ECOLOGICAL FACTORS AFFECTING THE DISTRIBUTION OF *Pseudomonas solanacearum* IN KENYA

BY

JOHNSON B. NYANGERI

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DECLARATION

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

..... 12/8/82
J.B. Nyangeri Date

This thesis has been submitted for examination with our approval as University supervisors.

..... 12/8/82.
Dr. E.M. Gathuru Date

..... 12/8/82
Dr. D.M. Mukunya Date

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ABSTRACT

One hundred and thirty-eight potato farms were examined for the presence of bacterial wilt in Central, Eastern and Rift Valley Provinces. The altitude of these areas ranged from 1390 m to 2790 m above the sea level and the mean annual temperatures ranged from 12.1°C to 21.2°C. Twenty-one per cent of the farms were found infested with bacterial wilt. The highest percentage of infested farms lay between an altitude of 1520 m and 2120 m. The disease incidences decreased with elevations outside this zone.

Disease symptoms were used to identify diseased plants in the field. Thirty-three isolates collected were classified as Pseudomonas solanacearum biochemical type II or race 3 of Buddenhagen, et al. (1962). Potatoes (Solanum tuberosum) were infected exclusively by this biochemical type. Biochemical type III was isolated from egg-plant (S. melongena). This biotype was not isolated from potatoes and was not found pathogenic to potatoes.

The optimum growth temperature of P. solanacearum in vitro was 31°C. The minimum and maximum temperatures were around 13°C and 35°C respectively. No growth was obtained at 12°C and 37°C. The in vivo growth-chamber inoculation experiments showed an increase in the rate of wilt development as temperatures increased from 14°C to 24°C. At higher temperatures, the rate of wilt development decreased. Dose

response experiments in the greenhouse during four seasons of the year showed that the concentration of viable cells required for 50% of inoculated plants to wilt decreased with an increase in temperature from 16.9°C to 20.7°C.

The pathogen survived best in soil at 24°C followed by 20°C and then 18°C. At these temperatures the bacteria multiplied, reached a peak in the 14th day, then declined slightly. The bacterial population remained almost constant for 28 days at 14°C while at 28,30 and 33°C, the concentrations of bacteria declined.

Most plants inoculated with the pathogen at high altitude had latent infection. When tubers from these plants were kept in humid conditions at 30°C, 20% showed disease symptoms. P. solanacearum was isolated from 3% of externally symptomless tubers and 14.9% of plants raised from symptomless tubers developed wilt symptoms under favourable conditions.

P. solanacearum is a good immunogen. Antisera prepared against the bacterium had titres ranging from 1/128 to 1/1024. The antisera were found to be specific and could be used in the laboratory for quick identification of P. solanacearum.

1. INTRODUCTION

Potato, (Solanum tuberosum), originated in the Chilean area of South America (Salaman, 1949). The plant was introduced into Kenya during the late 19th century by English travellers. Early white settlers started growing potatoes in Kenya highlands in the Rift Valley Province as soon as they settled there in the late 19th century. The African farmers in Kenya began the cultivation of the crop around 1918 purely for home consumption as there was no provision for marketing their produce. The areas growing potatoes subsequently expanded from the Rift Valley into Kiambu, Murang'a and Nyeri districts of Central Province (Waithaka, 1975).

Potatoes have assumed great importance in Kenya both as a subsistence food item and a cash crop. The crop is cultivated on large and small scale farms mostly in Central, Rift Valley and Eastern Provinces. Some potatoes are also grown in Taita-Taveta district in Coast Province and Kisii district in Nyanza Province. The total area under potato in Kenya is estimated at 75,000- 100,000 hectares producing between 400,000 and 500,000 metric tonnes of the crop each year, most of which is consumed locally (Durr, and Lorenzl, 1980).

The crop grows best at an altitude between 1200 and 2800 m. These areas receive an average annual rainfall of between 850 and 1200 mm. The rainfall is distributed in two rainy seasons, usually referred to as long-rain and short-rain

seasons. The long-rain season starts in March and continues to June and the short-rain season starts in October and ends in December. The rainfall during the long-rains is, on the average, higher than during the short-rains. At the beginning of each season, a potato crop is planted.

In Kenya, one of the objectives of potato production is to achieve an increased production of the crop (Min. of Agric. Kenya, 1974). This objective, however, is not adequately achieved because the potato crop is usually adversely affected largely by disease causing agents such as fungi, bacteria, viruses and nematodes. Some of these agents cause losses both in the field and during storage. A list of some of these diseases is as follows:-

late blight caused by Phytophthora infestans
early blight caused by Alternaria solani
bacterial wilt caused by Pseudomonas solanacearum
bacterial soft-rot caused by Erwinia spp.
a mild mosaic caused by Potato virus X
a mosaic caused by Potato virus Y
leaf-roll, stunted growth caused by potato leaf roll virus
root-knot caused by Meloidogyne spp. etc.

The most destructive diseases are: late blight and bacterial wilt.

Bacterial wilt of potato was first reported in Kenya in 1940 by Nattrass (1945 and 1946). He observed that the

symptoms of the disease appeared to be identical with those of the Ring-rot caused by Corynebacterium sepedonicum spp. Attempts were made to demonstrate a gram-positive organism since Corynebacterium spp. show gram-positive reaction, but this failed. The identity of the pathogen, therefore, remained doubtful for some time. Cultures of the organism were studied by Dowson (1949) who identified the organism as an "atypical" strain of Pseudomonas solanacearum. He called the strain "atypical" because the Kenyan strain was found not to discolour vascular bundles of potato host plants as had been observed in other countries.

Crop losses are mainly due to the nature of damage caused by the disease on crop plants. The foliage of the infected plant become prematurely yellow and dies gradually or may wilt suddenly without much change in colour. The stems droop and shrivel and there is usually brown discolouration of the vascular system. The infected tubers show discolouration of vascular system. No tubers are formed in plants which are attacked early during the stages of growth. The tubers on plants infected in the middle of the growing season are small and few and are found at harvest time in all stages of decay.

The wilt pathogen can survive for several seasons in the soil and can be a cause of new outbreaks. In soil the bacterium can survive as resting cells even at the wilting point which in the potato growing areas is around 25% moisture (Min. of Agric. Kenya, 1966). The pathogen has also

been found to survive in crop debris, roots of alternative hosts and volunteer tubers from earlier crops. These factors make it difficult to control Pseudomonas solanacearum by conventional means. It is possible, however, to control bacterial wilt to a certain extent by a combination of planting wilt free seed produced in areas cold enough for the disease development and reducing soil contamination through crop rotation.

In Kenya, some investigations on the disease have been carried out by various workers. The work of Nattras (1945 and 1946) has been outlined above. In 1960, surveys of the potato crop were carried out in Central Province and the results showed that incidences of the disease in Nyeri and Kiambu districts were 75 and 30% respectively. Results of two more surveys carried out later in the province showed that incidences of bacterial wilt increased from 40% in 1961 to 55% in 1962 (Min. of Agric. Kenya, 1960, 1961 and 1962). Only two strains of P. solanacearum were found to be present in Kenya (Robinson and Ramos, 1964). The strains reported are biochemical type II and biochemical type III or race 3 and race 1 respectively of Buddenhagen et al., (1962).

Statistical analysis of the relationship between soil temperature, ambient temperature and rate of wilting were carried out by Ramos in 1971. He found that ambient temperature was less influential in causing wilt as compared to soil temperature. The soil temperature at which rates of wilting of potato approached zero were calculated using regression

equations and was found to be 13.31°C. Other workers found that altitude influenced the incidences of the disease (Harris and Michieka, 1972 and Harris 1976). The results of the surveys on the potato crop which they carried out in Central and Eastern Provinces, showed that wilt was found in 2/3 of the crop examined in low zones (1300 m to 2100 m). In higher zone (2100 m to 2700 m), wilt was found in 5% of the fields examined. The results clearly indicated great reduction of incidences at high altitudes.

Although some work on the bacterial wilt has been done in Kenya, adequate information still lacks on the ecological factors affecting distribution of P. solanacearum in the country. Since the disease is a major constraint in potato production in this country, the study was undertaken with the following objectives:-

1. to investigate the influence of altitude on P. solanacearum biochemical type II race 3
2. to determine the possibility of latent infection
3. to investigate the occurrence of new bacterial strains in various potato growing areas in Kenya, and
4. to suggest suitable altitudes for certified potato seed production based on the findings of this investigation.

2. LITERATURE REVIEW

2:1 Introduction

Little was known about the incidence of bacterial wilt of potatoes caused by Pseudomonas solanacearum prior to the later part of the nineteenth century (Kelman, 1953). The disease was first recognized and reported by Comes in Italy in 1884. In 1890, Burrill described very briefly a disease of potatoes which had attracted his attention as something new and which was probably this disease. He was the first person to make pure cultures of the bacterium and inoculate the organism into the potato. On the basis of the results he obtained from the inoculations, Burrill concluded that the bacterium was specific cause of tuber rot (Smith, 1914).

Following a brief study of wilt disease on tomato and potato in Southern Mississippi, Halstead (1890 and 1891) concluded that the causal agent was a bacterium. He suggested that cucurbits were also attacked by the same bacterium. This suggestion aroused the interest of Smith (1914) who began his study of the disease in 1895. In 1896, he published the first adequate description of the pathogen and demonstrated its pathogenicity to potatoes.

2:2 Economic Importance

Bacterial wilt affects crops such as potatoes (Solanum tuberosum), tomatoes (Lycopersicon esculentum), bananas (Musa sp.),

tobacco (Nicotiana tabacum) etc, which are of economic importance in practically every region between latitudes 45°N and 45°S of the world (Kelman, 1953, and Anonymous, 1977). The disease causes heavy losses due to the destruction of the affected crop plants. Premature death of plants and reduction in yield are the main ways in which bacterial wilt causes economic loss in crops. Secondary cause of decreased revenue from potato is the rotting and decay of tubers during storage and when in transit (Smith, 1914 and Kelman 1953). In 1914, Smith observed that the disease had destroyed a great number of fields of potatoes and tomatoes in the South-eastern region of United States and had put an end to commercial growing of these crops in Southern Mississippi, Alabama and parts of Florida. In 1931, about 11% of tobacco seed-beds on 65 estates in Sumatra were discarded because of the bacterial wilt problem. There were also heavy losses on potato and tomato as a result of which the cultivation of these crops was discontinued in the same region (Kelman, 1953).

Surveys carried out in Kenya (Min. of Agric. Kenya, 1960, 1961 and 1962) showed that the disease was of economic importance in the country. It was found that 75% of the potato crop in Nyeri and 30% of the crop in Kiambu districts were infected in 1960. When the disease incidences in Central Province in 1961 and 1962 were compared, it was found that the number of crops infected rose from 40% in 1961 to 55% in 1962. These figures indicated that the disease was becoming serious in Kenya and needed attention.

2:3 Symptomatology

The mode of entry of Pseduomonas solanacearum into the host tissue is usually through the root system (Kelman, 1953). A wound is considered to be necessary for entrance of the pathogen. Such a wound may be caused by parasitic fungi, nematodes, insects and tools used during weeding. Following entrance of the pathogen into host tissue, the length of time which elapses before symptoms appear in the suscept is extremely variable. It is dependent on the age, species of plant attacked and environmental factors. Smith (1914), found that the young succulent plants developed critical disease symptoms much more rapidly than older plants.

The disease symptoms caused by P. solanacearum are those of general wilt followed by complete collapse of affected plants (Smith, 1914; Dowson, 1949; Kelman, 1953; and Harrison, 1960). The first symptom, usually noticed on a warm day, is slight drooping of the tip of one or two of the lower leaves which may easily be mistaken for a temporary shortage of soil moisture (Harrison, 1960). The affected leaves may recover their turgidity towards evening. By the third day, two or three leaves of the affected plant are usually permanently wilted. The lamina roll upwards and inwards from the margins until the leaves are completely inrolled (Harrison, 1960). Sometimes only one branch of a potato hill may show flacidity. In other cases if onset of the disease is rapid, foliage of an

entire hill may quickly droop and wilt without change in colour (Smith, 1914; Kelman, 1953 and Harrison, 1960).

Harrison (1960), observed that the stems of affected plants were visibly infected only for a distance of 5 or 8 cm from the old seed. When the stems were cut transversely at a point above the ground level, there were no macroscopic signs of the disease. He also found that even when considerable pressure was applied to the exterior of the stem, there was no oozing of bacteria from the vascular ring. This is contrary to what Smith (1896, 1914) reported.

Symptoms on infected tubers are brown discolouration of the vascular ring with exudation of dirty-white, slimy masses of bacteria when a cut tuber is gently pressed (Smith, 1914). Harrison (1960) did not find any sign of disease externally except for badly affected tubers. However, when the tubers were cut transversely they showed white to creamy-white pockets of diseased tissue in the vascular ring. The disease tissue was of putty-like consistency and was not slimy. Identical symptoms were described by Nattrass (1945) in Kenya where an atypical strain of P. solanacearum was reported. Similar strains were reported in Portugal and India.

At the eyes or stolon end of a severely decayed potato, a sticky exudate form which mixes with the soil and causes it to adhere to the tuber surface (Kelman, 1953). Plants attacked early during the growing season form no tubers. The tubers on plants infected in the middle of the season are small and few and are found at harvest time in all stages of decay (Smith, 1914).

2:4 Etiology

In 1896, Smith published the first edequate description of the causal agent of bacterial wilt of solanaceous plants and demonstrated its pathogenicity to potato. He named the bacterium Bacillus solanacearum E.F Smith, 1896. A number of generic names have been suggested since then.

The first generic name (Bacillus) was proposed by Smith (1896) because he thought the bacterium had peritrichous flagella. Later, Smith placed the organism in the genus Bacterium. In 1914, Smith suggested that the organism be known as either B. solanacearum or, following Migula's classification, as P. solanacearum because the pathogen was found to be motile with a single polar flagellum.

The organism was again reclassified and placed in the genus Phytomonas (Bergey, 1923), but Dowson (1939 and 1943) transferred it to a new genus Xanthomonas, because it differed from typical members of the earlier group in certain cultural characteristics. Later, it was found that members of the genus Xanthomonas were unable to utilize asparagine as a sole source of carbon and nitrogen in a synthetic medium, whereas P. solanacearum was able to utilize this compound. This characteristic plus the absence of the typical yellow pigment in its colonies raised questions as to the reason of Dowson's suggested change in classification. As a result, Dowson (1949) transferred the organism back to the genus Pseudomonas. This is in agreement with the classification adopted in Bergey's Manual of

Determinative Bacteriology.

Below is a list of synonyms:

Pseudomonas	solanacearum	E.F. Smith, 1914
Bacillus	solanacearum	E.F. Smith, 1896
Bacterium	solanacearum	Chester, 1898
Phytomonas	solanacearum	(E.F. Smith) Bergey et al, 1923
Xanthomonas	solanacearum	(E.F. Smith) Dowson, 1939

2:5 Characteristics of Pseudomonas solanacearum

Smith (1896 and 1914) was the first person to describe the causal agent of bacterial wilt of potato. He observed that single cells were rod-shaped and had rounded ends. Many of these rods were 0.5 x 1.5 µ. The sizes were, however, variable and were influenced by the conditions under which growth was obtained. Rods taken from young cultures in broth or nutrient agar were found to be longer than those taken from old cultures.

Pseudomonas solanacearum is described in Bergey's Manual of Determinative Bacteriology as gram-negative rods, measuring 0.5 - 0.7 by 1.5 - 2.5 µm and motile by one to four polar flagella. The bacterium shows distinct bipolar staining with methylene-blue, carbol fuchsin or any of the alkaline aniline dyes (Smith, 1914). Hayward (1960) indicated that the lack of affinity for basic dyes which result in bipolar staining was caused by the presence in the cells of massive inclusions of poly-β-hydroxybutyrate.

In determining the characteristic appearance of colonies of

P. solanacearum, conditions prevailing during incubation and the type of substrate used are of great importance since they influence bacterial growth (Smith, 1914). Smith observed that colonies on solid media were usually irregularly round, slightly raised, smooth and measured 3-5 mm in diameter. Appearance of colonies of the bacterium have since been described by various workers as irregular in shape, fluidal, slightly raised, slimy and creamy-white with pink-orange centres (Dowson, 1949; Kelman, 1954; Harrison, 1960; Hayward 1960 and French and Sequeira, 1970).

2:6 Races and strains of Pseudomonas solanacearum

It has been observed that (1) there is, in some cases, apparent immunity of plants in a given area considered to be suscepts elsewhere; (2) inoculations with pure cultures to known hosts may fail to cause disease; and (3) atypical cultural or physiological traits in certain isolates have been detected (Kelman, 1953). These observations indicate the possibility of the existence of different strains within the species Pseudomonas solanacearum E.F. Smith. Strain determination in this species, however, is difficult. The numerous hosts affected by this pathogen, its wide geographic distribution, the intrinsic complexities of strain differentiation in this variable species, have resulted in the development of several methods of classification at subspecific level, including races, strains and pathotypes, on the basis of widely different criteria (French and Sequeira, 1970).

Harrison and Freeman (1960), after considerable cross-precipitation purification, were able to distinguish an Australian potato strain of P. solanacearum from a Rhodesian isolate on the basis of differences in physiological reactions. They did not obtain most specific antibody formation since nucleoproteins were not used as immunogens. Natural host range, reaction of differential hosts and colony appearance on tretrazolium medium, were used to classify several hundred P. solanacearum isolates obtained from a wide range of hosts in Central and South America into three races. The races were characterized as: race 1 which affects tobacco (Nicotiana tabacum) and other solanaceous hosts; race 2 which causes wilt of bananas, Heliconia spp; and other Musaceous hosts; and race 3 which affects potato (Solanum tuberosum), (Buddenhagen, et al. 1962). The work of Sequeira and Averre (1961), showed that race 1 could be distinguished from race 2 isolates by the intense brown pigment produced by the former when grown on a medium containing tyrosine. Race 3 isolates produced small amount of this pigmentation in the same medium.

Based on various physiological characteristics, Hayward (1964), classified 95 isolates of P. solanacearum into major biochemical type I,II,III and IV. He found that all the isolates obtained from potato formed a single group on physiological reactions. He called the group biochemical type II which could be differentiated from three other groups by its ability to oxidize maltose, lactose and cellobiose and inability to oxidize mannitol, sorbitol and dulcitol. This biotype

corresponds to race 3 of Buddenhagen et al., (1962) and it attacks potatoes. Potatoes can also be affected by the broad-range race 1 or biochemical type III (Buddenhagen and Kelman, 1964). Buddenhagen and Kelman (1964) also noted that in most classification schemes, colony morphology and virulence of different isolates on inoculated hosts could be used to distinguish not only the three major races but also certain strains within these races.

In 1966, Morton et al., differentiated the three races of P. solanacearum by serological methods. They found that P. solanacearum was not closely related to other plant pathogenic bacteria such as Corynebacterium michiganense, P. savastanoi, P. syringae and Xanthomonas vesicatoria. Coleno et al., (1976) observed that serotype scheme could be used for identification of P. solanacearum.

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

In Kenya, only two races of P. solanacearum have been reported. These are race 1 and race 3. Nattrass (1945 and 1946) had reported that there were two strains of P. solanacearum both of which attacked potatoes in Kenya. However, in 1964, Robinson and Ramos reported that only one strain attacked potatoes. The strain which was found to attack potatoes was biochemical type II. They also found that adequate differentiation between the biotypes was possible with only six carbohydrates. The carbohydrates they used were: glucose, lactose, mannitol, sorbitol and cellobiose. Salicin was used as a negative control. The work of Harris (1976) indicated that the strain which causes the disease on potatoes in Kenya is a low temperature strain.

2:7 Effect of Temperature on Distribution and Survival of Pseudomonas solanacearum

Temperature is one of the environmental factors which influence growth of bacteria. In 1896, Smith studied the influence of temperature on the growth of P. solanacearum and reported temperatures of 35-37°C as most favourable for growth of the bacterium. Since then, other workers have found the optimum temperature to be slightly lower. Thus, Harrison (1960) observed that the optimum temperature of the potato isolates he worked on was between 27°C and 28°C, the maximum temperature for the isolates was 39°C and the thermal death point lay between 47 and 51.5°C. Hayward (1964), observed that isolates from potato produced only a trace of growth at 37°C. Laboratory

experiments done by Harris (1976), showed that the growth rate of the bacterium was highest at 30°C and declined to zero between 15 and 12°C. He also observed a conspicuous effect on altitude on incidences and severity of the bacterial wilt of potatoes in Kenya. The results of the surveys he conducted indicated that at an altitude of 2,400 m and above, the disease was rare. In 1971, Ramos studied the temperatures at which infection and wilting occured using statistical analysis. He found that ambient temperature was far less influential in causing wilt as compared to soil temperature. The soil temperature at which rates of wilting approached zero were calculated using regression lines and were found to be 13.31°C for potatoes (Ramos, 1971). Ramos (1976) also found that biochemical type III race 1 had greater soil survival ability than type II race 3.

Temperature plays an important role in the geographic distribution of the organism since it is rarely found in areas where the mean temperature falls below 10°C (Kelman, 1953). The optimum temperature listed by Meier and Link (1923) for development of bacterial wilt of potatoes was between 25°C and 36°C. Below 12°C they found that the disease was inhibited.

Bacterial wilt of potatoes is an important disease in Australia (Lloyd, 1976). In his investigations, Lloyd found that the disease occured in cool temperate regions of New South Wales. It was found that in such a cool temperate climate, unlike tropical climate, alternative weed hosts were of little consequence and therefore, the bacterium appeared to survive in soil for 2-3 years in pieces of debris. The same findings were

observed by Graham *et al.*, (1979) who,showed that the pathogen could survive in plant debris and in latently infected tubers. McCarter (1976), also studied the persistence of *P. solanacearum* in artificially infested soils and found that the pathogen persisted at least for 4 years.

3. MATERIALS AND METHODS

3:1 A Survey of Bacterial Wilt of Potatoes in Central, Eastern and Rift Valley Provinces

A survey was carried out on the bacterial wilt of potatoes in Central, Eastern and Rift Valley Provinces where potatoes are grown in abundance. The survey was done during the long and short rain seasons of 1980/81. The aim of the survey was to determine the distribution and severity of bacterial wilt disease in relation to altitude and also to investigate the possibility of occurence of new strains of Pseudomonas solanacearum in various potato growing areas in Kenya. Information was gathered by asking farmers questions which had been previously prepared and the responses were recorded on questionnaire forms. Field observation were also recorded.

Selected routes were followed using a vehicle. At intervals of 10-15 kilometres, stops and visits were made on potato farms. The altitudes of areas were measured using an altimeter and the acreages of farms were roughly estimated and recorded in hectares. Each farm was examined for the presence of any plants showing wilt symptoms and the results were recorded as "wilt present or absent" as applicable. Rows of potato crops were then randomly selected and then healthy and diseased plants were counted using a hand counter.

The disease symptoms as described by Kelman (1953) and Harrison (1960), were used to differentiate a diseased plant

from a healthy one.

Samples of diseased materials were collected, labelled, placed in polythene bags and brought into the laboratory at National Agricultural Laboratories for isolation and identification of bacteria. Samples from other solanaceous crops, such as egg-plants and tomatoes, which were found wilting in the field were also collected, brought to the laboratory and isolations were made from them.

3:2 Identification of *Pseudomonas solanacearum*

3:2:1 Isolation and Storage

In the laboratory, the samples were washed thoroughly in running tap water and then sectioned using alcohol-flame sterilized scalpel and forceps. The sectioned pieces were placed in sterile screw-capped tubes containing 5 ml sterile distilled water. These were allowed to stand for 15-30 min to allow bacteria to ooze out of the pieces. The resulting suspensions of bacterial ooze were streaked on Kelman's tetrazolium (TZC) medium (Jenkins and Kelman, 1976) using a sterile wire-loop.

After incubating the cultures at 31°C for 48 hr, discrete single colonies with characteristic appearance resembling those described by Kelman (1954) were picked using a sterile wire-loop and suspended in sterile distilled water in small screw-capped bottles. Sub-cultures were made on fresh Kelman's TZC media to obtain pure cultures. A total of 33 cultures were isolated

(Table 1) and were given serial numbers in order of their collection. Culture No. 13 was obtained from Commonwealth Mycological Institute (C.M.I.) and it was run as a standard culture in all identification tests.

Pure cultures were stored in sterile distilled water in screw-capped bottles at room temperature (18-20 ^{o}C) as described by Kelman and Person, (1961). Routine checks were made at 3 months intervals on stored cultures by sub-culturing on Kelman's TZC medium to determine the stability of the virulent type.

3:2:2 Cultural Characteristics of Pseudomonas solanacearum

Bacterial suspensions of pure cultures were streaked on Kelman's TZC medium in petri-dishes, incubated for 48 hr at 31 ^{o}C and then colonies were observed visually for appearance in colour, consistency, shape, formazan deposit and size. The diameters of colonies were determined by superimposing a ruled scale upon the longest axis of each colony (Kelman, 1954; Hayward, 1960 and 1964; Harrison, 1960 and French and Sequeira, 1970).

Cultures of the isolates were used for the Gram-stain reaction. For each isolate a dilute suspension was made in a small drop of sterile distilled water.

Table 1: Potato, tomato and egg-plant isolates of *Pseudomonas solanacearum* collected from Nairobi area, Central, Eastern and Rift Valley Provinces during the long and short rain seasons in 1980/81.

Isolate number	Location	Host	Season
1.	National Agricultural Laboratories (NAL)	Potato (*Solanum tuberosum*)	Short rains
2.	Kiambu (Central Province)	"	"
3.	" " "	"	"
4.	N.A.L. (Nairobi)	"	"
5.	Murang'a (Central Province)	"	"
6.	" " "	"	"
7.	" " "	"	"
8.	N.A.L. (Nairobi)	"	"
1o.	" "	"	"
11.	Kiambu (Central Province)	"	"
12.	" " "	"	"
15.	Bomet (Rift Valley Province)	"	"
16.	" " " "	"	"
17.	" " " "	"	"
18.	" " " "	"	"
19.	N.A.L. (Nairobi)	"	"
20.	Murang'a (Central Procince)	"	"
21.	Embu (Eastern Province)	"	"
22.	" " "	"	"
23.	" " "	"	"
24.	" " "	"	"

Table 1: cont...

Isolate number	Location	Host	Season
25.	Embu (Eastern Province)	Potato (*Solanum tuberosum*)	Short rains
26.	Meru (Eastern Province)	"	Long rains
27.	" " "	"	"
28.	" " "	"	"
29.	" " "	"	"
30.	" " "	"	"
31.	Nyandarua (Central Province)	"	"
32.	" " "	"	"
33.	" " "	"	"
34.	" " "	"	"
9.	N.A.L. (Nairobi)	Egg-plant (*Solanum melongena*)	Short rains
13.	C.M.I.		-
14.	Yatta Scheme (Eastern Province)	Tomato (*Lycopersicon esculentum*)	Short rains

The suspension was spread thinly on a grease free, clean microscope slide, and then let to dry in air. To fix the smear, the slide was passed through a flame on a burnsen burner 2-3 times. The smears were gram stained and then observed under the microscope, using 100x objective, for the gram-reaction and the morphology of the cells. The sizes of the cells were measured

in µm using an occular micrometer previously calibrated on the same microscope with a stage micrometer.

3:2:3 Biochemical Characteristics of Pseudomonas solanacearum

The following tests were carried out to determine biochemical characteristics of each isolate (Table 1). Isolate No.13 was used as a standard culture.

Test on oxidation of carbohydrates by the isolates was done according to the method of Hayward (1964). Oxidation of the following monosaccharides, disaccharides and hexose alcohols were determined: glucose, sucrose, maltose, lactose, cellobiose, mannitol, sorbitol and dulcitol. Salicin was used as a negative control.

A 10% aqueous solution in distilled water was made from each test carbohydrate. These were sterilized in 5 ml aliquots in small screw-capped bottles at 1 atmosphere pressure for 20 minutes. To make the desired test medium, 5 ml of a particular carbohydrate was poured in 45 ml of sterile molten semi-solid basal medium (Appendix 1) contained in a 100 ml conical flask. The test medium was thoroughly mixed and then poured, under sterile conditions, into previously sterilized 150 mm x 10 mm internal diameter test tubes each of which was half filled. The medium was allowed to set. Each test-tube was stab inoculated with a pure bacterial culture. The results were read after 14 days incubation at 31°C. Oxidation was indicated when the medium turned yellow gradually from the top downwards. The

control tubes remained olivaceous green in colour.

Nitrate reduction test was done on all isolates to test the presence of the enzyme nitrate reductase in the bacterial isolates. The nitrate reductase cause the reduction of nitrate to nitrite in the presence of a suitable electron donor. The isolate cultures were inoculated into peptone water containing potassium nitrate (KNO_3). The tests were kept for 48 hr at 31 °C and then 1 ml of test reagent was added to the test cultures. A red colour developing within a few minutes indicated the presence of nitrite and hence the ability of the organism to reduce nitrate.

Investigation on the ability of the bacterium to denitrify was done by covering the inoculated test cultures in test tubes with sterile liquid paraffin. The gas produced was trapped under oil indicating the ability of the bacterium to denitrify.

The method of Kovacs (1956) was used to test for the presence of oxidase in the bacterial isolates. The test depends on the presence in bacteria of certain oxidases that catalyse the transport of electrons between electron donors in the bacteria and a redox dye-tetramethyl-p-phenylene-diamine. The dye is reduced to a deep purple colour in positive tests. Whatman's No.1 filter papers were soaked in a freshly prepared 1% solution of tetramethyl-p-phenylene-diamine dihydrochloride. The colony to be tested was picked with a sterile wire-loop and was smeared on the moist area. A positive reaction was indicated by an intense deep-purple colour appearing within 5-10 sec.

To demonstrate the presence of catalase in the bacterial isolates, a loopful of a 48 hr bacterial growth on Kelman's TZC medium was placed on a clean microscope slide then a drop of 20% hydrogen peroxide was added using a dropper. A positive reaction was indicated by production of gas bubbles. In the reaction, catalase enhances the production of oxygen from hydrogen peroxide.

Hydrogen sulphide production ability by the bacterial cultures was also tested because some organisms decompose sulphur-containing amino acids to form hydrogen sulphide among other products. A positive test is demonstrated by the formation of black insoluble ferrous salt. Whatman's No.1 paper strips were soaked in lead acetate solution and let to dry. The strips were then suspended over peptone water cultures which were then incubated for 96 hr at 31 ^{o}C and observed daily for positive reaction.

Arginine hydrolysis test was done to test the presence of arginine dihydrolyase in the bacteria cells. Arginine broth was inoculated with the isolates and incubated at room temperature (18-20^{o}C). The change of colour from bright pink to brown in this test indicated hydrolysis of arginine and consequently the presence of the pertinent enzyme.

The ability of the bacterium to hydrolyse starch was tested by inoculating the bacterial cultures on starch agar and looking for the presence of a clear zone around the inoculum that would indicate starch hydrolysis.

3:3 Identification of Races and Biochemical types of Pseudomonas solanacearum

To identify the isolates (Table 1) into races and biochemical types, the following characters were used:-

1. Biochemical characteristics
2. Colony characteristics and
3. Tobacco leaf infiltration reactions

Biochemical type determination of the isolates was done according to the method of Hayward (1964), with some modification, using the basal medium with the composition shown in appendix 1.

Five ml of 10% (W/V) sterile solution of test carbohydrate was added to 45 ml of molten, cooled basal medium. The solution was then dispensed in sterile plugged tubes (1 x 12.5 cm) to a depth of about 4 cm. The medium was labelled according to the carbohydrate added. The carbohydrates used were: glucose, sucrose, maltose, lactose, cellobiose, sorbitol and dulcitol. Salicin was used as a negative control.

The media were stab inoculated with pure bacterial cultures of the isolates. The results were observed after 3,7 and 14 days incubation at 31°C. Oxidation was indicated when the medium turned yellow starting at the top and progressing downwards.

The nitrate reduction test was carried out on all isolates as described earlier.

Detailed colony characteristics according to the method of French and Sequeira (1970) were used to group the isolates into races. Pure isolates (Table 1) were cultured on Kelman's TZC medium and after incubation at 31°C for 48 hr, colony characteristics were observed visually and described.

Tobacco leaf infiltration test was conducted using the method of Lozano and Sequeira, (1970). This test was useful in that it gave different tobacco leaf reactions for different races Nicotiana tabacum L cv. "White burley" plants were used for inoculations 30-45 days from the date of transplanting. The fully expanded leaves were infiltrated by injecting water suspensions of bacteria into the intercellular spaces with a hypodermic syringe fitted with a gauge 30 needle. The bacterial suspension contained approximately 2×10^8 cells/ml. For each isolate two plants were tested. The leaves of the control plants were infiltrated with sterile distilled water. The inoculated plants were incubated under green-house conditions and leaf reactions recorded after 24,48,72 and 96 hr.

3:4 Pathogenicity

All isolates (Table 1) were tested for their pathogenicity on potato (Solanum tuberosum L. cv. Kerr's Pink) and egg-plant (S. melongena). For each isolate three plants from the two species above were tested. Root-wounding method of Winstead and Kelman (1952) was used for inoculations with some modification.

The plants were grown in 17 cm diameter plastic pots filled

with sterile soil. Potato plants were inoculated when they were 4 weeks old or when the 8th leaf was fully expanded and the egg-plants were inoculated 4 weeks after transplanting. Inocula of isolates were prepared by growing the cultures in Kelman's medium, without tetrazolium salt for 48 hr at 31°C. The bacteria were harvested by washing the surfaces of the medium with sterile water into 50 ml conical flasks. The suspensions were then well shaken and their optical densities (O.D.) adjusted to read 0.5 using a portable colorimeter and a blue filter. At this O.D., the concentration of bacteria was approximately 10^9 cells/ml. This had been calculated previously using the viable cell count method.

Inoculations were made by cutting the lateral roots with a scalpel along one side of the plant to a depth of approximately 4 cm and pouring 10 ml of a bacterial suspension over the severed roots. Wilting symptom and reisolation of P. solanacearum from the wilted plants were used as signs of pathogenicity.

Pathogenicity tests were also done on the following varieties of potato using P. solanacearum biochemical types II and III: Annet, Kenya Baraka, Desiree, Feldeslolin, Roslin Eburu (B53), Roslin Tana and Roslin Gucha.

3:5 Temperature

The investigations below aimed at finding out the effect of temperature on the following:-

in vitro growth of *Pseudomonas solanacearum*

in vivo " " " " and

populations of *P. solanacearum* in the rhizosphere .

The findings may help to explain the effect of temperature on wilt development in the field.

3:5:1 Effect of Temperature on Growth of *Pseudomonas solanacearum* on Kelman's Medium

Isolates of *P. solanacearum* Nos. 8 and 9 (Table 1) were used for these investigations. Isolate No.8 was randomly selected to represent Hayward's (1964) biochemical type II or race 3 of Buddenhagen *et al.*, (1962) and isolate No.9 was selected to represent biochemical type III or race 1 of Buddenhagen *et al.*, (1962). The isolates were streaked separately on Kelman's TZC medium using a sterile platinum wire-loop. The plates were incubated at known temperatures until descrete single colonies of reasonable sizes had grown. The length of time taken for such colonies to grow was recorded in hours.

A known number of colonies were then removed by cutting off a piece of the medium on which the colonies were growing. These were shaken in 10 ml sterile distilled water, in small screw-capped bottles, for 3-4 minutes. The process was replicated four times and the suspensions were used as follows:

six ten-fold serial dilutions were made from each of the four replicates. Samples (0.1 ml) from each of the last three dilutions, of each replicate, were spread on each of 3 Kelman's TZC agar plates. After incubation for 48 hr at 31°C, the number of colonies growing on each plate were counted. The number of cells per colony, generation time, the number of doublings and number of generations were calculated. Curves based on the number of doublings in 48 hr against temperature were plotted.

The optimum temperature for P. solanacearum biochemical type II is 31°C (Harris, 1976). The bacterium grows poorly at 37°C (Harrison, 1960) and the lower limit for growth of the bacterium is 15°C while at 13°C no growth is obtained (Harris, 1976). Because of these findings the following temperatures were selected for this investigation:

Isolate No.8: 13,15,17,19,21,23,25,27,30,31,33,35 and 37°C

Isolate No.9: 14,18,22,26,30,34,36 and 38°C

3:5:2 Effect of Temperature on Development of Bacterial Wilt in Inoculated Potatoes kept under Greenhouse and Growth Chamber Conditions.

(a) Greenhouse experiments on the effect of temperature on the development of bacterial wilt:

Greenhouse experiments to test the effect of temperature on the development of wilt were carried out in November/December, 1980, February/March, April/May and June/July, 1981. Certified

potato tubers, cultivar "Kerr's pink", were grown and used during each season. The potatoes were grown in plastic pots, 16 cm diameter filled with sterilized soil. When the plants were 4 weeks old they were considered ready for inoculation and were arranged into 10 blocks each containing 8 plants. Five blocks were inoculated with P. solanacearum biochemical type II (culture of isolate No.8 in Table 1) and the remaining 5 blocks were inoculated with biochemical type III (culture of isolate No.9 in Table 1).

Inocula of biotypes were obtained from 48 hr bacterial cultures grown on Kelman's TZC medium without tetrazolium salt to avoid pigmentation. Original suspensions of the biotypes were made by washing the cultures with sterile distilled water into separate 100 ml sterilized conical flasks. These were well shaken then the O.D. adjusted to 0.5 using a portable EEL colorimeter. Such a suspension contained approximately 10^9 bacterial cell per ml.

Six ten-fold serial dilutions from each of the original suspensions were prepared to give the following concentrations:

10^9 (original), 10^8, 10^7, 10^6, 10^5, 10^4, and 10^3 cells per ml. Each potted plant in a block was inoculated randomly with one of these concentrations. The remaining plant in each block, was used as a control. For the inoculations, root-wounding method of Winstead and Kelman, (1952) was used as described earlier. Observations were made daily and the number of days taken for each plant to wilt were recorded. Soil temperatures were recorded daily at the following times:

9 A.M. 12 M., 3 P.M., 8 P.M., and 3 A.M.

Curves based on wilting time against temperature were plotted for each season and LD 50 estimated.

(b) Effect of Temperature on development of wilt in a Growth Chamber:

Potato plants were raised in plastic pots as described earlier. When the plants were 4 weeks old, 15 of them were transferred into a growth-chamber set at a known temperature and 80 - 100% humidity. Ten of the plants were inoculated each with 10 ml of 10^9 cells/ml of P. solanacearum biochemical type II using the root-wounding method of Winstead and Kelman, (1952). The rest of the plants were used as controls. The number of days taken for each test plant to wilt were recorded, a curve based on the mean days to wilting against temperature was plotted. The experiment was run at the following temperatures:

14, 18, 20, 24, 28, 30 and 33°C

3:5:3 Pseudomonas solanacearum Populations in the rhizosphere at Various Temperatures

Potato plants, cultivar "Kerr's Pink", were placed in the growth-chamber at known temperatures and inoculated as described earlier. Soil samples (about 1 g each) were collected from three separate pots having test plants and the 4th sample was collected from one of the control pots. Samples were collected at the 7th, 14th, 21st and 28th day after inoculation. A stock borer 0.75 cm in internal diameter was used to collect the soil at a depth of about 10 cm from the surface. These samples were taken to the

laboratory and the bacterial population determined as follows: Each sample was shaken for 3-4 min in 10 ml sterile distilled water and then six ten-fold serial dilutions were prepared. Samples (0.1 ml) from each of the dilutions at 10^{-4}, 10^{-5} and 10^{-6} were spread on 3 Kelman's TZC agar plates. After incubation for 48 hr at 31°C, colony counts were made and the number of bacteria per g of dry soil calculated. Curves based on mean population density/g of soil against time in days were plotted.

3:6 Effect of Altitude on Wilt Development

3:6:1 Potato Wilt Assessment at Low and High Altitudes

The objective of the experiment was to investigate the effect of altitude on wilt development in inoculated potato plants. For this, experiments were laid at Kamae Forest Station and National Agricultural Laboratories. The site at the National Agricultural Laboratories is approximately 1760 m and was selected to represent low altitude. Kamae Forest Station is located at 2480 m and it served as the high altitude site. At this site the land was under afforestation and therefore considered relatively free from the pathogen.

"Kerr's Pink" certified potato seed tubers were planted at each site in 3 plots each 6x3 m, with paths 1.5 m between them. Diammonium phosphate fertilizer was applied during planting at the rate of 500 Kg/ha. The spacing between plants was 30 cm and 0.74 m between rows. There were 5 rows in each plot and 20 plants in each row. Plants were sprayed with

Mancozeb (Zinc Manganese ethylene bis-dithiocarbamate) every two weeks to control late blight. The spraying started when the crop was 1 week old after emerging and continued until it matured.

Inocula of P. solanacearum biochemical type II and III were obtained from the 48 hr bacterial growth on Kelman's TZC media without tetrazolium salts. The suspensions were made in sterile distilled water and adjusted to O.D. 0.5 using a colorimeter. All plants in the first plot at each site were inoculated with P. solanacearum biochemical type II and the plants in the second plots were inoculated with biochemical type III. Plants in the third plots were inoculated with sterile distilled water to serve as controls.

Inoculations were made by the stem puncture method of Winstead and Kelman, (1952). A drop of the inoculum was placed on the axil of the third fully expanded leaf from the top, and the stem was pierced by thrusting a scalpel-tipped dissecting pin downward through the inoculum drop. All stems of each plant were inoculated. The number of plants which showed wilting symptoms were recorded.

The experiments were done in two seasons (during short rainy season, 1980, and long rainy season, 1981). New plots were used in the second season.

3:6:2 Assessment of Bacterial Infection in Potato Tubers

The aim of the experiment was to investigate the possibility of latent infection of tubers which appeared healthy

externally. Attempts were made to isolate P. solanacearum from 100 externally symptomless tubers obtained from infested experimental plots at Kamae. Sections of potato tuber tissue were placed in sterile water contained in small screw-capped bottles. These were stood on the bench for 15-30 min and the resulting suspensions were streaked on Kelman's TZC medium. The plates were incubated for 48 hr at 31°C. The resulting bacterial growth was examined and the suspected colonies were identified using identification methods described earlier. The number of tubers from which P. solanacearum was isolated were recorded.

In another observation, 562 externally symptomless tubers from Kamae experimental plots were stored in the laboratory at room temperature (18-20°C) for 3 weeks. The tubers were then examined for exudate from the "eyes". The number of tubers which revealed exudation were recorded. One hundred of the tubers which remained healthy in external appearance were incubated at 30°C in a moist desiccator according to the method of Graham, et al., (1979). The tubers were examined daily for bacterial ooze from the "eyes" and the record taken.

Another 150 tubers were planted in plastic pots 16 cm diameter filled with sterilized soil. The plants were kept in the greenhouse and were observed daily for disease symptoms. As soon as the symptoms appeared, plants were cut at ground level and stem sections were checked for streaming of bacterial ooze. Suspensions obtained in this way were streaked on Kelman's TZC medium to verify the presence of P. solanacearum. The number of the plants which wilted was recorded.

3:7 Development of a Serological Laboratory Test For Detection of P. solanacearum in Potato Tubers of Infected Plant.

P. solanacearum can be isolated, in the laboratory, from diseased material by plate-streaking. The bacterial cultures obtained are then identified by techniques for studying cultural, morphological and biochemical characteristics of the bacterium. This takes a long time and, therefore, there exists need for a quick laboratory test to identify P. solanacearum.

P. solanacearum has been found to be a good immunogen (Digat and Cambra, 1976; Harrison and Freeman, 1960 and Morton et al., 1966). These workers have shown that antiserum against P. solanacearum can be prepared and used for agglutination tests.

This experiment is an endevour to prepare antiserum which could be used in the laboratory for quick identification of P. solanacearum in potato tubers of infected plant.

3:7:1 Preparation of Antigens

Two types of methods were used for the preparation of antigens. Heat treated antigen was prepared according to the method of Harrison and Freeman, (1960). P. solanacearum was grown on Kelman's TZC medium without tetrazolium salt for 48 hr at 31°C. The growth was washed off with normal saline, and after centrifugation for 10 min, the sediments were resuspended

in fresh normal saline, boiled for 2 hr and adjusted to a density of approximately $4x10^6$ organisms per ml. This was achieved by adjusting the O.D. of the bacterial suspension to 0.5 and then diluting this to about a half the concentration. Formalin (0.25%) was added as a preservative and the suspensions were stored at $4^{\circ}C$ until required.

Sonicated antigen was prepared by washing the organisms in physiological saline three times and then adjusting the density to $5x10^8$ cells per ml. These were disintegrated with sonifier at 9-10 amps for one minute. After adding formalin (0.25%) as a preservative, the antigen was kept at $4^{\circ}C$ until required for rabbit injection (Morton, et al., 1966).

3:7:2 Preparation of Antisera

Two rabbits were used for each batch of antigen. Before inoculations, a sample of blood was taken from the marginal ear vein of each rabbit for normal sera preparation which were preserved with equal volumes of glycerol and then stored at $4^{\circ}C$ until required.

Heat treated antigen was injected into rabbits Nos.1 and 2 according to the schedule of Harrison and Freeman, (1960). The antigens were injected via a marginal ear vein using a syringe fitted with a 25 gauge needle as follows: 0.5 ml, 0.5 ml, 1 ml, 1 ml, 2 ml, 2 ml at 3 day intervals, 5 ml 8 days later and a final injection of 3 ml after a further 12 days. Seven days after the last injection each rabbit was bled completely and the clotted blood was centrifuged at 4000 revolutions per

minute (r.p.m.) to separate antisera. These were preserved with equal volumes of glycerol and stored at 4°C.

To prepare the antisera using sonicated antigens, rabbits Nos 3 and 4 were injected intravenously on 1st, 4th, 8th 12th and 19th day with 1,2,3,4, and 5 ml of antigen respectively. The animals were bled on the 29th day and the antisera were prepared and preserved as indicated above. The antisera were labelled numerically in respect to the No. of rabbit from which they were prepared.

3:7:3 Determination of Antisera Titres

The agglutination tests were conducted to determine the titres of antisera prepared. The tests were carried out in Dreyer's tubes by mixing a dilution series of antiserum with a suspension of a 24 hr old culture of P. solanacearum. The dilution series of antiserum were prepared as follows:

Twelve Dreyer's tubes were arranged in a metal rack and numbered 1 to 12. Physiological saline (0.5 ml) was added to all tubes except tube No.1. Into tube 1 and 2, 0.5 ml of antisera was added. The dilution of antiserum in tube 2 was, therefore, $\frac{1}{2}$; after thorough mixing 0.5 ml was withdrawn from the tube 2 into tube 3, making the dilution of antiserum in tube 3 to be $\frac{1}{4}$. This was continued till a dilution of 1/1024 was obtained in tube 11. From this tube, 0.5 ml was withdrawn and discarded. Into tube 12, a control system, 0.5 ml of normal serum was added and after thorough mixing 0.5 ml was withdrawn and discared. Into each tube, 0.5 ml of a 24 hr old culture of

P. solanacearum suspended in physiological saline was added. The dilutions, therefore, doubled. Thus, the dilution in tube 1 was ½ and that in tube 11 was 1/2048.

The contents were well shaken and then incubated overnight in a water-bath at 52°C. The highest dilution which showed agglutination was recorded as the titre of the antiserum.

3:7:4 Tube Agglutination Tests

The objective of this experiment was to test the specificity and the laboratory use of the antisera prepared above.

Five Dreyer's tubes, in each row, were arranged in 4 rows in a metal rack. Into each tube in the first row, 0.5 ml of P. solanacearum suspended in physiological saline was added, Also into each tube in the 2nd, 3rd and 4th rows, suspensions of Corynebacterium michiganense, sap extracted from infected potato tubers and sap extracted from healthy potato tubers were added respectively.

The antisera prepared above were diluted in ½ with sterile physiological saline and then 0.5 ml of antisera labelled 1 was added into all tubes, in the 1st column. Into the 2nd, 3rd and 4th columns, 0.5 ml of antisera labelled 2,3 and 4 were added into each tube respectively. Into all tubes in the 5th column 0.5 ml of control normal serum was added. The contents were well mixed, incubated overnight at 52°C in a water-bath and then results of agglutinations were recorded.

3:7:5 Slide Agglutination Tests

The objective of these tests was to find out whether P. solanacearum in infected potato tubers could be detected by the antisera prepared above.

A clean grease-free microscope slide was divided into two sections with a grease pencil. Diseased potato tubers were cut transversely and then squeezed to extract tuber sap which was removed by use of a sterile wire-loop and placed on the two sections of the slide. A drop of antiserum labelled No 1 was added on to the sap on one portion of the slide while a drop of normal serum was added to the sap in the second portion of the slide to serve as a negative control. The contents were thoroughly mixed and let to stand at room temperature for 2-5 minutes and then the observations recorded. The procedure was repeated for antisera labelled Nos. 2,3 and 4.

Tuber sap extracted from healthy tubers was also tested in the same way and results recorded. This served as negative controls.

The specificity of the antisera Nos. 1,2,3 and 4 were tested using suspensions of P. solanacearum and Corynebacterium sp. The former bacterium served as a positive control while the latter served as a negative control.

To determine the highest dilution of the bacterium which could give observable agglutination reaction, a 24 hr culture of P. solanacearum was washed with physiological saline then placed

in a screw-capped bottle. After shaking for 2-3 min, the bacterial concentration in the suspension was adjusted to 10^9 cell/ml. This was serially diluted and agglutination tests performed on each dilution using all antisera. The results gave the highest dilution of the bacterium which could give observable agglutination reactions.

4. RESULTS

4:1 Survey Results

During the short-rainy and long-rainy seasons of 1980/81, a total of 138 farms were visited in Central, Eastern and Rift Valley Provinces and examined for the presence of bacterial wilt of potatoes. Among these, 29 farms were found infested with P. solanacearum. This accounted for 21% of the total number of the farms visited. By grouping the farms into different altitude zones (Table 2), it was found that high percentages of the infested farms lay between 1520 m and 2120 m above the sea level. The incidences of the disease decreased considerably at an altitude of 2420 m and above. The survey also indicated infection in the following potato cultivars: Roslin Eburu (B53), Kenya Akiba, Anett, Kerr's Pink, Roslin Tana, Roslin Gucha, Feldesiolin and Desiree.

The mean annual temperatures in Kenya are closely related to ground elevation. For any given locality the mean annual air temperature (T) can be calculated with reasonable precision from the formula:

$$T = 30.2 - 6.5 \times E \ (^{o}C)$$

Where E is the elevation above mean sea level in thousands of metres (Anonymous, 1970). When this formula was applied, it was possible to present the data in Table 2 in relation to temperature (Table 3). Results of this calculation indicated that areas with mean annual air temperature between

16.4°C and 20.3°C had high percentage of farms infested. The percentage of infested farms outside these temperatures declined.

Table 2: Incidence of bacterial wilt of potatoes in relation to altitude in Kenya

Altitude zone in metres	No. of plots examined	% of infested plots	Total area examined in Hectares	% acreage in severity category*		
				0	1	2
Above 2580	37	8.1	61.8	99.1	0.9	0
2420-2580	25	4.0	34.7	98.0	2.0	0
2270-2420	14	7.1	13.6	94.9	5.1	0
2120-2270	20	25.0	14.6	70.5	28.8	0.7
1970-2120	9	33.3	4.9	67.4	22.4	10.2
1820-1970	12	58.3	5.9	28.5	23.7	47.8
1670-1820	7	42.9	0.7	57.1	14.3	28.6
1520-1670	6	50.0	1.8	66.6	16.7	16.7
Below 1520	8	37.5	1.9	73.7	26.3	0

*Severity Category:

0 represents no plant wilting

1 " 1-5% plants wilting

2 " > 5% plants wilting

Table 3: Incidence of bacterial wilt of potatoes in relation to temperature in Kenya.

Temperature range in °C	No. of plots examined	% of infested plots	Total area examined in Hectares	% acreage in severity category*		
				0	1	2
Below 13.4	37	8.1	61.8	99.1	0.9	0
13.4 - 14.5	25	4.0	34.7	98.0	2.0	0
14.5 - 15.5	14	7.1	13.6	94.9	5.1	0
15.5 - 16.4	20	25.0	14.6	70.5	28.8	0.7
16.4 - 17.4	9	33.3	4.9	67.4	22.4	10.2
17.4 - 18.4	12	58.3	5.9	28,5	23.7	47.8
18.4 - 19.4	7	42.9	0.7	57.1	14.3	28.6
19.4 - 20.3	6	50.0	1.8	66.6	16.7	16.7
Above 20.3	8	37.5	1.9	73.7	26.3	0

*Severity Category:

0 represents no plants wilting

1 " 1-5% plants wilting

2 " > 5% plants wilting

4:1:1 Field symptoms of the disease

Symptoms on potatoes above ground level

In some plants, the whole hill was affected while in others one or two stems were involved. Foliage of affected plants had drooped and wilted without much change in colour. The laminae were rolled upwards and inwards from the margins. Some leaves, especially the lower ones showed slight yellowing. Plants which were not severely infected had one or two wilted leaves. These were plants in which infection had just started.

The stems of badly infected plants had shrivelled. When such stems were sectioned transversely, a brown discolouration of the vascular ring was observed. The stems of recently attacked plants were still standing upright and cross-sections did not reveal any discolouration of the vascular ring.

Symptoms of the disease on under ground parts of potatoes

Some of the potato tubers from diseased plants had no visible external symptoms of the disease. At the "eyes" or stolon end of the tubers there was a sticky exudate which mixed with the soil and adhered to the surface of the tubers. When the tubers were cut into halves, brown discolouration of the vascular bundles was revealed in some tubers. On applying slight pressure on the cut tubers, greyish-white bacterial slime oozed out of the vascular rings. In some cases putty-like bacterial mass came out of vascular rings on application of slight pressure to the cut tubers.

Symptoms of the disease on tomatoes and egg-plants

The infected tomato plants wilted suddenly. Distinct epinasty of the petioles prior to wilting were apparent if the onset of the disease was slow. Adventitious roots developed on the stems. Wilting symptoms appeared rapidly on egg-plants. Symptoms on the foliage were similar to those produced by scorching due to high temperatures.

From the samples which were collected during the survey, 33 isolates of P. solanacearum were obtained most of which were isolated from potatoes. The 34th isolate was a standard culture obtained from C.M.I. (Table 1). Isolate No.9 was obtained from egg-plants which were grown in Nairobi at about 1760 m above sea level while isolate No.14 was obtained from tomatoes which were grown in Yatta Scheme at an elevation of approximately 1000 m above sea level.

4:2 Identification of Pseudomonas solanacearum

4:2:1 Isolation and Storage

In some cases pure cultures were obtained with the first plate-streaking and there was no need of sub-cultures. Characteristic colonies of P. solanacearum appeared after 48 hr incubation at 31°C on Kelman's TZC agar plates. Pure cultures which were stored in small screw-capped bottles containing sterile water at room temperature (18 - 20°C), remained viable and virulent up to six months.

4:2:2 Cultural characteristics

The morphology of 48 hr cultures of all isolates (Table 1) incubated at 31°C on Kelman's TZC agar were observed and described (Table 4). The colonies were slightly raised, slimy and appeared creamy-white with pink-orange centres. They were irregular in shape, and 2-5 mm in diameter. However, there were also smaller and larger colonies than the ones described above. The colonies were fluidal in their consistency. The bacterium was strictly aerobic, non-fluorescing and non-fastidious. Thus, it could grow on synthetic medium containing glucose and an inorganic nitrogen source such as ammonium chloride. The cells were gram-negative, non-sporulating, straight rods with rounded ends and 1.2 - 1.7 x 0.5 - 0.6 µm in size.

4:2:3 Biochemical characteristics

The pathogen oxidized the following carbohydrates: glucose, sucrose, maltose, lactose and cellobiose. Sorbitol, dulcitol and mannitol were not oxidized. The latter are alcohols. Nitrate was reduced to nitrite but denitrification did not occur. The bacterium was catalase and oxidase positive, argenine dihydrolase negative and did not produce hydrogen sulphide or hydrolyse starch (Table 5).

According to Bergey's Manual of Determinative Bacteriology (1974) the pathogen was classified and placed in the genus Pseudomonas solanacearum E.F. Smith.

Table 4: Colony Characteristics of *Pseudomonas solanacearum* on Kelman's TZC medium after 48 hours incubation at 31°C

Isolate number	Colony shape	Colony size in mm	Colour	Consistency	Formazan formation
1 - 8 and 10 - 34	irregular, smooth slime deposition, entire edges, raised smaller colonies were somewhat round	2 - 5	Creamy white	Fluidal	Pink formazan deposit at the centre
9	irregular, smooth slime deposition, entire edges, raised	2 - 4	creamy white	Fluidal	Pink intense deposition toward centre

Table 5: Some biochemical characteristics of 34 isolates of P. solanacearum collected from potato growing areas in Kenya

Isolate No.	Salicin	Glucose	Sucrose	Maltose	Lactose	Cello-biose	Mannitol	Sorbitol	Dul-citol	Nitrite from Nitrate	Gas from Nitrate	Oxi-dase Test	Catalase Activity	H_2S Test	Arginine Dihydrolase	Race	Bio-type
1	-	+	+	+	+	+	-	-	-	+	-	+	+	-	-	3	II
2	-	+	+	+	+	+	-	-	-	+	-	+	+	-	-	3	II
3	-	+	+	+	+	+	-	-	-	+	-	+	+	-	-	3	II
4	-	+	+	+	+	+	-	-	-	+	-	+	+	-	-	3	II
5	-	+	+	+	+	+	-	-	-	+	-	+	+	-	-	3	II
6	-	+	+	+	+	+	-	-	-	+	-	+	+	-	-	3	II
7	-	+	+	+	+	+	-	-	-	+	-	+	+	-	-	3	II
8	-	+	+	+	+	+	-	-	-	+	-	+	+	-	-	3	II
9	-	+	+	+	+	+	+	+	+	+	±	+	+	-	-	1	III
10	-	+	+	+	+	+	-	-	-	+	-	+	+	-	-	3	II
11	-	+	+	+	+	+	-	-	-	+	-	+	+	-	-	3	II
12	-	+	+	+	+	+	-	-	-	+	-	+	+	-	-	3	II
13	-	+	+	+	+	+	-	-	-	+	-	+	+	-	-	3	II
14	-	+	+	+	+	+	-	-	-	+	-	+	+	-	-	3	II
15	-	+	+	+	+	+	-	-	-	+	-	+	+	-	-	3	II
16	-	+	+	+	+	+	-	-	-	+	-	+	+	-	-	3	II
17	-	+	+	+	+	+	-	-	-	+	-	+	+	-	-	3	II
18	-	+	+	+	+	+	-	-	-	+	-	+	+	-	-	3	II
19	-	+	+	+	+	+	-	-	-	+	-	+	+	-	-	3	II
20	-	+	+	+	+	+	-	-	-	+	-	+	+	-	-	3	II
21	-	+	+	+	+	+	-	-	-	+	-	+	+	-	-	3	II
22	-	+	+	+	+	+	-	-	-	+	-	+	+	-	-	3	II
23	-	+	+	+	+	+	-	-	-	+	-	+	+	-	-	3	II
24	-	+	+	+	+	+	-	-	-	+	-	+	+	-	-	3	II
25	-	+	+	+	+	+	-	-	-	+	-	+	+	-	-	3	II
26	-	+	+	+	+	+	-	-	-	+	-	+	+	-	-	3	II
27	-	+	+	+	+	+	-	-	-	+	-	+	+	-	-	3	II
28	-	+	+	+	+	+	-	-	-	+	-	+	+	-	-	3	II
29	-	+	+	+	+	+	-	-	-	+	-	+	+	-	-	3	II
30	-	+	+	+	+	+	-	-	-	+	-	+	+	-	-	3	II
31	-	+	+	+	+	+	-	-	-	+	-	+	+	-	-	3	II
32	-	+	+	+	+	+	-	-	-	+	-	+	+	-	-	3	II
33	-	+	+	+	+	+	-	-	-	+	-	+	+	-	-	3	II
34	-	+	+	+	+	+	-	-	-	+	-	+	+	-	-	3	II

KEY: + represents positive reaction
- " negative "

4:3 Biochemical types and Races of *Pseudomonas solanacearum* isolated

Buddenhagen, *et al.*, (1962) grouped *P. solanacearum* into 3 races. The species has also been divided into four biochemical types by Hayward (1964) based mainly on acid formation from sugars and denitrification. Based on these characteristics, 33 of the isolates collected from different parts of Kenya including the one from C.M.I. (Table 1), gave characteristic reactions of biochemical type II, or race 3 of Buddenhagen *et al.*, (1962). Only one isolate was characterized as biochemical type III or race 1 of Buddenhagen *et al.*, (1962). The biochemical types were differentiated on the basis of utilization of carbohydrates and denitrification. Thus, biochemical type II oxidized all carbohydrates tested except Dulcitol, Mannitol and Sorbitol and the type did not produce gas from nitrates. Biochemical type III oxidized all the carbohydrates and produced some gas from nitrate (Table 5). Colony characteristics as a means of differentiating races of *P. solanacearum* are shown in Table 4.

The reactions of the tobacco leaf infiltration test showed that there were two races of *P. solanacearum* in Kenya. Isolate No.9 was the only one which gave a different reaction from the rest. The reaction it gave appeared about 40 hr after the leaf had been infiltrated. There was a brownish necrotic reaction in the area infiltrated. A yellow zone surrounded the brownish necrotic area forming a halo (plate No.1). The reaction resembled the one described by Lozano and Sequeira, (1970) for *P.*

Plate 1: The reaction of Nicotiana tabacum cv. White burley 40 hr after being infiltrated with a suspension of Pseudomonas solanacearum biochemical type III.

Plate 2: The reaction of Nicotiana tabacum cv White burley 48 hr after being infiltrated with a suspension of Pseudomonas solanacearum type II

solanacearum biochemical type III or race 1. The other isolates showed yellowish discolouration of the infiltrated areas, 48 hr after infiltration of the leaves. The size and colour of the lesion did not increase or change after 96 hr (Plate No.2). These reactions resembled those described by Lozano and Sequeira, (1970) for P. solanacearum biochemical type II or race 3 of Buddenhagen et al., (1962). The inoculated leaves of control plants remained symptomless until the experiment was discontinued 2 weeks after infiltration.

4:4 Pathogenicity of Pseudomonas solanacearum on Potatoes and Egg-plant

All isolates except isolate No.9 (Table 1) caused wilt symptoms on potato plants 9-11 days after inoculation and the bacterium was recovered from the infected plants. P. solanacearum was not re-isolated from potato plants inoculated with isolate No.9 (biochemical type III race 1).

There were no bacterial wilt symptoms in the following cultivars of potatoes which were inoculated with the culture of isolate No.9: Kerr's Pink, Anett, Roslin Eburu (B53), Kenya Baraka, Desiree, Feldeslolin, Roslin Tana and Roslin Gucha.

All the isolates (Table 1) including isolate No.9 were pathogenic to egg-plant. Such plants wilted 5-12 days after inoculation and the bacterium was re-isolated from them (Plate No.3).

Plate 3: Egg-plants showing wilting symptoms after inoculation with Pseudomonas solanacearum biochemical type II and III.

4:5 Temperature

4:5:1 Influence of Temperature on *in vitro* Growth of *Pseudomonas solanacearum*

The minimum temperature for growth of *P. solanacearum* biochemical type II was found to be 13°C. Growth was not obtained at 12°C. It was also found that the maximum temperature for growth was 35°C and no growth was obtained at 37°C (Table 6). The optimum temperature for biochemical type II was found to be 31°C, while that of biochemical type III was 30°C (Tables 6 and 7).

The region of increased multiplication rate as shown by the relationship of doubling time and temperature (Table 6) was between 25°C and 31°C. This region also represented the temperatures at which generation time was lowest. This pattern of the influence of temperature on biochemical type II was similar to that of biochemical type III (Table 7). Figure 1 is a plot of growth/temperature relationship of both *P. solanacearum* biochemical type II and III.

4:5:2 Influence of Temperature on *in vivo* Growth of *Pseudomonas solanacearum*

April/May and June/July 1981 periods were cooler than November/December 1980 and February/March 1981 periods (Table 8). The average number of days taken before disease symptoms appeared in inoculated plants increased as the bacterial concentration in

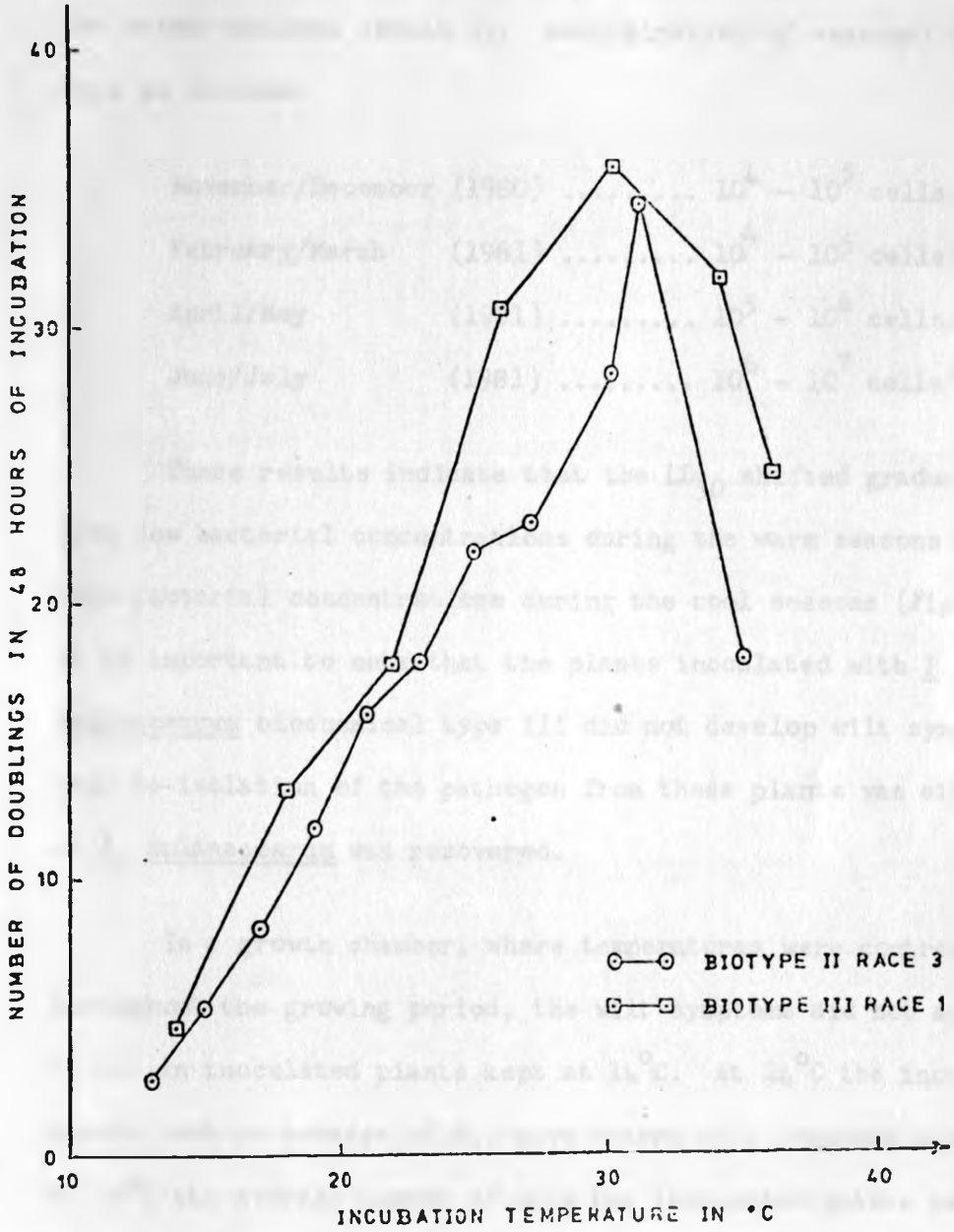
Table 6: The effect of temperature on growth of Pseudomonas solanacearum biochemical type II race 3 on Kelman's TZC medium

Temperarute ^{o}C	Number of cells/ colony after 48 hours	Generation Time in hours	Number of doub-lings after 48 hours
12	No growth	-	-
13	6	17.9	2.7
15	39	9.0	5.3
17	316	5.8	8.3
19	3,300	4.1	11.7
21	65,500	3.0	16.0
23	228,200	2.7	17.8
25	3,651,400	2.2	21.8
27	7,826,900	2.1	22.9
30	308,353,000	1.7	28.2
31	21,151,100,000	1.4	34.3
35	228,200	2.7	17.8
37	No growth	-	-

Table 7: The effect of Temperature on Growth of *Pseudomonas solanacearum* Biochemical type III race 1 on Kelman's TZC medium

Temperature °C	Number of cells/ colony after 48 hours	Generation Time in hours	Number of doublings after 48 hours
	No growth	-	-
14	24	10.56	4.6
18	10,100	3.60	13.3
22	244,600	2.68	17.9
26	1,627,500,000	1.57	30.7
30	52,080,100,000	1.35	35.6
34	3,255,000,000	1.52	31.6
36	25,429,700	1.95	24.6
38	No growth	-	-

Fig. 1: Growth Temperature Relationship of Pseudomonas solanacearum on Kelman's Medium.



the inocula used decreased (Table 9). The average number of days taken before disease symptoms appeared on plants for each concentration was higher during the cooler seasons than during the warmer seasons (Table 9). Determination of seasonal LD_{50} were as follows:

November/December	(1980)	$10^4 - 10^5$ cells/ml
February/March	(1981)	$10^4 - 10^5$ cells/ml
April/May	(1981)	$10^5 - 10^6$ cells/ml
June/July	(1981)	$10^6 - 10^7$ cells/ml

These results indicate that the LD_{50} shifted gradually from low bacterial concentrations during the warm seasons to high bacterial concentrations during the cool seasons (Fig.2). It is important to note that the plants inoculated with P. solanacearum biochemical type III did not develop wilt symptoms. When re-isolation of the pathogen from these plants was attempted, no P. solanacearum was recovered.

In a growth chamber, where temperatures were controlled throughout the growing period, the wilt symptoms did not appear at all in inoculated plants kept at 14°C. At 24°C the inoculated plants took an average of 8.9 days before wilt symptoms appeared. At 30°C the average number of days the inoculated plants took to show wilt symptoms was higher than that at 24°C. This means that the inoculated plants took long to wilt at 30°C, very short time to wilt at 24°C while the ones placed at 14°C never wilted (Table 10). Fig.3 indicates that the optimum temperature for wilt development was around 24°C.

Table 8: The mean soil temperature in the greenhouse at 15cm deep at various times of the day during experimental seasons.

SEASONS	T I M E					AVERAGE
	9 AM	12 M	3 PM	8 PM	3 AM	
Nov./Dec. 1980	17.5	23.6	23.4	18.8	16.5	20.0
Feb./Mar. 1981	18.4	24.9	23.9	19.3	16.9	20.7
Apr./May 1981	16.8	20.1	21.4	18.7	16.6	18.7
June/July 1981	14.2	18.1	19.9	18.1	14.4	16.9

Key: Temperature recorded in ^{o}C.

Table 9: The average number of days taken for plants inoculated with different concentration of Pseudomonas solanacearum bio-chemical type II to wilt during different experimental seasons.

BACTERIAL CONCENTRATIONS (TREATMENTS)	AVERAGE NO. OF DAYS BEFORE DISEASE SYMPTOMS APPEARED			
	NOV/DEC.1980	FEB/MAR 1981	APR/MAY 1981	JUNE/JULY 1981
(Original Suspension) No. of cells/ml = 10^9	14.4	14.6	11.0	14.6
10^8	16.4	15.2	16.2	15.2
10^7	15.5	17.0	15.2	18.6
10^6	19.8	19.0	17.8	18.4
10^5	21.0	25.0	23.0	14.0
10^4	22.3	18.0	24.0	>30
10^3	>30	>30	>30	>30
0 (controls)	>30	>30	>30	>30

Key: > represents more than

Fig. 2: DETERMINATION OF LD 50 OF Pseudomonas solanacearum IN GLASS HOUSE INOCULATION EXPERIMENT DONE DURING THE FOUR SEASONS OF THE YEAR.

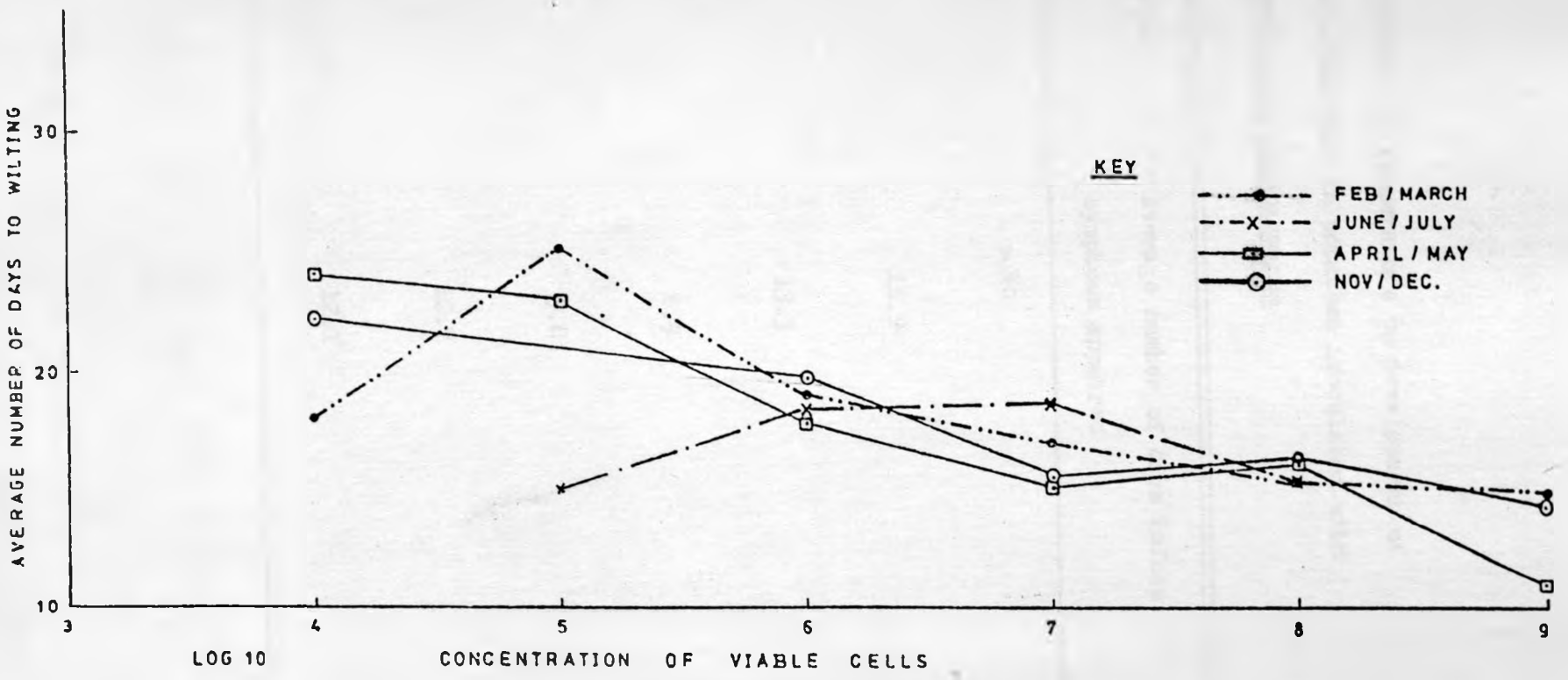
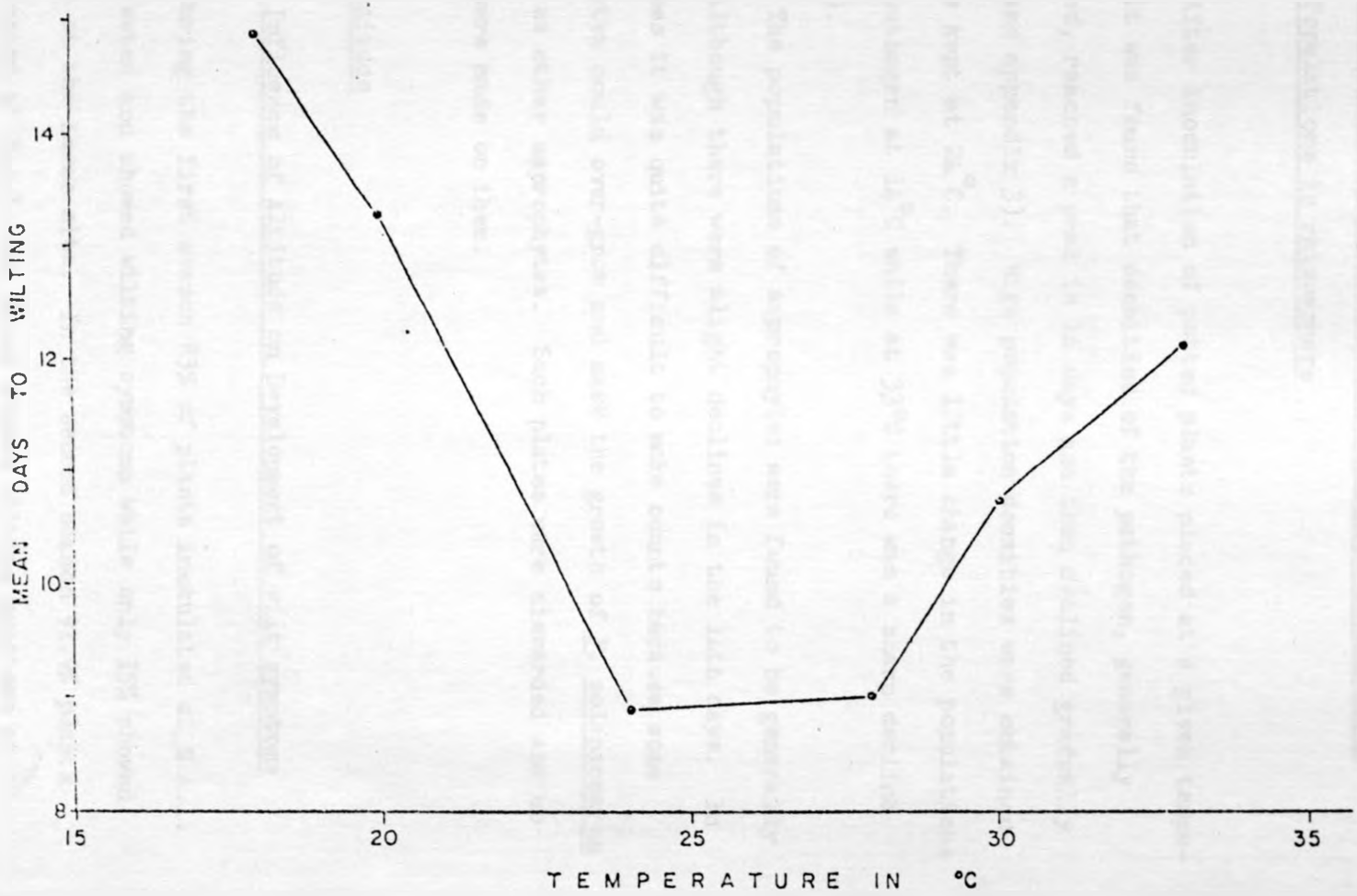


Table 10: Influence of temperature on development of bacterial wilt in potatoes inoculated with Pseudomonas solanacearum

Temperature in ^{o}C	Average number of days before symptoms appeared
14	>30
18	14.9
20	13.3
24	8.9
28	9.0
30	10.7
33	12.1

Fig. 3. Effect of Temperature on the Mean Days to Wilting of Potato Plants Inoculated with *P. solanacearum* at Various Temperatures.



4:5:3 Influence of Temperature on *Pseudomonas solanacearum* Populations in rhizosphere

After inoculation of potted plants placed at a given temperature it was found that densities of the pathogen, generally increased, reached a peak in 14 days and then declined gradually (Fig.4 and appendix 3). High population densities were obtained in soils kept at 24°C. There was little change in the populations of the pathogen at 14°C while at 33°C there was a sharp decline (Fig. 4).

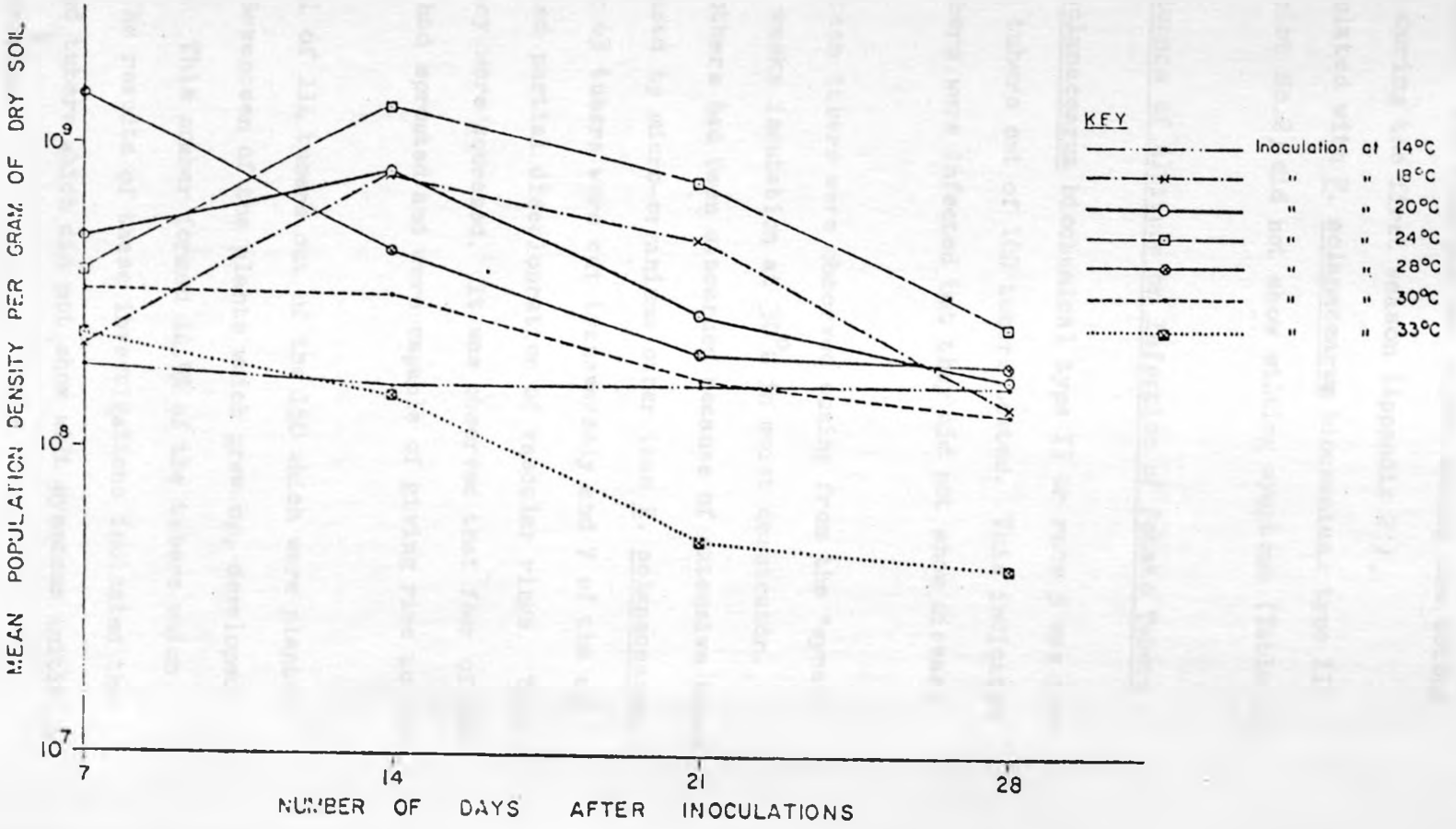
The populations of saprophytes were found to be generally stable although there were slight declines in the 14th days. In some cases it was quite difficult to make counts because some saprophytes could over-grow and mask the growth of *P. solanacearum* as well as other saprophytes. Such plates were discarded and no counts were made on them.

4:6 Altitude

4:6:1 Influence of Altitude on Development of wilt symptoms

During the first season 83% of plants inoculated at N.A.L. were infected and showed wilting symptoms while only 15% showed symptoms at the Kamae site. In the second season 91.9% plants were infected at N.A.L. and 26.4% showed wilting symptoms at Kamae (Table 11). This indicated that the percentage of infection was higher in low altitudes than at high altitudes. The difference of percentage infection in the two seasons could be attributed to differences in rainfall and temperatures during

Fig. 4: Population Density Trends of Pseudomonas solanacearum after Inoculations at Various Temperatures.



the two seasons. The rainfall was higher during the second season than during the first season (Appendix 2).
Plants inoculated with P. solanacearum biochemical type III (Plants in plot No.2) did not show wilting symptoms (Table 11).

4:6:2 Influence of Altitude on Infection of Potato Tubers

P. solanacearum biochemical type II or race 3 was isolated from 3 tubers out of 100 tubers tested. This indicated that some tubers were infected but they did not show disease symptoms.

Thirteen tubers were observed oozing from the "eyes" during the 4 weeks incubation at 30°C in moist dessicator. Twenty-four others had been discarded because of extensive tuber breakdown caused by micro-organisms other than P. solanacearum. The remaining 63 tubers were cut transversely and 7 of the cut tubers revealed partial discolouration of vascular rings. They oozed when they were squeezed. It was observed that four of the seven tubers had sprouted and were capable of giving rise to new plants.

A total of 114 tubers out of the 150 which were planted, germinated. Seventeen of the plants which grew up, developed wilt symptoms. This number formed 14.9% of the tubers which germinated. The results of these investigations indicated that some plants and tubers which did not show wilt symptoms initially had latent infection.

Table 11: Infection percentage of potato by Pseudomonas solanacearum at Kamae and National Agricultural Laboratories during experimental seasons

SEASON	SITE	PLOT NO. 1			PLOT NO. 2			PLOT NO. 3		
		No. of plants inoculated	No. of plants wilted	% infection	No of plants inoculated	No. of plants wilted	% Infection	No. of plants inoculated	No. of plants wilted	% Infection
1st Season	N.A.L.	100	83	83	98	0	0	100	0	0
2nd Season		99	91	91.9	96	0	0	100	0	0
1st Season	KAMAE	92	14	15.2	83	0	0	86	0	0
2nd Season		87	23	26.4	89	0	0	91	0	0

Plot 1 : Plants in this plot were inoculated with biochemical type II

Plot 2 : Plants in this plot were inoculated with biochemical type III

Plot 3 : Plants in this plot were inoculated with sterile distilled water to serve as controls.

4:7 Serology

4:7:1 Antisera Titres

The antisera titres were determined by tube agglutination method and are shown in Table 12. Rabbits No.1 and 2 produced antisera with 1/1024 and 1/512 titres respectively. Rabbits No.3 and 4 both produced antisera with 1/128 titre.

4:7:2 Use And Specificity of The Antisera

The results of tube agglutination tests are shown in Table 13. No antisera gave serological reaction to Corynebacterium michiganense and the sap extracted from healthy potato tubers. These two were included in the agglutination tests to serve as negative controls. Bacterial suspension of P. solanacearum and sap extracted from infected potato tubers gave homologous serological reactions against all the antisera. The normal serum gave no reaction. The same pattern of results was obtained when slide agglutination tests were used (Table 13).

The highest bacterial dilution to give observable agglutination reaction was 10^6 cells/ml (Table 14).

Table 12: Titres of antisera produced in rabbits injected with *Pseudomonas solanacearum* biotype II

Antiserum Number	Antiserum titre
1	1/1024
2	1/512
3	1/128
4	1/128

Table 13:

Reaction of Agglutination tests for *Corynebacterium michiganense* and *Pseudomonas solanacearum* biotype II against *P. solanacearum* biotype II antisera

Bacterial cultures and sap extracts from potato tubers	Antisera number				Normal serum
	1	2	3	4	
Corynebacterium michiganense	-	-	-	-	-
Pseudomonas solanacearum biotype II	+	+	+	+	-
Sap extracted from infected potato tubers	+	+	+	+	-
Sap extracted from healthy potato tubers	-	-	-	-	-

Table 14:

Agglutination reactions of serial dilutions of Pseudomonas solanacearum biochemical type II vegetative cells against P. solanacearum antisera prepared from four rabbits.

Bacterial dilutions	Antisera				Normal serum
	1	2	3	4	
10^9 cells/ml	+	+	+	+	-
10^8 cells/ml	+	+	+	+	-
10^7 cells/ml	+	+	+	+	-
10^6 cells/ml	-	-	-	±	-
10^5 cells/ml	-	-		-	-

5. DISCUSSION AND CONCLUSIONS

In Kenya, potatoes (Solanum tuberosum L), are grown throughout a wide agroclimatic belt extending from 1200 m up to 2800 m above sea level (Harris, 1975). Results of the survey on bacterial wilt, carried out during 1980/81, covering the potato growing zone, showed that at elevations below 1520 m the disease was less serious. Between 1520 and 2270 m above sea level, the incidences of the disease were very high while above 2270 m the disease decreased with the increase in altitude. Above 2580 m the disease occurence was rare (Table 2). The same trend of the disease occurence was observed by Harris in 1976.

When temperature ranges were substituted for altitude zones, it was observed that bacterial wilt was severe in areas with temperatures of about 18°C (Table 3). The incidence of occurence of bacterial wilt decreased with decrease in temperature. Remarkable decrease in the disease incidences was observed in areas with mean annual air temperature of 15.5°C. These temperatures have been found to be limiting in wilt development. The optimum temperature for the disease development is 21°C (Harris, 1976). The disease distribution, therefore, is obviously not as a result of direct effect of altitude but reflects the influence of correlated climatic factors such as temperature and rainfall. The reason why wilt declines in lower altitudes where soil temperatures are high, however, is not known but it is possible that other factors such as reduced rainfall become limiting (Harris, 1976).

Based on the survey results and earlier investigations by other workers such as Harris, it could be suggested that potato seed tubers should be produced in areas which have mean annual soil temperatures of 15°C and below. At this temperature, wilt does not develop and, therefore, there are high chances of producing clean seed in order to eliminate dissemination of the disease pathogen. Areas with an altitude of 2270 m and above meet the requirements. There are many areas in potato growing areas in Kenya which can be used for seed production. The following areas are suitable: Kibirichia in Meru District, most of Nyandarua District and Molo area in Rift Valley Province.

The wilt disease symptoms in the field were found to be satisfactory in the identification of the diseased potato plants. Plants at various stages of disease development were observed. Most of the affected plants retained the green colour after wilting. Most of the farmers could recognize the diseased plants in the field and a good number of them knew the consequences of the disease. Such farmers knew the steps to take to control it.

The isolates collected during the surveys showed the same characteristics as those described by Kelman (1954), for Pseudomonas solanacearum on Kelman's TZC agar (Table 4). The clear cut correlation between the characteristic colony morphology on Kelman's TZC agar and pathogenicity, made it possible to identify the bacterium on colony morphology alone. Following the classification according to the Bergey's Manual of Determinative Bacteriology, the bacterium was placed in the nomenspecies

Pseudomonas solanacearum E.F. Smith.

Buddenhagen, et al., (1962) grouped P. solanacearum into three races. The species has also been divided into four biochemical types by Hayward (1964) on the basis of acid formation during the oxidation of some carbohydrates and denitrification. Based on these criteria, reactions of tobacco leaf infiltration tests and the differentiation of races and strains of P. solanacearum on Kelman's TZC medium by the method of French et al., (1970), it was found that 33 of the isolates shown in Table 1 were P. solanacearum biochemical type II or race 3 of Buddenhagen et al., (1962), and only one isolate was biochemical type III or race 1. Biochemical type II isolates were isolated from potato (S. tuberosum) and tomato (Lycopersicon esculentum Mill), and the biochemical type III was isolated from egg-plant (S. melongena).

Previous work in Kenya on P. solanacearum by Nattrass (1945 and 1946), Robinson and Ramos (1964) and Harris (1976) showed that there were only two races of the pathogen present in the country. These workers reported the presence of race 1 and race 3. They found race 3 in high altitude areas and it attacked potatoes, tomatoes and egg-plants. This race has also been found in the Coast Province at Taita-Taveta hills. Although Taita hills are in the Coast Province, the area is 1000 - 1500 m above sea level and potatoes and other solanaceous plants are cultivated. Some infected potato tubers might have been taken there and that might be the reason why biochemical type II of the pathogen is found at the coast. For the same reason the pathogen is found in Yatta Scheme in Eastern Province which is at about 1000 m above sea

level. Race 1 is mainly found in low altitudes and it attacks egg-plants, bringals, pepper and other solanaceous species. At high altitudes this race is rare or absent.

In this work, only these two races were found present in the country. This confirmed the previous findings. It was also found that potatoes were attacked by race 3 only. This specificity was verified by the fact that the isolates collected from the field during the survey (Table 1) were from different potato cultivars grown in Kenya and all of them were infected with P. solanacearum biochemical type II or race 3 of Buddenhagen et al., (1962) (Table 5). The cultivars from which the pathogen was isolated are Roslin Eburu (B 53), Roslin Tana, Roslin Gucha, Kenya Akiba, Anett, Kerr's Pink, Desiree, Kenya Baraka and Feldeslolin.

Biochemical type III or race 1 was not isolated from potatoes. When the nine potato cultivars above were inoculated with the bacterium, they did not develop the disease symptoms and the bacterium was not recovered from the plants in re-isolation experiments. This indicated that the biochemical type III strain found in Kenya does not attack potatoes.

The in vitro growth rate of P. solanacearum was highest at 31°C and declined to zero at 12°C and 37°C (Table 6 and 7). Fig. 1 shows the curves obtained from plots of number of doublings in 48 hr incubation of P. solanacearum against incubation temperatures. The number of doublings were highest at the incubation temperature of 31°C and lowest at 13°C. This corresponds to the

findings of the growth rate shown here.

Temperature change frequently produces prompt, direct and proportional alteration in the rate of biological processes such as enzyme reactions, respiration and others. At temperatures not far outside the normal range (e.g. 37°C for mammals), biological processes are retarded or completely inhibited. This phenomenon of the effect of temperature on biological processes was clearly demonstrated by P. solanacearum on Kelman's TZC medium. It is, therefore, thought that such in vitro observations reveal the role of temperature in the development of bacterial wilt in nature. Thus, the growth rate of the bacterium is retarded or completely inhibited at 12°C and 37°C and therefore, disease does not develop, while at around the optimum temperatures the growth rate is high and therefore, the bacterial populations quickly reach the magnitude at which the disease development is faster.

At soil temperatures of around 14°C in vivo experiments, wilt did not develop while it developed at the soil temperatures around 24°C (Table 10 and Fig.3) and it declined at higher temperatures. Harris (1976) indicated that the ceiling for wilt corresponded with temperatures around 15°C and the optimum of about 21°C. The results of this work indicated that the bacterial growth rate was inhibited at 14-15°C and it was highest at 21-24°C.

It was found that the concentration of the viable cells needed for wilt development depended on temperature. Results in Tables 8 and 9 and Fig.2 indicate that cool seasons such as April/May and June/July require high concentration of inoculum to cause

wilt than warm seasons such as November/December and February/ March. These findings could be explained as being due to high rate of growth of the pathogen at optimum temperatures and low rates of growth at temperatures outside the optimum.

P. solanacearum survived best in soil at 24°C followed by 20°C and 18°C. At these temperatures the population densities in the rhizosphere increased to a peak at the 14th day and then declined slightly. The concentration of bacteria remained constant for 28 days at 14°C soil temperature. At 28,30 and 33°C the concentration of bacteria declined (Fig. 4). These results can be explained as follows: At the optimum temperatures (18-24°C) the bacterial growth rate was high and the infected tubers as well as roots produced bacterial ooze which mixed with the soil thus increasing the population. At 14°C the bacterial growth was inhibited while at 28,30 and 33°C, the populations declined because the bacterial death rate was higher than the growth rate. The results show the same trend of P. solanacearum population density study carried out by Ramos (1976). Ramos found that densities of both biochemical types increased reaching a peak in 3-5 days, and then sharply declining for biotype II and slowly for biotype III. The differences in days at which the populations reached the peak in the two experiments might have been due to the differences in the experimental methods. Ramos carried out the bacterial population studies on bare soil columns and field plots devoid of host covers while in this work the studies were carried out using soil on which potato plants were growing. It can be concluded that population density and survival of P. solanacearum in soil are influe-

nced by soil temperature and that the soil temperature in which P. solanacearum survives best is from 18°C to 24°C.. Under such conditions wilt development is expected to be most rapid.

At low temperatures potato plants may be latently infected and therefore symptom expression fails. This phenomenon occurred at Kamae (Table 11). It means that the soil temperatures at Kamae did not allow the bacterial population to build up to the magnitude that would cause the disease symptoms to appear, although the plants were infected. The latent infection was, however, revealed when potato tubers, obtained from Kamae experimental plot No. 1 during the two seasons, showed disease symptoms under favourable conditions. Thus, 3% of tubers obtained from apparently healthy plants were found infected with P. solanacearum during the isolation experiments. Twenty per cent of externally symptomless tubers kept under conditions of high humidity and at 30°C exuded bacterial ooze from the eyes or had their vascular rings discoloured. Latent carriers of P. solanacearum regenerated as healthy tubers do but produced heavily infected plants. About 14.9% of plants raised from externally symptomless tubers wilted under greenhouse conditions.

It is thought that the low soil temperatures at Kamae caused some plants to be latently infected. When the tubers of these plants were grown under favourable conditions, they produced infected plants. Latently infected tubers may cause serious disease outbreaks in farms where the pathogen was previously absent. The danger of using latently infected tubers as seed can be overcome by screening potato seed lots before planting.

Results of serological experiments indicate that P. solacearum is a good immunogen which can be used in antiserum production and can be detected in homologous reactions. The difference in antisera titres of rabbits No.1 and 2 which received heat treated antigens, suggest that there occured different serological responses in the test animals (Table 12).

Cross-linking of Multivalent protein antigens by antibody leads to precipitation whereas cross-linking of cells of large particles by antibody directed against surface antigens leads to agglutination. In this work agglutination reactions were observed since bacterial cells were used. Most cells are electrically charged, therefore, a reasonable number of antibody links between two cells before the mutual repulsion is overcome. It was found that maximum agglutination for this serological system occured at antisera dilution of 1/4.

Tube and slide agglutination test results indicated that P. solanacearum is not closely related to Corynebacterium michiganense. The observation indicate that there exists potential value of serology in identifying P. solanacearum rapidly in the laboratory. This method can, therefore, be used for quick screening of potato seed lots for the presence of P. solanacearum before use. It was found that the antisera prepared could pick P. solanacearum at a 10^6 cells/ml dilution. This means that if sap extracted from the infected material has 10^6 cell/ml, then they can be detected serologically.

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7.

A P P E N D I C E S

A P P E N D I X 1

FORMULAE OF MEDIA AND CHEMICAL SOLUTIONS

1. Formula for preparation of Kelman's tetrazolium chloride (TZC) medium

(a)	Peptone	10 g
	Glucose	10 g
	Casein hydrolysate	1 g
	Distilled Water	0.5 L
(b)	Agar No. 3	10 g
	Distilled water	0.5 L

(c) 1% solution of Tetrazolium chloride:

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

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

2. Peptone Water

Peptone	10 g
Sodium chloride (Nacl)	5 g
Water	1000 ml

Adjust pH to 8.0-8.4 and boil to dissolve. Filter and adjust pH to 7.2-7.4. Sterilize at 1 atmosphere for 15 minutes.

3. Physiological Saline

Sodium Chloride (Nacl)	0.85 g
Distilled water	100 ml

Dissolve and sterilize at 1 atmosphere pressure for 15 minutes.

4. Bromothymol Blue

Bromothymol blue	0.75 g
Alcohol (95%)	12.5 ml
Distilled water	37.5 ml

Dissolve the bromothymol blue in alcohol and then make up to required volume with distilled water.

5. Biochemical basal medium

$NH_4H_2PO_4$	1 g
Kcl	0.2 g
$MgSO_4\ 7H_2O$	0.2 g
Peptone	1 g
Bromothymol blue	10 - 20 ml
Distilled water	1 L

Adjust pH to 7 with 40% NaoH. Then add Agar 1.5 g. Dispense in 45 ml aliquotes in 50 ml flasks plugged with cotton wool. Sterilize at 1 atmosphere for 20 minutes.

6. Arginine broth

Tryptone	5 g
Yeast extract	5 g
K_2HPO_4	2 g

L-Arginine monohydrochloride	3 g
Dextrose	0.5 g
Distilled water	1 L

Dissolve by heating, adjust to pH 7, dispense in 5-10 ml amounts and sterilize at 1 atmosphere for 10 minutes.

7. Nitrate reduction test medium

Potassium nitrate, KNO_3 (nitrite free)	0.2 g
Peptone	5.0 g
Distilled Water	1 L

Dispense in 5 ml aliquots and sterilize at 121°C for 15 min.

Test reagents*

Solution A. Dissolve 8.0 g of Sulphanitic acid in 1 litre of 5N acetic acid.

Solution B. Dissolve 5.0 g. of α-naphthylamine in 1 litre of 5N acetic acid.

*Immediately before use, equal volumes of solutions A and B were mixed to give the test reagent.

Appendix 2

Meteorological data for Kimakia Station in South Kinangop near Kamne, from September 1980 to July 1981

DATE		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
SEPT. 1980	MAX. TEMP.	16.2	18.2	18.2	18.0	14.5	16.7	19.5	20.5	19.3	17.5	19.2	19.5	19.6	19.5	17.0	16.5	19.7	20.7	22.5	19.5	18.5	17.0	18.2	17.2	17.0	17.6	17.6	18.0	19	20	
	MIN. TEMP.	5.1	4.4	5.7	5.7	6.7	4.0	4.0	3.0	3.0	6.2	5.0	4.2	3.0	2.4	4.0	7.0	3.2	1.2	3.8	4.0	4.7	5.6	7.0	7.5	2.5	5.0	7.3	7.2	2.2	6.0	
	RAINFALL	Nil	Nil	Nil	8.0	1.0	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Trace	Nil	Nil	Nil	Nil	Nil	Nil	Nil	39.3	7.6	Nil	Nil	2.0	Nil	Nil	Nil	
OCT. 1980	MAX. TEMP.	18.0	19.5	18.7	20.5	18.2	12.2	17.0	19.0	19.2	19.7	18.5	18.6	18.5	17.5	18.1	19.5	21.0	21.0	19.0	18.5	19.0	20.0	20.1	20.3	20.0	20.0	20.0	18.6	17.0	17.0	19.0
	MIN. TEMP.	4.5	2.0	4.0	3.0	4.5	3.0	4.2	4.8	7.2	6.5	7.2	5.6	8.2	7.0	6.5	7.5	5.0	8.0	4.5	8.6	7.0	4.5	6.5	7.7	4.5	3.7	5.5	7.0	7.5	5.0	7.0
	RAINFALL	Nil	Nil	Nil	Nil	0.8	1.0	15.0	Nil	Nil	1.0	16.5	23.6	2.4	1.8	Nil	Nil	Nil	18.9	22.0	4.8	Nil	Nil	Nil	Nil	Nil	Nil	20.7	22.0	17.0	0.6	1.0
NOV. 1980	MAX. TEMP.	18.5	16.0	15.3	16.0	17.2	17.0	18.5	15.8	18.0	17.2	17.0	19.0	20.5	19.4	19.0	18.0	18.5	16.8	19.8	19.0	18.7	17.4	19.2	18.3	19.1	18.5	15.7	19.1	20.0	21.5	
	MIN. TEMP.	6.5	8.0	8.0	7.2	8.0	7.9	7.0	8.4	2.5	8.2	8.0	7.0	7.5	7.6	4.8	8.0	7.0	7.8	6.7	8.0	5.2	5.5	7.5		8.0	8.0	8.8	6.2	6.2	7.0	
	RAINFALL	16.0	29.5	27.1	5.2	18.7	1.4	35.0	3.6	16.8	0.6	43.3	15.0	1.93	14.7	28.0	10.0	33.2	2.8	0.7	5.8	11.0	5.6	3.0	9.5	1.0	Nil	7.6	Nil	Nil	Nil	
DEC. 1980	MAX. TEMP.	20.5	20.5	20.5	20.0	17.0	15.8	16.5	16.5	18.5	17.5	19.6	19.6	19.3	18.0	19.6	20.0	20.0	22.9	18.2	18.2	20.0	22.7	19.6	19.2	19.5	20.2	20.2	20.5	20.0	21.6	20.7
	MIN. TEMP.	5.0	5.0	5.4	10.0	10.5	11.0	10.3	10.1	10.5	10.0	5.5	7.6	10.5	9.8	8.8	6.4	9.0	10.6	2.0	4.2	6.5	6.0	7.8	6.8	9.2	10.0	6.7	4.0	6.1	4.0	5.7
	RAINFALL	Nil	Nil	Nil	25.0	19.0	2.3	8.2	3.8	Nil	Nil	12.0	1.0	Nil	Nil	Nil	3.0	9.3	12.3	0.5	Nil	2.8	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
JAN. 1981	MAX. TEMP.	20.0	19.7	19.5	16.0	19.5	18.3	19.2	19.5	19.3	20.0	19.9	20.2	21.0	21.0	20.0	20.5	19.5	19.5	21.6	21.0	21.5	21.0	20.5	21.8	21.0	22.2	20.5	21.8	22.4	22.6	19.6
	MIN. TEMP.	7.3	7.5	6.3	10.0	10.0	10.0	9.3	2.3	2.7	5.5	8.0	8.0	8.6	5.8	6.5	6.0	10.0	5.0	3.6	5.0	4.0	7.2	7.4	7.8	6.6	7.0	8.4	5.0	3.7	5.4	8.0
	RAINFALL	Nil	Nil	7.0	14.4	Nil	3.8	3.4	Nil	Nil	Nil	1.0	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	2.2	Nil	0.4	Nil	Nil	Nil	Nil	Nil	Nil	12.2	0.5
FEB. 1981	MAX. TEMP.	20.8	21.5	21.5	21.3	18.5	21.3	20.7	22.5	23.7	22.5	22.6	22.7	20.7	19.6	20.0	17.6	18.5	20.0	20.0	20.2	21.2	22.2	23.7	23.0	21.0	21.7	23.0	23.0			
	MIN. TEMP.	6.2	6.0	6.0	3.0	5.7	6.7	5.0	5.2	6.0	9.5	5.5	6.0	9.0	11.2	11.8	11.0	11.0	7.9	10.0	9.2	4.0	5.6	8.5	8.5	7.5	3.8	5.7	3.5			
	RAINFALL	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	13.5	15.5	15.5	8.4	2.0	10.8	8.4	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil			
MARCH 1981	MAX. TEMP.	22.6	20.7	21.5	22.5	22.0	21.0	20.2	20.7	21.0	21.5	19.5	19.0	20.0	19.2	19.2	21.5	19.5	18.7	18.8	18.5	19.3	19.5	20.0	20.3	21.5	18.3	18.0	18.0	19.5	16.5	19.0
	MIN. TEMP.	8.5	9.5	2.2	7.6	5.0	7.0	10.7	6.3	8.5	9.0	11.3	10.9	11.0	4.0	11.2	8.3	11.2	9.8	10.2	10.7	7.6	10.5	10.1	11.3	10.8	10.5	10.5	9.3	9.2	10.0	10.0
	RAINFALL	Nil	Nil	Nil	Nil	Nil	9.5	Nil	Nil	Nil	3.4	Nil	0.2	5.6	1.9	1.0	13.9	12.2	15.2	18.4	17.0	0.6	41.3	18.8	5.2	13.5	4.0	20.3	1.8	23.0	10.2	15.0
APRL 1981	MAX. TEMP.	19.5	18.3	19.0	20.0	19.0	18.0	20.0	21.6	19.5	19.3	19.0	18.5	18.5	19.0	18.5	16.8	16.5	16.5	18.2	17.6	18.5	18.6	20.0	19.5	18.0	16.5	18.0	19.0	19.2	21.2	
	MIN. TEMP.	9.3	10.0	9.6	8.2	9.2	7.0	6.2	6.0	7.6	9.0	10.0	11.0	9.9	10.0	5.0	11.5	11.7	11.5	10.0	9.3	7.4	5.5	6.0	11.2	9.0	11.4	9.8	7.5	9.0	4.9	
	RAINFALL	25.7	3.7	2.8	1.0	5.6	0.3	9.5	37.5	21.1	4.3	31.2	91.8	27.6	16.9	26.5	27.2	27.3	18.0	Nil	Nil	Nil	Nil	18.0	15.5	32.0	4.2	5.1	0.5	Nil	Nil	
MAY 1981	MAX. TEMP.	19.5	18.0	19.0	17.8	18.5	17.0	18.0	17.0	14.5	18.0	19.2	18.0	17.2	18.0	17.0	18.0	17.5	16.0	14.0	14.6	17.3	16.6	18.0	17.0	16.5	16.5	19.0	18.5	18.7	16.5	
	MIN. TEMP.	9.0	10.5	3.0	8.6	9.0	5.0	4.0	10.2	3.4	10.8	11.7	7.0	8.1	11.0	8.0	10.0	8.0	4.0	10.0	3.0	3.0	6.2	8.0	5.7	7.6	7.5	5.5	4.5	7.0	8.9	8
	RAINFALL	14.0	2.2	Nil	6.4	14.0	43.6	44.3	6.0	20.0	3.4	4.0	40.4	26.0	62.0	4.5	26.7	18.0	24.1	16.0	3.7	1.6	Nil	Nil	1.1	Nil	Nil	Nil	Nil	1.0	Nil	Nil
JUNE 1981	MAX. TEMP.	16.5	17.0	15.0	19.0	22.5	15.6	18.0	16.5	17.0	16.2	16.0	16.1	16.2	16.5	17.5	19.0	16.5	16.0	16.5	17.5	17.6	20.5	19.2	15.0	16.0	17.0	16.0	20.0	18.2	17.2	
	MIN. TEMP.	9.2	7.5	6.6	8.5	7.0	8.8	7.5	9.9	9.3	9.0	10.0	5.0	6.5	7.0	4.2	7.0	7.0	8.8	5.5	4.3	3.7	8.3	8.5	9.0	7.5	8.4	8.0	8.0	8.1	5.3	
	RAINFALL	Nil	Nil	0.5	Nil	2.0	2.0	4.4	10.3	1.0	2.1	Nil	Nil	Nil	Nil	Nil	0.6	Nil	Nil	Nil	Nil	1.0	Nil	2.0	0.3	Nil	5.8	11.1	13.4	4.7	Nil	
JULY 1981	MAX. TEMP.	18.0	18.0	16.0	16.0	17.0	17.5	15.0	18.8	15.6	15.2	13.6	15.2	13.2	15.6	15.0	15.8	17.0	13.5	14.3	15.0	15.1	12.8	14.0	16.8	15.2	13.0	11.7	12.5	15.1	14.8	15.5
	MIN. TEMP.	5.7	6.0	5.4	5.2	3.0	7.5	8.6	8.0	7.5	9.3	9.0	7.0	9.0	6.9	8.8	8.5	7.8	7.6	7.5	8.0	7.3	7.4	7.4	4.8	6.4	7.4	8.5	8.0	5.7	8.0	8.2
	RAINFALL	Nil	6.4	Nil	Nil	Nil	1.2	Nil	Nil	2.0	2.5	21.4	5.7	3.0	1.5	2.2	4.0	3.0	1.2	1.5	1.4	Nil	0.5	Nil	Nil	Nil	14.0	1.3	Nil	8.0	6.5	Nil

APPENDIX 2 Cont.

Meteorological data for National Agricultural Research Laboratories,
Nairobi, from September 1980 to July 1981

DATE		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
SEPT. 1980	MAX.TEMP.	23.0	24.5	24.5	24.5	21.0	23.0	26.5	24.0	26.0	23.0	25.5	25.0	26.5	26.5	21.5	24.0	27.0	26.0	27.0	26.5	24.0	24.5	24.0	23.0	24.0	25.0	24.5	25.0	25.2	26.3	
	MINI.TEMP.	12.0	11.5	13.5	13.0	12.8	9.5	10.5	10.0	10.0	12.0	13.0	12.5	11.5	11.0	11.5	12.0	12.5	11.0	12.0	12.5	12.5	13.0	13.3	13.4	12.5	12.5	12.5	13.0	12.0	15.2	
	RAINFALL	Nil	Nil	Nil	2.3	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	0.89	Nil	Nil	Nil	Nil	Nil	Nil	Nil	
OCT. 1980	MAX.TEMP.	24.5	26.0	25.2	26.5	26.5	26.5	26.0	25.0	26.0	26.0	27.0	26.0	24.5	25.0	25.0	26.0	27.0	28.0	27.5	26.0	26.0	26.0	27.0	27.0	27.5	27.0	27.0	26.0	23.5	24.5	25.0
	MINI.TEMP	16.0	17.2	17.0	10.2	18.2	14.8	10.5	14.5	13.5	13.0	13.5	13.5	13.5	14.5	14.0	13.5	13.5	15.0	15.5	14.5	14.2	13.8	13.6	13.0	14.5	13.2	14.5	15.0	14.0	17.5	11.5
	RAINFALL	Nil	Nil	Nil	Nil	Nil	Nil	Nil	8.4	Nil	Nil	Nil	8.3	Nil	2.4	Nil	Nil	Nil	0.3	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	6.6	Nil	Nil	Nil
NOV 1980	MAX.TEMP.	21.5	25.0	21.5	21.0	21.5	22.5	23.0	21.5	23.5	23.0	23.0	23.0	24.0	25.0	24.5	24.0	25.0	21.5	22.0	22.5	24.5	24.0	23.0	23.1	25.0	24.0	22.0	19.0	23.0	24.0	
	MINI.TEMP.	11.5	12.5	14.0	12.5	13.5	14.5	15.0	14.0	14.5	13.5	12.5	14.0	14.5	14.0	15.0	14.0	16.5	16.5	16.5	16.5	18.5	15.0	17.5	14.5	16.0	14.5	16.0	12.5	14.0	16.0	
	RAINFALL	Nil	Nil	12.0	23.0	10.16	Nil	28.0	5.2	2.6	Nil	4.0	Nil	15.0	1.7	Nil	48.3	18.5	Nil	2.0	1.0	24.0	5.8	5.3	1.0	Nil	2.4	9.3	Nil	Nil	Nil	
DEC 1980	MAX .TEMP.	24.5	23.5	23.5	23.0	23.0	20.0	22.5	20.5	23.0	22.5	24.0	24.0	23.5	22.5	22.5	23.5	24.6	23.0	22.0	23.0	23.5	24.5	23.5	23.5	23.5	23.5	24.0	24.0	24.0	25.0	25.0
	MINI.TEMP.	12.5	12.5	14.0	15.0	15.0	15.0	15.0	13.5	14.5	14.5	13.0	14.0	14.5	16.5	14.5	13.0	14.5	15.5	14.5	13.5	13.5	13.3	13.0	14.5	18.2	17.5	12.5	18.5	11.0	11.5	13.5
	RAINFALL	Nil	Nil	Nil	Nil	22.0	4.5	5.5	7.5	0.7	0.05	Nil	0.2	4.3	Nil	Nil	Nil	7.4	10.5	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
JAN. 1981	MAX.TEMP.	24.0	24.5	24.0	22.5	24.5	25.0	24.5	24.0	24.0	24.0	24.5	25.0	25.5	25.0	25.0	25.5	25.0	24.5	26.0	26.0	26.5	26.5	25.5	25.5	25.5	26.5	26.0	26.5	27.5	27.5	24.5
	MINI.TEMP	13.0	12.5	14.0	14.0	14.0	14.5	15.0	12.0	10.5	11.0	13.0	13.0	13.5	13.0	14.0	14.0	14.0	14.0	18.5	17.5	19.5	17.0	16.5	19.5	14.5	14.0	15.5	13.0	11.5	14.0	14.0
	RAINFALL	Nil	Nil	Nil	1.5	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	3.2	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
FEB 1981	MAX.TEMP	26.0	26.0	27.0	26.5	23.2	26.2	26.0	27.3	28.0	28.0	28.0	28.0	24.0	26.5	24.0	26.0	25.0	27.5	26.5	27.5	27.0	28.0	28.5	29.0	27.5	28.2	29.0	29.0			
	MINI.TEMP.	12.5	19.0	12.5	21.2	16.7	17.0	19.0	19.0	20.0	15	12	13.0	16.0	16.0	15.5	16.5	15.5	16.0	11.5	16.0	9.0	10.0	14.0	8.0	10.0						
	RAINFALL	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	2.5	Nil	Nil	1.7	0.5	0.4	0.3	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil			
MARCH 1981	MAX.TEMP	27.5	27.0	27.0	27.0	28.0	27.0	26.0	27.0	27.0	27.0	26.5	26.0	26.5	27.0	25.0	27.5	25.5	23.0	24.5	25.5	25.0	25.0	27.0	25.5	25.5	24.0	24.0	24.0	26.0	23.5	23.5
	MINI.TEMP.																															
	RAINFALL	Nil	Nil	Nil	Nil	29.0	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	7.5	2.8	8.4	5.0	6.0	Nil	7.3	1.5	4.5	15.5	5.8	Nil	5.2	3.6	2.0
APRIL 1981	MAX.TEMP	25.5	24.0	24.0	24.5	23.5	21.5	24.0	22.5	26.0	24.5	21.5	23.0	23.5	24.0	22.5	21.0	20.5	20.5	22.5	22.5	22.5	23.5	24.5	24.2	22.5	22.5	22.5	21.0	23.0	25.0	
	MINI.TEMP.																															
	RAINFALL	27.0	3.2	19.5	Nil	34.5	7.8	Nil	Nil	5.8	27.7	23.6	32.0	23.5	5.0	81.1	16.0	42.3	4.2	0.05	Nil	Nil	5.3	Nil	Nil	Nil	4.0	Nil	0.5	Nil	3.0	
MAY 1981	MAX.TEMP.	23.5	23.0	23.5	22.5	22.7	23.0	23.0	22.0	22.0	23.0	23.0	24.0	23.0	23.0	22.0	23.0	23.0	23.0	22.0	23.0	22.0	23.0	22.0	22.5	21.5	21.5	22.3	22.3	22.5	23.0	20.0
	MINI.TEMP.																															
	RAINFALL	0.1	3.6	1.8	2.7	0.2	8.7	20.5	34.3	32.8	Nil	Nil	1.3	20.0	13.3	0.7	7.5	Nil	Nil	6.5	4.0	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	7.0	Nil
JUNE 1981	MAX.TEMP.	20.5	22.5	21.5	22.0	22.0	22.5	22.2	22.	22.2	21.2	21.5	22.0	21.5	22.0	22.5	24.0	21.5	22.5	20.5	23.5	23.0	24.0	24.5	21.5	22.5	22.5	20.5	22.0	23.0	22.0	
	MINI.TEMP										12.0	14.5	20.5	16.0	11.0	9.5	12.5	12.5	12.5	10.0	9.0	8.5	11.0	10.5	12.5	10.0	9.5	11.5	10.0	13.0	11.5	
	RAINFALL	0.5	Nil	Nil	Nil	1.9	Nil	1.5	Nil	Nil	1.3	0.7	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	1.8	Nil	Nil	2.0	Nil	
JULY 1981	MAX.TEMP.	21.0	22.0	21.0	21.0	21.5	19.5	20.5	24.0	21.0	21.0	13.5	20.5	17.5	21.5	19.5	13.0	21.5	16.0	18.0	16.5	20.5	19.0	17.5	23.5	21.0	21.5	18.0	18.0	22.5	21.5	20.0
	MINI.TEMP	9.0	9.0	8.0	3.0	9.5	10.0	12.0	12.0	12.2	12.0	11.0	12.5	12.5	12.0	12.5	12.0	10.0	12.0	11.5	10.5	9.5	10.0	9.5	7.5	8.0	10.0	9.5	12.5	15.0	14.0	12.0
	RAINFALL	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	2.5	1.5	Nil	3.0	Nil	Nil	Nil	Nil	Trace	Nil	Nil	Trace	Trac.	1.1	Nil	Nil	Nil	Nil	Nil	Nil

Appendix 3

Population density trends of Pseudomonas solanacearum around the rhizosphere of inoculated potted Potato plants at seven soil temperatures.

TEMPERATURE in °C	Mean time to wilting (in days)		P.solanacearum/g of soil at day 7	14	21	28	saprophytic bacteria /g of soil at day 7	14	21	28
14	∝	Total	9.9990×10^{13}	1.860×10^{9}	2.0130×10^{9}	1.9770×10^{9}	2.630×10^{9}	1.4040×10^{9}	3.520×10^{9}	4.160×10^{9}
		Mean	2.722×10^{8}	2.0666×10^{8}	2.236×10^{8}	2.1966×10^{8}	2.922×10^{8}	1.56×10^{8}	3.9111×10^{8}	4.622×10^{8}
		SD	4.5215×10^{7}	8.8317×10^{7}	1.43×10^{8}	1.6879×10^{8}	3.4197×10^{8}	1.1374×10^{8}	1.735×10^{8}	1.85×10^{8}
18	14.9	Total	3.110×10^{9}	8.000×10^{9}	6.100×10^{9}	1.310×10^{9}	3.746×10^{9}	2.820×10^{9}	4.870×10^{9}	4.050×10^{9}
		Mean	3.455×10^{8}	8.8889×10^{8}	6.7778×10^{8}	1.455×10^{8}	4.155×10^{8}	3.1333×10^{8}	5.4111×10^{8}	4.50×10^{9}
		S.D	2.8076×10^{8}	2.1774×10^{8}	5.7342×10^{8}	2.5145×10^{8}	2.2283×10^{8}	2.122×10^{8}	4.548×10^{8}	3.80197×10^{8}
20	13.3	Total	6.200×10^{9}	8.020×10^{9}	4.0300×10^{9}	2.060×10^{9}	5.220×10^{9}	2.250×10^{9}	2.316×10^{9}	3.440×10^{9}
		Mean	6.8889×10^{8}	8.9111×10^{8}	4.4778×10^{8}	2.289×10^{8}	5.80×10^{8}	2.50×10^{8}	2.5666×10^{8}	3.82×10^{8}
		SD	4.411×10^{8}	8.315×10^{8}	4.124×10^{8}	3.5698×10^{8}	2.082×10^{8}	3.949×10^{8}	2.4469×10^{8}	6.554×10^{8}
24	8.9	Total	5.23×10^{9}	1.052×10^{10}	7.700×10^{9}	3.620×10^{9}	4.820×10^{9}	2.370×10^{9}	3.320×10^{9}	4.410×10^{9}
		Mean	5.8111×10^{8}	1.1688×10^{9}	8.555×10^{8}	4.022×10^{8}	5.3555×10^{8}	2.633×10^{8}	3.688×10^{8}	4.9×10^{8}
		SD	2.6955×10^{8}	9.169×10^{8}	5.2946×10^{8}	3.5046×10^{8}	2.7600×10^{8}	2.819×10^{8}	3.92×10^{8}	4.703×10^{9}
28	9.0	Total	4.513×10^{10}	5.780×10^{9}	2.830×10^{9}	2.510×10^{9}	5.70×10^{9}	6.830×10^{9}	4.790×10^{9}	4.20×10^{9}
		Mean	1.6811×10^{9}	6.4222×10^{8}	3.144×10^{8}	2.7889×10^{8}	6.333×10^{8}	7.5889×10^{8}	5.3222×10^{8}	4.666×10^{8}
		SD	6.2455×10^{8}	8.3633×10^{7}	1.431×10^{8}	1.2424×10^{8}	1.8668×10^{8}	5.8831×10^{7}	1.6184×10^{8}	2.114×10^{8}
30	10.7	Total	4.690×10^{9}	4.660×10^{9}	2.120×10^{9}	1.410×10^{9}	1.990×10^{9}	3.80×10^{9}	3.85500×10^{9}	3.760×10^{9}
		Mean	5.2111×10^{8}	5.1778×10^{8}	2.3556×10^{8}	1.5667×10^{8}	2.2111×10^{8}	3.6222×10^{8}	4.277×10^{8}	4.2×10^{8}
		SD	3.028×10^{8}	2.1022×10^{8}	1.0418×10^{8}	3.00×10^{7}	1.3596×10^{8}	1.5098×10^{8}	1.8773×10^{8}	7.106×10^{7}
33	12.1	Total	3.190×10^{9}	1.710×10^{9}	6.360×10^{8}	5.660×10^{8}	2.470×10^{9}	1.40×10^{9}	1.880×10^{9}	2.260×10^{9}
		Mean	3.5444×10^{8}	1.900×10^{8}	7.0667×10^{7}	6.2889×10^{7}	2.7444×10^{6}	1.5555×10^{6}	2.0888×10^{3}	2.5111×10^{8}
		SD	1.7222×10^{8}	4.77×10^{7}	3.423×10^{7}	2.529×10^{7}	1.5613×10^{8}	4.2459×10^{7}	3.723×10^{7}	8.852×10^{7}

KEY ∝ represents infinity

SD represents standard deviation