Survival of Tropical Xanthomonas oryzae in Relation to Substrate, Temperature, and Humidity

S. P. Y. Hsieh and I. W. Buddenhagen

Graduate Student and Professor, respectively, Department of Plant Pathology, University of Hawaii, Honolulu 96822. Present address of senior author: National Chung Hsing University, Taichung, Taiwan.

Portion of a Ph.D. thesis submitted to the University of Hawaii by the senior author.
Hawaii Agricultural Experiment Station, Journal Series Paper No. 1757.
Accepted for publication 25 November 1974.

ABSTRACT

Survival of a tropical isolate of *Xanthomonas oryzae* was studied in different substrates in relation to temperature and humidity, using a streptomycin-resistant mutant and direct isolation on a streptomycin agar. Survival was longer at low than at high relative humidities (RH) and temperatures; but higher temperatures with very low RH also permitted long survival. *X. oryzae* survived less than one month in warm (30 C), flooded, or moist soil or in leaves buried in such soil. In ooze droplets, or in diseased leaves in air at warm temperatures and 100% RH, the pathogen survived only 5-40 days. Lowering RH to 0-20% lengthened such survival to almost one year. The pathogen survived only briefly in warm rice paddy water. These data indicate the field disease cycle should be breakable in the wet tropics by having hosts absent for two months. In regions with dry or cold off-seasons, the disease cycle should continue regardless of periodic host absence.

Phytopathology 65:513-519

Additional key words: bacterial ecology.

In the Philippines, India, and other parts of tropical Asia, bacterial blight continues to reappear in renewed rice crops under many diverse climates and crop handling practices. The disease occurs annually in most second-crop rice in Taiwan, especially in the eastern coastal areas of the island. The source of initial inoculum which reinitiates the disease remains uncertain. In Japan, *Leersia oryzoides* var. *japonica* has been reported to be the main overwintering site of the bacterium (8, 16). Although some other plants have been reported as hosts in tropical countries (4, 22), no naturally diseased host plant other than *Oryza* species has yet been found in the

tropics (6). Seed may be infested by the bacterium; however, the role of seed infestation in the disease cycle has not yet been substantiated (14), despite reports of its importance (3, 7, 25).

Many observations have been made on survival of *Xanthomonas oryzae* (Uyeda and Ishiyama) Dowson in soil, irrigation water, diseased plants, alternate hosts, and seeds (7-9, 15, 16, 19, 21-29, 31-33). The reports were mostly from Japan and often were based on disease reappearance in a new crop, or on indirect methods indicating possible pathogen presence. Few studies have involved survival under specific conditions of temperature and humidity. An early Japanese study showed that the bacterium survived the winter in soil for 4 months (17); however, different results obtained in later studies indicated survival of only 1-2 months under various soil conditions (15, 19, 32, 33). The only report (23) on survival in soil in the tropics indicated a survival of only 5 days, but was based on nonselective methods which should not enable recovery of *X. oryzae*.

References are in general agreement that *X. oryzae* could not survive long (less than 1 month) in irrigation water when mixed with other microorganisms or in sterile distilled water, the latter with one exception (24), in which survival was stated to be 12 months or more. In living stubble and in infected straw kept indoors or piled outdoors protected from rain water the bacterium may survive until spring in Japan (15, 26-28). Work on survival in the rhizosphere has been limited (30), and data are inconclusive.

A streptomycin-resistant mutant of *X. oryzae* was used in the present studies of survival of *X. oryzae* in bacterial ooze, diseased leaves, soil, and in paddy water under various known conditions, to better understand the possible pathogen ecology and disease cycle in cultivated rice in the tropics.

MATERIALS AND METHODS.—A streptomycin-resistant mutant (VNS) of *X. oryzae,* originating from isolate VN from Vietnam, was used (13). The mutant selected was as virulent as the parent isolate when tested on 13 rice cultivars, grew on normal media as rapidly, and otherwise appeared to be indistinguishable from the parent isolate. Rice cultivar IR8 at the maximum tillering stage was inoculated by a needle-prick method to obtain diseased leaves and ooze droplets. Wakimoto's medium (30) supplemented with 1.0% streptomycin sulfate (w/v) and 100 μg/ml nystatin was used to grow VNS. Serial-dilution surface plating was carried out to determine quantitative survival in some substrates. Diseased leaves were ground in a mortar before serial dilution. Although on routine media different yellow saprophytes grow which are easily confusable with *X. oryzae,* in the presence of 10,000 ppm streptomycin *X. oryzae* VNS usually was the only yellow organism which appeared. Sometimes from some substates different-looking yellow colonies appeared. Representative colonies of each type were tested for pathogenicity on IR8 rice and for many physiological characters (12). Only one type, easily recognizable by its mucoid growth (14), was both pathogenic and physiologically a *Xanthomonas*. Colony testing was repeated sufficiently to be certain of correct identification. Relative humidites (RH) were controlled in desiccators following the method of Asuyama et al. (1). Calcium sulfate powder and distilled water were used to provide 0 and 100% RH. Saturated solutions of sodium acetate, calcium nitrate, or potassium acetate provided RH at 20 C, of 76, 54, and 20%, respectively. At 30 C, saturated potassium nitrate and saturated calcium chloride solutions provided RH of 68 and 30%, respectively.

RESULTS.—*Survival of X. oryzae in ooze droplets.*—Four weeks after inoculation diseased plants

TABLE 1. Survival of *Xanthomonas oryzae* in ooze droplets at different temperatures and relative humidities (RH)

	Percent ooze droplets containing viable *X. oryzae*										
	1-4 C	10 C	20 C					30 C			
Days	0% RH	0% RH	0	20	54	76	100	0	30	68	100
0	100[a]	100[a]	100[a]	100[a]	100[a]	100[a]	100[a]	100[a]	100[a]	100[a]	100[a]
5	100	100	100	100	100	100	70	100	100	100	20
10	100	100	100	100	80[c]	75[c]	0[b]	100	100	80	0[c]
30	100	100	100	100	75	55	0	100	100	60[b]	0
60	100	100	100	100	50	25	0	100[b]	100[b]	20	0
90	100	100	100	100	35	0	. . .	60	67	13	. . .
120	100	100	50	100	0	0	. . .	53	60	0	. . .
150	100	90	40	70	0	. . .	. . .	20	33	0	. . .
180	100	80	20	40	. . .	. . .	. ..	0	27	. . .	. . .
210	90	70	20	30	. . .	. . .	. . .	0	13	. . .	. . .
240	90	80	20[b]	30	. . .	. . .	. . .	. . .	0	. . .	. . .
270	90	60	13	6.7[b]	. . .	. . .	. . .	. . .	0	. . .	. . .
300	80	67	13	0	. . .	. . .	. . .	. . .	. . .	. . .	. . .
330	80	47	6.7	6.7	. . .	. . .	. . .	. . .	. . .	. . .	. . .
360	60	47	0	0	. . .	. . .	. . .	. . .	. . .	. . .	. . .
390	60	40	0	0	. . .	. . .	. . .	. . .	. . .	. . .	. . .
420	40	27	0	0	. . .	. . .	. . .	. . .	. . .	. . .	. . .
760	20[b]	20[c]	. . .[d]	. . .	. . .	. . .	. . .	. . .	. . .	. . .	. . .

[a]Entries in columns at this level until footnote designations b or c were based on 10 samples.
[b]Entries at this level and below were based on 15 samples.
[c]Entries at this level and below were based on 20 samples.
[d]Indicates no ooze was tested for the 760-day 20 C, 0% RH treatment combination.

were transferred to a growth chamber maintained at 100% RH by shutting off the fan and light. With 12 hours of such conditions, ooze exuded from diseased leaves, after which the fan and light were turned on, which reduced the RH to about 40%. Dry ooze droplets were collected and kept briefly in a desiccator at 8 C before they were subjected to different conditions for longevity tests. Ooze droplets were placed on a cellophane sheet in open petri dishes in the desiccators. Periodically, 10-20 ooze droplets from each treatment were tested for viability by sowing them directly on plates. Results were recorded as percentage of droplets containing viable *X. oryzae*. Due to the strong hydrophilic nature of the ooze, droplets placed in 100% RH absorbed water, lost their original shape, and stuck flat to the cellophane sheet. The cellophane sheet with such attached droplets was placed on the medium. When most cells were viable, growth appeared in 2-3 days; as the experiment progressed and many bacterial cells lost viability, 4-7 days were required to reveal growth of *X. oryzae* from the ooze. Readings were taken daily up to 10 days of incubation before final readings were made.

Viability of the bacteria in ooze was completely lost within 10 days at 30 C in 100% RH (Table 1). At 20 C, survival was increased as RH decreased, reaching a maximum of 330 days at 0 and 20% RH. At 30 C, the survival period was generally less than at 20 C, reaching a maximum of 210 days at 30% RH. At low temperatures of 1-4 and 10 C, *X. oryzae* was viable in ooze droplets kept at 0% RH for at least up to 760 days, when the experiment was terminated.

In another experiment, the effect of sunlight on survival was tested. Saturated potassium nitrate and calcium chloride solutions were separately poured into desiccators which were placed in a greenhouse where temperatures fluctuated from 27 to 43 C. The RH that were attained with these chemicals varied with changes of temperature, around levels of 68% and 30%. Half of the ooze droplets were exposed to sunlight by being placed on aluminum foil in petri plates in the desiccators, while the other half were in shade beneath the aluminum foil.

TABLE 2. Survival of *Xanthomonas oryzae* in ooze droplets exposed to sunlight or under shade with fluctuating relative humidity (RH) around 30 or 68%, in greenhouse at 27 to 43 C

	Percent ooze droplets containing viable *X. oryzae*			
	30% RH (fluctuating)		68% RH (fluctuating)	
	Exposed to		Exposed to	
Days	Shade	Sunlight	Shade	Sunlight
0	100[a]	100[a]	100[a]	100[a]
5	100	100	100	70
15	100	100	60	10[c]
30	100	13[b]	33[b]	0
60	70	0	0	. . .
90	33[b]	. . .[d]	. . .	. . .
120	0	. . .	. . .	. . .

[a]Entries in columns at this level until footnote designations b or c were based on 10 samples.
[b]Entries at this level and below were based on 15 samples.
[c]Entries at this level and below were based on 20 samples.
[d]Indicates no ooze was tested for the 90-day, 30% RH, sunlight-exposed treatment combination.

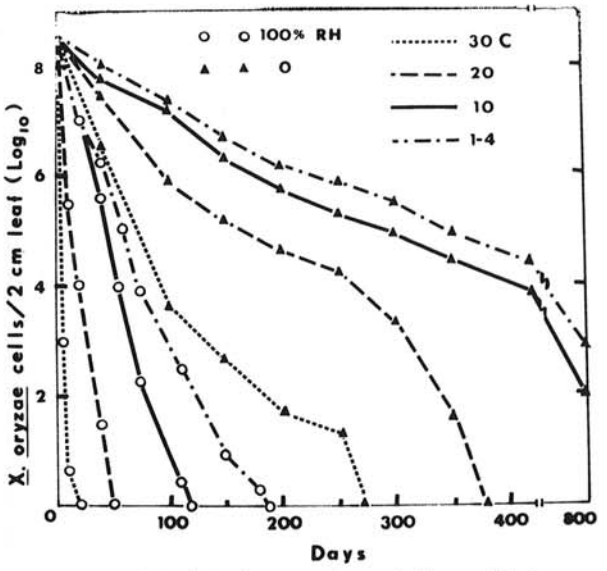

Fig. 1. Survival of *Xanthomonas oryzae* in diseased rice leaves at different temperatures at 0% and 100% relative humidities.

Sunlight, with accompanying temperature effect and filtered through greenhouse glass, shortened the longevity of *X. oryzae* in ooze droplets (Table 2). The bacterium was recovered at 30 (but not at 60) days when shaded, and at 15 (but not at 30) days when exposed to sunlight in RH fluctuating around 68%. Likewise, the bacterium was isolated from ooze held up to 90 days of incubation under shade, and only up to 30 days when exposed to sunlight when kept at RH fluctuating around 30%.

Survival in diseased leaves.—The leaf blade of diseased leaves having uniform long lesions was removed from the midrib and cut into 2-cm portions. The two portions at the ends of the lesions were ground and diluted for quantitative assay of bacterial cells. If both samples contained 10^8 cells or more (per 2-cm), the intervening 2-cm sections were subjected to various treatments and sampled later. The environments used for testing survival in diseased leaves were the same as those used for ooze droplets. Periodically, five diseased leaves were sampled for each treatment.

X. oryzae survived longest at 0% RH in diseased leaves, and progressively longer with decreasing temperature (Fig. 1.). After incubating at 1-4 and 10 C for 800 days, 10^2 to 10^3 cells/2-cm leaf section were detected, reduced from an original level of about 10^8 cells. The bacterium was recovered up to 360 days after incubating at 20 C. At 30 C, the population declined faster than at lower temperatures, although some bacteria survived 8 months.

Diseased leaves kept in 100% RH were rapidly colonized by fungi, with more rapid colonization at higher temperatures. The bacterial population in these diseased leaves declined much faster than at 0% RH and this decline was faster with increasing temperatures. At 100% RH, the longest periods during which the bacterium could be isolated were 185, 110, 40, and 10 days at 1-4, 10, 20, and 30 C, respectively (Fig. 1).

At 20 C, *X. oryzae* could be recovered, 40, 110, 125, 340, and 360 days after incubating in 100, 76, 54, 20, and

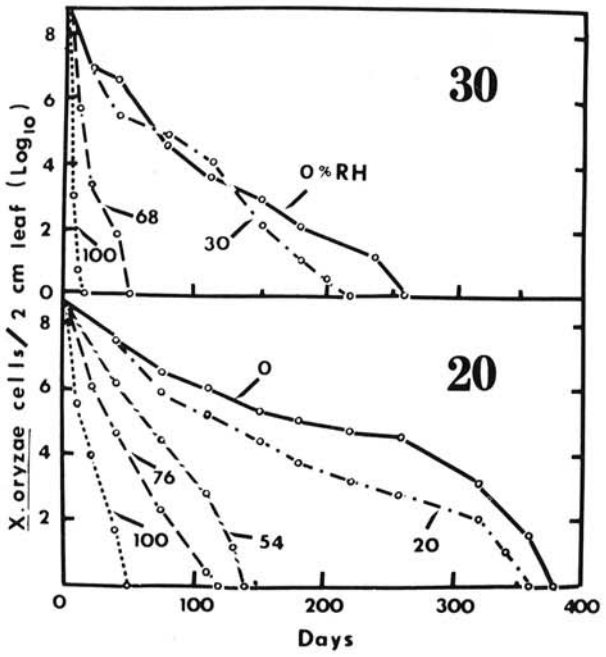

Fig. 2. Survival of *Xanthomonas oryzae* in diseased rice leaves in different relative humidities at 20 and 30 C.

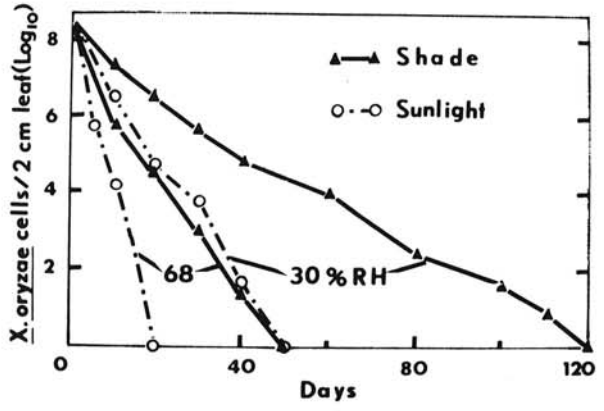

Fig. 3. Survival of *Xanthomonas oryzae* in diseased rice leaves exposed in a greenhouse to sunlight or shade with relative humidities fluctuating around 30 or 68% as temperature fluctuated from 27 to 43 C.

0% RH, respectively. At 30 C, longevity was less, but the same pattern was obtained with decreasing humidity resulting in increasing survival time (Fig. 2).

Sunlight shortened the longevity of *X. oryzae* in diseased leaves (Fig. 3). The bacterium survived 110 days in shade and only 40 days in sunlight at RH fluctuating around 30%. With humidities around 68%, the bacterium was recovered up to 40 days from shaded leaves, and up to only 10 days from sunlit leaves.

Survival of X. oryzae in diseased leaves buried in soil.—Waimanalo soil was sifted through 0.79-mm (24-mesh) screen. Distilled water was added to the soil to make 20, 40, and 80% water content. Oven-dried (80 C, 48 hours) soil without added water was considered to be 0% water content. The 80% water content of the soil provided a flooded condition, as occurs in wetland rice cultivation. Diseased leaves were buried in soil in 1,000-ml plastic beakers which were individually wrapped in plastic bags. Two sets of beakers of the different water contents were prepared, one incubated at 20 C, the other at 30 C. Five diseased leaves were sampled from each treatment.

The pathogen population in diseased leaves in flooded soil, and in soil at 40% moisture content, declined very rapidly and disappeared completely within 12 and 20 days at 30 and 20 C, respectively (Fig. 4). In leaves buried in soil at 20% moisture content, the bacterium survived 40 and 60 days at 30 and 20 C, respectively. The bacterium survived significantly longer in diseased leaves buried in soil considered close to 0% water content. It could be recovered 180 and 360 days after burying in this dry soil at 30 and 20 C, respectively.

Survival of X. oryzae in soil.—A bacterial suspension of 5×10^8 cells/ml was sprayed evenly on dry sifted soil to give final water contents of 20 and 40%. Theoretically, the pathogen population levels in these two soils were 8.3×10^7 cells/g and 1.7×10^8 cells/g. The same suspension was stirred into soil to provide a flooded soil condition. In another treatment, a bacterial suspension of 10^9 cells/ml was sprayed evenly on dry-sieved soil which was immediately transferred to a desiccator with several changes of silica gel to absorb the water within the first day of treatment. This treatment was considered as 0% water content, even though some very tightly held moisture must have been present. Immediately after applying bacterial suspensions to the soil, 1.0 g of soil was taken from each treatment and subjected to dilution plating for estimation of the recoverable population at zero time. A 1-ml sample was taken by pipet from the flooded soil which was shaken before sampling. The soils of each treatment were divided into four plastic beakers which were individually wrapped with plastic bags and incubated at 1-4, 10, 20, and 30 C. Periodically, soil from each treatment was randomly sampled in triplicate.

X. oryzae did not survive long in soil, especially when the water content of the soil was high in combination with high incubation temperatures (Fig. 5). The bacterium was detected from soil under flooded condition up to only 4, 12, 31, and 62 days after incubation at 30, 20, 10, and 1-4 C, respectively. The bacterium was detected from soil at 40% water content 4, 32, 80, and 92 days after incubation at 30, 20, 10, and 1-4 C, respectively. In soil at 20% water content, the bacterium was no longer recoverable at 15, 48, 130, and 170 days after incubation at 30, 20, 10, and 1-4 C, respectively. In soil with negligible water content, survival was slightly longer.

Survival of X. oryzae in rice paddy irrigation water.—An isolate of *X. oryzae* from the Philippines incubated for 48 hours on slants was suspended in distilled water, centrifuged at 1,085 *g* for 10 minutes, resuspended in distilled water to a concentration of 4×10^9/ml, and mixed with fresh Philippine rice paddy irrigation water of pH 6.5 at a ratio of 1:9. These preparations were incubated at different temperatures and sampled periodically.

At 30 C, the highest temperature used, the bacterium survived less than 6 days. At 20 C, it survived less than 12 days; at 10 and 1-4 C survival was extended to 37 and 60

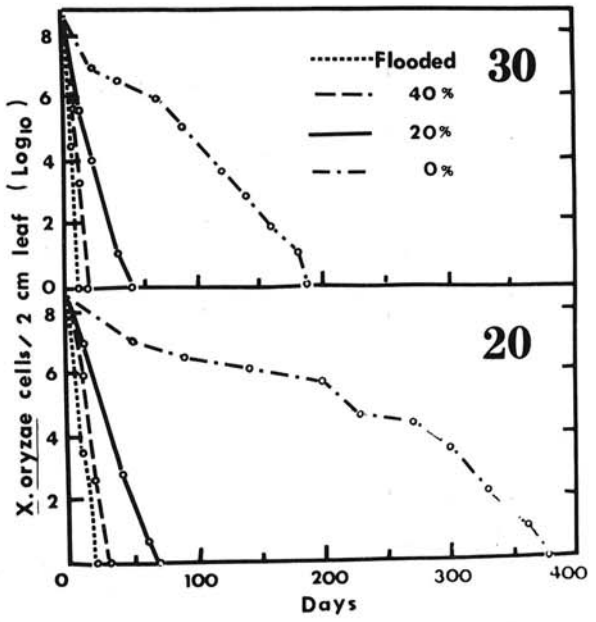

Fig. 4. Survival of *Xanthomonas oryzae* in diseased rice leaves buried in Waimanalo soil of different water contents at 20 and 30 C.

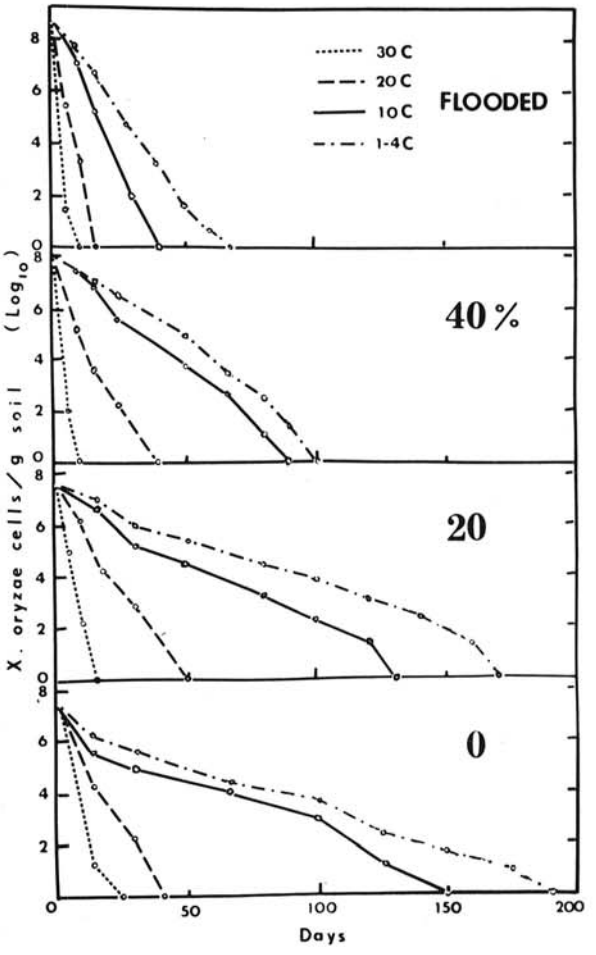

Fig. 5. Survival of *Xanthomonas oryzae* in Waimanalo soil at different temperature and water contents.

days. When the normal paddy-water microflora was removed by Millipore filtration, *X. oryzae* populations decreased only slightly in the 12-day test period used for temperatures of 20 and 30 C.

DISCUSSION.—In general, *X. oryzae* survived longer under conditions of low relative humidities and temperatures, with increasing survival times as these parameters were progressively lowered. Our results indicate that the bacterium in diseased leaves in soil might survive long enough to attack a succeeding crop in both temperate, and in some, but not all, tropical areas. In many areas of the tropics, the bacterium would have to survive several months of dry season between crops. With increased dryness its chances for survival would increase. At 30 C and 30% RH, *X. oryzae* survived more than 6 months in diseased leaves in air. But, in diseased leaves buried in soil, such long-term survival at 30 C occurred only when soil moisture was close to 0%. Diseased rice leaves in soil of high water content decomposed rapidly at high temperatures, and *X. oryzae* in these disintegrating tissues soon lost viability. Thus, in constantly humid and warm tropical climates, its chances of surviving a long break without living host (or nonhost?) tissue would appear to be small. It has been shown that *X. campestris* (5), *X. malvacearum* (11), *X. pelargonii* (20), *X. phaseoli* (10), and *X. translucens* (2) can survive long enough in the soil environment to be alive at the time of planting or sowing the next season's crop, but probably only in debris of diseased plants in the soil.

In the free state in soil, *X. oryzae* survived for a much shorter period of time than when in diseased leaves buried in soil. In soil with negligible water content, free *X. oryzae* cells could be recovered only up to 60 and 30 days at 20 and 30 C, respectively, while those protected in diseased leaves in the same soil survived much longer, being detectable for 360 and 180 days at 20 and 30 C, respectively. However, free *X. oryzae* cells could survive 120 and 80 days at low temperature of 10 C or below in soil at 20 and 40% water content, respectively.

Winter is the main gap between two succeeding rice crops in temperate regions. The temperatures in winter in temperate Asian countries are low, mostly below 10 C, at which *X. oryzae* can survive in diseased leaves for more than 800 days in 0% RH, and for 110 days in 100% RH. This is in accord with earlier findings that *X. oryzae* does survive and over-winter until the next spring in soil in Japan (15, 27, 33). On the other hand, *X. oryzae* in soil could not survive long at conditions suitable for the rice crop; at 30 C *X. oryzae* in the free state survived only 4 and 15 days in soil of 40 and 20% water content, respectively. Its corresponding survival in diseased leaves was less than 10 days in soil at 40% or 80% (flooded) water content. These data indicated that long-term survival in the wet tropics would require either living host tissue, or possibly commensal survival on paddy grasses or weeds. No direct data for these latter sites exist from the tropics.

On the other hand, millions of acres of single-crop rice exist in the tropics where the off-season is very dry. In India, this long off-season ranges from extremes of very

cold (< 0 C) to very hot (> 40 C). Either way, relatively long survival appears to be possible, as long as conditions are arid. Our data should help explain the heretofore perplexing yearly reappearance of the disease in such regions.

However, the soil survival data in the present study were obtained from only one soil type, a hydro-humic latosol soil derived from volcanic rocks in Hawaii. Thus, although these data from a study of a specific soil may indicate probable trends as affected by humidity and temperature, broad generalizations may not be justified. Certainly, rice is grown in a great variety of soils, some of which could be expected to affect survival of *X. oryzae* differently. This is especially likely, since it appears that microflora activity is the direct cause of poor survival.

Paddy water, at temperatures normally occurring in the tropics, appears very unfavorable for survival of *X. oryzae*. Contrary to some earlier suppositions, paddy water with its normal microflora appears to be a dead-end for *X. oryzae* splashed into it, unless transmission to other rice plants occurred within a few days. Thus, reinfection from this source might be difficult (3).

Practical application of these findings to the control or "management" of bacterial blight in the field in the tropics should be possible to minimize disease reappearance in a renewed rice crop. Flooding and ploughing the soil 20 days, or preferably longer, before sowing or transplanting should minimize disease reappearance, because *X. oryzae* is unable to survive long in submerged conditions at high temperatures, either in the free state or in decomposing host tissues. Farmers often plow and prepare paddy fields just before transplanting or sowing, without early flooding which could eliminate the pathogen. It would be especially important and simple to eliminate *X. oryzae* from seed-bed locations by these means, in order to prevent re-establishment throughout fields on transplants. This should bew successful only if seed itself is not re-introducing the pathogen, even if only as an epiphyte, a point still unresolved because of confusion with yellow saprophytic bacteria, which are ubiquitous in seed (14).

Direct sowing of the ooze droplets on plates was found to be a very useful method for testing survival of the bacterium in ooze. A single plate could be used to test as many as 40 ooze droplets. In ooze, *X. oryzae* is not readily killed by desiccation at 0% RH. This may be due to the protective effects of the polysaccharide slime, as claimed for *X. phaseoli* (18). However, the ooze is strongly hydrophilic and under short periods of high humidity it absorbs water, soon becoming colonized by other bacteria or fungi. Under these conditions, *X. oryzae* in ooze loses viability within a few days. Therefore, it seems that ooze could not readily serve as a primary source of inoculum for succeeding crops.

Because *X. oryzae* can survive at or below 10 C in diseased leaves and ooze for more than 2 years at 0% RH, a convenient method to preserve different isolates of *X. oryzae* would appear to be in such leaves or ooze at these temperature and humidity levels. *X. oryzae* bacteria isolated from long term preservation in diseased leaves and ooze droplets were found to maintain their original high degree of virulence.

Sunlight (acting directly, or indirectly through a temperature effect) has a distinct negative effect on the viability of *X. oryzae*. Survival of *X. oryzae* in infested straw scattered in the field, in ooze on the surface of soil, or in diseased stubble standing in the field directly exposed to sunlight and rain water, would be shorter where solar radiation is high, than under shade and in dry sites protected from sunlight and rain water. In the tropics, the conditions under which survival in the field and in seed is sufficient to provide primary inoculum for the subsequent crop still require investigation for each environment and crop-cycle system.

We know of no studies reported or underway in the tropics to obtain direct data on these key aspects of pathogen ecology. The lack of a selective medium, the slow-growing nature of the pathogen, and the common confusion with yellow saprophytes, essentially preclude a successful approach to such investigations. The use of methods employed here, and especially the use of a streptomycin-resistant isolate plated on a streptomycin-containing medium, appears to enable such studies to be undertaken. Caution in interpreting the results is appropriate, however, because of the possible differing survival rates of the wild type and the streptomycin-resistant mutant, a point which would require investigation in each case.

LITERATURE CITED

1. ASUYAMA, H., H. MUKO, and N. SUZUKI. 1962. Laboratory guide for plant pathologists. (2nd ed.) Japan Plant Prot. Assoc., Tokyo. 843 p.
2. BOOSALIS, M. G. 1952. The epidemiology of Xanthomonas translucens (J. J. and R.) Dowson on cereals and grasses. Phytopathology 42:387-395.
3. CHAKRAVARTI, B. P., and M. RANGARAJAN. 1967. A virulent strain of Xanthomonas oryzae isolated from rice seeds in India. Phytopathology 57:688-690.
4. CHATTOPADHYAY, S. B., and N. MUKHERJEE. 1968. Occurrence in nature of collateral hosts (Cyperus rotundus and C. defformis) of Xanthomonas oryzae, incitant of bacterial blight of rice. Curr. Sci. 37:441.
5. CLAYTON, E. E. 1924. A progress report on black-rot investigations, with special reference to cauliflower on Long Island. Phytopathology 14:24 (Abstr.).
6. DALMACIO, S. C., and O. R. EXCONDE. 1967. Host range of Xanthomonas oryzae in the Philippines. Philippine Agric. 51:283-289.
7. EAMCHIT, S., and S. H. OU. 1970. Some studies on the transmission of bacterial blight of rice through seed. Philippine Agric. 54:33-45.
8. GOTO, K., R. FUKAZU, and K. OHATA. 1953. Overwintering of the causal bacteria of rice leaf blight in the rice plant and grasses. Agric. Hortic. 28:207-208.
9. GOTO, M. 1969. Ecology of phage-bacteria interaction of Xanthomonas oryzae (Uyeda et Ishiyama) Dowson. Bull. Fac. Agric., Shizuoka Univ. 19:31-67.
10. GRAHAM, J. H. 1953. Overwintering of three bacterial pathogens of soybean. Phytopathology 43:189-192.
11. HARE, J. F., and C. J. KING. 1940. The winter carry-over of angular leaf spot infection in Arizona cotton fields. Phytopathology 30:679-684.
12. HSIEH, S. P. Y. 1973. Ecological studies of Xanthomonas oryzae, the causal organism of bacterial blight of rice. Ph.D. Thesis, Univ. of Hawaii, Honolulu. 169 p.
13. HSIEH, S. P. Y., and I. W. BUDDENHAGEN. 1974. Suppressing effects of Erwinia herbicola on infection by Xanthomonas oryzae and on symptom development in rice. Phytopathology 64:1182-1185.

14. HSIEH, S. P. Y., I. W. BUDDENHAGEN, and H. E. KAUFFMAN. 1974. An improved method for detecting the presence of Xanthomonas oryzae in rice seed. Phytopathology 64:273-274.
15. INOUE, Y., K. GOTO, and K. OHATA. 1957. Overwintering and mode of infection of leaf blight bacteria of rice plant. Bull. (Div. Plant Breed.) Tokai-Kinki Agric. Exp. Stn. 4:74-82.
16. ISAKA, M., and E. MIYAKOSHI. 1967. Overwintering of Xanthomonas oryzae in wild grasses. Ann. Phytopathol. Soc. Jap. 33:330 (Abstr.).
17. ISHIYAMA, S. 1922. Studies on bacterial leaf blight of rice. Rep. Agric. Exp. Stn., Tokyo. 45:233-261.
18. LEACH, J. G., V. G. LILLY, H. A. WILSON, and M. R. PURVIS, JR. 1957. Bacterial polysaccharides: the nature and function of the exudates produced by Xanthomonas phaseoli. Phytopathology 47:113-120.
19. MIZUKAMI, T. 1961. Studies on the ecological properties of Xanthomonas oryzae (Uyeda et Ishiyama) Dowson, the causal organism of bacterial leaf blight of rice plant. Agric. Bull., Saga Univ. 13:1-85.
20. MUNNECKE, D. E. 1956. Survival of Xanthomonas pelargonii in soil. Phytopathology 46:297-298.
21. NAKAZAWA, M., and K. KATO. 1958. Studies on the surviving place of Xanthomonas oryzae in infected tissue of rice plant in paddy field. Proc. Assoc. Plant Prot. Kansai 1:12-14.
22. RAO, P. S., and H. E. KAUFFMAN. 1971. A new Indian host of Xanthomonas oryzae, incitant of bacterial leaf blight of rice. Curr. Sci. 40:271-272.
23. SINGH, R. N. 1971. Perpetuation of bacterial blight disease of paddy and preservation of its incitant. II. Survival of Xanthomonas oryzae in soil. Indian Phytopathol. 24:140-144.
24. SINGH, R. N. 1971. Perpetuation of bacterial blight disease of paddy and preservation of its incitant. I. Survival of Xanthomonas oryzae in water. Indian Phytopathol. 24:153-154.
25. SRIVASTARA, D. N., and Y. P. RAO. 1964. Seed transmission and epidemiology of the bacterial blight disease of rice in North India. Indian Phytopathol. 17:77-78.
26. TAGAMI, Y., H. FUJII, S. KUHARA, and T. KURITA. 1960. Overwintering of Xanthomonas oryzae in stubble. Ann. Phytopathol. Soc. Jap. 25:68 (Abstr.).
27. TAGAMI, Y., S. KUHARA, T. KURITA, H. FUJII, N. SEKIYA, and T. SATO. 1964. Epidemiological studies on the bacterial leaf blight of rice, Xanthomonas oryzae (Uyeda et Ishiyama) Dowson. 2. Successive change in the population of the pathogen in paddy fields during rice growing period. Bull. Kyushu Agric. Exp. Stn. 10(1):23-50.
28. TAGAMI, Y., S. KUHARA, T. KURITA, H. FUJII, N. SEKIYA, S. YOSHIMURA, T. SATO, and B. WATANABE. 1963. Epidemiological studies on the bacterial leaf blight of rice, Xanthomonas oryzae (Uyeda et Ishiyama) Dowson. 1. The overwintering of the pathogen. Bull. Kyushu Agric. Exp. Stn. 9(1):89-122.
29. WAKIMOTO, S. 1955. Overwintering of Xanthomonas oryzae on unhulled grains of rice. Agric. Hortic. 30:1501.
30. WAKIMOTO, S. 1955. Studies on the multiplication of OP1 phage (Xanthomonas oryzae bacteriophage). Sci. Bull. Fac. Agric., Kyushu Univ. 15:151-160.
31. WAKIMOTO, S. 1956. Overwintering of the rice leaf blight bacteria in the soil. Agric. Hortic. 31:1413-1415.
32. WAKIMOTO, S., and K. TAMARI. 1956. Studies on the overwintering of Xanthomonas oryzae in dried state (prelim. rep.). Proc. Assoc. Plant Prot., Kyushu 2:107-109.
33. YOSHIMURA, S. 1963. Diagnostic and ecological studies of rice bacterial leaf blight, caused by Xanthomonas oryzae (Uyeda et Ishiyama) Dowson. Bull. Hokuriku Agric. Exp. Stn. 5:27-182.