SURVIVAL OF

XANTHOMONAS CAMPESTRIS f.sp. PANICI AND
XANTHOMONAS CAMPESTRIS f.sp. PASPALI
To design effective control measures, it is necessary to know where the plant pathogenic bacteria survive in nature during cold or dry seasons. Such reservoirs or hiding places of plant pathogenic bacteria are found in soils, seeds, infected plant debris, living plant tissues, alternate hosts, weeds or in some cases even in insects. In case of xanthomonads, the generalisation that can be drawn from various studies is that they do not multiply or survive in soil in the free state. It was shown experimentally that the following bacteria declined rapidly and reached extinction within days or weeks after their inoculation into soil: *Xanthomonas pelargonii* (Munnecke, 1956), *X. phaseoli* (Graham, 1953; Sabet and Ishaq, 1969; Schuster, 1967, 1970; Schuster et al. 1973), *X. translucens* (Boosalis, 1952; Wallin, 1946), *X. malvacearum* (Brinkerhoff and Fink, 1964; Russell, 1955), *X. citri* (Goto, 1970), *X. campestris* (Schaad and White, 1974), *X. vesicatoria* (Peterson, 1963) and *X. oryzae* (Mizukami and Wakimoto, 1969). Of the xanthomonads, only *X. alfalfa* has been reported to overwinter in soil apparently free of debris (Claflin and Stuteville, 1973). However, part of these tests
was conducted with soil steamed before inoculation and thus do not present natural conditions.

Most xanthomonads are seed-borne and survive in or on seeds for varying periods of time. With *X. malvacearum*, the bacterium has been reported as a surface contaminant (Wickens, 1953) and as residing internally in the seed (Brinkerhoff and Hunter, 1963). *X. oryzae* overwinters on unhulled rice grains (Wakimoto, 1955). The variation in the reported survival times of xanthomonads in seeds is in part presumably a function of the sensitivity of the isolation method, the strains of the bacterium examined, and the storage conditions for the seeds. For example, *X. phaseoli* has been reported to survive for three (Basu and Wallen, 1966) and 15 years (Schuster and Coyne, 1974), whereas *X. phaseoli* var. *sojensis* was stated to survive for 6-30 months (Graham, 1951).

When a xanthomonad is detected in soil, it probably represents the vestige of a declining population in association with infected plant debris from diseased plants. The capacity of xanthomonads to survive in plant debris may enable the bacterium to overwinter under certain conditions. Since the
population become undetectable soon after infested debris is decomposed, survival is dependant upon the rate of decay of infested plants and will vary markedly depending upon environmental conditions. However, when infested plant debris was sorted dry, xanthomonads survived from 18 months to 8 years (Claflin and Stuteville, 1973; Sabet and Ishag, 1969; Schnathrost, 1964). With ordinary cultivation methods and with normal rotations, it is unlikely that the bacterium will survive long enough to infect new plants in most localities. The importance of the soil environment in influencing survival is shown by comparing the work by Sabet and Ishag (1969) who found that *X. phaseoli* did not survive long in bean debris because of rapid decomposition, with that of Schuster (1967, 1970) who found that it overwintered in infested host and non-host debris, especially when the debris was on the soil surface and decomposition was minimal. Other examples of xanthomonads surviving in infested host debris are: *X. phaseoli* var. *sojensis*, overwintering in bean straw on soil surface (Graham, 1953); *X. translucens*, overwintering on wheat straw (Boosalis, 1952); *X. pelargonii* surviving on geranium debris for 6 months (Munnecke, 1956); *X. vesicatoria*, overwintering in tomato debris (Peterson, 1963); *X. malvacearum*. 
overwintering in cotton debris (Brinkerhoff and Fink, 1964) and X. alfalfa overwintering in alfalfa debris (Claflin and Stuteville, 1973). X. oryzae also survived in rice straw but principally when stored under dry condition (Inone et al., 1957; Tagami et al., 1963; Wakimoto, 1964). When the straw was incorporated into the soil, the bacterium did not survive for more than several months. However, the pathogen overwintered in stubble in warm areas of Japan when the fields were left fallow (Tagami et al., 1963). Singh (1971 a,b) found that it did not survive in field or pond water or overwinter in composts.

Some xanthomonads may survive on the roots of susceptible hosts. X. translucens was shown to overwinter on winter wheat, winter rye, quack grass and broom grass (Boosalis, 1952). This inoculum was capable of causing new infections in the spring when susceptible crops were planted in the overwintering areas. X. oryzae was detected as a saprophyte on roots and in rhizospheres of three species of wild grasses by using phage technique (Goto et al., 1953; Inone et al., 1957; Tagami et al., 1963,1964; Yoshimura, 1963; Yoshimura et al., 1956). These 3 species, Leersia oryzoides and the variety japonica,
and *Zizania latifolia* are common in areas where disease occurs. *Leersia* spp. in general appear to be as overwintering sources of inoculum. Tagami et al. (1963) showed that the population of *X. oryzae* on these weed hosts declines in winter and increases in spring coincident with development of above-ground plant parts. The possibility that bacteria may colonize roots or establish a resident population in the rhizosphere or rhizoplane has interesting implications for seed-borne diseases. The transfer of the pathogen from the seed to roots could enable the bacterium to persist until the environment is conducive for invasion and infection of above-ground plant parts.

Little research has been done on the extent or importance of survival of bacterial plant pathogens on rhizoplanes or rhizospheres of non-host plants. There are, however, several pathogenic bacteria which overwinter and survive as residents in such locations, although the importance of this as a source of inoculum is not clear. *X. citri* was detected on the rhizoplanes of various weeds collected near infected citrus trees prior to new infections in the spring (Goto, 1970). The infestations were postulated to have originated from rain-splashed inoculum from old lesions on citrus
leaves. Goto and Ohta (1971) also demonstrated the presence of low populations of *X. citri* almost year round on rhizones and roots of *Zoysia japonica*. However, the majority of strains from citrus and *Z. japonica* differed physiologically and in phage sensitivity. The evidence, therefore, is still lacking that the low populations of *X. citri* in the rhizospheres or rhizoplane of non-host persist indefinitely or provide primary inocula for citrus infections.

Other xanthomonads have been found in association with non-host plants. *X. oryzae* was found on roots of 11 wild grasses and 16 other weeds out of 44 weeds tested (Isaka, 1969). However, some of the grasses may have been wild hosts of the pathogen. *X. malvacearum* was isolated in summer from roots of 14 of 161 types of weeds growing near infected cotton plants, but during winter some weeds were free of *X. malvacearum*, thus negating evidence that weeds served as sources of inoculum (Smith, 1962).

In the present investigation the survival of *X. campestris* f. sp. *panici* and *X. campestris* f. sp. *paspali* in soil and in host tissue has been studied.
Materials and Methods

Fresh pathogenic isolates of *X. campestris* f.sp. *panici* and *X. campestris* f.sp. *paspali* were obtained and used in these studies.

Survival of the organisms added to the soil

A representative sample of soil was collected from Waghai. Fifty grams of soil was filled in two Erlenmeyer flasks of 250 ml capacity. One flask containing soil was sterilized under 15 psi for 30 minutes at 121°C on three consecutive days. The other flask containing soil was not sterilized. The pathogens were grown on sucrose-peptone-agar slants for 48 hours. Seven ml of the bacterial suspension, containing approximately $10^9$ organisms ml$^{-1}$ was added to soil in both the flasks. The flasks were kept at room temperature, 28° - 30°C.

Every week, one g of soil from each of the flasks was drawn aseptically and mixed with sterile distilled water. The survival of the pathogens was examined by isolating them on sucrose-peptone-agar plates or by spraying the suspension on the respective
host plants and then trying to isolate the pathogens from the infected portions.

Survival of the organisms in host tissues

In one set of experiment, small pieces of dried infected leaves, from which pathogenic cultures were isolated, were mixed with sterilized and unsterilized soil separately in earthen pots. The pots were kept in open under natural weather conditions.

In another set of experiment, small pieces of dried infected leaves were mixed with soil and filled in perforated plastic containers (height 5" and diameter 4"). The containers were then placed at the depths of 6" in the field. From both these sets host tissues samples were drawn at random at weekly intervals. The survival of the pathogens was examined by crushing the host tissues in sterile distilled water and then streaking the suspension on sucrose-peptone-agar plates or by spraying the suspension on respective host plants and then trying to isolate the pathogens from the infected portions.
Air dried diseased leaves of *Panicum miliaceum* L. and *Paspalum scrobiculatum* L. were stored at room temperature, 28° - 30°C. The specimens were observed at monthly intervals for the typical bacterial ooze. Isolation of the pathogens was carried out on sucrose-peptone-agar plates.

The stubbles and straw of *Panicum miliaceum* L. and *Paspalum scrobiculatum* L. left over for 6-7 months in the field at Waghai after harvest were obtained and examined for the presence and survival of the pathogens in the infected tissues as mentioned above.
**iii Results**

A

Isolates of the pathogens from the sample drawn from the flask containing unsterile soil could be successfully obtained only during the first 2-3 weeks, whereas from the sample drawn from the flask containing sterile soil, the pathogens could be isolated for 7-8 months.

B

In the pot experiments both the pathogens when mixed with sterilized soil survived in the host tissue for about 6 months. But when mixed with unsterilized soil in the pots, they survived for only few days.

When the infected specimens mixed with soil were placed in the plastic containers at the depths of 6" in the field, the pathogens could be successfully isolated for 6-8 weeks from the host tissue specimens drawn from the containers.

C

The typical bacterial ooze could be observed and the pathogens isolated for more than a year from
the infected leaves of the host plants stored at room temperature, 28° - 30°C.

The pathogens could be isolated from the stubbles and straw of *Panicum miliaceum* L. and *Paspalum scrobiculatum* L. left over in the field after harvest for 6-7 months.
iv Discussion

A

The results of the studies on the survival of *X. campestris* f.sp. *panic* and *X. campestris* f.sp. *paspali* have shown that the pathogens could perpetuate for about 7-8 months when inoculated in sterile soil. But when unsterile soil was used they survived only for 2-3 weeks. This is probably due to the antagonistic effect of soil microflora on the pathogens in the latter case.

B

The survival of both the pathogens in the infested plant debris mixed with unsterile soil, for short duration is due to the decomposition of the infested plant debris by soil microflora. Survival of the pathogens is dependant upon the rate of decay of infested material and varies markedly depending upon environmental conditions. Similarly faster loss of the pathogens under buried conditions was closely correlated with microbial decomposition of the plant material.
The survival of both the pathogens in the infected material stored at room temperature, 28°-30°C for more than a year and in the stubbles and straw left over in the field for 6-7 months is probably due to the protection offered by the intact host tissues to the pathogens. The stubbles and straw left over in the field were open to the hazards of environmental conditions.

The study revealed that *X. campestris f.sp.paspali* and *X. campestris f.sp. panicl* survived and overwintered in the respective plant debris and stubble and straw on the soil surface under field condition.

It is, therefore, to be concluded that the undecomposed infected plant material, stubbles and straw on the soil surface, may carry over the pathogens from one season to another and become the primary source of inoculum.