OCCURRENCE, VARIABILITY AND MANAGEMENT OF *Ralstonia solanacearum* IN POTATO PRODUCTION SYSTEMS IN KENYA.

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DECLARATION AND RECOMMENDATION

Declaration

This thesis is my original work and has not been presented for a degree in any other University

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ABSTRACT

Production of potato (*Solanum tuberosum* L.) in Kenya is constrained by many biotic, abiotic and socio-economic factors. Among the biotic constraints, bacterial wilt, caused by *Ralstonia solanacearum* (Yabuuchi *et al*., 1995) presents major management difficulties. Attempts to combat the disease have been difficult due to the complex heterogeneous genetic make-up of the pathogen, comprising different races and biovars, its survival mechanisms and its large number of hosts, including weeds. A study on occurrence and variability of the pathogen, alongside investigations on its interactions with various plant species was conducted to help understand the pathogen and set a basis of determining the potential hosts to avoid or crop species to include in rotational pattern programmes that would minimize effects of the disease. Population structure was determined by isolating the pathogen from collected samples. The distribution and genetic variability were determined in isolates using tobacco leaf infiltration, biochemical tests and polymerase chain reaction (PCR) with repetitive sequences, using enterobacterial repetitive intergenic concensus (ERIC), and BOX repetitive primer sets. Weeds were artificially inoculated with the pathogen to test if they were hosts. Latent infection of weeds was determined using enzyme-linked immunosorbent assay (ELISA). To determine the effect of crop rotation on potato bacterial wilt incidence and tuber yields, one and two-season rotation experiments were conducted using maize, beans, cabbage, potato and rhodes grass.

Sixty eight isolates of *R. solanacearum* were obtained from 70 samples collected. All of the suspected potato and tomato (*Lycopersicon esculentum* L.) samples were found to be infected with *Ralstonia solanacearum* while only 33% of weed samples were infected. *Ralstonia solanacearum* biovar 2 and biovar 3 (race 3 and race 1, respectively) were found to be present. *R. solanacearum* biovar 2 was widely distributed in many areas, while biovar 3 was isolated from a restricted area (UM3 agro-ecological zone) in Murang'a. Both biovars 2 and 3 were isolated from potato and tomato samples. Some weeds were infected by both *R. solanacearum* biovars 2 and 3. Other weeds were exclusively infected with either biovar 2 or biovar 3. Some of the weeds were found to be latently infected. One-season rotation with maize reduced wilt incidence from 46.7% to 5%, while two-season rotation with maize, followed by beans or cabbage reduced wilt incidence from 46.7% to 1.0% and 2.0%, respectively. Planting two different crops was superior to planting the same crop in two subsequent seasons in reduction of bacterial wilt. Potato yields improved with the reduction of incidence of bacterial wilt.

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ABBREVIATIONS AND ACRONYMS

Aq	: Aqueous
ASAL	: Arid and Semi Arid Lands
ASARECA	: Association for Strengthening Agricultural Research in Eastern and Central Africa
BOX	: A primer for repetitive polymerase chain reaction
bv	: Biovar
CIP	: International Potato Centre
CPG	: Casamino- peptone- glucose
CTAB	: Cetyl (Alkyl) trimethyl ammonium bromide
CL	: Coastal Lowlands
DAP	: Diammonium phosphate
DAPG	: Days after Planting
DAS – ELISA	: Double antibody sandwich – enzyme linked Immunosorbent assay
EG	: Endoglucanase
ELISA	: Enzyme linked immunosorbent Assay
EDTA	: Ethylene diamine tetra acetate
EPS	: Extracellular Polysaccharides
ERIC	: Enterobacterial Repetitive Intergenic Concensus
GPS	: Geographic positioning system
ha	: Hectare
KARI	: Kenya Agricultural Research Institute
Kb	: Kilobase
LH	: Lower Highlands
LM	: Lower Midlands
MBE	: Beta Mercaptoethanol
NARL	: National Agricultural Research Laboratories
NCM – ELISA	: Nitrocellulose membrane enzyme-linked immunosorbent assay
NPRC	: National Potato Research Centre
OD	: Optical density

PCR	: Polymerase chain reaction
PG	: Polygalacturonase
rep	: Repetitive
REP	: Repetitive extragenic palindromic
rpm	: Revolutions per minute
SE	: Standard error
SMSA	: Semi- selective Medium South Africa
S-N-K	: Student Newman Keuls
TA	: Tropical Alpine
TAE	: Trizma base sodium Acetate, EDTA (tris-acetic acid-EDTA)
TBE	: Trizma base Boric acid, EDTA (tris-boric acid-EDTA)
TE	: Tris HCl, EDTA
TZC	: Tetrazolium Chloride
UH	: Upper Highlands
UPGMA	: Unweighted pair-group method with arithmetic averaging
Uv	: Ultra-violet
UM	: Upper Midlands
v	: Volume
V	: Volts
W	: Weight

CHAPTER ONE

1.0 INTRODUCTION

Introduced to East Africa by British farmers in the 1880s, potato (*Solanum tuberosum* L.) has assumed great importance in Kenya both as a staple food item and as a cash crop over the past 30 years (FAO, 2007). The crop is presently cultivated in Rift-valley, Central and Eastern Provinces. Potato is also grown in Taita-Taveta in Coast Province and Kisii in Nyanza Province. Measured by quantity harvested, potato now ranks second as the country's food crop, after maize, with production in 2007 totalling around 800,000 tons (FAO, 2007). Most growers of the crop are smallholders living in densely populated and intensively cultivated highland regions. They own land measuring between 0.25 and 5 hectares. There are a few larger farms but the bulk of marketed potato produce is by smallholders. Yields have persistently remained below 10t/ha in farmers' fields in comparison with over 40t/ha attainable under research conditions (Lung'aho *et al.*, 1997). There is, therefore, need for increased and sustainable production of the crop since it contributes a great deal towards local food security.

Potato production in Kenya is constrained by many biotic, abiotic and socio-economic factors (Lung'aho *et al*., 1997). Among the biotic constraints, bacterial wilt caused by *Ralstonia solanacearum* (Yabuuchi *et al*., 1995) stands out as a major challenge, which presents management difficulties. *Ralstonia solanacearum* is a phytopathogenic bacterium that causes lethal wilting in over two hundred known plant species. Important crops infected by the pathogen include potato, tomato, tobacco, ground-nuts and bananas. No single control strategy is known to be effective against the disease due to the pathogen's complex heterogeneity, wide host range, longevity in soil after infestation, unavailability of clean seed tubers and decreasing land holding sizes that encourage continuous cropping with potatoes. This practice favours build-up of disease inoculum within potato cultivation systems. Consequently, incidences and losses due to bacterial wilt have been increasing over the years (Nyangeri *et al*., 1984; Ajanga, 1993; Barton *et al*., 1997; Ateka *et al*., 2001; Kinyua *et al.*, 2001). Integrated management strategies are advocated for in dealing with the bacterial wilt menace. However, several attempts have been made with limited success (Lemaga *et al.*, 1997; Mienie and Urquhart, 1997; Lemaga *et al*., 2004; Lemaga *et al*., 2005). The development of any effective management strategy should consider a thorough understanding of the pathogen's population structure, its survival and interaction with various plant species in target production ecosystems.

Occurrence of *Ralstonia solanacearum* in cooler, high altitude areas where potato seed used to be produced may be as a result of changes in agricultural practices or emergence of a novel bacterial strain, better adapted to cooler conditions. Smith *et al.* (1995) recognized the existence of biovars 2A and N2 among strains isolated from Kenya's major potato growing regions. These findings had earlier been alluded to by Dawson (1949) and Robinson and Ramos (1964) when they reported the occurrence of "typical" and "atypical" strains of the bacterium. Additionally, several distinct profile types have been reported among Kenyan biovar 2 strains (Smith *et al*., 1995). Although some work on genetic diversity has been done in Kenya, all the 46 isolates worked on were from potato hosts (Smith *et al*., 1996). This means that it is not known whether the same intraspecific groups are found in other hosts including weeds. It is also not known whether there are many more of these groups which have not yet been detected. Since no countrywide study has been carried out on the population structure of *R. solanacearum* in Kenya to encompass all potato growing regions and other possible hosts of the pathogen, a lot of information is still lacking. Such knowledge would help in overcoming some of the challenges being faced in the management of the disease, particularly with respect to breeding for resistance to the pathogen and probable survival abilities. Consequently, there is need to identify isolates of *Ralstonia solanacearum* to intra-sub-specific levels, whereby their biological properties might be more predictable so as to enable the development of effective disease management strategies.

Studies on population structure of the pathogen would remain incomplete without deliberate tests of pathogenicity of identified strains on a wide array of plants. Numerous plant species which are attacked by *R. solanacearum* include diverse groups of weeds. Some of these weeds are susceptible; others are tolerant, while still others are latent to the pathogen infection. These carriers could serve as primary inoculum source for subsequent susceptible crop. There are sheltered sites for the pathogen, which include deep soil layers and the rhizosphere of roots of weed hosts (Jackson and Gonzalez, 1981; Granada and Sequeira, 1983). The significance of weed hosts varies in different environments and cropping systems. Little is reported on the magnitude of such significance in the Kenyan cropping environment. Therefore, there is need for thorough investigations on the significance of weed hosts in relation to the perpetuation of the pathogen in the local environment. Pathogenicity testing would give information on situations where bacterial wilt is observed after planting potatoes on land that had no history of a

solanaceous crop. The study would also help in identification of non-hosts and deterrents to the pathogen.

One of the challenges facing the implementation of crop rotation programmes in the control of bacterial wilt has been lack of alternative crops of identified economic value to farmers. This has constrained the diversification of cropping systems in potato-growing areas, leading to continuous planting of potato on the diminishing land holdings. Coupled with the severe shortage of disease-free tubers, these factors have contributed to the perpetuation of the bacterial wilt menace (Ajanga, 1993; Barton *et al*., 1997; Ateka *et al*., 2001; Kinyua *et al.*, 2001; Lemaga *et al.*, 2001a; Lemaga *et al.*, 2004). Research to identify crops that should be avoided in rotations on the basis of their ability to encourage persistence of *R. solanacearum*, and those of high economic value that prevent inoculum from building-up, is an important starting point in any systematic pattern of crop rotation recommendation for the management of bacterial wilt. In the Kenyan situation, little of such work has been carried out. Therefore, there is need to carry out such investigations in order to get workable recommendations for the management of bacterial wilt in Kenya.

1.1 Statement of the problem

Bacterial wilt, caused by *R.solanacearum*, has been a persistent challenge to potato production in Kenya and many other countries. Recent work in Kenya has indicated that there is an increase of incidences of bacterial wilt of potato. The disease has spread to all potato growing zones and has steadily build-up over the years, causing losses and decline in yields (Ajanga, 1993; Barton *et al*., 1997; Ateka *et al*., 2001; Kinyua *et al*., 2001). Ajanga (1993) estimated yield losses due to bacterial wilt at 50% in ware and 75% in seed potato in Kenya. Average farm yields have been recorded at less than 10t/ha in comparison to over 40t/ha obtainable under research farm conditions. The low yields are attributed to build-up of pests and diseases among other factors (Lung'aho *et al*., 1997). Actual yield losses caused by bacterial wilt, however, have not been established.

Previous attempts to combat the disease have not had much impact due to the heterogeneous genetic make-up of the pathogen, its partially understood survival mechanisms and its large number of hosts including weeds. Whether such heterogeneity and intraspecific groupings of *Ralstonia solanacearum* are present in Kenya has not been systematically determined. The wide

variation in the characteristics of the pathogen has resulted in several sub-specific classifications based on different criteria. This is a major problem which has discouraged research workers from identification of strains of the pathogen, which is necessary for the determination of varietal resistance to the disease and, therefore, control of the disease. Information about the ecology, and hence knowledge about interactions with various local plant species in potato production ecosystems, is lacking. There is need to determine population structure, potential hosts to avoid or crop species to include in rotation programmes aimed at minimizing the disease.

1.2 Justification

Bacterial wilt is a major constraint to potato production in Kenya. Continuous cropping of potato on small scale farms, combined with shortage of clean seed, provides ideal conditions for spread of the disease. The disease could be controlled and even eliminated from potato farms by combination of clean seed, good crop rotation pattern and strict sanitation. However, rotation crops and a systematic pattern of crop rotation need to be determined for any given region. This demands a comprehensive understanding of the population structure as well as the ability of various plant species to host or support the survival of *R.solanacearum.*

The understanding of the structure of *R. solanacearum* population, the role other hosts, particularly weeds, play in the survival of the pathogen and perpetuation of the disease would be useful in devising proper control strategies of the disease.

1.3 Research objectives

On the basis of the knowledge gaps on the population structure, the role of weeds in potato production ecosystems in relation to *Ralstonia solanacearum* problem and the need for effective management strategies to control bacterial wilt in Kenya, a study was carried out with the following objectives:

1.3.1 Broad objective of the study:

To study the diversity and characteristics of *Ralstonia solanacearum* causing bacterial wilt in various host plant species and its possible management strategy in Kenya.

1.3.2 Specific objectives:

1.3.2.1 To determine the occurrence and variability of *Ralstonia solanacearum* present in potato-production ecosystems in Kenya.

1.3.2.2 To establish the pathogenic characteristics of *Ralstonia solanacearum* isolates obtained from potato-production ecosystems in Kenya.

1.3.2.3 To identify crop rotation sequences those are effective in the management of bacterial wilt.

1.4 Hypotheses

1.4.1 *Ralstonia solanacearum* strains affecting potato and other plant species are not the same in phenotypic, biochemical and genomic characteristics.

1.4.2 Strains of *Ralstonia solanacearum* isolated from weeds and other hosts are not pathogenic to potato.

1.4.3 Crop rotation sequences have no effect in the management of bacterial wilt of potato.

1.5 Scope and limitation

Sampling of diseased plant materials from potato-growing regions of Kenya was carried out during the March to July 2006 growing season. Additionally, other plant hosts and weeds were collected and analyzed in the laboratory for infection. This revealed whether the weeds provided sheltering sites and perpetuated the disease. The sampling area covered most of the potato growing regions in Kenya.

Laboratory and glasshouse studies were conducted at NARL on all isolates to determine their races and biovars. This revealed the population structure of *Ralstonia solanacearum* in the country. Pathogenicity studies on various weeds were included in the study to find out the role played by weeds in the survival of the pathogen. Pathogenicity and pathogenic strains of the isolates were determined. Double antibody sandwich (DAS) enzyme linked immunosorbent assay (ELISA) and nitrocellulose membrane (NCM) enzyme linked immunosorbent assay, Semi-selective medium - South Africa (SMSA), and Kelman's Tetrazolium Chloride (TZC) medium were used in these investigations.

A certain sequence or pattern of the rotation has to be followed in order to succeed in controlling bacterial wilt. This has not been done for Kenya and therefore this work was undertaken to establish the pattern of crop rotation needed for Kenya. Experimental plots were laid out at NARL after fencing off the plots and contaminating them with *R. solanacearum*. Planting materials were sourced from KARI's National Potato Research Centre -Tigoni and the

Kenya Seed Company's outlet in Nairobi. The experiments were carried out during the long-rains and short rains seasons for four seasons (September-December 2005; March-July 2006, September 2006-January 2007 and March-July 2007).

The limitations encountered in this study were unpredictable weather conditions that affected field trials in 2005. This necessitated discontinuation of experiments due to severe drought conditions, causing some delay. Fortunately, this was at the start of the trials and after the drought ended, the conditions remained favourable throughout the period of the trials.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Early history of bacterial wilt disease

Little was known about the incidence of bacterial wilt caused by *Ralstonia solanacearum* prior to the latter part of the nineteenth century. It is possible that full recognition of the disease awaited the intensification of the culture of susceptible solanaceous hosts such as tomato, potato and tobacco in the subtropical, tropical and warm temperate zones of the world (Kelman, 1953). The earliest record of this disease may have been made in Japan. The occurrence of a serious tobacco disease (called "neguchi") in the tobacco growing districts was mentioned in an old Japanese book written in 1683 (Kelman, 1953).

The first reports of the disease in the United States were made in 1890 and 1891 in connection with an unidentified bacterial pathogen affecting potato tubers from the southern states (Kelman, 1953). Upon receipt of diseased tomato plants from Ocean Springs, Mississippi in 1895, Smith began investigations on the problem which resulted in the first adequate description of the disease and the causal agent. On a field trip through South Carolina, he saw whole fields of potatoes and tomatoes destroyed by bacterial wilt. Farmers indicated to him that the disease was well known to them and that it was not a new problem in the area. However, the number of years that it had caused losses was not known (Kelman, 1953).

In Java, the disease on peanuts was apparently a minor problem prior to 1890. When markets for peanuts in China became lucrative and wide cultivation of this crop followed, bacterial wilt soon became a major threat to peanut production.

Observations of the presence of the disease on potatoes in Queensland, Australia were made in 1894. In India, reports on the disease of potato wilt in the Poona District in 1892 were probably the first record of the disease on potato. The earliest report from Europe on the disease was made by Comes in 1884 when he described a bacterial disease affecting tomato in Naples (Kelman, 1953).

2.2 Origin and diversity of the pathogen

The origin of the nearly world-wide wilt disease of potato, tobacco, tomato, and many other members of various dicotyledonous families is lost in antiquity. It is not known from which continent *Ralstonia solanacearum* arose or whether it evolved separately in many

locations on related ancestors of modern plants. Reports of severe wilt in crops planted for the first time in virgin soil in Indonesia, Central America and Florida indicate that the pathogen has long been present in widely separated areas (Buddenhagen and Kelman, 1964; Hayward, 1991). In the United States, the disease is infrequent North of Maryland and Virginia and West of the Appalachian mountains and is absent from much of this area, especially the western states (Buddenhagen and Kelman,1964).

Since the disease was first observed about the same time in so many different areas of the world and was apparently well established in most of these regions when scientific studies were first initiated, it is difficult to draw any conclusions as to the region of origin and subsequent dissemination of the pathogen (Kelman, 1953). Most evidence at the present time supports the hypothesis that strains of *R. solanacearum* are the product of long evolution occurring independently in various areas on different hosts. The possibility that some soil Pseudomonads could be reverted easily to *R. solanacearum* by rapid selection pressure is most improbable (Buddenhagen and Kelman, 1964). Kelman (1954) suggested that in investigating possible progenitors of *R. solanacearum* from saprophytic soil inhabitants, the non-virulent butyrous mutants should be considered. These essentially saprophytic mutants are part of the species, *R. solanacearum*, but their fate in soil is unknown. Whether they might exist in soil and have the potential to "revert" to the virulent fluidal form is not known.

The apparent complexity and world-wide distribution of *R. solanacearum* has prompted questions as to whether the pathogen evolved separately in different geographical locations (Buddenhagen, 1985). The many reports of bacterial wilt following the introduction of exotic crop plants in virgin areas indicate that *R. solanacearum* is endemic on the native flora in many parts of the world (Kelman, 1953). Race 3 is the most homogenous group within *R. solanacearum* in terms of host range and physiological parameters and is synonymous with biovar 2. Race 3 strains are limited primarily to potato and are thought to have evolved on native tuber-bearing solanum species in the Andean region of South America. Although it is likely that race 3 occurs in many potato growing areas of the world because it was dispersed in infected tubers, there is speculation that it may have originated outside of South America (Buddenhagen, 1985).

Despite continued efforts to unravel the relationships among members of *R. solanacearum*, no substantial progress has been made since the implementation of the race and

biovar systems of classification. Questions regarding the origin and evolution of *R. solanacearum* remain unanswered. Modern molecular biology techniques, however, provide the means to determine the relatedness of bacterial strains on a more exact basis (Cook *et al*., 1989).

Recently, a better understanding of the intraspecific groupings within *R. solanacearum* has been done through a number of molecular techniques (Cook *et al.*, 1989; Hayward, 1991; Hayward *et al*., 1991; Gillings and Fahy, 1993; Smith *et al*., 1995; Smith *et al*., 1996; Smith *et al*., 1997; Seal e*t al.*, 1999; Pastrik and Maiss, 2000; Scortichini *et al.*, 2000; Timms-Wilson *et al.*, 2001; Genin and Boucher, 2002). These studies have indicated that *R. solanacearum* evolved as two major and distinct groups as a result of geographic separation. Division 1 comprising biovars 3, 4, and 5 originated in Asia and Australia and division 2 comprising biovars 1, 2 and N2 originated in the Americas. Several of the above investigations employing molecular methods have confirmed this dichotomy within *R. solanacearum* (Fegan and Priou, 2005). Sequencing of the 16s-23s rRNA gene intergenic spacer region (ITS), the polygalacturonase gene and the endoglucanase gene has supported the existence of the two divisions as well as the existence of the group of strains originating in Indonesia. Polylogenetic analysis of the endoglucanase and *hrp*B genes has confirmed the presence of a group of strains originating in Africa (Fegan and Priou, 2005).

The occurrence of intra-specific groups outside their centre of origin probably resulted from human activity. This is particularly true for the presence of race 2 and 3 outside of Central and South America, respectively. These studies corroborate broadly and indicate that race 1 is the most and race 3 the least genetically diverse of all the races of the pathogen. Smith *et al.* (1995) indicated that few studies had been carried out on delineation of genetic diversity within these subdivisions. They observed that the delineation of bacterial populations was a prerequisite to studying the epidemiology of pathogens and, ultimately, the development of control strategies.

2.3 Economic importance of bacterial wilt disease

The wilt disease caused by *Ralstonia solanacearum* is one of the most important plant bacterial diseases in the world. As is the case with many other plant pathogens which have both a wide host range and extensive distribution, it is difficult to make an accurate estimation of the economic losses caused by *R. solanacearum* (Graham, Jones and Lloyd, 1979). The disease causes losses due to the destruction of affected crop plants. Premature death of plants and

reduction in yield are the main ways in which bacterial wilt causes economic loss in the crop. Secondary cause of decreased revenue from potato is the rotting and decay of tubers during storage and transit (Kelman, 1953).

The disease is of great significance in the agricultural economy of many regions. With reference to early losses in the South-Eastern United States, Smith wrote; "This disease has destroyed a great many fields of potatoes and tomatoes in the South, and has put an end to commercial tomato growing in certain sections, e.g. Southern Mississippi, Southern Alabama and parts of Florida". During the period 1920 to 1940, the sociological and economic impact of wilt at Granville County was felt by hundreds of farm families. Due to loss of income from their main cash crop, many farmers were forced to sell their farms and leave Granville County in search of new employment (Kelman, 1953).

There is little general information on the economic impact of *R. solanacearum* worldwide. Direct yield losses vary widely according to host, cultivar, climate, soil type, cropping practices and pathogen strain. Bacterial wilt of potato has been estimated to affect some three million farm families (about 1.5 million Ha) in around 80 countries with global damage estimates currently exceeding $950 million annually (Elphinstone, 2005). China, Bangladesh, Bolivia and Uganda were thought to be among the most seriously affected countries. In Bolivia, yield loss estimates at harvest ranged from 30%-90% and losses during storage were as high as 98% (Coelho and Nutter, 2005). Current reports from Bangladesh quote some regions as having more than 30% of potato crops affected by bacterial wilt with over 14% reduction in yield (Elphinstone, 2005). In Nepal, tuber rotting occurred in an average of 10% of stored potato and a maximum of 50% in some cases. Crop losses in small farms in the Nepalese hills were up to 100%, mainly due to poor cultural practices and planting seed from infected crop (Elphinstone, 2005).

Potato yield losses due to bacterial wilt in Uganda are estimated at 30% (Alacho and Akimanzi, 1993), with occasional losses of 100% (Opio, 1988; Kakuhenzire *et al.*, 1993). A survey conducted under the African Highland Initiative in Kabale, the major potato producing district in south-western Uganda, estimated the yield loss due to bacterial wilt at 26% (Lemaga *et al.*, 1997). Research work in Kenya revealed that there is an increase of incidences of bacterial wilt of potato causing losses and decline in yields (Ajanga, 1993, and Barton *et al*., 1997.

Ajanga (1993) estimated yield losses due to bacterial wilt at 50% in ware and 75% in seed potato.

2.4 Infection and symptomatology

The mode of entry of *R. solanacearum* into the host tissue is usually through openings in the root system. A wound is considered to be necessary for entrance of the pathogen. Such a wound may be caused by parasitic fungi, nematodes, insects and tools used during weeding. incubation period before symptoms appear in the susceptible plant varies, Following entrance of the pathogen into a host, depending on species of plant attacked, age of the plant and environmental factors. Young succulent plants develop critical disease symptoms much more rapidly than older plants (Kelman, 1953).

Symptoms caused by *R. solanacearum* are wilt followed by complete collapse of affected plants (Dowson, 1943; Dowson, 1949; Harrison, 1960). The first symptom, usually noticed on a warm day, is slight drooping of the tip of one or two of the lower leaves, which may easily be mistaken for a temporary shortage of soil moisture (Harrison, 1960). The affected leaves may recover their turgidity towards evening. By the third day, two or three leaves of the affected plant permanently wilt. The lamina rolls upwards and inwards from the margins until the leaves completely rolled in (Harrison, 1960). Sometimes only one branch of a potato hill may show flaccidity. In other cases, if onset of the disease is rapid, foliage of an entire hill may quickly droop and wilt without change in colour (Kelman, 1953; Harrison, 1960).

The wilting of foliage is due to the interference with water movement due to formation of extracellular polysaccharides in vessels and tracheids, bacterial cells plus tyloses. The yellowing of foliage is due to the breakdown of chlorophyll resulting from decreased supply of mineral nutrients and water, plus unknown effects of host and pathogen metabolites. Sometimes the foliage shows marginal necrosis, which is due to decreased supply of water and other factors. Distinct epinasty of the petioles prior to wilting may be apparent if the onset of the disease is slow. Leaf epinasty may also occur in potato and African marigold (Kelman, 1953). Leaf epinasty is due to increased levels of indole-acetic acid (IAA).

Adventitious roots commonly develop on the stems of infected tomato plants, when plants wilt gradually or when plants are inoculated with isolates of low virulence. However, these structures frequently develop naturally on healthy plants under moist conditions. Similarly, formation of adventitious roots following inoculation has been observed on tobacco, African

marigold and sunflower. The cause of the growth of adventitious roots may be the increase in IAA and interference with downward movement of materials in the phloem, due to pathogen effects (Kelman, 1953; Buddenhagen and Kelman, 1964). Stunting is one of the symptoms observed in infected plants. Gradual defoliation is also another characteristic of infected plants. The leaves may turn yellow slowly and roll or curl before they drop (Kelman, 1953).

Examination of a cross-section of the plant stem during early stages of infection reveals light tan to yellow-brown discolouration of the vascular tissue. A valuable diagnostic sign of the disease on any host is the appearance of slimy viscous ooze in the form of tiny, dirty-white to brownish glistening beads on the cut stem with vascular strands severed (Kelman, 1953).

Ralstonia solanacearum is highly pathogenic and infects plant tissues through natural openings or via mechanical wounds on roots. On entry, bacterial cells penetrate the xylem vessels and spread throughout the plant establishing foci of infection. The colonizing bacteria cause rot and tissue disintegration through the action of lytic enzymes. The vascular discolouration is due to tyrosinase of the pathogen and polyphenoloxidase of the host. Dissolution of pectic substances in the middle lamella is caused by pectin methylesterase and polygalacturonase. The degradation of cellulose in the cell walls is caused by cellulases produced by the pathogen (Husain and Kelman, 1958). Extracellular polysaccharides (EPS), endoglucanase (EG) and endopolygalacturonase (PG) are the major virulence factors.

Harrison (1960) observed that the stems of affected potato plants were visibly infected only for a distance of 5-8cm from the old seed. When the stems were cut transversely at a point above this distance, there were no macroscopic signs of the disease.

Symptoms on infected tubers are brown discoloration of the vascular ring with exudation of dirty-white, slimy masses of bacteria when a cut tuber is gently pressed (Harrison, 1960). At the eyes or stolon end of a severely decayed potato, a sticky exudate form, mixes with the soil and causes it to adhere to the tuber surface. Plants attacked early during the growing season form no tubers. The tubers of plants infected in the middle of the season are small and few and are found in various stages of decay (Kelman, 1953).

Not all tubers produced on plants which show above-ground symptoms are affected. However, diseased tubers are occasionally found on plants whose foliages show no apparent evidence of infection. Root decay that is not accompanied by foliar wilting is often the most characteristic manifestation of the disease in some host plants. This is true for less susceptible

host plants such as Castor bean and weeds such as *Leptilon canadense, Ambrosia trifida* and *Solanum carolinense.*

2.5 Etiology

Early etiological work was described by Kelman (1953) as follows: "In 1896, Smith published the first adequate description of the causal agent of bacterial wilt of solanaceous plants and demonstrated its pathogenicity to potato. The bacterium was named *Bacillus solanacearum*. A number of generic names have been suggested since then").

The first generic name, *Bacillus* was proposed by Smith who thought the bacterium had peritrichous flagella. Later, Smith suggested that the organism be known as either *Bacterium solanacearum* or following Migula's classification, as *Pseudomonas solanacearum* because the pathogen was found to be motile with a single polar flagellum.

The organism was again reclassified and placed in the genus *Phytomonas* (Bergey, 1923), but Dowson (1943) transferred it to a new genus, *Xanthomonas,* because it differed from typical members of the earlier group in certain cultural characteristics. Later, it was found that members of the genus *Xanthomonas* were unable to utilize asparagines as a sole source of carbon and nitrogen in a synthetic medium, whereas *Pseudomonas solanacearum* was able to utilize this compound. This characteristic plus the absence of typical *Xanthomonas* yellow pigment in its colonies raised questions as to the reason of Dowson's suggested change in classification. As a result, Dowson (1949) transferred the organism back to the genus, *Pseudomonas*. This is in agreement with the classification adopted in Bergey's Manual of Systematic Bacteriology (Palleroni, 1984). Below is a list of synonyms, which have been given to the pathogen:

Bacillus solanacearum Smith, 1896

Bacterium solanacearum Chester, 1898

Pseudomonas solanacearum Smith, 1914

Phytomonas solanacearum (Smith) Bergey, 1923

Xanthomonas solanacearum (Smith) Dowson, 1939

Burkholderia solanacearum (Smith) Yabuuchi, 1992

In 1992 a number of Gram-negative *Pseudomonas* spp. belonging to the rRNA group II was reclassified into the new genus, *Burkholderia (*Yabuuchi *et al.,* 1992*).* Since then, multiple changes have occurred within the genus, which is expanding rapidly and presently comprises 22 species. Some species (*Burkholderia pickettii and B. solanacearum*) have since been transferred

to another new genus *Ralstonia* (Yabuuchi *et al.*, 1995) on the basis of phenotypic characterization, cellular lipid and fatty acid analysis, phylogenetic analysis of 16s rDNA nucleotide sequences and RNA-DNA hybridization. The type species of the new genus is *R. pickettii*. The type strain of *R. solanacearum* is ATCC 10696T (Yabuuchi *et al*., 1995).

2.6 Morphology and cultural characteristics

Smith was the first person to describe the causal agent of bacterial wilt of potato. He observed that single cells were rod-shaped and had rounded ends. Many of these rods were 0.5 x 1.5µm. The sizes were, however, variable and were influenced by the conditions under which growth was obtained. Rods from young cultures in broth or nutrient agar were found to be longer than those from old cultures (Kelman, 1953). *Pseudomonas solanacearum* is described in Bergey's Manual of Systematic Bacteriology as gram negative rods, measuring 0.5-0.7 by 1.5-2.5µm and motile by one to four polar flagella. The bacterium shows distinct bipolar staining with methylene-blue, carbol fuchsin or any of the alkaline aniline dyes (Kelman, 1953). Hayward (1960) indicated that the lack of affinity for basic dyes which result in bipolar staining was caused by the presence in the cells of massive inclusions of poly-ß-hydroxybutyrate. It was observed that cells from virulent fluidal colonies of *R. solanacearum* produce poly β-hydroxybutyric acid. Polymer levels on a cell dry weight basis were higher for virulent types. However, this compound is also formed by saprophytic species and, therefore, any possible role in virulence is obscure. Research studies, however, indicate that this storage material plays an important role in carbon metabolism of many bacteria (Doudoroff and Stanier, 1959).

In determining the characteristic appearance of colonies of *R. solanacearum*, conditions prevailing during incubation and the type of substrate used are of great importance since they influence bacterial growth. Smith observed that colonies on solid media were usually irregular, round, slightly raised, smooth and measured 3-5mm in diameter (Kelman, 1953). Appearance of colonies of the bacterium have since been described by various workers as irregular in shape, fluidal, slightly raised, slimy and creamy-white with pink-orange centres (Dowson, 1949; Kelman, 1954; Harrison, 1960; Hayward, 1960; Hayward, 1976; French and Sequeira, 1970). With few exceptions, most authors agree that *R. solanacearum* is non-spore forming, non-capsulate, Gram-negative, and not acid-fast and reveals distinct bipolar staining.

2.7 Races, biovars and hosts

It has been observed that in some cases, apparent immunity of plants to infection by *R. solanacearum* in a given area may be considered to be susceptible elsewhere, inoculations with pure cultures to known hosts may fail to cause disease and atypical cultural or physiological traits in certain isolates exist (Kelman, 1953). Evidence of new or different strains based solely on the failure of inoculation experiments in one or more hosts may reflect the use of avirulent or weakly pathogenic strains. These observations indicate the possibility of existence of different strains within the species of *R. solanacearum*. Strain determination in this species, however, is difficult. The numerous hosts affected by the pathogen, its wide geographic distribution, and the intrinsic complexities of strain differentiation in this variable species, have resulted in the development of several methods of classification at sub-specific level, including races, strains and biovars, on the basis of widely different criteria (French and Sequeira, 1970).

There are three types of strains of bacteria in nature: (a) Strains specialized in pathogenicity only; (b) Strains specialized in pathogenicity and in other physiological or morphological characters which may or may not be related to pathogenicity; (c) Strains specialized in bacteriological characters only. A tentative classification scheme for *R. solanacearum* could be done using characters such as pathotype, colony type, biochemical type, isotype, serotype and bacteriocinotype. Once sufficient characters are known, then the isolate can be placed in a category or strain (Buddenhagen and Kelman, 1964). The term race has been applied in classification of *R. solanacearum*. The term has subtitle connotations of character as well as category, since it is mainly a subjective grouping of pathotypes.

Natural host range, reaction of differential hosts and colony appearance on tetrazolium medium were used to classify several hundreds of *R. solanacearum* isolates obtained from a wide range of hosts in Central and South America into three races. The races were characterized as: race 1 which infects tobacco (*Nicotiana tabacum*) and other solanaceous hosts; race 2 which causes wilt of bananas, *Heliconia* spp. and other Musaceae hosts; and race 3 which affects potato (*Solanum tuberosum*) (Buddenhagen *et al.,* 1962). Sequeira and Averre (1961) showed that race 1 could be distinguished from race 2 isolates by the intense brown pigment produced by the former when grown on a medium containing tyrosine. Race 3 isolates produced small amount of this pigmentation in the same medium.

Buddenhagen and Kelman (1964) noted that in most classification schemes, colony morphology and virulence of different isolates on inoculated hosts could be used to distinguish not only the three major races but also certain strains within these races. Race 3 has the narrowest host range, limited primarily to potatoes and to a lesser extent tomatoes. A further characteristic of race 3 is that it is mainly prevalent in warm temperate zones and thus lower optimum temperature favours its growth.

Tobacco leaf infiltration technique was used by Lozano and Sequeira (1970) to differentiate the races of *Ralstonia solanacearum*. Each race showed a different reaction at the infiltrated area. Race 1 isolates caused no visible symptoms 24 hours after inoculation, but a dark brown necrotic lesion appeared after 36 hours. Race 2 isolates induced hypersensitive reaction within 10-12 hours after infiltration and race 3 isolates caused a yellowish discoloration of the infiltrated area by 48 hours after inoculation. Colony morphology, melanin formation and pathogenicity tests are useful in classification of isolates into races and strains (French and Sequeira, 1970).

Morton *et al.* (1966) differentiated the three races of *R. solanacearum* by serological methods. They found that *R. solanacearum* was not closely related to other plant pathogenic bacteria such as *Corynebacterium michiganense, Pseudomonas savastanoi, Pseudomonas syringae* and *Xanthomonas vesicatoria.* Harrison and Freeman (1961), after considerable cross-precipitate purification, were able to distinguish an Australian potato strain of *R. solanacearum* from a Rhodesian isolate, corroborating differences based on physiological reactions. They, however, did not obtain most specific antibody formation since nucleoproteins were not used as immunogens.

On the basis of various physiological characteristics, Hayward (1964) classified 95 isolates of *R. solanacearum* from different hosts into major biochemical types I, II, III and IV. He found that all the isolates obtained from potato formed a single group on physiological reactions. He called the group biochemical type II which could be differentiated from three other groups by its ability to oxidize mannitol, sorbitol and dulcitol. The biochemical type II or race 3 is the only race/biotype that can be described by a single biovar class, biovar 2. It corresponds to race 3 of Buddenhagen *et al.* (1962). Buddenhagen and Kelman (1964) also noted that in most classification schemes, colony morphology and virulence of different isolates on inoculated hosts could be used to distinguish not only the three major races but also certain strains within these

races. The potatoes can also be affected by the broad-range race 1 or biotype 3 (Buddenhagen and Kelman, 1964).

Five races have been described and designated according to the host or hosts primarily affected (Buddenhagen and Kelman, 1964) and five biovars have been differentiated according to ability to utilize and/or oxidise several hexose alcohols and disaccharides (Hayward, 1964). Biovars 1 and 2 are less nutritionally versatile than biovars 3 and 4 and this difference is reflected in separation into distinct phenons by numerical taxonomy. There are some differences in electrophoresis pattern of the membrane proteins between biovars, and biovars 1 and 2 are distinct from biovars 3, 4 and 5 on the basis of DNA probes and restriction fragment length polymorphism (RFLP) analysis (Cook *et al.,* 1989). There are also marked differences in geographical distribution of biovars suggestive of separate evolutionary origin. In general, biovar 1 is predominant in the Americas while biovar 3 is predominant in Asia. Biovars 2, 3 and 4 occur in Australia, China, India, Indonesia and many other countries. The relationship between host specialization and phenotype is most clearly evident between race 3, the potato race, and biovar 2. In general, race 3 and biovar 2 are equivalent (Buddenhagen and Kelman, 1964). Biovar 2 has a limited host range being found mainly on potato and sometimes on tomato and a few weed hosts. The biovar is adapted to grow at low temperatures and has limited capacity for survival in fallow soil than biovar 3 (Buddenhagen and Kelman, 1964).

Nattrass (1945; 1946) reported that there were two strains of *P. solanacearum* both of which attacked potatoes in Kenya. However, Robinson and Ramos (1964) reported that only one strain attacked potatoes. The strain, which was found to attack potatoes, was biochemical type 2. They also found that adequate differentiation between the biotypes was possible with the use of only six carbohydrates. The carbohydrates they used were: glucose, lactose, mannitol, sorbitol and cellobiose. Salicin was used as a negative control. The work of Harris (1976) indicated that the strain which caused the disease of potato in Kenya was a strain that grew at low temperature. Nyangeri (1982) found that only biochemical type 2 affected potatoes in Kenya, however, biochemical type 3 was also present and was isolated from *Solanum melongena.* There have been reports that biochemical type 3 has been isolated from potato in some parts of the world.

It was reported that the strain which attacked potato in Portugal was similar to the isolate that was described by Nattrass (1946) in Kenya in that the vascular tissue of infected potato plants did not show brown discolouration. This was *Ralstonia solanacearum* biovar 2.

2.8 Intra-specific groups

Evidence from studies on the host range, colony morphology, biochemistry, physiology and molecular biology of *R. solanacearum* suggests that it is a complex and heterogeneous species (Kelman, 1953; Buddenhagen *et al.*, 1962; Hayward, 1964; Cook *et al.,* 1989; Hayward *et al*., 1991; Fegan and Priou, 2005). Methods for subdividing the species have been devised. The race scheme groups isolates according to host range and colony morphology and has classified the population into five races (Buddenhagen *et al*., 1962). The biovar scheme divides the species into five groups, based on the ability of strains to metabolize specific disaccharides and hexose alcohols (Hayward, 1964; Hayward, 1991).

The use of molecular techniques has afforded a greater understanding of intra-specific groupings within *R. solanacearum*. Cooksey and Graham (1989) showed that the species can be divided on the basis of restriction fragment length polymorphism (RFLP). Analysis of RFLP of isolates suggests that race 3 strains are highly conserved at the DNA level (Cook *et al.*, 1989). However, some strains reported to be widely distributed in the lowlands of the Amazon basin are metabolically more versatile than biovar 2 strains isolated in the Andean highlands. This versatile phenotype has been designated biovar N2 (Hayward *et al.,* 1991). Gillings and Fahy (1993) showed that biovar 2 and N2 strains differed in their distribution and metabolic properties when they analysed the organisms using total genomic DNA digested with restriction enzyme *Hae* III. Separation of these digests on 5% polyacrylamide gels and subsequent silver staining was shown to be a rapid and sensitive method of distinguishing between biovar 2 and N2 strains.

Recently, genetic diversity among isolates of *R. solanacearum* race 3 biovar 2 in Kenya was determined by using genomic fingerprinting protocols. Repetitive sequence (rep)-PCR involving the use of primer sets based on conserved repetitive bacterial DNA elements: enterobacterial repetitive intergenic consensus (ERIC), repetitive extragenic palindromic (REP) and BOX that yield genomic fingerprints specific to pathovars and strains of gram-negative bacteria were used (Smith *et al.,* 1995). The actual functions of these highly repeated and conserved elements remain mysterious. It has, however, been suggested that they are involved in stabilizing mRNA, translational coupling between genes, chromosome organization, DNA gyrase and DNA polymerase (De Bruijn, 1992). It has also been suggested that the elements have the potential to form stem-loop structures and may play an important role in the organization of bacterial genome. Genome organization is thought to be shaped by selection, and

thus the dispersion of the REP, ERIC and BOX sequences may be indicative of the structure and evolution of the bacterial genome (Louws *et al*., 1994). Smith *et al.* (1995) also used an alternative approach which relies on restriction endonuclease profiles of genomic DNA. They used rare-cutting restriction endonucleases and thereafter resolution of the macro fragments by pulsed-field gel electrophoresis (RC-PFGE). Analysis of the Rep-PCR data obtained revealed only 3 rep-PCR profile types at 95% similarity value. RC-PFGE identified 10 distinct profile types among Kenyan biovar 2 isolates.

A new taxonomic framework and methodology has been proposed which can allow identification of sub-specific groups within the *R. solanacearum* species complex. This reflects the known diversity within the *R. solanacearum* (Fegan and Priou, 2005). Under this classification, members of *R. solanacearum* can be subdivided into four phylotypes corresponding to the four genetic groups identified via sequence analysis. Phylotype 1 is equivalent to division 1 (Cook *et al.*, 1989) which includes all strains belonging to biovars 3, 4 and 5. Phylotype II is equivalent to division 2 and includes strains belonging to biovars 1, 2 and 2T. Phylotype II contains *R. solanacearum* race 3 potato pathogen. Phylotype III contains strains belonging to biovars 1, and 2T isolated from Africa and surrounding islands. Phlotype IV contains strains isolated from Indonesia belonging to biovars 1, 2 and 2T. This phylotype also contains the two close relatives of *R. solanacearum*, *R. syzygii* and blood disease of banana (BDB) pathogen (Fegan and Priou, 2005).

Each phylotype is composed of a number of sequevars. A sequevar, or sequence variant, is defined as a group of strains with a highly conserved sequence within the area sequenced. Each sequevar may be composed of a number of clone lines that may be identified using genomic fingerprinting methods such as pulsed-field gel electrophoresis (PFGE), amplified fragment length polymorphism (AFLP) or rep-PCR (Fegan and Priou, 2005).

2.9 Weed hosts

The numerous plants attacked by *Ralstonia solanacearum* include not only important economic hosts such as tobacco, potato, tomato, pepper, eggplant, peanut and banana, but also a number of ornamentals and a very diverse group of weeds. It was first believed that the pathogen affected only solanaceous plants, but it was soon found that the host range involved species in many other families (Kelman, 1953). The extensive host range of *R. solanacearum*

includes several hundred species representing 44 families of plants. The range and variety of weed hosts of *R. solanacearum,* therefore, is extensive. Some of the host plants are susceptible; others tolerant, while still others may act as latent or symptomless carriers (Granada and Sequeira, 1983). However, these behavioural characteristics of hosts vary in different environments. It is known that there are sheltered sites where the bacterium may survive between successive plantings of a susceptible crop. These include plant debris and latently infected potato tubers (Graham *et al*., 1979), the deeper soil layers and in the rhizosphere of roots of weed hosts (Jackson and Gonzalez, 1981; Granada and Sequeira, 1983). The significance of the weed hosts varies greatly in different environments and cropping systems. Such investigative work has not been done in our environment and therefore, these investigations will be beneficial.

In India, *Ageratum conyzoides* and *Ranunculus scleratus* showed no symptoms, but the pathogen was readily isolated from surface-sterilized roots (Hayward, 1991). In a cool temperate region of Australia, the perennial weed S*olanum cinereum* was identified as a symptomless carrier of *R. solanacearum* biovar 2 in potato cropping land, which could have significance in long term survival of the pathogen. Another perennial water course weed *Solanum dulcamara* has been shown to be a symptomless carrier of *R. solanacearum* biovar 2. The host continuously releases the bacterium into the water (Hayward, 1991). Other weeds growing in water courses such as *Urtica dioica* (stinging nestle) may play a similar role.

In the Philippines, *Portulaca oleraceae* was reported to be a symptomless host, while it was reported as a susceptible host to *R. solanacearum* biovar 3 in Queensland, Australia (Hayward, 1991). In the tropics annual weed hosts are available throughout the year, thereby increasing the population of the bacteria in the soil. These weeds may serve as alternative hosts whenever non-host plants are cultivated thus making crop rotation ineffective in reducing the bacterial population in the soil. In Philippines, the population of the pathogen gradually declined in both rhizosphere and non-rhizosphere soils of various crop and weed hosts, except in the rhizosphere soil of *Portulaca oleracea* where it gradually increased (Hayward, 1991).

It is generally considered that *R. solanacearum* does not survive for long periods in vegetation-free soil (Granada and Sequeira, 1983). Some soils are conducive to the survival of *R. solanacearum* and others are suppressive. However, bacterial wilt occurs throughout the world in soils of great diversity. A few investigations have been done on this topic but the

workers often do not define the soil type which they mention. The information is sometimes fragmented and contradictory (Kelman, 1953; McCater, 1976; Hayward, 1991).

Pradhanang *et al.* (2000b) have compared various methods for detection of *R. solanacearum* populations in soil. Knowledge of pathogen survival based on infection of some potential weed hosts is still incomplete and further investigation is justified (Pradhanang *et al.*, 2000a).

In the absence of potatoes, Tusiime *et al.* (1998) reported that many herbaceous weeds were latently infected with the bacterium. There are reports that the following weeds were found to be hosts of *R. solanacearum* in Kenya: *Cleome monophylla, Datura stramonium, Erlangea tomentosa, Portulaca oleraceae, Solanum incanum* and *solanum indicum* (Ondieki, 1973; Kung`u and Boa, 1997; Janse *et al*., 2005).

2.10 Crop rotation as a control measure of the disease

Various strategies have been developed for the control of bacterial wilt, but many are limited in general application. They are often crop or site specific or severely limited in application by socioeconomic conditions (Hayward, 1991).

Research reports indicate that crop rotation helps to significantly reduce or even eradicate potato bacterial wilt and it is therefore recommended as a component of integrated disease management (Kloos *et al.*, 1991). A two and half-year rotation with pasture coupled with healthy seed tubers and effective quarantine procedures were found sufficient to eradicate potato bacterial wilt (Lloyd, 1976). Barton *et al.* (1997) reported higher potato yields under crop rotation in Kenya. Granada and Sequeira (1983) indicated that crop rotation could be less useful for the control of bacterial wilt, particularly race 1 than was generally believed. This is because of the diverse nature of the organism and its ability to survive for a long time by infecting roots of non-host plants used in crop rotation. Jackson and Gonzalez (1981) reported that on naturally infested soil, rotations with maize, cowpeas and sweet potatoes did not reduce the relative inoculum potential of *P. solanacearum*, race 1. In the absence of potatoes, Tusiime *et al*. (1996) reported that many herbaceous weeds were latently infected with the bacterium which could render crop rotation ineffective. Verma and Shekhawat (1991) reported that a five-year rotation that included wheat, lupin and maize reduced wilt and increased yield. Lemaga *et al.* (2004), in their experience of African Highlands Initiative, found that in a mildly infested field (15-20%), a

one-season crop rotation was sufficient to significantly reduce bacterial wilt incidences and increase yields, whereas in heavily infested fields (>90%) a two season rotation was not sufficient to reduce bacterial wilt to economically acceptable levels. Lemaga *et al*. (2001a) found that rotation of crops reduced incidences of bacterial wilt and increased yields. In their soil amendment experiments, Lemaga *et al.* (2001b) also found that disease incidences declined with the use of fertilizers and the yields were increased.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Determination of population structure of *Ralstonia solanacearum*

3.1.1 Sampling

During the long rainy season, March to July 2006, potato farms in major potato growing zones in Kenya were visited and plants exhibiting symptoms typical of bacterial wilt disease were sampled. These farms were in agro-ecological zones including Upper midlands 3 (UM3) in Murang`a, Central Province and National Agricultural Research Laboratories in Kabete; Upper midland 2 (UM2) in Meru, Eastern Province; Lower highland 2 (LH2) in Bomet, Nakuru, Gituamba and Bahati in Rift Valley Province; Lower highland 1 (LH1) in Limuru and Kiambu in Central Province; Upper highland 2 and 3 (UH2 and UH3) in Nyandarua, Central province and Matharu in Rift Valley Province and Lower highland 3 (LH3) in some parts of Nyandarua (Figure 1). The altitudes, latitudes, and longitudes of sampling sites were recorded using Geographic Positioning System (GPS) equipment (GPS 48 Garmin, Garmin Corp.) for subsequent mapping of *Ralstonia solanacearum* occurrence using the programme Arc View GIS 3.2 (Environmental System Research Institute, California, USA).

Sampling was carried out with the assistance of agricultural extension officers in the respective areas. In each region, 10 farm sites were targeted, with farms being at least five kilometres from each other. At each site, the potato crops were examined for the presence of infected plants, some of which were then sampled using a line transect. Disease symptoms as described by Kelman (1953) and Harrison (1960) were used to identify diseased plants. Samples were not taken from uninfested farms. Some photographs of diseased plants and infested fields were taken. Stems and/or tubers from potato and stems from other plants showing disease symptoms were sampled, placed in marked paper bags and transported to the laboratory for further examination and isolation of *Ralstonia solanacearum*. Some weeds growing near the potatoes showing bacterial wilt symptoms were sampled and tomato plants having bacterial wilt symptoms were also sampled. A total of 70 samples suspected to be infected were collected, taken to the laboratory and analysed for infection by *Ralstonia solanacearum*. The names of the divisions or areas from where the samples were collected were recorded. The agro-ecological zones were noted and the cropping patterns of the collection areas were recorded.

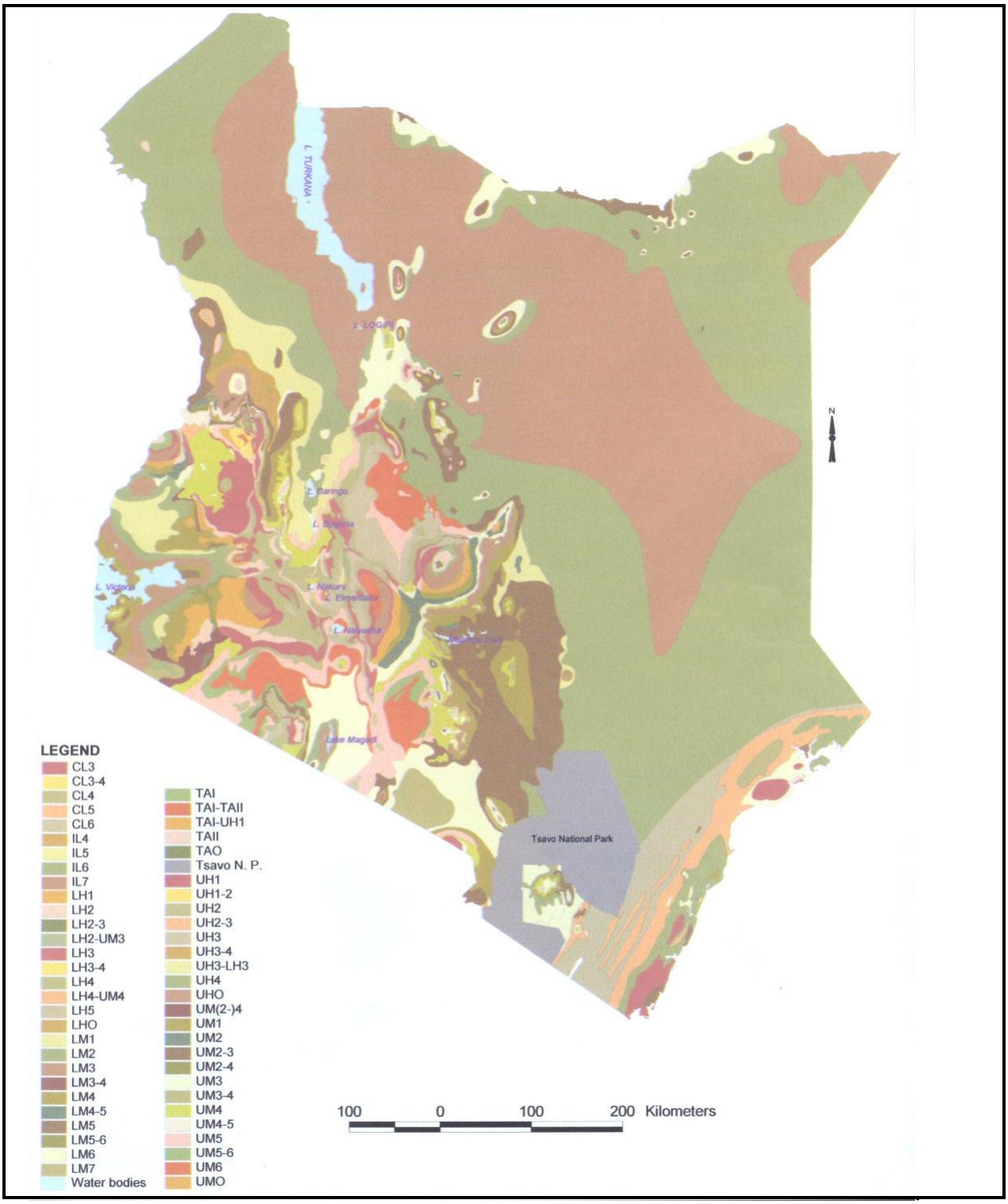


Figure 1. Agro-climatic zone map of Kenya. CL=Coastal Lowlands; IL=Inner Lowlands; LH=Lower Highlands; LM=Lower Midlands; TA=Tropical Alpine; UH=Upper Highlands; UM=Upper Midlands, Source: Sombroek *et al*. (1980).

3.1.2 Extraction of bacteria from infected plant material

Stem segments (3–5cm long) were cut from the lower part of the diseased plant materials. The segments were washed thoroughly in running tap water. The segments were then surface-sterilised by immersing in 70% ethanol and flamed off or blotted dry on paper towels for quick removal of the alcohol. Alternatively, the stem segments were submerged in 0.5% sodium hypochlorite (NaOCl) for 5 minutes rinsed three times in sterile distilled water and gently blotted dry on paper towels. After surface-sterilisation of the plant material, 0.5cm pieces were aseptically cut off from both sides of the segments using a sterile scalpel. From the remaining segments, 0.5–1.0cm sections were cut and put in 5ml sterile distilled water for 5 minutes to allow any bacterium present to ooze into the water. A cloudy appearance of the extract often indicated high numbers of bacteria.

In the case of potato tubers, the samples were dipped in 1% sodium hypochlorite for 5 minutes, and then air-dried on clean paper towels. Using sterile blades, the tubers were cut transversely at the stolon end and then small pieces of the vascular rings of approximately 2mm width and 1mm depth were removed using a sterile cuticle remover. The pieces were put in 5ml sterile distilled water to allow any bacterium present to ooze into the water. For both cases of stems and tubers, the segments were aseptically removed from the water to leave behind the bacterial suspension.

3.1.3 Isolation and purification

A loopful of the bacterial suspension was aseptically taken and streaked onto Semi-selective Medium, South Africa (SMSA), (French *et al.*, 1995), (Appendix I (a)). Another loopful of the suspension was streaked on Kelman's Tetrazolium Chloride (TZC) medium (Jenkins and Kelman, 1976) (Appendix 1 (c)). The streaking was duplicated for each sample and medium. The streaked plates were incubated in an inverted position at 28^0C for 48–72 hours. After the incubation period the plates were examined and the observed characteristics, for example, appearance in colour, consistency, shape, formazan deposit and size, were recorded. The colonies that were consistent with *Ralstonia solanacearum* characteristics were sub-cultured onto fresh SMSA or Casamino acid Peptone Glucose (CPG) agar: [(0.1%) peptone, (0.01%) casamino acids (Difco), (0.05%) glucose, and (1.5%) agar] (Appendix I (b)). The purified colonies were assigned isolate numbers and picked for storage.

3.1.4 Storage/ preservation of the isolates

Discrete colonies, irregular in shape, fluidal and slightly raised, slimy and creamy-white with pink-orange centres resembling those described by Kelman (1954); Harrison (1960); Hayward (1960); Hayward (1964) were given isolate numbers, picked by touching a discrete colony with a sterile wire loop, put in 5ml sterile distilled water in screw-capped bottles and stored at room temperature (18 – 20^0C) as described by Kelman and Person (1961) until needed. The stored cultures were sub-cultured every six months to maintain the isolates.

3.1.5 Race and biovar determination on isolates

3.1.5.1 Determination of races of *Ralstonia solanacearum*

Tobacco leaf infiltration tests were conducted on all isolates using the method of Lozano and Sequeira (1970) in order to group the isolates, collected during the long rainy season in March to July 2006, into races. *Nicotiana tabacum* L. var. White burley plants were used for inoculations, 42 days from the date of transplanting. Fully expanded leaves were infiltrated by injecting a suspension of bacterial cells into the intercellular spaces with a 25-gauge hypodermic needle. Two hundred microlitres (200µl) of the bacterial suspension at a concentration of approximately $2x10^8$cfu/ml were used per injection. Two leaves per plant were injected with the same isolate. Control plants were injected with 200µl of sterile distilled water. For every isolate, two tobacco plants were tested. The inoculated plants were incubated under greenhouse conditions and leaf reactions were recorded at 24, 48, 72 and 96 hours post-inoculation.

3.1.5.2 Determination of biovars of *Ralstonia solanacearum*

Determination of biovars of *R. solanacearum* was based on the utilisation, by the bacterium, of the disaccharides cellobiose, lactose and maltose, and the oxidation of the hexose alcohols dulcitol, mannitol and sorbitol (Hayward, 1964). Ten percent (10% W/V) solutions of the carbohydrates were prepared and dispensed in 10ml aliquots. Heating was done to dissolve these sugars. The hexose alcohols are relatively heat-stable and were therefore sterilised by autoclaving at 121^0C for 15 minutes. The disaccharides are heat labile and were therefore sterilised by filtration into pre-sterilised small flasks using 0.22 micron Millipore membrane and syringe. To 90ml molten basal medium (Appendix I (d)) cooled to about 50^0C, 10ml of a given

carbohydrate solution was added. After mixing, the medium was dispensed in 3-4 ml aliquots into previously sterilised test tubes (150mm x 10mm size), plugged with non-absorbent cotton. The process was repeated for all the carbohydrates.

After setting, the media were stab-inoculated in duplicates with pure bacterial cultures of the isolates from the samples collected in March to July 2006. A control was set-up without any carbohydrate (salicin, a non-carbohydrate was used). The tests were incubated at 28^0C in an incubator. Reactions were observed and recorded after 3, 5, 7, and 14 days according to the method described by Hayward (1964). Reactions are usually complete by the seventh day.

3.1.5.3 Differentiation of biovar 2A and 2T (N2)

Ralstonia solanacearum biovar 2 isolates obtained from the Kenya's potato production ecosystems were differentiated into phenotypes 2-A and 2T (N2) using the method by Hayward (1991) as follows:

Using the same basal medium for biovar determination (Appendix I (d)), 90ml aliquots were melted and then cooled to about 50^0C. Ten millilitres of 10% solutions of D (+) trehalose and L (-) tryptophan were added to the cool basal medium. The medium was dispensed in 3 – 4 ml aliquots into labelled sterile tubes (150mm x 10mm size) plugged with non-absorbent cotton. The media were stab inoculated in duplicates with bacterial cultures of isolates obtained from samples collected in March-July, 2006. The tests were incubated at 28^0C and observed after 3, 5, 7 and 14 days, according to the method by Hayward (1964), to determine the utilisation or non-utilisation of the carbohydrates.

3.1.6 Molecular analysis of isolates

3.1.6.1 Bacterial isolates

The bacterial isolates used in this study were obtained from samples collected in March to July 2006. They were obtained from diseased potato, tomato and a collection of weed plants. The isolates were cultured on Casamino acid Peptone Glucose (CPG) agar and incubated at 28^0C for 48 – 72 hours prior to the DNA extraction process. They were maintained in 5ml sterile distilled water in screw-capped bottles and routinely sub-cultured on CPG agar and incubated at 28^0C.

3.1.6.2 Extraction of bacterial DNA

Sterile loops were used to remove 40 – 60 mg bacterial biomass from 48 – 72 hours old cultures of *R. solanacearum.* For each isolate, the biomass was deposited in a marked 1.5ml Eppendorf tube. Five hundred microlitres (500µl) of warm (65^0C) cetyltrimethylammonium bromide (CTAB, Appendix VI (1)) extraction buffer were added into each tube and mixed by swirling gently. The tubes were incubated at 65^0C for one hour with continuous gentle rocking using a thermolyne 37600 mixer (Smith *et al*., 1995).

The tubes were removed from the incubator after one hour and cooled for 5 minutes at room temperature. Three hundred microlitres (300µl) of chloroform: octanol (ratio 24:1, Appendix VI (4)) were added to each tube and mixed by rocking gently for 10 minutes. The tubes were centrifuged for 10 minutes at 3500 revolutions per minute (rpm) using a mini spin plus centrifuge. The top aqueous layers were pipetted out into new marked 1.5ml Eppendorf tubes. Three hundred microlitres of chloroform: octanol solution was again added into each tube and the process was repeated.

Three microlitres (3µl) RNase were added to each tube which were then incubated at 37^0C for one hour. This was to digest off RNA present. Three hundred microlitres (300µl) of ice-cold absolute alcohol were added and then the contents were mixed by swirling gently. The tubes were centrifuged at 4000 rpm for 5 minutes. The resulting pellets were washed with 500µl of Wash One (Appendix VI (2)) for 20 minutes and then 500µl of Wash Two (Appendix VI (3)) for 10 minutes. The excess Wash Two buffer was drained dry from the tubes. The pellets were dissolved in 200µl of 1X TE and then the tubes were kept in the refrigerator at 4^0C until needed.

3.1.6.3 Quantification of bacterial DNA

To dilute the samples for optical density (OD) readings, 15µl of each DNA sample was added to 735µl TE in Eppendorf tubes then mixed well. A spectrophotometer, Ultrospec 2000, (Pharmacia Biotech, Cambridge, England) was used to read the optical densities of the samples at wavelengths 260nm and 280nm. The ratio was determined by dividing OD_{260nm} by OD_{280nm} for each sample in order to assess the purity of the samples. Ratios ranging from 1.8 to 2.0 indicated expected range for nucleic acids. Ratios less than 1.8 indicated possible contamination with proteins or any other UV absorbers, whereas ratios above 2.0 indicated possible contamination

with chemical carry-overs from buffers used such as chloroform or phenol. Such samples were re-precipitated and cleaned using sodium chloride.

The concentration of DNA in µg/µl was calculated using formula 1 below. Depending on the concentration in a sample, adjustments were made to get a uniform concentration for the purpose of PCR. This was done by adding TE before storing at 4^0C. The working concentration of the *R. solanacearum* DNA samples was 10ng/µl.

$$\text{DNA concentration } (\mu g/\mu l) = \frac{OD_{260} \times 50 \text{ (dilution factor)} \times 50 ug/\mu l}{1000} \quad \ldots\ldots\ldots\ldots\ldots\ldots 1$$

3.1.6.4 Amplification of DNA template (PCR)

The repetitive–element sequence PCR (rep–PCR) method employed in this study was adapted from Smith *et al*. (1995) with some modifications. The primers used were: ERIC (Enterobacterial Repetitive Intergenic Consensus) and BOX. The sequences for ERIC primers were: ERIC IR [5'–ATGTAAGCTCCTGGGGATTCAC–3'] and ERIC 2 [5'–AAGTAAGTGAC TGGGGTGAGCG–3'] synthesized by Eurofins MWG GmbH, while the sequence for BOX was: BOX A, a subunit of the BOX element, (BOX AIR) [5'– CTACGGCAAGGCGACGCTGACG–3']) synthesized by Applied Biosystems.

The PCR conditions applied were as described by De bruijn (1992) and Louws *et al.* (1994) and are summarised as follows: - Three micro litres of template DNA (30ng) were used per reaction. PCR reactions were carried out in a 20µl volume containing 50 pmol each of two opposing primers (ERIC), and 10µl Gotaq master mix (promega). The PCR amplifications were performed with a DNA thermal cycler (Bio Rad model cycler, USA). For ERIC and BOX primers, the cycles used were as follows: initial cycle at 95^0C for 7 minutes, 30 cycles of denaturation at 94^0C for 1 minute; annealing at 52^0C or 53^0C for 1 minute with ERIC or BOX primers, respectively, and extension at 65^0C for 8 minutes with a single extension cycle at 65^0C for 15 minutes and a final soak at 4^0C.

3.1.6.5 Separation of DNA bands (Agarose gel electrophoresis)

PCR amplification products were resolved using 1.5% ethidium bromide-stained agarose (Seakem LE agarose) gel electrophoresis. The agarose gel was prepared by weighing 1.5gm agarose and placing in a 250ml beaker containing 100ml of 1xTAE buffer, swirled gently to mix

before heating in a microwave oven until the agarose was molten. To the molten gel, 2µl of ethidium bromide were added under fume hood and swirled gently, then allowed to cool up to about 40^0C. This was then gently poured into midi gel tray fitted with suitable combs (2mm) and left to cast then combs removed. The tray with the gel was submerged into electrophoresis tank containing 1xTAE buffer. A total of 10µl (8µl DNA and 2µl loading dye) of each sample were loaded in appropriate wells. A 1Kb DNA ladder (1µl ladder and 2µl loading dye) was loaded in the first and last wells.

After running for two hours at 50V, the gel was placed against UV lamp and photographs taken using Sony cyber shot digital camera. The data were collected by scoring presence or absence of the bands. The presence or absence of a particular DNA fragment was converted into binary data, where presence = 1 and absence = 0. Similarity coefficients for all pair-wise combinations were determined by Nei's (1978) genetic distances and clustered using the unweighted pair-group method with arithmetic averaging (UPGMA).

3.2 Determination of pathogenic characteristics of *Ralstonia solanacearum* isolates

3.2.1 Pathogenicity on tomato and potato

All isolates obtained during the March to July 2006 sampling from different potato growing zones in Kenya were tested for their pathogenicity on potato (*Solanum tuberosum* var. Tigoni) and tomato (*Lycopersicon esculentum* var. Money-maker) in pot experiments under greenhouse conditions. For each isolate, three plants from the species above were used. Root-wounding method of Winstead and Kelman (1952) was used for inoculations.

The test plants were grown in plastic pots (17cm diameter by 15cm deep) filled with sterile soil. Potato plants were inoculated when they were four weeks old or when the 8^{th} leaf was fully expanded while the tomato plants were inoculated four weeks after transplanting. Inocula from isolates were prepared by culturing the stored isolates on Casamino acid Peptone Glucose (CPG) agar and incubating the cultures at 28^0C for 48 hours. The cultures (from 2-3 plates) were harvested by washing the surface of the media with sterile distilled water and passing a glass spreader over the colonies gently, and then the bacterial suspension was poured into marked 50ml conical flasks. The suspensions were well shaken, the concentration adjusted to approximately $10^7 – 10^9$ cfu/ml and final volume made up to 50ml.

Inoculations were made by cutting the lateral roots of the test plant with a sterile scalpel blade along one side of the plant to a depth of approximately 4cm. Ten millilitres of the bacterial suspension were then poured over the severed roots to ensure good contact. For each isolate, three test plants were used. Control plants were treated by pouring 10mls of sterile distilled water on the severed roots.

The plants were incubated under glasshouse conditions and observed regularly for wilting symptoms as described by Kelman (1953) and Harrison (1960). Data on time taken for the tomato and potato test plants to wilt were recorded. Plants exhibiting wilting symptoms were sampled, taken to the laboratory and bacterial isolations made. The characteristics of the cultures of *R. solanacearum* were recorded and compared with those described by Kelman (1954), Harrison (1960), Hayward (1960), and Hayward (1964) as slightly raised, slimy and creamy-white. They were irregular in shape and varying in sizes. Smaller colonies appeared somewhat stretched. The sizes ranged from 2mm to 5mm in diameter. They were fluidal in their consistency and smooth, with entire edges. The colonies formed pink whirling patterns at the centres.

An aliquot of each bacterial suspension was taken and using sterile test tubes, micro-pipettes, yellow tips and sterile water, 10 fold serial dilutions were made up to $10^{-8.}$ From dilutions 10^{-6} and 10^{-8}, 0.1ml were pipetted out and spread on marked SMSA plates using a glass spreader. This was replicated three times. The plates were incubated at 28^{0}C for 48 hours then the resulting colonies were counted. The number of viable cells per millilitre which were inoculated into test plants / pots was calculated.

3.2.2 Pathogenicity of *R. solanacearum* on selected weeds and crop plants

Common weeds that were found growing in the cultivated field at National Agricultural Research Laboratories (NARL) (where the experiments were carried out) were sampled and grown in pots in glasshouse conditions. The weeds were then tested to determine whether they were infected by the *R. solanacearum.* Some crop species were also tested (Table 1).

Table 1. Weed and crop species inoculated with *Ralstonia solanacearum*

Weed/crop species	Family	Common name
Galinsoga parviflora	Asteraceae	Gallant soldier
Oxalis latifolia	Oxalidaceae	Oxalis
Euphorbia hirta	Euphorbiaceae	Asthma weed
Sonchus oleraceus	Compositae	Sow-thistle
Amaranthus spp	Amaranthaceae	Pig-weed
Tagetes minuta	Compositae	African marigold
Bidens pilosa	Compositae	Black jack
Malva verticillata	Malvaceae	Mallow
Conyza bonariensis	Compositae	Fleabane
Datura stramonium	Solanaceae	Thorn apple
Portulaca oleraceae	Portulacaceae	Purslane
Oxygonum sinuatum	Polygonaceae	Double thorn
Nicandra physaloides	Solanaceae	Apple of Peru
Commelina benghalensis	Commelinaceae	Wandering Jew
Solanum nigrum	Solanaceae	Black nightshade
Eleusine indica	Gramineae	Finger millet
Asystasia schimperi	Acanthaceae	
Vigna unguiculata	Leguminosae	Cow-pea
Cucurbita maxima	Cucurbitaceae	Squash
Erucastrum arabicum	Cruciferae	
Ageratum conyzoides	Asteraceae	Goat weed
Cleome monophylla	Capparidaceae	Spider flower
Leonotis mollissina	Labiatae	Lion's tail
Rumex abyssinicus	Polygonaceae	Dock
Pelargonium zonale	Geraniaceae	Geranium
Solanum melongena	Solanaceae	Egg-plant, Brinjals
Capsicum annum	Solanaceae	Sweet pepper
Lycopersicon esculentum	Solanaceae	Tomato

The experiment was laid out in a complete randomised design (CRD) with three replicates. Twenty-two potted plants for each species were arranged into seven groups. Each group comprised of three plants. The remaining one plant for each plant species was used as control. Suspensions of four representative isolates (isolate No. 12 at 2 x 10^8 cfu/ml; isolate No. 15 at 1 x 10^8cfu/ml; isolate No. 16 at 1.9 x 10^8 cfu/ml; and isolate No. 18 at 4.6 x 10^8cfu/ml), all of which were representatives of *R. solanacearum* biovar 3, and three representative isolates (isolate No. 8 at 3.7 x 10^8cfu/ml; isolate No. 21 at 2.1 x 10^8cfu/ml; and isolate No. 54 at 4 x 10^8 cfu/ml), all of which were representatives of *R. solanacearum* biovar 2, were made after the isolates had been sub-cultured by streaking on CPG agar and incubated at 28^0C for 48 hours.

The cultures were harvested by pouring sterile distilled water on the media and a glass rod scrapped gently over the cultures. The contents were poured into 500ml marked conical flasks then the volumes were made up-to 500mls with sterile distilled water. The contents (inocula) were well mixed, then an aliquot taken for viable bacterial counts. This was to ascertain the concentration of each bacterial suspension as indicated above.

Inoculation of the plants was done according to the method by Winstead and Kelman (1952) described in section 3.2.1. For each of the seven isolates, three plants from each plant species were inoculated. The control plants were treated with sterile distilled water. The plants were incubated in the greenhouse conditions and observed regularly for any disease symptoms.

Plants which showed symptoms of bacterial wilt were sampled and taken to the laboratory for bacteriological analysis following the procedures outlined in Sections 3.1.2 to 3.1.4. The pathogenicity of the isolates from the test plants were tested using tomato (*L. esculentum* var Money-maker). Four-week old tomato plants were inoculated by stem puncture technique. A drop of inoculum, at a concentration of 10^6cfu/ml, was placed between 3rd and 4th internodes and then a slit was made longitudinally on the stem through the inoculum drop. This allowed the inoculum into the xylem. The process was repeated for all isolates. For control plants a drop of sterile water was used. The tomato plants were observed regularly for any wilting symptoms.

The plants which did not show any disease symptoms after six weeks from the date of inoculation were subjected to enzyme linked immunosorbent assay (ELISA) to determine whether the plants were latently infected. Two ELISA methods were used for the analysis namely, nitrocellulose membrane (NCM) ELISA and double antibody sandwich (DAS) ELISA. The double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) is an enzymatic assay that uses a micro titre plate as support for the samples and reagents. The protocol developed by Priou (2004) was followed and the kits used were sourced from CIP in Lima, Peru. Nitrocellulose membrane (NCM) ELISA employs a nitrocellulose membrane, instead of a micro titre plate, as support for the samples and reagents. The procedure employed was that developed by Priou (2001). NCM-ELISA kits were obtained from CIP, Lima, Peru.

3.2.3 Monitoring of natural infection of weeds in the field

Weed species found to be susceptible following artificial inoculations in the glasshouse were targeted in this investigation. *Ralstonia solanacearum* infested plots at the National Agricultural Research Laboratories (NARL) were regularly monitored for naturally infected dominant weeds (Table 2). Plants which were found showing wilting symptoms in the infested plots were sampled and brought into the laboratory and analysed for bacterial infection as outlined in Section 3.1.2 to 3.1.4. Five asymptomatic weeds from each species were sampled, brought into the laboratory and tested for *R. solanacearum* infection using the streaking method on SMSA and NCM – ELISA.

3.2.4 Infection of weeds under glasshouse conditions

Common weed species which were found growing in the cultivated field at NARL (Table 2) were collected and grown on infested soil in the glasshouse as described hereunder. Sterile soil was filled into five raised concrete troughs in the glasshouse. The troughs were raised 1m above the ground and measured 1.2m x 2.1m. Planting materials comprised of seeds and seedlings obtained from un-infested areas. The soil was sprayed with *Ralstonia solanacearum* bacterial suspension at a concentration of 1 x 10^7cfu/ml, using a Knap-sack sprayer. The troughs were partitioned and then marked according to the weeds planted in each portion. All plants were monitored regularly for the development of wilt symptoms records of which were taken. Plants which developed wilt symptoms were collected, taken to the laboratory for bacteriological analysis. The asymptomatic plants were collected after 60 days from the planting date and analysed for latent infection using DAS-ELISA.

Table 2. Weed species investigated for infection by *Ralstonia solanacearum* under glasshouse conditions

Weed	Common Name	Family	Planting Material
Galinsoga parviflora	Gallant soldier	Asteraceae	Seed
Oxalis latifolia	Oxalis	Oxalidaceae	Seedling/vegetative bulbs
Euphorbia hirta	Asthma weed	Euphorbiaceae	Seedling
Sonchus oleraceus	Sow-thistle	Compositae	Seedling
Amaranthus spp	Pig- weed	Amaranthaceae	Seed
Tagetes minuta	African marigold	Compositae	Seed
Bidens pilosa	Black jack	Compositae	Seed
Malva verticillata	Mallow	Malvacaea	Seed
Conyza bonariensis	Fleabane	Compositae	Seed
Datura stramonium	Thorn apple	Solanaceae	Seed
Portulaca oleraceae	Purslane	Portulacaceae	Seedling
Oxygonum sinuatum	Double thorn	Polygonaceae	Seedling
Nicandra physaloides	Apple of Peru	Solanaceae	Seed
Commelina benghalensis	Wandering Jew	Commelinaceae	Vegetative stolons
Solanum nigrum	Black night-shade	Solanaceae	Seed
Asystasia schimperi		Acanthaceae	Seedling
Erucastrum arabicum		Cruciferae	Seedling
Ageratum conyzoides	Goat weed	Asteraceae	Seedling/seed
Cleome monophylla	Spider flower	Capparidaceae	Seed
Leonotis mullissina	Lion's ear	Labiatae	Seedling
Rumex abyssinicus	Dock	Polygonaceae	Seedling

N.B: Use of seed or seedlings depended on ease of availability

3.3 Determination of effective crop rotation sequences

3.3.1 Glasshouse experiments

3.3.1.1 One season rotation experiment

A one-season rotation glasshouse experiment was conducted at NARL in a large glasshouse measuring 21m x 9m which was divided into two equal parts. One of the sides, with slightly raised floor, was filled with soil dug from a pit about 2m deep. The soil was mixed with manure in the ratio 3:1 (soil: manure). The area was demarcated into 12 plots each measuring 2.2mx1.1m separated by paths measuring 1m wide. The plots were sprayed uniformly with *R. solanacearum* suspension, consisting approximately $1x10^8$ cfu/ml, using a knap-sack sprayer.

Diammonium phosphate (D.A.P) (18:46) was applied at the rate of 500kg/ha (KARI, 2006). A potato crop was planted on all plots in the March to July 2006 season to confirm soil infestation by the pathogenic bacterium. Certified potato tuber seeds for both glasshouse and field experiments were sourced from the National Potato Research Centre-Tigoni. The maize, beans, cabbage and Rhodes grass seeds were sourced from a Kenya Seed Company outlet in Nairobi.

Rotational crops were then planted in October 2006 to February 2007 using CRD (Table 3), and spaced as follows: - maize (*Zea mays,* H513) at a spacing of 75cm x 20cm*;* beans (*Phaseolus vulgaris* var Rose coco) at a spacing of 30cm x 15cm*;* cabbage (*Brassica* spp.) at a spacing of 50cm x 50cm and potato (*Solamun tuberosum* var, Tigoni) at a spacing of 75cm x 20cm.

The crops were regularly watered and late blight and insects were controlled by spraying the potato crop with Ridomil-MZ and Dimethoate, respectively. The plants (potato crop on control plots) were observed regularly for any wilting symptoms. The numbers of wilting plants and the number of days taken to the onset of bacterial wilt were recorded.

Table 3. Treatments used in One-season rotation experiment in the glasshouse

Treatment	March to July 2006	October 2006 to February 2007	March to July 2007
1	Potato	Beans	Potato
2	Potato	Cabbage	Potato
3	Potato	Maize	Potato
4	Potato	Potato	Potato

During the second growing season (March to July 2007), potatoes were planted in all plots (Table 3). The crop was regularly watered and sprayed with Ridomil-MZ and Dimethoate to control blight and insects, respectively. The crop was observed regularly for any wilting symptoms. The numbers of wilting plants and the number of days taken to the onset of bacterial wilt were recorded. Wilt incidence for each treatment was calculated as a percentage of total number of plants that emerged.

3.3.1.2 Two seasons rotation experiment

A two-season rotation glasshouse experiment was conducted at NARL in the glasshouse described above. The side of a large glasshouse on which this experiment was carried out had concrete beds which were 1m high and filled with sterile soil prepared as indicated in section 3.3.1.1. The soil in the concrete beds was uniformly inoculated with *R. solanacearum* suspension of approximately 1 x 10^8 cfu / ml using a knapsack sprayer. The experiments were conducted in October 2005 to February 2006 (to check soil infestation), and in March to July 2006, October 2006 to January 2007 and March to July 2007 seasons.

Rotational crops were planted and replicated three times on 21 plots (raised concrete beds) in a complete randomised design. The treatments were as outlined in Table 4. The spacing of the crops was as follows: maize *(Zea mays*, H513), at spacing of 75cm x 20cm; beans (*Phaseolus vulgaris* var Rose coco), at spacing of 30cm x 15cm; cabbage (*Brassica* sp), at spacing of 50cmx50cm; Rhodes grass (*Chloris gayana*) by broadcasting; potato (S*olanum tuberosum* var Tigoni), at a spacing of 75cm x 20cm. Diammonium phosphate (D.A.P) (18:46) was applied at 500kg/ha (KARI, 2006). The crops were regularly watered and late blight and insects were controlled by spraying the potato crop with Ridomil-MZ and Dimethoate, respectively. The potato on the control plots were observed weekly for any wilting symptoms.
Potatoes were planted in all plots during the fourth season (March to July 2007). Potato plants were observed weekly and wilting potato plants and the days taken to the onset of bacterial wilt were recorded. Wilt incidence for each treatment was calculated as a percentage of total number of plants that emerged.

Table 4. Treatments used in two-season rotation experiment in the glasshouse

Treatments	October 2005 to Feb.2006	March to July 2006	Oct.2006 to Feb. 2007	March to July 2007
1	Potato	Maize	Beans	Potato
2	Potato	Cabbage	Maize	Potato
3	Potato	Beans	Cabbage	Potato
4	Potato	Maize	Maize	Potato
5	Potato	Beans	Beans	Potato
6	Potato	Potato	Potato	Potato
7	Potato	Grass	Grass	Potato

3.3.2 Field experiments

3.3.2.1 Field trial site

Field crop rotation experiments were conducted at the Kenya Agricultural Research Institute (KARI) at the National Agricultural Research Laboratories (NARL). NARL is in agro-ecological zone UM3 and at an altitude of 1737m a.s.l, and it receives an average rainfall of 973mm per annum (the actual recorded precipitation and temperatures during the experimental period are shown in appendix VII). The rainfall is bimodal. Mean maximum and mean minimum temperatures were 22^0C and 14^0C, respectively.

The soil at NARL is well drained, extremely deep, dusky red to dark reddish brown, friable clay; with inclusions of well drained, moderately deep, dark red to dark reddish brown friable clay over rock, *pisoferric* or *petroferric* material classified as *Eutric Nitisols* with *nito-chromic cambisols* and chromic *acrisols*, partly *pisoferric* or *petroferric* phase (Sombroek *et al*., 1980).

3.3.2.2 Experimental field identification and development

The experimental field measuring 70m x 20m which was identified for this work had been under sweet potato (*Ipomea batatas)* cultivation in the previous season. The plot was fenced using chain link. At the gate, there was a disinfectant trough for disinfecting the feet of anyone entering or exiting the trial site. The plot was ploughed and harrowed thoroughly. Tomato (*Lycopersicon esculentum*) seedlings sourced from Kamae area near Njabini were planted on the plot in June 2005. The tomato plants were inoculated with a suspension of *R. solanacearum* at a concentration of 1 x 10^8cfu/ml using the method of Winstead and Kelman (1952) described under section 3.2.1.

The plants were observed regularly and the wilting plants were recorded. At the end of the season, the trial site was cleared and the tomato plants were chopped into small pieces, which were spread uniformly all over the experimental field. The field was then dug to incorporate the tomato debris into the soil.

3.3.2.3 Experimental set-up

The experimental field was demarcated into 30 plots each measuring 6m x 4.5m with 1m paths on all sides. Twenty one (21) of the plots were for the two-season crop rotation experiment and the nine (9) plots were for one season crop rotation experiment. Three plots which were under potato crop throughout the trials were used as controls for both one-season and

two season rotations. Three clean plots, outside the contaminated field, measuring 6m x 4.5m were prepared and planted with potatoes for clean potato production and subsequent comparison of potato yields between the infested and the uninfested plots.

3.3.2.4 One-season rotation

The nine plots, which were for one-season trial, were planted with different crops (treatments) which are commonly grown in potato growing regions in Kenya, in a complete randomised block design with three replicates each (Table 5). The crops which were selected for this study were: Maize (Z*ea mays*, H513); beans (*Phaseolus vulgaris L*. var Rose coco); potato (*Solanum tuberosum L.* var Tigoni) and cabbage (*Brassica* sp). During June to September 2005, the experimental plots in the field were planted with tomatoes which were inoculated with *R. solanacearum* to contaminate the field.

All the crops were planted at the recommended spacing, which were 75cm x 30cm for potatoes and maize; 60cm x 15cm for beans and 60cm x 60cm for cabbage. Late blight and insects were controlled with Ridomil-MZ and Dimethoate, respectively, for the potato plots. Diammonium phosphate (D.A.P) (18:46) fertiliser was applied at the rate of 500kg/ha (KARI, 2006). The planting was done in early October 2005 during the short rainy season (Appendix VII).

At the onset of the long rainy season (March – July 2006) the demarcated plots were prepared for planting of the rotational crops (Table 5). Manure was spread evenly over the plots, and *R. solanacearum* (suspension of 10^8 cfu/ml) was sprayed uniformly on the plots and then raked in. This was done to boost the initial soil infestation.

Table 5. Treatments used for one- season rotation experiment in the field

June to September 2005	March to July 2006	October 2006 to January 2007
Tomato	Maize	Potato
Tomato	Beans	Potato
Tomato	Cabbage	Potato
Tomato	Potato	Potato

3.3.2.5 Two-season rotation

The twenty one (21) plots which were for the two-season trial were planted with different crops which are commonly grown in potato growing regions in Kenya, in a complete randomised block design with three replicates each (Table 6). The crops which were selected for this study were: Maize (Z*ea mays*, H513); beans (*Phaseolus vulgaris* L. var Rose coco); potato (*Solanum tuberosum* L. var Tigoni);cabbage (*Brassica* sp) and Rhodes grass (*Chloris gayana*). Recommended spacing for each crop as indicated in one season trial was used. Rhodes grass (*Chloris gayana)* was included in the two-season trial and was planted by broadcasting (Table 6). Late blight and insects were controlled with Ridomil-MZ and Dimethoate, respectively, for potato plots. Diammonium phosphate (D.A.P) (18:46) fertiliser was applied at the rate of 500kg/ha (KARI, 2006).

At the onset of the long rainy season (March to July 2006) the demarcated plots were prepared for planting of the rotational crops. Manure was spread evenly over the plots, and *R. solanacearum* suspension of 10^8 cfu/ml was sprayed uniformly on the plots and then raked in. This was done to boost the initial soil infestation.

Table 6. Treatments used for two-season rotation experiment in the field

June to September 2005	March to July 2006	October 2006 to January 2007	March 2007 to July 2007
Tomato	Maize	Beans	Potato
Tomato	Beans	Maize	Potato
Tomato	Cabbage	Cabbage	Potato
Tomato	Maize	Maize	Potato
Tomato	Beans	Beans	Potato
Tomato	Grass	Grass	Potato
Tomato	Potato	Potato	Potato

3.3.2.6 Data collection

During the growing seasons, potato plants were observed regularly for wilting symptoms and the number of wilting plants and time taken to onset of bacterial wilt were recorded.

Correlation between marketable yield and bacterial wilt incidence was also determined. Latent infection from one hundred (100) externally symptomless tubers obtained from each experimental plot was determined by using nitrocellulose membrane (NCM) ELISA. The yields from clean plots were compared with those from *R. solanacearum* infested plots.

CHAPTER FOUR

4.0 RESULTS

4.1 Population structure of *Ralstonia solanacearum*

4.1.1 Samples and ecological characteristics

The altitudes of the sampling areas ranged from 1678m to 2794m a.s.l, the latitudes ranged from $000^0$93.347'N to $001^0$08.651'S and the longitudes ranged from $034^0$47.561'E to $037^0$00.299'E (Table 7). Fifty four farms (representing 58.7% of the farms visited in the regions outlined under section 3.1.1) were found infested with *R.solanacearum*. From the surveyed regions (Figure 2), 70 samples collected comprised of 63 potato (*S. tuberosum*) (90%), 4 tomato (*Lycopersicon esculentum*) (5.7%) and 3 weeds (4.3%). The weeds were pig-weed (*Amaranthus* spp.), spider flower (*Cleome monophylla*) and fleabane *(Conyza bonariensis*). The potato samples were obtained from the following agro-ecological zones: Upper midlands 3 (UM3), at an altitude

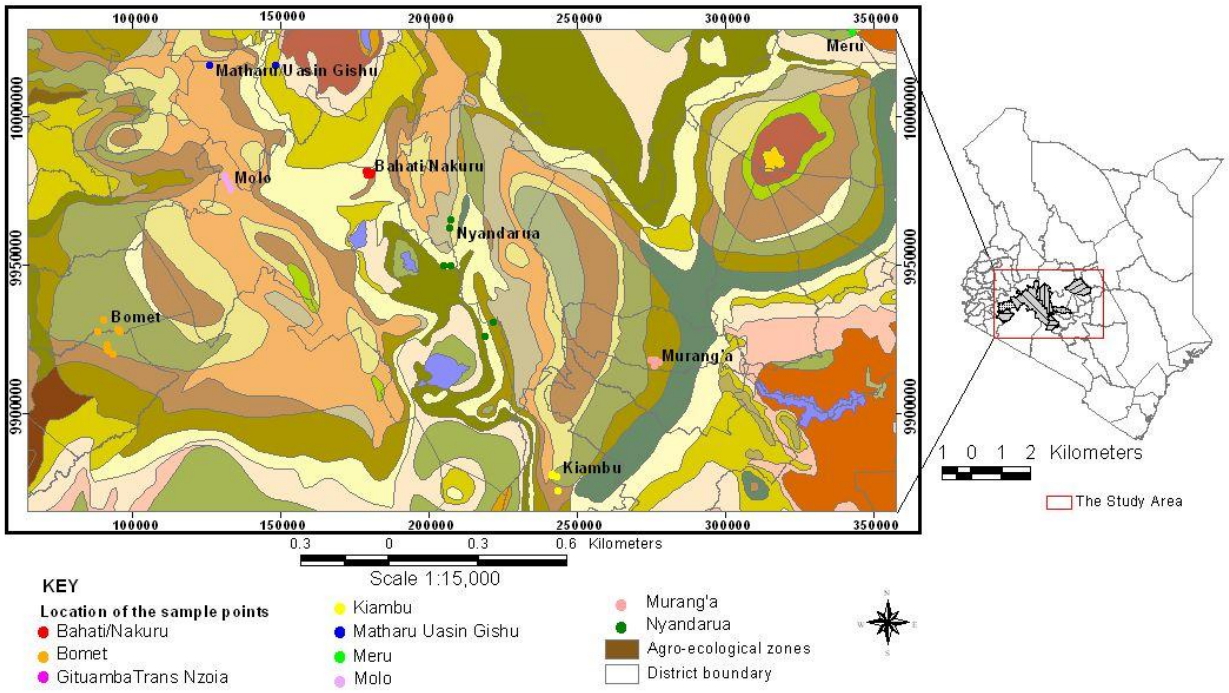


Figure 2. Mapping of *Ralstonia solanacearum* occurrence in Kenya using programme Arc View GIS 3.2.

ranging from 1678m to 1737m a.s.l. (13 samples); Upper midland 2 (UM2), at an altitude ranging from 1750m to 1943m a.s.l (9 samples); Lower highlands 2 (LH2), at an altitude range from 1944m to 2093m a.s.l. (18 samples); Upper highlands 3 (UH3), at an altitude of 2194m a.s.l. (1 sample); Lower highlands 1 (LH1), altitude ranging from 2195m to 2262m a.s.l. (8 samples) and Upper highlands 2 (UH2), altitude ranging from 2352m to 2794m a.s.l. (14 samples). Mapping of R. solanacearum occurrence in Kenya using programme arc view GIS 3.2 is shown in Figure 2.

The potato production regions mapped included Nyandarua, Murang'a, Molo, Kiambu, Meru, Bomet, Nakuru, Uasin Gishu and Trans Nzoia (Figure 2 and Table 7).

Table 7. Diseased plant samples collected from potato growing agro-ecological zones in Kenya during the long rainy season March to July 2006.

Sample No.	Host	Location of sampling site	Agro-ecological zone	Altitude in metres	Latitude	Longitude
1	Potato	Kiambu	LH1	2223	$001^0$06.061'S	$036^0$41.573'E
2	Potato	"	LH1	2217	$001^0$06.088'S	$036^0$41.595'E
3	Potato	"	LH1	2257	$001^0$05.732 'S	$036^0$40775'E
4	Potato	"	LH1	2257	$001^0$05.736'S	$036^0$40.778'E
5	*Amaranthus* spp	"	LH1	2257	$001^0$05.736'S	$036^0$40.778'E
6	Potato	"	LH1	2257	$001^0$05.716'S	$036^0$40.751'E
7	Potato	"	LH1	2258	$001^0$05.706'S	$036^0$40.752'E
8	Potato	"	LH1	2261	$001^0$05.691'S	$036^0$40.752'E
9	Potato	"	LH1	2262	$001^0$05.688'S	$036^0$40.752'E
10	Potato	"	LH2	1989	$001^0$08.651'S	$036^0$41.820'E
11	Potato	"	UM3	1678	$000^0$45.121'S	$037^0$00.085'E
12	Potato	Murang'a	UM3	1681	$000^0$45.145'S	$037^0$00.299'E
13	Potato	"	UM3	1680	$000^0$45.152'S	$037^0$00.173'E
14	Potato	"	UM3	1680	$000^0$45.191'S	$037^0$00.173'E
15	Potato	"	UM3	1680	$000^0$45.191'S	$037^0$00.173'E
16	Potato	"	UM3	1708	$000^0$45.880'S	$036^0$59.413'E
17	Potato	"	UM3	1707	$000^0$44.880'S	$036^0$59.412'E
18	Tomato	"	UM3	1707	$000^0$44.880'S	$036^0$59.413'E
19	Potato	"	UM3	1743	$000^0$44.646'S	$036^0$59.714'E
20	Tomato	"	UM3	1743	$000^0$44.647'S	$036^0$58.714'E
21	Potato	Nyandarua	UH3	2222	$000^0$40.336'S	$036^0$28.657'E
22	Potato		UH3	2222	$000^0$40.336'S	$036^0$28.657'E

23	Potato	"	UH2	2466	000^{0}37.893'S	036^{0}30.140'E
24	Potato	"	UH2	2466	000^{0}37.893'S	036^{0}30.140'E
25	Potato	"	UH3	2194	000^{0}27.456'S	036^{0}21.233'E

Table 7. Cont.

Sample No.	Host	Location of sampling site	Agro-ecological zone	Altitude in metres	Latitude	Longitude
26	Potato	Nyandarua	UH2	2352	000^{0}19.001'S	036^{0}22.397'E
27	Potato	"	UH2	2356	000^{0}19.003'S	036^{0}22.397'E
28	*Cleome monophylla*	"	UH2	2356	000^{0}19.003'S	036^{0}22.397'E
29	Potato	"	UH2	2354	000^{0}20.513'S	036^{0}22.286'E
30	*Conyza bonariensis*	"	UH2	2340	000^{0}19.001'S	036^{0}22.401'E
31	Potato	Bomet	LH2	2079	000^{0}43.545'S	035^{0}21'.069'E
32	Potato	"	LH2	2077	000^{0}43.595'S	035^{0}21.069'E
33	Potato	"	LH2	2093	000^{0}43.030'S	035^{0}20.285'E
34	Potato	"	LH2	2093	000^{0}43.032'S	035^{0}20.285'E
35	Potato	"	LH2	2029	000^{0}41.966'S	035^{0}20.014'E
36	Potato	"	LH2	2031	000^{0}42.735'S	035^{0}20.020'E
37	Potato	"	LH2	2029	000^{0}39.432'S	035^{0}18.314'E
38	Potato	"	LH2	2027	000^{0}37.414'S	035^{0}19.270'E
39	Potato	"	LH2	2030	000^{0}39.117'S	035^{0}21.811'E
40	Potato	"	LH2	2025	000^{0}39.427'S	035^{0}22.327'E
41	Potato	Molo	UH2	2531	000^{0}11.169'S	035^{0}41.331'E
42	Potato	"	UH2	2530	000^{0}11.296'S	035^{0}41.390'E
43	Potato	"	UH2	2536	000^{0}12.447'S	035^{0}42.057'E
44	Potato	"	UH2	2536	000^{0}12.447'S	035^{0}42.057'E
45	Potato	"	UH2	2542	000^{0}13.394'S	035^{0}42.379'E
46	Potato	Bahati/ Nakuru	LH2	1958	000^{0}10.339'S	036^{0}07.434'E
47	Potato	"	LH2	1948	000^{0}10.937'S	036^{0}07.123'E
48	Potato	"	LH2	1950	000^{0}10.119'S	306^{0}07.014'E
49	Potato	"	LH2	1944	000^{0}10.384'S	036^{0}08.011'E
50	Potato	"	LH2	1958	000^{0}10.405'S	036^{0}08.090'E
51	Potato	"	LH2	1961	000^{0}10.883'S	036^{0}07.929'E
52	Potato	"	UM3	1937	001^{0}15.520'S	036^{0}46.289'E
53	Potato	"	UM3	1937	001^{0}15.520'S	036^{0}46.289'E
54	Potato	"	UM3	1937	001^{0}15.520'S	036^{0}46.289'E
55	Potato	"	UM3	1937	001^{0}15.520'S	036^{0}46.289'E

Table 7. Cont.

Sample No.	Host	Location of sampling site	Agro-ecological zone	Altitude in Metres	Latitude	Longitude
56	Tomato	"	UM3	1937	$001^0$15.520'S	$036^0$46.289'E
57	Tomato	"	UM3	1937	$001^0$15.520'S	$036^0$46.289'E
58	Potato	Gituamba/ Trans Nzoia	LH2	2324	$000^0$09.347'N	$034^0$07.561'E
59	Potato	"	LH2	2324	$000^0$93.347'N	$034^0$07.561'E
60	Potato	Meru	UM2	2070	$000^0$17.431'N	$037^0$35.180'E
61	Potato	"	UM2	1970	$000^0$15.183'N	$037^0$35.487'E
62	Potato	"	UM2	1783	$000^0$38.421'N	$037^0$35.842'E
63	Potato	"	UM2	1761	$000^0$38.471'N	$037^0$36.043'E
64	Potato	"	UM2	1784	$000^0$44.574'N	$037^0$36.256'E
65	Potato	"	UM2	1768	$000^0$37.35'N	$037^0$36.314'E
66	Potato	"	UM2	1750	$000^0$44.131'N	$037^0$24.551'E
67	Potato	"	UM2	1719	$000^0$45.820'N	$037^0$32.136'E
68	Potato	"	UM2	1706	$000^0$52.251'N	$037^0$51.658'E
69	Potato	Matharu UasinGishu	UH2	2794	$000^0$09.255'N	$035^0$50.506'E
70	Potato	"	UH2	2777	$000^0$09.255'N	$035^0$50.506'E

Disease symptoms observed on infected potato plants in the field were as follows: withering of leaves, wilting and collapse of affected plants. During the early stages of infection a few leaves were found to be drooping. In some cases only a few stems in a potato hill were wilting while the rest of the branches appeared unaffected. In most cases foliage of the entire hill were found wilted without change in colour (Plate 1). Other symptoms found in infected plants were yellowing of leaves, stunting of plants and discolouration of the vascular system. At the stolon end of severely infected potato tubers, sticky exudates formed and mixed with soil, causing it adhere to the surface of the tubers (Plate 2). Infected tubers exhibited a brown discolouration of the vascular rings when cut transversely.

Plate 1. Symptoms of bacterial wilt on an infected potato plant.

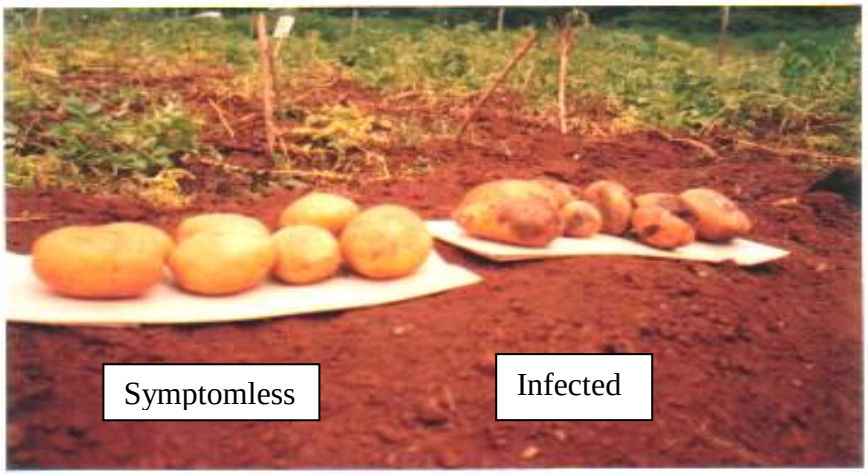


Plate 2. Symptomless (left) and infected (right) potato tubers.

The major cash crops in the potato growing regions varied from one area to the other. However, they were mainly pyrethrum (*Chrysanthemum cinerariaefolium*), coffee (C*offea arabicum*), tea (*Camellia sinensis*), wheat (*Triticum aestivum*), maize (Z*ea mays*) and potato (S*olanum tuberosum*). Other crops included beans (*Phaseolus vulgaris*), cabbage (*Brassica* sp), peas (*Pisum sativum*), and tomato (*Lycopersicon esculentum*). The general cropping patterns in the sampling areas were: maize-beans-potato; maize-cabbage-potato; beans-cabbage-potato (Table 8). Intercropping of maize and beans, maize and peas, maize, beans and peas, potato and beans planted in coffee plantation. Potato and maize were common in many areas in Kiambu and Murang`a regions.

Table 8. Cropping patterns in some of the sampling areas.

Location	Cropping pattern
Kiambu and Murang'a	Intercropping of potato with cabbage and peas; maize and beans; maize, beans and peas; potato in coffee; potato and beans in coffee; pure stands of potato, maize, cabbage, coffee and tea.
Nyandarua	Inter-cropping of cabbage and peas; maize and beans; maize and peas; pure stands of maize, cabbage, potato, carrots and pyrethrum.
Meru	Inter-cropping of potato with maize and beans; potato, beans and peas in coffee; beans and kale; maize and peas, pure stands of maize, potato, and coffee.
Bomet	Inter-cropping of maize and beans; pure stands of maize, potato, cabbage, tomato and tea.
Molo	Inter-cropping of maize and beans; maize and peas; pure stands of maize, potato, cabbage and pyrethrum.
Trans Nzoia / Uasin Gishu	Pure stands of maize, wheat, and potato.

4.1.2 Description and identification of isolates

Sixty eight pure isolates of *R. solanacearum* were obtained from the samples which were collected in March to July 2006 from potato production agro-ecological zones in Kenya. This was from 97.1% of the total plant samples which were collected after being suspected to be infected with *R. solanacearum* in the field. The isolates were assigned the numbers which corresponded to the sample codes (Table 7). There was no isolate obtained from two weed samples, named and coded as *Amaranthus* spp. (No. 5) and *Conyza bonariensis* (No. 30).

On both SMSA and Kelman's TZC agar, *R.solanacearum* colonies were observed to be slightly raised, slimy and creamy-white. They were irregular in shape and varying in sizes. Smaller colonies appeared somewhat stretched. The sizes ranged from 2mm to 5mm in diameter. They were fluidal in their consistency and smooth, with entire edges. The colonies formed pink whirling patterns at the centres. Based on the above observations and the descriptions by Kelman (1954), Harrison (1960), Hayward (1960), Hayward (1964) and French and Sequeira (1970), the isolates were identified as *Ralstonia solanacearum*.

On Casamino acid-Peptone-Glucose (CPG) agar, the *R. solanacearum* colonies had the same characteristics as described above but did not produce pink whirling patterns at the centres and the smaller colonies did not appear stretched as in the case of SMSA medium (Plate 3).

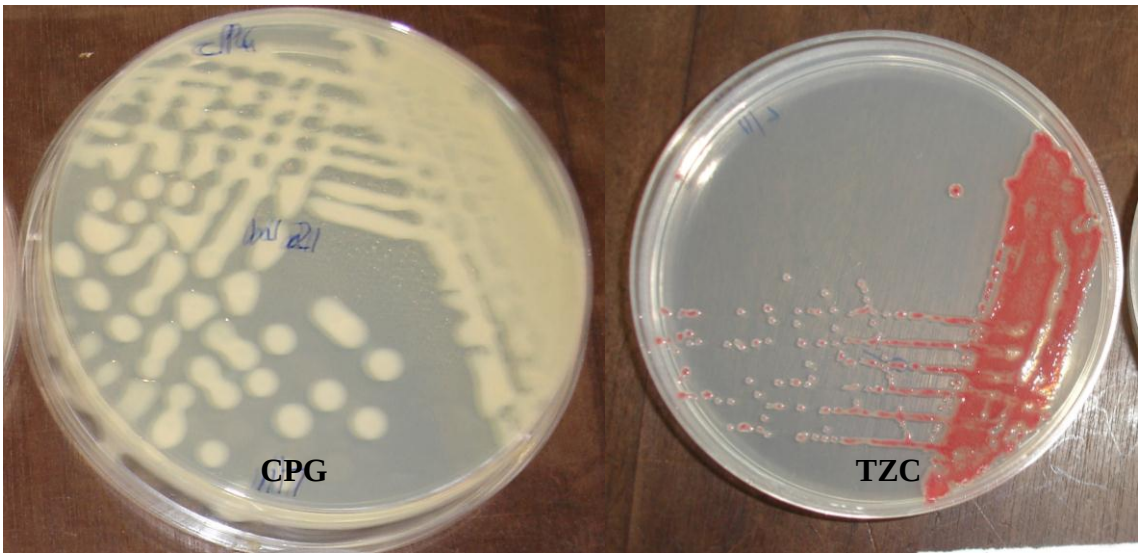


Plate 3. Cultures of *Ralstonia solanacearum* colonies on Casamino- Peptone- Glucose (CPG) agar and Kelman`s Tetrazolium Chloride (TZC) agar.

4.1.3 Races of *Ralstonia solanacearum*

In the tobacco leaf infiltration test, eight out of the 68 isolates (11.8%) showed brownish, leathery necrotic reactions on the leaf in the infiltrated area. Yellow zones surrounded the necrotic areas forming a halo (Plate 4). The areas bordering the leaf veins did not show yellow discolouration.

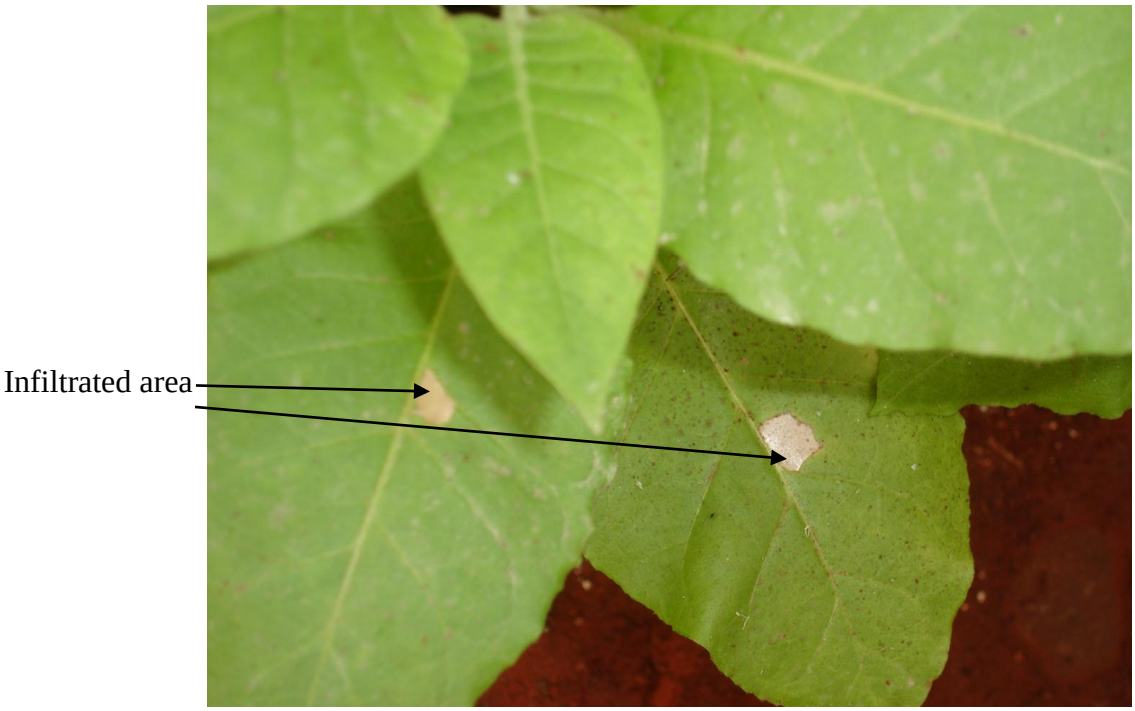


Plate 4. Brown, leathery necrotic reaction on tobacco leaf infiltrated with *Ralstonia solanacearum* isolate number 13 obtained from potato.

The isolates which showed these reactions were from Murang'a region which was 1678m to 1743m a.s.l. The isolates were obtained from potato (*Solanum tuberosum* L.*)* and tomato (*Lycopersicon esculentum* L.) (Tables 7 and 9, and Appendix V). Based on the above observations and the descriptions by Lozano and Sequeira (1970), these isolates were identified as *Ralstonia solanacearum* race 1.

Sixty out of the 68 isolates (88.2 %) gave a yellowish discolouration of the infiltrated areas about 48 hours after infiltration. The sizes and the colour of the lesions did not increase or change after 96 hours (Plate 5).

Plate 5. Yellowish discolouration on tobacco leaf infiltrated with *Ralstonia solanacearum* isolate number 52 obtained from potato.

The isolates which showed these reactions were obtained from high altitudes (1743m to 2777m a.s.l) in potato production agro-ecosystems. The hosts from which isolations were made were *Solanum tuberosum* (94.9%), *Lycopersicon esculentum* L. (3.4%) and *Cleome monophylla* (1.7%) (Tables 7 and 9, and Appendix V). Based on the above observations and the descriptions by Lozano and Sequeira (1970), the sixty isolates were identified as *Ralstonia solanacearum* race 3 of Buddenhagen *et al.* (1962).

4.1.4 Biovars of *Ralstonia solanacearum*

When *R. solanacearum* utilised the disaccharides under the test conditions, the colour of the medium changed from olivaceous green to yellow which indicated that there was change of pH from alkaline to acidic range.

When *R. solanacearum* oxidised the hexose alcohols, the pH and colour of the medium changed from alkaline pH or olivaceous green colour to acidic pH or yellow colour. The hexose alcohols took 3 to 5 days to be oxidised, while it took a few days longer (7-9 days) for the

disaccharides to be utilized. Sixty out of 68 isolates (88.2%) utilised disaccharides but did not oxidize hexose alcohols (Table 9, Plate 6 and Appendix II).

The isolates were obtained from altitudes between 1743m and 2794m a.s.l. The hosts from which the isolates were obtained were *Solanum tuberosum* L. (95%), *Lycopersicon esculentum* L. (3.3 %) and *Cleome monophylla* (1.7%).

Based on the above observations and the biochemical typing by Hayward (1964), the sixty isolates were classified as biochemical "type 2" (biovar 2).

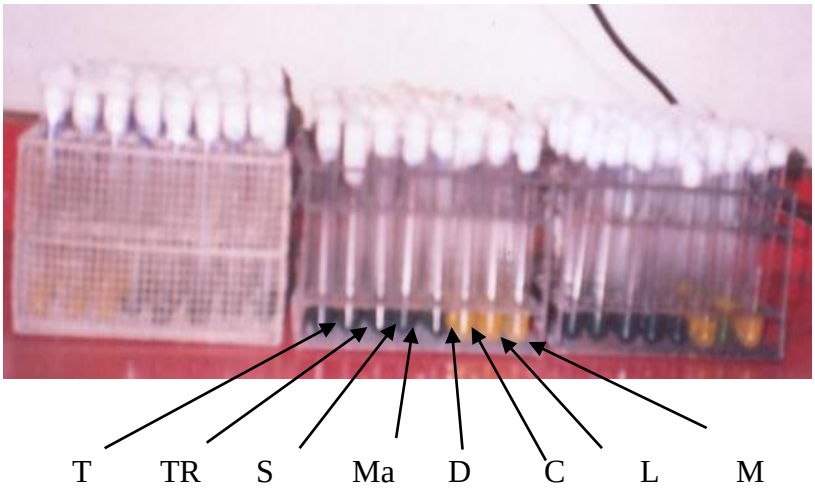


Plate 6. Utilization of disaccharides and oxidation of hexose alcohols by *Ralstonia solanacearum* isolates. T=Trytophan, TR= Trehalose, S=Sorbitol, Ma=Mannitol, D =Dulcitol, C=Cellobiose, L =Lactose, M=Maltose.

Eight out of the 68 isolates (11.8%) utilised disaccharides and oxidized hexose alcohols (Table 9 and Appendix II). The isolates were from plants sampled from Murang'a region at altitudes between 1678m and 1743m a.s.l. This is in the Upper Midland 3 agro-ecological zone (UM3). The hosts from which the isolates were obtained were: *Solanum tuberosum* L. (87.5*%)* and *Lycopersicon esculentum* L. (12.5%). Based on the biochemical typing by Hayward (1964), the eight isolates were classified as biochemical ''type 3" (biovar 3).

When the isolates, which were classified as biovar 2, were tested for oxidation of D (+) trehalose, all the tests were negative. Likewise when the isolates were tested for utilisation of L (-) tryptophan, all the tests were also negative (Table 9 and Appendix IV). On the basis of these observations and descriptions by Hayward (1991) and French *et al.* (1995), all the isolates were classified as metabolically less active Andean phenotypes.

Table 9. Biochemical characteristics and classification of isolates of *Ralstonia solanacearum* collected from potato growing areas of Kenya in March to July 2006 season.

Sample	Host	Maltose	Lactose	Cellobiose	Dulcitol	Mannitol	Sorbitol	Salicin	Trehalose	Trytophan	Tobacco Leaf reaction	Race	Biovar
1	Potato	+	+	+	-	-	-	-	-	-	Chlorosis 48hrs after infiltration	3	2A
2	Potato	+	+	+	-	-	-	-	-	-	"	3	2A
3	Potato	+	+	+	-	-	-	-	-	-	"	3	2A
4	Potato	+	+	+	-	-	-	-	-	-	"	3	2A
5	*Amaranthus* spp	N.I	N.I	N.I	N.I	N.I	N.I	N.I	N.I	N.I	N.I	N.I	N.I
6	Potato	+	+	+	-	-	-	-	-	-	Chlorosis 48hrs after infiltration	3	2A
7	Potato	+	+	+	-	-	-	-	-	-	"	3	2A
8	Potato	+	+	+	-	-	-		-	-	"	3	2A
9	Potato	+	+	+	-	-	-	-	-	-	"	3	2A
10	Potato	+	+	+	-	-	-	-	-	-	"	3	2A
11	Potato	+	+	+	-	-	-	-	-	-	"	3	2A
12	Potato	+	+	+	+	+	+	-	N.D	N.D	Brownish, Leathery necrotic lesion in infiltrated areas	1	3
13	Potato	+	+	+	+	+	+	-	N.D	N.D	"	1	3
14	Potato	+	+	+	+	+	+	-	N.D	N.D	"	1	3
15	Potato	+	+	+	+	+	+	-	N.D	N.D	"	1	3

Table 9 contd.

Sample	Host	Maltose	Lactose	Cellobiose	Dulcitol	Mannitol	Sorbitol	Salicin	Trehalose	Trytophan	Tobacco Leaf reaction	Race	Biovar
16	Potato	+	+	+	+	+	+	-	N.D	N.D	"	1	3
17	Potato	+	+	+	-	-	-	-	-	-	Chlorosis 48hrs after infiltration	3	2A
18	Tomato	+	+	+	+	+	+	-	N.D	N.D	Brownish, Leathery necrotic lesion in infiltrated areas	1	3
19	Tomato	+	+	+	+	+	+	-	N.D	N.D	"	1	3
20	Potato	+	+	+	+	+	+	-	N.D	N.D	"	1	3
21	Potato	+	+	+	-	-	-	-	-	-	Chlorosis 48hrs after infiltration	3	2A
22	Potato	+	+	+	-	-	-	-	-	-	"	3	2A
23	Potato	+	+	+	-	-	-	-	-	-	"	3	2A
24	Potato	+	+	+	-	-	-	-	-	-	"	3	2A
25	Potato	+	+	+	-	-	-	-	-	-	"	3	2A
26	Potato	+	+	+	-	-	-	-	-	-	"	3	2A
27	Potato	+	+	+	-	-	-	-	-	-	"	3	2A
28	*Cleome monophylla*	+	+	+	-	-	-	-	-	-	"	3	2A

Table 9 contd.

Sample	Host	Maltose	Lactose	Cellobiose	Dulcitol	Mannitol	Sorbitol	Salicin	Trehalose	Trytophan	Tobacco Leaf reaction	Race	Biovar
29	Potato	+	+	+	-	-	-	-	-	-	"	3	2A
30	*Conyza bonariensis*	N.I	N.I	N.I	N.I	N.I	N.I	N.I	N.I	N.I	N.I	N.I	N.I
31	Potato	+	+	+	-	-	-	-	-	-	Chlorosis 48hrs after infiltration	3	2A
32	Potato	+	+	+	-	-	-	-	-	-	"	3	2A
33	Potato	+	+	+	-	-	-	-	-	-	"	3	2A
34	Potato	+	+	+	-	-	-	-	-	-	"	3	2A
35	Potato	+	+	+	-	-	-	-	-	-	"	3	2A
36	Potato	+	+	+	-	-	-	-	-	-	"	3	2A
37	Potato	+	+	+		-	-	-	-	-	"	3	2A
38	Potato	+	+	+	-	-	-	-	-	-	"	3	2A
39	Potato	+	+	+	-	-	-	-	-	-	"	3	2A
40	Potato	+	+	+	-	-	-	-	-	-	"	3	2A
41	Potato	+	+	+	-	-	-	-	-	-	"	3	2A
42	Potato	+	+	+	-	-	-	-	-	-	"	3	2A
43	Potato	+	+	+	-	-	-	-	-	-	"	3	2A
44	Potato	+	+	+	-	-	-	-	-	-	"	3	2A
45	Potato	+	+	+	-	-	-	-	-	-	"	3	2A
46	Potato	+	+	+	-	-	-	-	-	-	"	3	2A
47	Potato	+	+	+	-	-	-	-	-	-	"	3	2A

Table 9 contd.

Sample	Host	Maltose	Lactose	Cellobiose	Dulcitol	Mannitol	Sorbitol	Salicin	Trehalose	Trytophan	Tobacco Leaf reaction	Race	Biovar
48	Potato	+	+	+	-	-	-	-	-	-	“	3	2A
49	Potato	+	+	+	-	-	-	-	-	-	“	3	2A
50	Potato	+	+	+	-	-	-	-	-	-	“	3	2A
51	Potato	+	+	+	-	-	-	-	-	-	“	3	2A
52	Potato	+	+	+	-	-	-	-	-	-	“	3	2A
53	Potato	+	+	+	-	-	-	-	-	-	“	3	2A
54	Potato	+	+	+	-	-	-	-	-	-	“	3	2A
55	Potato	+	+	+	-	-	-	-	-	-	“	3	2A
56	Tomato	+	+	+	-	-	-	-	-	-	“	3	2A
57	Tomato	+	+	+	-	-	-	-	-	-	“	3	2A
58	Potato	+	+	+	-	-	-	-	-	-	“	3	2A
59	Potato	+	+	+	-	-	-	-	-	-	“	3	2A
60	Potato	+	+	+	-	-	-	-	-	-	“	3	2A
61	Potato	+	+	+	-	-	-	-	-	-	“	3	2A
62	P otato	+	+	+	-	-	-	-	-	-	“	3	2A

Table 9 contd.

Sample	Host	Maltose	Lactose	Cellobiose	Dulcitol	Mannitol	Sorbitol	Salicin	Trehalose	Trytophan	Tobacco Leaf reaction	Race	Biovar
63	Potato	+	+	+	-	-	-	-	-	-	"	3	2A
64	Potato	+	+	+	-	-	-	-	-	-	"	3	2A
65	Potato	+	+	+	-	-	-	-	-	-	"	3	2A
66	Potato	+	+	+	-	-	-	-	-	-	"	3	2A
67	Potato	+	+	+	-	-	-	-	-	-	"	3	2A
68	Potato	+	+	+	-	-	-	-	-	-	"	3	2A
69	Potato	+	+	+	-	-	-	-	-	-	"	3	2A
70	Potato	+	+	+	-	-	-	-	-	-	"	3	2A

N.D = Not done because they were biovar 3 while the test was to differentiate biovar 2s; N.I = No isolate was obtained; + = Positive; - = Negative; "= Same as above.

4.1.5 Genetic variability of *Ralstonia solanacearum* isolates

4.1.5.1 Spectrophotometer readings of *Ralstonia solanacearum* sample DNA

The ratio of the spectrophotometer sample readings at OD_{260} and OD_{280} ranged from 1.68 to 2.20. The ratio of 1.8–2.0 indicated that the absorption was due to nucleic acids (Appendix VIII). The purity of the sample DNAs, based on the ratio is shown on Figure 3. Letter C in Figure 3 represents control where there was no OD reading since there was no nucleic acid in the sterile distilled water used. When the purity of samples DNA was checked on the gel, a single band was obtained, signifying high quality DNA (Plate 7).

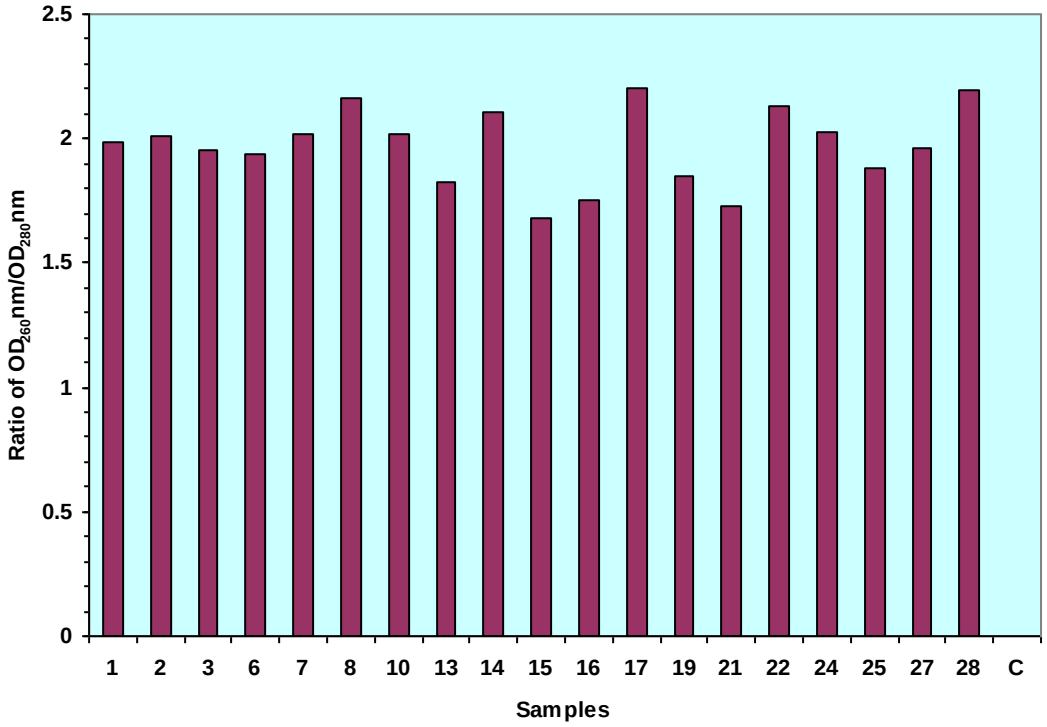


Figure 3. Variation in the ratio of optical densities of *Rastonia solanacearum* DNA measured at OD_{260nm} and OD_{280nm}.

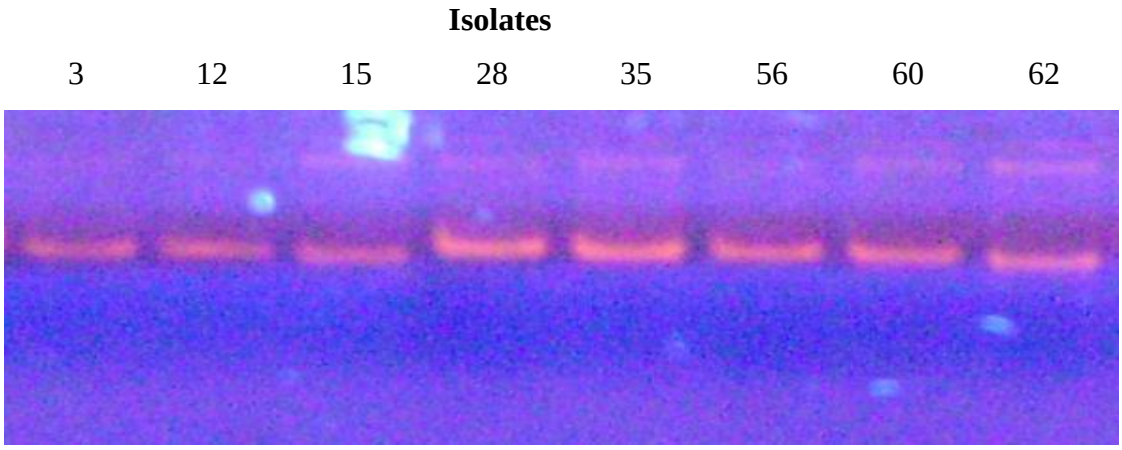


Plate 7. Ethidium bromide-stained agarose gel of *Ralstonia solanacearum* DNA viewed under UV light.

The concentrations of the *R. solanacearum* DNAs which were quantified using Ultrospec 2000 spectrophotometer (Pharmacia Biotech, Cambridge, England) at optical densities OD_{260} and OD_{280} are shown on Figure 4 and Appendix VIII. The concentrations ranged from 4.1µg/ml to 25.0µg/ml. The control marked with letter C did not have any DNA.

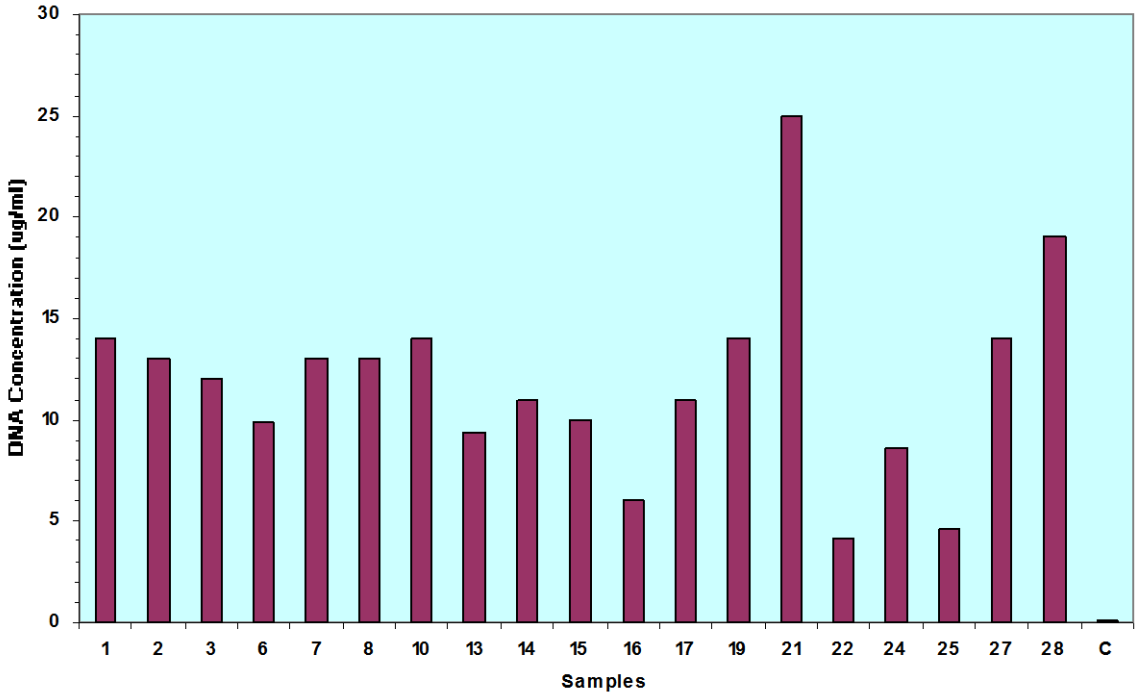


Figure 4. Variation of concentrations (µg/ml) of DNA in 19 selected samples.

Adjustment of sample DNA to 10.0ng/µl resulted in uniform concentrations of *Ralstonia solanacearum* DNAs as shown in Plate 8.

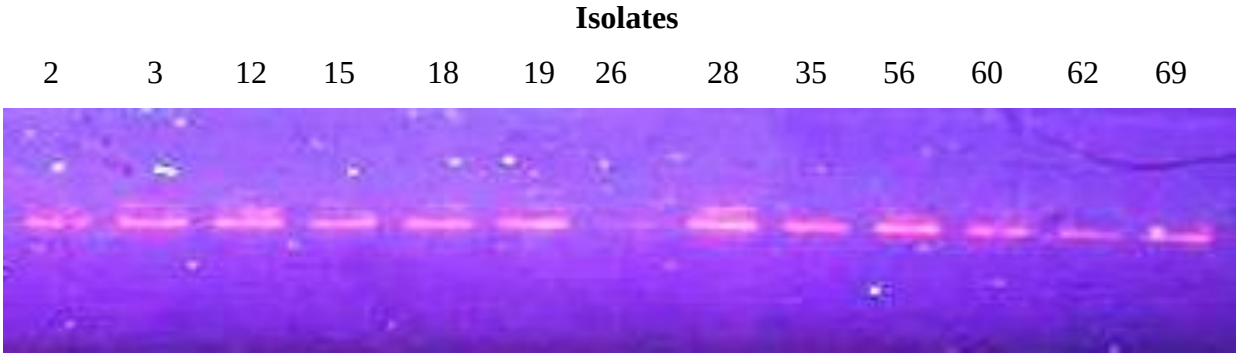


Plate 8. Ethidium bromide-stained agarose gel of DNA extracted from isolates of *Ralstonia solanacearum* and diluted to a uniform concentration.

4.1.5.2 PCR Amplification products

The primer sets gave clear genomic PCR profiles that were reproducible. The ERIC primer sets yielded multiple distinct DNA products of various sizes from both biovar 2 and biovar 3 isolates (Plate 9). The patterns generated were found to be different between the two biovars. Biovar 2 isolates revealed three types of profiles. Sample 43 on its own had a profile with the least number of bands (2 bands). Samples 26 and 51 had a profile with four DNA bands. The rest of the biovar 2 samples formed another profile with seven DNA bands. *Ralstonia solanacearum* biovar 3 samples formed a distinct profile of four faint bands.

Isolates

M 12 15 16 18 19 2 3 26 28 35 38 40 43 47 51 56 60 62 69

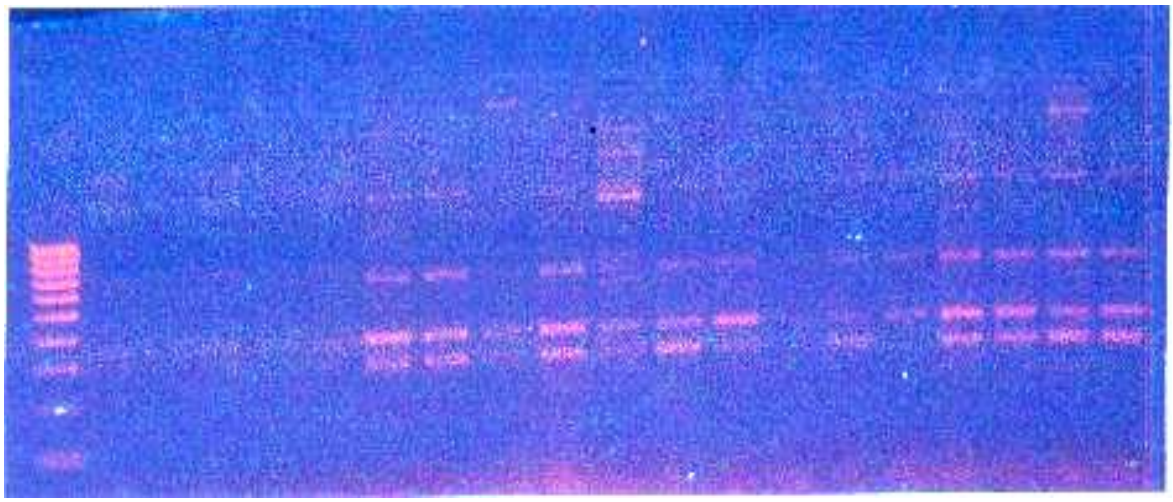


Plate 9. Amplification products of *Ralstonia solanacearum* DNA using ERIC primer sets. Lane 1=1kb ladder (Bioline); Lanes 2-6=isolates 12,15,16,18 and 19, respectively (representing *R. solnacearum* biovar 3); Lanes 7-20=isolates 2, 3, 26, 28, 35, 38, 40, 43, 47, 51, 56, 60, 62 and 69, respectively (representing *R. solanacearum* biovar 2).

The BOX primer sets gave a single genomic PCR profile of four DNA bands for *R. solanacearum* biovar 3 (Plate 10, lanes 1 -3). In the biovar 2 samples, the BOX primer sets gave a genomic PCR profile of 7 DNA bands (lanes 4-8).

Isolates

12 15 18 2 3 26 28 35 M

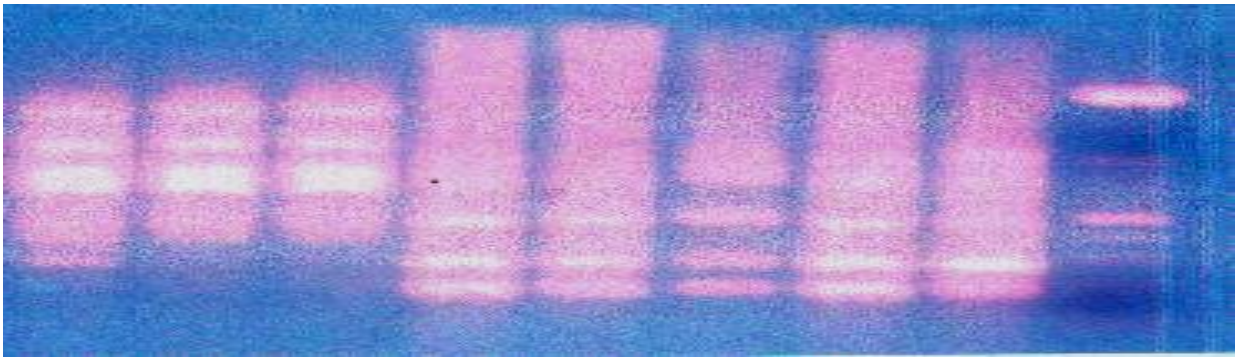


Plate 10. Amplification products of *Ralstonia solanacearum* DNA using BOX primer set. Lane 1-8= samples 12, 15, 18, 2, 3, 26, 28 and 35, respectively. Lanes 9= ladder.

Plate 11 was generated using primer set BOX which gave clear genomic patterns of mainly *R. solanacearum* biovar 2 isolates. Isolate 13 in lane 4 was, however, *R. solanacearum*

biovar 3. This isolate gave three DNA bands two of which were faint. Isolate 31 in lane 7 exhibited different characteristics in banding and it was thought that although there was DNA, it might have not been amplified and therefore the DNA remained as a single band. Lane 17 was negative control and had no DNA. Isolates from weed samples (samples 71, 72 and 74) exhibited different banding. Their amplification revealed five genomic bands. All the other isolates exhibited about seven bands of DNA fractions.

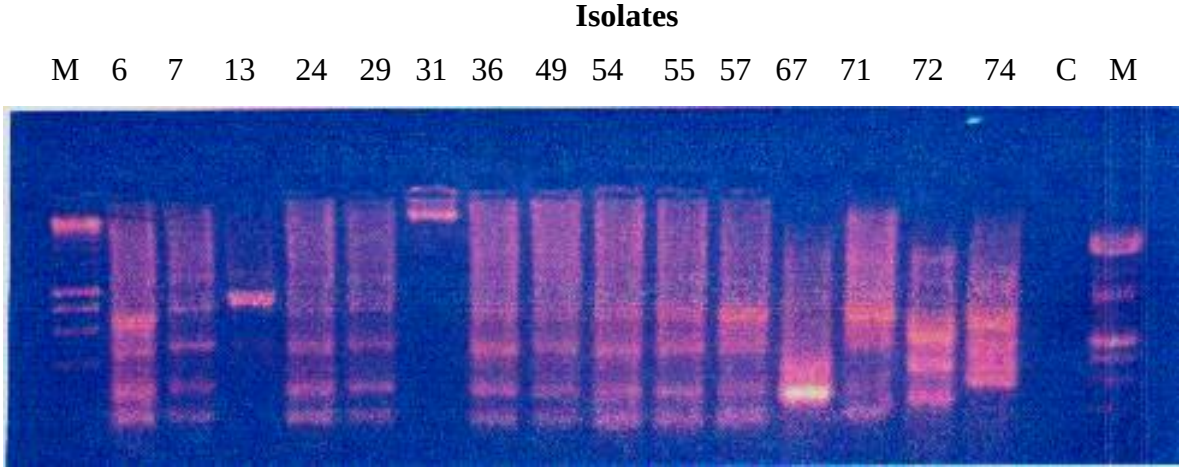


Plate 11. Amplification products of *Ralstonia solanacearum* DNA using BOX primer set. Lanes 1 and 18= DNA Ladder 1 kb and 100bp respectively; Lanes 2-3 and 5-16= *R. solanacearum* biovar 2. Isolates 71, 72 and 74 were obtained from weeds. Lane 17=negative control.

The clustering of representative isolates strongly suggested relationships among the isolates (Figures 5 and 6). The dendrogram generated using ERIC primer sets data showed that the isolates 12, 15, 16, 18 and 19 were similar. Isolate 13 was also similar to the above isolates although the relationship was a bit distant. This was true with the biochemical classification which grouped these isolates as *Ralstonia solanacearum* biovar 3. ERIC primer sets also showed that all the other isolates were similar. This group comprised *R. solanacearum* biovar 2. The dendrogram generated by BOX primer set also grouped isolates 13, 15, 16 and 18 together. The biochemical classification grouped all the other isolates into *Ralstonia solanacearum* biovar 2. However, the molecular finger-printing and clustering using arithmetic average showed that some isolates were closer in their relationships than others (Figures 5 and 6). Isolates 28, 71 and 74 were obtained from weeds. In the dendrogram generated using ERIC primer sets, isolates 71 and 74 were grouped close to one another. The isolates were, however, distantly related to the rest of the isolates.

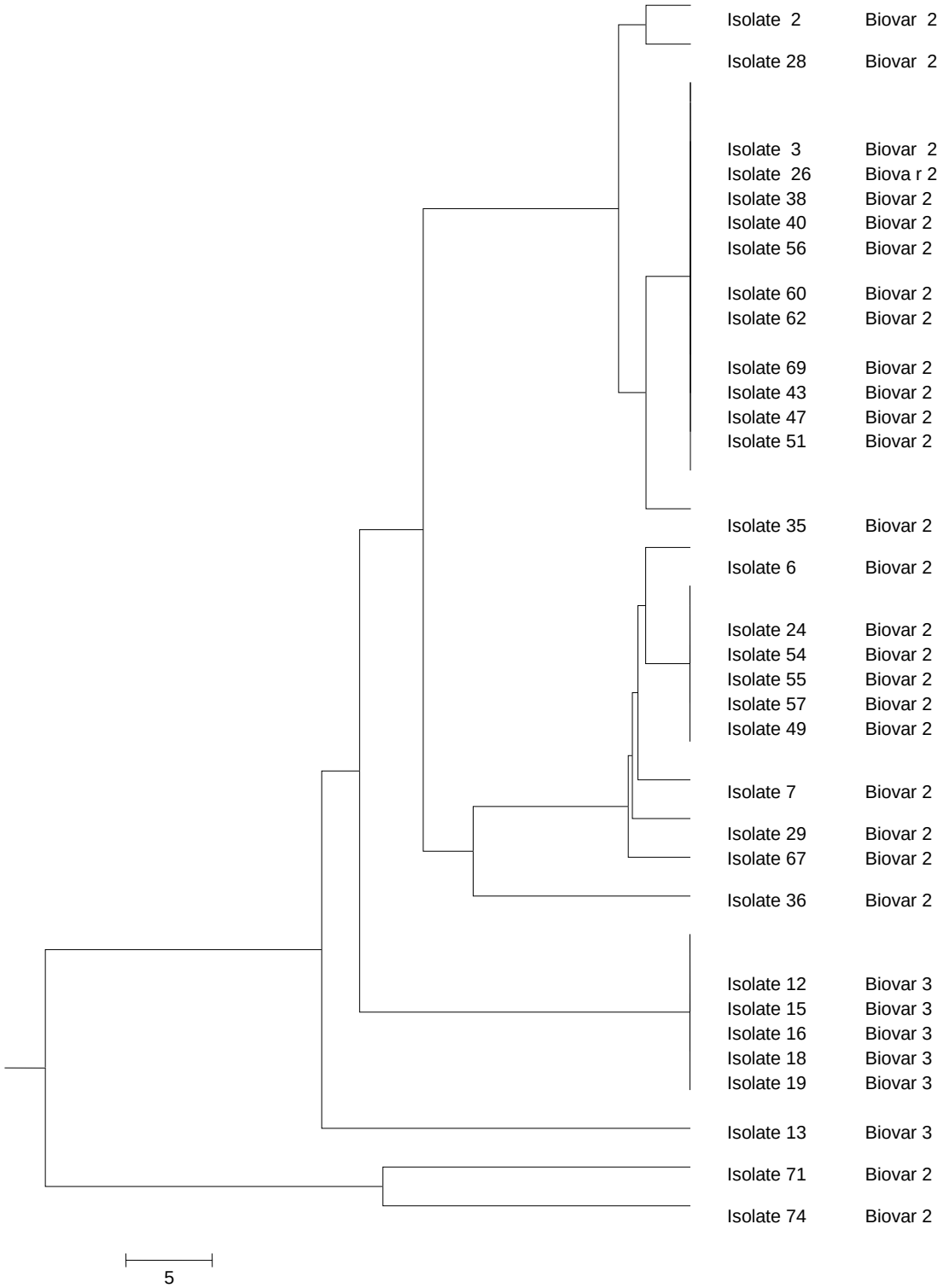


Figure 5. Dendrogram generated from DNA amplification products using ERIC primer sets showing relationships among *Ralstonia solanacearum* biovar 2 and *Ralstonia solanacearum* biovar 3. The scale indicates similarity distance (%).

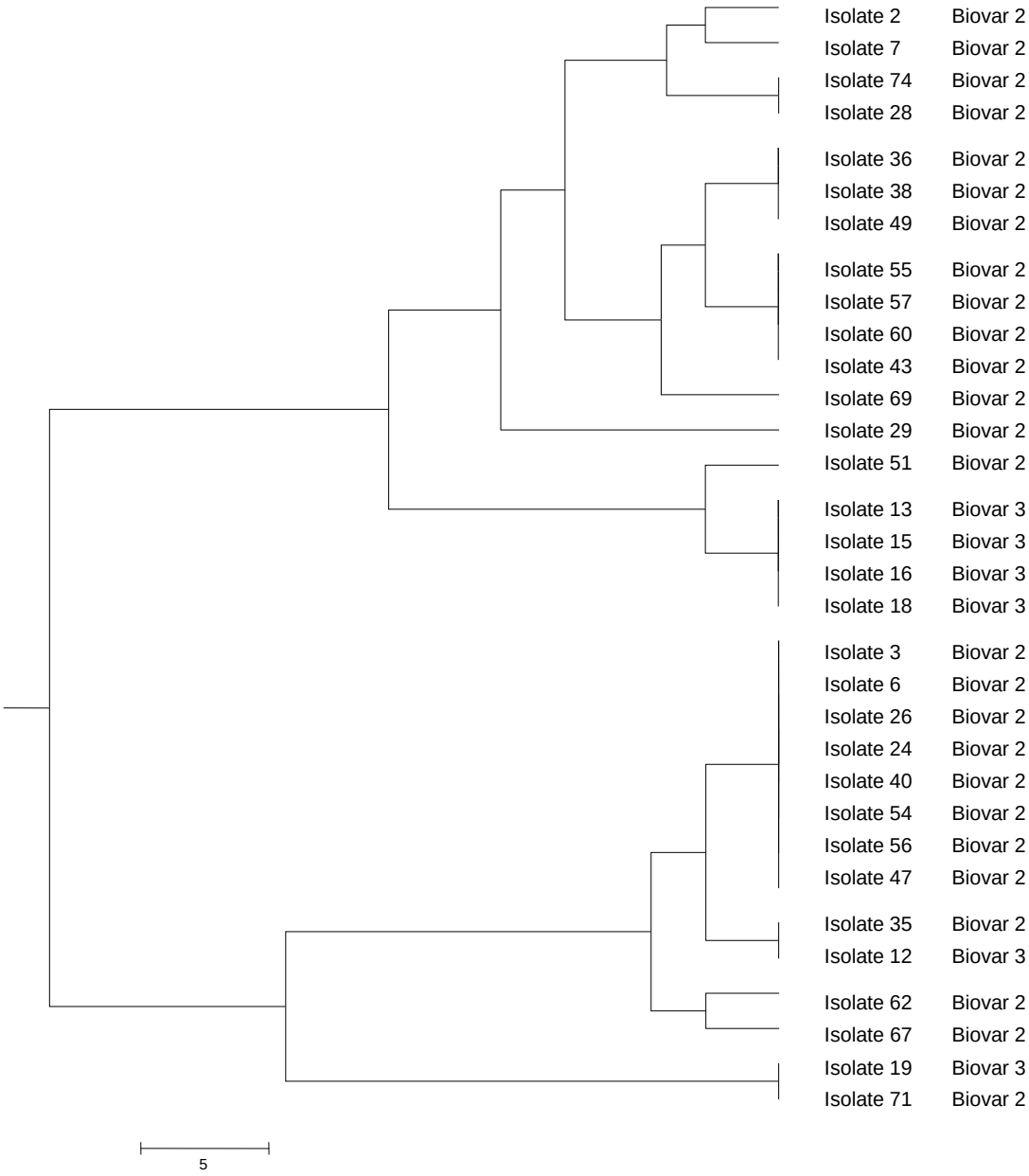


Figure 6. Dendrogram generated from DNA amplification products using BOX primer set showing relationships among *Ralstonia solanacearum* biovar 2 and *Ralstonia solanacearum* biovar 3. The scale indicates similarity distance (%).

4.2 Pathogenic characteristics of *Ralstonia solanacearum* isolates

4.2.1 Pathogenicity on potato and tomato

Fifty nine replicated tomato (*Lycopersicon esculentum* var Moneymaker) test plants (86.8%) and 59 replicated potato (*Solanum tuberosum* var Tigoni) test plants (86.8%) wilted after inoculation with isolates of *R. solanacearum* which had been isolated from collected samples. This indicated that the isolates were pathogenic to both potato and tomato plants. The first wilting symptoms on tomato plants started to appear eight days from the date of inoculation, while potato plants started to show wilting symptoms 12 days after inoculations.

Tomato plants showed the wilt symptoms on an average of 14 days after inoculation, which was a significantly shorter period than the average of 17 days taken by the potato plants. Therefore, there was a significant difference in the number of days to wilting ($F_{[1, 58]} = 10.12$, $P \leq 0.01$) between the hosts. There was also significant difference in the number of days to wilting ($F_{[1, 58]} = 2.024$, $P \leq 0.01$) among the isolates causing the wilt in potato and tomato (Table 10). Isolates 1, 2, 4, 19, 29, 35, 53, 54, and 63 were among the isolates which took the least number of days to cause wilt in the hosts, while isolates 6, 8, 15, and 24 were among those which took the highest number of days to cause wilting of the hosts (Table 10).

The plants inoculated with *R. solanacearum* biovar 2 took a mean of 15 days to develop wilt symptoms in tomato, while it took a mean of 20 days to develop disease symptoms in potato. It was observed that test plants inoculated with *R. solanacearum* biovar 3 took a longer time (a mean of twenty seven days) to develop wilt symptoms on potato while it took a mean of 17 days to develop wilt symptoms on tomato (Table 10). *Ralstonia solanacearum* was recovered from the wilting test plants after streaking the bacterial ooze suspension on SMSA and Kelman's media and incubating at 28°C for 48-72 hours.

The cultures had the colonies which were slightly raised, slimy with entire edges, and creamy-white. The colonies were irregular in shape and varying in sizes. They were fluidal in their consistency, smooth and they formed pink whirling patterns (whorls) at the centres. On the basis of the above observations and the descriptions by Kelman (1954), Harrison (1960), Hayward (1960), Hayward (1964), French and Sequeira (1970) for *R. solanacearum,* the isolates obtained from the pathogenicity test plants were identified as *R. solanacearum*.

Table 10. Time to first wilt symptom appearance in potato and tomato.

Isolate	Mean number of Days	
	Potato	Tomato
1	10.0	10.0
2	10.0	10.0
3	18.0	21.0
4	10.0	10.0
6	20.0	24.5
7	13.5	16.0
8	19.5	31.5
9	17.0	13.5
10	13.5	13.0
12	30.0	24.0
13	21.5	19.0
14	20.0	17.0
15	27.0	21.5
16	27.0	17.0
17	21.0	13.0
18	16.0	13.5
19	11.0	10.0
21	14.0	16.0
22	14.5	16.0
23	13.0	10.5
24	26.0	24.0
25	13.0	10.0
27	15.0	14.0
28	14.5	12.5
29	11.0	9.5
31	14.5	10.5
32	20.5	22.0
33	13.0	10.0
35	10.0	9.5
36	14.5	19.0
37	16.0	16.0
38	16.5	10.0
43	21.0	19.0
44	13.0	10.5
46	16.0	16.5
47	19.0	16.0
48	17.5	16.0
49	16.0	13.0
50	14.5	10.5
51	14.5	13.5

Table 10 cont.

	Mean number of Days	
Isolate	**Potato**	**Tomato**
52	11.5	12.5
53	14.5	10.0
54	11.5	10.0
55	14.5	19.0
56	11.5	13.5
57	13.0	13.0
58	19.0	16.0
59	16.0	16.0
60	16.0	13.0
61	10.0	10.0
62	11.5	10.0
63	10.0	9.5
64	11.5	10.5
65	14.5	13.5
66	16.0	16.0
67	11.5	13.0
68	17.5	13.5
69	17.5	21.5
70	17.5	21.5
Mean*	17.0^{a}	14.0^{b}

***** Means followed by same superscript letter are not significantly different at 5% probability level according to Student-Newman-Keuls (S-N-K) test.

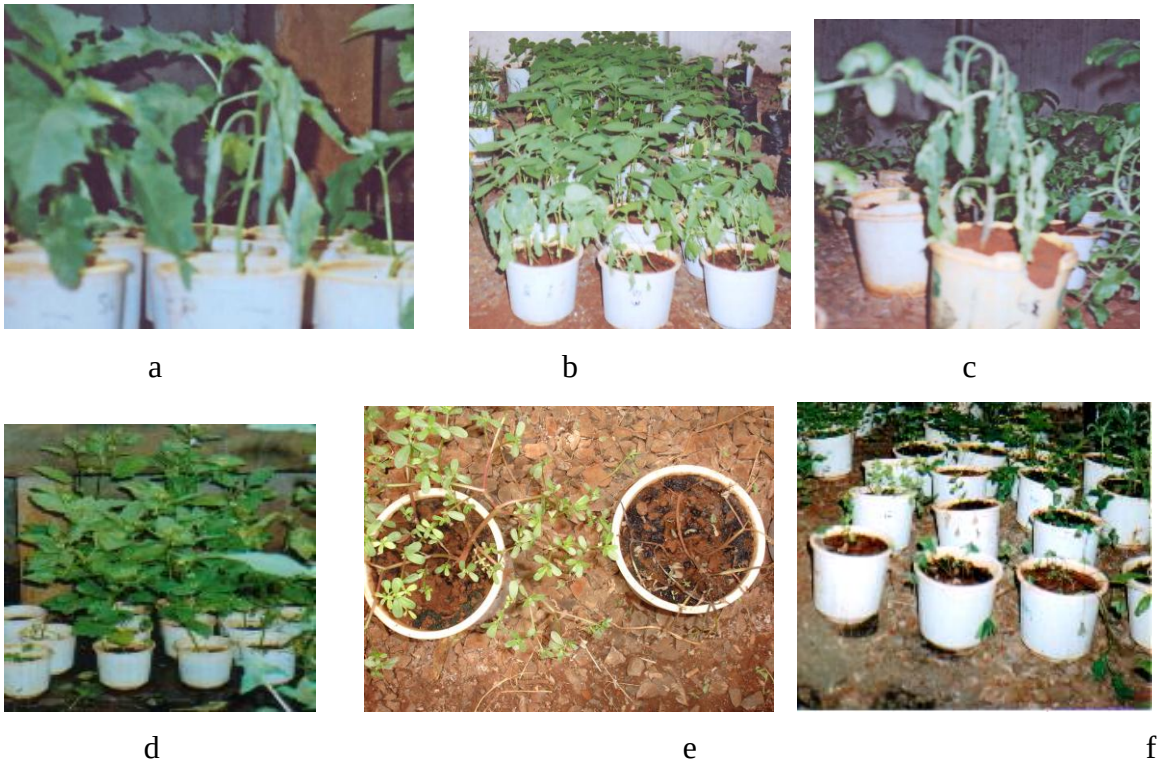
At the end of the pathogenicity test, all test plants had wilted or showed other bacterial wilt symptoms. The symptoms observed in the glasshouse were withering of leaves. During the early stages of infection, a few leaves were found to be drooping. In most cases foliage of the entire plant wilted without change in colour. Other symptoms found in infected plants were yellowing of leaves, and discolouration of the vascular system. Stunting of the plants was not observed.

4.2.2 Pathogenicity of *R. solanacearum* on selected weeds and crop plants

The most dominant weed species (Table 1) in the cultivated field at NARL and a few selected crops which were screened showed various wilting symptoms as described below.

4.2.2.1 Symptomatic test plants

The following plant species exhibited wilting symptoms in which all or some test plants wilted, collapsed and died: *Datura stramonium, Solanum nigrum, Cleome monophylla, Portulaca oleraceae, Oxalis latifolia, Nicandra physaloides, Solanum melongena, Capsicum annuum and Lycopersicon esculentum* (Plate 12).



a b c

d e f

Plate 12. Plants showing bacterial wilt symptoms after inoculation with *Ralstonia Solanacearum*. a=*Datura stramonium*, b=*Solanum nigrum*, c=*Lycopersicon esculentum*, d= *Nicandra physaloides*, e=*Portulaca oleraceae*, f=*Oxalis latifolia*.

Solanum nigrum, Lycopersicon esculentum and *Solanum melongena* were highly susceptible to both *R. solanacearum* biovar 2 and biovar 3. *Datura stramonium, Nicandra physaloides, Capsicum annuum, Oxalis latifolia* and *Portulaca oleraceae* were more susceptible to *R. solanacearum* biovar 3 than they were to *R. solanacearum* biovar

2. *Cleome monophylla* was highly susceptible to *R. solanacearum* biovar 2 than it was to *R. solanacearum* biovar 3. When the plants which showed wilting symptoms were tested using *Ralstonia solanacearum* biovar 2 and biovar 3 the results obtained are shown in Tables 11 and 12 and Figure 7.

Table 11. Mean number of plants that wilted after artificial inoculation with *Ralstonia solanacearum* biovars 2 and 3.

	Means*	
Species	**Biovar 2**	**Biovar 3**
Datura stramonium	0.3[c]	1.5[b]
Portulaca oleraceae	0.3 [c]	0.5 [c]
Solanum nigrum	2.7 [a]	2.5 [a]
Oxalis latifolia	0.3 [c]	0.8 [c]
Nicandra physaloides	0.3 [c]	0.8 [c]
Solanum melongena	1.3 [b]	1.5 [b]
Capsicum spp.	0.7 [b]	0.8 [b]
Cleome monophylla	2.3 [a]	0.3 [c]
Lycopersicon esculentum	2.7[a]	2.5 [a]

***** Means followed by same superscript letter in columns are not significantly different at 5% probability level according to Student-Newman-Keuls (S-N-K) test.

Table 12. Number of weed and crop plants, out of three, exhibiting wilt symptoms upon inoculation with different *Ralstonia solanacearum* isolates

	Biovar 2			Biovar 3					
Species	**Isolate 8**	**Isolate 21**	**Isolate 54**	**Isolate 12**	**Isolate 15**	**Isolate 16**	**Isolate 18**	**Control**	**Mean***
Capsicum spp.	1	1	0	1	2	0	0	0	1 [b]
Cleome monophylla	2	3	2	0	0	0	1	0	1 [b]
Datura stramonium	0	1	0	2	1	2	1	0	1 [b]
Lycopersicon esculentum	3	3	2	2	3	3	2	0	2 [a]
Nicandra physaloides	0	1	0	0	2	0	1	0	1 [b]
Oxalis latifolia	0	1	0	1	2	0	0	0	1 [b]
Portulaca oleraceae	1	0	0	2	0	0	0	0	1 [b]
Solanum melongena	2	1	1	1	2	1	2	0	1 [b]
Solanum nigrum	3	3	2	3	3	1	3	0	2 [a]
Mean*	1 [b]	2 [a]	1 [b]	1 [b]	2 [a]	1 [b]	1 [b]	0[c]	

***** Means followed by same superscript letter are not significantly different at 5% probability level according to Student-Newman-Keuls (S-N-K) test.

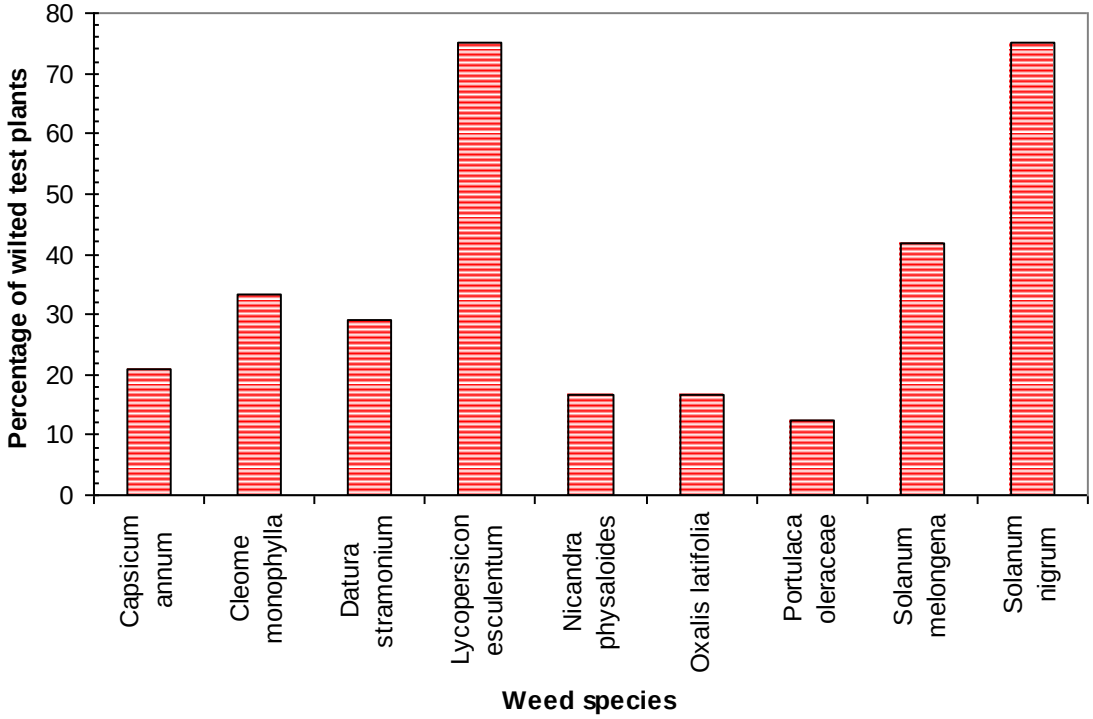
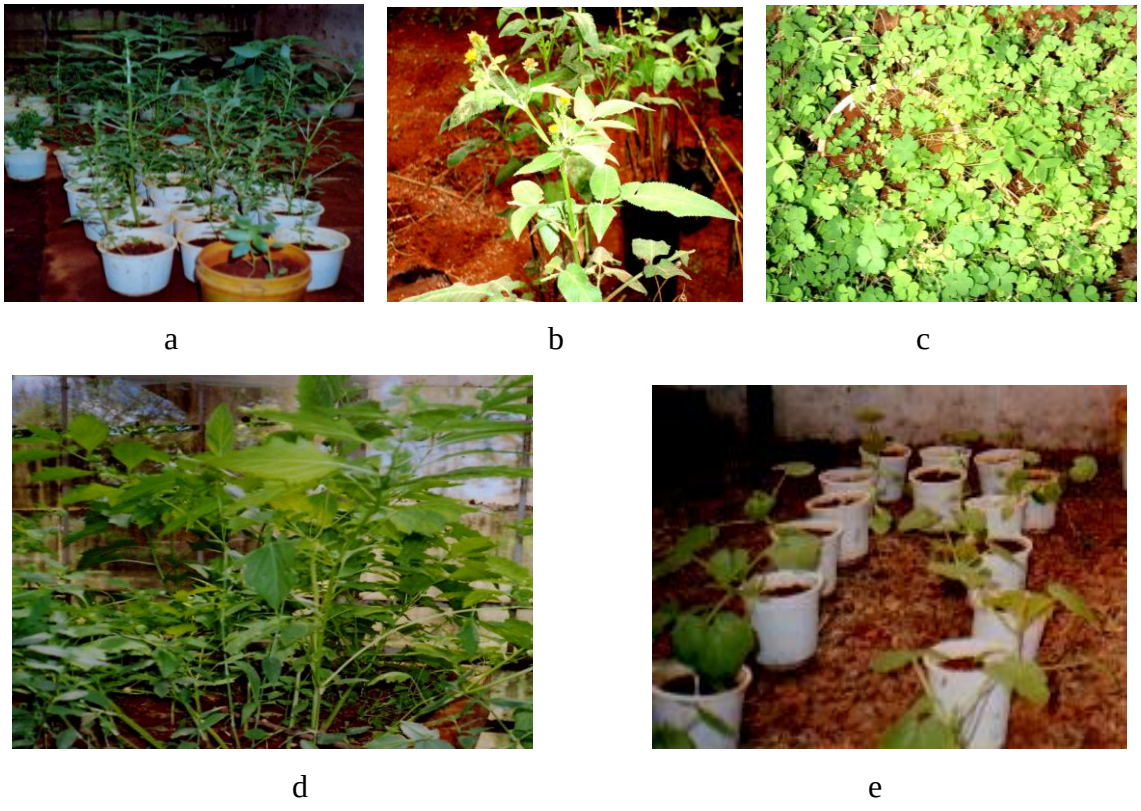


Figure7. Percentage of weed and crop plants exhibiting wilt symptoms after inoculation with *Ralstonia solanacearum.*

Ralstonia solanacearum was isolated from the wilted test plants. When the isolates were tested for their pathogenicity on tomato (*Lycospersicon esculentum* var. Money-maker), some of the test tomato plants wilted. Control plants did not wilt (Plate 13)

Plate 13. Tomato pathogenicity test using isolates obtained from weeds

The following artificially inoculated plant species showed yellowing of the leaves: *Amaranthus* spp., *Bidens pilosa*, *Cucurbita maxima*, some of the *Oxalis latifolia*, *Nicandra physaloides* and O*xygonum sinuatum*. *Cucurbita* spp. showed the highest number of plants with yellowing of leaves followed by *Amaranthus* spp., *Bidens pilosa*, *Nicandra physaloides* and *Oxalis latifolia. Datura stramonium* had the lowest number of plants with yellowing of leaves (Plate 14, Table 13, and Figures 8).



a b c

d e

Plate 14. Plants infected with *Ralstonia solanacearum* showing yellowing of leaves.
a=*Amaranthus* spp., b=*Bidens pilosa*, c=*Oxalis latifolia*, d=*Nicandra physaloides*, e=*Cucurbita máxima.*

There was a significant difference in the number of plants which showed yellowing of leaves among different plant species ($F_{[6, 42]} = 4.136$, $P \leq 0.001$). There was also a significant difference in the number of plants showing yellowing of leaves when they

were inoculated with different isolates of *Ralstonia solanacearum* ($F_{[7, 42]} = 2.231$, $P \leq 0.05$) (Table 13 and Figures 10 and 11).

Table 13. Means of plant species showing yellowing of leaves after inoculation with different isolates of *Ralstonia solanacearum*

Species	Isolate 8	Isolate 12	Isolate 15	Isolate 16	Isolate 18	Isolate 21	Isolate 54	Control	Mean*
Amaranthus spp	2	3	0	2	1	0	1	0	1[b]
Bidens pilosa	1	1	3	1	2	1	0	0	1[b]
Cucurbita maxima	3	3	2	2	3	2	3	0	2[a]
Datura stramonium	1	0	0	0	0	0	2	0	0[c]
Nicandra physaloides	1	0	1	2	1	2	1	0	1[b]
Oxalis latifolia	3	1	0	1	0	1	0	0	1[b]
Oxygonum sinuatum	1	1	2	0	0	0	0	0	1[b]
Mean *	2[a]	1[b]	1[b]	1[b]	1[b]	1[b]	2	o[c]	

***** Means followed by same superscript letter are not significantly different at 5% probability level according to Student-Newman-Keuls (S-N-K) test.

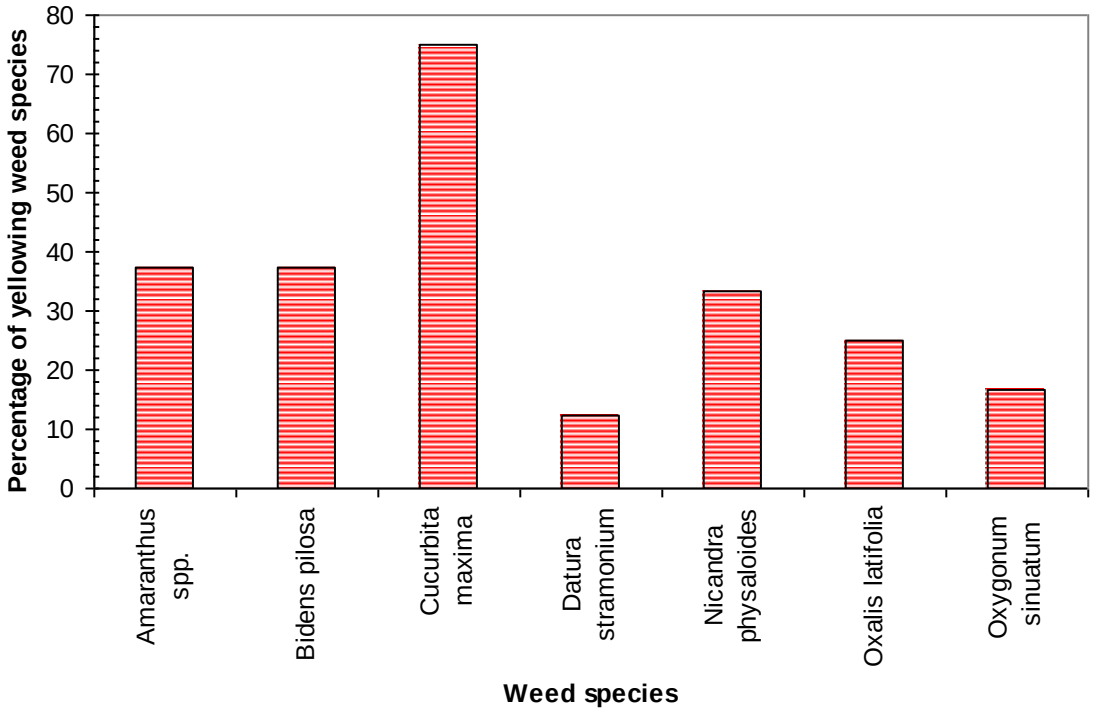


Figure 8. Percentage of plants showing yellowing of leaves after inoculation with *Ralstonia solanacearum*.

Ralstonia solanacearum was isolated from stems and bulbs of inoculated *Oxalis latifolia*, stems of *Oxygonum sinuatum* and *Nicandra physaloides*. *Ralstonia solanacearum* was not isolated from stems of *Amaranthus* spp. *Bidens pilosa* and *Cucurbita maxima* although these plant species showed leaf yellowing symptoms.

Defoliation and yellowing of plants were observed in *Nicandra physaloides* and *Datura stramonium* species only. There was a significant difference on defoliation of plants ($F_{[1,7]} = 18.78$, $P \leq 0.01$) between the two species. There was also a difference among the isolates in causing defoliation ($F_{[7,7]} = 2.27$, $P < 0.05$) (Table 14).

Table 14. Number of defoliated *Datura stramonium* and *Nicandra physaloides* plants out of three, after inoculation with different isolates of *Ralstonia solanacearum*

Species	Isolate 8	Isolate 12	Isolate 15	Isolate 16	Isolate 18	Isolate 21	Isolate 54	Control	Mean*
Datura stramonium	2	0	0	0	0	1	1	0	1 [b]
Nicandra phyaselodes	3	3	1	2	3	2	3	0	2 [a]
Mean*	3 [a]	2 [b]	1 [c]	1 [c]	2 [b]	2 [b]	2 [b]	0 [d]	

***** Means followed by same superscript letter are not significantly different at 5% probability level according to Student-Newman-Keuls (S-N-K) test.

Stunted growth was observed in some *Tagetes minuta*, *Bidens pilosa*, *Portulaca oleraceae*, *Nicandra physaloides*, *Galinsoga parviflora*, *Cucurbita maxima* and *Solanum nigrum* plants. There was a significant difference in the number of stunted plants ($F_{[6,42]} = 7.806$, $P \leq 0.001$) among the species. There was also a significant difference among the isolates ($F_{[7,42]}=7.369$, $P \leq 0.001$) in causing stunting. The difference came about when the control plants in which there was no stunting of the plants were compared with the test plants (Table 15).

No isolation of *R. solanacearum* was made from *Galinsoga parviflora*, *Bidens pilosa* and *Cucurbita maxima*. Control plant species which were inoculated with sterile distilled water did not show any of the wilting symptoms described above.

Table 15. Mean of stunted weed species after inoculation with different isolates of *Ralstonia solanacearum*

Species	Isolate 12	Isolate 15	Isolate 16	Isolate 18	Isolate 21	Isolate 54	Isolate 8	Control	Mean*
Bidens pilosa	3	3	3	3	2	2	3	0	2 [b]
Cucurbita maxima	3	2	2	3	2	2	3	0	2 [b]
Galinsoga parviflora	3	3	3	3	2	3	1	0	2 [b]
Nicandra physaloides	3	0	2	1	2	1	2	0	1 [c]
Portulaca oleraceae	1	3	2	3	2	0	2	0	2 [b]
Solanum nigrum	0	0	2	0	0	1	0	0	0 [d]
Tagetes minuta	3	3	3	3	3	2	3	0	3 [a]
Mean*	2 [a]	2 [a]	2 [a]	2 [a]	2 [a]	2 [a]	2 [a]	0 [b]	

***** Means followed by same superscript letter are not significantly different at 5% probability level according to Student-Newman-Keuls (S-N-K) test.

4.2.2.2 Asymptomatic plants after artificial inoculation

The plants in this category showed no wilting symptoms up to six weeks after inoculation with the bacteria *R. solanacearum*. After 60 days, the asymptomatic plants were tested using ELISA technique and some of the test plants were found to be positive for *Ralstonia solanacearum* (Table 16, Plates 15, and 16 and Figure 9).

The asymptomatic plants which were found to be ELISA positive for *R. solanacearum* were *Galinsoga parviflora, Commelina benghalensis, Erucastrum arabicum, Ageratum conyzoides, Oxygonum sinuatum* and *Rumex abyssinicus*. Some of the results obtained in the nitrocellulose membrane (NCM) ELISA and double antibody sandwich (DAS) ELISA are shown below. In DAS ELISA results, some *Galinsoga parviflora* samples were negative, while other samples of the same species were positive for *R. solanacearum*. Also, while some of the plants belonging to *Tagetes minuta, Portulaca oleraceae, Nicandra physaloides, Oxalis latifolia, Bidens pilosa,* and *Amaranthus* spp. were DAS and NCM ELISA positive for *R. solanacearum,* others in the same species were negative for *R. solanacearum* (Figures 10, 11, 12 and 13 and Plates 15 and 16).

Table 16. Number of plants, out of three per species, which were DAS ELISA positive for *Ralstonia solanacearum* after artificial inoculation with different isolates of *Ralstonia solanacearum*

Species	Isolate 8	Isolate 12	Isolate 15	Isolate 16	Isolate 18	Isolate 21	Isolate 54	Mean*
Ageratum conyzoides	3	3	3	3	3	0	0	2 [b]
Amaranthus spp	3	0	3	0	3	0	0	1[c]
Asystasia schimperi	0	0	0	0	0	0	0	0 [d]
Bidens pilosa	3	3	3	0	0	0	0	1 [c]
Cleome monophylla	3	3	0	0	0	3	0	1 [c]
Commelina benghalensis	3	0	3	0	3	0	0	1 [c]
Conyza bonariensis	0	0	0	0	0	0	0	0[d]
Cucurbita maxima	3	3	3	0	3	0	0	2 [b]
Datura stramonium	0	3	3	3	3	3	0	2 [b]
Eleusine indica	0	0	0	0	0	0	0	0 [d]
Erucastrum arabicum	0	3	0	0	3	3	3	2 [b]
Euphorbia hirta	0	0	0	0	0	0	0	0 [d]
Galinsoga parviflora	3	3	0	3	0	0	0	1 [c]
Leonotis mullissina	0	0	3	0	0	3	0	1 [c]
Malva verticillata	0	0	0	0	0	0	0	0 [d]
Musa spp.	0	0	0	0	0	0	0	0 [d]
Nicandra physaloides	0	3	3	3	0	3	0	2 [b]
Oxalis latifolia	3	3	3	3	3	0	0	2 [b]
Oxygonum sinuatum	0	0	3	3	0	3	3	2 [b]
Pelargonium zonale	0	0	0	0	0	0	0	0 [d]
Portulaca oleraceae	3	0	3	0	3	3	0	2 [b]
Rumex abyssinicus	0	3	0	3	3	0	3	2 [b]
Solanum nigrum	3	3	3	3	3	3	3	3 [a]
Sonchus oleraceus	0	0	0	0	0	0	0	0 [d]
Tagetes minuta	3	3	0	0	3	3	0	2 [b]
Vigna unguiculata	0	0	0	0	0	0	0	0 [d]
Mean*****	1[a]	1[a]	1[a]	1[a]	1[a]	1[a]	1[a]	

***** Means followed by same superscript letter are not significantly different at 5% probability level according to Student-Newman-Keuls (S-N-K) test.

Twenty five percent of *Oxalis latifolia* and 87.5% of *Tagetes minuta* plants which were tested using DAS ELISA were positive for *R. solanacearum*. In the same test, 12.5% percent of *Bidens pilosa* were found to be positive for bacterial infection while 100% of *Galinsoga parviflora* and *Conyza bonariensis* weed plants were negative. In the case of *Portulaca oleraceae,* 87.5% of plants were found to be infected whilst 37.5% of

Nicandra physaloides were infected. Fifteen percent of *Commelina benghalensis* test plants were found to be infected while 25% of *Amaranthus* spp. was infected (Plate 15 and Figure 9).

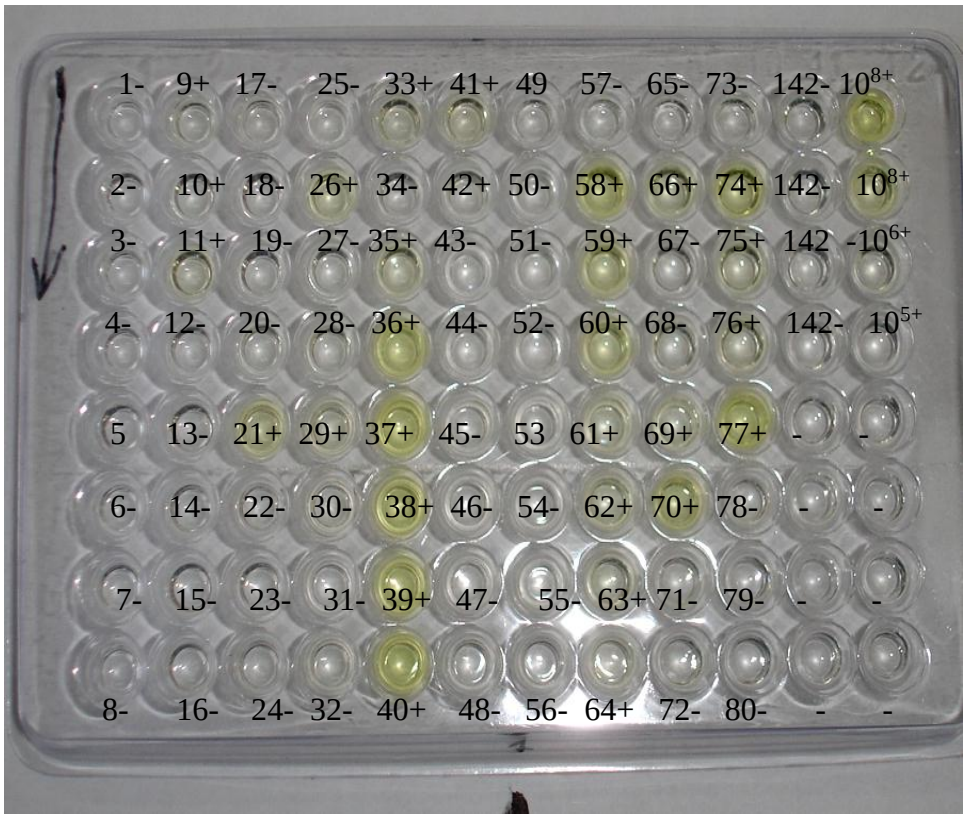


Plate 15. Plate of double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) showing positive (yellow colour) and negative reactions on various plant samples, 1-80. 1–8=*Galinsoga parviflora,* 9–24=*Oxalis latifolia,* 25–32=*Amaranthus* spp., 33–40=*Tagetes minuta,* 41–48=*Bidens pilosa,* 49–56=*Conyza bonariensis,* 57–64=*Portulaca oleraceae,* 65–72=*Nicandra physaloides,* 73–80=*Commelina benghalensis,* 142(-)=Negative controls and samples 10^{8}-10^{5} (+)=positive controls.

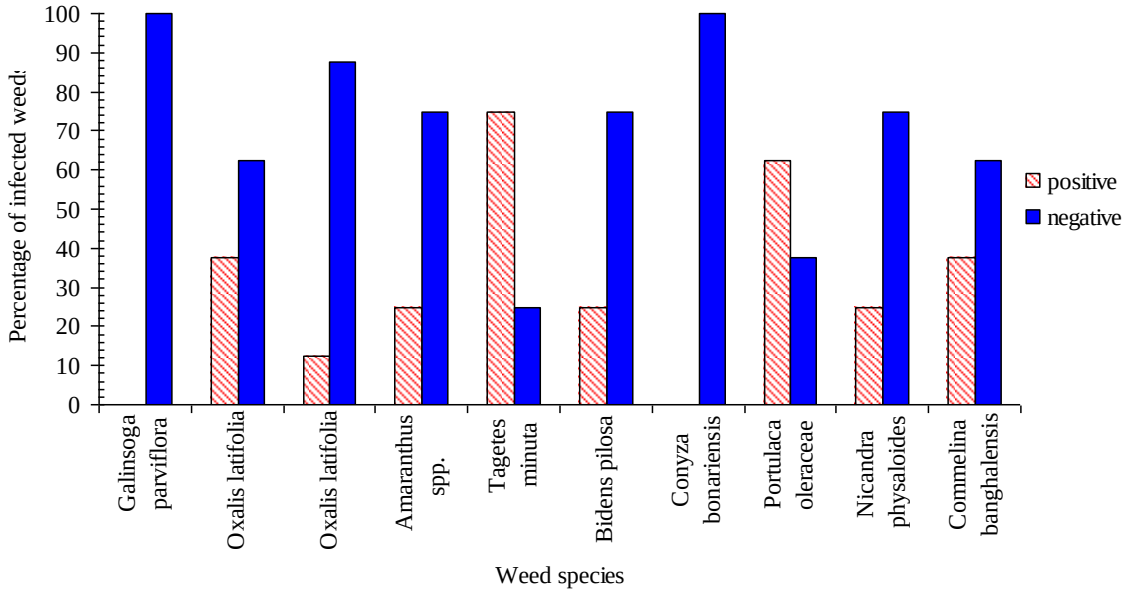


Figure 9. Percentage of weeds which were DAS- ELISA positive and negative for *Ralstonia solanacearum* infection. Blue bars represent DAS-ELISA negative while orange bars represent DAS-ELISA positive.

In another DAS ELISA test, 87% of *Solanum nigrum* were found to be infected with *R. solanacearum* while *Eleusine indica, Pelargonium zonale, Euphobia hirta, Sonchus oleraceus,* and *Malva verticillata* were negative. About 37.5% of *Ageratum conyzoides,* and 75% of *Cucurbita maxima* were found to be positive for *R. solanacearum* infection. All plants tested in the following species were also found positive: *Galinsoga parviflora, Datura stramonium, Oxalis latifolia., Amaranthus* spp. and *Tagetes minuta* (Figure 10).

Some plant species which were screened in these investigations did not exhibit wilting symptoms with any of the isolates used. Such plants were also ELISA test negative for *R. solanacearum* as shown in Figures 9, 10, 11 and 12; Plates 15 and 16 and Table 16. These plant species were: *Conyza bonariensis, Sonchus oleraceus, Asystasia schimperi, Malva verticillata, Eleusine indica, Euphorbia hirta, Vigna unguiculata, Pelargonium zonale* and *Musa* spp.

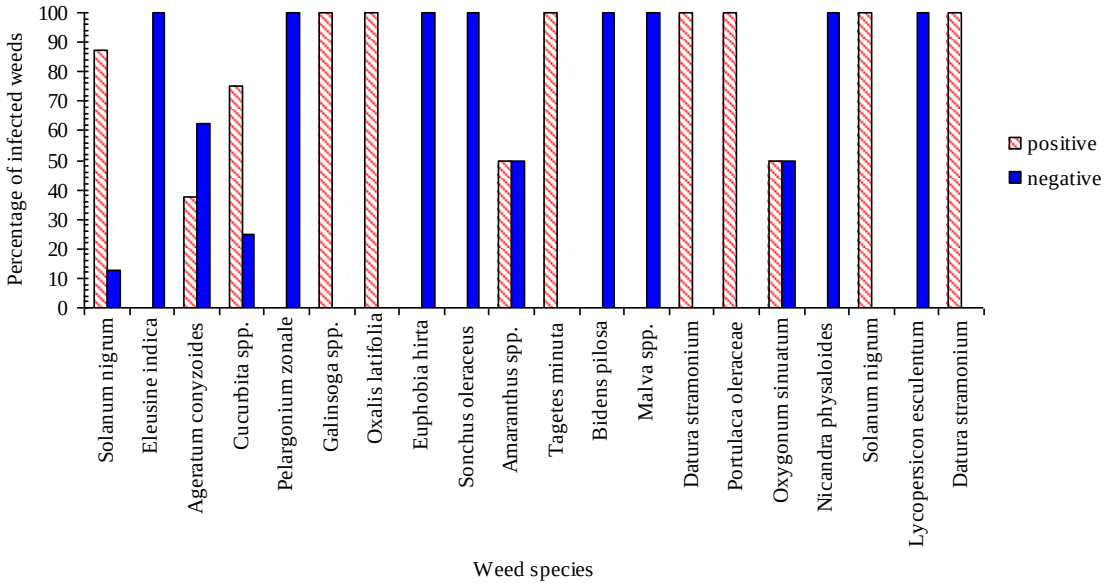


Figure 10. Percentage of weeds which were DAS- ELISA positive and negative for *Ralstonia solanacearum* infection

When NCM- ELISA was used to test for *R. solanacearum* infection on various plant species, the results which were obtained are as shown in Plate 16 and Figures 11 and 12.

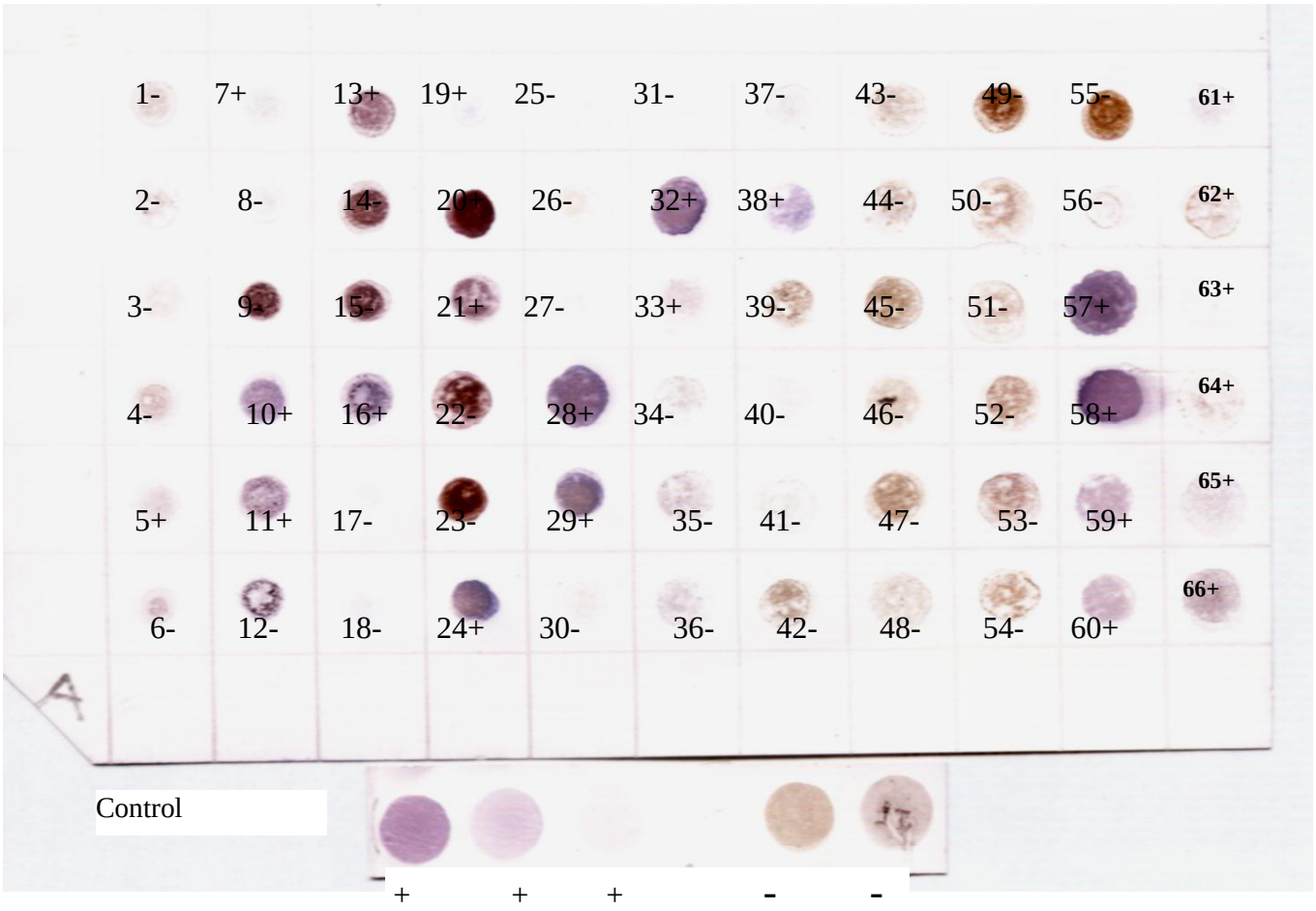


Plate 16. **Plate of nitrocellulose membrane-enzyme linked immunosorbent assay (NCM-ELISA) showing positive (purple colour) and negative reactions on various plant samples, 1-66.** No. 1–8=*Galinsoga parviflora*, *9*–24=*Oxalis latifolia*, 25–31=*Amaranthus* spp, *33*–39 =*Tagetes minuta*, *41*–48=*Bidens pilosa*, 49–55=*Conyza bonariensis*, *57*–64=*Datura stramonium*, 65–66=*Portulaca oleraceae*, (-) =Negative, (+) = positive

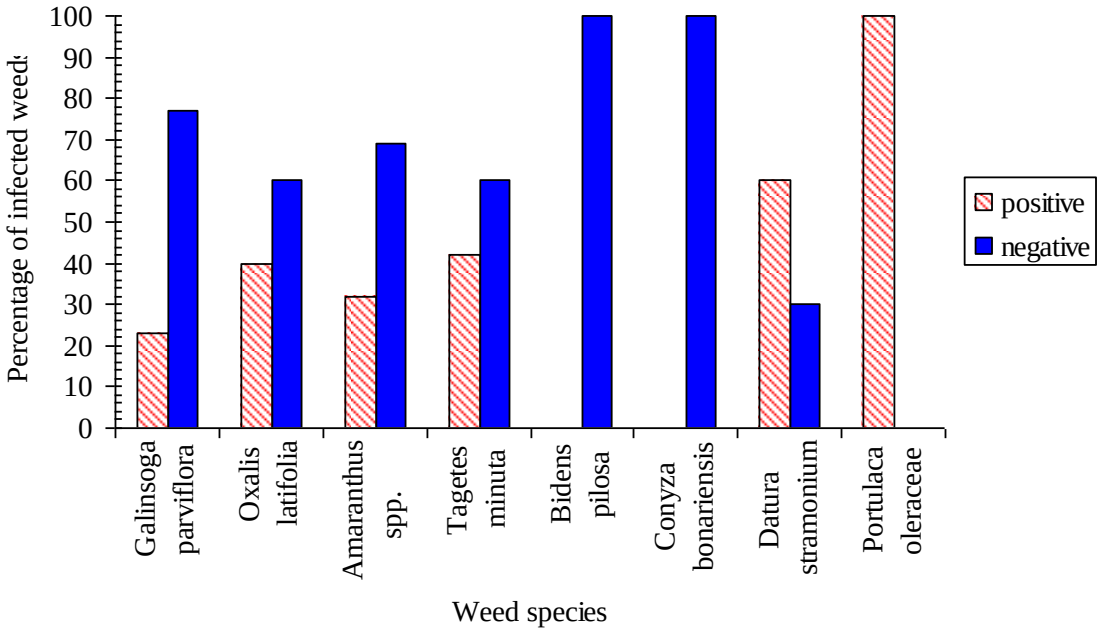


Figure 11. Percentage of weeds which were nitrocellulose membrane-enzyme linked immunosorbent assay (NCM- ELISA) positive and negative for *Ralstonia solanacearum* infection

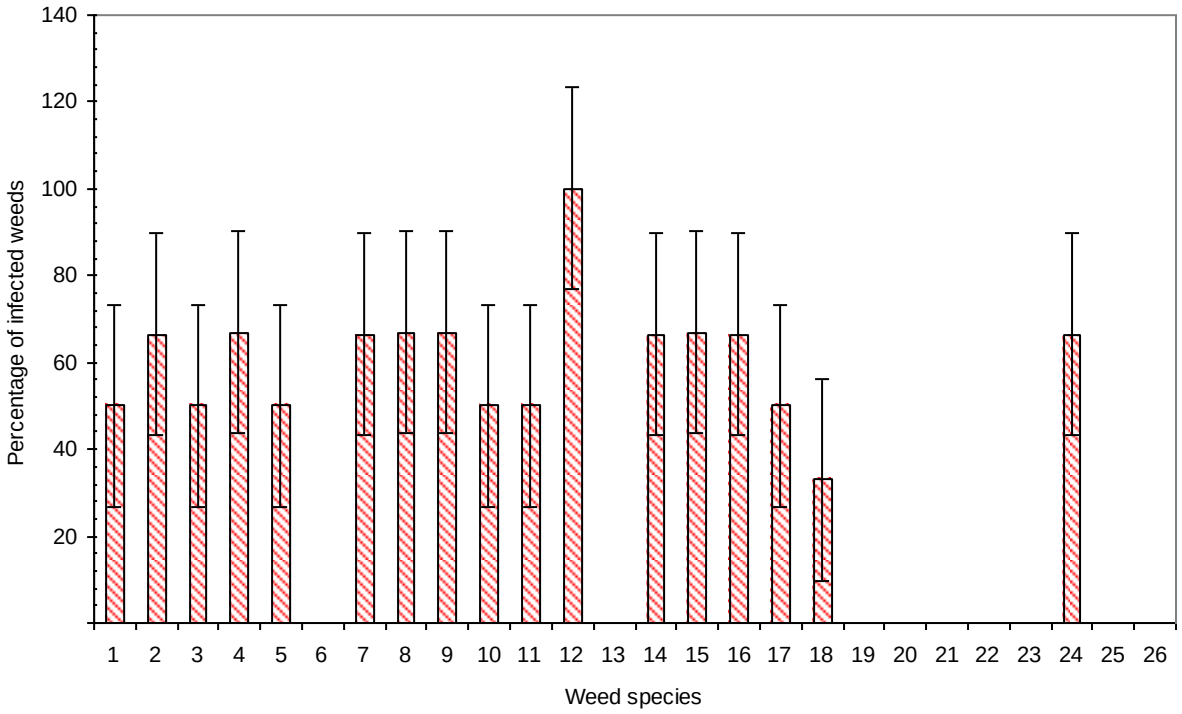


Figure 12. **Percentage of plants which were NCM- ELISA positive and negative for *Ralstonia solanacearum*.** 1=*Galinsoga parviflora*, 2=*Oxalis latifolia*, 3=*Amaranthus* ssp, 4=*Tagetes minuta*, 5=*Bidens pilosa*, 6=*Conyza bonariensis*, 7=*Datura stramonium*, 8=*Portulaca oleraceae*, 9= *Oxygonum sinuatum*, 10=*Nicandra physaloides*, 11=*Commelina benghalensis*, 12=*Solanum nigrum*, 13=*Eleusine indica*, 14=*Cucurbita maxima*, 15=*Erucastrum arabicum*, 16=*Ageratum conyzoides*, 17=*Cleome monophylla*, 18=*Leonotis mollissina*, 19=*Sonchus oleraceus*, 20=*Euphorbia hirta*, 21=*Malva verticillata* 22=*Asystasia schimperi*, 23=*Vigna unguiculata*, 24=*Rumex abyssinicus*, 25=*Pelargonium zonale*, 26=*Musa* spp.

Analysis of variance (ANOVA) showed that there was a significant difference ($F_{[25, 25]} = 3.74$, $P \leq 0.001$) in the number of infected weeds among the weed species. It also showed that there was a significant difference ($F_{[1, 25]} = 10.547$, $P \leq 0.01$) in the number of infected weeds by biovar 2 and biovar 3 of *Ralstonia solanacearum* (Figure 12 and Table 17).

Table 17. Mean number of plants, out of the three inoculated, which were infected by different biovars of *Ralstonia solanacearum* in various species.

Species	Biovar 2	Biovar 3	Mean*
Ageratum conyzoides	1.00	3.00	2.00[b]
Amaranthus spp	1.00	1.50	1.25[c]
Asystasia schimperi	0.00	0.00	0.00[d]
Bidens pilosa	1.00	1.50	1.25 [c]
Cleome monophylla	2.00	0.75	1.38 [c]
Commelina benghalensis	1.00	1.50	1.25 [c]
Conyza bonariensis	0.00	0.00	0.00 [d]
Cucurbita maxima	1.00	2.25	1.63[b]
Datura stramonium	1.00	3.00	2.00 [b]
Eleusine indica	0.00	0.00	0.00 [d]
Erucastrum arabicum	2.00	1.50	1.75[b]
Euphorbia hirta	0.00	0.00	0.00 [d]
Galinsoga parviflora	1.00	1.50	1.25 [c]
Leonotis mullissina	1.00	0.75	0.88 [c]
Malva verticillata	0.00	0.00	0.00 [d]
Musa spp.	0.00	0.00	0.00 [d]
Nicandra physaloides	1.00	2.25	1.63 [b]
Oxalis latifolia	1.00	3.00	2.00[b]
Oxygonum sinuatum	2.00	1.50	1.75[b]
Pelargonium zonale	0.00	0.00	0.00 [d]
Portulaca oleraceae	2.00	1.50	1.75 [b]
Rumex abyssinicus	1.00	2.25	1.63[b]
Solanum nigrum	3.00	3.00	3.00[a]
Sonchus oleraceus	0.00	0.00	0.00 [d]
Tagetes minuta	2.00	1.50	1.75[b]
Vigna unguiculata	0.00	0.00	0.00 [d]
Mean*	0.92 [b]	1.62[a]	

***** Means followed by same superscript letter are not significantly different at 5% probability level according to Student-Newman-Keuls (S-N-K) test.

DAS-ELISA on asymptomatic pathogenicity test tomato plants indicated that 81.8% of the tomato test plants were latently infected with *Ralstonia solanacearum* (Table 18). The pathogen was isolated from 61.5% of the stems and 53.8% of the roots of test plants. The plants which were ELISA test negative for *R. solanacearum* in both the stems and roots formed 18.2% of the plant species (Table 18).

Table 18. Table showing DAS-ELISA positive for *Ralstonia solanacearum* on asymptomatic tomato plants inoculated with *Ralstonia solanacearum* isolates obtained from weeds

Source of *Ralstonia solanacearum*	Symptoms on the host weed plants	Symptoms on the tomato test plants	DAS- ELISA on 1gm of inoculated tomato plant parts	
			Stem	**Root**
Galinsoga parviflora	Stunting	no wilting symptoms	+	+
Oxalis latifolia	stunting and wilting	no wilting symptoms	+	+
Amaranthus spp	yellowing of leaves	no wilting symptoms	+	-
Tagetes minuta	Stunting	no wilting symptoms	+	+
Bidens pilosa	stunting, adventitious roots	no wilting symptoms	-	-
Datura stramonium	wilting and stunting	no wilting symptoms	+	+
Portulaca oleraceae	wilting and stunting	Yellowing	+	+
Oxygonum sinuatum	yellowing of leaves	no wilting symptoms	+	-
Nicandra physaloides	wilting and yellowing of leaves	no wilting symptoms	-	+
Commelina benghalensis	no wilting symptoms	no wilting symptoms	-	-
Solanum nigrum	Wilting	no wilting symptoms	+	+

+ = positive ELISA result for *R. solanacearum*, - = negative ELISA result for *R. solanacearum*

4.2.3 Monitoring of natural infection of weeds by *R. solanacearum* in the field

Some weed species were found wilting in the field infested with *R. solanacearum* at NARL. These weed species were *Cleome monophylla, Leonotis mullissina* and *Nicandra physaloides*. The weeds were considered to be natural hosts (Plate 17 and Table 19). The isolates which were obtained from such plant species with wilting symptoms (*Datura stramonium, Tagetes minuta, Portulaca oleraceae, Cleome monophylla, Nicandra physaloides, Leonotis mullissina* and *Oxalis latifolia)* had typical cultural characteristics on SMSA and Kelman TZC medium. The colonies were fluidal, irregular in shape, slightly raised, creamy in colour with pink whorls at the centres. On the basis of the above observations and the descriptions by Kelman (1954), Harrison

(1960), Hayward (1960), Hayward (1964), French and Sequeira (1970), the isolates were identified as *R. solanacearum*.

a b

Plate 17. *Cleome monophylla* (a) and *Leonotis mollissina* (b) found wilting in a field infested with *Ralstonia solanacearum* at NARL.

Four out of five plants tested in the laboratory for *R. solanacearum* infection in each of *Portulaca oleraceae* and C*leome monophylla* species were found to be positive. This formed 80% of the number of plants which were tested. Twenty percent of the weed plants tested in each of *Datura stramonium, Nicandra physaloides, Leonotis mullissina* and O*xalis latifolia* were positive, while 40% of *Tagetes minuta* species were found to be positive. All the five samples of *Amaranthus* spp. and *Galinsoga parviflora* which were tested were negative for *R. solanacearum* (Table 19).

Table 19. Detection of *Ralstonia solanacearum* infection in naturally growing weed species in plots with wilted potato at KARI- NARL

Natural host	Family	Symptoms at sampling time	% of plants from which isolations were made *
Datura stramonium	Solanaceae	stunting & defoliation	20
Tagetes minuta	Asteraceae	Stunting	40
Portulaca oleracea	Portulacaceae	Yellowing	80
Cleome monophylla	Capparadidaceae	wilting and stunting	80
Galinsoga parviflora	Compositae	stunting and yellowing	0
Nicandra physaloides	Solanaceae	wilting, defoliation	20
Leonotis mollissina	Labiatae	wilting, yellowing, defoliation	20
Amaranthus spp.	Amaranthaceae	Stunting	0
Oxalis latifolia	Oxalitaceae	Yellowing	20

* Five plants from each species were analysed for infection

4.2.4 Infection of plant species under glasshouse conditions

Fifty two percent of the plant species which were tested for natural infection were found to be infected with *R. solanacearum* when SMSA medium was used, while 76% of the plant species were found to be infected when DAS ELISA was employed (Table 20).

Some of the weeds investigated in this study are shown in appendix XI.

Table 20. Infection of weeds by *Ralstonia solanacearum* under glasshouse conditions.

Plant species	Nature of propagation	Isolation of *R. solanacearum* on SMSA	DAS-ELISA result
Galinsoga parviflora	seed	-	+
Oxalis latifolia	seedling/vegetative	-	+
Euphorbia hirta	seedling	-	-
Sonchus oleraceus	seedling	-	-
Amaranthus spp	seed	-	+
Tagetes minuta	seed	+	+
Bidens pilosa	seed	-	+
Malva verticillata	seed	-	-
Conyza bonariensis	seed	-	-
Datura stramonium	seed	+	+
Portulaca oleraceae	seedling	+	+
Oxygonum sinuatum	seedling	-	+
Nicandra physaloides	seed	+	+
Commelina benghalensis	vegetative	-	+
Solanum nigrum	seed	+	+
Asystasia schimperi	seedling	-	-
Erucastrum arabicum	seedling	-	+
Ageratum conyzoides	seedling/seed	+	+
Cleome monophylla	seed	+	+
Leonotis mullissina	seedling	+	+
Rumex abyssinicus	seedling	-	+

+ = Positive isolation on SMSA and positive DAS-ELISA for *R. solanacearum*; - = No isolation on SMSA and negative DAS-ELISA for *R.solanacearum*

4.3 Determination of effective crop rotation sequences

4.3.1 One-season rotation in glasshouse

There was no significant difference ($P>0.05$) in time to the onset of bacterial wilt symptoms. It took a mean of 46.2 days after planting for bacterial wilt symptoms to appear in all the treatments (Potato-beans-potato, Potato-cabbage-potato and Potato-maize-potato). The control treatment (Potato-potato-potato), however, had a slightly earlier onset (37.7 days) of bacterial wilt symptoms (Table 21).

One-season rotation treatments exhibited significantly lower wilt incidence ($P\leq 0.01$) than the control treatment where potato was planted for three consecutive seasons. Rotation with maize reduced the wilt incidence from a baseline of 25% at the beginning of the experiment to 20% at the end of the experiment (or by 57.2%) while rotation with

beans and cabbage reduced the wilt incidence to 22.2% and 23.7%, respectively, (or by 52.5% and 42.8%, respectively) from the incidence of 46.7% at the beginning of the rotational experiment. The reductions were, however, not significantly different from each other (Table 21).

Table 21. Effect of one-season rotation on time to onset of bacterial wilt and bacterial wilt incidence under glasshouse conditions.

Treatment	Time to onset of wilt symptoms (days)*	Incidence (%) *
Potato-Beans-potato	49.0[a]	22.2[b]
Potato-Cabbage-Potato	48.7[a]	23.7[b]
Potato-Maize-Potato	49.3[a]	20 .0[b]
Potato-Potato-Potato	37.7[b]	57.7[a]
SE	2.681	3.692

***** Means followed by same superscript letters in columns are not significantly different at 5% probability level according to Student-Newman-Keuls (S-N-K) test.

4.3.2 One season rotation in the field

A bacterial wilt incidence of 46.7% was exhibited by the tomato plants inoculated for inoculum enhancement. There was no significant difference ($P > 0.05$) on the time to the onset of bacterial wilt among the treatments, where maize, beans or cabbage were planted before potato. However, it took the control the least period (35 days) before the onset of bacterial wilt. Tomato-cabbage-potato treatment took 51 days (the longest duration) to the onset of bacterial wilt (Table 22).

Table 22. Effect of one-season rotation on time to onset of bacterial wilt, bacterial wilt incidence and potato yields in a field at NARL, Kenya.

Treatment	Incidence*	Time in days to onset of wilt*	Yield (t/ha) *					% marketable yield	% increase in marketable yield
			Ware	Seed	Chatt	Total	Marketable		
Tomato-Beans-Potato	6 b	44 a	3.4a	17.7 a	2.4 a	23.4 a	21	90	740
Tomato-maize-Potato	5 b	45 a	2.0 a	13.7 ab	2.2 a	17.9 ab	15.7 ab	88	528
Tomato-Cabbage-Potato	7 b	51 a	3.7 a	23.6 a	1.8 a	29.1a	27.3 a	94	992
Tomato-Potato-Potato-Potato	73 a	35 a	0.4 b	2.1 b	0.4 b	3.0 b	2.5 b	84	
SE	2.665	6.218	1.4	3.283	0.302	4.35	4.406		

***** Means followed by same superscript letter in columns are not significantly different at 5% probability level according to Student-Newman-Keuls (S-N-K) test.

There was a significant difference (P≤0.001) in bacterial wilt incidence among the treatments. The positive control (tomato-potato-potato) treatment was significantly different (P≤0.001) from other treatments in incidence of bacterial wilt. This treatment had 73% incidence of bacterial wilt compared to other treatments all of which had wilt incidences of 7% or less (Table 22, Figure 13 and Appendix IX, ANOVA table (a).

A one-season rotation with cabbage, beans or maize significantly reduced the wilt incidence from 46.7% to less than 7% in both treatments. The rotations significantly increased total tuber yields (P≤0.01) as compared to the control. Total tuber yields of 29.1, 23.4 and 17.9t/ha were obtained after rotation with cabbage, beans and maize, respectively, as compared to that of the control at 3.0t/ha (Table 22, and Appendix IX, ANOVA table (b)-one season).

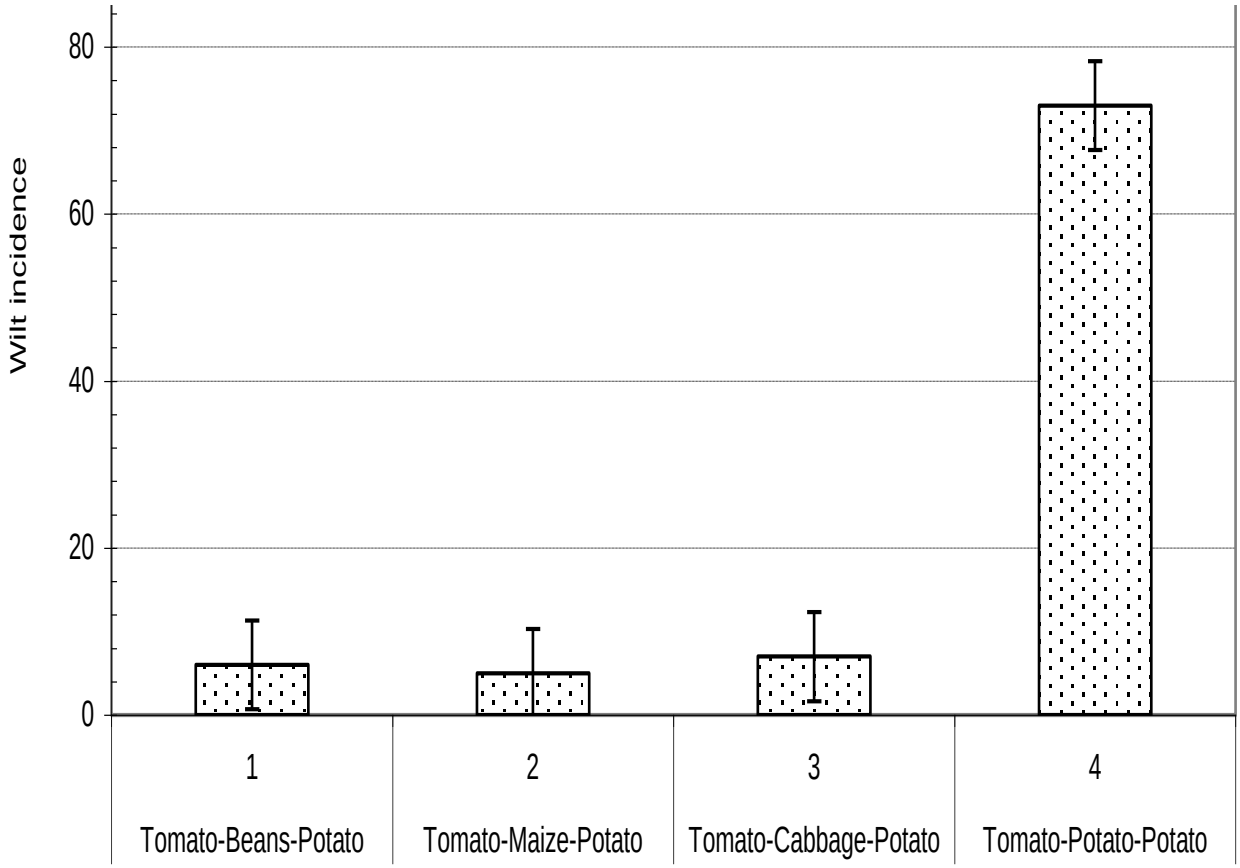


Figure 13. Effect of one-season rotation on field incidence of bacterial wilt

Ware potato yields, among all the treatments, showed no significant difference (P>0.05). However, rotations with cabbage, beans and maize yielded 3.7, 3.4 and 2.0 t/ha, respectively as

compared to the control which yielded 0.4 t/ha (Table 22). The control had a significantly lower ($P \leq 0.05$) seed yield compared to the other treatments. Rotations with cabbage, beans and maize had seed yields of 23.6, 17.7 and 13.7t/ha, respectively, as compared to the yield from the control at 2.1t/ha (Table 22).

There was significant difference ($P \leq 0.05$) in chatt yield among the treatments. The rotations with beans, maize and cabbage had 2.4, 2.2 and 1.8t/ha chatt yield, respectively compared to the control which yielded 0.4t/ha (Table 22).

There was a significant difference ($P \leq 0.05$) in marketable yields among the treatments. Rotations with cabbage, beans and maize had marketable yields of 27.3, 21 and 15.7t/ha, respectively, as compared to significantly low marketable yield (2.5t/ha) of the control (Table 22 and Appendix IX, ANOVA table (c)). There was no significant difference ($P > 0.05$) in percent marketable yield among the treatments. However, the percentage of marketable yield in the control was slightly lower (83%) than those of the other treatments which ranged from 88-94% (Table 22 and Appendix IX, ANOVA table (d)). The percent increase in marketable yield was highest in rotations with cabbage (992%) followed by beans (740%) and then maize at 528% (Tables 22).

Bacterial wilt developed very fast in the control treatment rising from 5.6% on the 35th day after planting to 69.6% on the 91st day after planting (DAPG) (Figure 14. Wilt development in the rotation with beans was slightly higher than that of maize treatment. Wilt in both treatments rose from 0.3% on the 35th day to 6.2% for beans and 5.0% for maize on the 91st day after planting. Wilt development in rotation with cabbage was very slow compared to other treatments but on the 91st day it was 6.4%. Bacterial wilt significantly (R^2=0.581) reduced the yield of marketable potato tubers (Figure 15).

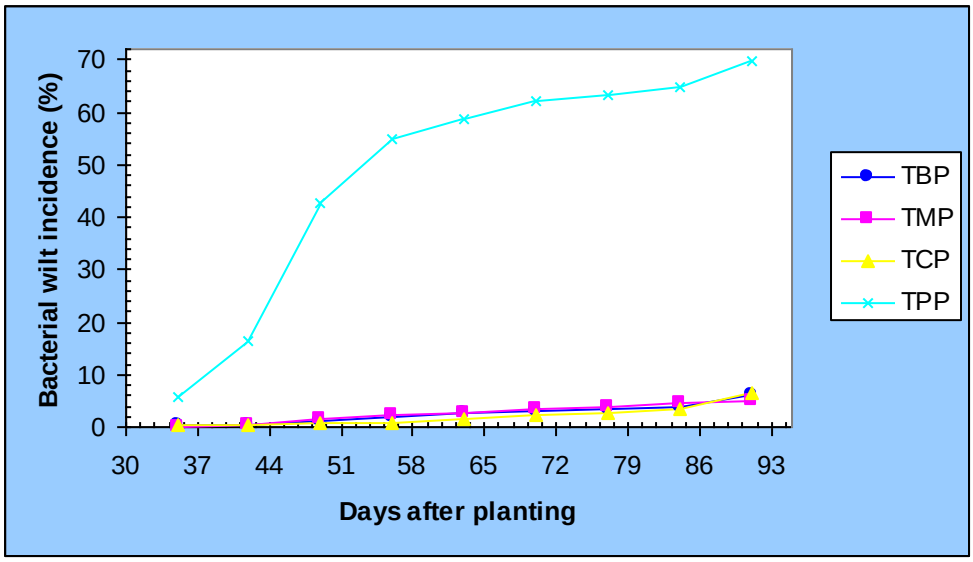


Figure 14. **Effect of one season rotation on bacterial wilt development.** TBP=tomato-bean–potato, TMP=tomato-maize-potato, TCP=tomato–cabbage–potato, and TPP=tomato–potato–potato.

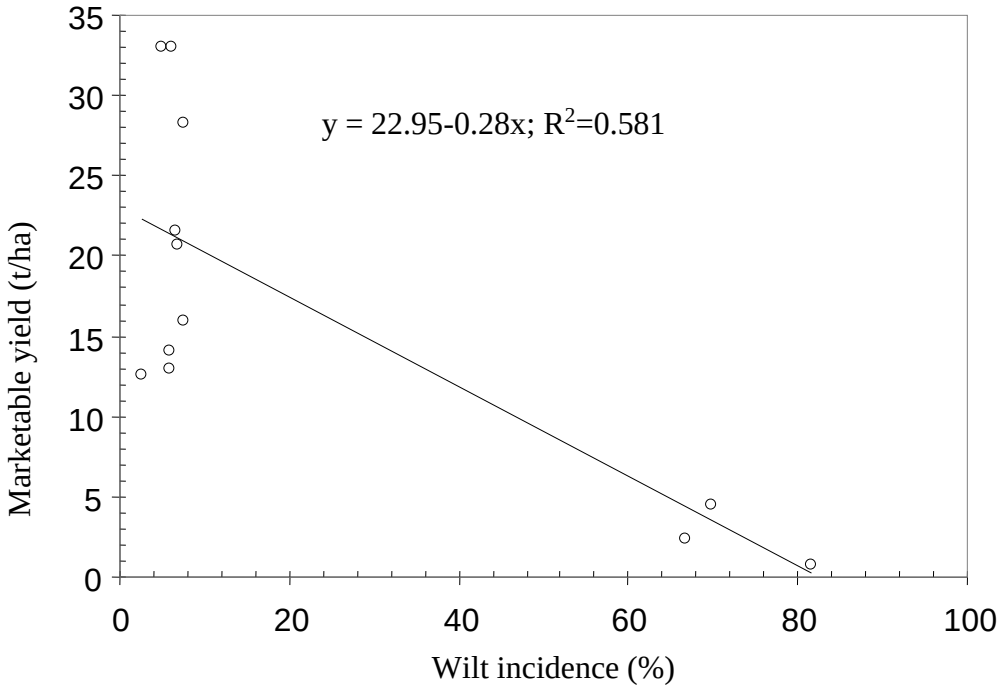


Figure 15. **Relationship between marketable yield and bacterial wilt incidence in one-season rotation in the field.**

The control treatment (tomato-potato-potato) had the highest percentage (21%) of latently infected tubers in one season rotation. This was followed by rotation with cabbage (10%) and then rotation with beans at 9%. Rotation with maize had latent infection of tubers at 7%, indicating that this rotation gave the lowest bacterial wilt incidence, best quality and a higher quantity of potato tubers (Table 23). During the long rainy season (March to July 2006) the infested plots (control rotation plots: tomato-potato-potato) had 58.3% incidence of *R. solanacearum*.

Table 23. Infection of potato tubers obtained from one-season rotation experiment

Treatment*	No. of tubers incubated at 18-20° C	No. of tubers showing bacterial wilt symptoms							
		Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Total	% infection
TMP	100	3	0	0	2	1	0	7	7
TBP	100	4	2	0	1	1	0	9	9
TCP	100	0	4	1	2	1	2	10	10
TPP	100	5	3	2	5	1	2	21	21

*TBP=tomato-bean – potato, TMP=tomato-maize-potato, TCP=tomato–cabbage–potato, and TPP=tomato– potato–potato

There was significant difference ($P \leq 0.05$) in total yields between yields obtained from clean plots and those obtained from infested plots. The total yield from clean plot was 24.8t/ha, while that from infested plots was 12t/ha. The seed yields were significantly different ($P \leq 0.05$) between treatments. The clean plots yielded 8.9t/ha, while the infested plot yielded 4.2t/ha (Table 24).

The marketable yields showed significant difference ($P \leq 0.05$) between the treatments. The clean plot produced 18.6t/ha of marketable tubers while the infested plots produced 6.4t/ha which was a significantly lower yield. The percent marketable yield of total for the clean plot was 75%, while that of infested plot was 53% and the percent increase in marketable yield for the clean plot was 191% for the clean plot (Table 24).

Table 24. Comparison of yields between un-infested plot and plots infested with *R. solanacearum* (March 2006 to July 2006)

Total yield (t/ha) *						% Marketable yield	% Increase in marketable yield
Plots	**Ware**	**Seed**	**Chatt**	**Infected**	**Marketable**		
Clean	9.7a	8.9[a]	5.2[a]	1.0[a]	18.6[a]	75	191
Infested	2.2[b]	4.2[b]	1.0[b]	4.6[b]	6.4[b]	53	
SE	1.867	0.579	0.776	0.811	1.547		

***** Means followed by same superscript letters in columns are not significantly different at 5% probability level according to Student-Newman-Keuls (S-N-K) test.

4.3.3 Two-seasons rotation in glasshouse

There were significant differences ($P \leq 0.01$) among the treatments in the incidence of bacterial wilt. The control treatment (potato-potato-potato-potato) had a significantly ($P \leq 0.01$) higher incidence of bacterial wilt of 33.4% than all the other treatments (Table 25). The potato-beans-beans-potato treatment was neither significantly different from the other treatments nor was it different from the control. The treatment recorded 20% incidence of bacterial wilt down from 25% at the beginning of the experiment (Table 25).

There was no significant difference ($P>0.05$) on the time to onset of bacterial wilt symptoms among the treatments. The mean time to the onset of bacterial wilt was 48.7 days. However, in the control treatment the onset of bacterial wilt was 43 days after planting. The plots which had Rhodes grass growing for two seasons before planting potato in the final experiment, took 45 days before the onset of bacterial wilt. These plots had also the lowest incidence (2.2%) of bacterial wilt (Table 25).

Table 25. Effect of two season rotation on time to onset of bacterial wilt symptoms and bacterial wilt incidence under glasshouse conditions.

Treatment	Days to onset of wilt symptoms*	Incidence (%)*
Potato-Maize-Beans-Potato	50[a]	8.9[b]
Potato-Cabbage-Maize-Potato	48[a]	6.7[b]
Potato-Beans-Cabbage-Potato	53[a]	11.1[b]
Potato-Maize-Maize-Potato	52[a]	4.5[b]
Potato-Beans-Beans-Potato	50[a]	20.0[ab]
Potato-Potato-Potato-Potato	43[a]	33.4[a]
Potato-Grass-Grass-Potato	45[a]	2.2[c]
SE		4.497

***** Means followed by same superscript letters in columns are not significantly different at 5% probability level according to Student-Newman-Keuls (S-N-K) test.

4.3.4 Two-season rotation in the field

There was a significant difference ($P \leq 0.001$) in the incidence of bacterial wilt among treatments. The control treatment (tomato-potato-potato-potato) was significantly different from the others. The rest of the treatments showed no significant difference ($P > 0.05$) in incidence of bacterial wilt (Table 26, Figure 16 and Appendix IX ANOVA table (e).

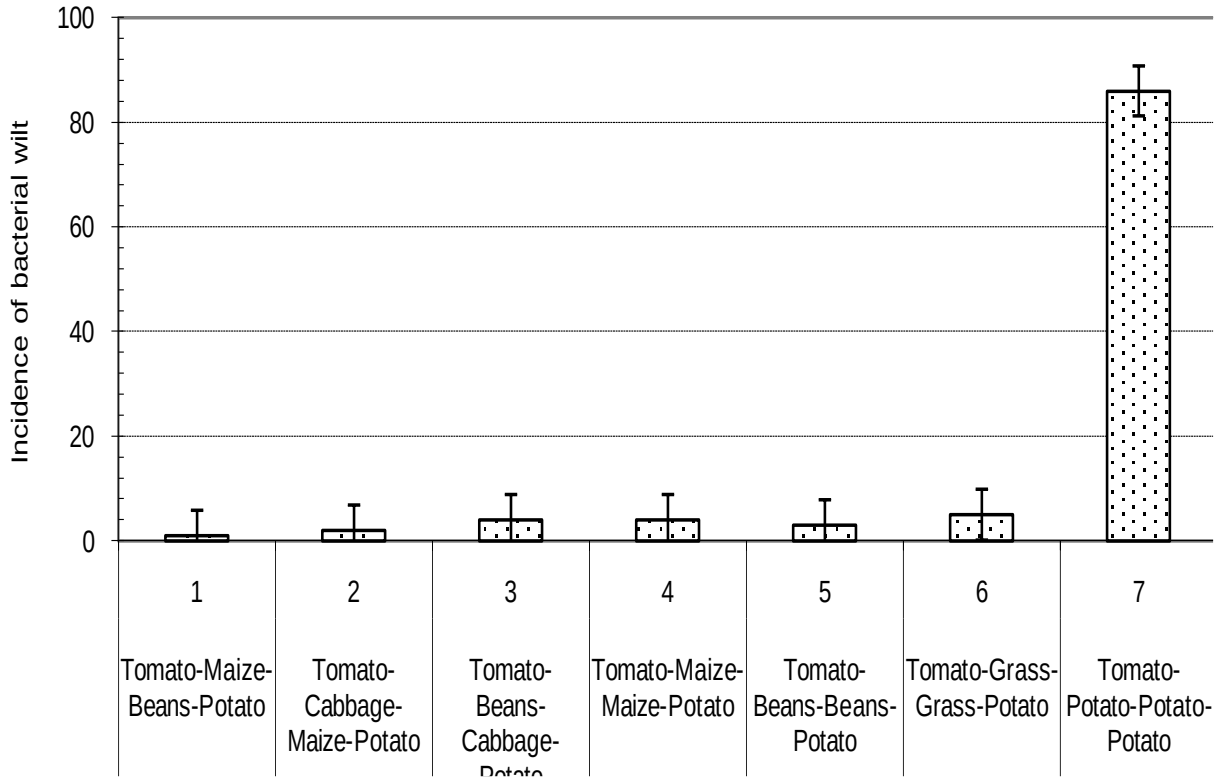


Figure 16. Effect of two-season rotation on field incidence of bacterial wilt

Apart from the control, all the other treatments showed marked reduction of incidence of bacterial wilt. Rotations in which beans were planted after maize reduced bacterial wilt incidence to 1% whilst rotations involving cabbage followed by maize reduced the disease incidence to 2% from the initial level of 46.7% on the tomato crop.

The ware potato yield was significantly different ($P \leq 0.01$) among the treatments. The tomato-beans-cabbage-potato treatment showed significant difference in ware yield. The treatment yielded 12.8t/ha compared to the rest of the treatments which were almost similar (Table 26). There was also significant difference ($P \leq 0.01$) in seed yield among treatments (Table 26). The control had a significantly lower seed yield than the other treatments. The total yield was significantly different ($P \leq 0.01$) among the treatments. The tomato-bean-cabbage-potato showed significant difference among the treatments, yielding 18.9t/ha compared to the rest of the treatments which yielded 10t/ha and below. The control gave the lowest yield which was 1.6t/ha (Table 26 and ANOVA table (f)).

There was no significant difference (P>0.05) in chatt yield among the treatments. However the control gave the lowest yield in chatts (0.6t/ha) compared to other treatments which yielded 1 to 1.9t/ha. The marketable yield showed significant difference (P≤0.01) among the treatments. Treatment with tomato-beans-cabbage-potato yielded the highest (18.0t/ha) marketable produce compared to all other treatments which yielded 8.4t/ha or less (Table 26).

Table 26. Effect of two-season rotation on bacterial wilt incidence and potato yields in a field at NARL, Kenya.

Treatment	incidence of bacterial wilt*	Yield (t/ha) *					% increase in marketable yield	% marketable yield
		Ware	Seed	Chatt	Total	Marketable		
TMBP	1 [b]	4.1[b]	3.4 [a]	1.9[a]	9.4 [b]	7.5[b]	650	80
TCMP	2 [b]	2.9[b]	3.3 [a]	1.2[a]	7.5 [b]	6.3[b]	530	84
TBCP	4 [b]	12.8[a]	5.2 [a]	1.0[a]	18.9[a]	18.0[a]	1700	95
TMMP	4[b]	3.5[b]	3.6[a]	1.4[a]	8.4 [b]	7.0[b]	600	83
TBBP	3 [b]	4.5 [b]	4.0 [a]	1.6[a]	10.0 [b]	8.4[b]	740	84
TGGP	5 [b]	3.7 [b]	2.7 [a]	1.3[a]	7.8 [b]	6.4[b]	540	82
TPPP	86 [a]	0.6 [b]	0.4[b]	0.6[a]	1.6 [b]	1.0[c]		63
SE	2.4	1.552	0.646	0.3	1.957	0.354		

***** Means followed by same superscript letter in columns are not significantly different at 5% probability level according to Student-Newman-Keuls (S-N-K) test.

Treatments
*TMBP=Tomato-Maize-Beans-Potato, TCMP=Tomato-Cabbage-Maize-Potato, TBCP=Tomato-Beans-Cabbage-Potato, TMMP=Tomato-Maize-Maize-Potato, TBBP=Tomato-Beans-Beans-Potato, TGGP=Tomato-Grass-Grass-Potato, TPPP=Tomato-Potato-Potato-Potato.

The control yielded the lowest (1.0t/ha) among the treatments (Table 26). The marketable yield increase over the control ranged from 530% to 1700%. The control treatment had the lowest proportion (63%) of marketable yields of the total (Table 27). This indicated that the quantity of potato for the control was lowest and also the quality of tubers produced was the poorest.

Bacterial wilt developed very fast in the control treatment from 9.2% on the 32nd day from the planting date to 81.3% on the 88th day from the date of planting (Figure 17). Wilt development in the rotation with grass rose from 0.3% on the 39th day to 5.1% on the 88th day from the date of planting. Tomato-maize-maize-potato, tomato-beans-cabbage-potato and

tomato-beans-beans-potato gave almost similar curves of wilt development. Rotations with tomato-cabbage-maize-potato and tomato-maize-beans-potato gave the lowest percentage (1 and 2% respectively) of wilt development (Figure 17).

Bacterial wilt significantly ($R^2 = 0.2979$) reduced the yield of marketable potato tubers (Figure 18).

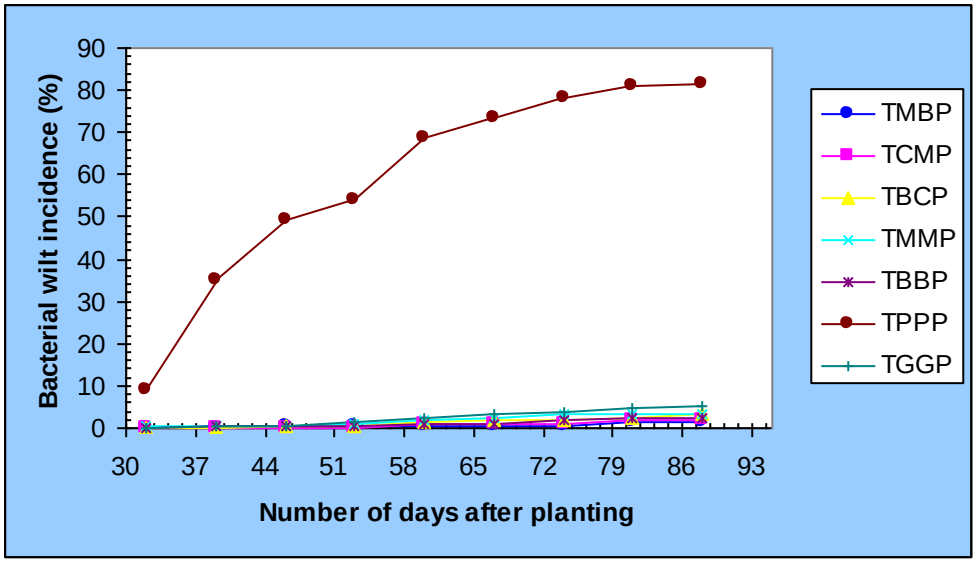


Figure 17. Effect of two season rotation on bacterial wilt development. TMBP=Tomato-Maize-Beans-Potato, TCMP=Tomato-Cabbage-Maize-potato, TBCP=Tomato-Beans-Cabbage-Potato, TMMP=Tomato-Maize-Maize-Potato, TBBP=Tomato-Beans-Beans-Potato, TPPP-Tomato-Potato-Potato-Potato, TGGP=Tomato-Grass-Grass-Potato.

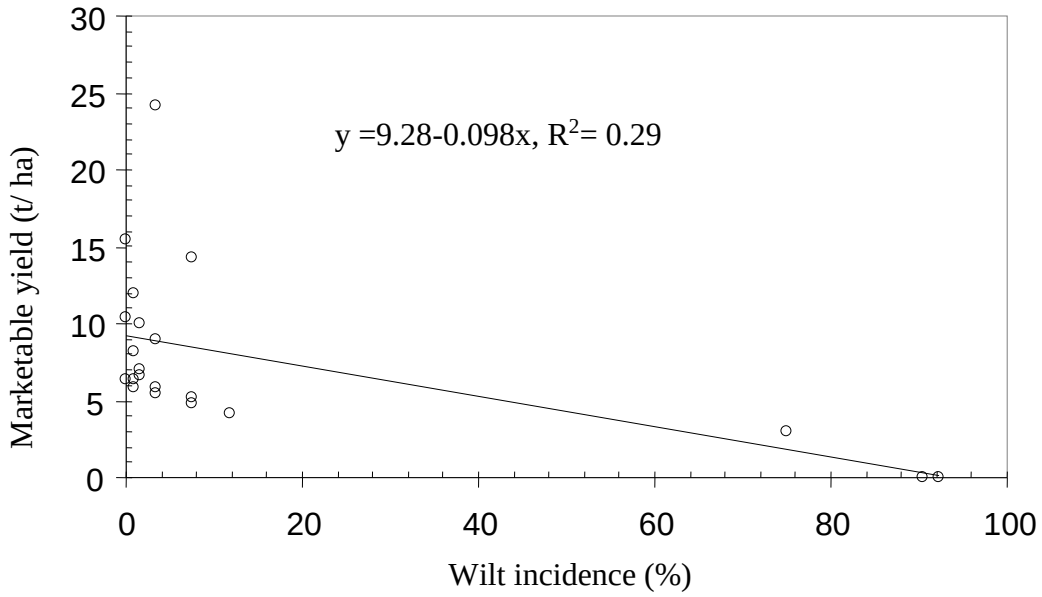


Figure 18. Relationship between marketable yield and bacterial wilt incidence in two-season rotation in the field.

In the two season rotations, the non-rotational treatment gave the highest percentage (27%) of latently infected tubers, while the treatment with grass and maize had the lowest percentages (2%) of latently infected tubers. Three percent (3%) of tubers obtained from rotation with maize followed with beans were latently infected with *R. solanacearum*. Rotations with cabbage followed with maize and that of beans followed with cabbage each had 5% of the tubers latently infected. Rotation with beans followed with beans had 7% of the tubers latently infected with *R. solanacearum* (Table 27).

During the short rainy season (October 2006 to February 2007) the potato crop in the clean plot did not show any infection by *R. solanacearum*. The control plots had 72% incidence of bacterial wilt. The wilt incidence during this season compared with the previous season was higher by 14%. This indicated that the population of *R. solanacearum* in the infested plots had increased.

Table 27. Infection of potato tubers obtained from two-season rotation experiment.

Treatment	No. of tubers incubated at 18-20°C	Number of tubers showing bacterial wilt symptoms							
		Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Total	% Infectio
TMBP	100	0	1	0	2	0	0	3	3
TCMP	100	1	0	0	1	2	0	5	5
TBCP	100	1	1	2	0	0	1	5	5
TMMP	100	0	0	1	0	1	0	2	2
TBBP	100	0	0	3	1	2	0	7	7
TGGP	100	1	1	0	0	0	0	2	2
TPPP	100	6	2	5	1	1	3	27	27

Treatments
TMBP=Tomato-Maize-Beans-Potato, TCMP=Tomato-Cabbage-Maize-Potato, TBCP=Tomato-Beans-Cabbage-Potato, TMMP=Tomato-Maize-Maize-Potato, TBBP=Tomato-Beans-Beans-Potato, TGGP=Tomato-Grass-Grass-Potato, TPPP=Tomato-Potato-Potato-Potato.

There was significant difference ($P \leq 0.05$) in marketable yields between those obtained from clean plots and those obtained from infested plots. The clean plot yielded 30.0t/ha compared to 2.5t/ha yields from infested plot. The seed yield showed marked difference between the two treatments. The clean plot yielded 24.5t/ha while the infected plot yielded 2.1t/ha. The increase in marketable yield of total for clean plot was 90%. The percent marketable yield of total for the clean plot was 90% while that of the infected plot was 56% and the percent increase in marketable yield for clean plot was 1100%. The results show that the clean plot yielded higher quantity and better quality of tubers compared to the infested plots (Table 28).

Table 28. Comparison of yields between uninfested plot and plots infested with *Ralstonia solanacearum* (October 2006 to February 2007).

Treatment	Total yield (t/ha)*					%	% increase in
Plot	**Ware**	**Seed**	**Chatt**	**Infected**	**Marketable**	**Marketable yield**	**marketable yield**
Clean	5.5[a]	24.5a	1.8[a]	1.5[a]	30.0[a]	90	1100
Infested	0.4[b]	2.1[b]	0.4[b]	1.6[a]	2.5[b]	56	
SE	1.089	4.318	0.225	0.459	5.401		

***** Means followed by same superscript letters in columns are not significantly different at 5% probability level according to Student-Newman-Keuls (S-N-K) test.

CHAPTER FIVE

5.0 DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Discussion

Ralstonia solanacearum was found in all potato growing regions in Kenya where sampling was carried out. The regions covered agro-ecological zones extending from 1678m up to 2794m a.s.l., latitudes ranging from 000^{0}93.347'S to 001^{0}08.651'S and longitudes ranging from 034^{0}47.561'E to 037^{0}00.299'E. These are the regions where the bulk of Kenya's potato is produced. The presence of the disease in 58.7% of the farms visited indicates that the disease is wide-spread in the potato growing areas. The presence of *R. solanacearum* in upper highlands UH2, altitudes ranging from 2352m to 2794m a.s.l. show that the pathogen has spread to high altitude areas where clean potato seed is produced. Such areas are Nyandarua and Molo. This means that it is no longer safe to rely on seed produced in high the altitudes areas as clean. It is important to note that this can also be an impact of climate change.

The wilt disease symptoms as described by Kelman (1953) and Harrison (1960) were found to be satisfactory in the recognition of the diseased plants in the field. Thus it was confirmed that all the potatoes and tomatoes collected were infected when they were analysed in the Laboratory. However, only one out of the three (33.3%) suspected weeds was infected by *R. solanacearum*. This formed 33% of weed plants which were positive for *R. solanacearum*. It was easy to recognize infected potato and tomato plants in the field by using descriptive disease symptoms. However, it was not easy to use the same descriptions to recognize infected weeds in the field. It was observed that symptom expression in plants were better during hot and sunny days.

Many farmers who inter-cropped potato with other crops such as peas (*Pisum sativum*), beans (*Phaseolus vulgaris*) and planted potato in coffee plantations owned small holdings. The farmers with larger farms, however, cultivated potato in pure stands. The cropping pattern is important in crop rotation for the control of bacterial wilt of potato in our region as indicated in the investigations in this work. The inter-cropping of potato with other crops has variable effects. It is known that beans fix nitrogen and therefore nitrify the soil. The nitrification of soil is beneficial to crops. It is also known that beans are potential hosts of *R. solanacearum*. Beans

have also been reported to be symptomless carriers of the bacterium (Granada and Sequeira, 1983; Kloos *et al.,* 1991). Thus, the bean crop can cause quick increase of the inoculum and increase infection potential in an infested field. Maize has been found to be more effective in reducing wilt incidence due to the presence of *Pseudomonas cepacia,* which is antagonistic to *R. solanacearum* in the rhizospheres (Elphinstone and Aley, 1992). This can be a beneficial aspect in inter-cropping maize with other crops in bacterial wilt infested soil.

The isolates obtained from diseased plant materials collected during the long rainy season (March to July 2006) were identified as *R. solanacearum*. The characteristics observed largely conformed to published descriptions given by various authors such as Kelman (1954), Harrison (1960), Hayward (1960), Hayward (1964) and French and Sequeira (1970). In general isolates grew well on Semi-selective medium South Africa (SMSA), Kelman's tetrazolium chloride (TZC) and Casamino acid Peptone Glucose (CPG) media used for this study.

Tobacco leaf infiltration technique was useful in differentiation of the races of *R. solanacearum*. The reactions of the tobacco leaves resembled those described by Lozano and Sequeira (1970) for *R. solanacearum* race 1 and race 3 of Buddenhagen *et al.* (1962). In this study, only two races were identified. The races identified and therefore present in Kenya were race 1 and race 3.

The biochemical characterization revealed that there were only two biovars of *R. solanacearum* in Kenya. Although there are five (5) biovars of *R.solanacearum* worldwide, only two of them were found present in Kenya. These were biovar 2 and biovar 3. This is true according to the previous findings by earlier investigators (Robinson and Ramos, 1964; Nyangeri, 1982) and, therefore, this indicates that new introduction of the pathogen into the country has not occurred. The term "biochemical type'' is synonymous to the term "biovar'', which is the term mainly used in this study. In the earlier findings, potatoes were found infected by only *R. solanacearum* biovar 2, which has a narrow host range limited primarily to potatoes and, to a lesser extent, tomatoes (Robinson and Ramos, 1964; Nyangeri, 1982; Smith *et al*., 1995). Smith *et al.* (1995) reported that potato could be infected by *R. solanacearum* biovar 3. The current study revealed that *Ralstonia solanacearum* biovar 3 also infected potato, tomato as well as many weeds in Kenya. This is contrary to other findings that it only infects brinjals (*Solanum melongena*), pepper (*Capsicum annuum*), *Capsicum frutescens, Solanum indicum, Solanum nigrum* and many weed plants (Kelman, 1953; Pradhanang *et al.,* 2000a). The biovar 3

isolates were obtained from Murang'a region at altitudes between 1678m and 1743m a.s.l. These investigations revealed that *Ralstonia solanacearum* biovar 3 was found in the lower altitudes from where diseased plant materials were obtained. The biovar was absent from higher altitudes (1750m -2794m a.s.l.), whereas biovar 2 was spread in all altitudes from where the samples were collected. These findings indicate that environmental factors influence the distribution of *R. solanacearum* biovars in Kenya. Although biovar 3 was found localized in its distribution in Murang'a region, there is a possibility of the biovar spreading to other areas through potato tuber seed.

When the isolates obtained in this study were subjected to tests to differentiate the biovar 2 isolates, all the isolates were found to be metabolically less active Andean phenotype, biovar 2-A. Although biovar N2 or 2-T, which is metabolically more active in tropical lowlands had been reported to be present in the country by Smith *et al*. (1995), this biovar was not detected in this study. Biovar 2-A was also found in *Cleome monophylla,* a weed which was among the collected samples (sample number 28). This weed was collected from a spot where there was an infected potato plant, indicating that there could be cross infection of the weeds and potato plants by the same organism. This indicates that weeds can be infected by the pathogen and perpetuate wilt disease of potato even when potato crop is rotated with non-solanaceaous crops. This fact is not well known by many farmers in this region and therefore, there is need to educate them in this area. Field hygiene is therefore necessary if the wilt problem is to be managed.

The rep-PCR is capable of differentiating isolates of *Ralstonia solanacearum* due to banding patterns. The products of the rep-PCR with chromosomal DNA were found to generate very characteristic patterns when separated on agarose gels. Thus, rep-PCR may constitute a useful method of fingerprinting and identifying bacterial genomes (De Bruijn, 1992). The results from this study show that both ERIC and BOX-like sequences are present in the genomes of *Ralstonia solanacearum* and thus can be used as markers for molecular analysis.

The results showed that there are two biovars of *Ralstonia solanacearum* present in Kenya. These are *Ralstonia solanacearum* biovars 2 and 3. Both of these biovars were detected by the biochemical and molecular fingerprinting. Biovar 2 was isolated mainly from potatoes and biovar 3 was isolated from potato, tomato and some weeds. The biovar 2 strains were spread throughout all potato growing zones, while biovar 3 was found in lower altitudes of the potato growing areas.

The dendrograms generated by use of ERIC and BOX primers showed some genetic similarities and differences among the isolates. This can be attributed to differences in genetic make-up of the organisms. Indeed the banding patterns of the isolates showed differences among the isolates. The clustering of representative isolates strongly suggested close relationships among the isolates. The dendrogram generated using ERIC primer sets data showed that the isolates 12, 15, 16, 18 and 19 were related. Isolate 13 was also related to the above isolates although the relationship was a bit distant indicating diversity nature of the pathogen. This was true with the biochemical classification, which grouped these isolates as *Ralstonia solanacearum* biovar 3. ERIC primer sets also showed that all the other isolates were related, however, their relationship showed great diversity. This group comprised *R. solanacearum* biovar 2. The dendrogram generated by BOX primer set also grouped isolates 13, 15, 16 and 18 together. The biochemical classification grouped all the other isolates into *Ralstonia solanacearum* biovar 2. The molecular finger-printing and clustering using arithmetic average showed that some isolates were closer in their relationships than others. Isolates 28, 71 and 74 were obtained from weeds. In the dendrogram generated using ERIC primer sets, isolates 71 and 74 were grouped close to one another. The isolates were, however, distantly related to the rest of the isolates. The dendrogram showed that isolates with similar genetic make-up were grouped together. Thus, biovar 3 isolates were clustered together; similarly biovar 2 isolates were also clustered together. The clusters also indicate the distance between the groups and intra-specific groups. The groupings also reflect the genotypic variations within the species and their diversity.

All the isolates tested for pathogenicity were pathogenic on potato and tomato. Some isolates were more virulent than others and therefore it took the host a shorter period of time to exhibit disease symptoms. Most of the *R. solanacearum* biovar 2 isolates were pathogenic to the test plants. *R. solanacearum* biovar 3 isolates were less virulent and therefore it took the inoculated test plants a bit longer to exhibit disease symptoms. This is due to genetic variation of the pathogen. It is, therefore, important that in order to succeed in breeding for resistance for this pathogen, breeders must breed for resistance to specific biovar.

The extensive host range of this pathogen includes several hundred species in about 44 plant families. The plant species include many weed species (Kelman, 1953). Some of the hosts are susceptible, others are tolerant, while still others act as latent or asymptomatic carriers (Granada and Sequeira, 1983). The investigations in this work revealed that in Kenya, many

weed species found in potato production ecosystems are hosts of *R. solanacearum*. The families of weed species which were found to be hosts of *R. solanacearum* are Solanaceae, Asteraceae, Oxalidaceae, Amaranthaceae, Compositaea, Portulacaceae, Polygonaceae, Commelinaceae, Cruciferaceae, Capparidaceae and Lobiataceae. Research workers in other parts of the world have found the above families and many more to be hosts of this pathogen (Kelman, 1953; Tusiime *et al*., 1998; Pradhanang *et al*., 2000a). This is an unrealised and unknown fact to the Kenyan potato farmers.

Some plant species exhibited wilting symptoms and the plants eventually collapsed and died. It was observed that *Solanum nigrum*, *Solanum melongena* and *Lycopersicon esculentum* were highly susceptible to *R. solanacearum* and that both biovar 2 and biovar 3 were pathogenic to the plant species. *Cleome monophylla* was more susceptible to *R. solanacearum* biovar 2 than it was to *R. solanacearum* biovar 3. All the plant species above were, therefore, grouped under "susceptible hosts" group. It is important to note that most of the plants in this group were solanaceous plants. However, plants from other families (Oxalidaceae, Portulacaceae, and Capparatidaceae) also wilted and eventually collapsed and died. Infection of *Oxalis latifolia* and *Nicandra physaloides*, which also wilted, collapsed and died along-side the above plants, as found in these investigations, was the first report in Kenya. These findings indicate that the above weeds can harbour the pathogen and, therefore, the pathogen can survive for a long period of time in the soil in infested area. The implication of this on disease management is that without good sanitation and weeding, it is difficult to control the disease even with good crop rotation.

Some plant species exhibited other symptoms resembling those caused by *R. solanacearum*. The symptoms were yellowing of leaves, defoliation and stunting. The plant species which exhibited such symptoms were grouped under "tolerant" group. It is important to note that some individual plants in this group had wilted and therefore this group was not very distinct from the first group. The yellowing of the leaves is due to the breakdown of chlorophyll, resulting from decreased supply of water and other nutritional factors to the infected plants. Plant species in which there was defoliation included *Datura stramonium* and *Nicandra physaloides*; yellowing of leaves was found in *Oxalis latifolia, Amaranthus* spp., *Oxygonum sinuatum*, and *Bidens pilosa*. These plants were found to be infected with *R. solanacearum* since the pathogen was isolated from them. This is the first report in Kenya on infection by *R. solanacearum* of the

following weed plants: *Oxalis latifolia, Nicandra physaloides, Amaranthus* spp., *Oxygonum sinuatum* and *Bidens pilosa.*

There was a third group of plants which were asymptomatic, but when they were subjected to ELISA test, they were positive for *R. solanacearum* infection. This group included *Galinsoga parviflora, Commelina benghalensis, Erucastrum arabicum, Ageratum conyzoides, Oxygonum sinuatum and Rumex abyssinicus.* These plant species were placed in the "latently-infected group''. The latently infected plants did not exhibit disease symptoms, although they were infected. Infective agent was isolated from such plants although they were symptomless. The finding that the above plants were infected by *R. solanacearum* was the first report in Kenya. These findings indicate that latently infected plants can support and perpetuate the survival of *R. solanacearum,* which can cause bacterial wilt disease on susceptible plants. It is, therefore, important that such plants should be carefully removed from potato growing farms in order to control bacterial wilt.

The fourth group of plants which were screened in this investigation were those which did not exhibit wilt symptoms and when they were subjected to ELISA test they were negative for *R. solanacearum*. These plants were placed in a "non-hosts" group. These plant species included *Conyza bonariensis, Sonchus oleraceus, Asystasia schimperi, Malva verticillata, Eleusine indica, Euphorbia hirta, Vigna unguiculata, Pelargonium zonale* and *Musa* spp. Plants which are not hosts of an organism are said to be resistant to the disease caused by that organism.

Some of the ELISA-negative plants for *R. solanacearum* in this investigation have been reported elsewhere as hosts of *R. solanacearum*. These plants were *Vigna unguiculata* (Kelman, 1953); *Euphobia hirta* (Kelman, 1953); *Eleusine indica* (Pradhanang *et al*., 2000); *Pelargonium zonale* (Janse *et al*., 2005); and *Musa* spp., which is infected by *R. solanacearum* biovar 1 or race 2. In the case of *Musa* spp., the *R. solanacearum* race 2, which causes disease in banana, has not yet been detected in the country. The biovars 2 and 3 which were found present in the country did not infect the banana test plants in these investigations. The *Pelargonium zonale* plants, which were inoculated with *R. solanacearum* did not show any wilt symptoms and *R. solanacearum* was not recovered from them. However, this species has been reported in other countries as hosts of *R. solanacearum*. Cuttings of *P. zonale* which were imported to North America from Kenya were found to be infected by this pathogen (Janse *et al.,* 2005) indicating

that the pathogen infects this plant species. The experimental plants may have not been infected because they were resistant variety.

When the "bacterial isolates" that were obtained from wilting plants were inoculated into the tomato plants, they caused wilt symptoms on some tomato test plants. Other tomato plants showed stunting, yellowing of leaves and adventitious roots production. When the stems and roots of such tomato plants were subjected to ELISA test, some of them tested positive for *R. solanacearum*. This observation indicated that the plants were latently infected and the strains that caused bacterial wilt disease on weeds and other plant species could also cause disease on tomato, potato and any other susceptible plant.

Plants which were found wilting in the field were grouped as susceptible hosts. These plant species included *Cleome monophylla, Nicandra physaloides, Leonotis mullissina* and *Portulaca oleraceae*. Placed in the same group of "naturally infected hosts" were plant species which were naturally growing in the infested field, and from which *R. solanacearum* was isolated. However, there were plant species which did not exhibit wilting symptoms in the field. These plant species included *Datura stramonium, Tagetes minuta* and *Oxalis latifolia*. These plants were latently infected. Only one plant (*Leonotis mullissina*), which was found wilting in the field was found to be infected with *R. solanacearum*. This means that the rate of infection of this family would be low. The finding that *Leonotis nullissina* and *Tagetes minuta* were infected by *R. solanacearum* was the first report ever made in Kenya.

In the glasshouse more plant species were found to be latently infected by *R. solanacearum* although they did not exhibit symptoms. These were *Galinsoga parviflora, Amaranthus spp., Bidens pilosa* and *Ageratum conyzoides*. This is the first report of bacterial wilt infection in these plant species in Kenya. These results indicated that the role of weed hosts in the persistence of *R. solanacearum* in the environment may have been underestimated. The investigations revealed that *R. solanacearum* infects and multiplies in the weeds studied above. However, many of the tolerant and latently infected weeds did not have bacteria oozing from stem sections when they were suspended in water. This supports the observations by Granada and Sequeira (1983) that populations of bacteria released from latently infected weeds are not likely to be as high as from wilted potato or tomato plants and therefore the bacterial ooze is not easily detected.

It is generally considered that *R. solanacearum* does not survive for long periods in vegetation-free soil (Granada and Sequeira, 1983). *R. solanacearum* biovar 2 is thought to survive for less time than other biovars in the absence of potato due to its narrow host range. In artificially inoculated soils, biovar 2 survived for only 8-11 weeks (Graham *et al*., 1979; Granada and Sequeira, 1983). However, under natural conditions the pathogen has reportedly survived in the absence of potato for long periods (Pradhanang *et al.,* 2000a). The persistence of the pathogen in soil is attributed to its survival in roots of weeds. It is, therefore, important for farmers to weed their farms well as a means of controlling the disease.

This first in-depth study has shown that *Ralstonia solanacearum* has a very wide weed host range in Kenya. Some of these weeds exhibited the wilting symptoms, others were tolerant and still others were latently infected. This kind of finding has been reported by other investigators who worked in other regions (Tusiime *et al.,* 1996; Tusiime *et al*., 1998; Pradhanang *et al.,* 2000a).

In this study, it was revealed that in the absence of a potato crop the organism can survive in weed hosts from where it could then infect the next crop of potato. The finding also explains why bacterial wilt of potato occurs in fields, which have not been under solanaceous crop cultivation previously.

Crop rotation reduced bacterial wilt incidence. Thus maize reduced bacterial wilt from the baseline wilt incidence at the beginning of one-season glasshouse experiment from 25% to 20% by the end of the experiment. The wilt reduction was, therefore, 20%. The reductions by beans and cabbage rotations were from 25% to 22.2% and 23.7%, respectively. The wilt reduction was, therefore, 11.2% and 5.2%, respectively. In the field the same treatments resulted in rotation with maize reducing wilt from 47.0% to 5.0% while bean and cabbage rotations reduced wilt to 6% and 7%, respectively. The wilt reduction was, therefore, 89.4%, 87.2% and 85.1% for maize, beans and cabbage, respectively. However, there was an increase of bacterial wilt incidence from the baseline bacterial wilt incidence of 46.7% to 73%, a percent increase of 56.3% in the control treatment. The results show that maize reduced bacterial wilt better than beans and cabbage. However, both treatments reduced wilt significantly. In a one season rotation, it is better to use maize as a rotational crop. Research has shown that negative effect of rotation crops on bacterial wilt is due to the decrease in virulent *R. solanacearum* population

(Kloos *et al*., 1991), and presence of antagonistic bacteria in their rhizosphere for maize (Elphinstone and Aley, 1992).

The two-season rotation treatments in the field reduced bacterial wilt from the baseline incidence of 46.7% by the 97.8% in tomato-maize-bean-potato treatment, 95.7% in tomato-cabbage-maize-potato treatment, 91.4% in tomato-bean-cabbage-potato treatment, 91.4% in tomato-maize-maize-potato treatment, 93.6% in tomato-bean-bean-potato treatment and 89.3% in tomato-grass-grass-potato treatment. Bacterial wilt incidence in the control treatment (tomato-potato-potato-potato) increased from the baseline incidence at 46.7% to 86%, a percent increase of 84.2%. This confirms research reports which indicate that crop rotation helps to significantly reduce or even eradicate potato bacterial wilt and that it is therefore recommended as a component of integrated management strategies (Kloos *et al.,* 1991).

Potato yields improved with the reduction of incidence of bacterial wilt. The control treatment (potato-potato-potato and tomato-potato-potato-potato) resulted in low total yields (3.3t/ha and 1.63t/ha for one season and two-seasons rotations respectively) as compared to other treatments. This is so because in higher incidences of bacterial wilt many tubers are infected and rot causing low yields.This finding agrees with the findings of Verma and Shekhawat (1991). It has been reported by other workers that the negative effects of rotation crops on bacterial wilt should be associated with decrease in virulent *R. solanacearum* population (Kloos *et al.*, 1991), and presence of antagonistic bacteria in their rhizospheres for maize (Elphinstone and Aley, 1992). They reported that the presence of *Pseudomonas cepacia* which is antagonistic to *R. solanacearum* in the rhizosphere of maize could be responsible for the reduced bacterial wilt in a potato-maize rotation.

Good results in bacterial wilt reduction were obtained in one-season rotation with maize in the field, which reduced wilt incidence from a baseline of 46.7% to 5%. Additionally, the two seasons rotation with maize followed by beans or cabbage gave the lowest bacterial wilt incidence. These treatments reduced wilt incidence from a baseline of 46.7% to 1.0% and 2.0%, respectively. It could not be explained why treatment with Rhodes grass for two seasons in the field resulted in a 5.1% bacterial wilt incidence which was higher than other treatments, while two-season rotation in the glasshouse, the treatment (potato-grass-grass-potato), resulted in the lowest percentage (2.2%) of bacterial wilt incidence. Probably this could be because the

pathogen survived and multiplied in the rhizosphere of the roots of the grass and other weeds in the field. Weeds in the glasshouse were well controlled than in the field.

It was found that all the two-season rotations reduced bacterial wilt incidence but planting two different crops was superior to planting the same crop in two consecutive seasons in a rotation. This is in agreement with the findings by Lemaga *et al*. (2001). The high percentage of wilt incidence in the control treatment was attributed to increase in pathogen populations in the plots. In rotations with beans there was elevated incidence of bacterial wilt and this was attributed to the fact that beans are potential hosts of *R. solanacearum* (Granada and Sequeira, 1983). Therefore, the bacterial population may have multiplied in the beans as a host plant and thereafter infected the soil and increased infection percentage.

The percentage of latent infection of potato tubers was higher in the control plots in both one-season and two-season rotations. In latently infected plants the bacterial population may be low and therefore the host plants fail to exhibit disease symptoms. The plants are therefore latent carriers of *Ralstonia solanacearum.* It was possible to show that the tubers were infected by incubating them and examining them regularly to see whether they were oozing bacteria from the "eyes". Cutting the tubers on the stolon end revealed oozing from the xylem vessels. Nyangeri *et al*. (1984) found that latently infected tubers, when used as seed, regenerated and produced heavily infected plants and hence spread the pathogen into previously uninfested soil. Such tubers were therefore a means of spread for bacterial wilt of potatoes in Kenya.

During the long rainy season (March to July 2006) the marketable yield of potato from clean plot was lower (18.7t/ha) than the yield during the short rainy season (October 2006 to February 2007) (30.0t/ha). Marketable yield can be defined as the produce that can be sold as ware and seed. The contaminated plots had lower yield (6.4t/ha during long rains and 2.5t/ha during short rains) and were significantly different from those of clean plots. Yields declined, in the contaminated plots, with the increase of wilt incidences. During all seasons the clean plots did not have any wilt incidence, while the contaminated plots had wilt incidence of 58.3% during the first season (March to July 2006) and 72% wilt incidences during the second season (October 2006 to February 2007).

5.2 Conclusions

From the findings of the present study, it can be concluded that the population structure of *R. solanacearum* in Kenya comprises of *R. solanacearum* biovar 2 and biovar 3. Biovar 2 is

found in low as well as high altitudes, while biovar 3 is found only in low altitudes. The biovar 3 infects potatoes in low altitudes only. Both biovars 2 and 3 infect potato (*Solanum tuberosum*), tomato (*Lycopersicon esculentum*) and many weeds such as *Cleome monophylla, Targetes minuta, Galinsoga parviflora, Oxalis latifolia, Amaranthus* spp*., Bidens pilosa, Datura stramonium, Portulaca oleraceae, Oxygonum sinuatum, Nicandra physaloides, Commelina benghalensis, Solanum nigrum, Ageratum conyzoides, Rumex* spp. etc in Kenya.

Isolates from weeds do infect potatoes and vice versa. However *R. solanacearum* biovar 3 infect weeds more than *R. solanacearum* biovar 2. There are intraspecific profiles of *R. solanacearum* biovars in Kenya as demonstrated in the molecular analysis. *R. solanacearum* pathogen has a great number of hosts including crops and weeds. The hosts are from diverse number of plant families e.g. Asteraceae, Oxalitaceae, Compositae, Solanaceae, Commelinaceae, Graminaceae, Leguminaceae, Cucurbitaceae Cruciferaceae, Capparatidaceae, Polygonaceae, Geranaceae, Portulacaceae etc. Weeds play a big role in the survival of *R. solanacearum*, thus providing sheltering sites in which the pathogen survives and by extension spreading bacterial wilt disease.

Kenya does not have a recommended crop rotation pattern for potato. This study has established a crop rotation pattern which can be used by the farmers in Kenya to control the bacterial wilt menace. The pattern is potato-maize-potato for one-season rotation and potato-maize-beans-potato for two-season rotations.

Good cultural practices, especially good hygiene, are important in production and control of bacterial wilt of potato. Crop rotation reduces or eradicates bacterial wilt disease and in so doing increases yield and quality of potato. However, practising crop rotation *per se* without good hygiene might not eradicate the disease due to the presence of weeds which can provide survival or sheltering sites for the bacterium. It is also concluded that planting two different crops is superior to planting the same crop in two subsequent seasons in a rotation. A cereal followed by a legume gives better reduction of bacterial wilt in a rotation.

5.3 Recommendations

In any breeding for bacterial wilt resistance, it is recommended that breeding for resistance for both race 1 biovar 3 and race 3 biovar 2 be considered. Both biovars infect potatoes and they are different in their genetic make-up and virulence.

Since many weeds are hosts of *Ralstonia solanacearum*, it is recommended that good field hygiene should be maintained in potato and all other crops cultivated to avoid the survival of bacteria in sheltered sites (e.g. weed hosts).

Farmers should be informed about the spread of potato bacterial wilt through weed hosts, latently infected potatoes and other host plants. This activity should be carried out by agricultural extension workers who should be encouraged to grasp the research findings so that they can pass the new knowledge to the farmers.

Deliberate effort should be made to produce clean planting materials for the farmers who knowingly or unknowingly continue to use infected potato seed. This should be done by contracting selected farmers to be paid a higher premium for their produce.

For effective and economically sound potato production, crop rotation is recommended as an important component of an integrated management strategy. In any crop rotation pattern, maize crop (cereal crop) should be included since it seems to control the wilt better. Cabbage and beans should be included in crop rotation pattern in order to improve on the yield and quality of potato.

It is recommended that further research should be carried out on weeds which were not covered in this study to find out whether they are hosts of *R. solanacearum.*

Further research on the effects of other rotational crops on bacterial wilt is recommended. The research should cover crops in the region which were not covered in the current study.

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APPENDICES

APPENDIX I. Media for the cultivation of *Ralstonia solanacearum*

(a) Semi-selective Medium, South Africa (SMSA), (French *et al*., 1995).

Bactopeptone (Difco)	10g
Glycerol	5ml
Casamino acids (Difco)	1g
Bacto agar (Difco)	15g
Distilled water	1000ml

Sterilize for 15 minutes at 121^{o}C.

Add to 250ml of melted medium at a temperature of 50^{o}C.

1% polymyxin B sulphate Sigma	2.5ml
(Final, conc., 100ppm)	
1% crystal violet	125µl
(Final concentration 5ppm)	
1% Tetrazolium salts (sigma)	1.25ml
(Final concentration, 50ppm)	
1% Bacitracin (Sigma)	625µl
(Final concentration 25ppm)	
1% penicillin (sigma)	125µl
(Final concentration 0.5ppm)	

When additional inhibition of fungal contaminants or soil inhabitants is desired add:

1% cycloheximide (sigma)	2.5ml
(Final concentration, 100ppm)	

(b) Casamino Peptone Glucose Agar (CPG)

Bacteriological peptone	10g
Glucose	10g
Casamino acid (Amicase)	1g
Oxoid Agar	18g
Distilled water	1L

For use dissolve part (b) by steaming and mix with an equal volume of part (a) (100mls of each part). Add 1ml of sterile part (c). Mix and pour plates.

(c) Kelman's Tetrazolium Chloride Agar (TZC), Jenkin and Kelman (1976)

(i)	Peptone	10g
	Glucose	10g
	Casein hydrolysate	1g
	Distilled water	0.5 litre
(ii)	Agar No.3	10g
	Distilled water	0.5 litre
(iii)	1% solution of Tetrazolium chloride:	

Dissolve all parts separately and dispense part (a) and (b) in 100ml aliquots in 250ml screw-capped bottles. Sterilize all parts at 1 atmosphere at 121 ^{o}C for 20 minutes and store at room temperature.

(d) Carbohydrate Media for Biovar (bv) Determination (Hayward, 1964; Hayward, 1976).

Basal medium

$NH_4H_2PO_4$	1g
KCl	0.2g
$MgSO_4.7H_2O$	0.2g
Peptone	1.0g
Bromothymol blue	10-20ml
Distilled water	1L

Adjust pH to 7.0 with 40% NaOH. Then add 3.0g of Agar. Dispense in 45ml aliquots in 50ml flasks plugged with cotton wool. Sterilize at 1 atmosphere for 20 minutes.

Carbohydrates used were cellobiose, lactose and maltose and hexose alcohols used were dulcitol, mannitol and sorbitol.

Prepare 10% solutions of the carbohydrates in 10ml amounts. Heating may be needed to dissolve these sugars. The hexose alcohols are relatively heatable and can be autoclaved at 110^{o}C for 20 minutes. The disaccharides are heat-labile and should be sterilized by filtration

(Seitz or 0.22 micron Millipore membrane) into pre-sterilized test tubes or small flasks or by tyndalization (steaming at 100 ^{o}C for 20 minutes on successive days).

To the melted basal medium cooled to about 60 ^{o}C , add the carbohydrate solution. Dispense 3-4ml into previously sterilized test tubes (150mm x 10mm size) using a sterile cotton stoppered pipette.

Biovar determination is based on the following:-

Bv1 = Utilization and oxidation tests negative

Bv2 = Utilizes disaccharides; does not oxidize the alcohols

Bv3 = Utilization and oxidation both positive

Bv4 = Utilization of disaccharides negative; oxidizes the hexose alcohols.

Bv5 = Utilizes disaccharides; oxidizes mannitol but not dulcitol or sorbitol.

APPENDIX II. Classification of *R. solanacearum* into biovars based on the ability to utilize disaccharides and oxidize hexose alcohols, producing acid when positive (+).

Physiological tests		Biovars				
		1	**2**	**3**	**4**	**5**
Utilization of disaccharides						
Cellulose		-	+	+	-	+
Lactose		-	+	+	-	+
Maltose		-	+	+	-	+
Oxidation of alcohols:	Dulcitol	-	-	+	+	-
	Mannitol	-	-	+	+	+
	Sorbitol	-	-	+	+	-

APPENDIX III. Definition of races of *Ralstonia solanacearum* by host range and biovars determination in each race.

Race	Natural hosts	Biovars
1	Many solanaceae, some diploid bananas, numerous other crops and weeds in many families	1, 3 or 4
2	Triploid bananas, certain *heliconias*	1 or 3
3	Potato, tomato, and rarely, a few other hosts	2
4.	Mulberry	5

APPENDIX IV. Differentiation of *Ralstonia solanacearum* biovar 2 isolates into the metabolically less active Andean phenotype bv 2-A, race 3 and metabolically more active tropical lowlands bv 2-T

Test	Bv 2-A	Bv 2-T
Acid from D (-) ribose	-	+
Acid from D (+) trehalose	-	+
Utilization of L (-) tryptophan	-	+
Utilization of L (+) tartrate	-	+

APPENDIX V. Race determination in *Ralstonia solanacearum* by tobacco leaf infiltrated with the pathogen

Treatment	Rase 1	Rase 2	Rase 3
Stem inoculation	● Wilting	No reaction	No reaction
Leaf inoculation	● Necrosis (48hrs) ● Wilting 7-8 days	Hypersensitivity Reaction 12-24hrs	Chlorosis 2-8 days

APPENDIX VI. Reagents for DNA analysis

1. CTAB extraction buffer[1]

Stock	Final	Reaction volume (10ml)	50ml
Double distilled water (ddH_20)	-	6.5ml	32.5
1M Tris – 7.5	100mM	1.0ml	5ml
5M Nacl	700mM	1.4ml	7ml
0.5M EDTA – 8.0	50ml	1.0ml	5ml
CTAB[2]	1%	0.1g	0.5g
14M BME[3]	140mM	0.1ml	0.5ml

[1] Use freshly made; warm buffer to 60 – 65ºC before adding the CTAB and BME.

[2] CTAB – mixed alkyltrimethyl – ammonium bromides (Sigma m – 7635)

[3] Add BME (B-mercaptoethanol) just prior to use, under a fume hood.)

2. Wash 1. 76% Ethyl alcohol, 0.2 M NaOAC

Stock (100ml)

Absolute ethanol	76ml
2M NaOAC	8ml
Distilled water	16ml

Dissolve sodium acetate in double distilled water. Sterilize by autoclaving.

3. Wash II. 76% Ethyl alcohol, 10mM NH_4OAC

Stock (100ml)

Absolute ethanol	76ml
1M NH_4OAC	1ml
Distilled water	23ml

4. Chloroform/Octanol mixture (24:1)

Stock (100ml)

Chloroform	96ml
Octanol	4ml

5. Tris HCL/EDTA buffer (TE buffer)

Stock (100ml)

1M Tris.HCl –pH 7.4	1ml
0.5 M EDTA 0.2ml	0.2ml
Sterile distilled water	

6. TAE buffer (Tris HCL, glacial acetic acid, EDTA)

Stock (50X)

Trizma base	242g
EDTA (0.5M)	57.1ml
Glacial acetic acid	100ml

Mix Tris with double distilled water by stirring, add EDTA and acetic acid then bring the final volume to 1 litre with double distilled water.

7. Ethidium bromide (10mg/ml)

8. Electrophoresis running dye

0.25% (w/v aq.) bromophenol blue

0.25% (w/v aq.) xylene cyanol FF

30% (v/v aq.) glycerol

9. Agarose (Seakem LE agarose)

APPENDIX VII. Actual rainfall (pentad), 66% probability (dekad), maximum and minimum temperatures 2005-2007.

MONTH	Pentad (5-day)	66% prob.	Rain in mm 2005	Rain in mm 2006	Rain in mm 2007	Max in ^{0}C 2005	Max Temp. in ^{0}C 2006	Max Temp in ^{0}C 2007	Min Temp in ^{0}C 2005	Min Temp. in^{0}C 2006	Min in ^{0}C 2007
JAN	1	0	0	2.1	26.7	26.8	24.8	22.5	19.1	17.2	18
	2	NA	0	0	1	27.5	28	24.4	19.1	17.6	18.8
	3	1	0	0	0	27.1	27.4	24	19.4	19.6	18
	4	NA	0	6	0	27.5	26	26.2	19.5	17.7	17.3
	5	0	0	0	0	28.9	26	26	20	18	18
	6	NA	102.5	0	0	26.9	27.3	26	19.1	14.6	15.3
FEB	7	0	2.5	0	32.8	26.5	25.3	27	19.2	18	18.8
	8	NA	0	0	0	27.1	27.6	28	19.5	19.2	18.4
	9	0	0	0	31.5	27.1	22.7	27.6	19.4	19.6	17.8
	10	NA	3.8	0	0	28.6	29.5	27	19.7	18.4	18
	11	0	0	0	0	31.1	24.2	28.8	17.8	21.4	20
	12	NA	0	12.3	23.6	30.3	21.4	27.7	21	17.5	18.2
MAR	13	16	31	73	0	28.4	26.4	29.3	21.6	17	12
	14	NA	0	84.2	1.9	27.2	26.5	29.3	18.5	19.3	11.5
	15	5	0	2.9	12.8	26.5	26.8	29	18.1	18.1	12.5
	16	NA	68.1	0.6	19	27.9	26.7	27.8	19.5	19.6	14.4
	17	12	35.3	12.4	2.9	25.9	26.8	25	20	19.1	14
	18	NA	0	8	13.9	27.5	27.3	24.4	20.1	18.8	14.5
APR	19	31	9.3	46.8	0	27	23.2	27.5	20.9	18	16.4
	20	NA	37.3	105.2	2.3	24.9	22.9	26.8	19.4	16.5	18.3
	21	52	30	4.5	60.8	24.8	25	25.5	17.9	18.3	15.8
	22	NA	3.3	68.8	121.1	25.3	24.2	26	19.7	18.4	16.4
	23	46	0	33.9	57.6	26	24	24	18	18.5	17.4
	24	NA	14	18.2	76.4	26.6	22.8	24.6	19	17.6	19.9
MAY	25	36	85.3	74.8	0	23.7	23	25.3	18.7	17.5	18

APPENDIX VII. Continued

MONTH	Pentad (5-day)	66% prob.	Rain in mm 2005	Rain in mm 2006	Rain in mm 2007	Max in ^{0}C 2005	Max Temp. in ^{0}C 2006	Max Temp in ^{0}C 2007	Min Temp in ^{0}C 2005	Min Temp. in^{0}C 2006	Min in ^{0}C 2007
	26	NA	63.1	65	0	22.5	23	24	18.4	17.1	15.8
	27	NA	4.6	29.7	11.7	24	24.4	24.5	17.7	17.5	16.8
	28	21	18.6	0	71.4	22	25.6	25.2	18.4	16.5	17
	29	NA	88.2	0	0	20	24.1	25	16.4	16.5	16
	30	22	73.3	6.3	0	21.5	24	24	17.4	16.1	17
JUN	31	NA	0	1.2	0	22.2	23.3	NR	16.6	16	NR
	32	8	1.6	3	0	20.1	23.4	27.2	16.2	17.2	18.2
	33	NA	0	0.7	18	21.8	24.2	28	15.5	16.6	16
	34	1	2.5	0	0	22.8	23.8	25.6	15	16.4	16.6
	35	NA	24.2	0	0	22.2	23.3	23.3	14.9	16.8	15.8
	36	2	0	0	0	20.7	21.9	25.6	14.8	12.8	15.6
JUL	37	NA	0	0	0	22.2	25.1	22.3	14.8	14.4	13.4
	38	0	3.7	0	0	20.1	19.1	20	13.7	14.3	14
	39	NA	0	0	0	21.8	19.9	19.6	13.1	12.8	12
	40	2	3.5	0.9	0	22.8	17.9	19.5	13.6	14.1	12.9
	41	NA	0	4.5	0	22.2	23.2	22.7	13.2	15.4	13.6
	42	0	7.8	1.5	10.2	20.7	19.9	24.3	13.8	13.7	16.7
AUG	43	NA	0	28	10.9	19.8	24.9	22.3	12.1	16.5	14.1
	44	0	0	0	3.5	21.6	22	20.2	13.5	15	12.1
	45	NA	2.7	0	2.7	20.8	23.5	22.3	13.5	15.5	13.2
	46	2	1.7	0	25.1	20.9	25.6	23.2	15.3	9.2	14.6
	47	NA	0	0	0	26.5	22.3	23.6	16.6	15	17.8
	48	1	7.6	46	0	22.7	22.3	24	14.5	13.7	14.2
SEP	49	NA	1.2	0	0	24.8	16.5	19.6	15.2	14.5	13.9
	50	4	24	0	22.1	24	NR	23.5	13.5	13.8	14.5
	51	NA	0.9	0	53.8	24.6	15.3	17.4	15.8	NR	12.8
	52	1	0	32.6	0	22.5	19.5	25.8	15	18.5	12.6
	53	NA	0	NR	0	26.2	20	24.5	17.4	19	14.8
	54	2	0	NR	0	27.1	NR	26.7	17.7	NR	13.3
OCT	55	NA	0	0	NR	26.5	28.8	26.7	17.6	18.5	NR
	56	0	0	0	NR	28.3	28.3	NR	17.8	17.4	NR
	57	NA	0	2.9	NR	27.3	25.3	NR	18.8	14	NR
	58	0	11.4		NR	25.1	NR	NR	17	NR	NR
	59	NA	15.4	35.4	NR	25.8	26.3	NR	16.8	19.6	NR
	60	19	10.2	0	NR	25.6	25.8	NR	19.1	17.2	NR
NOV	61	NA	27.5	5.1	NR	26.5	25.5	NR	19.1	15.6	NR

APPENDIX VII. Continued

MONTH	Pentad (5-day)	66% prob.	Rain in mm 2005	Rain in mm 2006	Rain in mm 2007	Max in ^{0}C 2005	Max Temp. in ^{0}C 2006	Max Temp in ^{0}C 2007	Min Temp in ^{0}C 2005	Min Temp. in^{0}C 2006	Min in ^{0}C 2007
	62	21	29.8	42.4	NR	24.9	23.1	NR	17.7	15.5	NR
	63	NA	12.6	140.4	NR	22.7	23.6	NR	16.8	15.3	NR
	64	20	0	38.1	NR	23.2	24.8	NR	18.9	16.5	NR
	65	NA	14.5	52.5	NR	23.4	23	NR	17.3	NR	NR
	66	27	5	33.5	NR	23.2	26	NR	17.2	NR	NR
DEC	67	NA	0	8	NR	24.9	25.6	NR	16.5	NR	NR
	68	21	1.2	4.1	NR	27.3	25.7	NR	18.6	NR	NR
	69	NA	0	0	NR	26.3	22.7	NR	18.6	NR	NR
	70	7	0	0	NR	25.6	NR	NR	17.8	NR	NR
	71	NA	0	23.8	NR	23.8	24.5	NR	16.3	NR	NR
	72	2	0	24.5	NR	22.2	23.7	NR	17.4	NR	NR

NR=Not recorded, NA=not available; (Courtesy: KARI – NARL)

APPENDIX VIII. Spectrophotometer readings of *R. Solanacearum* DNA at OD_{260} and OD_{280}, ratio of the readings and DNA concentrations of representative samples

Number	Representative sample	OD_{260}	OD_{280}	Ratio	Concentration in µg/ml
1	1	0.297	0.157	1.986	14.0
2	2	0.277	0.146	2.008	13.0
3	3	0.263	0.145	1.952	12.0
4	6	0.217	0.121	1.941	9.9
5	7	0.275	0.145	2.016	13.0
6	8	0.279	0.135	2.161	13.0
7	10	0.308	0.162	2.021	14.0
8	13	0.197	0.112	1.825	9.4
9	14	0.225	0.110	2.106	11.0
10	15	0.231	0.147	1.683	10.0
11	16	0.127	0.076	1.750	6.0

APPENDIX VIII. Continued

Number	Representative sample	OD260	OD280	Ratio	Concentration in µg/ml
12	17	0.221	0.103	2.204	11.0
13	19	0.313	0.182	1.845	14.0
14	21	0.559	0.346	1.732	25.0
15	22	0.082	0.390	2.132	4.1
16	24	0.181	0.930	2.024	8.6
17	25	0.106	0.063	1.878	4.6
18	27	0.297	0.163	1.964	14.0
19	28	0.389	0.179	2.193	19.0
20	C	0.003	0.004	0.001	0.1

APPENDIX IX. ANOVA Tables

(a). ANOVA table for % incidence of bacterial wilt –one season rotation

Source	df	Sum of squares	Mean square	F	Significance
Rep	2	12.080	6.040	0.284	0.763
trt	3	10020.687	3340.229	156.807	0.000***
Error	6	127.809	21.302		
Total	11	10160.576			

(b). ANOVA table for total yield – one season rotation

Source	df	Sum of squares	Mean square	F	Significance
Rep	2	12.33	6.166	0.109	0.899
trt	3	1133.74	377.914	6.657	0.025*
Error	6	340.64	56.773		
Total	11	1486.71			

(c). ANOVA table for marketable yield –one season rotation

Source	df	Sum of squares	Mean square	F	Significance
Rep	2	2.060	1.030	0.018	0.983
trt	3	1,000.590	333.530	5.728	0.034*
Error	6	349.380	58.230		
Total	11	1,352.030			

(d). ANOVA table for % marketable yield of total –one season rotation

Source	df	Sum of squares	Mean square	F	Significance
Rep	2	61.385	30.692	0.872	0.465
trt	3	154.763	51.588	1.465	0.315ns
Error	6	211.290	35.215		
Total	11	427.438			

(e). ANOVA table for % incidence of bacterial wilt –two season rotation

Source	df	Sum of squares	Mean square	F	Significance
Rep	2	131.552	65.776	3.805	0.053
trt	6	17,656.408	2,942.735	170.234	0.000***
Error	12	207.437	17.286		
Total	20	17,995.396			

(f). ANOVA table for total yield –two season rotation

Source	df	Sum of squares	Mean square	F	Significance
Rep	2	0.046	0.023	0.002	0.998
trt	6	476.139	79.357	6.908	0.002**
Error	12	137.861	11.488		
Total	20	614.046			

(g). ANOVA table for the analysis of weed and crop species artificially inoculated with *R. solanacearum* and wilted

Source	Df	Sum of Squares	Mean Square	F	Sig.
Weed_spp	8	33.528	4.191	7.178	0.000***
Biovars	7	18.431	2.633	4.51	0.000***
Error	56	32.694	0.584		
Total	71	84.653			

(h). ANOVA table for the analysis of weed species inoculated with *R. solanacearum* showing yellowing of leaves

Source	df	Sum of Squares	Mean Square	F	Sig.
Species	6	18.357	3.06	4.136	0.002**
Biovars	7	11.554	1.651	2.231	0.050*
Error	42	31.071	0.74		
Total	55	60.982			

(i). ANOVA table for the analysis of leaf defoliation

Source	df	Sum of Squares	Mean Square	F	Sig.
Species	1	10.563	10.563	18.778	0.003**
Biovars	7	8.938	1.277	2.27	0.151ns
Error	7	3.938	0.563		
Total	15	23.438			

(j). ANOVA table on ELISA analysis of plant species artificially inoculated with *R. solanacearum* biovar 2 and 3

Source	df	Sum of Squares	Mean Square	F	p-values
Weeds	25	55.231	2.209	3.74	0.001
Biovar	1	6.231	6.231	10.55	0.003
Error	25	14.769	0.591		
Corrected Total	51	76.231			

(k). ANOVA

Source	df	Sum of Squares	Mean Square	F	p-values
Weeds	25	55.231	2.209	3.74	0.001
Biovar	1	6.231	6.231	10.547	0.003
Error	25	14.769	0.591		
Corrected Total	51	76.231			

APPENDIX X (a and b). DAS- ELISA READER DATA, DAS and NCM- ELISA RESULTS

Biohit Plc

Assay:_Quick Read
Wavelength:405

Date:09/10/06
Time:15:27:17
Temp:

Lot: /
Operator:
Plate ID:01

COMMENTS

	1	2	3	4	5	6	7	8	9	10	11	12
A												
CALL												
CalcOD	0.218	0.269	0.194	0.155	0.260	0.283	0.171	0.162	0.170	0.148	0.155	0.591
Well	SMP1	SMP9	SMP17	SMP25	SMP33	SMP41	SMP49	SMP57	SMP65	SMP73	SMP81	SMP89
RSLT												
B												
CALL												
CalcOD	0.226	0.236	0.179	0.378	0.177	0.165	0.161	0.612	0.317	0.959	0.141	0.313
Well	SMP2	SMP10	SMP18	SMP26	SMP34	SMP42	SMP50	SMP58	SMP66	SMP74	SMP82	SMP90
RSLT												
C												
CALL												
CalcOD	0.185	0.392	0.146	0.159	0.310	0.167	0.137	0.467	0.108	0.379	0.126	0.203
Well	SMP3	SMP11	SMP19	SMP27	SMP35	SMP43	SMP51	SMP59	SMP67	SMP75	SMP83	SMP91
RSLT												
D												
CALL												
CalcOD	0.171	0.126	0.157	0.187	0.542	0.167	0.154	0.468	0.165	0.241	0.128	0.117
Well	SMP4	SMP12	SMP20	SMP28	SMP36	SMP44	SMP52	SMP60	SMP68	SMP76	SMP84	SMP92
RSLT												
E												
CALL												
CalcOD	0.138	0.159	0.383	0.194	0.456	0.117	0.107	0.146	0.173	0.302	0.102	0.105
Well	SMP5	SMP13	SMP21	SMP29	SMP37	SMP45	SMP53	SMP61	SMP69	SMP77	SMP85	SMP93
RSLT												
F												
CALL												
CalcOD	0.124	0.143	0.129	0.153	0.420	0.109	0.130	0.182	0.353	0.128	0.099	0.101
Well	SMP6	SMP14	SMP22	SMP30	SMP38	SMP46	SMP54	SMP62	SMP70	SMP78	SMP86	SMP94
RSLT												
G												
CALL												
CalcOD	0.122	0.132	0.116	0.104	0.711	0.118	0.190	0.251	0.131	0.125	0.101	0.112
Well	SMP7	SMP15	SMP23	SMP31	SMP39	SMP47	SMP55	SMP63	SMP71	SMP79	SMP87	SMP95
RSLT												
H												
CALL												
CalcOD	0.168	0.152	0.130	0.172	0.306	0.131	0.111	0.163	0.115	0.113	0.101	0.130
Well	SMP8	SMP16	SMP24	SMP32	SMP40	SMP48	SMP56	SMP64	SMP72	SMP80	SMP88	SMP96
RSLT												

APPENDIX X (b). DAS and NCM- ELISA

(i) NCM -ELISA

Test No	Isolate	Biovar	Plant spp	NCM-ELISA		Test No	Isolate	Biovar	Plant spp	NCM-ELISA
1	12	bv 3	*Galinsoga parviflora*	-		25	12	bv 3	*Amaranthus spp*	-
2	15	bv 3	"	-		26	15	bv 3	"	-
3	16	bv 3	"	-		27	16	bv 3	"	-
4	18	bv 3	"	-		28	18	bv 3	"	+
5	8	bv 2	"	+		29	8	bv 2	"	+
6	21	bv 2	"	-		30	21	bv 2	"	-
7	54	bv 2	"	-		31	54	bv 2	"	-
8	C			-		32	C+			+
9	12	bv 3	*O.latifolia*	-		33	12	bv 3	*T. minuta*	-
10	15	bv 3	"	+		34	15	bv 3	"	-
11	16	bv 3	"	+		35	16	bv 3	"	-
12	18	bv 3	"	-		36	18	bv 3	"	-
13	8	bv 2	"	+		37	8	bv 2	"	-
14	21	bv 2	"	-		38	21	bv 2	"	+
15	54	bv 2	"	-		39	54	bv 2	"	-
16	C+			+		40	C-			-
17	12	bv 3	*O. latifolia*	-		41	12	bv 3	*Bidens pilosa*	-
18	15	bv 3	"	-		42	15	bv 3	"	-
19	16	bv 3	"	-		43	16	bv 3	"	-
20	18	bv 3	"	-		44	18	bv 3	"	+
21	8	bv 2	"	-		45	8	bv 2	"	-
22	21	bv 2	"	-		46	21	bv 2	"	-
23	54	bv 2	"	-		47	54	bv 2	"	-
24	C+			+		48	C			-

NCM –ELISA. Continued

Test No	Isolate	/Biovar	Plant spp	NCM-ELISA		Test No	Isolate	/Biovar	Plant spp	NCM-ELISA
49	12	bv 3	*Conyza bonariensis*	-		73	12	bv 3	*Oxygonium sinuatum*	-
50	15	bv 3	"	-		74	15	bv 3	"	+
51	16	bv 3	"	-		75	16	bv 3	"	+
52	18	bv 3	"	-		76	18	bv 3	"	-
53	8	bv 2	"	-		77	8	bv 2	"	-
54	21	bv 2	"	-		78	21	bv 2	"	+
55	54	bv 2	"	-		79	54	bv 2	"	+
56	C			-		80	C+			+
57	12	bv 2	*Datura stramonium*	+		81	12	bv 3	*Nicandra physaloides*	+
58	15	bv 3	"	+		82	15	bv 3	"	-
59	16	bv 3	"	+		83	16	bv 3	"	+
60	18	bv 3	"	+		84	18	bv 3	"	-
61	8	bv 2	"	-		85	8	bv 2	"	-
62	21	bv 2	"	-		86	21	bv 2	"	+
63	54	bv 2	"	-		87	54	bv 2	"	-
64	C			-		88	C			-
65	12	bv 3	*Portuca oleraceae*	-		89	12	bv 3	*Commelina benghalensis*	-
66	15	bv 3	"	+		90	15	bv 3	"	-
67	16	bv 3	"	-		91	16	bv 3	"	-
68	18	bv 3	"	+		92	18	bv 3	"	-
69	8	bv 2	"	-		93	8	bv 2	"	-
70	21	bv 2	"	+		94	21	bv 2	"	-
71	54	bv 2	"	-		95	54	bv 2	"	-
72	C			-		96	C			
97	12	bv 3	*Ageratum conyzoides*	-		121	15	bv 3	"	-
98	15	bv 3	"	+		122	16	bv 3	"	-
99	16	bv 3	"	-		123	18	bv 3	"	-
100	18	bv 3	"	-		124	8	bv 2	"	-
101	8	bv 2	"	+		125	21	bv 2	"	-
102	4	bv 2	"	-		126			*Solanum tuberosum*	-
103	54	bv 2	"	-		127	Plot 22		"	-

NCM –ELISA. Continued

104	C			-		128			“	-
105	12	bv 3	*Ipomea purpurea*	-		129			“	-
106	15	bv 3	“	-		130	Plot 23		“	-
107	16	bv 3	“	-		131	“		“	-
108	18	bv 3	“	-		132	“		“	-
109	8	bv 2	“	-		133	“		“	-
110	21	bv 2	“	-		134	Plot 24		“	+
111	54	bv 2	“	-		135	“		“	-
112	C			-		136	“		“	-
113	12	bv 3	*Erucastrum arabicum*	+		137	“		“	-
114	15	bv 3	“	-		138	Plot 25		“	-
115	16	bv 3	“	-		139	“		“	-
116	18	bv 3	“	+		140	Plot		“	-
117	8	bv 2	“	-		141	“		“	-
118	21	bv 2	“	+		142	Plot 26		“	-
119	54	bv 2	“	+		143	“		“	-
120	12	bv 3	*Sonchus oleraceus*	-		144	“		“	-

NCM- ELISA. Continued

Test No	**Isolate**	**/Biovar**	**Plant spp**	**NCM-ELISA**		**Test No**	**Isolate**	**/Biovar**	**Plant spp**	**NCM-ELISA**
145	“		“	-		155	“		“	-
146	Plot 27		“	-		156	“		“	-
147	“		“	-		157	“		“	-
148	“		“	-		158	Plot 30		“	-
149	“		“	-		159	“		“	-
150	Plot 28		“	-		160	“		“	-
151	“		“	-		161	“		“	-
152	“		“	-		162	*Cleome monophylla*		“	+
153	“		“	-		163	“		“	+
154	Plot 29		“	-		164	“		“	+

NCM- ELISA. Continued

Test No/Plot	Isolate/ Biovar	Plant spp	NCM-ELISA		Test No/Plot	Isolate/ Biovar	Plant spp	NCM-ELISA
1	Potato	*Solanum tuberosum*	-		25	18 bv 3	"	-
2	"	"	+		26	8 bv 2	"	-
3	"	"	+		27	26 bv 2	"	+
4	"	"	+		28	58 bv 2	"	+
5	"	"	-		29	15 bv 2	"	-
6	"	"	-		30	16 bv 3	"	+
7	"	"	-		31	18 bv 3	"	-
8	"	"	-		32	12 bv 3	*Amaranthus spp.*	-
9	"	"	-		33	15 bv 3	"	-
10	"	"	+		34	16 bv 3	"	-
11	"	"	+		35	18 bv 3	"	-
12	"	"	-		36	8 bv 2	"	-
13	"	"	-		37	26 bv 2	"	+
14	"	"	-		38	58 bv 2	"	+
15	"	"	+		39	12 bv 3	"	+
16	"	"	+		40	15 bv 3	"	+
17	"	"	+		41	16 bv 3	"	-
18	"	"	+		42	18 bv 3	"	+
19	"	"	+		43	8 bv 2	"	-
20	"	"	-		44	26 bv 2	"	-
21	"	"	-		45	58 bv 2	"	+
22	12 bv 3	*Galinsoga parviflora*	+		46	12 bv 3	*Bidens pilosa*	-
23	15 bv 3	"	-		47	15 bv 2	"	-
24	16 bv 3	"	-		48	16 bv 3	"	-

NCM –ELISA. Continued

Plot	Isolate/ Biovar	Plant spp	NCM-ELISA		Plot	Isolate/ Biovar	Plant spp	NCM-ELISA
49	18 bv 3	“	-		68	18 bv 3	“	-
50	8 bv 2	“	-		69	26 bv 2	“	+
51	26 bv 2	“	-		70	58 bv 2	“	-
52	58 bv 2	“	-		71	12 bv 3	*Rumex abyssinicus*	+
53	12 bv 3	*Portulaca oleraceae*	-		72	15 bv 3	“	-
54	15 bv 3	“	-		73	16 bv 3	“	+
55	16 bv 3	“	+		74	18 bv 3	“	+
56	12 bv 3	*Oxygonum sinuatum*	+		75	58 bv 2	“	+
57	15 bv 3	“	+		76	12 bv 3	*Commelina benghalensis*	-
58	16 bv 3	“	+		77	15 bv 3	“	+
59	18 bv 3	“	+		78	18 bv 3	“	-
60	8 bv 3	“	+		79	26 bv 2	“	-
61	26 bv 2	“	-		80	12 bv 3	*Ageratum conyzoides*	+
62	58 bv 2	“	-		81	18 bv 3	“	+
63	12 bv 3	*Nicandra physaloides*	+		82	18 bv 3	*Erucastrum arabicum*	+
64	15 bv 3	“	+		83	12 bv 3	*Commelina benghalensis*	-
65	16 bv 3	“	-		84	15 bv 3	“	-
66	12 bv 3	*Leonotis mullissina*	-		85	18 bv 3	*S. nigrum*	+
67	15 bv 3	“	+					

(ii) DAS -ELISA

Test No	Isolate	/Biovar	Plant spp	DAS-ELISA		Test No	Isolate	/Biovar	Plant spp	DAS-ELISA
1	12	Bv.3	*Galinsoga parviflora*	-		25	12	Bv 3	*Amaranthus spp*	-
2	15	Bv 3	"	-		26	15	Bv 3	"	+
3	18	Bv 3	"	-		27	18	Bv 3	"	-
4	8	Bv 2	"	-		28	8	Bv 2	"	-
5	25	Bv 2	"	-		29	25	Bv 2	"	+
6	50	Bv 2	"	-		30	50	Bv 2	"	-
7	53	Bv 2	"	-		31	53	Bv 2	"	-
8	58	Bv 2	"	-		32	58	Bv 2	"	-
9	12	Bv 3	*Oxalis latifolia*	+		33	12	Bv 3	*Tagetes minuta*	+
10	15	Bv 3	"	+		34	15	Bv 2	"	-
11	8	Bv 3	"	+		35	18	Bv 3	"	-
12	8	Bv 2	"	-		36	8	Bv 2	"	+
13	25	Bv 2	"	-		37	25	Bv 2	"	+
14	50	Bv 2	"	-		38	50	Bv 2	"	+
15	53	Bv 2	"	-		39	53	Bv 2	"	+
16	58	Bv 2	"	-		40	58	Bv 2	"	+
17	12	Bv 3	"	-		41	12	Bv 3	*Bidens pilosa*	+
18	15	Bv 3	"	-		42	15	Bv 3	"	+
19	18	Bv 3	"	-		43	18	Bv 3	"	-
20	8	Bv 2	"	-		44	8	Bv 2	"	-
21	25	Bv 2	"	+		45	25	Bv 2	"	-
22	50	Bv 2	"	-		46	50	Bv 2	"	-
23	53	Bv 2	"	-		47	53	Bv 2	"	-
24	58	Bv 2	"	-		48	55	Bv 2	"	-

DAS ELISA. Continued

Plot	Isolate	/Biovar	Plant spp	DAS-ELISA		Plot	Isolate	/Biovar	Plant spp	DAS-ELISA
49	12	Bv 3	*Conyza bonariensis*	-		83	18	Bv.3	"	+
50	15	Bv 3	"	-		84	8	Bv2	"	+
51	18	Bv 3	"	-		85	25	Bv.2	"	+
52	8	Bv 2	"	-		86	50	Bv.2	"	-
53	25	Bv 2	"	-		87		Bv.2	"	+

							53			
54	50	Bv 2	"	-		88	58	Bv.2	"	+
55	53	Bv 2	"	-		89	12	Bv 3	*Eleucine indica*	-
56	58	Bv 2	"	-		90	15	Bv 3	"	-
57	12	Bv 3	*Portulaca oleraceae*	-		91	18	Bv 3	"	-
58	15	Bv 3	"	+		92	8	Bv 2	"	-
59	18	Bv 3	"	+		93	25	Bv 2	"	-
60	8	Bv 2	"	+		94	50	Bv 2	"	-
61	25	Bv 2	"	+		95	53	Bv 2	"	-
62	50	Bv 2	"	+		96	58	Bv 2	"	-
63	53	Bv 2	"	+		97	12	Bv 3	*Ageratum conyzoides*	-
64	58	Bv 2	"	+		98	15	Bv 3	"	-
65	17	Bv 3	*Nicandra physaloides*	-		99	18	Bv 3	"	-
66	15	Bv 3	"	+		100	8	Bv 3	"	+
67	18	Bv 3	"	-		101	25	Bv 2	"	+
68	8	Bv 2	"	-		102	52	Bv 2	"	+
69	25	Bv 2	"	+		103	53	Bv 2	"	-
70	30	Bv 2	"	+		104	58	Bv 2	"	-
71	53	Bv 2	"	-		105	12	Bv 3	*Cucurbita maxima*	+
72	55	Bv 2	"	-		106	15	Bv 3	"	+

DAS ELISA. Continued

Plot	**Isolate**	**/Biovar**	**Plant spp**	**DAS-ELISA**		**Plot**	**Isolate**	**/Biovar**	**Plant spp**	**DAS-ELISA**
73	12	Bv 3	*C.benghalensis*	-		107	18	Bv 3	"	+
74	15	Bv.3	"	+		108	8	Bv 2	"	+
75	18	Bv.3	"	+		109	25	Bv 2	"	-
76	8	Bv2	"	+		110	Blank			

77	25	Bv.2	"	+		111	53	Bv 2	"	+
78	50	Bv2	*M. verticillata.*	-		112	58	Bv 2	"	+
79	53	Bv2	"	-		113	12	Bv 3	*P.zonale*	-
80	58	Bv.2	"	-		114	15	Bv 3	"	-
81	12	Bv.3	*S. nigrum*	+		115	18	Bv 3	"	-
82	15	Bv.3	"	+						

DAS ELISA. Continued

Test No/Plot	**Weeds**	**Plant spp**	**DAS-ELISA**		**Test No.**	**Weeds**	**Plant spp**	**DAS-ELISA**
116	Pathogenicity test isolates	*G.parviflora*	+		130	S	*M. verticillata*	-
117	R	"	+		131	R	"	-
118	S	*O. latifolia*	+		132	S	*D.stramonium*	+
119	R		+		133	R	"	+
120	S	*Euphobia hirta*	-		134	S	*P.oleraceae*	+
121	R		-		135	R	"	+
122	S	*S. oleraceus*	-		136	S	*O. sinuatum*	+
123	R	"	?+		137	R	"	-
124	S	*Amaranthus spp*	+		138	S	*N. physaloides*	-
125	R	"	-		139	R	"	+
126	S	*T. minuta*	+		140	S	*S.nigrum*	+
127	R	"	-		141	R	"	+
128	S	*B. pilosa*	-		142	Tomato Control	*L. esculentum*	-
129	R	"	-		143	*D.stramonium*		+

APPENDIX XI. SOME OF THE WEED SPECIES INVESTIGATED

Nicandra physaloides

Commelina benghalensis

Conyza bonariensis

Bidens pilosa

Portulaca oleraceae

Amaranthus spp.

Tagetes minuta

Ageratum conyzoides

Datura stramonium

Galinsoga parviflora

Solanum nigrum

Oxalis latifolia