CHAPTER 2

LITERATURE REVIEW

1. Introduction

The potato (*Solanum tuberosum* L.) originated from the Peruvian and Bolivian Andes mountains in South America and wild potato plants can still be found in Central America, Mexico and as far North as Colorado (Aartappelreeks, 1974; Steyn, 1999). It has been cultivated for thousands of years prior to the Spanish conquests of the Americas. Archaeological evidence credits the natives of Peru with cultivating the earliest forms of potatoes approximately 4500 years ago. The importance of the potato in the lives of Andean natives is evident in the religious ceremonies created with the tuber. The Inca people worshiped potato gods and celebrated rituals to ensure the success of their crops (Zuckerman, 1998).

In 1532, Spanish explorers arrived in what is modern day Peru, where amongst others they "discovered" the potato (Brown, 1993). Thirty years later it was shipped to Europe and introduced into Spain (Brown, 1993; Zuckerman, 1998). From Spain it was taken to Italy, then England in 1596 and subsequently Germany in 1601. From Europe, the potato spread to North America and the rest of the world including South Africa where it was initially planted during the late 1600's to provide food for sailors on their way to the Far East (Aartappelreeks, 1974).

Once introduced it became known as the food for the poor and was treated with distrust and fear, that lasted centuries. It was not before the mid 1700s that its potential as a staple food was fully realised (Zuckerman, 1998). Unlike the major cereals such as maize and wheat that are fast approaching the practical limits of their production potential, the yield potential of the potato is still largely under exploited (Zandstra, 1997). Changing consumption patterns in Africa and Asia are resulting in a move away from traditional staple foods such as corn and rice to potato.

2. Potato Production in South Africa

Potatoes are produced throughout the greater part of South Africa with 14 production regions stretching from coastal areas to 2000 m above sea level (Steyn, 1999). In some regions like the Sandveld, potatoes are produced throughout the year. Other regions such as the North Eastern Cape is characterised by a limited production season. Because of South Africa's wide climatic spectrum, potatoes can be produced throughout the year, providing a continuous source of fresh tubers (Potatoes South Africa, 1998/99).

Among the 14 production regions of South Africa, the Northern Province had the highest production of 19.2% during the period of 1998/1999 followed by the eastern Free State (16.3%) and western Free State (14.2%). The total hectares planted during this time reflected the percentage production of the various regions (Potatoes South Africa, 1998/99).

South Africa is the largest potato producer in Africa and approximately 1.6 million tons of potatoes are produced annually (Potatoes South Africa, 1998/99). Worldwide it is the 29th largest producer of potatoes (Encarta, 1999). China is currently the largest producer (42 million tons). It is followed by countries such as Russia (40 million tons), Poland (27 million tons) and the United States of America (22 million tons). Production in South Africa has steadily increased since 1992 from 1.25 million tons to 1.6 million tons in 1999. This increase was mainly due to improved irrigation practices, better farm management and research. Since 1991 the amount of hectares planted decreased from 67 thousand hectares to 59 thousand hectares, indicating more efficient farming systems.

The average market price of potatoes has increased from R7/10kg (1993) to almost R12/10kg in 1999 (Potatoes South Africa, 1998/99). For the 1999 season, the gross value of the potato crop was R1 127 970 m (Matlala, 2001).

Fresh produce markets are the most important distribution channels for potatoes, and of the annual crop only 6.8% is exported. Export has slowly increased from 110 000 to 120 000 tons annually as a result of the free trade agreement between South Africa and some of its neighbouring states. Currently, potatoes are mainly exported to neighbouring countries such Namibia, Botswana, Mozambique, Angola and Swaziland. Small quantities are sporadically exported to other countries in East and Central Africa. In 1998/99 almost 250 tons were exported

to England when the phytosanitary prohibition on South African potatoes was temporarily lifted (Potatoes South Africa, 1998/99).

Each of the different production regions is subject to characteristic climatic conditions and specific diseases, and therefore various cultivars have been identified best suited for each area. Other factors that will influence cultivar selection for production regions are: consumer preferences, planting date, soil type and availability of planting material (Visser, 1999). Although cultivars such as Up-to-Date, BP1, Vanderplank and Buffelspoort are traditionally the best known and widely cultivated seed potatoes in South Africa, various other cultivars are also available and institutes such as the Agricultural Research Council, Roodeplaat, Vegetable and Ornamental Plant Institute are constantly evaluating new cultivars suited for South African conditions.

3. Potato Bacterial Wilt

3.1. Symptom Expression

Potato bacterial wilt is caused by *Ralstonia solanacearum* (Kelman, 1953). The pathogen affects both above and belowground plant parts and damage can occur in two ways; premature wilting of top growth, and rotting of tubers either in the soil or in storage (Shekhawat *et al.*, 1992). According to them the time taken for symptom development depends on various factors such as age of the host plants and environmental factors and will vary from one situation to the next.

3.1.1. Aboveground Symptoms

The first symptoms usually appear during the warmer periods of the day. A slight drooping of the tip of one or two of the lower leaves can be seen midday but, towards the evening, the affected leaves may recover. These symptoms can easily be mistaken for a temporary shortage of soil moisture. However, a few days after the first symptoms have been noticed, wilting will extend to the lower leaves which will be unable to recover (Harrison, 1961). According to Conroy (1969) initially some stems in a potato hill will be wilted while others may appear healthy. Eventually all stems will wilt and die (Fig.1).

In initial stages of wilting, the leaves retain their healthy green colour but, in advanced stages, the foliage turns brown and dies (Conroy, 1969). According to Kelman (1953), a slight yellowing of the lower leaves becomes evident when the first leaflets begin to droop. Wilted leaves may fade to a pale green colour and will finally turn brown. Leaves can also roll upwards and inwards from the margins until they are completely inrolled. This inrolling can be accompanied by the slight yellowing of the foliage (Harrison, 1961).

According to Shekhawat *et al.* (1992) vascular browning is not always present. Harrison (1961) also reported that the vascular tissue appeared normal without any signs of discolouration. He concluded that microscopic examination was required at that point to detect the bacteria.

The presence of the pathogen can easily be demonstrated in the field by placing a cut stem section from the diseased plant in a container of water. Within a few minutes fine milky-white strands that are composed of masses of bacteria, will stream from the margin of the tissue (Kelman, 1953; Shekhawat *et al.*, 1992).

Figure 1. Typical symptoms on aboveground plant parts (Stander L. unpublished data).

3.1.2. Symptoms on Tubers

Tubers may or may not show external symptoms. Where the disease is well advanced, the presence of a greyish brown discolouration is visible through the periderm (Kelman, 1953). According to Harrison (1961), external symptoms vary from small brownish-grey depressions on

the tuber near the point of attachment of the stolon, to large lesions that cover the greater part of the surface of the tuber. In the later stages of disease development bacteria pass through the vascular tissue and will emerge at the eyes as sticky, dirty-white, often bubbly masses to which the soil readily adheres.

Very often when a diseased tuber is cut in half, distinct brown discolouration and localised decay is visible in the vascular ring (Fig.2). If slight pressure is applied to the cut tuber, typical greyish-white bacterial slime will ooze from the vascular ring (Shekhawat *et al.*, 1992). At a later stage the parenchymatous tissue can also be affected where cavities filled with millions of bacteria and decomposed tissue are formed. These cavities constantly expand and ultimately merge. At this stage secondary-rotting organisms may occur, turning large parts of the tuber tissue into a foul-smelling mass (Janse, 1996).

According to Harrison (1961) tuber symptoms do not always correlate with aboveground symptoms. Sometimes tubers from apparently healthy plants show typical vascular discolouration while tubers from wilted plants show no symptoms. Shekhawat *et al.* (1992) suggested that vascular browning in the host might depend on the ability of the invading bacterial strain to produce brown pigments.

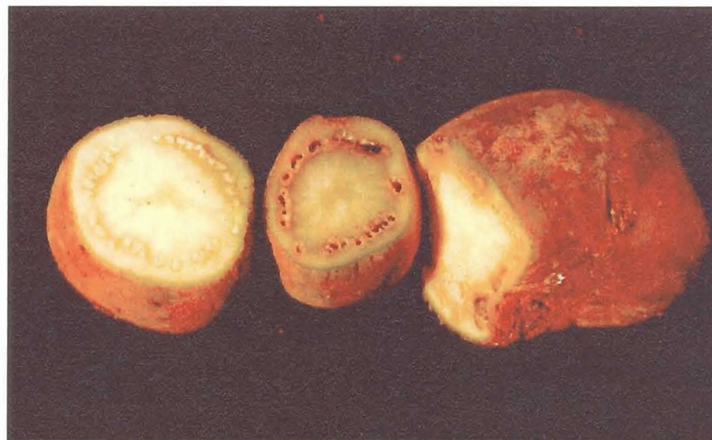

Figure 2. Severe symptoms on infected tubers (Stander L. unpublished data).

3.2. Occurrence of Potato Bacterial Wilt

Bacterial wilt of potatoes was first reported in the United States in 1890 (Kelman, 1953). Currently it occurs in most potato-producing regions throughout the world and is especially a problem in tropical and semitropical areas (Martin *et al.*, 1981a). It is a very important limiting factor in potato production and is one of the most serious bacterial diseases occurring on potatoes. In South Africa it was first reported by Doidge in 1914, but it was not until 1978 that research on this disease gained momentum after its presence was established in a number of seed potato crops (Swanepoel and Young, 1988). Although this disease occurs in temperate regions form time to time, it is endemic in the low-altitude, subtropical areas of Mpumalanga and the Northern Province, the costal regions of KwaZulu-Natal and the south western parts of the Western Cape. In other regions such as high-altitude areas of Mpumalanga and the eastern Free State this disease does not occur often but may be a problem from time to time (Aartappelreeks, 1975).

Little information about the occurrence and distribution of this disease in South Africa could be found. According to Cilliers (1992), confirmed cases of bacterial wilt of potatoes drastically increased during 1988 to 1991. However, this data was obtained through routine testing and did not represent the true occurrence and distribution of the disease. It did however raise concern about the increased occurrence of the disease and in 1996 an improved certification scheme for seed potatoes was introduced in order to control further spread of potato bacterial wilt (Nortje, 1997).

4. *Ralstonia solanacearum*

4.1. General Description

Ralstonia solanacearum is a Gram negative, aerobic, chemo-organotrophic rod with rounded ends and does not form spores or capsules (Kelman, 1953; Shekhawat *et al.*, 1992). The bacterial cells are motile but conflicting reports exist as to the number of flagella present on a single cell. According to Bergey's Manual of Determinative Bacteriology (Holt *et al.*, 1994) more than one flagellum occur, while authors such as Shekhawat *et al.* (1992) described virulent isolates as non-motile and non-flagellate and avirulent isolates as highly motile with one to four polar flagella.

Depending on the strain, the optimum temperature for growth varies between 27 and 37°C with maximum and minimum temperatures of approximately 39°C and 10 to 15°C respectively (Shekhawat *et al.*, 1992). The bacterium also lacks fluorescent, phenazine and carotenoid pigments.

4.2. Taxonomy

The causal agent of bacterial wilt was first isolated in 1896 and identified and described as *Bacillus solanacearum* by Erwin F. Smith (Kelman, 1953). In 1914, the name was changed to *Pseudomonas solanacearum.* For almost 80 years the pathogen was grouped within the genus *Pseudomonas.* By 1973 this genus was divided into five homology groups. Based on rRNA-DNA hybridisation, *P. solanacearum* was placed in homology group II (Hayward, 2000).

In 1992, a new genus *Burkholderia* was proposed based on 16S rRNA sequences, DNA-DNA homology values, cellular lipid and fatty acid composition and phenotypic characteristics (Yabuuchi *et al.*, 1992). Seven species from the original homology group II were transferred to this new genus, including *P. solanacearum.* In 1993, the new genus *Burkholderia*, was validated and *P. solanacearum* became known as *Burkholderia solanacearum* (Hayward, 2000).

In 1995, a new genus derived from *Burkholderia* was proposed by Yabuuchi *et al.* (1995). According to them, *B. picketti* and *B. solanacearum* showed similarities that were different from the remaining five *Burkholderia* species. Based on the results of phenotypic characterisation, cellular lipid and fatty acid analysis, phylogenic analysis of 16S rDNA nucleotide sequences and rRNA-DNA hybridisation three species (*B. picketti*, *B. solanacearum* and *Alcaligenes eutrophus*) were transferred to the new genus, *Ralstonia.* This proposal was validated in 1996 and *B. solanacearum* changed to *Ralstonia solanacearum* (Hayward, 2000).

Difference in host range, geographical distribution, pathogenicity, epidemiological relationships and physiological properties made *R. solanacearum* a very heterogeneous species (Hayward, 1991). Infra subspecific classification was therefore needed in order to define and categorise this diversity especially in targeted plant breeding, epidemiological investigations and quarantine (Gillings and Fahy, 1993a).

A binary system has been used since the early 1960's (Hayward, 1991). This system reflected two different approaches to subspecific classification. The one classified strains into different races according to host affinity, while the other used selected biochemical properties as the basis for separation into biovars. These were informal groupings at the infra subspecific level and were not governed by the Code of Nomenclature of Bacteria.

In 1962, Buddenhagen *et al.* proposed the race classification system. Since then five races have been distinguished based primarily on difference in host range and pathogenicity (Table 1) and, to a lesser extent differences in colony form and pigmentation (Buddenhagen *et al.*, 1962; Walker and Stead, 1993; Hayward, 2000).

Using differences in carbon source utilisation and nitrate metabolism, strains were alternatively divided into five biovars (Table 2). This system has been used since 1964 (Hayward, 1964; Hayward, 1991; Hayward, 2000) and according to Hayward (1994b), was specially designed in the context of epidemiology rather than taxonomy.

It was however found that biovar 2 isolates were not as uniform in phenotype as was previously thought. According to Gillings and Fahy (1993a), biovar 2 strains collected in the Amazon basin had different phenotypic properties both in terms of pathogenicity on various *Solanum* species and in metabolic activity compared to those isolated in the Andean highlands. The second metabolically more versatile phenotype had been designated biovar N2 (Hayward *et al.*, 1990; Gillings and Fahy, 1993b).

There was however, little apparent correlation between these two systems (Sequeira, 1993). According to him, the only real comparison could be drawn between race 3 (the potato race) and biovar 2, although all biovar 2 strains did not necessarily belong to race 3. This lack of concordance was due to the fact that the race system was based on ecological categories while the biovar system was based on classical phenotypic characters (Gillings and Fahy, 1993a).

In 1989, 28 restriction fragment length polymorphism (RFLP) groups were identified, using a selection of DNA probes that specified virulence or the hypersensitive response (Gillings and Fahy, 1993a). According to Hayward (2000), the number of RFLP groups has increased to 46 and will continue to grow as more isolates are examined.

According to Sequeira (1993), the RFLP groups previously described by Cook *et al.* in 1989 could be separated into two divisions that were genetically distinct from each other, sharing only 13.5% similarities. Division 1 consisted of the metabolically more versatile biovars 3, 4 and 5, while division 2 consisted of the other metabolically less versatile biovars 1, 2 and N2. Within each division, the coefficients of similarity were very high (78% for division 1 and 62% for division 2).

Another approach that has been followed, involves the partial sequencing of the *hrpB* and endoglucanase genes. Using these techniques, scientists could subdivide the isolates into three clusters. Cluster 1 contained all isolates of biovars 3, 4 and 5 and was equivalent to division 1. Cluster 2 contained isolates of biovars 1, 2 and N2 from Africa, the Antilles, USA and central and South America, and was equivalent to division 2. Cluster 3 contained isolates of biovars1 and N2 from Africa and the islands of Reunion and Madagascar. Cluster 3 has previously been found to fall close within either division 1 or 2 depending on the method employed to measure diversity. These findings again highlighted the diversity within *R. solanacearum* and were just one more way to sub-classify different isolates (Poussier et al., 2000).

Table 1 Classification of races according to primary hosts affected (Walker and Stead, 1993; Hayward, 2000)

Race	Host(s) primarily affected
1	Solanaceous hosts, diploid bananas and a variety of non-solanaceous hosts
2	Triploid banana and *Heliconia* spp.
3	Primarily potato, to a lesser extent tomato and a few weed hosts such as black nightshade
4	Mainly ginger
5	Mulberry

Table 2 Comparison between different biovars of *Ralstonia solanacearum* (Hayward, 1994b)

Property	Biovar 1	Biovar 2	Biovar 3	Biovar 4	Biovar 5
Utilisation of: mannitol	-	-	+	+	+
Sorbitol	-	-	+	+	-
Dulcitol	-	-	+	+	-
Trehalose	+	- [1]	+ [2]	+ [2]	+
Oxidation of: lactose	-	+	+	-	+
Maltose	-	+	+	-	+
cellobiose	-	+	+	-	+
Gas from nitrate (de-nitrification)	- [1]	- [1]	+	+	+

[1] Uncommon isolates give a positive reaction.
[2] Uncommon isolates give a negative reaction.

4.3. Worldwide Distribution

Ralstonia solanacearum has been reported on every major continent and islands from the warm temperate to tropical regions of the world. Few other diseases of plants are comparable in terms of its geographical distribution (Kelman *et al.*, 1994). Most records of bacterial wilt were within latitudes 40°N and 40°S with high summer temperatures and high rainfall (Hayward, 2000).

However, it is not clear where the disease originated from and if it was from one continent or if it evolved separately in many locations (Buddenhagen and Kelman, 1964).

It is presumed that the disease originated from South America. However, authors such as Hayward (1991) and Sequeira (1993) postulated that there might have been separate evolutionary origins since there are marked differences in the geographical distribution of different biovars. In general, division 2 (i.e. biovars 1, 2 and N2) has evolved primarily in the Americas and division 1 (i.e. biovars 3, 4 and 5) in Asia (Sequeira, 1993). Biovar 1 is the only biovar that has been reported from Florida to North Carolina. In Asia biovar 3 is predominant, with biovar 1 being absent from most parts. Biovars 2, 3 and 4 occur in Australia, China (together with biovar 5),

Indonesia, Papua New Guinea and Sri Lanka. While biovars 1 to 4 have been found in the Philippines (Hayward, 1991).

In future, genomic fingerprinting could be used to shed more light on the evolution and population structure of *R. solanacearum* (Gillings and Fahy, 1993a). Data obtained through techniques such as RFLP analysis might help to clarify the origins and natural hosts of particular strains. For example, Poussier *et al.* (2000) used partial sequencing of the *hrpB* and endoglucanase genes to divide isolates into 3 clusters. Clusters 1 and 2 corresponded to the previously defined divisions 1 and 2 but cluster 3 included biovar 1 and N2 isolates which originates from Africa. These isolates were clearly different from the other strains suggesting a separate evolution (Poussier *et al.*, 2000).

4.4. Host Range and Specificity

With the exception of *Agrobacterium tumefaciens*, the causal agent of crown gall, no other bacterial pathogen attacks as many different plant species as does *R. solanacearum.* Several hundred species, representing more than 50 plant families, have been identified as hosts of this pathogen with the number of new host species still increasing. Some of the most important agricultural crops affected by *R. solanacearum* include tomato, potato, pepper, tobacco, eggplant, groundnuts and bananas. A number of ornamental plants, woody perennials as well as a very large group of weed species can also be infected (Kelman, 1953; Buddenhagen and Kelman, 1964; Hayward, 2000).

Where hosts are concerned, it is important to realise that various factors such as temperature, soil type and rainfall may coincide to influence susceptibility of the host and symptom development (Hayward, 1991). According to him, another reason for the erratic occurrence of bacterial wilt on certain hosts in specific areas could also be due to strain differences in the pathogen. For example, *R. solanacearum* affects *Eucalyptus* only in Brazil and China but not in Australia. Similarly, bacterial wilt of cassava is confined to Indonesia although the host is widely cultivated in other countries where the pathogen is endemic. According to Kelman (1953), hosts do not necessarily show typical wilt symptoms and latent infections are not an uncommon phenomenon.

Apart from potatoes, other Solanaceous crops of economic importance include tomato, eggplant, tobacco and pepper (Hayward, 2000). According to Hayward (1994a), bananas, plantains and ginger are other economically important hosts. Some ornamental plants include bird-of-paradise, geranium and anthurium (Hayward, 1994a) and chrysanthemum (Shekhawat *et al.*, 1992). Leguminous hosts include groundnuts as well as cowpea and some beans. Trees and shrubs can also be affected and perennial fruit and nut crops include custard apple, cashew, cassava and mulberry, with *Eucalyptus* spp. representing woody perennial hosts. Other plants affected are strawberry, sweet potato, papaya, radish and cucumber (Hayward, 1994a). Various weed species can also be infected. Common South African weed species are blackjack, the common thorn apple and black nightshade (Swanepoel and Young, 1988; Swanepoel, 1992).

4.5. Epidemiology

4.5.1. Survival

There are conflicting results regarding the ability of *R. solanacearum* to survive in soil and its persistence in the field cannot be predicted (McCarter, 1976). Survival of the pathogen in soil can be affected by many factors such as initial inoculum concentration, whether the land is left fallow or cropped with a non-susceptible host or the physical, chemical and biological properties of the soil (Moffett *et al.*, 1983). The organism can survive in some soils for long periods even in the absence of host plants, whereas in other soils its population declines rapidly in spite of cultivation with susceptible crops. Race 1 of *R. solanacearum* has been shown to survive in bare-fallowed field plots for at least four years and race 3 for one to two years (Graham *et al.*, 1979). According to Lloyd (1978) there are a number of "sheltered sites" where the bacterium might survive in infected soil between successive plantings of a susceptible crop. These sites are alternative weed hosts, infected host debris, self-sown potato tubers and deeper soil layers.

There have been numerous reports of crop plants and weed species that act as alternative hosts for the pathogen. These plants can be infected and not express any symptoms (Shekhawat *et al.*, 1992). However, since plant debris rapidly decomposes in warm, moist soils, it would provide only temporary sheltered sites for the pathogen. Infected tubers on the other hand can provide shelter for a longer period (Graham *et al.*, 1979). According to Granada and Sequeira (1983) the pathogen also survives by colonising the rhizospheres of non-host plants.

Graham and Lloyd (1979) reported that the pathogen could be detected at all soil depths between 15 and 75 cm but not in the 0 to 15 cm zone, probably due to desiccation during dry weather prior to soil sampling. This could explain why various soil disinfectants performed poorly in the control of bacterial wilt. Martin *et al.* (1981b) were able to detect the pathogen in the first 30 cm of the topsoil. They stated that the vertical distribution of *R. solanacearum* is influenced by various factors such as soil type and rainfall and varies from one site to the next.

4.5.2. Source of Inoculum and Dispersal

Ralstonia solanacearum is regarded as a soilborne pathogen with primarily two sources of inoculum, namely infested soil and infected tubers (Shekhawat *et al.*, 1992). Infected seed tubers are the most effective inoculum source for short and long distance dispersal. Developing countries often lack seed certification programs and farmers are forced to retain tubers from plants grown in infested soils in order to have sufficient seed for the next crop. This practice will promote the problem and spread the disease even further (Ciampi *et al.*, 1980).

Tubers can carry the pathogen in three different ways: in vascular tissues, on the tuber surface and in lenticels (Shekhawat *et al.*, 1992). It was found that during storage the bacterial population decreased rapidly on the tuber surface to non-detectable levels, but survived in vascular tissues for at least eight months at 10 to 15°C and at room temperature. Only when temperatures dropped to 1.6°C, did the pathogen loose its virulence and viability in tubers and only if it was stored for long periods (Nielsen, 1963). Sunaina *et al.* (1989) could still detect the pathogen in lenticels and vascular tissue after 240 days of storage at 4°C.

Infected plant material decaying in soil releases masses of bacterial cells in a slime layer. These slime masses adhere to soil particles and form pellets, enhancing soil survival (Shekhawat *et al.*, 1992).

According to Shekhawat *et al.* (1992), secondary spreading of the disease from infected plants takes place by root contact, irrigation water, farm implements and people and animals moving through infected fields. Kelman and Sequeira (1965) concluded that infection by *R. solanacearum* can occur in roots without any external wounding of the root system if the number of cells in the inoculum is relatively high. Under high soil moisture levels, the pathogen emerges

or is released from the roots of infected plants and spread to adjacent roots of healthy plants, particularly when the roots are in close proximity. Where tubers are cut for planting, cutting knives can also spread the pathogen to healthy tubers (Shekhawat *et al.*, 1992). It was suggested that the pathogen could have an epiphytic phase in its life cycle and together with infected alternative host debris such as weed species can provide alternative inoculum sources (Buddenhagen and Kelman, 1964; Hayward and Moffett, 1978; Graham *et al.*, 1979).

4.5.3. Conditions Favourable for Disease Development

Ralstonia solanacearum predominates in tropical and subtropical regions where warm, humid conditions enhance disease development. The optimum temperature for development of bacterial wilt of potato is between 25 and 36°C (Kelman, 1953). According to Thurston (1963) infection occurred at a soil temperature of 12.8°C but symptoms did not develop unless the soil temperature remained at 21.1°C or higher for several days. Lower-temperature strains of *R. solanacearum* exist and are capable of causing disease under cooler conditions. Although disease incidence is usually lower under cooler conditions, significant losses may still occur (Elphinstone, 1996).

High soil moisture levels favour disease development and the pathogen cannot withstand desiccation. Temperature and soil moisture have a synergistic effect and wilt incidence will decline when soil moisture levels drop to 8 to 10% of water holding capacity. Wilt incidence will decline when the maximum/minimum temperature falls below 20/15°C (Shekhawat *et al.*, 1992).

4.5.4. Mechanism of Infection and Colonisation

According to Kelman (1953), *R. solanacearum* is a typical wound pathogen on roots. Under field conditions the pathogen can also infect plants by penetrating the natural gaps between primary and secondary roots (Kang *et al.*, 1994). Wounding can occur during cultural practices or through nematodes (Shekhawat *et al.*, 1992). Moffett *et al.* (1981) demonstrated that the organism entered certain hosts through stomata but, to a lesser extent.

After entry, the pathogen rapidly colonises the intercellular spaces of epidermal tissue, damaging the middle lamellae of cells and degrading the cell walls. This result in the destruction of xylem

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tissues and production of tyloses that block the vascular system and cause the plants to wilt (Kang *et al.*, 1994).

In 1978, Wallis and Truter conducted a detailed study on the histopathology of tomato plants infected with *R. solanacearum*. According to them the initial colonisation of host tissue did not occur in the xylem vessels of the roots as was first expected, but in small diameter cells adjacent to the large xylem vessels. Twenty-four hours after inoculation, stimulation of tyloses formation was noted in invaded as well as non-invaded cells. According to Agrios (1997), tyloses form in xylem vessels of most plants under various conditions of stress and during invasion by most of the xylem-invading pathogens. These structures are overgrowths of the protoplast of adjacent living parenchymatous cells that protrude into xylem vessels through pits, inhibiting further spread of the pathogen. Increased production of indole acetic acid and other growth substances are believed to be responsible for their formation (Sequeira and Kelman, 1962; Sequeira, 1965).

During their study, Wallis and Truter (1978) noted that bacteria migrated from the infected cells through a narrow, slightly elongated neck and enters the lumina of the tyloses. During the next 24 hours most tyloses matured and collapsed, possibly due to extracellular enzyme activity of the pathogen, releasing the bacteria into the xylem vessels. After their release, the bacterial concentration increased steadily and it was noted that the bacterial cells were often imbedded in a fine granular matter. This granular matter increased until many xylem vessels were filled, plugging the vessels and preventing normal water uptake and resulting in rapid wilting of the host plant.

According to Schell (1996), *R. solanacearum* disrupts cell walls to facilitate spreading through the vascular system into the rest of the host. One of the possible enzymes released is cellulase and according to Ofuya and Wood (1981), it reaches its maximum activity at the advanced stages of infection.

Techniques such as immunofluorescence (IF) microscopy using specific antibodies can be used to visualise the infection process of bacterial cells (McGarvey *et al.*, 1999).

4.5.5. Influence of Environmental factors on the Virulence of *Ralstonia solanacearum*

Ralstonia solanacearum has many specialised genes relating to interactions with its host and it has been postulated that host-pathogen interactions are strongly regulated by the environment (Denny *et al.*, 1998). The pathogen has presumably evolved to recognise selected environmental signals that are important for its survival or successful host-colonisation. It reacts upon these signals to regulate its virulence. Exactly what these environmental signals are and how they interact, are still not clear.

a) Phenotypic Conversion

One of the characteristics of *R. solanacearum* is the rapid loss of pathogenicity once it is maintained in culture. The pathogen therefore has to be inoculated into host plants and re-isolated to maintain virulence (Kelman, 1953). When the pathogen undergoes a change in colony morphology from mucoid to non-mucoid, it shows a greatly reduced capacity to cause wilt in plants. This phenomenon where virulence is lost is also referred to as phenotypic conversion (PC) and is thought to be regulated by the pathogens environment. According to Denny *et al.* (1994) these PC-type strains still retain pathogenicity because they are able to infect plants and cause stunting and proliferation of adventitious roots and should not be called avirulent.

Phenotypic conversion-types are easily recognised on TZC medium (Denny *et al.*, 1993). The colonies are round, dark red and butyrous, which contrast sharply with the typical irregularly round, mucoid, pink or white, fluidal virulent colonies. True PC-type strains are also markedly more motile. According to Denny *et al.* (1993), these changes in colony type and subsequent reduced virulence of the pathogen are mainly due to the loss of one or more components of extracellular polysaccharides (EPS) and the reduction in extracellular endoglucanase activity.

These types routinely appear when the pathogen is cultured for five to seven days in glucose containing broth with an organic nitrogen source without shaking (Denny *et al.*, 1993). They also arise during prolonged culture on agar plates and in wilted plants, which suggests that there may be natural conditions that favour their appearance or multiplication. The large number of PC-type mutants that arise under certain conditions may be because they have a selective advantage over the wild type. According to Denny *et al.* (1994) the oxygen limitation in unshaken broth cultures

appeared to select PC-mutants due to their higher motility and positive aerotaxis while a high-salt mineral medium allowed PC cells to grow better than the wild type.

Denny *et al.* (1994), hypothesised that *R. solanacearum* normally exist as the PC-type in soil but can switch to the wild type when a suitable host is found. According to them this shift in phenotype occurs when some endogenous inducer (EI) exceeds a critical concentration. Exactly what this inducer is and how it regulates PC is still not clear but according to Schell (1996), 3-OH PAME (3-hydroxypalmitic acid methyl ester) might be one example of such an inducer. In soil, the bacteria survive as microcolonies with relatively low cell densities and slow rates of multiplication that might prohibit sufficient 3-OH PAME levels to accumulate, thereby favouring the PC-type. These PC-type cells can conserve energy and cellular resources because of their reduced production of extracellular polysaccharides and proteins, enhancing survival. When the pathogen encounters a host plant, it begins to multiply and later reaches a sufficient cell density to produce high enough levels of 3-OH PAME to switch on virulence gene expression (Fig.3).

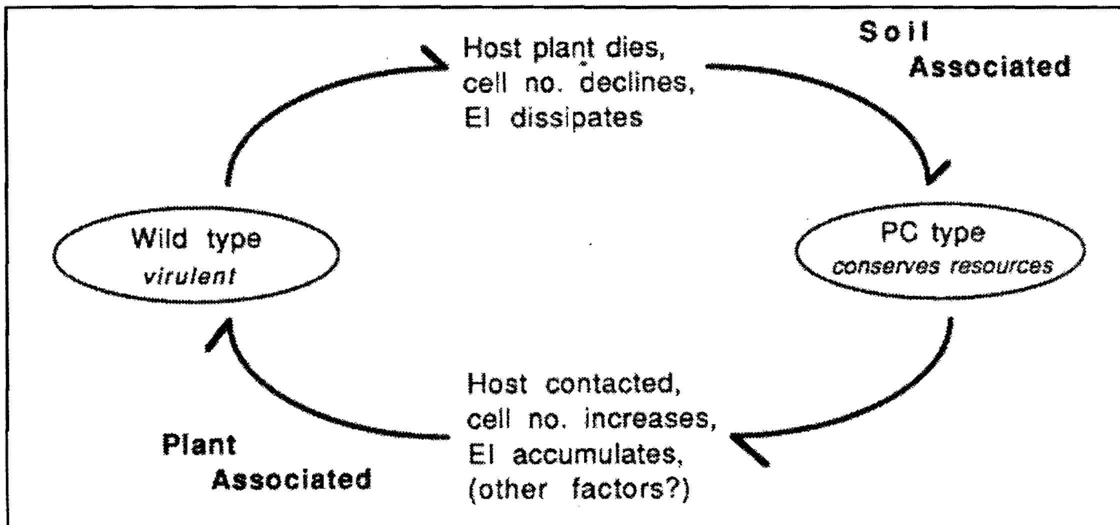

Figure 3. Postulated switching between the wild type and phenotypic conversion-type that might occur when bacteria move between soil and host plants. EI = endogenous inducer (Denny et al., 1994).

b) Virulence Factors

In order to understand how the control of virulence genes functions, it is necessary to understand the biochemistry and possible physiological functions of the pathogen's individual virulence factors. According to Denny *et al.* (1993), the extracellular polysaccharide (EPS I) is the primary virulence factor. Extracellular proteins (EXPs) such as cell wall-degrading enzymes; endoglucanase and *endo*-polygalacturonase enzymes are relatively minor virulence factors (Denny *et al.*, 1993; Denny *et al.*, 1998).

Despite many years of investigation, the exact role of extracellular polysaccharides (EPS) in the pathogenesis of plant-pathogenic bacteria remains unclear (Kao *et al.*, 1992). According to them the slime is thought to play a role in virulence by occluding the vascular system of the plant and reducing water transport and by protecting the bacteria from plant defence compounds.

The extracellular polysaccharide, EPS I, is the major known virulence factor of *R. solanacearum* and is released extracellularly in large quantities (Schell, 1996). It is an unusual nitrogen-rich polysaccharide and is composed of a trimeric repeating unit of N-acetyl-galactosamine, 2-N-acetyl-2-deoxy-L-galacturonic acid and 2-N-acetyl-4-N-(3-hydroxybutanoyl)-2,4,6-trideoxy-D-glucose (McGarvey *et al*, 1998). Quantification of EPS I in infected plants, using an enzyme-linked immunosorbent assay (ELISA), revealed that the bacterial populations in susceptible tomato cultivars produced greater amounts of EPS I per plant than those in resistant cultivars (McGarvey *et al.*, 1999). According to Huang *et al.* (1995), the production of EPS I requires the 18-kb *eps* gene cluster, which encodes several membrane-associated and soluble polypeptides involved in its biosynthesis and export. The *eps* gene cluster contains at least 12 genes that are probably transcribed as an operon from a single environmentally-regulated promoter (Schell, 1996). According to him, extracellular polysaccharide synthesis also involves portions of the *ops* gene cluster, which may encode synthesis of a common sugar precursor.

The pathogen also produces many EXPs that are likely or proven virulence factors. Some of these EXPs are plant cell wall-degrading enzymes such as endoglucanase (Egl), polygalacturonases (PglA and PglB) and pectin methylesterase (Pme) (Denny *et al.*, 1998). According to them, extracellular proteins as a whole have a major role in pathogenesis. However, once inside the host, *R. solanacearum* does not necessarily require these individual enzymes for

wilting. It is more likely that they function in root invasion, spreading into the vascular system or acceleration of disease development (Huang *et al.*, 1995; Schell, 1996).

c) Regulation of Virulence

According to Denny *et al.* (1993), the virulence of *R. solanacearum* is a trait regulated by a sophisticated, multi-component network that is sensitive to the environment. According to Schell (1996), the pathogen must cope with two very different environments, namely soil and plant. Denny *et al.* (1994), hypothesised that the pathogen normally exist as the PC type in soil but when it encounters a suitable host plant, it begins to multiply and later reaches a sufficient cell density to start producing extracellular virulence factors (Fig.3).

The production of virulence factors is controlled by a complex network that contains at least 12 regulatory genes (Schell, 1996). This network coordinates the expression of the different virulence genes in simultaneous response to multiple cues encountered during the various stages of pathogenesis and during soil survival, working together primarily to direct the syntheses of EPS I. Exactly how this network is regulated is still not known but is projected to function as illustrated in Fig.4.

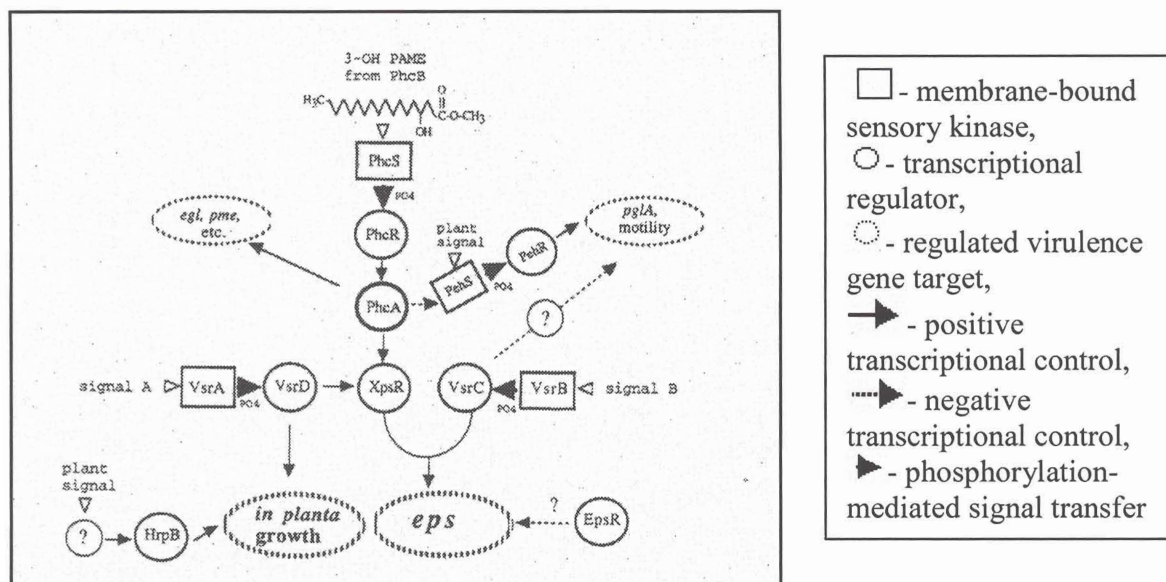

Figure 4. Schematic presentation of the organisation and operation of the virulence gene regulatory network (Schell, 1996).

4.6. Detection and Isolation

Various semi selective media can be used for the detection of *R. solanacearum*. In 1954, Kelman developed a semi selective medium containing tetrazolium chloride. On this medium, virulent colonies are fluidal, slightly raised, slimy and appear creamy-white with or without pink centres. These centres are typically comma-shaped. Avirulent colonies on the other hand are round, butyrous in texture and dark red (Kelman, 1954; Shekhawat *et al.*, 1992). Kelman's TZC medium is still used for soil and tissue isolations with moderate success. Other well-known media were developed by Graham and Lloyd (1979) and Granada and Sequeira (1983). These were modified versions of Kelman's TZC medium, containing antibiotics such as penicillin, chloramphenicol, polymixin B sulphate and bacitracin as well as crystal violet. The antibiotics and crystal violet reduced background population, resulting in more selective media. Karganilla and Buddenhagen (1972) and Chen and Echandi (1981) also modified TZC medium, adding various antibacterial as well as antifungal substances, resulting in extremely expensive media. A major limitation of all these media was that low population densities of the pathogen were not detectable (Nesmith and Jenkins, 1979). Especially in soil samples, the concentration of pathogen cells must exceed 10^4 cfu ml^{-1} soil sample before it can be detected (Karganilla and Buddenhagen, 1972; Nesmith and Jenkins, 1979; Chen and Echandi, 1981). According to Karganilla and Buddenhagen (1972), it appeared that the performance of a selective medium depended on the types of pathogen strains present in a region as well as on the soil itself.

Indicator plants can be used to detect the presence of the pathogen in soil (Graham and Lloyd, 1978). The indicator plant species should show disease symptoms within a short period of time following infection. Tomato and potato seedlings have been used as indicator plants. It takes two to three weeks for symptom development and slightly lower concentrations (10^4 cfu ml^{-1} soil sample) could be detected (Karganilla and Buddenhagen, 1972; Graham and Lloyd, 1978).

Detection methods based on immunological and nucleic acid based technologies have also been developed for the isolation of *R. solanacearum*. Although these techniques are generally more expensive, they are more accurate, -rapid and -sensitive than traditional plating techniques (Seal and Elphinstone, 1994). Serological detection techniques include ELISA and IF antibody staining. These techniques are commonly used to monitor the presence of *R. solanacearum* in host plants. Various ELISA kits have been developed for the detection of *R. solanacearum* primarily in tissue samples. Bellstedt and van der Merwe (1989) described two ELISA methods

for the detection of *R. solanacearum* biovar 2 and 3 in South Africa. These systems were based on a biotin/avidin system. In this system the high affinity of avidin for biotin was utilised. Where the two systems were combined, all strains of *R. solanacearum* from various localities in South Africa could be detected. Although ELISA techniques are useful for rapid and repetitive diagnostic tests, improvements are still required to extract the bacteria that adhere to soil particles and to inhibit non-specific reactions between antibodies and soil or plant material (Seal and Elphinstone, 1994).

Although DNA-based methods offer highly specific and sensitive means to detect the pathogen, they are more expensive than serological techniques (Seal, 1994). The polymerase chain reaction (PCR), one of the most well known DNA-based methods for the detection of *R. solanacearum*, is not dependent on the use of purified DNA (Gillings and Fahy, 1993a). Various primers have been designed to detect *R. solanacearum*. These primers were based on the published DNA sequence of the gene for endopolygalacturonase (*peh* A). The DNA fragments produced by the various PCR techniques can be further classified using RFLP techniques. Restriction fragment length polymorphism analysis is especially useful in distinguishing among different biovars or races of *R. solanacearum* (Seal, 1994). Although RFLP analysis is very discriminatory, it is time-consuming and not suitable for rapid screening of isolates (Seal and Elphinstone, 1994).

According to Black *et al.* (1998), the BACTID kit and Biolog system can be used to detect or confirm *R. solanacearum.* The BACTID kit is used to eliminate saprobic bacteria and to achieve preliminary identification (usually to the genus level) with as few tests as possible using media in micro-centrifuge tubes. The system includes software for identifying bacteria from test results. The system can partly differentiate between biovars of *R. solanacearum*. The high costs however, makes it too expensive for routine laboratory analysis especially in less-developed countries. The limited shelf life of plates is a further drawback.

4.7. Control

Due to its extremely wide host range and its ability to survive for prolonged periods in the soil, control of *R. solanacearum* is complicated and no single control measure can be used on its own to eradicate this pathogen. Bacterial wilt is particularly a problem because there is no chemical registered for the use on potatoes although various chemicals such as Bavistin and Biltox have

been tested (Shekhawat *et al.*, 1992). An integrated control program should therefore be implemented where a variety of control methods be combined and applied during different stages of cultivation in order to exclude the pathogen from all phases of potato production. The primary objective of such an integrated control strategy would be to limit the survival and multiplication of the pathogen as well as alternative hosts and interacting nematodes by means of cost-effective programs. The efficiency of a particular system would be location-specific since it must be adapted to suit certain climatic conditions, soil type, pathogen strain, farming system as well as the socio-economic situation of a specific area (Elphinstone and Aley, 1993; Mienie, 1997).

In developing countries many of the available control strategies that have been developed against *R. solanacearum* are not cost-effective and cannot be applied (Hayward, 1991). According to him, the disease can only be contained in these countries, unlike in developed countries where the use of resistant cultivars, pathogen-free seed, and adequate crop rotations are used to fight the battle against bacterial wilt. Various cultural practices can however be manipulated with little extra costs to inhibit growth and development of the pathogen without affecting growth of the crop. Some of these practices include: control before planting (i.e. field selection and preparation), control during planting and crop development and control during harvesting and thereafter.

4.7.1. Field Selection and Preparation

a) Field Selection

The field where the potato crop is planted must be bacterial wilt free. Various techniques such as PCR and ELISA are available to detect the pathogen in soil. Apart from high costs these techniques are impeded by inadequate soil sampling and are usually not an economic option for farmers. Another more affordable method for the detection of the pathogen in soil is to plant susceptible indicator plants and to evaluate them for symptom development over a period of time (Graham and Lloyd, 1978).

According to Kelman (1953), losses can also be minimised to some extend if farmers select well-drained sites or avoid low places in fields where the disease has been detected before. Farmers should also avoid fields where tomato, eggplant or tobacco has been grown in the past three years (Ali, 1995).

b) Crop Rotation, Bare Fallow Practices and Intercropping

Crop rotation with non-host crops has widely been investigated as a control measure where the disease is known to be present. According to Hartman and Elphinstone (1994), rotation with maize, rice and finger millet can reduce the incidence of wilt to some extent. Various other crops such as sorghum, wheat, cowpea, onion, garlic and carrot have also been used in different combinations to suppress pathogen levels within the soil (Shekhawat *et al.*, 1992). Scientists still do not agree on how long crop rotation programs should be. According to Shekhawat *et al.* (1992) two to three years of rotation should be enough while others such as Mienie (1997) suggested longer periods of four to five years.

Crop rotation can be combined with bare fallow practices (Jackson and Gonzals, 1979). During the bare fallow period all plants (i.e. weeds and volunteer potato plants) should be eradicated. The failure to eliminate all alternative hosts in crop rotation has been considered as one of the major factors that accounts for the insufficient control of bacterial wilt. The level of disease control with the use of crop rotation and bare fallow methods was however very variable. It appeared to be dependent on several factors such as the ability of the pathogen to survive in the absence of a host, climatic conditions and soil type (Akiew *et al.*, 1993).
Intercropping can be used to reduce the incidence of bacterial wilt as well as root-to-root transmission of the pathogen. In Burundi, intercropping with beans has been used successfully while corn and cowpea proved to be the most effective intercrops in the Philippines (Hayward, 1991). Autrique and Potts (1987) reported that intercropping potato with maize or haricot beans markedly reduced the incidence and rate of disease development in the potato crop.

c) Soil Preparation

Infected soil can be treated to some extend prior to planting. Since *R. solanacearum* is sensitive to desiccation, soil should be deep ploughed after the last potato harvest and left exposed preferably to high temperatures (35 to 40°C). Deep ploughing during a rotation system disturbs the soilborne inoculum of the pathogen, resulting in a decrease in inoculum levels and infection potential (Shekhawat and Chakrabarti, 1995; Mienie and Theron, 1999) and one or two deep ploughings of infested soil reduced wilt incidence by about 70% (Shekhawat *et al.*, 1992).

Soil amendments can be used with some degree of suppression although most have only been tested in pot culture experiments and are not yet commercially applicable (Hartman and Elphinstone, 1994). Chellemi *et al.* (1992), had some success in greenhouse experiments where they used composted organic amendments such as mushroom compost, poultry broiler litter and yard waste in different combinations to suppress bacterial wilt. Mixtures containing urea and calcium (S-H mixtures) and even seashell grit have also been used with various degrees of success and large nitrogen inputs, particularly sewage sludges, also helped to suppress the pathogen (Hartman and Elphinstone, 1994). Soil can also be disinfected with chemicals such as stable bleaching powder. However, most of such chemicals proved to be detrimental to the crop (Shekhawat *et al.*, 1992).

Steam was used for soil disinfection where temperatures were maintained at 85°C for 15 minutes but this method was not practical over large areas and only proved to be effective in seedbeds or greenhouses (Shekhawat *et al.*, 1992). According to Hartman and Elphinstone (1994) fumigants like chloropicrin have been tested for control of bacterial wilt on tomatoes but were not economically feasible over large areas. Although crop losses could be reduced, complete control or eradication could not be obtained.

Soil solarisation has been evaluated as a possible control measure but only a slight reduction in population levels could be obtained. Saumtally *et al.* (1993) suggested that higher temperatures might prove to be more effective and different combinations of transparent and dark plastic should be investigated.

The burning of vegetation can reduce the bacterial wilt incidence by spreading straw in furrows and set alight. However, this practice is dangerous and not often used (Shekhawat *et al.*, 1992).

4.7.2. Planting and Crop Development

a) Pathogen-free Propagative Material

It is crucial to use pathogen-free propagative material to minimise the concentration of initial pathogen inoculum. *R. solanacearum* can easily be disseminated through infected propagative material such as potato tubers (Hayward, 2000). A major problem is the ability of the pathogen to infect tubers without symptom expression. This latent infection of tubers is particularly

problematic in developing countries where certified seed is not readily available (Ciampi *et al.*, 1980). If there is no proper seed certification program, farmers have no alternative but to use potentially infected tubers for their next crop.

South Africa has a very effective seed certification program. According to Nortje (1999) seed potatoes are produced in all the provinces of South Africa with the Western Cape currently producing 35% of all certified tubers. More than 70% of South Africa's potatoes are produced from certified tubers.

The South African seed certification program functions according to the Plant Improvement Act (Act 53 of 1976). It was primarily implemented to reduce the incidence of tuber-carried pathogens and to provide farmers with high quality seed potatoes. This program is based on a true generation concept and was implemented on 1 June 1995. According to this concept, seed potatoes should be phased out of the program within eight generations to limit pathogen population build-up. Selected material from the National Cultivar Collection is multiplied *in vitro* and certified as generation 0 (G0). The first field multiplication is certified as G1 with subsequent generations certified up to G8. After each field multiplication seed potatoes advance to the next generation until it is phased out after eight generations. With each successive generation the probability that the tubers are infected with one or more pathogens increases. Each generation has three quality classes built in to compensate for diseases. If a seed crop exceeds a given diseases index, it is graded in a lower class. The third class is automatically phased out, despite the generation (Nortje, 1997). The latter class is considered as standard grade seed and is only suitable for the production of table potatoes.

A zero tolerance for *R. solanacearum* is maintained (i.e. if this organism is detected, farmers are immediately notified and their crop rejected). This ensures that no infected tubers can be sold as seed potatoes. However, once rejected as seed potatoes, farmers are still allowed to sell tubers as table potatoes. If these tubers should wrongfully be used as seed potatoes, the danger of spreading the disease still exists.

b) Resistant Cultivars

The use of resistant potato cultivars plays an important role in the integrated control of bacterial wilt. Unfortunately the complexities of host-pathogen-environment interaction make breeding for

resistance extremely difficult (Tung *et al.*, 1990) and no immunity has as yet been identified in potato (Hayward, 1991).

The effect of temperature on resistance to wilt caused by different strains and races of *R. solanacearum* have been investigated (Tung *et al.*, 1990; Tung and Schmiediche, 1995). Resistance was found to be temperature sensitive as well as strain specific. A wide range of genes for resistance needs to be incorporated into a genotype to provide adequate resistance. This should reduce strain specificity and improve the stability of resistance under changing environmental conditions (Tung *et al.*, 1990).

Since the early 1970's, *Solanum phureja* Juz. & Buk., a diploid cultivated species, has been used extensively in breeding programs by authorities such as the International Potato Centre in Peru, as the major source of resistance to bacterial wilt. It has, however, not been successful in all environments (Hayward, 1991). Its resistance is temperature sensitive and it is therefore best suited for higher elevations or cooler climates (Laferriere *et al.*, 1998). Resistant genes from other diploid potato species have been identified and used in breeding programs. Some of these species are: *S. chacoense* Bitt., *S. ciliatum* Lam., *S. jamesii* Torr., *S. multidissectum* Hawkes, *S. pinnatisectum* Dun., *S. raphanifolium* Hawkes, *S. sisymbrifolium* Lam., *S. sparsipilum* Bitt. and *S. stenotomum* Juz. & Buk. (Madalageri and Patil, 1995). Other *Solanum* spp. evaluated were: *S. acaule* Bitt., *S. berthaultii* Hawkes, *S. blanco-galgosi* Ochoa, *S. boliviense* Dun., *S. brachycarpum* Ochoa, *S. chomatophilum* Bitt., *S. demissum* Lindl., *S. polytrichon* Rydb., *S. stoloniferum* Schlechtd and *S. sucrense* Hawkes (Hartman and Elphinstone, 1994). According to Laferriere *et al.* (1998) another source of resistant genes was *S. commersonii* Dun., a diploid wild potato species from south-eastern South America. Attempts to incorporate this species (as with many other) into potato breeding programs were however thwarted by its sexual incompatibility with tetraploid *S. tuberosum*. To bypass this obstacle, protoplasts from the two species were electro fused where after the fused protoplasts multiplied to form callus tissue. This tissue differentiated to give rise to shoots that were used in further breeding programs. The stability of resistance of these plants under different field conditions and temperature regimes is still unknown and has to be evaluated.

According to French and De Lindo (1982), resistance to bacterial wilt in potato is a partially dominant character and is more of a polygenic type. Tung and Schmiediche (1995) agreed on the dominant character of resistance and suggested that only a few genes control it although the

number of genes involved is still unknown. Tung *et al.* (1990) and Tung and Schmiediche (1995) suggested that the genes for resistance in the host evolved independently from the pathogen and that a gene-for-gene relationship does not seem to be applicable to bacterial wilt.

Whether or not resistance to *R. solanacearum* is controlled by minor or major genes is not considered to be a point of great concern in practical breeding (Tung *et al.*, 1990). According to them, the inheritance and expression of resistance seem to be complex and new methods other than conventional breeding techniques should be evaluated to increase and stabilise resistance. Genetic engineering techniques are therefore being used to strengthen the basic composition of the host plant through the incorporation of suitable genes from other sources. Antimicrobial genes coding for lytic enzymes such as cecropins isolated from the lepidopteran *Hyalophora* were used for the control of bacterial diseases of plants and showed great potential against *R. solanacearum* (Montanelli *et al.*, 1995). According to Hayward (1991), lysozyme and other potent antibacterial proteins derived from insects can also be introduced into potatoes to increase resistance.

c) Time or Season of Planting

The pathogen can also be escaped by delaying the date of planting until temperatures are lower in summer- and autumn-production regions. In winter- and spring-production regions, early planting and harvesting can reduce bacterial wilt and tuber rot. Short duration early maturing potato varieties would be ideal for this practice since the time for crop development is reduced to escape the disease (Shekhawat *et al.*, 1992). However, this only applies for the production of table potatoes since the danger of latent infection still exists (Mienie and Theron, 1999). Seed carrying the latent pathogen can enhance disease epidemics when planted under conditions favourable for disease development (Hayward, 1991). In Brazil, changing of planting dates to avoid the rainy season was also suggested as a mean to decrease losses in potato crops (Kelman, 1953).

d) General Cultural Practices

In an attempt to reduce cost of planting material, farmers in developing countries often use tuber cuttings as seed (He, 1995). Cutting of seed tubers increases the risk of bacterial infection. During the cutting process the pathogen can be spread from a diseased tuber to healthy ones by

contaminated blades. When whole tubers are used the incidence of bacterial wilt is lower (Shekhawat *et al.*, 1992). According to them when cut tubers were used, disease incidence increased by more than 2.5 times and yield decreased by more than 40%.

Before any seed lot is handled, all containers, tools and implements should be thoroughly washed and disinfected. Labourers should also be informed about the impact of the disease and be issued with detergents for personal hygiene to prevent spread of the disease by their boots and hands (Mienie, 1998). All implements used in infected fields should be cleaned to get rid of excess soil and properly disinfected before being used in other fields. Sodium hypochlorite (3.5 to 5%) can be used for general disinfection and 4% formalin and steam (Conroy, 1969) or 0.5% carbolic acid (Jeyes Fluid) (Mienie and Theron, 1999) is suitable to clean and disinfect machinery. Movement through infected fields should also be limited since soil attached to vehicles, shoes and animal hooves can spread the pathogen to adjacent fields (Swanepoel and Bosch, 1988).

Due to the wounding caused by nematodes (*Meloidogyne* spp.), *R. solanacearum* would be able to infect tubers easier where nematodes are present (Elphinstone and Aley, 1993). Controlling nematodes will result in a decrease in the number of infection loci and control the spread and development of bacterial wilt (Kelman, 1953). According to Akiew *et al.* (1993) effective control methods include nematode-resistant varieties, chemical soil treatment, soil fumigation and rotation with crops that are resistant to nematodes.

Removing and destroying wilted plants limits the spread and development of bacterial wilt since these plants act as inoculum sources. Potato plants should be carefully monitored throughout the season and wilted plants should be removed and burned immediately (Mienie, 1997).

Where infection is very high, irrigation should be temporarily suspended to avoid further spread to adjacent fields. Farmers should also avoid the use of irrigation water that might be contaminated by *R. solanacearum* (i.e. water from irrigation dams near infested fields) (Swanepoel and Bosch, 1988).

Since root injuries facilitate infection, injury to the roots should be avoided during cultivation. Where intercropping is applied, care should also be taken not to damage the roots of potato plants (Kelman, 1953). Post-emergence earthing-up of potatoes is a common practice. It should be carefully applied without injuring potato plant parts. General post-emergence tillage should also

be restricted to an absolute minimum, limiting damage (Shekhawat *et al.*, 1992). According to Chakrabarti *et al.* (1995) earthing-up should be applied immediately after planting, when damage to plant parts should be minimal.

e) Chemical Control

Currently there is no chemical registered in South Africa for the control of potato bacterial wilt. Some of the chemicals that have been tested elsewhere against *R. solanacearum* include Bavistin, Emisan 6, plantomycin and Biltox (Shekhawat *et al.*, 1992). Most of these have been tested on other hosts such as tobacco, jute and ginger and have not been tested on potatoes. According to Hartman and Elphinstone (1994), an experimental bactericide, Terlai, showed potential in reducing wilt in pot culture experiments. Where it was applied in conjunction with *Pseudomonas fluorescence* it reduced wilt significantly (Machmud and Machmud, 1994).

Studies on the use of antibiotics as control agents have been performed with varying success. An antibiotic C-6, similar to erythromycin, was found to effectively inhibit the pathogen in diseased potato tubers. Pre-treatment of seed tubers with 500 to 1000 ppm of the antibiotic followed by two foliar sprays at the same concentration effectively controlled the disease and also increased the yield per unit area by approximately three times (Shekhawat *et al.*, 1992).

f) Biological Control

The primary agents used to control bacterial wilt biologically include antagonistic rhizobacteria and avirulent mutants of *R. solanacearum.* These organisms are invasive but non-wilt inducing and compete for vascular colonisation of xylem vessels and induce host resistance (Hayward, 2000). Various actinomycetes and bacteria such as *Pseudomonas fluorescens* and *Bacillus polymyxa* have been reported to be active control agents. These organisms have been found to delay the development and reduce the incidence of bacterial wilt (Shekhawat *et al.*, 1992). *Pseudomonas cepacia*, commonly isolated from maize roots, showed antagonistic potential in culture and pot trials (Hartman and Elphinstone, 1994). Other organisms such as *B. mesentericus*, *B. megaterium*, *B. subtilis*, *B. mycoides* and even *Erwinia* spp. have been reported potential biocontrol agents for *R. solanacearum* (Hartman *et al.*, 1993).

According to Hayward (1991) the mechanisms involved in biocontrol include induced resistance, or competitive exclusion. The active colonisation of the rhizosphere with antagonistic soil bacteria or bacteriocin- and bacteriophage-producing strains of *R. solanacearum* can protect the plant from virulent infections. The induction of host plant resistance as the result of artificial inoculations with heat-killed virulent strains, avirulent mutants or incompatible strains of *R. solanacearum* in roots, stems and leaves of host plants has also been reported (Trigalet *et al.*, 1994). According to them, the factors involved in the resistance response of the host plant are very complex and although biocontrol seems promising under controlled conditions, it has not proven effective in natural environments.

In an attempt to limit the factors that influence biocontrol efficacy, endophytic antagonists derived from the wild-type pathogen have been evaluated (Trigalet *et al.*, 1994). Once established within the plant it provides continuous protection. Avirulent mutants of *R. solanacearum* are deficient in production of exopolysaccharides and appeared to be the most likely candidates for endophytic biocontrol agents. Although these organisms had the ability to multiply within susceptible host tissue upon inoculation by the stem puncture technique, their systemic spreading was limited and their populations declined after a while (Trigalet *et al.*, 1994).

According to Hayward (1991), few of the tested approaches to biological control of bacterial wilt have reached a stage of commercial application and much more research is needed. Biocontrol strategies developed in laboratories often failed under natural conditions either since root colonisation of the biocontrol agent was too poor or too dependent on environmental greenhouse conditions (Trigalet *et al.*, 1994). Mienie (1998) evaluated *Pseudomonas resinovorans* as potential control agent. Results indicated that treatment with the antagonist significantly reduced the severity of bacterial wilt. *P. resinovorans* is currently being registered as biocontrol agent for potato bacterial wilt in South Africa. Although not yet registered, it has already been widely distributed and used amongst local potato farmers (Pers. Comm. N.J.J. Mienie, Potatoes South Africa, Seekoegat).

4.7.3. Harvesting and Storage

a) Sanitation Practices

According to Mienie (1998), all storage and seed handling equipment should be properly cleaned and disinfected after harvest to prevent survival and build up of inoculum on surfaces or in potato debris. All tubers, vines, soil, old boxes and bags should be removed and destroyed (Chakrabarti *et al.*, 1995). Storage bins, walls and floors should be washed with hot soapy water, thoroughly rinsed and disinfected.

b) Time of Harvest

Kelman (1953) suggested that the harvest date of potatoes grown in infested soil or in seasons when bacterial wilt is severe should be advanced. Trials conducted in India indicated that early harvesting reduced bacterial wilt and tuber rot (Shekhawat *et al.*, 1992). This is not always applicable because harvesting tubers when they are not fully developed may present marketing problems.

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