Races and Survival of *Pseudomonas syringae* pv. *phaseolicola* in Northern Tanzania

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ABSTRACT

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The pathogenic variation among 118 isolates of *Pseudomonas syringae* pv. *phaseolicola* associated with halo blight of bean (*Phaseolus vulgaris*) in the Arusha and Kilimanjaro regions of northern Tanzania from November 1988 to February 1990 was examined with four differential bean cultivars. The three races recovered were race 2 (52.5%), race 1 (45.0%), and race 3 (2.5%). Some strains of race 2, designated as 2P, produced a brown, diffusible pigment on agar media. Some race 2P strains grew at 34 C, whereas the non-pigment-producing strains of race 2 and of races 1 and 3 failed to grow at this temperature. Race 2 strains obtained from bean debris from Monduli in the Arusha region induced systemic chlorosis and stunting on the usually resistant differential bean cultivar Edmund. The ability of *P. s. phaseolicola* to survive in bean debris and in dead standing bean plants varied according to race, geographic location, depth in the soil, and bean genotype.

Pathogenic variation among populations of *Pseudomonas syringae* pv. *phaseolicola* (Burkholder) Young et al, cause of halo blight of bean (*Phaseolus vulgaris* L.), is well known. Jensen and Livingstone (12) were the first to demonstrate host specificity among 13 strains in *P. s. phaseolicola*. Patel and Walker (20) reported the occurrence of races 1 and 2 in the United States on the basis of the reaction of bean cultivar Red Mexican U.I.-3, which is resistant to race 1 but susceptible to race 2. The occurrence of these two races has since been reported in Tanzania and many other countries (2,9,14,25,28). The existence of a third race in Nebraska was first suggested by Schuster et al (23) and Coyne et al (4), who recovered strains of halo blight bacteria that were virulent to the usually resistant Great Northern bean cultivars such as Great Northern U.I.-59 and California Pink. Recently, Taylor et al (28) confirmed the existence in Africa of race 3 of *P. s. phaseolicola*. Race 3 causes a hypersensitive reaction in cv. Tendergreen, which is susceptible to races 1 and 2. Resistance to race 3 is governed by a single dominant gene, which is also present in several cultivars of U.S. origin, including Seafarer and Tendercrop (10).

Breeding for halo blight resistance is generally considered the best method of disease control and has been used successfully in several areas of the world (1,5,8,24). The development and deployment of resistant cultivars requires a knowledge of the distribution of races of the pathogen in the affected region. Therefore, the purpose of this study was to identify the races of *P. s. phaseolicola* prevailing in northern Tanzania and to determine their survival ability under different field conditions. Information generated from this study will be useful in breeding programs for development of resistant bean cultivars in the country.

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MATERIALS AND METHODS

Sampling and isolation. Bean plants with symptoms resembling those of halo blight were collected from farmers' fields in the Arusha and Kilimanjaro regions of northern Tanzania from November 1988 to February 1990. Pieces of diseased tissue and small areas of surrounding healthy tissue were excised and surface-sterilized for 1 min in 2.6% NaOCl and rinsed in sterile distilled water. The pieces were crushed on flame-sterilized glass slides containing one or two drops of sterile distilled water. The resulting suspensions were streaked onto King's medium B (KB) (13). Fluorescing colonies were purified by a series of single colony transfers and their identities confirmed by biochemical and physiological tests (LOPAT) (16): pathogenicity tests on Canadian Wonder bean seedlings; carbon source utilization tests with mannitol, sorbitol, and inositol (22); ice nucleation activity at −5 C (17); and sensitivity to bacteriophage and serological tests. A total of 118 strains were identified as *P. s. phaseolicola*. Reference strains (882, 1281A, 1299A, 1301A, 1302A, and 1375A), bacteriophages, and antisera were provided by J. D. Taylor (Institute of Horticulture, Wellsbourne, Warwick, England). Each test was done three times, with similar results. Cultures were maintained in glycerol and 0.1 M phosphate buffer (pH 7.2) (1:1, v/v) and on nutrient agar (NA) slants at 0 C. Cultures were also stored in bean leaf powder in sterile vials at room temperature (22 C) in darkness.

Bacteriophage tests. Sensitivity of bacterial strains to bacteriophages 11P, 12P, and 48P was tested by the method of Taylor (26) and J. D. Taylor and D. M. Teverson (*personal communication*). Log phase cultures (48–72 hr) of each isolate were grown on KB and NA. Thick bacterial suspensions were prepared by adding 4 ml of nutrient broth to each slant culture. Two or three drops of each bacterial suspension were thoroughly mixed with 2.5 ml of sterile soft glycerol agar (5 g/L of proteose peptone, 3 g/L of yeast extract, 20 ml of glycerol, 7 g/L of Bacto agar) at 45–50 C. The mixture was then poured on cool, solidified NA plates, and the plates were swirled gently to evenly distribute a thin layer of bacterial suspension. After 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 hr, after which results were recorded. Each strain was replicated three times. Clear zones (plaques) resulting from lysis of bacterial cells by phages indicated a positive identification of *P. s. phaseolicola*. Reference strains 1299A and 1375A were included as positive controls.

Serology. Antisera specific for *P. s. phaseolicola* were provided by J. D. Taylor and used in the agglutination method of J. D. Taylor and D. M. Teverson (*personal communication*). One 0.07-ml drop of the antiserum was placed on a clean glass microscope slide, and a small amount of 24- to 48-hr bacterial growth was picked up with a platinum wire loop and mixed into the drop of the antiserum. Agglutination observed against a light-colored background was recorded as positive.

Race identification. Races were determined from pod and foliage reactions of four differential bean cultivars: Canadian Wonder (universal susceptible), Edmund (universal resistant), Red Mexican U.I.-3 (resistant to race 1), and Tendergreen (resistant to race 3). Pathogen-free seeds of these differential bean cultivars, provided by M. J. Silbernagel (USDA-

ARS, Prosser, WA), were multiplied at Lyamungu Agricultural Research Station, Moshi, Tanzania.

Plants were grown in a screenhouse in 16-cm-diameter sterile plastic pots or in 7 × 8 × 22 cm sterile plastic flats containing a sterile mixture of forest soil and sand (2:1, v/v). Soil was heat-sterilized by exposure to a high-temperature (198 C) kerosene flame in a Terra Force Terralizer (Kent Horticultural Engineers, Kent, England). Plants were maintained at temperatures of 19–27 C and a natural 12 ± 0.5 hr day length and watered as required with tap water.

Inoculum was prepared from 24- to 48-hr cultures grown on KB at 24 C. The cultures were suspended in 0.01 M phosphate buffer (pH 7.2). Concentrations of bacterial suspensions were adjusted turbidimetrically to contain about 10^7 to 10^8 cfu/ml.

For determination of leaf reaction, 7- to 10-day-old seedlings with fully expanded primary leaves were injected with bacterial suspensions at the first node with a sterile 25-gauge needle attached to a sterile 10-cc hypodermic syringe. Next, a 0.5-L hand-operated plastic atomizer was used to spray bacterial suspensions on the abaxial surfaces of the leaves, avoiding soaking. Inoculated plants were covered with a plastic bag for 24 hr in the screenhouse and observed for development of halo blight during the next 10 days. Each plant was considered a replicate and six plants were used for each strain; two experiments were conducted.

Pod reactions were determined by the procedure of Ekpo (6). Pods at the flat stage were harvested, treated with 2.6% NaOCl for 1 min, rinsed, and dried aseptically on sterile filter papers. A 5-μl drop of inoculum was placed at three sites on each of three pods of each differential bean cultivar, and the pod was pricked five times through the inoculum drop to a depth of about 1 mm with a sterile 25-gauge disposable needle. Inoculated pods were placed, inoculated side up, in 7 × 8 × 22 cm plastic flats containing moist filter papers, then incubated for 7–10 days. The experiment was done twice.

Temperature studies. The effect of temperature on growth of races of *P. s. phaseolicola* was determined by inoculating, in quadruplicate, KB agar plates with different serial dilutions of each race. The plates were then placed upside down in incubators without light at 24, 28, and 34 C, ± 2 C; at 28 and 34 C, humidity in the incubator was adjusted to 70% to avoid excessive drying of agar plates. Growth was evaluated after 4–5 days. The effect of temperature on the ability of isolates to produce a brown, diffusible pigment on KB and NA was also determined subjectively.

Survival studies. Survival of races 1 and 2 of *P. s. phaseolicola* was studied with procedures developed by Saettler et al (21) and Groth and Braun (7). Three sites were chosen to represent different ecological environments in which beans are grown. These included a field cultivated to maize the previous year at Monduli (a dry area) and two fields at Lyamungu (a wet area). One of the fields at Lyamungu had been in fallow during the previous 3 yr and the other was cropped as a coffee/banana association. Three cultivars—Canadian Wonder (susceptible), Masai Red (moderately resistant), and GO 7928 (resistant)—were grown in 10-m rows in the field at Lyamungu and Monduli in March 1989. Plants were inoculated twice (18 and 30 days after planting) with cultures of race 1 or race 2. Control plants grown 25 m away were left uninoculated. Severely diseased plants were collected in early June, air-dried at 22 ± 2 C, and separated into leaves and stems. Some plants were left standing in the field. Stems were cut into pieces 2–3 cm long. Samples of leaves (0.5 g) and stems (10 pieces) were placed in color-coded fine-mesh nylon bags and taken to the field at the end of June, the finish of the main bean growing season. One-half of the bags were buried 2–5 cm beneath the soil surface and the remaining were placed 25 cm deep, the normal plowing depth. Samples of each race were separated by 10 m. Healthy control samples were placed 5 m away from diseased samples. Diseased samples were also stored in the laboratory at 22 ± 2 C for a similar period.

At monthly intervals starting in July, when beans are usually harvested, three leaf and stem samples for each race were retrieved. Because leaves of standing plants had fallen to the ground, leaf samples for this treatment were recovered from the soil surface. Samples were ground in dry sterile mortars, and 10 ml of sterile phosphate buffer (pH 7.2) was added. The ground debris was steeped for 15 min, then pelleted with a 2-min centrifugation at 20,000 *g*. The supernatants were diluted serially and plated in triplicate on KB agar containing cycloheximide (100 μg/ml). Plates were observed under ultraviolet light after 4–5 days of incubation. For each sampling period, five presumed colonies of *P. s. phaseolicola* were purified by single colony transfers and tested for sensitivity to bacteriophage and for pathogenicity on 7- to 10-day-old Canadian Wonder bean seedlings. In the pathogenicity tests, stems were injected and leaves were infiltrated with approximately 10^7 cfu/ml. Plants were incubated in the screenhouse and observed for disease over a 14-day period.

Volunteer bean plants. During the bean growing season, volunteer bean plants with severe symptoms of halo blight in farmers' fields and fields around farmers' backyards in Monduli (November–December) and in Lyamungu (September–October) were sampled. Isolations were attempted as described earlier. Colonies that produced fluorescent pigment in KB were subjected to biochemical, pathogenicity, and bacteriophage tests for species identification. Confirmed cultures of *P. s. 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

Identification of isolates of *P. s. phaseolicola*. On the basis of diagnostic test results, 118 cultures of bacteria were identified as *P. s. phaseolicola*. The bacteria were positive for levan sucrase and negative for oxidase, arginine dihydrolase, ice nucleation activity, and pectolytic activity and produced a rapid hypersensitive reaction on tobacco leaves. These strains did not utilize mannitol, sorbitol, or inositol as carbon sources. They were sensitive to bacteriophages 11P, 12P, and 48P and agglutinated with *P. s. phaseolicola* antiserum.

Prevailing races. Within the two regions surveyed (Fig. 1), three pathogenic races of *P. s. phaseolicola* were distinguished on the basis of the reaction of the four differential bean cultivars to inoculation with the collection of strains (Table 1). Race 2 was slightly more prevalent than race 1, and race 3 occurred at a very low frequency. Besides producing the usual blue-green, diffusible pigment, some race 2 strains also produced a brown, diffusible pigment, which was more pronounced on NA than on KB. Such strains, designated as race 2P, occurred infrequently and were restricted to the Lambo, Kilimanjaro, region and Monduli and Selian in the Arusha region. Race 2P strains were obtained from bean cv. Canadian Wonder, the breeding line FB-GP307-2, and other local cultivars.

Production of brown, diffusible pigment by strains of race 2P was detected as early as 48 hr on NA and after 4–5 days on KB. In biochemical and physiological tests and in reaction of foliage and pods of the four differential bean cultivars, the race 2P strains producing this pigment were not distinguishable from race 2 strains that did not produce the pigment.

All race 2 strains isolated from bean debris collected from farmers' fields in Monduli in November 1988 and September 1989 produced very large water-soaked lesions on the cultivar Tendergreen. In cv. Edmund, the strains produced a hypersensitive reaction in leaves but induced stunting and systemic chlor-

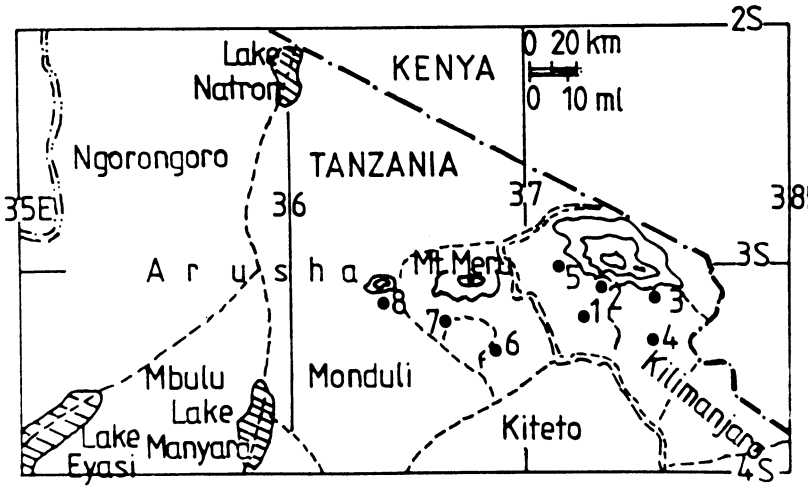

Fig. 1. Villages in northern Tanzania whose environs were surveyed for *Pseudomonas syringae* pv. *phaseolicola*: 1 = Lambo, 2 = Lyamungu/Narumu, 3 = Kilema, 4 = Miwaleni, 5 = Sanya Juu, 6 = Tengeru, 7 = Selian, 8 = Monduli.

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

Location	Altitude (m)	Number isolated	Number of strains per race			
			1	2	2P	3
Arusha region						
Monduli	1,630	39	8	26	3	2
Selian	1,387	45	27	16	1	1
Tengeru	...[a]	2	2	0	0	0
Kilimanjaro region						
Lambo	1,020	13	3	8	2	0
Lyamungu	1,268	13	12	1	0	0
Kilema	1,422	4	0	4	0	0
Narumu	1,268	1	0	1	0	0
Sanya Juu	1,400	1	1	0	0	0
Total		118	53	56	6	3

[a] Information lacking.

Table 2. Survival of *Pseudomonas syringae* pv. *phaseolicola* races 1 and 2 in infected bean debris (standing plants and debris buried for 6 mo at two depths) under field conditions at Monduli in the Arusha region and at Lyamungu in the Kilimanjaro region of northern Tanzania

Location	Race	Cultivar or genotype[a]	Plant survival (mo)					
			Standing		Buried 2–5 cm		Buried 25 cm	
			Leaves	Stems	Leaves	Stems	Leaves	Stems
Monduli[b]	1	CW	4	5	4	5	2	3
		MR	2	3	2	2	1	1
		G0 7928	0	0	0	0	0	0
	2	CW	5	6	5	6	2	4
		MR	2	3	2	2	1	1
		G0 7928	0	0	0	0	0	0
Lyamungu[c]	1	CW	1	2	0	0	0	0
		MR	0	2	0	0	0	0
		G0 7928	0	0	0	0	0	0
	2	CW	2	3	0	0	0	0
		MR	1	2	0	0	0	0
		G0 7928	0	0	0	0	0	0
Lyamungu[d]	1	CW	2	3	1	1	0	0
		MR	1	2	0	0	0	0
		G0 7928	0	0	0	0	0	0
	2	CW	2	3	1	1	0	0
		MR	2	2	0	1	0	0
		G0 7928	0	0	0	0	0	0

[a] CW = Canadian Wonder (susceptible), MR = Masai Red (moderately resistant), G0 7928 = breeding line (resistant).
[b] Field was cultivated to maize the previous year.
[c] Field was under coffee/banana association and had never been planted to beans.
[d] Field was under fallow the previous 3 yr.

osis in stems and did not cause water-soaked lesions. In contrast, the strains of race 2 isolated from bean plants growing in the same field and infected with halo blight produced no chlorotic symptoms on Edmund.

All races tested (two strains of race 1, two of race 2, and three of race 2P) grew at 24 ± 2 and 28 ± 2 C. However, two of the three strains of race 2P were able to grow at 34 ± 2 C. These strains also produced the brown, diffusible pigment at all three temperatures.

Survival studies. Survival of *P. s. phaseolicola* in bean debris buried in the soil and in standing bean plants in the field varied according to race, geographic location, depth of debris placement, and bean cultivar (Table 2). Within the same bean genotype, *P. s. phaseolicola* tended to survive longer in stems than in leaf debris and longer in standing bean plants and in bean debris 2–5 cm deep than in debris buried at 25 cm. For example, at Monduli, race 1 survived for 5 mo in stem debris of cv. Canadian Wonder and 4 mo in foliage debris, whether debris was of standing plants or buried 2.5 cm deep, but survived only 3 mo in stem debris buried 25 cm deep. At the same location, race 2 tended to survive longer than race 1 in stems of Canadian Wonder. This susceptible cultivar supported longer survival of halo blight bacteria than the moderately resistant Masai Red, while neither race survived even for 1 mo in GO 7928, the highly resistant line. Pathogenicity tests and sensitivity to bacteriophages 11P, 12P, and 48P confirmed the identity of recovered bacteria as *P. s. phaseolicola*.

In the coffee/banana intercrop environment at Lyamungu, populations of *P. s. phaseolicola* declined rapidly (Table 2). Race 1 was not recovered from leaves or stems of standing plants of cvs. Canadian Wonder and Masai Red at 2- or 3-mo sample dates, whereas race 2 remained viable for 2 mo in foliage and 3 mo in stems of standing plants. Neither race was recovered from debris of either bean genotype that had been buried 2–5 or 25 cm deep for 1 mo or longer.

In the field left fallow the previous 3 yr at Lyamungu, races 1 and 2 of *P. s. phaseolicola* were recovered after 2 and 3 mo in leaves and stems, respectively, of cv. Canadian Wonder left standing in the field. The pathogen apparently survived for only 1 mo in samples buried 2.5 cm deep and not at all in samples buried 25 cm deep. Race 2 survived for 1 mo in stem pieces of Masai Red. As in the coffee/banana intercrop environment, *P. s. phaseolicola* did not survive even 1 mo in samples buried 25 cm beneath the soil surface.

In the laboratory, both races were detected at very low numbers in the resistant line GO 7928 and only at the beginning of the experiment in June; thereafter, the pathogen was not de-

tected. However, these bacteria remained viable for more than 8 mo in infected dry debris of cvs. Canadian Wonder and Masai Red stored in the laboratory.

Lyamungu received rain throughout the survival study period (June–December 1989) (Fig. 2), whereas Monduli received little or no rain until November and December (Fig. 3). 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.

Volunteer plants. Races 1 and 2 of *P. s. phaseolicola* were readily recovered from lesions from diseased foliage of volunteer bean plants in two farmers' fields in Monduli and around several backyards at Lyamungu. Shatter-loss bean seeds germinated almost immediately after harvest (July/August) at Lyamungu, whereas most of the shatter-loss bean seeds at Monduli remained dormant in the field until October/November. Soil moisture at harvest was much higher at Lyamungu than at Monduli.

DISCUSSION

In the current survey, three races of *P. s. phaseolicola* exist in the Arusha and Kilimanjaro regions of northern Tanzania. Race 2, including race 2P, was detected at a somewhat higher frequency (52.5%) than race 1 (45.0%), and race 3 (2.5%) was detected infrequently. These findings agree with those of Taylor et al (28) 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 (28) observed that it is the dominant race in the neighboring country of Rwanda. Because of their prevalence in northern Tanzania, races 1 and 2 appear to be the most threatening races to bean production in that area. However, the increased movement of bean germ plasm between countries and the need for continued exchange of bean breeding material between research stations within countries may inadvertently result in spreading other races of *P. s. phaseolicola* in areas where they do not now occur.

There appeared to be variability within race 2. Some strains, designated as race 2P, produced a brown, diffusible pigment on NA and KB and were found over a broad range of altitudes, from 1,020 to 1,630 m above sea level (Table 1). In addition, at least two of three race 2P strains were able to grow at a higher temperature (34 ± 2 C) than the non-pigment-producing race 2 strains or the race 1 and race 3 strains. However, we do not know whether such strains also tolerate high temperatures under natural conditions, whether the brown, diffusible pigment is produced under natural conditions, and whether the ability to grow at high temperature in vitro is associated with pigment production. The delayed appearance of the pigment on KB might be due to the masking effect of the blue-green, diffusible pigment that is produced in large quantities on this medium. The only other phytopathogenic bacterium known to produce a brown, diffusible pigment in vitro is *Xanthomonas campestris* pv. *phaseoli* var. *fuscans* (3).

King et al (13) observed that bacteria of the genus *Pseudomonas* produce a number of pigments on medium A and B, including fluorescein, pyocyanin, and pyorubin. In addition, a dark brown pigment was also produced by some strains, which King et al believed was not an oxidation-reduction product of pyocyanin, fluorescein, or pyorubin. Because the species or pathovar that produced the dark brown pigment was not specified, we do not know whether the brown, diffusible pigment observed in the current investigation is similar to that reported by King et al (13).

Race 2 strains of *P. s. phaseolicola* obtained from debris in Monduli were consistently more aggressive than those from growing bean plants. This was manifested by their ability to produce systemic chlorosis and stunting on the usually resistant differential bean cultivar Edmund and by their ability to produce large water-soaked lesions on Tendergreen. These results suggest that virulence of race 2 strains may increase during survival in the bean debris under dry environmental conditions such as exist in Monduli. We do not know whether the increased virulence is due to mutation or to selection of a subpopulation whose increased virulence is associated with the ability to survive in debris. Spontaneous mutations involving increased aggression in *P. s. phaseolicola* and *P. s. pisi* have been observed by some researchers (27). Thus, the survival environment may have a greater effect on the selection of genes, or a virulent population, than the host cultivars (15). Such a phenomenon has also been suggested to occur in *X. c. oryzae* in Japan, in which increased virulence involved factors other than the host cultivars grown (11).

The finding that survival of *P. s. phaseolicola* in bean debris in the soil and in standing bean plants in the field varied with race, geographic location, depth of placement in the soil, and bean genotype indicates that management of halo blight should take into account such variations. The survival of the bacterium was favored in soils with a long dry per-

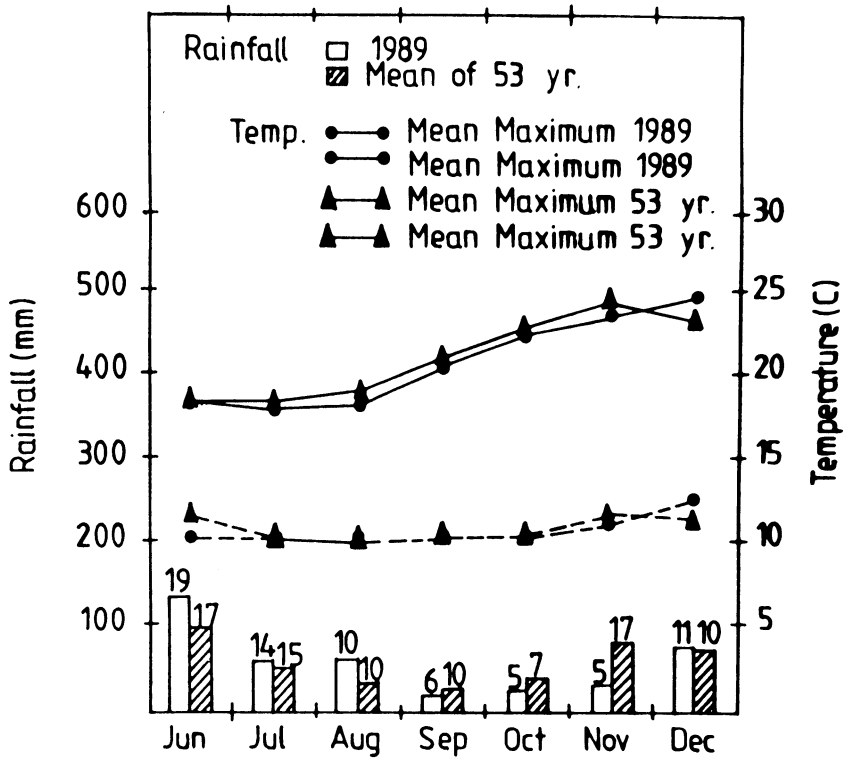

Fig. 2. Monthly rainfall and mean monthly maximum and minimum temperatures from June through December at Lyamungu for 1989 and for previous 53 yr (1935–1988). Number above each bar represents number of days with rain during that month.

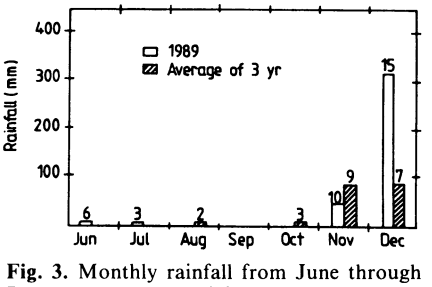

Fig. 3. Monthly rainfall from June through December at Monduli for 1989 and for previous 3 yr (1986–1988). Number above each bar represents number of days with rain during that month.

iod, as in Monduli, where race 2 survived for at least 6 mo in stems of dead, standing bean plants and in buried (2–5 cm deep) debris of the susceptible bean cultivar Canadian Wonder. At Monduli, race 2 tended to survive longer than race 1, and at Monduli and Lyamungu, survival of either race was generally longer in stems than in leaves. These results suggest that debris from highly susceptible cultivars constitutes a source of primary inoculum for halo blight under field conditions and confirm previous reports that the survival of *P. s. phaseolicola* is favored by dry soils (18,19). Soil moisture affects the longevity in the soil of many phytopathogenic bacteria by increasing microbial activity (19).

Under high soil moisture conditions and low pH such as the case at Lyamungu, survival of *P. s. phaseolicola* did not exceed 2–3 mo, especially in the coffee/banana environment, where soil moisture tended to remain high. However, diseased bean plants of the susceptible cultivar Canadian Wonder left standing in the field may act as sources of inoculum when beans are grown in pure stands, since two or three bean crops may be planted every year in areas such as Lyamungu. Resistant cultivars supported survival of *P. s. phaseolicola* for only 0–3 mo, depending on the degree of host resistance (Table 2). The use of such cultivars would be expected to reduce the amount of inoculum that will survive in bean debris in the field.

Volunteer bean plants provided an alternative survival site for *P. s. phaseolicola* in northern Tanzania, bridging the gap between the two bean-growing seasons, March–June and October–December. The greater prevalence of race 2 than of race 1 in northern Tanzania may be related to the ability of race 2 to survive longer under the same conditions in the field. However, differences in bean seed infection and transmission may also be involved. Studies are needed to determine whether any differences exist and to relate such differences to race prevalence in specific regions.

Results of this study suggest that control measures for *P. s. phaseolicola* in northern Tanzania should include, in addition to the use of resistant cultivars, such sanitation measures as burning infected debris in the field and around backyards after the bean crop is processed, especially in dry areas such as Monduli. In wet areas such as Lyamungu, plowing under volunteer bean plants when they are still very young will facilitate decomposition and thus reduce the amount of inoculum persisting between crops.

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