CHAPTER 2

SURVIVAL OF *RALSTONIA SOLANACEARUM* BIOVAR II IN ARTIFICIALLY INFESTED SOIL UNDER DIFFERENT CROPPING SYSTEMS

ABSTRACT

Soil survival of bacterial wilt was investigated in a field artificially infested with *Ralstonia solanacearum* biovar 2, race 3. Four cultural practices (maize monoculture, potato monoculture, bare-fallow and weed-fallow) were applied. To monitor *Ralstonia* populations in the soil, samples were taken at various intervals and viable colony counts were performed on semi-selective media. Inconclusive results lead to the introduction of the indicator plant technique. After a three-year and five-year cropping sequence, potato plants were planted in a designated region across all treatment plots. Percentage wilting of the indicator plants in the potato monoculture plots was 88% and 96% after three and five years respectively. The pathogen's ability to survive without the presence of a host was demonstrated by the high wilt index recorded in the fallow plots (58% and 42%). Maize is thought to suppress *R. solanacearum* populations in soils, yet more than 40% wilting was recorded in these plots after five years monoculture. Less wilting occurred in weed-fallowed plots (31% and 27%), even though some host species were present. Survival of this organism in the soil of all treatments exceeded the two-year period generally believed to be sufficient for eradication.

INTRODUCTION

Bacterial wilt or Brown rot is a destructive disease present in most potato growing regions of Africa, including South Africa. The disease can cause severe crop losses due to wilting in the fields which ultimately result in reduced yields and tuber rot in storage. Since it is known that the causal agent can survive in soil, it renders the soil unsuitable for subsequent potato production. Bacterial wilt is caused by the bacterium *Ralstonia solanacearum*, previously known as *Burkholderia solanacearum* or *Pseudomonas solanacearum* (Yabuuchi *et al.*1992, 1995). Various isolates of this pathogen exist which differ in their ability to affect various hosts, and in their biochemical metabolism. The term race (of which there are five) refers to classification according to hosts primarily affected, whereas the biovar system (biovar 1-5) is based on biochemical reactions (Hayward, 1994). To date, only biovar 2 (race 3) and biovar 3 (race 1) have been found in South Africa with biovar 2 being prevalent in the potato production regions. Although the intensity of bacterial wilt outbreaks varies from season to season, eradication has not been possible. This in spite of the fact that bacterial wilt has been a focal point in potato seed certification programs, information drives and research projects. Although measures such as planting of certified seed, use of land with a history of being disease-free and good sanitary practices have helped to reduce new infections, uncertainty still exists on the management of infected fields and for how long these fields could sustain the viable organism.

Research into the survival of *Ralstonia* in soil has gained considerable attention and has highlighted the fact that the survival period is influenced by a range of environmental factors, making universal predictions almost impossible. Probably the most crucial factor is the biovar or race of the pathogen that is involved, since this affects both host range and its survival in soil as a free-living organism. Biovar 3 is known to survive for up to eight years, probably due to its wide host range and higher aggressiveness. Biovar 2 on the other hand is generally believed to be less adapted to soil survival with eradication being possible within two years.

Pathogen survival, however, depends further on the initial population levels in the soil; biological, chemical and physical soil properties; soil tillage; temperature, moisture and oxygen levels and importantly, the type of cropping system used. This determines the availability of host material, type of plant debris that can serve as shelter sites, and microbial activity governing antagonistic or synergistic actions.

Several studies have been conducted on the impact of cultural practices on bacterial wilt populations in soil, with varying results. The potato strain, biovar 2 race 3, is generally believed to be controlled more readily by means of rotation programs than biovar 1 or 3. A two and a halve -year pasture rotation could reduce race 3 populations to an undetectable level in the cooler regions of Australia. Similarly, planting maize or a tolerant potato variety for several seasons after a six-month fallow period was sufficient to eradicate the pathogen (French, 1994). In Costa Rica, rotation with maize, bean or sweet potato intercropped, did not diminish the inoculum potential in the soil. Leaving the soil bare fallow for five months and applying herbicides reduced the incidence of wilt (Jackson & Conzales, 1979). Martin *et al.* (1981) were able to demonstrate that a bare fallow period of 140 days is not enough to eliminate race 3 from the soil. By means of indicator plants they were able to detect the wilt organism in upper (0-30cm), lower (30-60cm) and deeper (60-90cm) soil layers. Survival periods of between one and two years in bare fallow or weed-fallowed fields have been reported in the cooler regions of Australia (Graham *et al.*, 1979). Little is known on the survival of the potato race in warmer regions and how rotation or fallowing influences population levels.

The aim of this study was therefore to investigate the survival of *R. solanacearum*, biovar 2, in soils of an artificially infested field to which four cultural practices have been applied over a period of five years.

MATERIALS AND METHODS

Infestation

During the 1994/1995 season, a field of 20m by 48m was selected on the Experimental Farm of the University of Pretoria and prepared for planting. The soil was a clay-loam that drains relatively well with pH of 6.2. Inoculation procedure was conducted by the Agricultural Research Council (Roodeplaat). *Ralstonia solanacearum* biovar 2 race 3 was cultured on Kelman's tetrazolium chloride agar (TZC) (Kelman, 1953) for 48 hours. A bacterial suspension was prepared (concentration not stated) in sterile distilled water and was injected into a wound made in certified seed potatoes. The tubers were dipped in a separate biovar 2 suspension and planted at a high density. Wilt was monitored throughout the growing season and the diseased crop was ploughed in to ensure uniform and severe infection levels.

Application of cultural practices

In the 1995/1996 season the field was subdivided into twelve plots representing three replicates of four treatments, namely maize monoculture, potato monoculture, bare fallow and leaving the plots under weeds which could serve as alternative hosts. The field layout is depicted in Figure 2.1 and Figure 2.2. To prevent cross-contamination between plots metal plates were placed 1 m deep into the soil. After rotovating the soil and applying herbicide, the potato and maize plots were planted and together with the weed-fallowed plots, they were monitored weekly for a period of twelve weeks for any wilt symptoms. Certified seed potatoes were planted throughout the trial. Although not all weed species were identified in the weed-fallowed plots during the first season, *Datura* (a known host of biovar 3) and some *Portulaca* (a symptomless carrier of the potato race 3) were present. During the period of 1998-2000 further identifications were performed on weed and grass species. Isolations were performed

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during 1998 from roots and stems of *Galinsoga parviflora*, *Conyza sumatrensis*, *Tragopogon dubius*, *Datura strammonium*, *Schuria pinnata*, *Cyperus rotundus*, *Tagetes minuta*, *Ciclospermum leptophyllum* and *Bidens pilosa*. Plants were rinsed and surface sterilized in 1% sodium hypochlorite, 20 minutes for roots and 15 minutes for stems. This was followed by three rinses in distilled water and blended in sterile water. A dilution series ($10^1 - 10^8$) was prepared and plated on TZC. Isolations from fifteen randomly selected maize plants were performed in a similar manner. During the winter months plant growth was removed from the potato, maize and fallow treatments (Figure 2.3). The weeds were cut back. During the1996/1997 season half of each bare fallow plot was tilled to ensure greater exposure of soil to the sun. These cultural practices were continued yearly. Weeds were removed manually. Plots were irrigated with sprinklers during the crop season.

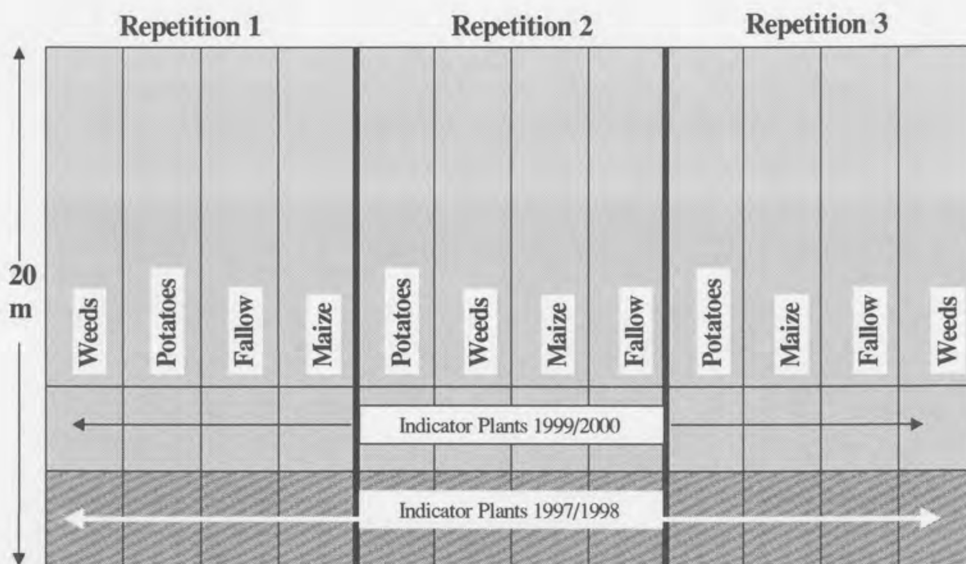

Figure 2.1 Layout of the field infested with *Ralstonia solanacearum*

Figure 2.2 Bacterial wilt infested field during cropping season

Figure 2.3 Field during the winter months

Soil samples

To monitor *Ralstonia* populations in the various treatment plots, soil samples were taken during July and September 1996, and February 1998 and 1999. In July and September 1996, four soil samples per plot were collected at a depth of 15-20 cm and

sent to Agricultural Research Council at Roodeplaat for analysis. A semi-selective modified TZC medium containing antibiotics, referred to as SMSA (Engelbrecht, 1994) was used to determine viable counts of *Ralstonia,* expressed as colony-forming units per gram soil (cfu/g).

During February 1998, soil samples were drawn in the proximity of plants where possible, both in the upper soil profile (0-30 cm) and at deeper levels (30-75 cm). Five sites were sampled per plot, totaling 120 soil samples. A soil sample of 100g was shaken in 100ml sterile Phosphate buffer solution (PBS), instead of using the standard 10g/90ml water. This modification was used to enhance the chance of detection. The dilution series was prepared and plated on SMSA as described above and colony counts were performed after 72 hours.

A different approach was followed in February 1999. At the end of the growing season, soil was removed from three sample sites per plot at a depth of up to 30 cm. Soil from each site was placed into five pots in which potato mini tubers were planted. From one pot per site a 100g soil sample was taken, placed in an Erlenmeyer flask containing 100ml PBS and shaken for 30 minutes. A dilution series was prepared and plated on TZC for determining *Ralstonia* populations. Wilting of potted plants was monitored throughout the growing season. Isolations were performed from wilted plants; from plants grown in pots from which sub-samples had been drawn for direct soil isolations; and from randomly selected symptomless plants.

Monitoring by means of indicator plants

Due to large fluctuations in *Ralstonia* counts obtained from the soil samples, the use of indicator plants was introduced during the 1997/1998 season. A 5m area of the field across all treatments was demarcated and prepared for planting. Certified seed potatoes were planted in five rows of ten plants each in each treatment plot. Wilting was monitored, milk-flow from cut stems of wilted plants was observed and bacterial isolations were performed from some infected stems to confirm bacterial wilt disease. Stems were surface sterilized in 1% sodium hypochlorite for 10 minutes and rinsed in

distilled water. A dilution series was prepared and plated on TZC. All plant material was removed from this area after twelve weeks to minimize inoculum build-up. The area was separated from the rest of the field the following season by means of a wire barrier to prevent movement through the area. This procedure was repeated during the 1999/2000 season in the demarcated region of five meters next to the previous indicator region (Figure 2.1).

Plant samples

Random samples of wilted potato plants were taken during each season and stems analysed on TZC to confirm the presence of *Ralstonia* as described for isolations from weeds. During the 1999/2000 season 25 isolates obtained from wilted indicator plants randomly collected across all plots were subjected to biovar identification tests to confirm that the causal organism of the wilt is biovar 2. The identification was based on the isolate's ability to utilise three hexose alcohols, namely mannitol, sorbitol, dulcitol and, to produce acids from the three disaccharides, lactose, maltose and cellobiose as described by Hayward (1994).

Statistical Analysis

Wilt indices of potato plants in the potato monoculture plots over a five-year period were statistically analyzed using the General Linear Models Procedure of the Statistical Analysis System (SAS®)[1] to determine variations between seasons. Wilt indices of indicator plants in all treatment plots of both the 1997/1998 and 1999/2000 seasons were analyzed to determine the effect of cultural practices. Tukey's Studentized Range at the 5% significance level was used to identify significant differences.

[1] SAS® System is an integrated system of software providing complete control over data management, analysis, and presentation, and may be obtained from SAS Institute South Africa (Pty) Ltd. 93 Central Street, Houghton, P.O. Box 2837, Houghton, 2041, Republic of South Africa

RESULTS AND DISCUSSION

Application of cultural practices

The average percentage wilt observed in potato plots over a period of five years is depicted in Figure 2.4. During the 1995/1996 season, onset of wilt was rapid with 18% of plants showing wilt symptoms within four weeks. The wilt index increased exponentially over the next eight weeks to a devastating 90%. Variation between replicates was not statistically significant. The wilt patterns clearly demonstrate that a severe and uniform infestation was achieved. Similar patterns could be observed in all seasons. The wilt indices observed in the potato monoculture plots over the five year period did not differ statistically expect for the lower wilt percentages recorded in the 1996/1997 season.

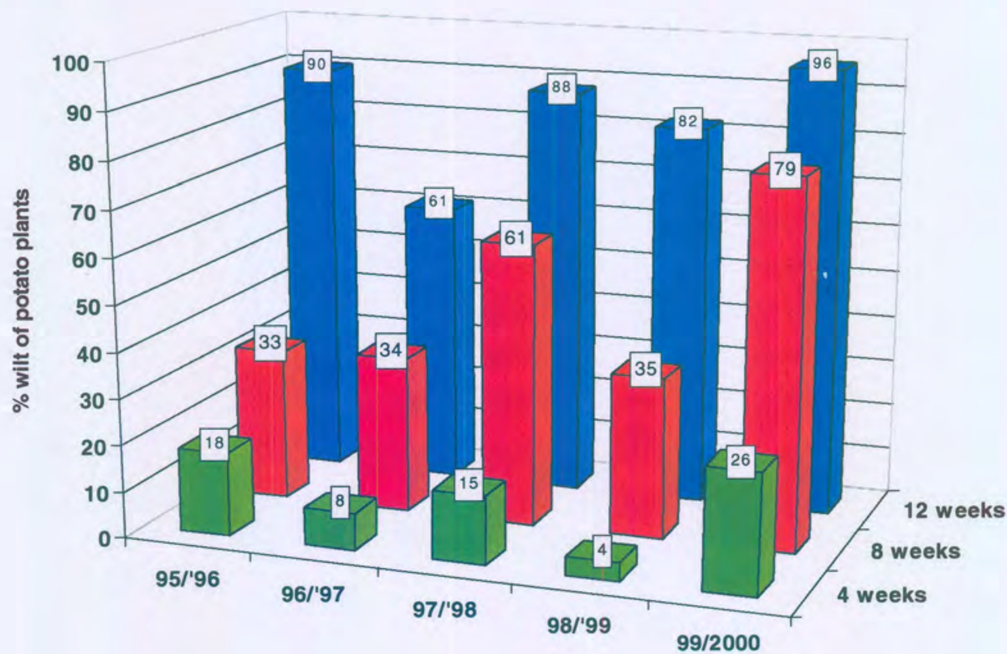

Figure 2.4 Percentage wilt in potato monoculture plots over a five-year period

The ability of *R. solanacearum* biovar 2 organism to maintain its population levels in soil, even in the absence of host cover during the eight months of non-cultivation, is reflected in the high incidence of wilt that was recorded every season. Infested plant material is not removed from these plots and therefore the debris can serve as shelter

sites during the dry winter months. Graham *et al.* (1979) found that potato debris could remain infested with the potato race for 233 days, which amounts to almost eight months. The intense expression of wilt symptoms during the 1999/2000 season may be ascribed to moist and humid conditions and lower light intensities that prevailed throughout the growing season.

No wilt was observed in maize plants nor could the pathogen be detected in the roots or stems of the 15 sampled plants. This indicates that either the pathogen does not invade and colonize maize tissue or that its soil population was too low in these plots, thereby escaping detection. A separate trial where maize is cultivated in highly infested soil would provide more accurate information. Root infection of maize plants has been reported (Granada & Sequeira, 1983).

Identification of the weeds on the weed-fallowed plots indicated a highly diverse population including families such as *Amaranthaceae*, *Asteraceae*, *Commelinaceae*, *Cyperaceae*, *Euphorbiaceae*, *Portulacaceae* and *Solanaceae*. Species such as *Datura ferox*, *D. strammonium* and *Portulaca oleracea* have previously been reported as hosts of the potato race pathogen. Several of the other identified weed species have been reported hosts to biovar 3 strain. However, several literature sources do not identify the strain involved. Only one of five sampled *D. strammonium* plants was infected with the bacterial wilt pathogen. Poor recovery of the pathogen from *D. strammonium* samples can be ascribed to the lower inoculum potential in the soil of weed-fallowed plots, as was demonstrated by the potato indicator plants. This highlights the necessity to determine host range in controlled pot trials. A very low concentration of *Ralstonia* was obtained in one of the stem-samples of *Tragopogon dubius* and *Cyperus rotundus* as well as in a root and stem sample of *Tagetes minuta*. These positive isolations could have been due to insufficient surface sterilisation or a rare incidence of root invasion and need to be confirmed by reliable pot trials.

The weed and grass species that have been identified in the weed-fallowed plots are listed in Table 2.1. *Chloris pycnothrix* and *Sporobolus africanus* are prevalent in all three replicates. *Eragrostis curvula* is prominent in the first repetition and occurs to a lesser degree in the other replicates. Broad-leaved weeds are interspersed between the

grasses. The more common ones present during several seasons include *Amaranthus* spp., *Bidens pilosa*, *Conyza sumatrensis*, *Tragopogon dubius* and *Taraxacum officinale*.

E. curvula has been identified in South Africa as a host of biovar 3, but not of biovar 2 (Swanepoel, 1992). Neither *C. pycnothrix* (spiderweb chloris) nor *S. africanus* (rat's tail dropseed) have been reported as hosts. Members of the *Poaceae* family have been successfully used in crop rotation programs to reduce the incidence of bacterial wilt (Akiew & Trevorrow, 1994).

Table 2.1 Weed and grass species identified in weed-fallow plots during 1998-2000

Botanical name	Common name
Amaranthus spp.	Common pigweed
Bidens pilosa	Blackjack
Chenopodium carinatum	Green goosefoot
Chamaesyce prostrata	Hairy creeping milkweed
Chloris pyconthrix	Spiderweb chloris
Conyza sumatrensis	Tall fleabane
Conyza boariensis	Flax-leaf fleabane
Commelina benghalensis	Benghal wandering jew / Common signal grass
Cyperus rotundus	Purple nutsedge
Ciclospermum leptophyllum	Wild celery
Datura ferox	Large thornapple
Datura strammonium	Common thornapple
Eragrostis curvula	Weeping love grass
Erigeron cannedensis	Horseweed
Euphorbia hirta	Red milkweed
Euphorbia inaequilatera	Smooth creeping milkweed
Galinsoga parviflora	Gallant soldier
Hibiscus trionum	Bladder weed
Hypochoeris radicata	Hairy wild lettuce

Botanical name	Common name
Lepidium africanum	Pepper weed
Portulaca oleracea	Common purslane
Pseudognaphalium luteo-album	Cudweed
Schkuhria pinnata	Dwarf marigold
Sisymbrium thellungii	Wild mustard
Sonchus oleraceus	Sowthistle
Sporobolus africanus	Rat's tail dropseed
Tagetes minuta	Khaki weed
Taraxacum officinale	Common dandelion
Tragopogon dubius	Yellow goats beard
Tribulus terrestris	Devil's thorn / Dubbeltjie

Soil samples

Ralstonia populations of both the July and September 1996 samplings are given in Table 2.2. The results of the first sampling showed a very low presence and even absence of the pathogen in all treatments except for the potato field. A high *Ralstonia* count was obtained in one of the maize monoculture replicates, resulting in maize treated plots having the highest average. This could be due to the accidental inclusion of old potato debris or slime masses remaining from the infestation process. The lowest population of *R. solanacearum* was noted in the weed-fallowed plots. In the September sampling, bacterial wilt counts per gram soil were generally higher than in the July sampling. Variation between the treatments was also less. The population recorded in the weed-fallowed plots was very similar to that found in the potato plots. The maize treatment had the lowest population. The ranking of treatments according to *Ralstonia* population detected in the soil samples differs between July and September. Such a shift in ranks over such a short period is highly unlikely.

Table 2.2 *Ralstonia solanacearum* counts (cfu/g) in soil samples collected from 12 plots managed through four different cultural practices for July and September 1996

July 1996			
Treatment	**Repetition 1**	**Repetition 2**	**Repetition 3**
Fallow	5.3×10^2	6.5×10^1	0
Maize	2	6.8×10^4	6×10^1
Weeds	3	1×10^2	0
Potatoes	3.1×10^2	3.7×10^3	5.9×10^2
September 1996			
Treatment	**Repetition 1**	**Repetition 2**	**Repetition 3**
Fallow	6.9×10^2	2.8×10^2	6.4×10^2
Maize	5.4×10^2	8×10^2	1×10^2
Weeds	3.1×10^3	8×10^2	1.2×10^1
Potatoes	3×10^3	7.2×10^2	5.6×10^2

The soil sample results obtained in February 1998 were unreliable (Table 2.3). In the potato monoculture plots only four of the total 30 samples were positive. Samples analysed from one repetition of the potato monoculture plots could not detect the pathogen, yet during that specific growing season the incidence of wilt in that plot was 86%. In the second and third potato replication *Ralstonia* could only be isolated from one of the five sites that were sampled albeit at both depths. Analysis of samples from the maize treatments resulted in one positive isolation, whereas in weed-fallowed plots the pathogen was detected in two of the thirty samples, although both samples had been drawn from the same replication in the field. In the fallow plots only two positive samples were obtained, one from soil collected at a depth of 0-30cm, the other from the 30-75cm layer. Although no difference could be observed in the amount of positive samples obtained from the upper layer in comparison to the lower layer, *Ralstonia* populations (cfu/g soil) were higher in samples taken from the upper layer.

Table 2.3 *Ralstonia solanacearum* counts in soil samples from four cultural practices for February 1998 (cfu/g)

Treatment:	Repetition 1		Repetition 2		Repetition 3	
	0-30 cm	30-75 cm	0-30 cm	30-75 cm	0-30 cm	30-75 cm
Potato monoculture						
Site 1	-	-	-	-	-	-
Site 2	-	-	-	-	-	-
Site 3	-	-	-	-	1×10^3	2×10^1
Site 4	-	-	2×10^3	1×10^1	-	-
Site 5	-	-	-	-	-	-
Bare-fallow						
Site 1	5.1×10^3	-	-	-	-	2×10^1
Site 2	-	-	-	-	-	-
Site 3	-	-	-	-	-	-
Site 4	-	-	-	-	-	-
Site 5	-	-	-	-	-	-
Maize monoculture						
Site 1	-	-	-	-	-	-
Site 2	-	-	-	-	-	-
Site 3	-	-	-	4×10^1	-	-
Site 4	-	-	-	-	-	-
Site 5	-	-	-	-	-	-
Weed cover						
Site 1	-	-	-	-	-	-
Site 2	-	-	-	-	-	-
Site 3	-	-	-	-	-	-
Site 4	-	-	-	-	2.2×10^2	-
Site 5	-	-	-	-	-	4×10^1

In February 1999 neither the use of indicator potato plants in pots nor direct isolation from soil on TZC plates was effective in detecting *Ralstonia*. (Table 2.4). Only eight of the 36 samples analyzed by the plating technique were positive: three from potato plots, two from a maize plot, two from the fallow plots and one from a weed plot. Mild wilt symptoms were observed in seven of the 180 indicator plants. *R. solanacearum* was isolated successfully from these stems. Random isolations from healthy appearing stems revealed, however, that several plants were latently infected. This demonstrates that observing symptoms alone is not sufficient to determine the presence of the pathogen in soil of such pot trials. Isolations obtained within the same pot from soil by the plating technique correlated relatively well to those obtained from the stem of plant. In one instance though, a positive isolation was obtained from the indicator plant but not from the direct soil sampling. Another case was observed

where the situation was reversed. The size of the soil sample taken from the field plays a role in successfully detecting bacterial wilt.

Martin *et al.* (1981) had conducted a similar trial, although sampling was performed at 0-30cm, 30-60cm and 60-90cm and ten instead of five indicator plants per site sampled were used. They were able to detect *Ralstonia* up to a depth of 90cm by both the plating technique as well as by planting indicator plants in the collected soil. The usefulness of indicator plants in increasing the detection rate was also observed in their study. At one location, the highest population was in the 0-30 cm layer, and in the other the highest was in the 30-60 cm zone.

Table 2.4 Detection of *Ralstonia* in soil samples collected during February 1999 by means of plating on TZC and use of indicator plants in pots

	Weed-fallow Replication 1					Weed-fallow Replication 2					Weed-fallow Replication 3				
Site 1	Pot 1	2	3	4	5	Pot 1	2	3	4	5	Pot 1	2	3	4	5
S.A	P					N					N				
Wilt	P	N	N	N	N	N	N	N	N	N	N	N	N	N	N
S.I	P	N			N	N				N	N	N			
Site 2															
S.A	N					N					N				
Wilt	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
S.I	N			N		N	N				N	N		P	
Site 3															
S.A	N					N					N				
Wilt	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
S.I	N				N	N			N		N	N			
	Maize Monoculture Replication 1					**Maize Monoculture Replication 2**					**Maize Monoculture Replication 3**				
Site 1	Pot 1	2	3	4	5	Pot 1	2	3	4	5	Pot 1	2	3	4	5
S.A	N					P					N				
Wilt	N	N	N	N	N	P	N	N	P	N	N	N	N	N	N
S.I	N		N	N		P	N	N	P		N	N	N		
Site 2															
S.A	N					N					N				
Wilt	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
S.I	N					N				N	N		N		
Site 3															
S.A	P					N					N				
Wilt	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
S.I	P					N		N	N		N		N	N	
	Bare Fallow Replication 1					**Bare Fallow Replication 2**					**Bare Fallow Replication 3**				
Site 1	Pot 1	2	3	4	5	Pot 1	2	3	4	5	Pot 1	2	3	4	5
S.A	N					N					N				
Wilt	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
S.I	N			N		N		N			N		N		N
Site 2															
S.A	N					N					N				
Wilt	N	N	N	N	N	N	N	N	N	N	N	N	N	P	N
S.I	N					N			N	N	N			P	
Site 3															
S.A	N					P					P				
Wilt	N	N	N	N	N	N	N	N	N	N	P	N	N	N	N
S.I	N	P				P			N		P		N		
	Potato Monoculture Replication 1					**Potato Monoculture Replication 2**					**Potato Monoculture Replication 3**				
Site 1	Pot 1	2	3	4	5	Pot 1	2	3	4	5	Pot 1	2	3	4	5
S.A	P					N					N				
Wilt	P	N	N	P	N	N	N	N	N	N	N	N	N	N	N
S.I	P			P	N	N		N			P			N	
Site 2															
S.A	N					N					P				
Wilt	N	N	N			N	N	N	N	N	N	N	N	N	N
S.I	N					N	N				N				N
Site 3															
S.A	N					N					P				
Wilt	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
S.I	N	P		N		N			N	P	P		P	N	N

S.A. = Analysis of soil from one pot per site sampled in the field
Wilt = Wilt observed in potted plant
S.I. = Isolation from stem of potted plant
P = Positive isolation of *R. solanacearum*
N = No *R. solanacearum* isolated

The use of indicator plants in collected soil in pots, direct plating technique on TZC and planting indicator plants directly in the field is compared in Figure 2.5 by using data obtained from potato monoculture plots. The success of indicator plants in the field could possibly be attributed to larger volumes of soil that are evaluated and to less disturbance in populations during collection and handling of samples. The importance of the number of samples taken is evident from the 23% increase in detection that was observed if five pots per site was evaluated in comparison to only one pot.

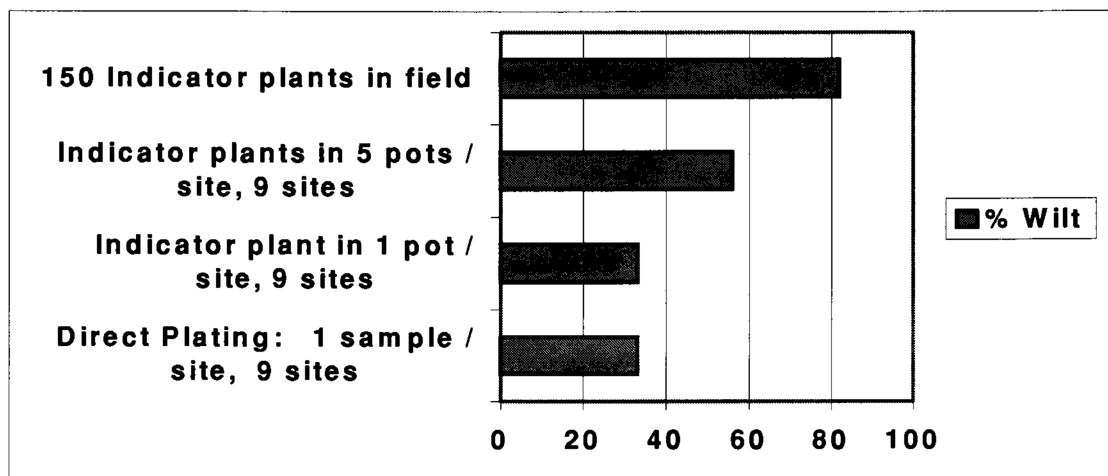

Figure 2.5 Comparison of three techniques used to detect *Ralstonia* populations in potato monoculture plots during the 1997/1998 season.

Monitoring by means of indicator plants

The wilt patterns of the indicator plants observed after a three-year rotation period is depicted in Figure 2.6. Within four weeks after emergence wilt was observed in all treatments. The highest incidence of wilt (15%) was recorded in potato monoculture plots, whereas the bare-fallow plots had the lowest (1%). Wilt incidences increased rapidly during the subsequent four weeks, especially in the bare fallow and potato plots. At twelve weeks the weed-fallowed plots had the lowest occurrence of wilt (31%), bare fallow plots the second highest (58%) and potatoes as expected, the highest (88%).

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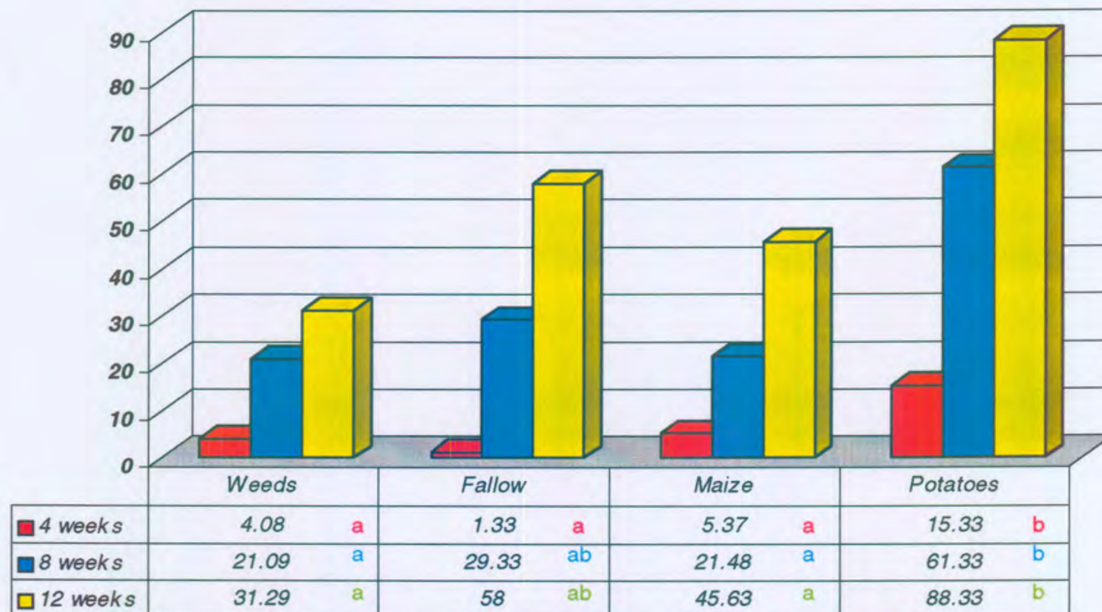
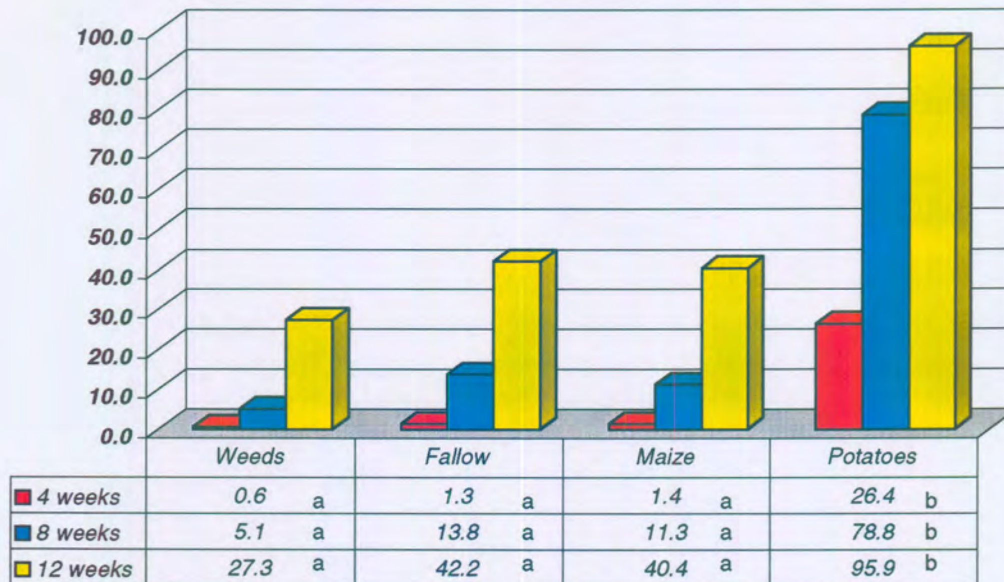


	Weeds		Fallow		Maize		Potatoes	
4 weeks	4.08	a	1.33	a	5.37	a	15.33	b
8 weeks	21.09	a	29.33	ab	21.48	a	61.33	b
12 weeks	31.29	a	58	ab	45.63	a	88.33	b

Figure 2.6 Percentage wilt of indicator plants after three years of cultural practices. Percentages followed by the same letter within each period are not significantly different according to Tukey's Studentized Range (P= 0.05)

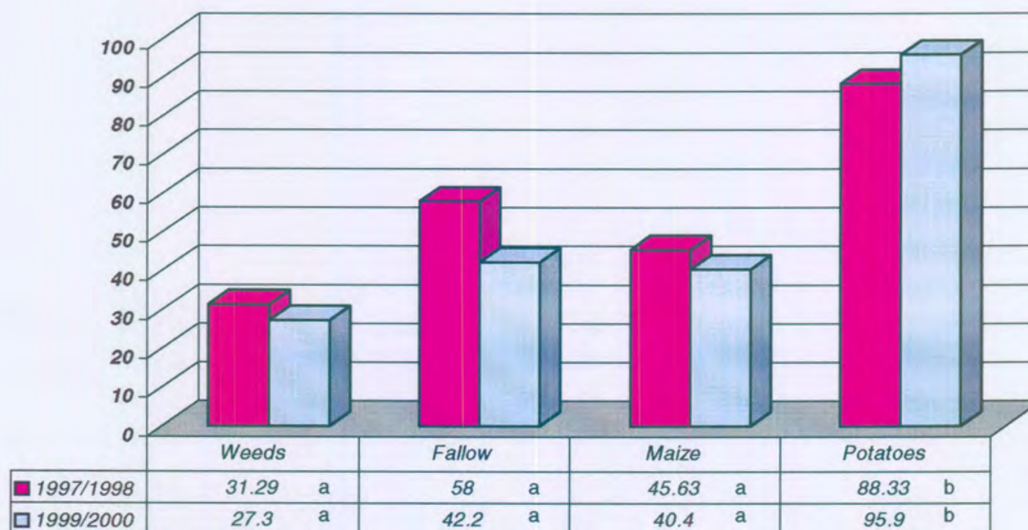
A similar pattern of wilt incidences in indicator plants emerged after the fifth year of rotation (Figure 2.7 & Figure 2.8). Wilting plants were visible in all treatments within four weeks after emergence. The ranking in wilt indices among the four treatments during the first weeks was different, however, with the least wilt (0.6%) observed in weed-fallowed plots instead of in bare fallow plots. The ranking of the wilt indices at twelve weeks did not differ from the previous trial. Potato plots had the highest wilt incidence, followed by bare fallow and maize monoculture. The lowest incidence was noted in weed-fallowed plots. The percentage wilt observed in the bare fallow plots did differ significantly from the potato plots during this season. No difference in percentage wilt could be observed between the tilled and non-tilled regions of each bare fallow plot (Data not presented).

The percentage wilt observed in all treatments except in potato monoculture plots had decreased, though not statistically significant since the previous evaluation.



	Weeds	Fallow	Maize	Potatoes
4 weeks	0.6 a	1.3 a	1.4 a	26.4 b
8 weeks	5.1 a	13.8 a	11.3 a	78.8 b
12 weeks	27.3 a	42.2 a	40.4 a	95.9 b

Figure 2.7 Percentage wilt of indicator plants in treatment plots after five years of cultural practices. Percentages followed by the same letter within each period are not significantly different according to Tukey's Studentized Range (P=0.05)



	Weeds	Fallow	Maize	Potatoes
1997/1998	31.29 a	58 a	45.63 a	88.33 b
1999/2000	27.3 a	42.2 a	40.4 a	95.9 b

Figure 2.8 Comparison of percentage wilting indicator plants in treatment plots after three and five years of rotation. Percentages followed by the same letter within each treatment are not significantly different according to Tukey's Studentized Range (P=0.05)

After both three- and five-year rotation, onset of wilt in bare fallow plots was relatively slow and the argument that survival of *Ralstonia* in the absence of a host is poor, seemed to hold true. In the subsequent weeks however, wilt increased rapidly surpassing wilt patterns in both maize and weed-fallow plots. The difference in incidence of wilt between potato monoculture and bare fallow plots was not even statistically meaningful after the three-year rotation period. Similar results have been reported by Akiew & Trevorrow (1994) where the incidence of tobacco wilt after two-year bare fallow was not significantly lower than after continuos tobacco. Martin *et al.* (1981) reported a drastic reduction of bacterial wilt population after a short bare-fallow period of 140 days. Whether this reduction is statistically meaningful at P=0.05 is not mentioned. After five years, however, percentage wilt in the bare fallow plots was statistically less than observed in the potato plots. Desiccation of soil is considered as a major factor in reducing soil population of the wilt organism, and is enhanced with bare fallow treatment. The disease pattern observed in both seasons in the bare fallow plots indicate that either insufficient desiccation occurred or that *Ralstonia* populations deeper in the soil profile remained less affected. It is also possible that the lack of plant material resulted in a decrease in general soil organisms, thereby reducing competition and suppression.

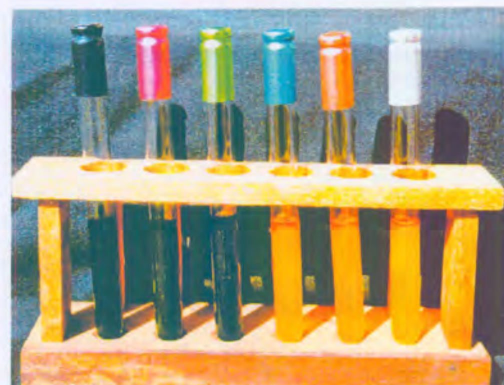
Removal of weeds is considered vital in integrated management of bacterial wilt since these can serve as hosts or shelter sites for long term survival (French, 1994; Jackson & Conzales, 1979). Yet potato plants in the weed-fallow plots exhibited the least wilt symptoms, although host species such as *Datura strammonium* and *Portulaca* were present. This may be an indication that certain weeds or grasses resulted in the suppression of the wilt organism either by harboring antagonistic microorganisms or by releasing inhibitory substances. It is important to determine which of the weeds that have been identified in these plots serve as hosts. Suppression is probably more likely a result of the grass species that dominate in the plots, than a specific weed. Weeds tended to be interspersed with the major cover being provided by grasses. In Australia forage sorghum, signal grass and Rhodes grass, are often used in rotation programs (Akiew *et al.*, 1993; Arthy & Akiew 1999).

Crop rotation with maize has often been applied as a technique to reduce bacterial wilt populations in the soil (French 1994). The information obtained by using indicators does indicate that maize monoculture is more effective in reducing the incidence of wilt than leaving the field bare fallow, though not statistically significant. However, wilt in the maize plots after the fifth season was still relatively high (40%). It is known that some strains of bacterial wilt can cause localized infections in maize roots. The total bacterial population released from these plants is however far lower than from true hosts, explaining the gradual decrease over time (Granada & Sequeira, 1983a). No infections were observed in the 15 randomly selected maize plants, indicating the inability of the local strain to invade and colonize the roots. Controlled trials where inoculum potentials are higher and more evenly distributed would be required to confirm these findings.

Plant samples

R. solanacearum could be isolated from the stems of all sampled potato plants. In most instances a clear bacterial flow could be observed when cut stems were placed in water. Ring symptoms were observed in some of the progeny tubers. All 25 *Ralstonia* isolates placed on biovar identification medium produced the same pattern of biochemical reaction as shown in Figure 2.9.

Carbohydrate	Biovar 2	Reaction of all 25 isolates
Lactose	+	+
Maltose	+	+
Cellobiose	+	+
Mannitol	-	-
Sorbitol	-	-
Dulcitol	-	-



Biovar 2

Figure 2.9 Identification of biovars of *R. solanacearum* according to utilization and/or oxidisation of certain carbohydrates (Hayward, 1994)

The results the field trial demonstrate that an adequate population of *R. solanacearum* biovar 2 survived in the soil after five years of fallowing or maize cultivation to cause serious infection in a subsequent potato crop. Survival clearly exceeded the two-year period suggested to be sufficient in eradicating this organism from soil (Elphinstone, 1996).

REFERENCES

AKIEW, E. & P.R. TREVORROW. 1994. Management of bacterial wilt of tobacco. In: A.C. Hayward and G.L. Hartman (eds) Bacterial wilt: The disease and its causative agent, *Pseudomonas solanacearum.* Cab International United Kingdom, pp 179-198

AKIEW, E., TREVORROW, P.R. & P.E. TONELLO. 1993. Management of bacterial wilt of tobacco. In: Hartman, G.L. and Hayward, A.C. (eds) Bacterial Wilt. Proceedings of the 1st international conference on the bacterial wilt, Kaohsiung, Taiwan, 28-31 October 1992. Canberra, Australia, ACIAR Proceedings 45: 270-275

ARTHY J. & S. AKIEW. 1999. Effect of short term rotation on *Ralstonia solanacearum* populations in soil. *Bacterial Wilt News Letter 16: 13-14*

ELPHINSTONE, J.G. 1996. Survival and possibilities for extinction of *Pseudomonas solanacearum* (Smith) Smith in cool climates. *Potato Research 39: 403-410*

ENGELBRECHT, M.C. 1994. Modification of a semi-selective medium for the isolation and quantification of *Pseudomonas solanacearum. Bacterial Wilt Newsletter 10: 3-5*

FRENCH, E.R. 1994. Strategies for integrated control of bacterial wilt of potatoes. In: A.C. Hayward and G.L. Hartman (eds) Bacterial wilt: The disease and its causative agent, *Pseudomonas solanacearum.* Cab International United Kingdom, pp 199-207

GRAHAM, J., JONES, D.A. & A.B. LLOYD. 1979. Survival of *Pseudomonas solanacearum* race 3 in debris and latently infected potato tubers. *Phytopathology 69:1100-1103*

GRANADA, G.A. & L. SEQUEIRA. 1983. Survival of *Pseudomonas solanacearum* in soil, rhizosphere, and plant roots. *Canadian Journal of Microbiology 29:433-440*

HAYWARD, A.C. 1994. Systematics and phylogeny of *Pseudomonas solanacearum* and related bacteria. In: A.C. Hayward and G.L. Hartman (eds) Bacterial wilt: The disease and its causative agent, *Pseudomonas solanacearum.* Cab International United Kingdom, pp 123-136

JACKSON, M.T. & L.C. CONZALEZ. 1979. Persistance of *Pseudomonas solanacearum* in an inceptisol in Costa Rica. In: Developments in Control of Potato Bacterial Diseases. Report of a Planning Conference, 12-15 June, 1979, CIP, Lima, pp 66-71

KELMAN, A. 1953. The bacterial wilt caused by *Pseudomonas solanacearum. Technical Bulletin No. 99 of the North Carolina Agricultural Experimental Station*

MARTIN C., TORRES, E. & U. NYDEGGER. 1981. Distribution of *Pseudomonas solanacearum* in tropical soil of Peru. Proceedings of the Fifth International Conference on Plant Pathogenic Bacteria, Bali, pp 185-194

SWANEPOEL, A.E., 1992. Survival of South African strains of biovar 2 and biovar 3 of *Pseudomonas solanacearum* in the roots and stems of weeds. *Potato Research 35: 329-332*

YABUUCHI, E., KOSAKO, Y., OYAIZU, H., YANO, I., HOTTA, H., HASHIMOTO, Y., EZAKI, T. & M. ARAKAWA. 1992. Proposal of *Burkholderia* gen. Nov. and transfer of seven species of the genus *Pseudomonas* homology group II to the new genus, with the type species *Burkholderia cepacia* (Palleroni and Holmes 1981) comb. Nov. *Microbiology and Immunology Vol. 36(12): 1251-1275*

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YABUUCHI, E., KOSAKO, Y., YANO, I., HOTTA, H. & Y. NISHIUCHI. 1995. Transfer of two *Burkholderia* and an *Alcaligenes* species to *Ralstonia* Gen. Nov.: Proposal of *Ralstonia pickettii* (Ralston, Palleroni and Doudoroff 1973) Comb. Nov., *Ralstonia solanacearum* (Smith 1896) Comb. Nov. and *Ralstonia eutropha* (Davis 1969) Comb. Nov. *Microbiology and Immunology Vol. 39(11): 897-904*

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CHAPTER 3

THE ROLE OF WEEDS IN THE PERPETUATION OF BACTERIAL WILT

ABSTRACT

Twenty-two weed and three grass species were investigated for their susceptibility to *Ralstonia solanacearum* biovar 2 and biovar 3 infection. The biovar 2 strain could be isolated from *Datura ferox*, *D. stramonium*, *Portulaca oleracea* and *Hibiscus trionum* although wilting was observed only in some *P. oleracea* plants. Host range for biovar 3 was wider and included *Amaranthus* spp., *Bidens bipinnata*, *Chamaesyce prostrata*, *Chenopodium album*, *Chenopodium carinatum*, *Cyperus rotundus*, *Datura ferox*, *Datura stramonium*, *Eragrostis curvula*, *Hibiscus trionum*, *Portulaca oleracea*, *Sonchus oleraceus*, *Tragopogon dubius*. Since very low infection rates were obtained for *Pseudognophalium luteo-album* and *Tagetes minuta*, their host status is questionable. A preliminary *in vitro* study was conducted to determine suppressiveness of some weeds/grasses. It appears the microbial activity associated with the plant could be involved in suppression of the wilt organism.

INTRODUCTION

The role of weeds in the survival of the bacterial wilt organism in the absence of cultivated hosts has long been recognized (Kelman, 1953; Amat *et al.*, 1978; Tusiime *et al.*, 1998). More than 230 weed species (Table 1.3) have been reported as hosts of *Ralstonia solanacearum* and consequently weed control is regarded essential in the management of bacterial wilt. Susceptibility of weed hosts ranges from highly susceptible in which typical wilt symptoms are expressed, to tolerant ones where often no symptoms are observed. It is also possible that certain species such as *Phyllanthus niruri* L. can serve both as a symptomless carrier and under different circumstances as a true host with typical wilt symptoms (Hayward, 1994). The specific race or biovar of *R. solanacearum* present often determines whether a plant will be infected or not. In South Africa *Amaranthus hybridus* L. is a host of biovar 3 (race 1), but not of biovar 2 (race 3) (Swanepoel, 1992). Race 1 (solanaceaceous strain) is known to have an extensive host range, affecting weeds not only from the Solanaceae family but also amongst others from the Asteraceae (Ansari, 1990), Capparaceae (Harris, 1976), Portulacaceae (Harris, 1976) and Urticaceae (Olsson, 1976). Race 3 (potato strain) has a limited host range including only a few weed species mainly from the Solanaceae.

Non-conformity in the occurrence of bacterial wilt in certain weeds does occur even if the same race or biovar of the pathogen is involved. Wild gooseberry (*Physalis* spp.) and *Bidens pilosa* L. are hosts of biovar 3 (race 1) in Australia (Akiew & Trevorrow, 1994) but not in South Africa where *Physalis angulata* L. is in fact susceptible to biovar 2 (race 3) (Swanepoel, 1992). This differential host pattern can be due to evolution of specific strains that are pathogenic for certain hosts in certain parts of the world and that are not found elsewhere. Alternatively, the plants may become susceptible if a number of environmental factors conducive to disease expression coincide (Hayward, 1994).

Bacterial wilt of potato in South Africa is caused mainly by the biovar 2 (race 3) isolate although biovar 3 (race 1) has also been isolated from this crop. Once bacterial wilt has been identified in a field, control revolves around effective implementation of

management strategies such as isolating the infested areas and preventing contaminated water to drain off the field, sterilising farming equipment and applying rotation programs.

Weed control is considered essential in the integrated management of this disease. Most findings indicate that weeds promote the survival of *Ralstonia* in the soil, transmit the pathogen to the next crop, and reduce the success of rotation practices (Jackson & Conzales, 1981; Shekhawat & Perembelon 1991; Tusiime, *et al.*, 1998). Akiew *et al.* (1993) found a significant increase in *Ralstonia* populations in weed-fallowed soil during the summer months when *Bidens pilosa* was abundant and rainfall frequent. They also observed a 100% wilt incidence on susceptible tobacco grown in summer in a field kept under weed-fallow for 18 months. Smith & Godfrey (1939), however, found no correlation between presence of susceptible weeds in one season and the severity of wilt in the next. An extensive list of weed hosts that have been reported world-wide is given in Table 1.3 of the literature review. In South Africa little work has been done to identify the host range for both strains. Those that have been reported as hosts include *Datura ferox*, (biovars 2 & 3) (Swanepoel & Young, 1988), *Datura stramonium* L. (biovar 3), *Amaranthus hybridis* L. (biovar 3), *Bidens bipinnata*, (biovar 3), *Eragrostis curvula* (Schrad.) Nees. (biovar 3), *Physalis angulata* L. (biovar 2), *Ricinus communis* L. (unspecified) and *Solanum nigrum* L. (biovar 3) (Swanepoel, 1992). Swanepoel (1992) also evaluated *Chenopodium album* L., *Galinsoga parviflora* Car., *Tagetes minuta* L. and *Nicotiana glauca* for host status and found them not to be susceptible to either biovar.

An investigation into the impact of cultural practices on the longevity of the potato race was conducted at the University of Pretoria (Chapter 2). Four cultural practices, namely maize and potato monoculture, bare-fallow and weed-fallow were applied over a period of five years. Indicator potato plants planted across all treatments after the third and fifth year, revealed that weed-fallowed plots had for both years the lowest incidence of wilt. The field results suggest these weed-fallowed plots to be suppressive to *R. solanacearum*. An investigation was undertaken to determine the host status of a range of weeds for biovar 2 and biovar 3, and to probe the hypothesis of suppressive interaction between certain weeds and the pathogen.

MATERIALS AND METHODS

The experiments on the role of weeds in the perpetuation of bacterial wilt focussed on three aspects. Firstly the possible host range was investigated by screening the weed species occurring on the field plot in a glasshouse trial. In the second phase the rhizophere of some weeds was evaluated for possible suppression of *Ralstonia solanacearum*. One experiment involved weeds grown in pots, the second weeds cultured in nutrient solution. The third phase of the investigation involved two experiments on *in vitro* suppression of *R. solanacearum* with weed extracts or leaching. These were conducted at different intervals, the latter involving three different techniques.

Phase 1: Determination of host range

Three grasses and 23 weeds were evaluated for host status of biovar 2 and biovar 3 in a glasshouse trial. Selected species that coincided with those identified in the bacterial wilt field trial (refer to Chapter 2) were: *Amaranthus* spp., *Bidens pilosa*, *Chamaesyce prostrata*, *Chenopodium carinatum*, *Chloris pycnotrix*, *Commelina benghalensis*, *Conyza albida*, *Cyperus rotundus*, *Datura ferox*, *Datura stramonium*, *Eragrostis curvula*, *Hibiscus trionum*, *Hypochoeris radicata*, *Lepidium africanum*, *Portulaca oleracea*, *Pseudognophalium luteo-album*, *Schkuria pinnata*, *Sisymbrium thellungi*, *Sonchus oleraceus*, *Sporobolus africanus*, *Tagetes minuta* and *Tragopogon dubius*. *Bidens bipinnata* and *Chenopodium album* was present at the edge of the field trial and since they are both common weeds in South Africa they were included in the host range studies. *Opuntia stricta* although not present in the bacterial wilt field trial was included in the investigation, as it is becoming a pest in certain areas.

A biovar 2 and biovar 3 isolate (originally isolated from potato) was cultured on Kelman's tetrazolium medium (TZC) (Kelman, 1953) for 48 hours. A bacterial suspension was prepared in 5 L of sterile distilled water. A dilution series was prepared and plated on TZC. Colonies were counted after 48 hours and the concentration was determined at 2 x 10^6 cfu/ml.

Vermiculite was soaked in the inoculum for an hour at a ratio of 3 g vermiculite to 10 ml inoculum. Pots were filled with 2 kg of a sterile 1:1 vermiculite and soil mix (v:v) and inoculated by incorporating 40 g of soaked vermiculite. This method allows the inoculum to be spread more uniformly throughout the soil than by adding 30 ml of inoculum to each pot.

Seedlings from each of the 26 species were carefully collected from the field to minimize root injury. Ten seedlings were transplanted into 5 pots inoculated with biovar 2, another ten into 5 pots inoculated with biovar 3 and two were planted into non-inoculated soil to serve as controls. Plants were watered daily and monitored for wilt symptoms. Seedlings not adapting to the transplantation were excluded from the trial.

Once wilt was established, isolations were performed to confirm the presence of *R. solanacearum*. After six weeks isolations were performed on all plants. The stems and roots were washed thoroughly, surface sterilized in 1% sodium hypochlorite for 10 minutes and rinsed three times in sterile water. Each sample was either blended or cut finely in sterile water depending on the size of the sample and a dilution series was prepared. These were plated on TZC and incubated for 48 hours.

Phase 2: Preliminary evaluation of different weeds for suppression of *R. solanacearum* in the rhizosphere

Experiment 1

In this investigation the rhizosphere soil from certain weeds used in the host range study was analyzed to determine whether a reduction or increase in *R. solanacearum* population had occurred in the six-week period. Soil was shaken from the rhizosphere of some plants of *Amaranthus* spp., *C. prostrata*, *C. pycnotrix*, *E. curvula* and *D. ferox* and allowed to air dry for 72 hours. A 10 g sample of the soil was suspended in 100ml sterile water and placed on a shaker for 30 minutes. A dilution series was prepared and plated on modified TZC (Elphinstone *et al.*, 1996) without the addition of bacitracin. Colony counts were performed after 72 hours.

Experiment 2

Due to poor recovery of the pathogen from the soil a follow-up trial was initiated to avoid the soil phase. Erlenmeyer flasks containing 90 ml sterile nutrient solution were inoculated with 10 ml *R. solanacearum* biovar 2 (6 x 10^5 cfu/ml) inoculum. Six small plantlets of *S. africanus*, *E. curvula* and *C. picnotrix* were collected from the field and rinsed in distilled water. The plants were transferred to the Erlenmeyer flasks and kept in a plant growth chamber for three weeks. Isolations were performed weekly on TZC from the solution of two flasks per species and from two control flasks with no plants. Colony counts were performed after 48 hours.

Phase 3: *In vitro* suppression of *R. solanacearum* with weed extracts

Experiment 1

Tagetes minuta, Conyza albida, Lepidium africanum and *Opuntia stricta* plants were collected in the field, rinsed to remove most soil particles, surface sterilized in 1% sodium hypochlorite for 15 minutes and rinsed three times in sterile water. The plant material was weighed and blended in sterile water at a ratio of 1 g plant material to 10 ml water. Supernatant of the extract was sterilized through microfiltration using a Millipore filter. The sterilized filter paper discs were impregnated with the sterile extract and allowed to air dry in a sterile petri-dish. Discs impregnated with a 1% sodium hypochlorite solution and sterile water were used as controls. Seeded TZC plates were prepared as follows. Standard TZC plates were poured and the medium was allowed to stall. An additional two 200ml bottles TZC medium was prepared with only half the standard amount of agar. Once cooled to below 40 °C, 20 ml *R. solanacearum* biovar 2 inoculum of concentration 3.1 x 10^5cfu/ml was added to one bottle, gently agitated and a thin layer of inoculated cooled TZC medium was poured onto standard TZC plates. To the other bottle, 20 ml of biovar 3 inoculum (2.0 x 10^5 cfu/ml) was added and poured onto standard TZC medium. Discs were placed in a random design on the seeded TZC (10 replications) and incubated for 72 hr. Inhibition zones were measured. This technique is similar to the one described by Korsten (1984).

Experiment 2

Plants of *T. dubius*, *E. curvula*, *S. africanus* and *H. radicata* were rinsed and soaked in sterile distilled water (ratio 1 g: 10 ml) for 16 hours without surface sterilization. Surface sterilization removes microbial activity and could interfere with the plant leaching (C.F. Reinhardt, pers. comm.)[2]. Suppression of *R. solanacearum* by these weeds was evaluated with three different techniques:

Technique A: Paper disc

The disc technique on seeded TZC as described above was used.

Technique B: Poisoned medium

Theron (1999) found in his studies on *Fusarium* species that diffusibility of certain fungicides was not effective when applying the paper disc technique and suggests the poisoned medium technique (Jones & Eheret, 1976) as an additional screening technique. For this reason filter sterilized weed leaching (obtained as described above) were incorporated into cooled TZC medium at a ratio of 20ml leaching to 500ml TZC medium, prior to pouring. *R. solanacearum* biovar 2 inoculum (1.5×10^3cfu/ml) was plated onto this medium and as control onto normal TZC medium in which the leaching was replaced with sterile water.

Technique C: Culture of R. solanacearum in leaching

In the third evaluation, 9 ml leaching was added to a sterile test tube with 1 ml *R. solanacearum* biovar 2 inoculum (2.5×10^6cfu/ml), giving a final bacterial population of 2.5×10^5 cfu/ml. In control tubes sterile water and potato leaching were used. Five repetitions of each treatment were incubated on a shaker for five days. A dilution series was prepared and plated onto TZC medium. Number of colonies formed was compared. This technique takes microbial activity associated with the weed into account.

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[2] Prof. C.F. Reinhardt, Dept. Plant Production & Soil Science, University of Pretoria, Pretoria 0002, South Africa

Statistical Analysis

General Linear Models Procedure of the Statistical Analysis System (SAS®)[3] was applied to determine the effect of weed extracts on the growth of *Ralstonia solanacearum*. Tukey's Studentized Range at the 5% significance level was used to identify significant differences.

RESULTS

Phase 1: Determination of host range

Adaptation of the small seedlings in the glasshouse was good and only five plants in total were lost. Figure 3.1 portrays some of the weeds grown in pots for the determination of host status. The Kremer & Unterstenhöfer (1967) method of plant scoring is often used to evaluate the susceptibility of plants to wilt. It was however not applied in this trial since it does not permit the removal of plants for isolation prior to completion of the trial period. Host status of the 23 weeds and three grasses are given in Table 3.1. None of the three grass species and only a few weed species were susceptible to biovar 2 infection. A high percentage of *D. stramonium* and *D. ferox* plants were infected although none showed wilt. *Portulaca oleracea* was also highly susceptible to biovar 2 with 20 % plants showing wilt symptoms. Infection rate of *Hibiscus trionum* to biovar 2 was very low.

One of the grass species and 14 weed species were susceptible to biovar 3. Wilt symptoms were observed in some plants of *D. stramomium*, *D. ferox*, *B. bipinnata*, *C. prostrata* and *P. oleracea*. A high percentage of plants from these species were infected. High infection rates to biovar 3 were also obtained in *Chenopodium album*,

[3] SAS® System is an integrated system of software providing complete control over data management, analysis, and presentation, and may be obtained from SAS Institute South Africa (Pty) Ltd. 93 Central Street, Houghton, P.O. Box 2837, Houghton, 2041, Republic of South Africa

C. carinatum, *Amaranthus* spp., and *Cyperus rotundus* plants. Lower infection rates (50% or less) were observed in *Eragrostis curvula*, *Sonchus oleraceus*, *Tragopogon dubius*, *Hibiscus trionum*, *Tagetes minuta* and *Pseudognophalium luteo-album*.

Figure 3.1 Weeds cultured in pots for determination of susceptibility to *R. solanacearum* (biovar 2)

Table 3.1 Evaluation of 21 weed and 3 grass species for susceptibility to *Ralstonia solanacearum* biovar 3 (race 1) and biovar 2 (race 3)

Plant species	Common Name	Percentage plants wilting			Percentage plants infected		
		Biovar 2	Biovar 3	Control	Biovar 2	Biovar 3	Control
Amaranthus spp.	Pigweed	0	0	0	0	90	0
Bidens bipinnata	Spanish blackjack	0	50	0	0	90	0
Bidens pilosa	Common blackjack	0	0	0	0	0	0
Chamaesyce prostrata	Hairy creeping milkweed	0	20	0	0	100	0
Chenopodium album	White goosefoot	0	0	0	0	70	0
Chenopodium carinatum	Green goosefoot	0	0	0	0	100	0
Chloris pycnotrix	Spiderweb chloris	0	0	0	0	0	0
Commelina benghalensis	Wandering Jew	0	0	0	0	0	0
Conyza albida	Tall fleabane	0	0	0	0	0	0
Cyperus rotundus	Purple nutsedge	0	0	0	0	90	0
Datura ferox	Large thorn-apple	0	20	0	80	100	0
Datura stramonium	Common thorn-apple	0	60	0	100	100	0
Eragrostis curvula	Weeping love grass	0	0	0	0	30	0
Hibiscus trionum	Bladder hibiscus	0	0	0	10*	50	0
Hypochoeris radicata	Hairy wild lettuce	0	0	0	0	0	0
Lepidium africanum	Pepperweed	0	0	0	0	0	0
Opuntia stricta	Australian pest-pear	0	0	0	0	0	0
Portulaca oleracea	Common purselane	20	40	0	70	100	0
Pseudognophalium luteo-album	Cudweed	0	0	0	0	10	0
Schkuria pinnata	Dwarf marigold	0	0	0	0	0	0
Sisymbrium thellungi	Wild mustard	0	0	0	0	0	
Sonchus oleraceus	Sowthistle	0	0	0	0	40	0
Sporobolus africanus	Rat's tail dropseed	0	0	0	0	0	0
Tagetes minuta	Khakiweed	0	0	0	0	10 *	0
Tragopogon dubius	Yellow goat's beard	0	0	0	0	30	0

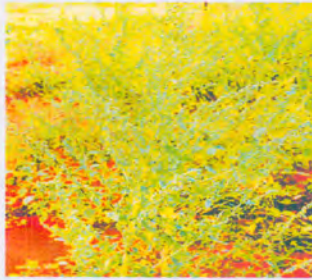
* These findings must be re-evaluated to confirm infection of host plant

Weeds identified as hosts of bacterial wilt (biovar 2 or biovar 3) during this trial are depicted in Figure 3.2.

Amaranthus spp. biovar 3

Bidens bipinnata biovar 3

Chamaesyce prostrata biovar 3


Chenapodium album biovar 3

Chenapodium carinatum biovar 3

Cyperus rotundus biovar 3

Datura ferox biovar 2 & 3

Datura stramonium biovar 2 & 3

Eragrostis curvula biovar 3

Hibiscus trionum biovar 2 & 3

Portulaca oleracea biovar 2 & 3

Sonchus oleraceus biovar 3

Tragopogon dubius biovar 3

Pseudognophalium luteo-album biovar 3 (re-evaluate)

Tagetes minuta biovar 3 (re-evaluate)

Figure 3.2. Weeds identified as hosts of *Ralstonia solanacearum* biovar 2 and/or biovar 3

Phase 2: Preliminary evaluation of different weeds for suppression of *R. solanacearum* in the rhizosphere

Experiment 1

The recovery of *R. solanacearum* from rhizosphere soil of *Amaranthus* spp., *Chamaesyce prostrata*, *Chloris pycnotrix*, *Eragrostis curvula* and *Datura ferox* was very poor, even when *R. solanacearum* had been isolated from the plant itself (Table 3.2). Growth of saprophytes on the plates was high. The reason for this is not clear since the selective modified TZC medium (Elphinstone *et al*., 1996) had been used. It is possible that the exclusion of bacitracin could have affected saprophytic growth.

Table 3.2 Rhizosphere population of *R. solanacearum* in plants of five weed species six weeks after inoculation

Species	Biovar	Plant	cfu/g soil	Plant status
Amaranthus spp.	3	1	4×10^3	infected
	3	4	0	infected
	2	6	0	negative
Chamaesyce prostrata	2	2	2×10^2	negative
	3	8	0	infected
Chloris pycnotrix	2	5	1×10^1	negative
	2	9	0	negative
	3	2	0	negative
Eragrostis curvula	2	1	0	negative
	3	3	0	infected
	3	2	0	negative
Datura ferox	3	5	0	infected
	2	4	2×10^1	infected

Experiment 2

R. solanacearum biovar 2 populations deteriorated to an undetectable level within the three weeks when co-cultured with *E. curvula*, *S. africanus* and *C. pycontrix* plantlets

in nutrient solution. The plantlets are shown in Figure 3.3. The pathogen maintained its viable population level in the otherwise sterile nutrient solution (Table 3.3). A strong growth of bacterial saprophytes was observed on the TZC plates prepared from the nutrient solution of the grass samples. Several of these saprophytes were cross-streaked against *R. solanacearum* biovar 2 on TZC plates to observe inhibition zones. Three unidentified bacteria inhibited the growth of *Ralstonia*.

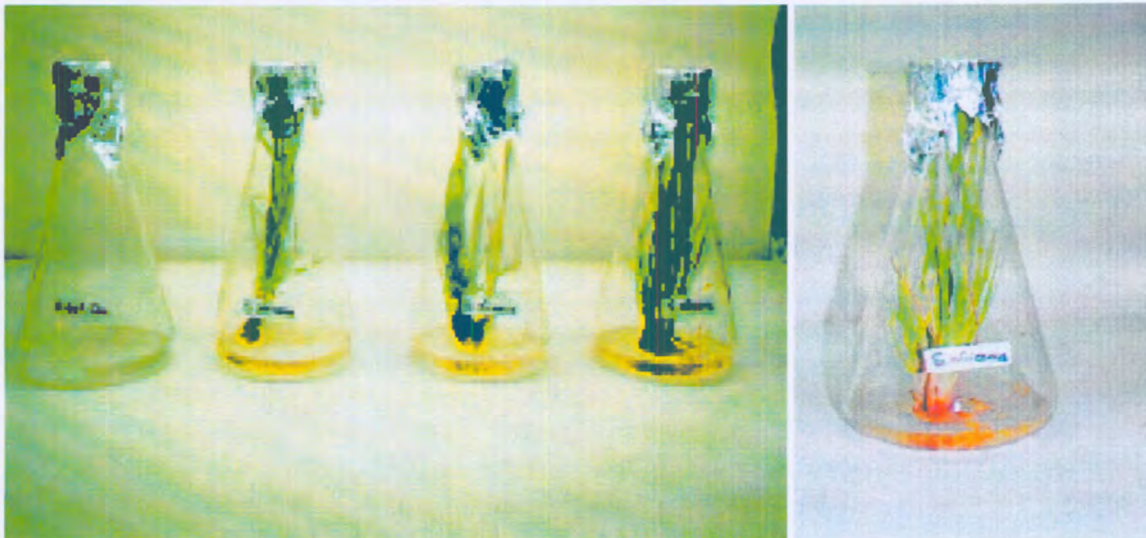


Figure 3.3 *Sporobolus africanus* plantlets co-cultured with *Ralstonia solanacearum* in nutrient solution

Table 3.3 *Ralstonia solanacearum* biovar 2 populations after co-cultivation with three different grasses in nutrient solution for a period of three weeks

	***Ralstonia solanacearum* populations (cfu/ml)**			
	Week 0	**Week 1**	**Week 2**	**Week 3**
Sporobolus africanus	3×10^5	1×10^1	0	0
Eragrostis curvula	7.4×10^5	2×10^2	0	0
Chloris pycnotrix	5.5×10^5	0	0	0
Control (nutrient solution)	7.3×10^5	5.4×10^5	8.7×10^5	6.4×10^5

Phase 3: *In vitro* suppression of *R. solanacearum* with weed extracts

Experiment 1

No statistically significant growth inhibition of *R. solanacearum* could be observed from paper discs impregnated with sterile weed extracts of *Tagetes minuta*, *Conyza albida*, *Lepidium africanum* and *Opuntia stricta* (Table 3.4). Only discs impregnated with the 1% NaOCl resulted in a consistent inhibition zone being formed (Figure 3.4).

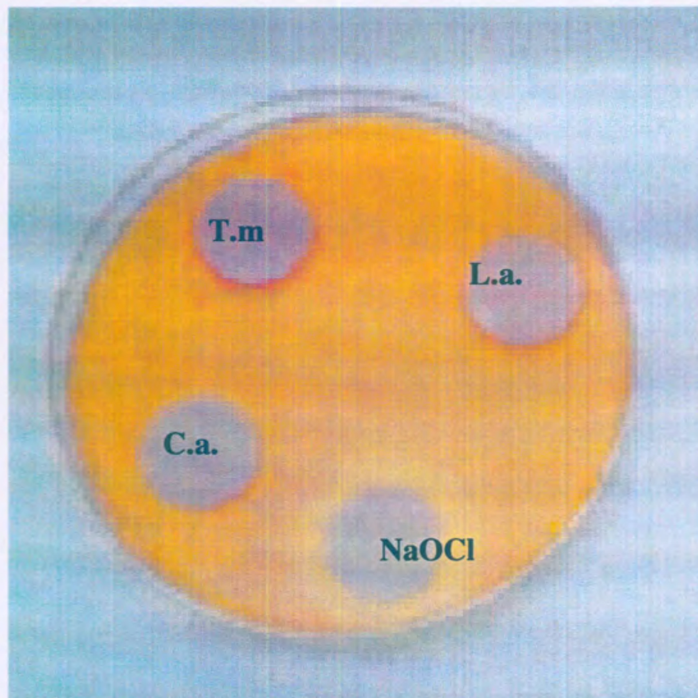


Figure 3.4 Inhibition zones on TZC plates seeded with *Ralstonia solanacearum*, biovar 2 inoculum

Experiment 2

Technique A: Paper disc

Paper discs impregnated with leaching of *Tragopogon dubius*, *Eragrostis curvula*, *Sporobolus africanus* and *Hypochoeris radicata* did not result in the formation of significant inhibition zones (Table 3.4).

Table 3.4 Mean *Ralstonia solanacearum* inhibition zones surrounding paper discs impregnated with weed extracts and controls, measured after 72 hours

Weed extract	Mean inhibition zone (mm) biovar 2	Mean inhibition zone (mm) biovar 3
Tagetes minuta	0.5 b	0.5 b
Conyza albida	0.2 b	0.2 b
Lepidium africanum	0.6 b	0.2 b
Opuntia stricta	0.8 b	0.5 b
Eragrostis curvula	0.4 b	0.2 b
Sporobolus africanus	0.5 b	0.5 b
Tragopogon dubius	0.6 b	0 b
Hypochoeris radicata	0.8 b	0.4 b
1% NaOCl	4.2 a	4.4 a
Sterile dH_2O	0.2 b	0.3 b

Data values followed by the same letter are not significantly different according to Tukey's Studentized Range (P=0.05)

Technique B: Poisoned medium

Incorporating sterile leaching of *E. curvula*, *S. africanus*, *T. dubius* and *H. radicata* into the TZC medium did not statistically reduce the number of *R. solanacearum* biovar 2 colonies formed after 48-hour incubation (Figure 3.5).

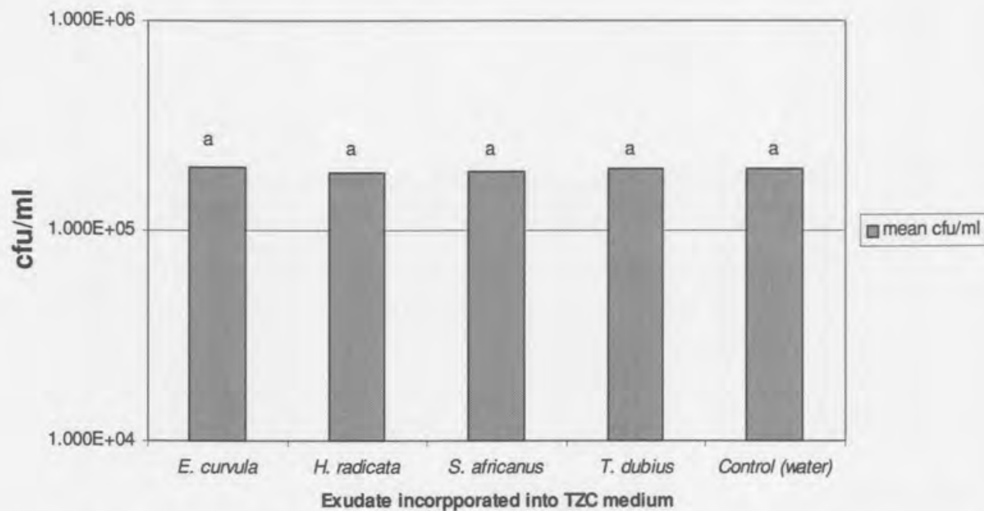


Figure 3.5 Logarithmic presentation of growth (cfu/ml) of *Ralstonia solanacearum* (biovar 2) on TZC medium poisoned with plant leaching of *E. curvula, H. radicata, S. africanus* and *T. dubius*. Data points with the same letter are not significantly different according to Tukey's Studentized Range (P=0.05)

Technique C: Culture of R. solanacearum in leaching of weeds

Virtually no *R. solanacearum* populations could be detected in the inoculated leaching of *E. curvula*, *S. africanus*, *T. dubius* and *H. radicata* after 5-day incubation. However, platings from the inoculated potato leaching also demonstrated a lower colony count (Table 3.5). Saprophytic growth was high. Counts obtained from the inoculated sterile water showed no reduction or increase in population levels.

Table 3.5 *R. solanacearum* populations in inoculated leaching of *E. curvula*, *S. africanus*, *T. dubius* and *H. radicata* after a 5-day incubation

Inoculated leaching from:	Repetitions (cfu/ml)					Average (cfu/ml)
	1	2	3	4	5	
E. curvula	0	1×10^1	0	2×10^2	0	4.2×10^1
S. africanus	0	0	2×10^1	0	0	4
T. dubius	3×10^1	0	3.4×10^2	0	0	7.4×10^1
H. radicata	0	0	0	0	1×10^1	2
Potato	5×10^2	3×10^1	0	6×10^3	0	1.3×10^3
Sterile water	9.8×10^4	1.5×10^5	2.1×10^5	8.7×10^4	5.2×10^5	3.2×10^5

DISCUSSION

The field study on longevity of the bacterial wilt pathogen in soil under different cultural practices (Chapter 2), revealed weed-fallowing to be more effective in reducing wilt incidence than maize monoculture and bare-fallowing. This prompted an investigation in to the susceptibility of 23 weed and three grass species to *Ralstonia solanacearum* and whether any of these weeds and grasses plays a role in the suppression of the pathogen. The investigation consisted of three phases. During the first phase host status to biovar 2 and biovar 3 of *R. solanacearum* was determined. Several weeds have been identified for the first time as hosts for biovar 2 (race 3) or biovar 3 (race 1) in South Africa. *Portulaca oleracea* has been reported as a host of race 1 and race 3 in Kenya (Harris, 1976), but has not previously been evaluated in South Africa. Its host status to both races in South Africa has now been

established with plants expressing wilt to both races. Biovar 3 could be isolated from 50% of the *Hibiscus trionum* plants although none had wilt symptoms. Infection with biovar 2 was very rare (10%) and results must be confirmed before considering this weed species a host of biovar 2. *H. trionum* has not yet been reported as a host of bacterial wilt. Other species of the *Hibiscus* genus such as H. *cannabinus* Linn. (Abdullah, 1993) and H. *sabdariffa* L. (Kelman, 1953) have been reported as hosts of *Ralstonia solanacearum* (biovar or race unspecified).

Chamaesyce prostrata (*Euphorbia chamaesyce*) was susceptible to biovar 3. This is a first report of this host in South Africa. *Euphorbia geniculata* L., *Euphorbia hirta* L. and *Euphorbia maculata* L. have been reported as hosts in India (Shekhawat *et al.*, 1992), and *Euphorbia prunifolia* in Malaysia (Abdullah, 1993). Other weeds that are reported as hosts of biovar 3 for the first time in South Africa include *Sonchus oleraceus*, *Tragopogon dubius*, *Cyperus rotundus*, *Bidens bipinnata* and *Chenopodium carinatum*. Although *S. oleraceus* has not been recorded as a host elsewhere, *S. arvensis* has been reported in India. *C. rotundus* and *B. bipinnata* are also hosts in India (Shekhawat *et al.*, 1992). *T. dubius* and *C. carinatum* have not been reported elsewhere as hosts of *R. solanacearum*. Although *Tagetes minuta* is a host in Australia (Akiew *et al.*, 1993) and in Uganda (Tusiime *et al.*, 1998), the low percentage of plants infected with biovar 3 in this trial necessitates further investigations to ascertain whether it a host to the local strain. Similarly the low incidence of infection to biovar 3 found in *P. leuteo-album* could be fortuitous and needs to be confirmed.

Datura stramonium has been reported in South Africa as a host of biovar 3 but not of biovar 2 by Swanepoel (1992). During this trial biovar 2 and biovar 3 was isolated from *D. stramonium* plants. Findings regarding hosts status of *Eragrostis curvula* were similar to those obtained by Swanepoel (1992). A relatively low percentage (30%) of plants was infected with biovar 3 and none with biovar 2. A higher infection rate (90%) of *Amaranthus* spp. was obtained in comparison to Swanepoel's (1992) findings (35%). Swanepoel (1992) was also not able to obtain infection of *Chenopodium album* with biovar 3, whereas in this trial 70% of plants were infected with this organism. This differential host pattern observed could be due to different

environmental and biological factors that prevailed. Swanepoel (1992) grew weeds from seeds in seedling trays and transplanted them once they reached a height of 5 cm. In this trial 8 – 10 cm sized weeds were transplanted from a field with clay-loam soil into a sterile vermiculite and soil mix. Some form of root wounding would occur with both techniques during transplanting. The technique used in this trial allows microbes associated with the roots of the weed to be transferred to the pots, since the soil around the roots was not removed. It is possible that transfer from the field to the glasshouse could have increased susceptibility resulting in more species serving as host than might have been encountered in natural circumstances.

In the second phase the rhizophere of some weeds was evaluated for possible suppression of *R. solanacearum*. One experiment involved weeds grown in pots, the second weeds cultured in nutrient solution. Although rhizosphere populations of *R. solanacearum* have been determined successfully by several authors (Granada & Sequeira, 1983a; Shekhawat *et al.*, 1992; Terblanche & de Villiers, 1998), repeated efforts to obtain stable population counts from the rhizosphere soil have failed in this investigation. Growth of saprophytic organisms on the agar plates was high, possibly obscuring the presence of *Ralstonia*. Elphinstone & Aley (1993) using another selective medium containing crystal violet and antibiotics (Granada & Sequeira, 1983b), experienced similar problems with saphrophic growth when isolating the pathogen from soil. Presence of bacterial antagonists can also inhibit the growth of *R. solanacearum* on agar plates (Elphinstone, 1996).

The use of a hydroponic system to evaluate the effect of root exudates on pathogen populations has merits if adapted and refined. From the data obtained it is not clear whether *E. curvula*, *S. africanus* and *C. pycontrix* plantlets cultured in inoculated nutrient solution had a direct effect on *Ralstonia* populations or whether detection of *Ralstonia* was simply hampered by the overgrowth of saprophytes and/or the presence of antagonistic bacteria. The antagonistic effect that was observed when three bacterial isolates isolated from the grass exudates were streaked against *R solanacearum* biovar 2 does not necessarily relate to an antagonistic activity under natural conditions but could be limited to the agar environment (Trigalet *et al.*, 1994). To create an aseptic environment in which the chemical nature of suppression can be

investigated plants can be cultured *in vitro* or aseptically from seed before being transferred to an inoculated hydroponic system. Surface sterilization of roots would reduce microbial populations, but could affect normal root activities.

During the third phase *in vitro* suppression of *R. solanacearum* with weed extracts or leaching was investigated. The results obtained with the disc technique did not demonstrate any inhibitory interaction between the sterile weed extract or leaching and colony growth of *R. solanacearum* on TZC medium. Similarly, inhibition was also not observed with the poisoned medium technique. It is possible that the concentration of the extract or leaching was not sufficient to demonstrate inhibition. Both the disc and poisoned medium techniques do not permit investigation of volatile substances. The apparent suppression of *R. solanacearum* biovar 2 observed in inoculated leaching of *E. curvula*, *S. africanus, T. dubius*, and *H. radicata* can probably partly be ascribed to the inability to detect the pathogen on TZC plates due to growth of other bacteria. This is highlighted by the fact that there was a substantial reduction of *R. solanacearum* colonies observed on TZC in inoculated potato leaching.

Elphinstone (1996) mentions that cereal and grass crops are often recommended in crop rotation programs since they inhibit the development of weeds, allow the use of selective herbicides and because natural bacterial populations in cereal rhizospheres are often antagonistic to bacterial wilt. Soil fumigation for the control of bacterial wilt is often not recommended, since this practice does not affect *R. solanacearum* in lower soil layers but affect antagonistic populations in the upper layers (Elphinstone, 1996). Preliminary results obtained by Arthy & Akiew (1999) indicate that rotation with Rhodes grass (*Chloris gayana*) may be effective reducing the incidence of wilt.

Further studies are required to determine whether high levels of antagonistic bacteria are present in the rhizosphere of the three grasses or the two weeds that occurred abundantly in the weed-fallowed plots. If so, it would partly explain the suppression that had been observed in these plots. Further investigations should be conducted into whether chemical compounds present in the grasses or weeds could be directly involved in the suppression of bacterial wilt.

REFERENCES

ABDULLAH, A. 1993. Bacterial wilt in Malaysia: Hosts, Disease Incidence and Geographical Distribution. In: Hartman, G.L. and Hayward, A.C. (eds) Bacterial Wilt. Proceedings of the 1st international conference on the bacterial wilt, Kaohsiung, Taiwan, 28-31 October 1992. Canberra, Australia, ACIAR Proceedings 45: 334-337

AKIEW, E., TREVORROW, P.R. & P.E. TONELLO. 1993. Management of bacterial wilt of tobacco. In: Hartman, G.L. and Hayward, A.C. (eds) Bacterial Wilt. Proceedings of the 1st international conference on the bacterial wilt, Kaohsiung, Taiwan, 28-31 October 1992. Canberra, Australia, ACIAR Proceedings 45: 270-275

AKIEW, E. & P.R. TREVORROW. 1994. Management of bacterial wilt of tobacco. In: A.C. Hayward and G.L. Hartman (eds) Bacterial wilt: The disease and its causative agent, *Pseudomonas solanacearum*. Cab International United Kingdom, pp 179-198

AMAT, Z., A. ALBORNOZ, M. HEVESI, M. STEFANOVA. 1978. *Pseudomonas solanacearum* detected in a naturally infested soil containing a new wild host. Proceedings of the Fourth International Conference on Plant Pathogenic Bacteria, Angers, pp 869-873

ANSARI, M.M. 1990. *Cosmos sulphureus* – a new host of *Pseudomonas solanacearum* race-1 from Andamans. *Indian Phytopathology 43: 438-439*

ARTHY, J. & S. AKIEW. 1999. Effect of short term rotation on *Ralstonia solanacearum* populations in soil. *Bacterial Wilt Newsletter 16:13*

ELPHINSTONE, J.G. 1996. Survival and possibilities for extinction of *Pseudomonas solanacearum* (Smith) Smith in cool climates. *Potato Research 39: 403-410*

ELPHINSTONE, J.G. & P. ALEY. 1993. Integrated control of bacterial wilt of potato in the warm tropics of Peru. In: Hartman, G.L. and Hayward, A.C. (eds) Bacterial Wilt. Proceedings of the 1st international conference on the bacterial

wilt, Kaohsiung, Taiwan, 28-31 October 1992. Canberra, Australia, ACIAR Proceedings 45: 276-277

ELPHINSTONE, J.G., HENNESSY, J., WILSON, J.K. & D.E. STEAD. 1996. Sensitivity of different methods for the detection of *Pseudomonas solanacearum* (Smith) Smith in potato tuber extracts. EPPO/OEPP Bulletin Vol.26.

GRANADA, G.A. & L. SEQUEIRA. 1983a. Survival of *Pseudomonas solanacearum* in soil, rhizosphere, and plant roots. *Canadian Journal of Microbiology 29:433-440*

GRANADA, G.A. & L. SEQUEIRA. 1983b. A new selective medium for *Pseudomonas solanacearum. Plant Disease 67: 1084-1088*

HARRIS, D.C. 1976. Bacterial wilt in Kenya with particular reference to potatoes. Proceedings of the First International Planning Conference Workshop on the Ecology and Control of Bacterial Wilt caused by *Pseudomonas solanacearum.* North Carolina State University, Raleigh, USA, pp 84-88

HAYWARD, A.C. 1994. Systematics and phylogeny of *Pseudomonas solanacearum* and related bacteria. In: A.C. Hayward and G.L. Hartman (eds) Bacterial wilt: The disease and its causative agent, *Pseudomonas solanacearum.* Cab International United Kingdom, pp 123-136

JACKSON, M.T. & L.C. CONZALEZ. 1981. Persistance of *Pseudomonas solanacearum* (race 1) in a naturally infested soil in Costa Rica. *Phytopathology 71: 690-693*

JONES, A.L. & EHERET, G.R., 1976. Isolation and characterization of benomyl-tolerant strains of Monilia fructicola. Plant Disease 60: 765-769

KELMAN, A. 1953. The bacterial wilt caused by *Pseudomonas solanacearum. Technical Bulletin No. 99 of the North Carolina Agricultural Experimental Station*

KORSTEN, L. 1984. Bacteria associated with bark canker of avocado. MSc-thesis submitted to the Department of Microbiology and Plant Pathology, University of Pretoria, Pretoria

KREMER, F.W. & G. UNTERSTENHÖFER. 1967. Computation of results of crop protection experiments by the method of Townsend and Heuberger. *Pflanzenschutz-Nachrichten Bayer* 20: 625-628

OLSSON, K. 1976. Overwintering of *Pseudomonas solanacearum* in Sweden. Proceedings of the First International Planning Conference Workshop on the Ecology and Control of Bacterial Wilt caused by *Pseudomonas solanacearum*. North Carolina State University, Raleigh, USA, pp 105-107

SHEKHAWAT, G.S. & S.K. CHAKRABARTI. 1993. Integrated management of potato bacterial wilt. In: Hardy, B. & E.R. French (eds) Integrated Management of Bacterial Wilt. Proceedings of an international workshop, New Dehli, India, October 11-16, 1993, pp 87-92

SHEKHAWAT, G.S, CHAKRABARTI, S.K. & A.V. GADEVAR. 1992. Potato bacterial wilt in India. Technical Bulletin No 38. Central Potato Research Institute, India

SHEKHAWAT, G.S. & M.C.M. PEROMBELON. 1991. Factors affecting survival in soil and virulence of *Pseudomonas solanacearum*. *Journal of Plant Disease and Protection 93:258-267*

SWANEPOEL, A.E. & B.W. YOUNG. 1988. Characteristics of South African strains of *Pseudomonas solanacearum*. *Plant Disease* 72: 403-405

SWANEPOEL, A.E. 1992. Survival of South African strains of biovar 2 and biovar 3 of *Pseudomonas solanacearum* in the roots and stems of weeds. *Potato Research 35: 329-332*

TERBLANCE, J. & D.A. DE VILLIERS. 1998. The suppression of *Ralstonia solanacearum* by marigolds. In:. P.H. Prior, Allen, C. & J. Elphinstone (eds) Bacterial wilt disease: Molecular and ecological aspects. Reports of the second

international bacterial wilt symposium, Gosier, Guadeloupe, France, 22-27 June 1997, pp 325-331

THERON, D.J., 1999. Fusarium dry rot of potatoes : etiology, epidemiology, toxicity and control. PhD thesis, University of the Orange Free State, Bloemfontein

TRIGALET, A., FREY, P & D. TRIGALET-DEMERY. 1994. Biological control of bacterial wilt caused by *Pseudomonas solanacearum*: State of the art and understanding. In: A.C. Hayward and G.L. Hartman (eds) Bacterial wilt: The disease and its causative agent, *Pseudomonas solanacearum*. Cab International United Kingdom, pp 225-233

TUSIIME, G., ADIPALA, E., OPIO, F. & A.S. BHAGSARI. 1998. Weeds as latent hosts of *Ralstonia solanacearum* in Highland Uganda: implications to development of an integrated control package for bacterial wilt. In: Prior, P.H., Allen, C. and J. Elphinstone (eds) Bacterial wilt disease: Molecular and ecological aspects. Reports of the second international bacterial wilt symposium, Gosier, Guadeloupe, France, 22-27 June 1997, pp 413-419

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CHAPTER 4

MAIZE ROTATION AS CONTROL STRATEGY OF BACTERIAL WILT ON POTATOES

ABSTRACT

The effect of maize on *Ralstonia solanacearum* populations in soil was evaluated in a pot trial. Populations declined faster in soil in which maize was cultured than in fallow soil. Within six weeks no *R. solanacearum* could be detected in either treatment. A gradual decline was also observed in soil in which potato was grown. No bacterial wilt could be isolated from the stems or roots of maize plants, indicating maize to be a non-host. Maize plants were cultured *in vitro* and used in a soilless system to further investigate whether maize is suppressive to the wilt organism or whether it hosts the pathogen. Three separate trials were conducted and findings varied. In the first two trials no *R. solanacearum* could be detected in the maize plants. *R. solanacearum* populations also deteriorated rapidly to an undetectable level in the inoculated nutrient solution in which the plants were cultured. In the control treatments, populations remained stable. High populations of an antagonistic bacterium were detected in maize plants and in the nutrient solution of the first trial. This bacterium was tentatively identified as *Chromobacterium violaceum* according to the Biolog MicroLog system. During the second trial different saprophytes were noted, some being mildly antagonistic to the pathogen on tetrazolium chloride medium. During the third trial, however, high bacterial wilt populations were observed in some of the maize plants and in the corresponding nutrient solution. In one of the plants in which R. *solanacearum* was not detected, *C. violaceum* was observed. These results indicate that microbial populations present in this case in the seed, could play a role in whether a maize plant becomes susceptible or not.

INTRODUCTION

Smith (1896) had suggested that several years of rotation with crops immune to bacterial wilt could assist in disease control. Maize (*Zea mays* L.) was one of the first crops to be evaluated. Impressive results were obtained. When tobacco was planted after four years of continuos maize culture, only 3.7% plants wilted, whereas in the continuos tobacco crop, wilt was 81.3% (Kelman, 1953). In a separate study it was found that the wilt incidence in tobacco after weed-fallow or bare-fallow was not significantly lower than after continuos tobacco. There was however a marked reduction after maize rotation (Kelman, 1953). Throughout the years maize has been included in rotation programs, either as a single rotation crop or in combination with other non-host crops (Jackson & Conzalez, 1981; Shekhawat *et al.*, 1992; Fortnum & Martin, 1998). The level of disease control achieved with maize rotation varies. Jackson & Conzalez (1981) found no effect with maize cropping, indicating that the efficiency of a specific cropping system is not universal. Granada & Sequeira (1983) were able to isolate *R. solanacearum* from roots of maize plants. Infections remained localized in the roots and bacterial release into the soil was less from these plants than from true host plants. Infection rate of the maize plants was also lower in that not every individual plant became infected. Similar findings were reported by Shekhawat *et al.* (1992) who also noted that *Ralstonia* populations were not high enough to exude from the root tissue into the soil. The low infection rates and the lack of bacterial release could explain why *Ralstonia* populations are often reduced after a rotation with maize. They do however not explain why in some cases rotation is more effective than a bare-fallow treatment.

Elphinstone & Aley (1993) reported *Ralstonia* populations in maize rizosphere soil to decline more rapidly to an undetectable level than in soil taken from herbicide treated fallow or weed fallow. When bacterial content of maize roots was examined, the authors could not detect *R. solanacearum* but high levels of another bacterium, later identified as *Pseudomonas cepacia*, were observed. This bacterium was antagonistic to *R. solanacearum* on both King's B medium and potato dextrose agar. Analysis of weed rhizospheres and soil taken from the herbicide treated fallow revealed significantly lower *P. cepacia* populations than found in the maize rhizosphere,

indicating an interaction between the multiplication of the antagonist and the decline of *R. solanacearum* in maize rhizosphere. Elphinstone & Aley (1993) noted that although *Ralstonia* populations fell below detectable levels with maize culture, sufficient residual populations remained to induce disease in a subsequent crop with susceptible tomatoes. Adhikari & Basnyat (1998) were able to delay the onset of wilt with one to three weeks and reduced the incidence of wilt in susceptible tomato with 20-26% by rotating with maize, okra, cowpea or resistant tomato. Abd-El-Ghafar (1998) found high populations of avirulent *R. solanacearum* in soil after rotation with maize, garlic, faba bean, soya bean and wheat, whereas after potato, tomato and *Capsicum annum* virulent populations were high and avirulent ones low. A three-year rotation with the nonhost crops reduced the incidence of wilt greatly, whereas a yearly or two-year rotation resulted in only a small reduction.

The field study conducted at the Experimental Farm of the University of Pretoria on the longevity of the biovar 2 strain (Chapter 2), revealed that the incidence of wilt was reduced to 40% after a five-year maize rotation, in comparison to the 95% wilt observed in potato monoculture. Maize (40%) appeared as efficient in reducing wilt as bare fallow (42%) but was less efficient than weed-fallow (27%). The aim of this study is to investigate whether maize is a carrier of the local biovar strain and whether maize cultivation could have a suppressive effect on the pathogen.

MATERIALS AND METHODS

The investigation into the role of maize culture on *Ralstonia* populations was subdivided into two phases, the first consisting of a pot trial in which populations in soil from pots planted with maize were compared to those from fallow soil pots and pots planted with potato. The latter involved three consecutive trials in which maize was cultured in nutrient solution.

Phase 1: Effect of maize on *Ralstonia* populations -pot trial

Fifty pots were filled with 2 kg of autoclaved sand:peat mixture (1:1). *Ralstonia solanacearum* biovar 2 inoculum was prepared from virulent 48-hour colonies and the concentration determined at 4 x 10^8 cfu/ml by means of serial dilution on TZC medium.Twenty ml of the inoculum was added to each pot and mixed into the soil. Maize seeds were planted in 15 pots (two per pot) and at seedling stage plants were reduced to one per pot. Certified potato tubers were planted in another 15 pots and the other 20 pots were kept fallow.

After one day five fallow pots were used to determine whether *Ralstonia* populations could be re-isolated from the soil. A 200 g soil sample was suspended in 200 ml sterile water and placed on a shaker for 30 minutes. A serial dilution series was prepared and plated on modified TZC (Elphinstone *et al.*, 1996) without the addition of bacitracin. Colony counts were performed after 72 hours.

The remaining pots were placed in a randomized block design of five blocks each with three repetitions. Maximum and minimum temperatures in the glasshouse were maintained at an average of 29° C day/16° C night. Wilting was monitored in the potato plants. Soil from three pots of each treatment was analyzed fortnightly for a period of ten weeks as described above. Soil was also shaken from the maize roots, weighed, suspended in sterile water at a ratio of 1g soil:2 ml water and analyzed as above. The bottom two-node piece of each of the three maize stems was cut off and roots removed. Root and stem sections were weighed, surface sterilized in 1 % sodium hypochlorite for 10 minutes, rinsed thoroughly and blended in sterile water at a ration of 1:2. After 30 minutes, a dilution series was prepared and plated on TZC (Kelman, 1953). Isolations from potato stems were done as described in Chapter 2 to confirm infection.

Phase 2: Effect of maize on *Ralstonia* populations –soilless system

Trial 1

Fifty test tubes with 10 ml Joerdens-Roettger medium (Joerdens-Roettger, 1987) were prepared and autoclaved. Maize seed was surface sterilized in 1% sodium

hypochlorite solution for 10 minutes, rinsed in sterile water and aseptically transferred to test-tubes. Test tubes were placed in the *in vitro* growth chamber and germination was monitored daily. Ten days after placing the seeds on medium, the maize plantlets were ready for transfer. Culture tubes were sterilized and 9 ml sterile nutrient solution was added. In 30 tubes 1 ml *R. solanacearum* biovar 2 inoculum was added to give a final concentration of 1.7×10^5 cfu/ml. Fifteen of these tubes served as controls (Treatment 1) to determine the growth of the pathogen. Maize plantlets were transferred aseptically to the other 15 tubes, ensuring that the roots were submerged in the nutrient solution (Treatment 2). In another 15 tubes, 1 ml sterile water was added to the nutrient solution instead of the inoculum and maize plants transferred (Treatment 3). Culture tubes were kept in a growth chamber and placed on a shaker at slow rotation to allow aeration of the nutrient solution. Evaluations were done weekly using three tubes per treatment. The volume of the solution in each tube was made up to 10 ml with sterile nutrient solution before a serial dilution was prepared. *Ralstonia* populations were determined from solutions of treatment 1 and 2 on TZC, whereas general bacterial populations were observed on TZC and nutrient agar from solution in treatment 3. Sterile paper discs were impregnated with filter-sterilized solutions from treatment 3 and placed on TZC seeded with *R. solanacearum*. Isolations were made from all maize plants after surface sterilization in 70% ethanol for one minute and plated on TZC and nutrient agar.

Trial 2

Due to contamination in some of the tubes and lack of space for plant growth, the first trial was terminated after three weeks. In the second trial, treatments were kept the same: treatment 1 consisting of pathogen in solution only, treatment 2 containing pathogen and maize plant, treatment 3 containing only maize plants in solution. Erlenmeyer flasks (500ml) containing filter paper and 10 ml nutrient solution were autoclaved. Maize seeds, surface-sterilized in 1% NaOCl and rinsed, were placed on the filter paper, one seed per flask. Fourty flasks were prepared. This method eliminates the risk of contamination during transferal of plants. In fifteen flasks no seeds were placed (for treatment 1). After fifteen days, maize plants were sturdy and inoculation began. Sterile nutrient solution was inoculated with *R. solanacearum* biovar 2 and 100 ml was added to each flask of treatment 1. The final *Ralstonia*

population in the flask was determined at 8.7 $\times 10^4$ cfu/ml by means of plating a dilution series on TZC medium. Fifteen flasks with maize plantlets were selected and a 100ml of inoculum was added for treatment 2. A 100ml sterile nutrient solution without *Ralstonia* was added to another fifteen flasks for treatment 3. Flasks were placed in a growth chamber and swirled gently daily for aeration. Evaluations were performed as described for trial 1, except that volumes were made up to 100ml with sterile nutrient solution before preparing a serial dilution.

Trial 3

This trial was performed in the same manner as trial two with the following exceptions: The inoculum concentration in the flasks at commencement was 1.3 x 10^5 cfu/ml. No *in vitro* inhibition by means of the paper disc technique was evaluated.

Co-culture of antagonist with *R. solanacearum*

The antagonist isolated from the maize plantlets in the soilless system and *R. solanacearum* biovar 2 were cultured on TZC plates for 48 hours. Inoculum was prepared from both cultures and the concentration determined at 1.2 x 10^8 cfu/ml and 9.5 x 10^6 cfu/ml respectively, by plating the serial dilution. Six test tubes with 9 ml sterile water were inoculated with 1 ml *Ralstonia* inoculum; another six with 1 ml antagonist inoculum and six were inoculated with both 1 ml antagonist and *Ralstonia* inoculum. The test-tubes were placed on a shaker for 30 minutes after which isolations were performed from three test tubes of each treatment. The remaining tubes were placed back on the shaker for gentle aeration. After ten days isolations were performed on TZC medium.

RESULTS

Phase 1: Effect of maize on *Ralstonia* populations -pot trial

An average *R. solanacearum* population of 2 x 10^5 cfu/g could be re-isolated one day after inoculation from four pots. In the fifth replicate high numbers (10^6) of bacterial saprophytes were present. Average bacterial counts obtained fortnightly from soil of the three treatments are presented in Figure 4.1. After six weeks, attempts to isolate the pathogen from fallow and maize culture soil were unsuccessful, partly due to high saprophytic growth. Similarly, *Ralstonia* could not be detected after eight weeks in soil from potato pots. In maize rhizosphere soil *Ralstonia* populations deteriorated to an undetectable limit within four weeks. No positive isolations were obtained from maize stems or roots, as had been observed by Shekhawat *et al.* (1992) and Granada & Sequeira (1983). Wilting of some potato plants was observed as early as two weeks after inoculation and bacterial wilt was positively isolated from two of the three plants tested after two weeks. After four weeks, *Ralstonia* could be isolated from all potato stems.

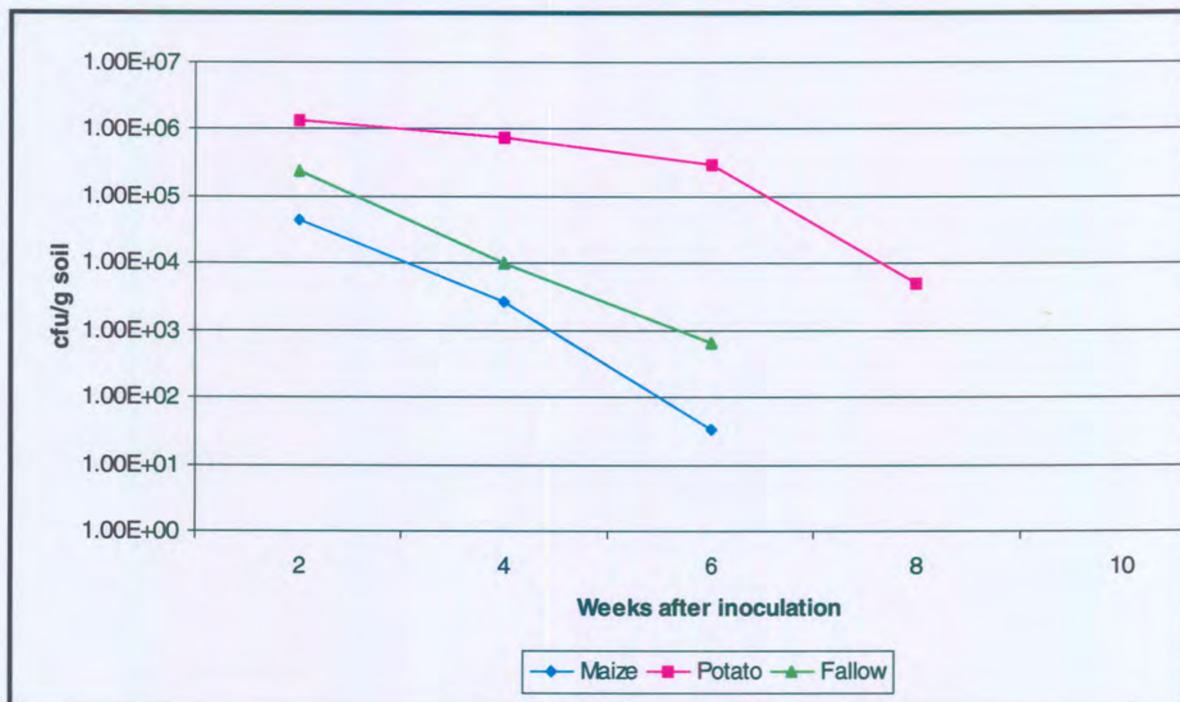

Figure 4.1 Logarithmic presentation of *R. solanacearum* (biovar 2) populations in soil (cfu/g) from pots in which maize or potato was grown or which was kept fallow

Phase 2: Effect of maize on *Ralstonia* populations –soilless system

Trial 1

The germination and development of maize plantlets *in vitro* on Joerdens-Roettger medium (1987) is depicted in Figure 4.2. Results obtained from platings of the solutions from the three treatments are given in Table 4.1.

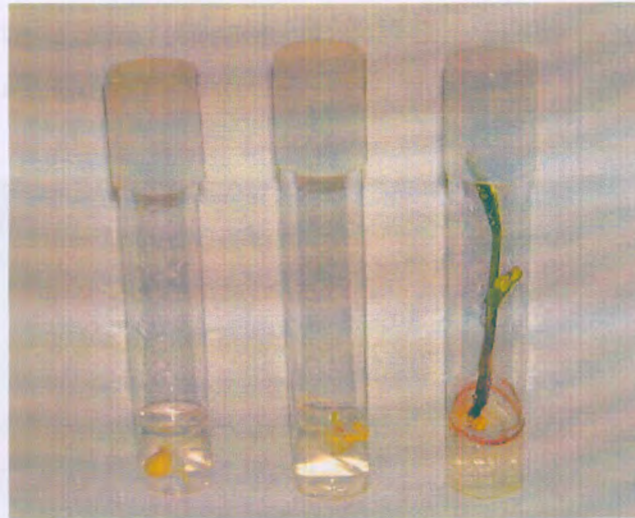

Figure 4.2 Germination and development of maize on Joerdens-Roettger (1987) medium

Table 4.1 Bacterial growth in the solutions containing only inoculum, inoculum and maize plants, and only maize plants of trial 1

	T 1: Pathogen only *R. solanacearum* counts (cfu/ml)	**T 2: Path. & Maize** *R. solanacearum* counts (cfu/ml)	**T 3: Maize only** Saprophytic growth
Day 0	1.74×10^5 (concentration of inoculum)		
Day 7	8.82×10^5	Below detection – saprophyte "type 1" *	"type 1" *
Day 14	5.69×10^5	Below detection – little other saprophytes, "type 1"* dominant	"type 1"*, little other
Day 21	7.68×10^5	Below detection – different saprophytes, "type 1"* dominant	Various saprophytes, "type 1"* in 2 flasks

* "type 1" – bacterium later identified tentatively as *Chromobacterium violaceum*

A prominent bacterium was consistently observed on tetrazolium plates prepared from solution containing maize plants. Discs impregnated with Millipore-sterilized solution

from flasks containing only the maize plants, did not create inhibition zones on seed TZC plates. No *R. solanacearum* could be isolated from any of the nine maize plantlets. From six of the nine plants, the same prominent bacterium was isolated. When this bacterium was cross-streaked against *R. solanacearum* biovar 2 or biovar 3 on TZC medium, strong antagonism was observed (Figure 4.3). The bacterium was later tentatively identified as *Chromobacterium violaceum* according to the Biolog MicroLog 1 4.01A system (Department of Microbiology and Plant Pathology, University of Pretoria and by K. Serfontein, ARC-Roodeplaat, Plant Protection).

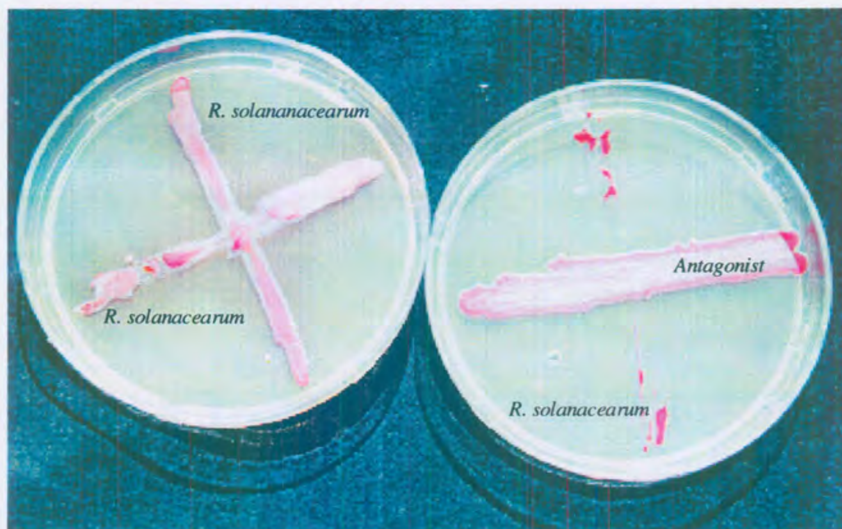

Figure 4.3 ***R. solanacearum*** **biovar 2 cross-streaked against antagonist tentatively identified as** ***Chromobacterium violaceum*** **isolated from maize plant on TZC (Kelman, 1953) medium**

Trial 2

Similar findings were made as in trial 1. *R. solanacearum* remained relatively constant in control flasks. No *Ralstonia* colonies could be isolated from the inoculated solution containing the maize plantlets. The antagonist isolated previously could however not be detected except in two instances. Instead, different unidentified saprophytes were observed on plates. No inhibition zones were observed on seeded plates. No *R. solanacearum* could be isolated from maize plants. Several saprophytes were observed on both TZC plates and nutrient agar. Two of five saprophytes cross-streaked against the bacterial wilt organism were mildly antagonistic. No attempt was

made to identify these. After 14 days contamination was observed in the control flasks containing the pathogen only and the trial was terminated.

Trial 3

The results obtained from this trial varied considerably from the previous two findings. *Ralstonia* populations in the nutrient solution appeared to be boosted in some of the flasks containing maize plantlets. *R. solanacearum* was also isolated from several maize plants. Results regarding *Ralstonia* populations in nutrient solution of treatment 1 and 2 as well as isolations made from maize plants of treatment 2 are presented in Table 4.2. In one of the maize plants and the corresponding nutrient solution from which no bacterial wilt could be isolated, the same antagonist as found in trial 1, was detected in relatively large numbers. The trial was discontinued after 21 days.

Table 4.2 *Ralstonia solanacearum* populations in the solution from treatment 1 (flasks with nutrient solution inoculated with *R. solanacearum*) and treatment 2 (flasks with inoculated nutrient solution containing maize plantlets) and presence of *Ralstonia* in the maize plants of treatment 2

	T 1: Pathogen only *R. solanacearum* (cfu/ml)	**T 2: Path. & Maize** *R. solanacearum* (cfu/ml)	**T 2: Maize plants** Presence of *R. solanacearum* in maize plantlet
Day 0	1.3×10^5 (initial inoculum concentration)		
Day 7			
Flask 1	1.17×10^5	1.07×10^7	Positive
Flask 2	1.14×10^5	4.66×10^7	Positive
Flask 3	1.26×10^5	6.93×10^6	Positive
Day 14			
Flask 1	8.08×10^4	4.53×10^4	Positive
Flask 2	1.22×10^5	0	Negative
Flask 3	8.58×10^4	3.1×10^7	Positive
Day 21			
Flask 1	9.08×10^4	0	Negative
Flask 2	5.75×10^4	3.5×10^2	Negative
Flask 3	3.66×10^5	2.82×10^8	-

Co-culture of antagonist with *R. solanacearum*

Results of the bacterial counts obtained after 30 minutes and 10 days from tubes containing only the pathogen and only the antagonist as well as their co-culture is presented in Table 4.3. When co-cultured for ten days, *R. solanacearum* was only detected in two of the three samples on only one plate of the dilution series. This makes it difficult to assess the true population.

Table 4.3 Average bacterial counts (cfu/ml) of *R. solanacearum* and the antagonist from controls and co-culture obtained after 30 minutes and 10 days

Treatment/ Time	*Ralstonia* only (cfu/ml)	Antagonist* only (cfu/ml)	Co-culture: *Ralstonia* (cfu/ml)	antagonist *(cfu/ml)
30 minutes	8.79×10^5	1.08×10^6	8.33×10^4	1.09×10^6
10 days	3.49×10^5	2.15×10^6	2.0×10^2	2.24×10^6

* Antagonist later tentatively identified as *Chromobacterium violaceum*

DISCUSSION

The role of maize culture on *Ralstonia* populations was investigated in two phases. Firstly a pot trial was conducted in which populations in soil from maize pots were compared to those from fallow pots and potato pots. During the second phase three consecutive trials were conducted in which maize was cultured *in vitro* in nutrient solution. Results obtained from the pot trial and those from the three *in vitro* trials were not consistent.

Ralstonia solanacearum populations in the soil from the maize pots declined more rapidly than in fallow soil and in soil of potato pots. This data coincides with the findings obtained from the field study (Chapter 2) and supports those of Elphinstone & Aley (1993). The general survival of *R. solanacearum* was however poor in the pot trial. After six weeks no positive isolations could be obtained from the fallow and maize soil and after eight weeks none from the potato soil. This is surprising since potatoes were still actively growing in those pots. Strong saprophytic growth could have hampered the detection of the pathogen. Akiew (1986) and Granada & Sequeira

(1983) report that the bacterium could survive at least for 33 weeks in the presence of maize plants. The bacterial wilt pathogen could not be isolated from the maize stems and roots in the pot trial, suggesting that maize is not a host to the local biovar 2 strain. This confirms the results obtained previously from isolations performed from maize plants in the field (Chapter 2).

The three *in vitro* trials provided varying results regarding the ability of maize to serve as a carrier or host of *R. solanacearum.* In the first two trials no bacterial wilt could be isolated from the maize plants. *R. solanacearum* populations in the nutrient solution containing maize plants deteriorated rapidly to an undetectable level, whereas populations in control flasks remained stable. In the first trial an antagonist, later tentatively identified as *Chromobacterium violaceum*, was consistently found in the solution as well as in the maize plants. Further preliminary studies indicate that this bacterium is relatively successful in suppressing *Ralstonia* populations. Although this bacterium was not detected in the second trial, several other saprophytic bacteria were observed on the medium and some of these appeared mildly antagonistic. Once again, no *R. solanacearum* was isolated from maize plants.

During the third *in vitro* trial, high bacterial wilt populations were observed in some of the maize plants and in the corresponding nutrient solution. In one of the plants in which R. *solanacearum* was not detected, *C. violaceum* was observed. These results indicate that microbial populations present in this case in the seed, could play a role in whether a maize plant becomes susceptible or not. Elphinstone & Aley (1993) found high populations of *Pseudomonas cepacia* in maize roots that appear antagonistic to *Ralstonia*. They reason that the presence of *P. cepacia* or similar bacteria may assist in explaining why Autrique & Potts (1987) reported a reduction of wilt when potatoes were intercropped with maize. The hypothesis that certain antagonistic bacteria present in soil could be transmitted to seed and that some of these could have a protective effect on developing maize plants should be further investigated.

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REFERENCES

AKIEW, E. 1986. Influence of soil moisture and temperature on the persistence of *Pseudomonas solanacearum*. In: Persley, G.J. (ed) Bacterial wilt disease in Asia and the South Pacific. Canberra, Australian Centre for International Research, Australia, ACIAR Proceedings 13, pp 77-79

ABD-EL-GHAFAR, N.Y. 1998. Control of potato bacterial wilt using crop rotation. *Annals of Agricultural Science Cairo 43:2,575-587*

ADHIKARI, T.B. & R.C. BASNYAT. 1998. Effect of crop rotation and cultivar resistance on bacterial wilt of tomato in Nepal. *Canadian Journal of Plant Pathology 20:283-287*

AUTRIQUE, A. & M.J. POTTS. 1987. The influence of mixed cropping on the control of potato bacterial wilt (*Pseudomonas solanacearum*). *Annals of Applied Biology 111:125-133*

ELPHINSTONE, J.G. & P. ALEY. 1993. Integrated control of bacterial wilt of potato in the warm tropics of Peru. In: Hartman, G.L. and Hayward, A.C. (eds) Bacterial Wilt. Proceedings of the 1st international conference on the bacterial wilt, Kaohsiung, Taiwan, 28-31 October 1992. Canberra, Australia, ACIAR Proceedings 45: 276-283

ELPHINSTONE, J.G., HENNESSY, J., WILSON, J.K. & D.E. STEAD. 1996. Sensitivity of different methods for the detection of *Pseudomonas solanacearum* (Smith) Smith in potato tuber extracts. EPPO/OEPP Bulletin Vol.26.

FORTNUM, B.A. & S.B. MARTIN. 1998. Disease management strategies for control of bacterial wilt of tobacco in the Southeastern USA. In: Prior, P.H., Allen, C. and J. Elphinstone (eds) Bacterial wilt disease: Molecular and ecological aspects. Reports of the second international bacterial wilt symposium, Gosier, Guadeloupe, France, 22-27 June 1997, pp 394-402

GRANADA, G.A. & L. SEQUEIRA. 1983. Survival of *Pseudomonas solanacearum* in soil, rhizosphere, and plant roots. *Canadian Journal of Microbiology 29:433-440*

JACKSON, M.T. & L.C. CONZALEZ. 1981. Persistence of *Pseudomonas solanacearum* (race 1) in a naturally infested soil in Costa Rica. *Phytopathology 71: 690-693*

JOERDENS-ROETTGER, D. 1987. Seed potato production in the Philippines: *in vitro* culture and rapid multiplication techniques, pp 48-52

KELMAN, A. 1953. The bacterial wilt caused by *Pseudomonas solanacearum. Technical Bulletin No. 99 of the North Carolina Agricultural Experimental Station*

KUMAR, M., CHATURVEDI, V.K. & D.P. SINGH. 1999. *Chromobacterium violaceum* – an unusual pathogen of swine. *Indian Veterinary Journal 76:869-871*

SHEKHAWAT, G.S., CHAKRABARTI, S.K. & A.V. GADEVAR. 1992. Potato bacterial wilt in India. Technical Bulletin No 38. Central Potato Research Institute, India

SMITH, E.F. 1896. A bacterial disease of the tomato, eggplant and Irish potato (*Bacillus solanacearum* nov. sp.). United States Department of Agriculture, Division of Vegetable Physiology and Pathology, Bulletin 12: 1-28

CHAPTER 5
GENERAL DISCUSSION

Potato is one of South Africa's most important food crops with approximately 1.6 million tons being produced annually. More than 5000 ha are under production in 14 regions situated throughout the country (Potatoes South Africa, 1999). Potatoes are not only produced for fresh consumption locally, but also for export and for the processing industry. To satisfy the increased demand it is essential that production factors be manipulated to optimize the crop environment and minimize the effects of pests and diseases. Bacterial wilt or brown rot of potato caused by *Ralstonia solanacearum* has affected the potato industry in South Africa since 1914 (Doidge, 1914). The disease occurred sporadically in plantings but in the 1980's serious outbreaks of bacterial wilt were reported. Although both biovar 2 and biovar 3 were isolated from diseased potato plants the former was prevalent in the potato production regions of South Africa. Control of bacterial wilt became essential for the survival of the potato industry in South Africa. According to the Agricultural Pest Act, 1983, *R. solanacearum* is a prohibited organism in South Africa and a zero tolerance for bacterial wilt in certified seed tubers is applied. Testing of seed tubers of generations 1-4 was implemented in 1991 and in 1995 compulsory testing of all registered seed tuber plantings (G0-G8) was introduced. Once the disease is confirmed in a registered seed planting, the registration is withdrawn (Swanepoel & Theron, 1999). Although the implementation of the certification scheme has resulted in a reduction in the number of confirmed cases in seed potatoes, eradication of the disease has not been achieved. The warm climatic conditions in South Africa favour disease development and the absence of cold winters enhances its survival in fields. Control of bacterial wilt is difficult and depends greatly on integrated management strategies.

Research into the survival of *Ralstonia* in soil has gained considerable attention and has highlighted the fact that a range of environmental factors influences the survival period, making universal predictions almost impossible. Probably the most crucial

factor is the biovar or race of the pathogen that is involved, since this affects both host range and its survival in soil as a free-living organism. Biovar 3 is known to survive for up to eight years, probably due to its wide host range and higher aggressiveness. Biovar 2 on the other hand is generally believed to be less adapted to soil survival with eradication being possible within 2-3 years. Success in reducing the incidence of wilt with rotation programs has varied, depending on location, type of rotation and duration of the program (Shekhawat *et al.*, 1992; Akiew *et al.*, 1993; Akiew & Trevorrow, 1994; French, 1994). Little information is available on the longevity of *R. solanacearum* in soil under South African conditions and how it is influenced by cultural practices.

Soil survival of bacterial wilt was investigated in a clay-loam field artificially infested with *R. solanacearum* biovar 2, race 3 to which four cultural practices were applied. These were maize and potato monoculture, bare-fallow and weed-fallow. *Ralstonia* populations in the soil were monitored after a three-year and five-year cropping sequence by planting potato in a designated region across all treatment plots as indicators of wilt. Percentage wilt observed in the potato monoculture plots was 88% and 96% respectively. After both three- and five-year rotation, onset of wilt in bare fallow plots was relatively slow and the argument that survival of *Ralstonia* in the absence of a host is poor, seemed to hold true. In the subsequent weeks however, wilt increased rapidly surpassing wilt patterns in both maize and weed-fallow plots. The difference in incidence of wilt between potato monoculture and bare fallow plots was not even statistically meaningful after the three-year rotation period. Similar results have been reported by Akiew & Trevorrow (1994) where the incidence of tobacco wilt after two-year bare fallow was not significantly lower than after continuos tobacco. After five years, however, percentage wilt in the bare fallow plots was statistically less than observed in the potato plots. Desiccation of soil is considered as a major factor in reducing soil population of the wilt organism, and is enhanced with bare fallow treatment. The disease pattern observed in both seasons in the bare fallow plots indicate that either insufficient desiccation occurred or that *Ralstonia* populations deeper in the soil profile remained less affected. It is also possible that the lack of plant material resulted in a decrease in general soil organisms, thereby reducing competition and suppression.

Maize has been used in rotation programs since the early nineteen hundreds (Kelman, 1953), yet a relatively high percentage wilt (40%) was recorded in maize plots after five years of monoculture. Granada & Sequeira (1983a) and Shekhawat *et al.* (1992) were able to isolate *R. solanacearum* from the root tissue of maize. Infections remained localized in the roots and bacterial release into the soil was less from these plants than from true host plants. Infection rate of the maize plants was also lower in that not every individual plant became infected. These findings indicate why an overall reduction of wilt can still be experienced when applying rotation with maize.

Roughing of weeds is considered vital in integrated management of bacterial wilt since these can serve as hosts or shelter sites for long-term survival of the pathogen (French, 1994; Jackson & Conzales, 1979). Yet potato plants in the weed-fallow plots exhibited the least wilt symptoms, even though host species such as *Datura stramonium* and *Portulaca* were present. This may be an indication that certain weeds or grasses resulted in the suppression of the wilt organism either by harboring antagonistic microorganisms or by releasing inhibitory substances. Weeds tended to be interspersed with the major cover being provided by grasses. Thirty species were identified in the weed-fallow plots over several seasons. In Australia, forage sorghum, signal grass and Rhodes grass, are often used in rotation programs (Akiew *et al.*, 1993; Arthy & Akiew 1999).

The field study clearly demonstrated that *R. solanacearum* biovar 2 can survive much longer in soil than generally believed, irrespective of the cultural practice applied. This information may assist the South African potato industry in formulating regulations for the certification scheme. In 1999 the regulations stated that no seed tubers may be cultivated on infected fields for a period of eight years when biovar 2 is isolated and never when biovar 3 is isolated (Swanepoel & Theron, 1999). The study on longevity of the bacterial wilt pathogen was however conducted in a clay-loam soil, a type not commonly found in most potato production regions. Since soil type in known to influence the survival period of *R. solanacearum* (Moffet *et al.*, 1983; Shekhawat & Perombolon, 1991), the information obtained from this study cannot be extrapolated to situations where lighter sandy soils were infected. The University of

Pretoria has recently commenced with a similar soil survival study in which a sandy soil was infected with biovar 2.

In light of the findings from the field study, an investigation was undertaken to determine the host status of twenty-two weed and three grass species for *R. solanacearum* biovar 2 and biovar 3. *Datura ferox*, *D. stramonium*, *Portulaca oleracea* and *Hibiscus trionum* were infected with the biovar 2 strain although wilting was observed only in some *P. oleracea* plants. Infection of *H.* trionum with biovar 2 was very rare (10%) and results must be confirmed. Portulaca *oleracea* has been reported as a host of race 1 and race 3 in Kenya (Harris, 1976), but has not previously been evaluated in South Africa. Its host status to both races in South Africa has now been established with plants expressing wilt to both races. Host range for biovar 3 was wider and included *Amaranthus* spp., *Bidens bipinnata*, *Chamaesyce prostrata*, *Chenopodium album*, *Chenopodium carinatum*, *Cyperus rotundus*, *Datura ferox*, *Datura stramonium, Eragrostis curvula*, *Hibiscus trionum*, *Portulaca oleracea*, *Sonchus oleraceus*, *Tragopogon dubius*.

H. trionum has not yet been reported as a host of bacterial wilt. Other species of the *Hibiscus* genus such as H. *cannabinus* Linn. (Abdullah, 1993) and H. *sabdariffa* L. (Kelman, 1953) have been reported as hosts of *Ralstonia solanacearum* (biovar or race unspecified). *Chamaesyce prostrata* (*Euphorbia chamaesyce*) is reported for the first time as a host in South Africa. *Euphorbia geniculata* L., *Euphorbia hirta* L. and *Euphorbia maculata* L. have been reported as hosts in India (Shekhawat *et al.*, 1992), and *Euphorbia prunifolia* in Malaysia (Abdullah, 1993). Other weeds that are reported as hosts of biovar 3 for the first time in South Africa include *Sonchus oleraceus*, *Tragopogon dubius*, *Cyperus rotundus*, *Bidens bipinnata* and *Chenopodium carinatum.*

Although *S. oleraceus* has not been recorded as a host elsewhere, *S. arvensis* has been reported in India. *C. rotundus* and *B. bipinnata* are also hosts in India (Shekhawat *et al.*, 1992). *T. dubius* and *C. carinatum* have not been reported elsewhere as hosts of *R. solanacearum.* Although *Tagetes minuta* is a host in Australia (Akiew *et al.*, 1993) and in Uganda (Tusiime *et al.*, 1998), the low percentage of plants infected with

biovar 3 in this trial necessitates further investigations to declare it a host to the local strain. Similarly the low incidence of infection to biovar 3 found in *P. leuteo-album* could be fortuitous and needs to be confirmed.

Preliminary studies were conducted to determine suppressiveness of some weeds/grasses to the bacterial wilt pathogen. In one facet of the investigation the rhizophere of some weeds was evaluated for possible suppression of *R. solanacearum*. One experiment involved weeds grown in pots, the second weeds cultured in nutrient solution. The other facet involved two experiments on *in vitro* suppression of *R. solanacearum* with weed extracts or leaching, the latter involving three techniques namely the impregnated paper disc on seeded medium, poisoned medium and culture of the pathogen in weed leaching. Although rhizosphere populations of *R. solanacearum* have been determined successfully by several authors (Granada & Sequeira, 1983a; Terblanche & de Villiers, 1998), repeated efforts to obtain stable population counts from the rhizosphere soil have failed in this investigation. Growth of saprophytic organisms on the agar plates was high, possibly obscuring the presence of *Ralstonia*. Elphinstone & Aley (1993) using another selective medium containing crystal violet and antibiotics (Granada & Sequeira, 1983b), experienced similar problems with saphrophic growth when isolating the pathogen from soil. Presence of bacterial antagonists can also inhibit the growth of *R. solanacearum* on agar plates (Elphinstone, 1996).

The use of a hydroponic system to evaluate the effect of root exudates on pathogen population has merits if adapted and refined. From the data obtained it is not clear whether *Eragrostis curvula*, *Sporobolus africanus* and *Chloris pycnotrix* plantlets cultured in inoculated nutrient solution had a direct effect on *Ralstonia* population or whether detection of *Ralstonia* was simply hampered by the overgrowth of saprophytes and/or the presence of antagonistic bacteria. The antagonistic effect that was observed when three bacterial isolates isolated from the grass exudates were streaked against *R. solanacearum* biovar 2 does not necessarily relate to an antagonistic activity under natural conditions but could be limited to the agar environment (Trigalet *et al.*, 1994). To create an aseptic environment in which the chemical nature of suppression can be investigated plants can be cultured *in vitro* or

aseptically from seed before being transferred to an inoculated hydroponic system. Surface sterilization of roots would reduce microbial populations, but could affect normal root activities.

The results obtained with the disc technique did not demonstrate any inhibitory interaction between the sterile weed extract or leaching and colony growth of *R. solanacearum* on TZC medium. Similarly, inhibition was also not observed with the poisoned medium technique. It is possible that the concentration of the extract or leaching was not sufficient to demonstrate inhibition. Both the disc and poisoned medium techniques do not permit investigation of volatile substances. The apparent suppression of *R. solanacearum* biovar 2 observed in inoculated leaching of *E. curvula*, *S. africanus*, *T. dubius*, and *H. radicata* can probably partly be ascribed to the inability to detect the pathogen on TZC plates due to growth of other bacteria. This is highlighted by the fact that there was a substantial reduction of *R. solanacearum* colonies observed on TZC in inoculated potato leaching.

Elphinstone (1996) mentions that cereal and grass crops are often recommended in crop rotation programs since they inhibit the development of weeds, allow the use of selective herbicides and because natural bacterial populations in cereal rhizospheres are often antagonistic to bacterial wilt. Soil fumigation for the control of bacterial wilt is often not recommended, since this practice does not affect *R. solanacearum* in lower soil layers but affect antagonistic populations in the upper layers (Elphinstone, 1996). Preliminary results obtained by Arthy & Akiew (1999) indicate that rotation with Rhodes grass (*Chloris gayana*) may be effective reducing the incidence of wilt.

Further studies are required to determine whether high levels of antagonistic bacteria are present in the rhizosphere of the three grasses or the two weeds that occurred abundantly in the weed-fallowed plots. If so, it would partly explain the suppression that had been observed in these plots. Further investigations should be conducted into whether chemical compounds present in the grasses or weeds could be directly involved in the suppression of bacterial wilt.

The effect of maize on *R. solanacearum* populations was evaluated in a pot trial as well as in hydroponic culture. In the pot trial populations in soil from maize pots were compared to those from fallow pots and potato pots. Pathogen populations declined faster in soil from maize pots than in fallow soil. A gradual decline was also observed in soil in which potato was grown. This data coincides with the findings obtained from the field study and supports those of Elphinstone & Aley (1993). The general survival of *R. solanacearum* was however poor in the pot trial. After six weeks no positive isolations could be obtained from the fallow and maize soil and after eight weeks none from the potato soil. This is surprising since potatoes were still actively growing in those pots. Strong saprophytic growth could have hampered the detection of the pathogen. No bacterial wilt could be isolated from the stems or roots of maize plants, suggesting that maize is not a host to the local biovar 2 strain.

During the second phase three consecutive trials were conducted in which maize was cultured *in vitro* in nutrient solution. Varying results were obtained regarding the ability of maize to serve as a carrier or host of *R. solanacearum*. In the first two trials no bacterial wilt could be isolated from the maize plants. *R. solanacearum* populations in the nutrient solution containing maize plants deteriorated rapidly to an undetectable level, whereas populations in control flasks remained stable. In the first trial an antagonist, later tentatively identified as *Chromobacterium violaceum*, was consistently found in the solution as well as in the maize plants. Further preliminary studies indicate that this bacterium is relatively successful in suppressing *Ralstonia* populations. Although this bacterium was not detected in the second trial several other saprophytic bacteria were observed on the medium and some of these appeared mildly antagonistic. Once again no *R. solanacearum* was isolated from maize plants. During the third *in vitro* trial, high bacterial wilt populations were observed in some of the maize plants and in the corresponding nutrient solution. In plants not infected either *C. violaceum* or other bacterial populations were observed. These results indicate that microbial populations present in the maize plants could play a role in the susceptibility of maize. Antagonistic bacteria associated with some maize plants or maize rhizophere could be partly responsible for the suppression of wilt that has been reported. Elphinstone & Aley (1993) found high populations of *Pseudomonas cepacia* in maize roots that appear antagonistic to *Ralstonia*. They reason that the presence of

P. cepacia or similar bacteria may assist in explaining why Autrique & Potts (1987) reported a reduction of wilt when potatoes were intercropped with maize. The hypothesis that certain bacteria (antagonistic and otherwise) present in soil could be transmitted to seed and that some of these could have a protective effect on the developing maize plant should be investigated further.

Continuation of both basic and applied research is essential in order to gain an understanding into the behavioral patterns of this organism and how it can best be controlled. Hopefully this dissertation can contribute to the knowledge of this disease.

REFERENCES

ABDULLAH, A. 1993. Bacterial wilt in Malaysia: Hosts, Disease Incidence and Geographical Distribution. In: Hartman, G.L. and Hayward, A.C. (eds) Bacterial Wilt. Proceedings of the 1st international conference on the bacterial wilt, Kaohsiung, Taiwan, 28-31 October 1992. Canberra, Australia, ACIAR Proceedings 45: 334-337

AKIEW, E. & P.R. TREVORROW. 1994. Management of bacterial wilt of tobacco. In: A.C. Hayward and G.L. Hartman (eds) Bacterial wilt: The disease and its causative agent, *Pseudomonas solanacearum.* Cab International United Kingdom, pp 179-198

AKIEW, E., TREVORROW, P.R. & P.E. TONELLO. 1993. Management of bacterial wilt of tobacco. In: Hartman, G.L. and Hayward, A.C. (eds) Bacterial Wilt. Proceedings of the 1st international conference on the bacterial wilt, Kaohsiung, Taiwan, 28-31 October 1992. Canberra, Australia, ACIAR Proceedings 45: 270-275

ARTHY J. & S. AKIEW. 1999. Effect of short term rotation on *Ralstonia solanacearum* populations in soil. *Bacterial Wilt News Letter 16: 13-14*

AUTRIQUE, A. & M.J. POTTS. 1987. The influence of mixed cropping on the control of potato bacterial wilt (*Pseudomonas solanacearum*). *Annals of Applied Biology 111:125-133*

DOIDGE, E.M., 1914. Some diseases of the potato. *Union of South Africa. Agricultural Journal 7: 698-703*

ELPHINSTONE, J.G. 1996. Survival and possibilities for extinction of *Pseudomonas solanacearum* (Smith) Smith in cool climates. *Potato Research 39: 403-410*

ELPHINSTONE, J.G. & P. ALEY. 1993. Integrated control of bacterial wilt of potato in the warm tropics of Peru. In: Hartman, G.L. and Hayward, A.C. (eds) Bacterial Wilt. Proceedings of the 1st international conference on the bacterial wilt, Kaohsiung, Taiwan, 28-31 October 1992. Canberra, Australia, ACIAR Proceedings 45: 276-277

FRENCH, E.R. 1994. Strategies for integrated control of bacterial wilt of potatoes. In: A.C. Hayward and G.L. Hartman (eds) Bacterial wilt: The disease and its causative agent, *Pseudomonas solanacearum.* Cab International United Kingdom, pp 199-207

GRANADA, G.A. & L. SEQUEIRA. 1983a. Survival of *Pseudomonas solanacearum* in soil, rhizosphere, and plant roots. *Canadian Journal of Microbiology 29:433-440*

GRANADA, G.A. & L. SEQUEIRA. 1983b. A new selective medium for *Pseudomonas solanacearum. Plant Disease 67: 1084-1088*

HARRIS, D.C. 1976. Bacterial wilt in Kenya with particular reference to potatoes. Proceedings of the First International Planning Conference Workshop on the Ecology and Control of Bacterial Wilt caused by *Pseudomonas solanacearum.* North Carolina State University, Raleigh, USA, pp 84-88

JACKSON, M.T. & L.C. CONZALEZ. 1979. Persistance of *Pseudomonas solanacearum* in an inceptisol in Costa Rica. In: Developments in Control of Potato Bacterial Diseases. Report of a Planning Conference, 12-15 June, 1979, CIP, Lima, pp 66-71

KELMAN, A. 1953. The bacterial wilt caused by *Pseudomonas solanacearum*. *Technical Bulletin No. 99 of the North Carolina Agricultural Experimental Station*

MOFFET, M.L., GILES, J.E. & B.A. WOOD. 1983. Survival of *Pseudomonas solanacearum* biovars 2 and 3 in soil: effect of moisture and soil type. *Soil Biology and Biochemistry. 15: 587-591*

POTATOES SOUTH AFRICA, 1999. The South African potato industry: a review 1998/1999. This report was compiled and published by Potatoes South Africa, Potato House, 529 Belvedere Street, Arcadia, Pretoria 0083

SHEKHAWAT, G.S. & M.C.M. PEROMBELON. 1991. Factors affecting survival in soil and virulence of *Pseudomonas solanacearum. Journal of Plant Disease and Protection 93:258-267*

SHEKHAWAT, G.S., CHAKRABARTI, S.K. & A.V. GADEVAR. 1992. Potato bacterial wilt in India. Technical Bulletin No 38. Central Potato Research Institute, India

SWANEPOEL, A.E. & D.J. THERON. 1999. Control measures for bacterial wilt, caused by *Ralstonia solanacearum*, as applied by the South African potato certification scheme. In: Leone, A., Foti, S., Ranalli, P., Sonnino, A., Vecchio, V., Zoina, A., Monti, L. & L. Frusciante (eds) 14th Triennial Conference of the European Association for Potato Research. Abstracts of Conference Papers, Posters and Demonstrations, Sorrento, Italy, 2-7 May 1999, pp 241-242

TERBLANCE, J. & D.A. DE VILLIERS. 1998. The suppression of *Ralston*ia *solanacearum* by marigolds. In:. P.H. Prior, Allen, C. & J. Elphinstone (eds) Bacterial wilt disease: Molecular and ecological aspects. Reports of the second international bacterial wilt symposium, Gosier, Guadeloupe, France, 22-27 June 1997, pp 325-331

TRIGALET, A., FREY, P & D. TRIGALET-DEMERY. 1994. Biological control of bacterial wilt caused by *Pseudomonas solanacearum*: State of the art and understanding. In: A.C. Hayward and G.L. Hartman (eds) Bacterial wilt: The

disease and its causative agent, *Pseudomonas solanacearum*. Cab International United Kingdom, pp 225-233

TUSIIME, G., ADIPALA, E., OPIO, F. & A.S. BHAGSARI. 1998. Weeds as latent hosts of *Ralstonia solanacearum* in Highland Uganda: implications to development of an integrated control package for bacterial wilt. In: Prior, P.H., Allen, C. and J. Elphinstone (eds) Bacterial wilt disease: Molecular and ecological aspects. Reports of the second international bacterial wilt symposium, Gosier, Guadeloupe, France, 22-27 June 1997, pp 413-419