Pseudomonas syringae pv. *phaseolicola* Populations and Halo Blight Severity in Beans Grown Alone or Intercropped with Maize in Northern Tanzania

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ABSTRACT

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In tests in northern Tanzania, populations of *Pseudomonas syringae* pv. *phaseolicola* on and in bean (*Phaseolus vulgaris*) foliage were higher, severity of halo blight on foliage and pods was greater, and incidence of seed infection was higher when beans were intercropped with maize (*Zea mays*) than when beans were grown alone. Maize leaves did not support high populations of the pathogen and thus did not seem to provide additional inoculum for the bean crop when the two were grown in association. However, bean leaves in the intercrop system took longer to dry after rainfall and were wetter than those in the monoculture. Simultaneous measurements of parameters related to the pathogen, disease, and weather are therefore needed to understand the effect of a bean/maize intercrop on incidence and severity of disease.

The majority of the bean (*Phaseolus vulgaris* L.) crop in Africa and other tropical areas is produced by small farmers in complex associations with other crop species, notably maize (*Zea mays* L.). Depending on the country, companion crops may include bananas, cassava, sweetpotatoes, yams, peas, and coffee. Such crop associations on the same land and at the same time are referred to as intercrops (5). During the last 10 yr, there has been an increasing awareness that the impact of the green revolution on small holder farming in developing countries has remained rather limited (4). Hence, certain current research has been focused on an analysis and improvement of traditional cropping systems. So far, traditional intercropping systems appear to be better adapted than monocropping systems to the ecological, socioeconomic, and sociocultural conditions of tropical agriculture (4,6,21,25).

Various researchers have noted that plant disease epidemics are favored by morphologically and genetically uniform crops grown on large areas of land (2,3,20). In 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 to large populations (1,3,4,6). Intercropping of bean with maize in the tropics has been reported to reduce disease severity in bean crops (11,12,17,18,27). However, this may not always be true. Much depends on such other factors as the host range of the pathogen, the sowing time, and the spatial geometry of the associated crops (6). In addition, disease is affected in intercrop systems by a complex function of the reproductive rate of the pathogen on or in the component crops and the rate of propagule transfer between host components (4,6). For example, anthracnose, caused by *Colletotrichum lindemuthianum*, may be more severe on beans intercropped with maize than on beans in a monocrop because of increased humidity and lower canopy temperature (6,16). Angular leaf spot, caused by *Phaeoisariopsis griseola*, may be more severe on beans intercropped with maize than on beans intercropped with cassava (6,16). In contrast, common bacterial blight, caused by *Xanthomonas campestris* pv. *phaseoli*, and halo blight, caused by *Pseudomonas syringae* pv. *phaseolicola* (Burkholder) Young et al, were more severe in beans grown in association with maize than in a bean monocrop system (11,17,27,28).

The effect of the maize intercrop on disease severity has been based on disease severity ratings, and disease ratings have not been related to changes in pathogen populations. The size of pathogen populations may provide an estimate of the degree to which a given agricultural system is ecologically suited to a particular pathogen, and measurements of populations have been used to describe various bacteria/plant interactions (22). The ob-

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jective of the research described herein was to relate populations of *P. s. phaseolicola* with severity of halo blight in bean grown as a monoculture and bean intercropped with maize.

MATERIALS AND METHODS

Location. Experimental fields were located at Lambo (altitude 1,020 m) and Lyamungu (altitude 1,268 m), latitude 3°14'S and longitude 37°15'E, in 1989 during the long rainy season (March to June). The two locations were about 13 km apart on the southern side of Mount Kilimanjaro. Both locations were in the Hai district of the Kilimanjaro region and had been planted to maize the previous two seasons.

Experimental design. Maize cultivar Kilima and bean cultivar Canadian Wonder were grown in 3 × 4 m plots arranged in a randomized complete block design with three replications. Pathogen-free seeds were provided by B. Gondwe (Agricultural Research Institute, Lyamungu). The three treatments were: 1) beans grown alone, spacing of 75 cm within rows and 20 cm between rows, two plants per hill; 2) maize grown alone, spacing of 75 × 25 cm, two plants per hill; and 3) beans intercropped with maize, i.e., single rows of beans alternating with single rows of maize, spacings as described. The two crops were sown simultaneously. Planting and all other agricultural practices were done by manual labor and as recommended for the region. Before planting, samples of soil, weeds, and maize debris were taken, following the stratified sampling design of Delp et al (9). Samples were placed in plastic bags and sent to the laboratory to assay for *P. s. phaseolicola* within 24–36 hr.

Inoculation of plants. The *P. s. phaseolicola* (race 1, strain 6) used in this study originated from a lesion on *Neonotonia wightii* (Graham ex Arnott) Lackey. The strain was inoculated to bean cultivar Canadian Wonder and reisolated from lesions. For inoculum preparations, 48-hr cultures on King's medium B (KB) (13) plates were suspended in glass-distilled water. Concentrations were adjusted turbidimetrically (OD = 0.04–0.05 at 620 nm) to contain about 10^7 to 10^8 cells per milliliter. A hand-operated atomizer was used to spray-inoculate plants with the cell suspensions on both sides of the leaves to runoff, with slight water-soaking. Maize plants were also sprayed with cell suspensions to determine if the bacteria multiplied or remained in that crop. The plants were inoculated when 19 days old, when the second trifoliolate leaf was fully open on beans and the fifth leaf was just unfolding on maize.

Bacterial population trends. Leaf samples were collected between 8 and 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, six to eight leaves were sampled at random and immediately transported on ice to the laboratory, where they were kept at 5 C until processed within 24–36 hr. Populations of *P. s. phaseolicola* were estimated with procedures similar to those of Smidt and Vidaver (23). Leaves were added to a sterile 0.01 M phosphate buffer (pH 7.2) containing 0.01% Tween 20 in conical flasks. The flasks were shaken for 30 min with a wrist-action shaker at 20–22 C. The amount of sterile phosphate buffer varied with each sample to ensure that the sample was immersed completely. Samples of the liquid phase were diluted with sterile phosphate buffer in a serial 10-fold manner, and 0.1-ml portions were removed and plated on KB supplemented with 100 μg of cycloheximide per milliliter. Bacterial colonies were counted after 4–5 days of incubation at 24 C. The washed leaves were blotted dry, then traced on paper, and the areas were measured using a LI-3100 area meter (LI-COR, Inc., Lincoln, NE). Colony counts were expressed as numbers per square centimeter of leaf. Only symptomless leaves were used for determination of epiphytic populations.

For measurements of internal populations of *P. s. phaseolicola*, leaves were submerged in 2.6% NaOCl for 3 min to kill surface microbes, then 10 disks (2 cm diameter) were punched out with a sterile cork borer from leaves free of visible lesions, placed on sterile filter papers, weighed, and homogenized in 3–5 ml of phosphate buffer. Homogenates were prepared, serially diluted, and plated as described above. Bacterial counts were expressed as numbers per gram of fresh tissue.

At each sample date, 10 presumed colonies of *P. s. phaseolicola* were randomly selected and tested for pathogenicity on 7- to 10-day-old bean seedlings by stem injection and spray inoculation of the foliage. Inoculated plants were covered with polyethylene bags for 24 hr in a screenhouse. Plants were maintained at 19–27 C with natural light (12 ± 0.5 hr day length) during the next 10 days.

Evaluation of disease reaction. Halo blight severity was evaluated with the CIAT scale of 1–9 (7), in which 1 = absence of symptoms and 9 = very severe disease. Leaf disease reaction was estimated at 10 and 25 days after inoculation, whereas pod symptoms were rated at physiological maturity. All plants within a plot were examined, and a mean rating was assigned to the whole plot. Maize plants were also examined.

Leaf wetness studies. The period required for bean and maize leaves to dry after rain was determined to understand the effect of the two cropping systems on moisture retention in the canopy. On 10 different days of rainfall at Lyamungu, leaves were examined for visible free moisture at 30-min intervals after rain stopped.

Bean seed infection assay. Rainfall and temperature data for Lambo and Lyamungu were obtained from the Agrometeorology Section at the Agricultural Research Institute, Lyamungu, Moshi. Mature dry pods were harvested manually from each plot, kept separate, allowed to dry under the shade, and then hand-shelled. A random sample of 600 bean seeds was drawn from each replicate and soaked in 2.6% NaOCl for 3 min. After surface-sterilization, seeds were aseptically plated, hilum down, on KB-

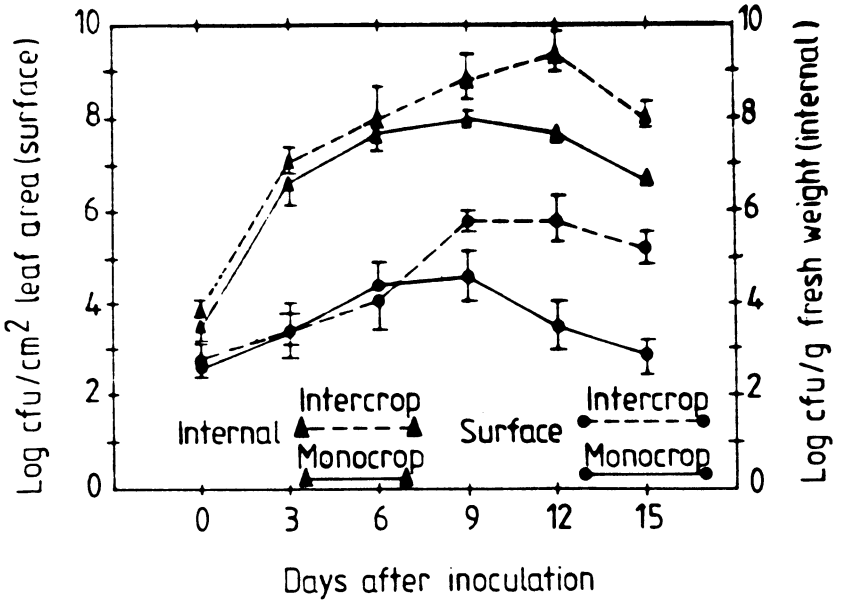

Fig. 1. Internal and surface population dynamics of *Pseudomonas syringae* pv. *phaseolicola* race 1 on and in bean foliage in a bean monocrop and a bean/maize intercrop system at Lambo in the Kilimanjaro region of northern Tanzania. Bars indicate standard errors of the means.

cycloheximide agar, six seeds per 10-cm-diameter glass petri dish. After 5 days plates were observed under ultraviolet light in the darkness. The presence of fluorescence was presumptive evidence for infected or infested seed. A series of single-colony transfers on KB agar plates were made from colonies associated with the seed and tested for pathogenicity as described above. Two separate determinations of seed infection were made for the same plot using the same sample size.

Data analysis. Statistical computations were made on a microcomputer with 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 with Student's *t* test. Data for percentage bean seed infection were arcsine transformed before analysis (15).

RESULTS

Population dynamics at Lambo. Populations of *P. s. phaseolicola* on and in bean leaves were almost the same in the monocrop as in intercrop system for the first 6 days after inoculation (Fig. 1). At days 9, 12, and 15, however, surface populations in the bean/maize intercrop system were higher than those in the bean monocrop system. Populations of *P. s. phaseolicola* inside leaves increased faster than those on the surface during the first 3 days (Fig. 1), but differences between the cropping systems were not seen until day 9. 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 populations reached a maximum of 6.3×10^7 cfu/g fresh weight at day 9 in the bean monocrop system and 4.0×10^9 cfu/g fresh weight at day 12 in the bean/maize intercrop system, after which populations gradually declined. Surface populations also decreased after day 12.

Population dynamics at Lyamungu. Surface populations increased slightly over the first 6 days in both cropping systems, then increased rapidly, reaching maximums of 5×10^4 and 5×10^5 cfu/cm^2 of leaf area at day 9 in the bean monocrop and bean/maize intercrop systems, respectively. Thereafter, surface populations declined sharply; monocrop populations reached a level significantly lower than that of intercrop populations at day 12, then suddenly increased to a level not significantly different ($P = 0.05$) from that of the intercrop system at day 15 after inoculation. The internal population increased rapidly up to day 6, then increased at a slower rate. The population tended to be slightly greater in the bean/maize intercrop system than in the bean monocrop throughout the sampling period (Fig. 2), but these differences were not significant ($P = 0.05$) except at day 15. Internal populations reached a maximum of 2×10^8 cfu/g fresh weight at day 12 in the monocrop system, then declined. In the intercrop system, levels increased to 1×10^8 cfu at day 15.

Populations of *P. s. phaseolicola* on and in maize leaves. Populations on maize leaves in the monocrop system declined rapidly and were not detected at day 6 after inoculation. In the intercrop plots, however, surface populations on maize leaves remained about 6.3×10^2 cfu/cm^2 of leaf area until day 9, then declined. Internal populations of *P. s. phaseolicola* in maize plants grown alone or in association with bean also decreased throughout the sampling period at both Lambo and Lyamungu. At Lambo (Fig. 3), practically no internal bacteria were detected in maize leaves by day 6 in both cropping systems.

At Lyamungu, populations of *P. s. phaseolicola* in maize were similar to those at Lambo. Surface populations increased to a maximum of about 2.5×10^3 cfu/cm^2 at day 3 after inoculation, then declined. The bacterium was not detected on maize after day 9 in the maize monocrop but remained at about 1×10^2 cfu/cm^2 through day 12 in the bean/maize intercrop system. Internal populations, on the other hand, decreased rapidly and were not detected after day 6 (Fig. 4). Internal populations in the

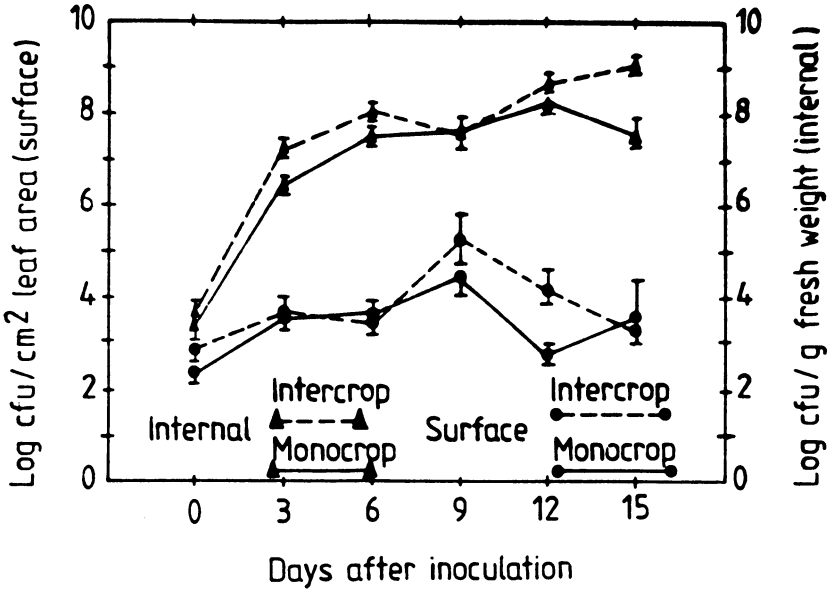

Fig. 2. Internal and surface population dynamics of *Pseudomonas syringae* pv. *phaseolicola* race 1 on and in bean foliage in a bean monocrop and a bean/maize intercrop system at Lyamungu in the Kilimanjaro region of northern Tanzania. Bars indicate standard errors of the means.

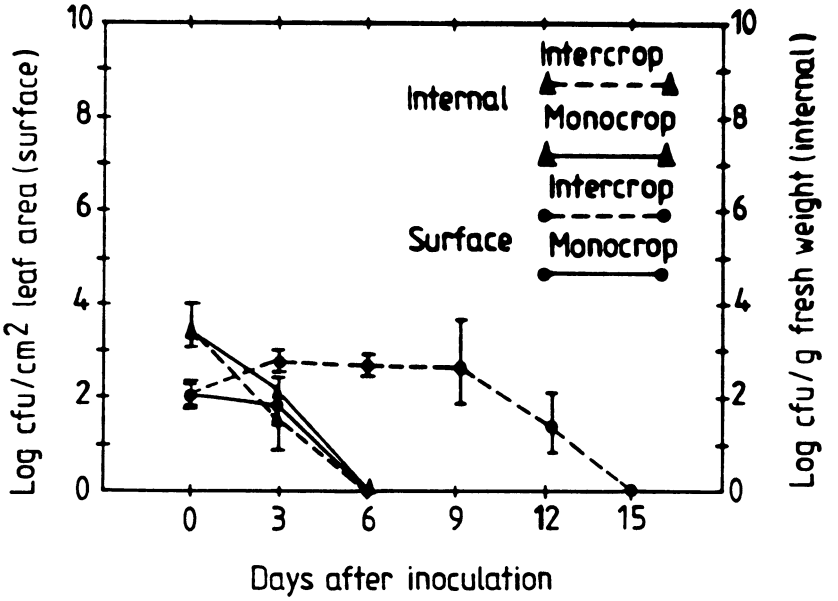

Fig. 3. Internal and surface population dynamics of *Pseudomonas syringae* pv. *phaseolicola* race 1 on and in maize foliage in a monocrop and a bean/maize intercrop system at Lambo in the Kilimanjaro region of northern Tanzania. Bars indicate standard errors of the means.

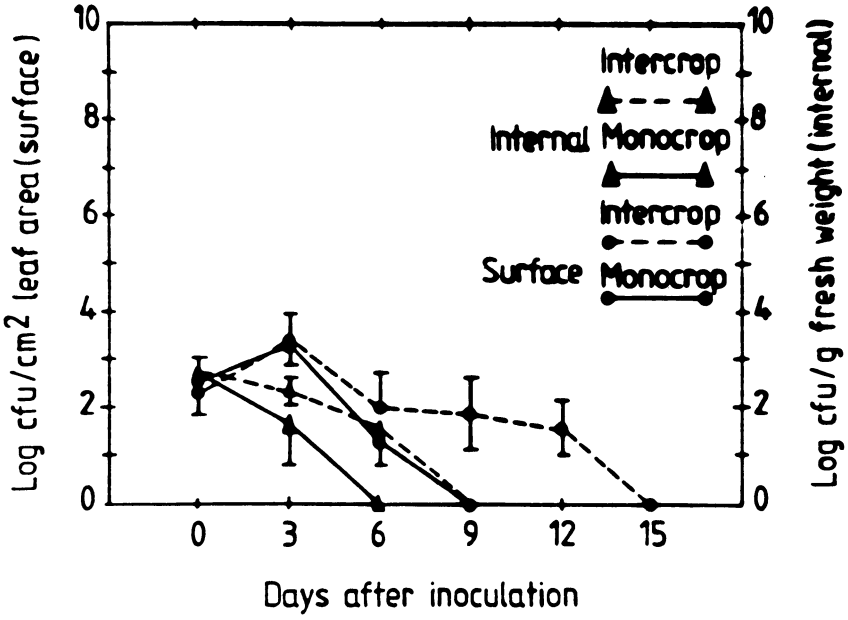

Fig. 4. Internal and surface population dynamics of *Pseudomonas syringae* pv. *phaseolicola* race 1 on and in maize foliage in a monocrop and a bean/maize intercrop system at Lyamungu in the Kilimanjaro region of northern Tanzania. Bars indicate standard errors of the means.

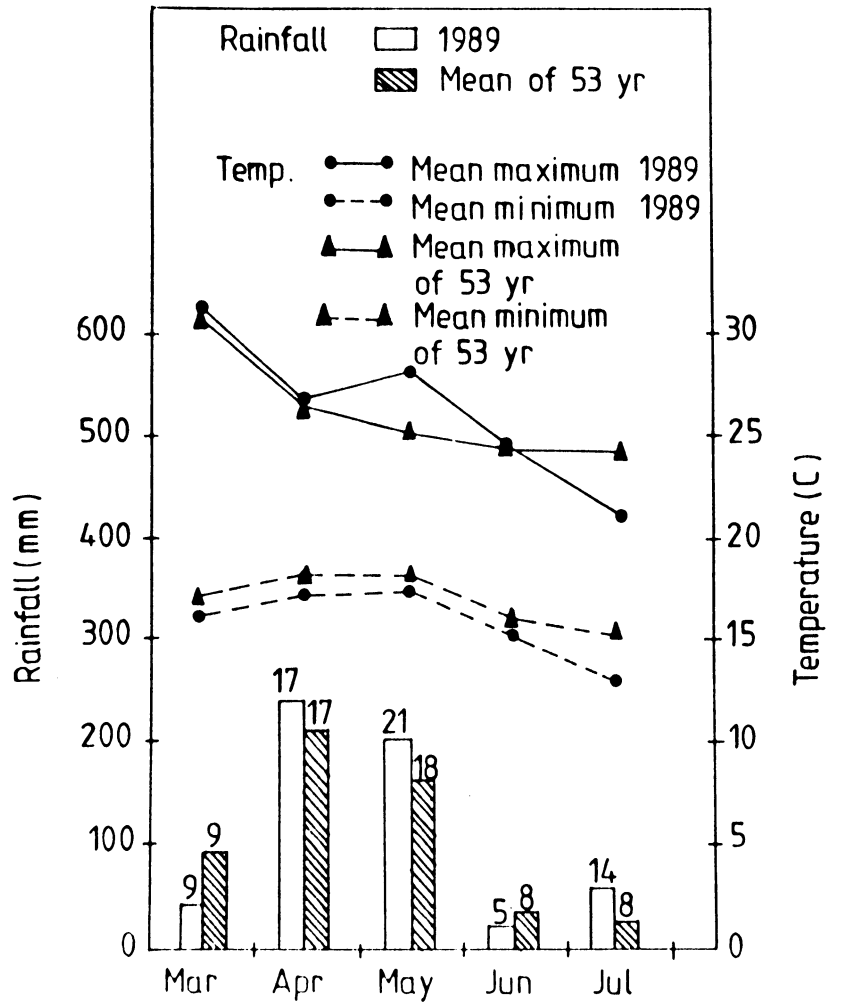

Fig. 5. Monthly rainfall and mean monthly maximum and minimum temperatures from March through July at Lambo for 1989 and for previous 53 yr (1935–1988). Number above each bar represents number of days with rain during that month.

bean/maize intercrop system declined at a slower rate and were not detected after day 9. Each presumptive colony of *P. s. phaseolicola* tested for pathogenicity on Canadian Wonder bean seedlings was pathogenic and produced typical halo blight symptoms within 8–10 days after inoculation.

Leaf and pod disease ratings. Halo blight severity on bean leaves at day 10 and day 25 was not significantly different ($P = 0.05$) between the two cropping systems at both Lambo and Lyamungu. However, at Lyamungu, disease tended to be more severe in the bean monocrop system than in the bean/maize intercrop system. Pod disease at both locations tended to be more severe in the bean/maize association than in the monocropping system, although differences were not significant ($P = 0.05$). At Lambo, the proportion of pod area covered with lesions in the intercropping system exceeded that in the monocrop system by 24%, whereas the difference at Lyamungu was 20%.

Bean seed infection. The incidence of *P. s. phaseolicola* was lower in seed from the pure stands than in seed from the intercrop, but the differences were not statistically significant ($P = 0.05$) except for experiment 2 at Lambo (Table 1). 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. phaseolicola* from infected bean seed were pathogenic when tested on Canadian Wonder bean seedlings.

Retention of moisture on leaves. Leaves of beans growing in the intercrop system required 2.8 ± 0.2 hr to dry after rain, whereas leaves of beans in the monocrop system needed 2.0 ± 0.2 hr, a difference of 40%. Such differences were not observed for maize, which re-

Table 1. Percentage of bean cultivar Canadian Wonder seed infected with *Pseudomonas syringae* pv. *phaseolicola* in a bean monocrop and a bean/maize intercrop system in northern Tanzania

Location / Experiment	Seed infection (%)[y]	
	Monocrop	Intercrop
Lambo		
1	16.8 ± 8 a[z]	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	32.9 ± 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.

quired 4.3 ± 0.2 hr to dry in both cropping systems.

Climatological data. At Lambo, rainfall totals for April and May 1989 were 242 and 201 mm, respectively (Fig. 5). The 53-yr rainfall average was 209 mm for April and 162 mm for May. Temperatures at Lambo ranged from 17 to 27 C in April, which were close to the 53-yr means of 18–27 C. In May, however, temperatures ranged from 17 to 28 C, compared with 18–25 C for the 53-yr means. Lyamungu, on the other hand, received 303 and 811 mm of rain for April and May, respectively (Fig. 6). The means of 53-yr rainfalls for the same months were 502 and 414 mm, respectively. Temperatures at Lyamungu were cooler than those at Lambo, ranging from 15 to 24 C for April and from 15 to 22 C for May. The 53-yr average ranged from 16 to 25 C for April and from 13 to 22 C for May. Rainfall and temperatures at both locations decreased progressively toward the end of the growing season. Population studies of *P. s. phaseolicola* were conducted during April and May.

DISCUSSION

Data from this study suggest that intercropping beans with maize favored multiplication of *P. s. phaseolicola* in bean leaves. Although there were no significant differences in severity of foliage disease between the two cropping systems, the greater population of the pathogen on bean in the intercrop tended to result in a higher incidence of contaminated seed than when beans were grown in a pure stand. Thus, disease severity on foliage may not always be adequate to assess the advantage of a given cropping system for disease control, particularly if a portion of the seed is to be planted for subsequent crops.

Populations of *P. s. phaseolicola* in the intercrop system may have been favored by the increased moisture retention in the canopy in the intercrop plots. Beans in the intercrop system retained moisture longer than beans grown alone. In addition, maize leaves took longer to dry than did bean leaves after rain ceased. This effect may be magnified because of longer maize canopy when beans are planted in an already established maize crop, as is sometimes the case in northern Tanzania and in other areas of the country. Bacterial populations, especially epiphytic ones, have been reported to increase when plants are wet (14,19,24). Increased moisture retention may also result in a lowered foliar temperature. Therefore, in areas where high temperature and reduced moisture are limiting factors for halo blight development, such as at Lambo, intercropping beans 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. Small farmers save seed to plant crops in the next season, and the higher the incidence of the pathogen in the seed, the greater the potential for disease development in the crop.

The greater population of *P. s. phaseolicola* at Lambo than at Lyamungu was probably due to differences in temperatures, which were about 3 C higher at Lambo than at Lyamungu during the assay period; temperature differences could have been greater within the canopy. Differences in the amount of rainfall may also have been involved in the population dynamics at the two locations. Although phytopathogenic bacteria on plant surfaces often multiply faster after rain (10), excessive rainfall (as was the case at Lyamungu) combined with low temperature may result in a net negative effect on population size.

Our results contrast with those of previous workers (17,28), who reported that halo blight was generally less severe among beans grown in association with maize than in those in pure stands. However, some of these studies were restricted to observations made in agronomy experiments (17,26,27), depending on natural infection that does not ensure uniform initial inoculum in the two bean cropping systems. If 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 (4,17). But population dynamics and percentage of seed infection, as quantitative measures of ecological fitness of the pathogen, were not considered in previous studies, nor were moisture retention differences measured. When these factors were taken into account and a uniform amount of initial inoculum was provided in the two bean cropping systems, the environment in the bean/maize intercrop system was found to favor increased multiplication of halo blight bacteria and to result in increased seed infection.

Contradictory reports of the effect of intercropping beans with maize on disease severity exist for bean anthracnose (6,8,17) and bean rust (17). As van Rheenen et al (27) suggested, the environmental differences between monocropping and intercropping and their influ-

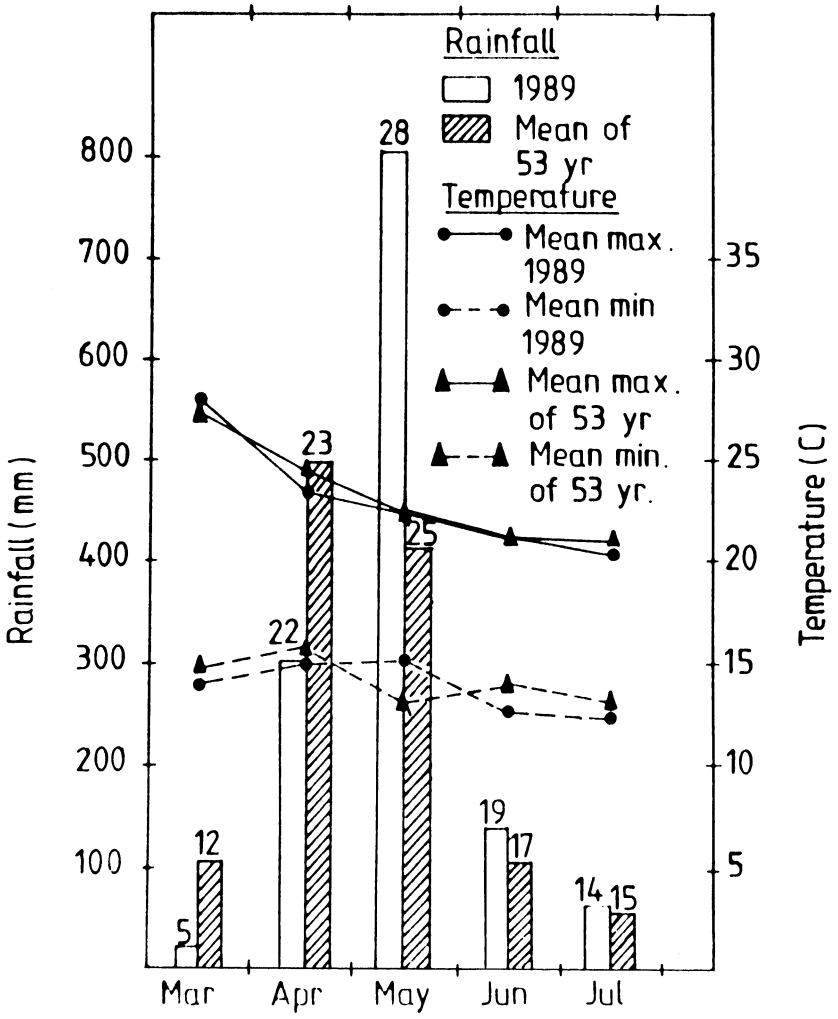

Fig. 6. Monthly rainfall and mean monthly maximum and minimum temperatures from March through July at Lyamungu for 1989 and for previous 53 yr (1935–1988). Number above each bar represents number of days with rain during that month.

ence on disease are far too complex for generalization. Better understanding will require simultaneous measurement of parameters related to the pathogen, including population size, disease incidence and severity, and weather variables such as moisture and temperature.

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