Etiology and integrated control of common scab on seed potatoes in South Africa

BY

REINETTE GOUWS

A dissertation submitted in partial fulfilment of the requirements for the degree of
MAGISTER SCIENTIAE: PLANT PATHOLOGY

IN THE FACULTY OF NATURAL AND AGRICULTURAL SCIENCES,
DEPARTMENT MICROBIOLOGY AND PLANT PATHOLOGY

UNIVERSITY OF PRETORIA, PRETORIA, SOUTH AFRICA

April 2006

I hereby declare that this dissertation submitted to the University of Pretoria for the degree of MSc (Agric) has not previously been submitted by me in respect of a degree at any other University.

Reinette Gouws
April 2006

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ACKNOWLEDGEMENTS

To my heavenly father for providing me with the wisdom and strength to fulfil my dream.

To my loving parents, in-laws and friends for all their support and encouragement.

To my promoter, Prof. F.C. Wehner, for his immeasurable guidance and expertise.

To my mentor, Albert de Klerk, your passion for this research and your technical guidance was the start of my relationship with common scab, thank you!

To my dear friend and colleague, Adele Macleod for her support and valuable inputs.

To my colleagues at ARC for their constant encouragement and friendship.

To my assistants, Kate Phetla and William Monareng, for all their hard work on the project.

To Potatoes South Africa for financial support.

To the Agriculture Research Council for the opportunity to further my studies.

To R. Loria (Cornell University Ithaca, New York, USA) for kindly providing strains, R. R. King (Potato Research Centre, Agriculture and Agrifood, Frederickton, Canada) for providing thaxtomin A standards and all the potato producers and agrochemical agents who assisted in supplying the samples.

And last but not least, to my dearest husband and son for all their love, support, prayers and patience, I would not have been able to do it without you!

ETIOLOGY AND INTEGRATED CONTROL OF COMMON SCAB ON SEED POTATOES IN SOUTH AFRICA

by

REINETTE GOUWS

SUPERVISOR:	F. C. Wehner
DEPARTMENT:	Microbiology and Plant Pathology
DEGREE:	MSc (Agric)

RESUMÉ

Common scab, as the name implies, is one of the most common and widespread diseases affecting production of potato in virtually all parts of the world where the crop is grown. In South Africa, the percentage of bags containing scab-infected seed tubers averages 32 %, with a corresponding rejection or decertification of the seed. The disease also reduces the cosmetic value of ware potatoes and, with the growing demand for blemish-free produce, increasingly results in the downgrading of consignments on the ware market. Control is mostly attempted by means of agrochemicals. *Streptomyces scabiei* is generally considered as the main cause of common scab, also in South Africa. However, various other *Streptomyces* species are known to be associated with the disease, either parasitically or saprophytically, but no attempt has yet been made to determine if any of them occur in South Africa. The purpose of this study was to elucidate the etiology of common scab in the country and to evaluate some strategies that could be included in an integrated control programme for managing the disease. The results indicated the following:

Isolation and characterisation of streptomycetes from symptomatic seed potato tubers from the five potato-production areas in South Africa worst affected by common scab, showed that the disease is caused by three phenotypically distinct *Streptomyces* groups, designated 1, 2 and 3, whereas a further three groups were

saprophytically associated with scabby tubers. *Streptomyces* group 1, which corresponded morphologically to the description of *S. scabiei*, was by far the most common, representing 52 % of all and 82 % of the pathogenic isolates, and occurred in all the regions surveyed. The two other *Streptomyces* groups containing pathogenic isolates may represent atypical *S. scabiei* isolates, or could be separate species. Analysis for the production of thaxtomin A, an important pathogenicity factor, confirmed a positive correlation between the pathogenicity of isolates and their ability to produce thaxtomin A. However, 14 % of the pathogenic isolates did not produce thaxtomin A, whereas 6 % of the non-pathogenic isolates tested positively for production of the toxin. Another interesting observation was the ability of 15 % of both all and of the pathogenic isolates to grow at a pH of 4.0, which is atypical of *S. scabiei*.

In the greenhouse, densities of *S. scabiei* were significantly lower in sand artificially infested with the pathogen and planted to triticale or cabbage than in fallow sand or sand planted to soybean, spinach, maize, pumpkin or sunflower, three months after planting of the crops. Survival in sand planted to rye and pea was also relatively low, albeit not significantly lower than the other crops. Roots of soybean, spinach, rye, sunflower, pea and cabbage were colonised significantly less by *S. scabiei* than those of pumpkin and maize. Cluster analysis separated the crops into two groups according to their effect on *S. scabiei*. The first group, comprising pumpkin, maize, soybean, sunflower and spinach, had little impact on survival of the pathogen. The second group, consisting of rye, triticale, pea, and cabbage, showed a distinct suppressive effect and on average supported only 0.23 % of the *S. scabiei* population sustained by the first group.

Incorporation of fresh and dry cabbage residues at rates of 0.25 and 0.1 % (m/v) into soil naturally infested with *S. scabiei* reduced common scab in the greenhouse by a significant 32 and 41 %, respectively. Amendment of scab-infested soil in the field with dry residues of cabbage, cauliflower, broccoli and Brussels sprouts at 0.33 % (m/v) resulted in reduction in disease of approximately 90 %.

CHAPTER 1

GENERAL INTRODUCTION

Potato (*Solanum tuberosum* L.) is the world's fourth most important food crop after wheat (*Triticum aestivum* L.), maize (*Zea mays* L.) and rice (*Oryza sativa* L.), and provides a balanced source of starch, vitamins and minerals to many communities in the global village (Rowe, 1993). With an annual yield of about 1.7 million tonnes and a gross value of R2 billion (approximately $320 million), potatoes rank third as staple food in South Africa after maize (R8.4 billion) and wheat (R2.7 billion) (http://www.nda.agric.za). Although this yield represents less than 1 % of the world's potato production, it is the highest in Africa (http://www.potatoes.co.za). The total area presently under potato production in South Africa is approximately 51 000 ha, of which 40 500 ha is under irrigation. Yield is on average 34.7 tonnes ha^{-1} under irrigation and 19.8 tonnes ha^{-1} under dry land cultivation. Sixteen per cent of the entire area under potatoes is devoted to the production of seed, which constitutes 12.9 % of the total yield. Of the ware crop, 16.1 % is sold directly, 47.5 % is supplied to local fresh produce markets, 6.6 % is exported and 16.9 % is processed. Processed commodities include dried chips (37 %), frozen chips (44 %), fresh chips (17 %), canned potatoes (1 %) and mixed vegetables (1 %) (http://www.nda.agric.za). Calculated from the above, per capita consumption of potatoes in the country averages about 31 kg per annum.

Of all primary food crops, potatoes suffer the greatest losses due to disease (Agrios, 1997). Potato diseases occurring in South Africa involve 30 fungal, 10 viral and three bacterial pathogens (Doidge, 1950; Doidge *et al*., 1953; Gorter, 1977; Denner *et al*., 1993; Theron, 1999; Crous *et al*., 2000; Millard, 2003), whereas seven insect and four nematode species are regarded as serious pests of the crop (Visser, 2005). Of the various diseases, common scab is one of the oldest. It was first recorded in South Africa by Pole Evans (1906), and has subsequently been described as common and widespread by Doidge & Bottomley (1931), Doidge *et al.* (1953) and Gorter (1977).

The etiology, epidemiology and control of common scab have been investigated extensively in many countries and the disease has been the topic of various reports in South Africa during the first half of the previous century (Pole Evans, 1908; Doidge, 1918; Dippenaar, 1931, 1933a,b, 1935, 1943; Wager, 1931; Bottomley, 1941). Since then, however, common scab has for many years been regarded as of little economic significance in the country. More recently, Anonymous (1975) referred to the existence of superficial, raised and deep-pitted lesions on common scab-infected tubers in South Africa, whereas Anonymous (1975) and Frean (1975) indicated that common scab occurs locally in soils with a pH as low as 4.25. Slabbert *et al.* (1994) reported that 45 % of 103 isolates of *Streptomyces scabiei* from four of the 14 potato production areas in South Africa produced thaxtomin A, a phytotoxin commonly associated with pathogenicity of the common scab pathogen (King *et al.*, 1991).

Currently, common scab is one of the more important factors limiting successful production of potatoes in the country (Nortje *et al.*, 2000). Between 1996 and 2004, the percentage of bags containing scab-infected seed tubers averaged 32 %, with a corresponding rejection or decertification of the seed. The disease also reduces the cosmetic value of ware potatoes and, with the growing consumer-demand for blemish-free produce, increasingly results in the downgrading of consignments on the ware market. Research focuses primarily on the screening of genotypes for resistance (Marais & Vorster, 1988; Marais & Visser, 1989; Nortje *et al.*, 2000) and recommendations accordingly. A seed certification scheme, which allows between 0 and 8 % infected tubers, depending on the generation and class, is also in place (Republic of South Africa, 1998). The only chemical that is presently registered for the control of soilborne inoculum of the pathogen is quintozene, whereas flusulfamide and mancozeb are registered as tuber treatments (Nel *et al.*, 2003). Frean (1975) recommended rotation with wheat, rye (*Secale cereale* L.), oats (*Avena sativa* L.), soybean (*Glycine max* (L.) Merr.) and lucerne (*Medicago sativa* L.) for the reduction of common scab. Various aspects relating to common scab in South Africa nevertheless remain unclear. For instance, although *S. scabiei* is the main causal organism of the disease worldwide (Loria, 1994), and has been shown to be prevalent in four of the 14 production areas in South Africa (Slabbert *et al*., 1994), it is not known if any of the other *Streptomyces* species associated with the disease (Archuleta & Easton, 1981; Lambert & Loria, 1989; Faucher *et al*., 1992; Goyer *et al*., 1996; Miyajima *et al*., 1998; Bouchek-Mechiche *et al*., 2000; Park *et al.,* 2003) are present. Also, the rotation crops recommended by Frean (1975) for the control of common scab are not necessarily economically and agronomically feasible in rotation systems with potato, or suitable for the management of other pathogens and pests. Lastly, although the chemicals registered against common scab can be effective, the cost of particularly soil applications is extremely high and control not always consistent (De Klerk & Engelbrecht, 1996). Growing consumer-resistance to, and the negative environmental impact of, synthetic pesticides furthermore necessitate the consideration of alternative control strategies.

The purpose of this study therefore was to (i) elucidate the identity of the common scab pathogen in selected potato production areas, (ii) identify rotation crops compatible with potato growing, and (iii) investigate the potential of biofumigation, i.e. rotation with glucosinolate-containing brassicaceous crops or incorporation of their residues into soil (Brown & Morra, 1997), as an affordable and environmentally-acceptable alternative to agrochemicals for the control of common scab.

The report comprises four chapters, besides the general introduction (**Chapter 1**). **Chapter 2** provides an overview of the etiology, ecology, epidemiology, parasitology and control of the disease. **Chapter 3** relates a survey of common scab pathogens associated with seed potatoes in South Africa. The effect of selected rotation crops on the survival of *S. scabiei* is reported in **Chapter 4**, whereas **Chapter 5** describes the control of common scab by means of biofumigation.

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CHAPTER 2

LITERATURE REVIEW

2.1. Causal organism

Scab diseases of potato (*Solanum tuberosum* L.) and certain other root crops are caused by streptomycetes. *Streptomyces* spp. are aerobic, filamentous, Gram-positive prokaryotes belonging to the order *Actinomycetales*, suborder *Streptomycineae*, family *Streptomycetaceae* and genus *Streptomyces.* According to Kutzner (1981), the family *Streptomycetaceae* is characterised by the following:

1. Extensively branched, unfragmented, vegetative mycelium, which gives rise to aerial hyphae that each terminate in the formation of chains of 5–50 conidia or "arthrospores" with a thin fibrous sheath.
2. Cell walls that contain L,L-diaminopimelic acid with glycine in interpeptide bridges.
3. G + C content ranging from 69 to 73 mol %.

Most described *Streptomyces* species are soil saprophytes. However, four species have developed the means to incite disease on subterranean parts of economically important tuber crops. These species, *Streptomyces scabiei* (synonym *S. scabies*), *Streptomyces acidiscabies, Streptomyces turgidiscabies* and *Streptomyces ipomea* are unrelated based on several criteria, including 16S ribosomal DNA sequence, DNA-DNA relatedness, morphological and biochemical attributes (Lambert & Loria, 1989a,b; Healy & Lambert, 1991; Takeuchi *et al.*, 1996; Miyajima *et al.*, 1998). The best studied of the plant-pathogenic species is *S. scabiei* which is the main cause of common scab on potato tubers. *S. acidiscabies* also causes disease on potato tubers (acid scab), but is limited to the Northeastern United States (Bonde & McIntyre, 1968; Lambert & Loria, 1989b), Canada (Faucher *et al.*, 1992), Hokkaido in Japan (Takeuchi *et al.*, 1996) and Korea (Kim *et al.*, 1998b). The disease symptoms caused by the above two pathogens are indistinguishable and both species can also infect taproots of radish (*Raphanus sativus* L.), turnip (*Brassica rapa* L.) and other root crops (Loria *et al.*, 1997). *S. turgidiscabies* was first reported in 1996 (Takeuchi *et al.*, 1996; Miyajima *et al.*, 1998) and is the third most important *Streptomyces* species that causes disease on potato. However, its distribution is limited to Japan, Finland, Sweden and Korea (Miyajima *et al.*, 1998; Kim *et al.*, 1998a; Kreuze *et al.*, 1999; Lehtonen *et al.*, 2004). *S. ipomea* is the main causal agent of sweet potato (*Ipomoea batatas* L.) soil rot, also referred to as pox, resulting from the black spots which occur on the feeder roots and underground portions of the stem. The disease causes a reduction in yield and a decrease in root quality, which can be more severe during dry weather and in poor soils (Loria *et al.*, 1997).

There is evidence that several other *Streptomyces* species may be able to cause disease on potato, e.g. *S. caviscabies* in Quebec, Canada (Goyer *et al.*, 1996), *S. aureofaciens, S. griseus, S. europaeiscabiei, S. reticuliscabiei* and *S. stelliscabiei* in France (Corbaz, 1964; Bouchek-Mechiche *et al.*, 2000), *S. cinereus, S. collinus* and *S. longisporoflavus* in India (Dey & Singh, 1983), *S. exofoliatus, S. rochei* and *S. violaceus* in Israel (Doering-Saad *et al.*, 1992), *S. luridiscabiei, S. niveiscabiei* and *S. puniciscabiei* in Korea (Park *et al.*, 2003), *S. flaveolus* in Northern USA (Millard & Burr, 1926), and *S. atrolivaceus, S. cinerochromogenes, S. corchorussi, S. diastatochromogenes, S. lydicus* and *S. resistomycificus* in Washington State, USA (Archuleta & Easton, 1981). The validity of some of the above species is uncertain since the early descriptions used for the identification of *Streptomyces* spp. were based on a very small number of characteristics.

S. scabiei was first described as *Oospora scabies* (Thaxter, 1891), a melanin-producing actinomycete bearing grey spores in spiral chains. Güssow (1914) renamed the species *Actinomyces scabies,* before it was given the name *Streptomyces scabies* by Waksman &

Henrici (1948). Unfortunately, a type culture of the original isolate was not maintained. When Waksman (1961) redescribed the species he designated a different isolate (IMRU 3018 = ISP 5078) as neotype (Elesaway & Szabo, 1979; Kutzner, 1981). Selection of this isolate apparently was based only on pathogenicity (Lambert & Loria 1989a). Elesaway & Szabo (1979) proposed a neotype culture (ATCC 33282) that fitted the original description of Thaxter, but the species was not included in the 1980 Approved List of Bacterial Names (Skerman *et al.*, 1980). Subsequently, Lambert & Loria (1989a) redescribed the common scab pathogen as *Streptomyces scabies* (Thaxter) Lambert & Loria (ATCC 49173, DSM41658, RL-34). This isolate conformed to Thaxter's description and its acceptance as type strain alleviated the confusion surrounding the identity and taxonomic validity of the organism. The nomenclature was amended once again by Trüper & De Clari (1997), who changed the epithet from the substantive noun (*scabies*) to its genitive form (*scabiei*).

2.2. Geographic distribution of common scab

Common scab of potato is one of the oldest known plant diseases and was first described in 1890 (Thaxter, 1891). Current information suggests that common scab is almost as widely distributed as the host itself (Loria *et al.*, 1997). It is present in all the potato-growing areas of North America and Europe (Keinath & Loria 1989a). Tashiro *et al.* (1983) reported the incidence of the disease in the Far East, whereas Mohanty *et al.* (1980) refer to a serious common scab problem in the coastal tracts of the Cuttack and Puri districts in India. A report by Pung & Cross (2000) confirmed the presence of the disease in Victoria, Australia, and particularly the island of Tasmania. It was previously reported in South Australia by Dillard *et al.* (1988). Common scab also occurs in Austria (Wenzl & Reichard, 1974), Denmark, (Oestergaard & Nielsen, 1979), France (Bouchek-Mechiche *et al.*, 2000), Finland (Heinamies & Seppanen, 1971), Germany (Koronowski & Massfeller, 1972), Greece (Alivizatos & Pantazis, 1992), Israel (Doering-Saad *et al.*, 1992), Hungary (Elesawy & Szabo, 1979), Ireland (Dowley, 1972), the Netherlands (Janse, 1988), Sweden

(Emilsson & Gustafsson, 1953), Norway (Bjor & Roer, 1980), Poland (Sadowski *et al.*, 1996), Arabia (Ali, 1987), Korea (Park *et al.*, 2003), Japan (Miyajima *et al.*, 1998) and the United Kingdom (Large & Honey, 1953; Read *et al.*, 1995). The disease is present in all potato production regions of South Africa, albeit at varying levels (Marais & Visser, 1989).

2.3. Host range

Common scab pathogens not only cause disease in potato, but can attack the fleshy roots of radish, turnip, beet (*Beta vulgaris* L.), groundnut (*Arachis hypogaea* L.), carrot (*Daucus carota* L.), rutabaga (*Brassica napus* L. var. *napobrassica* (L.) Rchb.), parsnip (*Pastinaca sativa* L.), mangel (*Beta macrorrhiza* Steven) and salsify (*Tragopogon porrifolius* L.) (Lutman & Johnson, 1915; Jones, 1953; Crete, 1980; Goth & Webb, 1986; Hooker, 1986; Sherf & MacNab, 1986; De Klerk *et al.*, 1997; Goyer & Beaulieu, 1997; Bouchek-Mechiche *et al.*, 2000). All these crops develop typical scab symptoms, including blisters, raised lesions and russet scab, and can therefore serve as reservoir of inoculum in the absence of potato. Various other monocotyledonous and dicotyledonous crops have also been reported to be negatively affected when artificially infected with *S. scabiei* (Hooker & Kent, 1946; Hooker 1949; Leiner *et al.*, 1996), probably due to toxic effects induced by thaxtomin A, a broad-spectrum phytotoxin produced by the scab pathogen (Leiner *et al.*, 1996). In South Africa, *S. scabiei* has been reported on potato, beet (Doidge *et al.*, 1953) and groundnut (De Klerk *et al.*, 1997). It is not a major pathogen on groundnut, but can be of economic significance where potatoes and groundnuts are grown in rotation in the same field.

2.4. Epidemiology and parasitology

2.4.1. Pathogenicity

There has been much speculation about the mechanisms of pathogenicity of common scab, particularly regarding exo-enzyme and toxin production (Lawrence *et al.* 1990; Healy, 1991). Extensive research in the late 1980's revealed the presence of esterase activity in culture extracts of some pathogenic strains of *S. scabiei* (McQueen & Schottel, 1986, 1987; Schottel *et al.*, 1989). It is believed that these esterases might assist the pathogen in degrading the protective waxes (suberin and cutin) covering the host periderm and thereby provide a point of entry (Raymer *et al.*, 1990). McQueen & Schottel (1987) also found that supplementing suberin with zinc resulted in optimal induction of esterase expression.

Although common scab-causing *Streptomyces* species are diverse based on physiological, morphological, DNA-DNA hybridisation and ribosomal analyses, similarities in host range and disease symptomology suggest a common mechanism of pathogenicity. All these species share the production of one or more of a family of phytotoxins referred to as thaxtomins, which can mimic disease symptomatology on host plants. According to Loria *et al.* (1997) speculation regarding the involvement of these phytotoxins dates back to 1926, although King *et al.* (1989) only succeeded in isolating and characterising the compounds 63 years later. Evidence of toxin production by pathogenic *Streptomyces* spp. was also reported by Lawrence *et al.* (1990) and the compounds identified as thaxtomin A and B. Thaxtomin A is the predominant phytotoxin produced by both *S. scabiei* and *S. acidiscabies* in potato tubers, although small amounts of ten other related compounds have also been isolated (King *et al.*, 1992; Loria *et al.*, 1997). Thaxtomin A has been shown to induce cell hypertrophy in proliferating plant tissue through the inhibition of cellulose biosynthesis (Fry & Loria, 2002; Scheible *et al.*, 2003). Therefore, the present hypothesis is that *Streptomyces* spp. penetrate into regions of rapidly growing potato tissue through the production of thaxtomin A that inhibits cell-wall development (Loria *et al.*, 2003).

Since the production of thaxtomin A is common to unrelated species, it was suggested that pathogenicity factors were transferred among *S. scabiei, S. acidiscabies* and *S. turgidiscabies* (Healy & Lambert, 1991; Bukhalid & Loria, 1997; Bukhalid *et al.*, 1998). In a study to determine the relationship between phytotoxin production and aerial mycelium formation by a scab-forming *Streptomyces* sp., IFO 13767, disease was induced but no evidence of thaxtomin A production could be found (Natsume *et al*., 1996). In a subsequent attempt to elucidate other pathogenicity determinants in *S. scabiei*, a putative virulence gene, *nec1*, was identified. It was sufficient to confer a necrogenic phenotype to the nonpathogen, *S. lividans* 66 Tk24 (Bukhalid & Loria, 1997). Shortly afterwards, Bukhalid *et al.* (1998) reported a strong correlation between thaxtomin production and the presence of *nec1* in *S. scabiei, S. acidiscabies* and *S. turgidiscabies*, and showed that the ORFtnp-*nec1* region was identical among strains of these pathogens. The genes for thaxtomin production as well as the *nec1* gene are located on a very large pathogenicity island (PAI) in *S. scabiei, S. acidiscabies* and *S. turgidiscabies* (Bukhalid *et al*., 1998, 2002; Healy *et al*., 2000; Kers *et al*., 2005). Current evidence strongly suggests that the PAI has been mobilised from *S. scabiei* to the two newly-emerged potato scab pathogens (Kers *et al*., 2005).

2.4.2 Mode of entry

To understand the mechanisms by which the scab pathogen causes infection, one should first consider the physiology of its host. The potato tuber is an adapted stem consisting of nodes and internodes (Adams & Lapwood, 1978). When tuber initiation takes place, the nodes are separated by the enlarging internodes. Lapwood & Adams (1975) suggested that internodes forming successively at the apex of a growing tuber go through a susceptible period when the stomata they bear, develop into young lenticels. The youngest internodes (referred to as A-1 and A-2) bear stomata that are resistant to common scab infection (Adams & Lapwood, 1978). Young lenticels with unsuberised filling cells on internodes A-3 to A-5, on the other hand, are very susceptible, whereas the older internodes with suberised lenticels appear to be resistant (Adams, 1975). During the first few weeks of tuber development new internodes form at a rate of two per week, therefore each internode passes through a susceptible phase of about 10 days from 1-2½ weeks after tuber initiation.

The common scab pathogen is not an efficient competitor for infection sites or food, but it is an excellent opportunist and has adapted itself to the host defences in an interesting way. After penetration the pathogen progresses intercellularly, primarily between cells within the developing lenticels (Adams & Lapwood, 1978), and as the cells die off it feeds on the dead tissue (Agrios, 1997). Mycelium occurs inside the host cells only in older areas of scab lesions (Labruyére, 1971; Hooker, 1981). Whilst multiplying, the pathogen secretes a biochemical substance (probably thaxtomin) that stimulates the cells surrounding the lesions to divide rapidly and to synthesise suberin at the site of infection (Babcock *et al.*, 1993). These suberised, corky layers cut off nutrients to living cells, thereby killing them and protecting the plant against moisture loss and microbial attack, hence providing the pathogen with further dead tissue to feed on (Fig. 2.1). The corky layer then pushes the infected area outwards, the potato periderm ruptures and scab forms.

2.4.3 Symptom development and expression

The common scab pathogen can infect all underground parts of the potato plant (Labruyére, 1971). Aboveground plant parts can also exhibit symptoms after artificial infection (Hooker, 1981). Initially the lesions appear as small (5-8 mm), reddish-brown, water-soaked lesions on the tuber periderm. As the pathogen continues to colonise the tuber, the host develops wound periderm resulting in slightly raised lesions composed of rough, corky tissue. These spots may coalesce to form irregularly shaped patches that are usually tan to brown in colour and rough in texture (Rich, 1983). The patches can become cracked as infection progresses and take on a star-like appearance. The disease can also extend into the tuber resulting in shallow or deep-pitted scab (Archuleta & Easton, 1981; Ndowara *et al.*, 1996). When the lesions are excised the underlying flesh appears straw-coloured and somewhat translucent (Rich, 1983). The tuber periderm stays intact over superficial lesions or is only slightly ruptured (Jellis, 1977). In deeper lesions, the tuber periderm is ruptured and up to three wound barriers are formed.

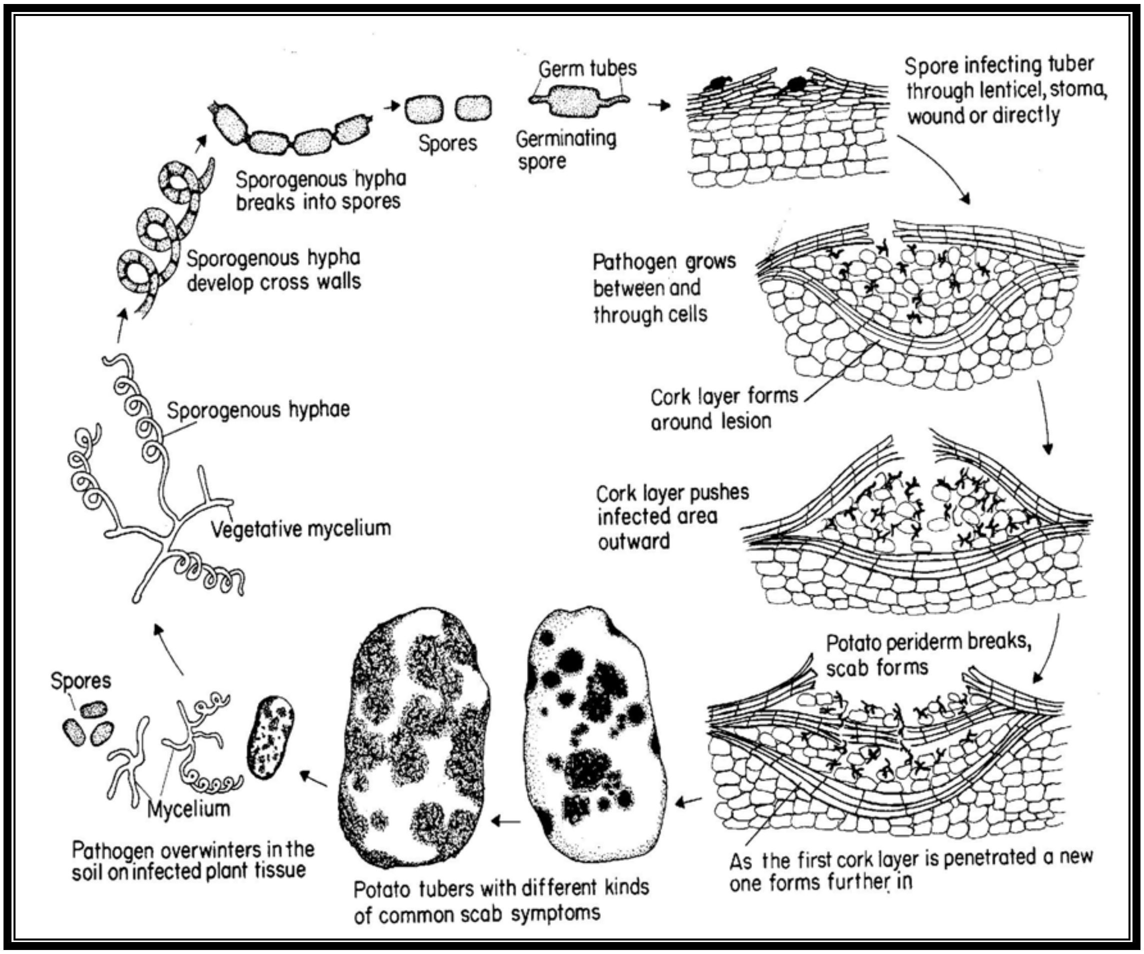
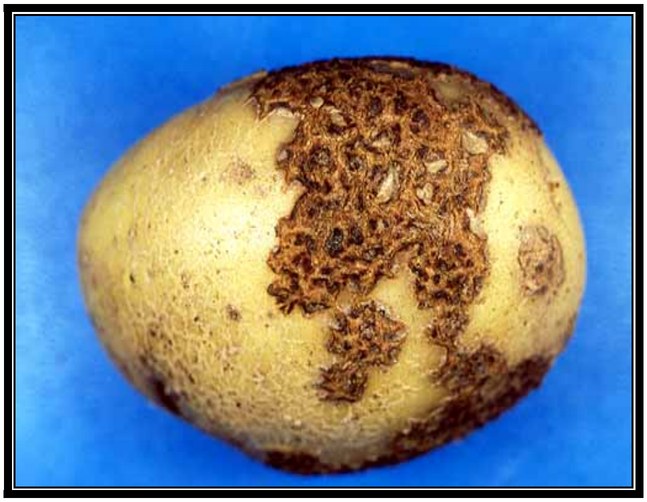


Fig. 2.1 Disease cycle of potato common scab (Agrios, 1997)

Some controversy exists regarding the classification of lesion types. In Great Britain, Millard & Burr (1926) distinguished between superficial (russet), ordinary, pitted, stud, tumulus and pimple scabs. In Sweden, Emilsson & Gustafsson (1953) described four classes, viz. superficial, ordinary, deep and elevated. Elevated lesions occurred quite frequently except in the most resistant varieties. Stevenson *et al.* (1942), in their extensive surveys in the United States, referred to only three pustule types, namely relatively large and deep, relatively large but superficial and small and superficial, which implies that elevated scab was uncommon.

Typical lesions occurring on potato tubers in South Africa are depicted in Fig. 2.2a-c. BP1, which is a very susceptible potato variety, usually exhibits deep-pitted lesions as

2a. Superficial **2b**. Deep-pitted



2c. Russet/netted scab

Fig. 2.2 Lesion types of common scab on BP1 potato tubers in South Africa (photographs by A. D. K. de Klerk)

well as superficial lesions that can cover the whole tuber, whereas Mondial, a more tolerant variety, only displays superficial lesions covering a small percentage of the tuber skin (A. F. Visser - personal communication).

Healy (1991) categorised potato scab into three types: russet, common and acid scab. Russet scab, also referred to as elephant hide, develops as a rather superficial layer of corky tissues covering large areas of the tuber surface and can be caused by weakly pathogenic strains of *S. scabiei*. Harrison (1962) considered common scab and russet scab occurring in Minnesota and North-Dakota to be separate diseases, based on infection and lesion type that occurred. 'Netted scab' was described by Scholte & Labruyere (1985) and seems to be the European version of russet scab. According to Loria *et al.* (1997), descriptions of russet and netted scab symptoms and the *Streptomyces* strains associated with them, suggest a varied etiology. The type of symptom is not consistent, but can be influenced by environmental variables, including cultivar. Observations on the expression of the various symptoms at different soil temperatures revealed that optimal soil temperature for common scab is 19-24 °C, for netted scab 13-17 °C, and for russet scab 23-27 °C (Healy, 1991). Another important difference is the influence of soil moisture. Low moisture levels aggravate common scab, whereas russet and netted scab lesions increase in severity with increasing soil moisture (Scholte & Labruyere, 1985).

Acid scab is caused by *S. acidiscabies,* the first scab pathogen capable of causing disease in soils with a pH below 5 (Lambert & Loria, 1989b). *S. acidiscabies* was isolated in Maine from potatoes with lesions similar to those of common scab (Manzer *et al.*, 1977). Physiological and morphological screening, however, showed that the causal organism was not *S. scabiei,* but a species identical to the *Streptomyces* sp. previously reported from potatoes in Maine by Bonde & McIntyre (1968). When grown on nutrient medium, *S. acidiscabies* produces flexuous spore chains, white to orange-red spore masses and a yellow or red soluble pigment (Lambert & Loria, 1989b). Unlike *S. scabiei*, it can grow on agar media with a pH of 4.0 and is incapable of utilising raffinose as source of carbon (Lambert & Loria, 1989b). *S. acidiscabies* has been isolated in Northeastern USA, Canada, Japan and Korea (Bonde & McIntyre, 1968; Lambert & Loria, 1989b; Faucher *et al.*, 1992; Takeuchi *et al.*, 1996; Kim *et al.*, 1998b). No incident of acid scab has been reported in South Africa to date, although common scab is known to occur locally in soils with a pH of 4.25 (Anonymous, 1975; Frean, 1975).

2.4.4 Vectors

Hopkins (1895) reported that the scab gnat (*Pnyxia scabiei* Hopkins) assists in the formation of deep-pitted lesions by burrowing into the tuber tissue, thus carrying scab inoculum deeper into the host. Schaal (1934) observed that feeding wounds of potato flea beetle (*Epitrix cucumeris* Harris) larvae provided entry for the scab pathogen. Larvae carried the pathogen internally and externally and could be involved in disseminating the inoculum from soil to tuber. Tamaki *et al.* (1976) found oligochaetes, mites and nematodes in scab lesions on potato tubers and Storch *et al.* (1978) isolated *Streptomyces* spp. from *Rhizoglyphus* mites and Collembola springtails (*Onychiurus subtenius* Folsom, *Folsomia fimetaria* L. and *Folsomia elongata* MacGillivray). Manzer *et al.* (1984) proposed that an understanding of the role of soil arthropods in the epidemiology of common and acid scab could provide a solution to the disease. No similar studies have been conducted in South Africa.

2.4.5 Longevity in soil

It is well-known that common scab can be present in “virgin” soils. Jones & Edson (1901) were of the first to document this phenomenon. Lutman (1914) later indicated that the normal microflora of practically all soils includes scab-producing *Streptomyces* species. The pathogen survives as spores or mycelium within crop debris. It can remain viable in soil from a decade (Kritzman *et al.*, 1996) to up to twenty years without any potato cultivation (Dippenaar, 1933). Common scab inoculum can be introduced into the soil by means of infected tubers and farming practices. Infested soil can spread by wind and rain to adjacent “clean” fields. The organism also survives passage through the digestive tract of animals and can be disseminated by manure (Rowe, 1993). Since *S. scabiei* is a monocyclic pathogen, i.e. completes only one disease cycle per growing season, initial inoculum density may play an important role in development of the disease (Keinath & Loria, 1991). Spores of *Streptomyces* species can survive in dry soil for long periods but the vegetative hyphae are intolerant of high moisture tensions (Mayfield *et al.,* 1972). The spores differ from hyphae in having a) an outer sheath, b) a thicker wall, c) greater resistance to heat, and d) resistance to drought. *Streptomyces* spores are not evenly distributed in the soil and occur in small, localised clusters (Mayfield *et al.,* 1972). These clusters are usually associated with debris derived from previous or existing crops. Germination of spores is enhanced by the close proximity of organic particles. Hyphae produced by the spores develop radially and it is thought that spread is facilitated in this way. *S. scabiei* tends to predominate in dry, neutral to alkaline soils (Hooker, 1986; Loria *et al*., 1986).

2.5 Control

Control of common scab has been attempted in various ways, e.g. by a) chemicals (Terman *et al*., 1948; Davis *et al*., 1974; Singh & Soni, 1987), b) irrigation (Lapwood *et al*. 1970, 1971, 1973), c) varietal resistance (Bjor & Roer, 1980; Marais & Vorster, 1988; Pemberton, 1994), d) pH and plant nutrients (McCreary, 1967; Williams *et al*., 1971), e) crop rotation and green manuring (Wood & Tveit, 1955; Pemberton, 1994), and f) biological control (Wood & Tveit, 1955; Anderson & Lorang, 1988; Hayashido *et al*., 1988; Gouws & Wehner, 2004). However, to achieve control of soilborne scab under all conditions of potato cultivation is not straightforward due to the complexity of factors affecting the development of the disease, viz. a) presence and density of the causal organism in the soil, b) number of potato crops produced in a field, c) potato variety grown, and d) environmental factors such as soil reaction, soil moisture and soil temperature (Dippenaar, 1933).

2.5.1 Chemical control

Common scab-causing *Streptomyces* species are usually introduced into potato fields by means of infected tubers (Dippenaar, 1933). Cases have also been reported where scab-causing *S. scabiei* was present in soils where no potato crop had previously been grown. Chemical control is thus directed at controlling the pathogen on seed tubers to limit soil infestation, as well as at reducing inoculum in previously infested soil.

2.5.1.1 Tuber treatment

Relatively little research has been conducted on the effect of tuber treatments on common scab. Kulikova (1982), Singh & Soni (1987), Somani (1988) and Fulton (1994) reported the use of copper sulphate, formaldehyde, mercuric chloride, borax, boric acid, tetracycline, plantomycin and quintozene. Fluazinam, flusulfamide, fludioxonil and mancozeb have provided control in Australia (Wilson *et al.*, 1999; Pung & Cross, 2000). Inconsistent results achieved with seed treatment have put a question mark on the efficacy of this approach.

The only seed treatment that has showed promise for the control of common scab in South Africa is flusulfamide dipping before planting (De Klerk & Engelbrecht, 1996). This chemical reduces the risk of common scab infection spreading from the mother tuber to the progeny and virgin soil. It is recommended that seed tubers showing signs of common scab, are of uncertain origin, or are planted to virgin soils, be treated before planting (A. D. K. de Klerk - personal communication). Unfortunately, treatment with flusulfamide has no effect on soilborne inoculum of *S. scabiei* and increases the risk of soft rot caused by *Erwinia carotovora* subsp. *carotovora* resulting from the dipping of tubers during treatment.

2.5.1.2 Soil treatment

The most widely used chemical method of controlling potato common scab is soil treatment with quintozene (PCNB) before planting (Erickson, 1960; Vashisth *et al.*, 1990). However, quintozene is persistent, could be carcinogenic, and may decrease yield or impart off-flavours to tubers (Labruyére, 1971). McIntosh (1977) found captafol as effective as quintozene with no reduction in potato yield, whereas Davis *et* *al.* (1974) investigated the effects of gypsum and sulphur for control of common scab, and concluded that both compounds reduce potato scab with only a slight change in soil pH.

Until 1993, quintozene and dichlorophen were the only soil treatments registered for the control of soilborne common scab inoculum in South Africa. Both provided inconsistant control, especially under high disease pressure conditions (De Klerk & Engelbrecht, 1996). Since 1993, fumigation with chloropicrin/ethylene dibromide has been applied successfully in South Africa, but is not cost-effective because of the quantities required. The fumigant also exhibits very slight mobility in soil and it is therefore important that the soil be properly prepared before fumigation (Erickson, 1960). Fumigation is not recommended for soils with a clay content higher than 20 %.

2.5.1.3 Foliar applications

McIntosh & Burrell (1980) reported that foliar sprays with ethionine (a protein synthesis-inhibiting amino acid) decreased the incidence of common scab caused by soilborne *S. scabiei* in greenhouse and field trials. Ethionine is a basipetally phloem-moving systemic compound. It has also been reported that the severity of the disease could be decreased by foliar spraying with the plant growth retardant, daminozide (N-dimethylamino-succinamic acid) (McIntosh, 1979; McIntosh & Bateman, 1979). Daminozide does not affect the pathogen directly but shortens the length of the internodes of the potato plant, thus decreasing the availability of *S. scabiei* infection sites (McIntosh, 1979). The anti-scab action thus seems more likely to lie in the suppression of symptom expression rather than prevention of primary infection (McIntosh & Bateman, 1979). Anti-scab action was also reported for 2,5-disubstituted benzoic acids (McIntosh *et al.*, 1988) and 3,5-dichlorophenoxyacetic acid (McIntosh *et al.* 1981, 1982). None of the above chemicals is registered for the control of common scab in South Africa (Nel *et al.*, 2003).

2.5.2 Irrigation

The incidence of common scab is determined by the level of inoculum present in the soil and on the surface of tubers, as well as factors such as soil moisture and pH (Booth, 1970). Lapwood & Lewis (1967) observed a close association between the incidence of common scab and low soil moisture during the early stages of tuber formation and applied this knowledge to control common scab by appropriate irrigation. In the United Kingdom and Europe, common scab is controlled largely by specified irrigation schedules (Lapwood, 1966; Lapwood *et al.*, 1970, 1971, 1973; Wellings & Lapwood 1971; Davis *et al.*, 1976; Adams *et al.*, 1987). The reason for this lies in the fact that actinomycetes grow best in pore spaces in the soil that are humid and air-filled (Williams *et al.,* 1972). Growth is retarded when pores become waterlogged. Waterlogging also increases available manganese, which can suppress the development of common scab (McGregor & Wilson, 1966). High soil moisture and low soil temperature after tuber initiation reduce the disease. Tubers become infected while still very young and if they can survive this period without infection they escape the disease (Lapwood & Hering, 1970). In commercial fields where tubers differ in age the susceptible period for the field as a whole lasts for about four weeks.

Various theories have been proposed to explain the mechanism of scab control by irrigation (Lapwood & Adams, 1975; Adams & Lapwood, 1978). Firstly, there is a direct influence on tuber susceptibility, e.g. lenticel proliferation. Soil moisture is necessary for the development of lenticels from stomata. With adequate soil moisture, susceptible stomata rapidly develop into resistant lenticels and are therefore protected from infection (Labruyère, 1971; Adams, 1975). Secondly, there are direct effects on the growth of the pathogen due to lowering of the soil temperature and reduced oxygen availability. Thirdly, irrigation indirectly affects disease by providing an environment conducive to antagonism (Lewis, 1971). Antagonistic bacteria move faster than scab-causing *Streptomyces* species in the water films in wet soil. The antagonistic bacteria colonise the lenticels first and compete with the pathogen for the niche. Fourthly, high moisture levels have been associated with a decrease in calcium levels in tuber tissue and it has been implied that increased calcium leads to increased scab susceptibility (Horsfall *et al.*, 1954; Davis *et al.*, 1976).

To control common scab effectively, irrigation must commence at tuber initiation (Lapwood *et al.*, 1971). It is important to know exactly when this process occurs with each planting. Tuber initiation of spring plantings starts as early as 7-10 days after emergence and in autumn plantings within 10-14 days after emergence. It is critical that the soil should remain moist for the first 4-10 weeks after the onset of tuber initiation (Lapwood & Hering, 1970). The specific cultivar and environmental conditions determine the critical period for infection. In South Africa the method of controlling common scab by means of irrigation is not as effective as in European countries. Most commercial potato cultivars in South Africa were selected for their high yield potential and therefore have a long tuber initiation period (A. F. Visser - personal communication) that complicates irrigation scheduling as control measure. Climatic conditions, particularly temperature, also influence the initiation period. Tuber initiation starts later and proceeds for longer when the temperature is high (Lapwood, 1972).

2.5.3 Varietal resistance

Chlorogenic acid in the periderm of potato tubers plays an important role in scab resistance and in the general protective mechanism of the plant (Johnson & Schaal, 1952). The mechanism is complex and depends on the amount of chlorogenic acid in the periderm and the presence of tyrosinase in the tuber tissue. The total concentration of chlorogenic acid is not as important as its localisation around the lenticels. The acid tends to accumulate in cells adjacent to injured areas, but it is not clear how the rate of accumulation of the acid is related to scab resistance (Johnson & Schaal, 1952). Tyrosinase is found at high concentrations in the same tissue as chlorogenic acid and oxidises the acid when the tissue is damaged. The oxidation products formed, such as quinones, may be toxic to the invading organism. This appears to be the main mechanism involved in scab resistance (Schaal & Johnson, 1955).

Resistance to common scab is also determined by the morphology of the lenticels (McKee, 1958, 1963). Lenticels of scab-resistant varieties are relatively small, set either deep in the skin of the tuber, or are covered by vestiges of the imperfectly ruptured cork rind, and usually have a dense accumulation of parenchyma. The lenticels of susceptible varieties, on the other hand, are not as well protected, because they are larger, contain more loosely massed parenchyma cells and never have protective layers of cork (Lutman, 1914). The tubers of resistant varieties transpire less than those of susceptible ones under the same conditions. Most of the potato cultivars planted commercially in South Africa are susceptible to common scab, although varieties such as Mondial can be regarded as tolerant (De Klerk & Marais, 1993). Breeding of resistant cultivars is therefore all the more important (Marais & Vorster, 1988).

2.5.4 pH and plant nutrients

Soil pH is the most reliable parameter for predicting common scab. The first investigations in this regard were conducted by Gillespie & Hurst (1918). In accordance with the *in vitro* growth response of *S. scabiei* to pH, disease development increases with soil pH from 5.0 to 8.0 (Goto, 1985). Maintaining the pH of soil at 5.0-5.2 can therefore significantly reduce common scab (Rich, 1983; Loria, 1991), but would obviously be ineffective against acid scab. The lower pH could also aggravate diseases caused by fungal pathogens (Alexander, 1961), and suppress beneficial bacteria (Williams *et al.*, 1971). It could furthermore result in reduced availability of nitrogen, calcium, magnesium, phosphorus, potassium, boron and maybe even sulphur, but increased availability of iron, manganese, zinc, aluminium, copper and cobalt (Brady, 1974). Although greater availability of manganese may have a restraining effect on common scab, excess quantities of manganese, iron and aluminium in low-pH soils could be phytotoxic. Lastly, some crops that are routinely rotated with potato, e.g. sweet clover (*Melilotus officinalis* (L.) Pallas), may not tolerate the low pH (Brady, 1974).

The management of common scab with plant nutrients has been extensively reviewed by Keinath & Loria (1989b). Mineral elements that have been implicated in the disease include calcium, nitrogen, phosphorous, sulphur, copper and manganese. Calcium, when added to the soil as lime (calcium carbonate), increases the pH and hence renders the soil more conducive to common scab (Dippenaar, 1933; Blodgett & Cowen, 1935; Odland & Arbritton, 1950; Goto, 1985; Lambert & Manzer, 1991). Increased severity of the disease has also been associated with high levels of calcium in the tubers (Davis *et al.*, 1976). Excess calcium therefore has to be avoided for the prevention of common scab. However, care should be taken not to deprive the crop of calcium as calcium deficiencies can cause physiological disorders in potato, e.g. wateriness, brown spot and skin cracks (Westermann, 1993). If calcium is required in a low-pH soil cultivated to potato, it is preferred to add gypsum (calcium sulphate) as it will not increase the pH, but still provide the plants with sufficient calcium to function normally (Goto, 1985).

Nitrogen affects common scab in different ways. Ammoniacal forms of nitrogen can acidify the soil, rendering it suppressive to disease (Terman *et al.*, 1948; Emilsson & Gustafsson, 1953). However, nitrogen also delays tuber initiation and development and consequently can increase scab incidence (Lapwood & Dyson, 1966). A third, albeit only hypothetical effect, is the inhibition of *S. scabiei* by ammonia. Ammonia is toxic to *S. scabiei* (Afafanasiev, 1937), but unlikely to be produced from ammoniacal fertilisers in soil (Brady, 1974).

Phosphorous has little direct effect on common scab. However, some forms of phosphorous such as basic slag, which has a high calcium hydroxide content, have been reported to increase both soil pH and scab (Wenzl & Reichard, 1974; Reichard & Wenzl, 1976). Triple-superphosphate, by contrast, was found to reduce the incidence of common scab (Davis *et al.*, 1976). The effect was ascribed to a reduction in the Ca:K ratio of tubers in response to the phosphate. However, Doyle & MacLean (1960) showed that Ca:K ratios, when manipulated independently of soil pH, were not correlated with scab rating.

Sulphur has been applied commercially for the control of common scab, albeit to a limited extent. Disease suppression mostly was due to lowering of the soil pH (Martin, 1920; Terman *et al.*, 1948; Hooker & Kent, 1950; McCreary, 1967), and the effect could be revoked by the application of lime (Larson *et al.*, 1938; Muncie *et al.*, 1944). Davis *et al.* (1974) indicated that reduction in soil pH by sulphur was not sufficient to account for disease suppression and implicated the lower levels of calcium in peels of tubers from sulphur-treated soil as reason for the reduction in disease. This is supported by Horsfall *et al.* (1954), who speculated that the reduction in pH resulting from the oxidation of sulphur to sulphuric acid could cause a reduction in replaceable calcium, leading to reduced tuber calcium and hence less scab. In soil, sulphur can be reduced anaerobically to hydrogen sulphide, which is toxic to *S. scabiei* (Vlitos & Hooker, 1951; Pavlista, 1992), but also to most other forms of life.

Copper is toxic to *S. scabiei* and was indeed one of the most effective compounds formerly tested against common scab (Halsted, 1895). However, due to the phytotoxicity of copper to potato it is not used commercially for scab control (Mortvedt *et al.*, 1963). Manganese also appears to have a direct suppressive effect on *S. scabiei* (Mortvedt *et al.*, 1961, 1963) but, like copper, can be toxic to potato. Control of common scab with manganese is inconsistent (Mortvedt *et al.*, 1961, 1963; McGregor & Wilson, 1964, 1966; Reichard & Wenzl, 1976) and seems to depend on both the rate of application and placement in the planting furrow (Keinath & Loria, 1989b).

Studies conducted with boron, iron and zinc have provided negative or inconclusive results (Keinath & Loria, 1989b). These elements, together with copper, are involved in lignin biosynthesis (Halliwell, 1978; Marschner, 1986) and may play a role in suberisation and lignification that confer resistance to potato tubers.

2.5.5 Crop rotation and green manuring

Common scab-causing *Streptomyces* species do not produce special survival structures, but can survive in soil for long periods on decomposing plant material, the roots of living plants and manure (Pemberton, 1994). Crop rotation with non-hosts and green manuring play an important role in the integrated management of common scab (Oswald & Lorenz, 1956; Pemberton, 1994). Sanford (1926) established the theory of inhibition of *S. scabiei* by microorganisms that are enhanced when green manuring is applied. Ploughing in rye (*Secale cereale* L.), mustard (*Brassica juncea* (L.) Czerniak) or vetches (*Vicia* spp.) decreases scab incidence (Millard, 1923; Millard & Taylor, 1927), whereas the addition of soybean (*Glycine max* (L.) Merr.) green manure reduces disease because of the lowering of pH (Atkinson & Rouatt, 1949; Oswald & Lorenz, 1956).

Rich (1983) reported that a crop rotation of four years incorporating soybean and lucerne (*Medicago sativa* L.) as green manure can decrease disease severity. Green manuring can also increase the nitrate content of the soil, which assists in reducing soil inoculum of the pathogen and furthermore enhances solubility of manganese in soil (McGregor & Wilson, 1964), thereby decreasing scab incidence. Planting of crops such as rye, sweet clover, wheat (*Triticum aestivum* L.), cowpea (*Vigna unguiculata* (L.) Walp.) and pearl millet (*Pennisetum glaucum* (L.) R. Br.) as green manure reduces the occurrence of common scab (Millard, 1923). Red clover (*Trifolium pratense* L.), although not a host of the common scab pathogen, increases disease incidence and should therefore be avoided as green manure (Rouatt & Atkinson, 1950). According to Rowe (1993) it is not recommended to apply animal wastes to soil because this practice could increase common scab. However, Lazarovits *et al.* (2001) reported that amending soil with ammonium lignosulfonate and liquid swine manure reduced common scab incidence in several trials.

2.5.6 Biological control

Biological control such as the utilisation of suppressive soils (Anderson & Lorang, 1988; Wilson, 1994), antibiotic-producing *Streptomyces* strains antagonistic to potato scab pathogens (Wood & Tveit, 1955; Alivizatos & Pantazis, 1992; Wilson, 1994) and biofumigation (Gouws & Wehner, 2004), offers an environmentally acceptable means of controlling common scab. It is important to realise that these methods can only be effective when applied as part of an integrated disease management system. *In vitro* studies with two suppressive strains of *Streptomyces* (*S. diastatochromogenes* strain PonSSII and *S. scabiei* strain PonR) showed both strains to significantly decrease scab (Liu *et al.*, 1995; Neeno-Eckwall *et al.*, 2001). The suppressive strains did not affect tuber yield and were reisolated from the tubers over the four years of the experiments. Hayashido *et al.* (1988) reported on a biofertiliser that suppressed *S. scabiei*. It was produced from swine dung and the suppressive effect was shown to be due to the presence of *Streptomyces albidoflavus* in the dung. Other bioactive compounds have also been studied as possible control agents e.g. antibiotic substances from the red alga, *Laurencia okamurae* Yamada (Horikawa *et al.*, 1996).

Biofumigation is an agronomic technique that exploits the enzymatic defence systems present in some plants (Brown & Morra, 1997). The plant species concerned are mainly members of the *Brassicaceae, Capparidaceae* and *Moringaceae* which possess the myrosinase-glucosinolate system. Tissues of these plants could be used as a soft, eco-compatible alternative to chemical fumigants and sterilants. Incorporating *Brassica* residues into soil is known to suppress a range of pest and disease organisms, including fungi (Chan & Close, 1987), nematodes (Mojtahedi *et al.*, 1991), insects (Brown & Morra, 1997, Mathiessen & Kirkegaard, 1998) and bacteria (Akiew *et al.*, 1996). Prior to the studies conducted in South Africa (Gouws & Mienie, 2000; Gouws & Wehner, 2004), no work has been done to determine the efficacy of biofumigation on common scab of potatoes.

Presently there is no single control measure that can prevent a serious outbreak of common scab and commercial control of the disease can only be achieved by an integrated crop management system. Such a system would include management of plant nutrients, seed and soil treatment, crop rotation, green manuring, irrigation timing and varietal resistance.

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CHAPTER 3

COMMON SCAB PATHOGENS ASSOCIATED WITH SEED POTATOES IN SOUTH AFRICA

ABSTRACT

Streptomycetes were isolated from potato tubers with symptoms of common scab from five of the potato production areas in South Africa worst affected by the disease. Results indicated that common scab of potato in South Africa is caused by three phenotypically distinct *Streptomyces* groups, designated 1, 2 and 3, and that a further three groups are saprophytically associated with the disease. *Streptomyces* group 1, which corresponded to the morphological description of *S. scabiei*, was by far the most common, representing 52 % of all and 82 % of the pathogenic isolates, and occurred in all the regions surveyed. The other *Streptomyces* groups (groups 2 and 3) containing pathogenic isolates may represent atypical *S. scabiei* isolates, or other *Streptomyces* species. Analysis for the production of thaxtomin A, an important pathogenicity factor, confirmed a positive correlation between pathogenicity and the ability to produce thaxtomin A. However, 14 % of the pathogenic isolates did not produce thaxtomin A, whereas 6 % of the non-pathogenic isolates produced the toxin. Another interesting observation was the ability of 15 % of both all and of the pathogenic isolates to grow at a pH of 4.0, which is atypical of *S. scabiei*.

3.1 INTRODUCTION

Common scab is a globally important constraint to the production of potato (*Solanum tuberosum* L.). The disease was recorded in South Africa almost a century ago by Pole Evans (1906), and has since become common and widespread in all potato production regions of the country (Doidge & Bottomley, 1931; Doidge *et al.*, 1953; Gorter, 1977). *Streptomyces scabiei* (synonym *S. scabies*) is regarded as the main causal agent of common scab (Lambert & Loria, 1989a; Loria *et al.*, 2003) and this assumption has been maintained in most research thus far conducted in South Africa (Dippenaar, 1933; Slabbert *et al.*, 1994; De Klerk *et al.*, 1997). However, there are several reports of other *Streptomyces* species that also cause potato scab, e.g. *S. caviscabies* in Quebec, Canada (Goyer *et al.*, 1996), *S. aureofaciens, S. griseus, S. europaeiscabiei, S. reticuliscabiei* and *S. stelliscabiei* in France (Corbaz, 1964; Bouchek-

Mechiche *et al*., 2000), *S. cinereus, S. collinus* and *S. longisporoflavus* in India (Dey & Singh, 1983), *S. exofoliatus, S. rochei* and *S. violaceus* in Israel (Doering-Saad *et al*., 1992), *S. turgidiscabies* in Japan, Finland, Sweden and Korea (Miyajima *et al*., 1998; Kim *et al*., 1998a; Kreuze *et al*., 1999; Lehtonen *et al*., 2004), *S. luridiscabiei, S. niveiscabiei* and *S. puniciscabiei* in Korea (Park *et al*., 2003b), *S. flaveolus* in Northern USA (Millard & Burr, 1926), *S. acidiscabies* in Northeastern USA, Canada, Japan and Korea (Bonde & McIntyre, 1968; Lambert & Loria, 1989b; Faucher *et al*., 1992; Takeuchi *et al*., 1996; Kim *et al*., 1998b), and *S. atrolivaceus, S. cinerochromogenes, S. corchorussi, S. diastatochromogenes, S. lydicus* and *S. resistomycificus* in Washington State, USA (Archuleta & Easton, 1981).

The validity of some of the above species is uncertain since the early descriptions used for the identification of *Streptomyces* spp. were based on a very small number of characteristics The need for a unified standard system for description of *Streptomyces* strains was first addressed in 1963 with the establishment of the International Streptomyces Project Committee (ISP), headed by E. B. Shirling. The ISP was a joint international effort to assemble and redescribe authentic type strains of the named species in the genera *Streptomyces* and *Streptoverticillium* (Kurylowicz *et al*., 1976). The ISP assembled a set of standardised tests and procedures, and a large number of detailed strain descriptions for the classification of *Streptomyces* species (Gyllenberg, 1976). Although these descriptions and identification techniques are very useful in the identification of scab-causing *Streptomyces* species today, DNA-based techniques (DNA-DNA hybridisation and 16S RNA sequence analysis), as well as cellular fatty acid analysis, have proved more valuable for the accurate description and identification of *Streptomyces* species (Healy & Lambert, 1991; Paradis *et al*., 1994; Ndowora *et al*., 1996; Takeuchi *et al*., 1996; Kreuze *et al*., 1999; Bouchek-Mechiche *et al*., 2000; Egan *et al.,* 2001). These studies have shown some diversity within strains morphologically identified as *S. scabiei,* as well as other phytopathogenic streptomycetes, and provided evidence that potato scab is caused by a polyphyletic group of *Streptomyces* species (Healy & Lambert, 1991; Paradis *et al*., 1994; Bouchek-Mechiche *et al*., 2000). *S. scabiei* nevertheless remains the predominant species causing scab (Loria *et al*., 1997).

Soil pH has traditionally played an important role in potato scab (Keinath & Loria, 1989). *S. scabiei* is usually found in dry, neutral to alkaline soils and generally does not grow below pH 5 (Lambert & Loria, 1989a). Therefore, one of the control measures for scab is to maintain the pH of potato field soils around 5.0-5.2 (Loria, 1991). However, this control method is not always successful since potato scab has been reported in acid soils in the USA, Canada, Japan and Korea (Bonde & McIntyre, 1968; Manzer *et al*., 1977; Faucher *et al*., 1992; Takeuchi *et al*., 1996; Park *et al*., 2003a). These scab problems are ascribed to *S. acidiscabies* rather than *S. scabiei* (Lambert & Loria, 1989b). *S. luridiscabiei,* and particularly *S. puniciscabiei* and *S. niveiscabiei,* isolated from raised corky lesions on potato tubers in Korea, are also capable of growing at a low pH (Park *et al*., 2003a,b). In South Africa, severe cases of scab have been reported from acid soils in Gauteng and KwaZulu-Natal (Frean, 1975; A. D. K. de Klerk, personal communication). The identity of these *Streptomyces* isolates has not been established and needs further investigation since soil acidifying practices such as application of sulphur, nitrogen and green manuring are fairly common in South Africa (Marais & Vorster, 1988).

The primary pathogenicity determinant in phytopathogenic streptomycetes is the phytotoxin thaxtomin A, which is produced by almost all scab-causing strains investigated to date (Lawrence *et al*., 1990; Loria *et al*., 1995, 2003; Healey *et al*., 2000). Thaxtomin A has been shown to induce cell hypertrophy in proliferating plant tissue through the inhibition of cellulose biosynthesis (Fry & Loria, 2002; Scheible *et al*., 2003). Therefore, the present hypothesis is that *Streptomyces* spp. penetrate into regions of rapidly growing potato tissue through the production of thaxtomin A that inhibits cell-wall development (Loria *et al*., 2003). The genes for thaxtomin production, as well as the *nec1* gene, another independent virulence factor, are located on a very large pathogenicity island (PAI) in *S. scabiei, S. acidiscabies* and *S. turgidiscabies* (Bukhalid *et al*., 1998, 2002; Healy *et al*., 2000; Kers *et al*., 2005). Current evidence strongly suggests that the PAI has been mobilised from *S. scabiei* to the two newly-emerged potato scab pathogens, *S. acidiscabies* and *S. turgidiscabies* (Kers *et al.,* 2005).

The polyphelytic group of *Streptomyces* species capable of causing potato scab has been shown to differ in virulence, host range, ecology, pH preference, toxin production and possibly mechanisms of pathogenicity (Millard & Burr 1926; Bonde & McIntyre, 1968; Gordon & Horan, 1968; Archuleta & Easton, 1981; Lambert & Loria, 1989a,b; Faucher *et al*., 1992; Miyajima *et al*., 1998; Park *et al*., 2003b). It is therefore important to verify the variation in *Streptomyces* isolates associated with scab lesions. The aim of this study was to determine the morphological and physiological diversity of *Streptomyces* isolates associated with common scab on seed potato tubers in South Africa. Isolates were characterised morphologically using the ISP method of classification. Subsequently, pathogenicity and thaxtomin production of each isolate were determined to establish whether a correlation exists between these two parameters.

MATERIALS AND METHODS

Common scab survey

A total of 145 seed potato tuber samples of the susceptible cultivars BP1 and Buffelspoort exhibiting superficial, raised and/or deep-pitted common scab symptoms were randomly collected from the five potato production areas in South Africa with the highest incidence of the disease (Fig. 3.1, Table 3.1). These areas represent 48.1 %, 64.5 % and 52.4 % of acreage under irrigated, dryland and total potato production in the country, respectively (Cilliers, 2003). The tubers were taken to the laboratory at the Agricultural Research Council-Roodeplaat in Pretoria where they were stored at 4 °C for a maximum of 2 weeks before being processed.

1. Isolation of streptomycetes associated with common scab lesions

Streptomyces spp. were isolated from tubers as described by Loria & Davis (1989). Whole tubers were surface-disinfested for 10 minutes in 0.6 % sodium hypochlorite and rinsed twice for 10 minutes in sterile distilled water (SDW). The corky layer of each lesion was lifted aseptically and about 5 mm^3 of straw-coloured tissue directly underneath the layer

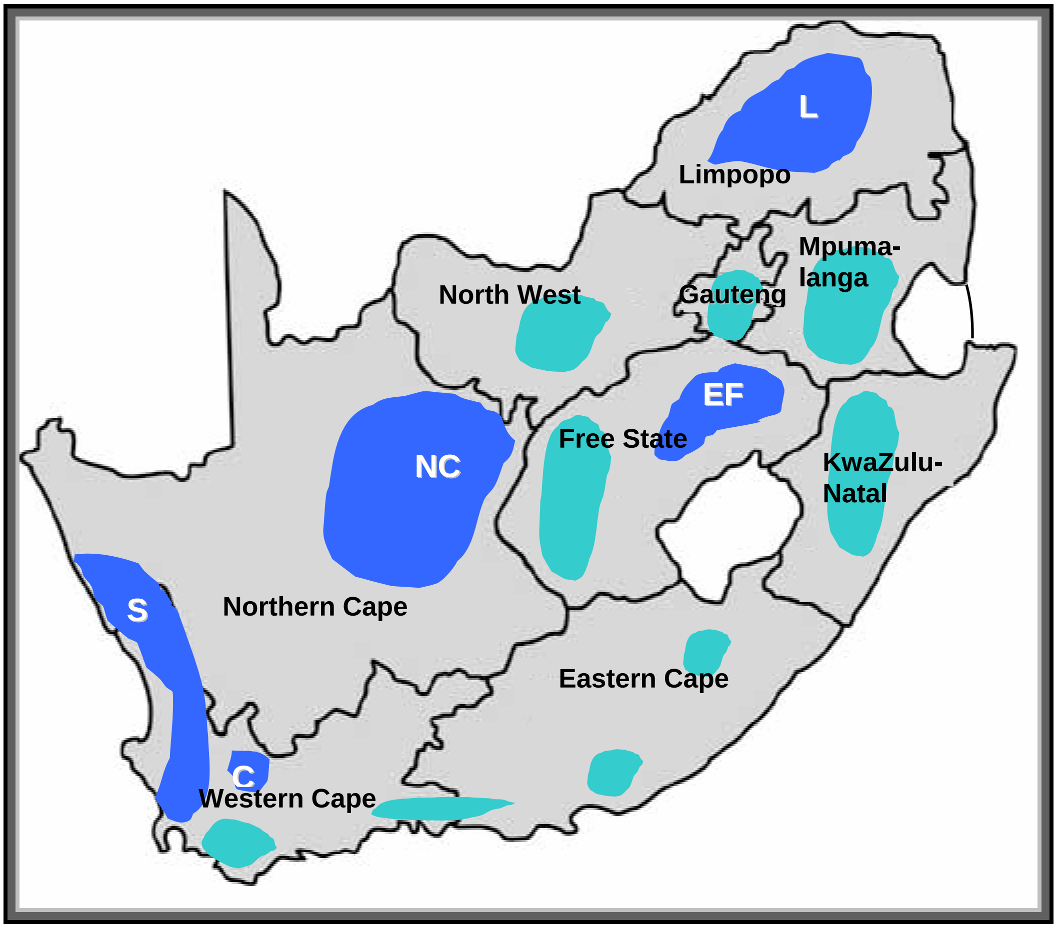

Fig 3.1 🟦 Potato production regions in South Africa included in the survey (**S=**Sandveld; **C=**Ceres; **NC=**Northern Cape; **EF=**Eastern Free State; **L=**Limpopo).
🟩 Potato production regions not included in the survey.

Table 3.1 Number of potato tuber samples collected from five potato production regions in South Africa.

Potato production region	Regional distribution of samples	No. of samples collected
Northern Cape	Douglas (34), Christiana (8), Barkley-Wes (15)	57
Ceres	Koue Bokkeveld (19)	19
Eastern Free State	Warden (10), Bethlehem (2)	12
Sandveld	Clanwilliam (18), Langebaan (7)	25
Limpopo	Polokwane (18), Soutpansberg (14)	32
TOTAL		**145**

was removed and triturated in 5 ml SDW with a pestle and mortar. After approximately 10 minutes, a drop of the suspension was plated on nystatin-polymyxin-penicillin-cycloheximide (NPPC) water agar. Plates were incubated at 30 °C for 10-14 days, where -after single colonies were transferred to yeast malt extract (YME - ISP medium 2) agar slants. Isolates were maintained on YME slants at 4 °C until being identified, and then stored at -70 °C in 20 % glycerol for reference purposes.

2. Morphological and physiological tests

Isolates were identified according to the ISP method (Shirling & Gottlieb, 1966), comprising the following:

a. Morphological characteristics

Aerial mass colour

Each isolate was cultured for 10-14 days in the dark at 30 °C on four different media, viz. YME, oatmeal agar (OA - ISP medium 3), inorganic sodium salts agar (ISSA - ISP medium 4) and glycerol asparagine agar (GAA - ISP medium 5). The colour of the mycelium and spores was recorded, and isolates were classified according to the Tresner-Backus colour wheel (Tresner & Backus, 1963).

Reverse pigmentation (colony colour)

Grouping was based on the production of characteristic diffusible pigments in the reverse of colonies on YME, OA, ISSA and GAA.

Spore chain morphology

To observe sporulation, the isolates were inoculated and grown around a round microscope cover slip obliquely inserted in YME plates. The cover slips were removed after sporulation and the adhering mycelium and spore chains were observed under a light microscope at 100x magnification.

b. Physiological characteristics

Carbon utilisation

Inoculum was prepared from 14-day-old cultures on OA. Spores and mycelium of each isolate were scraped from the medium and suspended in a test tube containing 5 ml

SDW. The contents of each tube was transferred to 25 ml tryptone yeast extract broth (TYB - ISP medium 1) in 125 ml Erlenmeyer flasks, and incubated for 48 hours in the dark at 25-28 °C on a rotary shaker. Sterile glass beads were added and the cultures were vortexed to disperse aggregated growth. Eight millilitres of each culture was transferred to a sterile centrifuge tube and centrifuged for 10 minutes at 3000 rpm. The precipitate was washed in several changes of SDW until a clear solution was obtained, which was resuspended in SDW to serve as inoculum.

L-arabinose, D-fructose, *i*-inositol, D-mannitol, raffinose, rhamnose, sucrose, D-glucose and D-xylose were dissolved in distilled water at 2 % (m/v). Each solution was filter-sterilised through a 0.22 µm Millipore filter and added to cooled autoclaved basal mineral salt agar (BSM - ISP medium 9) at 100 ml l^{-1}. Plates poured with the various sugar media were streak-inoculated in triplicate with each isolate. BSM without any sugar served as control. Plates were incubated for 10-14 days at 30 °C and growth was recorded as positive or negative. No growth was evident on the BSM control plates.

Melanin production

Peptone-yeast extract-iron agar (PYI - ISP medium 6) and tyrosine agar (TA - ISP medium 7) slants were inoculated in triplicate with each *Streptomyces* isolate. Uninoculated PYI and TA slants were included as reference. The slants were incubated at 28 °C in the dark and observed after 2 and 4 days. Isolates that produced a greenish brown to brownish black pigment on both media were considered to be melanin producers.

pH

Citrate-phosphate buffer solutions with a pH of 4.0, 4.5, 5.0, 5.5 and 6.0, respectively, were prepared as described by Cruickshank (1965). D-glucose (10 g), L-asparagine (0.5 g) and agar (15 g) were added per litre of each buffer and the various buffer solutions were autoclaved for 10 minutes at 120 °C. After confirming the pH of each solution it was dispensed into Petri dishes and allowed to solidify. The plates were inoculated in a similar manner as in the carbon utilisation test. Inoculated plates were incubated at 30 °C in the dark for 10-14 days and growth was recorded as positive or negative. The mean minimum pH at which growth occurred was calculated from the antilog functions of the pH values of the various isolates in each group/region. The number of isolates in each group/region capable of growing at pH 4 was also recorded. pH values (KCl) of representative irrigated potato soils in the regions surveyed were obtained from Kynoch/Yara in Randburg, GWK in Douglas and Omnia Fertilizers in Johannesburg, South Africa. Means were calculated as above and plotted against the mean minimum pH for growth of the pathogenic *Streptomyces* isolates from each region. All morphological and physiological tests were repeated twice and *S. scabiei* type strain ATCC 49173 and *S. acidiscabies* strain KM 4030 were included as controls.

Pathogenicity

Minitubers of potato cultivar BP1 were aseptically produced and maintained *in vitro* by implanting sterile sections from dark-grown sprouts in White's amended medium containing 8 % sucrose (King *et al.*, 1991). Inoculum was prepared from 14-day-old cultures of the various *Streptomyces* isolates on OA. When the cultures were sporulating profusely a few drops of SDW were added to each and a drop of inoculum was transferred to a 5-mm-diameter Whatman no.1 filter paper disc which was placed on the surface of a growing minituber in a sterile moist chamber at 25 °C. Each isolate was tested on eight minitubers. With pathogenic isolates, symptoms were noticeable within 4-10 days. The number of tubers that exhibited common scab lesions was recorded after 4 and 6 days, whereafter the filter paper discs were removed to allow the tissue underneath to dry. Tubers were evaluated for a third time on day 14. An isolate was considered to be pathogenic when at least 3 of the 8 tubers developed scab symptoms. Isolations were made from all lesions to confirm Koch's postulates. Pathogenicity testing was conducted twice and *S. scabiei* reference cultures ATCC 49173, NA 88 and EF 35 were included as positive controls.

Toxin production

Streptomyces isolates were grown for 14 days on OA at 28 °C. Agar cultures were homogenised in a Waring-Blender with acetone, filtered and washed twice with acetone. The acetone was evaporated and residues were extracted with chloroform. Crude extracts were compared with authentic thaxtomin A (kindly provided by R. R. King, Potato Research Centre, Agriculture and Agrifood, Fredericton, Canada) by means of thin-layer chromatography (TLC) according to Lawrence *et al.* (1990), as modified by Loria *et al.* (1995). Toxin production of all isolates were assessed twice.

Statistical analysis

Canonical variate analysis

Data were analysed using the statistical program GenStat (2003). Canonical variate analysis (CVA), also referred to as linear discriminant analysis, was used to determine which characteristics discriminated most between the taxonomic groupings. With CVA the variability in a large number of variables is firstly reduced to a smaller set of variables that account for most of the variability (Digby & Kempton, 1987). The new set of variables, called canonical variates, are linear combinations of the original measurements, and are thus given as vectors of loading for the original values. With this approach a set of directions are obtained in such a way that the ratio of between-group variability to within-group variability in each direction is maximised. A plot of the canonical variate means for each group shows the group positions relative to each other. In such a plot points closer together are similar and points further apart are dissimilar with respect to the variates that discriminate between them. The 95 % confidence region of the group means is calculated by circles with a radius of $2.4/\sqrt{n}$ around the means (Krzanowski, 1988) and when they overlap, the groups do not differ.

Chi-square analysis

Row x column (2 x 2) χ^2 analysis was performed on minimum pH and pathogenicity, thaxtomin A production and pathogenicity, as well as on mean pH of soils in the various regions and the minimum pH for growth of pathogenic isolates from the regions, to determine the correlation between these parameters. Yates' correction for 2 x 2 tables was applied (Snedecor & Cochran, 1980).

3.3 RESULTS

Characterisation of streptomycetes from scab lesions

The 145 isolates from deep, raised and superficial tuber lesions from the various potato production areas could be classified into six groups according to their morphological and physiological characteristics (Table 3.2; Appendices A-E). The 75 (52 %) isolates in group 1 produced brown colonies with grey spore masses borne in spiral chains. They synthesised melanin pigments when grown on PYI or TA medium and utilised all nine ISP sugars as sole source of carbon. The 17 (12 %) isolates in group 2 also produced brown colonies with grey spore masses borne in spiral chains and utilised all nine ISP sugars, but did not produce melanin pigments (Table 3.2).

Isolates of groups 3 (9 isolates – 6 %) and 4 (14 isolates – 10 %) produced brown colonies with grey spores borne in flexuous chains and utilised all nine ISP sugars, the only difference between them being melanin production, which was lacking in group 4. Group 5 (18 isolates – 12 %) comprised isolates that had a yellow-brown colony colour and produced grey-green spores in either spiral or flexuous chains. They utilised all ISP carbon sources but did not produce melanin pigments (Table 3.2). Group 6 (12 isolates – 8 %) included isolates with mostly black spores borne in flexuous spore chains, with no production of melanin pigments and utilisation of all ISP sugars (Table 3.2). Groups 1 and 2 were present in all the regions surveyed, group 4 in four, and groups 3, 5 and 6 each in three (Table 3.3).

The mean minimum pH at which isolates in the different groups grew varied from 5.34 (group 3) to 5.75 (group 4), with an overall mean of 5.48. Twenty-one (14 %) of the isolates, distributed among groups 1, 2, 4, 5 and 6, were able to grow at pH 4 (Table 3.2; Appendix F). Group 1 contained the highest number of isolates capable of growing at pH 4. The 14 isolates in group 1 showing growth at pH 4 included 11 (79 %) pathogenic isolates. Mean soil reaction ranged from neutral in the Northern Cape and Limpopo to strongly acid in Ceres and Eastern Free State and very strongly acid in the Sandveld (Fig. 3.2). Regions with a low soil pH yielded pathogenic isolates with a relatively low mean minimum for growth. The opposite applied to the neutral soils in Limpopo but not to the Northern Cape, and χ^2 analysis consequently showed no significant correlation between soil reaction and minimum pH for growth.

Table 3.2 Characteristics of the different groups of streptomycetes isolated from seed potato tubers with common scab symptoms in South Africa.

Characteristic	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Colony colour	Brown	Brown	Brown	Brown	Yellow-brown	Brown
Spore colour (aerial mass - OA)	Grey	Grey	Grey	Grey	Grey-green	Black
Chain morphology	Spiral	Spiral	Flexuous	Flexuous	Spiral/ flexuous	Flexuous
Melanin (PYI+TA)	+	-	+	-	-	-
Carbon utilisation						
L-Arabinose	+	+	+	+	+	+
D-Fructose	+	+	+	+	+	+
D-Glucose	+	+	+	+	+	+
i-Inositol	+	+	+	+	+	+
D-Mannitol	+	+	+	+	+	+
Raffinose	+	+	+	+	+	+
Rhamnose	+	+	+	+	+	+
Sucrose	+	+	+	+	+	+
D-Xylose	+	+	+	+	+	+
Growth at pH 4 (%) [a]	19	6	0	7	11	25
Mean minimum pH [b]	5.38	5.48	5.34	5.75	5.45	5.45
Pathogenicity (%) [c]	80	47	56	0	0	0
Thaxtomin A (%) [d]	85	88	100	0	0	0

[a] Percentage of isolates capable of growing at pH 4.

[b] Mean minimum pH at which isolates in the group grew.

[c] Percentage of isolates that were pathogenic according to a minituber pathogenicity assay.

[d] Percentage of pathogenic isolates producing thaxtomin A.

Table 3.3 Regional distribution and characteristics of streptomycete groups isolated from seed potato tubers with common scab symptoms in South Africa.

Characteristics	Northern Cape	Ceres	Eastern Free State	Sandveld	Limpopo
Group 1	26	17	7	14	11
Group 2	6	1	3	3	4
Group 3	5	1	0	0	3
Group 4	9	0	2	1	2
Group 5	6	0	0	6	6
Group 6	5	0	0	1	6
Growth at pH 4 (%) [a]	1.8	11	0	68	3.1
Mean minimum pH [b]	5.27	4.89	5.28	4.77	5.88
Pathogenicity (%) [c]	44	84	75	52	31
Thaxtomin A (%) [d]	100	100	100	46	80

[a] Percentage of isolates capable of growing at pH 4.

[b] Mean minimum pH of pathogenic isolates in the region.

[c] Percentage pathogenic isolates present within region.

[d] Percentage pathogenic isolates producing thaxtomin A.

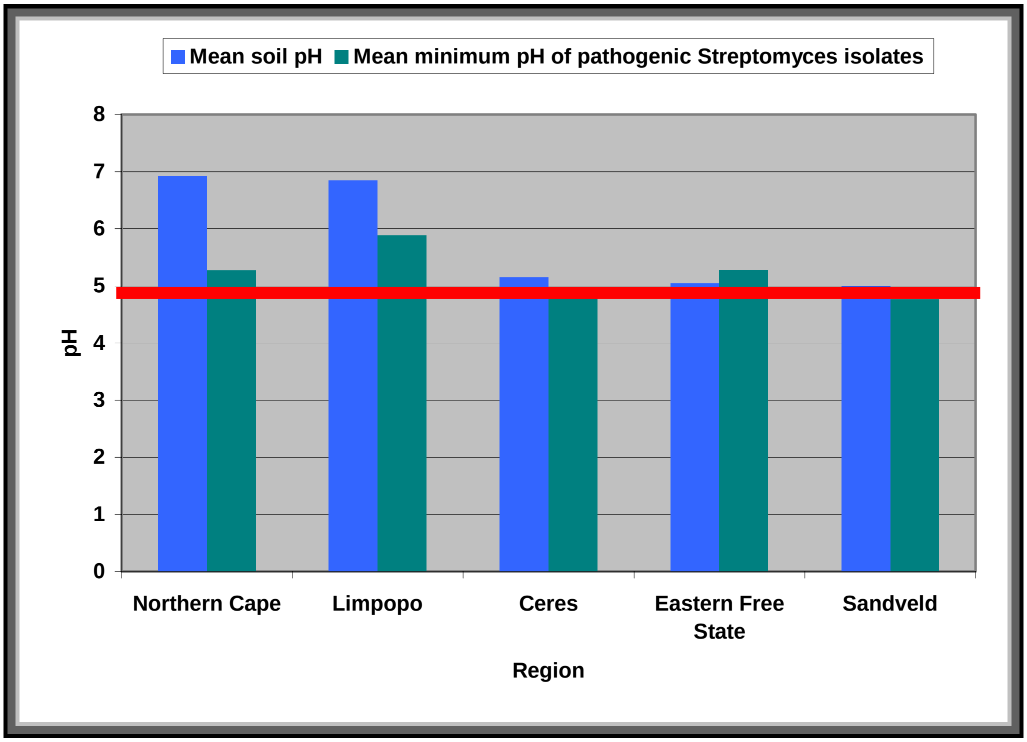

Fig. 3.2 Mean soil pH of regions sampled plotted against mean minimum pH for growth of pathogenic *Streptomyces* isolates from the various regions. The red line indicates the minimum pH of 5 at which *Streptomyces scabiei* is normally capable of growing (Lambert & Loria, 1989a).

Sandveld yielded the highest percentage of isolates capable of growing at pH 4 (Table 3.3). Indeed, 81 % of all and 91 % of the pathogenic isolates showing growth at pH originated from this region (Appendix F).

Pathogenicity

In total 73 (50 %) of the 145 *Streptomyces* isolates obtained from scab lesions were pathogenic. Sixty (80 %) of the isolates in group 1 produced common scab symptoms on minitubers, eight (47 %) of those in group 2 and five (56 %) in group 3 (Table 3.2). No symptoms were induced by any of the isolates in groups 4-6. Koch's postulates were confirmed for all the isolates that produced symptoms, including *S. scabiei* type strains ATCC 49173, NA 88 and EF 35.

Toxin production

Of the 60 pathogenic isolates in group 1, 51 (85 %) produced thaxtomin A (Table 3.2). The toxin was also produced by seven (88 %) of the eight pathogenic isolates in group 2 and all five the pathogenic isolates in group 3. In total 86 % of the 73 pathogenic isolates produced thaxtomin A (Appendices A-E). Among the 67 thaxtomin-producing isolates, four (6 %) were non-pathogenic. Seven of the 10 pathogenic isolates that did not produce thaxtomin A were capable of growing at pH 4 (Appendices B, D, E).

Statistical analysis

1. Canonical variate analysis (CVA)

The most important variates which discriminated between the six taxonomic groups were melanin production (r = 0.996), pathogenicity (r = 0.763) and thaxtomin A production (r = 0.606) as they correlated best with the CV scores.

Since the first latent root value was >1 (11.943) the CVA was meaningful and 95 % confidence regions indicated that groups 1 and 3 were not significantly different from each other, but differed significantly from groups 2, 4, 5 and 6 regarding melanin and thaxtomin A production (Fig. 3.3). Group 2 differed significantly from groups 4, 5 and 6 in respect of pathogenicity.

2. Chi-square analysis

No correlation was evident between minimum pH for growth and pathogenicity or, as indicated above, between minimum pH for growth and soil reaction. However, a positive correlation ($P \leq 0.0001$) existed between thaxtomin A production and pathogenicity.

3.4 DISCUSSION

A phenotypically diverse range of *Streptomyces* isolates was obtained from potato scab lesions in South Africa, half of them being saprophytic. The incidence of saprophytes associated with scab symptoms was lower, higher and approximately the same as the 90 %, 35 % and 53 % reported by Loria *et al.* (1986), Doering-Saad *et al.* (1992) and Faucher *et al.* (1992), from Northeastern USA, Israel and Canada, respectively. Pathogenic *Streptomyces* isolates in South Africa could be classified into three phenotypically distinct groups, designated 1, 2 and 3, while a further three groups (designated 4-6) were saprophytically associated with the disease.

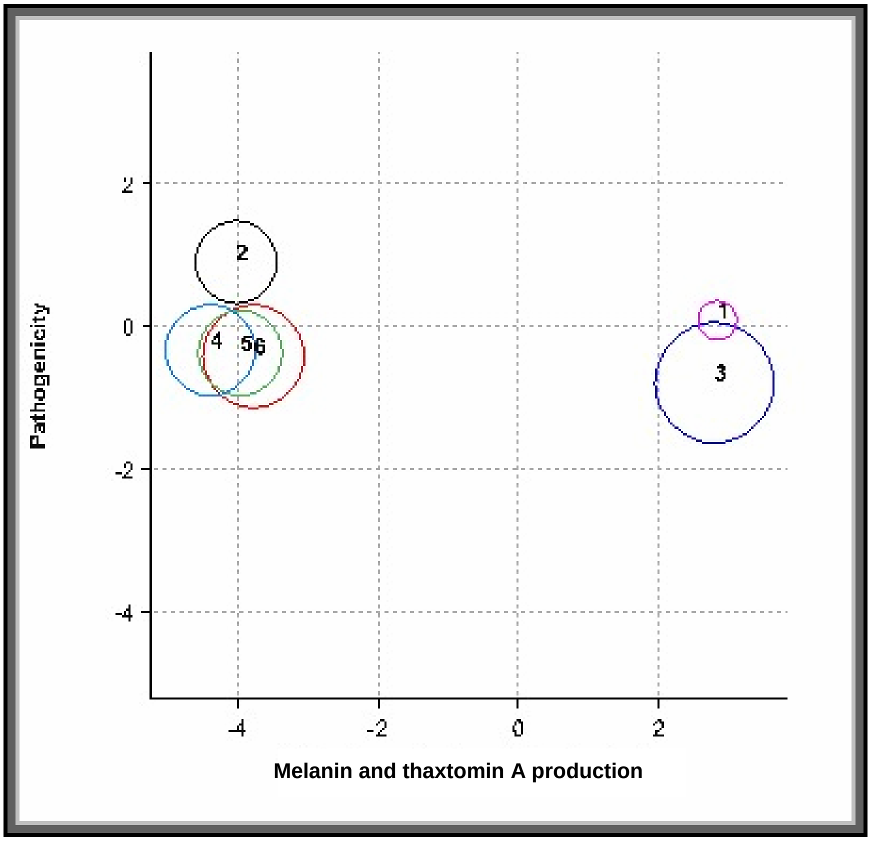

Fig. 3.3 Canonical variate analysis of streptomycete groups isolated from seed potato tubers with common scab symptoms in South Africa.

Streptomyces group 1 was the most common, representing 52 % of all and 82 % of the pathogenic isolates, and occurred in all the regions surveyed. Isolates in this group corresponded to the description of *S. scabiei* by Lambert & Loria (1989a), having smooth, grey spores borne in spiral chains, producing melanin and utilising all nine the recommended ISP diagnostic sugars as sole source of carbon. Bouchek-Mechiche *et al.* (2000) recently described two new species, *S. europascabiei* and *S. stelliscabiei,* that phenotypically resemble *S. scabiei*, but can only be distinguished from *S. scabiei* by means of DNA-DNA hybridisation. Consequently, the presence of *S. europaeiscabiei* and *S. stelliscabiei* among South African *S. scabiei* group 1 isolates cannot be ruled out until DNA-DNA hybridisation studies have been conducted.

Streptomyces group 2 was phenotypically identical to *S. scabiei* isolates in group 1, except that they did not produce melanin. Members of this group were also present in all five regions surveyed, but represented only 12 % of all and 11 % of the pathogenic isolates. It is possible that group 2 contained amelanogenic variants of *S. scabiei*. Lambert & Loria (1989a) considered melanin production as secondary, even though it is usually associated with pathogenicity. This is supported by Hollis (1952), who demonstrated the spontaneous mutability in melanogenic capacity of *S. scabiei*. Furthermore, a *S. scabiei* strain lacking melanin production has also been characterised in Finland using 16S rRNA analysis and morphological data (Lindholm *et al*., 1997; Kreuze *et al*., 1999). Alternatively, isolates in group 2 could have been *S. lydicus* (Locci, 1989), which has been associated with deep-pitted scab in Washington State, USA (Archuleta & Easton, 1981), but is now used commercially for the biological control of seed piece decay and rhizoctonia root rot in potato (Noel, 1997). The identity of isolates in group 2 should be investigated further through 16S rRNA sequence analysis as well as DNA-DNA hybridisation studies.

Isolates in group 3 differed from *S. scabiei* in producing their spores in flexuous instead of spiral chains. Representatives of group 3 were collected in only three of the regions and comprised 6 % of the total and 7 % of the pathogenic isolates. In Finland, *S. scabiei* isolates, characterised through 16S rRNA sequence data, have also been reported to contain flexuous instead of spiral chains (Lindholm *et al*., 1997; Kreuze *et al*., 1999). DNA-based studies will be required to elucidate the identity of South African isolates in group 3.

Streptomyces groups 4-6 did not include any pathogenic isolates, and had morphological characteristics clearly distinct from *S. scabiei.* Members of group 4 exhibited characteristics resembling those of *S. turgidiscabies* (Miyajima *et al*., 1998), having smooth grey spores in flexuous spore chains, lacking melanin or other diffusible pigment production and utilising all the ISP sugars. It will be interesting to determine through DNA studies whether these isolates are indeed genetically similar to *S. turgidiscabies*, but represent non-pathogenic isolates into which the pathogenicity island has not been mobilised. Morphologically, isolates in group 5 conformed to the description of *S. flaveolus* and *S. griseoflavus* (spiral/flexuous spore chains, grey-green spore mass, no melanin production), whereas those in group 6 most closely resembled *S. violaceusniger* (flexuous spore chains, grey to black spore mass, no melanin production). Only two of the above species, *S. flaveolus* and *S. violaceusniger,* are known to be associated with potato scab (Millard & Burr, 1926; Doumbou *et al.*, 2001).

In general, *S. scabiei* isolates do not grow at a pH below 5 (Lambert & Loria, 1998a). However, although the mean overall minimum pH for growth of South African *Streptomyces* isolates was 5.48, 33 % of all and 40 % of the pathogenic isolates were capable of growing at a pH lower than 5. Fifteen per cent of the pathogenic isolates, all from group 1, and the same percentage of the total number of isolates, even showed growth at pH 4. Phenotypically, the above pathogenic isolates corresponded to the description of *S. scabiei* and were clearly distinct from *S. acidiscabies*, *S. luridiscabiei* and *S. puniscabiei*, the only other common scab pathogens capable of growth at pH 4 (Lambert & Loria, 1989b; Park *et al.*, 2003a). This suggests a possible adaptation of some South African *S. scabiei* strains to acidic conditions. Further evidence in this regard is provided by the tendency, albeit not significant, for isolates from regions with acidic soils to have a relatively low minimum for growth. However, the fact that 81 % of all and 91 % of the pathogenic isolates capable of growing at pH 4 originated from the Sandveld region indicates that these acid-tolerant strains tend to be area-bound. Future surveys of scab pathogens in South Africa should obviously include determining the pH of all soils from which tuber samples are collected to elucidate the above phenomenon.

Analysis for thaxtomin production in South African *Streptomyces* isolates showed that 86 % of the pathogenic isolates and 6 % of the non-pathogenic isolates produced thaxtomin A. Thaxtomin A is the primary pathogenicity factor in potato scab-causing *Streptomyces* isolates (Healy *et al.,* 1997; Bukhalid *et al.*, 1998, Loria *et al.*, 2003). Therefore, the lack of thaxtomin production in 14 % of the pathogenic isolates was unexpected since most previous studies have found an absolute correlation between pathogenicity and thaxtomin A production (Lawrence *et al.*, 1990; Babcock *et al.*, 1993; Goyer, *et al.*, 1996; Healy *et al.,* 1997; Loria *et al.*, 1997, 2003; Bukhalid *et al.*, 1998), the only exceptions thus far being *S. luridiscabiei* and *S. puniscabiei* isolated from raised, corky lesions in acidic soils in Korea (Park *et al.*, 2003a,b). Furthermore, chemically mutagenised *S. scabiei* isolates with reduced or undetectable levels of thaxtomin A were non-pathogenic or had reduced virulence (Healy *et al.*, 1997; Goyer *et al.*, 2000). The presence of thaxtomin production in non-pathogenic isolates has not been reported previously.

Several arguments can be put forward to explain the lack of thaxtomin A production by some pathogenic South African *Streptomyces* isolates. In this study the production of thaxtomin was determined on OA, which is the standard substrate used for thaxtomin production (Loria *et al.*, 1995). However, Goyer & Beaulieu (unpublished data in Goyer *et al.* (1996)) reported that *S. caviscabies* isolates causing deep-pitted scab in Québec, Canada, only produced thaxtomin on potato peel or sweet potato peel medium and not on OA, albeit at very low levels. Therefore, the thaxtomin non-producing isolates in this study should also be tested on potato and sweet potato peel media to confirm the lack of thaxtomin production. Although thaxtomin A has been implicated as the primary pathogenicity factor in scab-causing *Streptomyces* isolates, the absence of thaxtomin A production in *S. luridiscabiei* and *S. puniscabiei* recently reported by Park *et al.* (2003a,b), suggests that there may be pathogenicity factors in addition to thaxtomin. Interestingly, Bukhalid *et al.* (1998) reported that a South African *S. scabiei* isolate (CEK 018) which produced thaxtomin A, lacked the *nec1* gene, an independent virulence factor that is linked to the genes for thaxtomin production on a large pathogenicity island (Kers *et al.*, 2005). This finding, along with the apparent lack of thaxtomin production in some isolates, suggest that a component of South African isolates either contain rearrangements or deletions within the pathogenicity island, or completely lack the pathogenicity island. Therefore, future studies should investigate the presence of *nec1*, thaxtomin biosynthetic genes and *ORFtnp* in South African isolates through Southern blot analysis. This will become increasingly important since colony polymerase chain reaction amplification for thaxtomin biosynthesis genes (*txtA*) is currently being used for discriminating pathogenic from non-pathogenic scab isolates (Wang & Lazarovits, 2004). What also needs to be established is whether there is a link between acid-tolerance and thaxtomin production. The high incidence of thaxtomin non-producers among the pathogenic acid-tolerant isolates in the present study, and the absence of thaxtomin production in *S. luridiscabiei* and *S. puniscabiei*, species capable of growing at pH 4.5 and 3.5, respectively (Park *et al.*, 2003a,b), suggest that such a possibility could exist. However, thaxtomin production by other acidophylic common scab pathogens such as *S. acidiscabies* and *S. niveiscabiei* (Lambert & Loria, 1989b; Park *et al.*, 2003a), indicates the contrary.

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CHAPTER 4

EFFECT OF POTENTIAL ROTATION CROPS ON THE SURVIVAL OF THE POTATO COMMON SCAB PATHOGEN, *STREPTOMYCES SCABIEI*

ABSTRACT

Nine crops were tested in the greenhouse for their ability to sustain or suppress *Streptomyces scabiei* in sand artificially infested with the pathogen. After a period of *ca*. 3 months, densities of *S. scabiei* were significantly higher in fallow sand and sand planted to soybean, spinach, maize, pumpkin or sunflower than in sand under triticale or cabbage, with no survival evident in the case of the latter crop. Survival in sand planted to rye and pea was also relatively low, albeit not significantly lower than the other crops. Roots of pumpkin, maize and, to a lesser extent, triticale yielded the highest number of *S. scabiei* propagules. Roots of soybean, spinach, rye, sunflower, pea and cabbage were colonised significantly less than those of pumpkin and maize, with roots of pea and cabbage being void of viable inoculum of the pathogen. Cluster analysis of data separated the crops into two groups according to their effect on *S. scabiei*. The first group, comprising pumpkin, maize, soybean, sunflower and spinach, had little impact on total densities of the pathogen. The second group, consisting of rye, triticale, pea and cabbage, showed a marked suppressive effect and on average supported only 0.23 % of the populations of *S. scabiei* sustained by the first group.

INTRODUCTION

Optimal production of potato (*Solanum tuberosum* L.), like most other field crops, can best be achieved by implementing a rotation programme that provides direct economic returns from each crop, as well as indirect benefits such as improvement of soil structure and fertility, and a reduction in pests and diseases (Thornton *et al.*, 1993). For the management of common scab caused by *Streptomyces scabiei* it is obvious that rotation with alternative hosts of the pathogen such as beet (*Beta vulgaris* L.), groundnut (*Arachis hypogaea* L.), carrot (*Daucus carota* L.), turnip (*Brassica rapa* L.), rutabaga (*Brassica napus* L. var. *napobrassica* (L.) Rchb.), parsnip (*Pastinaca sativa* L.), radish (*Raphanus sativus* L.), mangel (*Beta macrorrhiza* Steven) and salsify (*Tragopogon porrifolius* L.) (Lutman & Johnson, 1915; Jones, 1953; Crete, 1980; Goth & Webb, 1986; Hooker, 1986; Sherf & MacNab, 1986; De Klerk *et al.*, 1997; Goyer & Beaulieu, 1997; Bouchek-Mechiche *et al.*, 2000) should be avoided. All these crops develop typical scab symptoms, including blisters, raised lesions and russet scab, and can therefore serve as reservoir of inoculum in the absence of potato. Various other monocotyledonous and dicotyledonous crops have also been reported to be negatively affected when artificially infected with *S. scabiei* (Hooker & Kent, 1946; Hooker 1949; Leiner *et al.*, 1996), probably due to toxic effects induced by thaxtomin A, a broad-spectrum phytotoxin produced by the scab pathogen (Leiner *et al.*, 1996.).

Crops that are recommended for reducing common scab in rotation programmes include grasses, small grains, soybean (*Glycine max* (L.) Merr.), maize (*Zea mays* L.) and lucerne (*Medicago sativa* L.) (Millard, 1923; Hooker, 1949, 1956; Frean, 1975; Loria, 1991; Powelson *et al.*, 1993; Trench *et al.*, 1993; Gouws *et al.*, 2003). These recommendations have mostly been based on the incidence and/or severity of common scab symptoms on potatoes planted subsequent to the respective rotation crops. Only Hooker (1956) reported a reduction in total streptomycete populations in soil planted to onion (*Allium cepa* L.) and soybean, compared to maize and potato, but could not correlate these counts with the incidence of common scab on potato tubers. The direct effect of crops other than potato on soil populations of *S. scabiei* therefore remains unclear.

The purpose of the present study was to establish the extent to which soil inoculum of *S. scabiei* is sustained or suppressed under controlled conditions over a growing season by a selection of crops that are either recommended, not recommended, or have not previously been considered, for rotation with potato.

MATERIALS AND METHODS

Inoculum

Rifampicin resistance was confirmed for 12 pathogenic isolates of *S. scabiei* (Appendix G) by plating them on oatmeal agar (OA) supplemented with 10 μg ml^{-1} rifampicin (Elliot *et al.*, 1994). The isolates were then cultured for 10 days at 28 °C on OA with rifampicin and a pooled inoculum suspension containing 1.5 x 10^{8} cfu ml^{-1} was prepared from all 12 isolates.

Planting of crops

Sunflower (*Helianthus annuus* L.), rye (*Secale cereale* L.), triticale (*Triticosecale* Wittm.), pumpkin (*Cucurbita pepo* L.), pea (*Pisum sativum* L.), cabbage (*Brassica oleraceae* L. var *capitata* L.), spinach (*Spinacea oleracea* L.), maize and soybean were grown from surface-disinfested seed (1.5 % sodium hypochlorite for 3 minutes) in 15-cm-diameter plastic pots filled with heat-sterilised (200 °C overnight) river sand with a pH of 7.53 and coarseness of 0.6-1.5 mm. Twenty pots were planted to each crop and the pots were randomly arranged in a greenhouse with a day/night temperature of 8/25 °C.

Inoculation

When plants were *ca.* 2 weeks old, 10 pots of each crop was infested with *S. scabiei* by pipetting 10 ml of the above spore suspension around the stem of the plant into the sand. Sand in the remaining 10 pots of each crop was not infested and served as negative controls. A positive control comprising 10 pots with sterile sand infested with 10 ml of the *S. scabiei* spore suspension was also included. Plants were irrigated every fourth day by means of a micro-sprinkler system and 100 ml of a 0.1 % Chemicult® nutrient solution (19 % N; 8.2 % P; 15.8 % K; 0.09 % Mg; 0.035 % Zn; 0.1 % B; 0.007 % Mo; 0.0758 % Fe; 0.03 % Mn; 0.0075 % Cu) was added per pot every third week.

Harvesting

Plants were harvested at maturity (*ca.* 3 months) and their roots and shoots separated. The roots were rinsed in sterile distilled water (SDW), surface-disinfested with 1.5 % sodium hypochlorite for 3 minutes, washed again in SDW, and cut into 1 cm segments. One gram of root segments from each plant was air-dried on a laminar flow bench and homogenised in 9 ml of SDW in a Waring blender. Ten grams of soil from each pot was similarly suspended in 90 ml of SDW. A serial dilution series was prepared from each suspension and the 10^{-2} to 10^{-9} dilutions were plated in triplicate on OA with rifampicin. *S. scabiei* was enumerated on the plates after incubation for 14 days at 28 °C and representative colonies were streaked on OA and inorganic sodium salts agar for confirmation of their identity according to the ISP method (Shirling & Gottlieb, 1966). Logarithmic values were calculated for statistical analysis. Data were analysed using the statistical program GenStat (2003). As counts were generally Poisson distributed, differences between crops were tested by generalised linear modelling (Dobson, 1990). Means were separated by Fisher's protected *t*-test least significant difference. Soil and root counts were compared by means of regression analysis, whereas actual population densities were subjected to cluster analysis (Digby & Kempton, 1987), followed by analysis of variance, to separate the crops into categories according to their effect on the survival of *S. scabiei*.

RESULTS

Densities of *S. scabiei* were significantly lower in soil under triticale and cabbage than in fallow soil and soil planted to soybean, spinach, pumpkin, maize or sunflower (Fig. 4.1). No survival was evident in the case of cabbage. Survival in soil planted to rye and pea was relatively low, albeit not significantly lower than in other soils. Roots of soybean, spinach, rye and sunflower were colonised significantly less by *S. scabiei* than those of pumpkin and maize, whereas cabbage and pea roots did not contain viable propagules of the pathogen (Fig. 4.2). Soil and root counts did not correlate significantly at $P = 0.05$ ($r = 0.613 < 0.6319$), but cluster analysis indicated that the various crops could be separated into two distinct groups according to their effect on *S. scabiei*. One group comprising pumpkin, maize, soybean, sunflower and spinach, had little effect on densities of the pathogen, whereas the other group, consisting of rye, triticale, pea and cabbage, showed a marked suppressive effect (Fig. 4.3).

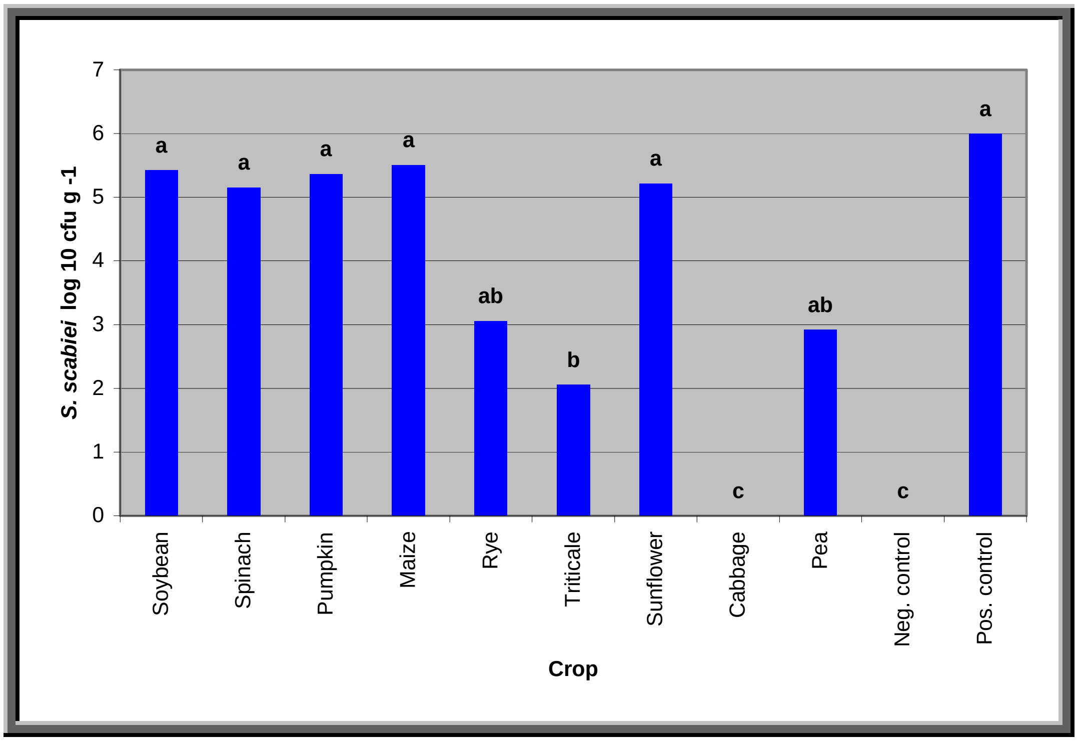

Fig. 4.1 Survival rate of *Streptomyces scabiei* in sand artificially infested with the pathogen and planted to different crops. Bars with the same letter do not differ significantly according to Fischer's protected *t*-test least significant difference ($P \leq 0.05$).

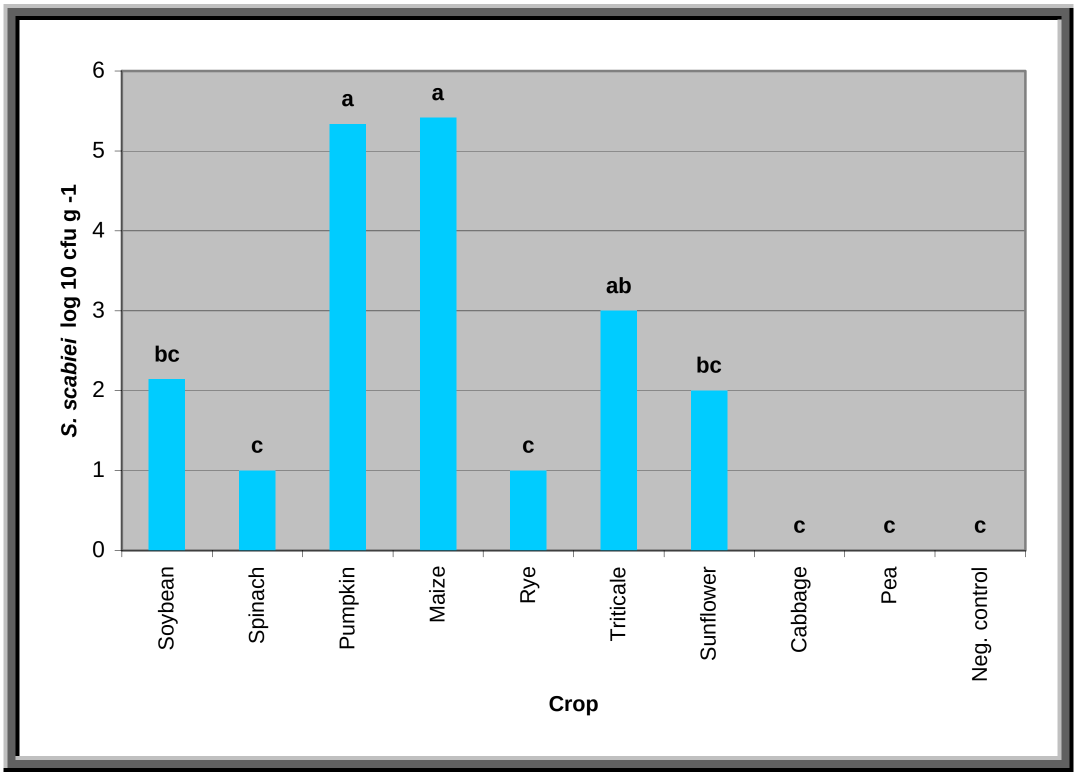

Fig. 4.2 Colonisation of roots of different crops by *Streptomyces scabiei* in sand artificially infested with the pathogen. Bars with the same letter do not differ significantly according to Fischer's protected *t*-test least significant difference ($P \leq 0.05$).

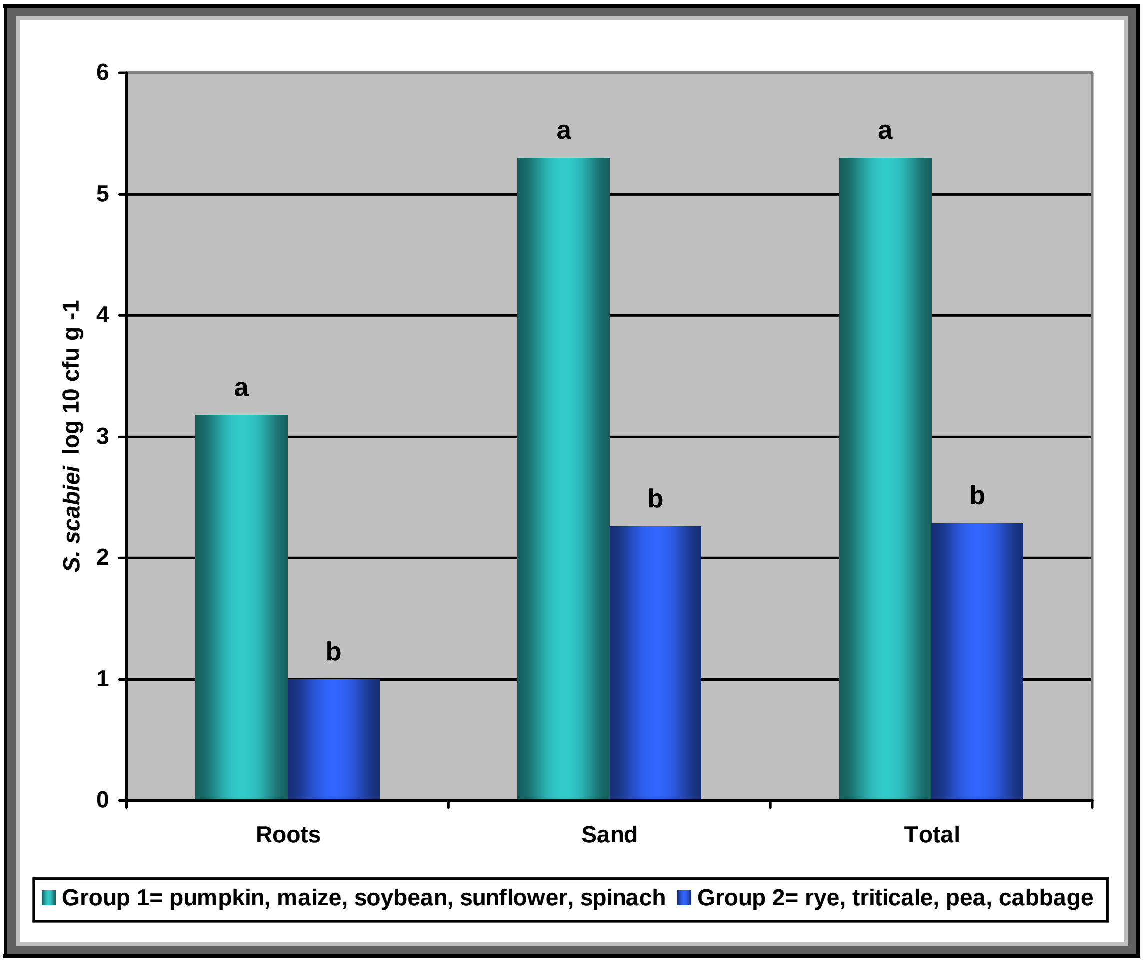

Fig. 4.3 Grouping of potential rotation crops according to cluster analysis and analysis of variance of their effect on the survival of *Streptomyces scabiei*. Bars with the same letter within population sources do not differ significantly according to the Fischer's protected *t*-test least significant difference ($P \leq 0.05$).

DISCUSSION

It is generally accepted that the length of time between potato plantings is more important than the type of rotation crop for the control of common scab in a rotation programme (Goss & Afanasiev, 1938; Blodgett, 1940; Hooker, 1956; Heeg & Richardson, 1958; Weinhold *et al.*, 1964). However, due to the profitability of potato farming, notwithstanding the rather precarious position commercial agriculture finds itself under the present political regime in the country, the majority of potato farmers in South Africa are reluctant to implement long rotations unless they can gain direct financial benefits, exceeding that provided by potato, from the rotation crop. A crop destined primarily for the reduction of a pest or disease will only be considered if there is sufficient evidence that it can eradicate or suppress the pest or pathogen as cost-effectively as chemical treatment, and in the shortest possible time. Accordingly, this study aimed at establishing the effect of various rotation crops on the scab pathogen within a growing season. The substrate used in the screening, coarse river sand, was selected because it favoured *S. scabiei* in having a low water-holding capacity and an alkaline pH (Loria *et al.*, 1991; Powelson *et al.*, 1993), and did not contain an established microbial community capable of competing with the scab pathogen, at least not during the initial phases of the experiment. That it sustained the pathogen is evident from the fact that populations of *S. scabiei* in the infested fallow control sand were approximately the same at termination of the experiment as at the beginning.

Compared to the infested fallow control, none of the rotation crops tested increased the inoculum level of *S. scabiei* in the sand, even when considering that infected root biomass constitutes a significant component of the inoculum of most soilborne pathogens. This is not entirely evident from the log values in the various figures, but in terms of actual counts the population of *S. scabiei* supported by maize, which yielded the highest sand and root counts, was only 62 % that of the infested control sand. This is in conflict with Hooker (1956), who found the total streptomycete populations in soil planted for two years to maize and soybean to be 206 % and 75 % higher, respectively, than in fallow soil. He could however, not correlate these counts with the incidence of scab on tubers of potato planted to the soil. Labruyère (1971) and Keinath & Loria (1989), by contrast, reported a positive correlation between scab severity and the population densities of melanin-producing and total actinomycetes, respectively, in potato soil. It therefore appears if total or melanin-producing actinomycete densities may reflect the inoculum potential of *S. scabiei* in soil under potato, but not necessarily in soils planted to different rotation crops. This seems quite logical since actinomycete populations differ quantitatively in the rhizosphere of different plant species (Abraham & Herr, 1964; Pinton *et al.*, 2001).

Root counts in this study also did not correspond with the reported sensitivity of seedlings of the various crops to *S. scabiei*. Hooker (1949), for instance, found roots of pumpkin and maize to be relatively resistant to attack by *S. scabiei*, whereas pea, soybean, cabbage, spinach and rye were severely affected within 13 to 30 days after planting of the crops in scab-infested soil. Leiner *et al.* (1996) similarly observed shoot growth of maize seedlings not to be impeded by *S. scabiei* six days after inoculating one-day-old seedlings with the pathogen, and that of pea and rye to be reduced significantly. In the present study, however, roots of maize and pumpkin yielded significantly higher counts of *S. scabiei* than the other crops tested, except for triticale. A possible explanation for this discrepancy is that infection of the roots could have been restricted by allowing the seedlings to develop some level of resistance to infection in the two weeks before infesting the sand with *S. scabiei*, and that the eventual counts therefore reflect saprophytic colonisation of the roots rather than parasitic infection.

The most interesting conclusion derived from this study was the separation of the various rotation crops into two distinct categories, one comprising summer crops passive towards *S. scabiei*, and the other consisting of winter crops suppressive towards the pathogen. According to actual densities, total populations of the pathogen supported by the suppressive group were on average only 0.23 % (range 0-0.8 %) that of the passive group. Of the crops in the passive category, only spinach is considered inappropriate in rotations aimed at reducing scab (Davis & Nunez, 2005), although no information in this regard is available for sunflower. The lack of suppressiveness of pumpkin, maize and soybean in the present study does not endorse their rating as particularly resistant to *S. scabiei* (Hooker, 1949; Leiner *et al.*, 1996), or as recommended rotation crops for scab control (Rich, 1983; Hooker, 1986, Loria, 1991; Powelson *et al.*, 1993), at least not in short rotations.

The reduction in populations of *S. scabiei* effected by rye and triticale, the two small grain crops in the suppressive category, supports the recommendation of this group of cereals for rotation with potato, not only for the management of scab (Loria, 1991), but also of various other diseases (Powelson *et al.*, 1993; Denner *et al.*, 2003). Neither is known to serve as host for any pathogen infecting potato in South Africa. Local demand for these two crops, however, is rather limited and any additional plantings will result in oversaturation of the market. The two remaining crops in the suppressive category, pea and cabbage, also do not constitute a major component of the agricultural industry in South Africa, but are nonetheless in greater demand and more profitable to cultivate than rye and triticale. They are, however, attacked by various fungal, bacterial and viral pathogens of potato (Gorter, 1977, 1982; Crous *et al.*, 2000), as well as nematodes (Keetch & Heyns, 1982; Westcott & Horst, 2001) and insects (Visser, 2005), including species known to transmit potato viruses (Blackman & Eastop, 1984; Brunt *et al.*, 1996), and could therefore sustain populations of these pests and pathogens in the absence of a potato crop. Cabbage nevertheless remains the only crop that totally eradicated the scab pathogen. This effect most likely was due to the biocidal action of the myrosinase-glucosinolate system inherent to the *Brassiceae* family (Brown & Morra, 1997). Further evidence in this regard is provided in Chapter 5.

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CHAPTER 5

CONTROL OF COMMON SCAB OF POTATO BY MEANS OF BIOFUMIGATION

ABSTRACT

Common scab, caused by *Streptomyces scabiei,* is a major disease of potato in South Africa and most other parts of the world. To determine if volatile compounds released from residues of brassicaceous crops have a reducing effect on the incidence of common scab, greenhouse and field trials were conducted with various brassica amendments. In the greenhouse, soil incorporation of fresh and dry cabbage residues at rates of 0.25 and 0.1 % (m/v), reduced common scab by a significant 32 and 41 % respectively. Amendment of naturally infested soil in the field with dry residues of cabbage, cauliflower, broccoli and Brussels sprouts at 0.33 % (m/v) resulted in a significant reduction in disease of approximately 90 %.

INTRODUCTION

Rotation with brassicaceous crops and incorporation of brassica residues into soil has been reported to suppress pest and disease organisms such as fungi, nematodes, insects and bacteria, as well as weeds (Brown & Morra, 1997). The suppressive effect is due to the presence of ß-D-thioglucosidic compounds known as glucosinolates (GSLs) in the *Brassicaceae* and other families of the order *Capparales* (Brown & Morra, 1997; Kirkegaard & Sarwar, 1998). GSLs *per se* are not toxic but are hydrolysed in the presence of water to biologically active compounds such as organic cyanides, ionic cyanates, oxazolidinethiones and isothiocyanates (ITCs), by the enzyme myrosinase (thioglucoside glucohydrolase), which occurs endogenously in brassica tissues (Brown & Morra, 1997). Myrosinase and GSLs are separated in intact plant tissue. Disruption of the tissues results in the mixing of the myrosinase and GSLs, and subsequent release of GSL degradation products. Of the various GSL hydrolysis products, ITCs are considered the most toxic. They are general biocides that interact non-specifically and irreversibly with proteins and amino acids (Fenwick *et al.*, 1983; Kawakishi *et al.*, 1983; Kawakishi & Kaneko, 1987). Since ITCs are volatile, the utilisation of brassicaceous crops in the control of pests and diseases has been termed "biofumigation" (Kirkegaard *et al.*, 1993; Angus *et al.*, 1994).

The profile, concentration and distribution of GSLs vary between *Brassica* species, type of tissue and with plant age, and consequently the concentration and type of hydrolysis products also vary (Mithen, 1992). Extracts from the same *Brassica* species may therefore be inhibitory or stimulatory to the target organism, depending on plant part, growth stage, or other conditions (Jiménez-Orsonio & Gleissman, 1987). Despite their non-specific action on proteins and amino acids, individual ITCs also vary in toxicity to different organisms (Brown & Morra, 1997). Efficacy of biofumigation can furthermore be affected by temperature (Borek *et al.*, 1995), soil type (Lehman, 1942; Mason-Sedun *et al.*, 1986; Matthiesson *et al.*, 1996), moisture and organic matter content (Borek *et al.*, 1995). Optimal control of a specific organism would thus depend on the *Brassica* species, type and age of the tissue, mode of application, and environmental and cultural variables.

ITCs have been applied successfully for the control of wireworms (Toba, 1984), root-knot nematode (Mojtahedi *et al.*, 1993), weeds (Boydston & Hang, 1995) and wilt caused by *Verticillium dahliae* Kleb. (Davis *et al.*, 1996) in potato (*Solanum tuberosum* L.) fields. Biofumigation has also been reported to suppress a number of pathogens known to cause disease in potato, e.g. *Fusarium sambucinum* Fuckel (Mayton *et al.*, 1996), *Sclerotium rolfsii* Sacc., *Pythium ultimum* Trow (Stapleton & Duncan, 1998), *Rhizoctonia solani* J. G. Kühn (Lewis & Papavizas, 1974; Sarwar *et al.*, 1998; Harding & Wicks, 2000), *Colletotrichum coccodes* (Wallr.) S. Hughes, *Phytophthora erythroseptica* Pethybr., *Phytophthora cryptogea* Pethybr. & Laff. (Harding & Wicks, 2000) and *Ralstonia solanacearum* (Akiew *et al.*, 1996). No report could be traced describing the effect of brassica residues on common scab of potato, caused by *Streptomyces scabiei*. Considering that *S. scabiei* is a

Gram-positive organism it should be relatively sensitive to ITCs (Fenwick *et al.*, 1983; Smelt *et al.*, 1989). This study aimed at establishing whether common scab can be controlled by soil incorporation of residues of brassicaceous crops and if *Brassica* species differ in efficacy in this regard.

MATERIALS AND METHODS

Greenhouse experiment

A naturally infested Hutton soil (pH 6.7) producing potato crops with a common scab index of 48 (see p98 for formula) was obtained from KwaZulu-Natal in South Africa. A portion of the soil was sterilised by autoclaving for 30 minutes at 120 °C on two consecutive days. The soil was dispensed into one hundred 25-cm-diameter plastic pots, 80 pots receiving non-autoclaved soil and 20 pots autoclaved soil. Cabbage (*Brassica oleracea* L. var. *capitata* L.) cv. Tenacity seedlings were planted, two seedlings per pot, to 60 of the pots with non-autoclaved soil. All the pots were maintained in a greenhouse at 8/25 °C (night/day) and received regular irrigation through a mini-sprinkler system, as well as 100 ml of a 0.1 % Chemicult ® nutrient solution (see p83 for formulation) per pot every third week. After 90 days, the heads of all plants were separated from the roots. Approximately 3 kg of the outer leaves (remaining after harvesting) was blended in a Waring blender while a further 3 kg was dried and ground in a laboratory mill. The soil was removed from all pots and the cabbage roots were sieved from soil planted to the crop. The soil was then treated or left as follows:

1) Dried cabbage leaves mixed at 1 g l^{-1} (0.1 % m/v) into non-autoclaved soil to which cabbage had previously been planted.
2) Fresh blended cabbage leaves mixed at 2.5 g l^{-1} (0.25 % m/v) into non-autoclaved soil to which cabbage had previously been planted.
3) Non-autoclaved soil to which cabbage had previously been planted.
4) Non-autoclaved soil not previously planted to cabbage.
5) Autoclaved soil not previously planted to cabbage.

The various soils were returned to the respective pots, 20 pots per treatment, and each pot was planted to two common scab-free minitubers of the potato cultivar BP1. Pots were randomly arranged in the above greenhouse and received irrigation and mineral nutrition as previously. The potatoes were grown to maturity (90 days), harvested, and the progeny tubers assessed for common scab according to the rating scale of Marais & Vorster (1988):

0 =	Clean
1 =	1-12 %
2 =	13-25 % scab
3 =	26-50 % scab
4 =	51-75 % scab
5 =	76-100 % scab
6 =	Superficial scab
7 =	Russet/netted scab
11=	Deep pitted scab

A value with a possible maximum of 55 was calculated for each tuber according to the formula:

Scab index = area covered with scab (0 - 5 above**) x lesion type (**6, 7, 11**)**

Scab indices between 0 and 55 are mostly skewly distributed and the median for each experimental unit was therefore used, as it is more reliable than the mean in such cases. The entire experiment was conducted twice. Trends observed in the two repeats of the experiment did not differ and data were therefore pooled for statistical analysis. Data were analysed by analysis of variance (ANOVA) and treatment means were separated using Tukey's multiple range test at 5 % level of confidence.

Field experiment

The effect of soil amendment with cabbage cv. Tenacity, cauliflower (*B. oleracea* L. var. *botrytis* L.) cv. Wallaby, broccoli (*B. oleracea* var. *italica* Plenck) cv. Arcadia and Brussels sprouts (*B. oleracea* var. *gemmifera* DC.) cv. Royal Marvel on common scab was evaluated in a naturally-infested field (common scab index of 50) at ARC-Roodeplaat, Pretoria, South Africa. The soil, a Hutton with pH 8.1, was under potato monoculture for ten consecutive seasons prior to the experiment. The experimental design comprised four random 6x1 m plots per treatment, i.e. each of the four brassica crops, and a control that remained fallow for the period that the brassicas were grown. Plots were separated by 50-cm-wide paths paved with concrete slabs.

Two rows spaced 30 cm apart were planted to the respective *Brassica* species at an intra-row spacing of 40 cm in each plot in April 2000. The crops were irrigated by an overhead sprinkler system according to evaporation in a class A evaporation pan. Fertiliser (2:3:2 (22) NPK) was applied through the sprinklers at planting at a rate of 2 g per seedling. Sprouts were harvested at maturity of the crops (approximately 90 days) and the residues (outer leaves and small heads) were collected, dried, and ground in a hammer-mill. The ground residues were evenly worked into the soil at 0.33 % m/v to a depth of about 20 cm in the respective plots in July 2000. Two weeks later, disease-free generation-one BP1 potato tubers were planted to all the plots at the same spacing as the brassica crops. Irrigation and fertigation were applied as above until October 2000, 90 days after planting, when the progeny tubers were harvested. One-hundred randomly-selected medium-sized tubers from each plot were assessed for common scab according to the above rating scale. Data were analysed statistically using GenStat (2003).

RESULTS

Greenhouse experiment

Incorporation of dry and fresh cabbage residues reduced common scab by a significant 41 % and 32 %, respectively (Fig. 5.1). Planting the soil to cabbage only resulted in a non-significant 18 % reduction in disease.

Field experiment

Residues of the four brassicaceous crops reduced common scab to the same extent when incorporated into the soil (*P*=0.0479) (Fig. 5.2). For all crops the percentage scabby tubers in the 0-5 category was significantly the highest, with a sharp decline in

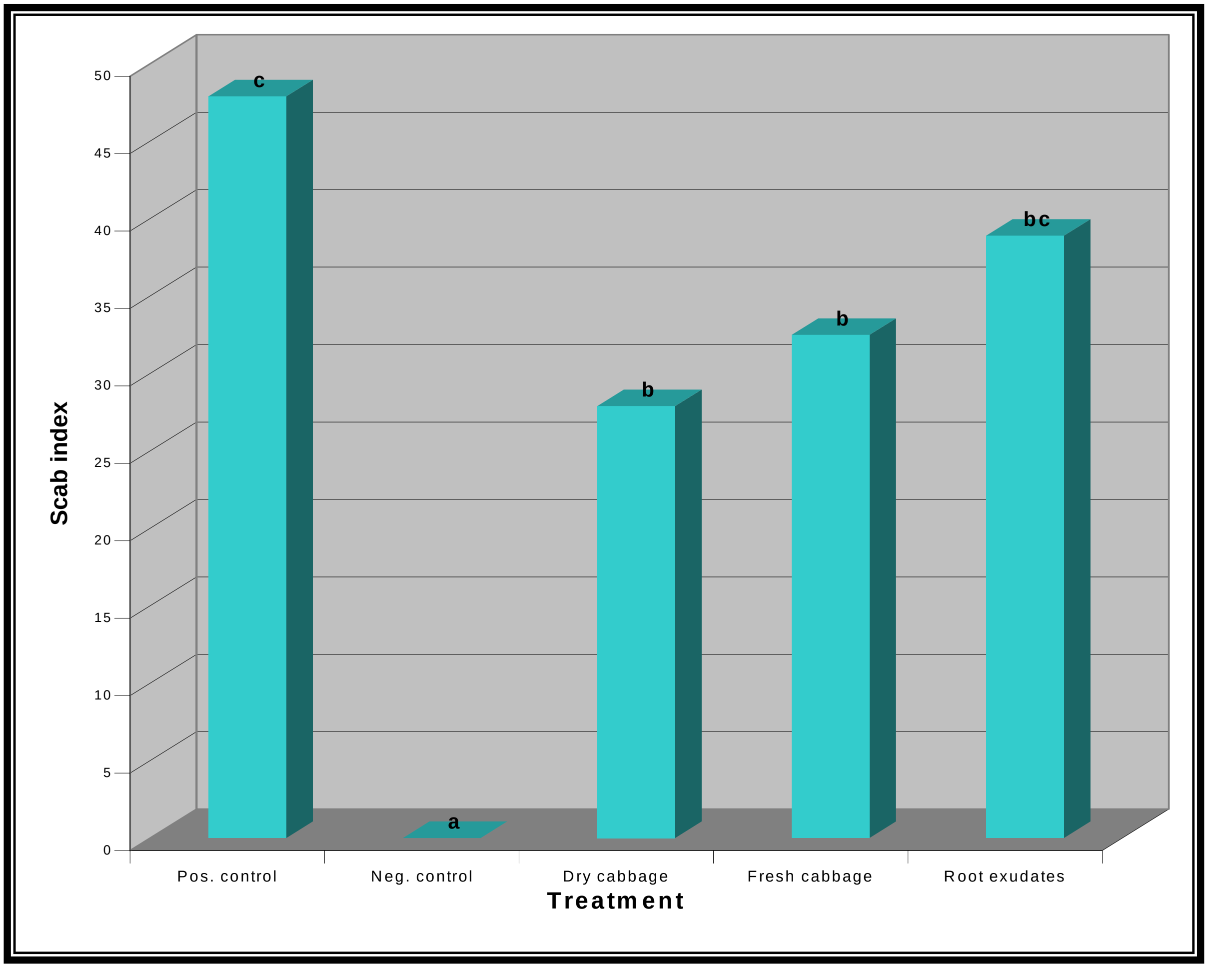

Fig. 5.1 Effect of cabbage amendments on common scab of potato in the greenhouse. Scab indices with the same letter do not differ significantly according to Tukey's multiple range test ($P \leq 0.05$).

incidence towards the 6-11 and subsequent ranks. Virtually all the control tubers had a scab rating of 36-55.

DISCUSSION

This study, of which the results have been published (Gouws & Mienie, 2000; Gouws & Wehner, 2004), is the first to show that common scab can be effectively controlled by biofumigation. Wiggens & Kinkel (2005) recently reported that green manuring of a field soil with canola (*Brassica napus* L. var. *oleifera* Delile) did not reduce percentage scab significantly compared to a fallow control in either of two consecutive seasons. However, they also failed to obtain control of *Verticillium* wilt caused by *V. dahliae*,

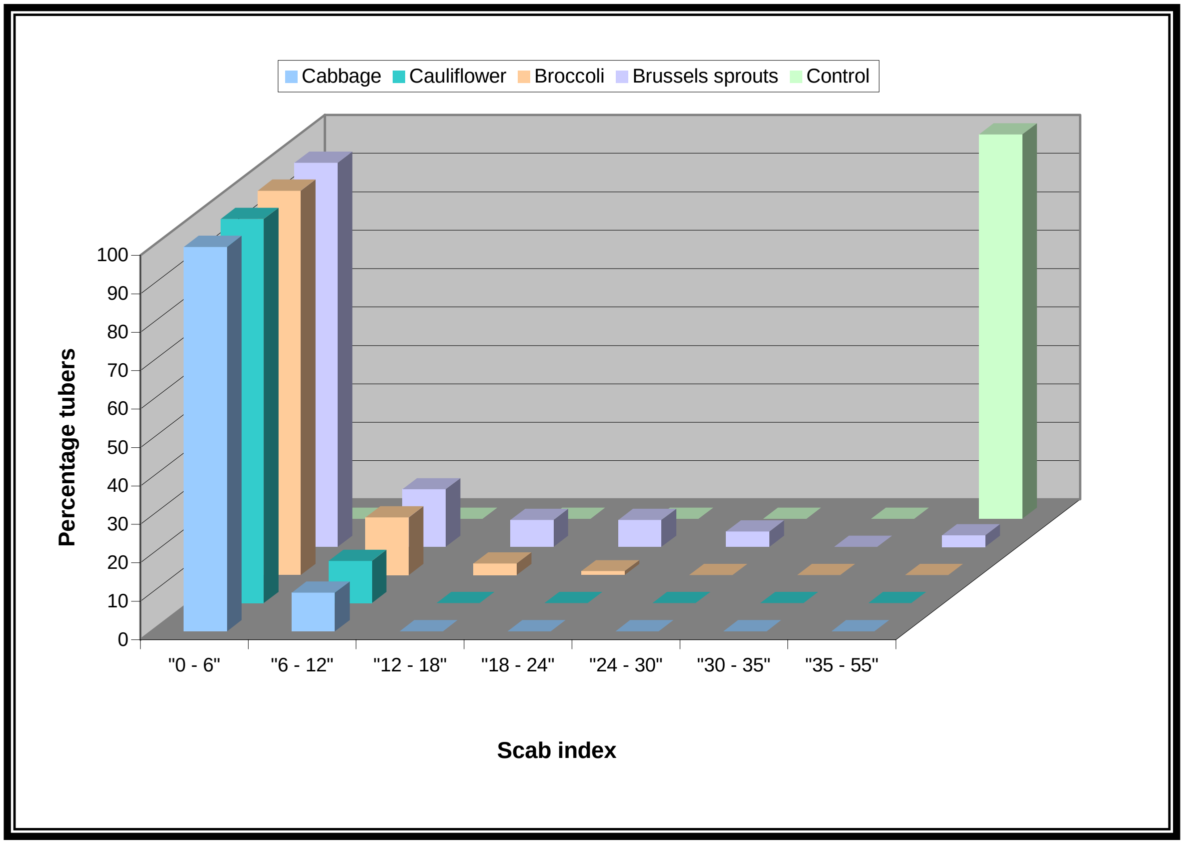

Fig. 5.2 Effect of soil incorporation of brassicaceous crop residues on common scab of potato in the field

despite the known sensitivity of this pathogen to biofumigation by brassicaceous crops, including canola (Davis *et al.*, 1996; Subbarao *et al.*, 1999; Koike & Subbarao, 2000; Millard & Wehner, 2001), which indicates that conditions were not conducive to biofumigation. Results of the present study suggest that the efficacy of biofumigation against *S. scabiei* is rate-related, considering the greater reduction (approximately 90 %) achieved in the field with an application rate of 0.33 % dry tissue than the 41 % reduction obtained in the greenhouse by amendment with 0.1 % dry material. Interestingly, total suppression of the scab pathogen, as achieved in Chapter 4 with root exudates of cabbage, could not be accomplished, probably because *S. scabiei* inoculum in the above chapter was artificially introduced into the substrate two weeks after planting of the cabbage, hence allowing the seedlings to produce and exude ITCs in sufficient quantities to render the substrate inhospitable to *S. scabiei* prior to arrival of the pathogen.

The type and quality of GSLs vary considerably between plants of different families, between plant in the same family, within a particular species and within organs of an individual plant (Clossais-Besnard & Larher, 1991; Brown & Morra, 1997; Kushad *et al.*, 1999). GSL concentrations furthermore fluctuate depending on tissue type, stage of development and environmental factors such as plant spacing, moisture regime and nutrient availability (Brown & Morra, 1997), making comparisons between separate studies difficult. It is nevertheless evident from the field experiment in this study that cabbage, cauliflower, broccoli and Brussels sprouts did not differ in efficacy towards *S. scabiei*. Subsequent field trials with the above crops, as well as with canola, showed that they suppressed common scab to the same extent, but with dry plant tissue consistently providing more effective control than fresh material or rotation with brassicaceous crops without incorporation of residues into the soil. The precise mode of action, however, is unclear and should be investigated further.

When considering biofumigation as a disease control option, cognisance should be taken of the fact that livestock poisoning is fairly common in animals consuming excessive quantities of brassicaceous plant material (Kingsbury, 1964). It is furthermore known that extracts of *Brassica* species are inhibitory to endomycorrhizal fungi (Vierheilig & Ocampo, 1990; Schreiner & Koide, 1993a,b) on which potatoes seem to be particularly dependent for optimal growth (Gerdemann, 1968), and that the *Brassicaceae* do not establish symbiosis with mycorrhizal endophytes (Gerdemann, 1968; Glenn *et al.*, 1985, 1988). More importantly, however, is the notoriety of GSL-containing plants to have a negative impact on successive plant communities or those growing in close proximity (Brown & Morra, 1997). Although potatoes seem to be amendable to ITC-producing fumigants (Toba, 1984; Mojtahedi *et al.*, 1993), a study by Leach *et al.* (1991) has shown potatoes after broccoli to have a significantly lower yield than after oats (*Avena sativa* L.), buckwheat (*Fagopyrum esculentum* Moench), pea (*Pisum sativum* L.) or lupin (*Lupinus* sp.) and also a very high incidence of secondary tuber growth. Phytotoxicity was not determined in the present study, but observations in the additional field trials referred to above revealed no adverse effect on growth or yield of potatoes planted to soil amended with up to 1.56 % dry brassicaceous material.

Probably the most important aspect of biofumigation is the logistics of such a control strategy. Of the various *Brassica* species tested, only cabbage is produced in significant quantities in South Africa. To apply biofumigation to the total area under potatoes in South Africa at a rate of 0.33 % dry residue would require the entire cabbage crop of six seasons, which simply does not make sense as cabbage can be cultivated more profitably than potato. However, when considering the broad spectrum of activity of biofumigation against fungal and bacterial pathogens, as well as nematodes, insects and weeds (Brown & Morra, 1997), it may eventually prove to be cost-effective on a commercial scale. In South Africa, biofumigation has already been shown to effectively control *Verticillium* wilt (Millard & Wehner, 2001) and rhizoctoniasis (Truter, 2005) in potato. Studies are currently in progress to establish optimal rates of application, the spectrum and persistence of activity, the efficacy of "new" and novel brassicaceous species such as mustard (*Brassica juncea* (L.) Czerniak) and to elucidate the mode of action involved.

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APPENDIX A

Characteristics of the 57 *Streptomyces* isolates from the Northern Cape common scab survey.

Isolate	Group	Minimum pH	Melanin production [a]	Spore chain morphology [b]	Pathogenicity [a]	Thaxtomin A Production [a]
DNK 005	1	5.5	+	Sp	+ (5/8)	+
DNK 007	1	4.5	+	Sp	+ (8/8)	+
DNK 016	1	5.5	+	Sp	+ (8/8)	+
DNK 020	1	4.5	+	Sp	+ (4/8)	+
DNK 021	1	5	+	Sp	+ (6/8)	+
DNK 023	1	5.5	+	Sp	+ (3/8)	+
DNK 025A	1	5	+	Sp	+ (3/8)	+
DNK 032	1	5	+	Sp	+ (3/8)	+
DNK 034	1	4.5	+	Sp	+ (3/8)	+
DNK 036	1	5	+	Sp	+ (7/8)	+
DNK 037	1	6	+	Sp	+ (5/8)	+
DNK 041	1	4.5	+	Sp	-	+
DNK 043	1	4.5	+	Sp	+ (8/8)	+
DNK 045	1	4.5	+	Sp	+ (4/8)	+
DNK 046	1	4.5	+	Sp	+ (4/8)	+
DNK 050	1	5	+	Sp	+ (5/8)	+
DNK 052	1	4.5	+	Sp	+ (5/8)	+
DNK 053	1	6	+	Sp	+ (4/8)	+
DNK 055	1	5	+	Sp	+ (8/8)	+
DNK 057	1	5.5	+	Sp	+ (3/8)	+
DNK 058	1	5	+	Sp	+ (7/8)	+
DNK 059	1	5	+	Sp	+ (6/8)	+
DNK 065	1	6	+	Sp	-	-
DNK 067	1	5	+	Sp	-	-
DNK 076	1	4.5	+	Sp	+ (8/8)	+
DNK 079	1	4.5	+	Sp	-	-

Isolate	Group	Minimum pH	Melanin production	Spore chain morphology	Pathogenicity	Thaxtomin A production
DNK 027A	2	6	-	Sp	-	-
DNK 039	2	4.5	-	Sp	-	-
DNK 025B	2	5.5	-	Sp	-	-
DNK 030	2	5	-	Sp	-	-
DNK 054	2	5	-	Sp	-	-
DNK 056	2	5	-	Sp	-	-
DNK 006	3	5	+	Rf	-	-
DNK 017	3	5	+	Rf	+ (5/8)	+
DNK 038	3	5	+	Rf	+ (8/8)	+
DNK 069	3	5	+	Rf	+ (8/8)	+
DNK 010	3	4.5	+	Rf	-	-
DNK 044	4	6	-	Rf	-	-
DNK 003	4	5	-	Rf	-	-
DNK 004	4	6	-	Rf	-	-
DNK 012	4	5.5	-	Rf	-	-
DNK 014	4	5.5	-	Rf	-	-
DNK 026	4	6	-	Rf	-	-
DNK 029	4	5.5	-	Rf	-	-
DNK 071	4	6	-	Rf	-	-
DNK 075	4	6	-	Rf	-	-
DNK 011	5	4.5	-	Rf	-	-
DNK 019	5	4.5	-	Sp	-	-
DNK 040	5	4.5	-	Rf	-	-
DNK 063	5	6	-	Rf	-	-
DNK 064	5	5	-	Rf	-	-
DNK 066	5	4.5	-	Rf	-	-
DNK 015	6	5.5	-	Rf	-	-
DNK 027B	6	5	-	Rf	-	-
DNK 033	6	4	-	Rf	-	-

Isolate	Group	Minimum pH	Melanin production	Spore chain morphology	Pathogenicity	Thaxtomin A production
DNK 048	6	5	-	Rf	-	-
DNK 061	6	6	-	Rf	-	-

a + = positive and - = negative melanin production / pathogenicity / toxin production.

b Sp = spiral spore chains and Rf = flexuous spore chains.

APPENDIX B

Characteristics of the 32 *Streptomyces* isolates from the Limpopo common scab survey.

Isolate	Group	Minimum pH	Melanin production[a]	Spore chain morphology[b]	Pathogenicity[a]	Thaxtomin A production[a]
VNT 023	1	5.5	+	Sp	+ (3/8)	+
VNT 033	1	6	+	Sp	-	-
VNT 035	1	6	+	Sp	+ (3/8)	+
VNT 039	1	5.5	+	Sp	-	-
VNT 041	1	6	+	Sp	+ (3/8)	-
VNT 047	1	6	+	Sp	-	-
VNT 048	1	6	+	Sp	-	-
VNT 049	1	6	+	Sp	-	-
VNT 065	1	6	+	Sp	+ (3/8)	+
VNT 073	1	6	+	Sp	+ (3/8)	+
VNT 083	1	6	+	Sp	+ (3/8)	+
VNT 043	2	6	-	Sp	+ (3/8)	-
VNT 046	2	6	-	Sp	-	-
VNT 064	2	5.5	-	Sp	+ (3/8)	+
VNT 079	2	4.5	-	Sp	+ (3/8)	+
VNT 024	3	6	+	[d]Rf	+ (3/8)	+
VNT 026	3	5.5	+	Rf	-	+
VNT 029	3	5	+	Rf	-	+
VNT 034	4	6	-	Rf	-	-
VNT 086	4	5	-	Rf	-	-
VNT 025	5	5.5	-	Rf	-	-
VNT 027A	5	5.5	-	Sp	-	-
VNT 027B	5	5.5	-	Sp	-	-
VNT 030	5	6	-	Rf	-	-

Isolate	Group	Minimum pH	Melanin production	Spore chain morphology	Pathogenicity	Thaxtomin A production
VNT 042	5	5.5	-	Rf	-	-
VNT 076	5	5	-	Rf	-	-
VNT 037	6	6	-	Rf	-	-
VNT 045	6	6	-	Rf	-	-
VNT 068	6	5.5	-	Rf	-	-
VNT 071	6	5	-	Rf	-	-
VNT 075	6	5	-	Rf	-	-
VNT 081	6	4	-	Rf	-	-

a + = positive and - = negative melanin production / pathogenicity / toxin production.

b Sp = spiral spore chains and Rf = flexuous spore chains.

APPENDIX C

Characteristics of the 12 *Streptomyces* isolates from the Eastern Free State common scab survey.

Isolate	Group	Minimum pH	Melanin production [a]	Spore chain morphology [b]	Pathogenicity [a]	Thaxtomin A production [a]
SOO 001	1	4.5	+	Sp	+ (3/8)	+
SOO 002	1	4.5	+	Sp	+ (5/8)	+
SOO 008	1	4.5	+	Sp	+ (3/8)	+
SOO 009	1	5.5	+	Sp	+ (3/8)	+
SOO 010	1	5.5	+	Sp	+ (4/8)	+
SOO 013	1	4.5	+	Sp	+ (3/8)	+
SOO 015	1	5.5	+	Sp	-	-
SOO 004	2	5.5	-	Sp	+ (3/8)	+
SOO 005	2	5.5	-	Sp	+ (3/8)	+
SOO 007	2	5.5	-	Sp	+ (3/8)	+
SOO 006	4	5.5	-	Rf	-	-
SOO 016	4	5.5	-	Rf	-	-

[a] + = positive and - = negative melanin production / pathogenicity / toxin production.

[b] Sp = spiral spore chains and Rf = flexuous spore chains.

APPENDIX D

Characteristics of the 19 *Streptomyces* isolates from the Ceres common scab survey.

Isolate	Group	Minimum pH	Melanin production [a]	Spore chain morphology [b]	Pathogenicity [a]	Thaxtomin A production [a]
CEK 001	1	4	+	Sp	-	-
CEK 004	1	5	+	Sp	+ (5/8)	+
CEK 006	1	5	+	Sp	-	-
CEK 007	1	5	+	Sp	+ (7/8)	+
CEK 010	1	5	+	Sp	+ (8/8)	+
CEK 016	1	4.5	+	Sp	+ (7/8)	+
CEK 018	1	5	+	Sp	+ (7/8)	+
CEK 022	1	5	+	Sp	+ (4/8)	+
CEK 028	1	5	+	Sp	+ (7/8)	+
CEK 031	1	5	+	Sp	+ (8/8)	+
CEK 034	1	4.5	+	Sp	+ (5/8)	+
CEK 036	1	5	+	Sp	+ (3/8)	+
CEK 037A	1	4.5	+	Sp	+ (7/8)	+
CEK 037B	1	5	+	Sp	+ (8/8)	+
CEK 038A	1	4.5	+	Sp	+ (7/8)	+
CEK 038B	1	4	+	Sp	+ (8/8)	-
CEK 002	1	5	+	Sp	-	-
CEK 033	2	5	-	Sp	+ (4/8)	+
CEK 008	3	5	+	Rf	+ (3/8)	+

[a] + = positive and - = negative melanin production / pathogenicity / toxin production.

[b] Sp = spiral spore chains and Rf = flexuous spore chains.

APPENDIX E

Characteristics of the 25 *Streptomyces* isolates from the Sandveld common scab survey.

Isolate	Group	Minimum pH	Melanin production [a]	Spore chain morphology [b]	Pathogenicity [a]	Thaxtomin A Production [a]
SAV 011	1	4	+	Sp	-	-
SAV 013	1	4	+	Sp	-	+
SAV 014	1	4	+	Sp	+ (8/8)	+
SAV 015	1	4	+	Sp	+ (5/8)	+
SAV 016	1	4	+	Sp	+ (4/8)	-
SAV 017	1	4	+	Sp	+ (6/8)	-
SAV 023	1	4	+	Sp	+ (3/8)	+
SAV 025	1	4	+	Sp	+ (4/8)	-
SAV 027	1	4	+	Sp	+ (3/8)	-
SAV 030	1	4	+	Sp	+ (3/8)	-
SAV 031	1	4	+	Sp	+ (6/8)	-
SAV 032	1	4	+	Sp	+ (5/8)	+
SAV 034	1	4.5	+	Sp	+ (5/8)	-
SAV 026	1	5.5	+	Sp	+ (3/8)	+
SAV 007	2	5.5	-	Sp	+ (4/8)	+
SAV 018	2	4	-	Sp	-	-
SAV 022	2	5	-	Sp	-	-
SAV 012	4	4	-	Rf	-	-
SAV 003	5	6	-	Rf	-	-
SAV 004	5	5	-	Rf	-	-
SAV 009	5	4	-	Rf	-	-
SAV 019	5	6	-	Rf	-	-
SAV 020	5	4	-	Rf	-	-
SAV 024	5	4.5	-	Rf	-	-
SAV 021	6	4	-	Rf	-	-

a + = positive and - = negative melanin production / pathogenicity / toxin production.

b Sp = spiral spore chains and Rf = flexuous spore chains.

APPENDIX F

Streptomyces isolates from the five potato production areas surveyed capable of growing at pH 4

Isolate	Group	Melanin production [a]	Spore chain morphology [b]	Pathogenicity [a]	Thaxtomin A production [a]
CEK 001	1	+	Sp	-	-
CEK 038B	1	+	Sp	+ (8/8)	-
SAV 011	1	+	Sp	-	-
SAV 013	1	+	Sp	-	+
SAV 014	1	+	Sp	+ (8/8)	+
SAV 015	1	+	Sp	+ (5/8)	+
SAV 016	1	+	Sp	+ (4/8)	-
SAV 017	1	+	Sp	+ (6/8)	-
SAV 023	1	+	Sp	+ (3/8)	+
SAV 025	1	+	Sp	+ (4/8)	-
SAV 027	1	+	Sp	+ (3/8)	-
SAV 030	1	+	Sp	+ (3/8)	-
SAV 031	1	+	Sp	+ (6/8)	-
SAV 032	1	+	Sp	+ (5/8)	+
SAV 018	2	-	Sp	-	-
SAV 012	4	-	Rf	-	-
SAV 009	5	-	Rf	-	-
SAV 020	5	-	Rf	-	-
SAV 021	6	-	Rf	-	-
DNK 033	6	-	Rf	-	-
VNT 081	6	-	Rf	-	-

a + = positive and - = negative melanin production / pathogenicity / toxin production.

b Sp = spiral spore chains and Rf = flexuous spore chains.

APPENDIX G

Rifampicin-resistant *Streptomyces scabiei* isolates included in the survival study (Chapter 4).

Isolate	Origin
DNK 037	Northern Cape
DNK 038	Northern Cape
DNK 043	Northern Cape
DNK 058	Northern Cape
DNK 076	Northern Cape
CEK 010	Ceres
CEK038B	Ceres
SAV013	Sandveld
SAV014	Sandveld
SAV015	Sandveld
SAV017	Sandveld
SOO 002	Eastern Free State