani, F. oxysporum* and *F. semitectum* Berk. & Rav. obtained from potatoes with or without dry-rot symptoms have been reported to produce trichothecenes (deoxynivalenol, DAS, fusarenone, nivalenol and neosolaniol), zearalenone, moniliformin, fusarochromanone and sambutoxin (Lafont *et al.*, 1983; El-Banna *et al.*, 1984; Logrieco

et al., 1987; Golinski *et al.*, 1988; Logrieco *et al.*, 1988; Latus-Ziętkiewicz *et al.*, 1988; Desjardins & Plattner, 1989; Perkowski, Foremska & Latus-Ziętkiewicz, 1989; Latus-Ziętkiewicz *et al.*, 1990; Wu, Nelson, Cook & Smalley, 1990; Vesonder, Goliński, Plattner & Zietkiewicz, 1991; Kim & Lee, 1994; Altomare *et al.*, 1995; Thrane & Hansen, 1995; Mulè *et al.*, 1997).

Mycotoxins have also been reported to be present in natural or artificially infected potato tubers. In France, Lafont *et al.* (1983) found several trichothecene toxins in potato tubers naturally and artificially infected with *F. sambucinum*. In Canada, El-Banna *et al.* (1984) reported low concentrations of trichothecenes in potato tubers artificially infected with an isolate of *F. sambucinum* and deoxynivalenol in tubers infected with *F. solani* [= *F. solani* (Mart.) Sacc. var. *coeruleum* (Sacc.) Booth] which had originally been isolated from potato tubers. Desjardins and Plattner (1989) reported beyond any doubt that isolates of *F. sambucinum* can produce trichothecenes, including 15-monoacetoxyscirpenol and 4,15-diacetoxyscirpenol as well as other minor trichothecenes, in artificially infected potato tubers, at levels up to 5 μg of toxin/g of rot fresh weight. This is a concentration higher than that allowed in Canadian grain destined for export and higher than that known to cause adverse effects in animals. Even apparently disease-free parts of infected potatoes contain the mycotoxins at levels of approximately 10% of those in the diseased portion. The removal of rotten parts will therefore not completely eliminate the toxin. Since trichothecenes are heat stable, it seems unlikely that these mycotoxins would be destroyed by the usual procedures of preparing potatoes for human consumption (Desjardins, 1989).

A newly described toxin, sambutoxin, (Kim & Lee, 1994) was also reported by Kim, Lee & Yu (1995) to be produced in potato tubers by *F. sambucinum* and *F. oxysporum* at levels of 15.8 to 78.1 ng/g of rot fresh weight. According to Kim *et al.* (1995) sambutoxin should be considered as a possible source of toxic effects on experimental and farm animals, although its toxicity is relatively low.

The fact that the South African isolates of *Fusarium* spp. from potato tubers proved to be toxic to ducklings, emphasizes the need to determine the type of toxins produced, particularly if these mycotoxins can be produced in colonized potato tubers. Potatoes infected with these *Fusarium* spp. should urgently be analysed for toxins, known to be produced by these species, in order to provide valuable information for toxin risk assessment of potatoes. The type of toxin produced, particularly by the *F. sambucinum* isolates, is also important as this might clarify the uncertainty concerning the identity of the potato isolates of this species in South Africa. According to PCR

analysis (Chapter 4, part 2), the majority of these isolates are apparently *F. venenatum*, a species which produces DAS exclusively (Altomare *et al.*, 1995; Thrane & Hansen, 1995). However, these isolates were not conspecific with *F. venenatum* on the basis of morphological characteristics and growth rates (Chapter 4, part 2). Consequently information on the mycotoxin(s) produced by these South African *F. sambucinum* isolates may provide a valuable tool to resolve their taxonomic position.

In addition to the potential hazardous effects on human consumers (Desjardins, 1989), the fact that discarded, diseased or damaged potato tubers are often fed to cattle, emphasizes the urgency of this kind of research. World-wide, this potential threat will become even more important as the demands on the available food supply increase because of increases in population. If food supply is limited, the mycotoxin hazard will increase on account of the fact that more fungus-damaged, potentially mycotoxin-containing tubers may be consumed rather than discarded and the fact that malnutrition enhances susceptibility to lower concentrations of food-borne mycotoxins (Marasas & Nelson, 1987).

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Table 1. *Fusarium* isolates from dry-rot and stem-end-rot lesions of potato tubers grown in South Africa

Fusarium species[a]	Isolate number	MRC[b] number	Geographical origin	Isolated from
	Pathogenic isolates			
F. acuminatum	O3/3/1P1	4793	Eastern Free State, SA	Stem-end rot
	O3/3/7D7	4794	Eastern Free State, SA	Dry rot
	D4/D15/5(4)	6449	Northern Cape, SA	Dry rot
	O3/1/4P8	6450	Eastern Free State, SA	Stem-end rot
	KB1/D17/5(2)	6451	Western Cape, SA	Dry rot
	KB5/P7/2	6452	Western Cape, SA	Stem-end rot
	KB5/D6/5	6453	Western Cape, SA	Dry rot
F. crookwellense	O7/3/1/D1	4639	Eastern Free State, SA	Dry rot
	K523/D1/6(5)	4643	Western Cape, SA	Dry rot
	O6/2/11D6	6444	Eastern Free State, SA	Dry rot
	O6/8/4D7	6445	Eastern Free State, SA	Dry rot
	O3/1/3P9	6446	Eastern Free State, SA	Stem-end rot
	F cr. P.2	6447	Eastern Free State, SA	Dry rot
	O6/2/4D6	6448	Eastern Free State, SA	Dry rot
F. culmorum	W15/D12/5	6838	Western Free State, SA	Dry rot
	W6/D1/5/3	6839	Western Free State, SA	Dry rot
	W13/D12/5	6840	Western Free State, SA	Dry rot
	W17/D2/5	6841	Western Free State, SA	Dry rot
	D6/D1/5	6842	Northern Cape, SA	Dry rot
F. equiseti	O2/2/10/D23	4675	Eastern Free State, SA	Dry rot
	N7/D7/5	6828	KwaZulu Natal, SA	Dry rot
	KB1/D18/4(2)	6829	Western Cape, SA	Dry rot
	O6/1/1/D1	6830	Eastern Free State, SA	Dry rot
	D5/D13/3(3)	6831	Northern Cape, SA	Dry rot
	D4/D9/4(2)	6832	Northern Cape, SA	Dry rot
F. graminearum Gr. I	T3/1/2/D6	4673	Mpumalanga, SA	Dry rot
	K864/P5/6	4672	Western Cape, SA	Stem-end rot
F. oxysporum	T3/1/1/D2	4640	Mpumalanga, SA	Dry rot
	O4/3/3D1	4798	Eastern Free State, SA	Dry rot
	N9/D6/2(4)	6379	KwaZulu Natal, SA	Dry rot
	N5/D4/2(4)	6380	KwaZulu Natal, SA	Dry rot
	O9/2/8D19	6381	Eastern Free State, SA	Dry rot
	N5/D6/2(4)	6382	KwaZulu Natal, SA	Dry rot
	D1/D4/2(5)	6383	Northern Cape, SA	Dry rot

F. sambucinum	O9/1/1/D2	4664	Eastern Free State, SA	Dry rot
	K630/D3/5	4665	Western Cape, SA	Dry rot
	K17/D3/3	4667	Western Cape, SA	Dry rot
	K17/D10/5	4669	Western Cape, SA	Dry rot
	K466/D6/5	4670	Western Cape, SA	Dry rot
	K466/D1/5(4)	4662	Western Cape, SA	Dry rot
	O3/1/15/4	4663	Eastern Free State, SA	Dry rot
	K17/D9/5(5)	4666	Western Cape, SA	Dry rot
	O3/1/1/D4	4668	Eastern Free State, SA	Dry rot
	O9/3/6/D4	4671	Eastern Free State, SA	Dry rot
	T1/1/4D12a	6454	Mpumalanga, SA	Dry rot
	T3/1/6D16	6455	Mpumalanga, SA	Dry rot
	O2/4/4D16	6456	Eastern Free State, SA	Dry rot
	O2/2/7P16	6457	Eastern Free State, SA	Stem-end rot
	KB1/D19/3(3)	6458	Western Cape, SA	Dry rot
F. scirpi	O2/1/3/P3	4676	Eastern Free State, SA	Stem-end rot
	T3/1/6/D16a	4677	Mpumalanga, SA	Dry rot
	T3/1/6D16	6838	Mpumalanga, SA	Dry rot
	KB1/D18/2	6839	Western Cape, SA	Dry rot
	O8/1/7D13	6840	Eastern Free State, SA	Dry rot
	F.sc P2	6841	Eastern Free State, SA	Dry rot
	O2/1/3P3	6842	Eastern Free State, SA	Stem-end rot
F. solani	O6/2/10/D18	4797	Eastern Free State, SA	Dry rot
	N9/D4/1(3)	6360	KwaZulu Natal, SA	Dry rot
	KB1/P3/1(5)	6358	Western Cape, SA	Stem-end rot
	N9/P2/1(4)	6359	KwaZulu Natal, SA	Stem-end rot
	No. 9	6361	Mpumalanga, SA	Dry rot
	D4/D5/5(3)	6362	Northern Cape, SA	Dry rot
Non-pathogenic isolates				
F. chlamydosporum	O4/4/5/2	4642	Eastern Free State, SA	Dry rot
	8/9	4684	Eastern Free State, SA	Dry rot
F. compactum	O4/2/1/D4	4641	Eastern Free State, SA	Dry rot
	O4/2/21/D2	4795	Eastern Free State, SA	Dry rot
F. moniliforme	K630/D6/2	4679	Western Cape, SA	Dry rot
	K869/P7/2	4680	Western Cape, SA	Stem-end rot
F. nygamai	O4/2/17/D4	4683	Eastern Free State, SA	Dry rot
	O9/3/10/D7	4796	Eastern Free State, SA	Dry rot
F. reticulatum	O2/2/3/P7	4681	Eastern Free State, SA	Stem-end rot
	O2/2/23/P4	4825	Eastern Free State, SA	Stem-end rot
F. subglutinans	K813/D3/6	4685	Western Cape, SA	Dry rot
	O4/1/D6	4799	Eastern Free State, SA	Dry rot

[a]Tubers were collected at sorting tables in 10 potato producing regions, surface disinfested (3% sodium hypochlorite, 15 min), cut in half and five discs (2 mm^3) were randomly dissected from the periphery of discoloured tissue and plated on potato dextrose agar.

[b]Accession numbers of representative isolates deposited into the culture collection of the Programme on Mycotoxins and Experimental Carcinogenesis (PROMEC), Medical Research Council (MRC), P. O. Box 19070, Tygerberg 7505, South Africa.

Table 2. Toxicity[a] to ducklings of *Fusarium* isolates from potato tubers in South Africa

Fusarium spp.	MRC[b] numbers	Toxicity to ducklings		
		Mortality	Day of death	Feed intake (g)
Pathogenic isolates				
F. acuminatum	4793	4/4	4.2	10
	4794	4/4	4.5	20
	6449	4/4	4.7	36
	6450	4/4	4.0	26
	6451	4/4	4.0	26
	6452	4/4	4.7	38
	6453	4/4	5.7	60
	Mean	**100%**	**4.5**	**30.9**
F. crookwellense	4639	4/4	5.0	48
	4643	4/4	6.0	48
	6444	4/4	5.3	30
	6445	4/4	6.3	150
	6446	4/4	6.3	90
	6447	1/4	5.0	798
	6448	4/4	5.5	58
	Mean	**89.3%**	**5.6**	**174.6**
F. culmorum	6838	4/4	7.0	98
	6839	4/4	5.0	78
	6840	4/4	5.0	26
	6841	4/4	5.0	46
	6842	4/4	4.7	60
	Mean	**100%**	**5.3**	**61.6**
F. equiseti	4675	4/4	7.2	202
	6828	2/4	4.5	1054
	6829	1/4	6.0	1378
	6830	2/4	6.5	508
	6831	2/4	6.0	524
	6832	2/4	6.5	868
	Mean	**54.2**	**6.1**	**755.7**
F. graminearum Gr. I	4673	0/4	-	1290
	4672	0/4	-	600
	Mean	**0%**	-	**945.0**
F. oxysporum	4640	1/4	-	1500
	4798	2/4	-	720
	6379	4/4	5.7	100
	6380	4/4	5.5	58
	6381	4/4	5.0	82
	6382	4/4	4.5	78
	6383	2/4	6.0	360
	Mean	**75%**	**5.3**[c]	**414.0**

F. sambucinum	4664	4/4	7.2	64
	4665	4/4	6.0	44
	4667	4/4	5.7	28
	4669	4/4	5.2	44
	4670	4/4	3.7	64
	4662	4/4	7.5	134
	4663	0/4	-	2100
	4666	4/4	6.0	20
	4668	4/4	5.2	58
	4671	4/4	4.7	56
	6454	4/4	5.5	62
	6455	4/4	5.7	70
	6456	4/4	4.5	36
	6457	4/4	5.3	48
	6458	4/4	3.7	54
	Mean	**93.3%**	**5.4**[c]	**192.1**
F. scirpi	4676	4/4	6.0	108
	4677	4/4	6.5	178
	6838	4/4	5.7	112
	6839	2/4	5.0	1000
	6840	4/4	6.5	116
	6841	4/4	5.0	50
	6842	4/4	6.0	132
	Mean	**92.9%**	**5.8**	**242.3**
F. solani	4797	1/4	-	-
	6358	4/4	5.5	102
	6359	4/4	4.7	70
	6360	3/4	5.0	1060
	6361	4/4	5.5	128
	6362	2/4	9.0	880
	Mean	**75%**	**5.9**[c]	**448.0**[d]
Non-pathogenic isolates				
F. chlamydosporum	4642	2/4	-	646
	4684	4/4	5.2	74
	Mean	**75%**	**5.2**[c]	**360.0**
F. compactum	4641	4/4	6.2	58
	4795	4/4	5.0	44
	Mean	**100%**	**5.6**	**51.0**
F. moniliforme	4679	4/4	5.0	80
	4680	4/4	4.2	68
	Mean	**100%**	**4.6**	**74.0**
F. nygamai	4683	4/4	2.7	12
	4796	4/4	3.0	16
	Mean	**100%**	**2.9**	**14.0**
F. reticulatum	4681	4/4	6.0	114
	4825	4/4	8.7	130
	Mean	**100%**	**7.4**	**122.0**
F. subglutinans	4685	4/4	5.0	136
	4799	4/4	7.5	120
	Mean	**100%**	**6.3**	**128.0**

[a]One-day-old Pekin ducklings fed on a diet consisting of equal weight of mouldy meal (autoclaved yellow maize kernels inoculated with *Fusarium* isolates, incubated at 25 ± 2 °C for 21 days in the dark and then ground) and commercial chicken mash.

[b]Number of representative isolates were deposited into the culture collection of the Programme on Mycotoxins and Experimental Carcinogenesis (PROMEC), Medical Research Council (MRC), P. O. Box 19070, Tygerberg 7505, South Africa.

[c]Mean day of death calculated on available data.

[d]Mean feed intake (g) calculated on available data.

SUMMARY

Eighteen *Fusarium* species were isolated from dry- and stem-end-rotted potato tubers in South Africa. Samples with dry-rot lesions were easily obtained, even though samples were taken directly after harvest and Fusarium dry rot is a post-harvest disease, highlighting the importance of this disease in South Africa. Nine species (*F. oxysporum, F. solani, F. sambucinum, F. acuminatum, F. crookwellense, F. graminearum, F. culmorum, F. scirpi* and *F. equiseti*) caused typical dry-rot lesions on artificially inoculated potato tubers. *Fusarium oxysporum* and *F. solani* were predominantly isolated and the most virulent. These results differ from those obtained in other parts of the world, especially the northern hemisphere, where *F. sambucinum* and *F. solani* are mainly associated with Fusarium dry rot.

Control of Fusarium dry rot with mancozeb or thiabendazole, the only fungicides registered for control of this disease in South Africa, was unsatisfactory. This was especially evident when *F. oxysporum* was the cause of the rotting. It is recommended that effective fungicides against *F. solani* and *F. oxysporum*, e.g. prochloraz, should be applied as soon as possible after harvest and that potatoes should be stored at 5 °C. Thiabendazole resistance, which is a problem in North America and Europe, appears to be absent in local isolates of *F. solani, F. oxysporum* and *F. sambucinum.* The use of seed pieces under local conditions is risky. When used due to scarcity of seed tubers or economic reasons, good quality seed tubers should be used and treated with carbendazim on the day prior to a spring planting under irrigation in soils with a temperature <18°C. *Fusarium solani* [= *F. solani* var. *coeruleum* and *F. coeruleum*] is commonly associated with dry rot of potatoes world wide. The local *F. solani* isolates were compared to foreign *F. solani*, *F. solani* var. *coeruleum* and *F. coeruleum* isolates. Local and foreign *F. solani* isolates did not differ morphologically and with respect to their optimum growth temperatures and matched the description of *F. solani*. However, foreign isolates referred to as *F. solani* var. *coeruleum* and *F. coeruleum* differed from the *F. solani* isolates. Grouping these isolates together under one species (*F. solani*) is thus disputable.

In contrast to other countries, *Fusarium sambucinum* [= *F. sulphureum* and *F. roseum* var. *sambucinum*], is not commonly associated with dry rot of potatoes in South Africa. The local isolates were compared with foreign isolates because they appeared to be atypical of *F. sambucinum sensu lato.* Moreover *F. sambucinum sensu lato* has been divided into three species

i.e. *F. sambucinum sensu srticto, F. torulosum* and *F. venenatum*. Morphologically and on the basis of optimum temperature for growth and dry-rot development, the local isolates could not be differentiated from the foreign isolates, but no vegetative compatibility occurred between them. Local and foreign isolates could be assigned to six and three vegetative compatibility groups, respectively. Sexual crosses between the local isolates and the *Gibberella pulicaris* strains, were unsuccessful. However, crossing some local isolates with each other resulted in fertile crosses, producing ascospores significantly larger than those produced when the *G. pulicaris* strains were crossed. This is the first report of South African *F. sambucinum* isolates from potatoes producing a teleomorph. Five of the local *F. sambucinum* isolates generated bands of the expected size with *F. venenatum* primers, indicating that these isolates are conspecific with *F. venenatum*. However, morphologically these isolates did not agree with the description of *F. venenatum* and their growth rates were faster. It appears that these South African isolates are members of a new species of *Fusarium* with a newly discovered *Gibberella.*

The genus *Fusarium* contains important mycotoxin-producing species which have been implicated in human and animal diseases. Diseased or damaged tubers are often fed to cattle, stressing the urgency to determine the possible threat posed to the industry. Except for *F. graminearum* Gr. I, isolates of the other 17 *Fusarium* species tested, caused death in one-day-old Pekin ducklings, indicating the involvement of mycotoxins. Differences in the toxicity of the various *Fusarium* spp., as well as within isolates of the same species, were evident. *Fusarium nygamai* appeared to be the most toxic, followed by *F. acuminatum* and *F. moniliforme*. The fact that South African dry-rot isolates proved to be toxic to ducklings emphasizes the need to determine the identity of toxins produced, particularly if these mycotoxins can be produced in colonized potato tubers.

This dissertation has made a contribution towards the better understanding of, and the development of an integrated control strategy for Fusarium dry rot of potatoes in South Africa.

OPSOMMING

Agtien *Fusarium* spesies is uit aartappels met droëvrot en puntjievrot simptome in Suid-Afrika geïsoleer. Alhoewel droëvrot 'n na-oes siekte is, is aartappels met droëvrotsimptome geredelik direk na oes, gevind. Dit bevestig die belangrikheid van Fusarium droëvrot in Suid-Afrika. Nege spesies (*F. oxysporum, F. solani, F. sambucinum, F. acuminatum, F. crookwellense, F. graminearum, F. culmorum, F. scirpi* en *F. equiseti*) het tipiese droëvrot simptome in kunsmatig geïnokuleerde aartappelknolle veroorsaak. *Fusarium oxysporum* en *F. solani* was die primêre asook die mees virulente spesies geïsoleer. Die resultate verskil van wat elders in die wêreld gevind word, veral lande in die noordelike halfrond, waar *F. sambucinum* en *F. solani* hoofsaaklik met Fusarium droëvrot geassosieer word.

Beheer van Fusarium droëvrot met mankoseb en tiabendasool, die enigste fungisiedes vir die beheer van die siekte in Suid-Afrika geregistreer, was teleurstellend, veral waar *F. oxysporum* die verrotting veroorsaak het. Fungisiedes wat tenminste teen beide *F. oxysporum* en *F. solani* effektief is, bv. prochloras, moet so gou moontlik na oes toegedien word waarna die aartappels by 5 °C opgeberg moet word. Bestandheid teen tiabendasool, wat 'n probleem in Noord-Amerika en Europa is, kon nie in plaaslike *F. oxysporum, F. solani* en *F. sambucinum* isolate gevind word nie. Die plant van gesnyde moere onder plaaslike toestande word nie aanbeveel nie. Indien gesnyde moere weens ekonomiese redes wel geplant word, moet slegs goeie kwaliteit moere, wat die dag voor plant gesny en met karbendasim behandel is, tydens lenteplantings in gronde onder besproeiing met temperature < 18 °C geplant word.

Fusarium solani [= *F. solani* var. *coeruleum* en *F. coeruleum*] word wêreldwyd met droëvrot van aartappels geassosieer. Plaaslike *F. solani* isolate is met buitelandse *F. solani*, *F. solani* var. *coeruleum* en *F. coeruleum* isolate vergelyk. Plaaslike en buitelandse *F. solani* isolate verskil nie morfologies en ten opsigte van hul optimum temperature vir groei van mekaar nie en stem met die beskrywing van *F. solani* ooreen. Die *F. solani* var. *coeruleum* en *F. coeruleum* isolate verskil egter van die *F. solani* isolate. Om die isolate dus onder een spesie (*F. solani*) te groepeer word bevraagteken.

In teenstelling met ander lande word *Fusarium sambucinum* [= *F. sulphureum* en *F. roseum* var. *sambucinum*] nie algemeen met droëvrot van aartappels in Suid-Afrika geassosieer nie. Die plaaslike isolate is met die buitelandse isolate vergelyk omdat hulle oënskynlik atipies van die *F. sambucinum sensu lato* is, en omdat lg. tans in *F. sambucinum sensu stricto, F. torulosum*

en *F. venenatum* opgedeel word. Morfologies en op grond van hul optimum temperature vir groei en droëvrot ontwikkeling, kon die plaaslike en die buitelandse isolate nie van mekaar onderskei word nie. Vegetatiewe verenigbaarheid was egter afwesig tussen die twee groepe isolate. Die plaaslike en buitelanse isolate kon in onderskeidelik ses en drie vegetatiewe verenigbaarheids groepe verdeel word. Geslagtelike kruisings tussen die plaaslike- en buitelandse *Gibberella pulicaris* isolate was ook onsuksesvol. Sommige plaaslike isolate kon egter suksesvol met mekaar gekruis word en dié askusspore geproduseer was betekenisvol groter as dié geproduseer tydens kruisings tussen die buitelandse *Gibberella pulicaris* isolate. Dit is die eerste geval waar Suid-Afrikaanse *F. sambucinum* isolate, vanaf aartappels, 'n teleomorf produseer. Vyf plaaslike *F. sambucinum* isolate het bande van verwagte grootte met *F. venenatum* voorvoerders geproduseer wat impliseer dat die isolate met die van *F. venenatum* ooreenstem. Morfologies stem die isolate egter nie met die beskrywing van *F. venenatum* ooreen nie en was hul groeispoed ook vinniger. Dit blyk dus dat die Suid-Afrikaanse isolate deel van 'n nuwe spesie van *Fusarium* is, waarvan 'n nuwe *Gibberella* ontdek is.

Belangrike mikotoksien produserende spesies kom in die genus *Fusarium* voor wat met mens- en diersiektes geassosieer word. Die feit dat beskadigde of besmette aartappelknolle algemeen vir vee gevoer word, beklemtoon die noodsaaklikheid om die gevaar wat dit vir die bedryf inhou, te bepaal. Met die uitsondering van die *F. graminearum* Gr. I isolate, was die ander 17 *Fusarium* spesies wat getoets is, in staat om dagoud Pekin eendjies te dood. Dit is 'n aanduiding dat mikotoksiene betrokke is. Toksisiteit verskille het tussen die *Fusarium* spesies, sowel as tussen isolate van die selfde spesie voorgekom. *Fusarium nygamai* blyk om die mees toksiese spesie te wees, gevolg deur *F. acuminatum* en *F. moniliforme.* Die feit dat Suid-Afrikaanse droëvrot-isolate toksies vir eendjies is, beklemtoon die noodsaaklikheid om die identiteit van die toksiene geproduseer te bepaal, veral sou die mikotoksiene in gekoloniseerde aartappels geproduseer word.

Die tesis het tot 'n beter begrip van, en die ontwikkeling van 'n itegreerde beheerprogram teen Fusarium-droëvrot van aartappels in Suid-Afrika bygedra.