UNIVERSITEIT VAN PRETORIA
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YUNIBESITHI YA PRETORIA

# CULTURAL PRACTICES FOR THE CONTROL OF BACTERIAL WILT OF POTATO

**By**

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# ABSTRACT

Bacterial wilt caused by *Ralstonia solanacearum* has affected the potato industry in South Africa since 1914. Control of bacterial wilt is difficult and depends greatly on integrated management strategies. Little information is available on the longevity of *R. solanacearum* in soil under South African conditions and how it is influenced by cultural practices. Information regarding local weeds which can serve as alternative hosts is limited.

Soil survival of *R. solanacearum* biovar 2 (race 3) was investigated in an artificially infested field managed by either one of four cultural practices, namely maize and potato monoculture, bare and weed-fallow. After a five-year period wilting of indicator plants was observed in all treatments with potato the highest, followed by bare-fallow, maize monoculture and lastly weed-fallow. Results demonstrated a greater ability of biovar 2 to survive in soil than generally accepted. Subsequently, the susceptibility of 22 weed and three grass species was investigated in the greenhouse. Five species were susceptible to biovar 2 and 13 to biovar 3. Preliminary *in vitro* studies conducted to determine suppressiveness of some weeds/grasses, indicate that microbial activity associated with some weeds could be involved in suppression of the wilt organism. Further studies are however required. The effect of maize on *Ralstonia solanacearum* populations was evaluated in a pot trial as well as in hydroponic culture. Results indicated that microbial populations present in the maize plant, could play a role in the susceptibility of maize to bacterial wilt infection. Antagonistic bacteria associated with some maize plants or with the maize rhizosphere could be partly responsible for suppression of wilt.

# PREFACE

Bacterial wilt caused by *Ralstonia solanacearum* E.F. is a destructive disease affecting a range of economically important crops grown in temperate, subtropical and tropical climates. In South Africa it was detected for the first time in 1914 on potato, thereafter on several other crops such as tomato, pepper, brinjals, peanuts and tobacco. Various isolates of this pathogen exist which differ in their ability to affect various hosts, and in their biochemical metabolism. The term race refers to the classification according to hosts affected, whereas the biovar system is based on biochemical reactions. To date, only biovar 2 (race 3) and biovar 3 (race 1) are found in South Africa. The former strain occurring mainly in temperate regions of the country and the latter in subtropical areas.

Potato is one of South Africa's most important food crops with approximately 1.6 million tons being produced annually. More than 50,000 ha are under production in 14 regions situated throughout the country. Potatoes are not only produced for local fresh consumption, but also for export and for the processing industry. The South African processing industry has increased by more than 100% over the last five years. To satisfy the increased demand, it is essential that production factors be manipulated to optimize crop environment and minimize the effects of pests and diseases. One of the diseases that has affected the South African potato industry since 1914 is bacterial wilt or brown rot of potatoes. 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. World-wide bacterial wilt is regarded as difficult to control or eradicate and therefore an integrated disease management approach is followed. In order to implement effective control strategies that are suitable for the region and for the specific race of the pathogen involved, it is essential to gain a good understanding of the disease and the pathogen that causes it. Chapter 1 represents a literature study in which some of the findings regarding the etiology, epidemiology and control of the bacterial wilt pathogen are summarised.

Bacterial wilt does not only cause severe crop losses due to wilting and tuber rot in storage, but it also renders the soil unsuitable for subsequent potato cultivation. When the role of infected seed tubers in the transmission of the disease was realized, compulsory testing of all registered seed tuber plantings was implemented. Confirmation of bacterial wilt in a planting would result in the withdrawal of registration. Availability of healthy seed, good sanitary practices and disease avoidance strategies have helped in reducing new infections, but uncertainty still exists on the management of infected fields and for how long these fields could sustain the organism. Chapter 2 reports on the investigation into the longevity of bacterial wilt (biovar 2) in artificially infested soils under different cultural practices. Maize and potato monoculture, and bare- and weed-fallow treatments were applied over a period of five years. To monitor *Ralstonia* populations, potato plants were planted in a designated region across all treatment plots after a three- and five-year cropping sequence.

Several weeds serve as alternative host to the bacterial wilt pathogen and thus play an important role in its survival in the absence of cultivated hosts. Consequently, weed control is regarded as an essential aspect of bacterial wilt management. Susceptibility of some weed species to bacterial wilt varies even if the same race or biovar of the pathogen is involved. In South Africa, little work has been done to identify the host range for both biovars. The study into the impact of cultural practices on the longevity of bacterial wilt suggested that the weed-fallowed plots are 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. The findings are reported in Chapter 3.

Maize has been included in rotation programs for may years, either as a single rotation crop or in combination with other non-host crops. The level of disease control achieved with maize rotation however, varies. In some instances *R. solanacearum* is capable of infecting maize, albeit at lower infection rates. In other instances, bacteria antagonistic to the wilt organism have been isolated from the maize rhizosphere. Maize monoculture in the above mentioned field study was slightly more efficient in

reducing wilt than bare fallow but was less efficient than weed-fallow. Chapter 4 reports on the investigation into maize as a carrier of the local biovar 2 strain and whether maize cultivation could have a suppressive effect on the pathogen.

# CHAPTER 1

# *RALSTONIA SOLANACEARUM*: ASPECTS OF ITS ETIOLOGY, EPIDEMIOLOGY AND CONTROL


## ABSTRACT

Bacterial wilt caused by *Ralstonia solanacearum* E.F. Smith, is a destructive seed and soil-borne disease affecting a range of economically important crops grown in temperate, subtropical and tropical climates. In South Africa the disease was first detected in 1914 on potato, thereafter several other crops such as tomato, pepper, brinjals, peanuts and tobacco have been affected. Its ability to infect a wide range of weed species enhances its survival during the non-cropping season. Bacterial wilt is difficult to control or eradicate and world-wide an integrated disease management approach is followed in reducing the severity of the disease. To develop effective control strategies it is essential to understand the organism and the disease it causes. This literature study summarises some of the findings regarding the etiology, epidemiology and control of bacterial wilt disease.

# INTRODUCTION

More than a century ago Erwin F. Smith described a bacterial disease affecting potato, tomato and eggplant. The generic name for this solanaceous wilt was proposed as *Bacillus*. E.F. Smith probably did not realise that the disease he had described would become one of the most important plant diseases causing severe economic losses world-wide. Since his publication in 1896 (Smith, 1896 as reported by Kelman, 1953) a continuous stream of publications have been released internationally, each shedding some light on this unusually complex disease. Commonly known as bacterial wilt, other names include brown rot (used especially in Europe on potatoes), Granville wilt (on tobacco), southern bacterial blight (on tomato, eggplant and tobacco), and moko disease on bananas.

Its ability to affect over 450 plant species (Prior *et al.*, 1998), its world-wide distribution and its destructive ability has resulted in this disease to be ranked as the most important bacterial plant pathogen (Kelman, 1998). The economic impact can not only be measured in terms of crop losses, but must also be assessed indirectly in terms of soils becoming unsuitable for subsequent crop production. This is important to large commercial farms and to small family fields. The introduction of different strains to different geographic regions increases the risk of more crops becoming susceptible to wilt within a particular region.

Bacterial wilt disease is considered difficult to control. Knowledge of the organism and the disease it causes are prerequisites for integrated management strategies. This literature study summarises some of the findings regarding the etiology, epidemiology and control of bacterial wilt disease.

# THE CAUSATIVE AGENT

The causative agent of bacterial wilt is a gram-negative, non-spore-forming, non-capsulate bacterium (Kelman, 1953). It is an aerobic organism with optimum growth temperatures ranging from 27-37 °C, depending on the strain. Maximum temperature for growth is about 39 °C and the minimum between 10-15 °C (Shekhawat *et al.*, 1992). There are conflicting reports on the amount of flagella present in a single cell. According to Bergey's Manual of Determinative Bacteriology (Holt *et al.*, 1994) this bacterium has more than one flagellum. Shekhawat *et al.* (1992) however describes virulent isolates to be non-flagellate and avirulent ones as having 1-4 polar flagella.

The shape and size of the causal organism was first described by E.F. Smith in 1896 as a small rod with rounded ends; its size varying according to growing conditions (Kelman, 1953). Bacteria isolated from infected tissues appeared as very short rods (0.3-0.6 x 0.4-1.2µ), those taken from young broths or cultures tend to be longer (ranging from 0.4-0.6 x 1.0-1.8µ), whereas those from old cultures have a short coccus-like form (Kelman, 1953).

Cultural characteristics of the colonies on tetrazolium chloride medium (Kelman, 1953) are often used to identify the bacterium. Virulent isolates produce fluidal, slightly raised colonies that are creamy white with or without pink centres. Colonies are rarely round (Shekhawat *et al.*, 1992). The pink centres often appear comma-like or half-moon in shape.

Different generic names have been used to classify the causal agent of bacterial wilt. Smith first proposed the name *Bacillus,* as he believed the bacterium to have peritrichous flagella. In 1898, Chester subsequently changed it to *Bacterium*; a name later also adopted by Smith (Kelman, 1953). With the finding that the pathogen actually had a single polar flagellum, it was placed under the classification as either *Bacterium solanacearum* or *Pseudomonas solanacearum.* Bergey (1923) reclassified the bacterium as *Phytomonas* although most continued to use the name *Bacterium.*

Studies and revision of the classification of gram-negative plant pathogens led to the provisional transferral of *P. solanacearum* to the new genus *Xanthomonas*. Since distinct cultural characteristics differed between *P. solanacearum* and the genus *Xanthomonas*, the pathogen was once again transferred back to the genus *Pseudomonas*. This agreed with the classification that was adopted in Bergey's Manual in 1948 (Kelman, 1953).

The classification of *P. solanacearum* was adopted for the next 44 years. Studies conducted by Yabuuchi *et al.* (1992) led to the proposal that *P. solanacearum* be transferred to the new genus *Burkholderia*. The seven species that were placed into this new genus differed from the type species of *Pseudomonas* in their oxidation and assimilation capabilities of several polyalcohols and disaccharides. Yabuuchi *et al.* (1992) also disagreed with the description of *P. solanacearum* given in Bergey's Manual (Holt *et al*., 1994) in that the type strain they identified was non-motile and without any flagellum.

Yabuuchi *et al.* (1995) reclassified *Burkholderia solanacearum* as *Ralstonia solanacearum*. This reclassification was based on studies involving phenotypic characterisation, rRNA-DNA hybridisation, phylogenic analysis of 16SrDNA nucleotide sequences, and analysis of cellular lipids and fatty acids.

# SUBSPECIFIC CLASSIFICATION

Several attempts have been made to find a suitable classification system for isolates of *R. solanacearum* as they often differ in host range, geographical distribution, pathogenicity, epidemiology and physiological properties. For almost thirty years two major approaches to differentiation were used, one based on hosts primarily affected resulting in the establishment of races, the other on selected biochemical properties conforming to distinct biovars. These classification systems do not abide by the Code of Nomenclature of Bacteria (Hayward, 1991a).

## Classification according to race

Subspecific classification of strains into 5 races is achieved by determining the hosts that are primarily affected.

Race 1: Strains affecting tobacco, tomato, a range of other solanaceous crops, some weeds and certain diploid but not triploid bananas.

Race 2: Those affecting triploid bananas, *Heliconia* spp. and other musaceous hosts.

Race 3: Strains affecting primarily potato and to a lesser extent tomato. It is weakly pathogenic on other solanaceous crops.

Race 4: Strains affecting mainly ginger.

Race 5: This race affects mainly mulberry.

## Classification according to biovar

According to Hayward (1994 b), five biovars can be identified based on ability to utilise three hexose alcohols, namely mannitol, sorbitol, dulcitol; and to produce acids from the three disaccharides, lactose, maltose and cellobiose (Table 1.1).

**Table 1.1 Identification of biovars of *R. solanacearum* according to utilisation and/or oxidisation of certain carbohydrates (Hayward, 1994 b)**

| Biovar/Carbohydrate | Biovar 1 | Biovar 2 | Biovar 3 | Biovar 4 | Biovar 5 |
|---|---|---|---|---|---|
| Lactose | - | + | + | - | + |
| Maltose | - | + | + | - | + |
| Cellobiose | - | + | + | - | + |
| Mannitol | - | - | + | + | + |
| Sorbitol | - | - | + | + | - |
| Dulcitol | - | - | + | + | - |

The distinction between race and biovar is not always clear except in the case of race 3 and biovar 2. Generally it can be said that race 3 (potato race) is equivalent to biovar 2, although the reverse is not necessarily true. In other words not all biovar 2 strains belong to race 3 (Sequeira, 1993). Biovar 2 strains that have been isolated in the Andean highlands seem to correspond to those of race 3 (Amat *et al.*, 1978). Biovar 2 strains from the Amazon basin, however, differ in phenotypic properties regarding pathogenicity on various *Solanum* species and metabolic activities. These have been designated as biovar N2 (Gillings & Fahy, 1994). Since biovar 2 isolates were found on potato or tomato, the correspondence of race 3 and biovar 2 seems true. Biovar N2, however, is probably not equivalent to race 3, as it was isolated from other *Solanum* species such as the nightshades. Biovar N2 also does not occur in the region where potatoes originated. Biovar 2 has a more limited host range than biovar 3, and is known to contain some strains that are adapted to pathogenesis at lower temperatures. It appears that its ability to survive in fallow soil is less than for biovar 3. These generalisations about the epidemiology and control of biovar 2 (race 3) set it apart from other races and biovars (Hayward, 1991a). Strains of race 1 affecting potatoes can consist of biovars 1, 3 and 4 (He *et al.*, 1987).

Within each of these races or biovars there are numerous subtypes that can be associated with certain geographical regions (He, 1983). This, together with the fact that *R. solanacearum* enjoys a world-wide distribution and that it is associated with indigenous plants in virgin soil supports the belief that this disease has been present in tropical soil for aeons (Sequeira, 1993).

More recent attempts to classify *R. solanacearum* strains involve molecular methods such as restriction length polymorphism (RFLP) analysis. Two major divisions could be identified, division 1 consisting of race 1, biovars 3, 4 and 5; and division 2 of biovar 1 (race 1 and 2) and biovar 2 (race 3) (Cook & Sequeira, 1994). These authors recognise only three traditional races. Fegan *et al.*, (1998) does not specifically mention the separation of the races into the two divisions but, describes division 2 as consisting of biovars 1, 2 and N2.

# DISEASE DEVELOPMENT AND SYMPTOMOLOGY

## Mode of entry

*Ralstonia solanacearum* usually enters its hosts via wounds in the root system (Johnson & Schaal, 1952). Cultural practices such as interplanting prior to harvest often lead to increased root damage (Kelman, 1953). The presence of root-invading parasitic fungi such as *Phytophthora* in the soil is believed to be another factor that may influence infection, although contradicting observations have been made in this regard (Kelman, 1953). The role of nematodes, especially the root-knot nematode (*Meloidogyne* spp.), in providing a wound for bacterial entry has been mentioned by several authors (Kelman, 1953; Buddenhagen & Kelman, 1964; Hayward, 1991a; Shekhawat *et al.*, 1992). Nematodes may also modify the host tissue making it more suitable for bacterial colonisation (Hayward, 1991a). Wilt resistant cultivars have been noted to become susceptible when attacked by nematodes. It is therefore not surprising that attempts were made to combine resistance to three of the *Meloidogyne* species and to *R. solanacearum* in potato plants (Gomez *et al.*, 1983).

Root decay caused by unfavourable soil conditions is believed to provide further entrance sites for the pathogen. Invasion through insect wounds has been noted on peanut roots and on potato tubers. Even infection of aerial parts via wounds has been reported under field conditions (Kelman, 1953).

Buddenhagen & Kelman (1964) reported transmission by several genera of insects visiting inflorescences of banana plants. Bacterial invasion does not occur directly through the flower, but occurs when open xylem vessels are exposed during natural dehiscence of bracts and male flowers.

Although wounding of some kind were usually regarded as a prerequisite for infection, documentation exists where this is not the case (Kelman & Sequeira, 1965). It is postulated that the bacterium could enter the host through points of secondary root emergence (Kelman, 1953; Buddenhagen & Kelman, 1964; Kelman & Sequeira, 1965). Kelman & Sequeira (1965) observed that relatively large numbers of bacteria are needed to infect unwounded roots. They suggest that massing of bacterial cells at points of secondary root emergence is required for enzymatic digestion of the mucilaginous sheath or other barriers.

## Histopathology of infected plants

The detailed study conducted by Wallis & Truter (1978) on the histopathology of tomato plants infected with *Ralstonia solanacearum*, shed considerable light on the spread of the pathogen within the host and the progressive destruction of its vascular tissue. Bacterial wilt was generally thought to be a vascular parasite confined initially to xylem vessels (Kelman, 1953; Buddenhagen & Kelman, 1964; Husain & Kelman, 1958a; Husain & Kelman, 1958b; Pegg & Sequeira, 1968). The Wallis & Truter (1978) study revealed, however, that initial colonisation of host tissue did not occur in the xylem vessels of the roots as expected, but in small diameter cells adjacent to large xylem vessels. Light microscopic examination could not identify whether these cells are tracheids, tracheid fibres or xylem parenchyma.

Wallis & Truter (1978) observed that within 24 hours after inoculation additional small diameter cells had become invaded and filled with bacteria. The bacteria within these cells showed marked pleomorphism and appeared to contain granules of storage products such as poly-B-hydroxybutric acid or volutin, indicating metabolic activity. In some cases, the bacteria were in close contact with the host cell wall and seemed to

orientate themselves toward the spaces between the bars of secondary thickening. In other cases they were contained in a bag-like structure, which effectively prevented them from affecting the primary wall. According to Wallis & Truter (1978) this concentration and orientation of bacteria towards bordered pits between vessels and adjacent cells during this stage of pathogenesis indicates that bacteria are either attracted by substances diffusing into the vessel from adjacent cells, or that they are drawn along an osmotic pressure gradient. This initial spread may be influenced by the method and the site of inoculation and may also indicate that at any particular stage of development of the host plant only certain cells are physiologically predisposed to invasion.

Stimulation of tyloses formation was noted in invaded cells and less frequently in non-invaded cells about 24 hours after inoculation, an observation absent in healthy plants. This indicates that although infection stimulates the production of tyloses, the actual presence of bacteria is not required. Kelman (1953) reported similar findings. The formation of tyloses is believed to be stimulated by increased production of indole acetic acid (IAA) and other growth substances (Sequeira & Kelman, 1962; Buddenhagen & Kelman, 1964; Sequeira, 1965; Pegg & Sequeira, 1968;).

Wallis & Truter (1978) observed a slow migration of bacteria during the first 48 hours after inoculation and no bacteria could be detected at a distance greater than 3,5 cm from the cut root-tip. No bacteria could be observed in the xylem vessels. During the next 24 hrs, however, disruption and collapse of tyloses had occurred, releasing the bacteria into the xylem vessels. Bacteria spread in root vessels above the region of tylose collapse. These bacteria increased steadily and in some cases the primary wall showed signs of degradation. Only at this stage of disease development were bacteria observed in the vessels of stems. During the first 72 hours after inoculation water uptake in inoculated plants had been 15 - 20% higher than in the control plants. Thereafter, however, the inoculated plants started to wilt and fluid uptake decreased relative to that in control plants. This observation correlates with the time when tyloses, after often obstructing vessels, collapsed, became disrupted and released bacteria into the xylem vessels (Wallis & Truter, 1978). After 144 hours the bacteria in the root vessel had reached such large numbers that they became compressed into

irregular shapes. Longitudinal spread in the stem was now rapid but compression of bacterial cells did not occur to such an extent as in the root vessels, nor did bacteria reach such large numbers. Tissue collapse was observed after 168 hours and various plugging substances were noted in the vessels and cells of diseased plants. Complete wilting of all test plants occurred about 192 hours after inoculation. A dense, darkly staining material, possibly of cell wall origin, accumulated in many vessels. This material was found where vessel walls had been dissolved by bacterial enzyme action and in lysigenous cavities formed by bacterial degradation of adjacent parenchyma cell walls (Wallis & Truter, 1978). It has also been reported that in some instances these parenchyma cells seem to enlarge and divide, causing partial collapse of infected vessels (Kelman, 1953).

According to Wallis & Truter (1978), the lack of damage to the walls of invaded root cells during the early stages of pathogenesis could possibly be ascribed to low levels of cellulolytic and pectinolytic enzyme activities as bacterial numbers are still relatively low. As bacterial numbers increased, degradation of wall material in the vessels was clearly visible. Kelman (1953) mentions that in solanaceous hosts, besides collapse of infected vessels, adjacent phloem areas can become infected and the cortex disorganized. The formation of a central cavity often results due to a breakdown of pith tissue. This general breakdown of walls and cells is, however, not found in stems of older plants that have well-developed secondary xylem.

## Visual symptom expression

Once the pathogen has entered the host, time elapses before visual symptoms appear. This incubation period varies greatly and depends on a variety of factors such as host species, environmental conditions, age of the host and level of resistance. The symptoms characteristic of bacterial wilt include wilting, stunting and yellowing of the foliage, epinasty and vascular browning in the stems (Kelman, 1953; Buddenhagen & Kelman, 1964; Shekhawat *et al.*, 1992). It has also been noted that climatic conditions can affect the type of visual symptoms expressed. In hot dry weather, infected plants may show irregular scalded areas on their leaves, which then

dry out and shatter at the edges (Kelman, 1953). Under moist conditions, however, the base of the stem, or leaf petioles may completely rot and break off (Kelman, 1953; Shekhawat *et al.*, 1992). In potatoes a slight yellowing of lower leaves is often noted as the first leaves begin to droop (Kelman, 1953). The extent to which yellowing occurs often depends on whether the onset of disease is rapid, in which case no real change in colour would occur.

Adventitious root formation on infected stems commonly occurs, especially if wilting is gradual (Kelman, 1953) or if an isolate of low virulence was responsible.

Potato tubers from infected plants do not always show external symptoms. The presence of external greyish brown discoloration indicates an advanced stage of disease, which upon further development leads to a bacterial exudate at the eyes or the stolen end of the tuber. Cross sections of infected tubers often show distinct brown discoloration and decay in the vascular ring (Shekhawat *et al.*, 1992).

## DISTRIBUTION

Bacterial wilt affects crops of economic importance in almost all the tropical, subtropical and warmer temperate regions of the world. Early reports of occurrence have often been incorrect, incomplete or not confirmed by proper identification techniques, making it difficult to truly assess its distribution.

The distinct differences in geographical distributions of biovars suggest a separate evolutionary origin (Hayward, 1991a). Biovar 2 presumed to have originated in South America (presumed site of origin of the potato) now has a wide spread distribution, indicating the ease with which it can be transmitted as latent infections in potato seed tubers. In many countries of Southern Europe such as Portugal, biovar 2 is the sole biovar. This is also true for the Mediterranean area, Argentina, Chile and Uruguay (Hayward, 1991a).

Biovars 1 and 2 are predominant in the Americas with biovar 3 being rare and biovar 4 not yet being identified with the exception of one case stated by Black & Sweetmore (1993). In Australia, however, biovar 3 predominates, biovars 2 and 4 occurring to a lesser extent. Biovars 2,3 and 4 also occur in India, Indonesia, Papua New Guinea, Sri Lanka and China (together with biovar 5). Only in the Philippines have all of biovars 1-4 been found and here as elsewhere in Asia, biovar 3 predominates in the lowland regions (Hayward, 1991a).

The classification into divisions as suggested by Cook & Sequeira (1994) corresponds, with a few exceptions, to the geographic distribution of the strains. In Division I 90% of the strains came from Asia and Australia, whereas 98% of those in Division 2 were from the Americas. This suggests that in early evolution *R. solanacearum* split into two groups which then evolved in geographical isolation to give rise to the strains typical of the Old World and the New World. All race 3 strains isolated in Africa, Asia and Australia belonged to the same RFLP groups which originated in the Andean region in South America.
With the aid of RFLP techniques more data could be obtained to support the concept of geographic isolation in the pathogens evolution (Sequeira, 1993). Strains from *Heliconia* are in RFLP groups that are restricted to the Americas, whereas strains from mulberry and ginger form distinct groups that are again restricted to certain regions in Asia.

Race 3 (potato race), although being widely distributed, consists of a very compact group originating in the Andean Region. Yet strains attacking bananas in the Asian continent seem to have evolved separately from those attacking bananas in the Americas (Sequeira, 1993). *R. solanacearum* has now been identified to be the cause of an old banana disease common in Asia, the so-called blood disease. RFLP patterns of these strains are so different from all other strains, that it was concluded that this group bears very little relationship to the American race 2 strains.

So far only biovars 2 and 3 have been reported in South Africa with biovar 2 having a major impact on the potato industry. Since neighbouring countries such as Angola, Mozambique and Zimbabwe are reported to have biovar 1 of *R. solanacearum*

(De Lourdes D'Oliveira, 1967) great care needs to be taken to prevent establishment of this biovar locally.

In Table 1.2 the world-wide distribution of bacterial wilt is demonstrated with mention of the specific biovars and/ or races involved. In several instances literature sources only reveal the occurrence of *R. solanacearum* without further differentiation into biovars or races. This list may be incomplete and in some mentioned countries, such as Spain, Poland and Sweden, the disease is believed to be eradicated (Elphinstone, 1996).

**Table 1.2 Distribution of bacterial wilt disease**

| COUNTRIES | BIOVARS | RACES | SOURCE |
|---|---|---|---|
| **AMERICAS** | | | |
| Argentina | 2 | 3 | Martin *et al.*, 1982 |
| Brazil | 1,2,3 | 1,2,3 | French *et al.* 1993 |
| Bolivia | 1,2 | | Smith *et al.* 1998 |
| British Honduras (Belize) | 1 | | Black & Sweetmore, 1993 |
| Canada | 1 | 1 | Hayward, 1991a |
| Chile | 2 | | Hayward, 1991 a<br>Ciampi-Panno, 1984 |
| Colombia | 1,2 | 1,3 | Martin *et al.*, 1982 |
| Costa Rica | 1,2,3 | 1,3 | Martin *et al.*, 1982 |
| El Salvador | | | Kelman, 1953 |
| Honduras | | 2 | Woods, 1984 |
| Mexico | 2<br>1<br>1 | 3<br>1<br>2 | Fucikovsky, 1978<br>Fucikovsky, 1978<br>Fucikovsky & Santos, 1993; Fucikovsky, 1978 |
| Panama | 2 | 3 | Martin *et al.*, 1982 |
| Peru | 1,2,3 | 1,3 | Martin *et al.*, 1981 |
| United States<br>- 1 literature source | 1<br>4 | 1<br>1 | Martin *et al.*, 1982<br>Black & Sweetmore, 1993 |
| Uruguay | 2 | 3 | Hayward, 1991 a |
| Venezuela | 2 | 3 | Martin *et al.*, 1982 |
| **WEST INDIES** | | | |
| Cuba | 3 | | Arnat *et al.*, 1978 |
| French West Indies | 1,2,3 | 2,3 | Prior & Steva, 1990 |
| Grenada | 1 | | Black & Sweetmore, 1993 |

| COUNTRIES | BIOVARS | RACES | SOURCE |
|---|---|---|---|
| Guadeloupe | | | Prior *et al.*, 1993 |
| Haiti | | | Kelman, 1953 |
| Jamaica | | | Kelman, 1953 |
| Martinique | 2,3 | 1,3 | |
| Puerto Rica | 1 | 1 | Cook & Sequeira, 1994 |
| Tobago | | | Hosein & Phelps, 1997 |
| Trinidad | 1 | 1 | Cook & Sequeira, 1994 |
| **EUROPE** | | | |
| Albania | | | Wenneker et al., 1998 |
| Austria | | | Kelman, 1953 |
| Belgium | 2 | 3. | Elphinstone, 1996 |
| Denmark | | | Wenneker *et al., 1998* |
| France | 2 | 3. | Elphinstone, 1996, Hayward *et al.*, 1998 |
| Greece | 2 | 3 | Walker, 1992 |
| Netherlands | 2 | 3 | Janse *et al.*, 1998 |
| Italy | 2 | 3 | Elphinstone, 1996 |
| Portugal | 2 | 3 | De Lourdes D'Oliveira, 1967; Elphinstone, 1996 |
| Rumania | | | Kelman, 1953 |
| Spain | 2 | 3 | Elphinstone, 1996 |
| Sweden | | | Olsson, 1976 |
| United Kingdom | 2 | 3 | Elphinstone, 1996 |
| U.S.S.R | | | Walker, 1992 |
| Yugoslavia | | | Walker, 1992 |
| **ASIA** | | | |
| China | 2,3,4,5 | 1,3,4,5 | He, 1983 |
| India | 2,3,4 | 1,2,3 | Shekhawat *et al.*, 1992; Sinha, 1986 |
| Indonesia | 2,3,4 | 1,3 | Hayward, 1991; Machmud, 1986 |
| Iran | 2 | 3 | Danesh & Bahar, 1984 |
| Israel | | 3 | Alvarez *et al.*, 1993 |
| Japan | 1,2,3,4 | 1,3 | Tsuchiya & Horita, 1998 |
| Java | 2,3,4 | 1,2,3 | Cook & Sequeira, 1994 |
| Malaysia | 3,4 | . | Abdullah, 1993 |
| Nepal | 2,3 | 1,3 | Adhikari, 1993 |
| Pakistan | 2,3 | | Burney & Ahmad, 1997 |
| Philippines | 1,2,3,4 | | Valdez, 1986 |
| Sri Lanka | 2,3,4 | 1,3 | Velupillai, 1986; Martin |

| COUNTRIES | BIOVARS | RACES | SOURCE |
|---|---|---|---|
| | | | & Nydegger, 1982 |
| Taiwan | 3,2,4 | 1 | Hsu, 1991 |
| Thailand | 3,4 | | Titatarn, 1986 |
| Vietnam | | 1 | Hong & Mehan, 1993 |
| **AFRICA** | | | |
| Algeria | | | Kelman, 1953 |
| Angola | 1,2 | 3 | De Lourdes D'Oliveira, 1967 |
| Burundi | 2 | 3 | Berrios & Rubirigi, 1993 |
| Egypt | 2 | 3 | Gillings & Fahy, 1994 |
| Ethiopia | | | Kelman, 1953 |
| Kenya | 2,3,4 | 1,3 | Harris, D.C. 1976 |
| Malawi | | | Black *et al.* 1998 |
| Morocco | | | Kelman, 1953 |
| Mozambique | 1 | | De Lourdes D'Oliveira, 1967 |
| Nigeria | 2 | 3 | Cook & Sequeira, 1994 |
| Rwanda | 2 | 3 | Vander Zaag, 1986 |
| Somalia | | | Kelman, 1953 |
| South Africa | 2,3 | 1,3 | Swanepoel, 1992 |
| Tanzania | | | Black *et al.* 1998 |
| Uganda | | | Tusiime *et al.*, 1998 |
| Zaire | | 3 | French *et al.*, 1998 |
| Zimbabwe | 1,2 | | Robertson, 1998 |
| **AUSTRALIA** | 2,3,4 | 1,3 | Hayward, 1991b |
| **INDIAN OCEAN** | | | |
| Andaman & Nicobar Islands | | | Ramesh & Bandyopadhyay, 1993 |
| Madagascar | 1 | 1 | Lallmahomed *et al.*, 1988 |
| Mauritius | 3 | 1 | Saumtally *et al.*, 1993 |
| Reunion | 1,2,3 | 1,3 | Girard *et al.*, 1993 |
| **PACIFIC OCEAN** | | | |
| Fiji | | | Iqbal & Kumar, 1986 |
| Hawaii | 4 | 1,2 | Alvarez *et al.*, 1993; Hayward, 1986 |
| New Zealand | | | Kelman, 1953 |
| Papua New Guinea | 2,3,4 | 1,2 | Tomlinson, 1985; Tomlinson & Gunther, 1986; He, 1986 |
| **ATLANTIC OCEAN** | | | |
| Madeira Island | 2 | 3 | De Lourdes D'Oliveira, 1967 |

# HOST RANGE

*R. solanacearum* is known to have a very extensive host range including not only economically important crop plants such as potatoes, tomatoes, tobacco and bananas, but also ornamental plants, trees and weeds. Species from more than 44 plant families have been identified by Hayward (1991a) and more hosts are being recognised and described. Some of the more recent reports include onion, *Allium cepa*, (Girard *et al.*, 1993); custard apple, *Annona* spp., (Mayers & Hutton, 1987); florist geranium, *Pelagornium hortorum*, (Strider *et al.*, 1981); strawberry, *Fragaria* L. spp., (Hsu, 1991) and radish, *Raphanus sativus* L., (Hsu, 1991). *R. solanacearum*, biovar 3, has recently also been noted on cashew in Indonesia and the Alexandra palm in Queensland, Australia (Hayward, 1991a).

There appears to be some irregularity in the distribution of bacterial wilt in certain hosts. Cassava is cultivated in many countries were bacterial wilt is endemic, yet the disease on this host appears to be confined to Indonesia. Similarly bacterial wilt on sweet potato has only been reported in China (Hayward, 1991a). Eucalyptus was first reported as a host in Brazil and China. Eucalyptus in Australia appeared to be a non-host until recently, when biovar 3 was isolated from diseased plants (Hayward, 1994b).

Specific strains pathogenic for certain hosts may have evolved only in certain parts of the world and are not found elsewhere. This theory is supported by recent RFLP studies. An alternative theory is that these hosts may only be susceptible where a number of environmental factors conducive to disease expression coincide, such as temperature regime, rainfall, soil type, inoculum potential, and other biological factors such as nematode populations (Hayward, 1991a; Hayward, 1994b).

Hosts of *R. solanacearum* do not necessarily express symptoms but can serve as symptomless carriers. This is especially true for many of the weed hosts such as common purslane (Hayward, 1991a), single leaved cleome (Shekhawat *et al.*, 1992) and the apple of Peru (Olsson, 1976). The slow rate of colonisation and disease

progress in symptomless hosts allows the bacteria to stay viable longer, serving as an inoculum source for susceptible crops or wild hosts.

Studies conducted by Shekhawat *et al.* (1992) indicate that *R. solanacearum* can even survive symptomless in roots of weed-hosts and in plants considered to be non-hosts. Granada & Sequeira (1983a) have reported similar findings. Roots of bean and maize, both presumed non-hosts were invaded with bacteria. Infection was however localised and not all plants became infected.

More than 450 species have been reported as hosts or symptomless carriers (Prior *et al.*, 1998) of certain strains of *R. solanacearum*. In Table 1.3 host plants are listed with reference to the country of report and where possible the strain involved. The host range of individual strains of *R. solanacearum* differs considerably with race 1 (the solanaceous race) having the widest range. This race is more common in the sub-tropical and tropical climates where plant debris tends to decompose more rapidly thereby providing only temporary shelter to the bacteria. Alternative hosts might therefore play an important role in the pathogen's survival. Only about 30-40 species have been positively identified as hosts of biovar 2 (race 3).

**Table 1.3 Known hosts or symptomless carriers of *Ralstonia solanacearum***

| Weed Hosts | | | | | |
|---|---|---|---|---|---|
| **Scientific Name** | **Common Name** | **Biovar cited** | **Race cited** | **Country of Report** | **Literature Reference** |
| *Acanthospermum hispidum* DC | Upright starbur | | | India | Shekhawat *et al.*, 1992 |
| *Achyranthes aspera* Cooke | Rough Chaff flower | | | India | Shekhawat *et al.*, 1992 |
| *Aclypha boehmerioides* Miq. | | | | | Kelman, 1953 |
| *Aclypha hispida* | | | | India | Shekhawat *et al.*, 1992 |
| *Aclypha indica* | | | | India | Shekhawat *et al.*, 1992 |
| *Ageratum conyzoides* | White weed | 3 | | Indonesia<br>Thailand<br>India<br>Andaman & Nicobar Islands<br>Malaysia<br>Uganda | Machmud, 1986<br>Titatarn, 1986<br>Shekhawat *et al.*, 1992<br>Ramesh & Bandyopadhyay, 1993<br>Abdullah, 1993<br>Akiew *et al.*, 1993 |
| *Ageratum houstonianum* Mill. | Blue billy-goat weed | | | Australia | Akiew *et al.*, 1993 |
| *Amaranthus graecizans* L. | Kaffir spinach | | | India | Shekhawat *et al.*, 1992 |
| *Amaranthus hybridus* L. | Cape pig weed | 3 | 1 | R.S.A | Swanepoel, 1992 |
| *Amaranthus sp.* | Bayam | | | Malaysia<br>Andaman & Nicobar Islands<br>Uganda | Abdullah, 1993<br>Ramesh & Bandyopadhyay, 1993<br>Tusiime *et al.*, 1998 |
| *Ambrosia artemisiifolia* L. | Common ragweed | | | | Kelman, 1953 |
| *Ambrosia trifida* L. | Giant ragweed | | | | Kelman, 1953 |
| *Anthirrhinum sp.* | | | | India | Kishore *et al.*, 1993 |
| *Artemissia sp.* | | | | India | Kishore *et al.*, 1993 |
| *Arabidopsis thaliana* | | | | India | Kishore *et al.*, 1993 |

| Weed Hosts | | | | | |
|---|---|---|---|---|---|
| **Scientific Name** | **Common Name** | **Biovar cited** | **Race cited** | **Country of Report** | **Literature Reference** |
| *Aritica dioca* | | | | India | Kishore *et al*., 1993 |
| *Asclepias curassarica* | Blood-flower | | 2 | Honduras<br>Australia | Berg, 1971<br>Akiew *et al*., 1993 |
| *Atropa belladonna* | Belladonna | | | | Kelman, 1953 |
| *Bidens bipinnata* L. | Spanish blackjack | 3 | | RSA<br>India | Swanepoel, 1992<br>Shekhawat *et al*., 1992 |
| *Bidens pilosa* L. | Common blackjack (Cobblers peg) | 3<br><br>3 | <br><br>1 | Thailand<br>India<br>Australia<br>Brazil<br>Uganda | Titatarn, 1986<br>Shekhawat *et al*., 1992<br>Akiew & Trevorrow1994<br>Mariano, 1998<br>Tusiime *et al*., 1998 |
| *Brachiaria plantaginea* | | | | Brazil | Mariano, 1998 |
| *Calendula officinalis* L. | Calendula | | | India | Shekhawat *et al*., 1992 |
| *Calopogonium mucunoides* Desv. | Calopo | | | Australia | Akiew *et al*., 1993 |
| *Cannabis savita* L. (s) | Indian Hemp | | | India | Shekhawat *et al*., 1992 |
| *Capsella bursa-pastoris* | | 2 | 3 | Netherlands | Van Beuningen *et al*, 1998 |
| *Cardamine* spp. | | 2 | 3 | Netherlands | Van Beuningen *et al*, 1998 |
| *Cassia mimosoides* L. | Five leaf cassia | | | Australia | Akiew *et al*., 1993 |
| *Cecropia peltata* | | | 2 | Honduras | Berg, 1971 |
| *Celosia argentea* L. | Cockscomb | | | India | Shekhawat *et al*., 1992 |
| *Cerastium fontanum* | | 2 | 3 | Netherlands | Van Beuningen *et al*, 1998 |
| *Cerastium glomeratum* | | 2 | | Nepals | Pradhanang, 1999 |
| *Chenapodium album* | | 2 | 3 | Netherlands | Van Beuningen *et al*, 1998 |
| *Chenopodium ambrosoides* L. | Wormseed goosefoot | | | India | Shekhawat *et al*., 1992 |
| *Chenopodium murales* L. | Nettle-leaved goosefoot | | | India | Shekhawat *et al*., 1992 |
| *Cichorium endivia* | | | | Brazil | Mariano, 1998 |
| *Citrullua lanatus* | Paddy melon | | | Australia | Akiew *et al*., 1993 |
| *Cleome monophylla* L. | Single leaved cleome | <br>2 | 1,3<br>1,3 | Kenya<br>India | Harris, 1976<br>Shekhawat *et al*., 1992 |
| *Cleome speciosissima* | Cat's whisker | | | Malaysia | Abdullah, 1993 |
| *Commelina benghalensis* L. | Common signal grass | | | India<br>Brazil | Shekhawat *et al*., 1992<br>Mariano, 1998 |
| *Corchorus acutangulus L.* | Native jute | | | Australia | Akiew *et al*., 1993 |
| *Corchus olitarius* | Corchorus | | | Malaysia | Abdullah, 1993 |
| *Cosmos caudatus* | Ulam Raja | | | Malaysia | Abdullah, 1993 |
| *Cosmos* sp. | | | | Andaman & Nicobar Islands | Ramesh & Bandyopadhyay, 1993 |
| *Cosmos sulphureus* | | | 1 | Andaman | Ansari, 1990 |
| *Crassocephalum crepidioides* | | 3 | | Thailand<br>Indonesia<br>Australia, Java | Titatarn, 1986<br>Machmud, 1987<br>Hayward, 1994 |
| *Croton hirtus* | Croton | 3 | | Malaysia<br>Indonesia<br>Sri Lanka | Hayward, 1986<br>Machmud, 1986<br>Velupillai, 1986 |
| *Croton sperciflorus* | | | | India | Shekhawat *et al*., 1992 |
| *Cyperus rotundus* L. | Red nutgrass | 3 | | India | Shekhawat *et al*., 1992 |
| *Datura ferox* | Large thorn apple | 2,3 | 1,3 | RSA | Swanepoel & Young, 1988 |
| *Datura metel* | | | | India | Schmiediche, 1986 |
| *Datura stramonium* L. | Common thorn apple | <br>2,3<br><br>1,2,3<br><br><br>2 | 1<br><br><br><br><br><br>3 | Kenya<br>R.S.A<br>India<br>Peru<br><br>Sweden<br>Chile | Harris, 1976<br>Swanepoel, 1992<br>Kishore *et al*., 1993<br>Marín & El-Nashaar, 1993<br>Olsson, 1976<br>Fernandez, 1986 |
| *Datura* spp. | | 4 | | Kenya | Martin & Nydegger, 1982 |
| *Drymaria cordata* | | 2 | | Nepal | Pradhanang, 1999 |
| *Dysophylla auricularia* (L.) Blume | | | | | Kelman, 1953 |
| *Eclipta alba* (L.) | White eclipta | 3 | | India<br>Australia | Shekhawat *et al*., 1992<br>Akiew *et al*., 1993 |

| Weed Hosts | | | | | |
|---|---|---|---|---|---|
| **Scientific Name** | **Common Name** | **Biovar cited** | **Race cited** | **Country of Report** | **Literature Reference** |
| *Eclipta prostrata* | | 3 | | Thailand | Titatarn, 1986 |
| *Eleutheranthera ruderalis* (Schw.)Sch.Bip | | | | | Kelman, 1953 |
| *Eragrostis curvula* | Weeping love grass | 3 | | R.S.A | Swanepoel, 1992 |
| *Erechtites hieracifolia* Rafin. | | | | Malaysia<br>Brazil | Abdullah, 1993<br>Mariano, 1998 |
| *Erigeron cannadensis* | Horseweed | | | | Kelman, 1953 |
| *Erigeron floribundis (Conyza sumatrensis, C. albida)* | Tall fleabane | | | Uganda | Tusiime *et al.*, 1998 |
| *Erigeron hirta* L. | Asthma plant | | | Australia | Akiew *et al.*, 1993 |
| *Eupatorium cannabinum* L. | | | | Sweden<br>India | Olsson, 1976<br>Shekhawat *et al.*, 1992 |
| *Eupatorium oderatum* | | 3<br>3 | | Thailand<br>Costa Rica,<br>Andaman & Nicobar Islands | Titatarn, 1986<br>Black & Sweetmore, 1993<br>Ramesh & Bandyopadhyay, 1993 |
| *Euphorbia geniculata* L. | painted Euphorbia | | | India | Shekhawat *et al.*, 1992 |
| *Euphorbia hirta* L. | red Euphorbia | | | India<br>Malaysia | Shekhawat *et al.*, 1992<br>Abdullah, 1993 |
| *Euphorbia maculata* L. | | | | India | Shekhawat *et al.*, 1992 |
| *Euphorbia prunifolia* | Euphorbia | 3 | | Malaysia | Hayward, 1986 |
| *Fagopyrum sagittatum* | | | | Indonesia | Machmud, 1986 |
| *Galinsoga parviflora* | Small flowered quick weed | | | Uganda<br>Brazil | Tusiime *et al.*, 1998<br>Mariano, 1998 |
| *Galium aparine* | | | | India | Kishore *et al.*, 1993 |
| *Galphimia gracilis L.* | | | | Malaysia | Abdullah, 1993 |
| *Gomphrena* sp. | | | | India | Shekhawat *et al.*, 1992 |
| *Heliconia acuminata* | | | 2 | Costa Rica | Sequeira & Averre, 1961 |
| *Heliconia caribaea* | | | 2 | Costa Rica | Sequeira & Averre, 1961 |
| *Heliconia imbricata* | | | 2 | Costa Rica | Sequeira & Averre, 1961 |
| *Heliconia latispatha* | | | 2 | Costa Rica | Sequeira & Averre, 1961 |
| *Hibiscus cannabinus* Linn. | | | | Malaysia | Abdullah, 1993 |
| *Hyptis capitata* Jacq. | | | | Malaysia | Abdullah, 1993 |
| *Hyptis suavealens* | | 1 | 1 | Brazil | Quezando-Soares & Lopes, 1994 |
| *Hyptis suaveolens* | | <br>3 | | Sri Lanka<br>Thailand<br>Malaysia | Velupillai, 1986<br>Titatarn, 1986<br>Abdullah, 1993 |
| *Ipomea setosa* L. | | | | Malaysia | Abdullah, 1993 |
| *Ipomoea* sp. | | | | Andaman & Nicobar Islands | Ramesh & Bandyopadhyay, 1993 |
| *Jussiaea linifolia* | | 3 | | Thailand<br>Andaman & Nicobar Islands | Titatarn, 1986<br>Ramesh & Bandyopadhyay, 1993 |
| *Lablab purpureus* L. | Lablab | | | Australia | Akiew *et al.*, 1993 |
| *Lagosca mollis* | | | | India | Shekhawat *et al.*, 1992 |
| *Lathyrus sp.* | | | | India | Kishore *et al.*, 1993 |
| *Leonurus sibiricus* L. | | | | Malaysia | Abdullah, 1993 |
| *Leucas martinicensis* | | | | Uganda | Tusiime *et al.*, 1998 |
| *Luzula campestris* | | 2 | 3 | Netherlands | Van Beuningen *et al*, 1998 |
| *Marsypianthes chamaedrys* | | 1 | 1 | Brazil | Mariano *et al.*, 1998 |
| *Melampodium perfoliatum* | | 1 | 1 | Costa Rica, | Black & Sweetmore, 1993 |
| *Merremia hastata* (Desr.) Hall | | | | | Kelman, 1953 |
| *Merremia umbellata* (Mey.) Hall | | | | | Kelman, 1953 |
| *Merremia vitifolia* (L.) Hall | | | | | Kelman, 1953 |
| *Milleria quinqueflora* L. | | 3 | | Cuba | Arnat *et al.*, 1978 |
| *Nepata sp.* | | | | India | Kishore *et al.*, 1993 |
| *Nicandra physaloides* (L.) | Apple of Peru | | 2 | Sweden<br>India | Olsson, 1976<br>Shekhawat *et al.*, 1992 |
| *Nicotiana alata* Link & Otto | | | | Sweden | Kelman, 1953 |
| *Nicotiana glauca* | Wild tobacco | | | | Kelman, 1953 |
| *Nicotiana glutinosa* | | 2 | 3 | Peru<br>Colombia | Martin & French, 1995<br>Thurston, 1963 |
| *Nicotinana rustica* | Aztec tobacco | 2 | 3 | Peru<br>Colombia | Martin & French, 1995<br>Thurston, 1963 |
| *Oenothera rosea* | | | | India | Kishore *et al.*, 1993 |
| *Oxalis latifolia* | Red garden sorrel | | | Uganda | Tusiime *et al.*, 1998 |

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| Weed Hosts | | | | | |
|---|---|---|---|---|---|
| **Scientific Name** | **Common Name** | **Biovar cited** | **Race cited** | **Country of Report** | **Literature Reference** |
| *Oxalis sp.* | | | | India | Kishore *et al*., 1993 |
| *Oldenlandia corymbosa L.* | | | | India | Shekhawat *et al*., 1992 |
| *Parthenium hysterophorus L.* | | | | Cuba | Stefanova, 1998 |
| *Physalis angulata* L. | Wild gooseberry | 3 | | Costa Rica | Black & Sweetmore, 1993 |
| | | 2 | | R.S.A | Swanepoel, 1992 |
| *Physalis floridana* | | 2 | 3 | Chile | Fernandez, 1986 |
| *Physalis minima L.* | Bladder cherry | | | Malaysia | Abdullah, 1993 |
| *Physalis niruri* | | | 1 | India | Shekhawat *et al*., 1992 |
| *Physalis peruviana* | | | | Colombia | Thurston, 1963 |
| *Physalis* spp. | Wild gooseberry | 3 | 1 | Australia | Akiew & Trevorrow, 1994 |
| *Piper auritum* | | | 2 | Honduras | Berg, 1971 |
| *Piper peltatum* | | | 2 | Honduras | Berg, 1971 |
| *Polanisia viscosa* (L.) DC | | | | | Kelman, 1953 |
| *Polygala* | | | | India | Kishore *et al*., 1993 |
| *Polygonum hydropiper* L. | | | | India | Shekhawat *et al*., 1992 |
| *Polygonum napalense* | | | | Uganda | Tusiime *et al*., 1998 |
| *Polygonum sp.* | | | | India | Kishore *et al*., 1993 |
| *Portulaca oleracea* L. | Common purslane | | 1,3 | Kenya | Harris, 1976 |
| | | 3 | | Thailand | Titatarn, 1986 |
| | | | | India | Shekhawat *et al*., 1992 |
| | | | | Brazil | Mariano, 1998 |
| *Portulaca volarasia* | | | | Andaman & Nicobar Islands | Ramesh & Bandyopadhyay, 1993 |
| *Pultenaea villosa* | | 3 | | Australia | Li & Hayward, 1993 |
| *Ranunculus sceleratus* L. | | | | India | Shekhawat *et al*., 1992 |
| *Ricinus communis* L. | Casteroil plant | | | R.S.A | Swanepoel, 1992 |
| | | | | Phillipines | Persley *et al*., 1986 |
| | | | | India | Shekhawat *et al*., 1992 |
| | | | | Malaysia | Abdullah, 1993 |
| | | | | Brazil | Mariano, 1998 |
| *Rumex abyssinicum* | | | | Uganda | Tusiime *et al*., 1998 |
| *Rumex sp.* | | | | India | Shekhawat *et al*., 1992 |
| | | 2 | 3 | Neyherlands | Van Beuningen *et al*., 1998 |
| *Salpiglossis sinuata* R & P | | | | Sweden | Olsson, 1976 |
| *Salvia privoides* Benth. | | | | | Kelman, 1953 |
| *Scoparia dulcis* L. | Goutweed | | | | Kelman, 1953 |
| *Scuttellaria scandens* | | | | India | Kishore *et al*., 1993 |
| *Senecio sonchifolia* Moench. | | | | | Kelman, 1953 |
| *Sesbania exaltata* | | | | Indonesia | Machmud, 1986 |
| *Solanum nigrum* L. | Black nightshade | 2 | 3 | Australia | Graham & Lloyd, 1979 |
| | | | 1,3 | Kenya | Harris, 1976 |
| | | 3 | | South Africa | Swanepoel, 1992 |
| | | | 2 | Honduras | Berg, 1971 |
| | | 2 | | Papua New Guinea | Tomlinson, 1985 |
| | | 3 | | Thailand | Titatarn, 1986 |
| | | | | India | Shekhawat *et al*., 1992 |
| | | | | Réunion | Girard *et al*., 1993 |
| | | | | Andaman & Nicobar Islands | Ramesh & Bandyopadhyay, 1993 |
| | | | | France | Hayward *et al*., 1998 |
| | | | | Sweden | Olsson, 1976 |
| | | 2 | | Peru | Martin & French, 1995 |
| | | 1,2,3 | 3 | Peru | Marín & El-Nashaar, 1993 |
| | | 2 | 3 | Netherlands | Van Beuningen *et al*., 1998 |
| *Solanum capsicastrum* | | | | Sweden | Olsson, 1976 |
| *Solanum carolinense* L. | Horse nettle | | | United States | Hayward 1991 |
| *Solanum cinereum* | | 2 | 3 | Australia | Graham & Lloyd, 1979 |
| *Solanum dulcamara* | Bitter nightshade | 2 | 3 | Sweden | Olsson, 1976 |
| | | | | Netherlands | Janse *et al*., 1998 |
| | | | | France | Hayward *et al*., 1998 |
| | | | | U.K | Elphinstone *et al*. 1998 |
| *Solanum hirtum* | | | 2 | Honduras | Berg, 1971 |
| *Solanum incanum l.* | | | | India | Shekhawat *et al*., 1992 |
| *Solanum jamaicense* Miller | | | | Philippines | Valdez, 1986 |

## Weed Hosts

| Scientific Name | Common Name | Biovar cited | Race cited | Country of Report | Literature Reference |
|---|---|---|---|---|---|
| *Solanum khasianum* | | 3 | | Indonesia | Machmud, 1987 |
| *Solanum khasianum* Clarke | | | | India | Shekhawat *et al.*, 1992 |
| *Solanum luteum Mill.* | | | | Sweden | Olsson, 1976 |
| *Solanum muskiana l.* | | | | India | Shekhawat *et al.*, 1992 |
| *Solanum sarrachoides* | | 2 | 3 | Chile | Fernandez, 1986 |
| *Solanum sisymbriifolium* | Dense-thorned bitter apple | | | Brazil | Mariano, 1998 |
| *Solanum umbellatum* | | | 2 | Honduras | Berg, 1971 |
| *Solanum verbascifolium* | | | 2 | Honduras | Berg, 1971 |
| *Solanum xanthocarpum* | | | | India | Shekhawat *et al.*, 1992 |
| *Solaum indicum L.* | | | | India | Shekhawat *et al.*, 1992 |
| *Sonchus arvensis* | | | | India | Shekhawat *et al.*, 1992 |
| *Spergula arvensis* L. | Corn spurry | | 3 | India<br>Uganda | Shekhawat *et al.*, 1992<br>Tusiime *et al.*, 1998 |
| *Spigela anthelmia* | | | | Inonesia | Machmud, 1986 |
| *Stachytarphita jamaicensis* L. | Snake weed | | | Australia | Akiew *et al.*, 1993 |
| *Stellaria sennii* | | | | Uganda | Tusiime *et al.*, 1998 |
| *Stellaria media* | | 2<br>2 | <br>3 | Nepal<br>Netherlands | Pradhanang, 1999<br>Van Beuningen *et al.*, 1998 |
| *Synedrella nodiflora* Gaertn. | Pig's weed | | | Malaysia | Abdullah, 1993 |
| *Tagetes minuta* | | | | Australia<br>Uganda | Akiew *et al.*, 1993<br>Tusiime *et al.*, 1998 |
| *Tagetes* sp. | | <br><br>3 | | India<br>Philippines<br>Nepal | Shekhawat *et al.*, 1992<br>Persley *et al.*, 1986<br>Adhikari, 1993 |
| *Thaliana sp.* | | | | India | Kishore *et al.*, 1993 |
| *Thalictum javanicum* | | | | India | Kishore *et al.*, 1993 |
| *Trifolium sp.* | | | | India | Kishore *et al.*, 1993 |
| *Tropaelum lobbianum* Hort. | | | | India | Shekhawat *et al.*, 1992 |
| *Tropaeolum majus* L. | Garden nasturtium | | | R.S.A<br>Sweden | Swanepoel, 1992<br>Olsson, 1976 |
| *Urtica dioeca* L. | Bush stinging nettle | | <br>1 | India,<br>Sweden<br>Columbia | Shekhawat *et al.*, 1992<br>Olsson, 1976 |
| *Valeriana hardwickii* | | | | India | Kishore *et al.*, 1993 |
| *Verbesina alata* L. | | | | | Kelman, 1953 |
| *Vernonia chinense* Mill. | | | | | Kelman, 1953 |
| *Vernonia cinerea* | | 3 | | Thailand | Titatarn, 1986 |
| *Vicia sp.* | | | | India | Kishore *et al.*, 1993 |
| *Xanthium chinense* Mill. | Cocklebur | | | | Kelman, 1953 |
| *Xanthosomas roseum* (s) | | | 2 | Honduras | Berg, 1971 |

## Tree and Shrub Hosts:

| Scientific Name | Common Name | Biovar cited | Race cited | Country of Report | Literature Reference |
|---|---|---|---|---|---|
| *Aleurites moluccana* (L.) Willd. | Candlenut | | | | Kelman, 1953 |
| *Aleurites* sp. | Tung oil tree | | | | Kelman, 1953 |
| *Anacardium occidentale* L. | Cashew | 3 | | Indonesia, Réunion | Hayward, 1994 |
| *Annona* L. spp. | Custard apple | 3<br><br>3 | 1 | Australia<br><br>Taiwan | Mayers & Hutton, 1987, Hayward, 1986<br>Hayward, 1994 |
| *Archontophoenix alexandrae* | Alexandra palm | 3 | | Australia | Arnat *et al.*, 1978 |
| *Azadirachta indica* J.Juss. | Neem tree | 3 | | Australia | Hayward, 1994 |
| *Carica papaya* | Papaya | | | India | Shekhawat *et al.*, 1992 |
| *Casuarina equisetifolia* L. | Horsetail beefwood/casuarina | 3<br><br>3,4 | 1 | China<br>India<br>China, India, Mauritius | He, 1983<br>Shekhawat *et al.*, 1992<br>Hayward, 1994 |
| *Cyphomandra betacea* | Tree tomato | 2<br>1,2,3 | 3 | Peru<br>Peru<br><br>Réunion | Martin & Nydegger, 1982<br>Marín & El-Nashaar, 1993<br>Girard *et al.*, 1993 |
| *Diospyros digyna* Jacq. | Black sapote | 3 | | Australia | Hayward, 1994 |
| *Eucalyptus* L'Her. spp. | Eucalyptus | 1<br>3 | | Brazil,<br>China, | Hayward, 1994<br>Dianese & Dristig, 1993 |



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| Tree and Shrub Hosts: | | | | | |
|---|---|---|---|---|---|
| **Scientific Name** | **Common Name** | **Biovar cited** | **Race cited** | **Country of Report** | **Literature Reference** |
| | | 3 | | Australia | Hayward, 1994 |
| *Eucalyptus grandis* | | | | Brazil | Mariano, 1998 |
| *Eucalyptus urophylla* | | | | Brazil | Mariano, 1998 |
| *Eugenia javanica* Lam. | Java/wax apple | | | Taiwan | Hayward, 1994 |
| *Manihot esculenta* Crantz | Cassava | 3,4 | | Indonesia | Machmud, 1986 |
| *Manihot glaziovii* M. Arg. | Ceara rubber | | | | Kelman, 1953 |
| *Moring oleifera* Lam. | Horse radish tree | | | India | Estelitta *et al.*, 1997 |
| *Morus alba* L. | Mulberry | 5<br>3,5 | 4 | China<br>India | He, 1983<br>Mathew *et al.*, 1993 (b) |
| *Musa* spp. | Banana | <br>1<br><br><br><br><br>1<br><br><br>3 | 2<br>1<br>2<br><br><br><br><br><br><br>1 | Honduras<br>Philippines<br>Costa Rica, Venezuela,<br>Honduras<br>India<br>Malaysia<br>Indonesia<br>Brazil<br>Australia<br>Trinidad | Woods, 1984<br>Soguilon *et al.*, 1994<br>French & Sequeira, 1970<br>French & Sequeira, 1970<br>Shekhawat *et al.*, 1992<br>Abdullah, 1993<br>Black & Sweetmore, 1993<br>Mariano, 1998<br>Akiew & Trevorrow,1994<br>Black & Sweetmore, 1993 |
| *Myristica fragrans* L. | Nutmeg | | | India | Mathew *et al.*, 1993 (a) |
| *Olea europaea* L. | Olive | 3,4 | 1 | China | He, 1983 |
| *Plantago* sp. | Plantain | 1 | | Costa Rica | Martin & Nydegger, 1982 |
| *Pluchea indica* Less. | (type of Indonesian shrub) | | | India | Shekhawat *et al.*, 1992 |
| *Pogostemen patchouli L.* | Patchouli (used for essential oils) | | | India | Mathew *et al.*, 1994 |
| *Schinus terebinthifolius* | Pepper tree | 3 | | Réunion | Hayward, 1994 |
| *Syzigium aromaticum* | Clove tree | 3 | | Indonesia | Machmud, 1987 |
| *Tectona grandis* L. | Teak | 3 | | Malaysia, Indonesia | Hayward, 1994 |

| Leguminous Hosts: | | | | | |
|---|---|---|---|---|---|
| **Scientific Name** | **Common Name** | **Biovar cited** | **Race cited** | **Country of Report** | **Literature Reference** |
| | Snapbean | 3 | | Philippines | Valdez, 1986 |
| | Stringbean | 2 | | Philippines | Valdez, 1986 |
| *Albizzia falcata* Back. | | | | | Kelman, 1953 |
| *Arachis hypogaea* (L.) | Groundnut | 3,4<br>3,4,<br><br><br><br><br><br><br><br>3 | 1 | China<br>Vietnam, Hawaii,<br>Australia, Philippines,<br>Papua New Guinea,<br>Thailand, R.S.A,<br>Réunion,<br>Indonesia<br>Uganda etc.<br>India<br>Malaysia<br>Andaman & Nicobar Islands<br>Brazil | He, 1983<br>Persley *et al.*, 1986<br>Persley *et al.*, 1986<br>Persley *et al.*, 1986<br>Persley *et al.*, 1986<br>Persley *et al.*, 1986<br><br>Shekhawat *et al.*, 1992<br>Abdullah, 1993<br>Black & Sweetmore, 1993<br>Ramesh & Bandyopadhyay, 1993<br>Mariano, 1998 |
| *Canavallia ensiformis* DC. | Jack bean | | | | Kelman, 1953 |
| *Cassia tora* L. | Cassia/Senna | | | India | Shekhawat *et al.*, 1992 |
| *Cyamopsis speciousus* (sic.) | | | | | Kelman, 1953 |
| *Desmodium diffusum* | | | | India | Shekhawat *et al.*, 1992 |
| *Dolichos lablab* L. | Indian bean/Lablab bean | | | India | Shekhawat *et al.*, 1992 |
| *Glycine max* L. | Soybean | | | India<br>Vietnam<br>Sweden | Shekhawat *et al.*, 1992<br>Persley *et al.*, 1986<br>Olsson, 1976 |
| *Indigoferra arrecta* Hochst. | Natal indigo | | | | Kelman, 1953 |
| *Leucaena glauca* | Leucaena | | | | Kelman, 1953 |
| *Melilotus indica* | | | | India | Shekhawat *et al.*, 1992 |
| *Mucuna* sp. (*capitata*) | Mucana (vegetable) | | | | Kelman, 1953 |
| *Phaseolus aureus* Roxb. | | | | Malaysia | Abdullah, 1993 |
| *Phaseolus calcaratus* Roxb. | Rice bean | | | | Kelman, 1953 |
| *Phaseolus coccineus* L. | Scarlet runner bean | | | | Kelman, 1953 |
| *Phaseolus mungo* L. | Black gram | | | | Kelman, 1953 |

## Leguminous Hosts:

| Scientific Name | Common Name | Biovar cited | Race cited | Country of Report | Literature Reference |
|---|---|---|---|---|---|
| *Phaseolus vulgaris* | Bean (French/kidney)<br>(Yellow wax bean) | <br>3,4<br>3<br><br>1,3 | | India<br>Malaysia,<br>Uganda<br>Réunion,<br>Brazil<br>Sweden<br>Colombia | Shekhawat *et al.*, 1992<br>Hayward, 1994<br>Hayward, 1994<br>Girard *et al.*, 1993<br>Melo & Takatsu, 1997<br>Olsson, 1976<br>Thurston, 1963 |
| *Phaseolus vulgaris* L. var. *humulis* | bushbean | | | Sri Lanka | Velupillai, 1986 |
| *Pisum savitum* L. | Garden Pea | | | | Kelman, 1953 |
| *Psophocarpus tetragonolobus* | Winged bean | 3<br>3 | | Malaysia<br>Philippines<br>Sri Lanka<br>India | Hayward, 1986<br>Valdez, 1986<br>Velupillai, 1986<br>Shekhawat *et al.*, 1992 |
| *Sesbania bispinosa* | | | | India | Shekhawat *et al.*, 1992 |
| *Sesbania grandiflora* Pers. | | | | | Kelman, 1953 |
| *Sesbania rostrata* | | 4 | | Malaysia | Hayward, 1994 |
| *Stylosanthes humulis* HBK | Townsville lucerne | | | Australia | Hayward, 1986 |
| *Tephrosia vogellii* Hook. | Vogel tephrossia | | | | Kelman, 1953 |
| *Teraminus labialis* Spreng. | | | | India | Shekhawat *et al.*, 1992 |
| *Vicia faba* | Broad bean | | | India<br>Sweden | Persley *et al.*, 1986<br>Olsson, 1976 |
| *Vignia sinensis* Savi | Cowpea, Long bean | 1,2,3 | | Brazil<br>India,<br>Philippines<br>Malaysia | Melo & Takatsu, 1997<br>Hayward, 1994<br>Persley *et al.*, 1986<br>Abdullah, 1993 |
| *Vignia ungiculata* | | | | India<br>Brazil | Shekhawat *et al.*, 1992<br>Mariano, 1998 |
| *Voandzeia subterranea* Thou. | Earthpea | | | | Kelman, 1953 |

## Ornamental Hosts:

| Scientific Name | Common Name | Biovar cited | Race cited | Country of Report | Literature Reference |
|---|---|---|---|---|---|
| *Anthurium andreanum* | Anthurium | <br><br>3 | <br><br>1 | Sri Lanka<br>Réunion, Australia,<br>Mauritius | Hayward, 1986<br>Hayward, 1994<br>Banymandhub-Munbodh *et al.*, 1998 |
| *Antirhinum* sp. | Snapdragon | <br>3 | <br>1 | India<br>Australia | Shekhawat *et al.*, 1992<br>Akiew & Trevorrow, 1994 |
| *Argemone mexicana* L. | Mexican poppy | | | India | Shekhawat *et al.*, 1992 |
| *Aster chinensis L.* | | | | India | Shekhawat *et al.*, 1992 |
| *Aster pilosus* Willd. | Aster | | | | Kelman, 1953 |
| *Canna glauca* | Canna | | | India | Kelman, 1953 |
| *Canna indica* | | | | India | Shekhawat *et al.*, 1992 |
| *Centaurea cyanus* L. | Cornflower | | | India | Shekhawat *et al.*, 1992 |
| *Crysanthemum coronarium* | | | | Philippines | Valdez, 1986 |
| *Crysanthemum indicum* | | | | India | Shekhawat *et al.*, 1992 |
| *Crysanthemum* spp. | Crysanthemum | | | India | Shekhawat *et al.*, 1992 |
| *Dahlia coccinea L.* | | | | Cuba | Stefanova, 1998 |
| *Dahlia pinata* | | | | Brazil | Mariano, 1998 |
| *Dahlia rosea* Cav. | Dahlia | | | India<br>Malaysia | Shekhawat *et al.*, 1992<br>Abdullah, 1993 |
| *Dahlia* spp. | Dahlia | | | R.S.A | Swanepoel, 1992 |
| *Dahlia variabilis* | | | | India | Shekhawat *et al.*, 1992 |
| *Ensete ventricosum* | Ornamental banana | 3 | 1 | French West Indies | Prior & Steva, 1990 |
| *Euphorbia pulcherrima* | Poinsettia | | | Réunion | Girard *et al.*, 1993 |
| *Eustoma grandiflora* | Texas blue bell | 4 | 1 | Taiwan | Chao *et al.*, 1995 |
| *Gerbera* spp. | Barbaton daisy | | | | Kelman, 1953 |
| *Hedychium Koenig* spp. | Ornamental ginger | | | Hawaii | Hayward, 1994 |
| *Helychrysum bracteatum* Andr. | | | | Cuba | Stefanova, 1998 |
| *Helinconia* | Helinconia | <br>1,3 | 2<br>2,1 | Costa Rica, Columbia<br>Australia,<br><br>Hawaii | French & Sequeira, 1970<br>Akiew & Trevorrow, 1994<br>Hayward, 1994 |
| *Helinconia caribaea* Lam. | | | | Costa Rica | Hayward, 1994 |

## Ornamental Hosts:

| Scientific Name | Common Name | Biovar cited | Race cited | Country of Report | Literature Reference |
|---|---|---|---|---|---|
| *Helinconia latispatha* Benth. | | | | Columbia | Hayward, 1994 |
| *Impatiens balsamina* | Garden balsam | | | India | Shekhawat *et al.*, 1992 |
| *Nasturtium officinale* | | | | Brazil | Mariano, 1998 |
| *Pelagornium capitatum* | Rose geranium | | 1 | Réunion | Girard *et al.*, 1993 |
| *Pelagornium x asperum* | Rose geranium | 1 | | Réunion | Girard *et al.*, 1993 |
| *Pelargonium hortorum* | Florist geranium | 1 | | U.S.A<br>Australia | Strider *et al.*, 1981<br>Strider *et al.*, 1981 |
| *Petunia hybrida* | Garden petunia | | | India | Shekhawat *et al.*, 1992 |
| *Petunia* spp. | Petunia | | | Malaysia | Abdullah, 1993 |
| *Phlox drummondii* Hook | Phlox | | | Malaysia | Abdullah, 1993 |
| *Salvia farinacea* Benth. | Blue salvia | | | Malaysia | Abdullah, 1993 |
| *Strelitzia reginae* Banks | Bird of Paradise | 3 | 1 | Hawaii<br>Réunion, Hawaii, Japan, Taiwan, Australia | Hayward, 1994<br>Hayward, 1994<br>Hayward, 1994 |
| *Tagetea erecta* L. | African marigold | 1,3 | | India | Shekhawat *et al.*, 1992 |
| *Vinca rosea* L. | Madagascar periwinkle | | | India | Shekhawat *et al.*, 1992 |
| *Zinnia elegans* Jacq. | | | | India<br>Malaysia | Shekhawat *et al.*, 1992<br>Abdullah, 1993 |
| *Zinnia* sp. | Zinnia | | | India | Shekhawat *et al.*, 1992 |

## Other Crop Hosts:

| Scientific Name | Common Name | Biovar cited | Race cited | Country of Report | Literature Reference |
|---|---|---|---|---|---|
| | Chinese cabbage | 3 | | Philppines | Valdez, 1986 |
| | Sweet pepper | 2,3,4 | 1 | Japan | Tsuchiya & Horita, 1998 |
| *Allium cepa* | Onion | | | Réunion, Venezuela | Hayward, 1994 |
| *Avena savita* L. | Oats | | | India | Shekhawat *et al.*, 1992 |
| *Beta vulgaris* L. | Beetroot | 1,3 | | India<br>Brazil | Shekhawat *et al.*, 1992<br>Melo & Takatsu, 1997 |
| *Brassica napus* L.v. *napus* | Kohlrabi | | | Sweden,<br>India | Olsson, 1976<br>Shekhawat *et al.*, 1992 |
| *Brassica oleracea* var. *capitata* | Cabbage | 3<br>1,3 | | India<br>Brazil | Shekhawat *et al.*, 1992<br>Melo & Takatsu, 1997 |
| *Capsicum annum* L. | Bell pepper, chillies | 3<br>3<br>3<br>3,4<br><br><br>2,3 | 1<br><br>1<br><br><br><br>1,3 | French West Indies<br>Papua New Guinea<br>China<br>Philippines<br>Thailand<br>India<br>R.S.A<br>Réunion,<br>Andaman & Nicobar Islands<br>Brazil<br>Peru | Prior & Steva, 1990<br>Tomlinson, 1985<br>He, 1983<br>Valdez, 1986<br>Titatarn, 1986<br>Shekhawat *et al.*, 1992<br>Swanepoel, 1992<br>Girard *et al.*, 1993<br>Ramesh & Bandyopadhyay, 1993<br>Mariano, 1998<br>Martin & French, 1995 |
| *Capsicum frutescens* | Chillies<br>(Bell pepper) | 3 | | Réunion<br>Brazil<br>Colombia | Girard *et al.*, 1993<br>Mariano, 1998<br>Thurston, 1963 |
| *Capsicum pendulum* (s) | Pepper | | | India | Shekhawat *et al.*, 1992 |
| *Capsicum* sp. | | | | Sweden<br>Costa Rica | Olsson, 1976<br>Black & Sweetmore, 1993 |
| *Centella asiatica* | Indian pennywort | 3 | 1 | Sri Lanka | De Zoysa & Liyanage, 1994 |
| *Cichorium endivia* | Chicory | 1,3 | | Brazil | Melo & Takatsu, 1997 |
| *Citrullus vulgaris* Schrad. | Watermelon | | | India | Shekhawat *et al.*, 1992 |
| *Commelina nudiflora* L. | Baby dewflower | | | | Kelman, 1953 |
| *Coriandrum savitum* | Coriander | 1 | | Brazil | Melo & Takatsu, 1997 |
| *Cucumis savitus* | Cucumber | | | Japan | Hayward, 1994 |
| *Curcuma domestica* | Tumeric | | | Sri Lanka | Velupillai, 1986 |
| *Curcuma longa* L. | Tumeric | | | India<br>Sri Lanka | Shekhawat *et al.*, 1992<br>Hayward, 1994 |
| Curcurbita maxima x C. moschata | Pumpkin | 4 | 1 | Japan | Tsuchiya & Horita, 1998 |
| Curcurbita moschata Poit | Pumpkin | | | India | Mathew *et al.*, 1994b |

## Other Crop Hosts:

| Scientific Name | Common Name | Biovar cited | Race cited | Country of Report | Literature Reference |
|---|---|---|---|---|---|
| *Cucurbita pepo* var. *melopepo* | Zucchini | 1,2,3 | | Brazil | Melo & Takatsu, 1997 |
| *Daucus carota* | Carrot | 1,3 | | Brazil | Melo & Takatsu, 1997 |
| *Fragaria* L. spp | Strawberry | 3,4 | 1 | Japan, Taiwan | Hayward, 1994 |
| *Gossipium* sp. | Cotton | | | India | Shekhawat *et al.*, 1992 |
| *Helianthus annus* L. | Sunflower | | | India | Shekhawat *et al.*, 1992 |
| | | | | Malaysia | Abdullah, 1993 |
| | | | | Cuba | Stefanova, 1998 |
| *Hibiscus cannabinus* | Indian hemp | | | Malaysia | Abdullah, 1993 |
| *Hibiscus esculentus* L. | Okra | | | India | Shekhawat *et al.*, 1992 |
| *Hibiscus sabdariffa* L. | Roselle | | | | Kelman, 1953 |
| *Kaempferia galanta* | Medicinal plant | 4 | | China | He, 1986 |
| *Luffa cylindrica* | Loofah | 3 | 1 | Taiwan | Pan *et al.*, 1996 |
| *Lycopersicum chilense* | | | | Sweden | Olsson, 1976 |
| *Lycopersicum esculentum* | Tomato | 3,4 | | Papua New Guinea | Tomlinson, 1985 |
| | | | | India, Vietnam, Fiji, | Persley *et al.*, 1986 |
| | | | | China | Persley *et al.*, 1986 |
| | | | | Sri Lanka, Thailand etc. | Persley *et al.*, 1986 |
| | | 3 | 1 | R.S.A | Engelbrecht & Hattingh, 1989 |
| | | 4 | 1 | China | He, 1983 |
| | | 1,3,4 | | Philippines | Valdez, 1986 |
| | | 3 | | Nepal | Adhikari, 1993 |
| | | | | Malaysia | Abdullah, 1993 |
| | | 1,2 | 1,3 | Réunion | Girard *et al.*, 1993 |
| | | | | Andaman & Nicobar Islands | Ramesh & Bandyopadhyay, 1993 |
| | | 1,2,3,4 | 1 | Japan | Tsuchiya & Horita, 1998 |
| | | | | Brazil | Mariano, 1998 |
| | | | | Sweden | Olsson, 1976 |
| | | 2 | 3 | Peru | Martin & French, 1995 |
| | | 1,2,3 | | Peru | Marín & El-Nashaar, 1993 |
| | | | | Colombia | Thurston, 1963 |
| *Maranta arundinacea* L. | | | | Malaysia | Abdullah, 1993 |
| *Nicotiana tabacum* | Tobacco | | | Thailand | Titatarn, 1986 |
| | | | | Vietnam | French & Sequeira, 1970 |
| | | 3 | | Nepal | Adhikari, 1993 |
| | | | | Réunion, | Girard *et al.*, 1993 |
| | | | | Malaysia | Abdullah, 1993 |
| | | | | Andaman & Nicobar Islands | Ramesh & Bandyopadhyay, 1993 |
| | | 2,3 | 1 | Japan | Tsuchiya & Horita, 1998 |
| | | | | Brazil | Mariano, 1998 |
| | | 1 | | Colombia | Black & Sweetmore, 1993 |
| | | 1,3,4 | | Australia, | Swanepoel, & Young,1988 |
| | | 3 | 2 | R.S.A, | Black & Sweetmore, 1993 |
| | | 4 | | U.S.A, | Marín & El-Nashaar, 1993 |
| | | 1,2,3 | | Peru | Black & Sweetmore, 1993 |
| *Perilla crispa* | Perilla | 3 | | Taiwan | Hayward, 1994 |
| *Perilla ocymoides L.* | Perilla | | | Japan | Tsuchiya & Horita, 1998 |
| *Petroselium crispum* | Parsley | 1,3 | | Brazil | Melo & Takatsu, 1997 |
| *Piper hispidinervium* | Long pepper | 1 | | Brazil | Lopes *et al.*, 1998 |
| *Pogostemon cablin* | Medicinal plant | 3 | | China | He, 1986 |
| *Pomoea batatus* LAM. | Sweet potato | 4 | 1 | China | He, 1983 |
| *Raphanus sativa* L. | Radish | | | Taiwan, | Hayward, 1994 |
| | | | | India | Shekhawat *et al.*, 1992 |
| *Sesamum indicum* | Sesame, gingelly | 3 | 1 | China | He, 1983 |
| | | | | Sri Lanka | Velupillai, 1986 |
| | | | | Thailand | Titatarn, 1986 |
| | | | | India | Shekhawat *et al.*, 1992 |
| | | | | Andaman & Nicobar Islands | Ramesh & Bandyopadhyay, 1993 |
| *Setaria italica* Beauv. | Indian millet | | | India | Shekhawat *et al.*, 1992 |
| *Solanum auriculatum* | Bringellier marron | | | Réunion | Girard *et al.*, 1993 |
| *Solanum capsicastrum* | | | | Sweden | Olsson, 1976 |
| *Solanum gilo* | (Nigerian vegetable) | | | Brazil | Mariano, 1998 |

| Other Crop Hosts: | | | | | |
|---|---|---|---|---|---|
| **Scientific Name** | **Common Name** | **Biovar cited** | **Race cited** | **Country of Report** | **Literature Reference** |
| *Solanum melangena* | Eggplant | 3 | | Australia | Moffet *et al.*, 1983, |
| | | | | R.S.A | Engelbrecht & Hattingh, 1989 |
| | | 3 | 1 | Papua New Guinea | Tomlinson, 1985 |
| | | 4 | | China | He, 1983 |
| | | 1,3,4 | | Philippines | Valdez, 1986 |
| | | | | Thailand | Titatarn, 1986 |
| | | 3 | | Nepal | Adhikari, 1993 |
| | | | | India | Shekhawat *et al.*, 1992 |
| | | | | Malaysia | Abdullah, 1993 |
| | | | | Réunion | Girard *et al.*, 1993 |
| | | | 1 | Andaman & Nicobar Islands | Ramesh & Bandyopadhyay, 1993 |
| | | 2,3,4 | | Japan | Tsuchiya & Horita, 1998 |
| | | | | Brazil | Mariano, 1998 |
| | | | | Sweden | Olsson, 1976 |
| | | 1,2,3 | | Peru | Marín & El-Nashaar, 1993 |
| *Solanum tuberosum* | Potato | 2,3,4 | | Philippines | Valdez, 1986 |
| | | 2 | | Nepal | Adhikari, 1993 |
| | | | | Malaysia | Abdullah, 1993 |
| | | 1,2 | 1,3 | Réunion | Girard *et al.*, 1993 |
| | | | | Andaman & Nicobar Islands | Ramesh & Bandyopadhyay, 1993 |
| | | 1,2 | | Bolivia | Smith *et al.*, 1998 |
| | | 2,4 | 3,1 | Japan | Tsuchiya & Horita, 1998 |
| | | | | Brazil | Mariano, 1998 |
| | | | | U.K., Netherlands, Italy | Hayward *et al.*, 1998 |
| | | | | France, Latin American | Hayward *et al.*, 1998 |
| | | | | countries, Australia | Hayward *et al.*, 1998 |
| | | | | Sweden | Olsson, 1976 |
| | | 2,4 | | Papua New Guinea | Tomlinson, 1985 |
| | | 1,2,3 | | Peru | Marín & El-Nashaar, 1993 |
| | | 2,3 | | South Africa | Swanepoel & Young, 1988 |
| | | 2,3 | | Australia | Black & Sweetmore, 1993 |
| | | 1 | | USA | Black & Sweetmore, 1993 |
| | | 1 | | Indonesia | Black & Sweetmore, 1993 |
| *Symphytum sp.* | Forage crop | 3 | | China | He, 1986 |
| *Tetragonia expansa* | Spinach (N. Zealand) | 1,3 | | Brazil | Melo & Takatsu, 1997 |
| *Zingiber officinale* | Ginger | 4 | | Costa Rica | Martin & Nydegger, 1982 |
| | | 3 | | Mauritius | Hayward, 1986 |
| | | 4 | 1 | China | He, 1983 |
| | | 3,4 | | China | Hayward, 1986 |
| | | 1,3,4 | | Malaysia | Hayward, 1986 |
| | | 3,4 | | Australia | Hayward, 1986 |
| | | 4 | | Hawaii , Philippines | Hayward, 1986 |
| | | 3 | | India | Hayward, 1986 |
| | | | | Sri Lanka | Velupillai, 1986 |
| | | 3,4 | | Thailand | Hayward, 1994 |
| | | | | India | Shekhawat *et al.*, 1992 |
| | | | | Andaman & Nicobar Islands | Ramesh & Bandyopadhyay, 1993 |

| Other plant species | | | | | |
|---|---|---|---|---|---|
| **Scientific Name** | **Common Name** | **Biovar cited** | **Race cited** | **Country of Report** | **Literature Reference** |
| | Squash | 3 | | Philippines | Valdez, 1986 |
| | Fennel | 4 | | Philippines | Valdez, 1986 |
| | Pechay | 3 | | Philippines | Valdez, 1986 |
| | Plantain | | 2 | Honduras, Colombia,<br>Peru, Costa Rica | French & Sequeira, 1970<br>French & Sequeira, 1970 |
| *Hyoscyamus niger L. V. niger* | | | | Sweden | Olsson, 1976 |
| *Hyoscyamus niger L. V pallidus* | | | | Sweden | Olsson, 1976 |
| *Epipremun aureum* | pathos | 1 | 1 | USA | Valdez, 1986, Norman & Yuen, 1998 |
| *Alpinia* L. spp. | | 3 | | Australia | Hayward, 1994 |
| *Blainvillea rhomboidea* | | | | Brazil | Mariano, 1998 |
| *Browallia speciosa* Major | | | | India | Shekhawat *et al.*, 1992 |

| Other plant species | | | | | |
|---|---|---|---|---|---|
| **Scientific Name** | **Common Name** | **Biovar cited** | **Race cited** | **Country of Report** | **Literature Reference** |
| *Corchorus capsularis* | Jute | | | West Bengal | Hayward, 1986 |
| *Corchorus olitorius* | Jute | | | West Bengal | Hayward, 1986 |
| *Corchorus spp.* | | | | India | Shekhawat *et al.*, 1992 |
| *Corchorus trilocularis* | | | | India | Shekhawat *et al.*, 1992 |
| *Coriandrum savitum* | | | | India | Shekhawat *et al.*, 1992 |
| *Cosmos caudatus* | | | | Sarawak | Hayward, 1986 |
| *Cu,inum cyminum* | | | | India | Shekhawat *et al.*, 1992 |
| *Echinochloa crusgalli* Beauv. | | | | India | Shekhawat *et al.*, 1992 |
| *Eleusine coracana* Gaerlin. | | | | India | |
| *Eustoma russellianum* | Russel prairie gentian | | | Japan | Tsuchiya & Horita, 1998 |
| *Foeniculum vulgare* | | | | India | Shekhawat *et al.*, 1992 |
| *Galium aparine L.* | | | | India | Shekhawat *et al.*, 1992 |
| *Heliotropium indicum* | | | | India | Shekhawat *et al.*, 1992 |
| *Hyptis suaveolens (*L.) Poit. | | | | India<br>Brazil | Shekhawat *et al.*, 1992<br>Mariano, 1998 |
| *Ipomea obscura* Ker. | | | | India | Shekhawat *et al.*, 1992 |
| *Ipomoea setosa* | | | | Malaysia | Hayward, 1986 |
| *Kalanchoe blossfeldiana* v. Paellnitz | | | | Réunion | Hayward, 1994 |
| *Kalanchoe tubiflora* | | | | Brazil | Mariano, 1998 |
| *Launea aspleniifolia* DC | | | | India | Shekhawat *et al.*, 1992 |
| *Limonium spp.* | Statice | 3 | 1 | Japan | Tsuchiya & Horita, 1998 |
| *Ludwiga suffruticosa* | | | | Brazil | Mariano, 1998 |
| *Marsypianthes chamaedrys* | | | | Brazil | Mariano, 1998 |
| *Mimosa scabrella* | | | | Brazil | Mariano, 1998 |
| *Momordica charantia* | Bitter Gourd | 1,2,3 | | Philippines<br>Sri Lanka | Valdez, 1986<br>Velupillai, 1986 |
| *Obetia ficifolia* | Bois d'ortie | | | Réunion | Girard *et al.*, 1993 |
| | | | | | |
| *Parthenium hysterophorus* | | | | India | Shekhawat *et al.*, 1992 |
| *Solanum americanum* | | | | Brazil | Mariano, 1998 |
| *Trachyspermum amni* | | | | India | Shekhawat *et al.*, 1992 |
| *Urtica nivea* | | 3 | 1 | China | He, 1983 |
| *Verbena hybrida* | | | | India | Shekhawat *et al.*, 1992 |

# SOURCES OF INOCULUM AND MODES OF DISPERSAL

Two major sources of inoculum exist, namely infected planting material and infested soil. Infected plants decaying in the soil can release masses of bacterial cells in a slime layer. These slime masses can adhere to soil particles and form pellets enhancing its survival (Shekhawat *et al*., 1992). The populations in the soil can then increase or decrease depending on the presence of alternative hosts and cultural practices. The inoculum threshold for initiating disease depends highly on predisposing factors. Devi *et al*. (1982) observed that the inoculum reached a threshold of about $10^7$ cfu / g soil before infection started.

Infected planting material such as potato tubers is the most effective source of inoculum and means of dispersal. Since the pathogen can be carried latently within tubers, controlling the transmission of this pathogen is complicated. Tubers can carry the bacteria in three manners, namely externally on tuber surfaces, in lenticels and in the vascular tissues (Shekhawat *et al.*, 1992). Although surface carried bacteria can be eliminated by chemical treatments, internal infections remain a threat. A study conducted by Sunaina *et al.* (1989) showed that during storage, bacterial populations decreased rapidly on the tuber surface reaching a non-detectable limit within 30-60 days at 4°C and 60-90 days at room temperature. But in lenticels and vascular tissues *R. solanacearum* could still be detected after 240 days.

Irrigation water, root contact and insects such as in the case of Moko disease of banana can also spread the disease. Mechanical dissemination occurs mainly by infested equipment both during sorting of seed tubers as well as on the field, and by movement of people and animals through infected fields. Insect dissemination plays an important role in the banana industry and has been reported in Honduras, Costa Rica and Colombia. Bees (*Trigona* spp.), wasps (*Polybia* spp.), fruit flies (*Drosophila* spp.), and flies of other genera have been identified as transmitters (Buddenhagen & Kelman, 1964). Reports where *R. solanacearum* was disseminated by chewing insects on potato (Colorado potato beetle, *Leptinotarsa declimlineata* Say.) and eggplants (green beetle, *Diabrotica graminea* Baly) have been made (Kelman, 1953). Negative results have however also been reported where insects were allowed to feed on infected plants and then on healthy ones (Kelman 1953).

Some evidence suggests that *R. solanacearum* can have an epiphytic phase in its life cycle which contributes to its survival and provides another source of inoculum. Hayward & Moffet (1978) demonstrated that leaf spot disease of capsicum was caused by *R. solanacearum*. Further studies (Moffet *et al*, 1983) revealed that under conditions with relatively high humidity, epiphytic colonisation could occur, leading to the formation of lesions on leaves. Mist inoculation of eggplant, pepper and tomato cultivated in growth-chambers caused leaf spots and wilting (Kelman *et al.,* 1994). Although leaf infection has been reported, there is no evidence that the pathogen can survive as an epiphyte on leaf and other plant surfaces (Kelman *et al.,* 1994). Aerial

transmission through rain splash dispersal on tobacco has been noted in Japan (Hayward, 1991a).

Infected host debris is an important short-term shelter for *R. solanacearum* in soil (Graham *et al.*, 1979) allowing survival between growing seasons. It also serves as a transmission agent. This is especially so for race 3 which has a limited alternative host range.

Weeds serving as hosts are well-documented sources of inoculum and contribute greatly to the survival of *R. solanacearum* in the absence of a cultivated host. They may also serve as a source of infection when virgin lands are cleared for cultivation (Buddenhagen & Kelman, 1964, Martin *et al.*, 1981).

## PRESENCE OF RALSTONIA SOLANACEARUM IN VIRGIN SOILS

The occurrence of *R. solanacearum* in newly cleared lands or virgin soils has been cited in literature (Kelman, 1953; Sequeira & Averre, 1961; Martin *et al.*, 1981) and has been attributed to the presence of wild hosts in the indigenous flora. In earlier reports proof was not always presented that bacterial wilt was truly a natural component of the soil microflora, and the possibility of contamination through planting material, drainage water or other means was not eliminated. Several authorities have studied the outbreak of bacterial wilt of bananas in newly planted areas in Costa Rica. A comprehensive study conducted by Sequeira & Averre (1961) involving 20000 acres of virgin woodlands in Costa Rica revealed extensive infection of *Heliconia latispatha*, *H. acuminata*, *H. imbricata* with the banana strain (B strain) of *R. solanacearum*. *Eupatorium oderatum* was found to be infected with the weed strain of the pathogen (T strain). French *et al.* (1981) who summarised the findings of Buddenhagen (1960), Sequeira (1960), Sequeira & Averre (1961) and his own, refer to the strain causing rapid wilt of bananas as race 2, strain B and those causing slow wilt and distortion on helinconias as strain D of race 2. Repeated cutting back of

heliconias caused the disease to be spread mechanically to other clusters of heliconias. When bananas were planted in cleared jungle, they developed a slow wilt and distortion not typical of the banana strain.

In Costa Rica *Heliconia* were occasionally found diseased in virgin jungle showing distortion and slow wilt symptoms caused by D strains of race 2. Bananas planted in cleared jungle also developed distortion and slow wilt, symptoms not characteristic of the B strain wilt disease, which causes rapid wilting and was responsible for severe outbreaks. Continuous passage of the D strain through bananas resulted in a doubling of the disease index, leading to the conclusion that bacterial wilt of bananas arose by a selection pressure exerted by bananas upon strain D (French *et al.*, 1981).

Martin *et al.* (1981) found that biovar 1 (race 1) and biovar 2 (race 3) of the pathogen attacked potatoes grown in virgin soils in the Amazon basin. No potatoes or other wilt-susceptible crops had been planted before and infestation by contaminated water or by planting infected seed was excluded. This suggests that these strains were indigenous to the region.

Sneviratne (1969) found biovar 2 to occur in virgin soils at elevations of 1891m in Sri Lanka. He believes it unlikely that the disease could have been introduced by European seed material. In their study on persistence of *R. solanacearum* in soil in Georgia, Dukes *et al.* (1965) had cleared land of timber and brush and planted tomatoes and found a high incidence of bacterial wilt indicating that the organism was indigenous.

## SURVIVAL OF RALSTONIA SOLANACEARUM IN SOIL

*Ralstonia solanacearum* is a soil-borne pathogen that is found in various types of soils world-wide. Reports regarding its survival period are often conflicting. Bacterial wilt was found to survive in fallow soil for periods of 2 to 10 years, yet in a different soil poorer survival rates were reported despite the presence of host plants (Nesmith &

Jenkins, 1985). Information on soil survival was often gathered indirectly or from glasshouse trials, partly because detection of the organism in field soils is difficult (Moffet & Wood, 1984).

The survival of *R. solanacearum* in soil is affected by several factors such as the initial inoculum concentration, whether the land is left fallow or cropped to a non-susceptible host, as well as the biological, chemical and physical properties of the soil (Moffet *et al.*, 1983). The temperature, moisture and oxygen status of the soil is further factors that influence the longevity of the pathogen. Survival of *R. solanacearum* in soil can be measured on the basis of two parameters, namely, the ability to withstand soil conditions to remain viable and the segregation into virulent and avirulent populations (Shekhawat & Perombelon, 1991). Several authors, amongst others Nesmith & Jenkins (1983) measures survival in terms of detection of fluidal cells on selective media. These cells are referred to as virulent. Denny *et al.* (1994) reason, however, that in soil *Ralstonia* normally exits in the "avirulent" (phenotype conversion) form, in which reduced production of extracellular proteins and extracellular polysaccharides occurs. In this form the bacteria can conserve energy and cellular resources thereby increasing its chances of surviving. Once host material is available, bacteria multiply and once sufficient cell density is obtained the extracellular virulence factors are produced. This hypothesis would greatly affect measurement of survival rate and might explain some of the discrepancies regarding the longevity of bacterial wilt in soil.

## Influence of soil temperature

Temperature requirements for optimal growth are known to differ for the various strains. Biovar 2, race 3 isolates have a lower optimum growth temperature than strains of race 1 (Thurston, 1963). Disease development in terms of wilting and visible tuber infection, is known to occur at lower temperatures of 14/16 °C with biovar 2 than with biovar 3 (race 1) (Swanepoel 1990). Katayama & Kimura (1984) also found that at lower as well as intermediate temperatures of 24 °C, growth of biovar 2 (race 3) was better than that of race 1, biovar 4. Shekhawat & Perombelon

(1991) studied the survival rates of biovar 3 (race 1) and biovar 2 (race 3) at various temperatures and confirmed that race 1 is better adapted to a wider range of temperature for growth than race 3. In their study it appears that population decline and loss of virulence of both races was slowest between 10-30 °C, provided other soil factors were congenial. At 35 °C, race 1 population declined to an undetectable limit within 10 weeks, whereas populations of race 3 could not be detected after 8 weeks. At low temperature of 5 °C, population decline was the same for both races, reaching undetectable levels within 12 weeks. Granada & Sequeira (1983b) however reported that soil kept in plastic bags at 4 °C maintained bacterial wilt populations for 673 days. This indicates that long-term survival in deeper soil levels at low temperatures is possible. Soil temperatures of over 40 °C appear to be fatal to *Ralstonia* populations, depending on the period of exposure. Seneviratne (1988) found that the pathogen was able to survive in some of the soil samples kept at 40 °C for seven days, but not in those kept at 43 °C.

Survival of races 1, 2, 3 in soil kept at 28 °C and water potential of –2 bars varied with race 1 surviving longest. Race 2 and race 3 had shorter survival periods (Granada & Sequeira, 1983b).

## Influence of soil moisture

Soil moisture may influence at least four aspects of the bacterial wilt disease, namely the survival of the bacterium in its free state in soil, rate of infection, disease development after infection and spread through the soil. Native farmers in India have from an early date noted the relationship between soil moisture and bacterial wilt of tobacco and attributed the disease to high moisture levels (Shekhawat *et al.*, 1992).

Studies on the effect of soil moisture on the survival of *R. solanacearum* have provided conflicting evidence. This can partly be attributed to interactions with other soil factors. According to Shekhawat *et al.* (1992) soil moisture and temperature have a synergistic effect on disease development. High temperatures or high soil moisture alone will not induce symptoms. They found that in India, potato wilt was higher after

onset of the monsoon, even though high temperatures prevailed earlier in the season. Wilt incidence declined when soil moisture dropped to 8-10% of water holding capacity (WHC). Tanaka & Noda (1973) found that growth rate of *R. solanacearum* in sterile soil is higher at high soil moistures (80-100% water content) than in soil at 40% of water holding capacity. Under identical conditions but in non-sterilised soil no increase in growth was observed, regardless of water content.

Okabe (1971) found that *R. solanacearum* grew more actively in dry soil of 15-20% water content (WC) than in moist soil (40-50% WC) and reasoned that this pathogen had the specific nature to utilise small amounts of capillary water held among soil particles while growth of other micro-organisms was delayed. If, however, soil moisture were increased, re-growth of these micro-organisms would be stimulated leading to increased competitive colonisation of organic matter and antibiosis. This would reduce *R. solanacearum* populations. This theory could explain why Tanaka & Noda (1973) did not observe increase in growth in the non-sterilised soil at both moisture levels. Their moisture levels were higher than those investigated by Okabe (1971).

Shekhawat & Perombelon (1991) reported that population decline was at its lowest in a soil moisture at 60% of water holding capacity (WHC). Even after 13 weeks the pathogen could still be detected. A deviation in moisture led to an increased population decline. Greater population declines occurred at high moisture levels (80-100% of WHC) than at low moistures (20-40% of WHC). At the high moisture levels a shift towards avirulence was more pronounced when soil temperatures were low (5°C) (Shekhawat *et al.*, 1992). In dry soils the populations decreased to an undetectable limit within 6 weeks.

Hsu (1977) noted similar trends regarding high and low moisture contents. He reported a survival period of 30 days for dry soil (0.8-7.9% WC); 105 days for flooded soil; 150 days for very moist soil (43-47.4% WC); 225 days for moist soils (15.2-19.9% WC) and 390 days for moderately moist conditions (30.7-36.7% WC).

Moffet *et al.* (1983) performed a study in which pressure potential rather than moisture content was used to measure soil moisture, since they regard this a more accurate measure of the availability of water to micro-organisms. Sensitivity to degrees of drying varied with the biovar of the pathogen used and soil type, with biovar 2 decline being greater than biovar 3. Population decline for both biovars in the various soils (clay loam, sandy loam, and clay) was generally highest at -0,003 kPa.

Sensitivity to dry conditions has been reported to be a contributing factor in reducing *R. solanacearum* populations in fallow land (Sequeira, 1962). Moffet *et al.* (1983) found, however, that the rapid decline of both biovars that occurred with drying was slowed if the pressure potential was maintained at a constant value. They did note that *R. solanacearum* decreased the most in the driest soils in the initial stages of the trials, but that in the end the driest soil contained higher numbers of viable pathogens than did the wetter soils.

A rapid decline was observed in flooded or very dry soils, irrespective of soil type (Nesmith & Jenkins, 1985). Their study also revealed that antagonistic actinomycetes were most numerous in dry soil, whereas antagonistic bacteria were predominant in wet soils.

## Influence of soil type

Relation between soil type and incidence of bacterial wilt is not clear and contrasting reports have been obtained. In Indonesia bacterial wilt of peanuts is most severe in heavy clay soils whereas in China it is prevalent in sandy, especially gritty soil, and not in heavy clay or loam (Hayward, 1991a).

Nesmith & Jenkins (1985) found that soil type influenced soil moisture and antagonistic microbial populations, which in turn affected the *Ralstonia* populations. Soil bacteria are active primarily in soil of higher pressure potential and reduced activity is noted at –0.03 kPa and especially at –0.15 kPa (Cook & Papendick, 1970, as quoted by Moffet *et al.*, 1983). The activity of the microbial population associated

with organic matter varies with soil type and pressure potential. In a study on effect of moisture and soil type on survival of *R. solanacearum* in soil, Moffet *et al.* (1983) reported organic matter contents of 2,5% in clay loam, 1.7% in clay and 1.1% in sandy loam. They noted a greater *R. solanacearum* population decline in the clay loam than in clay or sandy loam at higher pressure potentials. The increased decline in the clay loam was attributed to the higher microbial activity associated with the organic matter. *R. solanacearum* populations thus have to compete with the soil microbes for nutrition and be exposed to increased microbiostasis. The finding that population decline was less in the clay than in the sandy loam, even though the microbial activity is expected to be higher in clay, could be due to adsorption of *Ralstonia* onto clay particles, protecting them from microbiostasis (Moffet *et al.*, 1983). The authors recorded greater decline of biovar 2 population on all of the three soil types in comparison to biovar 3 populations. This was ascribed to greater sensitivity of this biovar to microbiostasis or its poor competitive ability or to the intrinsic nature of the biovar 2 isolate.

Tanaka (1976) previously reported the relation between organic matter, microbes and *Ralstonia* populations. In surface soil with high levels of organic matter and microbial activity, the pathogen population declined faster than in the subsoil with a lower content of these, and that addition of manure to the subsoil reduced the populations considerably.

In a study conducted by Shekhawat & Perombelon (1991) population decline was slower in clay than in sand even under dry conditions. In dry clay, race 1 and race 3 were detectable after 5 weeks and 3 weeks, respectively, whereas in dry sand both races of the pathogen were undetectable within 3 weeks.

## Influence of depth of soil layers

Results of several authors (Mc Carter *et al.*, 1969, Okabe, 1971, Tanaka & Noda, 1973) suggest that *R. solanacearum* can survive in deeper layers of certain soils. Once the pathogen has entered the deeper layers it can survive in localised microsites

(debris or "free soil") where microbial activity is likely to be low (Lloyd, 1978). Subsequent infection of a host plant would then depend on penetration of the root system into these microsites. The survival of the pathogen in these sites would probably be limited to a few deep-rooted hosts such as potatoes and tobacco growing in favourable soil types such as loose sandy soils (Lloyd, 1978).

Vertical distribution of the bacterial wilt pathogen in the soil varies, ranging from the presence in topsoil to almost a meter deep. Martin *et al.* (1981b) investigated the presence and distribution of *R. solanacearum* at three soil depths (0-30 cm, 30-60 cm, and 60-90 cm). In one location the bacterial population were highest in the 0-30 cm soil layer, whereas in the other location the highest population was recorded in the 30-60 cm layer. The authors suggest that such varying results could be attributed to amount of rainfall, type of soil and differences in root development. Other authors (Okabe, 1971; Tanaka, 1976; Graham & Lloyd, 1979) reported highest concentrations of *R. solanacearum* at deeper soil layers. Tanaka (Tanaka, 1976) observed the distribution of the pathogen in the 0-80 cm layer of naturally infested sandy loam soil, and noted a high population at all depths even after one year of fallow. After two years, however, the pathogen had practically disappeared.

Graham & Lloyd (1979) studied the distribution of *R. solanacearum* in soil at five sites in a naturally infested field. At three sites samples were collected at soil depths of 0-15 cm, 15-30 cm, 30-45 cm, 45-60 cm and 60-75 cm, at the other two only the 30-45 cm and 60-75 cm layers were sampled. The pathogen was detected at all five sites, but not at all depths. *R. solanacearum* could not be detected in any of the 0-15 cm samples and at one site the bacteria were only present in the 15-30 cm layer. Presence was detected in the 60-75 cm layer in four of the five sampling sites. The authors regard desiccation of *R. solanacearum* cells due to dry weather prior to sampling as one of the reasons that the bacterium was absent from the 0-15 cm zone, but state that other factors could also be involved. Graham & Lloyd (1979) observed that their results contrasted with those obtained by Mc Carter *et al.* (1969) in a similar vertical distribution study. Mc Carter *et al.* (1969) recorded a high infestation in the top 30 cm layer, with markedly reduced or absent populations in the 30-45 cm zone. No *Ralstonia* populations could be detected deeper than 45-60 cm except for a low

presence in one sample. Graham & Lloyd (1979) considered root development as one of the causes of variation. The work done by Mc Carter *et al.* (1969) involved soils infested by diseased tomato transplants whose roots are not likely to penetrate to great depths. Their study, however, involved soils infested with diseased potato plants.

The study conducted by Sunaina *et al.* (1892) also supports the hypothesis that the depth of root systems of hosts might govern vertical distribution. They found that during the potato season population build-up was higher in the top 30 cm than in the deeper soil layers. During the non-cropping season the population declined much quicker in the top 30 cm as compared to deeper layers, and in the top 20 cm it decreased to an undetectable level. The pathogen survived at the 20-60 cm soil level even after the field had been kept fallow for 7 months. The longer survival of the pathogen in deeper soil layers is to be expected as the roots and bacterial exudates remain undisturbed.

## Influence of anaerobiosis of soil

Longevity of *R. solanacearum* is also affected by the oxygen status of the soil. Anaerobic conditions cause a more rapid population decline with undetectable levels being reached within 7 weeks, whereas 11 weeks where required under aerobic conditions to reduce the population to undetectable limits. Anaerobic conditions also favoured a shift to avirulence (Shekhawat & Perombelon, 1991).

*R. solanacearum* is an aerobic organism and conditions that reduce the availability of oxygen should affect its survival negatively. Flooding of soil has been reported to adversely affect the pathogen's survival (Kelman, 1953; Nesmith & Jenkins, 1985). Yet some reports indicate that flooding of rice fields for several weeks does not eradicate bacterial wilt (De S. Seneviratne, 1988; Shekhawat *et al.*, 1992). In some cases bacterial wilt incidence was higher in a potato crop that followed after paddy rotation. The soil in flooded rice paddies appears to be more oxygen-rich than flooded fallow soils. Aerobic and anaerobic microsystems exist in soil of paddies. The diffusion of oxygen through the roots of rice creates an aerobic environment in the

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rhizospere and rhizoplane, allowing *R. solanacearum* to survive Shekhawat *et al.* (1992).

## Influence of pH of the soil

Although the optimum pH for growth of *Ralstonia solanacearum in vitro* is about 6.8, bacterial wilt has been reported in both acidic and alkaline soils. In North Carolina a high incidence of potato wilt occurred in soil with a pH 4.5. Tobacco wilt in this state was more common in moderate acid soils (pH 5-5.5) (Kelman, 1953). Similarly, in Guadeloupe bacterial wilt was reported in soils of pH 5-5.5 (Prior *et al.,* 1993) and in Mauritius it was not common in alkaline soils (Kelman, 1953). Soil conditions in which this bacterium occurred in Japan and Ceylon were often alkaline. In one instance a soil pH of 8.5 was recorded (Kelman, 1953).

Shekhawat & Perombelon (1991) investigated the impact of different pH levels on race 3 (biovar 2) and race 1 (biovar 3) grown in broth culture. At pH 4.5 growth of both race 1 and race 3 decreased and virulence was completely lost. At pH 8.5 the virulence and growth of race 3 was reduced whereas race 1 grew well although virulence was reduced. At pH 5.5 and pH 7 race 3 grew well and maintained a high level of virulence. Race 1 also grew well but could maintain high virulence only at pH 7. It would therefore seem that race 3 is better adapted to retain virulence under low pH conditions.

# CONTROL OF RALSTONIA SOLANACEARUM

## Chemical Control

Several chemical formulations have been evaluated for the control of bacterial wilt, with limited success. Disinfectants such as potassium sulfide, copper acetate, potassium permanganate and formalin are not effective and often damage the crop and

pose a threat to the environment (Kelman, 1953; Shekhawat *et al.*, 1992). Control or eradication of bacterial wilt has been attempted with fumigants such as chloropicrin, ethylene dibromide and methyl bromide with varying results (Kelman, 1953; Enfinger *et al.*, 1979; Engelbrecht *et al.*, 1990; Melton & Powell, 1991; Chellemi *et al.*, 1997). Chloropicrin has been applied since the 1940's and in most instances crop losses could be reduced, but complete control or eradication could not be obtained (Kelman, 1953). Enfinger *et al.* (1979) evaluated the application of an array of chemical formulations, amongst them fumigants such as chloropicrin, methyl bromide, DD-MENCS (a mixture of methyl isothiocyanate, dichloropropane and dichloropropene), and Metham. Chloropicrin was the only formulation that provided significant control throughout the season. DD-MENCS and a mixture of methyl bromide and chloropicrin (67-33%) were less effective than chloropicrin on its own, but reduced wilt more than methyl bromide. Methyl bromide was found to control wilt only until midseason. Metham applied as fumigant gave moderate to poor control early in the season. Applied as fumigant it was more effective than applied as drench or when incorporated.

Dichloropropane and dichloropropene formulations have also been used since the 1940's to reduce the incidence of wilt. At high dosages where wilt was completely controlled, plants developed chlorine injury (Kelman, 1953). Engelbrecht *et al.* (1990) revealed that disease suppression was most effective if a mixture of ethylene bromide and chloropicrin (55:45 m/m) was applied at a rate of 120 L/ha. All chloropicrin treatments were found to reduce the disease incidence significantly. They also found at one of the trial sites that a chloropicrin/methyl bromide mixture was less effective than chloropicrin on its own, but that it did not differ significantly from methyl bromide application. In a trial where very high infestation levels were encountered, fumigation with ethylene bromide: chloropicrin 55:45 m/m at 120L/ha failed to suppress the disease for the entire growing season. Ethylene bromide has been banned in the United States since 1983 (Sittig, 1985).

Melton & Powell (1991) were not successful in reducing the wilt index in tobacco fields by fumigating with 1,3-dichloropropene and chloropicrin but yields were increased. Fortnum & Martin (1998) obtained both a reduction of the pathogen and an

increased yield by fumigating with 1,3-dichloropropene (78%): chloropicrin (17%) mixture with both in-row and broadcast applications. The authors suggest a waiting period of three weeks after application and warn that spring rains can interfere with the application program.
A bactericidal formulation "Terlai" which has an active ingredient similar to 2,2-dichloro-N (2 hydroxy-1(hydroxy-methyl)-2-(4-nitrophenyl) ethyl acetamide, significantly reduced the incidence of wilt, especially if applied in conjunction with *P. fluorescens* (Machmud & Machmud, 1994). Chlorosis on the leaves of tomato was noted, possibly due to high concentrations of "Terlai" applied.

Chemical compounds have also been used to treat plants to offer protection against infection with *R. solanacearum*. Infection in tobacco seedlings could be delayed slightly by treating the roots & stems with compounds such as hydroxymercuricchlorophenol, zinc sulphate plus lime, Bordeaux mixture and sulphur. Aldicarb, a systemic insecticide, was found to hasten the development of wilt in tomato plants, by altering the quality and quantity of extracellular polysaccharide (Shekhawat *et al.*, 1992). Rhizome treatment of ginger with Emisan 6 (an organomercurial) plus plantomycin for 30 minutes in addition to three spray applications resulted in 100% control of bacterial wilt. Plantomycin alone or in combination with Blitox was 95% effective (Shekhawat *et al.*, 1992).

Several studies on the use of antibiotics as control agents have been performed with varying success. Pretreatment of potato tubers with antibiotic C-6 (similar to erthromycin) followed by two foliar sprays resulted in control and a three-fold increase of yield. Dipping roots of eggplant seedlings into streptocycline prior to transplanting reduced the incidence of wilt (Shekhawat *et al.*, 1992). Engelbrecht *et al.* (1990) could not control tobacco wilt effectively with frequent application of streptomycin sulphate.

## Management through Cultural Practices

### *The use of disease free planting material*

Planting material free of bacterial wilt is vital in preventing the pathogen to be introduced into wilt-free soil. This is extremely difficult with potato where the pathogen is often harbored latently (Hartman & Elphinstone, 1994). The introduction of infected seed into cooler regions has often resulted in the production of latently infected seed on apparently disease-free fields. Such infected tubers have been known to cause severe outbreaks of the disease (French, 1994). In order to prevent the spread of the disease, cuttings from *in vitro* stocks were used on a large scale in Vietnam (Vander Zaag, 1986). Latent infections have also posed a major problem for plant breeders selecting for resistance to wilt. One example is the tolerant cultivar Cruza 148. Although plants do not show any external wilt symptoms or visible tuber infections, the bacterium is carried symptomless in the tuber (Hayward, 1991). Although such tolerance allows production of potatoes in infected regions, it assists in transmitting the disease.

*R. solanacearum* can be transmitted by peanut seed (Machmud & Middleton, 1991). In China groundnut seed is preserved over the winter period and Yongxiang *et al* (1993) reason that application of dry preservation measures could prevent transmission. A subsequent study showed that peanut seed with a water content of 10% or higher could transmit R. *solanacearum* (Dongfang *et al.,* 1994). These authors also suggested that under normal conditions of preservation, the pathogen might not survive. Moffet *et al.* (1981) obtained infected plants from tomato and pepper seeds that had been inoculated artificially. In order to investigate seed transmission Shakya (1993) germinated tomato seeds and found that 21% of the seedlings developed water-soaked brown discoloration on their roots. After 8-9 days these seedlings collapsed. Isolated bacteria were identified as *R. solanacearum.* Singh (1994) was able to confirm the transmission of bacterial wilt through tomato seed as well as through eggplant seed. Occurrence and survival of *R. solanacearum* biovar 3 on eggplant seed was reported by Chatterjee *et al.* (1994).

### *Use of whole seed versus cut seed material*

Many potato crops are grown from seed pieces cut from larger tubers, mainly to reduce the cost of planting material. Emergence from cut seed is usually more uniform across a field, since sprouting from whole seed within individual hills occurs over a longer period of time (Mosley & Chase, 1993). Cutting of seed tubers increases the risk of bacterial or fungal infections. During the cutting process the pathogen can be spread from a diseased tuber to healthy ones by contaminated blades. The cut surfaces also provide ideal entry points for soilborne pathogens. In order to reduce fungal seed decay fungicides are usually applied. The impact of cutting seed material on the incidence of bacterial wilt has been noted by Shekhawat *et al.* (1988). Wilt increased up to 12 times in potato crops where cut seed had been used in comparison to uncut seed. Treatment of the cut pieces with the fungicide Dithane M-45 did not reduce the wilt.

### *Hilling at planting*

Hilling is important to avoid sun damage of developing tubers, to protect tubers against potato tuber moth and to minimize late blight infection of tubers. In some production areas multiple hilling is performed as part of a weed control program, in others a single hilling is done after sprout emergence (Rowe & Secor, 1993). Shekhawat & Chakrabarti (1993) recommend that this procedure be completed at planting time since this minimizes injury to the potato plant. Wounds provide entry sites for bacterial wilt. Post-emergence ridging can increase injury by 10-15% (Shekhawat *et al.* 1992). Hilling three weeks after emergence does not appear to increase the incidence of wilt where low infestation levels were encountered. Where infestations were high, post-emergence hilling did increase the percentage of wilt (Kloos, 1986).

### *Control of nematodes and weeds*

The role of root-knot nematodes in the development of bacterial wilt by providing entry points and possibly assisting tissue colonization has been reported by several authors (Hayward 1991a; Shekhawat *et al.*, 1992). Practices for the control of nematodes include the planting of resistant varieties, using chemical soil treatments,

fumigation and rotation with crops that are resistant to both nematodes and bacterial wilt (Akiew *et al.*, 1993).

Weed control is vital since several species serve as hosts and allow survival in the absence of a host crop (refer to Table 6.1). In one instance, however, the presence of susceptible weeds had no influence on the severity of wilt in the next season (Akiew *et al.*, 1993). 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).

***Crop rotation and fallow***

Rotation with non-hosts as a means of reducing the disease incidence has been used successfully in several crops. The type of crop chosen for rotation varies greatly and depends on the region and the race of the pathogen involved. In India, a two-year rotation with wheat-lupin, wheat-maize or wheat-fallow was very effective in reducing wilt on potatoes. In other instances rotation with finger millet, horsegram, sorghum, wheat, cabbage, carrot, onion and garlic reduced wilt by more than 90% (Shekhawat *et al.*, 1992). Soybean, cowpea, velvet bean, redtop grass, maize and cotton are recommended in two to three year rotation programs to control tobacco wilt in the United States, provided no root-knot nematode infestation is present (Akiew *et al.*, 1993).

In Australia forage sorghum, signal grass and Rhodes grass are often used in the rotation program (Akiew *et al.*, 1993; Arthy & Akiew 1999). A seven-year rotation with signal grass reduced wilt of tobacco greatly, but when tobacco was cultivated for two consecutive years on these fields, wilt incidence increased again to 20 %. Combining the use of resistant or moderately resistant tobacco cultivars with a two to three year rotation sequence of grass fallow appears to be effective (Akiew & Trevorrow, 1994).

The potato strain of *Ralstonia* is reportedly brought under control with relative ease in comparison to other races. In the cool regions of Dorrigo, Australia, a 2½ -year pasture rotation was sufficient to eradicate race 3. Planting the tolerant variety Molinera or maize for several seasons after a six-month fallow eradicated the

pathogen in the Peruvian highlands (French, 1994). Rotation with maize, oats or barley resulted in a 50-75% reduction in the incidence of potato wilt in India. Cowpea and cabbage are often included in rotation programs in potato cultivation (Shekhawat *et al.*, 1988).

The success of rotation in comparison to bare fallow programs varies. A rotation program using maize and bean intercrop or sweet-potato cultivation did not reduce the soil inoculum potential in Costa Rica. A five-month bare fallow with application of herbicide for weed control was more successful in reducing the incidence of wilt (Jackson & Gonzales, 1979). In another case, rice cultivation prior to a tomato crop was more successful in reducing wilt than fallow or other crops (French, 1994).

Interestingly, Shekhawat *et al.* (1992) were able to isolate *R. solanacearum* from the root tissue of maize, wheat, paddy rice and bean. Populations of the wilt organism were however not high enough to exude from the root tissue into the soil. The lack of bacterial release could explain the experienced reduction in *Ralstonia* population after a rotation with these crops. Granada & Sequeira (1983a) also found that *R. solanacearum* could infect roots of presumed non-hosts such as 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 these presumed non-hosts was also lower in that not every individual plant of a given species became infected. These findings indicate why an overall reduction of wilt is experienced when applying rotation. They do however not explain why in some cases rotation is more effective than a bare-fallow treatment.

The level of control achieved with crop rotation varies greatly and is dependent on factors affecting the survival of *Ralstonia* in the absence of a host (Akiew & Trevorrow, 1994). A short-term rotation appears in most instances, especially where race 3 is not involved, not to be effective in either eliminating or controlling wilt effectively.

***Soil Amendmends***

Several soil amendments have been studied in the hope of reducing wilt incidence. It was generally believed that alkalinity would favor the pathogen and acidity suppress it, but increasing soil acidity by adding potassium sulphate, nitric acid or sulphuric acid did not effect the incidence of wilt noticeably (Kelman, 1953). A range of fertilizers has also been evaluated, including superphosphate, calcium cyanamide and sodium nitrate, without much success. Urea applied at 1000kg/ha did reduce wilt significantly (Kelman 1953). Michel & Mew (1998) amended four soils with urea (200kg N/ha) and CaO (5000kg/ha) and reported that the success in reducing wilt varied with soil type. Differential reduction in wilt was also noted by Chellemi *et al.* (1992) in a trial with composted organic amendments. Site specific soil properties may be responsible for this phenomenon. A 99.9% reduction in wilt of tomato plants in glasshouses was reportedly achieved by an amendment developed by Sun & Huang (1985). The soil was amended with 1% S-H mixture which consisted of 4.4% bagasse, 8.4% rice husks, 4.25% oyster shell powder, 8.25% urea, 1.04% potassium nitrate, 13.16% calcium superphosphate and 60.5% mineral ash.

***Other cultural practices***

The removal of rogue plants, stubble and diseased plant material forms a vital part in disease management (Graham *et al.*, 1979; Persley, 1986). Such plant material allows the pathogen to survive in the absence of a host crop and permit infection of a subsequent crop. The use of infected farming implements, lack of sanitation after handling diseased material, or moving through infected fields contribute to the risk of transmitting the disease.

Disease avoidance revolves around the date of planting. Planting during the cool season can reduce yield losses, since high temperatures favor the development of bacterial wilt. This method has been used successfully in Australia, Taiwan and India (Shekhawat *et al.*, 1992; Akiew & Trevorrow, 1994).

## Biological Control

The concern over toxicity of chemical compounds combined with their relative inefficiency in controlling bacterial wilt has motivated the search for biological agents as part of an integrated management program. Trigalet *et al.* (1994) define biological control in terms of direct microbial antagonism (competition, antibiosis) and indirect microbial antagonism (induced resistance in the host). Later the antagonistic effects of botanicals were included in this category (Trigalet & Urquhart, 1998).

Several bacterial species have been reported as being antagonistic towards *R. solanacearum*, amongst them *Pseudomonas fluorescens, P. glumae, P. cepacia, Bacillus polymyxa,* other *Bacillus* spp. and *Erwinia* spp. (Trigalet *et al.*, 1994; Shekhawat *et al.*, 1992). Avirulent mutants of *R. solanacearum* have also been identified as antagonists. These root colonizers antagonize the pathogen at the root infection site, resulting in reduced and delayed onset of wilt. Isolates of *P. fluorescens* reduced wilt by over 50 % and increased tuber yield. Similarly *Bacillus* spp. isolates have been reported to reduce wilt by almost 90% and tuber rot due to *Ralstonia* by more than 80% (Shekhawat *et al.*, 1992). Biocontrol results obtained in the laboratory with these types of antagonists are often difficult to reproduce in field conditions. Antagonism observed on agar plates could have resulted from a different set of parameters than found under natural circumstances. In field conditions, the biocontrol organisms must compete with other soil microbes and must contend with both biological and physical factors (Trigalet *et al.*, 1994). Since the plant is susceptible to infections for a long period of time, antagonists at the root infection site must be present continuously and without too many fluctuations in their population.

The development of a bio-agent that acts as an endophytic antagonist has the advantage that once it is established in the plant, it can provide continuous protection. The agent should not cause disease and must be able to colonize roots, penetrate xylem vessels and multiply within the vascular tissue (Trigalet *et al.*, 1994). For this reason several studies have been conducted on avirulent mutants of *R. solanacearum* (Trigalet *et al.*, 1998; Smith *et al.*, 1998). Problems that have been experienced with endophytic antagonism include limited systemic spread and population decline,

probably due to agglutination by plant lectins or by them being bound to host cell walls (Trigalet *et al.,* 1994).

Several botanicals have been evaluated for their ability to reduce bacterial wilt infections. Terblanche & de Villiers (1998) noted that French Marigolds (*Tagetes patula* L.) not only reduced *Ralstonia* populations in the soil, but also inhibited the development of wilt symptoms on tobacco plants. Two thiophenes were extracted from the roots, which proved inhibitory to *R. solanacearum* in *in vitro* tests. Similarly it was found that sugi bark (*Crytomeria japonica* D.Don), a substrate for horticultural crops in soilless culture, reduced the incidence of wilt on tomato plants (Yu *et al.* 1997). The inhibition was mainly attributed to volatile oils, phenolics and acidic substances. Injecting the volatile oils into rockwool (another soilless substrate) also suppressed the wilt incidence. The main components of the volatile oils were identified as isophyllodecene and ferruginol. Asafoetida (*Ferula foetida*) mixed with tumeric powder (*Curcuma longa*) at ratio 1.5g: 5g: 10 liter water and 1g: 5g: 10 liter water, controlled wilt disease by 70.3 % and 69% respectively (Mazumder, 1998). Soil drenching with a formulation of asafoetida and tumeric reduced the mortality of brinjals due to bacterial wilt, especially if three drenches were performed at 20, 50 and 80 days (Pun & Das, 1997).

## Resistant Cultivars

A practical and economic approach to managing bacterial wilt infested soils would be to plant resistant cultivars. According to Prior *et al.* (1998), the term resistance refers to "any measurable plant mechanism able to overcome completely or to limit the development of a pathogen or its effects". Tolerance is defined as "the overall ability for a plant to withstand development of a pathogen without major losses in yield".

Although resistance to bacterial wilt has been documented in some crop species such as tomato, obtaining stable resistance without the occurrence of latent infections is a major challenge. The specificity of the pathogen-host-environment interaction has prevented the success in obtaining a cultivar that is resistant under a range of

environmental conditions and to various strains of the pathogen. It appears that the genetic basis for resistance to *R. solanacearum* is different in each resistant species. It would be beneficial to obtain a broadspectrum resistance in which maximum genetic variability for the "wilt-resistance" trait has been combined (Schmiediche, 1986).

Tung *et al.* (1990, 1992a, 1992b) demonstrated that genes affecting adaptation are involved in conferring resistance to bacterial wilt or in conditioning their expression. A heat tolerant parent was found to produce a higher frequency of resistant progenies when combined with an indisputable source of resistance. There are even some heat tolerant clones with no known resistance that could withstand wilt better under very hot conditions, than could clones known to have some specific genes for resistance. Furthermore, resistance appeared to be most stable and highest in clones where the resistance had been established by combining various specific sources of resistance (thereby producing a wide genetic background), or where a source of resistance was combined with a good source of adaptation (Tung *et al.*, 1992b).

# DISCUSSION

Bacterial wilt caused by *Ralstonia solanacearum* is one of the most destructive and successful plant pathogens affecting several economically important crops. Although the disease is more common in the tropics, subtropics and the warmer temperate areas, it has also been reported in the cooler regions such as Sweden, Austria and the UK. During the last century, the pathogen has been reported in so many countries that its distribution can now be regarded as world-wide, though the strains encountered vary. The wilt organism is well adapted to survival, being able not only to infect a wide range of hosts but also being capable of remaining viable in the soil for several years. Reliable scientific data on soil survival under different circumstances is scarce. Its ability to survive in more than 450 different species has facilitated its survival even through harsh periods where soil conditions are not congenial for survival in its free state. It's success as a plant pathogen is favoured in the ready way it disseminates. Besides transmission through infected soil and infected plant material (often showing

no symptoms), it can spread by mechanical contacts, contaminated water and in case of banana wilt even by insects.

Control of bacterial wilt lies in an integrated approach. Good agronomic practices such as using disease-free soils, disease-free and uncut planting material, effective weed and nematode control, incorporating good crop rotation systems and fallowing can assist greatly in reducing the incidence of wilt. Soil amendments and chemical control can be beneficial under specific circumstances. The potential of biological control as part of the management system is vast and the research input into this area has increased dramatically. Another important strategy in managing bacterial wilt infested soils lies in the use of resistant varieties. Although resistance in varieties have been globally reported over the years, most of these genotypes tend to be tolerant to bacterial populations, rather than being immune and often transmitted the disease to the progeny tubers without necessarily causing visual symptoms. Planting of such latently infected tubers could lead to infestation of previously disease-free fields or result in yield losses. Another concern for plant breeders is that the expression of resistance is strongly affected by environmental factors.

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# CHAPTER 2

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

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## 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.

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# 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

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.

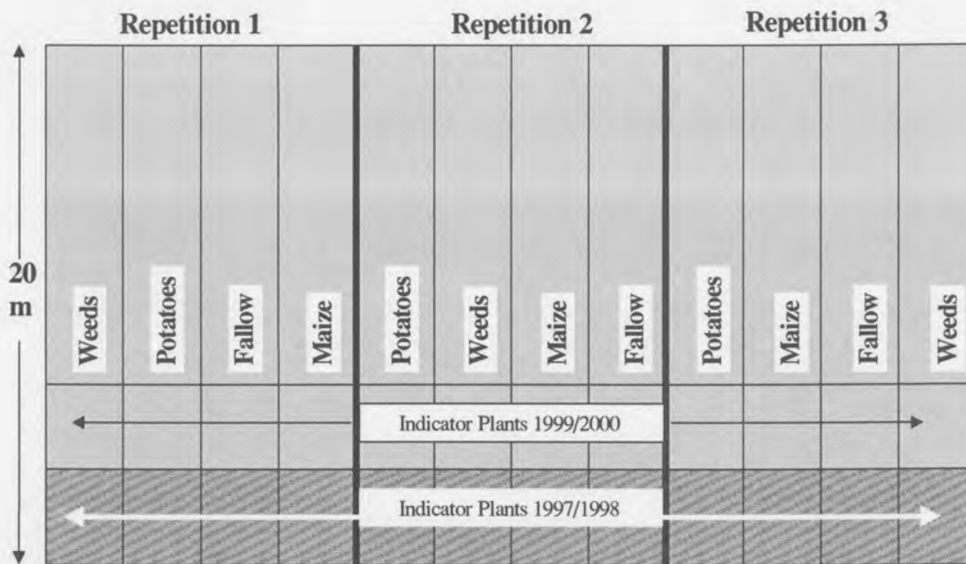


**Figure 2.1 Layout of the field infested with *Ralstonia solanacearum***

**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.

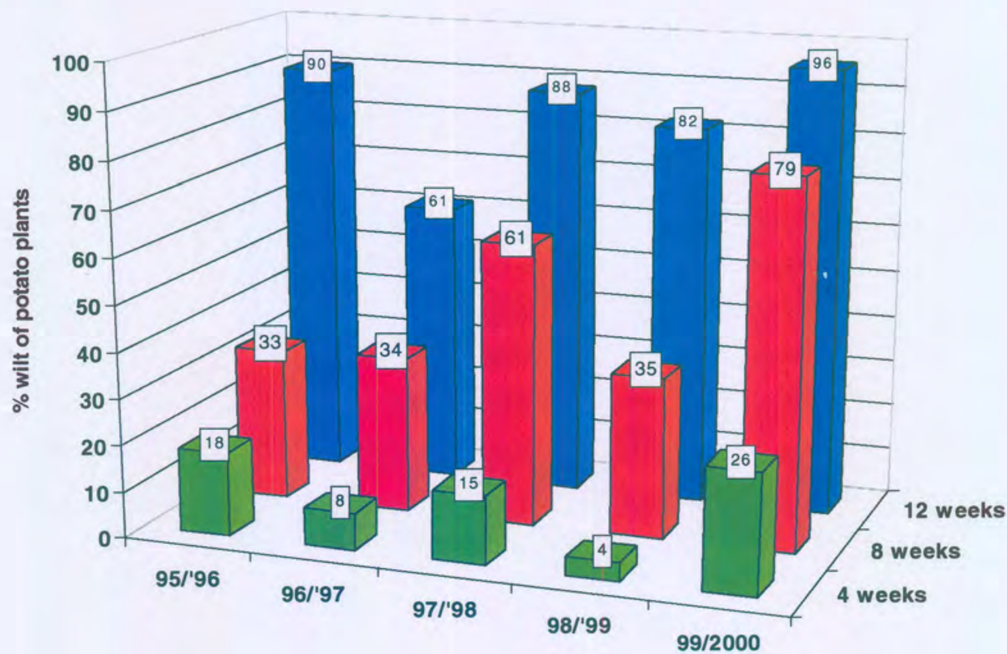


**Figure 2.4 Percentage wilt in potato monoculture plots over a five-year period**

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 |

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

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 | - | - | - | - | $1 \times 10^3$ | $2 \times 10^1$ |
| Site 4 | - | - | $2 \times 10^3$ | $1 \times 10^1$ | - | - |
| Site 5 | - | - | - | - | - | - |
| **Bare-fallow** | | | | | | |
| Site 1 | $5.1 \times 10^3$ | - | - | - | - | $2 \times 10^1$ |
| Site 2 | - | - | - | - | - | - |
| Site 3 | - | - | - | - | - | - |
| Site 4 | - | - | - | - | - | - |
| Site 5 | - | - | - | - | - | - |
| **Maize monoculture** | | | | | | |
| Site 1 | - | - | - | - | - | - |
| Site 2 | - | - | - | - | - | - |
| Site 3 | - | - | - | $4 \times 10^1$ | - | - |
| Site 4 | - | - | - | - | - | - |
| Site 5 | - | - | - | - | - | - |
| **Weed cover** | | | | | | |
| Site 1 | - | - | - | - | - | - |
| Site 2 | - | - | - | - | - | - |
| Site 3 | - | - | - | - | - | - |
| Site 4 | - | - | - | - | $2.2 \times 10^2$ | - |
| Site 5 | - | - | - | - | - | $4 \times 10^1$ |

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.

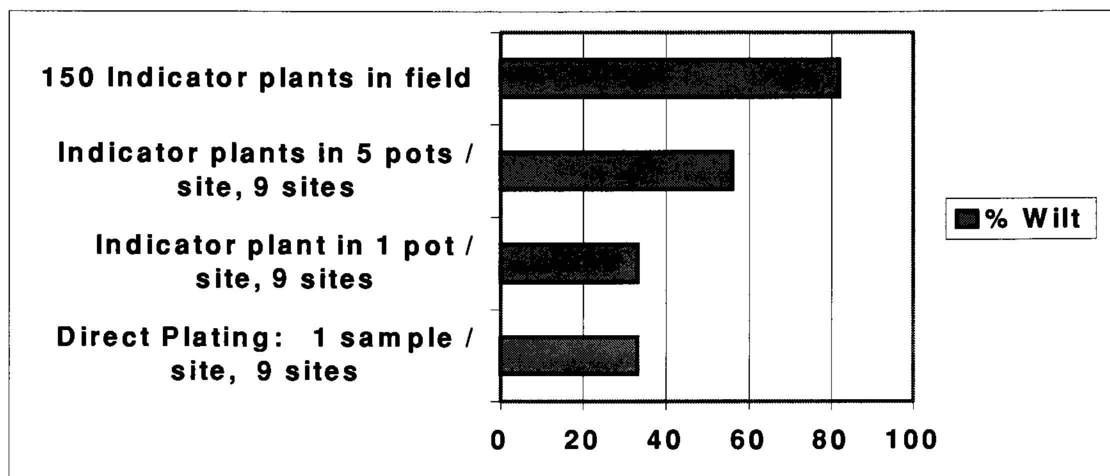


**Figure 2.5 Comparison of three techniques used to detect *Ralstonia* populations in potato monoculture plots during the 1997/1998 season.**

## 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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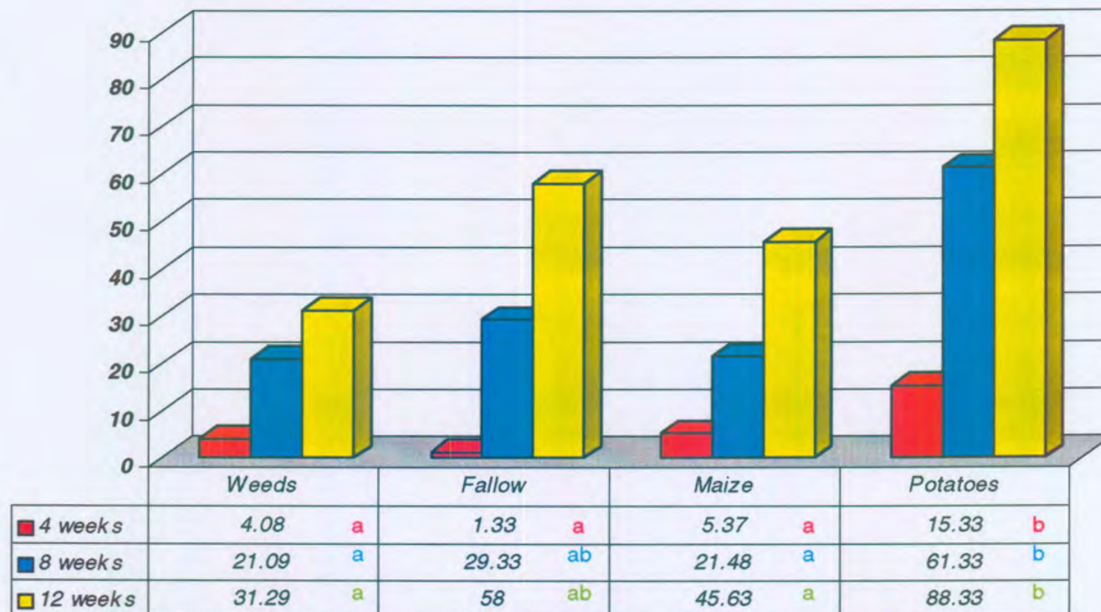


| | Weeds | | Fallow | | Maize | | Potatoes | |
|---|---|---|---|---|---|---|---|---|
| 4 weeks | 4.08 | a | 1.33 | a | 5.37 | a | 15.33 | b |
| 8 weeks | 21.09 | a | 29.33 | ab | 21.48 | a | 61.33 | b |
| 12 weeks | 31.29 | a | 58 | ab | 45.63 | a | 88.33 | b |

**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.

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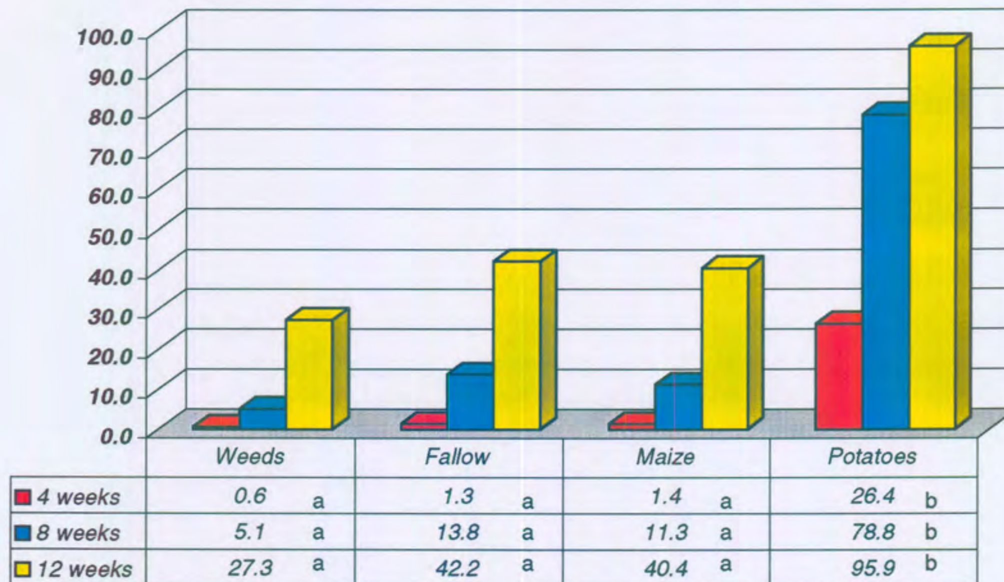



**Figure 2.7 Percentage wilt of indicator plants in treatment plots after five years of cultural practices.** Percentages followed by the same letter within each period are not significantly different according to Tukey's Studentized Range (P=0.05)

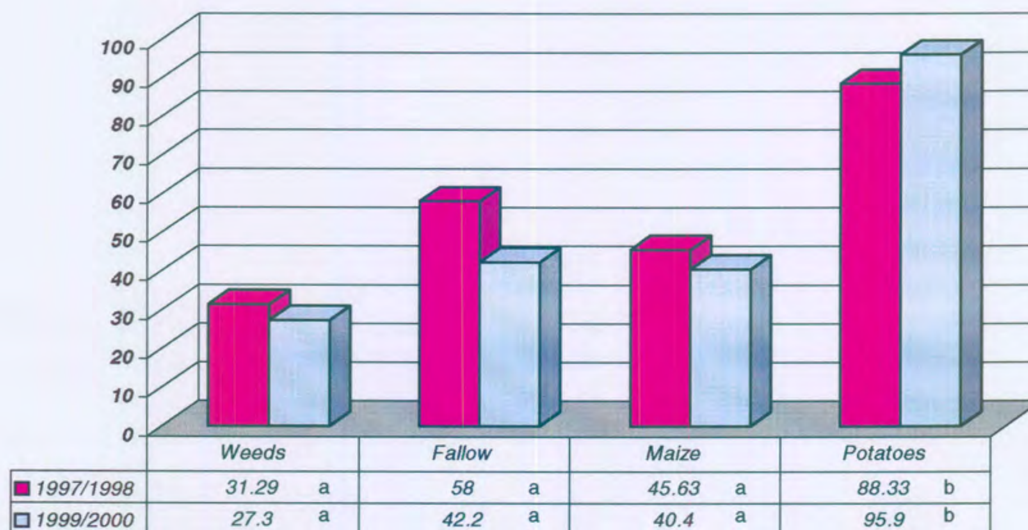



**Figure 2.8 Comparison of percentage wilting indicator plants in treatment plots after three and five years of rotation.** Percentages followed by the same letter within each treatment are not significantly different according to Tukey's Studentized Range (P=0.05)

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.

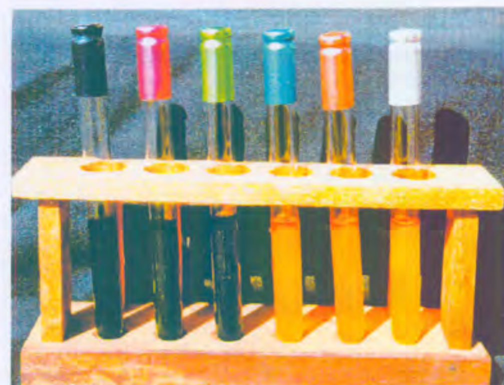
Removal of weeds is considered vital in integrated management of bacterial wilt since these can serve as hosts or shelter sites for long term survival (French, 1994; Jackson & Conzales, 1979). Yet potato plants in the weed-fallow plots exhibited the least wilt symptoms, although host species such as *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).

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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 $10^5$cfu/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 x $10^3$cfu/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 x $10^6$cfu/ml), giving a final bacterial population of 2.5 x $10^5$ 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*,

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[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.

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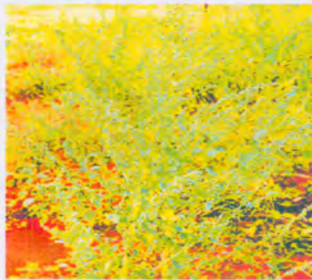
*Amaranthus* spp. biovar 3

*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 | $4 \times 10^3$ | infected |
| | 3 | 4 | 0 | infected |
| | 2 | 6 | 0 | negative |
| *Chamaesyce prostrata* | 2 | 2 | $2 \times 10^2$ | negative |
| | 3 | 8 | 0 | infected |
| *Chloris pycnotrix* | 2 | 5 | $1 \times 10^1$ | 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 | $2 \times 10^1$ | 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*.

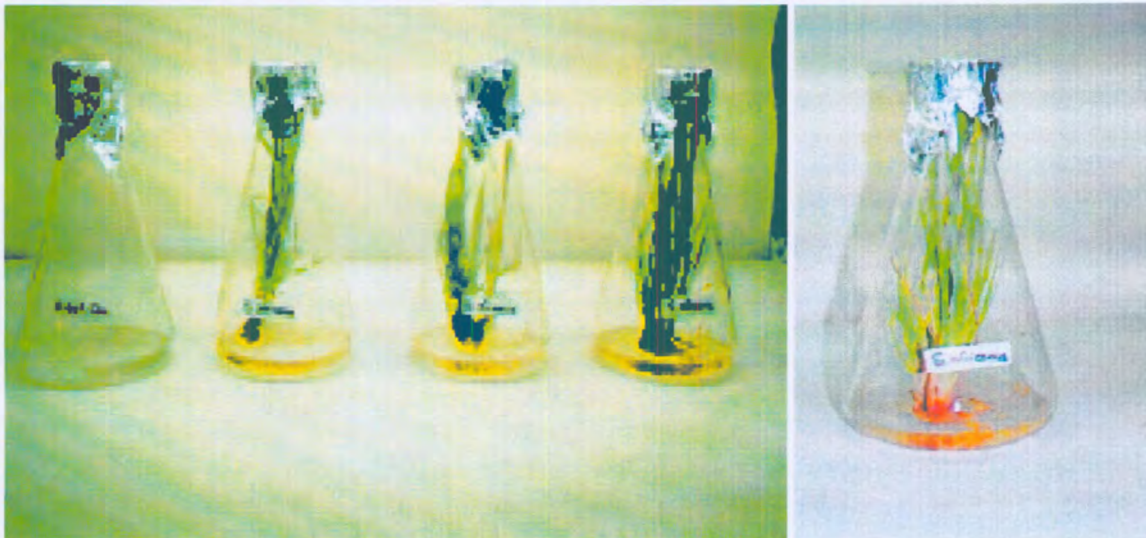


**Figure 3.3 *Sporobolus africanus* plantlets co-cultured with *Ralstonia solanacearum* in nutrient solution**

**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* | $3 \times 10^5$ | $1 \times 10^1$ | 0 | 0 |
| *Eragrostis curvula* | $7.4 \times 10^5$ | $2 \times 10^2$ | 0 | 0 |
| *Chloris pycnotrix* | $5.5 \times 10^5$ | 0 | 0 | 0 |
| Control (nutrient solution) | $7.3 \times 10^5$ | $5.4 \times 10^5$ | $8.7 \times 10^5$ | $6.4 \times 10^5$ |

## 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).

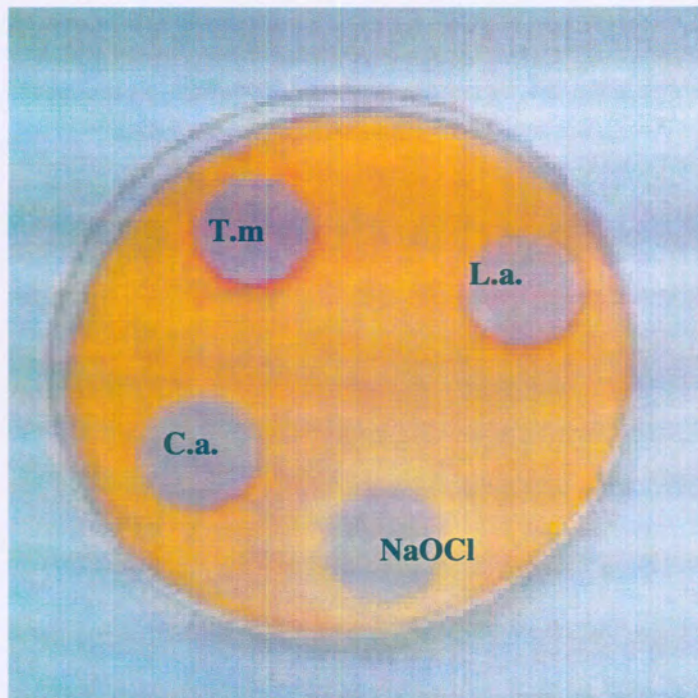



**Figure 3.4 Inhibition zones on TZC plates seeded with *Ralstonia solanacearum*, biovar 2 inoculum**

***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).

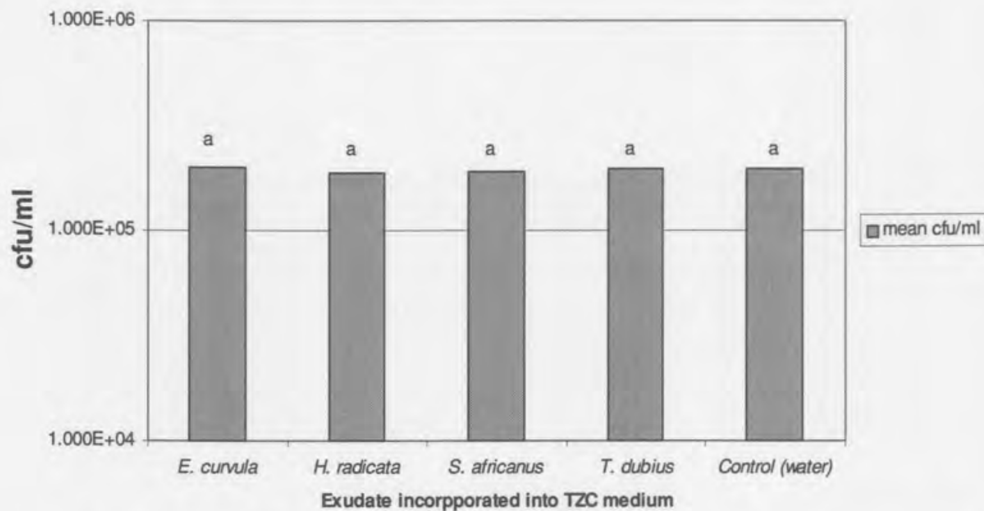



**Figure 3.5 Logarithmic presentation of growth (cfu/ml) of *Ralstonia solanacearum* (biovar 2) on TZC medium poisoned with plant leaching of *E. curvula*, *H. radicata*, *S. africanus* and *T. dubius*.** Data points with the same letter are not significantly different according to Tukey's Studentized 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 | $1 \times 10^1$ | 0 | $2 \times 10^2$ | 0 | $4.2 \times 10^1$ |
| *S. africanus* | 0 | 0 | $2 \times 10^1$ | 0 | 0 | 4 |
| *T. dubius* | $3 \times 10^1$ | 0 | $3.4 \times 10^2$ | 0 | 0 | $7.4 \times 10^1$ |
| *H. radicata* | 0 | 0 | 0 | 0 | $1 \times 10^1$ | 2 |
| Potato | $5 \times 10^2$ | $3 \times 10^1$ | 0 | $6 \times 10^3$ | 0 | $1.3 \times 10^3$ |
| Sterile water | $9.8 \times 10^4$ | $1.5 \times 10^5$ | $2.1 \times 10^5$ | $8.7 \times 10^4$ | $5.2 \times 10^5$ | $3.2 \times 10^5$ |

# 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.

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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.

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# 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 $1.7 \times 10^5$ 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 x$10^4$ 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.

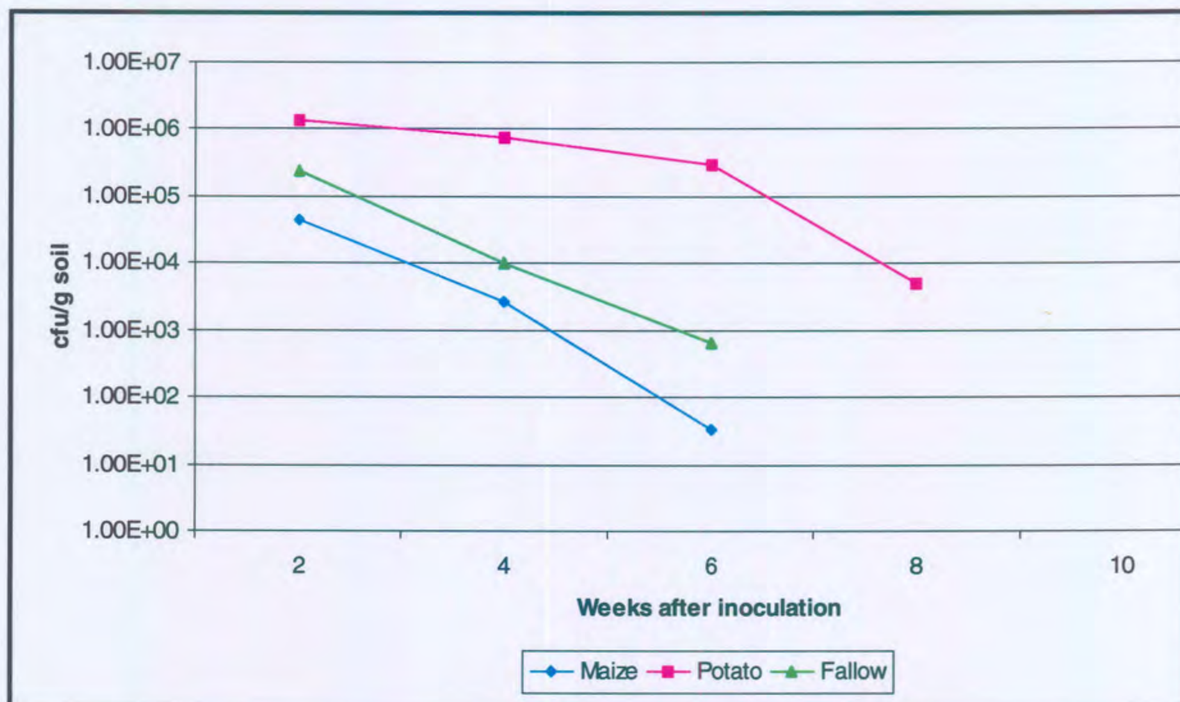


**Figure 4.1 Logarithmic presentation of *R. solanacearum* (biovar 2) populations in soil (cfu/g) from pots in which maize or potato was grown or which was kept fallow**

## 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.

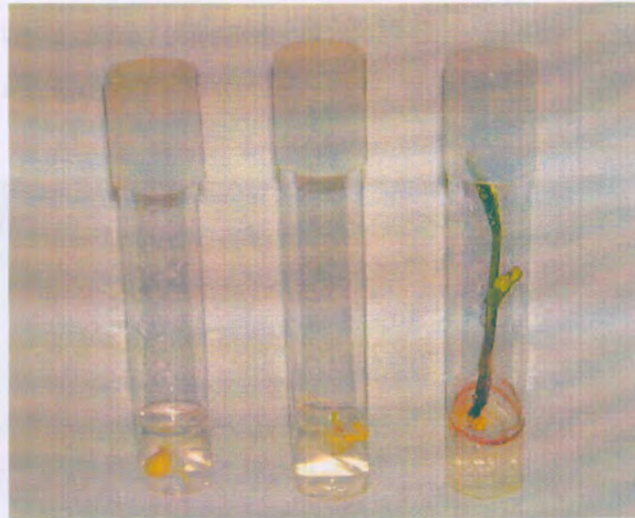

**Figure 4.2 Germination and development of maize on Joerdens-Roettger (1987) medium**

**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**<br>*R. solanacearum* counts (cfu/ml) | **T 2: Path. & Maize**<br>*R. solanacearum* counts (cfu/ml) | **T 3: Maize only**<br>Saprophytic growth |
|---|---|---|---|
| Day 0 | $1.74 \times 10^5$ (concentration of inoculum) | | |
| Day 7 | $8.82 \times 10^5$ | Below detection – saprophyte "type 1" * | "type 1" * |
| Day 14 | $5.69 \times 10^5$ | Below detection – little other saprophytes, "type 1"* dominant | "type 1"*, little other |
| Day 21 | $7.68 \times 10^5$ | 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

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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).

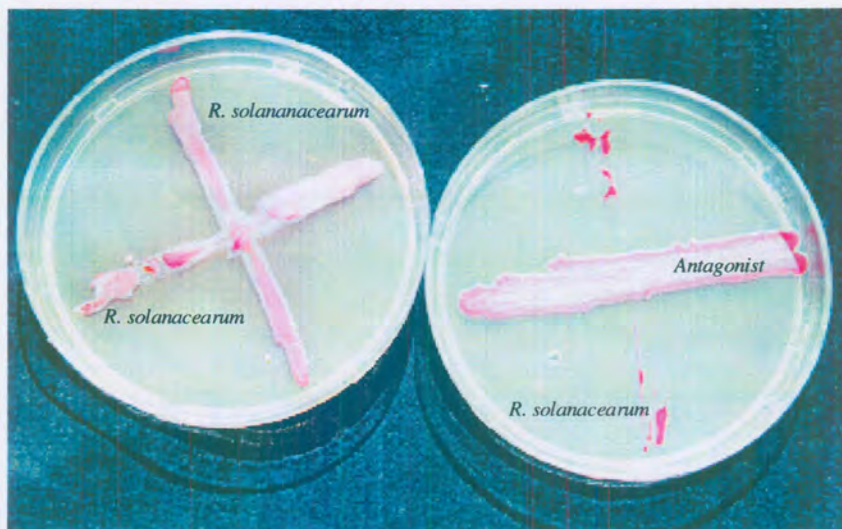


**Figure 4.3** ***R. solanacearum*** **biovar 2 cross-streaked against antagonist tentatively identified as** ***Chromobacterium violaceum*** **isolated from maize plant on TZC (Kelman, 1953) medium**

*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

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

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### 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 | $8.79 \times 10^5$ | $1.08 \times 10^6$ | $8.33 \times 10^4$ | $1.09 \times 10^6$ |
| 10 days | $3.49 \times 10^5$ | $2.15 \times 10^6$ | $2.0 \times 10^2$ | $2.24 \times 10^6$ |

* 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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# 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.

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