ECOLOGY, EPIDEMIOLOGY AND PATHOGENIC VARIABILITY OF *PSEUDOMONAS SYRINGAE* PV. *PHASEOLICOL*A IN THE SOUTHERN HIGHLANDS OF TANZANIA.

BY

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ABSTRACT

Investigations were carried out to determine races of *Pseudomonas syringae* pv. *phaseolicol*a existing in the Southern Highlands of Tanzania and the relationship between motility and virulence of the pathogen. The epiphytic survival of a brown diffusible pigment producing strain (9337- uncertain race type) and a non brown diffusible pigment producing strain (9359-race 6) of *P.s.phaseolicola* was also studied in bean genotypes Kablanketi and Uyole-90.

On the basis of cultural, biochemical and pathogenicity tests, 260 isolates were identified as *P.s.phaseolicola*. Using the Biolog Microplate identification system, other isolates of fluorescent pseudomonads encountered were confirmed to be *Pseudomonas fluorescens* A and *Pseudomonas syringae* pv. *syringae* A.

Race determination, using eight halo blight differential bean cultivars, showed that races 1, 2, 3, 4, 5, 6, 7b and 8 exist in the Southern Highlands of Tanzania. In addition, 7.6% of the strains characterized were of uncertain race type, suggesting occurrence of wider pathogenic variability than could be categorized using the current set of halo blight differential cultivars. Races 3 and 6 were the most prevalent. Race 3 occurred at a high frequency in Mbeya and was predominant in Nkasi and Sumbawanga districts. About 32% of the isolates collected produced the brown diffusible pigment *in vitro*. Some brown pigment producing strains were found to be race 3. Race 3 strains, which cause a very strong hypersensitive reaction associated with systemic necrosis on several bean genotypes including Tendergreen and A53, exist in the region.

Strains isolated from *N.wightii* and *Desmodium* sp. and characterized as race 7b were less virulent on bean cultivars A52 and A53 than race 7b strains originating from *P.vulgaris*.

Thirty-six germplasm accessions were tested for resistance to eight strains of *P.s.phaseolicola*. New sources of resistance to races 1, 3, 4, 5, 7b, and strains 9337 (brown diffusible pigment producing strain) and 14494 (closely related to race 9) were identified. Differences in motility between and within races of *P.s.phaseolicola* were also demonstrated.

Results from epiphytic population studies showed that there were differences between bean genotypes Kablanketi and Uyole-90 in supporting the epiphytic populations of the brown diffusible pigment producing strain 9337 and race 6 (strain 9359) of *P.s.phaseolicola*. The resulting halo blight disease severity scores were generally low but comparably higher on Kablanketi inoculated with race 6.

In vitro and *in vivo* studies under greenhouse conditions were conducted to determine the inhibitory effect of garlic bulb extract on growth of races 3, 6 and a brown diffusible pigment producing strain 9337 (uncertain race type) of *P.s.phaseolicola*. Antibacterial activity was shown as clear zones of inhibition. Halo blight disease incidence and severity on bean genotypes Uyole-90, Canadian Wonder and Kablanketi were significantly reduced.

Pseudomonas syringae pv. *phaseolicol*a strains 9337 (a diffusible brown pigment producer of an uncertain race type) and 9359 (race 6) survived in immature flat green pod tissues at 18 to 25^{0}C in the laboratory for nine months.

DECLARATION

I, **BETTY JAMES GONDWE,** do hereby declare to the Senate of Sokoine University of Agriculture that this thesis is my own original work and has never been submitted for a degree award in any other University.

8-12-1998
Date

Signature

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That this work is successfully completed is a testimony of **God's** faithfulness. To **HIM** I rest my hopes and I am ever grateful.

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To my daughter, **NAEMI BEATRICE.**

To the memory of,
my grandmother, the late **LONGILUMUTWA SEMULIGULA,** who taught me how to go about in times of difficulties;

My parents, the late **ANNA TIMOTHY LUPEMBE** and **JAMES GONDWE.**

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LIST OF ACRONYMS

BDP	Brown diffusible pigment
CFU	Colony forming units
EPS	Extracellular polysaccharides
GBE	Garlic bulb extract
GE	Germplasm evaluation
HR	Hypersensitive reaction
ISR	Induced systemic resistance
KB	Kings medium B.
MARTI	Ministry of Agriculture Research and Training
NA	Nutrient agar
PCA	Plate count agar
SDW	Sterile distilled water
SNA	Sucrose nutrient agar
STM	Semi solid tryptone media
SUA	Sokoine University of Agriculture
TSA	Tryptic soy agar

CHAPTER 1

INTRODUCTION

Halo blight caused by *Pseudomonas syringae* pv. *phaseolicola* (Burkholder) Young *et al.* is a systemic disease of beans *Phaseolus vulgaris* L. which was first recorded in Tanganyika by Wallace and Wallace (1949). In tropical areas the disease is most destructive in areas with heavy rainfall and moderate temperatures. Before 1991, very little research attention was given to the disease in the Southern Highlands of Tanzania. During the 1991 to 1995 bean growing seasons, severe outbreaks of halo blight disease were observed in farmer's fields in various parts of the Southern Highlands (Gondwe, unpublished). However, reasons for such severe outbreaks were not clear.

The use of resistant cultivars and cultural practices, such as pathogen-free seed that has been inspected during production and tested for absence of *P.s. phaseolicola*, are the most practical methods for halo blight disease management (Zaumeyer and Thomas, 1957). However, programmes to produce certified bean seed in Tanzania are lacking. Therefore, the majority of small scale farmers in the Southern Highlands, like in other parts of Tanzania, plant seed selected from the previous crop or use bean seed purchased from local markets. Although plant resistance is the most feasible option for managing halo blight disease for small scale bean growers (Beebe and Pastor-Corrales, 1991), the wide pathogenic variability of *P.s.phaseolicola* could render a cultivar that is resistant in one location, susceptible to halo blight in another location.

Occurrence of numerous races of the pathogen complicates the management of the halo blight disease. Therefore, constant monitoring of pathogenic variation of *P.s. phaseolicola* is essential if any strategy for breeding resistant bean varieties is to be effective.

Motility has been associated with virulence of bacteria. The work of Panopoulos and Schroth (1974) showed that the infectivity potential of *P.s.phaseolicola* could largely be influenced by its variation in motility. However, the relationship between motility and virulence of *P.s. phaseolicola* strains from the Southern Highlands of Tanzania has not been studied. Such information is important in understanding the role of motility in pathogenesis of *P.s. phaseolicola* in beans.

Pseudomonas syringae pv. *phaseolicola* is known to survive in infected seed, plant residues, on volunteer bean plants and on weeds (Mabagala and Saettler, 1992; Natti, 1967; Taylor, 1970). The bacterium has also been reported to grow epiphytically on aerial plant surfaces of beans (Legard and Schwartz, 1987; Stadt and Saettler, 1981). Furthermore, Stadt and Saettler (1981) found pronounced differences among bean cultivars in supporting epiphytic growth of the halo blight organism. These reports led to the suggestion that epiphytic populations of *P.s.phaseolicola* have the ability to initiate outbreaks of halo blight when suitable conditions prevail (Hirano and Upper, 1983). In the Southern Highlands of Tanzania, the majority of farmers grow beans as varietal mixtures. Sources of inocula of *P.s.phaseolicola* on the different bean cultivars and landraces grown in this part of the country have not been investigated.

Therefore, quantitative studies on epiphytic populations of *P.s. phaseolicola* on the most widely grown landraces and varieties in the Southern Highlands are needed to provide a better understanding of the complete actiology of *P.s.phaseolicola* in this area. Such information is useful in understanding the epidemiology and management of the halo blight disease.

The objectives of this study were therefore:

(a) To identify races of *P.s.phaseolicola* occurring in the Southern Highlands of Tanzania.

(b) To study the relationship between motility and virulence of *P.s.phaseolicola*.

(c) To study the epiphytic survival of the halo blight pathogen in bean (*Phaseolus vulgaris* L.) landraces and varieties grown by farmers in the Southern Highlands and assess the potential of epiphytic populations of *P.s.phaseolicola* as sources of inoculum.

CHAPTER 2

LITERATURE REVIEW

2.1 Occurrence and Importance.

Halo blight of beans (*Phaseolus vulgaris* L.) incited by *Pseudomonas syringae* pv. *phaseolicola* (Burk) Young *et al.*, was first studied by Burkholder in 1926 in New York. The disease has world-wide occurrence although in the tropics it is economically important in areas with moderate to cool temperatures (Beebe and Pastor-Corrales, 1991). In their report, Taylor *et al.* (1996) stated that in the Highlands of East, Central and Southern Africa, halo blight is prevalent in the medium to high altitude (1500-2500 masl) bean growing areas. However, in northern Tanzania halo blight has been found even at an altitude of 1020 masl. (Gondwe, 1987). In the Southern Highlands of Tanzania, halo blight disease develops early in the growing season and in the field, disease incidence may reach 100% on beans planted in November and December (Gondwe, 1990). Under epidemic conditions, halo blight disease can severely reduce bean yield. Seed yield losses of 42% have been recorded for the susceptible cultivar FB/GP 307-2 in field trials conducted at Lyamungo in Northern Tanzania (Mushi *et al.*, 1989). In Michigan, yield losses of 23 to 43% have also been reported (Schwartz, 1989). In snap bean production, losses due to halo blight disease are usually expressed as reduction in quality due to pod lesions (Webster *et al.*, 1983).

2.2 The Pathogen

Pseudomonas syringae pv. *phaseolicola* has been studied under various synonyms as listed by Bradbury (1986). The organism has rod-shaped cells occurring singly or in pairs. It is gram negative (Duodoroff and Palleroni, 1974) and oxidase-negative (Kovac's, 1956). Many strains of *P.s. phaseolicola* are motile by means of polar flagella (Bradbury, 1986). On nutrient agar, the colonies are circular and slightly raised (Lelliott and Stead, 1987). Rough and smooth colonies which differed serologically and in sensitivity to bacteriophage have been reported (Adam and Pugsley, 1934). *Pseudomonas syringae* pv. *phaseolicola* produces a water soluble fluorescent green pigment in medium B of King *et al.* (1954). The pigment fluoresces blue-green under ultra-violet light (254nm and 366nm). Some strains produce a brown diffusible pigment (BDP) *in vitro* (Gondwe, 1987; Mabagala and Saettler, 1992; Taylor and Teverson, 1985). Optimum temperatures for growth of *P.s.phaseolicola* range between 25 and 30°C. Some strains can grow at 4°C (Bradbury, 1986). The bacterium produces a phytotoxin called "phaseolotoxin" which is a virulence factor (Mitchell, 1978; 1984; Prosen *et al.*, 1993).

2.3 Symptomatology.

Halo blight disease can affect all aerial parts of the bean plant. On leaves, symptoms first appear as small water soaked lesions. Appearance of water soaked lesions has partially been associated with the occurrence of extracellular polysaccharides (EPS's), a less specific mechanism of pathogenesis (Denny, 1995). The EPS produced by bacteria in pathovars of *P.syringae* are loose slime layers. The slime produced (after infection) fills the intercellular spaces of the leaves and maintains a moist environment in which the bacteria can grow. It is postulated that this action causes the lesions to appear water soaked (El-Banoby and Rudolph, 1979; Fett and Dunn, 1989).

Haloes of greenish-yellow tissue may appear around the perimeter of the water soaked areas. During a severe epidemic, stems and pods may become infected. On stems, symptoms appear as reddish dashes extending longitudinally with the stem (Lelliott and Stead, 1987). On pods, halo blight disease symptoms appear as water-soaked spots which may enlarge to irregular blotches. Bacterial ooze may cover these spots. Bacteria on the pods may work their way through the tissue and infect the bean seeds. The bacterium may also enter the pods through the vascular system and infect the seed without causing lesions on the surface of the pods (Burkholder, 1926; Lelliott and Stead, 1987; Schwartz; 1989; Taylor *et al.*, 1979). Halo blight disease can infect the vascular system of bean plants, causing systemic chlorosis. The systemic chlorosis is caused by a phytotoxin called "phascolotoxin" (Ferguson and Johnston, 1980; Mitchell, 1976; 1978; 1984; Patel and Walker, 1963; Patil *et al.*, 1972; Patil, 1974; Skoog, 1952; Staskawicz and Panopoulos, 1979; Turner, 1986). Phaseolotoxin is an ornithine - alanine - arginine tripeptide carrying a phosphosulfamyl group (Mitchell, 1976; Peet *et al.*, 1984). The toxin inhibits the enzyme ornithine-carbamoyltransferase (Octase) involved in the metabolism of arginine and glutamate; and causes ornithine to accumulate in infected bean leaves (Ferguson and Johnston, 1980; Patel and Walker, 1963).

Pseudomonas syringae pv. *phaseolicola* is able to produce two detectable Octase activities, one sensitive and one resistant to phaseolotoxin *in-vitro*. The resistant activity is produced at 20°C but is undetectable at 30°C (Fuente *et al.*, 1993). The production of phaseolotoxin by *P.s.phaseolicola* is temperature regulated. It is produced optimally at 18 to 20°C. However, no detectable amounts of the toxin are produced at 30°C (Goss, 1940; Hoitink *et al.*, 1966; Skoog, 1952; Staskawicz and Panopoulos, 1979).

Lindgren *et al.* (1986), showed that *P.s.phaseolicola* possesses a group of genes designated hrp (phonetic "harp" for hypersensitive reaction and pathogenicity) that is essential for the induction of disease symptoms and for the elicitation of the hypersensitive reaction on resistant plants.

The known hrp genes in *P.s.phaseolicola* are clustered in a large genomic region that has been designated the hrp cluster. Organization of the hrp cluster from *P.s.phaseolicola* show that it is comprised of seven complementation groups designated as hrpL, hrpAB, hrpC, hrpD, hrpF, hrpE and hrpSR. The cluster encodes functions involved in the plant-bacterial interactions essentially throughout its length (Rahme *et al.*, 1991).

2.4 Host range

Phaseolus vulgaris is a major host species of *P.s. phaseolicola*. However, several other plant species have been reported to be hosts of this pathogen. In a report by CIAT (1987a), fifteen plant species that can be hosts of *P.s. phaseolicola* have been listed. In Tanzania, the pathogen has been found occurring naturally on *P. vulgaris*, *Dolichos* sp., *Vigna radiata*, *Cajanus cajan*, *Neonotonia wightii*, *Desmodium* sp., and *Phaseolus coccineus* (Gondwe, 1989; Mabagala and Saettler, 1992; Taylor and Teverson, 1988).

2.5 Pathogenic variation

Pathogenic variation in *P.s.phaseolicola* was first shown by Jensen and Livingstone (1944), in Nebraska, USA. Patel and Walker (1965) differentiated race 1 from race 2 on the basis of reaction of Red Mexican UI3. On this basis, races 1 and 2 have been reported by several different investigators (Boelema, 1985; Hale and Taylor, 1973; Kinyua *et al.*, 1981; Msuku, 1984; Wharton, 1967). Other workers have however, expressed some feelings that race designation was not valid. Schroth *et al.* (1971), for example, stated that, neither neither races 1 nor race 2 was homogeneous with respect to virulence when tested on a number of cultivars. They further stated that the separation of isolates into race 1 and race 2 on the basis of the Red Mexican cultivar was a separation of strains with different degrees of virulence. In addition, Schroth *et al.* (1971), felt that every strain could be designated as a different race.

They concluded that there may be an infinite number of races for *P. phaseolicola*. In central Nebraska where halo blight disease devastated a 600-acre plot of snap bean in 1964, strains which were more virulent than those reported for race 2 was found (Schuster *et al.*, 1965). Later, Coyne *et al.* (1979) and Schuster *et al.* (1979), suggested the existence of race 3 in the USA. When isolates of African origin were examined, a third race was confirmed (Taylor and Teverson, 1985). Race 3 was virulent towards *P. vulgaris* cultivars resistant to race 1, but caused hypersensitive reaction in cultivar Tendergreen. In Tanzania, race 3 has been reported to occur in Northern and Southern Highlands and in Kibondo district,Western Tanzania (Gondwe, 1987; Mabagala and Saettler, 1992; Taylor and Teverson, 1988a). Other characters have been used to identify races of *P.s.phaseolicola*. Rough and smooth colony types, which differed serologically and in sensitivity to bacteriophages were described by Adam and Pugsley (1934). The rough forms were characterized by lack of or weaker virulence compared to smooth colonies. In 1942, Jensen and Goss reported strains of halo blight, which induced only small haloless spots on Red Mexican but produced lesions with typical haloes on Red Kidney. Schuster and Coyne (1973), pointed out that these previous workers had essentially been working with strains of *P.s.phaseolicola* that would be relegated to race 1. In previous work, Gondwe (1990), characterized strains of P.s.phaseolicola from the Southern Highlands of Tanzania using cultivars Red Mexican UI3, Edmund, Tendergreen and 10 other *P.vulgaris* genotypes. Results showed that there were differences within and between the then existing three races of *P.s.phaseolicola*. Existence of additional races was reported by Teverson and Taylor (1991). These researchers subdivided the original race 1 into races 1, 5, 7 and 9 (all containing avirulence gene 1-A1; race 5 carries avirulence genes A2+A4; race 7, A1+A2 and race 9, A1+A5). Original race 2 was subdivided into races 2, 6 and 8 (race 2 contains avirulence genes A2+A5, race 8, A5 but race 6 had no avirulence genes; and attacked all the differential cultivars). Original race 3 was subdivided into races 3 and 4 (race 3 contains avirulence gene 3-A3 and race 4, avirulence genes A2+A3).

Races 8 and 9 were identified following further collections of halo blight diseased material in Malawi and Lesotho in 1989 and 1990. Race 3 which was originally reported not to be occurring outside Africa, was found in east Antioquia, Colombia in 1989 (Tamayo and Pastor-Corrales, 1991).

2.6 Motility

Pseudomonas syringae pv. *phaseolicola* is motile by means of flagella. Flagella motility in *P.s.phaseolicola* was associated with increased virulence on bean leaves when motile strains caused up to 12 times as many lesions as non-motile strains (Panopoulos and Schroth, 1974). However, Msuku (1984), found no relationship between motility and virulence of *P.s.phaseolicola* strains from Malawi. In nature, invasion of plant tissue by *P.s. phaseolicola* and other foliar bacterial pathogens generally occur through natural openings such as stomata, hydathodes or nectaries (Zaumeyer, 1932). Infection usually occurs during periods of free moisture such as following a rain or heavy dew or under high humidity (Watson *et al.*, 1973). It appears therefore, that under periods of free moisture, or high relative humidity, motile strains of *P.s. phaseolicola* would have considerable advantage over non-motile strains in the invasion of plants. Motility contributes to bacterial movement to internal surfaces of the plants and other protected sites (Hattermann and Ries, 1989). This allows the bacteria to avoid environmental stresses (Haefele and Lindow, 1987). Motility in bacteria can therefore be considered as an adaptation to avoid environmental stresses on leaf surfaces (Beattie and Lindow, 1995).

2.7 Survival

Infected bean seed is an important means of survival for *P.s. phaseolicola* in time and space. Therefore, several workers have made investigations aiming at developing assays for detection of the pathogen in seed lots (Audy *et al.*, 1996; Guthrie *et al.*, 1965; Jansing and Rudolph, 1990; Katznelson and Sutton, 1951; Taylor, 1970; Trigalet and

Rat, 1975; Wharton, 1967). The survival period for *P.s.phaseolicola* in seed can be influenced by conditions under which the seed is stored. The pathogen will survive poorly when seed is stored dry at 75% RH (Leben, 1965). Other studies have shown that *P.s. phaseolicola* can survive beyond the germination ability of the infected seed (Taylor *et al.*, 1979). Crop residues provide another major means by which *P.s. phaseolicola* survives. The pathogen can overwinter in undecomposed stems, pods and leaves of bean plants left standing in the field (Natti, 1967). *Pseudomonas syringae* pv. *phaseolicola* can survive for 12 months within dried infected bean leaves stored at 24°C in the laboratory, as well as within pulverized infected bean leaves mixed with dry soil and stored at 5 and 24°C, and with moist soil at 5°C (Natti, 1967). The importance of bean straw in the overwintering of *P.s.phaseolicola* (race 1, race 2 and Nebraska 16) was reported by Schuster and Coyne (1974). Investigations carried out in Northern Tanzania showed that the ability of *P.s.phaseolicola* to survive in bean debris in the soil vary depending on race, geographic location, depth of placement of debris in the soil and bean genotype used (Mabagala and Saettler, 1992b). Survival of *P.s. phaseolicola* on perennial weeds such as *Neonotonia wightii* has also been reported in northern Tanzania (Mabagala and Saettler, 1992a).

2.8 The epiphytic or resident phase.

Several workers have shown that *P.s. phaseolicola* is capable of establishing a resident or epiphytic phase on beans (Hirano and Upper, 1983). Epiphytic bacteria have been defined as bacteria that are capable of living (multiplying) on surfaces of apparently healthy plants and that they can be removed from leaves by washing (Hirano and Upper, 1983), or killed by UV irradiation or leaf surface disinfecting by chemicals (Henis and Bashan, 1986). Epiphytic bacteria are usually enumerated by counting the bacteria in washes of leaves (Leben, 1965).

Epiphytic populations studies on *P.s.phaseolicola* were first reported by Ercolani *et al.* (1974), when they recovered *P.s.phaseolicola* (<10^3 viable cells/g fresh weight) from leaf surfaces of hairy vetch (*Vicia villosa*) both in the summer and in the fall. In 1981, Stadt and Saettler demonstrated the ability of a rifampicin resistant mutant of *P.s.phaseolicola* to establish an epiphytic phase on bean leaves. Legard and Schwartz (1987) reported that volunteer beans were infected and/or colonized by epiphytic populations of *P.s. phaseolicola* in Colorado, USA. However, such studies have not been conducted in the Southern Highlands, Tanzania.

2.9 Dispersal

Pseudomonas syringae pv. *phaseolicola* may spread from infected or infested to clean seed during handling operations between threshing and planting. Dried bacterial ooze can form flakes, which contaminate the seeds (Grogan and Kimble, 1967). Heavily infected seed usually do not germinate. When this happens, the pathogen can spread from seed which has failed to germinate to a nearby germinating seed or developing seedling (Schuster and Coyne, 1974). Another way by which *P.s.phaseolicola* bacterial cells can move between plants is splash dispersal by rain (or irrigation) (Vennete, 1982). Movement of the bacterium between plants has also been shown to occur by aerosols generated during dry, sunny, windy weather, human activities, insects and by aerosols dispersed into the troposphere followed by atmospheric scrubbing by rain (Constantinidou *et al.* 1990; Lindemann and Upper,1985; Venette, 1982; Walker and Patel, 1964).

2.10 Halo blight disease management strategies.

Various management strategies have been employed to reduce yield and quality losses caused by halo blight disease in beans.

2.10.1 Use of pathogen - free seed.

The use of pathogen-free seed is one of the practical management strategies for halo blight disease. *Pseudomonas syringae* pv. *phaseolicola* is seed-borne and therefore, many countries have strict quarantine laws regarding the movement of bean seed across the borders (Geng *et al*, 1983; Grogan and Kimble, 1967). In order for the use of pathogen-free seed to be effective, detection of *P.s. phaseolicola* in seed is essential. In Idaho, where 80% of the bean seed in the United States are grown, regulations require that the bean seeds be assayed by the State Department of Agriculture for freedom from *P.s. phaseolicola* contamination. Furthermore, all bean crops grown for seed are field inspected and if halo blight is found, the crop must be destroyed (Webster *et al.*, 1983). Various techniques for the detection of *P.s.phaseolicola* in bean seed have been developed (Audy *et al.*,1996; Lahman and Schaad, 1985; Prosen *et al.*, 1993; Webster *et al.*, 1983). Despite such efforts, bean seed produced in Idaho is not always free of the halo blight pathogen (Webster *et al.*, 1983). The use of pathogen-free seed can be complicated by the fact that *P.s. phaseolicola* may survive on the testa of dry seeds in a manner comparable to epiphytic saprophytic bacteria (Grogan and Kimble, 1967). In developing countries like Tanzania, farmers use seed saved from the previous crop or bought from neighbours or market place.Therefore, the use of pathogen-free seed to manage halo blight disease is far from immediate uses by the subsistence farmers in developing countries like Tanzania. Removal of infected seed based on coloration, as practised by some farmers, cannot eliminate all infected seed (Taylor,1970).

2.10.2 Chemical control.

Chemical control of halo blight is usually done using copper-based chemicals (Hagedorn *et al.*, 1969; Taylor and Dudley, 1977). However, the use of chemicals in the control of *P.s. phaseolicola*, has proved ineffective because of the development of resistance by the pathogen (Taylor and Dudley, 1977).

In addition, the current concern over the use of chemicals, especially in food crop production and the high cost of chemicals, make their use in the management of the halo blight disease unattractive.

2.10.3 Cultural control

Deep ploughing, crop rotation (Zaumeyer and Thomas, 1957), elimination of crop residues after harvest, destruction of weeds and alternate hosts (Mabagala and Saettler, 1992; Natti, 1967; Schuster and Coyne, 1974), have been suggested as cultural practices for halo blight disease management.

2.10.4 Biological control

Biological control of plant diseases is an alternative approach, which is likely to work best as a component of integrated disease management systems. Previous studies on the introduction of antagonists for biological control of plant pathogens, had used the root-colonizing pseudomonads which are common rhizosphere inhabitants (Evans, 1992). About 36 years ago, *Pseudomonas fluorescens* isolated from bean (as contaminants) was reported to provide excellent protection when applied to bean leaves by rubbing, spraying or using toothpick, before but not after, inoculation with *P.s.phaseolicola* (Teliz-Ortiz and Burkholder, 1960). Recently, (Alström, 1991; 1995) provided evidence of disease resistance induced by rhizosphere pseudomonads against *P.s. phaseolicola*. The concept of induced systemic resistance (ISR) involves the activation of the plants defence mechanism which lead to systemic protection (Fravel, 1988; Liu *et al.*, 1992; Tuzun and Kloepper, 1994; van Peer *et al.*, 1991; Wei *et al.*, 1991; Zdor and Anderson,1992). Associations of L-form (*P.s. phaseolicola*) bacteria with bean plants have also been shown to confer induced systemic resistance in bean plants. (Amijee *et al.* 1992).

2.10.5 Use of resistant varieties

Resistance to *P.s. phaseolicola* has been known for nearly 50 years now. For example the bean variety Hidatsa Red was found to be immune while Peerless was reported to be resistant to the halo blight disease (Burkholder, 1948). In 1965, Patel and Walker reported that PI 150414 was resistant to both races 1 and 2 of the halo blight bacterium. Later, Coyne *et al.* (1966), found resistance in cultivar GN Nebraska No 1 sel. 27. Hagedorn *et al.*, (1974), identified resistance in Wis HBR 40 and Wis HBR 72. Davis *et al.* (1986), reported that resistance to race 3 was present in several *P.vulgaris* materials of North and South American origin. In Tanzania, bean genotype Mwanga Chuchu (Karagwe) was reported to be resistant to races 3, 4 and 5 (races 1 and 3 in the original race structure). Other bean genotypes, such as Masai Red and TB 79/248, were reported to be resistant to race 5 (race 1 in the original race structure) (Gondwe, 1987; Taylor and Teverson, 1988b). These reports indicate the possibility that resistance to *P.s. phaseolicola* can be found in locally well adapted *P. vulgaris* materials grown in Tanzania.

CHAPTER 3

MATERIALS AND METHODS

3.1 LOCATION

This study was conducted at the Ministry of Agriculture Research and Training Institute, Uyole (MARTI-Uyole), Mbeya. MARTI-Uyole (formerly Uyole agricultural Centre), is located about 10 km North of Mbeya at an altitude of 1798 metres above sea level (masl) in the Southern Highlands of Tanzania. The Institute is at latitude 8° 83' South and longitude 33° 34' East. Rains fall from November to May. Rainfall pattern is unimodal with annual totals ranging from 600 to 1400 mm (1980 to 1993 monthly total rainfall data from meteorological station at Uyole). The weather is relatively cool, with monthly mean maximum temperature ranging between 21.8 and 24.8°C and monthly mean minimum ranging between 9.9 and 11.1°C. (1980 to 1993 monthly mean temperature data from meteorological station at Uyole). The lowest temperatures are recorded between June and August. Mbeya gets frost during this period. Farmers in the Southern Highlands of Tanzania plant beans either at the onset of rains (November to January) or during the February to March period.

3.2 Collection of infected samples

Bacterial isolates were obtained from halo blight and halo blight-like infected material collected from in and out of bean fields in 1993, 1994 and 1995. They came from more than 60 villages in Iringa, Mufindi, Njombe, Mbeya, Mbozi, Ileje, Nkasi and Sumbawanga districts in the Southern Highlands of Tanzania (Fig. 1).

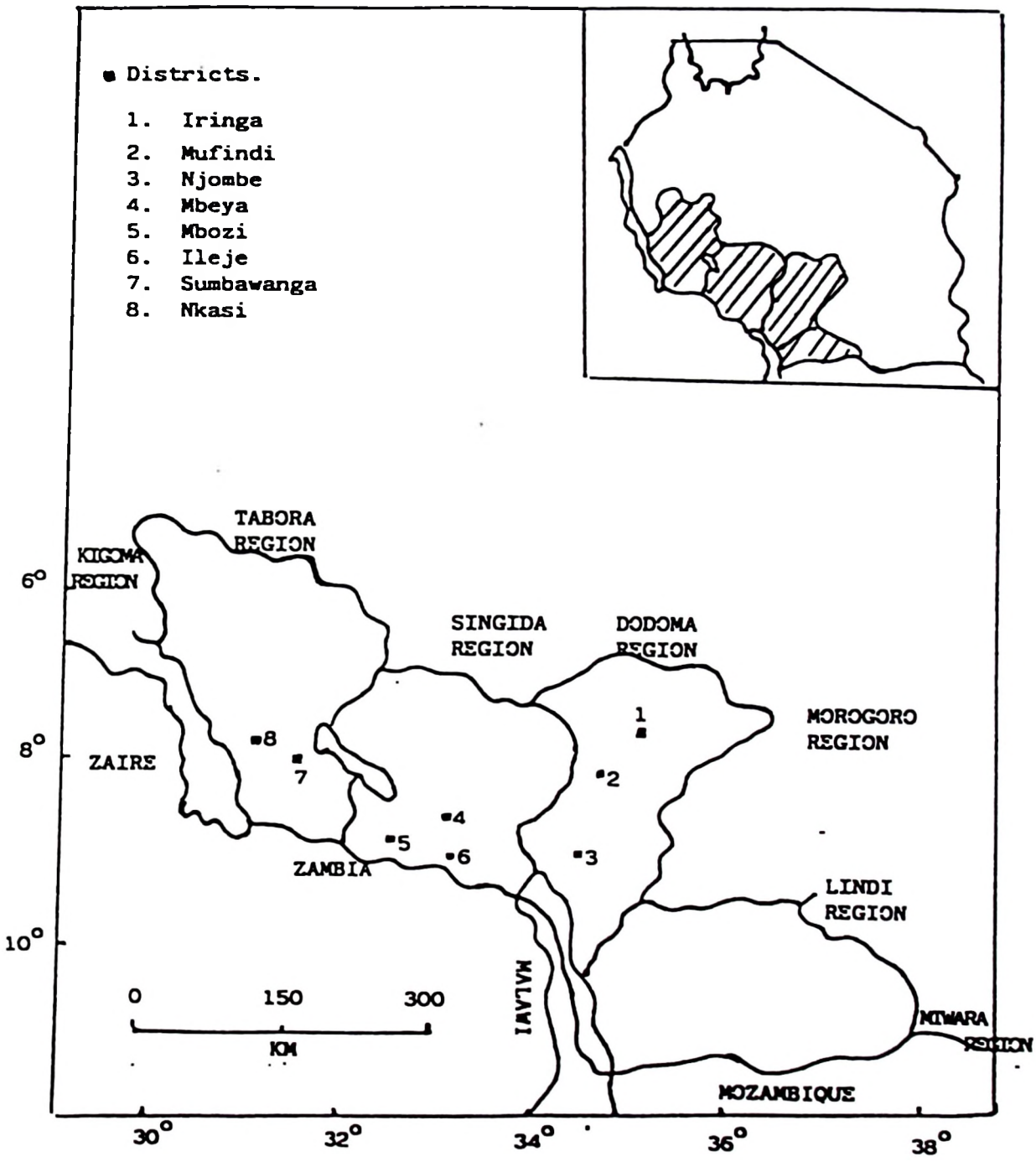


Figure 1. Map of the Southern Highlands of Tanzania showing areas from where samples which yielded *P.s.phaseolicola* and other pseudomonas isolates were collected. Insert shows the position of the Southern Highlands in Tanzania.

Aerial plant parts with characteristic symptoms of halo blight and other bacterial like symptoms were collected. Infected plant material were placed between clean absorbent sheets of paper and transported to the plant pathology laboratory at MARTI-Uyole for processing. Although attention was mainly placed on beans, other legumes were also sampled for assays. Such legumes included *Vigna* sp., *Neonotonia wightii*, *Desmodium* sp. *Clitoria ternatea*, *Cajanus cajan*, *Dolichos* sp., *Crotalaria* sp. and one unidentified shrub. *Trichodesma zeylanicum* and another weed belonging to the Asteraceae family were also sampled for assay. The latter two weed species were the most abundant in one halo blight severely infected bean field and exhibited water soaked or greasy necrotic lesions on the leaves.

3.3 Isolation

Microscopic examination for presence of bacteria was done when infected material collected showed halo blight-like disease symptoms. Isolation was done following procedures described by Bradbury and Kolkowisk (1984), and Lelliott and Stead (1987). Small portions of diseased tissue were removed aseptically from lesion margins, surface disinfected by quickly dipping into 0.1% NaOCl solution and rinsed three times in sterile distilled water. Portions of disinfected tissue were aseptically transferred to sterile plates containing sterile distilled water about five times their volume, and crushed to form a suspension. Loopfuls of the resulting suspension were streaked on to clean, well dried plates of nutrient agar (NA) and medium B of King *et al.* (KB) (1954). The wireloop was flamed with each streak, the aim being to separate the cells so that they produce individual colonies. Inoculated plates were incubated at 22 to 25^{0}C and examined daily for presence of *P.s.phaseolicola* like colonies for 3 to 5 days.

Purification of cultures. Well separated colonies (1 to 2) of bacteria that fluoresced green on KB were purified by repeated streaking on KB. Colonies that were consistently present in large numbers were sub-cultured for further tests.

Other characteristics such as colony colour, shape, size, texture, elevation, type of margin, consistency, translucency or opaqueness and rate of growth were further observed during purification of cultures.

3.4 Identification of bacterial isolates

Identification of bacterial isolates was done using Jensen's modified Gram stain method as described by Bradbury and Kolkowisk (1984). Production of fluorescent pigment under UV light, levan production, the oxidase test (Kovac's), arginine dihydrolase activity (Thornley, 1960), acid production from glucose (Bradbury and Kolkowisk, 1984) gelatin liquefaction (Lelliott and Stead, 1987) and carbon source utilization tests (Bradbury and Kolkowisk, 1984) were also used.

3.4.1 Carbon source utilization tests

3.4.1.1 Preparation of test media

Mineral base: The medium was prepared from a mineral base containing g/l $NH_4H_2PO_4$, 1.0; KCl, 0.2; $MgSO_4.7H_20$, 0.2; Agar, 15.0 (Ayers *et al.*, 1919). The solids were dissolved in distilled water and the pH adjusted to 7.2 with a few drops of 40% sodium hydroxide. Volumes of 45 ml were dispensed into bottles and autoclaved for 15 minutes at 121°C. This mineral medium without carbon sources was used as a negative control.

Carbon sources: The carbon sources used included mannitol, sorbitol and glucose. For each carbon source, 0.5 g was dissolved in 50 ml sterile distilled water. To avoid decomposition at high temperatures, the resulting solutions were filter-sterilized using 0.2 μm Whatman membrane filters.

Test media: The test media were prepared by adding 5 ml of the appropriate carbon source to 45 ml of molten basal medium at about 50°C. About 25 ml of medium were poured into each petri dish. After pouring, the plates were kept for 3 to 4 days at 18 to 22°C to allow drying and detection of any possible contaminants.

Inoculation of test plates. Isolates were tested in groups of nine. Inoculum from nine different isolates was prepared and transferred aseptically from the bottles into sterile metal bottle caps. Plates containing the various media were labelled with replicate number and marked. A flamed wire-loop was then lowered gently into the suspension in caps, very gently and carefully raised and transferred to contact the dry agar surface. Three plates of each test media were used for each set of nine isolates. Nine inoculum patches were made on each plate. To ensure correct orientation, the patches of inoculum were placed according to a map. This was achieved by the aid of the index mark made on both the map and the plate. Great care was taken to avoid cross contamination of the suspension by splash. Plates were checked to see if the inoculum patches had dried into the medium, before they were incubated at 22 to 25°C. Results were recorded at 3, 7 and 14 days. Growth was recorded as : - = no growth; + = growth present.

3.4.2 The tobacco hypersensitive reaction

The ability of each isolate to induce a hypersensitive reaction (HR) was tested on tobacco leaves, of greenhouse grown cultivar Kutsaga 51 plants, using the method described by Klement *et al.* (1964). Inoculations were performed with bacterial suspensions from 48 hour old cultures grown on NA. Bacterial suspensions of 10^8 colony forming units per millilitre were injected with a needle attached to a hypodermic syringe, into the intercellular spaces of intact leaves. Plants inoculated with sterile distilled water were included as negative controls. After inoculation, plants were kept in the greenhouse at 10 to 20°C. Isolates which induced necrosis on injected areas within 24 to 48 hours were recorded positive for HR on tobacco.

3.4.3 Pathogenicity test

Pathogenicity tests were done on 10 to 14 day old greenhouse grown bean seedlings cultivar Canadian Wonder by inoculating both the stem and the leaf. Bacterial suspensions were injected into stems using a 25 gauge needle attached to a disposable hypodermic syringe. In addition, primary leaves of the same plants were spray inoculated to run off without water-soaking using a hypodermic needle with its tip bent at right angles to produce a baffle for mist production. Plants were held about 15 cm away from the needle. Each isolate was tested using four to 10 plants. Tests were replicated twice. Inoculated plants were covered with polythene bags and incubated in the greenhouse for 48 hours at 10 to 20^{0}C. Thereafter, bags were removed and plants were observed daily for halo blight disease symptoms and any other reactions. Plants inoculated with sterile distilled water were included as negative controls. Appearance of typical halo blight symptoms confirmed *P.s.phaseolicola* identity.

Immature flat green pods from cultivar Canadian Wonder. Pathogenicity tests were also done on young immature flat green pods from greenhouse grown bean plants. Pods were picked, surface sterilized by dipping in 90% alcohol for 30 seconds and rinsed three times in sterile distilled water. Pods were dried using sterile absorbent papers and stab inoculated with a needle dipped in a bacterial suspension. Four to five inoculation points per pod were made. Each isolate was tested on two pods. The pods were incubated at 18 to 22°C in closed sandwich boxes lined with sterile damp absorbent papers and examined daily for development of halo blight symptoms. Pods inoculated with sterile distilled water were included as negative controls. Results were recorded 5 to 7 days after inoculation. Isolates causing characteristic water soaked necrotic lesions were considered to be pathogenic and therefore rated as positive (+) for *P.s.phaseolicola.* [Isolates inciting brown coloration at the inoculation point (noticeable at 24 hours after inoculation) or those producing no visible symptoms other than the

inoculation wound at the inoculation point, were not considered to be *P.s.phaseolicola* and therefore rated as negative (-)] .

Lemon fruits. Freshly picked lemon fruits were swabbed with 90% alcohol, washed in sterile distilled water three times and dried using sterile absorbent papers. Fruits were inoculated by stabbing using a needle loaded with a 48 hour old culture grown on NA. Lemon fruits stabbed with sterile needle dipped in sterile distilled water were used as negative controls. Inoculated lemon fruits were incubated in sandwich boxes lined with sterile damp absorbent papers. Evaluation was done 7 days after inoculation. Isolates producing black necrotic pits on lemon fruits were presumed to be *Pseudomonas syringae* pv. *syringae* (Lelliott and Stead, 1987) and were therefore scored negative (-) for *P.s.phaseolicola*.

Pear fruitlets. Immature pear fruitlets were obtained from Bulongwa, Makete district in Iringa region. Fruitlets were washed for 3 to 4 seconds in sodium hypochlorite, (1% solution) rinsed three times in sterile distilled water and dried using sterile absorbent papers. Each fruitlet was packed individually in a new plastic bag and stored in a refrigerator at 2 to 5°C. Pathogenicity test procedures were similar to those described above for lemon fruit. Evaluation was done 3 to 5 days after inoculation. Isolates inciting dark water-soaked necrotic spots at the inoculated site were considered to be *Pseudomonas syringae* pv. *syringae* (Lelliott and Stead, 1987) and were therefore scored positive (+) for the organism and negative (-) for *P.s.phaseolicola.*

3.4.4 Biolog Micro Plate identification System

The biolog identification system (Biolog Inc. Hayward, CA) for bacteria installed at the Department of Crop Science and Production, Sokoine University of Agriculture was used for further confirmation of the identity of some of the bacterial isolates. Pure cultures were grown on Trypticase soy agar (TSA) for 18 hours.

Inoculum was prepared by washing cultures in sterile distilled water to make bacterial suspensions with an optical density (OD_{590}) of approximately 0.4 - 0.6. Density of the suspensions was determined by eye (Black and Sweetmore, 1994) with turbidity standards provided by biolog. Biolog microplates were brought to room temperature (28 $\pm$ 2^0 C.) and each well was inoculated with 150 µl of bacterial suspension of an individual strain using a pipette (with sterile pipette tips). Plates were incubated at room temperature 28± 2°C and observed at 4 hours and readings taken at 16 hours as well as 24 hours. Microplate reading was done by eye as follows: + = fully positive. This indicated whole well diameter filled by deep violet. $\pm$ = Border line, indicating incompletely filled wells with deep violet. - = fully negative, also control : this was recorded where wells were clear and colourless. Data was entered manually into a computer for identification (Jones *et al.*1993).

3.5 Preservation of cultures

Cultures were preserved using the following methods:

Storage in nutrient agar slope under oil. The method described by Lelliott and Stead (1987), was used. Nutrient agar was prepared as recommended and poured in bijou bottles. The medium was allowed to set with bottles laid in a slanting position. Dry surface of the agar slants were streaked heavily with young, 48 hour-old-cultures grown on NA. Cultures were incubated at 22 to 25°C for 3 days and were then covered with sterile paraffin oil previously sterilized at 121°C for 15 minutes and dried for 12 hours in an oven at 100°C. The oil was poured to about 1 cm above the top of the agar. Cultures were maintained at room temperature (18 to 25°C.) for 9 months.

Storage in sterile distilled water. The method described by Lelliott and Stead (1987), was used. Forty-eight-hour old cultures grown on NA were used. A small growth was introduced aseptically in to sterile distilled water in bijou bottles.

The water had previously been double sterilized at 121°C for 15 minutes. Suspensions were stored at room temperature (18 to 25°C.) for 3 months before sub-culturing.

Immature flat green *P. vulgaris* pods. Inoculated bean pods from pathogenicity tests were air dried at room temperature (18 to 25°C) for 14 days and placed in brown paper envelops. Envelopes were labelled appropriately and stored in a drawer at room temperature.

3.5.1 Recovery of *P. s. phaseolicola* from storage

Cultures preserved in nutrient agar under oil. Using a wire loop, some bacterial growth was scrapped off and the wire loop withdrawn carefully through the oil layer making sure that the growth was not detached from the loop. Growth was streaked onto dry surface of KB and sucrose nutrient agar (SNA) plates and incubated at 22 to 25°C for 48 to 72 hours. Colonies were checked for purity (as described earlier) and increased on NA before use.

Cultures preserved in sterile distilled water. A few drops of the suspension were aseptically transferred on to a flamed microscope slide. A loopful of the suspension was streaked on to KB and SNA. Plates were incubated at 22 to 25°C and observed for 48 to 72 hours. Well separated colonies were checked for purity as described earlier and increased before further use.

Cultures preserved in immature flat green pod tissues. A small piece (approximately 4 mm^2) of the bean pod tissue was cut from the lesion and surface sterilized for 3 seconds in 1% solution of NaOCl. Tissues were rinsed three times in sterile distilled water, placed in a few drops of sterile distilled water on a sterile plate, and left to stand on the laminar flow cabinet for 30 to 60 minutes (depending on time under storage) to allow for the bacteria to ooze out. Using sterile needles, tissues were then teased apart and a loopful of the resulting suspension streaked onto SNA and KB. Plates were incubated at 22 to 25°C for 48 to 72 hours. Colonies were checked for purity as previously described and increased on NA before use.

3.6 Reference strains

Isolates of *P.s.phaseolicola* used as reference strains included, 1281A (race 1); 882 (race 2); 1301A (race 3); 1302A (race 4); 1375A (race 5); 1299A (race 6); 2656A (race 8) and 2709A (race 9). Reference strains were kindly provided by Dr. D.M.Teverson of the Natural Resources Institute, United Kingdom.

3.7 Inoculum preparation

Inoculum used in this study was prepared using the procedures of Bradbury and Kolkowisk (1984). Each bacterial isolate was grown on NA for 48 hours at 22 to 25^{0}C. Small growths of the cultures were subsequently suspended in sterile distilled water or 0.01M phosphate buffer, pH 7.2 as required. The resulting suspensions were adjusted turbidimetrically to a concentration of 10^8 colony forming units (CFU) per millilitre. Sterile distilled water was used as a diluent for inoculum used for *in-vitro* tests, unless otherwise stated. To overcome the problem of rendering bacterial cells susceptible to shock and death due to time and procedures involved in spray inoculation and other tests, phosphate buffer was used in preparing inoculum for field and greenhouse studies.

3.8 Growth of the test plants in the greenhouse

Bean plants used for pathogenicity tests were grown in 12 cm diameter plastic pots, and those for germplasm evaluation were grown in plastic trays 53 cm long, 33 cm wide and 6 cm deep. For epiphytic population studies, plants were grown in 15 cm diameter plastic pots. Four litre capacity plastic pots were used for growing plants required for production of immature flat green pods and for seed multiplication in the greenhouse. Steam sterilized forest soil from Kimondo area (about 2000 masl) in Mbeya was used for growing all the bean plants used in this study.

Temperatures and humidity in the greenhouse were measured using a Lambrecht hygrothermograph. Plants were watered twice daily in the morning and in the evening using tap water unless stated otherwise.

3.9 PATHOGENIC VARIATION STUDIES

3.9.1 Differential bean cultivars

The differential bean cultivars used in this study consisted of seven *P. vulgaris* and one *P. acutifolius*, and they included cultivars: Canadian Wonder, Tendergreen, A52, A53, Red Mexican UI3, A43, and Guatemala 196 and 1072. Original seed were supplied by Dr. J. D. Taylor of the Horticulture Research International, Wellesbourne, United Kingdom. Seed were initially multiplied in a screenhouse at SUA, Morogoro and later in the greenhouse at MARTI - Uyole, Mbeya.

3.9.2 Race identification

Bacterial isolates, which were identified as *P.s.phaseolicola*, were subjected to pathogenic variation tests. Bean seedlings were inoculated 10 to 14 days after planting using procedures described previously. Stems were inoculated by injecting them using a 25 gauge needle fitted to a 20cc hypodermic syringe. Both surfaces of leaves of each bean plant were spray-inoculated to runoff using a hand mist blower held at about 30 cm away from the plants. Inoculated plants were incubated under polyethylene bags for 48 hours. Bags were then removed and plants were observed daily for halo blight symptoms for up to 2 weeks after inoculation. The reactions of the eight differential bean genotypes to *P.s.phaseolicola* strains were recorded 7 and 10 days after inoculation using a scale of 1 - 9. Ratings of 1.0, 2.0 and 3.0 were considered to indicate resistance. 4.0, 5.0 and 6.0 intermediate resistance/susceptible and 7.0, 8.0 and 9.0 indicated susceptible reactions. Assignment of races to strains was based on the pathogenic patterns adapted from Teverson and Taylor (1991).

3.9.3 Screening 36 bean genotypes against eight *P.s.phaseolicola* strains

This study was conducted as an attempt to examine the bean accessions for resistance to *P.s.phaseolicola* strains and to observe any pathogenic variation in strains which could not be identified using the standard set of halo blight differential cultivars. To do this, eight strains representative of the races occurring in the Southern Highlands of Tanzania were used to screen a collection of 36 bean genotypes. The bean genotypes included landraces and breeding lines (Table 1) and were screened in two repeated experiments under greenhouse conditions. Experiments were arranged in a split plot design of a randomized complete block with four replicates. In both experiments, main plots were the eight strains and subplots contained 20 genotypes for the first experiment and 16 genotypes for the second experiment. Five bean seeds were used per replicate. The seeds were planted in steam sterilized soil in plastic trays of the size described previously and seedlings inoculated 10 to 14 days after planting using procedures described above for pathogenic variation. Reactions were recorded 10 and 14 days after inoculation using a 1 - 9 scale. 1 = highly resistant; 2 - 3 = resistant; 4 - 6 = intermediate (moderately resistant/susceptible); 7 - 8 = susceptible; 9 = highly susceptible (CIAT, 1987b).

3.9.4 Reactions of immature flat green pods from 14 bean cultivars to 128 strains of *P.s. phaseolicola*

Five experiments were conducted to determine pathogenic variation in *P.s.phaseolicola* using young immature flat green bean pods. In 1993, twenty four randomly selected *P.s.phaseolicola* strains were tested using pods from 28 greenhouse grown *P.vulgaris* cultivars in three repeated experiments. A split plot of a randomized block design with three replications was used. Strains were in the main plots and bean genotypes in the subplots.

Table 1. List of bean genotypes screened against eight strains of *P.s. phaseolicola* found in the Southern Highlands of Tanzania

Bean genotype[a]	Source
Canadian Wonder	Halo blight differential cv. HRI, UK
Edmund	Originally halo blight differential cv. HRI, UK
ZAA 840	Germplasm collection, MARTI Uyole
G5773	Germplasm collection MARTI Uyole
G3844	Germplasm collection MARTI Uyole
G2472	Germplasm collection MARTI Uyole
UAC G 435	Germplasm collection MARTI Uyole
UAC PBABL 43	Advanced bean line MARTI Uyole
UAC 161	Advanced bean line MARTI Uyole
CG 113	Germplasm collection MARTI Uyole
Tostado	Germplasm collection MARTI Uyole
LB 110	Advanced bean line ex Lyamungo, Moshi
LB465-1	Advanced bean line ex Lyamungo, Moshi
DRK-4	Advanced bean line ex Lyamungo, Moshi
FB/GP 264-3	Advanced bean line ex Lyamungo Moshi
Lyamungo-90	Released variety, Lyamungo, Moshi
Uyole-84	Released variety, MARTI Uyole
Uyole-90	Released variety, MARTI Uyole
Ilomba	Released variety, MARTI Uyole
Uyole-94	Released variety, MARTI Uyole
EGERM-74	YC 2 X Chipukupuku (cross) MARTI Uyole PBBL
226-3	Breeding line MARTI Uyole
HB 1-1	Advanced line SUA, Morogoro
Phaseolus coccineus	Local cultivar ex Iringa
Kigoma	landrace from Kigoma
Mdandu -White	Landrace from Mdandu, Njombe
Ludewa-yellow	Landrace from Ludewa
Masusu	Landrace from Mbeya
Chipukupuku	Landrace from Mbeya
Nyamuhanga	Landrace from Iringa
Kablanketi-red	A derivative of Kablanketi
Selection-8	Landrace from Arusha/Moshi
Kablanketi	Landrace from Mbeya
Samdzuha	Landrace from Mdandu, Njombe
EAI 2525	Bean line MARTI Uyole
Ikinimba	Beanline ex Rwanda

[a] Bean seeds were multiplied in the greenhouse at Uyole, Mbeya. Seeds from apparently halo blight-free plants were used in the screening tests. HRI=Horticulture Research International. MARTI=Ministry of Agriculture Research and Training Institute.

Each strain was tested on three pods using procedures previously described with slight modifications. Young immature flat green pods from greenhouse grown bean plants were picked, surface sterilized by dipping in 90% alcohol for 2 to 3 seconds and washed three times in three changes of sterile distilled water. Pods were placed in sandwich boxes lined with sterile absorbent papers. Inoculation was done by puncturing the pods with sterile tooth picks (4 to 5 sites per pod) and placing a drop of the appropriate inoculum over each injured site using sterile pipettes. Inoculated pods were maintained on the laminar flow cabinet to allow for the inoculum to be absorbed into the pod tissues. Pods inoculated with sterile distilled water were included as negative controls. The paper lining in the sandwich boxes were dampened by introducing sterile distilled water carefully from the sides of the boxes. Pods were incubated in closed sandwich boxes at 22 to 25°C. Observations were made daily for typical halo blight symptoms. Pod reactions were recorded as lesion width in millimetres, at 7 days after inoculation. In 1994 and 1995, a total of 94 strains were tested in two experiments. A randomized complete block design with three replications was used. Each strain was considered as a treatment and was tested using three pods from cultivar Canadian Wonder. Tests were repeated twice.

3.10 MOTILITY STUDIES

Experiments were conducted in the laboratory to study differences in motility between strains of *P.s. phaseolicola*. Strains used in this study were randomly selected from collections made previously. Each strain was considered as a treatment. A randomized complete block design with three replications was used. Experiments were repeated twice.

3.10.1 Test media

A semi solid tryptone media (STM) (Panopoulous and Schroth, 1974), was used to test *P.s.phaseolicola* strains for motility. STM contained 0.1 g, Tryptone; 0.1 ml, glycerol; 1.0 g, KNO_3; 1.18 g, K_2HPO_4; 0.44 g, KH_2PO_4 and 1.0 g, agar per litre of sterile distilled water. The medium was heated to dissolve the ingredients and portions of 20 ml each were then dispensed in sterile test tubes and autoclaved at 121^{0}C. for 15 minutes. The medium was kept in an air conditioned chamber with temperatures maintained at 17 ± 1°C until used (within 24 to 48 hours). Sixteen strains of *P.s.phaseolicola* were tested for motility using the semi solid tryptone medium contained in test tubes. Tests were carried out following the procedures described by Msuku (1984) with slight modifications. Each strain was tested in six test tubes . A 5 µl drop of the bacterial suspension to be tested was aseptically added to the medium in each tube. The inoculated test tubes were incubated in an upright position at 17± 1^0 C. Presence of a white band moving downwards was considered to indicate bacterial motility. Test tubes inoculated with sterile distilled water were used as controls. Data were recorded at 30 minutes, 1 and 4 hours after inoculation, as distance the bacterial band had moved downward in millimetres.

3.11 GROWTH OF *P.S.PHASEOLICOLA* ON MOTILITY MEDIUM

An experiment was conducted to determine growth rate of different strain of *P.s.phaseolicola*, using motility medium. The motility medium as described by Bradbury and Kolkowisk (personal communication) contained 0.5 g, K_2HPO_4; 0.2 g, $MgSO_4$.$7H_20$; 0.2 g, NaCl; 5.9g yeast extract; 20 g, agar in one litre of distilled water. The medium was preheated to dissolve the ingredients and autoclaved at 121^{0}C. for 15 minutes. About 25ml of the medium was poured into each petri plate. The plates were then kept for 3 to 4 days at 18 to 22^0 C to allow for drying and for detection of any possible contaminants.

Selection of the medium was based on the information that the motility medium enhanced motility in *P.s.phaseolicola.* Strains used in this study were selected randomly from collections made previously. Each strain was considered as a treatment. A randomized complete block design with three replications was used. Experiments were repeated twice. Twenty nine strains were used and each was tested using three plates containing the motility medium. Each plate represented one replicate. Using a flamed wire loop, inoculum for each strain was transferred to dry agar surfaces. Five isolated spots were made on each plate. Plates inoculated with sterile distilled water were used as controls. After the inoculum patches had dried into the medium, inoculated plates were incubated at 22 to 25^{0}C. Diameter of bacterial growth at each inoculated spot was measured at 1, 2, 3 and 4 days after inoculation.

3.12 EPIPHYTIC POPULATIONS STUDIES

The dynamics of epiphytic populations of *P.s.phaseolicola* on bean plants was studied in five experiments. Three experiments were conducted in research fields and two under greenhouse conditions at MARTI Uyole during 1993, 1994 and 1995 growing seasons. Experiments conducted in 1993 and 1994 were planted in fields, which had been under fallow for two consecutive years and not less than 500 metres away from any bean field. The 1995 experiment was planted in an isolated field, which had been under fallow for 5 years.

3.12.1 Field experiments

Genotype Uyole-90, a variety released by the bean programme at MARTI Uyole; and Kablanketi, a widely grown landrace originating from Mbeya, Tanzania, were used. Seeds were kindly provided by Dr. (Mrs) C. S. Madata (Bean Breeder at MARTI Uyole, Mbeya). A split plot design of a randomized complete block with three replications was used with bean genotypes as main plots.

Sub plots consisted of two *P.s.phaseolicola* strains 9337 (uncertain race type) and 9359 (race 6). *P.s.phaseolicola* strains were randomly selected from the 1993 collections. Plots consisted of four 3.5 metre rows at 50 cm apart. Bean seeds were planted at a distance of 10 cm apart, one seed per hole. Prior to planting, the bean seeds were surface disinfected using 1% NaOCl. The same seed was planted in the greenhouse to countercheck for germination percentage and freedom from halo blight and any other disease infection. To confine the inoculum to the borders, plots and replications were separated by ten and twenty rows of Triticale, respectively. Triticale was planted 2 weeks before the beans.

Inoculation of the bean plants. Bean plants were spray inoculated to-run off, three weeks after planting using a hand mist sprayer held about 30 cm away from the plants. Control plots were inoculated with sterile distilled water.

Assessment of surface bacterial population dynamics. Sampling started before inoculation, at 4 hours after inoculation and later at 3 days intervals for a period of 15 days. On each sampling day, leaflets were collected between 07.00 and 08.00 in the morning. Fifteen individual leaflets selected from the top of the plants were collected from each plot. The leaf samples were placed in plastic bags and transported in a cool box to the laboratory for processing. The dilution plate technique was used in estimating the bacterial populations. The leaflets were transferred to sterile 500 ml erlen-meyer flasks with 100 ml of sterile phosphate buffer (0.1 M pH 7.0). The samples were shaken for 30 minutes on a reciprocating shaker set at 200 rpm. Ten fold serial dilutions were prepared in sterile phosphate buffer and 0.01 ml portions from the serial dilutions were plated on KB containing cyclohexamide (100 µg/ml) to inhibit fungal growth. Cyclohexamide was prepared as 25 mg/ml stock solution in 12.5 methanol. Stock solutions were filter sterilized using 0.2 µm Whatman membrane filters and stored in a refrigerator (2-5^{0}C). Fluorescent colonies with characteristic colony morphology for *P.s.phaseolicola* were counted after 3 to 5 day incubation at 22 to 25°C. Presumed colonies of *P.s.phaseolicola* were restreaked on to KB to confirm purity.

Colonies were further tested for pathogenicity on young immature flat green pods obtained from bean cultivar Canadian Wonder. Bacterial cells counted, were transformed and expressed as log colony forming units (Log CFU) per cm^2 of leaf surface prior to data analysis.

Colony forming units (CFU) per cm^2 of leaf surface was estimated as:

$$\frac{\text{Cfu observed on plate x dilution of plate x amount of buffer used}}{\text{Leaf surface area}}$$

Estimation of leaf surface area. Leaf surface area was estimated using a graph paper. After washing, leaves were blot dried and traced on sterile paper. These were retraced on graph papers. The area was estimated by multiplying the number of squares each leaflet covered, by the area of a square.

3.12.2 Greenhouse experiments.

Bacterial strains, bean cultivars and inoculum preparation procedures were the same as previously described under field experiments. Seeds used had been multiplied under greenhouse conditions. To counter check that seeds for the tests were free from any disease, uninoculated plants were grown separately and were observed during the whole period of experimentation to see if they remained free of halo blight and any other disease. The experiments were carried out to determine whether bean seedlings emerging from *P.s.phaseolicola* infested soil would carry the bacterium on their leaf surfaces. Six treatment combinations were used as follows:

a. Cultivar Kablanketi planted and soil for covering the seeds premoistened using sterile distilled water (KAB-SDW).

b. Cultivar Kablanketi planted and soil for covering the seeds preinfested using a suspension from strain 9337 (uncertain race type) of *P.s.phaseolicola* (KAB - 37).

c. Cultivar kablanketi planted and soil for covering the seeds preinfested using a suspension from strain 9359 (race 6) of *P.s.phaseolicola* (KAB-59).

d. Cultivar Uyole-90 planted and soil for covering the seeds premoisteined using sterile distilled water (UY-90-SDW).

e. Cultivar Uyole-90 planted and soil for covering the seeds preinfested using a suspension from strain 9337 (uncertain race type) of *P.s.phaseolicola* (UY-90-37).

f. Cultivar Uyole-90 planted and soil for covering the seeds preinfested using a suspension from strain 9359 (race 6) of *P.s.phaseolicola* (UY-90-59).

Soil sterilization Forest soil from Kimondo (2000 masl) in Mbeya was packed in 1 kg quantities in cotton cloth bags and sterilized by autoclaving for 15 minutes at 121^{0}C. Soil was sterilized two times to minimize contamination.

Experimental procedures. Plastic pots (15 cm diameter) were filled to two thirds depth with sterile soil previously moistened (slightly) using sterile distilled water to facilitate absorption of water. Eighteen pots were used, each pot considered as a plot. Surface sterilized (1% NaOCl for 30 seconds) bean seeds (10 per pot) were placed on top of the soil and covered with a layer of soil previously infested by wetting using a 10^{8} bacterial suspension of *P.s.phaseolicola.* Pots were then placed in small plastic deep plates and placed on a bench in the greenhouse.(Plate 1) To avoid cross contamination, plants were watered from the bottom. This was done by pouring sterile distilled water in the small deep plates. Sterile distilled water was used for up to 7 days after planting.

Sterile distilled water was used to wet the soil in the control plots.

Plate 1. Set-up of pots on a greenhouse bench immediately after planting for the epiphytic populations studies experiment under greenhouse conditions. Watering was done from the bottom to avoid cross contamination. This was facilitated by standing the pots in small deep plastic plates in which water was poured.

Assessment of bacterial populations on primary leaves. Five primary leaves were randomly sampled from each pot 8 to 9 days after planting, when over 60% of the plants had emerged and the primary leaves were completely unfolded. External bacterial populations were estimated using the dilution plating technique, as described previously. For determination of internal populations of *P.s.phaseolicola*, symptomless leaves were first placed in 1% NaOCl for one minute to kill surface microoganisms. Leaves were rinsed three times in sterile distilled water and then homogenized in 10 ml of sterile phosphate buffer. Homogenates were serially diluted and plated as described earlier.
Disease rating. Disease incidences were evaluated by counting the number of plants exhibiting typical symptoms of halo blight. Results were expressed as a percentage of the total number of plants emerged. Disease severity was rated using a scale of 1 - 9 where 1.0 = No disease symptoms observed, and 9.0 = death of plants due to the halo blight disease (CIAT, 1987b).

3.12.3 Bacterial population in farmers bean fields

This study was carried out in order to find out whether the *P. fluorescens* A bacterial cells recovered from leaf surfaces of field grown bean genotypes Kablanketi and Uyole-90 also occurred in the farmers bean fields outside the MARTI-Uyole experimental area. It was an attempt to establish reasons for failure to recover *P.s.phaseolicola* strains 9337 and 9359 inoculated on to the two bean cultivars during the 1993 growing season.

Sampling procedure. Four fields, (each planted with a different bean cultivar) were chosen in Itezi (1725masl) Mbeya. According to the farmers, these fields had been under mixed cropping of potatoes and maize during the previous season. Sampling was done once on bean plants, which had been planted in March, 1993 and were at the flowering stage. Each field was divided into three plots prior to sampling. Three samples each of 15 leaflets were randomly picked from each of the four fields.

Leaf samples were packed in plastic bags placed in a cool box and transported to MARTI Uyole for analysis. External bacterial populations of green-fluorescent pseudomonads were estimated using the dilution plating technique, as described previously. Colonies of the most predominant fluorescent-pseudomonad were randomly selected and tested for levan (Bradbury and Kolkowisk,1984), oxidase (Kovac's, 1956), arginine dihydrolase activity (Thornley, 1960), tobacco hypersensitive reaction (Klement *et al.*, 1964), ability to liquefy gelatin in 3 days (Lelliott and Stead, 1987) utilization of carbon sources for growth (Bradbury and Kolkowisk, 1984) and pathogenicity on young immature flat green pods and on 10 to 14 day-old bean seedlings. In addition, the Biolog Microplate identification system installed at SUA, Morogoro, Tanzania was used to confirm the identity of the microoganisms recovered.

3.13 SURVIVAL OF TWO STRAINS OF *P.S.PHASEOLICOLA* ON YOUNG IMMATURE FLAT GREEN PODS

Investigations on survival of *P.s.phaseolicola* have demonstrated that the pathogen can overwinter in undecomposed stems, pods and leaves of bean plants left standing in the field. In addition *P.s.phaseolicola* was shown to survive for 12 months within dried infected bean leaves stored at 24^{0}C in the laboratory (Natti, 1967). However, no information was available on the effectiveness of immature flat green pods in the storage of *P.s.phaseolicola*. Therefore, survival of two strains of *P.s.phaseolicola* occurring in the Southern Highlands of Tanzania was studied by measuring the viable colony forming units in the pod tissues for a period of 9 months. Two bacterial strains, 9337 (uncertain race type) and 9359 (race 6) were used in a test to compare the ability of different strains of *P.s.phaseolicola* to survive on young immature flat green pods of bean cultivar Canadian Wonder in the laboratory. Pods from greenhouse grown plants were picked, surface sterilized by dipping in 90% alcohol for 30 seconds and rinsed in three changes of sterile distilled water. Pods were placed in sandwich boxes lined with sterile absorbent paper and left to dry on laminar flow cabinet.

Using sterile toothpicks, pods were pierced to make 4 to 5 wounds on each pod. A drop of the inoculum approximately 10^8 cfu per ml was transferred carefully on to each wounded site and left to be absorbed into the pod tissues. Pods inoculated with sterile distilled water were used as controls. The paper lining in the sandwich boxes were dampened by introducing sterile distilled water carefully from the sides of the boxes. Pods were incubated at 22 to 25^{0}C and observed daily for halo blight symptoms. To evaluate the survival of strains 9337 and 9359 of *P.s.phaseolicola* in bean pod tissues, the bacterial cells were recovered at 24 hours,7 days, one month and later at 3 months intervals for a period of 9 months. Bacteria were estimated using the dilution plating technique. A 4 mm^2 portion of the bean pod tissue was cut from a lesion and surface sterilized for 2 to 3 seconds in 1% solution of NaOCl. Tissue was rinsed in three changes of sterile distilled water, placed in 10 ml of sterile phosphate buffer on a sterile plate and left to stand on an laminar flow cabinet for 30 to 60 minutes (depending on time under storage) to allow for the bacteria to ooze out. The tissue was then teased apart using sterile needles, ten fold serial dilutions were prepared in sterile phosphate buffer and 0.01 ml portions from the serial dilutions were plated onto KB containing cycloheximide (100 g/ml) to inhibit fungal growth. Fluorescent colonies with the morphology for *P.s.phaseolicola* were counted after 3 to 5 days of incubation at 22 to 25^{0}C. The resulting pod tissue bacterial populations were transformed and expressed as log cfu per cm^2 as described previously then, graphed against time under incubation/storage.

3.14 THE POTENTIAL FOR GARLIC BULB EXTRACT TO INHIBIT GROWTH OF *P.S.PHASEOLICOLA.*

Several plant species have been examined for their potential to produce antibacterial compounds (Alström, 1992; Thakur *et al.*, 1991). In India, garlic bulb extract was shown to have antibacterial activity against *Xanthomonas campestris* pv. *vescatoria* (Mangamma and Srelamuulu, 1991).

Garlic is widely grown by farmers in the Southern Highlands of Tanzania. However, no information is available on its effect on plant pathogenic bacteria in this region. This preliminary study was therefore, conducted to investigate on the potential for garlic bulb extract to inhibit growth of *P.s.phaseolicola* as a component in the integrated management of halo blight disease of beans. The choice was based on the assumption that use of botanical pesticides was safe to the environment. The experiments were conducted in the laboratory and under greenhouse conditions.

3.14.1 *In vitro* studies

The potential for garlic bulb extract to inhibit growth of *P.s.phaseolicola* strains 9337 (uncertain race type) and 9359 (race 6) *in vitro* was investigated using plate count agar (PCA), NA and KB. Aqueous extracts of garlic bulb were prepared using the procedures of Mangamma and Sreelamulu (1991) with slight modifications. Garlic bulb after removal of their outer dried tissues were weighed and crushed using sterile mortal and pestle. Extracts were prepared by adding 100 ml of sterile distilled water to the crushed garlic and the resulting suspensions filtered through a double layer of sterile cheese cloth and subsequently Whatman filter paper. For *in vitro* tests garlic bulb extracts of 40% w/v concentration was used. To test for the bactericidal activity of garlic bulb, the agar diffusion method as described by Alström (1992), was used with some modifications. A dense suspension of the bacteria was prepared by washing off 48-hour-old cultures grown on NA using 10 ml of sterile distilled water. About 0.1 ml of bacterial suspensions were separately seeded on each of the media used (3 plates of the media for each of the strains tested) and left to dry on the laminar flow cabinet . A drop of about 0.02 ml of the garlic bulb extract was then transferred on agar medium inoculated with the bacteria. Two controls were included, one remained uninoculated to observe background growth of the bacteria. In another control a drop of sterile distilled water was used. After the drops of garlic bulb extract had dried into inoculated media, plates were incubated at 22 to 25^{0}C. The experiment was repeated two times.

The diameters of any resulting zones of inhibition were measured (mm) 48 hours after inoculation.

3.14.2 Greenhouse studies

Two experiments were conducted to investigate the effect of garlic bulb extract on halo blight disease incidence and severity. In the first experiment, the effect of garlic bulb extract at 40% w/v concentration was tested using bean cultivars Kablanketi and Uyole-90 and *P.s.phaseolicola* bacterial strains 9337 and 9359. Bean seeds for these experiments had been produced under greenhouse conditions and had been checked and found to have had a germination percentage of 99 to 100% and freedom from *P.s.phaseolicola* infection. In addition bean seeds were surface sterilized using 1% NaOCl prior to soaking. Bacterial suspensions of approximately 10^7 to 10^8 cfu per milliliter and the garlic bulb extract were introduced in the bean seeds by soaking at room temperature for 12 hours. All the seeds were rinsed in three changes of sterile distilled water before planting. Seeds from each treatment combination were then sown in 4 pots (15cm diameter) containing steam sterilized forest soil. The eight treatment combinations for garlic bulb experiment number one (GBE-1)were as follows: (a) bean seeds of cultivar Kablanketi, infested with *P.s.phaseolicola* strain 9359 (race 6) and subsequently soaked in garlic bulb extract of 40% w/v concentration; (b) bean seeds of cultivar Kablanketi infested with *P.s.phaseolicola* strain 9359 (race 6); (c)bean seeds of cultivar Uyole-90, infested with *P.s.phaseolicola* strain 9337 (uncertain race type) and subsequently soaked in garlic bulb extract of 40% w/v concentration; (d) bean seeds of cultivar Uyole-90 infested with *P.s.phaseolicola* strain 9337 (uncertain race type); (e) bean seeds of cultivar Kablanketi infested with *P.s.phaseolicola* strain 9337 (uncertain race type) and subsequently soaked in garlic bulb extract of 40% w/v concentration; (f) bean seeds of cultivar Uyole-90 infested with *P.s.phaseolicola* strain 9359 (race 6); (h) bean seeds of cultivar Uyole-90 infested with *P.s.phaseolicola* strain

9359 (race 6) and subsequently soaked in garlic bulb extract of 40% concentration. The second garlic bulb extract experiment (GBE-2) was conducted using bean seeds of cultivar Canadian Wonder. The objectives were : (a) to probe into the effect of garlic bulb extract on germination of bean seeds when used at concentrations lower than 40% w/v and (b) to find out if there were any differences in performance of garlic bulb extract introduced in the bean seeds before or after infection. Garlic bulb extract was prepared as described previously but at lower concentrations of 10 and 20% w/v. Two *P.s.phaseolicola* strains 7994 (race 3) and 12694 (race 6) were used. Bacterial suspensions and garlic bulb extract were introduced in bean seeds as described in the previous experiment.. Twelve treatment combinations were used as follows : (a) bean seeds infested with *P.s.phaseolicola* race 6; (b) bean seeds infested with *P.s.phaseolicola* race 3; © bean seeds soaked in garlic bulb extract of 10%w/v; (d) bean seeds soaked in garlic bulb extract of 20%w/v; (e) bean seeds infested with *P.s.phaseolicola* race 6 and subsequently soaked in garlic bulb extract of 10% w/v; (f) bean seeds infested with *P.s.phaseolicola* race 6 and subsequently soaked in garlic bulb extract of 20% w/v; (g) bean seeds soaked in garlic bulb extract of 10%w/v and subsequently infested with *P.s.phaseolicola* race 6; (h) bean seeds soaked in garlic bulb extract of 20% w/v and subsequently infested with *P.s.phaseolicola* race 6; (I) bean seeds infested with *P.s.phaseolicola* race 3 and subsequently soaked in garlic bulb extract of 10% w/v (j) bean seeds infested with *P.s.phaseolicola* race 3 and subsequently soaked in garlic bulb extract of 20% w/v; (k) bean seeds soaked in garlic bulb extract of 10% w/v and subsequently infested with *P.s.phaseolicola* race 3; (l) bean seeds soaked in garlic bulb extract of 20% w/v and subsequently infested with *P.s.phaseolicola* race 3. A randomized complete block design with four replications was used for both experiments. Results were recorded at 14 days after planting for both experiments, as well as at 28 and 42 days after planting for experiment GBE-2. Data collected included: number of bean seedlings emerged, number of plants showing halo blight symptoms and halo blight disease severity.

Halo blight disease severity was scored using a scale of 1-9, where 1= no disease symptoms and 9 = death of plants due to halo blight disease (CIAT, 1987b).

3.15 Data analysis

Data analysis was done using MSTAT-C programme (Michigan State University) and differences between means were separated using the LSD test.

CHAPTER 4

RESULTS

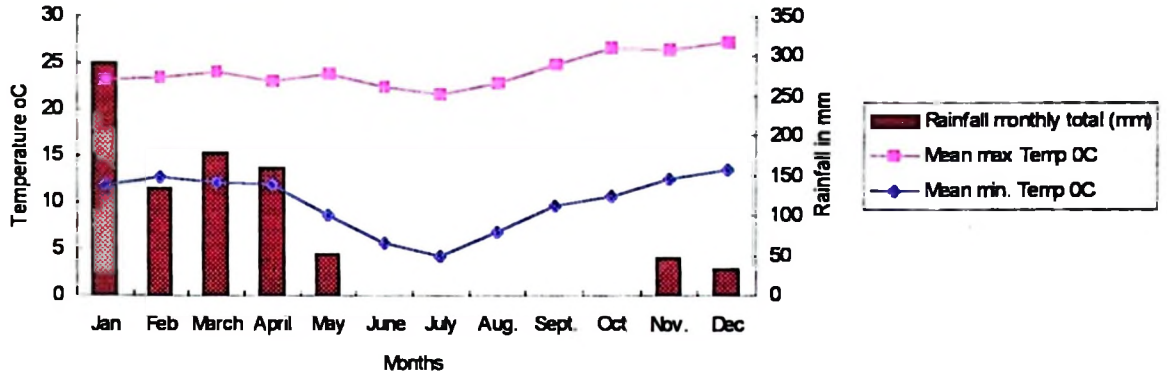
4.1 Weather conditions

The annual rainfall totals for MARTI Uyole were 893.9 mm in 1993, 717.3 mm in 1994 and 697.6 mm for 1995. The mean maximum and minimum temperatures were respectively 24.1°C and 9.9°C for 1993; 24.3°C and 10.6°C for 1994; 23.9°C and 10.3°C for 1995 (Fig. 2).

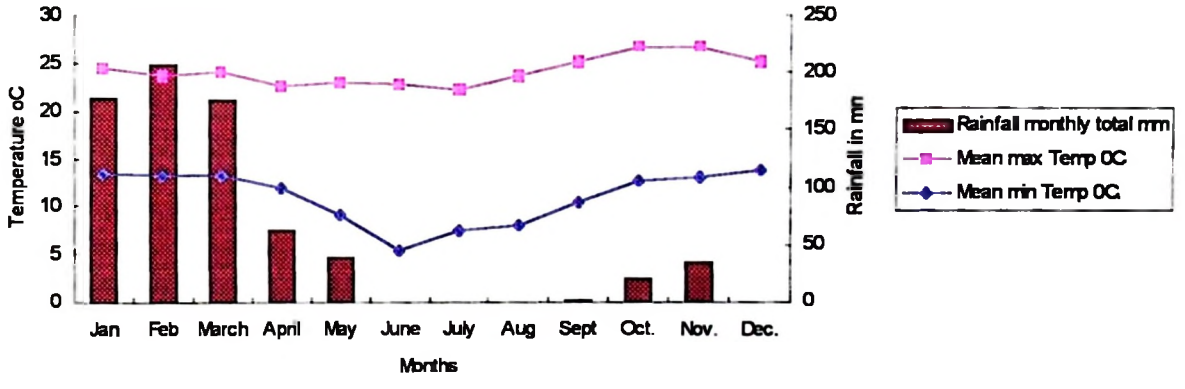
4.2 Identification of bacterial isolates

On the basis of cultural, biochemical and pathogenicity tests on bean cultivar Canadian Wonder foliage and young immature flat green pods, 260 isolates were identified as *P.s.phaseolicola*. Sources of bacterial isolates used in this study are shown in Table 2. The isolates produced a green diffusible pigment on KB, which fluoresced blue-green under UV light. They were positive for levan, negative for oxidase, gelatine liquefaction and arginine dihydrolase activity. These isolates were able to utilize glucose but not mannitol or sorbitol as sole carbon sources for growth and produced a hypersensitive response in tobacco leaves. Identification of these isolates was confirmed by pathogenicity tests. All were pathogenic on bean plants cultivar Canadian Wonder and gave typical halo blight symptoms on foliage and on young immature flat green pods.

A.



B.



C.

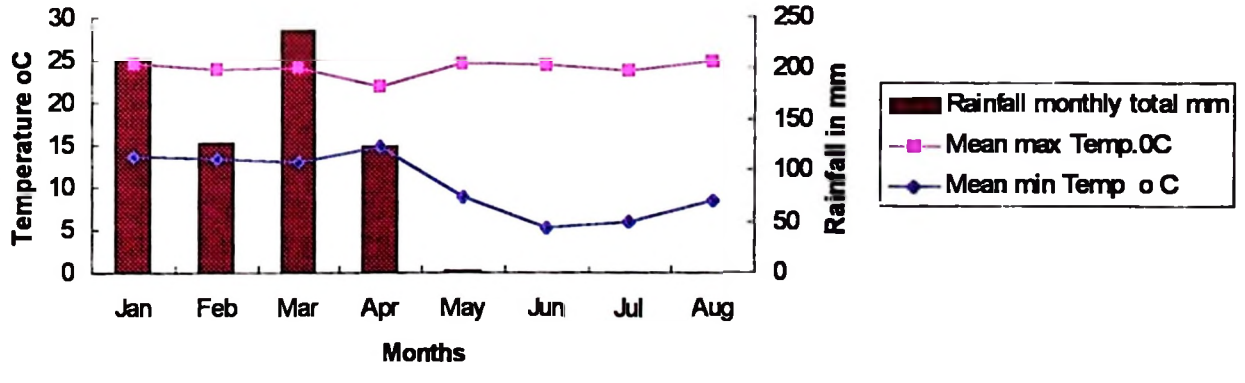


Figure 2. Total monthly rainfall, maximum and minimum temperature for MARTI Uyole for the years A = 1993; B = 1994; C = 1995.

Table 2. Sources of isolates of *P. s. phaseolicola* used in this study

District	Year	No of isolates	Altitude range	No of villages
Iringa	1993	12	1500 to 1650	3
	1994	38	1450 to 1600	12
	1995	1	1650	1
Mufindi	1993	0		
	1994	0		
	1995	9	1550 to 1580	3
Njombe	1993	5	1780	1
	1994	14	1650 to 1780	2
	1995	6	1400 to 1600	6
Mbeya	1993	22	1650 to 1750	5
	1994	96	1500 to 2000	18
	1995	34	1200 to 2000	15
Ileje	1993	0		
	1994	0		
	1995	2	1300	1
Mbozi	1993	0		
	1994	3	1525	1
	1995	3	1525	1
Nkasi	1993	0		
	1994	10	2000	1
	1995	0		
Sumbawanga	1993	0		
	1994	0		
	1995	5	2000	1
Total		260		71

Iringa, Mufindi and Njombe districts are in Iringa region. Mbeya, Mbozi and Ileje districts are in Mbeya region. Nkasi and Sumbawanga are in Rukwa region. In each village five to ten fields were surveyed. Infected plant samples were collected from inside and outside the fields.

One isolate (93124c) differed in that it produced rough colony types *in vitro*. The isolate was obtained from a volunteer plant growing on a garbage heap and showed dark brown to black, irregular, haloless greasy lesions. In addition to production of green diffusible pigment, 32% of the strains of *P.s.phaseolicola* collected also produced a brown diffusible pigment *in vitro*. These strains occurred in Mbeya, Mbozi and Iringa districts (Fig. 3).

4.3 Pseudomonads other than *P.s. phaseolicola*

Of the 10 other fluorescent pseudomonads examined, six were identified as *Pseudomonas fluorescens* A. The strains came from symptomless leaf surfaces of bean genotypes Chipukupuku, Kablanketi, Kigoma, Masusu and Uyole-90. *Pseudomonas fluorescens* A was also obtained from *Desmodium variagutum* (isolate 9369a), *Dolichos* species (isolate 93146), *Crotalaria incana* (isolate 93129) *Trichodesma zeylanicum* (isolate trc) and a weed species in the family Asteraceae (isolate ast-4). These plant species exhibited dark to almost black water soaked lesions on the foliage. Symptoms were clearly visible from the underside of the leaves. *Pseudomonas fluorescens* isolates showed variation in their reaction to levan (±). They were positive for oxidase, arginine dihydrolase and did not cause a hypersensitive reaction in tobacco leaves. The isolates were non pathogenic on bean plants cultivar Canadian Wonder foliage and immature flat green pods. They varied in their ability to utilize mannitol and sorbitol as sole carbon sources for growth. Isolates obtained from symptomless leaves sampled from *P. vulgaris* plants, utilized both mannitol and sorbitol while those from other leguminous plant species exhibiting dark water-soaked lesions utilized mannitol but not sorbitol as sole carbon source for growth.

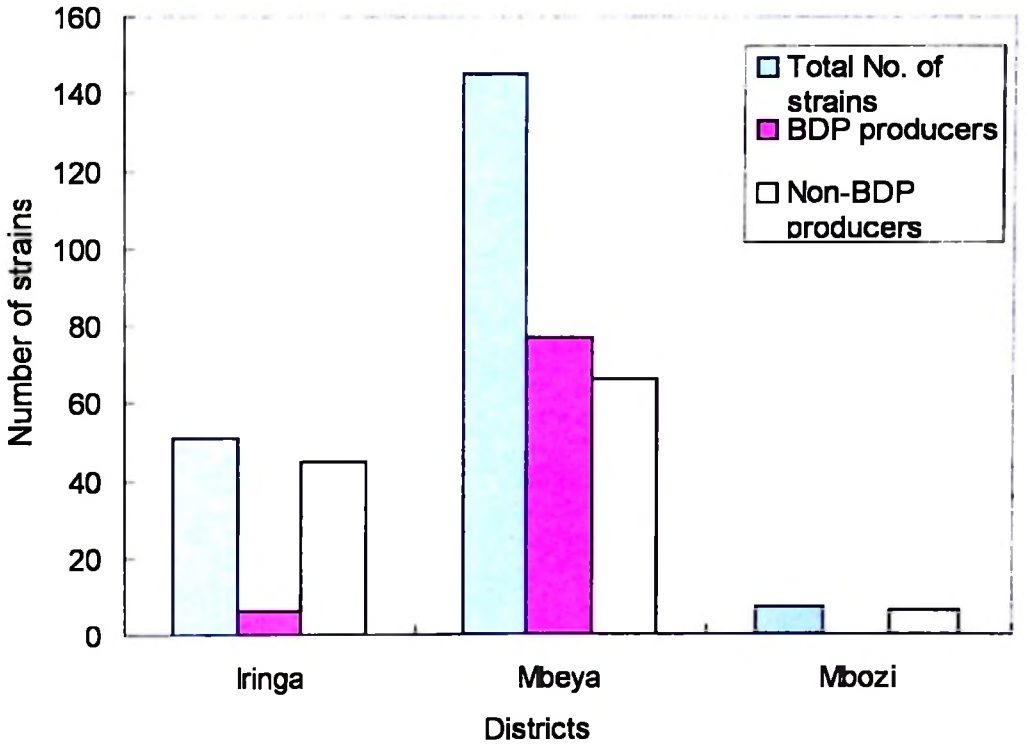


Figure 3. Proportion of *P. s. phaceolicola* strains producing the brown diffusible pigment (BDP) *in vitro*. The BDP strains were found in three out of the eight districts sampled during the period 1993 to 1995.

The other four isolates from *Vigna vexillata* (isolate vgn), *Clitoria ternatea* (isolate past-1 and past-2) and an unidentified wild legume (isolate 9360) were identified as *Pseudomonas syringae* pv. *syringae* A. The isolates were positive for levan and negative for oxidase and arginine dihydrolase activity. They were able to liquefy gelatin in 3 days and caused a hypersensitive reaction in tobacco leaves. The four isolates were able to utilize mannitol and sorbitol as sole carbon sources for growth and caused dark brown sunken necrotic lesions on lemon and pear fruitlets. Isolates 9360, past-1 and past-2 differed from isolate vgn in that bacterial growth for these three isolate was very slimy. In addition, the three isolates caused a very strong hypersensitive reaction in plants from nine bean genotypes. On immature flat green pods, the three isolates caused brown colouration noticeable at 24 hours after inoculation. After 3 days, the inoculated sites showed extensive dirty green water soaked lesions (Table 3).

Identification of *Pseudomonas syringae* pv. *syringae* A and *Pseudomonas fluorescens* A was further confirmed on the Biolog Microplate Identification system installed at the Department of Crop Science and Production, Sokoine University of Agriculture, Morogoro, Tanzania.

Table 3. Characteristics of selected *Pseudomonas* sp. isolates collected in the Southern Highlands of Tanzania.

Characteristics	9369a	12	ast-4	clnx	trc	vgn	past-1	past2	9360	9337	9359	7694	95015
Fluorescent pigment	+	++	++	++	+	+	+	+	+	+	+	+	+
Gelatin liquefaction	-	-	-	-	-	+	+	+	+	-	-	-	-
Acid production from glucose	nt	A	nt	nt	nt	nt	A	A	A	A	A	A	A
Arginine dihydrolase activity	+	+	+	+	+	-	-	-	-	-	-	-	-
Oxidase reaction	+	+	+	+	+	-	-	-	-	-	-	-	-
Pathogenicity on immature bean pods	-	-	-	-	-	-	HYP+	HYP +	HYP+	+	+	+	+
Pathogenicity on bean seedlings	-	-	-	-	-	-	HYP+[a]	HYP +[a]	HYP+?	+	+	+	+
Pathogenicity on pear fruitlets	-	-	-	-	-	+	+	+	+	-	-	-	-
Pathogenicity on lemon fruits	-	-	-	-	-	+	+	+	+	-	-	-	-
Tobacco hypersensitive reaction	-	-	-	-	-	nt	+	+	+	+	+	+	+
Utilization of carbon sources for growth													
Mannitol	++	++	+	+++	+	+	+	+	+	-	-	-	-
Sorbitol	-	++	-	++	-	+	++	++	++	-	-	-	-
Glucose	++	++	+	++	+	+	+	+	+	+	+	+	+

+ = positive reaction; ++ = stronger positive reaction; +++ = much more stronger positive reaction; - = negative reaction; nt = not tested; HYP+ = Hypersensitive reaction with dirty green water soaked necrotic lesions; clnx = isolate from external surfaces of bean leaves in the 1993 epiphytic populations field experiment;vgn = isolate from *Vigna* sp.; trc = isolate from*Trichodesma zeylanicum*; ast-4 = isolate from a weed species in the Asteraceae family; Past-1 and Past-2 = isolates from a pasture legume *Clitoria ternatea;* A = Acid production from glucose aerobically; HYP+? = Hypersensitive reaction associated with rapid necrosis of the plants; HYP+[a] = isolates showed a compatible reaction with bean cultivars Masusu and Uyole 94. Isolates 9369a, 12, ast-4,clnx and trc were identified as *P.fluorescens* A.Isolates vgn, past1,past2 and9360 were identified as *Pseudomonas syringae* pv. *syringae* A. 9337,9359,7694 and 95015 are code numbers for *P.s. phaseolicola* strains.

4.4 PATHOGENIC VARIATION IN *P. S. PHASEOLICOLA*

Considerable variation in the reaction patterns was observed on the halo blight differential bean cultivars, following inoculation with 250 *P.s.phaseolicola* strains. In some cases, identification of races was relatively easy, because reaction patterns were similar to those of the reference strains. In other cases, individual strains caused a mixture of reactions. Intermediates, which could not be defined as clearly resistant or clearly susceptible, were observed within the differential cultivars. Strains were, however, assigned to the races to which their reaction patterns most closely conformed.

4.4.1 Distribution of *P. s. phaseolicola* races identified

Proportions of *P.s. phaseolicola* races as they occurred in Iringa, Mbeya and Njombe districts in the Southern Highlands of Tanzania, are shown in Fig. 4, 5 and 6. Strains from Ileje district were all identified as race 2, while those from Sumbawanga and Nkasi districts were race 3. In Mufindi district, 3 of the nine strains collected were race 2, the other 3 were race 6 and the three remaining strains were of uncertain race type. Strains from Mbozi district were of races 3 and 4.

A strain determined as race 1 similar to reference strain 1281A, was obtained from *P.vulgaris* grown under irrigated conditions (vinyungu) in Iringa. Race 2, similar to reference strain 882, was found to occur in five out of the eight districts surveyed. Race 2 strains comprised 7.6% of the 250 *P.s.phaseolicola* strains characterized.

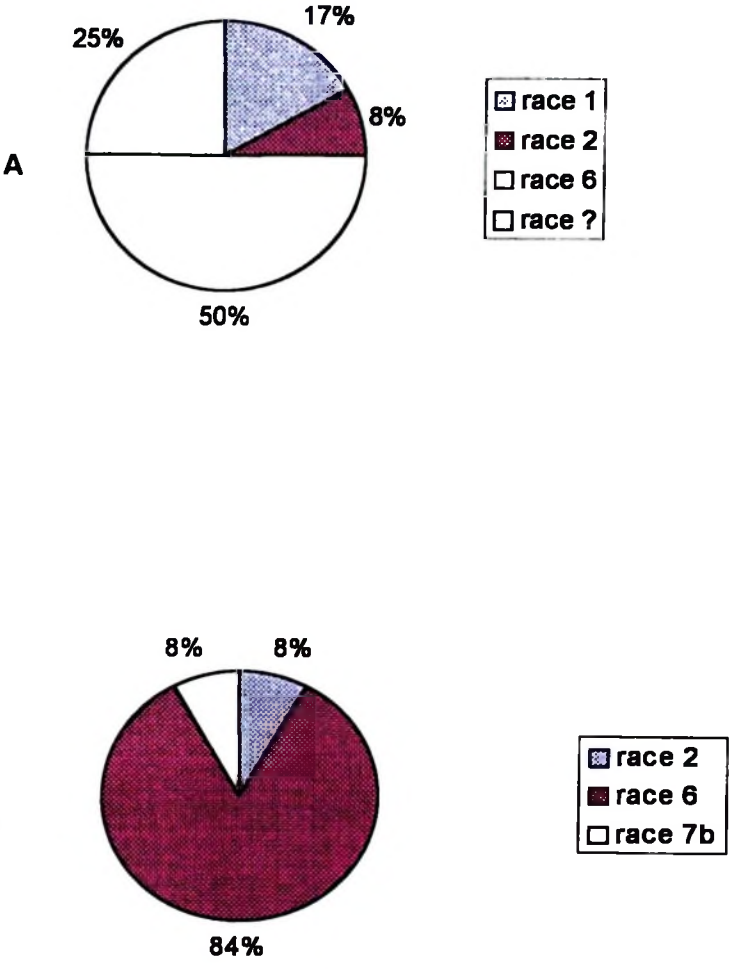


Figure 4. Proportion of various races of *P. s. phaseolicola* collected from Iringa district. A = strains collected in 1993; B = strains collected in 1994.

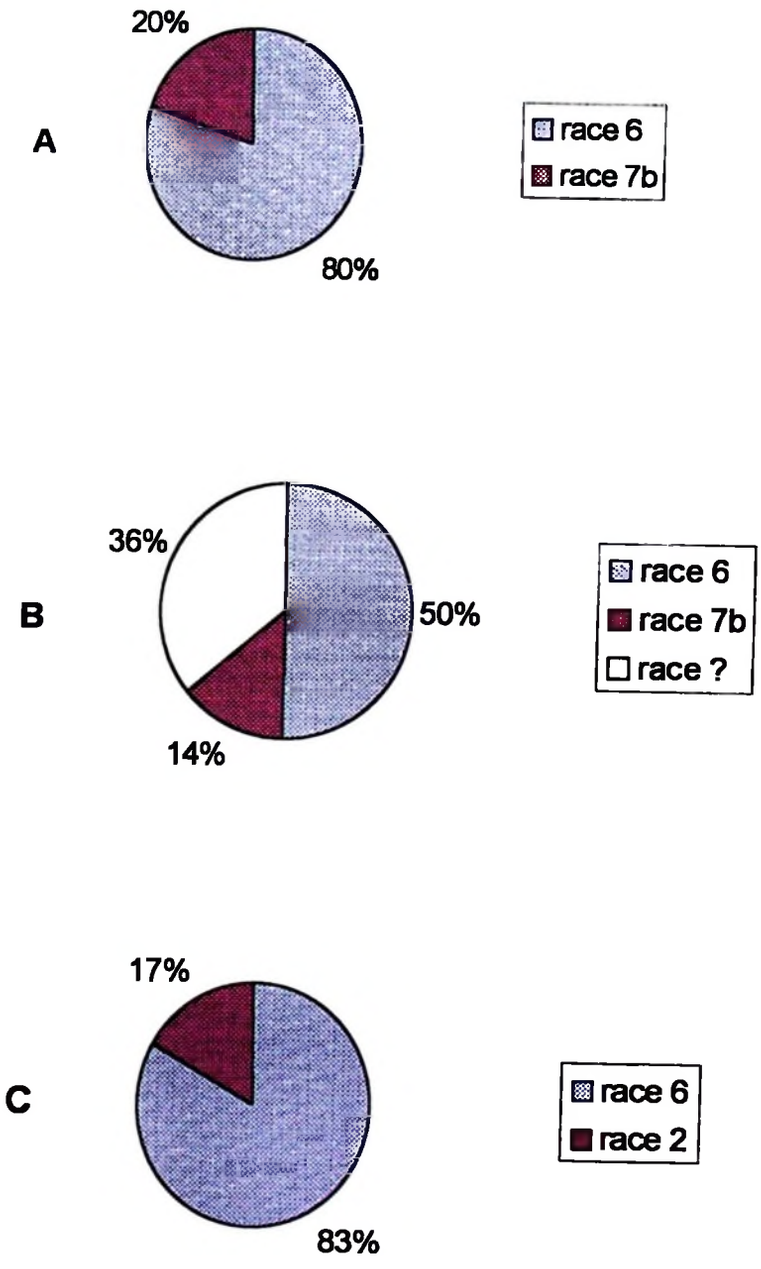


Figure 5. Proportion of various races of *P.s. phaseolicola* collected from Njombe district. A = strains collected in 1993; B = strains collected in 1994; C = strains collected in 1995.

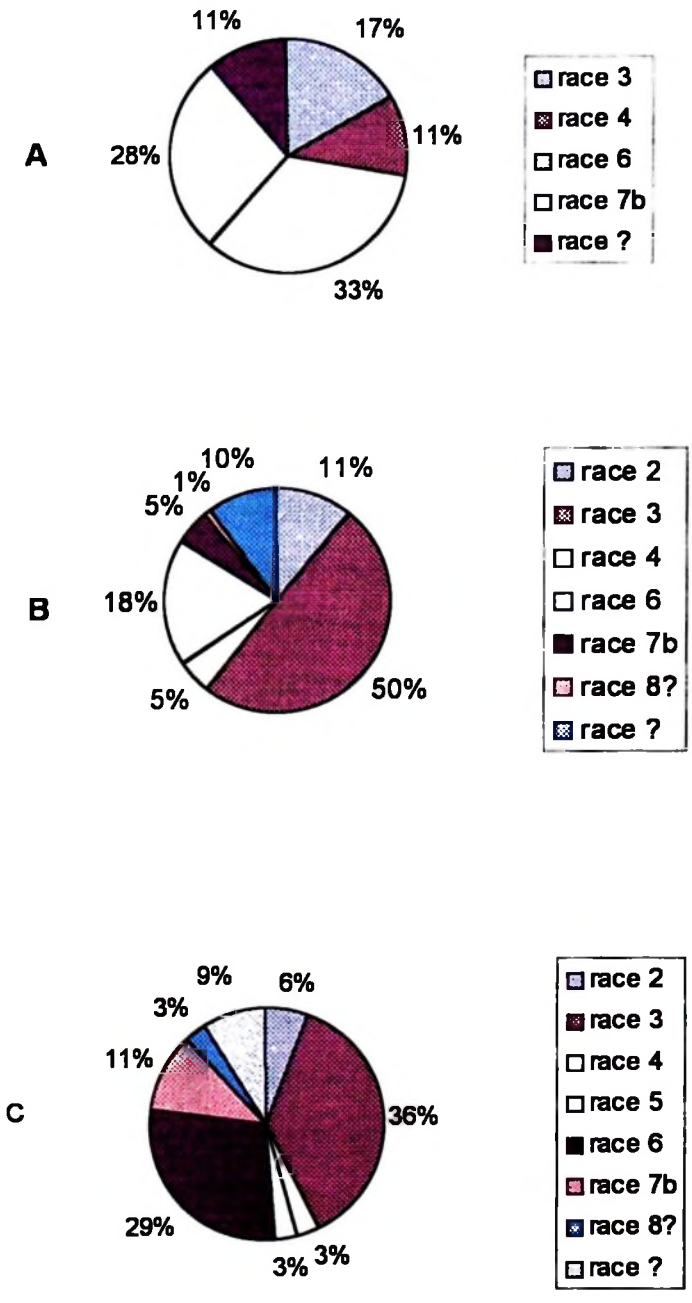


Figure 6. Proportion of various races of *P. s. phaseolicola* collected from Mbeya district. A = strains collected in 1993; B = strains collected in 1994; C = strains collected in 1995.

Race 3 strains were similar to reference strain 1301A and made the second largest component (32%) of the *P.s.phaseolicola* strains categorised. Race 3 was widely distributed in Mbeya and Mbozi districts and was the predominant race in Nkasi and Sumbawanga districts. Strains determined as race 4 similar to reference strain 1302 A occurred less frequently (6% of the total strains characterized). Race 5 similar to reference strain 1375A was found in only one disease incidence (0.4% of the strains categorized) and from *P.vulgaris* in Mbeya. Race 6 similar to reference strain 1299A, was the most frequently isolated and occurred for 35.6% of *P.s.phaseolicola* strains obtained from *P. vulgaris* in Iringa, Njombe, Mufindi and Mbeya districts. Eight percent of the strains were assigned to race 7b because the reference strain for comparison to previously identified race 7 (culture reference strain 1449 B) was received dead. Race 7b was found infecting *P.vulgaris, Neonotonia wightii* and *Desmodium* species in Iringa, Njombe and Mbeya districts. Two percent of the *P.s.phaseolicola* strains closely conformed to reference strain 2656A and were categorized as race 8. The strains were obtained from *P. vulgaris* in Mbeya. About 7.6% of the *P.s.phaseolicola* strains examined were of uncertain race type (R?). The strains caused reaction patterns, which did not fit in well with the identification scheme used.

4.4.2 Proportion of the brown diffusible pigment producers to non diffusible brown pigment producing strains in race types identified.

Strains producing the brown diffusible pigment *in vitro* formed relatively large proportions of strains categorized as races 3 and 6. In Mbeya district, 60 strains of *P.s.phaseolicola* were identified as race 3. Out of these 60 strains, 46 were BDP producers. This is about 1:1.3 BDP producers to non BDP producers. About 32 strains of *P.s.phaseolicola* from Mbeya district were identified as race 6. Out of the 32 strains 25 were BDP producers. This is 1:1.3 BDP producers to non-BDP producers. Five out of the 39 strains identified as race 6 in Iringa district were BDP producers.

This is 1:6.6 BDP producers to non BDP producing strains. In Mbozi district, the BDP producing strains encountered were race 3. The ratio of BDP producing to non-BDP producing strains belonging to race 3 in Mbozi was 1:2. There were no BDP producing strains from halo blight infected material collected in Ileje, Mufindi, Njombe, Nkasi and Sumbawanga districts.

4.4.3 Reaction of 36 bean genotypes to eight strains *P.s. phaseolicola*

The data, showing mean halo blight disease severity scores for thirty five *P.vulgaris* and one *P.coccineus* genotypes tested for reactions with eight strains representative of the races found to exist in the Southern Highlands of Tanzania are presented in Tables 4 and 5. These bean genotypes were evaluated in two experiments (GE-1 and GE-2). A set tested in experiment GE-1, consisted of 20 bean genotypes and the second set consisting of 16 bean genotypes was tested in experiment GE-2. All eight strains of *P.s.phaseolicola* used, were pathogenic to the 36 bean genotypes tested but their degree of pathogenicity varied significantly between the strains, the bean genotypes and even the bean genotype x strains interaction. All the 36 bean genotypes were highly susceptible to strains 7694 (race 2) and 12694 (race 6). Overall, average halo blight disease scores of 8.82 for race 2 and 8.61 for race 6 were recorded. Bean genotypes Uyole-90, G5773, LB465-1, Edmund and PBBL 226 showed high resistance to strain 7994 (race 3), with mean halo blight disease scores ranging from 1.13 to 2.0.

Table 4. **Reactions of 20 bean genotypes to eight strains of *P.s. phaseolicola* found in the Southern Highlands of Tanzania at 14 days after inoculation.**

Bean Genotype	Strains of *P. s. phaseolicola.*							
	7694 race 2	7994 race3	10794 race4	95015 race5	12694 race6	95014 race7	9337 race?	14494 Race 9?
Uyole-90	9.0	1.2	1.4	9.0	8.8	8.8	7.8	8.1[a]
CG 113	8.8	5.1*	6.0*	8.5	8.2	8.7	6.3*	8.2
P.coccineus	9.0	9.0	8.7	8.6	9.0	8.8	9.0	9.0
G 5773	9.0	1.3	1.5	8.7	8.5	8.1	7.5	8.7
HB 1-1	8.9	9.0	9.0	9.0	9.0	8.4	9.0	9.0
UAC 161	8.6	9.0	9.0	8.7	9.0	8.9	9.0	9.0
Tostado	8.8	6.0*	6.0*	8.3	9.0	8.5	7.6	9.0
MD-white	8.6	9.0	9.0	8.5	9.0	7.7	8.9	9.0
Nyamuhanga	9.0	8.6	8.6	8.6	8.8	5.0*	7.5	9.0
Masusu	8.9	8.8	9.0	8.4	8.9	7.5	6.9	9.0
Chipukupuku	8.5	8.1	9.0	8.1	8.6	8.3	5.7	9.0
Ludewa yellow	8.8	8.5	9.0	8.9	8.9	8.1	8.5	9.0
Kigoma	9.0	9.0	9.0	8.7	9.0	8.5	9.0	9.0
LB 110	9.0	4.9*	4.4*	6.0*	8.0	5.2*	8.7	8.8
G 2472	8.8	8.4	6.6*	8.3	8.1	8.6	8.3	8.9
UAC PBABL43	8.7	9.0	8.6	8.8	8.5	8.4	8.3	9.0
ZAA 840	9.0	6.6*	3.2	9.0	8.7	8.1	8.8	9.0
UAC G 435	9.0	9.0	8.0	8.7	8.6	7.9	7.9	8.7
G 3844	8.6	7.8*	5.6*	8.2	8.1	8.2	7.6	8.4
Canadian Wonder	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0
Mean	8.8	7.3	7.0	8.5	8.7	8.0	8.1	8.8
s.e.±				0.38				
cv %				9.17				

[a] Halo blight disease scores are based on a 1-9 scale where 1= no disease and 9.0 = death of the plants. Values are means of four replications, from repeated experiments carried out in the greenhouse. Data were analysed as a split plot of a randomized complete block design. Bacterial strains were in the main plots and bean genotypes were in the subplots.

*Some plants reacted with a very strong hypersensitive reaction, associated with suppression of plant growth and systemic necrosis.

Race? =Uncertain race type Race 9? = Closely related to race 9

Table 5. Reactions of 16 bean genotypes to eight strains of *P.s. phaseolicola* from the Southern Highlands of Tanzania at 14 days after inoculation.

Bean genotypes	Strains of *P.s. phaseolicola*							
	7694	7994	10794	95015	12694	95014	9337	14494
	race2	race3	race4	race5	race6	race7b	race?	race9?
Kabl. Red	8.5[a]	7.8	7.9	8.9	8.7	9.0	8.1	8.8
Selection-8	9.0	8.7	9.0	9.0	8.8	9.0	9.0	9.0
LB 465-1	8.1	2.0	2.1	3.0	7.6	2.0	3.9	8.6
DRK-4	9.0	8.9	9.0	9.0	9.0	9.0	9.0	9.0
FB GP 264-3	9.0	7.7	7.1	9.0	8.5	8.6	8.8	8.7
Edmund	9.0	1.1	5.7*	9.0	8.6	8.6	8.0	7.6
Ikinimba	8.7	6.1	7.6	2.5	7.0	2.3	7.1	2.7
Ilomba	9.0	8.8	8.4	8.0	9.0	5.1*	9.0	8.9
Uyole - 84	8.2	7.0	7.0	2.8	7.7	7.1	2.5	8.6
Kablanketi	9.0	8.6	7.8	7.6	8.9	8.3	6.7	9.0
Samdzuha	8.0	6.5*	9.0	7.9	8.2	8.8	8.0	9.0
Egerm-74	9.0	8.8	9.0	8.2	9.0	8.3	8.7	9.0
EAI 2525	9.0	7.9	8.7	3.6*	8.4	5.6*	8.1	8.8
PBBL 226	9.0	2.0	1.6	4.0	9.0	8.0	8.6	8.6
Lyamungo-90	8.8	9.0	8.3	2.2	9.0	1.0	8.1	9.0
Uyole - 94	9.0	8.5	9.0	8.1	8.8	8.2	8.6	8.8
Mean	8.8	6.8	7.3	6.4	8.5	6.8	7.6	8.4
S.E ±				0.38				
CV %				9.9				

[a] Halo blight disease scores are based on a 1-9 scale, where 1 = no disease and 9.0 = death of the plants. Values are means of 4 replications. Experiments were carried out in the greenhouse using a split plot of a randomized complete block design. Strains were in the main plots and bean genotypes in the subplots.
*Some plants in these genotypes reacted with a very strong hypersensitive reaction, which was associated with suppression of plant growth and systemic necrosis R?=uncertain race type. Race 9?=colsely related to race 9.
Race? = race type uncertain; Race9? = closely related to race 9; Kabl. Red = Kablanketi red

Bean genotypes CG 113, Tostado. LB 110 and ZAA 840 reacted to strain 7994 (race 3) with a very strong hypersensitive reaction which was associated with production of reddish brown necrotic lesions distributed over the entire leaf surfaces. Some plants within these genotypes showed suppressed growth (scores of 4.0 > 6.0) and systemic necrosis. Ikinimba reacted to strain 7994 with a slow disease development showing, a mean halo blight disease score of 6.07 at 14 days after inoculation. However, when incubated for longer than 14 days after inoculation, Ikinimba developed systemic chlorosis with necrosis. Samdzuha, a landrace from Mdandu, Njombe showed segregation in reaction to strain 7994 (race 3). Some plants showed typical water soaked lesions with necrosis while others showed less halo blight disease severity and developed new symptomless shoots noticeable at 14 days after inoculation. Four bean genotypes Uyole-90, G 5773, LB465-1 and PBBL 226 showed a high resistance to strain 10794 (race 4) with halo blight disease scores of 1.0 to 2.1. Genotype ZAA 840 showed some resistance to race 4 with a mean disease score of 3.25 while CG 113, Tostado, LB 110, G 2472, G 3488 and Edmund showed intermediate reactions with disease scores of 4.0 to 6.0 to strain 10794 (race 4). Ikinimba, LB 465-1, Uyole-84, EAI 2525 and Lyamungo-90 showed resistance to strain 95015 (race 5) with disease severity scores of 2.0 to 3.0. Two bean breeding lines, PBBL 226 and LB110, showed intermediate resistance with disease scores of 4.0 to 6.0 to strain 95015. Lyamungo-90, Ikinimba, and LB 465-1 showed resistance to a strain 95014 (race 7b) with disease scores of 1.0 to 2.0, while LB 110, Ilomba, EAI 2525 and Nyamuhanga, reacted with intermediate disease score of 5.0. Nyamuhanga, a landrace originating in Iringa, segregated for reactions to strain 95014 (race 7b).Some plants showed a hypersensitive reaction associated with systemic necrosis while others showed a hypersensitive reaction without systemic necrosis of the plants. When Nyamuhanga was inoculated with strain 95014 in repeated tests, two to four out of every 10 plants responded with a very strong hypersensitive reaction associated with systemic necrosis of the inoculated plants.

Uyole-84 showed resistance to strain 9337 (disease score of 2.5) while LB 465-1 reacted with a mean disease severity score of 3.8 indicating that some plants had intermediate resistance to strain 9337. The bean land races Masusu, Chipukupuku and kablanketi and an introduced bean line CG 113 also showed an intermediate reaction to strain 9337. The reactions of Masusu, Chipukupuku, and Kablanketi differentiated strain 9337 from the BDP producing strain (12694) characterized as race 6. The three landraces had shown high susceptibility to strain 12694. The reactions of these bean landraces to strain 9337 also cancels off the possibility of associating this bacterial strain to race 3. This is because none of these landraces (i.e. Masusu, Chipukupuku and Kablanketi) had responded with an intermediate reaction to race 3. Ikinimba was the only bean genotype, which showed resistance to strain 14494 and therefore, differentiated the strain from the other seven used in these experiments. Results presented in Tables 6 and 7 show that strains 7694 (race 2), 12694 (race 6) and 14494 (race 9?) were significantly more virulent to the bean genotypes tested while strains 7994 (race 3) and 95014 (race 7b) had consistently shown less virulence to the 36 bean genotypes. Analysis of variances indicated significant strain difference, genotype and strain x genotype, interaction differences ($P = 0.01$) (Tables 8 and 9). Overall, bean genotypes LB 110, Uyole-90, and G5773 (Table 10) as well as PBBL 226-3, Ikinimba, and LB465-1 (Table 11) showed significantly less halo blight disease severity. Sources of resistance identified have been summarized and presented in Table 12. Reactions of the landraces to the eight strains used in these tests are summarized in Table 13.

Table 6. **Mean halo blight disease severity scores for eight strains of *P.s. phaseolicola* from the Southern Highlands of Tanzania on 20 bean genotypes at 14 days after inoculation.**

Isolate code	Race	Mean disease score (1-9 scale)
7694	2	8.9[a] A
14494	9?	8.8 A
12694	6	8.7 AB
10794	5	8.5 B
9337	?	8.1 C
95014	7b	8.1 C
7994	3	7.4 D
13594	4	7.1 D
s.e.±		0.11

[a] Values are means of two experiments, each with four replications. Experiments were carried out in the greenhouse, data were analysed as a split plot of a randomized block design.

[b] Within a column, means followed by the same letter are not significantly different (P=0.05) by LSD.

?= Uncertain race type. Reactions do not fit well into the scheme used.

Table 7. **Mean halo blight disease severity scores for eight strains of *P.s.phaseolicola* found in the Southern Highlands of Tanzania on 16 bean genotypes at 14 days after inoculation**

Isolate code	Race	Mean disease score (1 - 9 scale)[a]
7694	2	8.77 A[b]
12694	6	8.51 B
14494	9?	8.38 C
9337	?	7.64 D
10794	4	7.33 E
7994	3	6.85 F
95014	7b	6.82 F
95015	5	6.44 G
s.e. ±		0.10

[a] Values are means of two experiments each with four replications. Experiments were carried out in the greenhouse; data were analysed as a split plot of a randomized block design.

[b] Within a column, means followed by the same letter are not significantly different (P=0.05) by LSD.

? =Uncertain race type, reactions do not fit in well on the scheme used.

Table 8. Mean squares from analysis of variance for reactions of 20 bean genotypes to eight strains of *P.s.phaseolicola* found in the Southern Highlands of Tanzania

Source	DF	Mean square
Replication	3	0.43
Strains	7	37.22**
Error	21	0.91
P.vulgaris x genotype	19	17.41**
Strains x genotype	133	6.32**
Error	456	0.56
Total	639	
cv%	9.17	

** Significant at the 0.01 probability level.

Two experiments were carried out in the greenhouse, each with four replications. Data were analysed as a split plot of a randomized complete block design.

Table 9. Mean squares from analysis of variance for reaction of 16 bean genotypes to eight strains of *P.s.phaseolicola* found in the Southern Highlands of Tanzania

Source of variation	DF	Mean Square
Replication	3	1.20
Strains	7	49.53**
Error	21	0.62
Bean genotypes	15	54.09**
Strains x genotypes	105	11.95**
Error	360	0.56
Total	511	
cv %		9.90

**Significant at the 0.01 probability level.

Two experiments were carried out in the green house, each with four replications.

Data were analysed as a split plot of a randomized complete block design.

Table 10. **Mean halo blight disease severity scores for 20 bean genotypes inoculated with eight strains of *P.s.phaseolicola* found in the Southern Highlands of Tanzania at 14 days after inoculation**

Entry No.	Bean genotype	Mean disease score (1 - 9scale)
20	Canadian Wonder	9.0[a] A[b]
5	HB 1-1	8.9 A
13	Kigoma	8.9 A
6	UAC 161	8.9 A
3	*Phaseolus coccineus*	8.9 A
8	Mdandu - white	8.7 AB
12	Ludewa-yellow	8.7 AB
16	UAC PBABL 43	8.7 AB
18	UAC G 435	8.5 BC
10	Masusu	8.4 BC
15	G 2472	8.3 CD
11	Chipukupuku	8.1 CDE
9	Nyamuhanga	8.1 CDE
7	Tostado	7.9 DE
19	G 3844	7.8 EF
17	ZAA 840	7.8 EF
2	CG 113	7.5 F
14	LB 110	6.9 G
1	Uyole-90	6.8 G
4	G 5773	6.7 G
s .e.±		0.13

[a]Values are means of two experiments each with four replications. Experiments were carried out in the greenhouse at MARTI Uyole Mbeya. Disease severity assessment was done at 14 days after inoculation. Data were analysed as a split plot of a randomized complete block design. Bacterial strains were in the main plots and bean genotypes were in the subplots.

[b] Within a column, means followed by the same letter are not significantly different (P = 0.05) by LSD.

Table 11. **Mean halo blight disease severity scores for 16 bean genotypes inoculated with eight strains of *P.s.phaseolicola* found in the Southern Highlands of Tanzania recorded at 14 days after inoculation**

Entry no.	Bean genotype	Mean disease score (1 - 9scale)
4	DRK-4	8.99[a] A[b]
2	Selection - 8	8.95 A
12	EGERM-74	8.77 B
16	Uyole-94	8.62 BC
1	Kablanketi-red	8.46 CD
5	FB GP 264 - 3	8.43 DE
8	Ilomba	8.28 EF
10	Kablanketi	8.24 F
11	Samdzuha	8.18 F
13	EAI 2525	7.52 G
6	Edmund	7.19 H
15	Lyamungo-90	6.93 I
9	Uyole-84	6.38 J
14	PBBL 226 - 3	6.35 J
7	Ikinimba	5.53 K
3	LB 465 - 1	4.66 L
s.e.±		0.13

[a] Values are means of two experiments each with four replications. Experiments were carried out in the greenhouse at MARTI Uyole Mbeya. Disease severity assessment was done at 14 days after inoculation. Data were analysed as a split plot of a randomized block design. Bacterial strains were in the main plots and genotypes were in the subplots.

[b] Within a column, means followed by the same letter are not significantly different (P=0.05) by LSD

Table 12. **Sources of resistance to races of *P.s.phaseolicola* found in the Southern Highlands of Tanzania during the 1993 / 95 period**

Bean genotype	Strains[a] /race types.								
	1281A race1	7694 race2	7994 race3	10794 race4	95015 race5	12694 race6	95014 race7b	9337 race?	14494 race9?
Uyole-90			1.25	1.49[b]					
G5773			1.30	1.56					
PBBL226			2.0	1.63					
Uyole84					2.83			2.5	
Ikinimba	(1.0)[c]				(1.0)2.54		2.34		(1.0)2.75
Edmund			1.13						
LB465-1			2.0	2.08	3.01		2.0	3.8	
Lyamungo90					2.25		1.0		

[a] Strain no. 1281Ais a reference strain. *P.s.phaseolicola* strains: 7694, 7994, 10794, 95015, 12694, 95014, 9337 and 14494 are collections from this study.

[b] Disease scores are based on a 1 - 9 scale. 1=no disease and 9.0 death of the plants. Values are halo blight disease severity means for two experiments each replicated four times. Assessment for disease severity was done at 14 days after inoculation.

[c] Disease severity scores in parenthesis show the reaction of cultivar Ikinimba to reference strains 1281 (race 1), 1375A (race 5) and 2709A (race 9). Values are means of two tests each done using ten plants of cultivar Ikinimba.

Table 13. **Reaction of ten bean landraces to eight strains of *P.s.phaseolicola* found in the Southern Highlands of Tanzania**

Bean genotype	Disease score (1-9scale)								
	R2[b]	R3	R4	R5	R6	R7b	R?	R9?	M
MD White	8.6	9.0	9.0	8.5	9.0	7.8	8.9	9.0	8.7[a]
Nyamuhanga	9.0	8.6	8.7	8.7	8.9	5.0*	7.5	9.0	8.2
Masusu	8.9	9.8	9.0	8.5	8.9	7.5	6.9*	9.0	8.4
Chipukupuku	8.6	8.2	9.0	8.1	8.6	8.3	5.7*	9.0	8.2
Ludewa- yellow	8.9	8.6	9.0	8.9	8.9	8.1	8.5	9.0	8.7
Kigoma	9.0	9.0	9.0	8.8	9.0	8.6	9.0	9.0	8.9
Kablanketi red	8.5	7.8	7.9	8.9	8.7	9.0	8.1	8.8	8.5
Selection 8	9.0	8.7	9.0	9.0	8.8	9.0	9.0	9.0	8.9
Kablanketi	9.0	8.6	7.8	7.6	8.9	8.3	6.7*	9.0	8.2
Samdzuha	8.0	6.5*	9.0	7.9	8.2	8.8	8.0	9.0	8.2
Mean	8.7	8.4	8.7	8.5	8.8	8.0	7.8	8.9	

a Values are means of four replications.

* Values indicating presence of resistant components in the landrace

b R2 = race 2; R3 = race 3 ; R4 = race 4; R5 = race 5; R6 = race 6; R7b = race 7b; R ? = uncertain race type; R 9? = strain closely related to race 9.

M = Mean

4.4.4 Reactions of immature flat *P. vulgaris* green pods to *P. s. phaseolicola.*

The results of a study on the interaction between 10 strains of *P.s.phaseolicola* with immature flat green pods from 14 bean genotypes are shown in Table 14. Strain 9357 (race ?), 93160 (race 3), 93150 (race 7b) and 93183 (race not characterized) caused incompatible reaction on pod tissues when inoculated on immature flat green pods from bean genotypes, Red Mexican UI3, A43, ZAA 840 and A53. At 24 h after inoculation, the pod tissues were noticeably brown. After 3 to 5 days the inoculated sites were dry. Overall, pods from ZAA 840, Red Mexican UI3 and A 53 showed significantly small lesions. Pods from Lyamungo-85, LB 110 and Kabanima had significantly large water soaked lesions (Table 15). On the basis of statistical mean separation tests, bean genotypes fell into five groups (Table 15). The 10 strains fell into six groups (Table 16). Results from two other experiments conducted in 1993 are shown in Tables 17 to 22. Analysis of variance for these experiments indicated significant strain, genotype and strain x genotype interaction differences (Appendices 1, 2 and 3). Results presented in Table 23 show reaction of immature flat green pods from genotype Canadian Wonder to 62 different strains of *P.s.phaseolicola.* There were significant differences (P = 0.05) between strains in lesion width. Strain 6294 (race 6) gave the largest mean lesion width and strain 4292 (race 3) gave the smallest lesion width. Lesion width ranged from 3.8 mm to 8.4 mm. The reaction of 32 strains of *P.s.phaseolicola* with immature flat green pods from Canadian Wonder, tested during the 1995 period are presented in Table 24. Pod lesion width ranged from 2.8 mm to 9.8 mm. The largest lesions were caused by strain 95023 (race 6) and the smallest lesions were caused by strain SMK (race 3? BDP producer).

Table 14. **Reaction (as lesion width in mm) of immature flat green pods from 14 bean genotypes, at 7 days after inoculation with ten strains of *P.s. phaseolicola.***

Bean		Strains of *P.s.phaseolicola.*									
Genotype	Code no.....	93170	9310a	93189	9357	9351	93189^	93153	93160	93183	93150
	Race.....	race6	race6	race2	race7?	race6	race2	nd	race3	nd	race7 b
Red Mexican UI 3		4.50	2.67	3.83	0.00	4.33	3.33	2.67	0.00	3.83	0.00[a]
Pinto		4.67	5.33	5.3	4.83	4.00	3.00	3.00	2.83	11.00	3.33
Kabanima		5.50	6.83	7.33	7.00	8.50	7.67	6.00	4.67	6.50	5.00
ZAA 840		1.00	3.50	4.00	0.00	6.33	3.67	3.67	0.00	2.00	1.67
A 43		5.83	5.17	6.33	0.00	6.17	7.83	3.17	0.00	3.17	3.33
A 52		4.83	4.42	4.81	5.08	4.31	5.89	3.78	5.06	4.89	4.42
LB 110		5.83	5.67	9.50	7.17	6.83	8.33	8.67	2.33	6.17	5.67
A 53		3.67	1.83	4.83	4.00	3.50	3.67	2.00	0.00	0.00	0.00
Tender green		4.17	3.67	2.33	5.50	3.33	5.67	3.50	3.33	3.00	4.17
Kablanketi		3.50	3.33	6.00	6.33	4.50	6.17	4.67	3.17	4.67	6.83
Lyamungo-85		6.17	7.17	6.00	7.17	10.00	7.67	4.33	8.00	5.17	6.50
Uyole 84		3.33	2.67	4.83	3.67	4.83	4.50	2.17	3.83	4.00	3.50
Lyamungo-90		6.00	6.83	6.33	3.17	8.33	7.33	4.50	6.00	4.50	3.83
Canadian Wonder		5.83	6.33	6.83	4.00	6.00	6.50	4.50	5.67	6.17	4.33
Mean		4.63	4.67	5.59	4.14	5.78	5.80	4.04	3.35	4.65	3.76
s.e.					0.49						
cv %					18.61						

[a] Values are means of two experiments, each with three replicates . The experiment was analysed as a split plot of a randomized complete block design. nd = race type not determined . 0.00 = pods showed only an inoculation wound at the inoculation site.
^ = code no. 93189-4

Table 15. **Mean lesion widths (mm) on immature flat green pods from 14 bean genotypes at seven days after inoculation with ten different strains of *P.s.phaseolicola*.**

Entry No.	Bean genotypes	Mean lesion width (mm)
11	Lyamungo-85	6.81[a] A[b]
7	LB110	6.62 A
3	Kabanima	6.50 A
13	Lyamungo-90	5.68 B
14	Canadian Wonder	5.62 B
10	Kablanketi	5.12 C
6	A 52	4.75 C
2	Pinto	4.73 C
5	A 43	4.10 D
9	Tender green	3.87 D
12	Uyole-84	3.73 D
4	ZAA 840	2.58 E
1	Red Mexican UI 3	2.52 E
8	A 53	2.35 E
s.e.±		0.16

[a] Values are means of two experiments, each with three replicates. Five inoculation points were made on each pod. Data were analysed as a split plot of a randomized complete block design.

[b]Within a column, means followed by the same letter are not significantly different (P = 0.05) by LSD.

Table 16. **Mean lesion width (mm) for ten strain of *P.s.phaseolicola* inoculated onto immature flat green pods from 14 bean genotypes as recorded at 7 days after inoculation.**

Isolate code	Race	Mean lesion width (mm)
93189-4	2	5.80[a] A[b]
9351	6	5.78 A
93189-1	2	5.59 A
9310a	6	4.67 B
93183	nd	4.64 B
93170	6	4.63 B
9357	7?	4.14 C
93153-b	nd	4.04 CD
93150	7b	3.75 D
93160	3	3.35 E
s.e ±		0.18

[a] Values are means of two experiments, each with three replicates. Five inoculation points were made on each pod. Data were analysed as a split plot of a randomized complete block design.

[b]Within a column, means followed by the same letter are not significantly different (P = 0.05) by LSD.

nd = race, not determined

Table 17. **Reaction (as lesion width in mm) of immature flat green pods from seven bean genotypes, at 7 days after inoculation with six strains of *P.s.phaseolicola.***

Bean genotype	Strains of *P.s.phaseolicola* [a]					
	9337[a] race?	9358 race6	93189 race2	9333a race?	9304 rac7b	9359 race6
Canadian Wonder	6.81	7.50	6.94	3.44	4.17	8.36[b]
Tender green	6.31	6.14	6.22	3.56	3.75	6.33
A43	4.67	5.17	5.17	3.58	3.72	4.03
A52	3.78	3.83	3.81	3.19	3.56	3.53
LB 110	3.33	5.39	5.53	3.61	4.08	6.17
Pinto	4.44	5.00	5.42	3.89	3.50	3.81
Lyamungo-90	4.86	5.44	5.69	4.78	3.11	5.31
Mean	4.89	5.50	5.54	3.72	3.70	5.36
s.c.$_{\pm}$			0.39			
cv %			14.7			

[a] Strains of *P.s.phaseolicola* used were randomly selected from collections made in 1993.

[b] Values are means of two experiments, each with three replicates. Five inoculation sites were made on each pod. Experiments were analysed as a split plot of a randomized complete block design.

? = uncertain race type

Table 18. **Mean lesion width (mm) on immature flat green pods from seven bean genotypes at 7 days after inoculation with six different strains of *P.s.phaseolicola.***

Entry No.	Bean genotype	Mean lesion[a] width (mm)
1	Canadian Wonder[b1]	6.20 A[c]
2	Tender green[b2]	5.38 B
7	Lyamungo-90	4.87 C
5	LB110	4.69 CD
3	A43[b3]	4.39 D
6	Pinto	4.34 D
4	A52[b4]	3.62 E
s.e.		0.16

[a] Values are means of two experiments, each with three replicates. Five inoculation points were made on each pod. Experiments were analysed as a split plot of a randomized complete block design.

b^1, b^2, b^3, and b^4 are halo blight differential bean cultivars. The remaining bean genotypes include a released variety and advanced breeding materials from the bean research programmes.

[c] Within a column, means followed by the same letter are not significantly different ($p = 0.05$) by LSD.

Table 19. **Mean lesion width (mm) for six strains of *P.s.phaseolicola* inoculated onto immature flat green pods from seven bean genotypes evaluated at 7days after inoculation.**

Isolate code	Race	Mean lesion width (mm)
93189-2	2	5.54 [a] A[b]
9358	6	5.49 A
9359	6	5.36 A
9337	?	4.88 B
9333a	?	3.72 C
9304	7	3.69 C
s.e.±		0.25

[a] Values are means of two experiments, each with three replicates. Five inoculation points were made on each pod. Data were analysed as a split plot of a randomized complete block design.

[b]Within a column, means followed by the same letter are not significantly different (P=0.05) by LSD.

Table 20. **Reactions (as lesion width in mm) of immature flat green pods from seven bean genotypes, at 7 days after inoculation with 18 strains of *P.s. phaseolicola***

Bean genotype	Strains/ races[b] of *P.s. phaseolicola.*																	
	93142	9353	9351	93188c	9341	93153b	93154	9357	93182	93184	93172	9358	93143c	93161	93140	93189-3	93181	93137
CW	6.33	6.83	3.97	6.33	4.17	3.67	4.83	3.00	3.67	2.67	3.67	3.50	4.00	4.17	3.17	4.67	4.67	3.50[a]
TG	2.17	3.67	3.78	6.83	3.00	4.00	3.83	3.00	3.50	2.67	3.33	3.50	0.00	3.83	4.50	6.00	0.00	3.67
RM UI-3	4.00	3.50	2.67	4.00	3.00	2.33	3.83	0.00	0.00	3.33	0.00	2.17	3.83	3.17	0.17	4.00	3.50	2.83
A 52	4.33	4.50	3.75	6.00	3.00	0.00	2.00	3.33	0.00	3.50	3.33	4.17	3.50	3.00	3.52	4.67	3.00	3.17
A 53	1.50	4.33	5.14	5.00	8.00	4.83	4.00	4.50	0.00	0.00	2.67	4.17	4.00	3.83	3.17	4.00	0.00	2.33
A 43	3.50	7.00	5.36	7.33	6.50	2.33	3.00	4.67	0.00	3.50	6.33	4.83	2.83	0.00	0.00	4.17	0.00	4.17
Kabl.	4.67	5.33	3.89	9.00	2.50	3.33	4.00	7.00	6.67	6.00	3.50	3.83	3.83	5.83	11.17	4.67	6.33	2.17
Mean	3.79	5.02	4.08	6.36	4.31	2.93	3.64	3.64	1.98	3.10	3.26	3.74	3.14	3.40	3.67	4.60	2.50	3.12
S.E ±	0.61																	
CV %	30.82																	

[a] Values are means of two experiments each with three replications. Experiments were analysed as a split plot of a randomized complete block design. [b] 93142/race 3; 9351 / race 6; 9353 / race 6; 93188c / race 6;9341 = race 6;93153b / race not determined; 93154 / race not determined; 9357 / race 7b; 93182 / race 4; 93184 /race not determined; 93172 / race 6; 9358 / race 6; 93143c / race not determined; 93161 / race 7b; 93140 / race 7b;93189-3 / race? (uncertain type); 93181 / race 4;93137 / race not determined. CW = Canadian Wonder TG = Tender green RM UI3 = Red Mexican UI3
Kabl. = kablanketi

Table 21. Mean lesion width (mm) on immature flat green pods from seven bean genotypes at 7 days after inoculation with 18 strains of *P.s.phaseolicola*

Entry no.	Bean genotype	Mean lesion width (mm)[a]
7	Kablanketi	5.20[a] A[b]
1	Canadian Wonder	4.26 B
6	A43	3.64 C
5	A53	3.41 C
2	Tender green	3.40 C
4	A52	3.26 C
3	Red Mexican UI3	2.57 D
s.e±		0.15

[a]Values are means of two experiments, each with three replicates. Five inoculation points were made on each pod. Data were analysed as a split plot of a randomized block design.

[b]Within a column, means followed by the same letter are not significantly different (p=0.05) by LSD.

Table 22. **Mean lesion width (mm) for 18 strains of *P.s.phaseolicola* inoculated onto immature flat green pods from seven bean genotypes evaluated at 7 days after inoculation.**

Race	Isolate code	Mean lesion width (mm)[a]
6	93188c	6.35[a] A[b]
6	9353	5.02 B
2	93189-3	4.59 BC
6	9341	4.31 CD
6	9351	4.08 CDE
3	93142	3.78 DEF
6	9358	3.73 DEFG
7b	93140	3.66 DEFG
7b	9357	3.64 DEFG
3	93154	3.64 DEFG
4	93161	3.40 EFGH
6	93172	3.26 FGH
3?	93143C	3.14 FGHI
nd	93137	3.11 FGHI
nd	93184	3.09 GHI
nd	93153b	2.92 HI
4	93181	2.50 IJ
4	93182	1.97 J
s.e ±		0.27

[a] Values are means of two experiments, each with three replicates. Five inoculation points were made on each pod. Data were analysed as a split plot of a randomized block design.

nd = race not determined; 3? = race type not confirmed.

[b] Within a column, means followed by the same letter are not significantly different

Table 23. **Reactions (as lesion width in mm) of immature flat green pods from bean cultivar Canadian Wonder to 62 strains of *P.s. phaseolicola* at 7 days after inoculation.**

Isolate Code	Race	Mean lesion width (mm)
0294	6	8.45 A
0594	6	8.45 A
2494	7b	8.36 AB
1294	6	8.20 ABC
3294	6	7.92 ABCD
2094	6	7.56 ABCDE
3494	6	7.44 ABCDE
1994	6	7.39 ABCDEF
1594	6	7.36 ABCDEF
2694	7b	7.33 ABCDEF
4194	3	7.33 ABCDEF
0894	6	7.22 ABCDEFG
3094	6	7.19 ABCDEFG
2794	6	7.11 ABCDEFG
0694	2	7.00 BCDEFGH
2894	7b	7.00 BCDEFGH
1194	6	6.86 CDEFGHI
0794	6	6.83 CDEFGHIJ
1394	6	6.75 DEFGHIJK
3394	6	6.67 DEFGHIJKL
1094	6	6.67 DEFGHIJKL
3194	6	6.36 EFGHIJKLM
14194	3	6.14 EFGHIJKLMN
2594	7b	6.14 EFGHIJKLMN
0394	7b	6.14 EFGHIJKLMN
2994	6	5.89 GHIJKLMNO
1694	6	5.89 GHIJKLMNO
13894	3	5.83 GHIJKLMNOP

Table 23. continued

Isolate code	Race	Mean lesion width (mm)
1794	7b	5.81 GHIJKLMNOP
0194	6	5.61 HIJKLMNOPQ
3594	6	5.58 HIJKLMNOPQ
5494	?	5.56 IJKLMNOPQ
1894	7b	5.56 IJKLMNOPQ
3694	6	5.45 IJKLMNOPQ
6394	3	5.42 JKLMNOPQ
3894	3	5.33 KLMNOPQR
7194	?	5.33 KLMNOPQR
3994	3	5.33 KLMNOPQR
4094	3	5.33 KLMNOPQR
6694	3	5.33 KLMNOPQR
5594	6	5.27 LMNOPQR
2194	6	5.17 MNOPQRS
5694	?	5.13 MNOPQRS
14694	3	5.11 MNOPQRS
5294	6	5.11 MNOPQRS
7494	2	5.08 MNOPQRS
0994	6	5.00 MNOPQRS
11494	3	5.89 NOPQRS
4794	?	4.82 NOPQRS
3794	3	4.80 NOPQRS
5194	6	4.80 NOPQRS
4994	?	4.71 OPQRS
7294	6	4.58 OPQRS
6994	?	4.58 OPQRS
7094	?	4.56 OPQRS
5394	?	4.55 OPQRS

Table 23
continued

Isolate code	Race	Mean pod reaction (Lesion width in mm)	
4894	?	4.42	PQRS
1494	6	4.36	QRS
5094	?	4.27	QRS
0494	7	3.94	RS
4294	3	3.82	S
Mean		5.94	
CV %			14.82

a Values are means of two experiments, each with three replicates. Five inoculation points were made on each pod. Data were analysed as a randomized complete block design.

Within column, means followed by the same letter are not significantly different (p=0.05) by LSD.

? uncertain race type.

Table 24. **Reaction (as lesion width mm) of immature flat green pods from bean cultivar Canadian Wonder to 32 strains of *P.s. phaseolicola* at 7 days after inoculation.**

Isolate code	Race	Mean lesion width (mm) [a]
95023	6	9.80 A[b]
95037	2	8.64 AB
95010	6	8.03 BC
95029	2	7.50 BCD
95003	6	7.45 BCDE
95024	6	7.40 BCDEF
95014	7b	7.40 BCDEF
95006	6	7.36 BCDEF
95004	6	7.25 BCDEF
95005	?	7.25 BCDEF
95 001	6	7.08 BCDEF
95007	6	7.08 BCDEF
7694	2	6.89 CDEF
ITZ-3	nd	6.25 DEFG
95031	6	6.15 DEFGH
95008	6	6.08 DEFGH
95015	5	6.06 DEFGH
95052	8?	5.81 EFGHI
95036	3	5.77 FGHI
95049	3	5.19 GHIJ
95042	3	5.11 GHIJK
95034	3	4.53 HIJKL
95002	3	4.39 IJKLM
95039	3	4.33 IJKLM
307-b	?	4.0 JKLM

Table 24. continued

Isolate code	Race	Mean lesion width (mm)[a]	
95046	3	3.92	JKLM[b]
95050	3	3.61	JKLM
95047	3	3.58	JKLM
95026	6	3.55	KLM
95048	3	3.22	LM
95044	3	2.95	LM
SMK-2	nd	2.83	M
Mean		5.83	
CV %		17.29	

[a] Values are means of two experiments, each with three replicates. Five inoculation points were made on each pod. Data were analysed as a split plot of a randomized complete block design.

Within a column, means followed by the same letter are not significantly different (P= 0.05) by LSD.

nd = information on race, lacking.

There were significant differences (P = 0.05) between strains as expressed by lesion width (Appendix 4). Tests for variation in pathogenicity using immature flat green bean pods show that, strains identified as race 2 and race 6 had consistently given the largest lesions. Strains identified as race 3 had for most of the tests caused small lesions. A few deviations from this trend were however, observed.

4.5 MOTILITY OF *P.S.PHASEOLICOLA*

4.5.1 Motility of *P. s. phaseolicola* as monitored in a semisolid medium

Significant differences in motility (P = 0.05), were observed between strains of *P.s.phaseolicola* as monitored in a semi solid medium. Fifteen out of the 16 strains tested were motile. Mean bacterial band movement (down the tube) measured in mm; ranged from 0 to 6.0 mm during the first 30 minutes following inoculation (Table 25). Results obtained at one hour after inoculation showed that 67% of the strains tested remained static. Rate of movement for the bacteria, which moved during the first hour following inoculation, ranged from 0 to 9.5 mm per hour. Four out of the six (67%) *P.s. phaseolicola* strains, which showed movement between 30 minutes and one hour after inoculation, were those characterized as race 6. The rate of movement recorded for the period between one and 4 hours, ranged from 0 to 8 mm per hour. The highest three rates of movement were recorded on strains 93188C (race 6); 93172 (race 6) and 93171 (race 7b). The lowest motility rates were observed on strains 9333a (uncertain race type), 9337 (uncertain race type) and 93137 (race not determined). Results also show that there are differences in rate of motility between strains of the same race.

The rate of motility for strains characterized as race 7b ranged from 0 to 6.5 mm per hour. Strains characterized as race 6 showed motility rates ranging from 3.6 to 8.0 mm per hour.

4.5.2 Growth of *P. s. phaseolicola* assessed using the motility medium

There were significant differences (P = 0.05) between strains of *P.s.phaseolicola* in the outward growth of the various strains on motility medium. Using the rate of increase in the outward growth to compare the strains tested, strains can be grouped into: (a) those which had a high initial rate of increase in growth during the first 24 hours; followed by a continuous decline in the rate of increase in growth during the second and third 24 hours of incubation. (b) those which had a low rate of increase in growth during the first 24 hours followed by an increase during the second 24 hours and a decline in the rate of increase in growth during the third 24 hours of incubation and © those which had a high initial rate of increase in growth during the first 24 hours followed by a decrease during the second 24 hours and an increase in rate of growth during the third 24 hours of incubation. Results indicate that all the strains tested increased in the outward growth from 24 hours of incubation (Table 26).

Table 25. **Motility of *P.s.phaseolicola* (as band movement down the tube) monitored in a semi-solid medium**

		Bacterial band movement down tube				
Isolate code	race	30min	1hour	rate[a]	4hours	rate mm/hr [b]
9304	7b	4.0	4.0	(0)	4.0	(0)
93189-4	2	4.0	4.0	(0)	18.8	(4.9)
93163	6	6.0	6.0	(0)	17.0	(3.6)
93104	6	0	5.0	(0.17)	22.4	(5.8)
93137	nd	5.0	5.0	(0)	13.0	(2.7)
9333a	?	6.0	6.0	(0)	13.5	(2.5)
9359	6	4.0	4.0	(0)	23.0	(6.3)
93171	7b	3.0	3.0	(0)	22.5	(6.5)
93160	3	5.0	5.0	(0)	22.6	(5.9)
9337	?	5.0	5.0	(0)	8.5	(1.2)
93140	7b	5.0	8.0	(0.1)	22.0	(4.7)
93182	4	4.0	9.5	(0.18)	21.5	(4.0)
lidb	nd	4.5	4.5	(0)	4.5	(0)
93189-3	2	5.0	5.5	(0.02)	20.0	(4.8)
93172	6	0	5.0	(0.16)	27.0	(7.3)
93188c	6	0	5.5	(0.18)	29.5	(8.0)
Mean		3.78	5.31			
cv %		14.43	15.37			

[a] Rate of increase in bacterial band movement was obtained by expressing the distance (in mm) the bacterial band had moved downwards in respect to the time (from 30 minutes to one hour) taken.

[b] Rate of increase for bacterial band movement was expressed as mm per hour.

nd= information on race type lacking. ? = uncertain race type.

Table 26. **Growth of *P.s. phaseolicola* (as spot diameter in mm) on motility medium.**

Isolate code	Growth of the bacterium (mm)			
	24 hours	48 hours	72 hours	96 hours
9341	7.200	7.733	8.400	9.533
LID - a	7.067	8.333	9.267	10.33
93142	6.867	8.200	9.267	10.13
93162	6.800	7.867	9.067	9.800
93188c	6.600	8.467	9.933	10.40
93150	6.533	7.533	8.667	9.467
93167	6.400	7.467	9.133	10.40
93189-4	6.333	7.933	8.933	9.533
9310a	6.267	7.867	8.867	10.07
93137	6.267	7.800	8.733	10.27
93164	6.200	7.133	8.600	9.267
Pyr-10	6.200	6.933	8.00	9.133
93171	5.867	7.267	9.200	10.07
9357	5.800	6.933	7.933	8.933
93172	5.733	7.200	9.067	9.467
93160	5.667	7.000	8.600	9.467
Mean	6.362	7.604	8.854	9.767
LSD (0.050)	0.589	0.500	0.715	0.830
CV%	5.55	3.94	4.85	5.10

[a] Values are means of bacterial growth from five isolated spots made on motility medium contained in petri plates. Bacterial suspensions of approximately 10^7 to 10^8 cfu/ml were spotted on the medium and plates incubated at 22 to 25^{0}C. After 24 hours of incubation diameters of bacterial growth evident, were measured and used to compare growth of the different strains in the motility medium.

4.6 EPIPHYTIC BACTERIA POPULATION STUDIES

4.6.1 Epiphytic bacterial populations on leaf surfaces of field grown beans

Leaf sampling started before inoculation, then at 4 hours after inoculation and later at 3 days intervals for a period of 15 days. During the 1993 growing season, *P.s. phaseolicola* was not recovered from leaf surfaces of field grown bean genotype Kablanketi and Uyole-90 inoculated with *P.s.phaseolicola* strains 9337 and 9359. A highly fluorescent bacterium was however, isolated from these bean leaf surfaces. On the basis of colony morphology, biochemical tests and the biolog identification system, the bacterium was identified as *Pseudomonas fluorescens* A. Pure cultures of *Pseudomonas fluorescens* A, produced rod shaped gram negative cells. The bacterium was positive for oxidase, negative for hypersensitive reaction on tobacco and was non-pathogenic on 10-day-old bean cultivar Canadian Wonder seedlings and on immature flat green pods.

4.6.2 Epiphytic populations of *Pseudomonas fluorescens* A on field grown bean genotypes Uyole-90 and Kablanketi

Mean epiphytic populations of *P. fluorescens* A. isolated from field grown beans during the 1993 growing season are presented in Table 27. Bacterial population of *P.fluorescens* A ranged from 5.0×10^1 to 3.0×10^2 cfu/cm^2. There were no significant differences (P = 0.05) between *P. fluorescens* A bacterial populations detected within the treatments.

Table 27. **Populations (per cm^2 of leaf surface) of *Pseudomonas fluorescens* A recovered from symptomless leaf surfaces of two bean genotypes grown in the field during the 1993 growing season at Uyole Research Institute, Mbeya, Tanzania at 6, 9, 12 and 15 days after inoculation with strains 9337 and 9359 of *P.s.phaseolicola*.**

Treatment combination	Mean CFU per cm^2			
	6days	9days	12days	15days
Kablanketi inoculated with sdw	6.4×10^1[a]	1.4×10^2	2.2×10^2	2.9×10^2
Kablanketi inoculated with 9337	5.0×10^1	1.7×10^2	2.3×10^2	2.9×10^2
Kablanketi inoculated with 9359	9.1×10^1	1.6×10^2	2.5×10^2	2.8×10^2
Uyole 90 inoculated with sdw	7.4×10^1	1.6×10^2	2.6×10^2	2.2×10^2
Uyole 90 inoculated with 9337	5.4×10^1	1.6×10^2	1.6×10^2	2.7×10^2
Uyole 90 inoculated with 9359	9.1×10^1	1.3×10^2	3.0×10^2	3.0×10^2
cv %	28.26	9.6	7.8	46.3

[a] Means are the average of three replicates from two different bean genotypes. Bacteria were removed from symptomless leaves by washing and enumerated by plating 10-fold serial dilutions on KB.

sdw = sterile distilled water

9337 and 9359 are isolate codes for *P.s.phaseolicola*

Strain 9337 is a BDP producer but of uncertain race type.

Strain 9359 is race 6.

4.6.2.1 Epiphytic populations of *Pseudomonas fluorescens* A from farmers' fields.

Failure to recover *P.s.phaseolicola* from leaf surfaces of field grown bean genotypes Kablanketi and Uyole-90, in the presence of *P.fluorescens* A, prompted a need to investigate the epiphytic bacterial populations on bean leaf surfaces in farmers' fields. The results show that *P. fluorescens* A was present in relatively large numbers on symptomless leaf surfaces sampled from bean genotypes Kigoma, Kablanketi, Chipukupuku and Masusu (Table 28). Isolation plates were almost pure and colony numbers of *Pseudomonas fluorescens* A ranged from 1.3×10^2 to 2.2×10^2 cfu/cm^2. Cultivar Kigoma gave least bacterial populations than genotypes Masusu, Kablanketi and Chipukupuku. The observation is interesting because the bean genotype Kigoma showed high susceptibility to all the *P.s.phaseolicola* pathogenic variants identified during this study (Table 4).

4.6.3 Epiphytic populations of *P.s. phaseolicola* from field experiments

4.6.3.1 Effect of bean genotypes Uyole-90 and Kablanketi

Populations of *P.s.phaseolicola* recovered from leaf surfaces of bean genotypes Uyole-90 and Kablanketi are shown in Fig. 7. Although the bacterial cell numbers recovered were relatively low, the results show different patterns for the bean genotypes Uyole-90 and Kablanketi in epiphytic populations of *P.s. phaseolicola.* The differences were more apparent during the initial period of colonization i.e. immediately after inoculation (4 hours to 3 days), and at 15 days after inoculation.

Table 28. Populations (per cm^2 of leaf surface) of *Pseudomonas fluorescens* A recovered from symptomless leaf surfaces of four bean genotypes sampled from farmers fields during the 1993 growing season in Itezi village Mbeya, Tanzania.

Bean genotype	Mean CFU per cm^2 leaf surface
Kigoma	1.3×10^{2a}
Chipukupuku	2.2×10^2
Kablanketi	1.4×10^2
Masusu	1.9×10^2
cv %	10.04

[a] Means are based on isolations from four bean genotypes each replicated three times in a randomized complete block design. Symptomless leaf samples were collected from uninoculated beans planted in March 1993 in farmers fields. Bacterial cells on leaf washes were detected by plating 10-fold serial dilutions on KB. Plates of KB streaked with pure cultures of *P.s.phaseolicola* were used for comparison with the dilution plates to assist in distinguishing bacterial cells when making the counts.

Immediately after inoculation, multiplication of *P.s.phaseolicola* bacterial cells on bean genotype Kablanketi was slow. In contrast, a sharp increase in bacterial cell numbers was observed on Uyole-90 during the period between 4 hours and 3 days after inoculation. From 9 to 15 days after inoculation, bacterial cell numbers on Kablanketi had increased and reached a plateau. Bacterial cells were more than those recovered immediately after inoculation. On Uyole-90 bacterial cell numbers observed from 12 to 15 days after inoculation had declined and populations recovered at 15 days were even lower than those observed at 4 hours after inoculation. (Fig. 7). A more or less similar trend was observed on field grown bean genotypes Kablanketi and Uyole-90 in 1995 (Fig. 8). However, bacterial cells recovered in the 1995 experiment, were very low and therefore, considered undetectable for most of the assays. This is because bacterial populations were less than 1.0 cfu/cm^2 of leaf surface. Statistical analysis showed that there were significant differences (P = 0.05) in bacterial populations recovered from the leaf surfaces of the two bean genotypes at 12 and 15 days after inoculation.

4.6.3.2 Effect of *P.s.phaseolicola* strains 9337 and 9359

Results on the effect of bacterial strains on the epiphytic populations of *P.s. phaseolicola* are shown in Figures 9 and 10. There were significant strain difference (P = 0.05) in populations recovered from the leaf surface. The results show that the two *P.s.phaseolicola* strains tested differ in their ability to survive epiphytically. During the first 4 hours to 3 days after inoculation, strain 9359 showed a slightly higher cell multiplication than strain 9337. At 6 days after inoculation, bacterial cells of strain 9359 decreased slightly but those of strain 9337 increased.

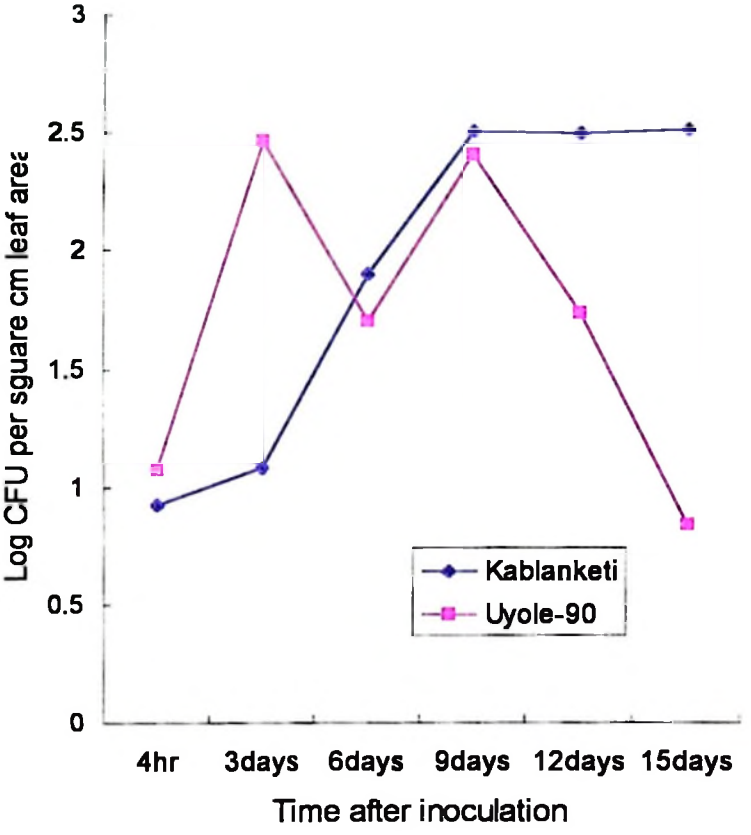


Figure 7. Epiphytic populations of *P .s. phaseolicola* on symptomless leaves of field grown beans genotypes Kablanketi and Uyole-90 during the 1994 growing season. Data are means of three replications. Leaves were spray – inoculated when bean plants were 21 days old.

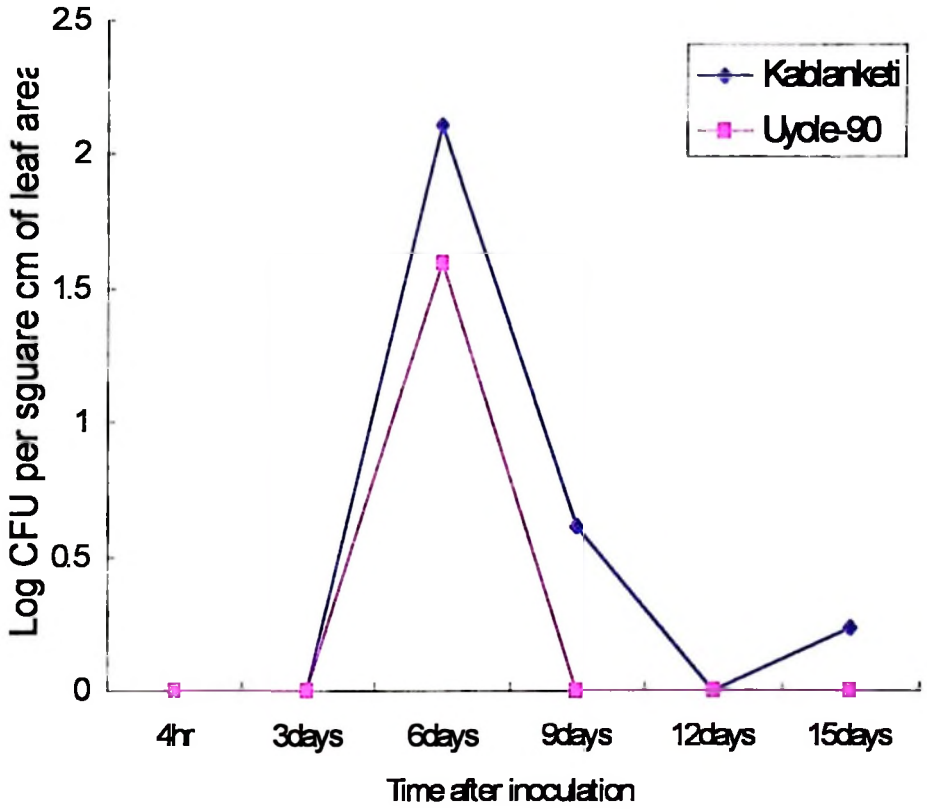


Figure 8. Epiphytic populations of *P. s. phaseolicola* on symptomless leaf surfaces of field grown bean genotypes Kablanketi and Uyole-90 during the 1995 growing season. Data are means of three replications. Leaves were spray – inoculated when bean plants were 21 days old.

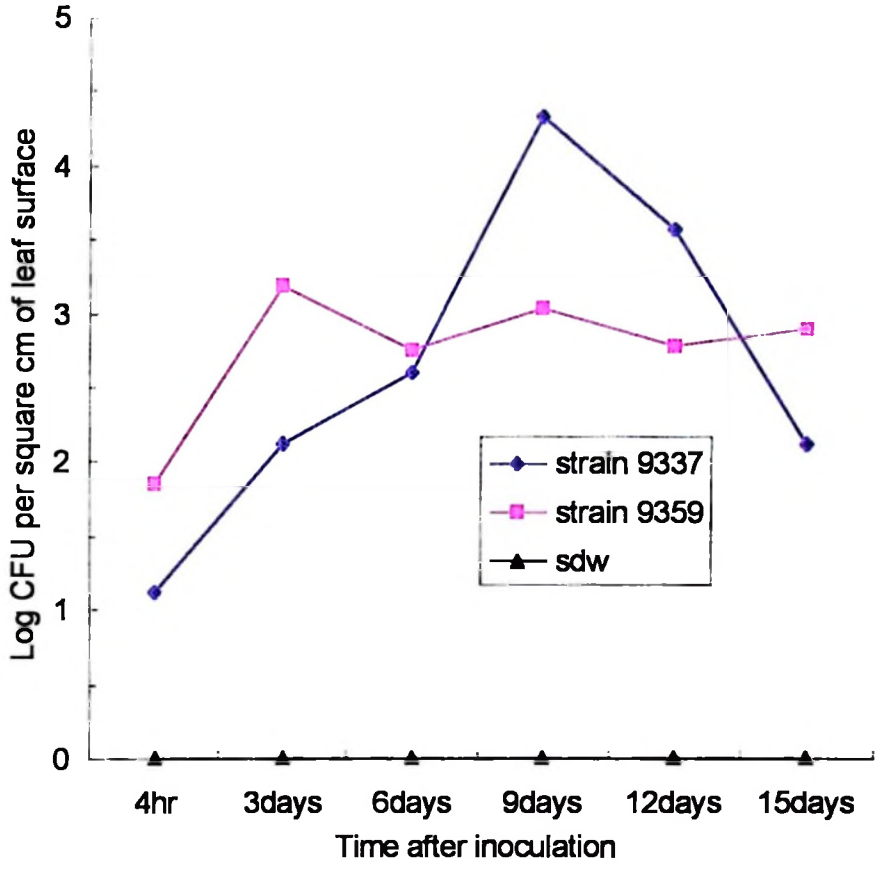


Figure 9. Epiphytic populations of strains of *P.s.phaseolicola* recovered from symptomless leaves of field grown bean genotypes Kablanketi and Uyole-90 during the 1994 growing season Leaves were spray-inoculated when bean plants were 21 days old. Control plots were inoculated with sterile distilled water (SDW).

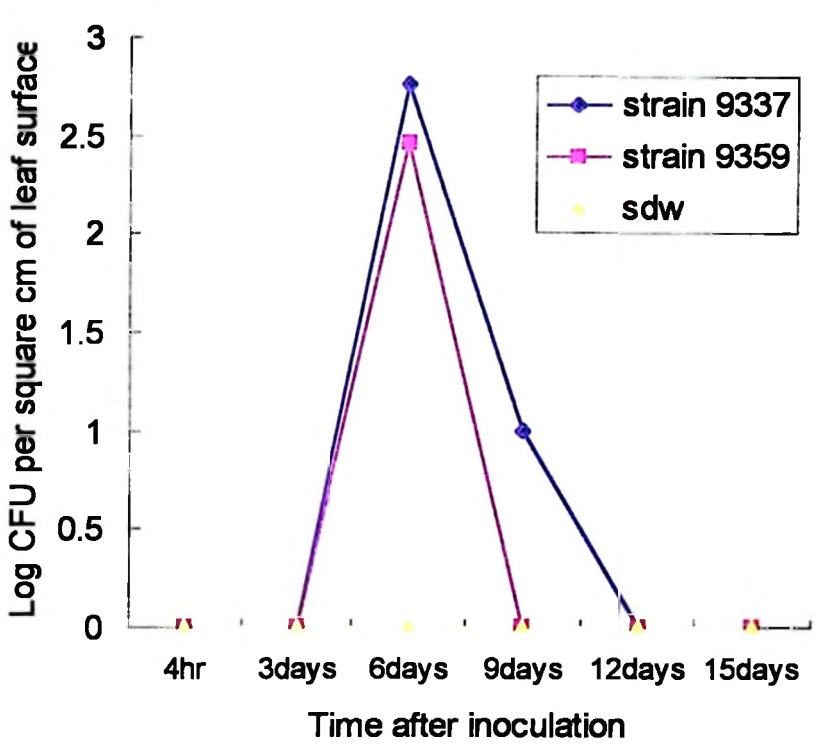


Figure 10. Epiphytic populations of *P. s. phaseolicola* from symptomless leaves of field grown bean genotypes Kablanketi and Uyole-90 during the 1995 growing season. Leaves were inoculated when plants were 21 days old.

Bacterial cells of strain 9359 increased steadily from 9 to 15 days after inoculation. Those of strain 9337, increased sharply at 9 days but decreased sharply again at 12 to 15 days after inoculation. Results from the 1995 experiment (Fig. 10) show that bacterial cells from both strains were undetectable (less than 1.0 cfu/cm^2) for most of the assays done. At 6 and 9 days, bacterial populations of strain 9337 were higher than those of strain 9359.

4.6.3.3 The interaction of bean genotypes and *P.s.phaseolicola* strains

Results of the interaction between bean genotypes Kablanketi and Uyole-90 x strains 9337 and 9359 of *P.s.phaseolicola* are shown in Figures 11 and 12. An interaction between Uyole-90 and both strains 9337 and 9359 showed a sharp increase in bacterial cell numbers between 4 hours and 3 days after inoculation. Bacterial cells on Uyole-90, dropped down sharply at 6 days, raised sharply at 9 days but declined again through the 12 to 15 days after inoculation. Bacterial cells recovered from Uyole-90 inoculated with strain 9359 at 15 days, were lower than those recovered at 4 hours after inoculation. During the period 4 hours to 3 days after inoculation, multiplication of both strains 9337 and 9359 on cultivar Kablanketi appeared to lag. At 9 days, bacterial cells of strain 9337 on Kablanketi increased sharply while those of strain 9359 on the same bean genotype declined slightly. From 12 to 15 days after inoculation, bacterial cells of strain 9359 on Kablanketi increased sharply while those of strain 9337 declined sharply.

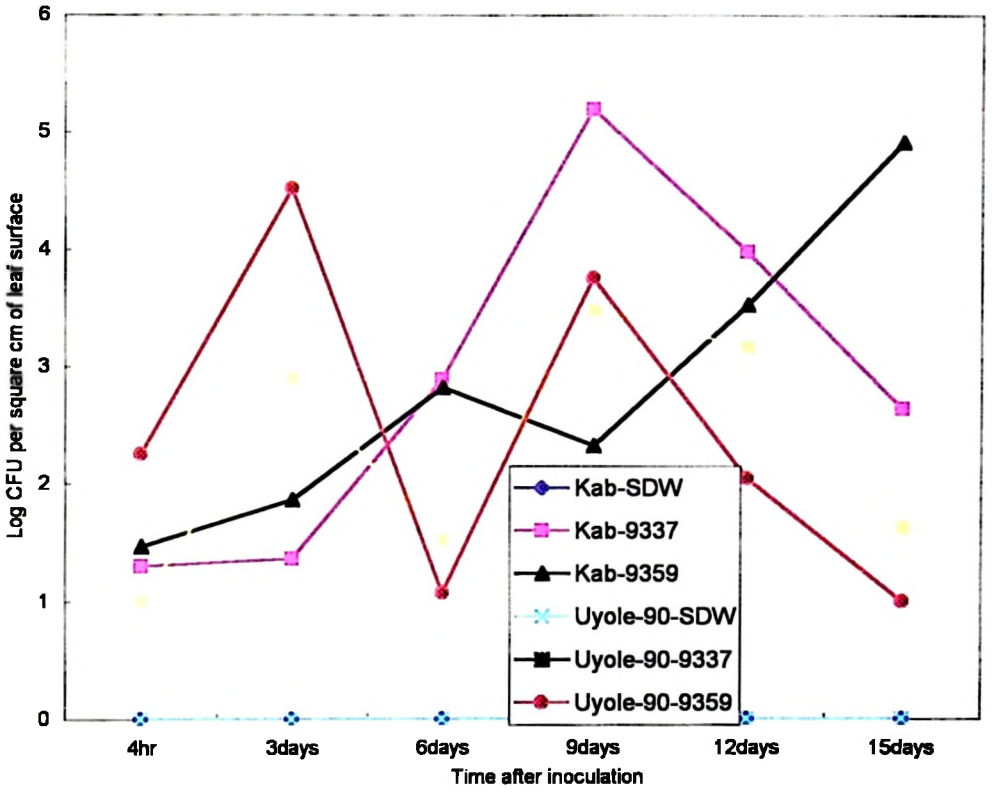


Figure 11. Epiphytic populations of *P.s.phaseolicola* recovered from symptomless leaves of field grown bean genotypes Kablanketi and Uyole -90, during the 1994 growing season. Leaves were spray-inoculated with suspensions from strains 9337 and 9359 when bean plants were 21 days old. Control plots were sprayed with sterile distilled water (SDW).

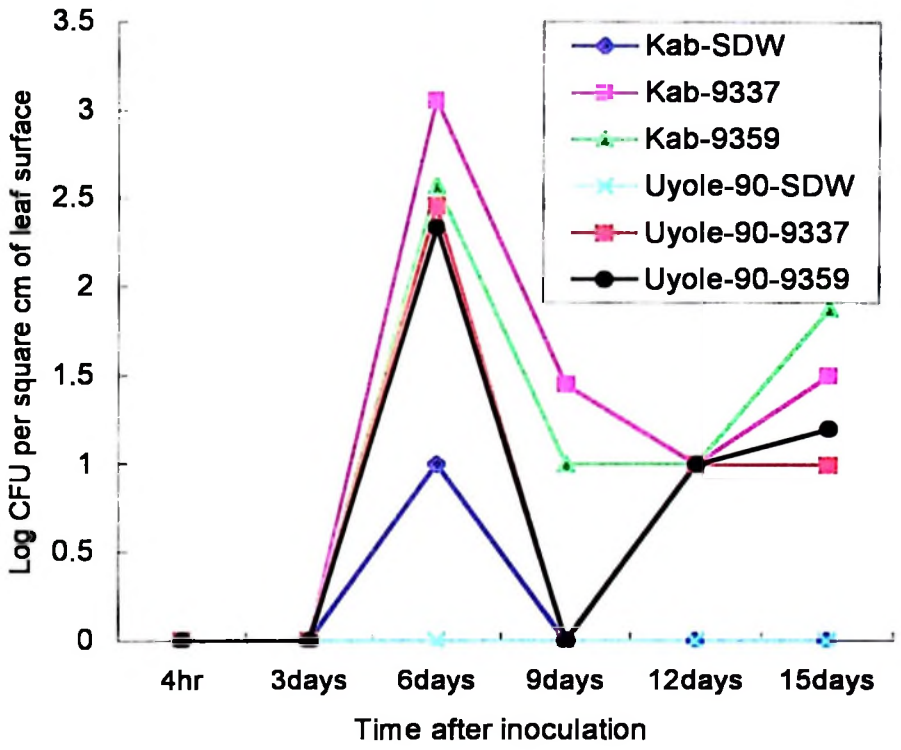


Figure 12. Epiphytic populations of *P.s. phaseolicola* recovered from symptomless leaves of field grown bean genotype Kablanketi and Uyole-90 during the 1995 growing season. Leaves were spray inoculated with suspensions from strains 9337 and 9359 when bean plants were 21 days old. Control plots were spray-inoculated with sterile distilled water (SDW).

4.6.4 Populations of *P.s. phaseolicola* determined under green house conditions

4.6.4.1 Epiphytic *P.s. phaseolicola* populations

The mean populations of *P.s.phaseolicola* recovered from the external leaf surfaces of bean genotypes Kablanketi and Uyole-90, planted and covered with soil infested with strains 9337 and 9359 were very low. The mean (actual) bacterial cell numbers were less than 10 cfu per leaf for all the treatments except on Kablanketi treated with strain 9359 which gave 25 cfu per leaf.

4.6.4.2 Endophytic *P.s. phaseolicola* populations

Bacterial populations of *P.s.phaseolicola* recovered from the internal surfaces of the leaf were also very low. The mean actual bacterial cell numbers (cfu/leaf) for all the treatments were less than 10 and therefore, considered undetectable.

4.6.5 Halo blight disease incidence

Results presented in Fig. 13, show halo blight disease incidence recorded from greenhouse experiments. The highest disease incidence of 68.3% was recorded on Kablanketi treated with strain 9359. Disease incidence on Uyole-90 treated with strain 9359, was less than 50%. The lowest disease incidence was recorded on Uyole-90 inoculated with strain 9337 of *P.s.phaseolicola*

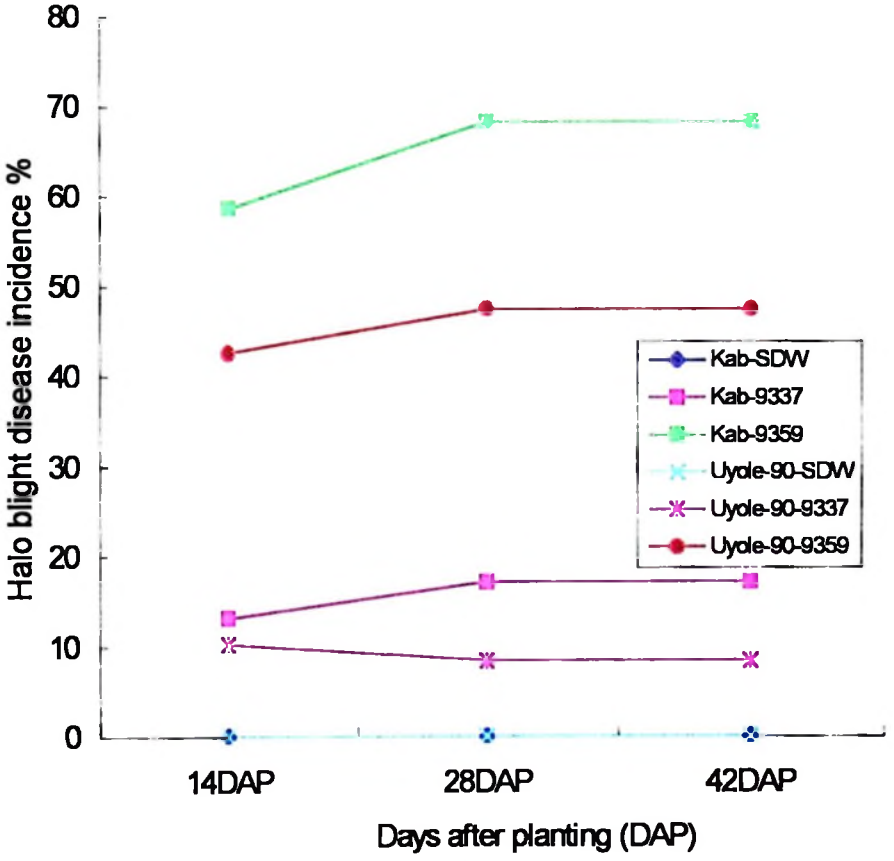


Figure 13. Halo blight disease incidence on greenhouse grown bean cvs. Uyole-90 and Kablanketi planted and covered with soil infested with suspensions from strains 9337 and 9359 of *P.s. phaseolicola*. Values are means of two experiments each replicated three times. Sterile distilled water (SDW) was used to moisten the soil in control plots.

4.6.6 Halo blight disease severity

Under field conditions, halo blight disease symptoms developed at 7 to 10 days after inoculation. The mean disease severity scores are presented in Fig. 14. Halo blight disease occurred on Kablanketi inoculated with either of strains 9337 and 9359. In 1995, some halo blight disease was observed on Uyole-90 inoculated with strain 9359. The mean halo blight disease severity scores ranged from 1.0 to 3.4 on the scale of 1.0 - 9.0. An interesting observation is probably the finding that although no epiphytic *P.s. phaseolicola* cells were recovered from the bean leaf surfaces during the 1993 field experiment, halo blight disease symptoms developed and were recorded. Isolates from diseased lesions were characterized and confirmed to be similar to previously inoculated *P.s.phaseolicola* strains 9337 and 9359. Mean disease severity scores recorded from the greenhouse experiments were higher than those recorded in experiments conducted under field conditions (Fig. 14 and 15). Disease severity patterns in both field and greenhouse experiments were however, similar. In greenhouse experiments, halo blight disease was first observed on the cotyledons and the primary leaves at emergence of the plants. The highest disease severity was observed on Kablanketi treated with soil infested with strain 9359. This was followed by Kablanketi planted and covered with soil infested with strain 9337. The mean disease severity on Uyole-90 treated with soil infested with strain 9359 was high at 14 DAP but declined at 28 DAP. Halo blight disease severity score on the Uyole-90 treated with soil infested with strain 9337 was low at 14 DAP, but increased and at 28 DAP, disease score was similar to the mean disease severity recorded on Uyole-90 treated with soil infested with strain 9359.

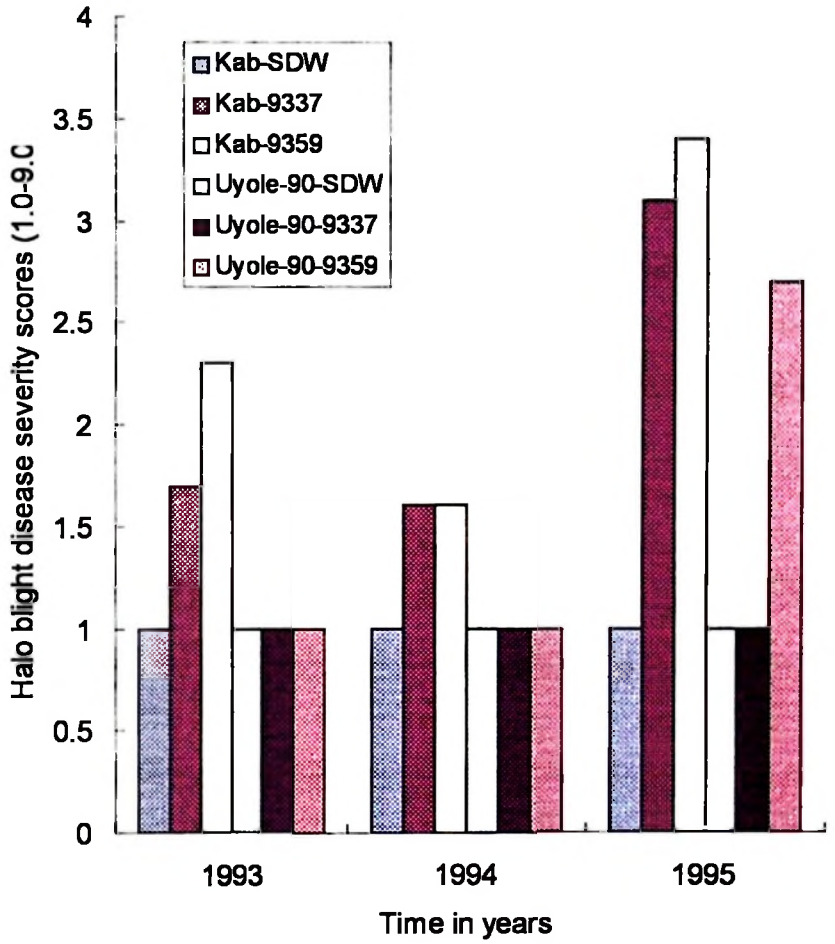


Figure 14. Halo blight disease severity on field grown bean genotypes Kablanketi and uyole-90 inoculated with suspensions from strains 9337 and 93 59 of *P.s. phaseolicola* during the 1993, 1994 and 1995 growing seasons. Disease scores are means of three replications. Control plots were spray-inoculated with sterile distilled water (SDW).

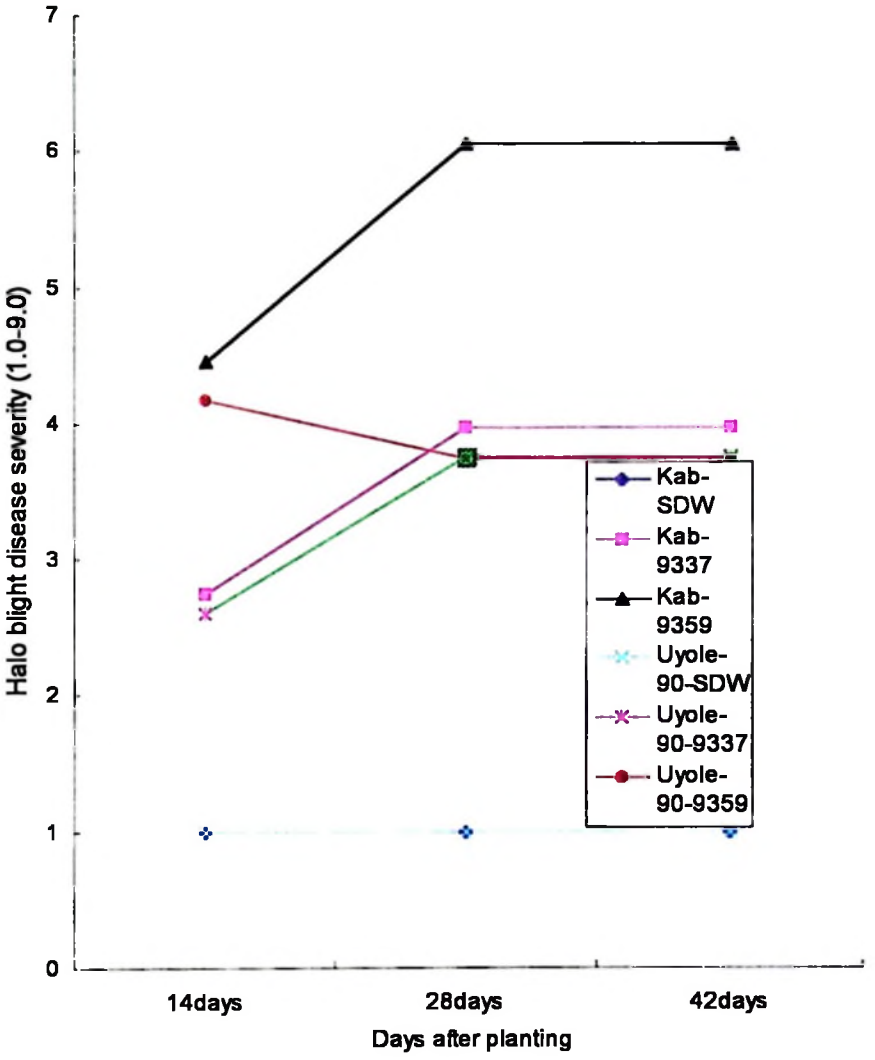


Figure 15. Halo blight disease severity scores on greenhouse grown bean genotypes Uyole-90 and Kablanketi planted and covered with soil infested with suspensions from strains 9337 and 9359 of *P.s. phaseolicola*. Values are means of two experiments each replicated three times. Sterile distilled water (SDW) was used to moisten the soil in control plots.

4.7 Survival of *P.s. phaselicola* in immature flat green pod tissues of cultivar Canadian Wonder

Results showing numbers of colony forming units per square centimetre of the bean pod tissue are presented in Fig.16. Bacterial cells of strains 9337 and 9359 of *P.s. phaseolicola* survived in bean pod tissues for up to 9 months. At 24 hours after inoculation, the numbers of bacterial cells recovered were relatively low. Data for an assay done at 7 days after inoculation is lacking because large numbers of coalesced bacteria made counting impractical. This observation may imply that bacterial multiplication in the pod tissues might have been very high at 7 days after inoculation and appeared to remain high for up to one month. Bacterial cells in the pod tissues started to decrease from 3 months of storage. The bacterial cell numbers in the bean pod tissues ranged from 2.3×10^1 to 2.8×10^5 cfu/ml. The two strains (9337 and 9359) of *P.s. phaseolicola* showed variation in ability to survive in Canadian Wonder bean pod tissues.

4.8 Effect of garlic bulb extract on growth of *P. s. phaseolicola in vitro*

Clear zones were evident on all the areas spotted with garlic bulb extract. No clear zones were observed on the plates spotted with sterile distilled water. Significantly ($P = 0.05$) larger inhibitory zones were recorded on plate count agar (PCA Difco.) (Table 29).

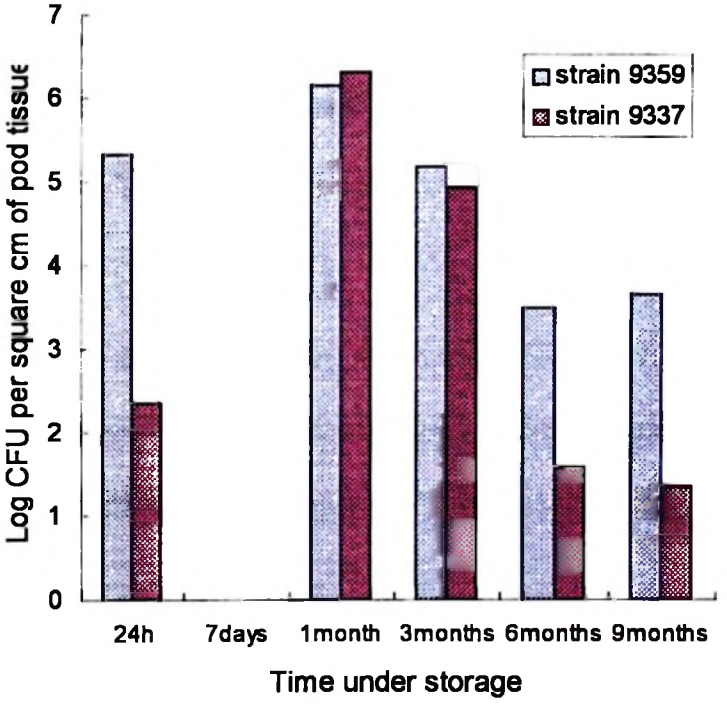


Figure 16. Colony forming units of *P.s. phaseolicola* strains 9337 (uncertain race type) and 9359 (race 6) recovered from artificially inoculated immature flat green bean (cv. CW) pods. Pods after inoculation were air dried and stored in manila envelopes in a drawer. At seven days, large numbers of coalesced bacterial cells made counting impractical.

Table 29. **Effect of garlic bulb extract on growth of *P.s.phaseolicola* measured as diameter of clear zones on three culture media**

Treatment combination	Diameter of clear zones (mm)
Race 3 on PCA	15.50 a^A
Race 6 on PCA	14.10 ab
Race 3 0n NA	13.51 b
Race 3 on KB	12.75 bc
Race 6 on NA	12.50 bc
Race 6 on KB	11.72 c
Mean	13.35
LSD	1.63
cv %	8.10

^A Values are means of three replicates. Five drops of garlic bulb extract were separately transferred on agar medium inoculated with suspensions of *P.s.phaseolicola.*

Within a column, means followed by the same letter are not significantly different (P=0.05) by LSD.

PCA = Plate count agar; NA = Nutrient agar; KB = Medium B of King *et al.* (1954).

Race 3 = *P.s.phaseolicola* strain no. 7994. Race 6 = *P.s.phaseolicola* strain no. 12694

4.8.1 Effect of garlic bulb extract on growth of *P.s.phaseolicola* under greenhouse conditions.

Results showing the mean, plant count after emergence, halo blight disease incidence and severity are presented in Table 30. An analysis of variance showed significant differences (P=0.05) between treatments. In these experiments, treatments involving infestation of the bean seeds with *P.s.phaseolicola* only were also considered as controls. Infestation of bean seeds with strain 9337 resulted into fewer bean seedlings that emerged. An average plant count after emergence of 1.5 for the Uyole-90 and 2.7 for Kablanketi was recorded. When bean seeds were inoculated with strain 9359, the number of bean seedlings emerged were slightly higher. Average plant counts after emergence were 5.5 for Uyole-90 and 3.7 for Kablanketi. In a treatment where garlic bulb extract (40%w/v) was applied to the bean seeds of Kablanketi after infestation with strain 9359 the mean number of bean seedlings that emerged was 4.2. When garlic bulb extract (40% w/v) was applied to the bean seeds of Kablanketi after infestation with strain 9337, a lower number of bean seedlings that emerged (a mean of 2.2) was recorded. Treatment of bean seeds of Uyole-90 with garlic bulb extract (40%w/v) after infestation with strain 9359 had a mean number of 4.2 bean seedlings that emerged, a number which was lower than the control (i.e. infestation with strain 9359 without garlic bulb extract). Application of garlic bulb extract to bean seeds of genotype Uyole-90 after infestation with strain 9337 showed a higher number of bean seedlings that emerged than the control. In general, data on plant count after emergence show that when garlic bulb extract (40%w/v) was applied after infestation with strain 9359, numbers of bean seedlings that emerged for Uyole-90 were less than the control (i.e.Uyole -90 inoculated with strain 9359).

An application of garlic bulb extract after infestation with strain 9337 showed a difference in number of bean seedlings that emerged between the two bean genotypes. Kablanketi had a lower number of seedlings that emerged (a mean of 2.2) than its control (a mean of 2.7). Uyole-90 had a higher number of seedlings that emerged (a mean of 2.5) than its control (a mean of 1.5). Results presented in Table 30 further show that application of garlic bulb extract after infestation with *P.s.phaseolicola* reduced halo blight disease incidence and severity. A deviation from this general observation was noted on Uyole-90 treated with strain 9359. In this treatment combination, application of garlic bulb extract after infestation did not reduce halo blight disease severity.

Further investigations were carried out using lower concentrations of the garlic bulb extract. The effect of garlic bulb extract at concentrations of 10 and 20% w/v was compared using the halo blight susceptible bean cultivar Canadian Wonder and the two most prevalent races (3 and 6) of *P.s.phaseolicola* in the Southern Highlands of Tanzania. Results are presented in Table 31. Bean seeds inoculated with suspensions from race 6 of *P.s.phaseolicola* had a mean of 1.5 bean seedlings that emerged (less by 8.5 seeds). When garlic bulb extract was applied (at both 10 and 20%w/v) after infestation with race 6 of *P.s.phaseolicola*, there was no bean seedling that emerged. However, when garlic bulb extract was applied before infestation with race 6 of *P.s.phaseolicola* an average of 8.5 and 7.7 bean seedlings emerged (Table 31).

Infestation of the bean seed with race 3 of *P.s.phaseolicola* had a mean of 6.7 seedlings that emerged. Treatment of bean seeds with garlic bulb extract after infestation with suspension from race 3 of *P.s.phaseolicola* had means of 4.7 and 4.2 seedlings that emerged for garlic bulb extract concentrations of 10 and 20% w/v respectively. These numbers are lower than those obtained when bean seeds were inoculated with race 3 of *P.s.phaseolicola* without garlic bulb extract.

Table 30. **Mean plant count after emergence, halo blight disease incidence and severity on bean genotypes and Uyole-90 and Kablanketi treated with garlic bulb extract and/ or suspensions from strains 9337 and 9359 of *P.s.phaseolicola***

Treatment combination	Plant count at 10DAP	Disease incidence % at 14DAP	Disease severity (1-9)y 14DAP
Kablanketi/Bacterilized (9359) + garlic 40%	4.2x	36.6	4.9 AB
Kablanketi/Bacterilized (9359)	3.7	100	8.8 A
Uyole-90/Bacterilized (9337) + garlic 40%	2.5	0	1.0 C
Uyole 90/Bacterilized (9337)	1.5	25.0	5.4 AB
Kablanketi/Bacterilized (9337) + garlic 40%	2.2	6.2	2.2 BC
Kablanketi/Bacterilized (9337)	2.7	42.8	5.4 AB
Uyole-90/Bacterilized (9359)	5.5	89.2	6.0 AB
Uyole-90/ Bacterilized(9359) + garlic 40%	4.2	73.7	6.6 A
Mean	3.3	46.7	4.9
cv%	59.69	51.3	50.09

x Values are means of two experiments each with 4 replications. Experiments were carried out under greenhouse conditions at MARTI UYOLE, Mbeya. Bacterial suspensions and or garlic bulb extract were introduced in the bean seeds by soaking for twelve hours.

Within a column, means followed by the same letter are not significantly different (P=0.05) by LSD.

y Disease severity score 1 = no disease symptoms; 9 = death of plants

DAP = days after planting

Table 31. **Plant count after emergence, halo blight disease incidence and severity scores on bean cv. Canadian Wonder treated with garlic bulb extract and/ or suspensions from race 3 and 6 of *P.s.phaseolicola***

Treatment combination	Mean		
	Plant count at 10DAP	Plants with HB symptoms 14DAP	Disease severity (1-9) [a] 14DAP
Bacterilization with race 6	1.5	1.5	8.5[b]
Bacterilization with race 3	6.7	6.2	7.1
Garlic extract at 10%	9.2	0	1.0
Garlic extract at 20%	6.0	0	1.0
Bacterilization (race 6)+garlic 10%	0	0	9.0
Bacterilization (race 6) + garlic 20%	0	0	9.0
Garlic 10% + Bacterilization (race 6)	8.5	5.2	7.3
Garlic 20% + Bacterilization (race 6)	7.7	3.5	6.3
Bacterilization (race 3) + garlic 10%	4.7	4.2	7.1
Bacterilization (race 3) + garlic 20%	4.2	3.2	7.6
Garlic 10% + Bacterilization (race 3)	8.2	6.0	6.9
Garlic 20% + Bacterilization (race 3)	7.5	5.0	6.4
Mean	5.4	2.9	6.4
cv %	23.3	47.3	15.7

[a] Disease severity score of 1 = no disease symptoms; 9 = death of plants due to disease.

[b] Values are means of two experiments each with 4 replications. Experiments were carried out under greenhouse conditions at MARTI Uyole, Mbeya. Bacterial suspensions and garlic bulb extract were introduced in the bean seeds by soaking for twelve hours.

DAP = days after planting

When garlic bulb extract was applied before infestation the number of bean seedlings that emerged was higher than in a control treatment (infestation with race 3) without garlic bulb extract. Data presented in Table 31 also show that the highest number of bean seedlings emerged were recorded when bean seeds were treated with garlic bulb extract at 10% w/v.

4.8.2 Effect of garlic bulb extract on expression of halo blight disease symptoms and halo blight disease severity

All the bean plants, which emerged from a treatment with race 6 of *P.s.phaseolicola* without garlic bulb extract, exhibited halo blight disease symptoms and a severe halo blight disease score of 8.5 (Table 31). Application of garlic bulb extract to bean seeds after infestation with race 6 of *P.s.phaseolicola* resulted into complete failure in germination and emergence of the bean seedlings. When soil covering these seeds was removed, masses of bacterial ooze were observed on the seed surfaces. Small growths of these bacterial masses were picked to confirm the identity of the bacteria. On the basis of cultural, biochemical and pathogenicity tests on the halo blight differential cultivars, the bacterium was confirmed to be *P.s. phaseolicola* race 6. It was also observed that a good number of the bean seeds inoculated with suspensions from race 6 and which failed to germinate, did not decay even at 14 days after planting. Application of garlic bulb extract before infestation with race 6 of *P.s.phaseolicola* showed that there was a reduction in halo blight diseased plants and a lesser disease severity compared to the bacterial inoculated treatment. Garlic bulb at 20% w/v showed a better effect on regulating the halo blight disease incidence and severity than garlic bulb extract at 10%.

However, treatments with garlic bulb extract at 20% showed lower numbers of bean seedlings that emerged. Results from treatments with race 3 showed that application of garlic bulb extract after infestation with race 3 had a lesser effect on development and expression of halo blight disease symptoms. When garlic bulb extract was applied before infestation with race 3 the number of plants showing halo blight symptoms and halo blight disease severity were however, minimized.

Results presented in Table 32 show that there was a decrease in the number of bean plants in treatments where bean seeds had been inoculated with race 3. The number of plants showing halo blight disease symptoms were less than those recorded at 14 days after planting, but the halo blight disease severity was higher. In treatments where bean seed had been inoculated with race 6, the number of plants showing halo blight disease were less than those recorded at 14 days after planting, and the halo blight disease severity was also reduced.

Result obtained at 42 days after planting are shown in Table 33. Data show that treatments with race 3 had fewer plants showing halo blight disease symptoms than those recorded at 14 and 28 days after planting. However, there was an increase in disease severity except for one treatment where garlic bulb extract at 20% w/v was applied before seed infestation with race 3. The garlic bulb extract applied after infestation with race 3 did not have much effect in reducing the halo blight disease incidence and severity. However, treating the bean seed with garlic bulb extract at 20% w/v before infestation with race 3, appeared to minimize the halo blight disease incidence and severity.

At 42 days after planting (Table 33), treatments with race 6 of *P.s.phaseolicola*, had lower numbers of bean plants than those recorded at 14 and 28 days. Halo blight disease incidence and severity on treatments with 10% w/v garlic bulb extract, were also reduced. There was a slight increase in disease severity on treatments with garlic bulb extract at 20% w/v. This was not expected and the reason for this observation is not clearly understood.

Table 32. **Mean plant count, halo blight disease incidence and severity scores on bean cv. Canadian Wonder treated with garlic bulb extract and or/ bacterial suspensions of race 3 and 6 of *P.s. phaseolicola* recorded at 28 days after planting.**

Treatment combination	Mean		
	Plant count 10DAP	Plants with HB symptoms 14DAP	Disease severity (1-9)[a] 14DAP
Bacterilization with race 6	0.5	0.5	8.5[b]
Bacterilization with race 3	5.7	5.75	7.8
Garlic extract at 10%	9.0	0	1.0
Garlic extract at 20%	6.5	0	1.0
Bacterilization (race 6) + garlic 10%	0	0	9.0
Bacterilization (race 6) + garlic 20%	0	0	9.0
Garlic 10% + Bacterilization (race 6)	9.0	4.0	6.8
Garlic 20% + Bacterilization (race 6)	7.0	3.2	5.0
Bacterilization (race 3) + garlic 10%	3.7	3.2	8.7
Bacterilization (race 3) + garlic 20%	2.7	2.2	8.2
Garlic 10% + Bacterilization (race 3)	7.2	5.0	7.7
Garlic 20% + Bacterilization race 3	7.2	5.0	7.4
Mean	4.86	2.5	6.7
cv %	29.97	60.95	18.82

[a] Disease severity score, 1 = no disease; 9.0 = death of plants due to halo blight disease.

[b] Values are means of two experiments each with 4 replications. Experiments were carried out under greenhouse conditions at MARTI Uyole Mbeya. Bacterial suspensions and aquous extract of garlic bulb were introduced in the bean seeds by soaking for twelve hours.

DAP = days after planting

HB= halo blight

Table 33. **Mean plant count, halo blight disease incidence and severity scores on bean cv. Canadian Wonder treated with garlic bulb extract and or/ suspensions of race 3 or 6 of *P.s. phaseolicola* recorded at 42 days after planting.**

Treatment combination	Plant count at 10DAP	Mean plants with HB symptoms 14DAP	Disease severity (1-9)[a] at 14DAP
Bacterilization with race 6	0.5	0.5	8.8[b]
Bacterilization with race 3	3.0	2.5	8.2
Garlic extract at 10%	9.0	0	1.0
Garlic extract at 20%	6.2	0	1.0
Bacterilization (race 6) + garlic 10%	0	0	9.0
Bacterilization (race 6) + garlic 20%	0	0	9.0
Garlic 10% + Bacterilization	6.0	2.0	5.2
Garli 20% + Bacterilization	6.2	2.5	6.5
Bacterilization (race 3) + garlic 10%	3.2	2.7	8.7
Bacterilization (race 3) + garlic 20%	3.2	1.7	9.0
Garlic 10% + Bacterilization (race 3)	7.0	5.2	8.3
Garlic 20% + Bacterilization (race 3)	5.5	3.2	5.7
Mean	4.1	1.7	6.7
cv %	39.23	62.19	19.95

[a] Disease severity score, 1.0 = no disease symptoms; 9.0 = death of plants due to halo blight disease.

[b] Values are means of two experiments each with 4 replications. Experiments were carried out under greenhouse conditions at MARTI Uyole Mbeya. Bacterial suspensions and or aquous extract of garlic bulb were introduced in the bean seeds by soaking for twelve hours.

DAP = days after planting

HB = halo blight

CHAPTER 5

DISCUSSION

The current investigations were an attempt to study the ecology, epidemiology and the pathogenic variation of *P.s.phaseolicola* population in the Southern Highlands of Tanzania. One of the objectives was to generate information on the occurrence and the distribution of races of the pathogen in the region. Results including detailed tests and characterization of races made on 250 strains of *P.s.phaseolicola* have been presented. The strains were collected from more than 70 different localities in eight districts of the Southern Highlands of Tanzania (Fig. 1; Table 2). The results on prevalence and distribution of *P.s. phaseolicola* in the Southern Highlands of Tanzania has particular significance in view of the fact that halo blight has been considered as a disease of minor importance in this region. Plant breeders, agronomists and the farming community need to be aware of the distribution of halo blight in the area because the disease can influence their efforts in introducing and adopting improved bean cultivars and other related management aspects.

5.1 Differentiation and distribution of *P.s.phaseolicola* races.

A sound knowledge of a pathogen, its variants or strains and their distribution, is a vital prerequisite for an effective breeding for resistance programme.

In this study, a considerable variation in the degree of virulence among 250 different strains of *P.s. phaseolicola* examined in detail was observed. Strains were assigned to races

according to their pathogenicity on seven *P.vulgaris* and one *P.acutifolious* differential bean cultivars. **Races 1, 2, 3, 4, 5, 6, 7b, and 8** exist in the Southern Highlands of Tanzania. The finding is consistent with those of Taylor *et al.* (1996) who reported that Tanzania had the largest number of halo blight races than any country in the world. However, results from the current investigations have, in addition, shown that 7.6% of the total strains of *P.s.phaseolicola* examined in detail were of uncertain race type. Strains were considered of uncertain race type because of lack of clear-cut characteristic symptoms when inoculated on some of the differential bean cultivars. Strains of uncertain race type were categorized and grouped as follows:

(a) Race 9 related strains (Race 9?). The strains (represented by strain 14494) would have been assigned to race 9 but incited an intermediate reaction in cultivar Red Mexican UI3. The reference strain for race 9 caused a clear-cut susceptible reaction to Red Mexican UI3. In Africa, race 9 has been reported to occur in Malawi (Taylor *et al.*, 1996) only. Race 9 related strains found in this study were predominantly from Makambaku area in Njombe district. Occurrence of a pathogenic variant, closely conforming to race 9 in Makambaku illustrates the diverse pathogenic variability in *P.s.phaseolicola* existing in the Southern Highlands of Tanzania.

(b) Race 5 or race 7 related strains. Strains in this group were mainly obtained from Ilembo-Usafwa and Iyawaya in the Mporoto Mountains in Mbeya.

The strains (e.g. 4794, 4894 and 5694) incited a hypersensitive reaction (a score of 1.0 HYP) on cultivar A43 and an intermediate reaction, with disease severity scores ranging from 4.0 to 6.0 on cultivar Red Mexican UI3. The strains appeared to be "in between" the strains described by Taylor *et al.* (1996) as race 5 and race 7. The Ilembo-Usafwa is believed to have been pyrethrum-peas-wheat-round potatoes growing areas (Madata, personal communication). Beans are a recent introduction in Ilembo-Usafwa. During the 1993 and 1994 growing seasons, severe outbreaks of halo blight disease were observed in these areas. A few factors were noted which could help explain the possible causes of these halo blight disease outbreaks. First, *Neonotonia wightii* and *Desmodium* sp. were observed to be growing wildly in these mountain areas. The two plant species are known to be hosts to *P.s.phaseolicola* (CIAT, 1987a; Gondwe, 1989; Mabagala and Saettler, 1992; Taylor *et al.* 1996). There were no plants exhibiting halo blight symptoms in close proximity of the infected bean fields. The literature suggests epiphytic growth of *P.s.phaseolicola* on weeds such as *Vicia villosa* (Ercolani *et al.*, 1974) and the potential for epiphytically surviving *P.s.phaseolicola* to serve as reservoirs of inoculum for susceptible host plants (Hirano and Upper, 1983; Hirano *et al.*, 1995). In addition, some of the bean cultivars introduced in these areas (e.g. UAC 161) were highly susceptible to the *P.s. phaseolicola* pathogenic variants occurring in the Southern Highlands of Tanzania (Table 10). Third, Ilembo-Usafwa lies over 2000 masl and is cool and wet. These conditions are favourable for development of the halo blight disease. Whether either one or an interaction of these factors was the cause of the halo blight disease outbreaks observed in the Mporoto Mountains remain unresolved. The possibility that seed could be the source of inoculum was doubtful because of the pattern of infection, which was very uniform. Under natural field conditions, halo blight disease incidence caused by seed-borne inoculum, appeared in patch-type pattern. Furthermore, same seed stock was planted at MARTI; Uyole and none of the strains collected from the research fields at Uyole gave halo blight disease scores similar to those

observed in strains in this category. Occurrence of such severe halo blight disease outbreaks further emphasizes the need for detailed information on the prevalence, distribution and importance of halo blight of beans as a disease. This is very important if rational decisions on expansion of bean production in the Southern Highlands of Tanzania are to be made. The finding that strains from Ilembo-Usafwa were closely related to strains previously reported as races 5 or 7 may further suggest that the source of inoculum for these disease outbreaks could be *N. wightii* and or *Desmodium* sp. This is based on the fact that the majority of strains characterized as race 7 has consistently been observed to originate from host species other than *P.vulgaris*. For example, Allen (1995) showed that all the strains originating from Tanzania and designated as race 7 came from host species other than *P.vulgaris*. Over 60% of the strains of African origin characterized as race 7 came from hosts other than *P.vulgaris* (Taylor *et al.*, 1996).

(c) Race 7b with less disease severity on cultivars A52 and A53. The strains incited intermediate disease reactions with scores of 5.0 to 6.0 and a low susceptibility score of 7.0 on differential cultivars A52 and A53 (e.g. strains 0394, 15094, 1794, 93171). Strains in this group were assigned to race 7b without a reference strain for comparison because reference strain 1449B was received dead. Inoculated plants from bean cultivars A52 and A53 were observed to recover by developing new symptomless growth from below the cotyledonary node. The reactions were more apparent when plants were incubated for up to 14 days after inoculation. This response was incited by strains originating from *N. wightii* and *Desmodium* sp. Strains originating from *P.vulgaris* and characterized as race 7b incited susceptible reactions with disease scores of 8.0 to 9.0. This finding suggests that strains from *P.vulgaris* characterized as race 7b may be better adapted to pathogenesis and survival on beans than the strains from *N. wightii* and *Desmodium* sp. In other disease systems, the behaviour was referred to as differential adaptation to host genotype (Lannou and Mundt, 1996). These workers explained this as being due to existence of selection for increased

reproductive ability on a given host genotype within parasites populations of the same virulence type. Other factors such as environmental interaction could be involved. Burkholder earlier suggested change in virulence when *P.s. phaseolicola* is transferred from one host species to another. He speculated that the halo blight bacterium had been imported on kudzu where it was causing little damage, but once on the bean it became a pathogen of extreme importance. The speculation by Burkholder (1948) differs from findings from the current study and an earlier report by Gondwe (1989) that race 1 (in the original race structure) of *P.s.phaseolicola* from *N. wightii* caused less halo blight disease severity when inoculated on bean cultivars such as LB 282, BAC 66 and LB 206. The view however, remains the same, that there is a change in virulence when races of *P.s.phaseolicola* are transferred from other plant species to some *P.vulgaris* genotypes.These results also suggest that *P.s.phaseolicola* population in the Southern Highlands of Tanzania is composed of races which can be isolated from almost one host species, and **races** which can be isolated from two or perhaps more than two host plant species. Therefore, races 1,2 ,3 ,4,5, 6, and 8 identified in this study can fall into the former race category because they were isolated from *P.vulgaris* only. Race 7b fall in the later race category because it was obtained from *Desmodium* sp., *Neonotonia wightii* and *P.vulgaris*.

Observations made in several locations in Mbeya during the 1993 to 1995 period showed that *N.wightii* and *Desmodium* sp. plants growing under established eucalyptus forest and uncultivated areas exhibited typical halo blight disease symptoms during the period starting October/November to June/July. In July, August and September, disease symptoms disappeared. When *N.wightii* was cut down to ground level or destroyed by uncontrolled fires set out during the months of July to September, the regrowth was observed to be free of any halo blight symptoms. However, this regrowth also exhibited typical halo blight symptoms immediately after even a single rain in October or November. These observations were made in isolated locations away from the bean fields. Therefore, the source of inoculum for halo blight disease symptoms in situations such as when *N.wightii* had been

destroyed by fire or cut down to ground level are not known. Leben (1983) reported that *P.s. phaseolicola* spread to distal portions of the root zone. However, it was not clear as to whether or not the bacteria was capable of multiplying and surviving in the roots. Scientific evidence on these aspects is considered necessary to better understand the ecology of *P.s. phaseolicola* particularly with respect to survival.

(d) Race 3 with a very strong hypersensitive reaction. Strains in this group (e.g. strains 95016, 95021, and 95048) incited a very strong hypersensitive reaction on differential cultivars Tendergreen and A53. The hypersensitive reaction was associated with suppression of growth and even systemic necrosis, which caused death of some of the inoculated plants. This reaction was not pronounced in differential cultivar A43. The strains in this group would have been assigned to either race 3 or 4 depending on whether or not *P. acutifolius* was infected. The work of Rahme *et al.*, (1991), indicated that *P.s. phaseolicola* possesses a group of genes designated hrp, phonetic "harp" that are essential for the development of disease symptoms and for the elicitation of the hypersensitive reaction on resistant plants. It is clear therefore, that the differential bean cultivars Tendergreen and A53 were resistant to the *P.s. phaseolicola* strain in-group (d). The suppressed growth observed would probably be due to a systemic invasion. This may imply that when certain strains of *P.s. phaseolicola* infects the vascular system of the host plants, they may behave differently in different bean cultivars. In 1990, Gondwe reported that one of the *P.s.phaseolicola* strains from Mbeya caused a hypersensitive reaction associated with severe suppression of growth of bean plants from 10 different bean cultivars. In addition, results reported previously (Tables 4 and 5) show that bean genotypes such a CG113, Tostado, LB110 and ZAA840 reacted to strain 7994 (race 3) with a hypersensitive reaction associated with suppressed growth and systemicnecrosis of some of the inoculated plants. Gopalan and He (1996), reported that the "avr" genes mediate genotype-specific incompatibility in resistant host plants.

The "avr" genes however, depend on functional hrp genes for expressing their phenotype. The possible explanations for such dependence are yet to be studied and resolved.

(e) Race 8 related strains. Strains in this group incited a hypersensitive reaction, which was associated with suppression of growth on differential cultivar A43. Strains in this group could have been assigned to race 8. The strains were obtained from bean research plots. Four of these strain (i.e. 95030, 95051, 95052 and 95054 came from test cultivars in the Southern Africa Regional Bean Evaluation Nursery (SARBEN entries 33 and 35). The SARBEN is comprised of breeding materials developed by different National programmes. The 1994 - 1995 SARBEN for example consisted of entries contributed by Zambia(3) Zimbabwe(6) South Africa(12) Tanzania(7) Malawi(71) (Chirwa and Aggarwal, 1995). Occurrence of race 8 is confined to some South African countries. This is supported by the work of Fourie (1995) who reported that race 8 was the dominant type of the races identified in South Africa during the period 1992 to 1994. Taylor *et al.* (1996), also reported finding race 8 mainly in Lesotho although one of the race 8 strains they found originated from Tanzania. The Tanzanian race 8 was identified as a result of re-classification of strain NCPPB 1647 collected in 1964 from *Dolichos* SP. Because this strain was obtained from Tanzania, it is possible that race 8 has been existing in the country for a long time. However, the area from which this strain was obtained was not specified. There are therefore two possible explanations to the sources of strains in category (e). One possibility is that the race has been existing in Tanzania for more than 30 years now. Alternatively, the strain was brought in with experimental seed material, from the Southern African countries. The variation in reactions observed between the previously identified race 8 and strains (race 8?) in this study could be due to an interaction between the bacterial-strain with environmental conditions such as rain, solar radiation and temperatures.

The interaction between the differential cultivar Red Mexican UI3 and certain strains in this study is of particular significance. Recently Taylor *et al.*, (1996), reported that the genetic analysis had indicated a possible difference in the RI gene in Red Mexican UI3 and Guatemala196-B. While this could be possible, results in the current study may also indicate that the resistance allele in Red Mexican UI3, could be more complex than those currently known to be. Earlier, Gondwe (1989), had reported on two *P.s.phaseolicola* strains from Lambo and Arusha in Northern Tanzania, which could not give clear-cut reactions on the differential bean cultivar Red Mexican UI3. Furthermore, results from this study show that, whereas the differential cultivar Red Mexican UI3 responded with an intermediate reaction to strain 14494, cultivar Ikinimba responded with a clear-cut resistant reaction. Thus, it appears that by using the current set of halo blight differential cultivars, an adequate description of the pathogenic variation existing in the *P.s.phaseolicola* population in the Southern Highlands of Tanzania cannot be fully obtained. A summary of the categories of the races considered to be of uncertain race types would show that the current halo blight differential set has some deficiencies in the description of (a) race 3, where a strong hypersensitive reaction associated with systemic necrosis was observed on differential cultivars Tendergreen and A53;. (b) race 7, where strains from *N.wightii* and *Desmodium* sp. incited a less severe reaction on bean cultivars A52 and A53; © races 5 and 7, where the differential cultivar Red Mexican UI3 reacted with an intermediate reaction and the differential cultivar A43 showed resistance with a hypersensitive reaction.

In terms of prevalence, race 6 was more common in Iringa followed by Njombe and Mbeya. Resistance to race 6 has not been found. However, during the surveys, it was observed that farmers in different localities in Iringa grew local bean cultivars other than Nyamuhanga. These ranged from small seeded white beans ("Ndongwekukye" in local language) to yellow and khaki medium sized beans. The fact that these beans have been existing in these areas despite this devastating *P.s.phaseolicola* race 6 and other pathogens highlight the possibility that these local materials have some resistance to various races or strains of

P.s.phaseolicola. Efforts should be made to search for resistance to these devastating races of *P.s.phaseolicola* in our local adapted bean materials.The frequency of occurrence and distribution of race 3 can be associated to bean germplasm exchange. Results show that race 3 was mostly found in Mbeya (Fig. 6) and it was the only race found to exist in Nkasi and Sumbawanga districts in Rukwa region. The reports by Taylor *et al.* (1996), that race 3 was mainly found in Burundi and, Gondwe (1989) that race 3 exist in Kibondo, Kigoma region may indicate that the predominant existence of race 3 in Rukwa region could be due to an exchange of bean germplasm between Burundi, Kigoma and Rukwa region.

5.1.1 Proportion of the brown diffusible pigmented strains in various races of *P.s.phaseolicola.*

In the present investigations, about 53 % of the BDP producing strains examined were race 3. Another 37 % were race 6, while the remaining 10% BDP producing strains (e.g. 9333a and 9337) were of uncertain race type. In the original race structure Taylor and Teverson (1985) showed that the BDP producing strains were race 2. Recently, the original races 1, 2 and 3 were sub-divided into nine races of halo blight (Teverson and Taylor, 1991). Race 2 has been sub-divided into races 2, 6 and 8. It would be expected therefore, that the BDP producing strains would be found in the current races 2, 6 and 8. However, results from this study show that the BDP producing strains were in race 6 with one or two in race 2 and none in race 8. Therefore, the BDP production cannot be associated to any race. The finding from the current investigations that some BDP producing strains existing in the Southern Highlands of Tanzania were race 3 further supports this. Occurrence of the BDP producing strains characterized as race 3 in this study further emphasizes the need for periodic surveillance for variation existing in *P.s.phaseolicola* in different regions. The BDP producing strains characterized as race 3 caused relatively smaller lesions when inoculated

on to immature flat green pods. Results also show that some of the BDP producing strains such as 9337 and 9333a had a low rate of motility (Table 25).

5.1.2 Sources of resistance to *P.s.phaseolicola*

In this study, efforts were made to evaluate germplasm from the National bean research programmes including a small collection of landraces grown by farmers in the Southern Highlands of Tanzania. The results of germplasm evaluation indicated that there were resistance to races 3, 4, 5, 7, strains 9337 and 14494 (Table 12). Resistance to races 3 and 4 occurred at the highest frequency of 13.8% and 11%, respectively. Resistance to race 3 was also demonstrated in pod inoculation tests through production of smaller lesions (Tables 16 and 24). High level of resistance to race 3 has also been reported by Davis *et al.*(1986).The presence of resistance to race 3 at a high frequency and the intermediate and low susceptibility reaction the race 3 strains incited on the bean germplasm tested (Tables 6 and 7) may indicate that race 3 of *P.s.phaseolicola* may coexist with beans in the Southern Highlands of Tanzania. The identification of new resistance sources is of direct value to farming needs because they can be incorporated into breeding programmes.

5.1.3 Reactions of landraces included in the germplasm evaluation.

The landraces were incorporated in the germplasm evaluation as an attempt to assess the degree of genetic diversity within and between landraces. Heterogeneity within these landraces was demonstrated. For example, a halo blight disease score of 5.0 was recorded when Nyamuhanga was inoculated with a strain characterized as race 7b in this study (Table 13). In repeated tests, an average of 20 to 40% of the components of Nyamuhanga showed a strong hypersensitive reaction with systemic necrosis. This is an indication that the different components in cultivar Nyamuhanga carry different gene products for resistance

to race 7b of *P.s. phaseolicola*. Some resistance was also found in Samdzuha, a landrace from Mdandu area in Njombe. Individual plants showed variations in disease reactions when inoculated with a strain characterized as race 3. The level of resistance (Tables 5 and 13) found in Samdzuha provides further evidence that resistance to *P.s. phaseolicola* may be found in our local bean materials. Individual plants in Masusu, Chipukupuku and Kablanketi gave halo blight disease severity scores ranging from 2.0 to 6.0 when inoculated with strain 9337. These landraces are widely grown in Mbeya. Therefore, the intermediate response to strain 9337 as observed on these bean genotypes might have several explanations. One possibility is that strain 9337 has not undergone sufficient natural selection for virulence. Alternatively, the strain has been coexisting with these bean cultivars. A third possibility is that strain 9337 was a mutant. Strain 9337 was obtained from *P.vulgaris* volunteer plants showing halo blight disease symptoms on primary leaves. Because volunteer plants came from spilt seed and spilt seed usually survive under adverse conditions, these conditions might have caused the bacterium surviving in the seed to mutate. Therefore, strain 9337 might as well be a mutant. The levels of resistance to some strains of *P.s. phaseolicola*, as found in landraces Nyamuhanga, Samdzuha, Masusu, Chipukupuku and Kablanketi (Tables 4 and 5), is of practical importance. Beebe and Pastor-corrales (1991) suggested that genetic diversity such as exists in landraces is the surest strategy for stable resistance to certain diseases and is of a traditional disease control mechanism that should not be abandoned.

An attempt was made to search for bean materials with resistance to halo blight for immediate use. Two breeding lines, PBUCT 91/22 and Uyole-84 x Kablanketi provided by Dr. C.S. Madata, Bean Breeder MARTI Uyole, Mbeya, Tanzania were evaluated separately. Mean disease scores of 6.0 and 6.5 were recorded on PBUCT 91/22 and Uyole-84 x Kablanketi, respectively, when inoculated with a strain (strain 12694) characterized as race 6. This result is important because sources of resistance to these breeding lines were sought

in local landraces and cultivars well adapted to the existing agro-ecological and other factors affecting bean production in the Southern Highlands of Tanzania. The result also indicates that resistance to race 6 may be obtained from our locally well adapted bean materials. Further evidence in support of the suggestion that resistance can be found in our local material is available in the report by Gondwe (1987) who showed that Mwanga-chuchu (Karagwe), a landrace from Kagera region, was resistant to race 3. This finding was confirmed by Taylor and Teverson (1988b). They also reported that Mwanga-chuchu (Karagwe) showed complete resistance (disease score of 1.0 on a scale of 1-5) to strain 1375A (race 1 in the original race structure but currently race 5) and to strains 1301A and 1302A (races 3 and 4, respectively). In addition, cultivars Masai Red (a landrace from Kilimanjaro/Arusha regions) and TB 79/248 (one of the landraces collected in 1979) were also shown to be resistant to strain 1375A (originally race 1 but currently race 5).

5.2 Is *Pseudomonas fluorescens* A inhibitory to strains 9337 and 9359 of *P.s.phaseolicola* ?

It has long been recognized that leaf surfaces are colonized by a diversity of bacteria. (Hirano and Upper,1983; Leben,1965). In this study investigations were carried out to probe into the potential of bean genotypes Kablanketi and Uyole-90, to support growth of *P.s. phaseolicola* strains 9337 and 9359 epiphytically. During the 1993 growing season, *P. fluorescens* A was detected in considerably large populations on cultivar Uyole-90 and Kablanketi under experimental conditions (Tables 27 and 28). Similarly, relatively high populations of *P. fluorescens* A were detected on leaf surfaces of bean landraces Kablanketi, Masusu, Chipukupuku and Kigoma sampled from farmers' fields (Table 29).

Pseudomonas fluorescens bacteria are frequently isolated from plant tissue or soil and reported as potential biocontrol agents of phytopathogens. For example,Teliz-Ortiz and Burkholder (1960) reported on *P. fluorescens* isolates, which were antagonistic to *P.s. phaseolicola*. Recently, *Pseudomonas* spp. - mediated induced systemic resistance (ISR) became evident in bean against *P.s. phaseolicola* (Alström, 1991; 1995). Failure to recover the inoculated strains 9337 and 9359 of *P.s.phaseolicola* from symptomless leaf surfaces of field grown Uyole-90 and Kablanketi during the 1993 season, in the presence of *P. fluorescens* A (Tables 27 and 28) may in part be attributed to antagonism. There are also possibilities that L-form bacteria were formed as a result of an interaction between certain metabolites of the *P.fluorescens*, A which existed on the bean leaves, and the inoculated cells of *P.s. phaseolicola*. The potential for biocontrol of halo blight disease by L-form bacteria has been reported by Amijee *et al.*, (1992). However, there is no direct evidence to ascertain this possibility. It may be worth mentioning here that halo blight disease of beans has been considered a minor disease in the Southern Highlands of Tanzania. Several factors could be the cause for this low ranking. One possibility is that the halo blight disease severity observed in the bean fields might have been low. The low halo blight disease severity in the fields in the Southern Highlands of Tanzania, despite conducive climatic conditions and high and variable pathogen populations, may suggest that *P.s.phaseolicola* could be subject to some form of natural biological control. Occurrence of *P. fluorescens* A, as reported herein, could be one among the several possible factors contributing to inhibition of growth and multiplication of *P.s.phaseolicola* in the bean fields. This might have minimized the intensity and severity of the disease and consequently made the disease less noticeable. Almost no information is available on the interaction between potential antagonists and natural epiphytic micro flora that might occupy the same ecological niche on plants, in the Southern Highlands of Tanzania. It is suggested therefore, that investigations be carried out to monitor epiphytic populations of *P.fluorescens* on bean leaf surfaces in the Southern Highlands of Tanzania.

The dynamics of the interaction of *P.fluorescens, P.s.phaseolicola*, bean genotypes, epiphytic micro flora and the environment need to be fully understood before *P.fluorescens* can be effectively applied as a biocontrol agent. This is particularly important now that there is a strong urge to utilize natural organisms for management of various diseases in this environmentally alert society.

5.3 Epiphytic survival of *P.s. phaseolicola*

Plant pathogenic bacteria live in commensal relationship with plants and exhibit different levels of intimacy with the leaves that harbour them (Beattie and Lindow, 1995). The results from this study add supporting evidence that *P.s.phaseolicola* can be found on symptomless bean leaf surfaces. An inherent cultivar to cultivar variability in its ability to support populations of *P.s.phaseolicola* was demonstrated during 1994 and 1995 growing seasons (Fig.7). Populations of *P.s.phaseolicola* on Kablanketi built up slowly and did not decline at 15 days after inoculation. This implies that *P.s.phaseolicola* on leaf surfaces of Kablanketi persists for a long time. The possibility that Kablanketi may carry the bacteria to the next crop is important in Mbeya where two successive bean crops can be produced during one rain season (November/December to June). Epiphytic populations of *P.s.phaseolicola* on cultivar Uyole-90 declined sharply at 15 days after inoculation. The ability of cultivar Uyole -90 to support *P.s.phaseolicola* populations epiphytically may not therefore, be important where two successive bean plantings are possible. Results from greenhouse studies indicated that *P.s.phaseolicola* bacterial cells detected on symptomless leaf surfaces of bean seedlings emerging from soil infested with strains 9337 and 9359 were very few. In this study, soil was infested by being moistened using bacterial suspensions prepared from agar culture. Under natural field conditions, sources of bacteria to the soil environment may include:

(a) bacterial suspensions formed as a result of bacterial ooze from halo blight disease lesions on aerial parts of infected bean plants; being hit and washed down by rain or excessive dew. Epiphytically surviving *P.s. phaseolicola* may also be washed off during the rains and form suspensions (Hirano et al.,1995). Bacterial suspensions may be carried down to non-infested soil in running water. This situation can occur in Mbeya where planting dates are stretched for about 4 weeks and two successive bean crops can be produced in one growing season.

(b) Bean seed, which is severely infected with *P.s.phaseolicola,* will not germinate. Previously, it was observed that bean seeds artificially inoculated with *P.s. phaseolicola* (race 6) failed to germinate. When soil covering the seed was removed, masses of *P.s.phaseolicola* (race 6) bacterial ooze were observed around the seed. The bacterial ooze can form bacterial suspensions when in contact with soil water films. Because most of the strains of *P.s.phaseolicola* are motile (Table 25), there are possibilities that the bacteria can move to nearby sown or spilt germinating seed. It thus follows that when bean seed is sown in *P.s.phaseolicola* infested soil, the emerging seedling will carry the bacterium either in an epiphytic phase on symptomless foliage or endophytically in lesions of diseased plants. The populations of *P.s.phaseolicola* from greenhouse experiments were very low. However, on the basis of infectivity titration studies done under controlled conditions, it is known that an individual bacterial cell can cause visible disease (Ercolani, 1973; 1984). This information may help explain why halo blight disease was observed in experiments where epiphytic populations of *P.s. phaseolicola* recovered from the leaf surfaces of the bean leaves were very low.

Results presented in Fig. 14 show that disease severity under field conditions was relatively low. Several studies have associated large epiphytic bacterial populations with amount of disease in a bean canopy. (Hirano and Upper, 1983; Mew and Kennedy, 1982; Lindemann *et al.*, 1984)

In the present investigations, amount of bacteria on the leaf surface was low but disease severity was relatively high (Fig. 15).

In the 1993 planting for example, *P.s.phaseolicola* was not recovered from symptomless leaf surfaces of the field grown bean cultivar Uyole-90 and Kablanketi. However, halo blight disease was manifested in plots planted with Kablanketi and inoculated with both strains 9337 and 9359 (Fig.14). Therefore, the amount of disease in this planting appears to be cultivar related. In the 1994 planting, *P.s.phaseolicola* populations on the two bean genotypes differed (Fig.7) with Kablanketi showing higher bacterial populations at 15 days after inoculation than cultivar Uyole-90. Occurrence of halo blight disease on Kablanketi only (Fig. 14) could be associated to both the *P.s.phaseolicola* populations and the bean genotype. Results from the 1995 planting show that epiphytic populations were very low except for those recovered at 6 days after inoculation (Fig. 8). Nevertheless, halo blight disease severity was slightly higher than for the other two previous plantings. Furthermore, halo blight disease was recorded on cultivar Uyole-90 inoculated with *P.s.phaseolicola* strain 9359. Halo blight disease severity during this season appeared to be both cultivar and bacterial strain associated. Therefore, the major factor that influenced disease development in the absence of large epiphytic populations of *P.s.phaseolicola* is probably the bean genotype. A comparison of the occurrence of rain (during the assay period) across the three plantings may show that the role of rain in the epiphytic population sizes is obscure. In the 1994 experiment for example, the highest bacterial populations were detected at 9 days after inoculation. The amount of rain on this day was 1.2 mm and on the preceding day 2.9 mm of rainfall was recorded. In 1995, the highest populations were recorded on the sixth day after inoculation. The amount of rain recorded on this day was 5.4 mm and, on the preceding day, 1.0 mm of rain was recorded. Overall, 81.0, 61.5 and 98.1 mm of rain fell during the whole assay period for the 1993, 1994 and 1995 plantings, respectively.

The highest halo blight disease severity scores were recorded in 1995 when the amount of rainfall was highest but the highest epiphytic populations of *P.s.phaseolicola* were recorded in 1994 when the amount of rain that fell during the assay period was lowest. These results may indicate that the intense rains that occurred in 1995 might have washed off of the leaf surface some cells of *P.s.phaseolicola.* The process might have reduced the pathogen populations as shown in Fig. 8. Given that an individual bacterial cell can cause visible disease (Ercolani, 1984), rain might have facilitated ingress of the remaining few *P.s.phaseolicola* cells into the intercellular spaces of the bean leaves where growth may have occurred and caused the halo blight disease severity observed (Fig. 14). The result that halo blight disease occurred in bean plants in the 1993 experiment in the absence of epiphytic populations of *P.s.phaseolicola* and in the presence of *P.fluorescens* A (Table 27) may also be associated with the rain. The intense rains may have facilitated ingress of the *P.s.phaseolicola* bacterial cells into the intercellular spaces of the bean leaves where multiplication occurred and therefore, caused the disease severity recorded (Fig. 14). Results obtained from greenhouse studies show that the halo blight disease severity scores especially on Kablanketi treated with strain 9359 was quite high (Fig. 15). However, both the epiphytic and the endophytic *P.s.phaseolicola* populations were very low. Because assays for greenhouse experiments were done on primary leaves and soon after emergence of the bean seedlings, age of leaf might have had an influence on the epiphytic and endophytic populations of *P.s. phaseolicola.* Legard and Schwartz (1987) also reported that age of plant may have an effect on epiphytic populations of *P.s.phaseolicola.* Disease incidence and severity were first recorded at 14 DAP. Hence, the relatively high disease severity scores recorded from the greenhouse experiments could be a result of multiplication of the few *P.s.phaseolicola* bacterial cells observed in the primary leaves. The high disease severity scores recorded in the greenhouse may be attributed to conditions, which might have favoured better development of the disease.

Although results show that both genotypes were able to support growth of *P.s.phaseolicola* epiphytically, amount of disease have, for most of the times, been more on Kablanketi treated with strain 9359 except under field conditions such as those observed in 1995.

5.4 Motility of *P.s.phaseolicola*

Results presented in Table 25 show that almost all the strains tested were motile. Motility was very rapid with some of the strains. For example strain 93188c (race 6) moved at a speed of 8 mm per hour. Results also show that motility was very slow with strain 9337 (uncertain race type). There are differences in motility between and within races of *P.s.phaseolicola*. For example, strains 93188c (race 6) and 93163 (race 6) show a difference of 4.4 mm in motility at 4 hours. Similarly, strains characterized as race 7b show differences in motility. The only strain, which was non-motile, was of race 7b, which originated from *N. wightii*. The other two strain of race 7b tested originated from *P.vulgaris* and were motile. These results may suggest that associating motility with virulence could be complicated because other factors such as the leaf environment may affect virulence of the bacterial strain.

5.5 The potential for garlic bulb extract to inhibit growth of *P.s.phaseolicola*.

This study was an attempt to generate information on the potential for garlic bulb extract to inhibit growth of *P.s. phaseolicola* as an input in the management of the halo blight disease. The potential for garlic bulb extract to inhibit growth of plant pathogens had earlier been investigated (Mangamma and Sreelamulu, 1991). It is clear from these results (Table 29) that *in vitro* an aqueous extract of garlic bulb at 40%^{w}/v, inhibited growth of *P.s.phaseolicola*. Larger inhibitory zones were recorded on PCA. (Table 29).

The reasons for this observation are not clearly known.Results from greenhouse studies may imply that although *P.s. phaseolicola* race 6 is more virulent than race 3, the antibacterial activity of garlic bulb extract produced more inhibitory effect on race 6 of *P.s.phaseolicola* than on race 3 (Table 30).The race 6 strain used in this study was a BDP producer. Properties of the BDP produced by some *P.s. phaseolicola* strains are not known. Similarly, compounds responsible for the antibacterial activity of garlic bulb extract are not clearly known (Mangamma and Sreelamulu, 1991). It can be speculated that the unidentified compounds present in the brown diffusible pigmentation and those in garlic bulb extract reacted and were responsible for the suppressive effect observed on treatment combinations associating race 6. Effect of garlic bulb extract appears to be influenced by race type and bean genotype. Extrapolating results presented in Table 30 , it can be seen that bean seed imbibed with race 6 of *P.s. phaseolicola* suspension (approximately 10^8 cfu/ml) could not germinate. This information can be used to explain losses due to seed-borne diseases, which are often times overlooked. Failure of bacterially infected bean seed to germinate can cause several losses including costs; in purchasing the seed itself, land preparation, planting and the detrimental effects of weeds which are likely to occupy the spaces resulting from seeds which failed to germinate. It is suggested therefore that, in addition to measuring and relating disease severity to yield in crop loss assessments in the Southern Highlands of Tanzania, these other costs should also be taken into consideration. The results presented in Table 31 show that about seven bean seedlings of cultivar Canadian Wonder inoculated with *P.s. phaseolicola* race 3 before planting emerged. This is approximately 67% of the total seeds sown, and about 33% loss due to the pathogen. In treatments where bean seeds were inoculated with race 6, a mean of 1.5 plants emerged. In percentage, this is a loss of about 85%. Comparably, it would appear that losses caused by race 3 are less than those caused by race 6.

Race 3 has consistently incited intermediate reactions on a number of bean genotypes on both the foliage and the pods (Tables 4, 5, 16). This intermediate reaction may indicate that despite its high frequency of occurrence, race 3 can coexist with beans in the Southern Highlands of Tanzania. The results are of practical importance to plant breeders when searching for bean materials with resistance to race 3.

5.6 Pseudomonads other than *P.s.phaseolicola*.

Results showing that large populations of *P. fluorescence* A occurred on symptomless leaf surfaces of several bean genotypes in the Southern Highlands of Tanzania (Tables 27 and 28) may be of practical value. The information can be used by the plant protectionists and may consequently benefit the farming community at large. The work of Teliz-Ortiz and Burkholder (1960) and the recent reports by Alström (1991; 1995) that induced systemic resistance to *P.s. phaseolicola* was found in plants treated with a plant growth promoting strain of *P.fluorescens* (597), provide a basis for the need for further investigations on this potential biological control approach.

Occurrence of *P.syringae* pv. *syringae* A is of particular significance. Three strains of this organism caused a very rapid necrosis onto bean cultivars artificially inoculated with the pathogen under greenhouse conditions. It is recommended that *P.vulgaris* should not be planted adjacent to *Clitoria ternatea*, a leguminous pasture legume from which two of the *P. syringae* pv. *syringae* A strains were obtained.

5.7 Survival of *P.s. phaseolicola* stored in bean pod tissues.

Data on survival of *P.s. phaseolicola* in bean pod tissues (Fig. 16) indicate the possibility of using immature flat green bean pods as a method for preservation or maintenance of *P.s.phaseolicola*.

The method can be appropriate especially in small pathology laboratories in developing countries like Tanzania as it is cheap in terms of materials required for incubation and storage. Plastic sandwich boxes are required for incubation of the pods after inoculation, and manila envelopes used for storage or maintenance of the dried pods. Furthermore, many strains can be kept in small drawers. The two strains (9337 and 9359) of *P.s. phaseolicola* tested survived in the bean pod tissues for 9 months. Again this is a considerably long period for maintenance of bacterial strains especially in areas of limited resources like Tanzania.

CHAPTER 6

CONCLUSIONS AND RECOMMENDATIONS

(a) The *P.s.phaseolicola* population in the Southern Highlands of Tanzania has shown a diverse pathogenic variability with 8 races closely related to the previously identified races found to exist in the region. About 7.6% of the 250 strains of *P.s.phaseolicola* examined in detail were of uncertain race type. This is because they failed to exhibit clear-cut characteristic symptoms on some differential cultivars. The results show that the current set of halo blight differential bean cultivars was inadequate in describing the pathogenic variation existing in *P.s.phaseolicola* population occurring in the Southern Highlands of Tanzania. The deficiencies are found in the following examples:
Some strains closely related to previously identified race 3, incite a very strong hypersensitive reaction associated with suppressed growth and systemic necrosis of plants in differential cultivars Tendergreen and A53 but not on A43.

Some strains related to previously identified races 5 and 7 caused less severe disease reaction on differential cultivars A52 and A53. Strains obtained from *N.wightii* and *Desmodium* sp. and designated as race 7b, were less virulent on *P.vulgaris*. Strains of the same race originating from *P.vulgaris* were more virulent on *P.vulgaris*, supporting the view that there is a change in virulence when *P.s.phaseolicola* strains are transferred from one species to another.

Some strains, which would have been characterized as race 1 or 5 or 7 or 9 caused intermediate reactions on the differential, bean cultivar Red Mexican UI3. Similar strains were found to occur in northern Tanzania by Gondwe (1989).

Results further suggest the need to find ways of dealing with intermediate or non clear-cut responses in pathogenic variation studies.

(b) Some *P.s. phaseolicola* strains which produce the brown diffusible pigment *in vitro*, are for the first time found to be race 3 and therefore, the production of the brown diffusible pigment cannot be associated to any race of *P.s.phaseolicola.*

(c) Race 3 of the *P.s.phaseolicola* was not found in various districts surveyed in Iringa region. Some level of resistance to race 3 was found in Samdzuha-a landrace from Mdandu, Njombe district, and Iringa region. The results raise a few questions. For example, why has race 3 not spread from Mbeya to Iringa?. Is the resistance to race 3 (such as that found in cultivar Samdzuha) present in local bean materials grown by farmers in Iringa and, therefore restricting the existence of the race in the region? Investigations towards resolving these issues are considered important.

(d) New sources of resistance to *P.s.phaseolicola* races 3, 4, 5, 7b, and strains 9337 and 14494 were identified. Nyamuhanga, a landrace originating from Iringa region was for the first time shown to have components with resistance to race 7b of *P.s.phaseolicola.* Results support the view that resistance to some strains of *P.s.phaseolicola* can be found in our locally well adapted bean germplasm.

(e) The bean genotypes Kablanketi and Uyole-90 supported and showed variation in epiphytic populations of strains 9337 and 9359 of *P.s.phaseolicola.* Differences were more apparent at 15 days after inoculation. The two bacterial strains also varied significantly in their ability to establish and maintain epiphytic populations. The role of epiphytic populations of *P.s.phaseolicola* on the epidemiology of the halo blight disease of beans was not as apparent.

Halo blight disease severity scores for field experiments were generally low and varied from season to season. However, halo blight disease severity patterns for both the greenhouse and field experiments showed higher disease severity on Kablanketi treated with strain 9359 of *P.s.phaseolicola.*

(f) *Pseudomonas syringae* pv. *syringae* A, occurs in the Southern Highlands of Tanzania. The significance of the pathogen in bean production in Tanzania has not been reported. Strains from *Clitoria ternatea* showed a very strong hypersensitive reaction associated with systemic necrosis on the differential and other bean cultivars. The strains also showed a compatible reaction with bean cultivars Masusu and Uyole-94. The finding is of practical importance because of the possible dissemination, infection and destruction of the bean crop by the *P.syringae* pv. *syringae* from *Clitoria ternatea.*

(g) *Pseudomonas fluorescens* A occurred as an epiphyte on different field grown bean cultivars. Failure to recover the *P.s. phaseolicola* bacterial cells previously inoculated onto the leaf surfaces of field grown bean Kablanketi and Uyole-90 was partially associated with the presence of the bacterium on the leaf surfaces. *Pseudomonas fluorescens* mediated induced systemic resistance on bean against *P.s.phaseolicola* has recently been evidenced (Alström, 1991; 1995). In addition, *P. fluorescens* has been reported to protect beans against halo blight when inoculated before but not after inoculation with *P.s.phaseolicola.* (Teliz-Ortiz and Burkholder,1960). Thus knowledge on the dynamics of the interaction of *P.fluorescens*, *P.s.phaseolicola*, bean genotypes, the other epiphytic micro flora and the environment in the Southern Highlands of Tanzania is considered essential for developing management strategies for the halo blight disease of beans.

(h) The antibacterial activity of garlic bulb extract against strain 9337 (uncertain race type), and races 3 and 6 of *P.s.phaseolicola* was demonstrated. The information is considered to be of practical importance in developing management strategies for the halo blight disease of beans.

(i) A BDP producing strain 9337 (uncertain race type) and a race 6 strain 9359 of *P.s.phaseolicola* survived for 9 months in artificially inoculated and air dried immature flat green pods stored at 19 to 25^0 C.

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APPENDICES

Appendix 1. Mean squares from analysis of variance for reaction of immature flat green pods from seven bean genotypes to six strains of *P.s.phaseolicola* at 7 days after inoculation.

Source	DF	Mean squares
Replication	2	0.69
Strains	5	15.65**
Error	10	1.26
P.vulgaris genotype	6	12.31**
Strains x *P.vulgaris* genotype	30	1.86**
Error	72	0.45
Total	125	
cv%		14.07

**Significant at 0.01 probability level.

Data was analysed as a split plot of a randomized complete block design with three replications.

Appendix 2. **Mean squares from analysis of variance for reaction of immature flat green pods from seven *P.vulgaris* genotypes to strains of *P.s.phaseolicola* at 7 days after inoculation**

Source	DF	Mean square
Replication	2	0.07
Strains	17	20.31**
Error	34	1.49
P.vugaris genotypes	6	37.97**
Strains x *P.vulgaris* genotypes	102	8.59**
Error	216	1.28
Total	377	
cv %		30.82

** Significant at the 0.01 probability level.
Data were analysed as a split plot of a randomized block design with three replications.

Appendix 3 **Mean squares from analysis of variance for reaction of immature flat green pods from bean cultivar Canadian Wonder to 62 strains of *P.s.phaseolicola* at 7 days after inoculation.**

Source	DF	Mean squares
Replications	2	0.31
Strains	61	4.35**
Error	122	0.77
Total	185	
cv%		14.82

** Significant at the 0.01 probability level.

Data was analysed as a randomized complete block design with three replications.

Appendix 4. Mean squares from analysis of variance for reaction of immature flat green pods from bean cultivar Canadian Wonder, to 32 strains of *P.s.phaseolicola* at 7 days after inoculation.

Source	DF	Mean square
Replication	2	1.07
Strains	31	9.97**
Error	62	1.02
Total	95	
cv %		17.29

** Significant at the 0.01 probability level.
Data was analysed as a randomized complete block design with three replications.