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ECOLOGY OF PSEUDOMONAS SYRINGAE PV. PHASEOLICOLA IN NORTHERN TANZANIA

By

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ABSTRACT

ECOLOGY OF PSEUDOMONAS SYRINGAE PV. PHASEOLICOLA IN NORTHERN TANZANIA

By

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This study was undertaken to investigate the ecology of Pseudomonas syringae pv. phaseolicola, the causal agent of halo blight of beans, in the Arusha and Kilimanjaro regions, northern Tanzania. A total of 118 isolates were collected from November 1988 to February 1990, and examined for pathogenic variability using four differential bean cultivars. Three races were found; race 2 occurred at a higher frequency (52.5%) than race 1 (44.9%) and race 3 (2.5%). Some isolates of race 2, designated race 2P, produced a brown diffusible pigment on agar media. Some race 2P isolates grew at a higher temperature (34 $\pm$ 2°C) than the non-pigment-producing race 2 isolates or race 1 and 3 isolates. Race 2 isolates obtained from bean debris in Monduli, Arusha region, were more virulent than those isolated from growing bean plants. They induced systemic chlorosis and stunting on the usually resistant differential bean cultivar, Edmund.

The ability of P.s. pv. phaseolicola to survive in bean debris and in dead standing bean plants varied depending on race, geographical location, depth of placement in the soil and the bean genotype used. Volunteer bean plants also provided an alternative survival site for halo blight bacteria. Of the 17 weed species belonging to 10 families, only Neonotonia wightii served as a perennial reservoir of P.s. pv. phaseolicola. The weed survived the dry periods on fences, hedge rows, by roadsides, on ditch banks near bean fields and in corners of farmers' fields. Only race 1 of the bacterium was recovered from N. wightii. However, under artificial inoculation the weed was susceptible to all three races.

Since race 1 of P.s. pv. phaseolicola was not seed-borne in N. wightii, this precludes the weed spreading the halo blight pathogen into new areas through seed.

Intercropping beans with maize favored multiplication of halo blight bacteria on and in bean leaves, and resulted in more severe disease on pods and in a higher percentage of seed infection, than occurred in beans grown in pure stands. Maize leaves did not support high populations of halo blight bacteria, and thus do not seem to provide additional inoculum for the bean crop when the two are grown in association.

In memory of my advisor Dr. A.W. Saettler and my uncle, Muhindi Musiba.

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Chapter 1

GENERAL INTRODUCTION

Beans (Phaseolus vulgaris L.) are an important source of dietary protein in many parts of the world, including Tanzania. Production occurs in a wide range of cropping systems and environments (Allen et al., 1989; CIAT, 1986). In Tanzania, beans are mainly grown in the highlands of northern, southern and western parts of the country where rainfall is adequate for bean production. However, yields have remained relatively low with an average yield of 600 kg/ha in farmers' fields (CRSP, 1990). Like many other tropical bean production regions, diseases, insect pests, and low fertility are the most important production constraints in the country.

Halo blight incited by Pseudomonas syringae pv. phaseolicola (Burk.) Young, Dye and Wilkie is one of the most important diseases of beans in Tanzania. The disease is very prevalent in high, cool, wet areas where beans are mainly grown, and losses due to halo blight can be severe (Silbernagel and Mills, 1987). Because of the widespread occurrence and the severity of halo blight in areas where the disease occurs in the country, it is assumed that the disease causes considerable yield reduction. However, the precise extent of losses has not been well established. Recently, in field trials conducted at Lyamungu, northern Tanzania, yield losses of up to 42% in susceptible cultivars were reported (CIAT, 1989). Breeding for halo blight resistance is generally considered the best method of

controlling the disease in small holder production systems in Tanzania, and efforts are being made to produce halo blight resistant varieties (Teri et al., 1990). However, the development of resistant varieties must take into account variability of both the pathogen and the genetic resistance in the host. In addition, successful halo blight control measures require a thorough understanding of the ecology of halo blight bacteria in the country.

While much information on P.s. pv. phaseolicola is readily available in other countries, little is known about the ecology of this bacterial pathogen in Tanzania. The objective of this dissertation was, therefore, to examine the ecology of P.s. pv. phaseolicola in northern Tanzania. The study was divided into three parts: pathogenic variation and survival in the soil of halo blight bacteria in northern Tanzania are covered in Chapter 2. Chapter 3 examines the role of weeds in survival of halo blight bacteria. In northern Tanzania, as in many other parts of the country, the majority of the bean crop is grown in association with maize and other crops. Chapter 4 is, therefore, devoted to population dynamics of P.s. pv. phaseolicola in beans when grown in pure stands and in association with maize.

LITERATURE REVIEW

Halo blight of beans is caused by the bacterium Pseudomonas syringae pv. phaseolicola (Burk.) Young, Dye & Wilkie (Young et al., 1978). The disease was first reported and described from the state of New York by Burkholder in 1926. Since then, the disease has been reported in many countries of Central and South America, U.S.A., Africa, Europe and Australia (Buruchara, 1983; Johnson, 1969; Msuku, 1984; Schwartz, 1980; Schwartz and Pastor-Corrales, 1989). In Tanzania and other tropical countries, halo

blight is more prevalent in high, cool, wet areas where beans are produced. In such areas, the disease can cause severe crop losses (CIAT, 1981; Karel et al., 1981; Silbernagel and Mills, 1987).

Occurrence and severity

Yield losses of 23-43 percent have occurred in research fields in Michigan (Saettler and Potter, 1970), and the disease has caused serious losses in Colorado (Schwartz and Legard, 1986). In eastern Africa, the disease can be of economic important in Malawi and Kenya, but rarely in Uganda, except at the highest altitudes of cultivation (Allen, 1983). Halo blight disease has generally been considered of minor importance in Tanzania, especially in some areas of the Kilimanjaro region. However, in recent years severe outbreaks of the disease have been reported in some parts of this region and in other regions such as Arusha, Mbeya and West Lake. These outbreaks have been suggested to be due to the occurrence of a new virulent race, probably race 3 of the pathogen (Gondwe, 1987).

Although it is well known that plant diseases constitute a major constraint to bean production in Tanzania and elsewhere in Africa, there are very few reliable data quantifying crop losses. In addition, it is difficult to extrapolate yield losses made under experimental conditions to those likely to be incurred in agricultural practice, where crop yields are limited by a complex of stress factors, of which diseases are but a part (Allen, 1983). Research geared toward estimating yield losses due to halo blight and other bacterial diseases in Tanzania is generally lacking. Such a situation has resulted in underestimating yield losses caused by halo blight disease.

Synonyms of the pathogen

Other names of Pseudomonas syringae pv. phaseolicola found in literature include: Phytomonas medicaginis (Sackett) Bergey et al., var. phaseolicola Burkholder; Bacterium medicaginis (Sackett) E.F. Smith var. phaseolicola (Burkholder) Link & Hull; Pseudomonas medicaginis Sackett var. phaseolicola (Burkholder) Stapp and Kotte; Bacterium puerariae Hedges; and Pseudomonas medicaginis Sackett f.sp. phaseolicola (Burkholder) Dowson (Bradbury, 1986; CMI, 1965).

Morphological and physiological characteristics

Pseudomonas syringae pv. phaseolicola exhibits very distinct morphological, physiological and nutritional characteristics. Cells appear as single straight rods (Sands et al., 1970), and are motile with peritrichous flagella. Cells are gram-negative, strictly aerobic and do not require growth factors. Poly-ß-hydroxybutyrate is not accumulated as an intracellular carbon reserve. In artificial media, especially those deficient in iron, cultures produce a diffusible fluorescent pigment. Arginine dihydrolase activity is absent (Doudoroff and Pallerozin, 1974), and it is oxidase negative (Kovacs, 1956).

The bacteria are capable of utilizing D-gluconate, L (+) arabinose, sucrose, succinate, DL-ß-hydroxybutyrate, transconitate, L-serine, L-alanine and *p*-hydroxy benzoate, but glutarate, mesotartrate, DL-glycerate, iso-ascorbate, erythritol, sorbitol, meso-inositol and N-caproate are not utilized. The optimum temperature for growth is 20-23°C. White to creamy colonies with a bluish hue are produced which may be either smooth or rough (Adam and Pugsley, 1934; Zaumeyer and Thomas, 1957).

It has been observed that the rough and smooth colony forms of P.s. pv. phaseolicola are different from one another serologically and in their reaction to

bacteriophages. The rough forms were later shown to produce less extracellular polysaccharides and to be less virulent than the smooth forms (Carey and Starr, 1957). Extracellular polysaccharides produced by Psp have been associated with pathogenicity on susceptible bean leaves (Buruchara, 1983; Epton et al., 1977).

Host range

Halo blight bacteria can infect various plant species. Moffett (1983), Schwartz, (1989) and Zaumeyer and Thomas (1957) included the following as hosts: common bean (Phaseolus vulgaris L.), lima bean (P. lunatus L.), tepary bean (P. acutifolius A. Gray var. acutifolius), Marcroptilium bracteatum (Nees ex Mart) Maréchal et Baudet, scarlet runner bean (P. coccineus L.), P. polyanthus Greenman, P. polystachyus (L.), pigeonpea (Cajanus cajan (L.) Millsp.), hyacinth bean (Lablab purpureus (L.) Sweet), soybean (Glycine max (L.) Merrill), Vigna angularis (Willd.) Ohwi et Ohasi, mung bean (V. radiata (L.) Wilczek var. radiata), Pueraria lobata (Willd.) Ohwi, glycine (Neonotonia wightii (Arn.) Lackey) and siratro (Macroptilium atropurpureum (DC) Urb. (CIAT, 1987).

Survival

Knowledge as to how a pathogen survives and is disseminated is very important for developing successful control strategies. Halo blight bacterium survives in infected seeds and plant residue on the soil surface, and has been found on volunteer beans in the field early in the growing season (Legard and Schwartz, 1987; Schuster and Coyne, 1975b, Schwartz, 1989). The bacterium survives in these habitats until environmental conditions become favorable for infection. P.s. pv. phaseolicola has also been reported to survive for nine months after passage through sheep which consumed infested plant debris (Starr and Kercher, 1969).

It has been observed that survival in bean plant debris buried under dry condition is often longer than when soil is saturated (Allen, 1983). Buddenhagen (1965) suggested that bacterial pathogens evolving away from the requirements of soil saprophytic existence have tended to develop sustained plant to plant infection cycles often through insect transmission. Coupled with survival in seed, they have thus lost the more complex requirements of survival in the soil. Thus, the halo blight pathogen has essentially been liberated from the requirements of soil phase through seed transmission or through association with a perennial host (Allen, 1983).

Schuster and Coyne (1975b) observed that the more virulent strains of P.s. pv. phaseolicola were better adapted for survival than the less virulent strains. This may be due to the fact that more virulent strains tend to multiply to higher numbers in the host than the less virulent strains. In vitro, P.s. pv. phaseolicola cells survived in liquid nitrogen at -172°C for 30 months or on silica gel at -20°C for 60 months (Leben and Sleesman, 1982; Moore and Carlson, 1975).

The bacterium has a tremendous potential to cause disease. As few as 0.1 percent of halo blight infected seed can, under conducive environmental conditions, lead to severe crop losses in susceptible bean cultivars (Patel et al., 1964, Schwartz, 1980, 1989). Under Wisconsin conditions, as few as 12 infected seeds per hectare may lead to an epidemic of halo blight disease (Walker and Patel, 1964). Zero tolerance of infected seed appears to be a necessity where rates of disease spread are likely to be high. Under British conditions a tolerance level of 1 infected seed per 5 kg has been estimated as the maximum allowable for the halo blight bacterium (Taylor et al., 1979). In tests of disease transmission from seed to seedlings, it has been found that heavily infected seeds usually failed to produce seedlings. Most infections developed from seeds with slight or no symptoms at all (Allen, 1983).

Since the halo blight bacteria survive and are transmissible by seed, in Tanzania it is more severe for farmers who do not purchase high quality seed but rather maintain a portion of their harvest for the next planting (Gondwe, 1987). Programs to produce pathogen-free seed by growing the crop under furrow irrigation are lacking.

Epiphytic phase of *P.s.* pv. *phaseolicola*

The epiphytic phase of P.s. pv. phaseolicola was first reported by Ercolani et al., (1974). Their studies indicated that halo blight bacteria multiplied to some extent on hairy vetch (Vicia villosa Roth, Leguminoseae) near bean fields, especially in field corners under Wisconsin conditions. Using a rifampicin-resistant mutant of P.s. pv. phaseolicola, Stadt and Saettler (1981) observed that the pathogen was capable of establishing an epiphytic phase on bean leaves in Michigan. Under Colorado conditions, Legard and Schwartz (1987) demonstrated that P.s. pv. phaseolicola can occur as an epiphyte on dry beans. They further observed that the bacterium became a predominant epiphyte late in the season when bean plants matured.

Penetration and population dynamics

P.s. pv. phaseolicola enters plants through wounds or stomata during periods of high relative humidity or free moisture (Saettler and Potter, 1970; Zaumeyer and Thomas, 1957). Maino (1972) reported that the bacterium produces hemicellulases which degrade host cell wall material during pathogenesis. The pathogen multiplies epiphytically on blossoms, pods and stem internodes under experimental conditions. P.s. pv. phaseolicola also multiplies rapidly on or near the surface of foliage with or without lesions in the presence of dew (Saettler and Potter, 1970; Stadt and Saettler, 1981).

Dissemination

Dissemination of P.s. pv. phaseolicola between plants occurs by water splash and winds during periods of rainfall. Insects, animals and people moving through the crop when the foliage is wet can also spread the pathogen (Walker and Patel, 1964). Pod infection with halo blight bacteria, especially of the dorsal vascular tissues can lead to internal seed contamination which is the primary means of dissemination. Contaminated seed is important for both local and long distance transport of P.s. pv. phaseolicla (Saettler and Potter, 1970; Stadt and Saettler, 1981).

Symptoms

The disease affects all parts of the plant above ground (Burkholder, 1926; Zaumeyer and Thomas, 1957). Characteristic leaf symptoms initially appear as small, brown, watersoaked spots on the abaxial surface of the leaves 3-5 days after infection (Omer and Wood, 1969). Later, a halo-like zone of greenish yellow tissue develops around the water-soaked areas. Patel and Walker (1965) observed that halos and pronounced systemic chlorosis develop more commonly at 16-20°C than at 24-28°C. They also noted that age and nutritional status of the host greatly influenced disease development. Older leaves were more tolerant than young leaves, while extreme low and high levels of nitrogen, phosphorus and potassium retarded the development of halo blight.

Systemic chlorosis may occur. Both the halo and systemic chlorosis are due to a non-host specific toxin called phaseolotoxin produced by the bacterium during infection (Coyne and Schuster, 1974; Coyne et al., 1971; Schuster and Coyne, 1975a; Schwartz, 1989; Zaumeyer and Thomas, 1957). Phaseolotoxin has been purified and characterized as an ornithine-alanine-arginine tripeptide carrying a phosphosulfamyl group (Ferguson and

Johnston, 1980; Mitchell, 1976; Mitchell and Bieleski, 1977; Patil et al., 1972; Turner and Mitchell, 1985).

The molecular genetics of phaseolotoxin production and immunity in P.s. pv. phaseolicola have been investigated by Tn5 mutagenesis and cosmid cloning procedures (Peet et al., 1985). These procedures indicate that soon after the tripeptide is secreted by bacteria into the plant, plant enzymes (peptidases) cleave the peptide bonds and release alanine, arginine and phosphosulfamylornithine. The latter is the biologically functional moeity of phaseolotoxin (Mitchell and Bieleski, 1977; Patil et al., 1972). The toxin affects cells by binding to the active site of, and inactivating, the enzyme ornithine carbamoyltransferase (OCTase) which normally converts ornithine to citrulline, a precursor of arginine. By its action on the enzyme, the toxin thus causes accumulation or ornithine and depleted levels of arginine (Mitchell and Bieleski, 1977; Patil et al., 1972; Turner and Mitchell, 1985).

Studies by Peet et al. (1985) indicate that a toxin insensitive OCTase activity found in toxigenic strains of the bacterium may be involved in the organism's natural immunity to its own toxin. These researchers also suggest that at least some of the genes involved in phaseolotoxin production, as well as structural gene(s) for OCTase may be clustered. Further research is, however, needed to determine whether the loci affecting phaseolotoxin production are structural genes involved in phaseolotoxin biosynthesis or in other pathways indirectly related to phaseolotoxin production.

There is evidence that phaseolotoxin production may be suppressed in infected bean plants possessing hypersensitive resistance but not in susceptible bean plants (Allen, 1983). However, it has also been reported that pretreatment of resistant cultivars with the toxin can suppress the hypersensitive response and phytoalexin accumulation. Thus, it is likely

that the factor which determines hypersensitive resistance to halo blight disease is associated with suppression of phaseolotoxin production (Gnanamanickam and Patil, 1976).

Stem and pod symptoms include typical greasy spots. On pods, lesions appear as green water-soaked spots which may enlarge and coalasce. Pod lesions in highly susceptible cultivars are water-soaked and may be colorless, while resistant cultivars may show less water soaking and reddish brown edges of restricted lesions (Schwartz, 1989; Stadt and Saettler, 1981; Zaumeyer and Thomas, 1957). Under humid conditions, a cream white exudate may be present in the center of the lesion and older lesions often have a shiny surface appearance indicating the presence of dried bacteria (Zaumeyer, 1932; Zaumeyer and Thomas, 1957). Infected developing pods may rot or become shrivelled and discolored. Stem girdling or joint rot occurs at nodes above the cotyledons when infection originates from contaminated seed (Schwartz, 1989; Zaumeyer and Thomas, 1957).

Very small spots may appear on the seed, or the entire seed may be destroyed. In pigmented seed, it is difficult to distinguish spots caused by halo blight bacteria from those caused by other bean pathogens. Such pathogens include Colletotrichum lindemuthianum (Sacc. et Magn.) Briosi et Cav., the causal agent of bean anthracnose, and Xanthomonas campestris pv. phaseoli (E.F. Smith) Dowson, the causative organism of bean common bacterial blight (Msuku, 1984). A greater proportion of infected seed occurs when infection occurs earlier in plant development (Schwartz, 1989).

Pathogenic variation

Pathogenic variation among populations of P.s. pv. phaseolicola is well known. Using 13 isolates, Jensen and Livingstone (1944) were the first to demonstrate pathogenic variation in P.s. pv. phaseolicola. However, no differences were observed in physiological

tests except for slight differences in growth rate. Both qualitative (Patel and Walker, 1965) and quantitative (Schroth et al., 1971) variation in the pathogenicity of P.s. pv. phaseolicola to beans have been reported. Qualitative differences (pathogenicity) have conventionally depended on the reaction of the bean cultivar Red Mexican UI-3 inoculated artificially.

Patel and Walker (1965) reported the occurrence of race 1 and 2 in the U.S.A. These races were characterized based on the reaction of the inoculated bean cultivar, Red Mexican IU-3, which is resistant to race 1 but susceptible to race 2. On the same basis, the occurrence of these two races has been reported again in the U.S.A. (Schuster et al., 1965); South America (Buruchara and Pastor-Corrales, 1981; Schuster and Coyne, 1975a; Schwartz, 1989); Great Britain (Epton and Deverall, 1965; Wharton, 1967); New Zealand (Hale and Taylor, 1973); Bulgaria (Poryazov, 1975); Kenya (Kinyua and Mukunya, 1981); Malawi (Msuku, 1984; Silbernagel and Mills, 1987); Tanzania (Taylor et al., 1987) and South Africa (Edington, 1990).

The existence of another race of P.s. pv. phaseolicola in the U.S.A. was first suggested by Schuster et al. (1979) and Coyne et al. (1979). Recently, Taylor et al. (1987) reported the existence in Africa of race 3, which was virulent toward bean cultivars with a single gene for resistance derived from the cultivar Red Mexican UI-3. Race 3 caused a hypersensitive reaction in the cultivar Tendergreen. Resistance to race 3 has subsequently been found to be governed by a single dominant gene, which is also present in several cultivars of U.S.A. origin, such as Seafarer and Tendercrop (Harper et al, 1987). Experiments examining the effect of bacterial numbers on lesion production have shown that fewer cells of race 3 than race 1 and 2 were required for symptom development following inoculation of pods. Antagonism between these isolates was not observed in vitro (Harper et al., 1987). D. J. Allen (personal communication) has indicated that race 3 of P.s. pv. phaseolicola occurs in Colombia.

Characterization of halo blight bacteria isolates into race groups has been based on leaf and/or pod reactions (Buruchara, 1983; Hale and Taylor, 1973; Patel and Walker, 1965) following inoculation with bacteria, and on bacteriophage tests (Hale and Taylor, 1973; Taylor, 1970). Schroth et al. (1971) indicated that possibly, there are many strains of P.s. pv. phaseolicola which vary in virulence. Neither race 1 nor race 2 were homogeneous with respect to virulence when tested on leaves of certain bean cultivars. They considered that the practice of separating isolates of P.s. pv. phaseolicola into race 1 and 2 on the basis of their reaction to Red Mexican UI-3 only distinguished strains of bacteria with different degrees of virulence. Similar observations were reported by Szarka and Velich (1979).

Some workers argue that the establishment of pathotypes on a host differential basis is subject to variation in inoculation techniques, environmental growth conditions, and the subjectivity of scoring for the disease (Coddington et al., 1987). Others feel that race designation is not valid because serological tests show that P.s. pv. phaseolicola antiserum is not race specific (Guthrie, 1968). A ^{32}P-labelled DNA probe carrying a gene(s) involved in phaseolotoxin production by P.s. pv. phaseolicola has been used to detect and identify the bacterium in pure and mixed cultures. Hybridization tests were highly reliable and no race specificity was reported (Schaad et al., 1989).

Quantitative differences (virulence) have been related to variation in toxin production and in motility (Patel et al., 1964; Russell, 1975; Saettler et al., 1981). Jensen and Livingstone (1944) and Johnson (1969) reported that halo-less P.s. pv. phaseolicola isolates were less virulent than those that produced halos. Studies by Mulrean and Schroth (1979) indicated that motility in this pathogen may be regulated by chemicals produced by plant tissues in potential infection sites on leaves. Some researchers have attributed the variation in virulence of strains of halo blight bacteria to differences in motility.

Panopoulos and Schroth (1974) observed that motile strains caused up to twelve times as many lesions as non-motile stains. However, Msuku (1984) found no relationship between motility and virulence in a study of four pathotype groups in Malawian isolates of halo blight bacteria. This study also indicated that most of the group 1 isolates were obtained from warmer areas as opposed to the more virulent isolates in groups 2, 3, and 4, which were obtained from cooler areas of Malawian bean growing regions. The author noted that with an exception of one isolate in pathotype 4, all Malawian isolates serologically shared the same antigenic characteristics.

Appearance of new virulent strains has been attributed to a number of factors. These include genetic changes in the pathogen that has long occupied a given area, introduction of an organism into a new area, and changes in the cropping systems which affect the ecological niche of the pathogen (Buruchara, 1983; Schuster and Coyne, 1975a). Studies have also indicated that the virulence of a bacterial population was increased through mutation and selection during passage through a resistant host (Buruchara, 1983). In other host-pathogen systems variation in pathogenicity may also evolve with the host genotype. Correa (1987), using isozyme studies, suggested that each pathogen population is subject to a selection pressure in favor of one allele or another, probably depending on the genotypes serving as hosts.

Synergism

Synergism between P.s. pv. phaseolicola and Uromyces phaseoli (Reben) Wint., the bean rust pathogen, has been reported (Schwartz, 1989). Yarwood (1969) observed that lesion size became larger when plants were infected with a bean rust pathogen followed by infection with halo blight bacteria. Lesion numbers may also be increased by inoculating P.s. pv. phaseolicola mixed with Achromobacter sp. (Maino, 1972).

Cultural control

Halo blight bacteria are known to survive between growing seasons in bean debris on the soil surface and on volunteer beans (Schuster and Coyne, 1975b). Deep plowing and crop rotation are thus recommended to reduce initial inoculum. The pathogen is also seed-borne. In order to reduce initial inoculum density, the use of pathogen-free seed produced under conditions unfavorable to the bacterium is highly recommended (Schwartz, 1989; Zaumeyer and Thomas, 1957). Clean seed production is a major method for controlling halo blight and other bean bacterial diseases in the U.S.A., where clean seeds are produced in the arid west under irrigation. The production of clean seed in the arid west (Idaho) depends on field inspection for visible evidence of plant infection, laboratory inoculation of susceptible pods with suspensions from seed lots, serological tests and quarantine to prevent importation of bean seed from areas where the pathogen exists (Schwartz, 1989).

Seeds should also be thoroughly cleaned of dust after threshing because they can be contaminated by halo blight bacteria present in powdered plant tissue. Contaminated seed can also be treated with chemicals, including antibiotics to kill bacteria present on the surface (Grogan and Kimble, 1967; Russell, 1975; Saettler et al., 1981). While field inspection and roguing of diseased plants may help to ensure that seeds are pathogen free, contamination of seed may still occur, even when halo blight incidence is negligible,even in certain resistant cultivars (Allen, 1983; Katherman et al., 1980; Stadt and Saettler, 1981).

Chemical control

Various researchers have indicated that halo blight can be controlled chemically by foliar applications of Bordeaux mixture, copper oxychloride, copper sulfate, copper oxide,

streptomycin sulfate, or dihydro-streptomycin sulfate. These chemicals may be applied at 7 to 10 day intervals at rates of 200-400 g per 1000 square meters. Application may also be done at first flower and pod-set at the rate of 0.1 percent a.i. per 675 liters per hectare, to prevent spread and development of halo blight on leaves and pods (Hagedorn et al., 1969; Ralph, 1976; Saettler and Potter, 1970; Taylor and Dudley, 1977, Zaumeyer and Thomas, 1957).

Russell (1975) and Schwartz (1989) suggest that application of antibiotics to the foliage may induce the development of resistant mutants, and their use should therefore be avoided. Moreover, control of halo blight disease by use of chemicals may not always be practical in tropical areas (Allen, 1983). In addition, chemicals are relatively expensive for small holder growers in Tanzania and other developing countries, and may also be difficult to obtain (Saettler et al., 1981), or to apply appropriately.

Plant resistance

The use of resistant varieties as a means to control halo blight has been successful in some areas of the world (Baggett and Frazier, 1967; Coyne et al., 1967; Schwartz, 1989; Zaumeyer and Meiners, 1975). However, development of resistant varieties must take into account variability of both the pathogen and genetic resistance in the host. Independent genes separately govern leaf resistance, pod resistance and plant systemic chlorotic reactions (Baggett and Frazier, 1967; Coyne and Schuster, 1974; Coyne et al., 1971). For example, pod susceptibility frequently occurs in plants which possess leaf resistance. However, linkage has been observed between the different genes that control leaf and plant systemic chlorotic reactions (Hill et al., 1972). Breeding programs should, therefore, select germplasm which provides resistant reactions against leaf and pod infection, and in which the pathogen is non-systemic (Coyne and Schuster, 1974).

Plant resistance to P.s. pv. phaseolicola has been reported to involve suppression of toxin production and inhibition of bacterial growth (Russell, 1977). Bean germplasm resistant to race 1 and 2 has been identified in field and greenhouse tests (Schwartz, 1989) and is under the control of both dominant and recessive genes. Dominant resistance to race 1 was reported in Red Mexican UI-3 by Patel and Walker (1966). Varieties Wisconsin HBR40 and 72 developed by Hagedorn et al. (1974) are reported to be resistant to both races 1 and 2 of Psp, Xanthomonas phaseoli (common bacterial blight); P.s. pv. syringae (bacterial brown spot), and various fungal pathogens. However, for successful long term control of halo blight disease, integrated control programs should be adopted (Schwartz, 1989).

In an integrated control program, several disease control methods are employed, including regulatory inspections for healthy seed production, quarantine measures, cultural practices (rotation, sanitation), biological control (resistant varieties) and chemical control (seed treatment and foliage sprays). The optimum use of each of these control measures makes the others relatively more effective. However, such integrated disease control measures are most successful and economical when all relevant information regarding the crop, its pathogen, the environmental conditions expected to prevail, locality, availability of materials, and costs are taken into account.

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Chapter 2

PATHOGENIC VARIATION OF PSEUDOMONAS SYRINGAE

PV. PHASEOLICOLA IN NORTHERN TANZANIA

INTRODUCTION

Pathogenic variation among populations of Pseudomonas syringae pv. phaseolicola is well known. Using 13 isolates Jensen and Livingstone (1944) were the first to demonstrate pathogenic variation in P.s. pv. phaseolicola. Patel and Walker (1965) reported the occurrence of races 1 and 2 in the U.S.A. These races were characterized based on the reaction of inoculated bean cultivar Red Mexican UI-3, which is resistant to race 1 but susceptible to race 2. On the same basis, the occurrence of these two races has been reported in Tanzania and many other countries (Buruchara and Pastor-Corrales, 1981; Hale and Taylor, 1973; Kinyua and Mukunya, 1981; Silbernagel and Mills, 1987; Taylor et al., 1987). The existence of a new race in Nebraska, U.S.A. was first suggested by Schuster et al., (1979) and Coyne et al., (1979), who recovered new strains of halo blight bacteria which were virulent to the usually resistant Great Northern bean cultivars such as Great Northern UI-59 and California Pink. Recently, Taylor et al., (1987) confirmed the existence in Africa, of race 3 of P.s. pv. phaseolicola, which was virulent on bean cultivars with a single gene for resistance derived from cultivar Red Mexican UI-3. Race 3 caused a hypersensitive reaction in the cultivar Tendergreen, which is susceptible to races 1 and 2. Resistance to race 3 has been found to be governed by a single dominant gene, which is also present in several cultivars of U.S.A. origin such as Seafarer and Tendercrop (Harper et al., 1987). D.J. Allen (personal communication) indicated that race 3 occurs in Colombia.

Breeding for halo blight resistance is generally considered the best method of control, and the use of resistant varieties as a means to control halo blight has been successful in some areas of the world (Baggett and Frazier, 1967; Coyne et al., 1967; Hagedorn et al., 1974; Schwartz, 1989). However, the development of resistant varieties must take into account variability of both the pathogen and the genetic resistance in the host. Therefore, a knowledge of the distribution of races of halo blight bacteria is important in relation to programs of breeding resistant bean varieties, including those in Tanzania. The purpose of this study, therefore, was to investigate races of P.s. pv. phaseolicola prevailing in northern Tanzania and their survival ability under different field conditions. Information generated from this study will be very useful for bean breeding programs for disease resistance, and their development will also provide an alternative management tool for halo blight disease in the country.

MATERIALS AND METHODS

Sampling and isolation

To determine the relative prevalence of races of P.s. pv. phaseolicola in Arusha and Kilimanjaro regions, northern Tanzania (Fig. 1) where halo blight is one of the important diseases of beans, a survey was conducted during the period of November 1988 to February, 1990. Samples were collected periodically at random from several farmers' bean fields. A total of 118 isolates were collected. Isolations were made from diseased plant material with halo blight-like symptoms. Pieces of diseased tissue including small areas of surrounding healthy tissue were excised and surface-sterilized for 1 minute in 2.6% NaOCl and rinsed in sterile distilled water. The pieces were crushed on flame-sterilized glass slides containing one or 2 drops of sterile distilled water. Using a sterile wire loop, the resulting suspensions

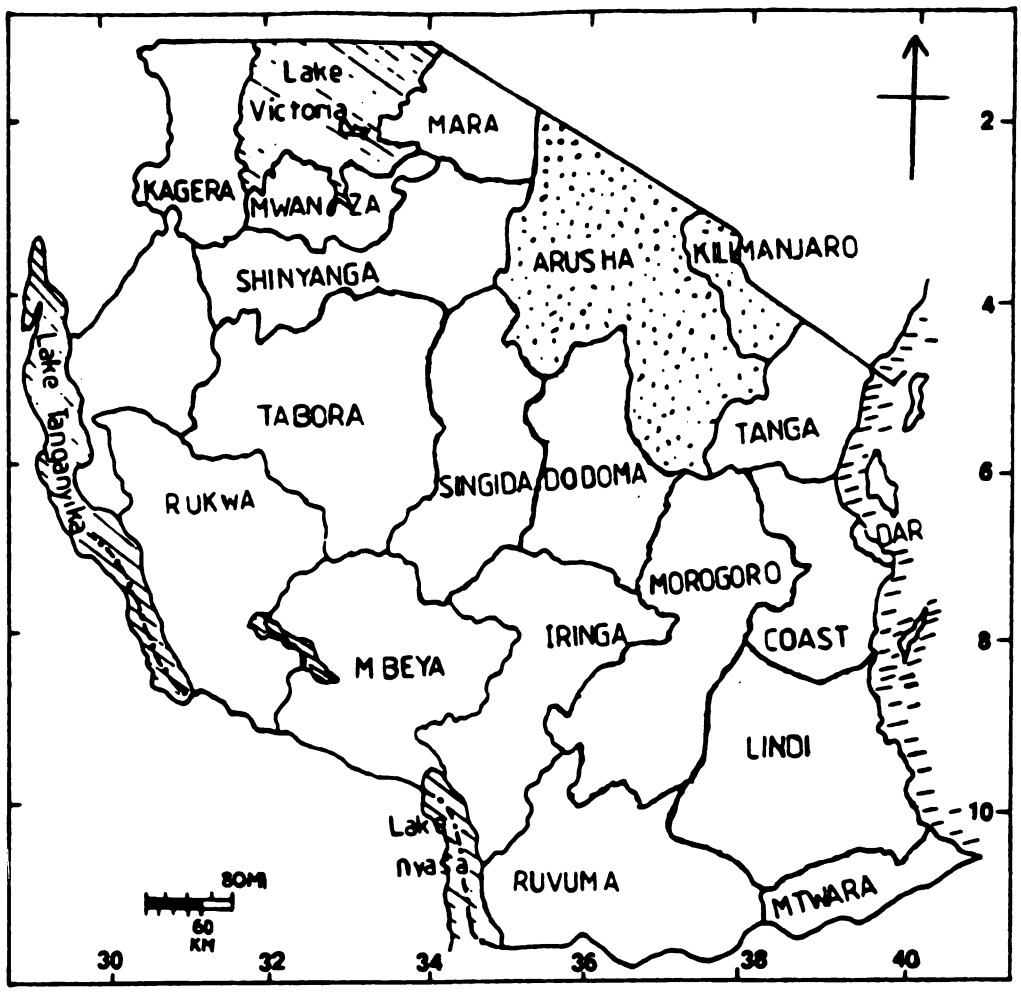


Fig. 1. Map of Tanzania showing location of Arusha and Kilimanjaro regions.

were streaked onto medium B of King et al. (1954) (KMB). Fluorescing colonies were purified by a series of single colony transfers and their identity confirmed by biochemical and physiological tests using procedures of Lelliot et al. (1966); pathogenicity tests on Canadian Wonder bean seedlings; carbon source utilization tests (Schaad, 1988) using mannitol, sorbitol and inositol; ice nucleation activity using the single temperature droplet method of Lindow (1988) and Lindow et al. (1978, 1982) at -5°C; sensitivity to bacteriophage and serology.

Bacteriophage tests

Sensitivity to bacteriophage was tested using the method described by J.D. Taylor and D.M. Teverson (personal communication), as follows. Three bacteriophages (11P, 12P, 48P), specific to P.s. pv. phaseolicola, were used. Log phase cultures of each isolate grown on KMB and nutrient agar were used. Thick bacterial suspensions were prepared by adding 4 ml nutrient broth to each slant culture. Two to 3 drops of the resulting bacterial suspension of each isolate were added to glass vials containing 2.5 ml of sterile soft glycerol agar (SGA) (g/l:proteose peptone (Sigma), 5.0; yeast extract (Difco), 3.0; glycerol, 20 ml; Bacto-agar, 7.0) maintained molten in a water bath at 45-50°C. Suspensions were thoroughly mixed and poured on cool, solidified nutrient agar plates, followed by swirling gently to allow a thin layer of SGA with bacterial suspension to form over the plate. Plates were dried in a laminar flow chamber. One 5-μl drop of each bacteriophage was applied to the surface of the medium at designated spots and the drops were allowed to dry on a laboratory bench. Plates were then incubated upside down for 24-48 hours after which results were recorded. Each isolate was replicated three times. Clear zones (plaques) due to lysis of bacterial cells by phages indicated a positive P.s. pv. phaseolicola identity. Three

tests were conducted for each bacterial isolate, and reference strains 1299A and 1375A were included as positive controls.

Serology

P.s. pv. phaseolicola antisera was provided by Dr. J.D. Taylor. The agglutination method of J.D. Taylor and D.M. Teverson (personal communication) was used. One 0.07-ml drop of the antiserum was placed on a clean glass microscope slide, and a small amount of 24-48 hour bacterial growth picked up with a platinum wire loop was mixed into the drop of the antiserum, which was stained pink. Agglutination was observed against a light colored background. Occurrence of agglutination was recorded as positive. Three tests were conducted for each isolate.

Reference strains

Reference strains of P.s. pv. phaseolicola (882, 1281A, 1299A, 1301A, 1302A and 1375A) were included in this investigation. Bacteriophage, antisera, and reference strains were provided by Dr. J.D. Taylor (Institute of Horticultural Research, Wellsborne, Warwick, England).

Storage of Isolates

Bacterial isolates were maintained as very thick suspensions in glycerol:0.1M phosphate buffer (50:50, v/v) and on nutrient agar slants at 0°C in an incubator (A. Gallenkamp and Co., London). Isolates were also stored in bean leaf powder in sterile vials at room temperature (22 $\pm$ 2°C) in darkness. The three storage methods were used to reduce the chances of loss of viability.

Soil sterilization

Soil used for screen house experiments was heat-sterilized by exposing soil to a high temperature kerosene flame (198°C) using a Terra Force Terralizer (Kent Horticultural Engineers, Kent, England). A forest soil/sand mixture (2/1, v/v) was used. The two soil components were mixed and sieved to remove gravel and debris before the sterilization process.

Growing of plants

All plants used in this study were grown in the screen house in 16 cm-diameter sterile plastic pots or in 7 x 8 x 22 cm^3 sterile plastic flats containing a sterile mixture of forest soil/sand. Plants were maintained at temperatures ranging from 19 to 27°C and a natural 12 $\pm$ 0.5 hour day-length, and watered as required with tap water. The halo blight susceptible cultivar, Canadian Wonder, was used as a control throughout the study.

Inoculum preparation

Each P.s. pv. phaseolicola isolate was grown on KMB for 24-48 hours at 24 $\pm$ 2°C. Suspensions were made by adding 0.01 M phosphate buffer, pH 7.2, to each culture and dislodging the bacteria. Concentrations of bacterial suspensions were adjusted turbidimetrically to contain about 10^7 -10^8 colony forming units (CFU) per ml.

Race identification

Races were determined from pod and foliage reactions of four differential bean cultivars. These were Canadian Wonder (universal susceptible), Edmund (universal resistant), Red Mexican UI-3 (resistant to race 1) and Tendergreen (resistant to race 3).

Pathogen-free seeds of these differential bean cultivars were provided by Dr. M.J. Silbernagel (USDA/ARS, Prosser, WA) and were then multiplied at Lyamungu Agricultural Research Station, Moshi, Tanzania. For leaf reaction, 7-10 day old seedlings containing fully expanded primary leaves were injected with bacterial suspensions at the first node using a sterile 25 gauge needle attached to a sterile 10cc hypodermic syringe. Following needle inoculation, bean seedlings were also spray-inoculated abaxially with bacterial suspensions without water-soaking, using a half-liter plastic hand-operated atomizer. After inoculation, plants were covered with a plastic bag for 24 hours in the screen house and observed for halo blight symptom development during the following 10 days. Each single plant was considered a replicate and six plants were used for each isolate; two experiments were conducted.

Pod reactions were determined using procedures of Ekpo (1975). Pods at the flat stage were harvested, surface-sterilized, rinsed and dried aseptically on sterile filter papers. Three pods from each differential bean cultivar were inoculated by placing a 5-μl drop of inoculum at three sites per pod, followed by pricking the pod five times through the inoculum drop to a depth of about 1.0 mm using a 25-gauge sterile disposable needle. Inoculated pods were placed side by side in 7 x 8 x 22 cm^3 plastic flats containing moist filter papers with the inoculated side up and incubated for 7-10 days. Data were compiled from two repeated experiments. Isolates of known P.s. pv. phaseolicola races and sterile phosphate buffer were included as positive and negative controls, respectively.

Temperature studies

To determine the effect of temperature on growth of P.s. pv. phaseolicola races, growth studies were conducted on KMB agar plates. Plates were inoculated in quadruplicate

with different serial dilutions of each race. After inoculation, plates were placed upside down in an incubator (Fi-totron 600H, Fisons Environmental Equipment, Loughborough, England) without light, at 24, 28 and 34°C with a temperature variation of ± 2°C. At 28 and 34°C, humidity in the incubator was adjusted at 70% to avoid excessive drying of agar plates. Colony diameter was measured after 4-5 days. The ability to produce a brown diffusible pigment at different temperatures on KMB and NA was also examined by growing the isolates producing the pigment at the above mentioned temperatures. Subjective pigment production determinations were recorded during the following 5 days.

Survival studies

Survival of races 1 and 2 of halo blight bacteria was studied following the procedures of Saettler et al. (1986) and Groth and Braun (1989). Three sites were chosen representing different ecological environments in which beans are grown in Arusha and Kilimanjaro regions. The coffee/banana intercrop environment was also included. Three cultivars, Canadian Wonder (susceptible), Masai Red (slightly susceptible) and GO 7928 (resistant) were grown in 10 m rows in the field at Lyamungu and Monduli in March, 1989. Plants were inoculated twice with race 1 (isolate #2) or race 2 (isolate #4) 18 and 30 days after planting. Control plants grown 25 m away were left uninoculated. Severely halo blight diseased plants were collected in early June, air-dried at room temperature and separated into leaves and stems. Some plants were left standing in the field. Stems with typical halo blight symptoms were cut into pieces 2-3 cm long. Samples of leaves (0.5g) and stems (10 pieces) were placed in fine-mesh nylon bags and tied with nylon threads. A different color of nylon-mesh was used for each race to avoid mixing the two races, and within a race threads of different colors were used to distinguish cultivars. Samples were then taken to

the field at the end of June, the end of the main bean growing season. Half of the samples were placed 2-5 cm beneath the soil surface and the remaining half placed at 25 cm depth. Samples of each race were separated by a distance of 10 m. Healthy control samples were placed 5 m away from diseased samples. Diseased samples were also stored in the laboratory at 22 $\pm$ 2°C for a similar period.

At monthly intervals starting in July, when beans are usually harvested, three samples for each race and for each plant part, including samples from standing plants, were retrieved. Samples for standing plants included stem tissue and fallen leaves. After removing the nylon mesh, samples were ground in sterile mortars and pestles and 10 ml of sterile phosphate buffer added. The resulting suspensions were left to stand for 15 minutes followed by a 2-minute centrifugation at 20,000xg using a Marusan centrifuge (Sekuma Seisakusho Ltd., Tokyo, Japan) to sediment the plant debris. The supernatants were diluted serially and plated in triplicate on KMB agar containing cycloheximide (100 μg/ml). Plates were observed under UV light after 4-5 days of incubation. For each sampling period, 5 presumed P.s. pv. phaseolicola colonies were purified by single colony transfers and tested for sensitivity to bacteriophage and for pathogenicity on 7-10 day-old Canadian Wonder been seedlings, by injecting stems and by infiltrating leaves with suspensions of approximately 10^7 cfu/ml. Plants were incubated in the screen house and observed for halo blight symptom development for up to 14 days.

Soil pH measurements

Soil pH for each placement site at each geographical location was measured electrometrically using a pH meter (EIL-7015, Kent Industrial Measurements Ltd., England) at the time of sample placement in the field and at monthly intervals when samples were retrieved. Three soil samples were taken for each placement depth.

Volunteer bean plants

During the bean growing season, farmers' fields with severe halo blight symptoms were identified. Volunteer bean plants from such fields and around farmers' backyards in Monduli (November-December) and at Lyamungu (September-October) were observed for the presence of halo blight symptoms. Isolation attempts were made from suspect plants as described earlier. Presumed P.s. pv. phaseolicola colonies which were fluorescent were subject to biochemical, pathogenicity and bacteriophage tests for species identification. Confirmed P.s. pv. phaseolicola colonies were tested on the four bean differential cultivars for race identification.

Weather data

Rainfall and temperatures for Lyamungu and Monduli were obtained from the agrometeorology section at the Agricultural Research Institute, Lyamungu, Moshi, and from the Monduli District Agricultural Development office, respectively.

RESULTS

Prevailing races

Within the two regions surveyed in northern Tanzania (Fig. 2), three pathogenic races of halo blight bacteria were distinguished based on the reaction of the four differential bean cultivars used. The distribution of these races in the surveyed area is shown in Table 1. Race 2 was the most prevalent of the three races in the area, followed by race 1; race 3 occurred at a very low frequency (Fig. 3). Besides producing the usual blue-green diffusible pigment, some race 2 isolates also produced a brown diffusible

pigment, which was more pronounced on nutrient agar than on KMB. Such isolates were designated as race 2P (Table 1 and Fig. 3). Race 2P isolates occurred infrequently, and were restricted to Lambo, Kilimanjaro region and Monduli and Selian in Arusha regions. The bean cultivars from which race 2P isolates were obtained included Canadian Wonder, the breeding line FB-GP307-2 and other local varieties.

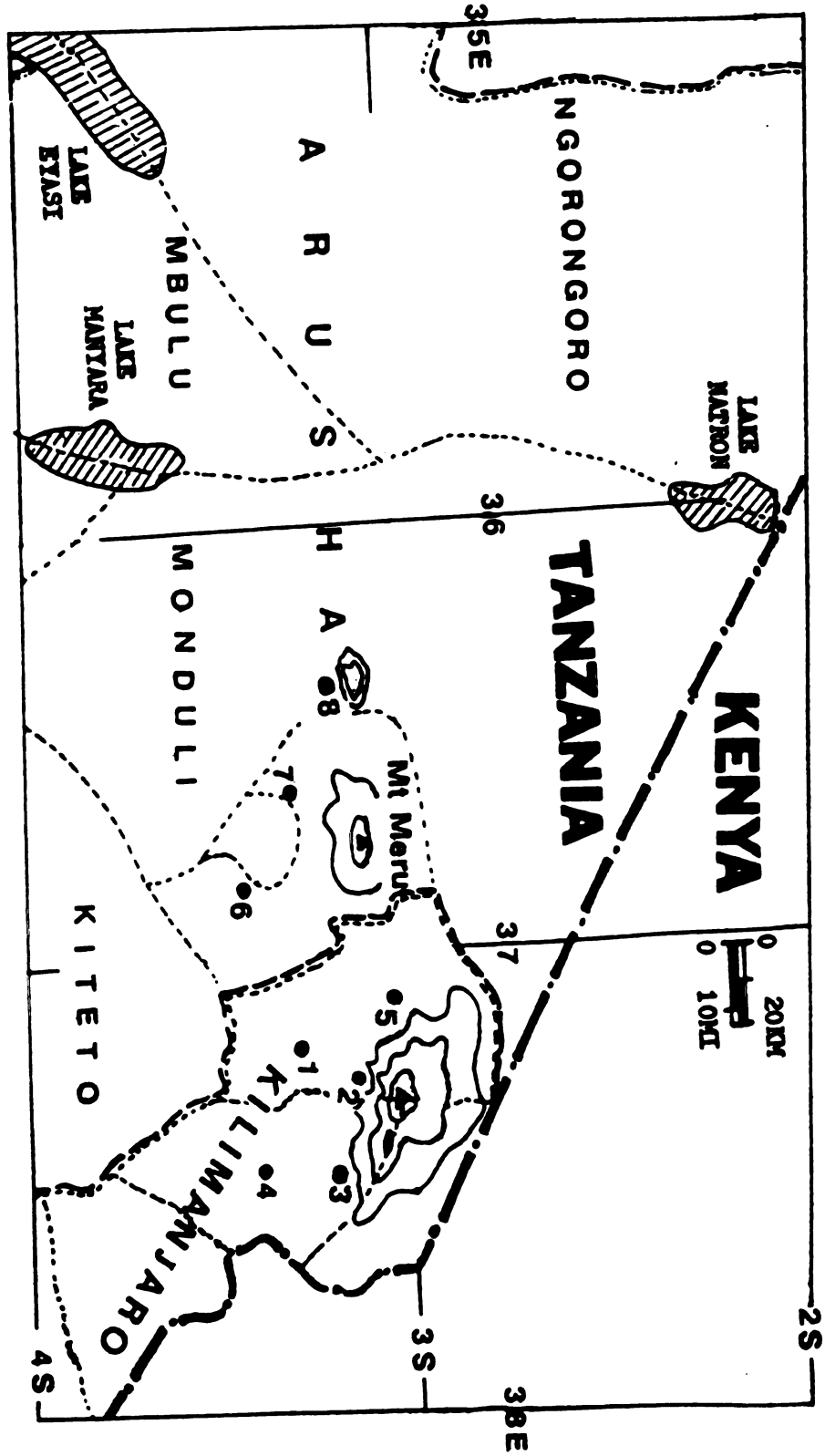
Brown diffusible pigment production could be detected as early as 48 hours on nutrient agar and after 4-5 days on KMB. The delayed appearance of the pigment on KMB might be due to the masking effect by the blue-green diffusible pigment which is produced in large quantities on this medium. The brown diffusible pigment-producing isolates were not distinguishable from other race 2 isolates that did not produce the pigment, based on biochemical and physiological tests. Race 2P isolates induced a reaction similar to that of race 2 on foliage and on pods of the four differential bean cultivars used.

All race 2 isolates obtained from bean debris collected from farmers' fields in Monduli in November, 1988 and September, 1989 were highly virulent and produced very large water-soaked lesions on the cultivar Tendergreen. They also induced stunting and extensive systemic chlorosis in the resistant differential cultivar Edmund. However, no water-soaked lesions were produced in Edmund and inoculated leaves produced a hypersensitive reaction (Fig. 4). By contrast, race 2 isolates obtained from halo blight-infected bean plants produced only a hypersensitive reaction on Edmund.

Temperature studies

The growth responses of different isolates of the three races of halo blight bacteria are shown in Table 2. Growth of all races did not differ much at 24 $\pm$ 2 and 28 $\pm$ 2°C. However, 2 out of 3 race 2P isolates were able to grow at 34 $\pm$ 2°C. These isolates also

Fig. 2. Origin of Pseudomonas syringae pv. phaseolicola isolates in northern Tanzania. 1 = Lambo, 2 = Lyamungu, 3 = Kilema, 4 = Miwaleni, 5 = Sanya Juu, 6 = Tengeru, 7 = Selian, 8 = Monduli.


3°S
3°E
LAKE EYASI
LAKE MANYARA
MBULU
ARUSHA
NGORONGORO
LAKE NATRON
TANZANIA
KENYA
MONDULI
KITETO
Mt Meru
KILIMANJARO
1
2
3
4
5
6
7
8
3°7
3°6
2°S
3°S
3°8E
4°S
0 20KM
0 10MI

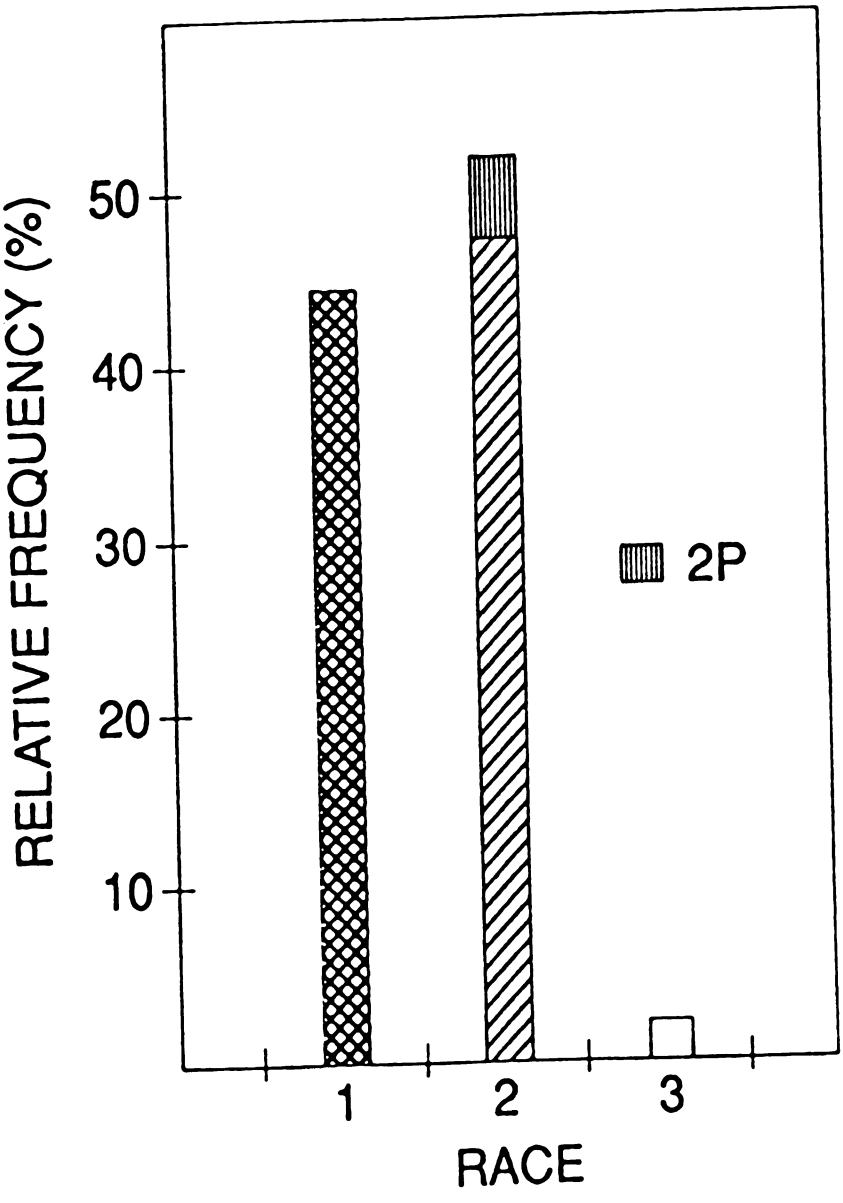


Fig. 3. Relative frequency of races 1, 2 and 3 of Pseudomonas syringae pv. phaseolicola in collections made in Arusha and Kilimanjaro regions, northern Tanzania from November, 1988 to February, 1990. Frequencies are expressed as percentages of the total number of isolates collected. 2P represents race 2 isolates producing brown-diffusible pigment.

Table 1. Distribution of races of Pseudomonas syringae pv. phaseolicola in Arusha and Kilimanjaro regions, northern Tanzania.

Location	Altitude (m)	Total No. of isolates	Number of isolates of race 1	2	2P	3
Arusha						
Monduli	1630	39	8	26	3	2
Selian	1387	45	27	16	1	1
Tengeru	?	2	2	—[a]	—	—
Kilimanjaro						
Lambo	1020	13	3	8	2	—
Lyamungu	1268	13	12	1	—	—
Kilema	1422	4	—	4	—	—
Miwaleni	765	—	—	—	—	—
Narumu	1268	1	—	1	—	—
Sanya Juu	1400	1	1	—	—	—
Total		118	53	56	6	3

? Information on altitude lacking.

[a]— Represents absence of indicated race among P.s. pv. phaseolicola isolates.

Fig. 4. Stunting and systemic chlorosis symptoms produced by race 2 isolates obtained from bean debris in Monduli, Arusha region northern Tanzania on a resistant differential bean cultivar, Edmund.

Table 2. Influence of temperature on growth of representative isolates of races of Pseudomonas syringae pv. phaseolicola on KMB.

Race	Isolate No.	Mean colony diameter (mm) at temperature (°C)[W] 24 ± 2	28 ± 2	34 ± 2
1	50	3.6 ± 0.2	2.7 ± 0.2	–[X]
1	70	2.5 ± 0.2	2.4 ± 0.1	–
2	12	2.7 ± 0.2	2.6 ± 0.1	–
2	882[Y]	2.2±0.4	2.1 ± 0.2	–
2	1299A[Y]	2.6 ± 0.0	2.4 ± 0.1	–
2P[Z]	5	2.3 ± 0.1	2.5 ± 0.1	3.0 ± 0.2
2P[Z]	19	2.5 ± 0.1	2.4 ± 0.0	2.3 ± 0.1
2P[Z]	44	2.5 ± 0.2	2.4 ± 0.0	–
3	46	2.6 ± 0.1	2.4 ± 0.1	–
3	1302A[Y]	2.6 ± 0.1	2.3 ± 0.2	–

[W]Values are means of four replications ± standard errors.

[X]– = No growth was observed.

[Y]Isolates were kindly supplied by Dr. J.D. Taylor, Institute of Horticultural Research, Wellsborne, Warwick, England.

[Z]2P = Race 2 brown-diffusible pigment producing isolates.

produced the brown diffusible pigment at all three temperatures. The pigment was easily seen on nutrient agar plates due to the absence of the blue-green diffusible pigment which occurs on KMB.

Survival studies

The ability of P.s. pv. phaseolicola to survive in bean debris buried in the soil and in standing bean plants left in the field varied depending upon the race, geographical location, depth of debris placement and the bean genotype used. Within the same bean genotype P.s. pv. phaseolicola survived for a longer period in stems than in leaf debris. For example, at Ngarash, Monduli, Arusha region, race 1 survived for 5 months in Canadian Wonder stem debris and only 4 months in foliage debris (Table 3). This race also survived well for 5 months at Monduli in standing bean plants and in bean debris placed in soil at a depth of 2-5 cm, while it survived for only 3 months when debris was placed 25 cm deep. On the other hand, at the same location race 2 survived in stems of cultivar Canadian Wonder one month longer than race 1 (Table 3). Race 2 was readily recovered from bean stem tissue samples of plants left standing in the field and those buried at 2-5 cm throughout the 6 month period (July to December). In all cases, the susceptible cultivar, Canadian Wonder, supported longer survival of halo blight bacteria, probably due to more inoculum present, than the other bean genotypes used. Pathogenicity tests and sensitivity to bacteriophages 11P, 12P and 48P confirmed the identity of recovered isolates as P.s. pv. phaseolicola.

In the coffee/banana intercrop environment at Lyamungu survival of halo blight bacteria declined rapidly in bean plants left standing after the harvest season and in bean debris buried in the soil. Race 1 was recovered after only 1 month in leaves and 2 months

Table 3. Survival of Pseudomonas syringae pv. phaseolicola races 1 and 2 in infected bean debris represented by standing plants and debris buried for 6 months at two depths under field conditions at Ngarash, Monduli, Arusha region and at Lyamungu, Kilimanjaro region, northern Tanzania.

			Survival duration (mon)					
			Standing plants		2-5 cm deep		25 cm deep	
Location	Race	Genotype[w]	Leaves	Stems	Leaves	Stems	Leaves	Stems
Monduli[x]	1	CW	4	5	4	5	2	3
		MR	2	3	2	2	1	1
		G07928	0	0	0	0	0	0
	2	CW	5	6	5	6	2	4
		MR	2	3	2	2	1	1
		G07928	0	0	0	0	0	0
Lyamungu[y]	1	CW	1	2	0	0	0	0
		MR	0	2	0	0	0	0
		G07928	0	0	0	0	0	0
	2	CW	2	3	0	0	0	0
		MR	1	2	0	0	0	0
		G07928	0	0	0	0	0	0
Lyamungu[z]	1	CW	2	3	1	1	0	0
		MR	1	2	0	0	0	0
		G07928	0	0	0	0	0	0
	2	CW	2	3	1	1	0	0
		MR	2	2	0	1	0	0
		G07928	0	0	0	0	0	0

[w]CW = Canadian Wonder (susceptible), MR = Masai Red (slightly susceptible), G07928 = breeding line (resistant).

[x]Field used for the study was cultivated to maize the previous year.

[y]The field was under coffee/banana association, and no bean crop had been grown in the field.

[z]The field used was under fallow the previous 3 years.

in stems of standing plants of cultivars Canadian Wonder and Masai Red, whereas race 2 remained viable for 2 and 3 months in foliage and stems of standing plants, respectively. The two races (1 and 2) were not recovered from debris of either bean genotype after 1 month burial at 2-5 cm and at 25 cm deep (Table 3).

In the field left fallow the previous 3 years at Lyamungu, races 1 and 2 of P.s. pv. phaseolicola were recovered after 2 and 3 months in leaves and stems, respectively, of cultivar Canadian Wonder left standing in the field. In samples placed at 2-5 cm depth, race 1 was viable for 1 month (July) only in Canadian Wonder, while race 2 survived for the same duration in Canadian Wonder and in stem pieces of Masai Red (Table 3). As in the coffee/banana intercrop environment, halo blight bacteria were not detected after 1 month in samples buried at 25 cm beneath the soil surface, the normal plowing depth.

At both geographical locations (Monduli and Lyamungu), the pathogen remained viable for a longer period in plants left standing in the field than in buried debris (Table 3). Comparing the two locations, however, P.s. pv. phaseolicola survived for a longer period at Ngarash, Monduli than at Lyamungu. In dry infected debris stored in the laboratory at 22 $\pm$ 2°C, the bacterium remained viable for more than 8 months in cultivars Canadian Wonder and Masai Red. However, in the resistant line, G07928, both races were detected at very low numbers only at the beginning of the experiment in June; thereafter the pathogen was not detected.

pH values

During the period of the survival study, the soil pH values at Ngarash, Monduli ranged from 6.8-6.9 at both placement depths. In the field under the coffee/banana intercrop at Lyamungu, pH values ranged from 4.6 in July to 6.2 in September for the

topsoil, and from 4.7 in July to 6.3 in September for the 25 cm depth. The field left fallow the previous 3 years had pH values ranging from 4.9 to 5.0 for the two placement depth for July and September, respectively. At Lyamungu the low pH in July may be attributed to ammonium sulfate which is applied as a nitrogen fertilizer for coffee.

Volunteer plants

P.s. pv. phaseolicola was recovered from infected foliage of volunteer bean plants in two farmers' fields in Monduli and around several backyards at Lyamungu. Due to high moisture content in the soil, shatter loss bean seeds germinated almost immediately after harvest (July/August) at Lyamungu. However at Monduli, where the soil moisture was very low due to lack of rain, most of the shatter loss bean seeds remained dormant in the field until October/November when soil moisture conditions allowed germination. Volunteer bean plants around farmers' backyards arose from seeds dropped or discarded during threshing.

Weather data

Rainfall and temperature changes at Monduli and at Lyamungu from June to December are shown in Fig. 5 and Fig. 6, respectively. Lyamungu received rain throughout the survival study period (June-December, 1989). On the other hand, Monduli remained dry for most of this period and received little or no rain until November and December (Fig. 5). The monthly mean maximum temperature at Lyamungu ranged from 20.5 to 26.5°C and the monthly mean minimum temperature ranged from 12.5 to 15°C.

Figure 5. Monthly rainfall from June to December at Monduli for 1989 and mean monthly rainfall for 3 years (1986-1988). Numbers above each bar represent number of rain days during the indicated month.

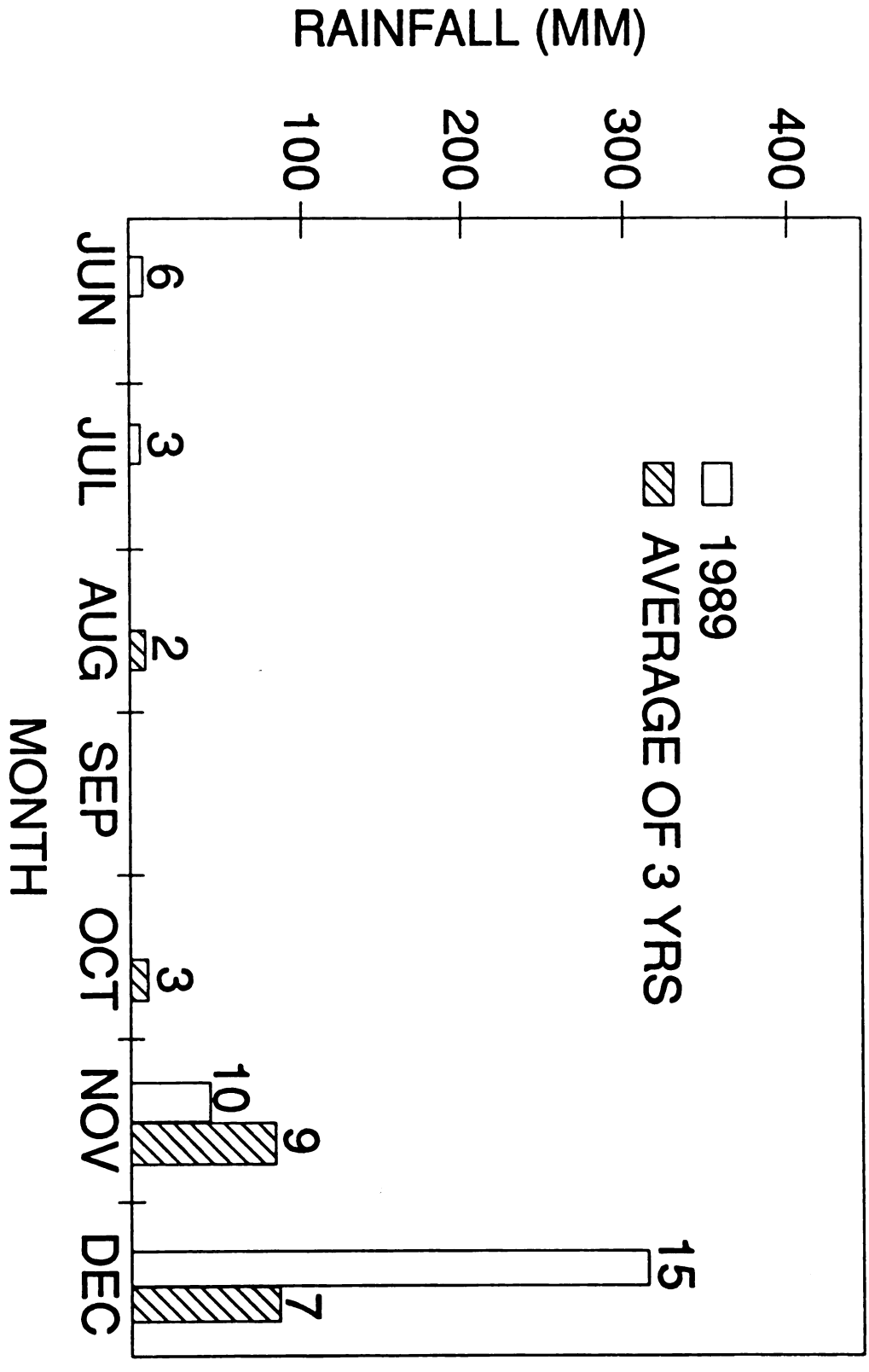
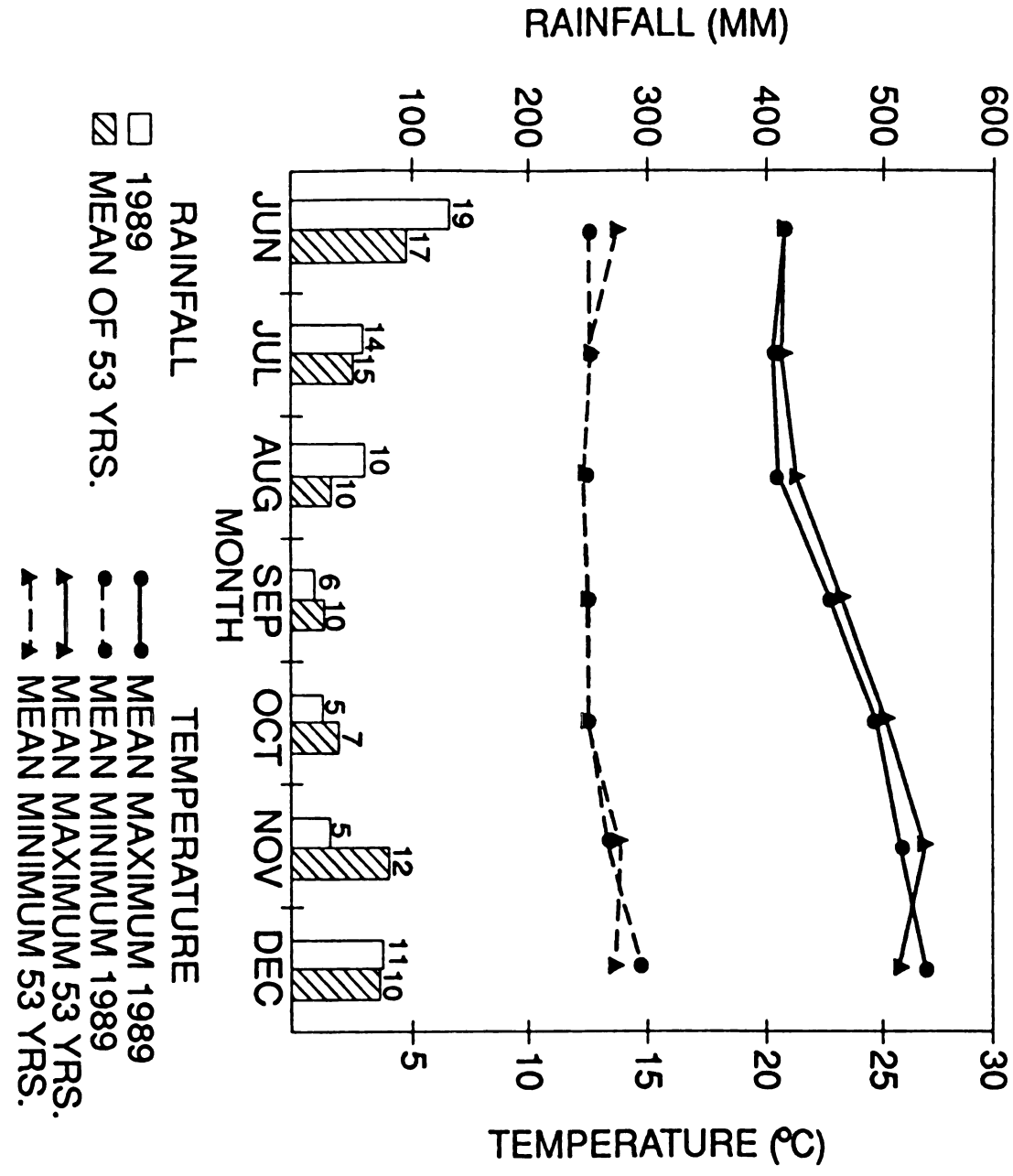

RAINFALL (MM)
100
200
300
400
1989
AVERAGE OF 3 YRS
JUN
JUL
AUG
SEP
OCT
NOV
DEC
MONTH
6
3
2
3
10
9
15
7

Figure 6. Monthly rainfall and mean monthly maximum and minimum temperatures from June to December at Lyamungu for 1989 and for 53 years (1935-1988). Numbers above each bar represent number of rain days during the indicated month.


RAINFALL (MM)
600
500
400
300
200
100
30
25
20
15
10
5
TEMPERATURE (°C)
19
17
14
15
10
10
6
10
5
7
5
12
11
10
JUN
JUL
AUG
SEP
OCT
NOV
DEC
MONTH
RAINFALL
1989
MEAN OF 53 YRS.
TEMPERATURE
MEAN MAXIMUM 1989
MEAN MINIMUM 1989
MEAN MAXIMUM 53 YRS.
MEAN MINIMUM 53 YRS.

DISCUSSION

The current survey has revealed that in Arusha and Kilimanjaro regions, three races of P.s. pv. phaseolicola exist, and race 2 including race 2P occurred at a relatively higher frequency (52.5%) than race 1 (44.9%) and race 3 (2.5%). These findings agree with previous work by Taylor et al. (1987) who reported the existence of three races of this pathogen in Tanzania and in other countries, especially around the African Great Lakes region. Although the frequency of race 3 was very low in northern Tanzania, Taylor et al. (1987) observed that it is the dominant race in the neighboring country of Rwanda. Due to its high prevalence in northern Tanzania, race 2 appears to be the most threatening race to bean production in that area. However, the increased movement of bean germplasm between countries and the need for continued exchange of bean breeding material between research stations within countries may assist in spreading other races of P.s. pv. phaseolicola in areas where they presently occur at low frequencies.

Within race 2, there appeared to be a great deal of variation, with some isolates producing a brown diffusible pigment on NA and KMB. Brown diffusible pigment-producing isolates were designated as race 2P, and were found at altitudes ranging from 1020 to 1630 m above sea level (Table 1). In addition, this study has revealed that at least 2 out of 3 race 2P isolates were able to grow at a higher temperature (34 $\pm$ 2°C) than the non-pigment-producing race 2 isolates or the race 1 and 3 isolates. However, it is not known whether such isolates may also tolerate high temperatures under natural conditions. Two important questions also remain unanswered. First, whether this brown diffusible pigment is also produced in vivo under natural conditions or whether it is a nutritional artifact of growth in vitro. Second, whether the ability to grow at high temperature in vitro is associated with pigment production. More studies on the physiology of brown diffusible

pigment production in race 2 isolates are needed in order to answer these questions. Phytopathogenic bacteria producing brown diffusible pigments are very rare in nature, and so far have been known to occur only in Xanthomonas campestris pv. phaseoli var. fuscans (CMI, 1965).

King et al. (1954) observed that bacteria of the genus Pseudomonas produce a number of pigments on medium A and B, including fluorescein, pyocynin and pyorubin. In addition, a dark-brown pigment was also produced by some strains, which they speculated was not an oxidation-reduction product of pyocyanin, fluorescein or pyorubin. Since these workers did not specify the species or pathovar that produced the dark-brown pigment, it is unknown whether the brown, diffusible pigment observed in the current investigation is similar to that reported by King et al. (1954).

This work also demonstrated that race 2 isolates of P.s. pv. phaseolicola isolated from debris in Monduli were more virulent than those isolated from growing bean plants. These more virulent isolates produced systemic chlorosis and stunting on the usually resistant differential bean cultivar, Edmund (Fig. 4). These results suggest that virulence of race 2 isolates may increase during survival in the bean debris under dry environmental conditions such as in Monduli. It is not known whether this is due to mutation or selection of a sub-population whose increased virulence is associated with ability to survive in debris. Spontaneous mutation involving increased virulence in P.s. pv. phaseolicola and P.s. pv. pisi has been observed by some researchers (Taylor et al., 1989). It is possible that environmental factors may have a greater effect on the selection of some pathogenicity and virulence genes than the host cultivars grown (Kiyosawa, 1980). Such a phenomenon has also been suggested to occur in Xanthomonas campestris pv. oryzae in Japan (Harino, 1981) but not in dried debris. Although it is difficult to measure precisely the effect on virulence

genes that may be caused by environmental changes, frequent surveys of different ecological areas in Tanzania will always provide information on the effect of the environment on virulence of halo blight bacteria.

The finding that the ability of P.s. pv. phaseolicola to survive in bean debris in the soil and in standing bean plants in the field varied depending on race, geographical location, depth of placement in the soil and the bean genotype used, indicate that halo blight disease management practices should take into account such variations. The survival of the bacterium was favored by soils with a high pH and a long dry period e.g., in Monduli, where it survived for 6 months in the susceptible bean cultivar, Canadian Wonder. These results suggest that debris from highly susceptible cultivars constitutes a source of primary inoculum for halo blight under field conditions in dry areas. These data agree with previous reports that the survival of P.s. pv. phaseolicola is favored by dry soils (Natti, 1967, 1970), although no pH values were given in these studies. This study did not distinguish the effect of soil moisture and soil pH on survival of halo blight bacteria. Soil moisture affects the longevity in the soil of many phytopathogenic bacteria by increasing microbial activity (Natti, 1970). However, the effect of soil pH on the survival of such bacteria has not been well studied.

Under high soil moisture conditions and low pH such as was the case at Lyamungu, survival of halo blight bacteria was considerably reduced, especially in the coffee/banana environment where soil moisture tended to remain high (Table 3). However, halo blight diseased bean plants of the susceptible cultivar, Canadian Wonder, when left standing in the field may act as sources of inoculum when beans are grown in pure stand, since in areas like Lyamungu, two to three bean crops may be planted every year. However, it is unlikely that standing bean plants left in the field may act as sources of primary inoculum for beans

grown in the coffee/banana intercrop environment. This is because copper-based chemicals used to control coffee rust (Hamileia vastatrix) may further decrease the ability of P.s. pv. phaseolicola to survive under such environments. Copper-based chemicals have been reported to significantly reduce halo blight bacteria populations in bean fields (Legard and Schwartz, 1987; Saettler and Potter, 1970; Zaumeyer and Thomas, 1957). Data indicate that resistant cultivars do not seem to support survival of P.s. pv. phaseolicola (Table 3). Such cultivars when grown, will reduce the amount of initial inoculum that may survive in bean debris in the field.

On the basis of this study, it was observed that volunteer bean plants also provide an alternative survival site for halo blight bacteria in northern Tanzania, bridging the gap between the long rain (March-June) and the short rain (October-December) bean crops. The prevalence of race 2 at a higher frequency in northern Tanzania may be related to its ability to survive longer than race 1 under the same conditions in the field (Table 3). It will be interesting to study differences in bean seed infection and transmission between races of halo blight bacteria to verify whether such differences exist and relate them to race prevalence in some regions.

Generally, this study has indicated that there is a great deal of pathogenic variation of P.s. pv. phaseolicola in northern Tanzania. In addition to the use of resistant varieties, control strategies should include sanitation such as burning of infected debris in the field and around backyards after processing the bean crop, especially in dry areas, like Monduli. This will reduce the amount of inoculum that can survive. Plowing under volunteer bean plants when they are still very young will allow faster decomposition and therefore reduce inoculum between crops in wet areas such as Lyamungu.

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CHAPTER 3

THE ROLE OF WEEDS IN SURVIVAL OF PSEUDOMONAS SYRINGAE PV. PHASEOLICOLA IN NORTHERN TANZANIA

INTRODUCTION

The role of weeds and non-host plant species as alternate sources of inoculum for disease epiphytotics has been documented for several plant pathogenic bacteria (Goto, 1972). The importance of cruciferous weeds as a reservoir of inoculum of Xanthomonas campestris pv. campestris causal agent of black rot of crucifers has been reported. The bacterium was observed to spread at least 12 meters from infected weeds to cabbage transplants in Georgia (Kuan et al., 1986; Morris and Knox-Davies, 1980; Schaad and Dianese, 1981). The bean bacterial brown spot pathogen, Pseudomonas syringae pv. syringae, highly virulent on beans, survived overwinter on leaves of hairy vetch (Vicia villosa) in Wisconsin. A correlation was established between the presence of high epiphytic populations of bacteria on this weed and outbreaks of bacterial brown spot in adjacent bean fields. High numbers of bacteria spread from hairy vetch plants to bean plants during rainstorms early in the summer (Ercolani et al., 1974).

Saprophytic survival of plant pathogenic bacteria on root surfaces was first revealed by Valleau et al. (1944). In an extensive survey these workers showed that Pseudomonas angulata (P. syringae pv. tabaci) overwintered in close association with the roots of several crops and weeds. Xanthomonas campestris pv. vesicatoria is another bacterium which Valleau et al. (1944) revealed to exist in association with roots of wheat under natural conditions, but they were unable to prove the same for P. syringae pv. phaseolicola and Xanthomonas campestris pv. phaseoli var. sojense. Laub and Stall (1967), and Kishun and

Sohi (1979), also observed that Xanthomonas campestris pv. vesicatoria survived on leaves of weeds, Solanum nigrum and Physalis minima.

Smith (1962) detected Xanthomonas campestris pv. malvacearum in roots and leaves of various weeds collected from blighted cotton fields by inoculating young cotton seedlings. Kikumoto and Sakamoto (1969) found that the growth of soft rot Erwinia was stimulated in the rhizospheres of weeds which were common in fields of chinese cabbage. These weeds included Agrostis perennans, Portulaca oleraceus, Chenopodium album, and Commelina communis. The effect of soil from the rhizospheres of weeds on the growth of Pseudomonas solanacearum has also been studied (Goto, 1972). The population of this pathogen in artificially infested soils collected from weed rhizospheres varied considerably depending on the kind of weed plants. This suggests that differences in quality and quantity of nutrients secreted from the roots of these plants can influence the pathogen by stimulating or depressing the microflora or by depressing or stimulating the pathogen.

In many parts of Tanzania, one crop season is well separated from the next by a dry season of varying duration depending on location and year. Most plant pathogens, therefore, are faced with a problem of survival between seasons because of the discontinuity of host crop plant populations. Weeds and wild plants may close this gap and thus contribute to outbreaks of plant diseases by bridging between seasons, between crops, and between locations. Weeds also constitute a reservoir of minor diseases which may be exchanged between them and cultivated crops (Dinoor, 1974). Moreover, there is no way yet to predict when and how a minor disease of a wild plant or weed will spread into cultivated crops and become a major problem.

Epiphytic survival of P.s. pv. phaseolicola was first reported by Ercolani et al. (1974). Their studies indicated that the bacterium multiplied to some extent on hairy vetch (Vicia

villosa), a leguminous weed growing near bean fields under Wisconsin conditions. Recently, Taylor et al. (1987) obtained several isolates of P.s. pv. phaseolicola from Neonotonia wightii (Arn.) Leakey in South America and in Africa, including Tanzania. While efforts are being made to control halo blight disease in the bean crop, the disease is not under control in weeds or wild plants. No studies have been conducted in Tanzania to determine the role of weeds and non-host species in survival and as sources of inoculum for halo blight bacteria. More information is therefore needed to predict the impact of P.s. pv. phaseolicola inoculum that develops and survives on weeds. With this view in mind, a survey was conducted to investigate the role of weeds in survival and as sources of inoculum for halo blight bacteria in northern Tanzania, which is the focus of this chapter.

MATERIALS AND METHODS

Sampling and isolation procedures

Isolations of P.s. pv. phaseolicola were attempted from weed plants with and without leaf lesions. These weed plants were collected from the bean growing areas of the Arusha and Kilimanjaro regions, northern Tanzania, from November 1988 to January 1990. Weed collections were made where halo blight was reported to be severe the previous growing season. In addition, samples were collected from hedges around backyards of farmers' houses, roadsides, and on hedges which commonly grow along irrigation channels. Weed samples were placed in plastic bags and sent to the laboratory where they were kept cold at 5°C until processed within 24-48 hours.

Before processing in the laboratory, samples were separated into leaves, stems, and roots. Ten to 15 g of each of these weed plant components were weighed and placed in 500 ml flasks containing 100-300 ml of 0.01 M phosphate buffer, pH 7.2, with 0.01 percent

Tween 20. Samples were shaken for 30 minutes on a wrist action shaker (Staurt Scientific Co. Ltd, England) adjusted at medium speed at 22-25°C. The supernatant liquids were 10-fold serially diluted four times in the same buffer and 0.01 ml portions from each dilution, including the original undiluted liquid, were plated on Medium B of King et al. (1954) (KMB) supplemented with 100 μg cycloheximide per ml to prevent fungal growth. Inoculated plates were incubated at 24 ± 2°C for up to 5 days. Plates were examined under short- (254 nm) and long-wave (366 nm) ultraviolet light. Fluorescing colonies were transferred and further purified on KMB using a series of single-colony transfers. Isolation was also attempted from weed leaves that contained lesions. Pieces of diseased tissue including small areas of adjoining healthy tissue were excised, surface-sterilized with 2.6% NaOCl for three minutes, and rinsed twice in sterile glass distilled water. The tissue pieces were then transferred onto flame-sterilized glass slides and crushed in one or two drops of sterile distilled water. The resulting suspensions were streaked on KMB-cycloheximide plates and incubated at 24 ± 2°C. Bacterial colonies resembling P.s. pv. phaseolicola were purified as described above.

Storage of isolates

After purification, isolates were maintained as very thick suspensions in glycerol: 0.1M phosphate buffer (50:50, v/v) and on nutrient agar slants at 0°C in an incubator (A. Gallenkamp and Co., London). Isolates were also stored in dry bean leaf powder at room temperature (22 ± 2°C) in darkness. Ten-day old bean seedlings, variety Canadian Wonder, grown in a screen house were spray-inoculated abaxially with suspensions of pure cultures of P.s. pv. phaseolicola isolates. Inoculated plants were maintained in the screen house at 19-27°C. Leaves with typical halo blight symptoms were harvested 10-14 days after

inoculation and air-dried at 22 ± 4°C. After drying leaves were ground to a fine powder using sterile mortars and pestles, and the powder containing each bacterial isolate was stored in sterile glass vials.

Sterilization and grinding of samples for detection of *P.s.* pv. *phaseolicola*

Samples were surface sterilized using 2.6% NaOCl for 2-3 minutes and rinsed in sterile glass distilled water. Samples were ground using sterile mortars and pestles, then mixed with the required volume of sterile 0.01 M phosphate buffer, pH 7.2, unless otherwise stated. The resulting suspensions were left to stand for 20-30 minutes followed by ten-fold serial dilutions in the same buffer, and 0.1 ml portions were plated on KMB or other specified media.

Identification of bacterial isolates

Identification procedures were based on Schaad's manual (1988) and those given by Hayward (1985). Isolates were observed for production of green diffusible fluorescent pigment on KMB (King et al., 1954) using short- (254 mn) and long- (366 nm) wavelength ultra-violet light. All isolates were tested for levan production, oxidase reaction, pectolytic activity, arginine dihydrolase activity and tobacco hypersensitivity (LOPAT) (Lelliot et al., 1966). For levan production, 24-hour old bacterial cells grown on KMB were streaked on NA containing 5% sucrose (w/v) and incubated for up to 5 days. Three replicates were used for each isolate, and NA plates without sucrose were used as controls. The appearance of domed mucoid colonies after 3-5 days of incubation was considered positive for levan production.

Oxidase reaction (Kovacs, 1956) was tested by smearing cells from a 24-hour KMB plate cultures of each isolate with a platinum wire loop on a filter paper containing an oxidase spot reagent (Difco). Three tests were conducted for each isolate. The appearance of a dark red color within 60 seconds was considered to be positive. Pectotytic enzymes activity was detected by pit formation on polypectate containing media, using the procedures described by Hildebrand and Schroth (1971). Bacterial cells from 24-hour old plate cultures were spot-inoculated in triplicate on plates of sodium polypectate medium (1 ml 1.5% alcoholic bromthymol blue, 6 ml 10% $CaCl_2 \cdot 2H_20$ and 22 g sodium polypectate in 1000 ml glass distilled water; 100 ml of 4% molten sterile agar was added after autoclaving. The pH was adjusted to 5.0 and 8.0 (Olive and McCarter, 1988) using HCl and NaOH). Inoculated plates were incubated for 6-7 days. High and low pH values were used to determine the ability of species to grow at the two specific pH values.

Arginine dihydrolase activity was tested for using Thornley's medium 2A (Thornley, 1960) (g/l:peptone, 1.0; NaCl, 5.0; K_2HPO_4, 0.3; agar, 3.0; phenol red, 0.01; arginine HCl, 10.0); pH 7.2. Ten ml of sterile medium in test tubes were stab-inoculated with bacterial cells, 24 hours old. After inoculation, the surface of the medium in each test tube was layered with 3 ml of 3% sterile molten agar (Fahy & Hayward, 1983) and incubated for up to five days. A color change to red (alkaline) was considered positive. Each bacterial isolate was replicated three times and test tubes stab-inoculated with a sterile loop wire were included as negative controls.

The ability of bacterial isolates to induce a hypersensitive reaction (HR) on tobacco (Nicotiana tabacum L.) was tested (Lelliot and Stead, 1987; Klement et al., 1964). Tobacco plants were inoculated with suspensions of each bacterial isolate by infiltrating the cell suspensions into leaves. Infiltration was done abaxially by pressing the end of a sterile 10

cc disposable hypodermic syringe against forefinger-supported tobacco leaves and slowly introducing the bacterial suspensions. Each isolate was replicated twice. P.s. pv. phaseolicola isolates 1299A and 1301A supplied by Dr. J.D. Taylor (Institute of Horticultural Research, Wellesbourne, Warwick, England), and sterile phosphate buffer were used as positive and negative controls, respectively. Inoculated tobacco plants were kept in the screen house and evaluated for HR after 24-48 hours.

Pathogenicity tests

All isolates were evaluated for pathogenicity on susceptible Canadian Wonder bean plants. Screen house-grown bean plants were inoculated when the first trifoliolate leaf was fully open (10-14 days old) by spraying abaxially with a hand operated plastic atomizer (Bayer Chemical Co., Nairobi, Kenya) to run-off without water-soaking. Each isolate was replicated three times using one bean plant per replicate. A known pathogenic P.s. pv. phaseolicola isolate (1299A) and sterile phosphate buffer were included as positive and negative controls, respectively. Inoculated bean plants were incubated in the screen house and observed daily for symptom development. Pathogenicity was evaluated 10-14 days after inoculation. Appearance of typical halo blight symptoms confirmed P.s. pv. phaseolicola identity.

Ice nucleation activity

Isolates were tested for ice nucleation activity (INA) using the single temperature droplet freezing method of Lindow (1988) and Lindow et al. (1978, 1982) at -5°C. Bacterial suspensions from cultures grown for 2-3 days were prepared in 0.1 M phosphate buffer, pH 7.0, and adjusted to an optical density of 0.1 at 620 nm using a

spectrophotometer. Suspensions were ten-fold serially diluted three times in the same buffer. INA was determined by placing 30 ten-μl droplets from each of the dilutions, including the original suspensions, on paraffin-coated aluminum weighing boats floating on 50% ethanol maintained at -5°C in the refrigerator (Lee Refrigeration PLC, Bognor Regis, West Sussex, England). The freezing of each individual droplet was determined visually at one minute time intervals up to three minutes. Droplets from blank phosphate buffer dilutions were included as negative controls.

Carbon source utilization

Stock solutions of mannitol, sorbitol and inositol (Sigma Chemical Co., St. Louis, MO) were prepared in glass distilled water following the procedures given by Schaad (1988). Stock solutions were filter sterilized using 0.22 μm pore size membrane filters (Millipore Product Division, Bedford, MA 01730) attached to a 60 ml hypodermic syringe, and added at 0.1% (w/v) final concentration to autoclaved and cooled (45-50°C) Dye's medium C (g/l of glass-distilled water: $NH_4H_2PO_4$, 0.5; K_2HPO_4, 0.5; $MgSO_4 \cdot 7H_2O$, 0.2; NaCl, 5.0; yeast extract, 1.0; Bacto-agar, 15.0). Bromocresol purple (1 ml of a 1.6% alcohol solution) was added before autoclaving. Log phase bacterial cultures grown on KMB were streaked on the medium in triplicate and plates were incubated for up to 10 days. Growth was compared to plates containing no added carbon sources.

Bacteriophage tests

Sensitivity to bacteriophage was tested using the methods described by Taylor (1970) and J.D. Taylor and D.M. Teverson (personal communication) as described in Chapter 2.

Serology

P.s. pv. phaseolicola antiserum was kindly provided by Dr. J.D. Taylor. The agglutination method of J.D. Taylor and D.M. Teverson (personal communication) described in Chapter 2 was used. Three tests were conducted for each bacterial isolate, and known P.s. pv. phaseolicola isolates 1299A and 1375A were included as positive controls.

Race identification

After species identity was confirmed, isolates of P.s. pv. Phaseolicola obtained from weeds were subjected to race identification using differential bean cultivars Canadian Wonder (universal susceptible), Edmund (universal resistant), Red Mexican UI3 (resistant to race 1) and Tendergreen (resistant to race 3). Both pod and plant leaf reactions were used for race determination as described earlier in Chapter 2.

Assay of *Neonotonia wightii* reproductive parts for *P.s.* pv. *phaseolicola* infection

Flower buds, open flowers and young flat pods of infected Neonotonia wightii plants were collected from Monduli district, Arusha region, and from Hai district, Kilimanjaro region, Tanzania, during 1989 and 1990. Samples were collected from reproductive branches containing severely infected leaves. Plants from each site were marked so that periodic sampling could be done on the same plants. Samples from each site were placed in separate plastic bags to allow the correlation of any seed infection with weather conditions, which were recorded for each site. After collection, samples were sent to the laboratory and kept at 5°C until processed within 24-28 hours.

For each sampling, 10 flower buds and 10 open flowers were weighed separately, surface-sterilized, rinsed, and ground in 10 ml of sterile phosphate buffer. Aliquots of 0.1 ml were plated on KMB-cycloheximide and incubated as earlier described for 5 days. To assay for the possibility of P.s. pv. phaseolicola infection in young flat pods, 10 pods for each sampling time from each site were weighed, surface sterilized, rinsed, comminuted and plated as above. Plates were incubated and observations made daily to detect the presence of fluorescing colonies.

Neonotonia wightii seed infection and transmission assay

Mature dry pods from Neonotonia wightii plants collected from selected sites were placed in plastic bags and sent to the laboratory where they were air-dried. After drying pods were shelled manually by hand; both seeds and shells were assayed for the presence of P.s. pv. phaseolicola. Transmission of P.s. pv. phaseolicola in seed was assayed using the method of Schuster and Coyne (1975). N. wightii seeds were surface-sterilized, rinsed three times and blotted dry on sterile filter papers in a laminar flow chamber. Drying was necessary to remove the sticky character of seeds when wet which made them difficult to handle. After drying, seed lots from each site were transferred into sterile test tubes containing 10 ml of phosphate buffer with 0.01% Tween 20. Samples were shaken for 30 minutes on a wrist action shaker, after which seeds were removed from test tubes aseptically and divided into two lots. One lot was dried aseptically, plated hilum down on KMB-cycloheximide agar and incubated for 5 days. Plates containing bacterial growth around seeds were observed under UV-light to detect any fluorescing bacterial colonies.

Fluid in which seeds were soaked was diluted and plated on the same medium. Portions of the seed soakings from each seed lot were also used to inoculate five, 10-15 day old Canadian Wonder bean seedlings by injection and leaf water-soaking methods. In the latter, inoculum was introduced into four infiltration sites on primary and fully open first trifoliolate leaves, and each plant was considered a replicate. Inoculated bean plants were maintained in the screen house and observed for halo blight symptoms for up to 14 days. Control plants were inoculated with dilute suspensions of P.s. pv. phaseolicola (10^3-10^4 CFU/ml) to simulate the low amount of inoculum which may occur in the soakings, and negative control plants were inoculated with sterile phosphate buffer.

Another seed lot from each test tube was planted in the screen house. Individual plants that germinated were examined for the presence of water-soaked lesions on stems and leaves. Starting from the primary leaf stage, 5 plants were sampled at random at 4 day intervals for three weeks to further assay for the presence of P.s. pv. phaseolicola. Sampled plants were separated into leaves, stems, and roots. The separate plant components were weighed, surface sterilized, rinsed, and ground in phosphate buffer. The suspensions were serially diluted and plated on KMB-cycloheximide. Plates were observed for up to 5 days.

Shells from mature pods of N. wightii were assayed for presence of P.s. pv. phaseolicola. From each site and for each sampling date, 3 g of pod shells were surface-sterilized, ground and mixed with phosphate buffer. Dilutions of suspensions were plated and observed for the presence of P.s. pv. phaseolicola-like colonies for up to 5 days.

Reaction of *Neonotonia wightii* to races 1, 2 and 3 of *Ps.* pv. *phaseolicola*

To test the reaction of N. wightii to the prevailing three races of P.s. pv. phaseolicola in the region, uninfected plants were identified from three locations,

Lyamungu, Lambo and Monduli, representing different weather conditions. Mature pods were harvested, placed in labelled plastic bags and air-dried under laboratory conditions at 22-27°C. Dry pods were then manually shelled by hand and seeds from each site were kept separate. To check for the absence of internal P.s. pv. phaseolicola infection, seeds from each location were surface-sterilized, mixed with 10 ml of phosphate buffer containing 0.01% Tween 20 and shaken for 30 minutes. Seeds were removed from test tubes and the resulting soakings were diluted and plated as described. Observations were made daily for the presence of P.s. pv. phaseolicola-like colonies for 5 days.

One hundred surface-sterilized pathogen-free seeds from each site were planted in 16 cm diameter sterile plastic pots, 20 seeds per pot. The experimental design was a split-split plot with five replicates, with location as whole plots, plant age as subplots and P.s. pv. phaseolicola races as sub-subplots. Three locations, two plant ages (9 and 15 days) and three isolates for each race were used. Two of the race 1 isolates (41, 83) were obtained from Phaseolus vulgaris, the third race 1 isolate (1281A) was obtained from P. coccineus and was supplied by Dr. J.D. Taylor. All race 2 isolates (7, 42, 106) were obtained from P. vulgaris, and isolate 106 produced a brown pigment. All race 3 isolates (46, 89, 1301A) were obtained from P. vulgaris.

Inoculum for each isolate was produced as described above. N. wightii plants, 9 and 15 days old, were spray-inoculated to run-off without water-soaking, using a hand operated atomizer. Control plants were spray-inoculated with race 1 isolated from N. wightii plants and sterile phosphate buffer. Inoculated plants were kept in the screen house and observed daily for symptom development for 15 days. The experiment was repeated twice.

Insects as pests of beans and *N. wightii*

Many Neonotonia wightii plants which were infected by P.s. pv. phaseolicola at Lyamungu Agricultural Research Station, were highly perforated by leaf chewing insects. Pod borers were also evident on dry pods. Therefore, efforts were made to determine the possibility of such insects attacking beans, thus disseminating halo blight bacteria to beans and N. wightii. Dry pods of N. wightii damaged by insects were collected at Lyamungu Agricultural Research Station and stored in 500 ml beakers covered with a nylon cloth, in the laboratory at 20-24°C. After 25-30 days, pods were shelled manually and adult weevils of an unidentified bruchids were collected and transferred to another container with sound dry pods of N. wightii where they were maintained. Weevils were then tested on bean cultivars Canadian Wonder, Masai Red and Kiburu (local); N. wightii seeds were included as controls.

The method of Giga and Smith (1987) was used to test the susceptibility of bean seeds to bruchids from N. wightii. Seeds were first sterilized by freezing for 10 days to kill eggs or insects present, then were equilibrated for about 3 weeks at 20 to 24°C. One hundred seeds of each test cultivar and about 300 seeds of N. wightii seeds were placed in sterile 250 ml flasks. Five weevils (2 males, 3 females) were placed in each flask and covered with finely perforated nylon cloth for aeration. Each test sample was replicated three times and kept in the laboratory at temperatures mentioned above, and observed periodically. After the introduced insects had died, samples were kept for a period of 28 days to allow any hatched eggs to develop. When emergence holes were observed on the control samples, the number of adult bruchids in each test sample were recorded. Samples were considered susceptible when the weevil count was higher than the original number in each flask. For leaf chewing insects, highly perforated plants were frequently visited at

different times of the day. The aim of such visits was to collect insects found on these plants and cage them on bean plants to find out possibilities that such insects could be bean pests, thus disseminate halo blight bacteria between N. wightii and beans. Visits were conducted for a period of four months beginning September, 1989.

RESULTS

A total of 17 weed species belonging to 10 families were collected and assayed for the presence of P.s. pv. phaseolicola for the period of November 1988 to January 1990 (Table 1). Only one weed species, Neonotonia wightii (formerly Glycine wightii), belonging to the family Leguminosae was found to be a natural host of halo blight bacteria. Symptoms were restricted to leaves. Characteristic leaf symptoms initially appeared as small, water-soaked spots on the undersides of young leaves. Later, a greenish-yellow halo zone developed around the water-soaked area (Fig. 1). Symptoms were more evident on young leaves a few weeks after the onset of the short rains (October to December). During the dry season, halo development around necrotic areas was very restricted, and sometimes did not occur.

P.s. pv. phaseolicola was isolated from N. wightii samples collected in Kilimanjaro and Arusha regions throughout the year because the weed survives dry seasons, mainly on fences (Fig. 2), hedge rows, by the roadsides, on ditch banks near bean fields and in corners of the farmers' fields. In some fields, halo blight symptoms occurred only on the foliage and pods of bean plants grown near N. wightii. They occurred especially on the leeward side of the hedges, indicating that P.s. pv. phaseolicola was disseminated from infected weeds to the bean crop during rains.

Table 1. Weed families and species assayed for presence of Pseudomonas syringae pv. phaseolicola in northern Tanzania.

Family/Weed species	Location	Symptoms
1. Asteraceae (Compositae)		
Bidens pilosa L.	Kilacha/Lyamungu/ Monduli	Leaf necrosis & chlorosis
Bothriocline laxa N.E.Br: (Erlangea laxa)(N.E.Br.)S.Moore	Lyamungu	Marginal necrosis on stems & leaves
Conyza sumatrensis (Retz) E.H. Walker	Miwaleni/Lyamungu	None
Galinsoga parviflora Cav.	Lyamungu/Monduli/ Sanya Juu	None
2. Boraginaceae		
Trichodesma zeylanicum (Burn.f.) R.Br.	Lambo/Monduli Kirua/Tengeru	Necrotic lesions on leaves
3. Commelinaceae		
Commelina benghalensis L.	Lambo/Monduli	None
4. Cyperaceae (Sedges)		
Cyperus lotundus L.	Lyamungu	Stem & leaf necrosis, water soaking, chlorosis
5. Leguminosae		
Neonotonia wightii (Wight & Arn.) Lackey: (Glycine wightii)	Lambo/Monduli/Sanya Juu/ Lyamungu/Tengeru	Leaf necrotic lesions, water soaking & halo
Pueraria sp.	Lyamungu	Necrotic lesions on leaves
6. Malvaceae		
Sida alba L.	Lambo	Stem & leaf necrosis, water soaking, chlorosis

Table 1. (cont.)

Family/Weed species	Location	Symptoms
7. Nyctaginaceae		
Boerhavia erecta L.	Lambo/Miwaleni	None
8. Poaceae (Grasses)		
Panicum sp.	Lambo/Selian	None
Setaria sp.	Kibosho/Lyamungu	Leaf necrotic lesions
9. Polygonaceae		
Oxygonum sinuatum (Meisn.) Dammer	Lambo/Monduli	None
10. Solanaceae		
Nicandra physalodes (L.) Gaertn	Lambo/Narumu/Sanya Juu/ Monduli/Tengeru	Leaf necrosis & halo
Solanum incunum L.	Sanya Juu/Lyamungu	Leaf necrotic lesions, chlorosis

Figure 1. Typical halo blight symptoms on Neonotonia wightii leaves.

Figure 2. Halo blight-infected Neonotonia wightii plants surviving the dry season on a fence at Lyamungu Agricultural Research Station, Tanzania.

Identification of bacterial isolates

Twenty seven isolates of fluorescent pseudomonads, of which 22 were obtained from N. wightii and 5 from Bothriocline laxa, were characterized. All isolates were fluorescent under both short- (254 nm) and long- (366 nm) wavelength ultraviolet light. All 22 isolates from N. wightii were positive for levan sucrase; negative for oxidase, arginine dihydrolase, INA, and pectolytic activity; and produced a rapid hypersensitive reaction on tobacco leaves. These isolates also did not utilize mannitol, sorbitol, and inositol as carbon sources, they were sensitive to bacteriophages 11P, 12P and 48P as evidenced by plaque formation, and agglutinated with P.s. pv. phaseolicola antiserum, confirming P.s. pv. phaseolicola identity. Bacterial isolates from B. laxa were positive for INA, oxidase, arginine dihydrolase, and 2 of the five isolates utilized the three carbon sources. The five isolates were negative for levan production and, pectolytic activity; they did not produce a hypersensitive reaction on tobacco, and were insensitive to the three bacteriophages used. These data indicated that the five isolates from B. laxa were P. fluorescens.

Pathogenicity tests

Identity of the 27 bacterial isolates was confirmed by pathogenicity tests. All isolates from N. wightii were pathogenic on susceptible Canadian Wonder bean plants, whereas those from B. laxa were not. Water-soaked lesions developed within 3-4 days of inoculation and typical halo blight symptoms developed 7-10 days after inoculation. Systemic chlorosis was also evident in young leaves.

Race identification

Leaf and pod reactions of bean differential cultivars to the 22 bacterial isolates obtained from N. wightii are shown in Table 2. On the basis of these results all isolates were typical of race 1 as shown by a resistant reaction on Red Mexican UI-3. All compatible reactions involved the development of large water-soaked lesions, especially on Tendergreen, and the production of bacterial ooze at the point of inoculation on the stem. Inoculation by leaf spraying and pod inoculation gave similar results. Rapid hypersensitive reactions were observed in leaves and pods of the cultivar Edmund as indicated by tissue browning at the inoculation point. The virulence of 10 race 1 isolates from N. wightii was compared with 10 race 1 isolates from bean sources on the differential varieties. There was no difference in incubation period and symptom development between the two groups of race 1 isolates.

Assay of *N. wightii* reproductive parts for infection by *P.s.* pv. *phaseolicola*

Throughout 7 months (August to December, 1989 and January to February, 1990) of testing, no flower bud, open flower, or flat pod samples were found infected with P.s. pv. phaseolicola, despite severe foliage infection of sampled plants. Similar results were obtained for all three locations studied, which also represented different weather conditions (Chapter 2). Even random samples of reproductive parts collected within 5-10 cm of highly diseased shoots were free of any detectable levels of the pathogen. Moreover, symptoms were not seen on any reproductive part examined for samples collected from all locations. It was also observed that pods on N. wightii plants were very hairy, a factor which may prevent the inoculum from reaching the pod surface, but this was not true for flower buds or parts of open flowers. The restriction of symptoms to foliage and lack of

Table 2. Race identification of Pseudomonas syringae pv. phaseolicola strains isolated from Neonotonia wightii in northern Tanzania.

Location	No. of isolates	Bean differential cultivar[a] CW Leaf/Pod	RM Leaf/Pod	TG Leaf/Pod	ED Leaf/Pod	Race
Lambo	3	+/+[b]	-/-	+/+	-/-	1
Lyamungu	11	+/+	-/-	+/+	-/-	1
Machame	1	+/+	-/-	+/+	-/-	1
Monduli	6	+/+	-/-	+/+	-/-	1
Tengeru	1	+/+	-/-	+/+	-/-	1

[a]1CW = Canadian Wonder RM = Red Mexican UI-3
TG = Tendergreen ED = Edmund

[b]+ = Susceptible reaction; - = Resistant reaction

Data were compiled from two experiments

detection of halo blight bacteria in flower buds, open flowers, and flat pods indicate that P.s. pv. phaseolicola was not able to move systemically in the vascular tissue to these reproductive tissues under different weather conditions that existed in these locations.

Neonotonia wightii seed infection and transmission assay

To determine whether halo blight bacteria were present in mature seeds of N. wightii, samples were taken monthly from three locations (Lambo, Lyamungu, and Monduli), representing differing weather conditions. All mature pods harvested from N. wightii plants with severe foliage symptoms of halo blight were symptomless. Seeds from these pods were also symptomless. Direct seed plating, plating of seed washings, and of shell pod suspensions all failed to detect the pathogen. Canadian Wonder bean plants inoculated with seed soakings did not develop visible halo blight symptoms unlike positive control plants which showed typical halo blight symptoms 12 days after inoculation. However, seeds at all three locations studied were infected with other bacteria. The dominant colony color of such bacteria was creamy-white; a few colonies were orange colored. The percentages of seed infected with these unidentified bacteria for each sampling period at different locations are shown in Table 3. None of the colonies observed fluoresced under UV-light and none were tested for pathogenicity.

In the screen house experiments, a total of 2860 seedlings were assayed for infection by Psp. None of the seedlings of N. wightii from seeds collected from plants naturally infected with halo blight bacteria showed any visible symptoms on stems or leaves. The bacterium was also never recovered from root, stem, and leaf samples from such seedlings.

Table 3. Percentage of Neonotonia wightii seed infected with Pseudomonas syringae pv. phaseolicola and other bacteria in northern Tanzania.

Sampling date	Mean # of seeds assayed	% Seed infection at[a]					
		Lambo		Lyamungu		Monduli	
		Psp[b]	Others	Psp	Others	Psp	Others
August 1989	652	0.0	55.7	0.0	75.9	0.0	62.2
Sept 1989	596	0.0	20.1	0.0	52.7	0.0	13.4
Nov 1989	990	0.0	37.0	0.0	31.1	0.0	64.2
Dec 1989	495	0.0	41.3	0.0	39.4	0.0	85.0

[a]For each sampling date, values are means of three replicates. Seeds were surface sterilized in 2.6% NaOCl for three minutes and plated on KMB supplemented with cycloheximide (100 mg/ml), and incubated for 5 days at 24 ± 2°C.

[b]Psp = Pseudomonas syringae pv. phaseolicola

Reaction of *Neonotonia wightii* to races 1, 2, and 3 of *P.s.* pv. *phaseolicola*

Table 4 presents the reaction of N. wightii seedlings to races 1, 2, and 3 of P.s. pv. phaseolicola under screen house conditions. Pathogen-free seeds used for the study were collected from three locations in northern Tanzania, representing different weather conditions. All three races produced susceptible reactions in plants inoculated at 9 and 15 days old. The development of halo blight symptoms was observed daily after foliage inoculation. There was no difference in incubation period and the kind of symptoms produced on these seedlings for all three races. Typical halo blight symptoms appeared 8 days after inoculation at 19-27°C; results were consistent for the two experiments conducted. Stunting of plants, chlorosis of young leaves, and presence of yellowish-green halos around water-soaked and necrotic lesions were evidence of toxin production by isolates of all three races tested.

Susceptibility of three bean cultivars to insect pests of *N. wightii*

An unidentified bruchid from N. wightii did not attack the bean cultivars: Canadian Wonder, Masai Red, and Kiburu (a local landrace). In contrast, there was a 60% increase in insect count on N. wightii (Table 5). This may indicate that female bruchid weevils were either not able to lay eggs on the tested bean cultivars, or eggs were laid and failed to hatch. Despite frequent visits to insect-damaged and halo blight-infected N. wightii plants to search for leaf chewing insects, no such insects were observed on the plants. It was, therefore, suspected that the damage may be caused by insects which could be nocturnal feeders.

Table 4. Reaction of Neonotonia wightii to races 1, 2, and 3 of Pseudomonas syringae pv. phaseolicola from northern Tanzania.

Plant age[a] (days)	Isolate numbers	Race	Seedling reaction for seed from		
			Lambo	Lyamungu	Monduli
9	41,83,1281A	1	+[b]	+	+
	7,42,106	2	+	+	+
	46,89,1301A	3	+	+	+
15	41,83,1281A	1	+	+	+
	7,42,106	2	+	+	+
	46,89,1301A	3	+	+	+

[a]Age at which Neonotonia wightii seedlings were inoculated. Inoculation was done as explained in the text. Two experiments were conducted for each plant age and each treatment was replicated five times, each replication consisting of 20 plants.

[b]+ = Susceptible reaction.

Table 5. Susceptibility of seed of three bean cultivars grown in northern Tanzania, and Neonotonia wightii seed, to bruchid weevils.

Cultivar	No. of insects introduced[a]	No. of newly emerged insects after 28 days[b]
Canadian Wonder	5	0
Masai Red	5	0
Kiburu	5	0
N. wightii	5	3

[a]Five insects (2 males/3 females) were introduced for each treatment.

[b]Insect counts are means of three replicates. Each replicate consisted of 100 bean seeds and 300 N. wightii seeds. Counts were taken 28 days after the introduced insects had died.

DISCUSSION

It has generally been considered that halo blight infected bean seed is the most important source of P. s. pv. phaseolicola inoculum, especially given that pathogen-free seed programs are lacking in Tanzania, and farmers retain portions of their harvest for the next planting (Gondwe, 1987). The current study indicated that at least one weed species, N. wightii can also serve as a perennial reservoir of P. s. pv. phaseolicola under northern Tanzania conditions. Halo blight bacteria were detected in this weed throughout the year, even during dry periods when beans were not growing in the field. These results suggest that N. wightii provides a suitable habitat for long term survival and multiplication of P. s. phaseolicola over much of the year.

The pathogen was readily disseminated from the weed to neighboring bean plants under different weather conditions. These findings may account for occasional outbreaks of bean halo blight which have been reported in the Kilimanjaro region. Since some of the farmers' bean crops were free of halo blight disease, it is suggested that infected seed is not always the source of initial inoculum in the region. Furthermore, halo blight was observed only at the edges of the fields in some areas in the region, another indication that N. wightii is an important source of supplementary initial inoculum for this pathogen. B. Gondwe, (personal communication) and farmers in northern Tanzania have noted that halo blight outbreaks were few in the region in years of little rain. Such observations have led farmers in Monduli to believe that halo blight is not a disease but a stress caused by excess rainfall. Gondwe (1985) speculated that occasional outbreaks of halo blight disease in the Kilimanjaro region were due to the occurrence of a new virulent race, probably race 3. However, in an extensive survey of the prevailing races in northern Tanzania, race 3 was not recovered in the Kilimanjaro region (Chapter 2).

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Since N. wightii is a common weed on fences and hedges around farmers' backyards, and most farmers thresh their bean crop in backyards, infected bean debris and diseased volunteer plants may be a source of primary inoculum for N. wightii weeds around such areas. In addition, the bacterium is also likely to be spread from prunings from hedges containing diseased weeds. N. wightii is also used as a feed for animals kept under the zero grazing system commonly employed in the Kilimanjaro region, a factor which may further assist in disseminating the bacterium. Moreover, halo blight bacteria have been reported to survive for 9 months after passage through sheep under Australian conditions (Starr and Kercher, 1969). Such may also be the case in the Kilimanjaro region, especially in dry areas.

The finding that only race 1 of P. s. pv. phaseolicola was recovered from N. wightii throughout the period of this study is surprising, but agrees with those reported by CIAT (1987) and in general by those reported by Taylor et al. (1987). Since both bean and N. wightii are susceptible to race 1 of the pathogen, the original host of this race is not clear. Screen house experiments revealed that races 2 and 3 could also cause disease in N. wightii when inoculated artificially. However, there are only two reports of race 2 isolates from the same weed under natural conditions, one from Rwanda and another from Tanzania (Taylor et al., 1987). Thus, the general trend seems to be clear, that under natural conditions susceptibility of N. wightii seems to be restricted to race 1. It is unknown why race 2 was not recovered from N. wightii in this study, despite its high frequency on bean in northern Tanzania (Chapter 2). One possible reason for lack of natural infection may be that the minimum bacterial population required for disease occurrence in the weed was not attained. Infectivity titration and host-pathogen relationship studies are needed to understand the relationship between races of P. s. pv. phaseolicola and N. wightii.

Based on the lack of seed infection in this weed, we can hypothesize that under field conditions a nearby infected bean crop acts as a source of initial inoculum for N. wightii plants. Once diseased, the weed remains so until the next season when it acts as a source of inoculum for a pathogen-free bean crop that may be grown nearby, or as a supplementary source of inoculum for the bean crop. The restriction of halo blight disease symptoms on foliage, and the failure to detect any internal infection in flower buds, blossoms, young pods, mature dry seeds and pod shells, indicate that the pathogen does not move systemically in the vascular tissue of this weed to the seed in the pod. The mechanism by which P. s. pv. phaseolicola is excluded from vascular transmission to the seed of this weed is unknown. Also, it is not known why other bacterial contaminants occurred in N. wightii seed at very high numbers, nor how they gained access to the seeds. It is epidemiologically significant that P.s. pv. phaseolicola is not seed-borne in N. wightii. This eliminates the danger of N. wightii spreading the halo blight pathogen into new areas through seed. Although this danger remains with infected bean seed, procedures of producing bean seed relatively free from halo blight bacteria are feasible (Schwartz, 1989).

Insect pests attacking N. wightii pods and seed do not appear to be bean pests. However, the possibility of cross attack by leaf chewing insects can not be eliminated. Moreover, contaminated insect pests from diseased N. wightii might, under high moisture conditions such as those at Lyamungu, deposit halo blight bacteria when walking on bean leaves. This possibility increases when an insect is a common pest of both beans and N. wightii. More experiments are needed to establish the possibility that insect pests may serve as vectors of P. s. pv. phaseolicola between the two hosts.

Attempts to control halo blight disease in northern Tanzania and in other areas must take into consideration infected N. wightii plants as reservoirs of the pathogen. Control measures should aim at disengaging the source of inoculum from the new bean crop by use of pathogen-free seed and sanitation measures. Because survival of N. wightii plants occurs primarily outside cultivated fields, destruction of such plants around bean fields would prevent a disease-free bean crop from infection by inoculum from weeds by shifting the source of inoculum further away from the bean fields. These disease control measures could also be supported by the introduction of halo blight-resistant bean varieties.

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CHAPTER 4

POPULATION DYNAMICS OF PSEUDOMONAS SYRINGAE PV. PHASEOLICOLA IN TWO BEAN CROPPING SYSTEMS IN NORTHERN TANZANIA.

INTRODUCTION

The majority of the bean crop in Africa and other tropical areas is produced by small farmers in complex associations with other crop species, notably maize. Depending on the country, companion crops may include bananas, cassava, sweet potatoes, yams, peas, and coffee. Such crop associations on the same land and at the same time are referred to as intercrops (CIAT, 1986a). The bean cultivars used in these cropping systems (landraces) usually represent years of natural evolution as the farmers have selected them for survival and production. The term landrace refers to a heterogeneous mixture of a predominantly self-pollinating species which is maintained by a subsistence farmer. Very low levels of hybridization may occur causing genetic recombination. The occurrence of such genetic drift and individual farmer selection serve to make each population genetically unique (Martin, 1984). Some of these landraces include a great mixture of seed types, while others may have a high proportion comprising generally similar seed types though of diverse phenotype, otherwise. Bean landraces can easily be separated into their components by planting progeny rows from single plants, and when this is done, differences in growth habit, disease resistance and yield potential between them are readily apparent. Some components are often so obviously inferior that it is difficult to understand why they have survived. Occasionally, one component may appear so outstanding in comparison with others that it is equally difficult to understand why its selection as a more or less pure line has been avoided (Leakey, 1970). Landraces are generally good competitors with weeds and other associated crop species (CIAT, 1986b, Leakey, 1970).

Until the 1960's there has been a tendency in East Africa and Africa in general, for agricultural workers to discourage intercropping and instead to promote monocropping. However, small farmers have persistently refused to abandon the system of intercropping (van Rheenen, et al., 1981). Moreover, during the last ten years, there has been an increasing awareness that the impact of the so-called green revolution on small holder farming in developing countries has remained rather limited. Hence, research has directed more attention to the analysis and subsequent improvement of traditional cropping systems including the more efficient use of limited resources. Research results so far clearly indicate that traditional intercropping systems are better adapted to the ecological, socio-economic and socio-cultural conditions of tropical agriculture than monocropping systems (Burdon, 1978; CIAT, 1986b; Norman and Baker, 1974; Steiner, 1984).

Various researchers have pointed out that plant disease epidemics are favored by morphologically and genetically uniform crops grown on large areas of land (Browning, 1957; Browning and Frey, 1969; National Academy of Science, 1972). By contrast, a combination of genetically different crops grown together in the same field does not provide the uniform substrate needed by the pathogen to multiply and acquire epidemic proportions (Borlaug, 1958; Browning and Frey, 1969; Burdon, 1978; CIAT, 1986b). Accumulating evidence also indicates that a reasonable proportion of the plant population will prove resistant to any host-specific pathogens that appear. Intercropping of bean with maize in the tropics has been reported to reduce disease severity in the bean crop (Katunzi, et al., 1987; Kikoka and Teri, 1988; Mukiibi, 1976; Msuku and Edje, 1982; van Rheenen, et al., 1981). Insect infestation has also been reported to be reduced (Altieri, et al., 1978). However, this is not always the case. Much depends on other factors such as the host range of the pathogen, the relative sowing time and the spatial geometry of the associated

crops (CIAT, 1986b). In addition, the behavior of pathogen populations in intercrop systems is a complex function of the reproductive rate of the pathogen and the rate of propagule transfer between host components (Burdon, 1978; CIAT, 1986b).

For example, anthracnose (Colletotrichum lindemuthianum) may be more severe on beans associated with maize than on beans in the monocrop system due to increased humidity and lower temperature in the canopy. Angular leaf spot (Phaeoisariopsis griseola) may also be more severe on beans intercropped with maize than on beans intercropped with cassava (CIAT, 1986b; Moreno, 1977). Bacterial diseases (Xanthomonas campestris pv. phaseoli and P. s. pv. phaseolicola) were reported to be less severe in beans in associations with maize than in a sole cropping system (Katunzi, et al, 1987; Msuku and Edje, 1982; van Rheenen, et al., 1981; Vermeulen, 1982).

Several mechanisms of disease reduction in the intercrop have been suggested (Burdon, 1978; Manners, 1982). These include: 1) The replacement of a high proportion of genotypes that in a pure stand would be susceptible plants by resistant ones reduces the amount of susceptible tissue per unit area of crop, and hence the amount of infection in the succeeding generation, 2) The average distance which the inoculum has to travel to reach a susceptible plant surface is increased, 3) The movement of inoculum from one susceptible plant to another may be hindered by the presence of intervening resistant plants and, 4) Cross protection may occur, i.e., pre-inoculation with a race to which the line is resistant may protect it from a race to which it is normally susceptible. However, the relative contribution made by each of these mechanisms is far from clear.

Research work on the effect of intercropping bean with maize in reducing disease severity has been based on visual disease ratings. However, no work has been done to relate visual disease severity ratings with changes in pathogen populations in the bean

monocrop and bean-maize intercrop systems. Population dynamics of plant bacterial pathogens may provide an estimate of the degree to which a given agricultural system is ecologically suited to a particular pathogen, and has been used to describe various bacteria-plant interactions (O'Brien and Lindow, 1988). The objective of the current investigations was, therefore, to examine the population dynamics of halo blight bacteria in the two bean cropping systems and relate bacterial populations to disease severity ratings.

MATERIALS AND METHODS

Location

Field experiments of bean (Phaseolus vulgaris) and maize (Zea mays) in monoculture and in association were conducted in experimental fields at Lambo and Lyamungu at altitudes of 1020m and 1268m, respectively, and at latitude 3°14'S and longitude 37°15'E, in 1989 during the long rainy season (March to June). The two locations were about 13km apart on the southern side of mount Kilimanjaro. Both locations were in Hai district, Kilimanjaro region, and were planted with maize the previous two seasons.

Experimental design

A randomized complete block design (RCBD) with three replications per treatment was used. Maize cultivar Kilima and bean cultivar Canadian Wonder were used. Pathogen-free seeds were kindly provided by B. Gondwe (Agricultural Research Institute, Lyamungu, Moshi, Tanzania). The three treatment combinations included i) Monoculture beans with intra- and inter-row spacings of 75 cm and 20 cm, respectively, 2 plants per hill; ii) Monoculture maize with a spacing of 75 cm x 25 cm, 2 plants per hill; and iii) Beans in

association with maize, i.e., single rows of beans alternating with single rows of maize; with spacings as indicated for i) and ii). The two crops were sown simultaneously. Each plot measured 4m x 3m. Planting and all other agricultural practices were accomplished by manual labor, and were carried out as recommended for the region. Before planting, samples of soil, weeds and maize debris were taken using the stratified sampling design of Delp et al. (1986). Samples were placed in plastic bags and sent to the laboratory for assay of P. s. pv. phaseolicola as described in Chapter 3.

Bacterial isolate

Race 1 isolate #6, used in this study, was obtained from N. wightii and reisolated from artificially inoculated bean cultivar Canadian Wonder. Inoculum was prepared from 48-hour-old cultures grown on KMB (King, et al, 1954) plates by rinsing the bacteria off the agar surface and suspending them in glass distilled water. Concentrations were adjusted turbidimetrically to contain about 10^7-10^8 cells/ml.

Inoculation of plants

Plants were spray-inoculated with bacterial suspensions on both sides of the leaves to run-off, with some slight water-soaking, using a hand-operated atomizer. Both bean and maize plants were inoculated so that the behavior of the pathogen on maize plants could also be studied. Plants were inoculated when 19 days old, when the second trifoliolate leaf was fully open on beans, and when the fifth leaf was just unfolding on maize plants.

Sampling of leaves

Leaf samples were collected between 8 to 10 a.m. before inoculation, immediately after inoculation when leaves had dried and thereafter, at 3-day intervals for up to 15 days. For each replicate, 6-8 leaves were sampled at random and were immediately transported on ice in plastic bags to the laboratory and kept at 5°C until processed within 24-36 hours.

Bacterial population trends

Epiphytic populations of P. s. pv. phaseolicola were estimated using procedures of Smidt and Vidaver (1986). Leaves were shaken in sterile 0.01M phosphate buffer containing 0.01% Tween 20 on a wrist action shaker adjusted at medium speed for 30 minutes at 20-22°C. The amount of sterile phosphate buffer varied with each sample to ensure that sufficient volume was used to completely immerse the samples. After appropriate 10-fold serial dilutions, suspensions were plated in triplicate on KMB supplemented with 100μg cycloheximide per ml. Bacterial colonies were counted after 4-5 days of incubation at 24 $\pm$ 2°C. After washing, leaves were blotted dry and traced on paper and the area measured using a LI-3100 area meter (LI-COR, Inc., Lincoln, Nebraska, U.S.A.). Colony counts were expressed as numbers per cm^2 of leaf. Only symptomless leaves were used for determination of epiphytic populations.

Internal populations of P. s. pv. phaseolicola were estimated from surface-sterilized leaves (3 minutes in 2.6% NaOCl). Ten 2-cm diameter disks were punched out with a sterile cork borer, placed on sterile filter papers, weighed, and homogenized in 3-5 ml of phosphate buffer. Homogenates were prepared, serially diluted and plated as described earlier. Bacterial counts were expressed as numbers per gram of fresh tissue.

For each sampling, 10 randomly chosen P. s. pv. phaseolicola colonies were tested for pathogenicity on Canadian Wonder bean seedlings by the three methods described in Chapter 3.

Evaluation of disease reaction

Bean plants in monoculture and in association with maize were evaluated for disease reactions on foliage and pods. Halo blight severity was evaluated using the CIAT scale of 1-9 (CIAT, 1987), where 1 represented absence of symptoms and 9 represented very severe disease. Leaf disease reaction was estimated at 10 and 25 days after inoculation, whereas pod symptoms were rated at physiological maturity. For each disease rating date, the canopy of each individual plant was visually inspected row-wise in every plot and a rating representing the whole plot was assigned. Maize plants were also observed for any presence of symptoms.

Leaf wetness studies

To understand the effect of the two cropping systems on moisture retention in the canopy, the period required for bean and maize leaves to dry after rain was determined. Leaves were examined for the presence of free moisture at 30-minute intervals following the cessation of rain at Lyamungu for 10 different days of rainfall.

Bean seed infection assay

After drying, bean plants were harvested manually. Pods from each plot were kept separate and allowed to dry under the shade. Following hand-shelling, a random sample of 600 bean seeds was drawn from each replicate using a wooden board with 100 holes.

After surface-sterilization, seeds were aseptically plated, hilum down, on KMB-cycloheximide agar, 6 seeds per 10 cm-diameter glass petri dish and incubated for 5 days. To identify P. s. pv. phaseolicola-infected seed, plates were observed under UV-light in the darkness. Pure cultures of the pathogen were made from infected seeds by a series of single colony transfers on KMB agar plates and tested for pathogenicity as described earlier (Chapter 3).

Two separate determinations of seed infection were made for the same plot using the same sample size.

Weather data

Rainfall and temperature data for the Lambo estate and Lyamungu, were obtained from the Agrometeorology Section at the Agricultural Research Institute, Lyamungu, Moshi, Tanzania.

Data analysis

Statistical computations were made using the MSTAT-C statistical package (Michigan State University). Bacterial populations were log-transformed to determine the effect of each cropping system on population size. Significant differences between cropping systems were estimated using Student's t-test. Data for percentage bean seed infection were arcsine transformed before analysis (Little and Hills, 1978).

RESULTS

Population dynamics at Lambo

Populations of P. s. pv. phaseolicola on and in bean leaves in the bean monocrop and in bean/maize intercrop systems at Lambo are shown in Fig. 1. From 0 to 6 days after

inoculation surface populations were almost the same in both cropping systems. However, at 9 days after inoculation surface populations were higher in the bean/maize intercrop system than in the bean monocrop system, and were significantly higher ($P = 0.05$) in the intercrop than in the monocrop system at 12 and 15 days after inoculation. A similar pattern was observed for internal populations of P.s. pv. phaseolicola in bean leaves. However, internal populations were much higher than surface populations throughout the monitoring period (Fig. 1).

Internal populations were higher in the bean/maize intercrop than in the monocrop starting three days after inoculation. The differences were statistically significant ($P = 0.05$) at 12 and 15 days after inoculation when the maize leaf canopy started shading the bean canopy. Internal bacterial populations reached a maximum of 7.8 and 9.6 $\log_{10}$ units/g fresh weight at 9 and 12 days after inoculation in the bean monocrop and bean/maize intercrop systems, respectively, after which there was a gradual decline in P.s. pv. phaseolicola populations. Such was also the case for surface populations (Fig. 1)

Population dynamics at Lyamungu

Differences in bacteria multiplication among the two cropping systems also were apparent at Lyamungu, but were less pronounced than at Lambo. The internal population of P.s. pv. phaseolicola on and in bean leaves tended to be greater in the bean/maize intercropping than in the bean monocropping system throughout the sampling period (Fig. 2). However, internal populations were not significantly affected ($P = 0.05$) by cropping systems except at the 15 day sampling period. Surface populations of P.s. pv. phaseolicola increased slightly during the first 6 days and then increased rapidly in both cropping systems reaching a maximum level of 4.7 and 5.6 $\log_{10}$ units/cm^2 leaf area at 9 days in the bean

Figure 1. Surface and internal population dynamics of Pseudomonas syringae pv. phaseolicola race 1 on and in bean foliage in two bean cropping systems at Lambo, Kilimanjaro region, northern Tanzania. Bars indicate standard errors of the means.

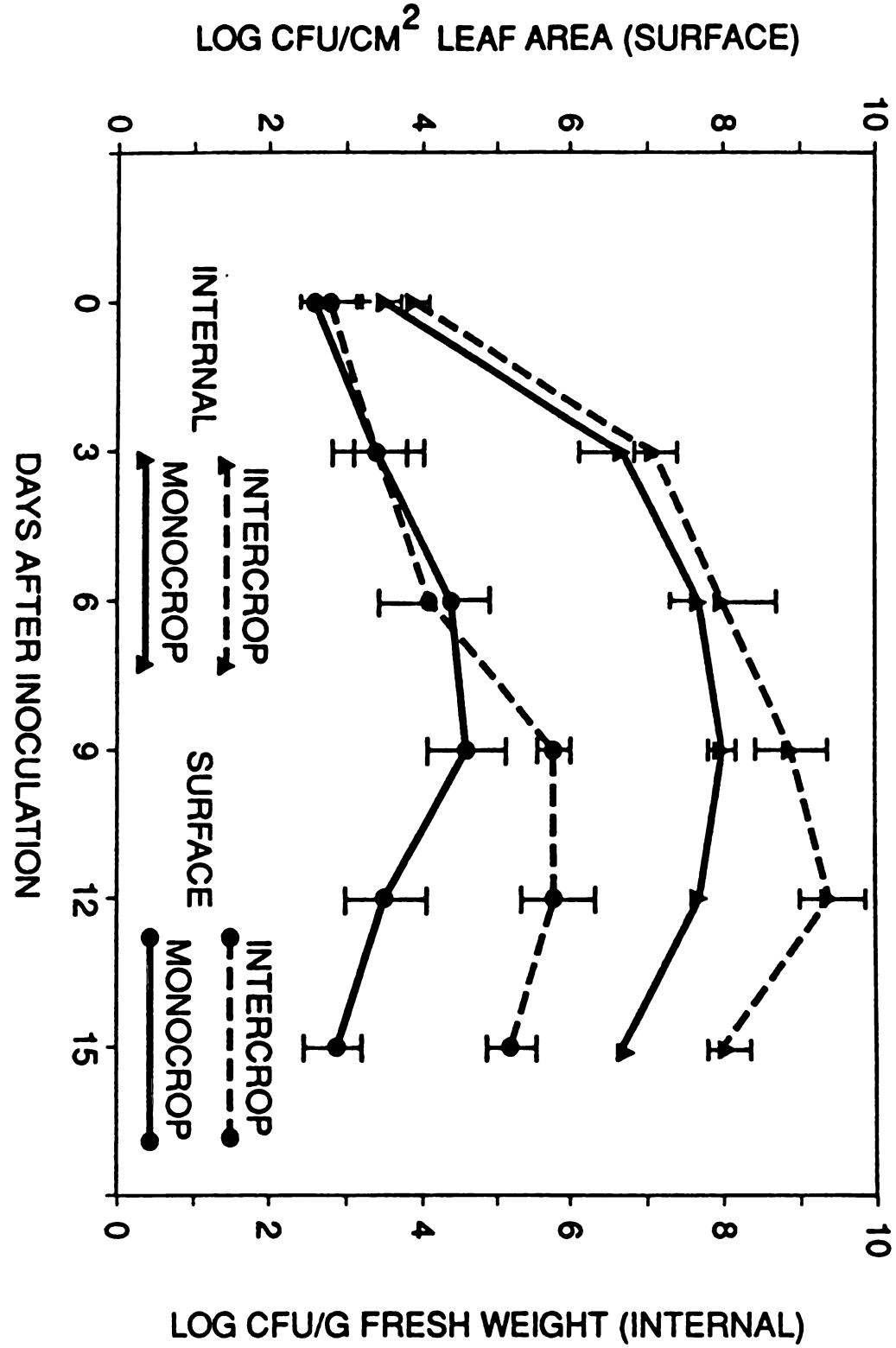
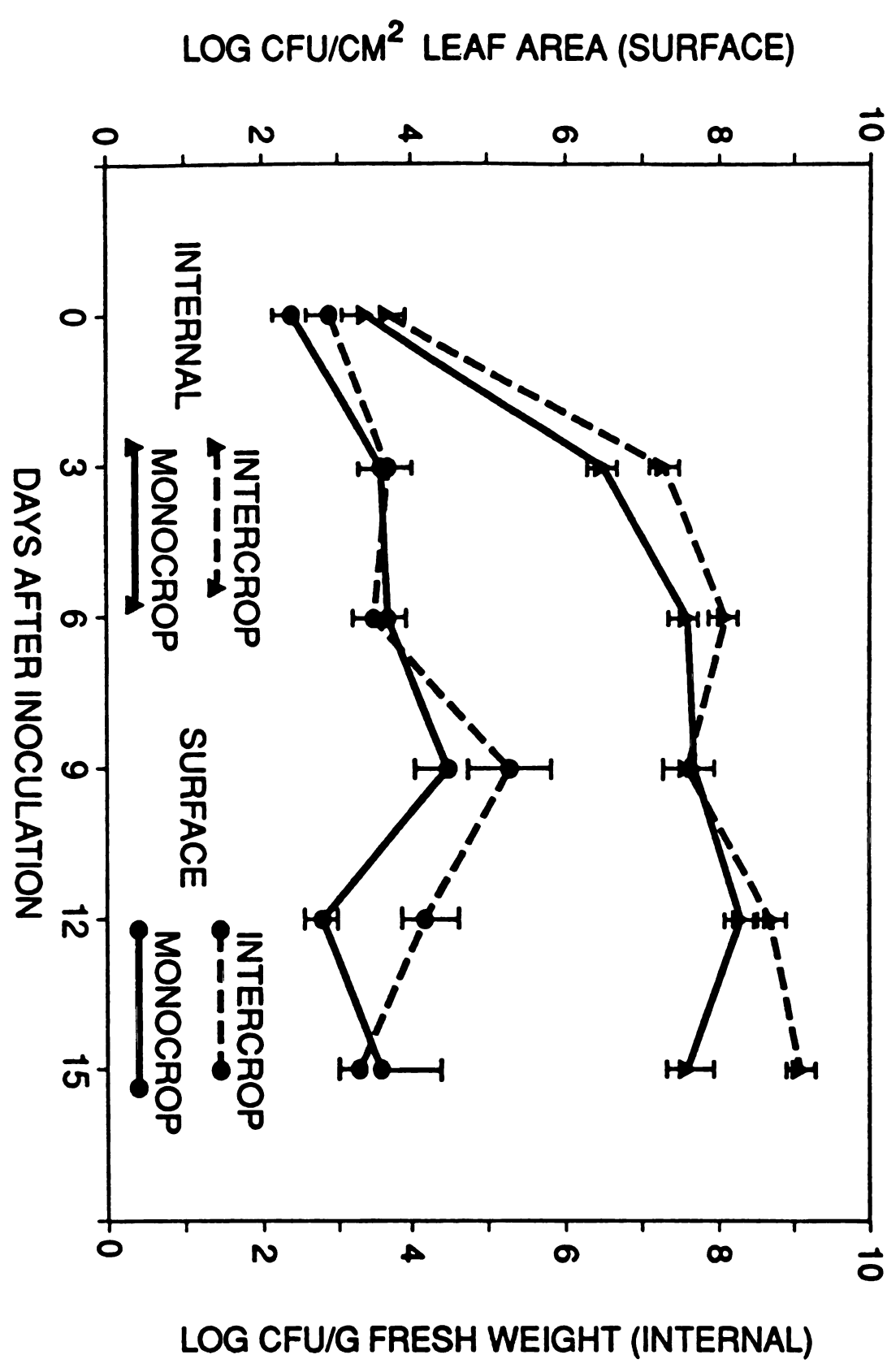

LOG CFU/CM^2 LEAF AREA (SURFACE)
0
2
4
6
8
10
INTERNAL
INTERCROP
MONOCROP
SURFACE
INTERCROP
MONOCROP
DAYS AFTER INOCULATION
0
3
6
9
12
15
LOG CFU/G FRESH WEIGHT (INTERNAL)

Figure 2. Surface and internal population dynamics of Pseudomonas syringae pv. phaseolicola race 1 on and in bean foliage in two bean cropping systems at Lyamungu, Kilimanjaro region, northern Tanzania. Bars indicate standard errors of the means.


LOG CFU/CM^2 LEAF AREA (SURFACE)
0
2
4
6
8
10
INTERNAL
INTERCROP
MONOCROP
SURFACE
INTERCROP
MONOCROP
DAYS AFTER INOCULATION
0
3
6
9
12
15
LOG CFU/G FRESH WEIGHT (INTERNAL)

monocrop and bean/maize intercrop systems, respectively. Thereafter, surface populations declined sharply; monocrop populations reached a level significantly lower than intercrop populations at 12 days, and then suddenly increased to a level not significantly different ($P = 0.05$) from that of the intercrop system at 15 days after inoculation. While internal population patterns were similar in both systems, maximum bacterial populations generally tended to be lower in the bean monocrop than in the bean/maize intercrop system. At 15 days after inoculation the difference was about 100-fold and was significantly different ($P = 0.05$). Internal populations reached a maximum of 8.3 $\log_{10}$ units/g fresh weight at 12 days after inoculation in the bean monocropping system, then declined at 15 days. Bacterial populations in bean leaves in the intercrop system increased to 9 $\log_{10}$ units at 15 days. In both cropping systems internal P.s. pv. phaseolicola populations increased steadily except at 15 days after inoculation.

Populations of P.s. pv. phaseolicola on and in maize

In contrast to behavior on and in bean leaves, internal populations of P.s. pv. phaseolicola in maize plants grown alone or in association with bean, decreased throughout the sampling period both at Lambo and at Lyamungu (Figs. 3 and 4). At Lambo, practically no internal bacteria were detected in maize leaves 6 days after inoculation in both cropping systems. In the maize monocropping system surface P.s. pv. phaseolicola populations also declined rapidly and were not detected 6 days after inoculation. However, in the bean/maize intercrop system, surface populations on maize leaves increased slightly 3 days after inoculation, then maintained a fairly constant population of 2.8 $\log_{10}$ units/cm^2 leaf area until 9 days and then declined to undetectable concentrations at 15 days.

Figure 3. Surface and internal population dynamics of Pseudomonas syringae pv. phaseolicola race 1 on and in maize foliage in monocrop and intercrop systems at Lambo, Kilimanjaro region,northern Tanzania. Bars indicate standard errors of the means.

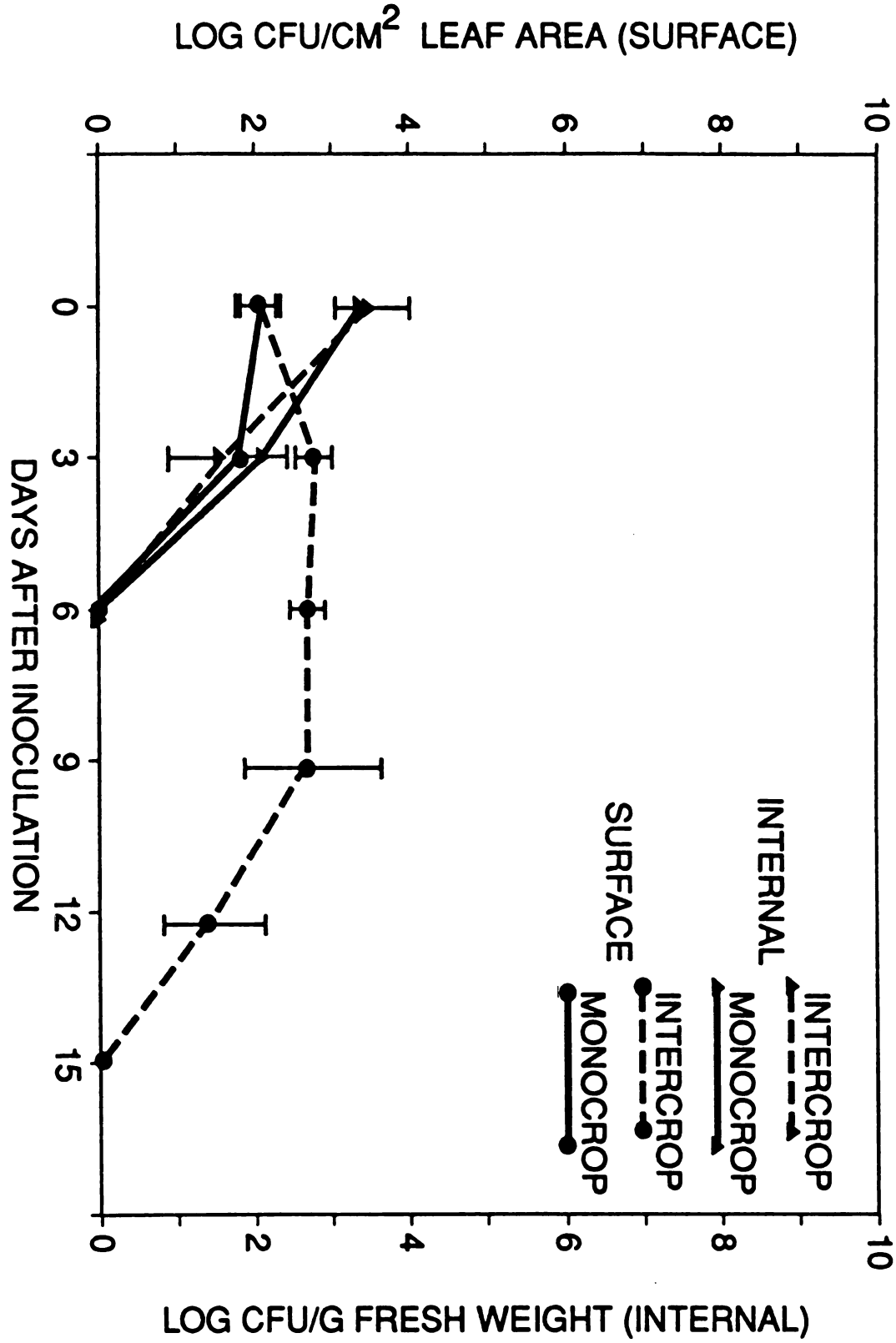
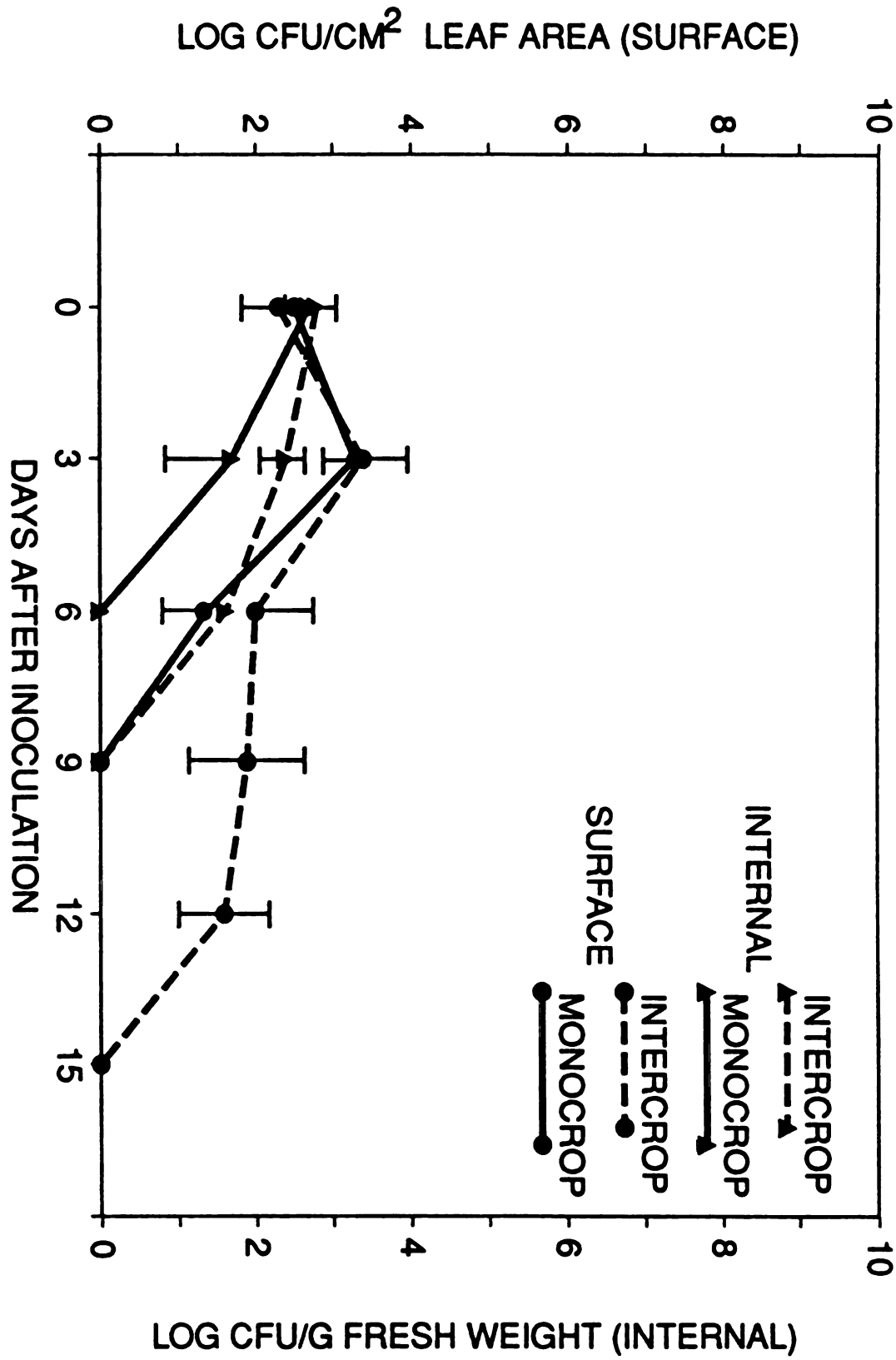

LOG CFU/CM2 LEAF AREA (SURFACE)
0
2
4
6
8
10
DAYS AFTER INOCULATION
0
3
6
9
12
15
INTERNAL
INTERCROP
MONOCROP
SURFACE
INTERCROP
MONOCROP
LOG CFU/G FRESH WEIGHT (INTERNAL)

Figure 4. Surface and internal population dynamics of Pseudomonas syringae pv. phaseolicola race 1 on and in maize foliage in monocrop and intercrop systems at Lyamungu, Kilimanjaro region, northern Tanzania. Bars indicate standard errors of the means.


LOG CFU/CM2 LEAF AREA (SURFACE)
0
2
4
6
8
10
DAYS AFTER INOCULATION
0
3
6
9
12
15
SURFACE
INTERNAL
INTERCROP
MONOCROP
INTERCROP
MONOCROP
LOG CFU/G FRESH WEIGHT (INTERNAL)

At Lyamungu, population dynamics of P.s. pv. phaseolicola were similar to those at Lambo. Internal populations in the maize monocrop decreased rapidly and were not detected after 6 days (Fig. 4). Internal populations in the bean/maize intercropping system declined more slowly, but were not detected after 9 days. On the other hand, surface populations increased to a maximum of about 3.4 $\log_{10}$ units/cm^2 at 3 days after inoculation and then declined (Fig. 4). The bacterium was not detected on maize after 9 days in the maize monocrop but remained at ca. 2 $\log_{10}$ units/cm^2 through day 12 in the bean/maize intercrop system.

In all cases colony samples of P.s. pv. phaseolicola which were taken randomly and tested for pathogenicity on Canadian Wonder bean seedlings were pathogenic and produced typical halo blight symptoms within 8-10 days after inoculation.

Leaf and pod disease ratings

Foliage disease severity was evaluated twice, at 10 and 25 days after inoculation, and pod disease severity was rated once at pod physiological maturity. The mean disease severity ratings plus/minus their standard errors for the two locations are shown in Table 1. Halo blight severity at Lambo was similar for the two cropping systems. However, at Lyamungu, halo blight was slightly, but not significantly, more severe ($P = 0.05$) in the bean monocrop system than in the bean/maize intercrop system. In contrast, however, pod disease severity at both locations was more severe in the bean/maize association than in the monocropping system, although differences were not significant. At Lambo, the proportion

Table 1. Halo blight disease severity ratings for foliage and pods of Canadian Wonder bean in two bean cropping systems in northern Tanzania.

			Mean disease severity ratings[x]	
Location	Plant part	Days[y]	Monocrop	Intercrop
Lambo	Leaves	10	4.3 ± 0.2	4.2 ± 0.2
		25	6.8 ± 0.4	6.8 ± 0.4
	Pods[z]		3.8 ± 0.2	5.0 ± 0.3
Lyamungu	Leaves	10	4.0 ± 0.0	3.8 ± 0.3
		25	7.2 ± 0.2	6.2 ± 0.2
	Pods[z]		4.8 ± 0.3	6.0 ± 0.3

[x]Values are means of three replicates ± standard errors of the means. Disease severity indices ranged from 1 = no disease to 9 = very severe disease, with 50% or more of leaf area covered with lesions.

[y]Days after inoculation.

[z]Disease severity rating on pods was done at pod physiological maturity with a scale of 1-9 where 1 = no disease and 9 = very severe disease, with 50% or more of pod area covered with lesions.

of pod area covered with lesions in the intercropping system exceeded that in the monocrop system by 24% while at Lyamungu the difference was 20%.

Retention of moisture on leaves

Leaves of beans growing in the intercrop system required 2.8 hours to dry after rain as compared with 2.0 hours for the bean monocrop, a difference of 40% (Table 2). Such differences were not observed for maize in the two cropping systems. Maize leaves required about 2 hours longer to dry than bean leaves.

Climatological data

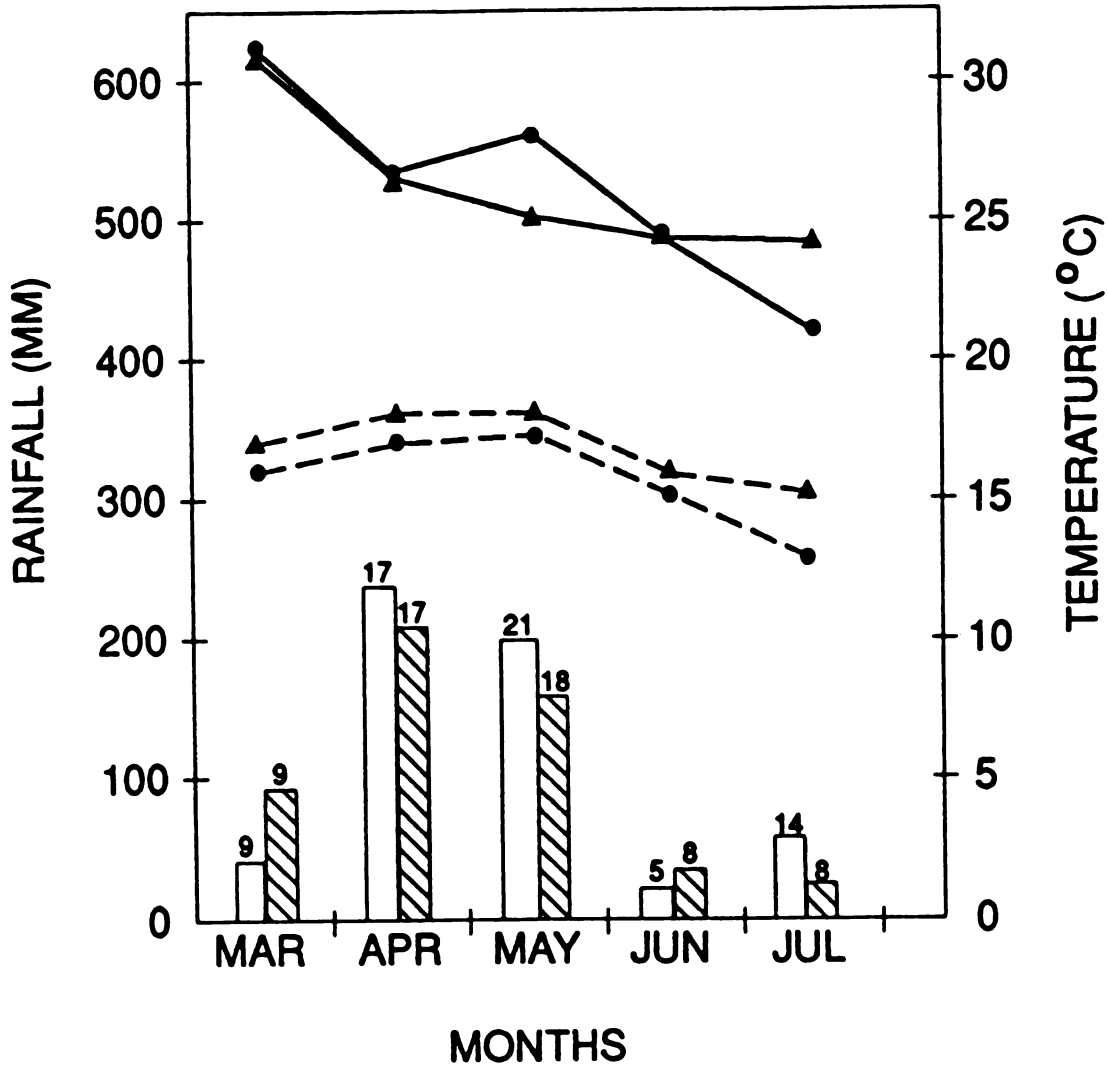
Rainfall totals for April and May at Lambo were 242.1 and 201.2 mm, respectively (Fig. 5). However, the 53 year rainfall averages were 209.0 mm for April and 161.7 mm for May. Temperatures at the same location in April ranged from 17.1 to 27.4°C, which almost agreed with the range of 53 years, which was 18.1 to 27.1°C. In May however, temperatures ranged from 17.4 to 27.7°C as compared to 17.6 to 24.9°C, the average range of 53 years. On the other hand, Lyamungu received 303.1 and 810.6 mm of rain for April and May, respectively (Fig. 6). The means of 53 year rainfalls for the same months were 502.3 and 414.1 mm, respectively. Temperatures at Lyamungu were cooler than those at Lambo and ranged from 15.2 to 23.9°C for April and 15.1 to 22.2°C for May. The 53 year average ranged from 15.8 to 24.6°C for April and 13.0 to 22.2°C for May. Rainfall and temperatures at both locations decreased progressively towards the end of the growing season. Population dynamics studies of P.s. pv. phaseolicola were conducted during the period of April and May.

Table 2. Time required for visible moisture on bean and maize leaves to dry following cessation of rain at Lyamungu, Kilimanjaro region, northern Tanzania.

Cropping system	Crop	Time (hours)[z]
Monocrop	Bean	2.0 ± 0.2
	Maize	4.3 ± 0.3
Intercrop	Bean	2.8 ± 0.2
	Maize	4.3 ± 0.2

[z]For 10 rain instances in a field with 3 replications (= 30 readings per value) plus/minus standard errors of the means.

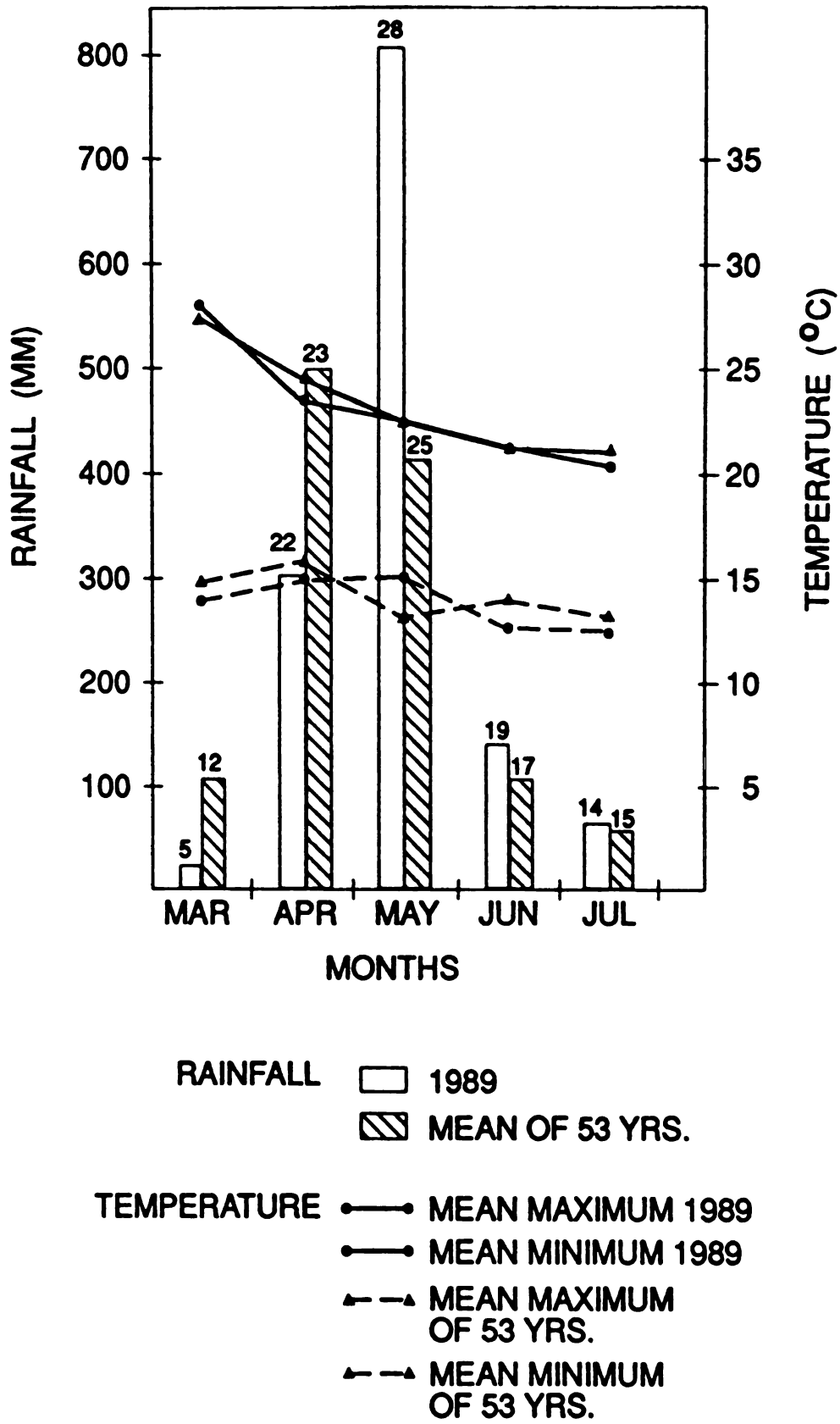
Figure 5. Monthly rainfall and mean monthly maximum and minimum temperatures from March to July at Lambo for 1989 and for 53 years (1935-1988). Numbers above each bar represent number of rain days during the indicated month.



RAINFALL
1989
MEAN OF 53 YRS.

TEMPERATURE
MEAN MAXIMUM 1989
MEAN MINIMUM 1989
MEAN MAXIMUM OF 53 YRS.
MEAN MINIMUM OF 53 YRS.

Figure 6. Monthly rainfall and mean monthly maximum and minimum temperatures from March to July at Lyamungu for 1989 and for 53 years (1935-1988). Numbers above each bar represent number of rain days during the indicated month.


RAINFALL (MM)
800
700
600
500
400
300
200
100
TEMPERATURE (°C)
35
30
25
20
15
10
5
28
23
25
22
19
17
14
15
12
5
MAR
APR
MAY
JUN
JUL
MONTHS
RAINFALL
1989
MEAN OF 53 YRS.
TEMPERATURE
MEAN MAXIMUM 1989
MEAN MINIMUM 1989
MEAN MAXIMUM OF 53 YRS.
MEAN MINIMUM OF 53 YRS.

Bean seed infection

The percentage of bean seed infected with P.s. pv. phaseolicola was lower for bean grown in pure stands than when grown in association with maize, but the differences were not statistically significant (P = 0.05) except for experiment 2 at Lambo (Table 3). In general, experiments at Lambo and at Lyamungu showed similar trends of seed infection. High levels of seed infected with halo blight bacteria in the bean/maize intercrop system were consistent with the high disease severity ratings on pods at both locations. Randomly selected pure cultures of P.s. pv. phaseolicola colonies from infected bean seed were all pathogenic when tested on Canadian Wonder bean seedlings.

DISCUSSION

When considered together, the data from this study suggest that intercropping beans with maize ecologically favors multiplication of P.s. pv. phaseolicola in bean leaves. Although there were no significant differences in foliage disease severity between the two cropping systems, the increased inoculum density in the bean/maize intercrop system resulted in more severe disease on pods and in a higher percentage of seed infection than when beans were grown in a pure stand. Results of this study further suggest that disease severity on foliage may not always be adequate to assess the advantage of intercrop systems in reducing disease because high numbers of infected seed may be produced in such systems.

The mechanism for increased P.s. pv. phaseolicola multiplication in the intercrop system seems to involve increased moisture retention in the canopy as indicated by the fact that maize leaves took longer time to dry after cessation of rain than bean leaves. In addition, bean in association with maize retained moisture longer than bean grown alone.

Table 3. Percentage of Canadian Wonder bean seed infected with Pseudomonas syringae pv. phaseolicola in two bean cropping systems in northern Tanzania.

		Seed infection (%)[y]	
Location	Experiment	Monocrop	Intercrop
Lambo	1	16.8 ± 8[z] a	28.3 ± 7 a
	2	11.8 ± 8 a	38.1 ± 5 b
Lyamungu	1	12.3 ± 2 a	20.6 ± 10 a
	2	12.4 ± 8 a	32.2 ± 9 a

[y]Values are means of three replicates ± standard errors of the means.

[z]Within each experiment, means followed by the same letter are not significantly different ($P = 0.05$) by Student's t-test. Data were arcsine transformed before analysis.

This effect may be magnified when beans are planted late in the maize crop as is sometimes the case in northern Tanzania and in other areas of the country because of the greater canopy development. Bacterial populations, especially epiphytic populations, have been reported to increase when plants are wet (Leben and Daft, 1967; Mulrean and Schroth, 1982; Smitley and McCarter, 1982). Increased moisture retention in the intercrop system may also result in a lowered temperature in the canopy. Therefore, in areas where high temperature and reduced moisture are limiting factors for halo blight development, such as at Lambo, intercropping bean with maize may increase halo blight severity, especially on pods. This is of practical significance in countries such as Tanzania, where programs to produce pathogen-free seed are lacking. Data from this work also indicate that maize leaves do not support high populations of halo blight bacteria. This reduces the risk of the maize canopy providing additional inoculum for the bean crop when the two are grown in association.

Comparison of data obtained from the two sites indicate that P.s. pv. phaseolicola multiplied to higher numbers at Lambo than at Lyamungu (Figs. 1 and 2). It is likely that differences in population dynamics were caused by differences in temperature. Temperatures were about 4 C lower at Lyamungu than at Lambo during the assay period. When combined with canopy effects, temperature differences between the two locations could even be increased beyond 4 C. Differences in the amount of rainfall may also account for differences in population dynamics between the two locations. Although multiplication of phytopathogenic bacteria on plant surfaces has been reported to increase after rain (Hirano and Upper, 1983), when excessive rainfall, such as was the case at Lyamungu, is combined with low temperature, a net negative effect on population size may occur. We can therefore hypothesize that when initial inoculum is not a limiting factor,

temperature and moisture availability in the associated crops become key elements in determining the balance between increasing and reducing disease severity in such cropping systems. However, when initial inoculum comes from outside the cropping system, the maize crop would act as a barrier and therefore, reduce the amount of initial inoculum available for the bean crop (Burdon, 1978; Msuku and Edje, 1982). But this may not be the case for seed-borne pathogens such as halo blight bacteria. The original source of initial inoculum, therefore, is another factor governing the balance between increased and reduced disease severity in intercrop systems.

Earlier studies (Msuku and Edje, 1982; van Rheenen, et al., 1981; Vermeulen, 1982) have indicated that halo blight is generally less severe when beans are grown in association with maize than in pure stands. Some of these studies, however, have involved only observations in agronomy experiments (Msuku and Edje, 1982; Stoetzer and Waite, 1984; van Rheenen, et al., 1981), depending therefore, on natural infection which does not insure uniform initial inoculum in the two bean cropping systems. Moreover, population dynamics, percentage seed infection and moisture retention differences have not been considered in previous studies as quantitative factors to measure ecological fitness of the pathogen in such systems. By using these parameters to quantify disease and introducing a uniform amount of initial inoculum in the two bean cropping systems, results of the current study generally do not agree with previous reports, because the environment in the bean/maize intercrop system ecologically favored increased multiplication of halo blight bacteria as well as increased seed infection.

Contradictory results of the effect of intercropping beans with maize on disease severity have also been reported for bean anthracnose (CIAT, 1986b; CIAT, 1989; Msuku and Edje, 1982) and on bean rust (Msuku and Edje, 1982). As van Rheenen et al. (1981)

suggested, the environmental differences between monocropping and intercropping, and their influence on disease are far too complex for generalization and are not well understood. In order to understand better the interaction of plant pathogens and crops grown in association, we need to quantify the interacting parts of the systems. This will involve simultaneous measurement of parameters related to the pathogen, which include population size, disease incidence and severity, and weather variables such as moisture and temperature (Hirano and Upper, 1983).

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