"SCREENING FOR RESISTANCE OF TOMATO CULTIVARS TO *CORYNEBACTERIUM MICHIGANENSE* (E.F.SM.) JENS. AND THE EFFECT OF *MELOIDOGYNE INCOGNITA* (KOFOID AND WHITE) CHITWOOD ON DEVELOPMENT OF BACTERIAL CANKER"

JAMES OMBIRI

A THESIS SUBMITTED IN PARTIAL FULFILLMENT FOR THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN PLANT PATHOLOGY, UNIVERSITY OF NAIROBI.

AUGUST, 1988

D E C L A R A T I O N

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

JAMES OMBIRI

Date: 10/11/89

This thesis has been submitted for examination with my approval as University Supervisor.

PROF. E.M. GATHURU

UNIVERSITY SUPERVISOR

DATE: 10/11/89

ACKNOWLEDGEMENTS

I wish to thank Prof. E.M. Gathuru, my supervisor for his constant guidance, advice and encouragement during the course of this work. Let me also take this opportunity to thank Prof. D.M. Mukunya of Crop Science Department for his words of encouragement and advice during the undertaking of this course. I would like to express my gratitudes to Dr. Buruchara, plant pathologist, University of Nairobi, for his helpful advice and enlightening discussions during the initial stages of the project.

I thank Dr. G.G. Madumadu, Plant Breeder and Mr. Kanyagia, nematologist both in National Horticultural Research Station, Thika for their helpful advice and enlightening discussions. May I also thank the technical staff in Crop Science Department for their assistance in the laboratory and field work.

I also wish to acknowledge my gratitudes to my sponsor, the University of Nairobi for providing the scholarship and to Mr. John Gitau, Animal Production Department, University of Nairobi who tirelessly typed this work. I would like to express my gratitudes to all my friends and relatives who helped me in one way or another during the undertaking of this course.

I wish to thank my mother Hellen for her continuous encouragement and prayer for me during the undertaking of this challenging course.

Finally, I wish to thank God who has been my ultimate help.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF APPENDICES

ABSTRACT

Investigations were carried out to establish the levels of resistance in tomato cultivars to *Corynebacterium michiganense* (E.F.Sm.) Jens., and to find out the effect of *Meloidogyne incognita* (Kofoid and White) chitwood on resistance. Variation in virulence of *C. michiganense* strains was observed with strain D being the most aggressive.

Screening of tomato accessions showed cultivars "MR4", "$CmVF_{232}$", "Monense", "Okitsusozai No. 1-20", and "Bulgaria" (P.I. 324708) to possess high levels of resistance to strain D. The cultivars "Bulgaria" (P.I. 324707), "Heinz 2990", "USA-Utah", "Victoria Dwarf", "Cervena Kapha", "Red Currant 1149K", and "Plovdiv 8/12" (Bulgaria 12) exhibited moderate resistance. Cultivars "Roma", "Moneymaker" and "Marglobe" were susceptible.

Bacterial canker-susceptible tomato cultivar, "Moneymaker" and resistant cultivar "MR4" inoculated at transplanting with *C. michiganense* and *M. incognita* developed more severe bacterial canker symptoms than those inoculated with *C. michiganense* alone.

1. INTRODUCTION

Tomatoes, *Lycopersicon esculentum* Mill. are undoubtedly one of the world's most popular vegetables. They are good sources of vitamins A and C and can help to alleviate deficiencies of these vitamins in many developing countries. In addition to its nutritional value, widespread cultivation of tomatoes could generate rural employment, stimulate urban employment, expand exports and increase income of farmers (Villareal, 1980).

Tomatoes are grown in Kenya mainly for local consumption as fresh or processed products. Substantial foreign exchange has been earned through export of tomatoes (Appendix 1). The present population growth rate of 4.3 percent in Kenya (Carrol, 1987) and the rapidly expanding tourist industry will further enhance the demand for tomatoes.

Tomato production in Kenya is concentrated in Eastern and Central Provinces (Appendix 2 and 3). It can be seen from appendix 2 that the area under tomatoes is increasing each year although there are some fluctuations depending on the year and season. The districts of Machakos, Kiambu, Muranga, Nyeri and Kirinyaga produce the most tomatoes.

There are many limitations to cultivation of tomatoes in the tropics. Production techniques in the tropics are generally rudimentary and many

important production inputs are lacking (Grubben, 1977). Adequate control of pests and diseases is extremely difficult and, in this respect, very few cultivars which are adapted to the climatic conditions of the wet tropics are available. Limited research on production techniques has been carried out. Consequently the level of production is very low.

Diseases are some of the biggest drawbacks in tomato production in Kenya. One of the diseases of tomatoes that has gained importance is bacterial canker caused by the bacterium *Corynebacterium michiganense* (E.F.Sm.) H.L. Jens. It is a severe disease of tomatoes and has been reported from most tomato growing areas of the world (Strider, 1969). Although it is sporadic in occurrence, it has significance because of its destructive economic effects. Nattrass and Ciccarone (1946) reported severe infection of as much as 80 percent of the crop in East Africa. Bates (1961) who first recorded the occurrence of bacterial canker of tomato in Kenya reported losses of 50-60 percent. Losses result primarily from the general decline or death of vascularly infected plants (Bryan, 1930).

Since its discovery in Grand Rapids Michigan (Smith, 1910), much has been learned about the causal organism and its relationship to its primary host, tomato. Bacterial canker is considered as one of the most devastating diseases of tomatoes for several reasons: 1) Seeds are carriers of the pathogen, 2) the pathogen persists in soil for several years

(Bryan, 1930) from whence infection readily occurs through wounded roots (Kendrick and Walker, 1948), 3) the disease is spread from plant to plant in normal production practices such as transplanting, prunning and tying, 4) the organism becomes rapidly systemic throughout the vascularly infected plants, 5) no practical curative treatment has been devised, 6) resistance in tomato has not been found, and 7) vascularly infected young tomato plants usually die or become economically unprofitable.

So far, seed certification and treatment has been the primary means of control. However, introduction and use of disease resistant cultivars coupled with rational disease management practices might be instrumental in alleviating disease constraints in production of tomatoes in Kenya.

It has been found that many wilt diseases caused by species of *Corynebacterium* are associated with specific plant parasitic nematodes (Hawn, 1963). De Moura *et al.* (1975) found that the combined pathological potential of root-knot nematodes and *C. michiganense* on tomato cultivar "Manapal" was greater than their individual effects. More work is needed to confirm and establish the effect that the root-knot nematodes can have on resistance of tomatoes to *C. michiganense*. The information obtained on root-knot nematodes and *C. michiganense* interaction will be useful in laying out control measures against the bacterial canker disease. Root-knot nematodes have been

reported in all tomato producing areas of Kenya (Madumadu, 1979). The commonest root-knot nematode species found in Kenya according to Whitehead *et al.* (1960) are *Meloidogyne incognita* and *M. javanica*. The other species are rare. The presence of root-knot nematodes in the roots of infested plants is associated with a characteristic galling of the affected roots. The plants remain stunted and in severe cases they can die. Hainsworth (1962) reported that 10% of the agricultural produce of Kenya was lost due to nematode attacks principally *Meloidogyne* species.

To develop more information on the subject of resistance of tomatoes to *C. michiganense*, and the effect that the root-knot nematodes can have on the resistance, this study was initiated with the following main objectives:

1) To study the virulence of isolates of *Corynebacterium michiganense* (E.F.Sm.) Jens. obtained from different tomato growing areas in Central and Eastern provinces of Kenya.

2) To establish the level of resistance in tomato cultivars from various parts of the world including some commercially grown cultivars in Kenya.

3) To study the influence of root-knot nematodes *Meloidogyne incognita* (Kofoid and White) Chitwood on the development of bacterial canker.

2. LITERATURE REVIEW

2.1 Taxonomy of *Corynebacterium michiganense*

Smith (1910) first gave this organism the generic name, *Bacterium* but after many more examinations, Smith (1914) concluded that it was non-motile and suggested the name *Aplanobacter michiganense*. In the meantime, the organism had been referred to in Steven's (1913) text as *Pseudomonas michiganense*, conforming to Migula's (1877-1900) older classification for bacteria having polar flagella. Bergey *et al.* (1923) placed the tomato bacterial canker organisms in yet another genus, *Phytomonas*, which was one of the two genera contained in the tribe Erwiniae, described as plant pathogens. Finally, although considerable controversy still exists, (Clark, 1940, 1951, 1952; Conn, 1947; Patel and Kulkarni, 1951; Jensen, 1934) drew attention to the basic similarity between soil corynebacteria and two plant pathogens with regards to gram reaction and physiology and suggested that the tomato canker organism be placed in the genus *Corynebacterium* Lehm. and Heum. Jensen (1934) suggested the current name, *Corynebacterium michiganense*.

2.2 Morphology of *Corynebacterium*

The salient features of the morphology of the genus *Corynebacterium* were summarized by Breed *et al.* (1957). As is true for other members of this genus, *C. michiganense* is characteristically pleomorphic.

Cell shape may be coccoid, elongated rods, branched and nodular cells or minute cells depending on the media upon which the bacterium is grown (Jensen, 1952; Ramamurthi, 1959). However, when examinations are made from fresh preparations taken from plant material, cells of *C. michiganense* are characteristically rod-shaped occurring singly or in pairs (Smith, 1910; Bryan, 1930; Stapp, 1930; and Ramamurthi, 1959). Reproduction is by binary fission, and this method of cell division in this genus is referred to as "snapping" growth which results in the characterstic angular arrangements of cells (Jensen, 1952).

Smith (1914) demonstrated in his pioneer studies that *C. michiganense* was gram positive, not acid fast and non-sporeforming. He also reported that the organism was non-capsulate and non-motile. These chacteristics have been confirmed many times. Electron micrographs taken by Wakimoto, Mukoo (1968) revealed no flagella.

2.3 Characteristics of growth in Culture

Colony characteristics are variable, being influenced by conditions of growth and the nature of the strain (Strider, 1969). A good description of the characteristics of growth of *C. michiganense* on several media was presented by Bryan (1930). Pigmentation is usually in shades of yellow on nutrient-rich media (Schaad, 1980) and a nutrient-broth yeast extract medium (NBY) is recommended as a medium of choice for

growth, pigmentation, and colony differentiation.

2.4 Symptomatology

Symptom expression varies and is influenced by various factors such as type of culture, environmental conditions, age of plants when infected and infection site (Strider, 1969). It is generally agreed that wilting is the most characteristic symptom of bacterial canker; moreover the irregular or one-sided wilting of leaflets (Plate 1) and leaves (Plate 2) is peculiar to this disease on tomato and is an especially useful diagnostic feature (Bryan, 1930; Kotte, 1930; Orth, 1937; Colquhoun and McCarthy, 1943; Nattrass and Ciccarone, 1946; Smith and Goss, 1946; Strider and Konsler, 1965). The disease usually proceeds from the base of the plant upwards causing lower leaves to die progressively up the stem (Smith and Goss, 1946). Death of leaves is preceded by a gradual wilting and upward rolling of leaflets commonly beginning on single leaflet and on leaflets on only one side of affected leaves. Diseased leaflets on one side of a leaf may shrivel and die, while the leaflets on the other side remain apparently healthy. Eventually the whole leaf shrivels and dies but does not readily become detached from the petiole which remains turgid and attached for considerable time afterwards.

Another characteristic feature of canker is the visible appearance of yellow to brown streaks in the water-conducting tissue of stems and petioles (Jones, 1938; Smith and Goss, 1946; Nattrass and Ciccarone,

1946) and reddish to brown discolouration in the pith (Jones, 1938). Splits sometimes occur along these areas producing cankers (Bryan, 1930), hence the name bacterial canker. From such affected portions a yellowish slime may be squeezed. This feature distinguishes canker from bacterial wilt which exudes a white slime (Orth, 1937; Nattrass and Ciccarone, 1946). The discolouration is most pronounced at the junction of petiole and stem. A cross section at this point reveals a crescent-shaped area of necrotic vascular tissue.

Layne and Rainforth (1966) reported that systemically infected, green-house grown plants produced mottled and malformed fruit. Surface infection of fruit initially appears as minute, white superficial spots which later enlarge becoming brown and slightly raised, surrounded by a white halo. These are known as bird's eye spots which have been described as an absolute diagnostic characteristic of bacterial canker (Smith and Goss, 1946).

2.5 Factors influencing the reaction of tomato seedlings to infection by *C. michiganense*

Many factors influence the reaction of tomato seedlings to infection by *C. michiganense*. Most significant are virulence of the bacterial strains, inoculation procedure and dose, age of inoculated seedlings, temperature, soil moisture and host nutrition. Strains from different geographical areas of the world have been shown to differ in virulence

(Thyr, 1972). Therefore most breeders tend to use strains from their own area. These may have low, medium or high virulence when inoculated to certain tomato cultivars.

Various inoculation methods have been used involving many parts of the tomato plant including roots, stems, petioles, leaves and cotyledons. Strider (1970) reported that root inoculation methods failed to produce uniform infection and were judged undesirable. Petioles are inoculated by introducing the bacteria with a contaminated needle (Yatzynina, 1941) or by severing petioles with a scapel or pair of scissors previously dipped in a bacterial suspension (Pine *et al*, 1955; Thyr, 1968; and Van Steekelenburg, 1984). Leaves have been inoculated by spraying with a bacterial suspension (Layne, 1967; Strider and Konsler, 1965). However, Ark (1944) and Grogan and Kendrick (1953) obtained no infection by spraying young tomato plants. A cotyledonary method of inoculation has been described by Hassan *et al*. (1968).

Stem inoculation has been reported as the most reliable (Dejong and Honma, 1976). However, Van Steekelenburg (1984) observed that cutting the petiole of the first true leaf of young plants with a contaminated pair of scissors is a fast and easy inoculation technique in screening for resistance to systemic infection of *C. michiganense*.

Strider (1970) reported that disease development

was rapid and uniform in plants that were stem inoculated at 14 days of age but that disease development was slower on plants inoculated at 28 days of age. Forster and Echandi (1973) observed that there were greater differences in disease ratings between resistant and susceptible accessions after inoculating five-week-old plants.

Various day/night temperatures have been used to screen tomato seedlings for resistance to *C. michiganense*. Strider (1970) used 24-32°C day and 18°C night temperature, while Emmatty and John (1973) used 25°/20°C day and night temperatures, respectively. It was observed by Forster and Echandi (1973) that five-week-old plants at 24°/18°C day and night temperatures showed greater differences in disease ratings than those at 20°/18°C, 28°/18°C or 32°/18°C. Kendrick and Walker (1948) observed that plants grown at optimum soil moisture succumb most rapidly. Also, the disease is apparently favoured by a high concentration of nutrients. Tomato plants have been reported to prefer nitrate nitrogen and at the same time *C. michiganense* growth in the plant is enhanced by nitrate nitrogen (Huber, 1980).

The optimal concentration of inoculum depends on the method of inoculation. With foliar application, 10^6 or more cells/cm^3 (Basu, 1966) are needed while use of root canal file or a pair of scissors dipped in bacterial suspension requires a concentration of 10^7 to 10^9 cells/cm^3 (Forster and Echandi, 1973; Van Steekelenburg, 1984).

2.6 Host range

In his early description of bacterial canker, Smith (1914) cited tomato (*Lycopersicon esculentum*) and *Solanum mammosum* as hosts. Haven (1945) added *Solanum douglasii* while Ark and Thompson (1960) added *Solanum nigrum* to the hosts attacked. Hassan *et al.* (1968) demonstrated by cotyledonary spotting a number of *Capsicum* and *Solanum* species to be possible hosts of *C. michiganense*. Ark (1944) reported tree *Cyphomandra betacea* (CaV.) Sendt. to be susceptible.

Tomato is the natural host of *C. michiganense*. The *Lycopersicon* species and hybrids that have been shown to be susceptible by vascular discolouration or wilting are many (Strider, 1969). Alexander *et al.* (1942) demonstrated *L. esculentum* x *L. pimpinellifolium*, *L. hirsutum* Var. *glabratum*, *L. peruvianum* Var. *dentatum* and *L. peruvianum* Var. *humifusum* to be susceptible hosts. Hassan *et al.* (1968) on the other hand, demonstrated *L. cheesmanii* Riley, *L. esculentum* Var. *cerasiforme*, *L. esculentum* Var. *pyriforme*, *L. esculentum* x *L. hirsutum*, *L. glandulosum* Muller and *L. hirsutum* HBK to be susceptible hosts.

2.7 Resistance

2.7.1 Nature and source of resistance

The cause of wilting has been a subject of study by several investigators (Patino-Mendez, 1966; Rai, 1968; Rai and Strobel, 1967). Rai and Strobel (1967) characterized a toxin which was heat resistant, acid-labile and water soluble polysaccharide, chemically described as a glycopeptide. Rai (1968) suggested that the key mechanism of wilting resulted from damage of the cell membrane system and that the toxin played a major role in disease development.

A resistant tomato plant must either be resistant to the glycopeptide toxin or the bacteria themselves. According to Thyr (1971), canker-resistant tomato accessions express their resistance by limiting bacterial growth. The resistance is multigenic in nature, which is relative and not a case of immunity.

Thyr (1968, 1969) reported moderate resistance in Utah 659, which he described as *L. esculentum* var. *Ceraforme* (Dunal) A. Gray and in line G14565 described as *L. esculentum* cultivar "Cervena Kapha". Thyr also listed cultivar "Bulgaria 12" which was developed by Elenkov (1965) who made a cross between a resistant *L. pimpinellifolium* and a susceptible *L. esculentum*. Thyr (1968, 1969) confirmed the high degree of resistance reported in this cultivar. Some degree of resistance occurs in other *Lycopersicon* species, principally in *L. pimpinellifolium* and some utilization of this

resistance in tomato varieties has been reported. Stamova and Yordanov (1986) reported a high level of resistance in "Corina", a line which resulted from crossing *L. pimpinellifolium* and *L. racemigerum*.

2.7.2 Screening for resistance

Limited utilization of resistant varieties has been reported. Yatznina (1951) examined 197 varieties, none of which were immune to canker. Some cultivars or lines were found to be more resistant than others, though essentially all the cultivars were susceptible that is, there was no complete resistance. The currant tomatoes (*L. pimpinellifolium*) examined were very resistant showing only slight symptoms which disappear later in the season. Elenkov's (1965) results were similar. She reported that *L. pimpinellifolium* was the only species that recovered completely after inoculation. Marglobe and other commonly grown varieties have been proven susceptible by other workers (Bryan, 1930; Fenner, 1930; Hassan *et al.* 1968).

After failing to find a source of resistance to canker in commercial varieties of tomatoes, workers have attempted to locate resistance in plant introductions (PI's) of *Lycopersicon esculentum* and other *Lycopersicon* species. The introductions (Cultivars) of *L. pimpinellifolium* (PI's 126940, 270441, 270447, and 272641) which were examined by Hassan *et al.* (1968) succumbed to the disease in typical fashion. All plants inoculated either wilted or died. They further confirmed the findings of

Alexander *et al.* (1942) in regards to susceptibility of *Lycopersicon* species. Hassan *et al* (1968) also reported finding resistance in *L. hirsutum* (P.I. 251305).

Thyr (1968, 1969) reported a high degree of resistance in three *L. pimpinellifolium* accessions designated "Utah 737", "Utah 20", and "LA 12156L". Canker resistant varieties have been developed in Russia and Bulgaria (Yatzynina, 1951; Elenkov, 1965; Stamova and Yordanov, 1986). After the success of Elenkov (1965) in transferring the resistance from *L. pimpinellifolium* to the tomato cultivar Plovdiv 8/12 a number of *L. esculentum* cultivars with resistance to the bacterium have been bred and are increasingly becoming sources of resistance. Included in this list are cultivars "H2990" (Ematty and John, 1973); "Monense" (Laterrot *et al.* 1978); "MR4", "$CmVF_{232}$", "Okitsu Sozai No. 1-20" (Kuriyama and Kuniyasu, 1974); "Corina" (Stamova and Yordanov, 1986).

Due to the variation in virulence of strains of the pathogen, a resistant cultivar in one geographical area of the world has not necessarily been identified to exhibit its resistance in other areas. Boelema (1980) found the accession P.I. 330727 (Bulgaria 12) no more resistant than "Roma VF", a reportedly susceptible cultivar in South Africa. Also, due to differences in virulence in strains in different geographical areas of the world, most breeders tend to use strains from their own areas.

2.8 Variations in virulence of Corynebacterium strains

Several investigators (Baines, 1947; Bryan, 1930; Fawcett and Bryan, 1934; Strider and Lucas, 1970; and Thyr, 1972) have reported variations in the virulence of strains of *C. michiganense*. Bryan (1930) discovered the non pigmented strain which had a similar virulence to the yellow strains. Fawcett and Bryan (1934) reported a pink and white strain. The pink strain showed less virulence when compared to the yellow and white strains. Bryan (1930) observed occassional small, round convex colonies that never spread. This colony type was produced only from older cultures and was weakly virulent or avirulent. Jensen (1934) on the other hand reported a non-pathogenic strain which grew more rapidly and whose growth was more fluidal than the pathogenic strain. It grew at a higher temperature and had higher proteolytic activity than the pathogenic strain.

Baines (1947) reported differential virulence of a bacterium similar to *C. michiganense* isolated from *Solanum douglasii*, that reinfected *S. douglasii* as well as tomato. However, a tomato strain failed to infect *S. douglasii*. More recently, Strider and Lucas (1970) showed variation in virulence of *C. michiganense* to occur on the tomato cultivar "Manapal".

Thyr (1972) compared and noticed differences in virulences of seven strains of *C. michiganense* from six geographical areas of United States. He suggested that highly virulent strains should be employed in a

breeding programme so that an acceptable level of resistance can be maintained.

2.9 Role of plant parasitic nematodes in bacterial diseases

Plant parasitic nematodes are rapidly becoming well known for their being associated with many fungal, bacterial and viral diseases of plants. They may act as initiators, co-operators, synergists or aggravators of fungal and bacterial diseases, especially those of soil borne nature. (Holdeman, 1954; Pitcher, 1961; Powell, 1961; Raski and Hewitt, 1961). Far more work has already been done on interaction between nematodes and fungi. By contrast, work on nematode-bacteria relationships has been sparse and sporadic. Relatively few bacterial diseases associated with nematodes have been investigated.

The earliest published work seems that of Hunger (1901) who showed that tomatoes were readily attacked by *Pseudomonas solanacearum* in nematode-infested soil, but remained healthy in nematode-free soil. In 1926, Carne reported on laboratory and field work which indicated that *Anguina tritici* acted as a vector of the wheat pathogen *Corynebacterium tritici*. Ten years later, Ark and Thomas (1936) suggested that the nematode now known as *Pratylenchus penetrans* enabled otherwise harmless bacteria to damage roots of apple. In the same year, Kalinenko (1936) stated that three nematode species were performing a similar role on roots of two rubber-bearing plants, but in this case

the bacteria were shown harmful to roots in the absence of nematodes.

In their studies, Lucas *et al.* (1955); and Stewart and Schindler (1956) reported that endoparasites of roots *Meloidogyne* spp. assist in development of Tobacco-Granville wilt caused by *Pseudomonas solanacearum* and Carnation wilt caused by *P. caryophylli* by wounding. The bacteria *P. solanacearum* and *P. caryophylli* are endoparasitic vascular pathogens, capable of entering the host through mechanical wounds. Lucas *et al* (1955) further stated that the nematodes act as incitants, presumably as wound agents; but they may also act in other capacities, for the most efficient incitants apparently are the root-knot nematodes which feed in the vicinity of the vascular tissues, causing marked changes in host cells.

In his report, Pitcher (1963) stated that all nematodes puncture plant cells to feed and so can act as inoculants, but the micropuncture made by a nematode stylet is not the type of wound most likely to favour the entry of bacteria, which enter more readily through gross wounds, such as those made by biting insects or mechanical injuries. He further stated that the feeding of ectoparasites resembling that of aphids, seems better adapted to virus transfer; but endoparasites entering the host body and perhaps, causing local necrosis seem better suited to aid establishment of bacteria. Hugh and Powell (1968) suggested that attention should be given to

physiological changes in nematode infected plants when disease interactions are studied. On their study of the influence of certain *Meloidogyne* spp. on *Fusarium* wilt development in flue-cured tobacco, Porter and Powell (1967) found that morphological changes such as hypertrophy and hyperplasia accompanied by physiological alterations, may play some role in predisposing plants to Fusarium wilt development. They also found that the contents of infected giant cells degenerated following bacterial invasion, leaving virtually empty cells. Presumably this could be due to the presence of materials in these tissues more easily utilized in bacterial metabolism than materials found in normal plant tissues.

According to Pitcher (1963) one should not overstress the aspect of mechanical injury because biochemical or physiological changes brought about in host by the nematodes may be of equal or greater importance than the mere introduction of the bacterium into the susceptible tissues of the host. Specific nematodes are associated with many wilt and root rot diseases caused by species of *Corynebacterium* (Hawn, 1963); Fusarium (Binder and Hutchinson, 1959; Bowman and Bloom 1966); *Phytophthora* (Wyllie and Taylor, 1960), and many other fungi and bacteria. In their study on interaction of *Meloidogyne* and *C. michiganense*, De Moura *et al* (1975) stated that, though both pathogens can be very destructive when acting alone, their combined pathological potential may be far greater than their individual effects.

More work is needed to confirm and establish the effect that nematodes can have on the development of plant diseases caused by other pathogens. This information will be useful in laying out control strategies for the disease in question.

3. MATERIALS AND METHODS

3.1 Sources of *Corynebacterium michiganense* isolates

Isolates of *Corynebacterium michiganense* used in these studies were obtained from some of the main tomato growing regions of Central and Eastern Provinces of Kenya, representing varying ecological conditions (Table 1). Isolation was made from naturally infected tomato (*Lycopersicon esculentum* Mill) plants growing in farmers' fields and at the Field Station farm (Kabete) of the University of Nairobi. A total of 10 isolates were used, all of which were collected during the period between July and September 1987. The isolates were designated with different letters (A to J) depending on their areas of origin.

3.2 Isolation and storage of isolates

To isolate bacteria from the host, parts of the stem of infected plant material showing vascular discolouration were cut. The pieces were surface sterilized by immersing in 70% alcohol and flaming them. The pieces were placed on a sterile petri dish and aseptically cut into small pieces in sterile distilled water. They were then macerated using a sterile glass rod. The bacterial suspension was then streaked on nutrient broth yeast extract agar (NBY) medium (Appendix 4), and incubated at 27°C. The bacterial cultures were purified by a series of single colony transfers and maintained in yeast extract dextrose calcium carbonate agar (Appendix 4) slants at 4°C.

Table 1: Areas of origin of *Corynebacterium michiganense* isolates and their climatological conditions.

Area of origin	Isolate	Altitude (m)	Mean annual Rainfall (mm/yr)	Mean annual temperature (°C)
Nairobi (Kabete)	A	1910.7	1028	18.1
Machakos (Yatta)	B	1230	841	20.0
Kiambu (Kiambaa)	C	1650	1014	19.0
Kiambu (Kiambaa)	D	1650	1014	19.0
Nyeri (Mathira)	E	1914	1152	16.8
Machakos (Yatta)	F	1230	841	20.0
Kirinyaga (Kibirigwi)	G	1394	977	21.0
Nyeri (Mathira)	H	1814	1152	16.8
Kiambu (Kiambaa)	I	1650	1014	19.0
Muranga (Kandara)	J	1590	1175	20.0

Source of climatological data: Farm Management handbook of Kenya (Natural conditions and farm management information); and Meteorological office (University of Nairobi's field Station - Kabete)

3.3 Identification of isolates

The isolates were identified as *Corynebacterium michiganense* (E.F. Sm.) Jens. by subjecting them to the following tests:

3.3.1 Cultural characteristics of the isolates

Suspension of pure cultures of each isolate were streaked on NBY medium in petridishes, incubated for 48 hrs at 27°C and then colonies were observed visually for appearance in colour, form, elevation, margin and growth.

3.3.2 Gram stain, acid-fast test and cell morphology

Young actively growing 24 hr old cultures were used to prepare smears on microscope slides. The staining procedures used for both tests are outlined in Ritchie *et al.* (1976) paper. During examination of the slides, note was taken of the size, shape and arrangement of the cells.

3.3.3 Biochemical tests

3.3.3.1 Catalase test

To demonstrate the presence of catalase in the isolates, a loopful of a 48 hr culture was placed on a clean microscope slide, then a drop of 3% 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.

3.3.3.2 Fermentation of Sugars

Test on fermentation of carbohydrates was carried out by the use of nutrients broth as a basal medium composed of beef extract (3.0 g) peptone (10.0 g) and distilled water (1 litre). The sugars tested included sucrose, lactose, glucose, mannitol and arabinose. These were added at a rate of 0.5% into separate conical flasks containing the basal medium. After dissolving, the pH was adjusted to 7.0 by use of 0.5N NaOH. Five millilitre aliquots of test media were poured into small screw-capped bottles. These were sterilized and the media allowed to set. Each bottle was inoculated with a pure culture of each isolate. The results were noted after 14 days incubation at 27°C. Fermentation was indicated when the medium turned yellow.

3.3.3.3 Oxidase test

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 donors in the bacteria and a redox dye-tetra methyl-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 dihydro-

chloride. The colony to be tested was picked with a sterile wire-loop and smeared on the moist area. A positive reaction was indicated by an appearance of an intense deep-purple colour appearing within 5-10 seconds.

3.3.3.4 Triphenyl tetrazolium chloride test

The bacterial isolates were tested for tolerance on nutrient agar containing 2% triphenyl tetrazolium chloride (TTC). 5 spot inoculations on TTC agar medium were made for each isolate. Incubation was done at 27°C and observations of growth were recorded after 3-5 days.

3.3.3.5 Sodium chloride tolerance

Each isolate was streaked in nutrient agar containing 1, 3, and 5% (W/V) sodium chloride. Incubation was done at 27°C and observation of growth made at 7 and 14 days after inoculation.

3.3.4 Pathogenicity test

Tomato cultivar "Moneymaker" which has been grown in Kenya for a number of years and found susceptible in the field (Madumadu, 1985) was used in this experiment . "Moneymaker" is an indeterminate, vigorous greenhouse tomato with small to medium sized round fruits. The seeds used in this experiment were obtained from Kenya Seed Company. Seedlings were raised in wooden flats using sterile potting soil

mixture of composition shown in Appendix 4. Two weeks after seeding, seedlings were individually transplanted into 15 cm diameter plastic pots filled with sterile potting soil mixture.

The potted seedlings were kept in a greenhouse with air temperatures ranging 26-37°C during the day and 14-17°C at the night. The plants were watered regularly and topdressed once every week using calcium ammonium nitrate (CAN) fertilizer. The ten isolates described in Table 1 (Section 3.1) were used in this study.

3.3.4.1 Inoculum preparation

Inoculum suspensions were prepared by suspending the bacteria in sterile saline solution (0.85% NaCl) from 48 hr old cultures grown on NBY medium. The suspensions were then adjusted turbidimetrically using a spectronic 20 spectrophotometer (Baush and Lomb Co.) at 620 nm to a concentration of about 10^7 colony forming units (CFU) per millilitre.

3.3.4.2 Inoculation procedure

Three week old seedlings were inoculated with each bacterial isolate. Inoculation was made by cutting the petiole of the first true leaf near the stem with a pair of scissors previously dipped in bacterial suspension.

3.3.4.3 Disease assessment

Plants showing the characteristic symptoms of bacterial canker after 2-3 weeks of inoculation were considered diseased. The symptoms included unilateral wilting of leaflets, canker formation on the stem and petiole and vascular discolouration.

3.4 Virulence of *Corynebacterium* isolates on *Lycopersicon esculentum*

The susceptible tomato cultivar "Moneymaker" and the ten bacterial isolates described in Table 1 were used in this experiment. Raising of seedlings, inoculum preparation and inoculation procedure was the same as described in Section 3.3. Ten, three-week-old seedlings of "Moneymaker" were inoculated with each isolate at inoculum concentration of 10^7 cells per millimetre. A completely randomized design was used with ten replicates per treatment. The experiment was conducted in the greenhouse with air temperatures ranging 29-35°C during the day and 14-16°C at night. From this experiment, the most virulent isolate was established and consequently used in screening for resistance of tomato cultivars to *C. michiganense*.

3.4.1 Rating Procedure

The seedlings were scored 28 days after inoculation. A slight modification of the disease index of Forster and Echandi (1973) was used. This involved the use of the wilting symptoms as indicated below.

1 = No wilting

2 = Less than half the leaves wilted

3 = Half to three-quarters of the leaves wilted

4 = More than three-quarters of the leaves wilted but the terminal leaves of the main shoot not wilted

5 = Terminal leaves of main shoot and most other leaves wilted or dead.

3.5 Screening of tomato cultivars for resistance to *Corynebacterium michiganense*

Twelve tomato cultivars reported as resistant to *C. michiganense* and three commercially grown cultivars "Moneymaker", "Marglobe" and "Roma" were used in this experiment. "Moneymaker" served as a susceptible control cultivar. The cultivars are described in Table 2.

3.5.1 Sources of seeds, raising of seedlings and inoculation

Seeds of reportedly resistant cultivars were obtained from National Horticultural Research Station, Thika. Seeds of commercial cultivars were obtained from Kenya Seed Company. Seedlings of the test material were raised in flats and transplanted to plastic pots as described in Section 3.3.4. Ten three-weeks-old seedlings of each cultivar were inoculated with the most virulent isolate of *C. michiganense* obtained from the experiment on virulence of *C.*

michiganense isolates on susceptible cultivar, "Moneymaker". The seedlings were inoculated using inoculum concentration of 10^7 cells per millimetre. The experiment was laid out in a completely randomized design. The potted seedlings were kept in the greenhouse with air temperatures ranging 31-37°C during the day and 12-16°C at night.

Table 2: Tomato cultivars screened for resistance to *Corynebacterium michiganense*

Cultivar	Reported level of resistance	Reference
Moneymaker	Susceptible	-
Plovdiv 8/12	resistant	Elenkov (1965)
MR4	resistant	Forster and Echandi (1975)
Cm VF_{232}	resistant	Kuriyama and Kuniyasu (1974)
H2990 (P.I.330727)	resistant	Ematty and John (1973)
Monense	resistant	Boelema (1980)
Okitsu Sozai No. 1-20	resistant	Boelema (1980)
Victoria Dwarf No. 1	resistant	-
Bulgaria (P.I.324707)	resistant	-
Bulgaria (P.I.324708)	resistant	-
Cervena Kapha(P.I.34095)	resistant	Thyr (1969)
Red Currant 1149K (P.I.344102)	resistant	Thyr (1969)
USA-Utah (P.I.344103)	resistant	Thyr (1976)
Marglobe	Suscpetible	-
Roma	Susceptible	-

3.5.2 Rating Procedure

The plants were scored 28 days after inoculation using the scoring procedure described in Section 3.4.

3.5.2.1 Rating the level of resistance

The following arbitrary scales were used to rate the level of resistance of the tomato cultivars to *C. michiganense*

Mean severity score	Level of resistance
1.0 - 1.9	High resistance
2.0 - 2.5	Moderate resistance
2.6 - 5.0	Susceptible

3.6 Influence of *Meloidogyne incognita* on the development of bacterial canker in tomato

3.6.1 Source of *M. incognita* inoculum

Heavily galled roots of the tomato cultivar "Moneymaker" were obtained from tomatoes grown under field conditions at the Field Station (Kabete). The infected roots were thoroughly washed with tapwater. Egg masses of *Meloidogyne* sp. were handpicked from the infected roots placed under stereoscopic microscope. The mature females bearing the respective eggmasses were extracted and used to cut the perineal patterns for identification purposes. All the patterns cut revealed that the nematodes were *Meloidogyne*

incognita. The eggmasses of *M. incognita* were incubated under water in watchglasses at 27°C for three days. Freshly hatched larvae of *M. incognita* were observed using a stereoscopic microscope and a known concentration made by diluting the original suspension to the required concentration of 1000 larvae per 5 ml of water. The suspension was applied to the roots of fifteen three-week-old seedlings of susceptible cultivar "Rutgers" grown in 15 cm diameter plastic pots containing sterilized soil media of composition shown in Appendix 4. The seedlings were kept in the greenhouse and regularly watered. After two and half months the infected roots were used as source of inoculum of *Meloidogyne incognita*.

3.6.2 Raising seedlings of "Moneymaker" and "MR4"

Canker-susceptible cultivar "Moneymaker", and resistant cultivar "MR4" were grown from seed in wooden flats filled with sterilized soil mixture (Appendix 4). They were transplanted when 14 days old into 5-cm diameter pots containing the same type of soil mixture used in wooden flats.

3.6.3 Preparation of inocula of *C. michiganense* and *M. incognita*

Inoculum of the virulent isolate D of *C. michiganense* was prepared following the method described in 3.3.4. Inoculum of *M. incognita* was prepared by thoroughly washing the heavily galled roots of tomato cultivar "Rutgers". The roots were cut

into small pieces and placed in water contained in watchglasses. After 3 days of incubation at 27°C, numerous freshly hatched larvae of *M. incognita* were observed. Required concentration of 1000 larvae per 5 ml of water was made using the dilution method.

3.6.4 Inoculation procedure

After five weeks, "Moneymaker" and "MR4" plants were transferred with soil and roots intact into the same type of soil mixture (Appendix 4) contained in 15-cm diameter plastic pots. Just before transplanting, 5 ml suspension containing 1000 larvae was added around the roots. Plants were then placed in the hole made to receive the intact root system, and soil pressed around the roots. Control plants were similarly treated, but received no nematodes. Plants were inoculated with *C. michiganense* by cutting the petiole of the first true leaf using a pair of scissors previously dipped in *C. michiganese* suspension containing 10^7 cells/ml. Ten plants were utilized per treatment in all the experiments. Treatments consisted of:

i) *C. michiganense* alone
ii) *C. michiganense* plus *M. incognita*
iii) *M. incognita* alone
iv) non inoculated control

The treatments were laid out in a completely randomized design in a greenhouse with air temperatures ranging between 31-37°C during the day and 14-16°C at night. Plants were watered regularly with tapwater and

topdressed using CAN fertilizer.

3.6.5 Rating procedures

Two methods of rating the disease severity were used:

i) The percentages of seedlings with a score of 3 or more (using the scoring procedure described in Section 3.4) were taken 29 days, 31 days, 33 days and 35 days after inoculation.

ii) Determination of Linear extent of internal vascular discolouration (LEIVD) 35 days after inoculation. This method used by Thyr (1971) involved the cutting of the stems longitudinally and measurements taken of the extent of vascular discolouration from the point of inoculation. The heights of the plants were also measured. The two parameters were used to calculate % LEIVD as follows:

$$\% \text{ LEIVD} = \frac{\text{LEIVD}}{\text{Plant height}} \times 100$$

4. RESULTS

4.1 Identification of isolates

4.1.1 Cultural characteristics of isolates

Colony growth of the 10 isolates on NBY agar was moderate, smooth, convex and pale yellow in colour after 48 hr. With time, they became deep yellow, opaque and glistening. Although all the isolates exhibited the characterstics described above, the coloration was in shades of yellow. Isolates A, C, D and E were yellow, whereas the rest were deep yellow 7 days after culturing. There was variation in the rate of growth among the isolates. Isolates B, C, E and J grew faster than isolates, A, D, F and I which exhibited moderate growth, and isolates G and H which showed slow growth.

4.1.2 Gram stain, acid-fast test and cell morphology

All the isolates were gram-positive and not acid-fast. Microscopical examination of the prepared slides of the isolates under oil immersion revealed that the cells were short, slender and pleomorphic rods, occurring singly or in pairs.

4.1.3 Biochemical tests

A summary of the results of biochemical tests obtained, using 10 bacterial isolates is presented on Table 3.

Table 3: Reaction of 10 bacterial isolates and expected reactions of *Corynebacterium michiganense* to some biochemical tests

Biochemical Tests	Reaction of 10 isolates	Expected reaction of *C. michiganense*
Catalase reaction	+	+
Oxidase reaction	-	-
Fermentation of carbohydrates:		
Sucrose	+	+
Lactose	+	+
Glucose	+	+
Arabinose	+	+
Mannitol	+	+
TTC tolerance	+	+
NaCl tolerance		
1% NaCl	+	+
3% NaCl	±	±
5% NaCl	-	-

Description of symbols:

+ = positive reaction

- = negative reaction

± = variable reaction

4.1.3.1 Catalase test

Addition of hydrogen peroxide on the 48 hr old culture of all the isolates was accompanied with an effervescence which lasted for about 3 minutes. The catalase enzyme converted H_2O_2 to oxyygen and water. The appearance of a gas (oxygen) was considered a positive catalase test.

4.1.3.2 Fermentation of sugars

All the sugars tested gave a positive test for all the ten isolates. Fermentation was indicated when the medium containing either of the sugars sucrose, lactose, glucose, arabinose and mannitol turned yellow. There was a variation in the fermentation rate among the isolates.

4.1.3.3. Oxidase test

There was no colour change on the filter papers impregnated with 1% tetramethyl-p-phenylene-diamine dihydrochloride after smearing them with cultures of all the isolates, indicating negative tests.

4.1.3.4 Triphenyl tetrazolium chloride test

All the isolates were able to grow on TTC agar. A variation in rate of growth among the isolates was observed.

4.1.3.5 Sodium chloride tolerance

There was no growth restriction on nutrient agar containing 1% NaCl. However growth of isolates B and F was restricted at 3% NaCl. Isolates B, F and G showed no growth at 5% NaCl, but the rest of the isolates exhibited restricted growth.

4.1.4 Pathogenicity test

Most of the plants inoculated with bacterial isolates showed unilateral wilting of the leaflets (Plate 1) 2 weeks after inoculation. Plants inoculated with isolates D and F showed the symptoms much earlier than the rest. The plants showed irregular or one-sided wilting of the leaves (Plate 2) 3 weeks after inoculation. Cankers were observed along the stems and petioles (Plate 3) of the diseased plants. When the stems of the diseased plants were cut longitudinally, a dark brown vascular discolouration was observed which extended from the point of inoculation (Plate 4). All the isolates were pathogenic, to the tomato cultivar "Moneymaker".

Plate 1: Tomato cultivar "Moneymaker" (A) non-inoculated control, and (B) seedling showing unilateral wilting of leaflets "C" 2 weeks after inoculation with isolate D of *C. michiganense*

Plate 2. Tomato Cultivar "Moneymaker" (A) Non-inoculated control, and (B) Seedling showing irregular one-sided wilting of leaves three weeks after inoculation with isolate D of *C. michiganense*.

Plate 3: Part of the tomato plant, Cultivar "Moneymaker" showing (A) Canker formed on the stem and (B) Canker formed on the leaf petiole four weeks after inoculation with isolate D of *C. michiganense*

Plate 4. Longitudinal section of the stem of tomato plants, cultivar "Moneymaker" (A) non-inoculated control (B) the discolouration of vascular tissues and the pith four weeks after inoculation with isolate D of *C. michiganense*

4.2 Virulence of Corynebacterium isolates on *Lycopersicon esculentum*

The results of the virulence of the ten isolates of *C. michiganense* on susceptible cultivar "Moneymaker" are shown in Table 4 and graphically in Figures 1 to 10. Bacterial isolate D had the highest mean severity score of 3.8 (Table 4, Figure 4) with no plant falling in severity class 1. 40% of plants inoculated with isolate D had the highest score of 5 which means the leaves including the growing point were all wilted or the plant was dead. In descending order of aggressiveness, isolate F followed isolate D with a mean severity score of 3.4 (Table 4, Figure 6); followed by isolate H (mean score 2.4, Table 4, Figure 8); B (mean score 2.2, Figure 2); A (mean score 2.1, Figure 1); C (mean score 2.1, Figure 3); E (mean score 1.9, Figure 5); G (mean score 1.7, Figure 7); I (mean score 1.7, Figure 9); and J (mean score 1.7, Figure 10). Isolate D was the most aggressive on "Moneymaker", while isolates C, I and J were the least aggressive. The analysis of variance (Appendix 5) showed that the differences in virulence of isolates were significant ($p<0.05$).

Table 4: Virulence of ten bacterial isolates of *C. michiganense* on tomato cultivar "Moneymaker"

Bacterial isolate	Mean severity Score*	Virulence rank
A	2.1_{bc}	4
B	2.2_{bc}	4
C	2.1_{bc}	4
D	3.8_{a}	1
E	1.9_{bc}	4
F	3.4_{a}	1
G	1.7_{c}	8
H	2.4_{b}	3
I	1.7_{c}	8
J	1.7_{c}	8

*Means within columns followed by the same letter(s) are not significantly different at 5% level according to Duncan's New Multiple Range Test.

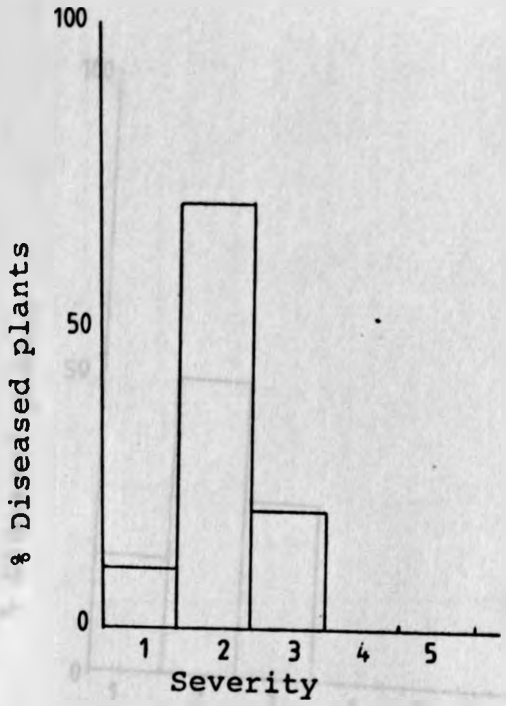

Fig. 1. Disease severity on susceptible tomato Cv. "Moneymaker" infected by Isolate A of C. michiganense.

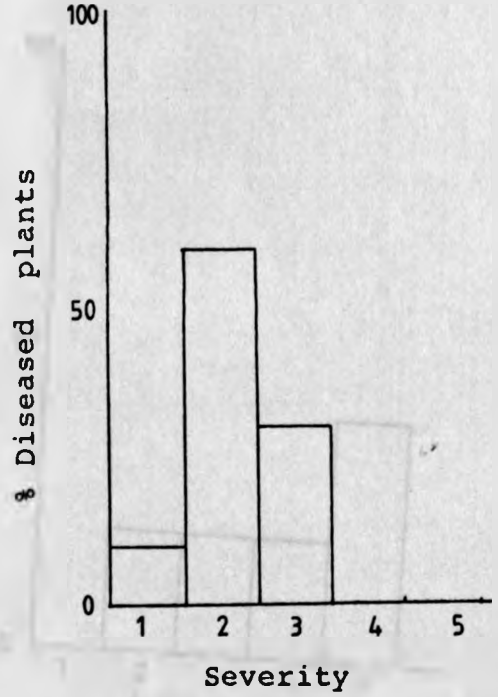

Fig. 2. Disease severity on susceptible tomato Cv. "Moneymaker" infected by Isolate B of C. michiganense.

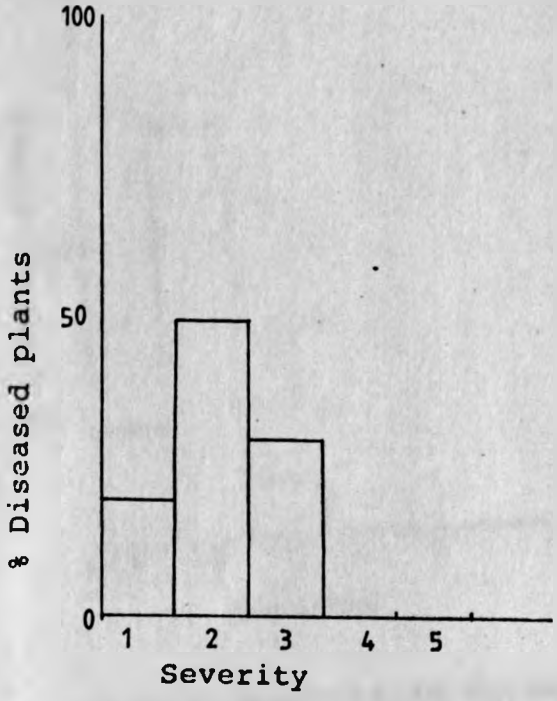

Fig. 3. Disease severity on susceptible tomato Cv. "Moneymaker" infected by Isolate C of C. michiganense.

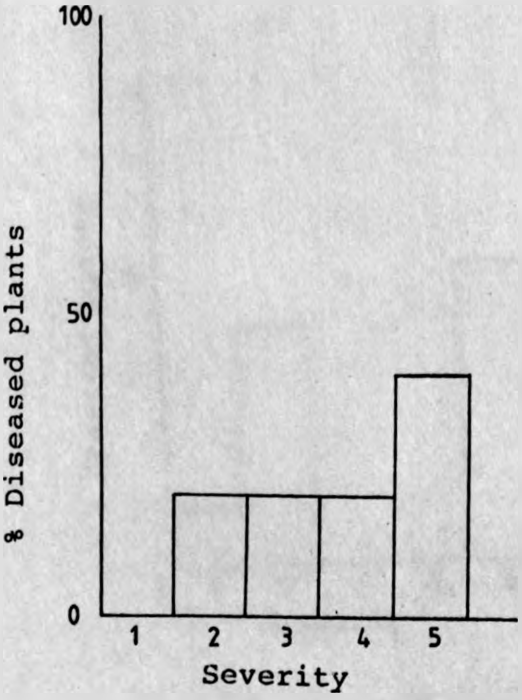

Fig. 4. Disease severity on susceptible tomato Cv. "Moneymaker" infected by Isolate D of C. michiganense.

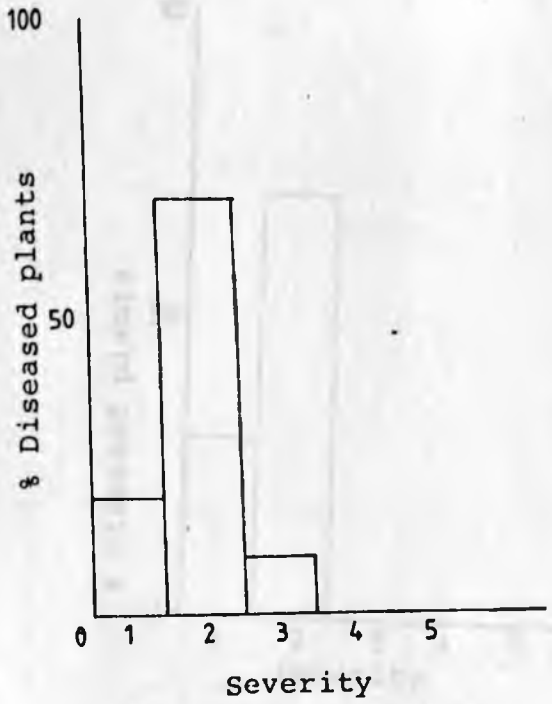

Fig. 5. Disease severity on susceptible tomato Cv. Moneymaker infected by Isolate E of C. michiganense.

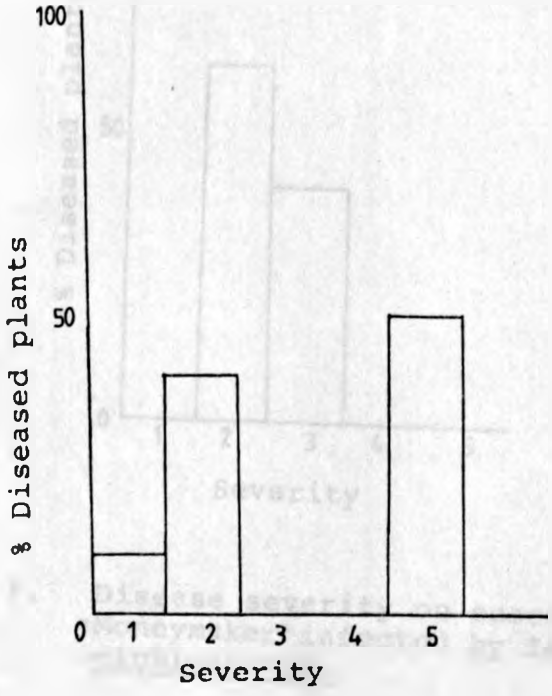

Fig. 6. Disease severity on susceptible tomato Cv. Moneymaker infected by Isolate F of C. michiganense.

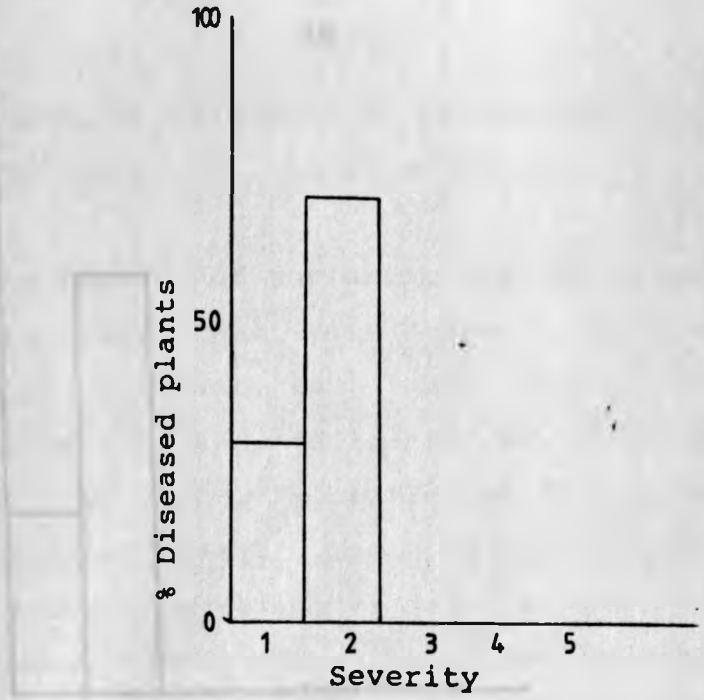

Fig. 7. Disease severity on susceptible tomato Cv. "Moneymaker" infected by Isolate G of C. michiganense.

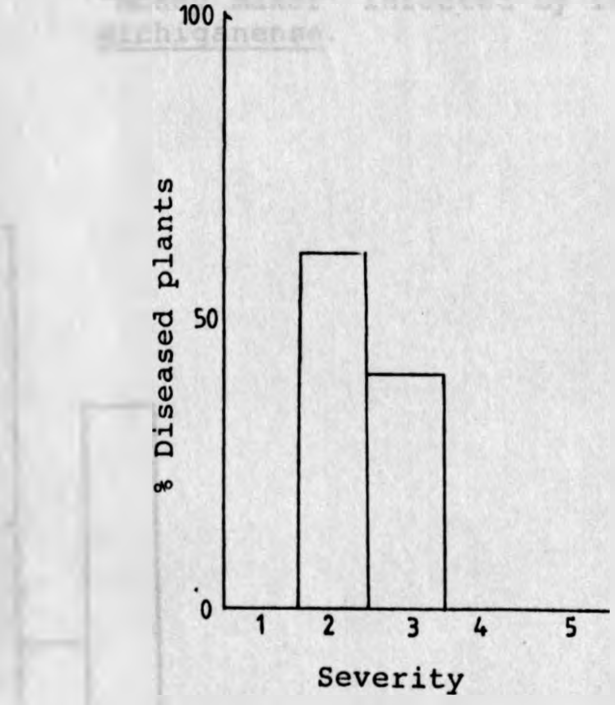

Fig. 8. Disease severity on susceptible tomato Cv. "Moneymaker" infected by Isolate H of C. michiganense.

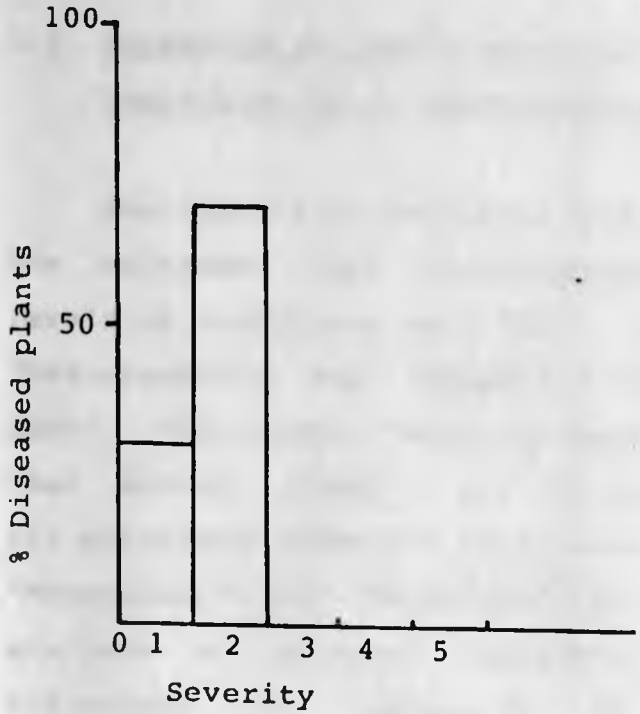

Fig. 9. Disease severity on susceptible tomato Cv. "Money maker" infected by isolate I of C. michiganense.

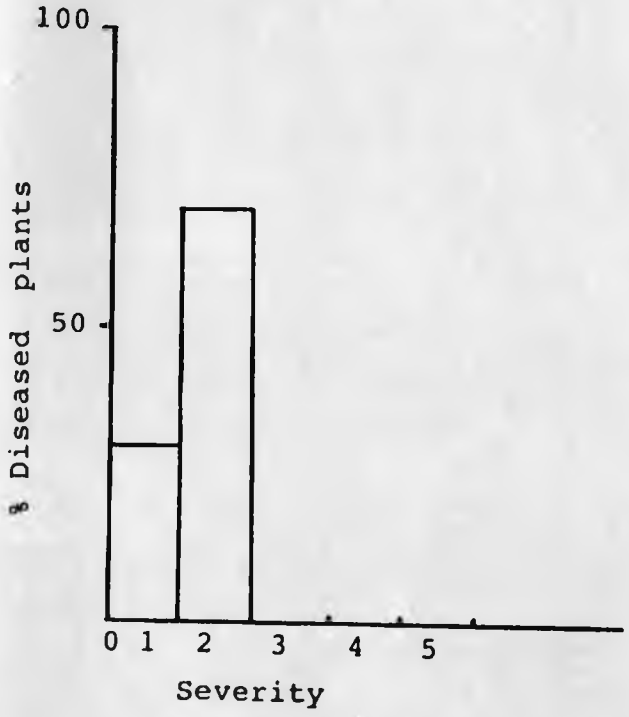

Fig. 10. Disease severity on susceptible tomato Cv. "Money maker" infected by isolate J. of C. michiganense.

4.3 Screening of tomato cultivars for resistance to *C. michiganense*

The results of screening are tabulated in Table 5. The cultivars that were demonstrated to have high levels of resistance were "MR4", "$CmVF_{232}$", "Monense", "Okitsusozai", and "Bulgaria" (P.I. 324708). "Heinz 2990", "USA-Utah", "Victoria Dwarf", "Cervena Kapha", "Red currant 1149K", and "Plovdiv 8/12" (Bulgaria 12) exhibited moderate resistance. Cultivars "Roma", "Moneymaker" and "Marglobe" were susceptible. The analysis of variance (Appendix 6) showed that the difference in resistance of cultivars to *C. michiganense* was significant at 5% level.

Table 5: Screening of tomato cultivars using the highly virulent isolate of *C. michiganense*

Cultivar	Mean Severity Score*	Level of resistance
MR4	1.4_a	High resistance
Cm VF_{232}	1.6_a	High resistance
Monense	1.8_a	High resistance
Okitsu sozai No.1-20	1.9_{ab}	High resistance
Bulgaria (P.I.324708)	1.9_{ab}	High resistance
Bulgaria (P.I.324707)	2.0_b	Moderate resistance
Heinz 2990 (P.I.330727)	2.1_b	Moderate resistance
USA - Utah (P.I.344102)	2.2_{bc}	Moderate resistance
Victoria Dwarf(P.I.319895)	2.2_{bc}	Moderate resistance
Cervena Kapha (P.I.34095)	2.2_{bc}	Moderate resistance
Red Currant 1149K (P.I.344102)	2.4_{bc}	Moderate resistance
Plovdiv 8/12 (Bulgaria 12)	2.4_{bc}	Moderate resistance
Roma	2.8_{cd}	Susceptible
Moneymaker	3.0_d	Susceptible
Marglobe	3.1_d	Susceptible

*Means within columns followed by the same letter(s) are not significantly different at the 5% level according to Duncan's New Multiple Range Test.

4.4 Influence of *Meloidogyne incognita* on the development of bacterial canker

Bacterial canker-susceptible cultivar "Moneymaker" and resistant cultivar "MR4" inoculated at transplanting with *C. michiganense* and *M. incognita*, developed more severe bacterial canker symptoms than those inoculated with *C. michiganense* alone. This is illustrated in Figures 11 and 12, and Table 6. Severe symptoms were first observed in plants of the canker-susceptible "Moneymaker" (Figure 11). It is interesting to note that there were no seedlings with a score of 3 or more, 29 days after inoculation of canker-resistant cultivar "MR4" with *C. michiganense* alone (Figure 12). It was also observed that, 35 days after inoculation, 80% of seedlings of "Moneymaker" inoculated with both pathogens had a score of 3 or more, whereas those inoculated with *C. michiganense* alone had only 40% of the seedlings with a score of 3 or more (Figure 11). Analysis of variance (Appendices 7 and 8) showed that the two treatments were significantly different for both the cultivars tested ($p<0.05$).

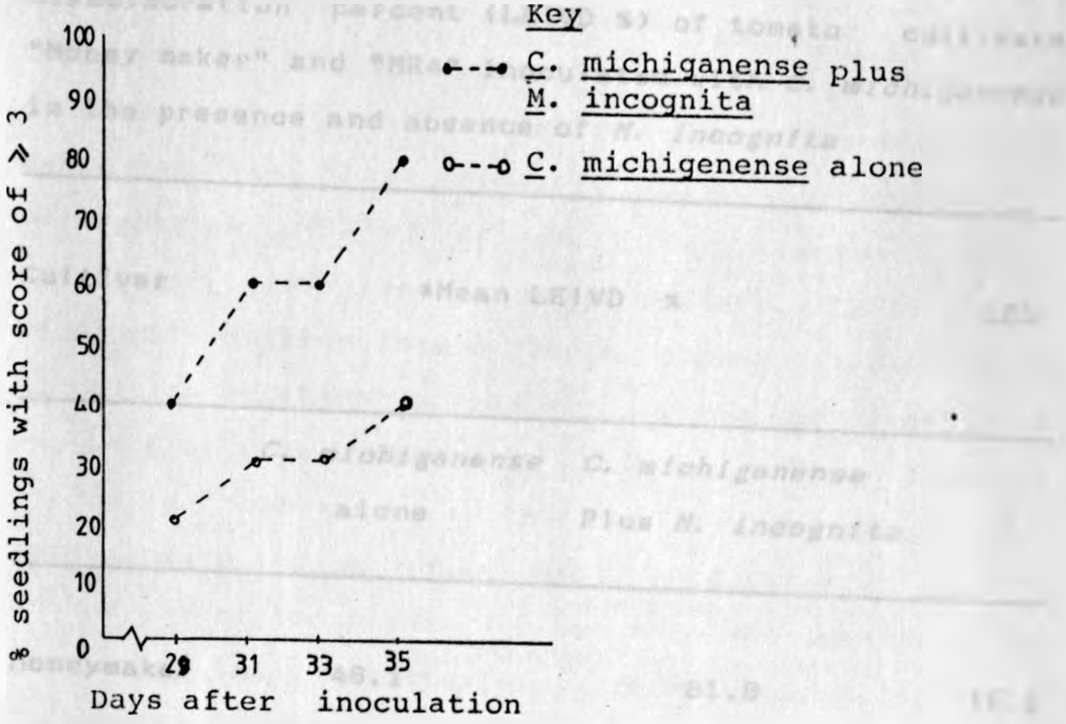

Fig. 11. % seedlings with score ⩾ 3 of "Moneymaker" inoculated with C. michiganense either alone or with M. incognita.

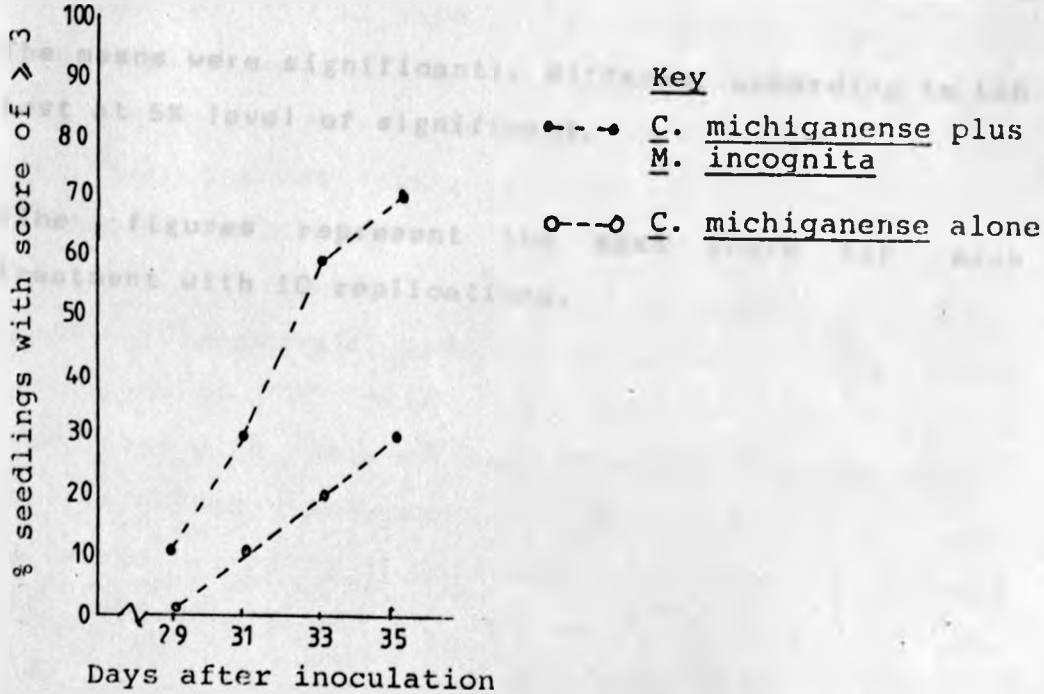

Fig. 12. % seedlings with score ⩾ 3 of "MR4" inoculated with C. michiganense either alone or with M. incognita.

Table 6: Mean linear extent of internal vascular discolouration percent (LEIVD %) of tomato cultivars "Money maker" and "MR4" inoculated with *C. michiganense* in the presence and absence of *M. incognita*

Cultivar	*Mean LEIVD %		LSD
	C. michiganense alone	*C. michiganense* Plus *M. incognita*	
Moneymaker	46.1	81.8	15.1
MR4	39.5	74.7	18.1

The means were significantly different according to LSD test at 5% level of significant.

*The figures represent the mean score for each treatment with 10 replications.

5. DISCUSSION

5.1 Identification of isolates of *C. michiganense*

The cultural characteristics of the bacterial isolates in NBY agar along with the biochemical and pathogenicity tests carried out gave results which conformed to those expected for *Corynebacterium michiganense* (Breed *et al.*, 1957; Jensen, 1952; Ramamurthi, 1959; and Vidaver and Starr, 1979). The bacterial isolates formed smooth, convex colonies with coloration in shades of yellow . There was a variation in rate of growth among the isolates. This confirms Strider's (1969) report, that colony characteristics are variable, being influenced by conditions of growth and nature of the strain. Given that the conditions of growth for the ten bacterial isolates were uniform, the variations in colony characteristics is likely to have arisen from differences in the nature of the strains. All the ten isolates tested were Gram-positive, non-acid fast cells that were short pleomorphic rods. They were oxidase negative, catalase positive, NaCl tolerant and fermenters of all the carbohydrates tested (Table 3). All the bacterial isolates exhibited positive reactions to TTC test.

In pathogenicity test, there was a variation among the isolates in their incubation periods. Plants inoculated with isolates D and F showed symptoms much earlier than the rest. The symptoms included unilateral wilting of leaflets, formation of cankers and discolouration of the vascular system. The

variation in incubation periods is most probably a reflection of differences in virulence of the *C. michiganense* isolates.

5.2 Virulence of *Corynebacterium michiganense* isolates on *Lycopersicon esculentum*

Based on the mean severity scores of the ten bacterial isolates (Table 4), it is possible to list the isolates under four virulence groups. Bacterial isolates D and F would fall under virulence group 1, the most virulent group; isolate H would fall under group 3; isolates A, B, C and E would fall under group 4, whereas isolates G, I and J would fall under group 8, the least pathogenic group. The results agree with those of previous reports (Baines, 1947; Bryan, 1930; Strider and Lucas, 1970; Thyr, 1972) that differences in virulence of bacterial strains of *C. michiganense* exist.

Variation in the virulence of strains can be attributed to: a) genetic changes in the pathogen that has long occupied a given area, b) introduction of an old organism to a new area or c) changes in the cropping systems which affect the ecological niche of the pathogen (Schuster and Coyne, 1975). Lincoln (1940) found that the virulence of a bacterial population increased through mutation and selection during passage through a resistant host. Anyone, or a combination of the above factors may probably have been responsible for variation in virulence among the bacterial isolates.

Differences in virulence of bacterial strains of *C. michiganense* may account for the susceptible reaction of some reported resistant tomato cultivars in other parts of the world (Boelema, 1980). This implies that a cultivar which is resistant in a particular area may not necessarily be resistant to *C. michiganense* in another area of the world. Out of the ten isolates tested, isolate D was found to be the most virulent (Table 4) and was therefore used to screen the tomato cultivars for resistance to *C. michiganense*. Use of highly virulent strains is imperative to any breeding program if an acceptable level of resistance is to be maintained.

5.3 Screening of tomato cultivars for resistance to *Corynebacterium michiganense*

The results of screening for resistance indicated five of the twelve reportedly resistant tomato cultivars to be highly resistant to strain D of *C. michiganense* (Table 5). The results obtained agree with many previous workers (Boelema, 1980; Ematty and John, 1973; Forster and Echandi, 1973; William and Metcalf, 1984) that "MR4", "Monense", "CmVF232", "Okitsu sozai No. 1-20" and "Bulgaria" (P.I.324708) are resistant. Cultivars "Bulgaria" (P.I.324707), "Heinz 2990", "USA-Utah", "Victoria Dwarf", "Cervena Kapha", "Red Currant 1149K" and "Plovdiv 8/12" (Bulgaria 12) exhibited moderate resistance. These cultivars have been reported as resistant by previous workers (Thyr, 1976; Alexander *et al.*, 1942; Elenkov, 1965). Many

s influence the reaction of tomato cultivars to
ion by *C. michiganense*. The most significant
s are virulence of the bacterial strain,
ation procedure and dose, age of inoculated
ngs and environmental temperature. Strains from
ent geographical areas of the world have been
to differ in virulence (Thyr, 1972). This is
ly one of the main causes of differences in
s of workers screening for resistance in tomato
ars in different parts of the world. Boelema
found the accession "Plovdiv 8/12" (Bulgaria 12)
ore resistant than "Roma VF" a reportedly
tible cultivar in South Africa.

Various inoculation methods have been used
ving many parts of the tomato plant including
, stems, petioles, leaves and cotyledons. However
inoculations and severing of petioles with
minated pair of scissors have been reported as
ble (De Jong and Honma, 1976; Van Steekelenburg,
. Difference in inoculation procedure can be a
e of difference in the results of screening for
tance (De Jong and Honma, 1976). Strider (1970)
ted that disease development was rapid and uniform
plants that were inoculated at 14 days of age but
se development was slower in plants inoculated at
days of age. Thus age factor can be a source of
erence in the results of screening for resistance.
was observed by Forster and Echandi (1973) that
-week-old plants at 24°/18°C day and night
eratures showed greater differences in disease
ngs than those at 20°/18°C, 28°/18°C or 32°/18°C.

Anyone, or a combination of the above discussed factors may probably have been responsible for variation in screening results by different workers. This may explain the reason why the reportedly resistant cultivars exhibited moderate resistance.

"Marglobe" and other commercially grown varieties have been proven susceptible by many previous workers (Bryan, 1930; Fenner, 1930; Hassan *et al.*, 1968; Yatzynina, 1951). The susceptibility of the cultivars "Marglobe", "Moneymaker" and "Roma" were confirmed. As shown in this study and elsewhere (Thyr, 1976) resistance to *C. michiganense* is only relative (not that of immunity).

5.4 The influence of *Meloidogyne incognita* on the development of bacterial canker of tomato

From the results shown graphically in Figures 11 and 12, bacterial canker symptoms were more severe in both tomato cultivars "Moneymaker" and "MR4" inoculated with *C. michiganense* and *M. incognita* than those inoculated with *C. michiganense* alone. Also, the mean LEIVD % (Table 6) for both cultivars were higher for plants inoculated with both the pathogens than those inoculated with *C. michiganense* alone. This result confirms the report by previous workers (De Moura *et al* 1975) who on their study of interaction of *Meloidogyne* and *C. michiganense* found that their combined pathological potential were far greater than the individual effects of *C. michiganense*. In addition to the higher level of severity exhibited by the plants

inoculated with both the pathogens, the development of bacterial canker was more rapid than that of *C. michiganense* alone (Figures 11 and 12).

Many previous workers (Carne, 1926; Kalinko, 1936; Holdeman, 1954; Raski and Hewitt, 1961) have reported nematodes as wound agents providing avenues for entry of either bacterial or fungal pathogens. But in this particular study *C. michiganense* was inoculated into the seedlings through the petioles of first true leaves whereas *M. incognita* inoculated through the roots. This implies that other factors in addition to the provision of avenues by nematodes through mechnical injury of the roots may be responsible for the lowering of the resistance of the host plants to bacterial canker. Pitcher (1963) stated that biochemical or physiological changes may be of equal or greater importance than mere introduction of the bacterium into the susceptible tissues of the host. These findings place additional stress on the importance of nematode control not only to decrease losses from this pathogen directly, but also for the control of bacterial canker. The most practical long-range root-knot control program is based on use of resistant varieties. Varieties resistant to root knot and bacterial canker can withstand both pathogens, thus reducing losses from complexes that may exist between them.

LITERATURE CITED

Alexander, L.J., R.E. Lincoln, and V. Wright, 1942. A survey of the genus *Lycopersicon* for resistance to the important tomato diseases occurring in Ohio and Indiana. Plant Disease Reptr. Suppl. 136: 51-85.

Ark, P.A., and H.E. Thomas, 1936. *Anguilla pratensis* in relation to root injury of apple and other fruit trees. Phytopathology 26: 1128-1134.

Ark, P.A., 1944. Studies on bacterial canker of tomato. Phytopathology 34: 394-400.

Ark, P.A., and Thompson, 1960. Additional host for tomato canker organism, *Corynebacterium michiganense*. Plant Disease. Reptr. 44:98-99.

Baines, R.C., 1947. Perenial night shade a host for *Corynebacterium michiganense*. Phytopathology 37: 359 (Abstr.).

Bates, G.R., 1961. Branch of Botany, Plant pathology and Seed Testing Rept. Ministry of Agric. Rhodesia Nyasaland, 1959/1960. pp. 50-57.

Basu, P.K., 1966. Conditions for symtomatological differentiation of bacterial canker, spot and speck on tomato seedlings. Canadian J. Plant Sci. 46:525-530.

Bergey, G.D.H.,F.C. Harrison, R.S. Breed, B.W. Hammer, and F.M. Huntoon, 1923. Bergey's manual of determinative bacteriology 1st Ed., P. 174. William and Wilkins Co., Baltimore.

Binder, E. and M.T. Hutchinson, 1959. Further studies concerning the effect of root-knot nematodes *Meloidogyne incognita acrita* on the susceptibility of the chesapeake tomato to Fusarium wilt. Plant disease Reptr. 43: 972-978.

Boelema, B.H., 1980. Resistance to *Corynebacterium michiganense* in tomato cultivars and breeding lines. Phytophylactica 12: 81-82.

Bowman, Pamela and and J.R. Bloom., 1966. Breaking resistance of tomato varieties to Fusarium wilt by *Meloidogyne incognita*. Phytopathology 56: 791 (Abstr.).

Breed, R.S., E.G.D. Murray, and A.P. Hitchens, 1957. Bergey's manual of determinative bacteriology. 7th Ed. Williams and Wilkins. Co., Baltimore, p. 1094.

Bryan, M.K., 1930. Studies on bacterial canker of tomato. J. Agric. 41: 825-851.

Bryan, M.K., 1930. An albino strain of *Alplanobacter michiganense*. Phytopathology 20: 141 (Abstr.).

Carrol, J., 1987. Economy. Africa South of Sahara. Europa, 1987: 556-563.

Clark, F.E.,1940. Notes on types of bacteria associated with plants roots. Kansas Acad.Sci.Trans. 43:75-84.

Clark, F.E., 1951. The generic classification of certain cellulolytic Corynebacteria. Soil Sci.Soc. Am. Proc. 15: 180-182.

Clark, F.E., 1952. The generic classification of the soil Corynebacteria. Intern. Bull. Bacterial. Nomen. and Taxon.2:45-56.

Colquhoun, T.T., and D.F. Mcarthy, 1943. The Grand Rapids disease of tomato. J. Dept. Agri. Southern Australia 46:310-313.

Conn, H.J., 1947. A protest against the misuse of the generic name *Corynebacterium*. J. Bacteriol. 54: 10.

De Jong, J., and S. Honma, 1976. Evaluation of screening techniques and determination of criteria for assessing resistance to *Corynebacterium michiganense* in tomato. Euphytica 25: 405-414.

De Moura, R.M., E. Echandi, and H.T. Powell, 1975. Interraction of *Corynebacterium michiganense* and *Meloidogyne incognita* on tomato. Phytopathology 65: 1332-1335.

Elenkov, Elnko, 1965. Breeding of tomatoes resistant to bacterial canker. Mezhunar sel'skokhoz, zh. 6: 40-44 (In Russian).

Ematty, D.A., and C.A. John, 1973. Evaluation of resistance to bacterial canker of H2990, a new tomato variety. Plant Disease Reptr. 57: 584-586.

Fawcett, D.H., and M.K. Bryan, 1934. Color in relation to virulence in *Aplanobacter michiganense*. Phytopathology 24: 308-309.

Fenner, L.M., 1930. Bacterial canker to tomato. Plant Disease Reptr. 14: 134-140.

Forster, R.L., and E. Echandi, 1973. Relation of age of plants, temperature, and inoculum concentration to bacterial canker development in resistant and susceptible *Lycopersicon* spp. Phytopathology 63: 773-777.

Grogan, R.G., and J.B. Kendrick, 1953. Seed transmission, mode of overwintering and spread of bacterial canker of tomato caused by *Corynebacterium michiganense*. Phytopathology 43: 473 (Abstr.).

Grubben, G.J.H., 1977. Tropical vegetables and their genetic resources. Edited by H.G. Tindall and J.T. Williams. Rome. pp. 25-30.

Hainsworth, E., 1962. An economic approach to nematode problems affecting Kenya Agriculture. Plant nematology conference, Kikuyu, 24-28 October, 1960, Proc. CCTA. Pub. No. 86, 35 pp.

Hassan, A.A., D.L. Strider and T.R. Konsler, 1968. Application of cotyledonary symptoms in screening for resistance to tomato bacterial canker and host range studies. Phytopathology 58: 233-239.

Haven, L., 1945. Nightshade, source of tomato infection. Seed world 57: 18.

Hawn, E.J., 1963. Transmission of bacterial wilt of alfalfa by *Ditylenchus dipsaci*. Nematologica 9: 65-68.

Holdeman, Q.L., 1954. Nematodes as possible members of disease complexes other than pathogens. Plant Diseases Reptr. Suppl. 226: 77-79.

Huber, D.M., 1980. Effect of nitrate nitrogen on growth of *C. michiganense* in the tomato plant. "Plant Disease" and Advanced Treatise" (J.G. Horsfall and E.B. Cowling, eds.), Vol. 5, pp. 381-405. Academic Press, New York.

Hugh, A. J., and N.T. Powell, 1968. Influence of Root knot nematodes on bacterial wilt development in flue-cured tobacco. Phytopathology 59: 486-491.

Hunger, F.W.T., 1901. Eeen bacterie-ziekte den tomaat. S. land Plantenuin meded 48: 4-57.

Jensen, H.L., 1934. Studies on Saprophytic *Mycobacteria* and *Corynebacteria Linnaen* Soc. New South Wales, Proc. 59: 19-61.

Jensen, H.L., 1952. The coryneform bacteria p. 77-90. In: Clifton, C.E.S. Raffee, and H.A. Baker (Editors). Ann. Rev. Microbiol. Vol. 6, 1954. Annual Reviews Inc., Stanford, California 492 p.

Jones, L.K., 1938. Bacterial canker of tomato. State Coll. Washington Ext. Bull. 242. 2P.

Kendrick, J.B., Jr. and J.C. Walker, 1948. Predisposition of tomato to bacterial canker. J. Agr. Res. 77: 169-186.

Kotte, W., 1930. The bacterial canker of tomato, a new plant disease for Germany. Zeitschr. Fur. Pflanzen-schutz 40:51-56.

Kovacs, N., 1956. Identification of Pseudomonas pyocyanea by the oxidase reaction. Nature (Lond.) 178: 763.

Kuriyama, T., and Kuniyasu, 1974. Studies on the breeding of disease resistant tomato by interspecific hybridization. III. On the

breeding of a new line resistant to bacterial canker caused by *Corynebacterium michiganense*. Bull, Veg. ornam. Crops. Res. Stn. Japan, Ser. A. No. 1: 93-107.

Laterrot, H.R. Brand, and Daunay, 1978. La resistance on *Corynebacterium michiganense* Chez la tomate. Etudi bibligraph: que. Ann. Amerlior Plantes 28: 579-591.

Layne, R.E.C., and J.R. Rainforth, 1966. A new symptom of bacterial canker resulting from systemic infection of tomato fruits and its implications in dissemination and seed transmission. Canadian J. Plant Sci. 46: 371-374.

Layne, R.E.C., 1967. Foliar trichomes and their importance as infection sites for *Corynebacterium michiganense* on tomato. Phytopathology 57: 981-985.

Lincoln, R., 1940. Bacterial Wilt resitance and genetic host parasitic interactions in maize. J. Agric. Res. 6: 217-239.

Lucas, G.B. J.N. Sasser, and A. Kelman, 1955. The relationship of root-knot nematodes to Grandville wilt resitance in tobacco. Phytopathology 45: 537-540.

Madumadu, G.G., 1979. Inheritance of resistance to root-knot nematode, *Meloidoyne javanica* (Treub.) chitwood, and some yield characters in tomato,

Lycopersicon esculentum Mill. Msc. Thesis.

Madumadu, G.G., 1985. Inheritance of resistance to *Corynebacterium michiganense* (E.F. Sm.) H.L. Jens. in tomato *Lycopersicon esculentum* and effect of host nutrition (H and Ca) on resistance. Ph.D. Dissertation.

Migula, W., (1897-1900). System der Bakteriea custav Fisher, Jera.

Mukoo, H., 1962. Bacterial canker of tomato. Plant Protect. (Japan) 16:453-456.

Nattrass, R.M. and A. Ciccarone, 1946. Bacterial canker of tomatoes in Kenya. East African Agric. J. 12: 26-29.

Orth, H., 1937. Investigations on the biology and control of the agents of bacterial wilt of tomatoes. (*Bact. michiganense* E.F.S.). Zbl. Bakt., Abt. 2. 96: 376-402.

Patel, M.K. and Y.S. Kulkarni, 1951. Nomenclature of plant pathogens. Indian Phytopathology 4: 74-84.

Patino-Mendez, G., 1966. Studies on the pathogenicity of *Corynebacterium michiganense* (E.F.Sm.) Jensen and its transmission in tomato seed. Ph.D. Thesis. Univ. Calif. (Dissertation Abstr. Sect. B. 2716): 1692 B - 1693B Dec. 1966).

Pine, T.S., R.G. Grogan, and W.B. Hewitt, 1955. Pathological anatomy of bacterial canker of young tomato plants. Phytopathology 45: 267-271.

Pitcher, R.S., 1961. Role of plant parasitic nematodes in bacterial diseases. Phytopathology 51: 35-39.

Porter, D.M. and N.T. Powell, 1967. Influence of certain *Meloidogyne* species on Fusarium wilt development in Flue-cured tobacco. Phytopathology, 57: 282-285.

Powell, N.T., 1961. Role of plant parasitic nematodes in fungal diseases. Phytopathology 53: 28-35.

Rai, P.V. and G.A. Strobel, 1967. Phytotoxins of *Corynebacterium*. Phytopathology 57: 1008 (Abstr.).

Rai, P.V., 1968. Chemical and biological properties of phytotoxic glycopeptides strains from *Corynebacterium michiganense* Ph.D. Thesis, Mont. State Univ. 66 p.

Ramamurthi, C.S., 1959. Comparative studies on some gram-positve phytopathogenic bacteria and their relationship to the Corynebacteria. Cornell Agr. Expt. Sta. Mem. 366: 52 p.

Raskai, D.J. and Wm. B. Hewitt, 1961. Plant parasitic nematodes as vectors of plant viruses. Phytopathology, 51: 39-47.

Richie, D.F., D.M. Weller and J.L. White, 1976. Isolation and identification of plant pathogenic bacteria. Department of Botany and Plant Pathology. Michigan State Unit. pp. 8-10.

Schaad, H.W., 1980. Laboratory Guide For Identification of Plant Pathogenic Bacteria.

Schuster, M.L. and D.P. Coyne, 1975. Genetic variation in bean bacterial pathogens. Euphytica 24: 143-147.

Smith, E.F., 1910. A new tomato disease of economic importance. Science (N.S.) 31: 794-796 (Abstr.).

Smith, E.F., 1914. The Grand Rapids tomato disease. In Smith's Bacteria in relation to plant disease. Cariegie institution. Wasington 8: 161-165.

Smith, W.P.C. and O.M. Goss, 1946. Bacterial canker of tomatoes. J. Dept. Agric. Western Australia 23: 147-156.

Stamova, L., and M. Yordanov, 1986. Source of high resistance to *Corynebacterium michiganense*. Acta Horticulture 190: 583-584.

Stapp, C., 1930. Contribution to the knowledge of *Bacterium sepedonicum* Speckerm. et. Koth., the causal organism of "bacterial ring root" of the

potato. Zeitschr. Fur. Parasitsukunde 2:756-823.

Steven N.E.,1913."The fungi which cause disease" P.80.

Stewart, R.N., and A.F. Schinder. 1956. The effect of some ectoparasitic and endoparasitic nematodes on the expression of bacterial wilt in cornations.

Strider, D.L., and T.R. Konsler, 1965. Cotyledonary symptoms of bacterial canker of tomatoes. Plant Disease Reptr. 49: 634-635.

Strider, D.L., T.R. Konsler, and A.A. Hassan, 1966. Bacterial canker of tomato. North Carolina Agr. Exp. Sta. Research and Farming 24: 13.

Strider, D.L., 1969. Bacterial canker of tomato caused by *Corynebacterium michiganense*. A literature review and bibliogaphy. North Carolina Agric. Exp. Sta. Tech. Bull. No. 193. 109 pp.

Strider, D.L., and L.T. Lucas, 1970. Variation in virulence of *Corynebacterium michiganense*. Plant Disease Reptr. 54: 976-978.

Strider, D.L., 1970. Tomato seedling inoculations with *Corynebacterium michiganense*. Plant Disease Reptr. 54: 36-39.

Thyr, B.D., 1968. Resistance to bacterial canker to tomato and its evaluation. Phytopathology 58:

279-281.

Thyr, B.D., 1969. Additional sources of resistance to bacterial canker of tomato *Corynebacterium michiganense* Plant Disease Reptr. 53: 234-237.

Thyr, B.D., 1972. Virulence of *Corynebacterium michiganense* isolates on Lycopersicon accessions. Phytopathology 62: 1082-1084.

Thyr, B.D., 1976. Inheritance of resitance to *Corynebacterium michiganense* in tomato. Phytopathology 6: 1116-1119.

Thyr, B.D., 1971. Resistance to *Corynebacterium michiganense* measured in six Lycopesicon accessions. Phytopathology 61: 972-974.

Van Steekelenburgg, N.A.M., 1984. Resistance to *Corynebacterium michiganense* in tomato genotypes. Eupphytica 34: 245-250.

Vidaver, A.K., and M.P. Starr, 1979. Phytopathogenic coryneform and related bacteria. In M.P. Starr, H. Stolp, H.G. Truper, A. Barbrvo, and H.G. Schelgel, eds. The Prokaryotes: A handbook on Habitats, Isolation and Identification of Bacteria.

Villareal, R. L. 1980. Tomatotes in the tropics. 1980. edition pg. XV.

Wakimoto, S. and T. Uematsu and H. Mukoo., 1968. Bacterial canker disease of tomato in Japan. Natt. Inst. Agric. Sci. (Japan) Series c, Bull. 22: 269-281.

William C., and J. Metcalf, 1984. Breeding for resistance to bacterial spot and bacterial canker in tomatoes. Hortscience 19: 69 (Abstr.). Phytopathology 46: 219-222.

Whyllie, T.D., and D.P. Taylor, 1960. Phytophthora root rot of soybeans as affected by soil temperature and *Meloidogyne hapla*. Plant Disease Reptr. 44: 543.

Yatzynina, K.N., 1941. Breeding for tomato varieties resistant to bacterial canker. *Aplanobacter michiganense* E.F. Smith. Rev. Appl. Mycol. 21: 473.

Yatzynina, K.N., 1951. Canker-resistant varieties of tomato. Hort. Abstr. 21: 3664.

7.

APPENDICES

Appendix 1: Horticultural Exports - 1986.

Production	Weight in Kgs.	Value in Kshs.
Tomatoes	230,718	1,984,174
Pineapples	863,723	5,182,338
Melon	21,660	314,070
Mangoes	2,941,232	50,000,944
Avocados	2,150,783	17,206,264
Strawberries	275,358	11,014,320
Passion fruits	645,732	11,231.176
Pawpaw	137,500	1,718,750
Limes/lemon	66,887	769,200
Misc. Fruits	160,510	1,861,916
Beans (French)	9,096,901	154,647,317
Beans (Bobby)	477,769	5,064,351
Capsicums	15,538	149,164
Okra	1,738,207	21,901,408
Anbergines	1,692,482	12,693,615
Karela	1,278,747	17,390,959
Mooli	7,100	60,350
Dudhi	867,660	9,289,536
Chillies	2,087,494	26,302,424
Valores	297,542	3,719,273
Guar	304,640	3,533,824
Other Asian vegetables	1,827,839	17,365,470
Courgettes	230,718	1,984,174
Leeks	13,391	115,162
Carrots	26,583	199,372
Misc. vegetables	527,547	6,594,337
Misc. root produce	57,851	439,667
Cutflowers	8.264.912	247.947.360
Totals	36,211,007	630,367,039

Source:Horticultural Crops Development Authority.

Appendix 2: Tomato Production estimate (hectares) in Kenya 1982-85

Province	1982	1983	1984	1985
Central	1835.5	2585.8	2321	4286
Eastern	1093.4	1027	1457.5	3282
Rift Valley	765	1130	1630	855
Western	651	850	532	-
Coast	459	-[a]	-	-
Nyanza	-	1077	1429.8	1740
North Eastern	-	20.25	85.6	32.2

Source: Provincial Director of Agriculture Annual reports.

[a] = A dash denotes no figures available.

Appendix 3: Tomato production estimates in Central and Eastern Provinces 1984/85

Province	District	1984		1985	
		Ha.	Tons	Ha.	Tons
Central	Kiambu	894	18420	2600	2600
	Nyandarua	60	1080	14	125
	Nyeri	295	8850	342	8535
	Muranga	657	10799	918	11061
	Kirinyaga	616	6240	412	8240
	Sub-total	2322	45389	4286	53961
Eastern	Kitui	-	-	-	-
	Machakos	944	13040	2273	29850
	Embu	130	400	680	720
	Meru	379	5029	305	7150
	Isiolo	4.5	36	10	80
	Marsabit	-	-	16	336
	Sub-total	1457.5	18505	3282	38136
	Total	3779.5	63894	7568	92097

Source: Provincial Director of Agriculture annual reports.

Appendix 4: Formulae of Media and Chemical solutions

1. Potting soil medium for glasshouse work

a)	Top Forest soil	40 tins*
b)	Horse manure	20 "
c)	Gravel Quarter inch	20 "
d)	Coffee hulls	20 "
e)	Dried animal blood	1 "
f)	Sulphate of ammonia	1.41 kg
g)	Single super phosphate	0.84 kg
h)	Muriate of potash	0.56 kg

*1 tin has a capacity of 24 cm^3

2. Yeast extract-dextrose - Calcium carbonate (YDC) medium

	g/l
Yeast extract	10.00
*Dextrose (glucose)	20.0
Calcium carbonate, light powder	20.0
Agar	15.0

*Should be autoclaved seperately.

3. Acid mercuric chloride solution

$HgCl_2$ -	12 g
Distilled water	80 ml
Conc. HCl	16 ml

4. Nutrient-broth yeast extract agar (NBY) medium

	g/l
Nutrient broth (Difco)	8.0
Yeast extract (Difco)	2.0
K_2HPO_4	2.0
KH_2PO_4	0.5
Agar	15.0

Add after autoclaving separately, 50 ml 10 % glucose, 1.0 ml 1 M $MgSO_4.7H_2$ after autoclaving adjust to pH 7.2.

Appendix 5: Analysis of variance of the results obtained in the study of virulence of *C. michiganense* isolates on tomato Cv. "Moneymaker"

Source of Variation	Degree of freedom	Sum of squares	Mean sum of squares	Fcal
Total	99	111		
Treatment	9	48	5.3	7.6*
Error	90	63	0.7	

*Significant at p = 0.05.

Appendix 6: Analysis of variance of the results of screening of tomato Cvs. for resistance of *C. michiganense*

Source of variation	Degree of freedom	Sum of squares	Mean sum of squares	Fcal
Total	149	82.6		
Treatment	14	32.9	2.35	6.35*
Error	135	49.7	0.37	

*Significant at p = 0.05.

Appendix 7: Analysis of variance of the interraction *Meloidogyne incognita* and *C. michiganense* on tomato Cv. "Moneymaker"

Source of variation	Degree of freedom	Sum of squares	Mean sum of squares	Fcal
Total	19	11008.1		
Treatment	1	6379.6	6379.6	24.8*
Error	18	4628.5	257.1	

*Significant at p = 0.05.

Appendix 8: Analysis of variance of the interraction of *Meloidogyne incognita* and *C. michiganense* on tomato Cv. "MR4"

Source of	Degree of freedom	Sum of squares	Mean sum of squares	Fcal
Total	19	12866.3		
Treatment	1	6184.6	6184.6	16.7
Error	18	6681.7	371.2	

*Significant at p = 0.05.