Quantification and characterization of the *Streptomyces* complex causing common scab of potatoes in South Africa

by

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Declaration

I, Estiene Jordaan, declare that the thesis, which I hereby submit for the degree Magister Scientiae (Agriculture) Plant Pathology at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

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Abstract

Common scab is a disease that results in corky lesions on the surface of potatoes. These lesions can be variable in size, shape and as a result of secondary infection may appear extremely cryptic. The disease is caused by *Streptomyces*, a group of Actinomycetes that are largely saprophytic. For this reason lesions may also contain saprophytic *Streptomyces* which makes isolation and detection of pathogenic species on potatoes difficult. Symptom expression is thought to be attributed to environmental factors and prevalence of pathogenic species.

Common scab causing *Streptomyces* isolates were collected from most of the potato producing regions in South Africa. These isolates were characterized based on morphology, physiology and sequenced to confirm identity and determine their closest related species. A diverse range of other Streptomycetes, similar in morphology and physiology to *Streptomyces scabiei* (Lambert & Loria), were frequently isolated during this study from common scab lesions. Most Streptomycetes isolated from common scab lesions were *S. scabiei* or *Streptomyces europaeiscabiei* (Bouchek-Mechiche). No *Streptomyces acidiscabies* (Lambert & Loria) or *Streptomyces turgidiscabies* (Miyajima) were found. *Streptomyces stelliscabiei* (Bouchek-Mechiche) appears to be more geographically restricted in South Africa.

The presence of *txtAB, tomA* and *nec1* genes were investigated within pathogenic as well as non-pathogenic isolates associated with common scab lesions. Some isolates were pathogenic but

lacked any of the pathogenicity / virulence genes; while others were non-pathogenic but showed the presence of at least one of the three genes. However it seems that the combined presence of all three these genes is a good indicator of the pathogenicity of an isolate when compared with the tuber slice assay for pathogenicity.

Although common scab does not decrease yield, it results in smaller progeny tubers. A complex interaction exists between common scab causing Streptomycetes and their soil environment – increasing soil pH from 6.5 to 8.5 decreases disease incidence and severity. Symptoms were not found to be linked to species, soil pH or soil moisture. An increase in initial inoculum contributes to an increase in disease incidence and severity.

In future uncertainty regarding the pathogenicity gene makeup of the South African *Streptomyces* complex must be resolved. This will include sequencing the most important genes related to pathogenicity and comparing South African isolates with the isolates from other countries. The detection of these genes with Thaxtomin A expression (HPLC) should also be examined. Should these two factors co-exist it will then be possible to design primers specific to South African pathogenic *Streptomyces* spp. that could possibly be used for quantitative detection pre-plant.

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CHAPTER 1

General introduction

Common scab is said to be caused by *Streptomyces scabiei* (Lambert & Loria). It is known to decrease processing potential and marketability of potatoes. Few *Streptomyces* species are pathogenic (Loria *et al.,* 2003). Some are very similar to *S. scabiei* based on morphology, physiology and genetic make-up but are not pathogenic on potatoes (*Solanum tuberosum* L.) (Doumbou *et al.,* 2001). Common scab is a problem world-wide under a wide range of environmental conditions, and cultural disease management is strongly relied upon for disease control.

This pathogen is also isolated and thus associated with the disease in South Africa (Doidge, 1950; Gouws, 2006). However, this is not the only Streptomycete isolated from scab lesions, which led us to believe that there may be a strong interaction between species or even different Streptomycetes at work. Other species of *Streptomyces* causing common scab are not uncommon, but the isolates associated with common scab in South Africa have not yet been characterized or quantified. The diversity of *Streptomyces* associated with common scab has lately received more attention (Doering-saad *et al.,* 1992; Takeuchi *et al.,* 1996; Lindholm *et al.,* 1997; Kreuze *et al.,* 1999; Wanner, 2006; Flores-González *et al.,* 2008; St-Onge *et al.,* 2008; Wanner, 2009; Leiminger *et al.,* 2012; Pánková *et al.,* 2012; Tashiro *et al.,* 2012; Dees *et al.,* 2013). During this study a large number of other *Streptomyces* (mostly non-pathogenic) were isolated from common scab lesions, but their role in disease incidence and severity is not yet fully understood or investigated. Most *Streptomyces* in South Africa isolated from tuber lesions resemble *S. scabiei* and although literature states that *txtAB* is the most reliable gene to associate pathogenicity with, it is becoming clearer that a combination of the three genes (*txtAB, tomA, nec1*) is a better indicator of pathogenicity in South Africa.

The environmental conditions associated with common scab are aerated and slightly alkaline soils (Loria *et al.,* 1997). These are however not the only environmental factors conducive to common scab. The role of other factors such as available nutrients in the soil and the soil texture may also be critical. All of these factors interact with one-another and it is becoming increasingly difficult to pinpoint the exact condition that will lead to common scab. Management strategies can be

adjusted to fit the unique *Streptomyces* spp. present in the soil, rather than staying focused on *S. scabiei* as the main causal organism; for instance increasing the soil moisture content or decreasing soil pH when the primary causal agents are *Streptomyces turgidiscabiei* or *Streptomyces acidiscabies,* which can tolerate these altered conditions and still cause disease.

Disease risk assessment is becoming more important, as knowledge of the soils' potentials and its limitations could help make optimal use of a limited resource (Cullen & Lees, 2007). Sparrow & Wilson (2012) extracted DNA from infected soil to determine the amount of common scab initial pre-plant inoculum, but found that almost no *Streptomyces* spp. could be detected, though there was 20% post-harvest incidence. Unfortunately they did not specify which gene they targeted for amplification. Khodakaramain & Khodakaramain (2012) coupled a completely different pathogenicity factor (other than *txtAB*) with deep-pitted symptoms. For this and numerous other reasons it is still difficult to rely on merely genetic, physiological or morphological methods of identification and quantification. It is important to first fully understand the factors that influence common scab and with it the complex of species causing symptoms and how they interact.

This study tries to better understand some of the factors involved in disease development of common scab. In **Chapter 3** the diversity of Streptomycetes associated with common scab lesions, from most of the potato production areas in South Africa, is determined and these isolates are characterized. **Chapter 4** focuses on the association between pathogenicity and the presence of pathogenicity / virulence factors identified in common scab causing isolates in other countries. The effect of initial inoculum, different pathogenic isolates, soil pH and soil moisture on disease incidence, severity, yield, number of tubers and symptom expression is examined in **Chapter 5**.

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CHAPTER 2

A literature review of common scab on potatoes

2.1 INTRODUCTION

The *Streptomyces* genus is one of a few groups of gram positive bacteria that are known to contain pathogenic species (Loria *et al.,* 2003). These pathogens cause scab and gall diseases of underground plant structures. The causal organism was first described in 1892 (Doering-saad *et al.,* 1992). Of the more than 400 described species, only a few are pathogenic (Loria *et al.,* 1997). *Streptomyces scabiei* (Thaxter) Waksman & Henrici is one of the well known and first described causal organisms of common scab on potatoes (*Solanum tuberosum* L.). Other species, differing in morphology and physiology, are also known to cause common scab symptoms on potatoes. Others however, that are morphologically and physiologically similar to *S. scabiei*, do not cause disease on potatoes (Doumbou *et al.,* 2001). For this reason, genetic characterization plays a critical role in differentiating between pathogenic and non-pathogenic species.

Common scab is a well known disease on potatoes throughout potato growing regions world-wide (Takeuchi *et al.,* 1996; Loria *et al.,* 1997; Bouchek-Mechiche *et al.,* 2000a; Lehtonen *et al.,* 2004). To date a variety of symptoms have been described, including shallow, raised, netted and deep pitted lesions, due to a range of species that are capable of causing disease under low or high soil moisture conditions as well as low or high soil temperatures (Loria *et al.,* 1997). The relationships between species, environment, cultivar and symptom expression are not clear. Pathogenicity is said to be acquired by non-pathogenic species through horizontal transfer of genes involved in pathogenicity and virulence. These genes cluster together in a region known as the pathogenicity island (PAI). Most of the disease management strategies have relied strongly upon chemical and cultural control (Loria *et al.,* 1997). The need for alternative measures is becoming increasingly important, as the agricultural industry is moving away from chemical control. There is also a tendency towards prevention rather than control, where disease risk assessment plays a critical role.

2.2 HOST

Most studies on pathogenic Streptomycetes have been on potato, but *Streptomyces* species can cause similar diseases on other tap root crops such as radishes (*Raphanus sativus* L.) and beet

(*Beta vulgaris* L.) (Agrios, 2005). *Streptomyces* spp. were also found to cause pod wart of peanut (*Arachis hypogaea* L.) in South Africa (Loria *et al.,* 1997). Carrot (*Daucus carota* L.) is another crop well known to be affected by *Streptomyces* spp. (Loria *et al.,* 1997; Pasco *et al.,* 2005). *Streptomyces ipomoeae* (Waksman & Henrici) causes the death of fibrous roots and cankers on storage organs of sweet potato (*Ipomoea batatas* (L.) Lam.), a disease known as *Streptomyces* soil rot (pox) (Loria *et al.,* 2001). Although scab pathogens are well adapted to potato tubers, they are not host specific. There is no potato cultivar immune to common scab (Bouchek-Mechiche *et al.*, 2000b). In South Africa cultivars such as Mondial were once resistant to common scab but are now susceptible nine percent of isolates obtained in this study were isolated from Mondial. Most of the popular South African varieties are susceptible (Visser, 1999).

2.3 PATHOGEN

In addition to the various *Streptomyces* species that are responsible for the multitude of common scab symptoms observed on potatoes, there is also disagreement in the naming of the first described and still widely recognized causal pathogen of this disease. This causes confusion in the agricultural sector and the scientific community. In 1890 Thaxter described this microorganism and named it *Oospora scabies* (Thaxter, 1892); he noted that pathogenic strains produce a soluble brown pigment (melanin) on various culture media (Loria *et al.,* 1997). It was later renamed *Actinomyces scabies* (Güssow, 1914) but the name was again changed to *Streptomyces scabies* (Waksman & Henrici, 1989). *S. scabies* was excluded from the Approved List of Bacterial Names in 1980 because the type strain described by Waksman and Henrici differed from other reference strains (Lambert *et al.,* 2007). The name was then changed to its Latin case, *Streptomyces scabiei* (Trüper & de' Clari, 1997). In 2007 there was a request for an opinion by Lambert *et al.* (2007), who recommended that the name *S. scabies* (Waksman & Henrici, 1948; Lambert & Loria, 1989) be conserved, as the name was validly published in 1989 as *S. scabies* ((*ex* Thaxter 1891) Lambert & Loria 1989) and is the more widely used epithet. Following the request by Lambert *et al.* (2007), Trüper (2008) strongly protested. He was of the opinion that microbiologists should not only accept changes in names of genera but also accept corrections in epithets. The correct epithet remains unclear, but will be used as *S. scabiei* in this dissertation.

2.3.1 Diversity

Not one, but a complex of *Streptomyces* species can cause common scab. *Streptomyces* species are diverse and abundant in soil, but only a few are plant pathogenic (Loria *et al.,* 2001).

Saprophytic species are often isolated from soil or tissue samples. Multiple species (in isolation or in combination) have been isolated and associated with common scab, so much so that these species are now referred to as the "*Streptomyces* complex" (Wanner, 2009).

The emergence of new pathogenic strains seems to have originated from the horizontal transfer of a pathogenicity island to non-pathogenic strains (Loria *et al.,* 2003). *S. scabiei* is found in almost every potato production area world-wide (Loria *et al.,* 1997; Cullen & Lees, 2007; Wanner, 2006). *Streptomyces europaeiscabiei* (Bouchek-Mechiche) and *Streptomyces stelliscabiei* (Bouchek-Mechiche) have been reported from Europe (Pasco *et al.,* 2005). *Streptomyces reticuliscabiei* (Bouchek- Mechiche) was recently found in Europe, Canada and Finland to cause netted scab (Pasco *et al.,* 2005; Wanner, 2006). Netted scab lesions are similar to superficial common scab lesions. *Streptomyces acidiscabies* (Lambert & Loria) was first found in 1953 in Maine (Loria *et al.,* 1997), causing a disease similar to that caused by *S. scabiei*, although at a lower pH. This pathogen differs morphologically, physiologically and immunologically from *S. scabiei,* but the symptoms are often indistinguishable from those caused by *S. scabiei* (Takeuchi *et al.*, 1996). *Streptomyces luridiscabiei, Streptomyces puniciscabiei* and *Streptomyces niveiscabiei* also cause scab under more acidic conditions (Cullen & Lees, 2007). Other scab-causing species are *Streptomyces caviscabies* (Waksman & Henrici) and *Streptomyces turgidiscabies* (Miyajima), which cause erumpent lesions under high moisture conditions (Kreuze *et al.,* 1999; Bouchek-Mechiche *et al.,* 2000b; Loria *et al.,* 2006). The russet scab strains from Canada are related to *Streptomyces aureofaciens*, but most strains are unclassified. *Streptomyces griseus* and *Streptomyces aureofaciens* are less virulent strains causing common scab (Takeuchi *et al.,* 1996). Table 2.1 summarizes species capable of causing common scab on potato.

2.3.2 Morphology and physiology

Streptomyces species are filamentous Actinomycetes (Loria *et al.,* 1997; Agrios, 2005; Madigan & Martinko, 2006). They produce aerial mycelium with hyphae that are approximately 1µm in diameter. Spores are formed on spiral or filamentous mycelium, which develops cross walls where the spores are then pinched off. They are gram-positive bacteria (Lambert & Loria, 1989). *Streptomyces* culture diversity can be brown to yellow colonies, spiral spore chains of grey to white spores as can be seen in Figure 2.1 and melanoid pigments (Doumbou *et al*., 2001). Keinath & Loria (1989) estimated that only about 10% of pathogenic *Streptomyces* potato isolates resemble *S. scabiei*.

Table 2.1: Diversity of *Streptomyces* species pathogenic on potato

***Streptomyces* spp.**	**Disease symptoms on tubers**	**Geographical occurrence**	**References**
S. scabiei	Deep pitted / shallow	All potato growing regions worldwide	Takeuchi *et al.* (1996); Loria *et al.* (1997); Bouchek-Mechiche *et al.* (2000a); Bouchek-Mechiche *et al.* (2000b); Lehtonen *et al.* (2004); Cullen & Lees (2007)
S. acidiscabies	Deep pitted / shallow	North America, Japan, Korea, Hokkaido Island, Finland, Canada	Takeuchi *et al.* (1996); Loria *et al.* (1997); Kreuze *et al.* (1999): Bouchek-Mechiche *et al.* (2000a); Bouchek-Mechiche *et al.* (2000b); Lehtonen *et al.* (2004); Hiltunen *et al.* (2005); Cullen & Lees (2007)
S. aureofaciens	Superficial / netted	Canada, Finland	Takeuchi *et al.* (1996); Loria *et al.* (1997); Kreuze *et al.* (1999)
S. griseus	Superficial	Hokkaido Island, Finland	Takeuchi *et al.* (1996); Loria *et al.* (1997); Kreuze *et al.* (1999)
S. europaeiscabiei	Deep pitted / shallow	France	Bouchek-Mechiche *et al.* (2000a); Bouchek-Mechiche *et al.* (2000b); Cullen & Lees (2007)
S. stelliscabiei	Star pitted	France	Bouchek-Mechiche *et al.* (2000a); Bouchek-Mechiche *et al.* (2000b); Cullen & Lees (2007)
S. luridiscabiei	Pitted / shallow	Korea	Cullen & Lees (2007)
S. puniciscabiei	Pitted / shallow	Korea	Cullen & Lees (2007)
S. niveiscabiei	Pitted / shallow	Korea	Cullen & Lees (2007)
S. caviscabiei	Pitted / shallow	Netherlands, France, Denmark, Canada, Finland	Kreuze *et al.* (1999); Bouchek-Mechiche *et al.* (2000a); Bouchek-Mechiche *et al.* (2000b)
S. turgidiscabiei	Pitted / shallow	Japan, Scandinavia, Finland, Hokkaido Island	Kreuze *et al.* (1999); Bouchek-Mechiche *et al.* (2000b); Lehtonen *et al.* (2004)
S. reticuliscabiei	Netted	France	Bouchek-Mechiche *et al.* (2000a); Bouchek-Mechiche *et al.* (2000b)
S. cinerochromogenes	Pitted / shallow	Finland	Kreuze *et al.* (1999)
S. corchorusii	Pitted / shallow	Finland	Kreuze *et al.* (1999)
Unclassified	Russet	United States, Japan	Bouchek-Mechiche *et al.* (2000a)

Many species of *Streptomyces* produce antibiotics (Madigan & Martinko, 2006) and thaxtomin toxins are said to be produced by the pathogenic species (Wanner, 2004; Agrios, 2005). The thaxtomin toxin was isolated in 1989 (King *et al.,* 1992; Loria *et al.,* 1997). There are at least 10 derivatives of this compound, but thaxtomin A is by far the most abundant and is mostly produced

by *S. scabiei* (Cullen & Lees, 2007). Thaxtomin production is determined by growth on oatmeal agar (OMA) followed by thin layer chromatography or high performance liquid chromatography (Loria *et al.,* 1995; Florez-González *et al.,* 2008).

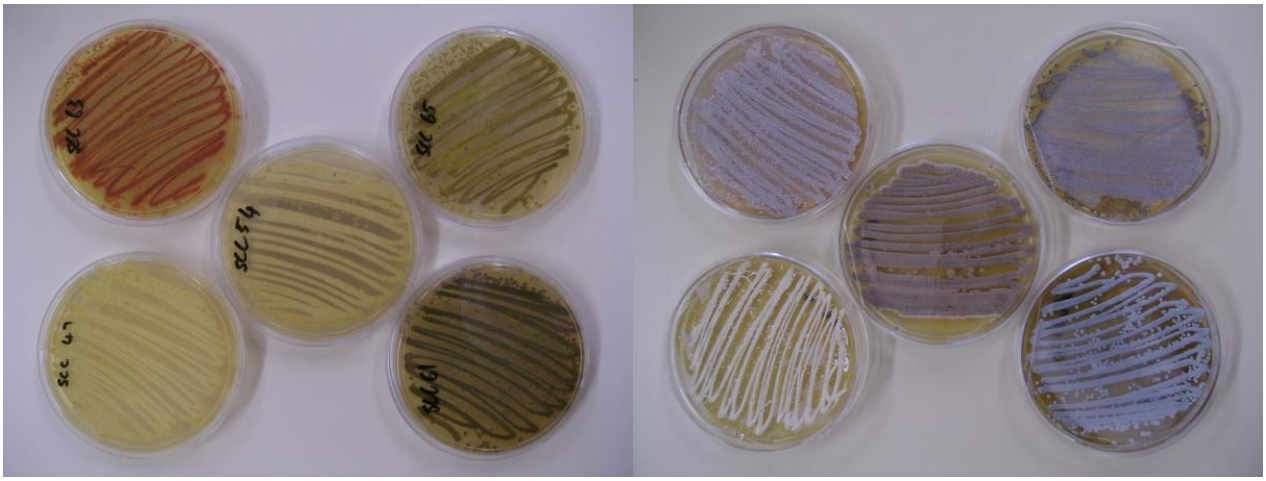

Figure 2.1: Diversity in colony and spore colour of *Streptomyces* spp. on yeast malt extract agar after 14 days.

Doumbou *et al.* (2001) found that *S. scabiei* produces on average 3mg of Thaxtomin A per gram of bacterial cells. Cullen & Lees (2007) cited an article by Conn *et al.* (1998) who reported on pathogenic *Streptomyces* strains that did not produce thaxtomin A, but caused deep-pitted lesions on tuber slices. Florez-González *et al.* (2008) on the other hand, stated that pathogenic species that do not produce thaxtomin are more the exception than the rule and are also geographically restricted. Pasco *et al.* (2005) proposed that the level of virulence is determined by the production of thaxtomins.

In literature the aetiology of common scab focuses mainly on three species; *S. scabiei, S. acidiscabies* and *S. turgidiscabies* (Loria *et al.,* 2006; Florez-González *et al.,* 2008). Although they are different in morphology, physiology and optimal environmental conditions, they show similar mechanisms for pathogenicity, as indicated by symptoms and host ranges. All three produce thaxtomin and it is believed that the thaxtomin synthesis gene is located on the pathogenicity island (Wanner, 2004; Florez-González *et al.,* 2008; Qu *et al.,* 2008).

Common scab-causing strains were (and in some instances still are) identified based on tests done according to the International *Streptomyces* Project (ISP) (Park *et al.,* 2003). Studies conducted on

the pathogenic Streptomycetes present in Finland (Lindholm *et al.,* 1997) and Korea (Park *et al.,* 2003) showed the phenotypic comparison criteria to be:

- Colony colour on YME – which could be gray to brown to yellow or variable
- Spore colour – gray, white, light yellow, pale orange and light gray
- Sporophore morphology – spiral or flexuous
- Melanin production on tyrosine and/or peptone agar
- Utilization of ISP sugars
- Minimum pH
- Resistance to various inhibitory substances
- Pathogenicity tests and
- Thaxtomin production

2.3.3 Genetic characterization

The morphological and physiological characteristics of pathogenic *Streptomyces* spp. are shared with non-pathogenic Streptomycetes, and this emphasizes not only the importance of molecular characterization, but also molecular detection (Doumbou *et al*., 2001). Two species, formerly classified as *S. scabiei* based on morphology, were recently genetically characterized as *S. europaeiscabiei* and *S. stelliscabiei* using DNA-DNA hybridization techniques (Bouchek-Mechiche *et al.,* 2000a).

DNA-DNA hybridization has shown that there is great variation in the *S. scabiei* species (Bouchek-Mechiche *et al*., 2000a). The strains differ in 16S rRNA sequences, reaction fragment length and repetitive element-PCR patterns (Wanner, 2004). Although phylogenetic analysis using the 16S rRNA gene sequence and DNA-DNA hybridization methods can be used to identify strains; it is not appropriate to use them for the study of populations in the soil, because it is expensive, time consuming and it cannot distinguish between pathogenic and non-pathogenic strains (Cullen & Lees, 2007).

In a Korean study Park *et al.* (2003) included a phylogenetic analysis based on 16S rRNA. Such phylogenetic analyses determine the similarities in 16S rRNA sequences of unknown strains compared to known strains and then group the unknown strains according to percentage similarity. Mun *et al.* (2007) emphasized practicing caution when using 16S rRNA sequence

analysis, as it may be misleading due to intraspecific variations and to a lesser extent the existence of multiple copy 16S rRNA genes with different sequences within a strain. They proposed the use of parts of the RNA polymerase (*rpoB*) gene as basis for phylogenetic differentiation.

The mobile conserved region known as the PAI contains pathogenicity genes and virulence factors that can be used to distinguish pathogenic from non-pathogenic species (Wanner, 2004; Florez-González *et al.,* 2008; Qu *et al.,* 2008; Bignell *et al.,* 2010). The gene region coding for thaxtomin production (*txtAB*), the *nec1* virulence gene (Cullen & Lees, 2007), *tomA* and *cfa6* virulence factors are all located on this island (Bignell *et al.,* 2010). As already mentioned it is believed that this PAI was, and is transferred from *S. scabiei* to other *Streptomyces* species (Wanner, 2004; Florez-González *et al.,* 2008; Qu *et al.,* 2008). There has been controversy as to which gene to target when identifying or quantifying common scab pathogen inoculum. Usually samples within a given geographical region are tested for all three genes to determine which gene is most prevalent, and this gene is then targeted for quantification. Recently a coronafacic acid-like gene region (*cfa*) was discovered by Bignell *et al.* (2010). This cfa molecule plays a role in plant-microbe interaction during infection in *Pseudomonas syringae* (van Hall) and *Pectobacterium atrosepticum* (Gardan) (Bignell *et al.,* 2010).

Although the exact role of the *nec1* gene is not clearly understood, it appears to be involved in the fitness of the pathogen, and it might allow the utilization of plant nutrients that would otherwise not be available to the pathogen (Cullen & Lees, 2007; Loria *et al.,* 2006). The presence of the thaxtomin synthesis gene and that of the Nec1 virulence factor is linked, but the nec1 protein is not needed for the production of thaxtomin. These two are thus said to be independent. Cullen & Lees (2007) proposed the use of the *nec1* for molecular detection of the pathogen, as this gene is absent in non-pathogenic strains. Florez-González *et al.* (2008) on the other hand stated that not all pathogenic strains have the *nec1* gene. The statement was based on the article by Bukhalid *et al.* (1998) and did not cite the article by Cullen & Lees (2007). Cullen & Lees did research on the pathogenic South African strains found by Bukhalid *et al.* (1998) that could not be detected by diagnostic techniques using the *nec1* gene. Cullen & Lees (2007) concluded that the *nec1* gene was in fact present and that the primers should have been used at a higher annealing temperature (64^0C). In addition Cullen & Lees (2007) mentioned other reasons for PCR failure and mentioned the presence of an insertion sequence located adjacent to the 3' end of the *nec1* gene that could also interfere with results.

2.4 THE DISEASE

Common scab of potatoes is found wherever potatoes are grown (Doering-saad *et al.,* 1992; Takeuchi *et al.,* 1996; Lindholm *et al.,* 1997; Kreuze *et al.,* 1999; Bouchek-Mechiche *et al.,* 2000a; Wanner, 2006; Flores-González *et al.,* 2008; St-Onge *et al.,* 2008; Wanner, 2009; Leiminger *et al.,* 2012; Pánková *et al.,* 2012; Tashiro *et al.,* 2012; Dees *et al.,* 2013). Loria *et al.* (1997) listed regions where common scab has been reported as Europe, South Africa, Australia, New Zealand, Israel, United States and Canada.

2.4.1 Economic importance

Potatoes are high in starch, vitamins and minerals and are regarded as the fifth most important food crop in the world (FAO, 2013) and third most important staple food in South Africa. The demand for quality on all markets is increasing (Wale, 2004), especially in the market for washed tubers (Pasco *et al.,* 2005). A disease such as common scab can decrease quality and thus marketability considerably. Severe lesions can render tubers unmarketable (Conn *et al.,* 1998). In 2010, on the fresh produce market in South Africa, 6% of tubers were downgraded due to common scab (2010 Annual Report, Potatoes South Africa). This can have significant implications for the potato supply chain and may result in a price increase.

Israel has also recently experienced an increase in scab due to conducive soils, host crops that are grown in succession and the lack of effective eradication methods. Tsror *et al.* (2007) found that seed lots imported into Israel from Northern Europe between 2004 and 2007 had an average of 26% common scab contamination. Common scab also decreases the storage life of the tubers and can increase the germination time or decrease the germination percentage (Krištůfek *et al.,* 2000). Florez-González *et al.* (2008) surveyed 19 cultivars over four years from five European countries (Netherlands, France, UK, Germany and Spain), and found that common scab is the most common bacterial disease in imported seed lots, with up to 83% incidence (Florez-González *et al.,* 2008).

2.4.2 Symptoms

Common scab lesions on tubers are initially observed as small, brown, raised spots that later enlarge, coalesce and appear corky (Agrios, 2005). Multiple symptoms are related to this disease and this is said to be attributed to different environmental conditions and the specific pathogenic species present (Loria *et al.,* 1997). The disease causes damage only to the skin of the tuber and is regarded as cosmetic, but in severe cases the lesions can affect the processing potential of the

tubers. The relationship between disease and symptomatology is not clear and it is unknown if the soil type, humidity, temperature, soil pH, species, potato cultivar or combinations of these factors can lead to specific symptoms. Lesions can be deep pitted, shallow, raised, star-shaped, netted or russet (Figure 2.2). In the case of deep pitted symptoms, lesions extend 3-4mm into the tuber. The tissue underneath the lesions is straw-coloured (Christ, 1996; Takeuchi *et al.,* 1996). The symptoms are also often confused with those of powdery scab (*Spongospora subterranea* f. sp. *subteranea* (Tomlinson)), Rhizoctonia russeting and insect damage.

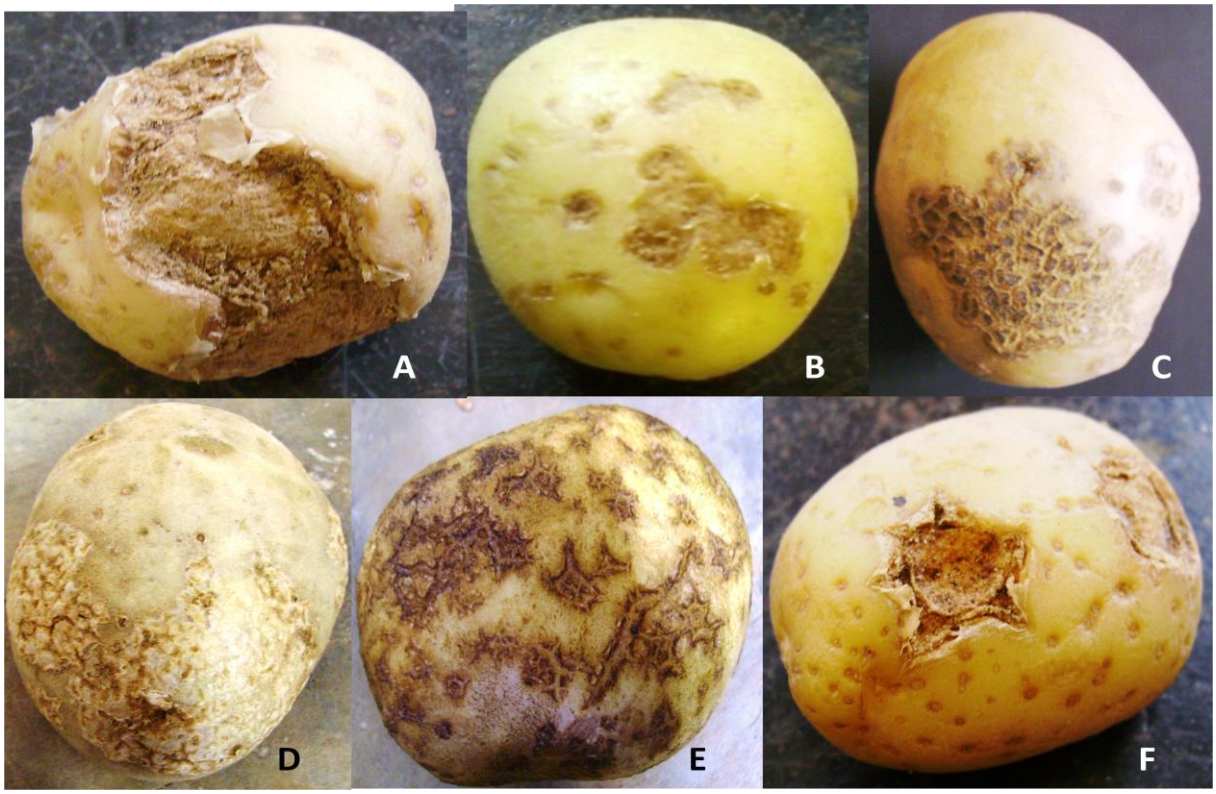

Figure 2.2: Common scab lesions: deep pitted (A), shallow (B), netted (C), raised (D), russet (E) and star-shaped (F) (Pictures: E. Jordaan).

2.4.3 Pathogenicity

Host-pathogen interactions are complex and occur on a molecular level (Loria *et al.,* 2003). The plant (host) has molecules (glycoproteins, oligosaccharides and peptides) that can sense microorganisms and respond in different ways. Despite differences in the distribution areas, phenotypic and genotypic traits, all the potato scab-causing strains show analogous mechanisms for pathogenicity (MPI) (Bukhalid *et al.,* 2002; Cullen & Lees, 2007; Florez-González *et al.,* 2008). Pathogenic *Streptomyces* all contain a PAI that not only harbours pathogenicity and virulence factors but also mediates its transfer to non-pathogenic species. Pathogenicity islands are defined

as regions of DNA encoding virulence, present in pathogenic strains but only partially present or absent in non-pathogenic genomes. These islands have variable G+C contents, distinct boundaries and genes related to mobile elements. Mobile elements include insertion sequences, integrases and transposases (Arnold *et al.,* 2003; Madhumita & Loria, 2007). *S. acidiscabies* (and other pathogenic strains) encodes a peptide synthase from *txtAB* (Arnold *et al.,* 2003; Wach *et al.,* 2007; Florez-González *et al.,* 2008). This is essential for thaxtomin production and pathogenicity. The pathogenicity island is 325-660kb long and contains multiple pathogenicity- / virulence-associated genes such as thaxtomin synthesis genes (*txt*), a necrosis gene (*nec1*), tomatinase gene homologues and mobile genetic elements (Cullen & Lees, 2007; Madhumita & Loria, 2007; Qu *et al.,* 2008).

The production of toxins is an important factor in disease development; sometimes these toxins contribute to virulence while other times they are required for pathogenicity (Hammerschmidt, 2007). One of the well known characteristics of *Streptomyces* spp. is the production of secondary metabolites such as phytotoxins and antibiotics (Loria *et al.,* 2003; 2008). *S. scabiei, S. acidiscabies* and *S. turgidiscabies*, among other species, produce thaxtomins, a group of dipeptide phytotoxins believed to be required for disease development. Thaxtomin can be extracted from lesions and also induces similar symptoms on immature tubers (Loria *et al.,* 2008). It appears that thaxtomin inhibits cellulose synthesis, or more specifically, inhibits the incorporation of ^{14}C glucose into cellulose (Hammerschmidt, 2007). Streptomycetes produce enzymes in addition to phytotoxins that degrade cellulose, lignin and chitin (Loria *et al.,* 2008). These phytotoxins cause plant cell necrosis at nanomolar concentrations (King *et al.,* 2003).

Thaxtomin plays an important role in host-pathogen interactions (King *et al.,* 1992; Fry & Loria, 2002). Cell volume in *Arabidopsis thaliana* is dramatically increased upon exposure to thaxtomin A. Thaxtomin can thus aid in penetration through compromising the cell wall integrity. This explains why the infection process occurs in rapidly growing tissue. The mechanisms of pathogenicity of *S. ipomoeae* and *S. reticuliscabiei* are unknown, although we do know that *S. ipomoeae* produces thaxtomin C (Loria *et al.,* 2003). Root growth inhibition is also a result of high thaxtomin concentrations and this suggests that thaxtomin can inhibit mitosis (Fry & Loria, 2002; Johnson *et al.,* 2007; Wach *et al.,* 2007). This toxin also interacts with microtubuls, actin and other components of the cytoskeleton, which induce dramatic cell wall hypertrophy (Loria *et al.,* 1997; Johnson *et al.,* 2007).

2.4.4 Disease cycle

Streptomyces spp. are disseminated through soil water, windblown rain or soil and through infected seed tubers (Loria *et al.,* 1997; Agrios, 2005) but are not spread in storage (Zitter & Loria, 1986). *S. scabiei* has been found to survive for extended periods in soil and on plant debris (Christ, 1996). Most *Streptomyces* spp. that cause common scab grow optimally in slightly alkaline soils with a pH between 5.2 and 8 (Loria *et al.,* 1997; Agrios, 2005), except for the scab-causing species found in acidic soils. Dry or aerated soils also promote disease development, and optimum growth occurs at a soil temperature of 30^{o}C for *S. scabiei* (Loria *et al.,* 1997). The incidence and severity of disease, as with any other disease, is dependent on the pathogen, host and environmental conditions (Agrios, 2005). Farmers are well practiced in the use of cultural methods to alter the environment to one that would result in the lowest amount of disease. Unfortunately this approach of changing the environment has led to the selection of *Streptomyces* spp. that have adapted to survive these conditions (Loria *et al.,* 1997). For example, *S. acidiscabies* grows optimally at a pH of 4.5.

Pathogenicity requires attachment, penetration, colonization and invasion by the pathogen, all of which elicit a response in the host (Loria *et al.,* 2008). The pathogen can only infect growing tissue and enters the tubers through natural openings or wounds (Agrios, 2005). Natural openings include stomata and lenticels. Adherence is the first step in this host-pathogen interaction. Loria *et al.* (2003) found an apparent attachment matrix on tuber surfaces colonized by *S. scabiei*. Scanning electron microscopy showed the track left after the removal of hyphae. Based on the findings of Clark & Matthews (1987) as sited in Loria *et al.* (2003), *S. scabiei* was found to grow and penetrate healthy potato tuber tissue in a similar manner to *S. ipomoeae,* a pathogen on sweet potato. Hyphal growth extends to form a network on the surface of the host. Within uniform distances in the hyphae there is a perpendicular branching of short hyphal growth. This suggests that these short hyphae are in fact penetration structures. From further electron microscopy studies it was clear that penetration occurs directly into plant cells or through fissures between cells; the hyphae then extend into the lumen, through cell walls and into adjacent cells. Pathogenic *Streptomyces* spp. can thus grow intracellularly and intercellularly (Loria *et al.,* 2008).

Once the tuber has been penetrated (usually during tuber initiation) the pathogen releases thaxtomins that cause plant cell hypertrophy (Agrios, 2005). The development of primary cell walls is altered and the cell does not go through the normal division cycles (Cullen & Lees, 2007). Mostly

only the outer cells are affected as the pathogen grows in the intercellular spaces of the parenchyma cells; these cells are later broken down and invaded. The lesions that form on the surface of the tubers are a result of plant defenses against attack. Just below the point of infection, cork layers start to form that are pushed outward. Through this the plant isolates the bacteria from further infecting the tuber. However, as these layers are produced the pathogen grows and multiplies as it utilizes the dead tissue (Agrios, 2005).

2.5 MANAGEMENT OF THE DISEASE

The disease triangle for common scab have multiple pathogenic species that cause disease under varying conditions (Wanner, 2009). In addition, because *Streptomyces* spp. are saprophytic they can survive on various plants or in the absence of a host. More importantly, *Streptomyces* spp. are commonly found in soils and pathogenicity islands can be transmitted to non-pathogens. These factors make this disease dynamic and unanswered questions remain regarding the etiology of the disease. The measures used to manage common scab include: chemical treatments, irrigation management, resistant varieties, pH adjustment of soil, crop rotation and biological control (Loria *et al.,* 1997, 2006).

Common scab is as much a tuber-borne disease as it is a soil-borne disease and for this reason the use of certified seed tubers will minimize the amount of inoculum due to infected seed (Christ, 1996; Agrios, 2005). Seed tubers can also be treated with pentachloronitrobenzene (PCNB) or maneb-zinc prior to planting (Wilson *et al.,* 1999; Agrios, 2005). Wilson *et al.* (1999) found that seed-borne inoculum plays a major role in disease development, in contrast to what was previously believed. New diagnostic techniques can rapidly determine if a seed lot is infected, even when no symptoms are visible.

Since most strains of *Streptomyces* spp. grow at a near-neutral pH, farmers are exploiting this by adjusting their soil pH to ±5.3 (Christ, 1996; Agrios, 2005). This practice will however not inhibit *S. acidiscabies*, which grows optimally at a slightly acidic pH. Another downside to the adjustment of soil pH is the limitation on the yield as well as on rotation crops suitable to grow at these lower soil pH levels (Loria *et al.,* 1997).

Crop rotation can also be used as a method of control (Agrios, 2005). It is important to choose the rotation crop with care. If crops that are also affected by *Streptomyces* spp. are grown, it may

increase the populations in the soil (Christ, 1996; Pasco *et al.,* 2005). Alfalfa, rye, and soy beans are recommended by Christ (1996).

Soil aeration is another environmental condition exploited by farmers as the pathogen needs this for optimal growth (Pasco *et al.,* 2005). Because infection is most likely to occur during the initial stages of tuber development, farmers tend to irrigate more frequently during these six weeks (Christ, 1996). In doing this aerated conditions are minimized and disease development due to *Streptomyces* spp. is considerably lower (Loria *et al.,* 1997; Agrios, 2005).

Resistant or tolerant potato varieties are also used in disease management (Agrios, 2005). Pasco *et al.* (2005) goes as far as to say that this is the best way to manage scab disease. Even though potatoes are bred for resistance to scab, not much is known about how it works or how it is inherited (Loria *et al.,* 1997).

Some soils seem to become suppressive to this disease through consecutive years of monoculture. *Streptomyces* spp. (Loria *et al.,* 1997) antagonistic to the pathogenic strains as well as bacteriophages are used as biological control agents (Agrios, 2005). It is believed that antagonistic *Streptomyces* spp. produce an antibiotic that inhibits other *Streptomyces* spp. (Loria *et al.,* 1997).

Grinstein *et al.* (1991) suggested the use of combined treatments, such as soil solarization combined with reduced amounts of formalin as fumigation treatment. Chemicals to control common scab are used to a lesser extent in Australia (and increasingly so in other countries as well) due to the potential hazardous effects thereof (Wilson *et al.,* 1999). Some of the chemicals used include Fluazinam, Fenpiclonil, Flusulfamide, and Bordic acid (Wilson, 1999).

An integrated approach to controlling any disease is often the ideal approach (Wale, 2004). It is crucial to monitor the development of soil-borne diseases during the growth of the crop in order to determine the potential for disease development. The success of a risk assessment can be determined by understanding: the pathogen, control practices, economics of potato production, practicality and the psychology of the farmer (Wale, 2004).

2.5.1 Risk assessment

Risk assessment involves the techniques followed to determine the probability of a disease occurring at a certain incidence or severity (Agrios, 2005). It is an early warning system to growers and can play a vital role in disease management. The amount of inoculum in the soil can be determined, and with known or predicted environmental conditions, the probability of the development of the disease can be determined (Wakeham & Kennedy, 2010). Accurate quantification of pathogenic species within any given field would strongly rely on the sampling techniques.

2.6 DETECTION AND QUANTIFICATION

2.6.1 Traditional

In order to determine the severity of disease, a scoring technique based on the outer appearance and tuber surface coverage is used. The scale usually reflects fixed amounts of disease (Stewart & Wastie, 1989; Marais & Vorster, 1988). Water agar (WA) can be used for isolation of Streptomycetes. Lesions are excised and macerated in sterile water (Florez-González *et al.,* 2008). The suspension is then streaked out onto the required media. Plates are incubated for eight to ten days at 27^{o}C. Through serial dilution the number of colony forming units per gram of soil or diseased tissue can be estimated.

Yeast malt extract (YME) agar is used to observe the colour of the spores and the colonies (Florez-González *et al.,* 2008). Peptone yeast-extract iron (PYI) media is used to determine if the isolates produce melanoid pigments. Different carbon utilization media are used to investigate the utilization of various carbon sources by the isolates. Ten days are normally required to observe any growth on the plates.

2.6.2 Molecular techniques

The reason behind developing molecular methods for detection of pathogens is to overcome the drawbacks encountered with traditional culturing methods (Damm & Fourie, 2005). Detection of pathogens with the use of molecular techniques is still improving (Wale, 2004). Molecular techniques are more sensitive in terms of quantification and more specific in terms of detection than traditional plating techniques (Florez-González *et al.,* 2008). Polymerase chain reactions (PCR) is a valuable tool in diagnostics of disease; it is sensitive, specific and simple (Florez-González *et al.,* 2008).

Although molecular based tests are accurate and quick, only small samples (for example 1g soil samples from a field) can be tested and there is a risk of not detecting the pathogen when it is in fact present (Wale, 2004). This can however, be overcome by appropriate sampling. It is also important to provide a cost-effective protocol (Damm & Fourie, 2005). Another problem encountered with the use of these techniques, is that humic substances are extracted with DNA. These inhibit the DNA-transforming enzyme during amplification, especially when using *Taq* polymerase (Damm & Fourie, 2005). This effect may be somewhat overcome with the use of a good extraction buffer, polyvinylpolypyrrolidone (PVPP) for example, prior to PCR and with the use of Sephadex or Sepharose columns. PVPP binds phenolic compounds. Agarose gel purification, caesium chloride density gradient centrifugation and PCR enhancers are other methods that will also remove inhibitors from DNA (Damm & Fourie, 2005). The biggest drawback of quantification with the use of PCR, however, is that the techniques take into consideration all DNA present in the sample regardless if it is dead or live material.

The real-time quantitative PCR (qPCR) approach allows for the quantification of DNA in a given sample (Moody, 2007). In quantitative PCR the product can be viewed in real time and quantified (Kaiser, 2007). This process, first described in 1996, is the most sensitive detection and quantification process for RNA and DNA. Absolute quantification determines the amount of DNA by comparing to a generated standard curve (Qu *et al.,* 2008). As few as three spores of *Streptomyces* per gram of soil can be detected using real-time PCR (Cullen & Lees, 2007). This technique can also be used to calculate the initial concentration of DNA, and results can be obtained within two hours. Another advantage is the fact that multiplex PCR assays can be designed, to include the detection of the *txtAB* gene for example or even other pathogens (Cullen & Lees, 2007). Factors related to pathogenicity are good markers for PCR detection because genes for pathogenicity are highly conserved among genetically different *Streptomyces* species (Florez-González *et al.,* 2008). On the other hand, as with any factor there is no guarantee that the selected factor will always be correlated with pathogenicity (Cullen & Lees, 2007).

2.7 CONCLUSION

Common scab is a disease that affects the underground parts of the plant and thus is of importance in taproot crops. It has been studied most extensively on potato. No cultivar in South Africa is resistant to this disease. Previously moderately resistant cultivars are now becoming susceptible.

Streptomycetes are filamentous spore-forming bacteria and produce antibiotics and in the case of pathogenic strains, thaxtomins. Multiple species of Streptomycetes cause scab-like symptoms on tubers and so the causal agent is now referred to as the *Streptomyces* complex. Through horizontal transfer of pathogenicity and virulence genes, non-pathogenic species can acquire factors needed to become pathogenic. Genetic characterization is needed to distinguish between species that are morphologically and physiologically similar. Pathogenicity and virulence determinants (*txtAB, nec1, tomA*) can be unique, or shared by strains.

This disease occurs in almost all potato producing areas and leads to the downgrading of tubers on the fresh produce market, decreasing of processing potential and even the rejection of seed tuber lots. Disease management is limited mostly to cultural practices and resistance breeding. Chemicals are continuously being removed from exposure to the environment; thus pre-plant risk assessment through quantification by molecular methods is being explored as an additional management practice.

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CHAPTER 3

Streptomyces species associated with common scab lesions in South Africa

ABSTRACT

One of the main reasons for downgrading of potato tubers in South Africa is common scab. *Streptomyces scabiei* is largely accepted as the causal organism, and other *Streptomyces* species associated with common scab are not often considered. This study therefore aims to determine the diversity and quantity of Streptomycetes associated with common scab on potatoes in South Africa. Isolates from 11 of the 16 potato producing regions in South Africa were characterized morphologically, physiologically and genetically. No *S. acidiscabies* or *S. turgidiscabies* were found, and as expected most pathogenic isolates were *S. scabiei* and *S. stelliscabiei.* Other species related to common scab of potatoes in South Africa may be important and require further research.

3.1 INTRODUCTION

Common scab is a blemish disease on potato tubers (*Solanum tuberosum* L.) reported throughout the world (Loria *et al.,* 1997). In South Africa this disease was found in 1906 by Pole Evans (Doidge, 1950). South Africa is fourth of the six largest potato producers in Africa, producing over two million tons a year (FAO, 2009). South Africa is the largest producer in tons per hectare in Africa (Potatoes South Africa, 2009). Potatoes are produced in 16 regions (Figure 3.1) throughout the country. Most of South African potato production is done under irrigation but some regions still have about 10% of their production under dry land conditions. In South Africa, the potato farming industry is one that is in most part, based on individual small to large scale farmers (Potatoes South Africa, 2013). Potatoes are a high input crop in terms of cost. Thus it is important for South African potato growers to be able to identify the diseases that threaten their crop, to know when and where best to control or prevent disease in their fields. Common scab is one of the main reasons for downgrading of tubers on the fresh produce market; thus directly influencing the growers' income.

Cultural practices remain the primary method for disease management of common scab (Dees & Wanner, 2012). Currently there are no resistant cultivars in South Africa (Gouws & Geldenhuis, 2011) and with the limitations on the use of products for the control of bacteria, it is becoming difficult to control this disease (Agrios, 2005). Tubers with common scab have been harvested

from virgin soils and from soils with no history of common scab (isolations made during this study). Based on environmental conditions and symptomatology it might be possible to predict the species involved in a specific situation, but cultural, physiological and molecular characterization remains an integral part of species identification (Leiminger *et al.,* 2012). Identifying the different species within a disease complex and under unique environmental conditions may give insight into better management or even preventative action for common scab.

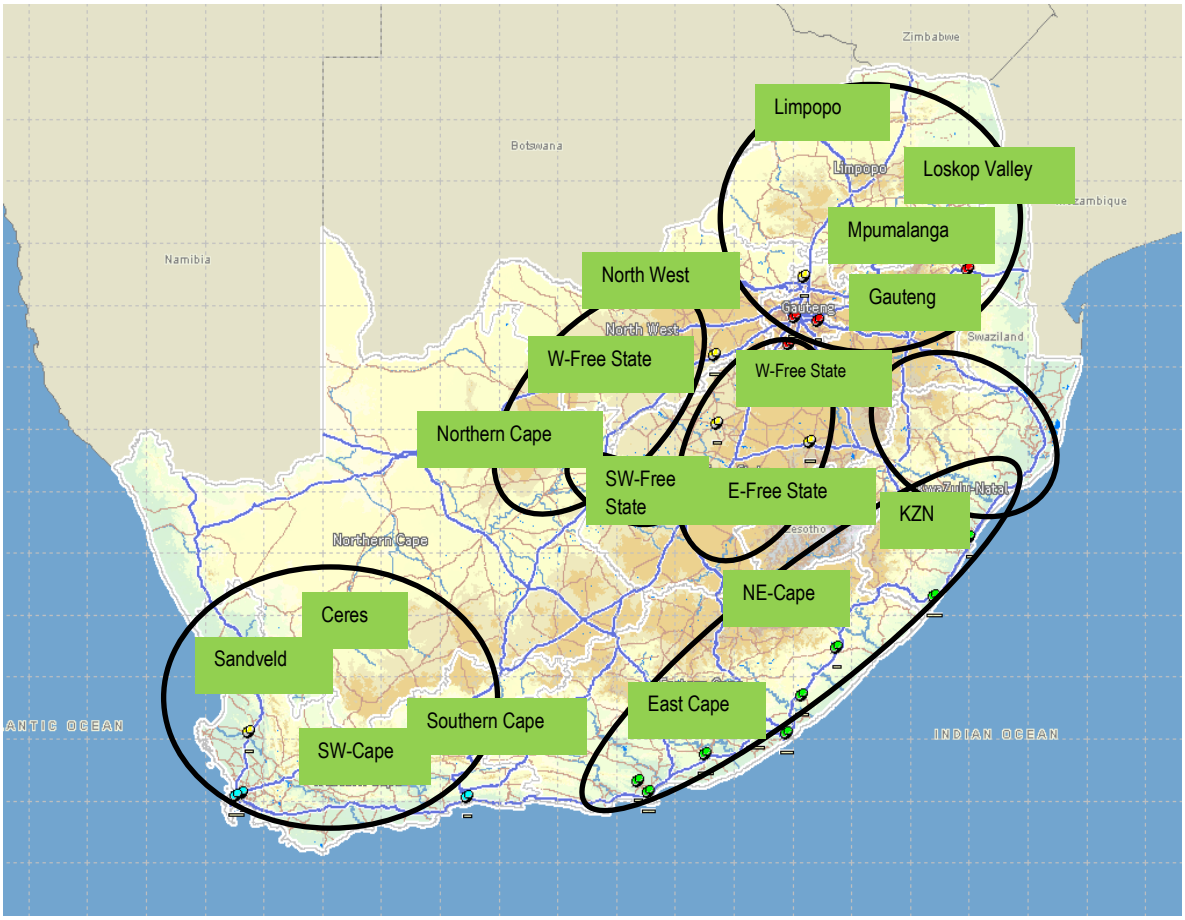

Figure 3.1: Potato production regions in South Africa (N-north, S-south, E-east, W-west) (Potatoes South Africa, 2009).

The interactions between the species complex, environment and host result in variable symptoms between regions and years, making this disease difficult to understand (Wanner, 2009). Common scab symptoms are mostly superficial in nature and the disease is regarded as cosmetic, although it also decreases the processing potential of the tubers (Loria *et al.,* 1997). Symptoms include deep pitted, shallow, netted, star shaped and russet type lesions; and more than one type of symptom may appear on a single tuber. Common scab symptoms form where the plant responds to

infection by producing corky layers in order to isolate the infection (Agrios, 2005). It is unclear if the Streptomycetes isolated at the end of the season represent the causal pathogen complex or if the complex of species changes during the season from first infection. Currently it is believed that common scab is not caused by a single species, but rather a "complex" of species within the *Streptomyces* genus (Loria *et al.,* 1997; Wanner, 2009). There are reports of various scab-causing species found within the same field and within the same lesion (Aittamaa *et al.,* 2008); which have led to investigations on species relatedness to specific symptoms and on the sensitivity of species to different environmental conditions (Lindholm *et al.,* 1997; Bouchek-Meciche *et al.,* 2000b; Krištůfek *et al.,* 2000; Wiechel & Crump, 2010; Khodakaramian & Khodakaramian, 2012; Tashiro *et al.,* 2012).

Streptomycetes are common in soils and of the known species only a few are pathogenic (Loria *et al.,* 1997). There are four well known causal species of potato common scab; *Streptomyces scabiei* (Lambert & Loria)*, Streptomyces europaeiscabiei* (Bouchek-Mechiche)*, Streptomyces turgidiscabies* (Miyajima) and *Streptomyces acidiscabies* (Lambert & Loria) (Loria *et al.,* 2006). These are not the only scab-causing species and reports of other species are geographically distinct. *S. scabiei* is found worldwide but is not common in Europe; while *S. europaeiscabiei* is more often found in Europe, France, Korea and North America; and *S. turgidiscabies* is frequently found in Japan and Finland (Lehtonen *et al.,* 2004; Wanner, 2009; Dees *et al.,* 2012).

A multitude of other, poorly described *Streptomyces* spp. have been isolated from lesions, but these are possibly not pathogenic (Taddei *et al.,* 2006). These include *Streptomyces caeruleus, Streptomyces echinoruber, Streptomyces geysiririensis, Streptomyces globosus, Streptomyces roseodiastaticus, Streptomyces tricolor, Streptomyces viridodiastaticus, Streptomyces crystallinus, Streptomyces niveoruber, Streptomyces celluloflavus* and *Streptomyces ghanaensis*. Their function in the disease complex is not understood and they make positive identification with PCR or culture methods of the causal species difficult (Song *et al.,* 2004; Taddei *et al.,* 2006). For example the diastochromogenes group contains *S. scabiei, S. europaeiscabiei, S. stelliscabiei, Streptomyces ipomoeae* (Waksman & Henrici) and *Streptomyces diastatochromogenes. Streptomyces stelliscabiei* (Bouchek-Mechiche) and *Streptomyces bottropensis* are 98.4% related based on their genetic makeup. *S. scabiei* and *S. acidiscabies* differ in morphology but their DNA is about 90% similar (Pánková *et al.,* 2012). *S. scabiei* and *S. turgidiscabies* often co-occur on the same lesions and it has been found that when isolating directly after sampling, more *S. scabiei* than *S.*

turgidiscabies was isolated, compared to when samples were left for a couple of days in storage (Aittamaa *et al*., 2008).

Not only is genetic characterization a challenge; physiological and morphological characterization is just as variable. *S. scabiei* and *S. europaeiscabiei* grow at pH above five (Linholm *et al.,* 1997). In Japan, isolates with flexuous spore chains that do not produce melanin and that grow at pH 4 are said to cause acid scab (Tashiro *et al.,* 2012). Conditions that normally cause common scab are dry and warm soils (Wanner, 2006). However, scab was also found to occur under irrigated conditions in Northern Europe, Israel and Canada (Doering-Saad *et al.,* 1992; Goyer *et al.,* 1996). Acidic conditions may be favourable for the development of acid scab caused by *S. acidiscabies*. Table 3.1 describes the characteristics of some of the well-known species.

Methods used for identification of pathogenic *Streptomyces* species include morphological, physiological and genetic evaluation of the isolates. Morphological characterization usually includes the spore and colony colours when grown on yeast malt extract agar (Tashiro *et al.,* 2012). The structure of the spore chain and sometimes the spores are examined under a microscope. Physiological testing involves growth at various pH levels, production of melanin in the presence of tyrosine, utilization of different sugars and resistance to various antibiotics (Loria *et al.,* 1995; Lindholm *et al.,* 1997; Bouchek-Mechiche *et al.,* 1998). Genetically the isolates are tested for the presence of the PAI genes and identified using different PCR based techniques, such as DNA-DNA hybridization and the use of species-specific primers (Leiminger *et al.,* 2012; Pánková *et al.,* 2012; Dees *et al.,* 2013).

Streptomyces scabiei was thought to be the only causal organism in South Africa until about 2000 (Bouchek-Mechiche *et al.,* 2000b; Gouws & Geldenhuis, 2011). However in 2000 Bouchek-Mechiche *et al.* (2000b) isolated *S. stelliscabiei* from a common scab lesion in South Africa. Until now the species associated with common scab in South Africa have not been fully understood or characterized. The aim of this study therefore was to determine the diversity of Streptomycetes associated with common scab lesions on potato tubers in South Africa.

Table 3.1: Morphological, physiological and genetic characteristics of well-known common scab causing species ([a]Lambert & Loria, 1989; [b]Lindholm *et al.,* 1997; [c]Miyajima *et al.,* 1998; [d]Bouchek-Mechiche *et al.,* 2000a; [e]Song *et al.,* 2004; [f]Loria, 2006; [g]Wanner, 2006; [h]Tashiro *et al.,* 2012)

Species	**Region**	**Lesion**	**Spore chain**	**Colony colour**	**Spore colour**	**Diffusable pigments**	**Growth at pH**	**Melanin production**	**Presence of *txtAB***
S. scabiei [abdefg]	World wide	Pitted	S	B	G	na	>5.2	+	+
S. europaeiscabiei [defg]	Europe, Korea	Pitted	S	ni	G	na	ni	+	+
S. acidiscabies [abdefgh]	USA, Korea, Japan	ni	F	B	W	R	<5.2	-	+
S. turgidiscabies [aefgh]	Japan, Finland, Korea, Norway	Erupt	F	Y	G	na	>4.5	+	+
S. stelliscabiei [defg]	France, South Africa, USA	Star	S	ni	G	na	ni	+	+
Streptomyces aureofaciens [ac]	ni	ni	F	B	G	na	>5	ni	ni
Streptomyces griseus [ac]	ni	ni	F	W	G	na	>6	ni	ni
Streptomyces setonii [ac]	ni	ni	F	Y	G	na	>4.5	ni	ni
Streptomyces tendae [ac]	ni	ni	F	Y	G	Y	>6	ni	ni
Steptomyces caviscabies [bdh]	Canada	Pitted	F	B	W	na	>5	-	ni
Streptomyces reticuliscabiei [deg]	France	Netted	F	ni	G	na	ni	-	ni
Streptomyces luridiscabiei [h]	ni	ni	F	ni	YW	ni	4.5-5	+	ni
Streptomyces puniciscabiei [h]	ni	ni	F	ni	O	ni	3.5-5	-	ni
Streptomyces niveiscabiei [h]	ni	ni	F	ni	W	ni	3.5-5	-	ni

F-flexuous, S-spiral, B-brown, Y-yellow, W-white, G-grey, YW-yellow-white, O-orange, R-red, ni-no information available, na-not any, +positive for, -negative for

3.2 MATERIALS AND METHODS

Sample collection

Five *Streptomyces* isolates were obtained from Dr. Tonya Weichel of the Department of Primary Industries, Potato Disease Research Division in Victoria, Australia. An additional 55 isolates were isolated from tubers showing common scab symptoms (deep pitted, superficial, netted and star shaped lesions) from 11 of the 16 potato production regions in South Africa. Tubers were collected from 2008 to 2010. Lesion type and disease score (Appendix A) were recorded prior to isolation (Table 3.2).

Table 3.2: *Streptomyces* isolation data

Date	Isolate #	Potato Production Region	Area	Cultivar	Score[1]	Lesion type
-	SCC 5	Limpopo	Polokwane	-	-	-
-	SCC 9	Limpopo	Tolwe	-	-	-
-	SCC 11	Limpopo	-	-	-	-
-	SCC 12	Limpopo	Tolwe	-	-	-
-	SCC 13	Limpopo	Tolwe	-	-	-
-	SCC 14	Limpopo	-	-	-	-
-	SCC 17	Limpopo	Tolwe	-	-	-
-	SCC 18	Limpopo	Polokwane	-	-	-
-	SCC 19	Limpopo	Polokwane	-	-	-
-	SCC 21	Limpopo	Polokwane	-	-	-
-	SCC 22	Limpopo	Polokwane	-	-	-
-	SCC 23	Limpopo	Polokwane	-	-	-
-	SCC 24	-	-	Mondial	-	deep pitted
-	SCC 26	-	-	-	-	-
-	SCC 27	-	-	-	-	-
-	SCC 28	Australia	Torquay	Coliban	-	-
-	SCC 29	Australia	Colac	Atlantic	-	deep pitted
-	SCC 30	Australia	Colac	Atlantic	-	netted
-	SCC 31	Australia	QLD Smiths	-	-	netted
-	SCC 32	Australia	Portland	Pontiac	-	raised
30/07/2009	SCC 36	Western Cape	Cape Town	-	2	deep pitted
-	SCC 37	North West	Vryburg	-	2	shallow
30/07/2009	SCC 38	Western Cape	Cape Town	-	5	deep pitted
01/06/2009	SCC 39	Western Cape	Sandveld	BP1	2	star
01/06/2009	SCC 43	Western Cape	Sandveld	BP1	2	superficial
01/06/2009	SCC 49	Western Cape	Sandveld	BP1	2	superficial
01/06/2009	SCC 52	Western Cape	Sandveld	BP1	6	netted
-	SCC 55	-	-	Mondial	-	-
-	SCC 57	Western Cape	Sandveld	BP1	-	deep pitted
07/2009	SCC 65	Mpumalanga	Groblersdal	Mondial	6	star
-	SCC 66	Western Cape	Ceres	-	2	netted
19/08/2009	SCC 67	Western Cape	Sandveld	BP1	-	deep pitted
27/10/2008	SCC 74	KZN	-	-	-	-
27/10/2008	SCC 76	Limpopo	Tolwe	Vivo	-	-
01/2010	SCC 80	Gauteng	Pretoria	-	6	deep pitted
06/08/2009	SCC 81	Free State	Christiana	-	2	shallow
25/03/2010	SCC 83	Mpumalanga	Lydenburg	BP1	3	superficial
27/10/2008	SCC 85	KZN	-	-	-	-
06/08/2009	SCC 86	Free State	Christiana	-	2	shallow
06/08/2009	SCC 87	Free State	Christiana	-	2	shallow
06/08/2009	SCC 90	Free State	Christiana	-	2	shallow
06/08/2009	SCC 96	Free State	Christiana	-	2	shallow
18/11/2009	SCC 109	Northern Cape	Douglas	-	2	shallow
18/11/2009	SCC 110	Northern Cape	Douglas	-	6	deep pitted
27/10/2008	SCC 111	KZN	-	-	-	-
18/11/2009	SCC 116	Northern Cape	Douglas	-	3	shallow star
25/03/2010	SCC 117	Northern Cape	Prieska	BP1	5	superficial
24/05/2010	SCC 119	KZN	Underberg	-	3	-
05/2010	SCC 120	Mpumalanga	Marble Hall	-	2	star lenticel
05/2010	SCC 121	Mpumalanga	Marble Hall	-	2	star lenticel
18/11/2009	SCC 124	Northern Cape	Douglas	-	6	deep pitted
05/2010	SCC 126	Mpumalanga	Marble Hall	-	2	star lenticel
05/2010	SCC 127	Mpumalanga	Marble Hall	-	2	star lenticel
27/10/2008	SCC 130	KZN	-	-	-	-
27/10/2008	SCC 132	KZN	-	-	-	-
01/2010	SCC 133	Gauteng	Pretoria	-	5	deep pitted
01/2010	SCC 136	Gauteng	Pretoria	-	6	deep pitted
01/2010	SCC 138	Gauteng	Pretoria	-	6	deep pitted
06/08/2009	SCC 146	Free State	Christiana	-	2	shallow
18/11/2009	SCC 149	Northern Cape	Douglas	-	6	deep pitted

[1] Refer to Appendix A for score determination, -: no information available

Bacterial isolates

Photographs were taken of each lesion prior to isolation. The scalpel was disinfected with 70% ethanol between samples to prevent cross contamination. From each lesion a 1cm^3 piece of the

edge of the lesion was excised including the straw coloured tissue directly underneath. Each piece was placed in a Bioreba macerating bag (Labretoria) and macerated with a rubber hammer. Nine millilitres of sterile distilled water was added to each bag containing the macerated tissue. A serial dilution was made to 10^{-5} dilution (previously determined to be the optimal dilution for isolating, results not shown here) and 100µl thereof plated in triplicate onto water agar (WA) (Loria *et al.*, 2001). Plates were incubated at 25-30°C, in the dark, for 12-20 days. Plates were examined at regular intervals during the incubation period for phenotypic *Streptomyces* colonies and those colonies were transferred to yeast malt extract agar (YME, ISP medium 2). Pure cultures were stored in 20% glycerol at -18°C and -80°C.

Morphological characterization

Following seven days of incubation at 25-30°C on YME, colony colour and spore colour for each isolate were noted. The presence or absence of diffusible pigments in the media was noted. Spore chain morphology was noted on WA with the use of a light microscope at 400x magnification, prior to re-plating onto YME.

Physiological characterization

Melanin production was determined after growth on tyrosine agar (TA, ISP medium 7) at 25-30°C after four, eight and 12 days of incubation. Isolates altering the clear colour of the medium with a dark diffusible pigment were considered as positive for melanin production. Growth at pH four, five, six and seven was determined by growing the isolates on YME medium with adjusted pH levels of four, five, six and seven; for 14 days at 25-30°C. The pH was altered by adding 1M hydrochloric acid or 1M sodium hydroxide to the media until desired pH was reached. A tuber slice assay as described by Loria *et al.* (1995) was used to give an indication of the pathogenicity of the isolates. Isolates were grown on oatmeal agar (OMA, ISP medium 3) at 28°C for 7-10 days to ensure optimal sporulation. Tuber slices ($2cm^2$ x 0.5cm thick) from the susceptible cultivar BP1 were sterilized in 0.05% calcium hypochlorite and 0.1% $CaCO_3$ for 3 minutes. Slices were then rinsed twice with sterile distilled water and placed into Petri dishes. Agar plugs from each isolate were placed inverted onto the tuber slice; this was done in triplicate in separate Petri dishes. Moist filter paper discs were placed in Petri dishes to ensure the tuber slices did not dry out. A sterile agar plug was used as control. Petri dishes were sealed and placed in the incubator in the dark for five days at 28°C. Tuber slices were examined for a necrotic area surrounding the agar plug.

Molecular characterization

Isolates were grown on YME for 7 days at 25-30^{o}C. Spores and mycelium were scraped from the cultures and DNA was extracted using the Zymo Soil DNA extraction kit (Inqaba) following the manufacturer instructions. DNA was kept at -18^{o}C until use. Primers 16S-1F (5'-CATTCACGGAGAGTTTGATCC-3') and 16S-1R (5'-AGAAAGGAGGTGATCCAGCC-3') (Bukhalid *et al.,* 2002) as described by Wanner (2006) were used to amplify the 16S rDNA gene. Products were viewed by agarose gel electrophoresis. Amplified DNA products were separated on 1% agarose gels (containing 0.5µg/ml ethidium bromide (Merck chemicals) and viewed under UV light. Clean-up was done with the Bioline Isolate PCR and gel kit (Celtic Diagnostic) as per manufacturer instructions. Sequencing was carried out on an Applied Biosystems ABI 3500xl automated capillary sequence analyzer (Life Technologies Carlsbad) for both the forward and reverse sequences. Subsequently, consensus sequences were obtained in BioEdit version 7.2.3 (Copyright© 1997-2013 Tom Hall) and blasted in the Information National Center for Biotechnology Information (NCBI) against prokaryotes.

3.3 RESULTS

Symptoms ranged from deep pitted and star shaped to shallow and raised. Tubers showing netted scab symptoms were only collected from the Western Cape region. A total of 149 isolates were collected from 11 of the 16 potato producing regions in South Africa, as seen in Table 3.2. Isolates with actinomycete spiral or flexuous spore chains on culture media were selected for further analysis. Of these 60 were selected for further morphological and physiological tests and 32 were randomly selected for species identification by sequencing of the 16S rDNA gene.

Most isolates were yellow-brown with grey spores on YME (Table 3.3). Morphology was variable as was found in a similar study in Israel (Doering-Saad *et al.,* 1992). The ability to produce pigmentation on YME media was lacking in most of the isolates. Physiological characteristics listed in Table 3.4 shows all the isolates grew at pH 7 while some could grow at pH 4 and above. Melanin pigments on tyrosine agar were only observed in half of the isolates; delayed or weak production of melanin was still noted as positive. Pathogenicity could not be linked to melanin production. Isolates with flexuous spore chains tend to not have all three of the pathogenicity / virulence genes, thus possibly ruling out the presence of flexuous spore chains as a characteristic of pathogenic isolates.

22% of the isolates selected for sequencing aligned to *S. scabiei* when blasted against the NCBI database. *Streptomyces stelliscabiei* comprised 3% of the sequenced isolates, *Streptomyces padanus* comprised 9% and *Streptomyces flavofuscus* 3%. No *S. acidiscabies* or *S. turgidiscabies* related sequences were found and no analyses were done to determine if some of the *S. scabiei* isolates are in fact *S. europaeiscabiei.* *Streptomyces stelliscabiei* was only isolated from the Western Cape region. GenBank accession numbers and genetic characterization for each isolate is shown in Table 3.5.

3.4 DISCUSSION

Species determination is not always clear from morphology and physiology, and distribution can better be determined genetically. However, genetic characterization alone cannot give a complete picture of pathogenicity. *Streptomyces scabiei* is described as a grey spore-producing actinomycete with spiral spore chains, which produces melanin on tyrosine containing media (Lambert & Loria, 1989). *Streptomyces europaeiscabiei* and *S. stelliscabiei* are morphologically similar to *S. scabiei* and also capable of producing melanin (Bouchek-Mechiche *et al.,* 2000a). *Streptomyces turgidiscabies* has smooth cylindrical spores on flexuous grey chains and does not produce melanin in the presence of tyrosine (Takeuchi *et al.,* 1996; Valkonen, 2004) and grows at pH 4.5 (Lindholm *et al.,* 1997). *Streptomyces reticuliscabiei* causes netted scab in France and is characterized as having grey flexuous spore chains and does not produce melanin (Bouchek-Mechiche *et al.,* 2000a). However, some netted scab isolates in the Netherlands are tyrosinase positive, while netted scab isolates in Denmark are tyrosinase negative (Bouchek-Mechiche *et al.,* 2000b). Also, the netted scab tyrosinase positive isolates in France were identified as *S. europaeiscabiei,* while the tyrosinase negative isolates causing netted scab were *S. reticuliscabiei.*

Various common scab symptoms have been described in South Africa and the variability in morphology and physiology of the Streptomycetes isolated from the lesions is great. As mentioned before, it is difficult to use morphological and physiological parameters to determine Streptomycete species as some non-pathogenic isolates with grey spores and producing melanin, resemble pathogenic *S. scabiei* (Faucher *et al.,* 1992) isolates. Although morphological characteristics are said to be more constant than physiological characteristics (Park *et al.,* 2003), the isolates tend to change in spore and colony colour after continuous re-culturing. Keinath & Loria (1989) estimated that the morphology and physiology of less than 10% of pathogenic isolates resemble that of *S. scabiei*.

Table 3.3: Morphological characterization results for the isolates in this study

Date	Isolate #	Spore chain	Colony colour[1]	Spore colour[1]	Diffusible pigments[1]
-	SCC 5	F	B	G	-
-	SCC 9	S	Y	G	-
-	SCC 11	S	Y	G	-
-	SCC 12	S	R	W	-
-	SCC 13	S	B	G	B
-	SCC 14	S	B	G	-
-	SCC 17	S	B	G	-
-	SCC 18	S	R	G	-
-	SCC 19	S	R	G	-
-	SCC 21	S	R	G	-
-	SCC 22	S	R	G	-
-	SCC 23	S	B	G	-
-	SCC 24	S	Y	G	-
-	SCC 26	S	Y	G	-
-	SCC 27	S	G	G	-
01/2001	SCC 28	F	Y	W	-
04/2001	SCC 29	S	B	G	GB
06/2001	SCC 30	S	Black	G	Black-gray
07/2001	SCC 31	S	Y	G	Y
07/2001	SCC 32	F	B	G	TB
30/07/2009	SCC 36	S	Y	B	-
-	SCC 37	S	B	G	-
30/07/2009	SCC 38	S	Y	B	-
01/06/2009	SCC 39	S	B	G	B
01/06/2009	SCC 43	S	R	G	-
01/06/2009	SCC 49	F	B	G	-
01/06/2009	SCC 52	S	B	G	GB
-	SCC 55	S	Y	G	-
-	SCC 57	S	Y	G	-
07/2009	SCC 65	F	Green	G	-
-	SCC 66	F	R	Y	R
19/08/2009	SCC 67	F	G	G	-
27/10/2008	SCC 74	F	Y	W	-
27/10/2008	SCC 76	F	Y	G	B
01/2010	SCC 80	S	B	G	B
06/08/2009	SCC 81	S	Y	W	-
25/03/2010	SCC 83	F	Y	G	-
27/10/2008	SCC 85	F	B	W	B
06/08/2009	SCC 86	S	B	G	B
06/08/2009	SCC 87	S	R	G	-
06/08/2009	SCC 90	S	B	G	-
06/08/2009	SCC 96	S	Y	W	-
18/11/2009	SCC 109	S	Y	G	-
18/11/2009	SCC 110	F	Y	W	-
27/10/2008	SCC 111	F	R	Y	R
18/11/2009	SCC 116	S	Y	G	-
25/03/2010	SCC 117	F	R	Y	R
24/05/2010	SCC 119	F	B	W	-
05/2010	SCC 120	S	B	G	-
05/2010	SCC 121	S	B	G	-
18/11/2009	SCC 124	S	B	G	B
05/2010	SCC 126	S	Y	G	-
05/2010	SCC 127	S	B	G	-
27/10/2008	SCC 130	F	Y	W	B
27/10/2008	SCC 132	F	Y	W	-
01/2010	SCC 133	F	W	W	-
01/2010	SCC 136	S	B	G	B
01/2010	SCC 138	S	Y	W	B
06/08/2009	SCC 146	F	Y	W	-
18/11/2009	SCC 149	S	Y	G	-

[1] as on YME agar

(F-flexuous, S-spiral, B-brown, Y-yellow, W-white, G-grey, YW-yellow-white, O-orange, R-red, GB-grey-brown, TB-tan brown, -: no pigments detected)

Table 3.4: Physiological characterization results for the isolates in this study

Date	Isolate #	Growth at pH				Melanin production			Pathogenicity[1]
		5	6	7	4	4days	8days	12days	
-	SCC 5	+	+	+	-	-	-	-	-
-	SCC 9	+	+	+	+	-	-	-	-
-	SCC 11	+	+	+	-	+	+	+	-
-	SCC 12	+	+	+	-	-	-	-	-
-	SCC 13	+	+	+	-	-	+	+	+
-	SCC 14	+	+	+	+	-	-	-	+
-	SCC 17	+	+	+	+	-	+	+	-
-	SCC 18	+	+	+	-	-	-	-	-
-	SCC 19	+	+	+	+	-	-	-	-
-	SCC 21	+	+	+	-	-	-	+	-
-	SCC 22	+	+	+	-	-	-	+	-
-	SCC 23	+	+	+	+	-	-	-	-
-	SCC 24	+	+	+	-	-	-	-	-
-	SCC 26	+	+	+	+	-	-	+	-
-	SCC 27	+	+	+	+	-	-	-	+
01/2001	SCC 28	+	+	+	+	-	-	+	-
04/2001	SCC 29	+	+	+	-	-	+	+	+
06/2001	SCC 30	+	+	+	-	-	-	+	-
07/2001	SCC 31	+	+	+	+	-	-	+	+
07/2001	SCC 32	+	+	+	+	-	-	-	-
30/07/2009	SCC 36	+	+	+	+	-	-	+	-
-	SCC 37	+	+	+	-	-	-	-	+
30/07/2009	SCC 38	+	+	+	+	-	-	+	+
01/06/2009	SCC 39	+	+	+	-	+	+	+	+
01/06/2009	SCC 43	+	+	+	+	-	-	-	+
01/06/2009	SCC 49	+	+	+	-	-	+	+	+
01/06/2009	SCC 52	+	+	+	-	+	+	+	nd
-	SCC 55	-	-	+	-	-	-	-	nd
-	SCC 57	+	+	+	-	+	+	+	+
07/2009	SCC 65	+	+	+	-	-	-	-	-
-	SCC 66	+	+	+	-	-	-	-	+
19/08/2009	SCC 67	+	+	+	-	-	+	+	-
27/10/2008	SCC 74	+	+	+	+	-	-	-	+
27/10/2008	SCC 76	+	+	+	-	+	+	+	+
01/2010	SCC 80	+	+	+	-	+	+	+	+
06/08/2009	SCC 81	+	+	+	-	+	+	+	+
25/03/2010	SCC 83	+	+	+	-	-	-	-	+
27/10/2008	SCC 85	+	+	+	-	+	+	+	+
06/08/2009	SCC 86	+	+	+	-	+	+	+	nd
06/08/2009	SCC 87	+	+	+	-	-	-	-	+
06/08/2009	SCC 90	-	-	+	-	+	+	+	+
06/08/2009	SCC 96	+	+	+	-	-	-	-	-
18/11/2009	SCC 109	+	+	+	+	-	-	-	-
18/11/2009	SCC 110	+	+	+	+	-	-	-	+
27/10/2008	SCC 111	+	+	+	+	-	-	-	-
18/11/2009	SCC 116	+	+	+	+	+	+	+	-
25/03/2010	SCC 117	+	+	+	-	-	+	+	-
24/05/2010	SCC 119	+	+	+	+	-	+	+	-
05/2010	SCC 120	+	+	+	-	-	-	-	-
05/2010	SCC 121	+	+	+	+	-	+	+	-
18/11/2009	SCC 124	-	+	+	-	+	+	+	+
05/2010	SCC 126	+	+	+	-	+	+	+	-
05/2010	SCC 127	+	+	+	-	-	-	-	-
27/10/2008	SCC 130	+	+	+	+	-	-	-	+
27/10/2008	SCC 132	+	+	+	-	-	-	-	+
01/2010	SCC 133	+	+	+	+	-	-	-	+
01/2010	SCC 136	+	+	+	-	-	-	-	nd
01/2010	SCC 138	+	+	+	-	+	+	+	nd
06/08/2009	SCC 146	+	+	+	+	-	-	-	nd
18/11/2009	SCC 149	+	+	+	+	-	-	-	nd

[1] Tuber slice assay

(+ indicates a positive reaction, - indicates a negative reaction and nd indicates it is not determined; -: under "Date" indicates no date available)

Table 3.5: Genetic characterization results for the isolates in this study

		16S rDNA characterization			
Date	**Isolate #**	**BLAST* identification**	**% Identity**	**E-value**	**GenBank accession number**
-	SCC 5	*S. spp*	100%	0.0	KF881291
-	SCC 9				
-	SCC 11	*Streptomyces iakyrus*	99%	0.0	KF881292
-	SCC 12				
-	SCC 13	*S. scabiei*	99%	0.0	KF881285
-	SCC 14				
-	SCC 17				
-	SCC 18	*Streptomyces coelicolor*	100%	0.0	KF881293
-	SCC 19	*S. spp.*	100%	0.0	KF881294
-	SCC 21	*Streptomyces fradiae*	100%	0.0	KF881286
-	SCC 22	*S. spp*	100%	0.0	KF881295
-	SCC 23	*S. spp.*	100%	0.0	KF881296
-	SCC 24				
-	SCC 26	*S. spp*			**
-	SCC 27				
01/2001	SCC 28	*S. flavofuscus*			**
04/2001	SCC 29	*S. scabiei*			**
06/2001	SCC 30				
07/2001	SCC 31	*S. padanus*			**
07/2001	SCC 32	*Streptomyces microflavus*			**
30/7/2009	SCC 36	*S. spp*	100%	0.0	KF881287
-	SCC 37				
30/7/2009	SCC 38	*S. padanus*	99%	0.0	KF881305
1/6/2009	SCC 39	*S. stelliscabiei*	100%	0.0	KF881288
1/6/2009	SCC 43				
1/6/2009	SCC 49	*S. spp*	100%	0.0	KF881306
1/6/2009	SCC 52				
-	SCC 55				
-	SCC 57				
7/2009	SCC 65	*Streptomyces fimicarius*	99%	0.0	KF881307
-	SCC 66				
19/8/2009	SCC 67	*S. spp*	100%	0.0	KF881289
27/10/2008	SCC 74				
27/10/08	SCC 76				
1/2010	SCC 80	*S. scabiei*	100%	0.0	KF881290
6/8/09	SCC 81				
25/3/10	SCC 83	*S. spp*	100%	0.0	KF881297
27/10/2008	SCC 85				
6/8/09	SCC 86	*S. scabiei*	100%	0.0	KF881298
6/8/09	SCC 87				
6/8/09	SCC 90				
6/8/2009	SCC 96				
18/11/09	SCC 109				
18/11/2009	SCC 110				
27/10/2008	SCC 111	*Streptomyces cyaneofuscatus*	100%	0.0	KF881299
18/11/09	SCC 116	*S. scabiei*	100%	0.0	KF881300
25/3/2010	SCC 117	*Streptomyces caviscabies*	100%	0.0	KF881301
24/5/2010	SCC 119	*S. microflavus*	100%	0.0	KF881308
5/2010	SCC 120	*Streptomyces rochei*	99%	0.0	KF881309
5/2010	SCC 121				
18/11/09	SCC 124	*S. scabiei*	100%	0.0	KF881302
5/2010	SCC 126				
5/2010	SCC 127				
27/10/08	SCC 130				
27/10/08	SCC 132				
1/2010	SCC 133	*S. padanus*	99%	0.0	KF881303
1/2010	SCC 136				
1/2010	SCC 138	*S. scabiei*	100%	0.0	KF881304
6/8/09	SCC 146				
18/11/09	SCC 149				

-: under “Date” indicates no date available

*Basic local alignment search tool of the NCBI database **Isolates from Department Primary Industries, Potato Disease Research Devision in Victoria, Australia

The morphology of most isolates in this study resembled that of *S. scabiei.* Although Bukhalid *et al.* (2002) found a South African *S. scabiei* isolate that produces flexuous spore chains, this characteristic was not observed in the isolates in this study. Most isolates grew at a pH close to 7 but some were able to grow at pH 4. Leiminger *et al.* (2012) found most isolates in their study to produce melanin; this is contrary to what was observed in this study where only half of the isolates were tyrosine positive. However we did find, isolates with delayed or weak melanin production, as did Taddei *et al.* (2006) in their study of *Streptomyces* spp. from Venezuelan soils. Isolates from netted scab in the Netherlands were tyrosinase positive, and considered identical to *S. scabiei* (Bouchek-Mechiche *et al.*, 2000b). Tyrosinase negative netted scab isolates were however also reported from Denmark and Sweden (but not studied to species level). All tyrosinase negative isolates from the Netherlands that caused netted scab lesions were identified as *S. reticuliscabiei* (Bouchek-Mechiche *et al.*, 2000b).

Only a few of the isolated strains in this study were found to be pathogenic, which is consistent with previous findings (Loria *et al.,* 1986; Lindholm *et al.,* 1997; Faucher *et al.,* 1992). Pathogenicity and melanin production could not be linked, which was also found in various other studies (Leiminger *et al.,* 2012). Leiminger *et al.* (2012) also observed melanin production by pathogenic and non-pathogenic isolates. Most studies base pathogenicity determination of isolates on the presence of *txtAB* and only conduct pathogenicity pot trials tests on selected isolates (Wanner, 2009; Leiminger *et al.,* 2012; Dees *et al.,* 2013). The largest number of isolates in this study are considered to be *S. scabiei* based on 16S rDNA characterization. Some of which do not have *txtAB,* but most possess all three genes associated with the PAI.

These results and other studies (Bouchek-Mechiche *et al.,* 2000b; Gouws, 2006; Gouws & Geldenhuis, 2011) show that *S. scabiei / S. europaeiscabiei* is the most abundant species associated with common scab of potato in South Africa and that *S. stelliscabiei* can also be isolated from the Western Cape production region. The South African distribution of pathogenic Streptomycetes associated with common scab of potato appears to be random, as no one species is restricted to a certain region. *S. stelliscabiei* was only isolated from the Western Cape, but the small sample sizes of some of the other regions could explain this and further investigation is needed to confirm if the Western Cape ecological niche selects for *S. stelliscabiei.* Gouws (2009) stated that different soil types may select for different scab-causing species and symptoms, suggesting that netted scab prevalence is related to heavier soils found in KZN. This is contrary to

what we found; tubers showing netted scab symptoms mostly originated from the Western Cape which includes the Sandveld region, known for its sandy soils. A previous study in South Africa showed that 56% of isolates were *S. scabiei* or *S. europaeiscabiei* and 0.01% *S. turgidiscaies*; no *S. acidiscabies* was found (Gouws, 2009).

This study revealed a population composition of 22% *S. scabiei* 3% *S. stelliscabiei* and 3% *S. flavofuscus.* Bukhalid *et al*. (2002) also found SA isolates that grouped close to *S. stelliscabiei*. In Germany *S. scabiei* is very seldom found: most isolates are *S. europaeiscabiei,* with *S. turgidiscabies, S. acidiscabies, S. stelliscabiei* and *S. bottropensis* also isolated from time to time (Leiminger *et al.,* 2012). No *S. turgidiscabies* or *S. acidiscabies* were isolated in this study. *S. scabiei* and *S. turgidiscabies* are often found on the same lesion (Hiltunen *et al.,* 2005). Washing tubers and even storing tubers before isolation of the pathogen can result in more *S. turgidiscabies* than *S. scabiei* DNA being isolated from the lesions (Lehtonen *et al.,* 2004; Valkonen, 2004). Dees *et al*. (2013) could only find *S. turgidiscabies* in a few countries but attributed this to the small sample size. They also stated that the lack of a specific geographical species distribution could be due to the use of infected seed tubers or that more than one species could naturally occur in a specific location (Lehtonen *et al.,* 2004; Wanner, 2009). A larger survey is thus needed to determine if *S. acidiscabies* and *S. turgidiscabies* are also associated with common scab of potatoes in South Africa. On the other hand Wanner (2009) found only two *S. turgidiscabies* isolates in a sample of 1074 *txtAB* positive isolates in North America. Climatic conditions may be the reason for this, as the largest number of *S. turgidiscabies* isolates have been obtained from Norway (Dees *et al*., 2012), Japan and Finland (Miyajima *et al.,* 1998; Kreuze *et al.,* 1999; Lehtonen *et al.,* 2004). There may also be competition between species (*S. scabiei* and *S. turgidiscabies*) in terms of ecology (Hiltunen *et al.,* 2009). Another explanation for the apparent absence of *S. turgidiscabies* and *S. acidiscabies* in South Africa could be that the association with potato as a host could be weak. Wanner (2009) stated that these two species are the furthest related to the most common species usually associated with common scab on potato and may have difficulty acquiring or maintaining the PAI.

The unidentified Streptomycetes associated with the lesions in this study comprised 32% of all isolates; similar to a study conducted by Bukhalid *et al.* (2002), in which 25% of the isolates were unidentified. Doumbou *et al.* (2001) found two unusual isolates associated with common scab lesions, but which are possibly not pathogenic: *Streptomyces grisecruber* and *Streptomyces*

violaceusniger. Other species found in our study that are also not pathogenic included *S. coelicolor, S. cyaneofuscatus, S. fimicarius, S. fradiae, S. iakyrus, S. microflavus, S. rochei*; and are seldom or never mentioned in studies done in other countries.

In addition to this; 9% of the isolates found in this study were identified as *S. padanus* based on 16S rDNA sequences; a species that is not regarded as a common scab pathogen. One of these isolates was capable of producing mild symptoms in a pot trial and all isolates were pathogenic in the tuber slice assay. However, none had any of the three pathogenicity / virulence genes usually associated with pathogenic Streptomycetes. Further studies are needed to determine if this is a new potato pathogen in South Africa. *S. caviscabies* and *S. fimicarius* were isolated in Canada from deep pitted lesions (Goyer *et al.,* 1996). However, only *S. caviscabies* has been associated with common scab. These two species were also identified in this study; and although *S. caviscabies* (SCC117) was not positive for pathogenicity with the tuber slice assay, *txtAB* and *nec1* were amplified using the TxtAtB1/ TxtAtB2 and Nf/Nr primers.

Our results suggest that the interactions between the pathogenic and non-pathogenic *Streptomyces* spp. within the lesions are not fully understood. This is the most comprehensive study of *Streptomyces* isolated from tubers post-harvest in South Africa. It still remains important to note that the soil microflora changes during the growing season and the *Streptomyces* responsible for the disease could thus also change during the season and post-harvest. Our study shows certain consistencies with a previous study in South Africa (Gouws, 2006), which focused on the seed tubers: the largest group of isolates resembled *S. scabiei* morphologically, pathogenic isolates not producing thaxtomin A were also isolated, non-pathogenic thaxtomin-producing isolates were found, pathogenic isolates capable of growth at pH 4 are also common. Previously Gouws (2006) suggested that isolates that grow at pH 4 are restricted to certain areas. However this no longer seems to be the case as isolates capable of growth at pH 4 were isolated from most of the regions.

3.5 CONCLUSION

This study is an estimation of the true population of Streptomycetes associated with common scab in South Africa. Future work should focus on a larger sample size to study the evolution and characterization of the Streptomycetes associated with common scab in South Africa. Some of the aspects that need re-evaluation are: the effect of the ratio of pathogenic and non-pathogenic

Streptomycetes on potato common scab lesions; why pathogenicity is acquired or lost; how Streptomycetes are distributed and the interactions between host and pathogen in different ecological niches.

These results may aid other researchers in understanding this pathogen and comparing their results with the fact that so many Streptomycetes (pathogenic and non-pathogenic) were isolated from this study shows that isolation and Koch's postulates alone is not enough to determine the pathogenic Streptomycetes responsible for common scab symptoms. Competition and evolution of this pathogen can result in the development of new pathogenic species. Seed-borne infection is important as it introduces species into new locations. It remains important to monitor the shift in population and to evaluate the effect of incidence and severity of common scab on potatoes; and ultimately to adjust management strategies accordingly. In the past, most research in South Africa was on *S. scabiei* as causal agent but other species may be just as important and also require research.

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CHAPTER 4

Streptomyces: Factors related to pathogenicity

ABSTRACT

Numerous *Streptomyces* species have been shown to cause common scab of potatoes world-wide. This cosmetic disease is characterized by multiple symptoms on the tubers. Symptom expression is thought to be attributed to environmental factors and pathogenic species found on the tuber. Pathogenicity within the *Streptomyces* genus is more the exception than the rule and is thought to be acquired through horizontal gene transfer of pathogenicity and virulence related genes (*txtAB, nec1* and *tomA*). Some of these genes come from closely related species within the *Streptomyces* genus and others are believed to come from other phytopathogenic bacteria. In this chapter we attempted to find a correlation between pathogenicity and the presence of three pathogenicity / virulence genes. Pathogenicity was determined with a tuber slice assay. The presence of *txtAB, nec1* and *tomA* was determined with conventional PCR. Some isolates were found to be pathogenic without any of the genes present, while others were not pathogenic with at least one of the genes. Pathogenicity could thus not be linked to the presence of a single one or a combination of two of the three genes. This suggests that other factors may also be involved in pathogenicity or that pathogenicity and virulence factors among isolates or within a given isolate, can be acquired or lost through mutations as they are required or unnecessary.

4.1 INTRODUCTION

Common scab has been reported in all the potato production areas in SA and causes downgrading of tubers on the fresh produce market and decreases processing potential (Gouws & Geldenhuys, 2011). This disease can also result in rejection of seed lots. Chemical control is limited and moderately resistant cultivars are now becoming susceptible, as is evident from the isolations in Chapter 3 from the moderately resistant cultivar Mondial. In addition a pathogenicity island (PAI) can be horizontally transferred from one species to another, thus making previously non-pathogenic strains pathogenic. There is controversy regarding which factor best represents pathogenicity within the *Streptomyces* complex.

Pathogenicity within actinomycetes is rare (Fry & Loria, 2002; Bignell *et al.,* 2010). A few Streptomycetes however cause necrosis of root and tuber crops (Bukhalid *et al.,* 2002; Kers *et al.,*

2005). Pathogenicity and virulence factors are clustered together on a PAI of the *Streptomyces* genome (Figure 4.1). Pathogenicity islands are defined as DNA regions containing pathogenicity and virulence genes, which are present in pathogenic strains (Arnold *et al.,* 2003) and almost always absent from non-pathogenic strains of the same or related strains. These regions have variable G+C content, mobile elements and distinct boundaries within the genome (Kers *et al.,* 2005). Horizontal transfer of the PAI to related species has been reported (Healy *et al.,* 2000; Bukhalid *et al.,* 2002). Thaxtomin biosynthesis genes, *nec1* and *tomA* are conserved among *Streptomyces scabiei* (Lambert & Loria)*, Streptomyces turgidiscabies* (Miyajima) and *Streptomyces acidiscabies* (Lambert & Loria), and are located on the PAI (Kers *et al.,* 2005; Wanner, 2009). Variations in virulence and the impact thereof on disease incidence and severity are not well understood (Wanner, 2004).

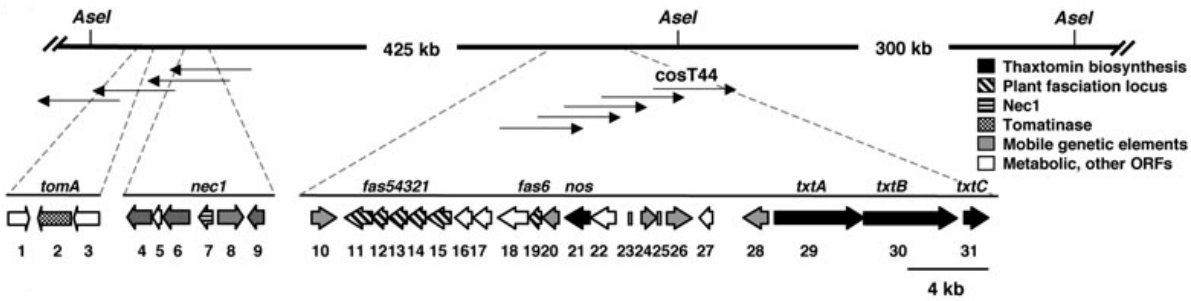

Figure 4.1: Pathogenicity island of *Streptomyces turgidiscabies* (Kers *et al.,* 2005).

Streptomyces scabiei, S. acidiscabies and *S. turgidiscabies* produce thaxtomins, which are phytotoxins that cause necrosis on immature potato tubers and tuber slices (Goyer *et al.,* 1998). Although the mode of action of thaxtomin and its specific target are not yet fully understood (Brochu *et al.,* 2010), it is suggested that these toxins inhibit root and shoot growth, cause hypertrophy, programmed cell death and affect the function of the plasma membrane (Ca^{2+} and H^{+} ion influx) of plant cells (Fry & Loria, 2002; Bignell *et al.,* 2010). These toxins are encoded by *txtAB* and *txtC* genes (Arnold *et al.,* 2003). Loria *et al.* (1995) hypothesized that thaxtomin biosynthesis and production of other secondary metabolites by *Streptomyces* spp. are regulated in a similar fashion. There are other related thaxtomins in the *Streptomyces* genus (Loria *et al.,* 1995; Kim *et al.,* 1999). Thaxtomin A is recognized to be produced by most pathogenic *Streptomyces* species (Loria *et al.,* 1995; Kim *et al.,* 1999). Thaxtomin A production exceeds that of thaxtomin B (second most abundant) in a 20:1 ratio (Loria *et al.,* 1995). Loria *et al.* (1995) demonstrated that two *S. scabiei* strains with differential virulence also differed in the amount of thaxtomin A produced. The strain producing more thaxtomin A was originally isolated from a deep pitted lesion

on a resistant cultivar, whereas the strain producing less was isolated from an erumpent lesion on a susceptible cultivar. Goyer *et al.* (1998) based their pathogenicity trials on growing potato seedlings in inoculated soil and harvesting the progeny tubers. They found that mutants producing 20 times less thaxtomin A than the wild-type strain were still pathogenic on potato. However, Goyer *et al.* (1998) found no distinct correlation between virulence and the quantity of thaxtomin A produced. The tuber slice assay is thus suitable for the evaluation of pathogenicity of Streptomycetes (Loria *et al.,* 1995; Kim *et al.,* 1999). It is however not sensitive enough to determine differences in virulence.

The nec1 protein is an independent virulence factor, as it is not required for thaxtomin production and plays a role in colonization of the host tissue (Kers *et al.,* 2005). Bukhalid *et al.* (2002) demonstrated that *nec1* has been transferred between *S. scabiei, S. acidiscabies* and *S. turgidiscabies.* They also found that the *nec1* sequence of two South African strains differed only in one base pair from the type strain.

Tomatinase is well-characterized in fungal pathogens of Solanaceous crops (Kers *et al.,* 2005). This enzyme is usually associated with loss of membrane integrity. Tomatinase mutations were found not to affect virulence (Seipke & Loria, 2008). The *tomA* gene is also found on the PAI in some pathogenic *Streptomyces* species (Kers *et al.,* 2005).

A secondary metabolite biosynthetic cluster, similar to coronafacic acid (CFA) in *Pseudomonas syringae* (van Hall) and *Pectobacterium atrosepticum* (Gardan) was discovered in the *S. scabies* 87-22 genome (Bignell *et al.,* 2010). Coronafacic acid forms part of coronatine, a phytotoxin that causes chlorosis, stimulation of ethylene production, hypertrophy and inhibition of root elongation. Studies conducted by Bignell *et al.* (2010) showed that the CFA-like biosynthetic cluster is important during colonization of the host and in virulence of *S. scabiei.* It is however not clear how CFA contributes to disease development, and does not appear to be present in *S. turgidiscabies* or *S. acidiscabies*. This gene cluster was also not found in *Streptomyces europaeiscabiei* (Bouchek-Mechiche), a species related to *S. scabiei.*

Pathogenicity of *Streptomyces* spp. is said to be transferred through horizontal gene transfer of pathogenicity and virulence genes (such as *txtAB, tomA* and *nec1*) located on a pathogenicity island (PAI), and it is likely that there has been a shift in the pathogenic population over time (Kers

et al., 2005; Wanner, 2009; Dees *et al.,* 2013). This could well be due to different cultural practices in managing the disease that have led to the selection of species capable of surviving under these altered crop conditions. Only about 10 species harbour all or part of this PAI (Loria *et al.,* 2006). It is widely accepted that if the *txtAB* gene is present, the isolate will be pathogenic (St-Onge *et al.,* 2008; Dees *et al.,* 2013).

The presence of the *txtAB, tomA* and *nec1* genes in South African *Streptomyces* isolates has not yet been investigated, but they are presumed to play a role in pathogenicity and virulence of *Streptomyces* species associated with common scab of potato in South Africa. For this reason we investigated the presence of these pathogenicity and virulence related genes in combination with pathogenicity tests, to determine which gene best corresponds to pathogenicity in *Streptomyces* species on potatoes in South Africa.

4.2 MATERIALS AND METHODS

Streptomyces isolation, growth media and DNA extraction

Two *Streptomyces* isolates used in this study were obtained from the Agricultural Research Council, Pretoria, South Africa; seven from Dr. Tonya Weichel of the Department of Primary Industries, Potato Diseases Research Division in Victoria, Australia and an additional 84 isolates were isolated from diseased tubers of both susceptible and moderately resistant cultivars obtained from 11 of the 16 potato production regions in South Africa from 2008 to 2010 according to the method of Wanner (2004) with minor modifications. The types of lesions isolated from were deep pitted, star shaped, superficial and netted. Plant material was kept at 4^{o}C until isolation. A list of isolates used in this study is given in Table 4.1. Diseased tubers were not surface sterilized in order to obtain all *Streptomyces* associated with the lesions. Pieces of the diseased tuber tissue were excised (including a piece of the healthy tissue underneath the lesion), placed into a maceration bag and macerated with a rubber hammer. Nine milliliters of sterile distilled water was added to the bag. The sample was then serially diluted to 10^{-5} and plated onto water agar. Following incubation at 28^{o}C for 14 days, colonies were viewed under a light microscope for characteristic spiral and flexuous spore chains. These colonies were plated onto yeast malt extract agar (ISP medium 2; YME) to obtain pure cultures. Isolates were stored in 20% glycerol at -20^{o}C. DNA was extracted (0.25g mycelium from YME plates) with the ZR Soil Microbe DNA kit from Zymo Research according to the manufacturer protocol and stored at -20^{o}C until use.

Table 4.1: Isolates used in this study, pathogenicity, presence or absence of *txtAB, tomA* and *nec1* genes and other relevant information associated with each isolate

	Isolation			Pathogenicity			Genes		
Isolate	**Date isolated**	**Growing Region**	**Cultivar**	**Score[1]**	**Lesion type**	**Pathogenic[2]**	***txtAB*[2]**	***tomA*[2]**	***nec1*[2]**
SCC 1	ni	Limpopo	ni	ni	ni	-	-	-	-
SCC 2*	ni	Limpopo	Jumbo	ni	deep pitted	-	-	-	-
SCC 5	ni	Limpopo	ni	ni	ni	-	-	+	-
SCC 6	ni	Limpopo	ni	ni	ni	-	-	-	-
SCC 7	ni	Limpopo	ni	ni	ni	-	-	-	-
SCC 8	ni	Limpopo	ni	ni	ni	-	-	-	-
SCC 9	ni	Limpopo	ni	ni	ni	-	-	+	-
SCC 11	ni	Limpopo	ni	ni	ni	-	-	-	-
SCC 12*	ni	Limpopo	ni	ni	ni	-	+	-	-
SCC 13	ni	Limpopo	ni	ni	ni	+	-	-	-
SCC 14	ni	Limpopo	ni	ni	ni	+	+	-	-
SCC 15*	ni	Limpopo	ni	ni	ni	-	-	-	-
SCC 17	ni	Limpopo	ni	ni	ni	-	+	-	-
SCC 18*	ni	Limpopo	ni	ni	ni	-	+	-	-
SCC 19	ni	Limpopo	ni	ni	ni	-	+	-	-
SCC 21*	ni	Limpopo	ni	ni	ni	-	+	+	-
SCC 22*	ni	Limpopo	ni	ni	ni	-	+	-	-
SCC 23*	ni	Limpopo	ni	ni	ni	-	-	-	-
SCC 24*	ni	-	Mondial	ni	deep pitted	-	-	+	-
SCC 25	ni	Limpopo	ni	ni	ni	-	-	-	-
SCC 26*	ni	-	ni	ni	ni	-	-	+	+
SCC 27	ni	-	ni	ni	ni	+	-	-	-
SCC 28*	01/2001	Australia[a]	Coliban	ni	ni	-	-	-	+
SCC 29*	04/2001	Australia[a]	Atlantic	ni	deep pitted	+	+	+	+
SCC 30*	06/2001	Australia[a]	Atlantic	ni	netted	-	-	-	+
SCC 31*	07/2001	Australia[a]	ni	ni	netted	+	-	-	-
SCC 32*	07/2001	Australia[a]	Pontiac	ni	raised	-	-	-	+
SCC 33*	10/2005	Australia[a]	Desiree	ni	raised	-	-	-	-
SCC 34*	10/2005	Australia[a]	Desiree	ni	raised	-	-	-	-
SCC 36	30/07/2009	Western Cape	ni	2	deep pitted	-	-	-	+
SCC 37*	ni	North West	ni	2	shallow	+	-	-	-
SCC 38*	30/07/2009	Western Cape	ni	5	deep pitted	+	-	-	-
SCC 39*	01/06/2009	Western Cape	BP1	2	star	+	+	+	+
SCC 40*	19/06/2009	Western Cape	BP1	ni	netted	-	-	-	-
SCC 41*	01/06/2009	Western Cape	BP1	2	superficial	-	-	-	-
SCC 42	01/06/2009	Western Cape	BP1	2	superficial	-	-	-	-
SCC 43	01/06/2009	Western Cape	BP1	2	superficial	+	-	-	-
SCC 44*	30/07/2009	Western Cape	ni	3	shallow star	-	-	-	-
SCC 45*	30/07/2009	Western Cape	ni	3	shallow	-	-	-	-
SCC 46	30/07/2009	Western Cape	ni	2	shallow star	-	-	-	-
SCC 47*	07/2009	Mpumalanga	Mondial	5	star	-	-	-	-
SCC 48*	07/2009	Mpumalanga	Mondial	6	star	-	-	-	-
SCC 49	01/06/2009	Western Cape	BP1	2	superficial	+	-	-	-
SCC 51	01/06/2009	Western Cape	BP1	6	netted	-	-	-	-
SCC 52	01/06/2009	Western Cape	BP1	6	netted	ni	+	+	+
SCC 53	01/06/2009	Western Cape	BP1	3	netted	-	-	-	-
SCC 54	07/2009	Mpumalanga	Mondial	7	star	-	-	-	-
SCC 55	ni	ni	Mondial	ni	ni	ni	+	-	-
SCC 57*	ni	Western Cape	BP1	ni	deep pitted	+	+	+	+
SCC 59	07/2009	Mpumalanga	Mondial	5	star	-	-	-	-
SCC 60	25/11/2009	Limpopo	Mondial	ni	ni	-	-	-	-
SCC 61	07/2009	Mpumalanga	Mondial	5	star	-	-	-	-
SCC 63	07/2009	Mpumalanga	Mondial	5	star	-	-	-	-
SCC 64	01/06/2009	Western Cape	BP1	3	netted	-	-	-	-
SCC 65	07/2009	Mpumalanga	Mondial	6	star	-	-	-	-
SCC 66	ni	Western Cape	ni	2	netted	+	-	-	-
SCC 67	19/08/2009	Western Cape	BP1	ni	deep pitted	-	-	-	-
SCC 71	19/08/2009	Western Cape	BP1	ni	netted	-	-	-	-
SCC 73	01/2010	Gauteng	ni	4	deep pitted	-	-	-	-
SCC 74	27/10/2008	KZN	ni	ni	ni	+	-	-	-
SCC 76	27/10/2008	Limpopo	ni	ni	ni	+	-	-	-

Table 4.1: Isolates used in this study, pathogenicity, presence or absence of *txtAB, tomA* and *nec1* genes and other relevant information associated with each isolate (continue)

Isolate	Date isolated	Growing Region	Cultivar	Score[1]	Lesion type	Pathogenic[2]	*txtAB*[3]	*tomA*[3]	*nec1*[3]
SCC 80	01/2010	Gauteng	ni	6	deep pitted	+	+	+	+
SCC 81	06/08/2009	Free-State	ni	2	shallow	+	+	+	+
SCC 82	06/08/2009	Free-State	ni	2	shallow	-	-	-	-
SCC 83	25/03/2010	Mpumalanga	BP1	3	superficial	+	-	-	-
SCC 85	27/10/2008	KZN	ni	ni	ni	+	-	-	-
SCC 86	06/08/2009	Free-State	ni	2	shallow	ni	+	+	+
SCC 87	06/08/2009	Free-State	ni	2	shallow	+	+	+	+
SCC 89	06/08/2009	Free-State	ni	2	shallow	-	-	-	-
SCC 90	06/08/2009	Free-State	ni	2	shallow	+	+	+	+
SCC 96	06/08/2009	Free-State	ni	2	shallow	-	-	-	-
SCC 105	03/08/2009	Western Cape	ni	2	shallow	-	-	-	-
SCC 109	18/11/2009	Northern Cape	ni	2	shallow	-	-	-	-
SCC 110	18/11/2009	Northern Cape	ni	6	deep pitted	+	-	-	-
SCC 111	27/10/2008	KZN	ni	ni	ni	-	-	-	-
SCC 116	18/11/2009	Northern Cape	ni	3	shallow star	-	+	+	-
SCC 117	25/03/2010	Northern Cape	BP1	5	superficial	-	+	-	+
SCC 119	24/05/2010	KZN	ni	3	ni	-	+	+	-
SCC 120	05/2010	Mpumalanga	ni	2	star lenticel	-	-	-	-
SCC 121	05/2010	Mpumalanga	ni	2	star lenticel	-	+	+	-
SCC 124	18/11/2009	Northern Cape	ni	6	deep pitted	+	+	+	+
SCC 126	05/2010	Mpumalanga	ni	2	star lenticel	-	-	-	-
SCC 127	05/2010	Mpumalanga	ni	2	star lenticel	-	-	-	-
SCC 129	27/10/2008	KZN	ni	ni	ni	-	-	-	-
SCC 130	27/10/2008	KZN	ni	ni	ni	+	-	-	-
SCC 132	27/10/2008	KZN	ni	ni	ni	+	-	-	-
SCC 133	01/2010	Gauteng	ni	5	deep pitted	+	-	-	-
SCC 136	01/2010	Gauteng	ni	6	deep pitted	ni	+	+	+
SCC 138	01/2010	Gauteng	ni	6	deep pitted	ni	+	+	+
SCC 139	01/2010	Gauteng	ni	6	deep pitted	-	-	-	-
SCC 146	06/08/2009	Free State	ni	2	shallow	ni	-	-	-
SCC 149	18/11/2009	Northern Cape	ni	6	deep pitted	ni	+	+	-

ni indicates that no information was available upon isolation

[1] percentage of the tuber surface covered in scab (see Appendix A)

[2] + pathogenic according to tuber slice assay; –non-pathogenic according to tuber slice assy;

[3] + indicates the detection of the product amplicon and – indicates that no product amplicon was detected

* repeated tuber slice assay; [a] Isolates received from Department of Primary Industries, Potato Diseases Research Division in Victoria, Australia

Pathogenicity tests

Pathogenicity was determined using the tuber slice assay according to Loria *et al.* (1995). Cultures were grown on oatmeal agar (ISP medium 3) for seven days at 28°C in the dark. Seed tubers (Generation 1) of a susceptible cultivar (BP1) were peeled and cut into disks (2cm^2 x 0.5cm thick). The disks were sterilized in 0.05% calcium hypochlorite and 0.1% calcium carbonate for three minutes and rinsed twice with sterile distilled water. Disks were placed in sterile Petri plates and an agar plug containing sporulating colonies was inverted onto the disk. Oatmeal agar plugs were used as control. Plates were then incubated at 28°C for five days and evaluated under dissection and light microscopes. Each isolate was replicated three times and the assay was repeated with selected isolates (31%) to confirm results.

PCR amplification of *txtAB, nec1* and *tomA* genes

Polymerase chain reaction (PCR) was used to determine the presence or absence of the three pathogenicity and virulence genes. Primers for the specific gene regions to be amplified (*txtAB, nec1* and *tomA*) were obtained from Whitehead Scientific as referenced by Wanner (2006) and Bukhalid *et al.* (1998). Primer pairs and conditions used in the PCR reactions are described in Table 4.2. The PCR analysis was carried out in 25µl reactions containing 5u/µl Taq DNA polymerase (recombinant) from Fermentas Life Science, 10xNH_4 reaction buffer (Bioline), 2.5mM dNTP mix (Bioline), 50mM $MgCl_2$ (Bioline) and 0.5µl template DNA. Amplification was carried out in an MJ Mini Personal Thermal Cycler (Bio-Rad) with an initial denaturation step at 95°C for 5min; followed by 35 cycles of denaturation at 95°C for 20 seconds, annealing for 30 seconds and extension at 72°C for 1 minute, followed by a final extension step at 72°C for 7 minutes and holding at 4°C. The annealing temperature for each primer pair is indicated in Table 4.2. Amplified DNA products were separated on 1% agarose gels (containing 0.5µg/ml ethidium bromide (Merck chemicals) and viewed under UV light.

Table 4.2: Primer pairs used in PCR for detection of pathogenicity / virulence genes and their associated PCR conditions

Gene	Primers	Annealing temperature (°C)	$MgCl_2$ concentration (mM)	Product size (bp)	Reference
txtAB	**TxtAtB1** 5'-CCACCAGGACCTGCTCTTC-3' **TxtAtB2** 5'-TCGAGTGGACCTCACAGATG-3'	50	1.25	385	Wanner, 2006
tomA	**Tom3** 5'-GAGGCGTTGGTGGAGTTCTA-3' **Tom4** 5'-TTGGGGTTGTACTCCTCGTC-3'	50	1.25	392	Wanner, 2006
nec1	**Nf** 5'-ATGAGCGCGAACGGAAGCCCCGGA-3' **Nr** 5'-GCAGGTCGTCACGAAGGATCG-3'	64	1.25	700	Bukhalid *et al.,* 1998

4.3 RESULTS

Pathogenic isolates comprised 29% of the 84 isolates tested (refer to Table 4.1), which was expected as only a few Streptomycetes are pathogenic, and other non-pathogenic Streptomycetes are frequently isolated from lesions (Song *et al.,* 2004; Taddei *et al.,* 2006). Figure 4.2 illustrates typical results found with the tuber slice assay. Pathogenicity was confirmed by a collapse of the cells directly underneath the agar block and a necrotic ring around the agar block.

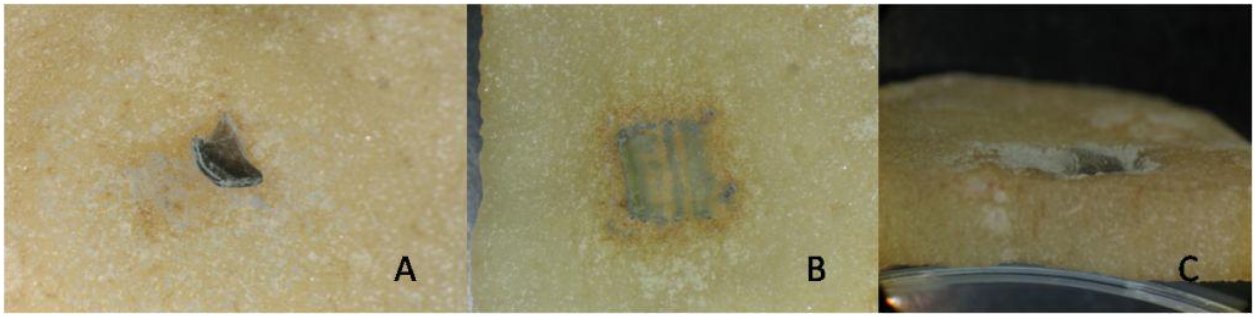

Figure 4.2: Potato tuber slice assay showing a non-pathogenic *Streptomyces* isolate (A), necrosis surrounding the agar plug taken as a pathogenic result (B), collapse of the tissue directly underneath the agar plug also taken as a pathogenic result (C).

The three genes were found in the same frequency among isolates. About 19% of the pathogenic isolates did not contain any of the three genes tested for. All isolates containing all three pathogenicity / virulence genes were pathogenic. One isolate had *txtAB* only and was pathogenic. However none of the other isolates containing just *txtAB* or a combination of two genes were pathogenic, which was surprising as *txtAB* is considered a determining factor for pathogenicity within the *Streptomyces* complex. Statistical analysis revealed that the presence of all three genes is a better indicator of pathogenicity ($P > 0.00005$).

4.4 DISCUSSION

Not one of the pathogenicity / virulence genes amplified in this study occurred more frequently than the others and it would appear that *txtAB* alone does not determine pathogenicity. Although one isolate (SCC14) was found to be pathogenic with only *txtAB*, the majority (89%) of isolates required the presence of all 3 genes for pathogenicity in the tuber slice assay. These results must be confirmed in pot trials. Only about 2% of the *Streptomyces* spp. described to date are pathogenic and there have been reports of pathogenic isolates lacking some pathogenicity determinants. Wanner (2004) found two isolates that lacked the *txtA* and *nec1* genes but which were pathogenic on radish. Pathogenic strains lacking the *nec1* gene have been reported in the United States, Japan, Hungary, South Africa and Korea (Bukhalid *et al.,* 1998; Kreuze *et al.,* 1999; Park *et al.,* 2003; Gouws, 2006). Lerat *et al.* (2010) pointed out that pathogenicity depends on the capacity of the species to synthesize the toxins. Although Park *et al.* (2003) reported two potato scab-causing isolates from Korea that did not produce thaxtomin.

Pathogenicity of common scab-causing isolates in South Africa, however, seems to be linked to the presence of all three pathogenicity / virulence genes. Pánková *et al.* (2012) did not find the haplotypes TNt, Tnt or tNT in their study focusing on plant pathogenic *Streptomyces* spp. in Central Europe. In this study we found at least one isolate of every haplotype. The haplotype composition of the South African population was as follows: haplotype tnt comprised 66% of the isolates; 12 TNT isolates (mostly deep pitted lesions but also some shallow lesions were associated with these isolates); 7 Tnt isolates; 4 tNt isolates (associated with various lesion types); 3 tnT isolates; 5 TnT isolates (mostly from shallow lesions); 1 TNt isolate (from a shallow lesion) and 1 tNT isolate. Similar to the findings of Pánková *et al.* (2012), the TNT haplotype was mostly associated with the deeper and more severe lesions. We also found that the TNT haplotype is associated with *S. scabiei* or *S. europaeiscabiei* and *S. stelliscabiei,* which is consistent with what Pánková *et al.* (2012) found for isolates in Central Europe. Dees *et al.* (2013) found the haplotypes TNT, TnT, TNt and Tnt in Norway; 60% of the population from this study in Norway did not have *nec1* but did have *txtAB*, while 37% did not have *tomA* but had *txtAB*.

Non-pathogenic *S. scabiei, S. acidiscabies* and *S. turgidiscabies* have been reported from Finland and America (Faucher *et al.,* 1992; Lindholm *et al.,* 1997; Wanner, 2006), which makes species identification alone insufficient to classify an isolate as pathogenic. Leiminger *et al.* (2012) and Wanner (2009) also isolated pathogenic but *txtAB*-negative *S. acidiscabies, S. stelliscabiei* and *S. bottropensis* from Germany and North America respectively. Genetic variation and evolution within species is not uncommon (Dees *et al.,* 2013). The *nec1* and *tomA* genes are at the opposite end of the chromosome to the *txtAB* gene and can be transferred and evolve independently (Aittamaa *et al.,* 2010). A good example of the evolution of pathogens and their ability to acquire genes from other pathogens is the horizontal transfer of the *nec1* gene in Streptomycetes.

Wanner (2009) proposed that the presence of *nec1* and *tomA* without the presence of *txtAB* might be due to non-pathogenic *Streptomyces* spp. acting as reservoirs for genes associated with pathogenicity. Following pathogenicity tests (growing selected isolates in pot trials at 18^{o}C, natural light and drip fertigation) Dees *et al.* (2013) found that symptoms within and between species was the same. The *txtAB* negative isolates did not cause symptoms in this study, similar to what was found by Leiminger *et al.* (2012). Of the *txtAB* negative isolates, Wanner (2009) found most had one or both of the *nec1* and *tomA* genes. *TxtAB* negative isolates produce mild symptoms and in some cases DNA extractions from common scab lesions did not show the presence of *txtAB*

(Wanner, 2009). It is however, still accepted in most parts of the world that only the presence of the *txtAB* gene in an isolate is enough to produce lesions (Wanner, 2009). Dees *et al.* (2013) found no correlation between species and geographical distribution or PAI and geographical distribution; also no correlation between disease severity and species or severity and PAI. Dees *et al.* (2013) did not find any non-pathogenic isolates containing the *nec1* or *tomA* genes, however pathogenicity was determined (for only 10 isolates) by PCR of the *txtAB* gene. The possibility exists that there are pathogenic isolates containing the *nec1* and *tomA* genes, while lacking the *txtAB* gene (Wanner, 2009). One of the putative *S. scabiei* isolates in the present study had an incomplete PAI; while another had none of the PAI genes.

Bignell *et al.* (2010) discusses the multitude of other *S. scabiei* (strain 87-22) genes that were found by comparative genomics. These genes were revealed to be conserved within other sequenced microbial pathogens and play a role in disease development and virulence. Some of these pathogenicity and virulence factors include coronafacic acid- and coronatine-like molecules, concanamycins, expansin-like proteins, cutinase, pathogenesis-related proteins and phytohormones. Natsume *et al.* (2005) isolated the 16-membered macrolide FD-891 as the causal phytotoxin of russet scab in Japan. Goyer *et al.* (1998) suggested that other factors (phytohormones and hydrolytic enzymes) may also be involved in disease processes. Kinkel *et al.* (1998) stated that 40% of variation in disease severity cannot be attributed to thaxtomin levels. Wanner (2004) stated that other pathogenicity determinants besides thaxtomin are involved in the disease caused by Streptomycetes on radish.

Comparative genomic analysis thus suggests that pathogenicity in *Streptomyces* spp. involves multiple genetic determinants (Bignell *et al.,* 2010). Comparative genomics also revealed 810 genes encoding secreted proteins, transcriptional regulators and membrane transporter enzymes for secondary metabolite biosynthesis. These genes were either shared or unique to pathogenic species on potato and sweet potato, suggesting that each species possesses its own unique set of pathogenicity and virulence genes.

Park *et al.* (2003) found two pathogenic strains that did not produce thaxtomin A. The *nec1* gene was amplified by PCR but a southern blot revealed the absence thereof. They then concluded that the *txtA* and *nec1* genes are not essential for pathogenicity or thaxtomin synthesis. Pánková *et al.* (2012) recorded *txtAB* negative isolates that was capable of causing the similar severity as *txtAB*

positive isolates. Non-pathogenic isolates in which the *txtAB* gene was amplified could contain mutations within the gene beyond the primer binding sites, as was found by Healy *et al.* (2000). Mutations in the thaxtomin A gene of a *S. acidiscabies* strain rendered that strain non-pathogenic.

Most characterized *S. scabiei* strains have been found to be pathogenic (Valkonen, 2004). Many *S. turgidiscabies* strains isolated from Finland are non-pathogenic (Lindholm *et al.,* 1997). On comparison of the *Streptomyces* pathogenomes of *S. scabiei* and *S. turgidiscabies*, Bignell *et al.* (2010) found genes that are specific to one species and others that are conserved between the two species. This suggests that each species has a unique set of genes contributing to pathogenicity, in addition to the conserved genes. Little is known about the molecular mechanisms involved in *Streptomyces* pathogenicity. Most researchers believe that similar mechanisms are shared by Streptomycete pathogens (Bukhalid *et al.,* 2002; Wanner, 2004; Cullen & Lees, 2007; Florez-González *et al.,* 2008; Qu *et al.,* 2008); however because of the rapid symptom development in the *Streptomyces*-potato pathosystem Loria *et al.* (2003) proposed multiple mechanisms of pathogenicity.

Although thaxtomin is important for disease expression, Tegg & Wilson (2010) did not find a relationship between resistance to common scab disease and tolerance to thaxtomin toxicity, which would suggest that reaction to this toxin is governed by many variables. Susceptibility in the glasshouse compared to field trials was found to be different. Results from this study indicate that cultivar resistance trials cannot be relied upon when only considering thaxtomin tolerance (Tegg & Wilson, 2010). It is thus not possible to use presence of the *txtAB* gene as the sole indicator of pathogenicity of *Streptomyces* strains.

South African Streptomycete isolates possibly contain rearrangements or deletions in the pathogenicity island pathogenicity and the presence of virulence and pathogenicity genes do not correlate well. This is also supported by various other studies where deletions and mutations have been identified (Goyer *et al.* 1998; Healy *et al.* 2000; Bignell *et al.* 2010). Similar to results in this study, Gouws (2006) also found pathogenic *Streptomyces* isolates from seed tubers that did not produce thaxtomin (14%), and non-pathogenic isolates that did produce thaxtomin (6%). Arnold *et al.* (2003) discusses the selfish operon model; where natural selection clusters genes related to affecting host fitness; and that these genes may be lost under relaxed conditions. Other factors, not yet determined, may also play a role in pathogenicity of South African common scab isolates.

Streptomyces padanus (SCC38 and SCC133) was frequently isolated from common scab lesions in South Africa (see Chapter 2) and even though they do not have any of the known pathogenicity genes, these isolates caused necrosis and collapse of cells during the tuber slice assay. *Streptomyces luridiscabiei* and *Streptomyces puniscabiei* are speculated by Park *et al.* (2003) to have a pathogenicity factor other than *txt,* as these isolates were also found to cause common scab on potatoes in Korea. This may well be the case in South Africa.

4.5 CONCLUSION

PCR reactions using *txtAB, nec1* and *tomA* specific primers revealed the presence of all three pathogenicity / virulence marker genes in South African common scab isolates; i.e. all haplotypes were present in the population tested. One putative *S. scabiei* isolate did not test positive for *nec1* and another putative *S. scabiei* did not test positive for any of the three pathogenicity / virulence genes. The co-occurrence of all three of the pathogenicity / virulence genes tested for in this study within an isolate could be linked to the pathogenicity of that isolate based on the tuber slice assay. Knowledge of the multitude of genes involved in pathogenicity and comparative genome sequences will lead to a better understanding of pathogenicity and virulence within *Streptomyces.* In this study, not one single gene could be linked to all pathogenic *Streptomyces* isolates in South Africa, but instead it appears as if all three genes need to be present for the isolate to be pathogenic. Optimization of quantification techniques of *Streptomyces* species for predicting common scab in South African potato production should be focused on *txtAB, nec1* and *tomA* together. Quantifying one gene alone may lead to overestimation.

Given the linear structure of the *Streptomyces* spp. chromosomes, it is possible that genes close to the edges can be lost because of instability of the chromosomes. This instability is related to the evolution of this genus based on the frequent structural changes of the chromosome (Kiss *et al.,* 2010). It would be wise to repeat this trial with the two non-pathogenic isolates containing *txtAB* and then to sequence the amplicon to determine how these two differ from one another. It is important to know the factors involved in pathogenicity as they can be used in in-vitro screening of germplasm for potato breeding programs. Future disease management programs will rely strongly upon finding these pathogenic species within populations and selecting for tolerance in potato cultivars. Further studies are needed to determine the pathogenic gene profile of South African isolates, to aid in common scab management programs and the possible use of genes in quantification of the inoculum within soil.

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CHAPTER 5

Potato common scab intensity related to isolates and soil conditions

ABSTRACT

The variability in disease-causing factors related to common scab on potatoes makes this disease difficult to control and even more difficult to predict. *Streptomyces scabiei* was first reported as the causal agent of common scab, and it has been widely accepted that this pathogen is prevalent under slightly alkaline and aerated conditions. However other *Streptomyces* spp. have been isolated and characterized that cause scab disease under low pH and higher moisture conditions. In this study *Streptomyces* isolates were evaluated in terms of symptomatology, yield reduction, disease incidence and severity; under high and low soil moisture conditions, alkaline soil and different initial soil inoculum levels. Results showed that there are complex interactions between isolates and prevailing soil pH conditions, which result in differences in disease incidence and severity. Nutrient elements such as Ca, K and Mg are of more importance than the soil pH itself. Symptomatology was not found to be linked to species, pH or soil moisture conditions alone. Although yield was not affected; inoculated plants produced more but smaller tubers than the control. Future studies should focus more on the species present and the various interactions that take place between different species under changing soil conditions in order to better utilize cultural disease management practices.

5.1 INTRODUCTION

Common scab is regarded as a complex disease caused by multiple species in the genus *Streptomyces* (Goyer *et al.,* 1996; Loria *et al.,* 1997). Severity varies depending on cultivar (Bjor & Roer, 1980), environmental conditions (Adams *et al.,* 1970), and even skin maturity (Hooker & Page, 1960); resulting in more than one type of symptom (Bouchek-Mechiche *et al.,* 2000). One of the most common causal species of common scab is *Streptomyces scabiei* (Lambert & Loria), which produces mostly deep pitted symptoms in aerated, neutral to slightly alkaline soils. The control of common scab has been largely based on cultural practices and the use of resistant or tolerant cultivars as effective chemical products are not available. Increasing the soil moisture content during tuber initiation (Davis *et al.,* 1976) deprives *Streptomyces* species from air they require for survival (Loria *et al.,* 1997; Agrios, 2005). The use of acid-producing fertilizers or sulfur applications lowers the pH of the soil to less favourable conditions for the pathogen (Loria *et al.,* 1997).

Typically *S. scabiei* does not grow at pH below 5 (Lambert & Loria, 1989; Valkonen, 2004). *Streptomyces* spp. that cause disease under lower pH and higher moisture conditions are geographically limited (Hiltunen *et al.,* 2005).

Soil moisture plays a role in common scab incidence and severity (Wharton *et al.,* 2007). When soil moisture is kept close to field capacity during tuber initiation it can inhibit infection. *Streptomyces acidiscabies* (Lambert & Loria) has been isolated from acidic soils in the USA and Canada (Hiltunen *et al.,* 2005; Lambert & Loria, 1989). An *S. acidiscabies* strain was also isolated from soils with a pH of 5.4 in the UK (Thwaites *et al.,* 2010). Han *et al.* (2004) observed that the most damage due to common scab is at pH 5.5 to 7.5. Soil pH could however be of importance due to the availability of nutrients required for disease development, rather than the direct effect of pH itself. Control strategies such as irrigation and altered soil pH often are not enough for common scab control because individual isolates vary in their virulence and ability to tolerate environmental conditions (Dees & Wanner, 2012).

Calcium is one of the elements to consider when evaluating the role of nutrients in the resistance of the plant to *Streptomyces* spp. (Horsfall *et al.,* 1954; Davis *et al.,* 1976; Lambert & Manzer, 1991). When $CaCO_3$ is added to culture media, the number of Streptomycetes isolated increases (Oskay, 2009). Free lime (or exchangeable Ca^{2+}) is believed to increase the risk of common scab (Wale, 2004). Severity of potato scab is positively correlated to the amount of exchangeable calcium in the soil (Horsfall *et al.,* 1954; Goto, 1985), and may be a better indicator for common scab severity than pH. However, Lambert & Manzer (1991) found that when considering post-harvest incidence, pH was a better indicator than exchangeable calcium. Krištůfek *et al.* (2000) conducted common scab trials in two areas with varying cation exchange capacities in the soil. They found a significant positive correlation between Ca/P ratio in the tuber peels and common scab severity. Some nutrients in the tuber periderm however can improve host physiology and so increase resistance (Keinath & Loria, 1989). The most Streptomycetes can be isolated from soils rich in nitrogen, phosphate, iron, manganese and calcium (Oskay *et al.,* 2009). Higher amounts of phosphate promote optimal growth of Streptomycetes. Kiss *et al.* (2010) found that available zinc ions can be used by scab-causing species as they require Zn for the formation of cell wall degrading enzymes. Potassium and magnesium balance in soil may also be a key factor; in an article by Rode (2012) Dr. G. Lazarovits stated that if the ratio of K:Mg is close to 0.4, common scab incidence is much lower than when this ratio is too high or too low.

Bouchek-Mechiche *et al.* (2000) found that *Streptomyces caviscabies* (Krainsky)*, S. acidiscabies* and *Streptomyces turgidiscabies* (Miyajima) cause disease under conditions different to those required for *S. scabiei.* In the same study they found that severity could not be linked with species, and that netted scab causing *Streptomyces reticuliscabiei* Bouchek-Mechiche *et al.* (2000) showed strong host specificity. On the other hand *Streptomyces europaeiscabiei* (Bouchek-Mechiche) was not host specific when causing netted scab (Bouchek-Mechiche *et al.,* 2000). A wide range of lesions was observed to be caused by *Streptomyces stelliscabiei* (Bouchek-Mechiche) and *S. europaeiscabies* (Bouchek-Mechiche *et al.,* 2000).

In France Bouchek-Mechiche *et al.* (2000) found *S. europaeiscabiei* and *S. stelliscabiei* to be associated with common scab-related symptoms normally produced by *S. scabiei. Streptomyces turgidiscabies* was so named because of the severe corky lesions it can produce on susceptible cultivars; but studies in Japan did not support the theory of species-specific symptoms (Takeuchi *et al.,* 1996). Symptomatology is believed to be determined by the environment, potato cultivar and the virulence of the isolates that are present (Hiltunen *et al.,* 2005). Therefore various scab symptoms will be caused by different pathogen species, which require different pH and soil moisture conditions. Although pathogenic Streptomycetes are not tissue or host specific, symptom expression is restricted to expanding host tissue (Bignell *et al.,* 2010). Bouchek-Mechiche *et al.* (2000) investigated the types of symptoms related to common scab and netted scab causing species: *S. scabiei, S. europaeiscabiei* and *S. stelliscabiei* grouped together (group 1), based on their ability to cause similar symptoms when compared on potato tubers, radish and carrots; *S. reticuliscabiei* (group 2) caused netted scab on potato tubers only and a third group, *S. europaeiscabiei,* produced either common or netted scab depending on cultivar or plant species. The symptoms caused by group 3 were found to be dependent on the soil temperature (Bouchek-Mechiche *et al.,* 2000). Wanner (2004) reported that moisture conditions and pH can have an effect on the expression of common scab symptoms.

Disease incidence is strongly related to the amount of initial inoculum in the soil (Keinath & Loria, 1991). Initial inoculum is a very important part of disease development of most soil-borne diseases. Common pre-plant practices can have an effect on the amount of initial inoculum, such as crop rotation that may reduce inoculum (Wharton *et al.,* 2007). Seed-borne inoculum is not enough to contribute to the severity of common scab, soil inoculum is more important, but seed-

borne inoculum can not be overlooked as this contributes to the infestation of new areas (Lehtonen *et al.,* 2004). Wanner (2007) showed that if the inoculum density is high, damage could be caused to the underground parts of the potato plant that could have a secondary effect on the above ground structures of the plant.

In this study three isolates from deep pitted and two isolates from netted scab lesions were selected. Following pot and field trials disease incidence and severity were scored, yields were measured and type of symptoms recorded at the end of the 2009 and 2010 growing seasons respectively. Pot trials were conducted in a greenhouse under high and low soil moisture; and two soil pH conditions. Field trials were conducted at the University of Pretoria experimental farm on mini plots. The aim of the study was to determine if different isolates will cause different symptoms, incidence and severity under varying pH, soil moisture conditions and initial inoculum; and whether or not the number of tubers and yield would be affected.

5.2 MATERIALS AND METHODS

Inoculum preparation

Reference isolates could not be obtained, for this reason the isolates selected were representative of those isolated from common scab lesions: pathogenic containing all three virulence and pathogenicity genes; not pathogenic with no virulence and pathogenicity genes present; pathogenic without any of the known virulence or pathogenicity genes. Inoculum was prepared for use in pot trials to determine the effect of soil pH and moisture on the number of tubers, yield, disease incidence and severity of disease for different isolates (SCC29, SCC30, SCC31, and SCC38; isolate information presented in Table 5.1) isolated from common scab lesions. Inoculum from isolate SCC57 was prepared for field trials to determine the effect of initial soil inoculum on yield, number of progeny tubers, disease incidence and severity. Isolates were obtained from lesions on scabby tubers by excising a small piece from the edge of the lesion and macerating it in sterile distilled water. This was then plated onto water agar (WA) for 21 days and pure colonies resembling *Streptomyces* were obtained on yeast malt extract agar (YME) and stored in glycerol at -20^{o}C. Two three-week-old cultures (YME) of each of four isolates (SCC29, SCC30, SCC31, and SCC38) were flooded with 5ml of sterile water. Spores were scraped off and mixed with 150g vermiculite and 750ml SAY solution (20g sucrose, 1.2g L-asparagine, 0.6g K_2HPO_4, 10g yeast extract in 1L water and adjusted to pH 7.2) (Wilson *et al*., 2009). Sterile YME plates were flooded with 5ml sterile water and used as control. Inoculated vermiculite was incubated at 28^{o}C for three weeks.

Inoculum for the field trial was obtained from growing 100µl from the glycerol store culture of isolate SCC57 on 90mm oatmeal agar (OMA) plates for 14 days at 28°C. Clean OMA plates were used as negative control in the field trial.

Table 5.1: Information on isolates used in this study

Isolate	Region	Date isolated	Pathogenicity[b]	Type of lesion Isolated from	Identification
SCC29	Australia[a]	04/2001	Pathogenic	Deep pitted	*Streptomyces scabiei*
SCC30	Australia[a]	06/2001	Non-pathogenic	Netted	*Streptomyces spp.*
SCC31	Australia[a]	07/2001	Pathogenic	Netted	*Streptomyces padanus*
SCC38	South West Cape, SA	07/2009	Pathogenic	Deep pitted	*Streptomyces padanus*
SCC57	South West Cape, SA	06/2009	Pathogenic	Deep pitted	*Streptomyces scabiei*

[a] Isolates obtained from Department of Primary Industries, Potato Diseases Research Division in Victoria, Australia

[b] Pathogenicity based on tuber slice assy (see Chapter 4)

Planting and growth conditions

The experimental layout of the pot trials was a randomized complete design with three replicates of each treatment combination. Treatment combinations consisted of four isolates and one un-inoculated control, two soil pH levels (pH 6.5 and pH 8.5) and two soil moisture levels (restricted plant available water and field capacity). For example the first three pots had a soil pH 6.5, watered to restricted plant available water and inoculated with the un-inoculated control; all treatment combinations are shown in Table 5.2. Seed tubers of the scab-susceptible cultivar BP1 were planted in plastic bags with a volume of five liters filled two thirds with a 10% vol/vol inoculated vermiculite-soil mix. Sterile vermiculite-soil mix was used for the control pots. A steam-sterilized sandy loam soil was used from the experimental farm at the University of Pretoria. A complete soil analysis was done prior to planting (Table 5.3) at the Department Plant Production and Soil Science at the University of Pretoria according to standard methods.

In order to achieve a pH of 8.5 in the calcium amended treatments, 90g of $CaCO_3$ was added per bag of soil. The soil pH value for the untreated bags was 6.5. The pH was determined by mixing a soil sample 1:2.5 (mass) with water and measured with a portable pH meter after 20 minutes. Restricted plant available water (rPAW) and field capacity (FC) were calculated pre-plant (Brady & Weil, 2002; Van der Linde *et al.,* 2007). Irrigation was done by hand. Watering stayed constant for the first three weeks after planting for all treatments (250ml three times a week), after which the restricted plant available water treatments were watered only twice a week with 250ml, while the

field capacity treatments were watered three times a week irrespective of soil moisture content at time of irrigation. The greenhouse compartment temperature fluctuated between 28°C and 30°C, with night temperatures seldom dropping below 28°C. Plants were fertilized with Multifeed (Plaaskem, South Africa) as per manufacturer instructions, every 10 to 14 days.

Table 5.2: Treatment combinations for pot trials (rPAW: restricted plant available water; FC: field capacity)

Treatment	Soil pH	Soil moisture
Un-inoculated control	pH 6.5	rPAW
		FC
	pH 8.5	rPAW
		FC
SCC29[a]	pH 6.5	rPAW
		FC
	pH 8.5	rPAW
		FC
SCC30[a]	pH 6.5	rPAW
		FC
	pH 8.5	rPAW
		FC
SCC31[a]	pH 6.5	rPAW
		FC
	pH 8.5	rPAW
		FC
SCC38[a]	pH 6.5	rPAW
		FC
	pH 8.5	rPAW
		FC

[a] Isolates described in Table 5.1

Table 5.3: Pre-plant soil analysis of the soil used in the pot trials

Year	Field No	pH	P (Bray I)	Ca	K	Mg	Na	NH_4	NO_3	Sand	Silt	Clay
		H_2O	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	%	%	%
2009	BE1	6.5	10.6	41	33	11	29	3.08	6.78	74.8	7.5	17.5

(Department of Plant Production and Soil Science at the University of Pretoria according to standard methods)

The field trials were carried out on 4 mini-plots (2m x 2.9m) on the experimental farm at the University of Pretoria. The trial layout was a randomized complete block design with 4 treatments and 4 replicates per treatment per block. The top soil was inoculated by removing the top 20cm of soil per row and mixed with the respective treatment inoculum. Soil for each control treatment row was mixed with one clean OMA plate (macerated); for the first treatment (low initial inoculum concentration) the soil in each row was mixed with one OMA plate (macerated) of sporulating cultures; for the moderate initial inoculum concentration treatment, soil in each row was mixed

with three OMA plates (macerated) of sporulating cultures; while for the high initial inoculum concentration treatment, five OMA plates (macerated) of sporulating cultures were mixed into each row. After mixing each row with its allocated inoculum the soil was placed back on top of the row. Planting density was 20cm between seed tubers and 50cm between rows. The first trial was planted with BP13 G6 seed tubers; while BP1 tubers were used in the repeat trial. Mini plots lay fallow for three years prior to planting. Soil analysis was carried out prior to planting and is presented in Table 5.4 at the Department of Plant Production and Soil Science at the University of Pretoria according to standard methods. Planting dates were 2009/10/16 (trial 1) and 2010/04/14 (trial 2), and harvest dates was 2010/02/04 and 2010/07/02 for trial 1 and trial 2 respectively. The mini plots were fertilized at planting with 600kg/ha 2:3:4(30); and plots were not re-inoculated prior to the repeat trial. Weather, especially rainfall was constantly monitored and plots were irrigated accordingly twice a week.

Table 5.4: Pre-plant soil analysis of the mini plots for the field trial

Year	Field No	pH	P (Bray I)	Ca	K	Mg	Na
		H_2O	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
2009	Mini plot 65	7.8	68.8	1306	149	395	70
2009	Mini plot 67	7.8	63.3	1291	86	383	56
2009	Mini plot 69	8.0	79.2	1277	100	386	36
2009	Mini plot 71	7.8	80.3	1348	72	382	75

Department of Plant Production and Soil Science at the University of Pretoria according to standard methods

Statistical analysis

Analysis of variance (ANOVA) was used to test for significant differences in severity, incidence, number of tubers and yield between the three treatments of the pot trials (pH, moisture and isolate) and interactions between them. ANOVA was also carried out on the field trial results for yield, number of tubers, incidence and severity. Treatment means were separated using Fisher's protected least significant difference (LSD) test at the 5% level of significance. Data were analyzed using the statistical program GenStat®. Type of symptoms from the pot trials were analyzed using a 2 x 2 contingency table and the chi-square test at $\alpha = 0.01$.

Soil analysis and disease evaluation post-harvest

Soil pH for the pot trials was measured post-harvest as described previously. Tuber yield per isolate was measured by pooling and weighing all the progeny tubers from plants in the same

treatment combination of the pot trials and for each inoculum level in the field trial. Incidence was noted as the percentage of tubers with scab symptoms and disease severity was determined using a severity key from Wenzel & Demel (1967) as cited in Krištůfek *et al.* (2000) with slight modifications (Appendix A). Types of symptoms (deep pitted, superficial, netted or star lesions; see Figure 2.2 in Chapter 2) observed for each isolate, pH and moisture treatment were noted for the pot trials only.

5.3 RESULTS

The data for yield and number of tubers was acceptably normal with homogeneous treatment variance for the pot and field trials, while angular transformation was needed for incidence and severity data of the pot trials. Inoculated plants in the pot trials showed yellowing of the leaves much sooner than the control plants, which only started yellowing at the time of natural senescence. Yields (weight in kg) and number of progeny tubers (counted) of individual treatments of the pot trials were compared to determine if different isolates decreased yield or number of progeny tubers. Specific isolates did not significantly reduce yield, and this was supported by the field trials where the yield increased rather than decreased as seen in Figure 5.1 (P=0.05). The yield in the pot trials was however significantly lowered by restricted plant available water treatments (P=<0.001). Isolates from the pot trials had a significant effect on the number of tubers produced (P=0.03), the number of progeny tubers was double in the treatment with lower pH and higher water content (pH 6.5 and FC) compared to the other treatment combinations (pH 8.5 and FC; pH 8.5 and rPAW; pH 6.5 and rPAW). The number of tubers produced by plants inoculated with *S. scabiei* SCC31 and SCC38 was significantly higher than that produced by plants inoculated with SCC30 (5% LSD=1.1) or the control, but the tubers were smaller. In our field trial inoculated treatments also showed an increase in number of progeny tubers, but decrease in size (P=0.05) when compared to the un-inoculated controls. However, there were no noticeable differences in yield when the trial was repeated (Figure 5.1).

In the pot trials, SCC30 and SCC38 produced only small shallow lesions at the lenticels. Small colonies of mycelium around the lenticels were also considered as scab during evaluation, but scored lower on the severity scale (Kiss *et al.,* 2010). SCC31 produced star and raised lesions on some of the tubers and *S. scabiei* produced star, deep pitted, raised or shallow lesions on most of the tubers. Soil moisture content and pH did not have an effect on the types of symptoms formed on the tubers based on visual observation (Figure 5.2). However, because isolate SCC29 showed a

variety of lesion types SCC29 was used for the chi-square analysis on soil moisture content and pH. Water had a significant effect on the type of lesion produced, while pH did not seem to influence the type of symptoms formed by SCC29 (Tables 5.5 and Table 5.6).

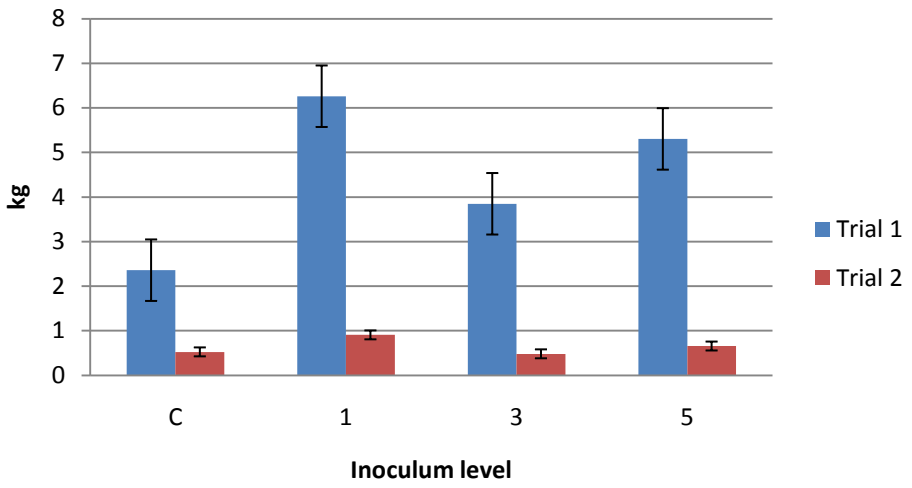

Figure 5.1: Average in yield (kg) for two field trials: 1 and repeated trial 2 on 4 mini plots at the University of Pretoria experimental farm. Inoculum levels were: C: un-inoculated control; 1,3,5: number of 90mm sporulating *S. scabiei* oatmeal extract agar plates added to the top 20cm of a 2.9m x 50cm row. Trial 1: SEM = 0.49; F-probability = 0.05; CV = 77%. Trial 2: SEM = 0.07; F-probability = 0.05; CV = 60%.

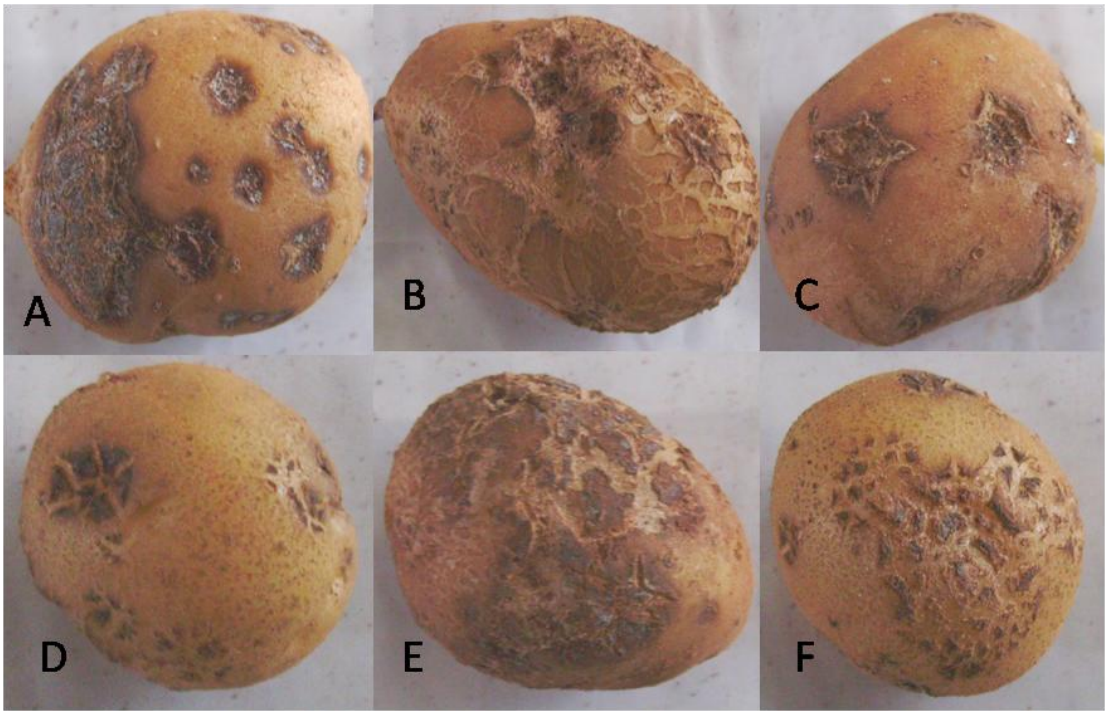

Figure 5.2: Deep pitted and star-like symptoms (A: rPAW & pH 8.5; B: FC & pH 8.5; C: FC & pH 6.5); raised and superficial symptoms (D: rPAW & pH 6.5; E: rPAW & pH 8.5; F: rPAW & pH 6.5) caused by isolate SCC29 (*Streptomyces scabiei)* in the pot trials.

Table 5.5: 2 x 2 contingency table with observed and expected (shown in brackets) frequencies as well as marginal frequencies for soil moisture and types of lesions with respective chi-square values (χ^2) and p-value

Lesions	**Field capacity**	**Restricted plant available water**	**Row Total**
Raised & superficial	4 (9.53)	14 (8.47)	18
Deep pitted & star	14 (8.47)	2 (7.53)	16
Column total	18	16	34*
χ^2_s	14.49		
$\chi^2(1)_{.001}$ & $\chi^2(1)_{.0001}$	10.83 & 15.14 respectively		
p-value	0.00005 < p < 0.0005		

* Grand total

Table 5.6: 2 x 2 contingency table with observed and expected (shown in brackets) frequencies as well as marginal frequencies for pH and type of lesions with respective chi-square values (χ^2) and p-value

Lesions	**pH 8.5**	**pH 6.5**	**Row Total**
Raised & superficial	12 (10.59)	6 (7.41)	18
Deep pitted & star	8 (9.41)	8 (6.59)	16
Column total	20	14	34*
χ^2_s	0.97		
$\chi^2(1)_{.2}$	1.64		
p-value	p > 0.2		

* Grand total

The K:Mg ratios of the mini plots in the field trial were 0.38 for plot 1, 0.22 for plot 2, 0.26 for plot 3 and 0.19 for plot 4. Plot 1 had the lowest disease incidence, while plot 4 had the highest disease incidence and severity. There were significant differences in disease incidence between each inoculum concentration treatments in the field trial (p=0.05). Plot 4 also had the highest amount of calcium and phosphate, characteristics also linked to an increase in disease severity (Krištůfek *et al.,* 2000).

Uninoculated controls did not show any symptoms in the pot trials. There was a significant interaction of pH and soil moisture with disease incidence (Table 5.7), but not with severity during the pot trials. Incidence appears to be much higher under field capacity and pH 6.5. Severity is seemingly not affected by a soil moisture-pH interaction effect.

Table 5.7: Mean incidence of potato common scab at different pH value and soil moisture treatment combinations (n=12)

pH & soil moisture combination	Mean Incidence (%)
6.5 & rPAW	21.79 a
8.5 & rPAW	22.50 a
8.5 & FC	26.60 a
6.5 & FC	62.07 b
SEM	7.39
LSD (5%)	21.28
CV %	77

SEM is the standard error of the means

LSD is Fisher's protected least significant difference test at the 5% level.

Means followed by the same letter do not differ significantly at the 5% level.

CV is the coefficient of variation

A significant effect of isolate on incidence and severity was also observed at the two different soil pH levels (Figures 5.3 and 5.4). At higher pH (pH 8.5) incidence and severity seemed to be lower. There were no significant differences in disease incidence or severity with pH for isolates *S. scabiei* and SCC30 during the pot trials. In contrast, isolates SCC31 and SCC38 appeared to be more sensitive to soil pH. There was no significant effect between isolate and soil moisture content on severity or incidence (Figures 5.5 and 5.6). In field trial 2 the season was drier during tuber initiation than during trial 1 and this increased disease incidence and severity (Figures 5.7 and 5.8); the effect was significant for incidence but not for severity.

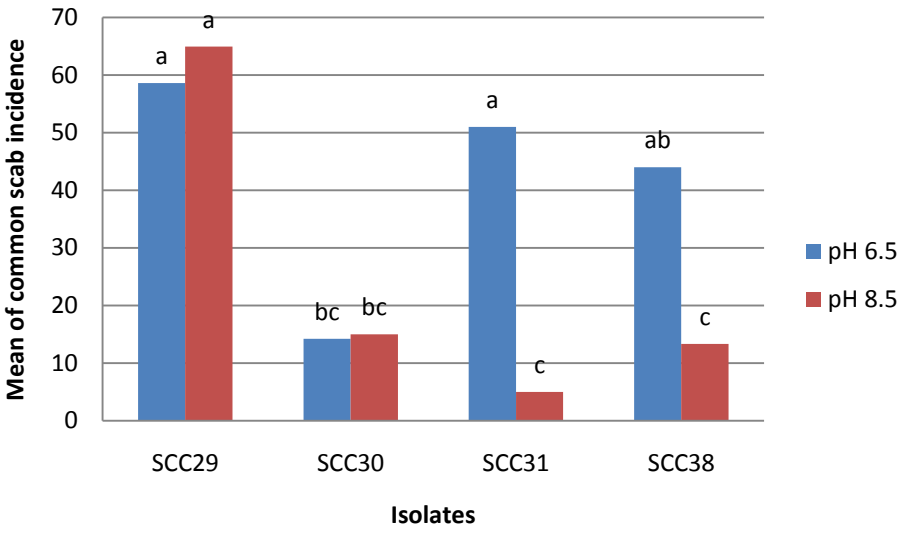

Figure 5.3: Mean disease incidence of potato common scab caused by four different isolates at high and low pH condition combinations. (Different letters above bars indicate significant differences between the treatment combinations of species and pH. SEM = 10.45; F-probability = 0.051; LSD (5%) = 30.1 and CV = 77%).

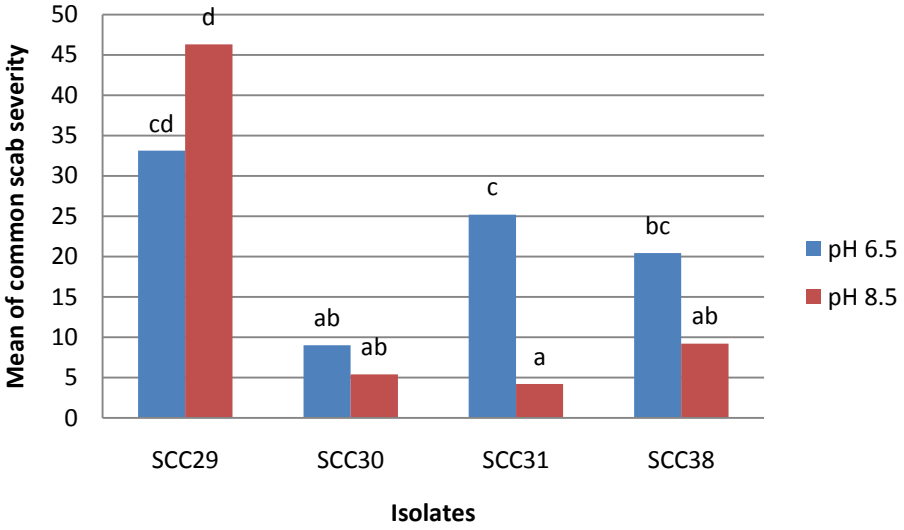

Figure 5.4: Mean disease severity of potato common scab caused by four different isolates at high and low pH condition combinations. (Different letters above bars indicate significant differences between the treatment combinations of species and pH. SEM = 5.28; F-probability = 0.02; LSD (5%) = 15.2 and CV = 67.6%).

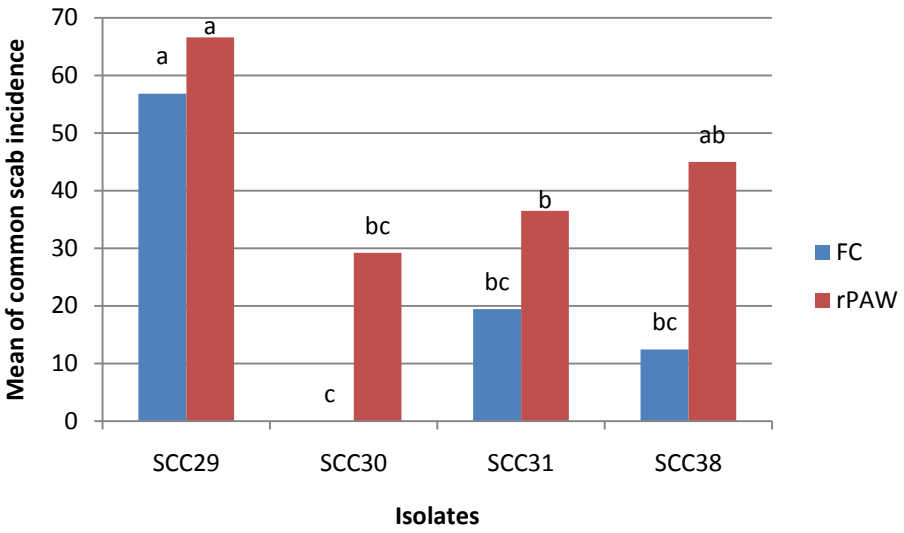

Figure 5.5: Mean disease incidence of potato common scab caused by four different isolates at field capacity (FC) and restricted plant available water (rPAW) treatment combinations. (Different letters above bars indicate significant differences between the treatment combinations of species and soil moisture conditions. SEM = 10.45; F-probability = 0.068; LSD (5%) = 30.1 and CV = 77%).

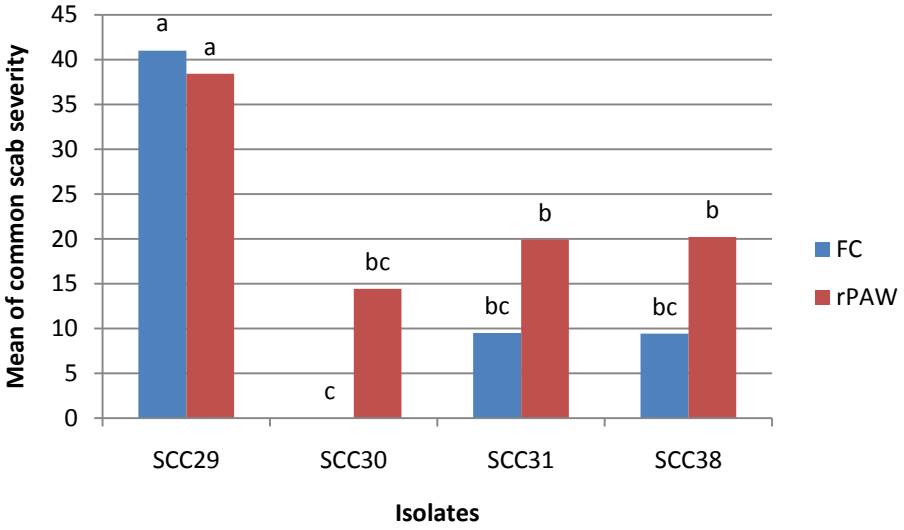

Figure 5.6: Mean disease severity of potato common scab caused by four different isolates at field capacity (FC) and restricted plant available water (rPAW) treatment combinations. (Different letters above bars indicate significant differences between the treatment combinations of species and soil moisture conditions. SEM = 5.28; F-probability = 0.4; LSD (5%) = 15.2 and CV = 67.6%).

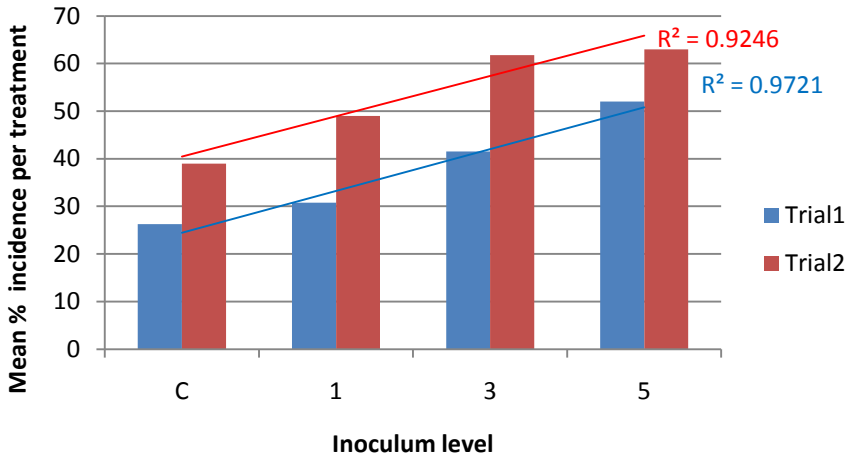

Figure 5.7: Mean incidence of common scab for two field trials: 1 and repeated trial 2 on 4 mini plots at the University of Pretoria experimental farm. Inoculum levels were: C: un-inoculated control; 1,3,5: number of 90mm sporulating *Streptomyces scabiei* oatmeal extract agar plates added to the top 20cm of a 2.9m x 50cm row. Trial 1: SEM = 3.19; F-probability = 0.05; CV = 61.2%. Trial 2: SEM = 8.91; F-probability = 0.05; CV = 42.8%.

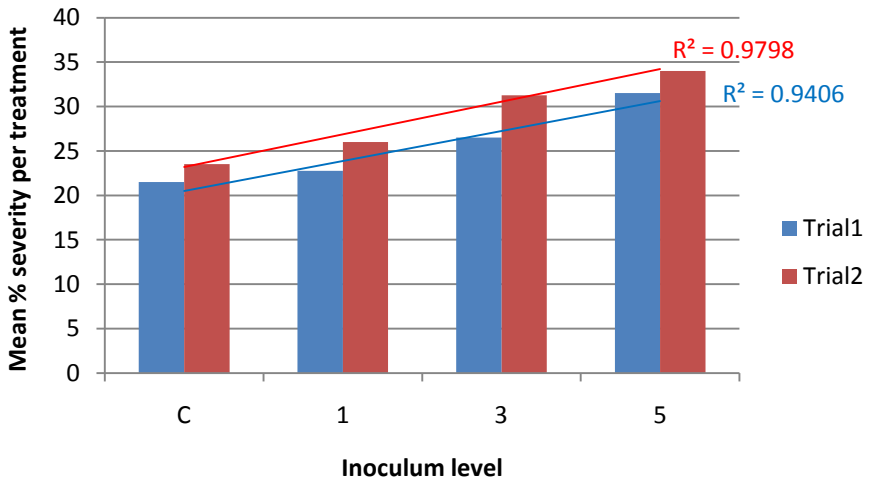

Figure 5.8: Mean severity of common scab for two field trials: 1 and repeated trial 2 on 4 mini plots at the University of Pretoria experimental farm. Inoculum levels were: C: un-inoculated control; 1,3,5: number of 90mm sporulating *Streptomyces scabiei* oatmeal extract agar plates added to the top 20cm of a 2.9m x 50cm row. Trial 1: SEM = 1.42; F-probability = 0.05; CV = 35.2%. Trial 2: SEM = 2.71; F-probability = 0.05; CV = 33.4%.

5.4 DISCUSSION

The yellowing of the inoculated plants noted during these trials requires further investigation. Streptomyces can cause scabby roots; which in turn causes yellowing or stunting due to the restriction of translocation of water and nutrients to the rest of the plant (Dees & Wanner, 2012). Common scab is widely accepted to be a cosmetic disease and usually no yield reduction is observed due to infection by the pathogen (Loria *et al.,* 1997; Agrios, 2005; Gouws & Geldenhuys, 2011). Our results are contrary to those of Han *et al.* (2004), who reported a reduction in yield due to common scab. Hiltunen *et al.* (2005) found a reduction in yield due to infection by *Streptomyces* in one pot trial; but this could not be found in a second pot trial. Our findings are consistent with these results, showing that common scab is a disease that does not necessarily affect yield. The increased yield observed in the field trials could be attributed to weak emergence of plants in the control rows or the increased number of smaller progeny tubers in treatment rows. Yield will however, be affected if the pH of the soil and available water are lowered to levels damaging to the crop itself (Maier *et al.,* 1997; Denner & Venter, 2011). This is consistent with the positive correlation we found between yield reduction and reduced moisture levels in the soil. A reduction in size but an increase in the number of progeny tubers was observed for the inoculated treatments compared to the uninoculated control. Valkonen (2004) and Hiltunen *et al.* (2005)

reported delayed emergence due to infection by pathogenic Streptomycetes, as well as a decrease in yield and increase in the proportion of small tubers, regardless of the potato cultivar. This was also observed in the first field trial we conducted, but could not be observed when the trial was repeated. This may be explained by various interactions of the pathogenic Streptomycetes with other soil microbes in the season between the two trials (the soil was not sterilized) or the difference in climatic conditions (Bouchek-Mechiche *et al.,* 2000; Larkin *et al.,* 2011). A severe infection by common scab pathogens might reduce yield, but we did not find supportive evidence for this as the disease severity in our pot trials was not high.

Common scab symptomatology does not appear to be strongly linked to pH or to pathogen species. *Streptomyces turgidiscabies* has been found to produce both erumpent and deep pitted lesions, which supports the theory that symptom type is not directly related to pathogen species (Hiltunen *et al.,* 2005). In a field trial we conducted during two growing seasons, the symptoms produced by a deep pitted isolate stayed the same even though the temperatures and rainfall were different. Additionally there were netted scab and deep pitted symptoms found concurrently on the tubers in both seasons. It was suspected that the netted scab-causing pathogen occurred naturally in the soil as the incidence and severity of netted scab did not vary between inoculum level treatments. In the pot trials however, more deep pitted and star shaped lesions developed under high soil moisture conditions than in lower soil moisture. This could be because the tubers expand quickly and result in more distinct symptoms under high soil moisture conditions, but as there is no mention of this in literature it has to be investigated further. The isolates from netted scab lesions (SCC30 and SCC31) did not reproduce netted scab on progeny tubers as was expected. This could be explained by the fact that the temperature in the trial was not optimal for netted scab pathogens, as these symptoms are usually reported at temperatures below those in this trial (Bouchek-Mechiche *et al.,* 2000). Different symptoms found on tubers can be attributed to the presence of more than one *Streptomyces* species. Symptoms may also be linked to the method of infection or even an additional stress on the plant itself. Crop physiology related to nutrients as well as soil moisture or a sudden change in environment can also be a determining factor in symptom expression. Various researchers have suggested that the type and severity of scab symptom is not a property of the species but rather of the cultivar (Hooker & Page, 1960; Bjor & Roer, 1980; Hiltunen *et al.,* 2005). Valkonen (2004) tested three cultivars known to have different levels of resistance to *S. scabiei* and *S. turgidiscabies,* and found similar induced symptoms suggesting causal organism could not be distinguished based on symptom type. It is thus difficult

or nearly impossible to determine the species of scab-causing Streptomycete based merely on the type of symptom.

Infection is dependent on the environmental conditions and the scab-causing species present (Oskay *et al.,* 2009; Kiss *et al.,* 2010). Different scab-causing species dominate under different environmental conditions such as soil moisture and pH (Hiltunen *et al.,* 2005; Dees & Wanner, 2012). The significant correlation between pH and isolate in the current study suggested that this interaction is important for disease incidence and severity, as shown in Figures 5.3 and 5.4. Disease incidence and severity caused by *S. scabiei* and SCC30 was not affected by pH while SCC31 and SCC38 seemed to be more sensitive to pH for disease incidence. Struz *et al.* (2004) found that with an increase in pH (from 5.5 to 7.5) there was an increase in the incidence of common scab symptoms. Previous studies have found that an increase in free Ca^{2+} is linked to an increase in severity (Horsfall *et al.,* 1954; Goto, 1985; Wale, 2004).

Davis *et al.* (1976) found a reduction in scab incidence with high soil moisture during tuber initiation. There is circumstantial evidence that *S. turgidiscabies* tolerates and may even cause severe disease symptoms under higher soil moisture conditions than *S. scabiei* (Valkonen, 2004). Data for and against control of common scab with irrigation and decreased soil pH exist (Lapwood *et al.*, 1973; Larkin *et al.,* 2011). Larkin *et al.* (2011) suggested that increasing irrigation may even increase common scab.

It is important to note that different scab-causing species may co-occur within the same field and even on the same lesion. For this reason the combined effect of multiple species on disease incidence and severity cannot be excluded (Hiltunen *et al.,* 2005). Higher incidence was observed under field conditions than in glasshouse trials conducted by Tegg & Wilson (2010); visually this seemed to be the case in our trials. Incidence may be more strongly linked to environment, while severity is linked to inoculum or species. Dees *et al.* (2013) agrees that species has a greater effect on severity than the pathogenicity / virulence genes present. In a field trial we conducted over two seasons severity stayed more constant between seasons than incidence. Incidence increased from season 1 to season 2 and could be attributed to inoculum build up in the soil. With the different levels of inoculum treatments in the field trials it was clear that the level of inoculum positively correlates to incidence and severity.

Nutrient elements within the soil may be more important in common scab disease development than pH, although pH would influence the availability of these nutrients. Based on previous results (Rode, 2012) the closer the K:Mg ratio is to 0.4 the lower the disease incidence. This is consistent with mini plot 1 which had a K:Mg ratio close to 0.4 and also the lowest disease incidence. Simmilar, plot 4 had a K:Mg ratio of 0.19 and had the highest disease incidence. Mini plot 4 also had the highest disease severity, which could be explained by the increased calcium and phosphate in the soil (Krištůfek *et al.,* 2000).

Anomalies in our results indicate that there might be another factor that plays a critical role in incidence and severity, such as temperature. A significant effect of temperature on isolates has been observed in previous studies, where intensity of the symptoms increased with temperature (Bouchek-Mechiche *et al.,* 2000; Han *et al.,* 2004). Maximum intensity of common scab symptoms for *S. europaeiscabiei* and *S. stelliscabiei* isolates was at 20/30^{o}C (night/day); but for *S. scabiei* the maximum was at a constant lower temperature of 20^{o}C (Bouchek-Mechiche *et al.,* 2000). Netted scab symptoms were most severe at lower temperatures of 17^{o}C (Bouchek-Mechiche *et al.,* 2000). Bouchek-Mechiche *et al.* (2000) also found that different cultivars express symptoms differently depending on the isolate. There could thus be an interaction effect between temperature and cultivar. Two cultivars from Finland differed in incidence of disease for different unknown Streptomycetes raising questions about the potato cultivar-dependent differences in biodiversity of Streptomyces within a given lesion (Valkonen, 2004).

5.5 CONCLUSION

Common scab has been described as a disease of varying symptoms caused by a group of Streptomycetes. In this study we investigated if soil moisture content and soil pH have an effect on disease incidence, severity, symptom expression and yield; and whether these effects are related to specific isolates. Though the variance was high it seems that common scab does not affect yield directly but can increase the number of smaller tubers. A single species cannot be linked to a specific symptom, but soil water content seemed to play a more important role than pH. It is possible that cultivar and environment are the drivers behind symptom type. High moisture and low pH will increase incidence, but do not affect severity. Severity seems to be a function of the isolate and optimal pH for pathogen growth and disease cycle.

For the industry, thorough knowledge of the environmental conditions that promote development of common scab can help to improve implementation of cultural control practices. It could also aid in the prediction of final disease intensity. This work provides a good basis for knowledge of *Streptomyces* species and symptomatology in South Africa. However, it is suggested that these trials be repeated with more replicates per treatment combination; this will ensure a lower coefficient of variance and will give a better fit to the true population. The susceptibility of various cultivars to the different scab causing pathogens, effect of available soil nutrients and soil temperatures, need to be investigated further.

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CHAPTER 6

General conclusion

Isolation and identification of isolates are time consuming due to the slow growth of *Streptomyces* spp. and difficult due to multiple species inhabiting the same lesion (Harrison, 1962). This could explain why the pathogen / disease complex of common scab has not received the attention it deserves. Little information on the distribution and frequency of pathogenic Streptomycetes is available; not only in South Africa but across the world. It may become increasingly important to study larger population groups across bigger areas to get a better picture of the true population of Streptomycetes involved in the common scab complex. There is still more to learn about the species involved and the effect of different environmental conditions on each species as well as on their interactions with each other and the environment. The species composition from planting to harvesting could be examined to determine if there is a shift in species diversity during the season and how this affects disease incidence and severity. The genetic characterization of the population could aid in PCR based techniques for early detection, which can prevent spread of species into new areas and pro-active cultural practices to keep incidence of common scab as low as possible.

In South Africa it appears that not one but all three pathogenicity and/or virulence factors are important in disease expression. Pánková *et al.* (2012) believes that the integrity of the pathogenicity island in central Europe, is more important than the presence of one pathogenicity related gene; and this also seems to be the case in South African isolates. With PCR techniques being developed for the detection and quantification of common scab pathogens in the soil, all three or even more genes need to be taken into consideration. Pathogenicity / virulence related genes are just as important for screening in order to breed new common scab resistant cultivars.

Common scab remains one of the reasons for downgrading of tubers on the fresh produce market and also decreases the processing potential of the tubers. Better control methods are needed. One would expect common scab disease studies in controlled environments to be less variable than field trials, but this is not often the case and it might be that the interactions between pathogen, host and environment are not yet fully understood. Bouchek-Mechiche *et al.* (2000) studied a deep pitted scab causing isolate and a netted scab causing isolate and revealed that due to the different climatic requirements of the two pathogens, one may be a problem in seasons or

areas less favourable for the other. Other factors such as nutrients available in the soil could be used in disease estimation but these avenues are only now being explored. It appears that basic epidemiological questions regarding common scab of potato remain unanswered. Cultural practices remain the primary method of disease control and in order to utilize these methods optimally the factors related to common scab disease need to be explored.

In future uncertainty regarding the pathogenicity gene makeup of the South African *Streptomyces* complex must be resolved. The attempt to do this will include sequencing the most important genes related to pathogenicity and comparing South African isolates with isolates from other countries. In addition, the presence of the *txtAB* gene should be correlated with Thaxtomin A expression (using HPLC). If these two factors co-exist in the South African population primers can be designed specific to South African pathogenic *Streptomyces* spp. that could possibly be used for quantitative detection pre-plant. More focus should also be placed on the plant nutrient composition in the soil and the tuber peels and the effect thereof on disease incidence and severity.

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Appendix A

1 = no symptoms (0%)

2 = ⅛ of ½ (6.25%)

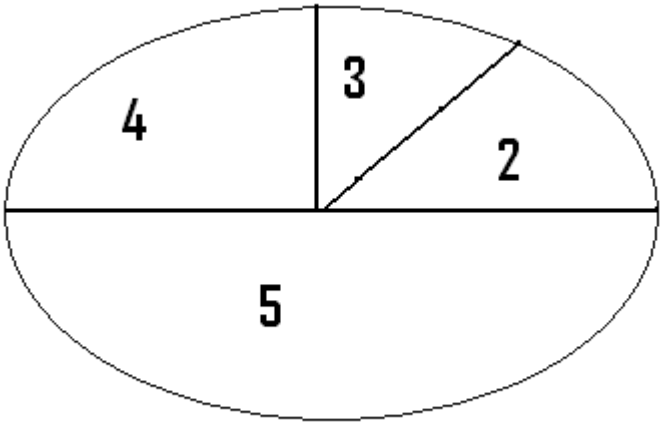
3 = ¼ of ½ (12.5%)

4 = ½ of ½ (25%)

5 = ½ (50%)

6 = ¾ (75%)

7 = whole tuber (100%)



$$\frac{(n \times 1) + (n \times 2) + (n \times 3) + (n \times 4) + (n \times 5) + (n \times 6) + (n \times 7)}{n \text{ sampled} \times 7} \times 100\%$$

Wenzel & Demel (1967) score

1 : 0%

2 : 0.8%

3 : 2.8%

4 : 7.9%

5 : 18%

6 : 34%

7 : 55%

8 : 77%

9 : 100%