BACTERIAL WILT *(RALSTONIA (PSEUDOMONAS) SOLANACEARUM)* OF POTATO IN SOUTH AND CENTRAL ETHIOPIA: DISTRIBUTION, LATENCY AND PATHOGEN CHARACTERIZATION

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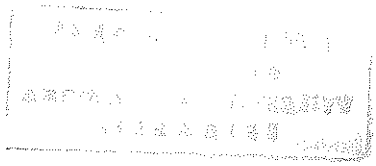

A THESIS SUBMITTED TO THE DEPARTMENT OF BIOLOGY, FACULTY OF SCIENCE, ADDIS ABABA UNIVERSITY AS PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE M.Sc. DEGREE IN BIOLOGY.

ADDIS ABABA UNIVERSITY

June, 1999

ADDIS ABABA, ETHIOPIA

ACKNOWLEDGEMENTS

I wish to express my deep appreciation and gratitude to Dr. Dereje Ashagari, Professor Endashaw Bekele, Dr. Fassil Assefa and Dr. Kifle Dagne, my advisors, for their genuine guidance, encouragement and invaluable criticisms.

My due acknowledgement goes also to the graduate school of the Addis Ababa University for financing the thesis work and to the staffs of biology department for their cooperation and encouragement.

I am also indebted to the Ethiopian Agricultural Research Organization (EARO) for giving me the opportunity to join program and for additional expenses covered. Sincere thanks are due to the Plant Protection Research Centre (PPRC), Ambo, and Ato Melaku Alemu, the centre manager, for arranging vehicle for field trips, provision of necessary materials and unreserved assistance throughout the study. The assistance of Ato Mohammed Dawud, the ex-centre manager of PPRC, in facilitating conditions at the centre during joining the program and his consequent cooperations were appreciable.

Sincere gratitude is also due to Dr. Bereketsehai Tuku and the staff members of Potato Improvement Program at Holleta Research Centre for their cooperation and provision of potato seed tubers used in the study.

I would like to express my deep gratitude to my colleague, Ato Takele Negewo, not only for his appreciable assistance during survey and sample collection, but also for his constant and unreserved cooperation throughout the study including typing the manuscript. I thank also Ato Chemeda Dilbo for his help in sample collection.

My deep appreciation goes also to my friends Ato Mesfin Bogale, Ato Taye Tessema, Ato Yohannes Lemma, Ato Adane Abraham Ato Solomon Nida and Ato Mulugeta Negeri for their encouragement and assistance during the study.

I also thank managers and experts of the agricultural bureaus of Shashemene, Jeldu, Ambo, Inchini districts and Bako Agricultural Research Centre for their cooperation during sampling and provision of important information and meteorological data.

Last, but not least, I would like to take this opportunity to sincerely thank the staff members of PPRC for their encouragement and cooperation during the study.

ABSTRACT

Survey of potato bacterial wilt caused by *Ralstonia solanacearum* was conducted in south and central zones of Ethiopia during 1996/97 cropping seasons and incidence, prevalence, latency and characteristics of strains were determined. Mean percentage wilt incidence of the disease was found to be relatively high in the irrigated areas of Bako (27.8%) and Ambo (18.2%) and in Shashemene district in both Meher (19.8%) and Belg (22.3%) produced potato crops. The percentage wilt incidence was low at Wondo Genet (7.2%), Jeldu (2.3%) and Inchini (1.3%). Mean percentage wilt prevalence of the disease was also high in Bako (87.5%), Shashemene (Belg, 75%; Meher, 70%), Ambo (70%) and Wondo Genet (62.5 %) and low in Jeldu (17.5%) and Inchini (7.5%).

Assessment of preceding crop on bacterial wilt development showed that successive potato cropping resulted in more disease development than preceded by non-host crop(s) and mean wilt incidences of, respectively, 35.3, and 19.1% were recorded.

Cultural, biochemical, carbohydrate utilization, hypersensitivity reaction and pathogenicity tests indicated that isolates belong to biovar 2 and race 3 of *R. solanacearum.* Relative virulence study showed that isolates from Bako and Ambo are more virulent while virulence of isolates from Jeldu and Inchini are least.

Mean percentage latent tuber infection was found to be relatively high in tubers harvested from infested crops at Jeldu (77.7%) and Shashemene (Meher 65.3%) followed by Wondo Genet (52.6%), Shashemene (Belg 53.7%), Ambo (51.2%) and Bako (45.7%), and least in tubers from Inchini (18.6%). In market bought tubers, high percentage infection was also recorded at Jeldu (53.5), Wondo Genet (50.0) and Shashemene (Meher ,48.5) and least was at Inchini (6.0).

On investigation of weed host plants as carriers of the pathogen, the pathogen was isolated from a common weed, *Galinsoga parviflora*. The bacterium was found pathogenic to potato and tomato., but not to *G. parviflora*. Hence, the weed is a latent carrier of *R. solanacearum*.

Generally, the disease is widespread and is a serious problem to potato production in the major growing areas of south and central zones of Ethiopia.

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1. INTRODUCTION

The history of mankind is tied up with agriculture which is the source of food for world's population and the backbone of most countries' economy.

It is paradoxical that, countries in which a high percentage of population is engaged in agriculture have the lowest agricultural out put; their people are living on a substandard diet and have the highest population growth rate. As a result of low technological development and scarcity of resources, millions of people in developing countries are undernourished. The major solutions often suggested are improvement of agricultural technologies and secure food self-sufficiency through increasing crop production and productivity as well as minimizing crop losses.

Plant diseases are among the major constraints to crop production that cause considerable crop losses contributing a lot to the world food deficit. Crop loss due to plant diseases is not limited to developing countries, rather, it is a global problem. For instance, in the United States alone, in 1978, out of the total crop losses estimated to 200 billion dollars, crop losses that amounted to 70 billion dollars were due to plant diseases (Agrios, 1978). This did not include losses caused by reduced quality of the harvested products and the cost of control measures applied to keep the losses at that level. Generally, it was estimated that in USA, each year, crops worth to 9.1, 7.7 and 6.2 billion dollars are lost, respectively, to diseases, insects and weeds (Agrios, 1988). Although, crop losses are a world wide problem, the condition is more painful to economically deficient countries than to developed ones. Such crop losses, however, can be minimized through investigation of the nature of the diseases and associated biotic and abiotic factors, and designing proper crop management strategy.

There are several types of diseases that affect cultivated plants. Bacteria are among the major causal agents of many plant diseases. However, in the early period of development of plant pathology, attention was not given to bacterial diseases. Even scientists who followed Leeuwenhoek's discovery of bacteria in 1676, did not suspect that bacteria could have something

to do with plant diseases. It was after the discovery of *Bacillus anthracis* by Louis Pasteur and Robert Koch in 1876 that, some plant pathologists started to suspect that bacteria might cause diseases in plants (Agrios, 1978).

Burrill was the first to relate a bacterium with plant disease. In 1880, he demonstrated that fire blight of pear and apple is also caused by a bacterium (Burrill, 1880 cited in Kiraly *et al.*, 1970). Soon after that, other workers have shown that, several other plant diseases are also caused by bacteria. Generally, about 200 species of bacteria are believed to be causative agents of many plant and animal diseases (Rangaswami and Rajagopalan, 1973).

Bacteria come only next to fungi as phytopathogens causing damage to economic crop plants. All plant pathogenic bacteria survive as parasites in the host plant and as saprophytes in the soil, and hence, are cultivable on artificial media. Almost all phytobacteria are rod-shaped with the exceptions of few species of *Streptomyces* which are filamentous (Agrios, 1978). Rod-shaped phytobacteria multiply with astonishing rapidity and their significance as pathogens stems primarily from the fact that they can produce a huge number of cells within a short period of time.

Ralstonia (Pseudomonas) solanacearum is one of the plant pathogenic bacteria that causes vascular wilt and tuber rot diseases. As reviewed by Kelman (1953), the bacterium is known by a variety of synonyms. In 1896, Smith identified the pathogen for the first time and named it *Bacillus solanacearum*. Later, in 1914, he revised and changed it to *P. solanacearum,* a name by which the pathogen has long been known. It is also known as *Burkholderia solanacearum.* Recently, it has been re-named *Ralstonia solanacearum*. The latter name has got acceptance among researchers and is now being used in publications and on scientific forums such as the International Bacterial Wilt Symposium recently held in France (Prior *et al.*, 1998). Hence, in this thesis, the name *R. solanacearum* is used instead of *P. solanacearum.*

R. solanacearum is a heterogenous species containing strains differing in distribution, biochemical, pathological and physiological characteristics. Based on these characteristics, the strains of the species are divided into subgroups, races (Buddenhagen and Kelman, 1964) and/or

biovars (Hayward, 1964).

The pathogen is dispersed mainly through planting materials. It can survive in soil and weed hosts and both can serve as sources of inoculum. The survival of strains in the soil and the activity in host plants , however, varies depending on biotic and abiotic factors. Temperature and moisture are the major abiotic factors that affect survival and action of *R. solanacearum* (Akiew, 1986).

There are numerous common names for the disease caused by *R. solanacearum* depending on the hosts of the pathogen and locality. They include brown rot of potato, Moko disease of banana (*Musa* spp), Granville wilt of tobacco (*Nicotiana tabacum*) (Kelman, 1953).

Bacterial wilt disease caused by *R. solanacearum* is a worldwide problem with a predominant distribution in the tropical, subtropical and warm temperate regions. It affects a wide range of plants as many as 200 plant species representing 35 families, but, primarily solanaceous plants among which potato is the major affected crop (French, 1986; Kelman, 1953).

Potato (*Solanum tuberosum* L.), along with rice, wheat and maize has become one of the four major crops of the world. It is an excellent food source; the tubers provide high energy and quality protein per unit of cultivated area and time as well as substantial amount of essential vitamins and minerals (CIP, 1984).

Crop productivity in general, and the production and productivity of potato in particular, is very low in the Third World. Hence, to satisfy the food demand of the increasing population, experts advise that in addition to increasing production of traditional crops (mostly cereals) technological change towards the use of other productive crops should also be promoted so that crop production exceeds population growth rate. Moreover, when famine occurs, widespread starvation can be averted by the introduction of high-yielding crop varieties. Potato is one of such crops that can serve the purpose.

The potential benefits of potato as a crop that can contribute to technological change come from its short growth period and high productivity per unit of time and area. Potato's adaptability is increased by the fact that planting materials can be multiplied in a variety of ways; it can be grown from true potato seeds, stem cuttings, and whole or cut seed tubers. As a result, today, potato production in the developing countries is expanding at an annual rate of 2.8% (CIP, 1995).

In Ethiopia, potato is one of the most widely used vegetable crop in the human diet. It is also an important cash crop for low income farmers. It grows more abundantly in the mid and highland parts of the country. Potato was first introduced to Ethiopia in 1858 by a German botanist called Schimper (Pankhurest, 1964). Then after, taking the advantage of its short growth period and especially due to the recurrent droughts that have been occurring in the country, the land under potato production has increased faster than any other food crops. In 1984, the area under potato production was estimated to exceed 50 thousand ha, but the yield per unit area is very low. The national yield of Ethiopia is estimated to be about 6 tons per ha (Berga Lemaga , 1986). This is extremely low as compared with the yields of other potato producing countries of the world, such as the Netherlands (40 tons/ha), German (28 tons/ha), Egypt (17.4 tons/ha) and Burundi (11 tons/ha) (Nganga, 1982). This is attributed to many factors among which diseases play the leading role.

Potato is susceptible to a number of diseases and pests. It is attacked by fungal diseases such as late and early blights, viral and nematode pathogens and insect pests. It is also attacked by several bacterial diseases; bacterial wilt (*R. solanacearum*), soft-rot of stem and tubers (*Erwinia carotovora*), common scab (*Streptomyces* spp.), brown ring (*Corynebacterium sepedonicum*),etc. Bacterial wilt caused by *R. solanacearum* is, however, the most destructive in the tropics and subtropics. It is the second most important disease of potato after late blight.

Although a comprehensive information on the economic importance of the disease is lacking, some fragmentary reports indicated that it causes severe damage in host crops throughout the world where the disease occurs. For instance, in India, in 1985, a loss of 70% was recorded on

potato (Sinha, 1986). In Burundi and Uganda losses of more than 60 and 80%, respectively, were reported on potato (Skoglund *et al.*, 1993).

In Ethiopia, bacterial wilt was first reported in 1956 by Stewart on potato and eggplant in Keffa region (Stewart, 1956). Later, in 1967 Stewart and Dagnachew Yirgou (1967) recorded the occurrence of the disease on potato, tomato and eggplant in Keffa, Shoa and Arsi regions. Then after, other workers also observed the disease mainly on potato and tomato in some central parts of the country (SPL, 1980; 1981;1986).

Sofar, no systematic loss assessment study has been done in Ethiopia. However, there is no doubt that the disease is economically important. According to a survey conducted in 1985 (SPL, 1986), wilt incidence of 0.8-21, 3.8-24 and 1.5-45% were recorded, respectively, at Tsedey, Bako and Ziway, on potato. Reports from farmers indicate that the disease is progressively spreading and causes severe damage and in some cases it is not uncommon to observe potato crops completely lost to bacterial wilt. Moreover, the progressive spread and interference with production of susceptible crops in infested fields indicate that the disease is highly important and needs due attention.

In Ethiopia, the problem of bacterial wilt is not well addressed. Research on bacterial wilt of potato has been started in the country recently and the progress is too little to solve the problem; mainly because, it lacks continuity. Sofar, some disease assessment works have been made in 1985 (SPL, 1986, Yaynu Hiskias, 1986) at some potato growing areas and prevalence of bacterial wilt was recorded on potato. Yaynu Hiskias (1989) analysed some isolates and reported that strains occurring in Ethiopia belonged to race 3 of biovar 2 of *R. solanacearum.* Some effort (Yaynu Hiskias, 1986) has also been made in screening potato cultivars for tolerance to the disease and some are in progress (PPRC, 1996). Apart from these, no systematic studies have been conducted on disease control and information on the occurrence and current status of the disease in major potato growing areas is not complete.

Today, production of potato is increasing greatly in the country, and along with this, due to the lack of proper recommended control measure coupled with the lack of disease-free seed supply systems and strict quarantine check in the country, it is obvious that the disease also spreads within and across localities into previously disease-free areas. Therefore, information on the existing status of the disease and nature of the strains of the bacterium occurring in the major growing areas is important.

Generally, disease survey is important for disease management and utilization of germplasm. Knowledge of disease distribution, incidence and characteristics of local strains is indispensable in designing appropriate and effective control strategies. Latently infected seed tubers are the frequent sources of infection and major disseminating agents of *R. solanacearum* in potato crops. Weed host plants are also reported to be among the inoculum sources of infection and make control measures such as crop rotation ineffective (Kishore *et al.*, 1995). Hence, information on latency in tubers and weed host plants is also crucial for proper management of the disease. Thus, this thesis work was initiated with the following objectives.

Objectives:

1) To asses incidence and prevalence of bacterial wilt caused by *R. solanacearum* on potato in major growing areas of south and central zones of Ethiopia.
2) To study biochemical, physiological and pathological characteristics of representative isolates.
3) To study latent infection in tubers.
4) To investigate some common weeds of potato crops and identify the possible carriers of the pathogen.

2. LITERATURE REVIEW

2.1. Taxonomy, Classification and Host range of *R. solanacearum*

In *Burgey's Manual of Determinative Bacteriology* (Kreig *et al.*,1986), the plant pathogenic bacteria were grouped under sections 4, 5 and 15 of Kingdom Prokaryote and represented by six genera namely: *Pseudomonas, Xanthomonas, Erwinia, Agrobacterium, Corynebacterium,* and *Streptomyces.*

About half of the plant pathogenic bacteria belong to the genus *Pseudomonas* (Dube, 1978). Pseudomonades are diverse in their characteristics. The majority of them produce a green diffusible fluorescent pigment on nutrient media, while others do not (Hayward, 1964). *R. solanacearum* was a member of those pseudomonades that do not produce the green fluorescent pigment. But, recent molecular investigations have brought nomenclatural change at generic level. Hence, it has been now re-classified in a separate genus, *Ralstonia.*

R. solanacearum produces massive extracellular polysaccharides on media containing utilizable carbohydrates (Hayward, 1964). It is a complex species containing strains differing in host range, distribution, physiological and biochemical characteristics. Using these differences, the strains in the species are characterized into subgroups.

The classification of plant pathogenic bacteria is still a debatable issue among bacteriologists. Accordingly, characterization of strains of *R. solanacearum* is not yet resolved. However, two principal approaches have gained acceptance by most researchers. These two approaches are race and biovar classifications suggested by Buddenhagen and Kelman (1964) and Hayward (1964), respectively.

The race classification is based on host range and geographical distribution of strains. Previously three races were identified. Race 1 is composed of strains that are widely distributed throughout the warmer zones of the world affecting a wide range of solanaceous and other plants, mainly potato, tomato, tobacco, eggplant, chilli, peanut, and several weeds. *R. solanacearum* race 2 consists strains primarily affecting musaceous hosts, such as plantains, bananas and *Heliconia* spp. It may also affect potatoes and occasionally tomato, but not other crops. Race 2 is indigenous to America and its native host is *Heliconia*. But the banana and plantains introduced from Asia and Africa provided the selection pressure for new strains to emerge from the indigenous population (French, 1986). Race 3 has a narrow host range being restricted to potato, tomato and a few weeds in nature. It primarily affects potato and hence, some times called the potato race. It occurs in cool climatic zones of the world. Later, two additional groups of strains have been reported, one from Philippines affecting ginger (Zehr, 1970a) and the other from China affecting mulberry (He *et al.*, 1983). The two groups were suggested to be called race 4 and 5, respectively, totalling the number of races to five.

The biovar classification is based on the physiological properties of the strains. Thus, the strains have been classified into five biovars according to their ability to oxidise three disaccharides, lactose, maltose and cellobiose; and three hexose alcohols, mannitol, sorbitol and dulcitol. Biovar 1 consists strains that oxidize neither group of carbohydrates. Strains that are capable of oxidizing the disaccharides but not the hexose alcohols are designated as biovar 2. Strains assigned in biovar 3 oxidize both carbohydrates. Strains capable of oxidizing the hexose alcohols but not the disaccharides are grouped as biovar 4. The strain from mulberry is found to be distinct in that it oxidizes the three disaccharides and mannitol, but not dulcitol and sorbitol and hence designated as biovar 5 (He *et al.*, 1983). Since the two classifications depend on different characters, a race may not fully correlate to a specific biovar except that race 3 is equivalent to biovar 2 (Buddenhagen, 1986). Even in this exception the reverse may not work because there are biovar 2 strains that are not race 3. Thus, researchers are compelled to find other better ways of classification of the species into subgroups.

For better understanding and characterization of *R. solanacearum,* the relationship of all strains of the biovars and races to each other and to other related species was studied and phylogenetic relationships were evaluated using restriction fragment length polymorphism (RFLP) (Cook and Sequeira, 1993) and polymerase chain reaction (PCR) (Li *et al.*, 1993). Similar results were obtained from both RFLP and PCR analysis involving, respectively, DNAs and 16 rRNAs. The RFLP pattern analysis revealed that strains of the species have common DNA fragments with two types of sequence orientations. Accordingly, all race 1 biovars 3,4, and 5 strains, designated as Division I, were very much alike with more than 78% sequence similarity. Race 1 biovar 1, and races 2 and 3 strains, designated as Division II, were also found to be alike with more than 62% similarity.

The similarity between the two divisions was, however, found to be 13.5%. Thus, it was suggested that the two groups may be potentially regarded as subspecies. The studies also showed that *R. solanacearum* is distantly related to other species and the closest relatives are *P. cepacia, P. pickettii* and *P. syzygii.* Thus, it was concluded that *R. solanacearum* strains are homogenous and distinct group that bears little relationship to other plant pathogenic pseudomonades.

2.2. Origin and Distribution of *R. solanacearum*

R. solanacearum is believed to be an ancient species whose origin is dated back to antiquity. According to Sequeira (1993), the worldwide distribution of *R. solanacearum,* its association with native plants growing in virgin soils and the large number of strains that exist in distinct geographical regions clearly indicate that it is an ancient species. There are, however, different views on its centre of origin. According to Buddenhagen (1986), the diversity of strains of the bacterium and wilt symptoms caused by the strains indicate that the origin of all the strains could not be the same. He suggested that the strains of the species have been originated from Latin America, Asia, and Australia.

Other workers, however, suggested that it has originated only once. Sequeira (1993) suggested that the strains form a distinct group that only remotely related to other pseudomonades and originated from a single location and evolve in different regions. More evidence come from molecular genetic analysis in support of this notion. The study (Cook and Sequeira, 1993) on DNA of various strains of *R. solanacearum* collected from different corners of the world comprising all known races and biovars showed that all the strains of the species share common DNA fragments that could be categorized only into two main groups or Divisions based on sequence similarity. The Divisions were corresponded to the geographic distribution of the strains and it was found that 90% of the strains in Division I were from Asia and Australia whereas, 98% of those in Division II were from America. Hence, it was concluded that *R. solanacearum* came from a common ancestor and early in evolution, it was divided into two groups which then evolved independently in geographic isolation giving rise to strains that are typical of the Old and New Worlds.

Bacterial wilt caused by *R. solanacearum* has been recognized as a major bacterial disease at least since the late nineteenth century. Kelman (1953) considered the earliest known record of bacterial wilt to be on tobacco in Indonesia in 1864, where entire fields were lost to bacterial wilt. It was also recorded on potatoes in the United States in 1890 (Kelman, 1953). Since these early reports, the disease has been discovered on a wide range of hosts in different ecological zones of the world (Persley, 1986).

Bacterial wilt is predominantly a disease of the tropical, subtropical and warm temperate regions. A few occurrences on potato in cooler temperate regions are known. The optimum temperature range for the pathogen is 25-35°C. The disease rarely occurs in areas where the mean monthly temperature is less than 10°C (Persley, 1986). In the few instances where bacterial wilt has been recorded in cooler climates, the disease has usually been associated with the introduction of infected planting materials such as potato tubers carrying latent infection of *R. solanacearum*.

R. solanacearum race 1 is commonly found in the lowlands of the tropics and warm temperate regions where it infests a wide range of solanaceous crops including potatoes and several weeds. The race is so variable that it has many pathogenic strains, each of which is pathogenic to several hosts including usually at least one solanaceous plant, the sum total of all hosts being estimated close to 200 species (French, 1986). Since these are present over a long growing season, they may contribute to the persistence of the race for a long period of time. In warmer climates, plant debris decompose more rapidly and infected debris would provide only temporary sheltered sites for *R. solanacearum* (Persley, 1986). Occurrence of race 1 in lowlands and warm temperate regions is, therefore, mainly due to its wide host range nature. Race 2 mainly occurs in Central and South America.

R. solanacearum race 3 is a low temperature adapted race which occurs principally in the temperate zones of subtropics and higher elevations of tropics. The distribution of race 3 in cool temperate areas is due to its close association with potato and its ability to survive under cool temperatures in the most suitable regions for potato cultivation. But in the past few years it has spread into more temperate regions and cooler highlands in the tropics. According to French (1986), this may have been the result of introduction of diseased seeds, with low temperature adaptation of the pathogen progressively taking place as selective pressure that resulted from progressive movement to cooler climates. Race 3 may have a limited distribution in warm temperate or tropical regions because of its restricted host range, its affinity to potatoes and the probable rapid decline of the pathogen population in warm moist soil where host debris are degraded rapidly. Thus, the occurrence of race 3 in tropical regions is also due to its introduction with potatoes rather than being a natural inhabitant of the environment (Persley, 1986). Generally, strains of *R. solanacearum* have worldwide distribution depending on host ranges and the ability to survive under different environmental conditions.

In Ethiopia, occurrence of the disease was first reported in 1956 (Stewart, 1956). Although information on the distribution of the disease in the country is not complete, the disease has been frequently observed in central regions of the country mostly around Ziway, Bako, Tsedey and

Ambo (SPL, 1980; 1981; 1986). The disease is progressively spreading into the previously disease-free areas with the introduction of infected potato seeds.

2.3. Source of Inoculum and Dissemination of *R. solanacearum*

Dissemination of *R. solanacearum* can be effected through various mechanisms. Infected planting material is one of the means of dispersal of the pathogen from place to place and from season to season, particularly in vegetative seed pieces of crops such as potato and ginger (Persley, 1986). Latent infections are common, especially in potato seed tubers and these are the major source of inoculum. Seed tubers produced in cool climates, such as tropical elevations, may not show any symptoms. But when planted in warmer locations, disease development may be severe. Hence, the introduction of infected seeds is the most common means of dispersal of the pathogen to previously disease-free areas (Martin and French, 1985).

True seed may be a more important source of inoculum and means for the long distance dispersal of *R. solanacearum* in vegetative crops such as tomato, capsicum, etc. Transplants such as tomato seedlings could also be responsible for long distance dispersal (Persley, 1986). The role of insects in the disease transmission is poorly understood except in race 2. It is well known that insects can transmit the disease among bananas and cause epidemics (Buddenhagen, 1986).

Localized dispersal of the pathogen by root-to-root spread was observed in tomato and tobacco (Kelman and Sequeira, 1965). The disease can also be disseminated by irrigation water. Mechanical transmission via contaminated farm implements can also be important (Buddenhagen and Kelman, 1964).

It is reported (Akiew, 1986) that, the pathogen can survive in soil where environmental conditions such as moisture are conducive. Hence, infested soil is one of the sources of inoculum. The pathogen may also overwinter in leftover plant debris which can serve as source of inoculum. Weed hosts harbour the bacterium and this may also be responsible to incite the

infection in the host plant. In general, the primary inoculum sources of bacterial wilt infection are infected plant materials and infested soil.

2.4. Survival of *R. solanacearum* in Soil

There are many reports on the soil-borne nature of the disease that differ widely regarding the longevity of the pathogen. According to Shamsudin *et al.*, (1978), race 3 could survive up to 2 years in soil under fallow conditions. Graham *et al.*, (1979) reported that race 3 survived in plant debris 33 weeks after harvest. In another study (Graham and Lloyd, 1979), it was found that race 3 survived better (82 days) in the deeper soil layers (55-65 cm) than in the topsoil (10-15 cm) (10 days). The finding led Graham and Lloyd (1979) to the conclusion that, in addition to survival in sheltered sites such as alternative weed hosts and infected debris, pockets of infestation in the deeper soil layers may serve as additional sites for the long-term survival of the bacterium.

Akiew (1986) studied the effects of temperature and soil moisture on survival of *R. solanacearum* and demonstrated that its population decreased sharply with increase in temperature and decrease in soil moisture. Hence, high soil moisture and low temperature appear to favour long-term survival of the bacterium in the soil, whereas, the effect of dry soil on bacterial viability appears to be a major factor for the absence of the bacterium or its failure to increase in hot dry areas. The same study also showed that, race 1 (biovar 2) strains survive longer and are more tolerant to desiccation than the strains of race 3 (biovar 2). Thus, the ability of both races to survive in soil devoid of host plant debris, but with high moisture and lower temperature, suggests that *R. solanacearum* can persist in deep soil layers where those conditions are likely to occur. Such findings, the persistence of the pathogen in the deeper soil layers, could explain the failure of soil disinfectants in the control of the disease (Persley, 1986). From these studies, it can be concluded that survival and longevity of *R. solanacearum* in the soil varies depending on the type of the strain and environmental factors.

2.5. Pathogenesis of *R. solanacearum* in the Host

Plant pathogens have developed various mechanisms to invade their hosts. One of the means of entry into the host is penetration of epidermal cells by specialized structures such as haustoria in fungi and stylet in nematodes. Bacteria lack these structures and hence are incapable of mechanical penetration into the cutinised plant tissues such as cuticle and periderm. Therefore, the sites of their entry into the plant are non-cutinised areas such as root hairs and stigmas, natural openings such as stomata, hydathodes, and lenticels and incidental wounds or those incited by insects, nematodes and other microorganisms (Rangaswami and Rajagopalan, 1973).

Infection of *R. solanacearum* is usually through the root system and the pathogen enters through wounds. Active invasion through non-cutinised surface, especially at the site of lateral root emergence, is also feasible by means of a large mass of cells functioning together. Kelman and Sequeira (1965) demonstrated that, the bacterium applied to healthy roots was able to incite the disease. Since emerging roots have pectinaceous sheath a pathogen has to have appropriate enzymes to digest it and get entry. It is believed that *R. solanacearum* might have the enzymes, because, several extracellular plant cell wall degrading enzymes including endoglucanase (cellulase) and polygalacturonase (Pectinase) which may degrade , respectively, cellulose and pectin were isolated from the bacterium (Allen *et al.*, 1993; Denny *et al.*, 1993). Besides, genes controlling pectinase synthesis were identified (Schell *et al.*, 1988). Hence, it was suggested that the pathogen can penetrate and enter through unwounded part of the roots using enzymes. However, high density of the bacterium is required to have more pectinase so that it can digest the cell wall effectively. Kelman and Sequeira (1965) reported that a population of at least 50, 000 bacterial cells /ml is necessary to cause infection in the absence of wound. The bacterium can get entry into the xylem tissue also by digesting the primary wall of the weakened cortical cells as well as tracheary elements using cellulase (Schell *et al.*, 1988).

Once *R. solanacearum* has entered the xylem vessel, it multiplies freely. To facilitate the micro environment in the xylem and to overcome conditions that can hamper its development, the

bacterium has developed certain mechanisms. One of such mechanisms involves avoidance of hypersensitive response induction. Hypersensitive response is basically one of the most important defence mechanisms in plants. It is induced in incompatible hosts. As the pathogen enters the uncongenial plant, the surrounding cells of the plant die and necrosis develop so that the pathogen is arrested there. Hypersensitivity response induction in *R. solanacearum* is controlled by *hrp* (hypersensitivity response pathogenicity) genes (Arlat *et al.*, 1993). The genes are required both for disease development and hypersensitivity response elicitation in plants. In the host plant, the genes do not cause hypersensitivity, rather involve pathogenesis. Moreover, the hypersensitivity responses observed sofar occur when *R. solanacearum* infiltrated into the intercellular spaces of leaves such as tobacco, not at the cortical tissue of the roots. Hence, the pathogen can develop in the host without inducing hypersensitivity.

The other mechanism involves prevention of its attachment to cell walls, particularly when multiplying in intercellular spaces. The attachment of the bacterium to the host cells is the interaction of the acidic lipopolysaccharide of the pathogen and the basic glycoprotein that is present at the surface of the host cell. It was demonstrated (Sequeira, 1993) that this interaction could be efficiently inhibited by the bacterial extracellular polysaccharides (EPS).

The other important mechanism involves facilitating nutritional requirement. The lumen environment of xylem is poor in nutrition and is largely water. Hence the nutritional requirement of the bacterium in the vessel is facilitated through its production of growth regulators. It was demonstrated (Akioshi *et al.*, 1987) that the bacterium produces growth regulators. These stimulate xylem parenchyma to re-differentiate and divide so that the nutrients are rerouted into the infected xylem. This mechanism enables the bacterium to obtain sufficient sugars and amino acids (Sequeira, 1993). Finally, using the enzymes, the bacterium moves laterally, form cavities and then released into the environment upon the collapse of the tissue. The most important event to the life cycle of the bacterium is the constant release of bacteria into the soil through the points of emergence of lateral roots so that the disease cycle continues (Sequeira, 1993).

2.6. Symptoms of Bacterial Wilt

Symptomatology of plant pathogenic bacteria generally depends on the site of development of the pathogen in the host plant tissue (Kiraly *et al.*, 1970). The development of *R. solanacearum* is in the vascular bundle. Wilt symptoms are produced by various bacterial action exerted on the host plant. The bacterium produces slimy substance surrounding the bacterial mass, this increase the viscosity of vessel fluid and movement of water declines resulting in wilt. Thus, the extracellular polysaccharides play a critical role in wilt induction. The action of pectic and cellulolytic enzymes may also contribute to wilting. Bacterial wilt caused by *R. solanacearum* affects both the above-ground and below-ground parts in all the hosts causing wilt in the former case and partial or complete rot in the latter.

Martin and French (1985) described that the first symptoms of the disease in the field are wilting, stunting and discolouration of the foliage. At an early stage of wilting, leaves and succulent top-portions of plants infected prematurely become flaccid and droop. This symptom can be seen during clear sunny and warm days and the plants recover during evenings. Later the wilting becomes permanent and is followed by drying of the leaves. Bronze discolouration of leaves may be observed in early wilting. Yellowing of lower leaves may occur before wilting in plants about to mature. In the initial stage, only part of a plant or only one stem or leaf or even only one side of a stem or leaf may wilt. On cross section of the affected stem, a white bacterial ooze may appear and the xylem vessels are seen discoloured dark brown. On longitudinal section, the vascular system may show dark narrow stripes. The brown staining of the vascular bundle could be seen as dark patches or streaks even from the surface of the stem in the latter stage of the disease. In some plants such as tomato, twisting of stems and epinasty may also be observed (Rangaswami and Rajagopalan, 1973).

In the below-ground plant part, roots and tubers are affected. Roots may show dark discolouration on cut. In the advanced stage of the disease the tubers may start rotting and the

bacterial mass may exudate from the eyes of the tubers to which soil adheres. Such tubers when cut open show localized decay in the vascular ring. All tubers of a wilted plant may not necessarily be infected, but occasionally infected tubers may be found in plants showing no wilting symptom (Sinha, 1986).

Generally, the disease is characterized by rapid wilt. The incubation period required for the first symptoms to appear after infection is very short, mostly within 48 hours in succulent annuals. In hardy perennials it may take longer, a month or more (Rangaswami and Rajagopalan, 1973). Symptom development may also depend on the strain type, host susceptibility and environmental factors.

2.7. Virulence and Variability of *R. solanacearum*

One of the most dynamic and significant aspects of biology is that characteristics of individuals within a species are not fixed in their morphology and physiology, but vary from one individual to another. This variation may be brought about by various genetic changes.

In bacteria, genetic variability arises mostly by mutation, cytoplasmic inheritance, conjugation, transformation and transduction. The change may involve a number of characteristics. According to Agrios (1978), mutations for virulence probably occur no more frequently than for any other inherited characteristic.

In *R. solanacearum*, shift in virulence and existence of both virulent and avirulent phenotypes is a common phenomenon. It may occur spontaneously or induced by various factors. It was observed that some virulent forms of the pathogen are capable of transforming spontaneously into avirulent forms and vice versa. The rate of reversion from avirulent to virulent is very low, but it was found to increase in the presence of some antibiotics such as tetracyclines (Gadewar *et al.*, 1993).

It was also shown that temperature variation may bring shift in virulence. In an experiment (Gadewar *et al.,* 1995) conducted on the two forms of *R. solanacearum,* shift of virulent strains to avirulent strains was observed when incubated at 37°C and the latter reverted to the former when incubated at 30°C.

When virulent strains shift or mutate to avirulent forms, they fail to produce wilting because, they lose some factors that enable them to induce infection. In the above mentioned temperature-induced avirulent forms of *R. solanacearum*, the cells developed afluidal colonies which indicates that they lost their ability to produce exopolysaccharide, the major wilt inducing factor in plants (Kao and Sequeira, 1992). This shows that in order to cause infection in the host plant, virulent strains render some means of attack or virulence factors.

Mechanisms of attack of plant pathogens can generally be grouped into mechanical and chemical. Chemicals are highly important throughout the process of pathogenesis of bacteria. Even in those pathogens that use mechanical force to penetrate plant tissues, their activities inside the plants are largely chemical in nature (Chaube and Singh 1991). Therefore, Agrios (1978) emphasized that the effects caused by pathogens on plants are almost entirely the result of biochemical reactions taking place between substances secreted by the pathogen and those present in or produced by the plant.

The main groups of substances or virulence factors secreted by bacterial pathogens and involve in production of disease in plants include, enzymes, toxins, growth regulators, and polysaccharides.

Production of these virulence factors is governed by the biochemical determinants or genetic constitution of a pathogen. The most important bacterial genes in the pathogenesis of *R. solanacearum* are the *hrp* genes. These genes have also been identified in most plant pathogenic gram-negative bacteria including *P. syringae, X. campestris* and *E. amylovora* (Boucher, 1988). Strains mutant for *hrp* genes were found non-pathogenic to compatible hosts (Arlat *et al.*, 1993).

In the investigated species, the genes were similar in size and in certain biological traits which are related to bacterial-plant interactions and associated mutations. This suggests that; 1) a common core of essential pathogenicity genes is shared among representatives of most gram-negative plant pathogenic bacteria, and 2) independent of the host-range and the type of symptoms they can induce (*i.e.,* wilt, necrosis, maceration or chlorosis), most gram-negative plant pathogenic bacteria have developed a common strategy of plant infection (Arlat *et al.*,1993). It is intriguing that this strategy is also shared by certain animal and human pathogens belonging to the genera *Yersinia* and *Shigella* (Gough *et al.*, 1992). The explanation as to how the corresponding genes have evolved in order to be present in such taxonomically distant organisms, however, is unclear.

In addition to the common *hrp*-encoded pathogenicity functions, it is clear that each individual pathogen must have a set of specific genes controlling functions such as the production of toxins, hydrolytic enzymes, polysaccharides or plant growth hormones which are probably directly responsible for the type of symptoms each organism induces on its own host plant(s). Nevertheless, phenotypic expression of these specific functions appears to be strictly dependent on the presence of a functional core of *hrp* genes (Arlat *et al.*, 1993).

An important virulence factor common to most phytobacterial pathogens is the extracellular polysaccharide. It gives mucoid fluidal shape to bacterial colonies. Polysaccharide serves as the primary virulence factor in wilt disease causing pathogens that invade the vascular system of a plant (Agrios 1978). In the vascular wilts, large polysaccharide molecules released by the pathogen in the xylem may be sufficient to cause a mechanical blockage of vascular bundles and thus initiate wilting. Together with the macro-molecular substances released in the vessels through the breakdown of host substances by pathogens, they reduce viscosity of the vascular fluid.

It has been reported (Trigalet-Demery *et al.*, 1993) that, in *R. solanacearum* the biosynthesis of EPS involves at least three gene clusters, the *eps* I and *eps* II (eps = Exopolysaccharide) and *ops* (ops = outer polysaccharides). The genes involved in regulation of EPS production are identified

as *phc*A, a transcriptional regulator, and *vsr*A and *vsr*S both serve as sensors (Huang *et al.*, 1994; Schell *et al.*, 1994). Strains that lack these genes were found to have a reduced virulence and the colonies were rough (butyrous) in shape.

Hydrolytic enzymes are also important virulence factors in the process of pathogenesis of *R. solanacearum*. Among these, polygalacturonases (PG) and endoglucanases (EG) are the major ones (Allen *et al.*, 1993). PG degrades pectic compounds present in the cell wall. There are two types of PGs; endopolygalacturonase which cleaves the polygalacturonate polymer internally, and exopolygalacturonase which removes one or a few galacturonate residues at a time from the end of the polymer.

Endoglucanase (Cellulase) is also an important virulence factor involved in the degradation of cellulose present in the cell wall (Roberts *et al.*, 1988). Genes called *cel* or *egl*,which are responsible for the production of this enzyme, were identified in *R. solanacearum* and other bacteria such as *Erwinia* spp. and *X. campestris* (Schell *et al.*, 1988).

Production of growth regulators by *R. solanacearum* is suggestive of the hormones as additional virulence factors used for obtainining nutrients (Akioshi *et al.*, 1987)

3. STUDY AREAS

The south and central zones of Ethiopia are among the most agriculturally important parts of the country. The region receives better rainfall and has diverse vegetation and climatic conditions. As a result, a wide range of crops are grown for local consumption and export. Almost all types of important food and cash crops including coffee, cereals, legumes, horticultural and oil crops are grown in the region. The major horticultural crops cultivated in the region includes potato, sweet potato, enset, pepper, tomato and many other vegetables. Although potato is cultivated throughout the region, mostly as garden crop, Shashemene (East Shoa zone), Wondo Genet (Sidama zone) and Ambo, Bako, Jeldu and Inchini (West Shoa zone) are among the major potato growing areas. Some of these areas differ in altitude, climatic conditions and farming practices that affect bacterial wilt development. Hence, these areas were selected for the present study.

3.1. Ambo

Ambo district is found in West Shoa zone. Ambo town is located about 125 km from Addis Ababa at 8^0 57'N latitude, 37^0 52'E longitude, and 2200m above sea level (a.s.l). The annual rainfall of 1997 was 1130.2 mm with mean minimum and maximum daily temperature of, respectively, 11.03 and 26.72^{0}C (Appendix 9). The soil type in the district is mostly vertisol, but in potato producing areas, it is dominated by sandy loam. Almost all types of cereals, legumes and oil crops are widely cultivated in the area. The major horticultural crops grown are potato and tomato. Both crops are produced under irrigation conditions during Bega, though, there is little potato cultivation during the main rainy season.

Late blight and bacterial wilt are the major disease problems of potato production. Bacterial wilt was recorded since 1980 (SPL, 1981). Production of potato was increased especially since 1988, but due to bacterial wilt problem, it is decreasing for the last three years (personal observation).

3.2. Bako

Bako, a town of Bako Tibe district of West Shoa zone, is situated 250 km from Addis Ababa. Disease assessment and sample collection was, however, made from two locations; Kejo (Alt. 1650 m a. s. l.) in East Wollega zone which is located only about 6 km away from Bako town to the west along Gibe river and Jato (Alt. 1800m a. s. l.) in Cheliya district of West Shoa zone. The locations are relatively hot. The 1997 daily mean minimum and maximum temperatures at Bako Research Centre were 14.2 and 28.2^{0}C, respectively, with annual rainfall of 1388.8 mm (Appendix 10). The soil is sandy loam.

Although, different types of cereal and legume crops are cultivated in the area, maize is the most extensively produced crop. Sugarcane and fruits including mango, orange and banana are also produced around Bako.The major horticultural crops grown in the area are pepper, potato and tomato. Production of potato takes place during Bega under irrigation. Bacterial wilt is one of the major disease problems of potato production. Occurrence of the disease has been recorded since 1979 (SPL, 1980) and since then, it has been observed causing damage to potato and tomato crops.

3.3. Jeldu and Inchini

Jeldu and Inchini are relatively highland districts with altitudes as high as 3100 and 2500m.a.s.l., respectively. They are situated in West Shoa zone about, respectively, 140 and 160 km from Addis Ababa. Both are characterised by low temperature and receive relatively high precipitation. The soil is sandy loam. Barley, wheat and potato are the major crops cultivated in the districts. Production of potato takes place during Belg. It is cultivated extensively as a rain-fed crop and planted with the start of rains in February or March. The major disease problem in potato production is late blight. Occurrence of bacterial wilt is not pronounced in these districts (MOA experts pers. comm.). Sample collection was made from farmers fields along the road.

3.4. Shashemene

Shashemene is a district situated about 250 km from Addis Ababa in East Shoa zone. Shashemene town is located at 7^0 13'N latitude and 38^0 35'E longitude. The altitude of the sampled areas ranges from 1800 to 2250m a. s. l. The soil is sandy loam.

Different cereal, legume and horticultural crops are widely cultivated during the main rainy season. Potato is the major horticultural crop cultivated in the district. It is one of the major cash crops of the local farmers. In the district, potato production takes place twice a year using both Belg and Meher rains. It is cultivated extensively in both seasons, although it is higher in Belg and covers about 10,000 ha of land annually (MOA experts pers. comm.).

Bacterial wilt along with late blight is the major disease problem. According to the local farmers, bacterial wilt has been occurring in the area for about two decades. Since then, although the severity varies, the disease is appearing at every season of potato production with extended distribution (MOA experts pers. comm.). Because potato is a profitable crop and some times farmers obtain some profitable yield and price from crops attacked at the later growth stage, production of potato is increasing in the area despite the disease problem. Sample collection was made from farmers fields throughout the district.

3.5. Wondo Genet

Wondo Genet is a small rural town in Awassa Zuria district, Sidama zone of South region. It is only 17 km from Shashemene town. The altitude ranges from 1650-1800m a.s.l. The location is rich in water resource, as a result, potato is mostly cultivated under irrigation conditions during

Bega. The major crops produced in the area include chat, coffee, sugarcane, fruits such as banana and orange, potato, sweet potato, enset, and different cereal, and legume crops.

Bacterial wilt and late blight are problems in the area to potato production. According to local farmers, late blight occurs and is severe if it rains during vegetative growth of the crop.

In addition to the mentioned major study areas, few wilted potato samples were collected from Tsedey farm where potato seed production has abandoned, except in few plots mainly for experimental purposes.

4. MATERIALS AND METHODS

4.1. Disease Assessment

Surveys were conducted in 1996/97 during the two rainy seasons of Ethiopia, 'Belg' (February-May) and 'Meher'(June-September), the short and main rainy seasons, respectively, and the dry season, 'Bega' (November-February). In the latter, potato production takes place under irrigation conditions.

Farmers fields of potato crops in the selected areas were visited during their growing season(s) and occurrence of bacterial wilt was assessed. Fields in locations around Wondo Genet, Bako and Ambo, were visited during Bega; fields at Jeldu and Inchini districts were assessed during Belg; Shashemene district was surveyed both during Belg and Meher cropping seasons. At each location, plants were visually inspected for bacterial wilt symptoms when the crop was at flowering stage and incidence (percent of plants with bacterial wilt symptom per field) and prevalence (percent of fields having plants with bacterial wilt symptoms) were recorded. In addition, farmers were interviewed and information on preceding cropping and disease history of the fields was recorded. Meteorological data of some locations were also collected.

4.1.1. Incidence Assessment

For bacterial wilt incidence assessment, five plots each with 100 plants per field were taken at random. In each plot, number of plants with bacterial wilt symptoms were counted and average of the five plots was taken as percent bacterial wilt incidence of a given field.

4.1.2. Prevalence Assessment

Randomly selected 30 to 40 potato crop fields per major study area or district were considered for prevalence assessment. Plants in each field were inspected for bacterial wilt symptoms and each field was recorded either as a field having plants with bacterial wilt infection or disease-free. The ratio of infested crop fields to the total considered fields was taken as percent prevalence of the disease in a given area.

4.1.3. Effect of Preceding Crops on Wilt Incidence

Effect of preceding crops on the incidence of potato bacterial wilt was assessed by collecting information on wilt history and preceding crops of some fields of potato crops along with their wilt incidence during the survey. Wilt incidences of representative two potato crops, one preceded by non-host crop(s) and one preceded by potato itself, taken from the same location and a total of ten crops of each category from ten different locations were used to estimate preceding crop effect on wilt incidence.

4.1.4. Diagnosis in the Field and Sample Collection

Potato plants with wilt symptoms were uprooted. Stems and/or tubers were cut and observed for signs of the disease. The signs include:

a) discolouration of vascular tissues, especially near the point of attachment between stolon and tuber or between taproot and stem.
b) presence of a creamy bacterial ooze that exudes from the stem or vascular ring of infected tuber.

In addition, a piece of tissue cut from a mid-portion of the base of a wilted stem was suspended in a glass of clear water for a while. Apperance of thread-like milky ooze that drifts downward from the vascular tissues was taken as confirmatory of bacterial wilt disease (Martin and French, 1985).

Diseased potato plants and tubers and some common weeds grown with infested potato crops were randomly sampled and brought to the laboratory for isolation and further investigations.Tuber samples for latent infection study were collected at harvest.

4.2. Isolation of the Pathogen

Isolation of the pathogen was carried out from stem, roots and/or tuber vascular tissues of potato plants and stems and roots of weeds following the procedure described by Jenkins and Kelman (1976). Leaves and petioles were removed from stems. The entire stem, root and tuber was thoroughly washed in running tap water. Using sterilized scalpel, the plant part was cut longitudinally and thin slices were taken from the discoloured part or from base portion of a suspected plant stem. The resulting pieces were briefly treated with denatured alcohol, rinsed with and placed in sterile distilled water and allowed to stand for a while until a turbid suspension was formed, or slices in distilled water were crushed using sterile mortar and pestle. In some oozing tubers the exudate was directly taken. Surface of the tubers was thoroughly washed using tap water, disinfected with 70% alcohol and rinsed with distilled water. Using sterile scalpel, the tuber was then cut longitudinally into two and the resulting halves were squeezed to release bacterial exudate. The exudate was either taken using sterilized loop and diluted in sterile distilled water or directly.

Turbid suspensions from samples or exudate of bacteria were streaked on triphenyl tetrazolium chloride agar medium and incubated at 30 ± 2°C. Colonies of *R. solanacearum* were then selected and used in the proceeding investigations of pathogen identification and characterization

of isolates. More than hundred isolates were recovered from potato and weed samples. Some of these are presented in Appendix 1.

4.3. Cultural Characteristics

Morphological features and colour of colonies of the isolates were determined using triphenyl tetrazolium chloride (TZC) medium composed of dextrose (10.0 g), peptone (10.0 g), casamino acid (1.0 g), agar (15.0 g) in a litre of distilled water and 0.005% triphenyl tetrazolium chloride (Kelman, 1954) and nutrient agar medium (NA) containing peptone (5.0 g), beef extract (3.0 g)

and agar (20.0 g) in a litre of distilled water (Sands *et al.*,1980). To determine colony size, the diameter of five well-separated colonies on each plate were measured by superimposing a ruler upon the largest axis of a colony and average of three duplicated plates was taken for an isolate. Fluorescent pigment formation was observed on King's medium B containing peptone (20.0 g), glycerol (15 ml), K_2HPO_4 (1.5 g), $MgSO_4.7H_2O$ (1.5 g), agar (20.0 g) in a litre of distilled water (King *et al.*,1954). Brown pigment formation was tested using Kelman medium without TZC, but supplemented with 0.01% L. tyrosine. Isolates were cultivated on each medium in duplicates.

For determination of levan formation, isolates were spread on nutrient agar medium supplemented with 5% sucrose (Hayward, 1964), incubated for 48 hours, and observed for large shining slimy colonies with specific offensive smell. Motility of isolates was observed from 48-72 hours old cultures diluted in sterile distilled water by hanging drop procedure (Bradshaw, 1979). A drop of the suspension was placed on a cover slide. The cover slide was placed on a cavity glass slide in an inverted position. Motility of the cells was observed under high power objective of a compound microscope.

4.4. Biochemical Characteristics

Gram-reaction was determined using potassium hydroxide solubility test (Suslow *et al.*, 1982). A loopful of bacterial culture was mixed with two drops of 3% potassium hydroxide solution with a toothpick on a glass slide for 5-15 seconds. The formation of milky thread upon lifting the toothpick was taken as an indication of the presence of the Gram-negative pathogen.

Oxidase test was conducted according to the procedure described by Kovac (1956). Seventy two hours bacterial cultures were used for the test. One loopful of bacterial culture grown on nutrient medium was transferred onto filter paper moistened with a solution of 1% N, N, N, N,- tetra methyl P-phenyl diamine dihydrochloride. Appearance of intense violet colour instantly (within 30 seconds) was taken as positive.

Salt tolerance of isolates was determined following the method described by Hayward (1964). Sodium chloride broth with 0.5, 1.0, 2.0 and 5.0%NaCl concentrations and salt-free of the medium were prepared and dispensed in test tubes. Each bacterial isolate was inoculated into each concentration of the medium in replications. Turbidity of the inoculated medium was taken as an indication of bacterial growth. Uninoculated tubes with salt and inoculated salt-free broth were used as, respectively, negative and positive controls.

Catalase activity was detected by adding 3% hydrogen per oxide to the surface of 48 hours old pure cultures and gas formation was observed (Lelliot and Stead, 1987).

Starch hydrolysis was determined by cultivating the isolates on 0.2% starch medium. After 3 days, cultures were flooded with Lugol's iodide solution and observed for colour change. Appearance of yellowish, clear zones around or under bacterial growth indicates hydrolysis of starch (Lelliot and Stead, 1987).

Nitrate reduction test was conducted using the nitrate semi-solid medium of Hayward (1976) which was prepared from peptone (10.0 g), NaCl (5.0 g), KNO_3 (2.0 g) and agar (3.0 g) in a litre of distilled water. Isolates were inoculated into the medium and incubated at 30°C. After four days, the production of nitrite (appearance of blue black colour) was detected by adding 3-4 drops of starch iodine reagent and 16% hydrochloric acid.

Poly-β-hydroxybutyrate (PBH) accumulation was determined by extracting dried bacterial cells with hot chloroform followed by filtration into three volumes of ether and observed for flocculent precipitate formation which is poly-β-hydroxybutyrate. Poly-β-hydroxybutyrate is a chloroform soluble but ether insoluble substance (Hayward, 1964).

4.5. Carbohydrate Utilization

The standard basal medium for oxidation and fermentation (Hayward, 1964) was used for the test. The basal medium was prepared from $MgSO_4.7H_2O$ (0.2 g), $NH_4H_2PO_4$ (10.0 g), KCl (0.2 g), peptone (1.0 g), agar (1.5 g) and bromo thymol blue (0.3 g) in a litre of distilled water. The medium was heated to boiling until the ingredients were melted and dissolved, adjusted to a pH of 7.1 and sterilized at 121° C for 30 minutes. The carbohydrates were prepared separately as 10% solutions and autoclaved at 110°C for 20 minutes except fructose, maltose, arabinose, galactose, and cellobiose which were filter-sterilized. Melted basal medium was dispensed in sterile flasks in 90 ml quantities. When it cooled to 60°C, 10 ml of each carbohydrate solution was added to make 1%. After mixing, this was dispensed in 4 ml volume in sterilized tubes.

The inoculum was prepared from 48 hours old bacterial culture. Suspensions of each isolate was prepared and added to each sugar in duplicates. One of the tubes was sealed with 3% water agar after stab inoculation. Uninoculated tubes were used as controls. All tubes were incubated at 28°C and monitored for 21 days for any colour change. A change to yellow colour was taken as positive for the test.

4.6. Hypersensitive Reaction Tests

Bacterial suspensions of each isolate were prepared from 48 hours old pure cultures at a concentration of 10^8 cells/ml (Klement *et al.*,1964). Using hypodermic syringes with fine needle, the suspensions were infiltrated into intercellular spaces of completely expanded leaves of tobacco, cv. White Burley, with replications. Control plants were infiltrated with sterile distilled water. Plants were kept at room temperature and reactions were observed and recorded daily.

4.7. Pathogenicity Tests

Susceptible cultivars of the major host plants of *R. solanacearum,* potato (*Solanum tuberosum* L. cv. CIP 383032.15), tomato (*Lycopersicon esculentum* Mill. cv. Marglobe), eggplant (*Solanum melongena* L cv. Black Beauty), tobacco (*Nicotiana tabacum* L. cv. White Burley), and pepper (*Capsicum annum* L. cv. Bako Local), were used for the test.

Pathogen free potato tubers, obtained from horticulture division of Holleta Research Centre, were raised in 20 cm pots (one plant per pot) filled with sterilized soil in greenhouse. Plants other than potato were obtained by sowing botanical seeds on sterilized soil containing trays and transplanting the resulting seedlings in 20 cm pots (one plant per pot) filled with sterilized soil.

Bacterial suspensions of isolates were prepared from 48-72 hours old culture and adjusted to a concentration of 10^8 cells/ml. Plants were inoculated at three leaf stage by stem puncture method (Winstead and Kelman, 1952). Suspension of each isolate was inoculated into the stems of each test plant at the axil of the second or third leaf from the apex in replications. Control plants were injected with sterile distilled water. A group of plants was set in an incubator with 28 ± 4^0C for a day before and three days after inoculation and transferred to greenhouse while another group, that is, replicates of incubated plants, was set in greenhouse with mean minmum and maximum temperatures of, respectively, 14.8 and 35.2^{0}C throughout the experiment. Host reactions were

assessed using 1-5 scale (French and Sequeira, 1970); 1=healthy; 2=epinasty, distortion and browning of stem or inoculated leaves; 3=wilting of one or two leaves; 4=wilting of half of the total number of leaves and 5=complete wilting (death) of the plant. Average reactions of three plants of each species per isolate was used for determining different levels of infection: none (1.0), low (1.1-2.5), medium (2.6-4.0) and high (4.1-5.0).

4.8. Relative Virulence

Relative virulence of representative isolates of study areas, obtained from potato, were evaluated on potato (cv.383032.15) and tomato (cv. Marglobe) plants. Two isolates with a stronger reaction on hypersensitivity reaction and pathogenicity tests were selected from each major study area and Tsedey. Growing conditions, inoculation of plants and disease rating were as previously described, but average of ten plants of each cultivar per isolate and mean of the two isolates was expressed in percentage as an index of relative virulence (severity) of isolates of a location.

4.9. Latent Tuber Infection

For the study of latent infection in potato, tubers were collected from three infested crops at harvest and bought randomly from local markets of each survey area. In addition, samples of seed tubers of three potato cultivars, each produced during Belg and Meher and stored for a season, were collected from Turufe kebele, Shashemene.

Percent latent tuber infection was determined by incubating about 100 to 200 healthy looking tubers per sample in incubators at 30°C for 30 days. Each tuber was then dissected and observed for vascular browning and/or bacterial ooze. A slice of tuber was also taken and analysed when necessary to confirm the presence of the pathogen. The ratio of positive tubers to the total tubers was used to calculate percentage latent tuber infection.

4.10. Weed Host Plants

To investigate the role of weed plants as possible carriers of *R. solanacearum*, isolation of the bacterium was attempted from commonly grown weed plants with potato crops. These were; *Amaranthus* spp., *Commelina* spp., *Cyperus* spp., *Guizotia scabra*, and *Galinsoga parviflora*. Isolates were subjected to cross inoculation of tomato, potato, tobacco, eggplant, pepper and *G. parviflora*. Besides, hypersensitivity reaction and other confirmatory tests including, cultural, biochemical and physiological tests were conducted following the previously described methods.

4.11 Analysis of data.

Because of wide interlocation variation in percent disease incidence, prevalence and tuber infection, collected data were transformed for statistical analysis using arcsine transformation (Fisher and Yates, 1943). Analysis of variance were carried out with MSTAT-C (Michigan State University).

5. RESULTS

5.1. Incidence and Prevalence of Bacterial Wilt

The percentage of plants and crops affected by bacterial wilt at the locations studied was variable (Table 1 and 2). Bacterial wilt was much more severe around Bako where potato crops were cultivated under irrigation conditions during Bega; wilt incidence ranged from 6 to 72% with a mean of 27.8% (Table 1). Wilt incidence was relatively low at Wondo Genet with a range of 1 to 15% and a mean of 7.2%. Incidence of the disease around Ambo area was intermediate between the two irrigation fed areas and a range and a mean wilt incidences of , respectively, 3 to 45 and 18.2% were recorded.

During Belg, the disease was more intense around Shashemene where a range of 2 to 51% and a mean of 22.3% wilt incidences were recorded. The disease was very minimal in Jeldu district and wilt incidence ranged from 1 to 4% with a mean of 2.3%. In Inchini, the occurrence of the disease was even negligible except for the traces of wilted plants that were observed in few fields; incidence ranged from 1 to 2% with a mean of 1.3%.

Intensity of the disease in Meher grown potato crops around Shashemene was comparable to Belg produced crops at the same district ; wilt incidence of 3 to 42% was recorded with a mean of 19.8%.

Prevalence of bacterial wilt (Table 2) was also higher in locations around Bako and 87.5% of potato crops were found infested. The disease was also prevalent in the other two irrigation fed areas, Ambo and Wondo Genet, where, respectively, 70 and 62.5% of the fields were found to have the disease.

Table 1. Incidence of potato bacterial wilt in the major growing areas of south and central zones of Ethiopia during 1996/97 cropping seasons

No	Administrative zone	District/ Major Location	Season	Incidence range (%)	Mean incidence			Altitude range (m.a.s.l.)
					Percent	Arcsine transformed	Back transformed	
1	East Shoa	Shashemene	Meher	3-42	19.8	24.9 (1.74)[a] ab[b]	18.4	1850-2250
		Shashemene	Belg	2-51	22.3	27.3 (1.74) ab	21.0	1850-2250
2	West Shoa	Jeldu	Belg	1-4	2.3	8.4 (3.28) d	2.6	2750-3050
		Inchini	Belg	0.5-2	1.3	6.5 (5.01) d	1.2	2350-2500
		Ambo	Bega	3-45	18.2	23.9 (2.75) b	16.5	2150-2250
		Bako	Bega	6-72	27.8	30.7 (2.75) a	26.0	1650-1800
3	Sidama	Wondo Genet	Bega	1-15	7.2	14.9 (2.75) c	6.8	1650-1700

( )[a] Standard error of means (S.E.M.)(df=83)

[b] Means followed by the same letters are not significantly different at p=5%

Table 2. Prevalence of potato bacterial wilt in the major growing areas of south and central zones of Ethiopia during 1996/97 cropping seasons

No	Administrative zone	District/Major Location	Season	No. of fields considered	Mean prevalence		
					Percent	Arcsine transformed	Back transformed
1	West Shoa	Bako	Bega	30	87.5	72.1a[a]	91.5
		Ambo	Bega	30	70.0	57.5b	71.2
		Jeldu	Belg	40	17.5	24.5c	17.2
		Inchini	Belg	40	7.5	11.3c	3.5
2	East Shoa	Shashemene	Meher	40	70.0	57.5b	71.2
		Shashemene	Belg	40	75.0	60.7ab	76.0
3	Sidama	Wondo Genet	Bega	30	62.5	52.3b	62.7

S.E.M.(df=21) 4.85

[a]Means followed by the same letters are not significantly different at p=5%.

Among Belg growing areas, distribution of the disease was high around Shashemene with average wilt prevalence of 75%. Occurrence of bacterial wilt in Jeldu and Inchini districts was very low and mean wilt prevalences of, respectively, 17.5 and 7.5% were recorded.

The disease was also prevalent in the Meher grown potato crops around Shashemene; the disease symptoms were observed in 70% of the total fields examined.

5.2. Effect of Preceding Crops on Wilt Incidence

Comparison of mean percentage wilt incidences in potato crops preceded by non-host crop(s) with those preceded by potato itself showed that the disease was relatively less severe in potato crops preceded by non-host crops; mean percentage wilt incidences were 19.1 and 35.3%, respectively (Table 3).

5.3. Cultural Characteristics

Bacterial isolates incubated at 30 ± 2°C showed variable colony morphologies and sizes depending on the type of medium used and the time of incubation (Table 4). On tetrazolium chloride agar culture, both virulent and avirulent colonies were observed. Virulent colonies presented broad fluidal whitish-grey and irregularly round margin with pink or red centre after 48 hours of incubation. Avirulent colonies were deep red with narrow white margin and small in size. On prolonged incubation, colour intensity of virulent colonies increased from grey to brown. Colony size taken at 72 hours, was also variable ranging from two to six mm in diameter, though, there were few that are less or greater than this range. The majority of them, however, fell between 3 and 5 mm. On nutrient agar medium, colonies were fluidal white-grey and relatively more round and smaller in size compared to the colonies cultivated on TZC.

Table 3. Effect of preceding crop(s) on bacterial wilt incidence of potato cultivated on some infested fields of the survey areas, 1996/97.

No.	District/Major location	Non-host crop rotation between potato crops			Successive potato cropping		
		No. of rotations and preceding crop(s)	Wilt incidence		Preceding Crop	Wilt incidence	
			Percent	Arcsine transformed		Percent	Arcsine transformed
1	Arabate	1, maize	3	10.0	potato	33	35.1
2	Awasho	1, maize	25	30.0	potato	40	39.2
3	Edola	1, maize	13	21.1	potato	31	33.8
4	Faji	1, maize	19	25.8	potato	18	25.1
5	Kejo	1, maize	21	27.3	potato	72	58.1
6	Abicha	2, beans, teff	40	39.2	potato	28	31.9
7	Faji Meti	2, wheat, teff	8	16.4	potato	37	37.5
8	Jato	2, maize, maize	6	14.2	tomato	20	26.6
9	Mutulu	2, maize, teff	45	42.1	potato	36	36.9
10	Sole	2, maize, beans	11	19.4	potato	38	38.1
Mean			19.1 (17.2)[a]	24.6*		35.3 (35.0)[a]	36.2*

S.E.M. (df=18) 3.09

*Significant difference at p=5%

()[a]Back transformed

Table 4. Cultural characteristics of isolates of *R. solanacearum*

Isolates code-RPP	on TZC medium		on NA[b] medium	Pigment production		Levan formation
	colony [a] colour	colony size[b] (mm)	colony size (mm)	fluores-cent	brown (intensity)	
488, 491, 494, 509, 519, 542, 552, 561, 562, 567, 568a, 632, 640, 648, 658	3+	4-6	2.5-4.5	0	H	+
487, 506, 520, 563, 569, 571, 599, 621,	3+	3.5-5	2-4	0	H	+
507, 508, 514, 516, 544, 546, 577, 603c, 605, 614, 622, 624	3+	3.5-5	2-4	nd	nd	nd
484a, 522, 524, 551, 564, 572, 576, 589, 612, 637, 685	2+	2.5-4	1.5-3	0	M	+
485, 550, 553, 597, 616, 618, 628	2+	2.5-4	1.5-3	nd	nd	nd
515, 565, 566, 574, 575, 579	1+	1.5-3	1-3	0	L	+
489, 518, 573, 578, 625, 626, 627, 655, 659	1+	1.5-3	1-3	nd	nd	nd

0= negative
+= positive
1+= red toward the centre with narrow margin
2+= light red toward the centre with broad margin
3+= pink toward the centre with broad margin
H= high
M= medium
L= low
nd= not done
[a] after 48 hours incubation
[b] after 72 hours incubation

All isolates produced brown diffusible pigment of variable intensities on Kelman's medium supplemented with thyrocine 0.01% L, but without TZC. Fluidal white-grey colonies appeared on the medium after 24 hours incubation at 28°C. After 72 hours, however, colour intensity has increased, but variably from grey to dark-brown. Sizes of most colonies ranged from 3 to 6 mm in diameter after 72 hours incubation on the same medium. On King's medium B, none of the isolates produced a green fluorescent pigment; they produced white-grey colonies. Generally, colonies cultivated on Kelman's medium were larger in size compared to the colonies grown on either TZC or NA media. Levan was formed on NA with 5% sucrose.The isolates were motile except RPP- 520, 567, 569, 614, and 616 which were apparently non-motile.

5.4. Biochemical Characteristics

Biochemical characteristics of the studied isolates are presented in table 5. All isolates were Gram-negative. Oxidase, catalase and nitrate reduction tests were found to be positive, but there was variation among isolates in oxidase activity showing from weakly to strongly positive reaction with the appearance of, respectively, light purple colour within 30 seconds and a strong purple colour within 10 seconds. The isolates were positive for poly-β-hydroxy butyrate, but failed to hydrolyse starch. They produced turbidity in 0.0,0.5 and1.0% NaCl medium, but none produced turbidity in 2 and 5% NaCl. Negative controls remained unchanged.

5.5. Carbohydrate Utilization

There was no marked difference among isolates in carbohydrate utilization (Table 6). All the isolates utilized the three disaccharides; lactose, maltose and cellobiose, though, there were some differences among isolates in the extent of utilization. Most isolates produced intense colour change of the medium containing lactose, cellobiose, maltose, inositol, glycerol, sucrose, galactose, and fructose, an indication of utilization of the carbohydrates, within 4 to 7 days, while some required 15 days. None of the isolates utilized the hexose alcohols,

Table 5. Biochemical characteristics of isolates of *R. solanacearum*

Isolates code-RPP	Gram-reaction	Oxidase	Catalase	PBH production	Starch hydrolysis	Nitrate reduction	Growth on NaCl medium at concentrations (%)			
							0.5	1.0	2.0	5.0
488, 491, 551, 552, 564, 568, 621, 648, 685	0	3+	+	+	0	+	+	+	0	0
514, 519, 553, 562, 567, 571, 599, 603c, 625, 626, 632, 640, 658	0	3+	+	nd	nd	nd	nd	nd	nd	nd
487, 494, 509, 522, 561, 612, 622	0	2+	+	+	0	+	+	+	0	0
485, 489, 507, 515, 516, 524, 544, 550, 572, 578, 589, 614, 616, 624, 627, 655	0	2+	+	nd	nd	nd	nd	nd	nd	nd
506, 520, 542, 546, 563, 569	0	1+	+	+	0	+	+	+	0	0
484a, 508, 597, 605, 618, 637, 659	0	1+	+	nd	nd	nd	nd	nd	nd	nd

+= positive
0= negative
1+= light purple colour
2+= intermediate between 1+and 3+
3+= strong purple colour within 10 seconds
nd= not done

mannitol, sorbitol and dulcitol. All the isolates utilized glucose oxidatively. Arabinose was utilized only slightly by few isolates.

5.6. Hypersensitivity Reaction

Infiltration of bacterial suspensions of isolates into intercellular spaces of tobacco leaves showed different reactions (Table 7). Most isolates produced yellow chlorosis within 24-48 hours among which some caused brown necrosis of the infiltrated part of the leaf within 3-5 days. Some produced water-soaked brown necrosis, typical hypersensitivity reaction, within 24-48 hours. None of the isolates induced wilting of the plant. Control plants were healthy.

5.7. Pathogenicity Tests

Pathogenicity results showed that all the isolates were pathogenic to potato and tomato (Table 8). The development of disease symptoms, however, varied with time, test plant, and isolate of *R. solanacearum*. Wilt symptoms that developed on tomato and potato plants was rapid and initiated on the leaf just below the injection site within 3 to 6 days. Disease severity was generally lower and the time required for symptom development was longer in plants set in the greenhouse than incubated. Incubated potato and tomato plants showed complete wilt within 7 to 13 days. Only some isolates caused complete wilt in both plants and required 10-20 days after inoculation in greenhouse. In response to most isolates, tomato plants exhibited excessive lateral root growths, epinasty, stunting and on dissection, browning of stems with variable dimensions ranging from 3 to 13 cm. The latter two symptoms were observed also on potato. Symptom development by eggplant and pepper required even longer time. Only few isolates caused complete wilt of eggplant, but none of the isolates caused wilt of pepper. Both plants, however, showed stunting and bleaching of leaves ,though, it was mild in pepper. Only isolates 494, 520 and 648 induced wilting in tobacco.

Table 6. Carbohydrate utilization of isolates of R. *solanacearum*.

Isolates code-RPP	Utilization of carbohydrates[a]										
	Glucose	Sucrose	Arabinose	Fructose	Galactose	Glycerol	Inositol	Cellobiose	Lactose	Maltose	Dulcitol Mannitol Sorbitol
488, 494, 520, 542, 546, 551, 552, 553, 561, 563, 571, 589, 603c, 618, 621, 622, 624, 625, 627, 632, 648, 658, 659	+(1)	+(1)	0	+(1)	+(1)	+(2)	+(2)	+(2)	+(2)	+(2)	0
519, 522, 544, 568a, 569, 626, 637, 640	+(1)	+(1)	+(3)	+(1)	+(1)	+(1)	+(2)	+(2)	+(2)	+(2)	0
487, 562, 567, 599, 655	+(1)	+(1)	0	+(1)	+(2)	+(2)	+(2)	+(2)	+(2)	+(2)	0
484a, 485, 597, 605, 612, 614, 616	+(1)	+(1)	0	+(2)	+(2)	+(2)	+(2)	+(2)	+(2)	+(3)	0
Controls	0	0	0	0	0	0	0	0	0	0	0

[a] No color change (0); complete color change : 3-7 days +(1), 7-14 days +(2), 14-21 days +(3).

Table 7. Reaction of *R. solanacearum* isolates to tobacco leaf infiltration within 1, 2, 3, 5 and 7 days.

Isolates code-RPP	Days after infiltration				
	1	2	3	5	7
494, 522, 542, 546, 551, 553, 563, 568a, 569, 648	HR	HR	HR	HR	HR
484a, 491, 520, 552, 562, 571, 621, 632, 640,	C	HR	HR	HR	HR
485, 487, 488, 561, 567, 597, 599, 612, 622, 624, 626, 637, 658	C	C	HR	HR	HR
507, 509, 515, 519, 567, 589, 603c, 605, 616, 625, 626, 655	C	C	C	HR	HR
485, 489, 506, 508, 514, 516, 518, 544, 564, 572, 573, 576, 578, 614, 618, 627, 659,685	C	C	C	C	C
Controls	NR	NR	NR	NR	NR

C= Chlorotic; HR= Water soaked / brown necrosis; NR=No reaction

5.8. Relative Virulence

Mean percentage of virulence of the representative isolates of the study areas is presented in Table 9. Isolates representing Bako, Ambo and Tsedey locations were highly virulent on both potato and tomato; and mean percentage virulences of, respectively, 90.5, 88.5, and 87.5 on potato and 93.0, 95.0, and 90.0 on tomato were recorded. Virulences of isolates from Shashemene were intermediate and of those from Wondo Genet, Jeldu and Inchini were low. Isolates from the latter three locations showed relatively lower virulence on tomato compared to potato.

5.9. Latent Tuber Infection

Occurrence of bacterial wilt infection in potato tubers collected from the study areas was investigated and mean percentage of latent tuber infection recorded (Table 10). The results showed that the highest tuber infection has occurred in tubers collected from Jeldu with a mean percentage of 77.7 in infested crops and 53.5 in market tubers; the least was in tubers from Inchini with 18.6% mean tuber infection in infested crops and 6.0% in market tubers.

In tubers from Shashemene potato producing areas, tuber infection was higher in tubers harvested from Meher crops than those of Belg and mean percentage of, respectively, 65.3 and 53.7% in tubers from crops with bacterial wilt symptoms and 48.5 and 37.0 in market tubers were recorded.

In tubers produced under irrigation conditions, percent infection was found to be high in tubers collected from infested crops of Wondo Genet with a mean of 61.3 and low in tubers from Bako with a mean of 45.7. Tubers from Ambo showed intermediate percent infection with a mean of 51.3. Market tubers from Wondo Genet also have shown higher infection (50.0) than either Bako (36.5) or Ambo (28.5).

The relative tuber infection in stored three potato cultivars produced in both seasons at Turufe, one of the sampling sites in Shashemene, was compared (Table 11). The result showed that among the three cultivars the highest mean percentage tuber infection (79.3) has occurred in Awash followed by Wechecha (67.0), and least in Genet (47.7).

Table 8. Pathogenicity of isolates of *R. solanacearum* on major hosts.

Isolates code-RPP	[a]Pathogenicity and host plants				
	potato	tomato	eggplant	tobacco	pepper
485, 487, 514, 516, 544, 551, 589, 612, 624, 685	M	M	L	0	0
488, 491, 542, 552, 562, 567, 568a, 571, 621	H	H	H	0	L
509, 546, 561, 564, 605, 632, 640, 658	M	H	M	0	L
489, 508, 519, 522, 553, 563, 569, 603c, 625, 627	H	M	L	0	0
506, 518, 572, 573, 576, 628	L	L	L	0	0
507, 524, 578, 597, 599, 613, 614, 618, 622, 655	M	L	M	0	0
616, 637, 659	L	M	L	0	0
494, 648	H	H	M	H	L
520	M	M	M	H	0
Control	0	0	0	0	0

[a]Pathogenicity measured 21 days after inoculation using 1-5 scale:
1=healthy; 2=epinasty, distortion and browning of stem or inoculated leaves; 3=wilting of one or two leaves; 4=wilting of half of the total number of leaves and 5=complete wilting (death) of the plant. Average reactions of three plants of each species per isolate was used for determining different levels of infection: no symptom (0) =1.0; Low (L) = 1.1-2.5; Medium (M) = 2.6-4.0; High (H) = 4.1-5.0

Table 9. Relative virulences of representative isolates of *R. solanacearum* (isolated from potato) to potato and tomato.

District/Major Location	Virulence index					
	Potato			Tomato		
	Percent	Arcsine transformed	Back transformed	Percent	Arcsine transformed	Back transformed
Bako	90.5	72.1a[a]	90.6	93.0	74.2ab[a]	92.6
Ambo	88.5	70.2a	88.5	95.5	77.8a	95.5
Shashemene	85.0	67.3ab	85.2	79.5	63.5b	80.2
Wondo Genet	76.5	61.2ab	76.8	57.5	49.3c	57.4
Jeldu	75.0	60.2ab	75.3	52.0	46.2cd	52.1
Inchini	55.5	48.3b	55.7	36.5	37.1d	36.4
Tsedey	87.0	69.0a	87.3	90.0	71.6ab	89.7
S.E.M. (df=13)		4.11			3.36	

[a]Means followed by the same letters in a column are not significantly different at p=5%.

Table 10. Mean latent tuber infection in market tubers and in tubers harvested from some representative infested potato crops and mean wilt incidence of the respective crops.

No	District/Major Location	Season	Latent tuber infection						Mean wilt incidence of crops sampled for tubers (%)
			Market tubers (%)	Tubers from infested crops					
				Number of crops sampled	Range (%)	Mean			
						Percent	Arcsine transformed	Back transformed	
1	Bako	Bega	36.5	3	43.3-48.1	45.7	42.5 (3.25)[a] cde[b]	45.7	30.6
2	Ambo	Bega	28.5	3	43.1-65.0	51.2	45.7 (2.52)bcde	51.3	24.0
3	Wondo Genet	Bega	50.0	3	50.3-71.4	61.3	51.5 (3.25) bc	61.1	9.1
4	Shashemene	Belg	37.0	3	41.7-60.0	53.7	47.2 (3.25)bcd	53.8	25.0
5	Jeldu	Belg	53.5	3	69.7-85.0	77.7	62.0 (3.25) a	78.0	2.7
6	Inchini	Belg	6.0	2	13.1-24.5	18.6	25.8 (3.98) f	19.0	1.5
7	Shashemene	Meher	48.5	5	50.0-73.6	65.3	54.0 (3.25) ab	65.5	20.4

( )[a] Standard error of means (S.E.M.)(df=15)

[b] Means followed by the same letters are not significantly different at p=5%

Table 11. Comparative latent tuber infection in three potato cultivars harvested from Turufe, Shashemene, during 1997 cropping seasons.

No	Cultivar	Latent tuber infection						
		Belg		Meher		Mean		
		Percent	Arcsine transformed	Percent	Arcsine transformed	Percent	Arcsine transformed	Back transformed
1	Awash	71.3	57.7	87.3	69.2	79.3	63.4a[a]	80.0
2	Genet	42.7	41.0	52.7	46.5	47.7	43.7b	47.5
3	Wochecha	68.7	56.0	65.3	53.9	67.0	54.9ab	67.0
S.E.M. (df=3)						3.73		

[a]Means followed by the same letters are not significantly different at p=5%.

5.10. Weed Host Plants

On investigation of weed plants as possible carriers of *R. solanacearum*, the bacterium was isolated only from *G. parviflora*. The isolates produced colonies typical of *R. solanacearum* in color and shape on TZC medium. The colonies grown on NA were also similar with those isolated from potato.

The isolates showed clear hypersensitivity reaction on tobacco infiltration. They were also found highly pathogenic to tomato and potato and induced wilting within 3 days. Inoculation of the isolates into *G. parviflora*, however, did not cause wilting of the weed or no disease symptom was observed.

6. DISCUSSIONS

6.1. Incidence and Prevalence of Bacterial Wilt

The results obtained in this study indicated that bacterial wilt of potato is a problem in the south and central region of Ethiopia and is generally widespread in mid-elevations (1650-2250m.a.s.l.) (Appendix Fig. 2). Among the surveyed areas, wilt incidence and prevalence are statistically higher (p=5%) at Bako, Shashemene and Ambo and low at Jeldu and Inchini.

Ambo

Potato bacterial wilt incidence in Ambo district is significantly higher than Wondo Genet, Jeldu or Inchini. Although, occurrence of the disease has been recorded since 1980 (SPL, 1981), the problem has become rampant since 1990 (pers. observation). As a result, most of the local farmers abandoned, or discontinued potato production limiting to homestead and a few fields. It seems that, the disease has become endemic. Farming practices including inadequate crop rotation, succesive potato cropping and use of non-selected (infected) planting materials are among the responsible factors for the relatively higher disease incidence and prevalence recorded.

Bako

Incidence and prevalence results showed that the disease is more severe in locations with a relatively lower altitudes. The areas studied around Bako, where the highest wilt incidence and prevalence are recorded, are located at a relatively lower elevation. The incidence and distribution of bacterial wilt in these locations may be attributed to local environmental conditions and agronomic practices of the local farmers. The disease has been occurring in the areas since a longer period of time, although, the first record was in 1978 (SPL, 1980) so that the pathogen might have been well adapted to the environment. Comparision of percent

wilt incidence (range 6-72, mean 27.8) recorded in this study to that recorded (range 3.8-24, mean 10.6) in 1985 (Yaynu Hiskias, 1986) at Bako also indicates the adaptation and build up of the pathogen population. It has been proved (He, 1990; Elphinstone and Alley, 1993: Machmud, 1993) that, long crop rotation and weed free fallowing of infested field reduce or eradicate the pathogen population from the soil. In these locations, however, crop rotation is usually short, mostly one or two non-host cropping between potato cropping, or farmers rotate potato with host crops mainly tomato. Successive potato cropping in the same field is also a common practice in the areas. These farming practices, however, are ineffective in reducing the inoculum potential from soil and some even contribute to its build-up. Studies have shown that (Saumtally *et al.*, 1993; Kishore *et al.*, 1995) warm temperature favours development of the pathogen. The relatively hot and humid climate around the areas might be conducive for the bacterium and enhance the infection. One of the means for the spread of the pathogen is irrigation water. Since farmers in the studied sites around Bako produce potato under irrigation conditions, it might have also contributed to the spread of the disease. Particularly, in one of the sites, Kejo, spread of the bacterium with irrigation water seems highly feasible, because, most of potato crops were situated side by side in a continuous manner without interruption with non- host crops or even border space so that the pathogen may reach adjacent fields from an infested field. Moreover, the area is sloppy lying along Gibe river. It seems therefore, the conditions allow easy movement of the pathogen within and across fields along with the irrigation water.

In conclusion, each of the above mentioned factors or their combined effect might have contributed to the relatively higher disease incidence and prevalence recorded in Bako area during 1996/97 cropping season.

Jeldu and Inchini

Despite the extensive potato cultivation, occurrence of bacterial wilt in Jeldu and Inchini districts was relatively minimal of all the studied areas. Mean wilt incidence is significantly (p=5%) low in both districts. Prevalence is even highly significantly (p=1%) low. These districts are known barley growing highlands with a relatively cool climatic condition. It has

been reported that occurrence of race 3 of *R. solanacearum* is limited in more cooler regions (French, 1986). The low bacterial wilt incidence and prevalence recorded were, therefore, due to the fact that the cold climatic condition of the areas may not be conducive for the development of the pathogen, or the pathogen might have been introduced recently so that it has not become well adapted to the environment, or it may be due to the combined effect of both of these factors.

Shashemene

Among the surveyed areas, production of potato takes place twice a year in Shashemene using Belg and Meher rains. Bacterial wilt incidences and prevalences recorded in both cropping seasons of 1997 indicated that the disease is relatively high and pauses a problem to potato production in the area.

Mean wilt incidence recorded during both seasons and prevalence during Meher are significantly higher than either of the studied areas except Bako. Although, the first record of occurrence of the disease in the district was in 1985 (SPL, 1986), local farmers believe that it has a longer history, probably more than two decades, and the disease occurs every season indicating that it has become endemic. Due to its high demand and price , however, local farmers produce potato extensively despite the disease problem. In Shashemene, crop rotation on potato harvested fields is usually short, one or two non-host cropping between potato croppings and these are inadequate to control the disease. Moreover, the production of potato twice a year consecutively on the same fields is a common practice that increases soil population of the bacterium (Kishore *et al.*,1995). These agronomic practices, a relatively warm climatic condition of the area and build up of the pathogen through time, therefore, seem to be among the responsible factors for the observed high disease incidence and prevalence.

Wondo Genet

In Wondo Genet, mean wilt incidence recorded is the least among the irrigated potato producing areas. Although, the information collected in this study is incomplete to explain this , the agronomic practices of the local farmers could probably be one of the contributing factors. Potato production in the area takes place only once during off-season of a year under irrigation. After harvest of potato, some fields are left fallow until the main rainy-season. During the main season, other non-host crops such as maize are cultivated on the fields. Moreover, Wondo Genet is one of the fruit growing areas. Sugarcane is also widely cultivated. These give long break to potato production. Studies have shown that, particularly, sugarcane is a non-host crop of *R. solanacearum* recommended to be used in effective crop rotation for bacterial wilt control (Saumtally *et al.*, 1993; Girard *et al.*, 1993). Such long break between potato croppings with non-host crops, therefore, reduces soil inoculum and may result in lower severity of the disease.

A common phenomenon observed in all areas considered in this study was that, most farmers use local or their own seeds as planting materials, though, some farmers at Bako used seeds produced at Jeldu. According to Shekhawat (1995), among the inoculum sources for potato bacterial wilt infection, the role played by seed tubers is the most important factor for the spread and recurrence of the disease; epidemics can occur more through seed-born inoculum than inoculum from other sources. It has been demonstrated that potato seed tubers carry the bacterium in vascular tissue, lenticels, and on the surface (Sunaina *et al.*, 1989; Bahal and Shekhawat, 1995). The use of infected local seeds particularly can result in more disease development than imported ones (Pradhanang *et al.*, 1993). Therefore, the higher wilt incidences and prevalences observed in most of the studied areas could also be explained by the use of local and non-selected (infected) seeds as planting materials.

Generally, results of wilt incidence and prevalence in the surveyed areas suggest that the disease is wide spread and is a problem to potato production particularly around Bako, Shashemene and Ambo areas and requires due attention.

6.2. Effect of Preceding Crops on Wilt Incidence

The survival of *R. solanacearum* in the soil as a source of inoculum for bacterial wilt infection has long been known (Graham and Lloyd, 1979; Akiew, 1986). The longevity of the pathogen, however, depends on such environmental factors as temperature and moisture (Akiew, 1986) as well as the presence of host plant debris and alternative hosts (French, 1994) and the strain of *R. solanacearum* (Akiew, 1986). It has been demonstrated (Graham, 1979; Graham and Lloyd, 1979) that, in fields devoid of host plants, survival of the pathogen is relatively shorter. Hence, crop rotation of the infested fields with non-host crops or fallowing is one of the recommended practices in the control of bacterial wilt infection (Akiew *et al.,* 1993; Elphinstone and Alley, 1993; He, 1990). The practice, in combination with other control measures, reduces the inoculum load or eradicate the pathogen from infested soil resulting in lower wilt incidence compared to successive host crop cultivation. Similar effect has been observed in this study too. Comparison of wilt incidences of potato crops preceded by non-host crops with those preceded by potato itself shows that successive cropping of potato resulted in significantly higher disease incidence and contributed 29.8% of the total wilt incidence recorded. The result shows that farming practices influence bacterial wilt development.

A number of studies have confirmed that extent of wilt incidence reduction due to crop rotation depends on the duration of non-host crop rotation or fallowing of the infested field. For instance, in one of related study (Sinha *et al.*, 1993), wilt incidence reductions of 55 and 67.5 %, were recorded, respectively, in two and three years non-host crop rotations as compared to successive potato cropping. Although, the duration and effectiveness of crop rotation varies and depends on several interacting factors such as strain of the pathogen, environmental conditions, and soil type (Saumtally, 1993), in general, with increasing the number of non-host crop rotations on infested field, survival of the pathogen in the soil also decreases resulting in a lower wilt incidence of the proceeding host crop. The data collected in this study, however, do not follow this general trend. In some potato crops cultivated on previously infested fields following two non-host crop rotations, wilt incidences were found to be higher than potato crops grown after one rotation. This could be the effect of such factors as using infected tubers as planting materials, or the involvement of weed host plants

in the persistence of the pathogen, or conduciveness of local environmental conditions for better development of the pathogen or their combined effect.

6.3. Cultural and Biochemical Characteristics

Triphenyl tetrzolium chloride medium of Kelman (1954) succesfully distinguishes isolates of *R. solanacearum*. Colony color and morphological characteristics presented by the isolates on the medium are similar to those reported by other workers (He *et al.,* 1983; Shekhawat and Gadewar, 1995). Production of levan and brown diffusible pigment are also in agreement with the characteristics described for the species (Hayward, 1964; Zehr, 1970a).

Oxidase, catalase, potassium hydroxide solubility and starch hydrolysis results, supplemented particularly with production of poly-ß-hydroxy butyrate, confirm that isolates belong to *R. solanacearum* (Zehr *et al.,* 1970; Sands *et al.,* 1980).

6.4. Carbohydrate Utilization

Results of oxidation and utilization of different carbohydrates obtained in this study were similar to those obtained in other countries (Hayward, 1976; He *et al.*,1983). Utilization of glucose oxidatively confirmed that isolates are aerobic which is one of the characteristics of *R. solanacearum*.

Oxidation of three disaccharides and three hexose alcohol sugars was the basis for the classification of strains of *R. solanacearum* into biovars (Hayward, 1964). The utilization of only the three disaccharides, but not the hexose alcohols, categorized isolates of the studied areas into biovar 2 of the species. The result is in agreement with that reported by Yaynu Hiskias (1989).

6.5. Pathogenicity and Hypersensitivity Reactions

All tested isolates were found pathogenic to potato and tomato plants, although, there were variations between the plants in the type of symptoms developed. Most isolates caused wilting of both host plants and few to eggplant. In response to some isolates, however, tomato plants showed symptoms such as development of adventitious roots , epinasty, irregular node development, and stunting. Such symptoms were reported to be caused by strains with reduced virulence (Shekhawat and Gadewar, 1995).

Wilt initiation of isolates in both potato and tomato was relatively more rapid and severe in incubated plants than plants kept in the greenhouse. Some of the wilted plants during incubation, however, rejuvenated and grew new leaves upon transfer to the greenhouse which indicates reduced virulence of the pathogen and tolerance of the host plants. The fluctuating greenhouse temperature, which was some times as high as 40°C and above, might have affected wilt development of the pathogen. Growth of plants was rapid and vigorous in the greenhouse, which could also be an alternative factor that affects wilt development. None of the isolates induced wilting in pepper that is observed in most of race 3 strains.

Tobacco host response was the characteristics used to differentiate between races (1-3) of *R. solanacearum* (Lazano and Sequeira, 1970). Race 1 strains induce necrosis and wilting of tobacco upon leaf infiltration while race 3 strains do not (Marin and Nashaar, 1993). Results of tobacco leaf infiltration and inoculation show variation among isolates that group them into two (Tables 4 and 5). Most isolates induced chlorosis of the infiltrated tobacco leaves which is a characteristics of strains of race 3. Meanwhile, some isolates induced necrosis within 2-3 days after infiltration of bacterial suspensions into tobacco leaves and few of these caused wilt in tobacco within 7 days after stem inoculation which are characteristics of race 1 and some low land race 3 strains (Marin and Nashaar, 1993). Therefore, since none of the isolates caused wilting upon infiltration into tobacco leaves, they are grouped under race 3.

However, according to Marin and Nashaar (1993), the above mentioned differences of tobacco responses to race 3 indicate that there might be phenotypic variation among isolates of the study areas, though it needs further investigation.

6.6. Relative Virulence

Among the major study areas, isolates from Bako and Ambo areas are found to be more virulent. Virulence of most isolates on potato is statistically similar except that isolates from Inchini showed significantly lower virulence than isolates from Ambo, Bako or Shashemene. This is probably because, the isolates are from the same host plant species, that is, potato. Virulence of isolates on tomato, however, varies. Isolates from Bako and Ambo are highly significantly aggressive compared to isolates from Wondo Genet, Jeldu or Inchini and significantly virulent than Shashemene isolates. Since both Ambo and Bako are relatively more tomato producing areas, the difference in virulence probably comes from better adaptation of isolates to the host plant. Mean percentage virulence of isolates from Tsedey is comparable to Bako and Ambo. Tsedey farm was producing potato seed tubers for many years both under irrigation and rainfed condions. But, due to bacterial wilt problem, the production was abandoned some ten years back, except in few plots mainly for experimental purposes.The farm also produces tomato which was also found attacked by the disease. The bacterium was isolated from few samples of wilted potato plants intercropped with Endod.The isolates are more virulent than the either two highland isolates, Jeldu and Inchini, particularly, on tomato wich indicates more adaptation of the isolates.

6.7. Latent Tuber Infection

Results of tuber study showed that in all the studied areas, mean percentage latent infection is lower in market tubers than in tubers collected from infested crops. This might be expected, since market tubers were bought randemly and there is a probability of coming from healthy crops as well. However, the fact that bacterial wilt-infested tubers are known to rot in transient and storage at high temperature, the result of market tubers could also be underestimated.

Among the studied areas, latent tuber infection is significantly high in both market and infested crops tubers produced at Jeldu, where wilt incidence was very low, and at

Shashemene in Meher produced tubers. It has been reported (Saumtally *et al.*, 1993) that, cool weather do not favor wilt development. Accordingly, the cold climatic condition of Jeldu perhaps affected wilt expression and tuber rotting effect of the pathogen so that the infection remained latent. When these tubers were incubated at higher ambient temperature, however, the activity of the pathogen increased and resulted in higher disease development. This finding is in agreement with the report of Gadewar and Chekrabarti (1995) in which, tubers harvested from cool regions and planted in warm regions have resulted in higher wilt incidence than tubers from warm regions cultivated in the same environment. On the contrary, the data shows that tuber infection is significantly lower in tubers harvested from study sites around Bako, where wilt incidence and prevlence records are highest, than tubers from Jeldu and Shashemene (Meher). Similarly, this could also be explained by temperature effect of the locations. The relatively warmer climate of the area could probably be favorable for the activity of the pathogen so that tuber tissues were disintegrated rapidly resulting in the elimination of some infected tubers. Hence, upon further incubation of the healthy looking tubers, the proportion of rotting tubers decreased resulting in a lower percentage of latent tuber infection. Therefore, although mean percentage tuber infection seems low, wilt incidence and prevalence records show that the disease is more severe around Bako than in Jeldu. The high tuber infection in Jeldu, however, alarms the potential spread and development of the disease in the district and even to other producing areas through the infected seeds.

The high percentage tuber infection coupled with the higher wilt incidence and prevalence recorded in Shashemene during both seasons indicates that the disease is widespread; the local conditions are conducive for the development of the pathogen and it has become a problem to potato production. Since seed tubers are the major disseminating agents of the disease, the result also suggests that the condition will be even more aggravated and severely hamper potato production.The same situation is observed in Ambo, though, tuber infection was not as high as that of Shashemene.

Tuber infection recorded from Inchini is least of all and is in agreement with wilt incidence and prevalence of the area indicating that occurrence of the disease in the district is scarce. Although occurrence of the disease is very low, detection of the pathogen suggests that

potentially the disease could be a problem to potato production in the district unless urgently checked.

It has been reported (Bahal and Shekhawat, 1995) that, latent tuber infection varies by season or year. In the study sites around Shashemene, infection in Meher-produced tubers was found to be significantly higher than Belg-produced tubers which might also be due to the effect of temperature differences between the two seasons.

Comparison of the three potato cultivars from Shashemene showed that tuber infection in Genet is significantly low. It indicates the variability of tuber infection by cultivar (Skoglund *et al.*, 1993). The result suggests, therefore, search for relatively tolerant varieties or cultivars to Ethiopian strains and minimizing losses incurred by the pathogen could be promising.

6.8. Weed Host Plant and Source of Inoculum

The possible sources of inoculum for bacterial wilt infection includes seed tubers, weed hosts, infested soil, irrigation water, cross contamination through farm implements, etc (Buddenhagen and Kelman, 1964; Martin and French, 1985; Akiew, 1986). The role of seed tubers, however, is more important in that it results in more infection and epidemics (Shekhawat, 1995). In this study, the disease symptoms observed in tubers and isolation of the pathogen from the tubers indicated that infected tubers are one of the sources of infection in the studied areas.

It has been demonstrated that weed host plants are involved in the persistence of *R. solanacearum* and serve as source of inoculum (Kishore *et al.*, 1995). They are problems in the control of the disease making crop rotation ineffective. On the studies of weed plants as possible carriers of *R. Solanacearum,* among five weed species investigated, a virulent strain of the bacterium was isolated from a healthy looking *G. parviflora*. The isolate exhibited typical characteristics of *R. solanacearum* on cultural, biochemical and physiological investigations. Hypersensitivity reaction on tobacco leaf infiltration and pathogenicity tests on major host plants also showed that the strain is virulent, similar to those isolated from the

proper host. Inoculation of the strain into healthy *G. parviflora*, however, didn't cause wilting or no visible wilt symptom was seen on the weed suggesting that it is only latently infected.

In conclusion, this study has revealed that *G. parviflora* is a latent carrier of *R. solanacearum* that could serve as a potential source of inoculum for the initiation of infection in host plants. In Ethiopia, though this is the only weed host sofar identified, there could be other carriers so that investigation on more common weeds is important.

Generally, this study indicated that the possible sources of inoculum for the observed infection in the surveyed areas could be seed tubers, infested soil and weed hosts among others.

7. SUMMARY AND CONCLUSIONS

Results of wilt incidence and prevalence as well as tuber infection show that bacterial wilt of potato caused by *R. solanacearum* is widespread and more severe in Bako, Shashemene and Ambo areas. The agronomic practices of farmers and local environmental conditions could probably be the reasons for the higher wilt incidences and prevalences observed in the areas. Non-host crop rotation practices of the infested fields are usually short and inadequate to reduce pathogen population of the infested soil. Moreover, rotation with other host plants and successive cropping of potato are among the contributing factors to the higher disease severity and distribution. The relatively warmer climate also seems favorable for the development of the pathogen in some of the areas. Spread of the pathogen with water could also be additional factor in the irrigated areas.

In Jeldu and Inchini, where the relatively lower wilt incidences and prevalences are recorded, the cooler climatic condition of the areas might have not been conducive to the pathogen for wilt expression or the pathogen may not yet adapted to the environment. As a result, the infection remains latent. This condition might have contributed to the relatively higher latent tuber infection observed in Jeldu, although, this may not be the case for Inchini.

In almost all studied areas, use of infected (non-selected) tubers as planting material seems the major factor for severe infections and progressive spread of the disease into previously disease-free areas.

Isolation of pathogenic strain of *R. solanacearum* from a healthy looking *G. parviflora* revealed that the weed is a latent carrier of the bacterium. It indicates that weed hosts also may serve as a source of inoculum for perpetuation of the pathogen in the studied areas.

Both phenotypes, virulent and avirulent forms of the pathogen, were detected on TZC medium with colony characters typical of *R. solanacearum* reported in other countries. Other cultural, physiological and biochemical investigations of isolates also showed similar characteristics described for the species. There was no marked difference among isolates in the utilization of the carbohydrates used to differentiate them into biovars. Consequently, all

isolates showed characteristics of biovar 2 of the species. Pathogenicity and hypersensitivity tests showed that, isolates are categorized into race 3, although, there are some differences among isolates in the latter test which needs further investigations.

Among the investigated isolates for relative virulence, those collected from Bako, Ambo and Tsedey were found to be more virulent on both potato and tomato. Virulence of the remaining isolates was even much lower on tomato than on potato.

Generally, bacterial wilt of potato is a widespread disease and is a problem to potato production in south and central zones of the country. Although, at present, the disease has become very serious in areas with higher incidence and prevalence record, the general trend of disease spread and detection of the pathogen suggests that the disease would potentially be a problem in other surveyed areas with lower severity and distribution too unless it is checked on time.

It is hoped that, this work may show the importance of bacterial wilt atleast in the studied areas. The disease is increasing in intensity, distribution into disease-free areas and parasitizing other hosts. Hence, to control the progressive spread of the disease and minimize the damage incured, future researches should focus on systematic studies that lead to design appropriate disease control and management strategies. The following activities are suggested as the main focus of future researches on the disease.

1) Distribution and severity of the disease in the remaining parts of the country on different hosts should be exaustively recorded.
2) Characteristics of all strains and races/biovars occurring in different hosts at different parts of the country should be elucidated using additional modern methods such as molecular techniques.
3) Investigations should be made on search for effective non-host crops and rotations against Ethiopian strains.
4) Search for resistant or tolerant cultivars to Ethiopian strains should be made.

5) Studies that lead to additional control measures, such as, on the ecology of the pathogen, and effect of sowing dates, chemicals and fertilizers should be conducted.
6) Depending on the nature of strains of the bacterium, appropriate and effective disease control and management strategies should be designed using integrated disease management (IDM) approach.

In the meantime, however, farmers should be aware of the disease and its control measures mainly of cultural practices such as crop rotations, use of clean seeds etc. Above all, since the major disseminating agents of the pathogen are seed tubers, establishment of healthy seed supply system is of paramount importance.

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APPENDIX

Appendix 1. Locations of some representative isolates of *R. solanacearum* recovered from potato and weed samples collected from the study areas.

Isolates code-RPP	Location	Altitude (m.a.s.l.)
484a, 485, 487, 488, 489, 491, 494, 542, 544, 546	Bako	1650-1800
506, 507, 508, 509, 514, 515, 516, 518, 519, 520, 522, (524), 685	Wondo Genet	1650-1750
550, 551, 552, 589, 597, 599, 603c, 605, (612), 614, 616, 618, (621), 622, 624, 625, 626, (628), 637, 640, 655, 658, 659	Shashemene	1800-2250
561, 562, 564, 565, 566, 567, 568a, 571, 574, 575	Ambo	2100-2250
569, 572, 573,	Inchini	2400-2550
553, 563, 576, 577, 578, 579	Jeldu	2750-3050
632, 648, 653	Tsedey farm	2500

Isolates in parenthesis are obtained from *G. parviflora,* the rest are from potato samples.

Appendix 2. Incidence of potato bacterial wilt in Ambo district, West Shoa zone, grown under irrigaion during Bega, 1996/97.

No.	Location	Field size (ha)	Wilt incidence (%)	Altitude (m.a.s.l.)
1	Mutulu(a)	0.30	11	2150
2	Mutulu(b)	0.50	36	2150
3	Mutulu(c)	1.00	45	2150
4	Birbirsa & Chirecha(a)	0.72	19	2100
5	Birbirsa & chirecha(b)	0.28	17	2100
6	Birbirsa & chirecha(c)	0.25	23	2100
7	Birbirsa & chirecha(d)	0.80	16	2100
8	MOA	1.00	3	2100
9	Maruf(a)	0.45	5	2200
10	Maruf(b)	0.40	7	2250
Mean			18.2	

Appendix 3. Incidence of potato bacterial wilt around Bako grown under irrigation during Bega, 1996/97.

No.	Location	Field size(ha)	Wilt incidence(%)	Altitude (m.a.s.l.)
1	Kejo	1.50	21	1675
2	"	0.40	35	1675
3	"	1.00	72	1675
4	"	0.75	8	1675
5	"	1.30	51	1650
6	"	0.60	29	1650
7	Jato	1.00	20	1800
8	"	0.50	12	1800
9	"	1.20	24	1800
10	"	0.40	6	1800
Mean			27.8	

Appendix 4. Incidence of potato bacterial wilt in Inchini district, West Shoa zone, during Belg, 1997.

No.	Location	Field size (ha)	Wilt Incidence (%)	Altitude (m.a.s.l.)
1	Buyoma kochore(a)	0.75	1.0	2400
2	Buyoma kochore(b)	1.00	1.0	2400
3	Buyoma Une	0.60	2.0	2550
Mean			1.3	

Appendix 5. Incidence of bacterial wilt in Jeldu district, West Shoa zone, during Belg, 1997.

No.	Location	Field size (ha)	Wilt incidence(%)	Altitude (m.a.s.l.)
1	Kata(a)	1.30	2	2750
2	Kata(b)	0.75	3	2750
3	Edensa galan	0.35	1	2850
4	Chilanko	0.80	1	2850
5	Galensa(a)	1.50	4	3050
6	Galensa(b)	0.75	2	3050
7	Sariti	0.50	3	2900
Mean			2.3	

Appendix 6. Incidence of bacterial wilt in Shashemene district, East Shoa zone,during Belg, 1997.

No.	Location	Field size (ha)	Wilt incidence (%)	Altitude (m.a.s.l.)
1	Abicha(a)	1.40	30	2075
2	Abicha(b)	0.60	28	2075
3	Abicha(c)	1.00	40	2075
4	Arabate(a)	1.00	20	2050
5	Arabate(b)	0.56	15	2050
6	Edola(a)	1.50	32	2050
7	Edola(b)	0.72	14	2050
8	Medo	0.32	8	1950
9	Turufe(a)Abiye Elamo	0.25	2	2000
10	Turufe(b)Abiye Elamo	1.20	31	2000
11	Jigessa	0.40	27	2100
12	Karara	1.00	28	2000
13	Melka Oda	1.00	5	2050
14	Woye Filicha	0.50	10	2050
15	Awasho Agamsa	0,50	25	2050
16	Awasho Dhanku	1.00	38	2150
17	Faji(a)	1.30	37	2250
18	Faji(b)	0.40	19	2250
19	Faji(c)	1.50	26	2250
20	Sole(a)	0.70	18	2075
21	Sole(b)	0.40	51	2075
22	Danaba(a)	0.80	10	1800
23	Danaba(b)	0.50	12	1850
24	Chabi Didagnata	0.50	18	1950
25	Siraro	0.75	14	1900
Mean			22.3	

Appendix 7. Incidence of bacterial wilt in Shashemene district, East Shoa zone, during Meher, 1997.

No.	Location	Field size (ha)	Wilt incidence (%)	Altitude (m.a.s.l)
1	Abicha(a)	1.25	15	2100
2	Abicha(b)	0.75	35	2100
3	Arabate(a)	0.72	33	2050
4	Arabate(b)	1.00	3	1950
5	Edola(a)	0.50	13	2050
6	Edola(b)	1.50	31	2050
7	Edola burka	0.25	14	2050
8	Medo	0.25	9	2050
9	Abiye elamo	o.75	16	2050
10	Elamo turufe	0.25	42	2000
11	Jigessa	0.75	18	2100
12	Karara	0.50	17	2000
13	Melka oda	0.25	13	1950
14	Woye filiche	0.50	19	2050
15	Awasho	2.00	40	2050
16	Faji(a)	0.50	8	2250
17	Faji(b)	1.00	18	2250
18	Faji(c)	0.45	30	2250
19	Sole(a)	1.50	11	2100
20	Sole(b)	0.75	21	2100
21	Sole(c)	0.60	37	2100
22	Toga(Danaba)(a)	0.50	18	1800
23	Toga(Danaba)(b)	0.25	7	1850
24	Chabididagnata	1.50	20	1950
25	Siraro	0.50	8	1900
Mean			19.8	

Appendix 8. Incidence of bacterial wilt around Wondo Genet, Sidama zone, grown under irrigation during Bega, 1996/97.

No.	Location	Field size (ha)	Wilt incidence(%)	Altitude (m.a.s.l.)
1	Wosha soyoma(a)	0.75	15	1650
2	" " (b)	1.30	6	"
3	" " (c)	0.60	9	"
4	" " (d)	0.50	4	"
5	Shasha kekele (a)	0.56	12	1650
6	" " (b)	0.35	3	"
7	" " (c)	0.50	6	"
8	Edo(a)	0.80	1	1750
9	" (b)	0.30	7	"
10	" (c)	0.50	9	"
Mean			7.2	

Appendix 9. Climatic Data of 1997 in Ambo Plant Protection Research Centre .

Month	Rain fall (mm)	Temperature (^{0}c)		Humidity (%)
		Min.	Max.	
January	27.8	10.6	26.8	68.5
February	0.0	10.0	28.1	58.4
March	26.7	12.9	29.5	50.1
April	157.0	11.9	27.8	60.9
May	79.2	11.7	28.8	59.6
June	204.5	11.5	26.0	73.2
July	276.5	11.5	23.2	83.6
August	128.2	11.2	23.8	85.2
September	64.5	10.1	26.4	76.8
October	118.7	10.7	26.1	75.1
November	42.6	10.7	26.0	66.5
December	4.6	9.6	28.1	53.1
Total	1130.2	132.4	320.6	811
Mean	94.18	11.03	26.72	67.58

Appendix 10. `Climatic Data of 1997 in Bako Research Centre .

Month	Rain fall (mm)	Temperature (^{0}c)		Humidity (%)
		Min.	Max.	
January	40.9	12.2	30.1	62.0
February	0.00	11.2	31.2	51.6
March	16.3	14.7	31.4	54.3
April	177.3	14.7	28.9	63.3
May	168.9	15.4	27.5	62
June	173.3	15.1	24.4	73.1
July	303.3	15.1	23.3	71.8
August	169.4	15.3	23.8	84.0
September	70.0	14.3	24.3	78.0
October	252.4	14.2	25.5	76.6
November	66.8	14.2	27.1	75.0
December	3.2	13.5	27.7	66.4
Total	1388.8	169.9	325.5	818.1
Average	115.7	14.2	27.1	68.2

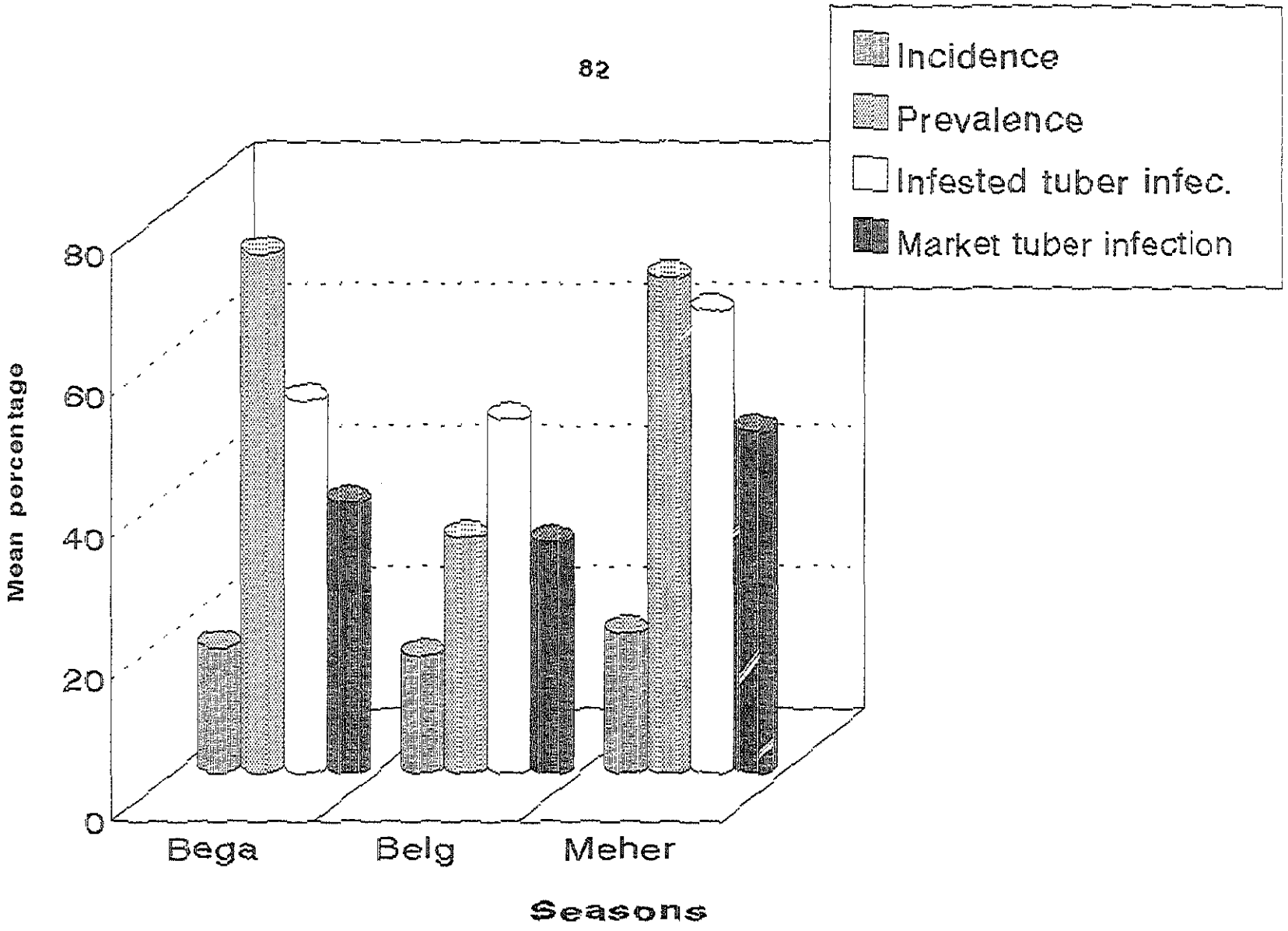

Fig. 1. Comparative seasonal mean incidence, prevalence and tuber infection of potato bacterial wilt in south and central zones of Ethiopia in 1996/97.

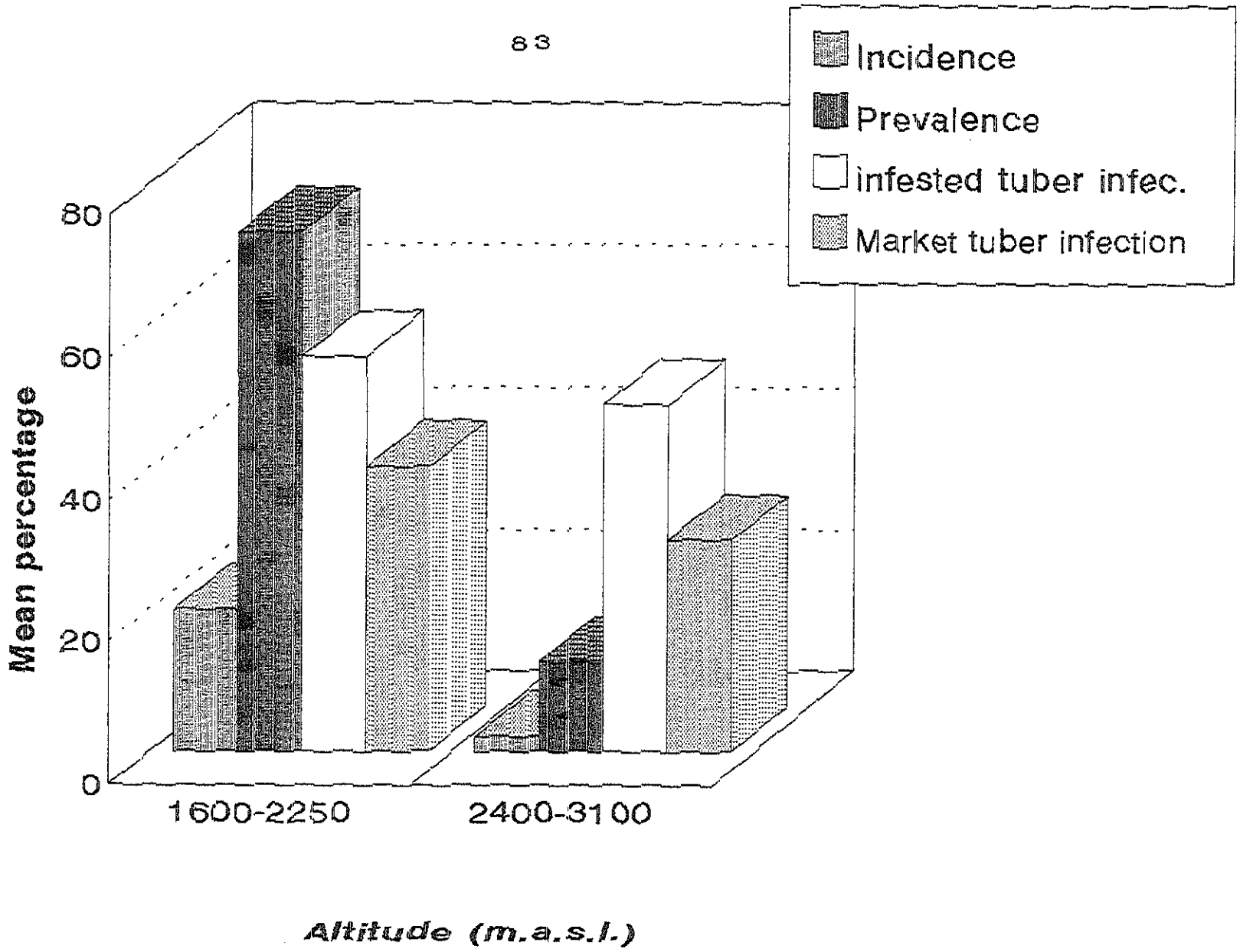

Fig. 2. Effect of altitude on mean percentage incidence, prevalence and tuber infection in south and central zones of Ethiopia in 1996/97.