Seed Transmission Studies of Xanthomonas oryzae in Rice

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ABSTRACT

Detailed studies on invasion of rice seed by *Xanthomonas oryzae* indicate that glumes of seeds readily become infected. Characteristic symptoms were observed on infected panicle branches, but not on infected seeds. Infected seed stored under natural conditions (temperatures ranging from 25 to 35 C) harbored viable bacteria for 2 months, after which no bacteria could be detected by the three methods tried. Seed transmission was not observed in freshly-harvested infected seeds grown under various conditions. In germinated seeds, bacterial populations declined and bacteriophage populations increased rapidly. Bacteriophages may play a role in reducing the bacterial population in germinating seed.

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Genetic susceptibility, high levels of nitrogen fertilizer, and intensive cropping of rice throughout the year in Asia have contributed greatly to the severity of bacterial blight disease, caused by *Xanthomonas oryzae* (Uyeda et Ishiyama) Dowson, on rice.

The possibility that bacterial blight is seed-transmitted causes concern because large quantities of seed of breeding material are distributed to many locations within Asia and to other continents for testing. Seed of IR8, the first high-yielding variety developed at the International Rice Research Institute in the Philippines, has been sent to more than 50 countries around the world.

Mizukami (8) noted the presence of the bacterium in husks of rice seed. Fang (3) and Srivastava (9) reported that the bacterium was also present in the endosperm of the grain. The reported percentage of seeds carrying the

pathogen varies from less than 1 percent to 100 percent (9).

The bacterium has been reported to survive for short periods of time in seeds (3, 9, 11). The length of time that it survives in the seed is critical in determining the potential importance of the seed as a primary source of inoculum. Although contaminated seeds have been reported to be an important primary source of inoculum in a few single crop areas of the tropics, seed-transmission has not been critically studied. In Japan, where the winters are much colder, the bacterium remained viable until the next growing season, but no seed transmission was reported (4, 10). Likewise, negative results from transmission studies were reported in Japan (10), the Philippines (2), and India (1).

We undertook experiments to answer the following questions. Do seeds carry the bacterium? If they do, how frequently? How long do they survive? If seed transmission occurs, what is the frequency and potential importance of seed as a source of primary inoculum? If seed transmission does not occur, why not?

MATERIALS AND METHODS.—Seeds used in this study were from naturally or artificially infected Taichung Native 1 (TN1) panicles. We artificially inoculated panicles by injecting 0.5 ml of bacterial suspension at the uppermost internode with a syringe several days after panicle emergence. All seeds were stored at room temperature of 25 to 35 C. Cultures of *Xanthomonas oryzae* were grown on peptone-sucrose agar medium for routine work (5) and on semi-synthetic potato-sucrose agar medium (11) for storage. The pathogenicity of bacteria isolated from seeds was assayed on five-week-old TN1 seedlings inoculated by the pin-prick inoculation method (7).

We tested several methods for isolating *X. oryzae* from the seed: (i) direct plating of the seeds, (ii) crushing of seeds in sterile water and streaking on agar plates, (iii) using micropipettes to remove bacterial ooze which streamed from the rachilla after the seed was detached. The ooze was placed in 1 ml sterile water, and streaked on agar plates.

We also used the concentrated suspension method (8), and the bacteriophage technique (11), to detect *X. oryzae* in the seeds. The concentrated-suspension technique consisted of bulking together either the ooze suspension or the homogenates from crushed whole seeds or from their individual parts. These were centrifuged to concentrate the bacterium and the sediment was inoculated to TN1 seedlings. The percentage of diseased leaves was used as an index of the relative numbers of bacteria in the concentrated suspension (Fig. 1).

For the phage technique, we assayed seed lots of 50 infected seeds during germination for residual phage, at the time of initial soaking and periodically for 10 days. In the absence of residual phages, we added a known amount of bacteriophages with a wide lysotype range to crushed seed or glume samples to determine the multiplication of the phage. The presence of *X. oryzae* was indicated after 12 hours of incubation if the phage population was greater in the test suspension than in the check.

RESULTS.—*Symptoms of infected panicles.*—The bases of diseased panicles sometimes became a straw yellow color before the grains became mature. In fields of severely diseased TN1 plants, we observed these symptoms on 5-10% of the panicles. In some cases the symptoms were only on one side of the panicle axis and the corresponding primary and secondary branches. When panicle parts were cut and placed in sterile water, bacterial ooze was readily observed and *X. oryzae* was easily isolated. In general, plants with low blight incidence at flowering had very few diseased spikelets, while those with high blight incidence had many infected spikelets.

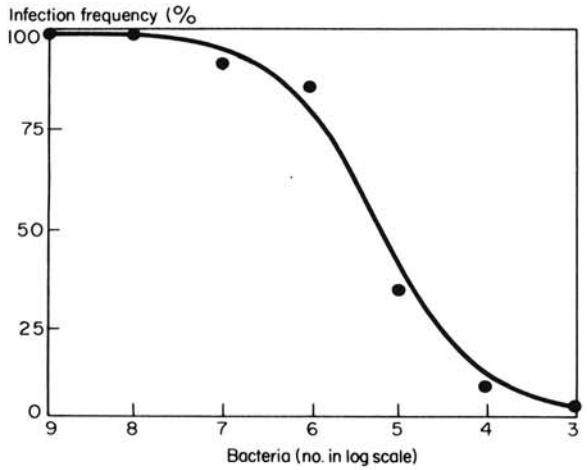

Fig. 1. Infection frequency of *Xanthomonas oryzae* on rice cultivar TN1 when inoculated with various concentrations of bacterial suspensions.

Detection of X. oryzae in seeds.—We could not isolate *X. oryzae* by directly plating whole or parts of surface-sterilized seeds on several media. Only fast-growing saprophytic bacteria were observed. By using the micropipette method, however, we isolated *X. oryzae* from as much as 90% of the husks of seeds which showed streaming immediately after harvest. We isolated *Xanthomonas oryzae* from 70% of the seeds 1 month after harvest, and from 40% at the end of 2 months. No *X. oryzae* could be isolated at the end of 3 months (Table 1).

Several yellow saprophytic bacteria routinely appeared within 24-48 hours. One species, similar to *X. oryzae*, appeared within 60-72 hours. This slow-growing species was distinguishable from *X. oryzae* only by its slightly greenish-yellow color. For definite diagnosis, however, pathogenicity tests were required.

TABLE 1. Detection of *Xanthomonas oryzae* in husks of rice seeds at various time intervals after harvest.

Detection method	Presence of *X. oryzae*[1]				
	Months after harvest				
	0	1	2	3	4
Isolation	++++	+++	++	0	0
Concentrated suspension	+++	++	+	0	0
Phage	++++	+++	++	0	0

[1] 0 = none; + = trace; ++ = low; +++ = medium; and ++++ = high.

The presence of the bacterium could be detected by the concentrated-suspension method and the phage technique up to 2 months after harvest. By the third month, however, no viable bacteria were detected (Fig. 2).

Transmission studies of infected seeds.—Since our results definitely established the presence of *X. oryzae* in glumes of seeds, we conducted experiments to see if the bacterium was transmitted to growing seedlings, and if it could cause disease.

Infected seeds were placed in glass beakers and allowed to grow for 3 weeks. The roots of some plants were periodically cut to allow bacteria present in the water to enter the plants. At no time did typical wilting symptoms occur in these plants. Phages, however, were detected in the water and on the leaves of the seedlings.

To create optimum conditions for seed transmission during the first 10 days after germination, large numbers of infected seeds and infected panicle branches were placed on sterile soil and high levels of nitrogen fertilizer were added. Pathogen-free seeds soaked in a heavy bacterial suspension and healthy seeds were germinated and grown under the same conditions. No seedlings were found infected with *X. oryzae* during the 6 weeks they were observed.

Freshly harvested, infected and healthy seeds were also sown in small plots of soil in which rice had never been grown before during the monsoon seasons of 1969, 1970, 1971. Twenty-one days later, the seedlings were transplanted into the same area where the nursery was located. Heavy doses of nitrogen fertilizer were applied to the plots to stimulate good growth. The plants were allowed to grow to maturity. No seedlings became diseased, and no symptoms appeared on the plants at any stage of growth during the three seasons.

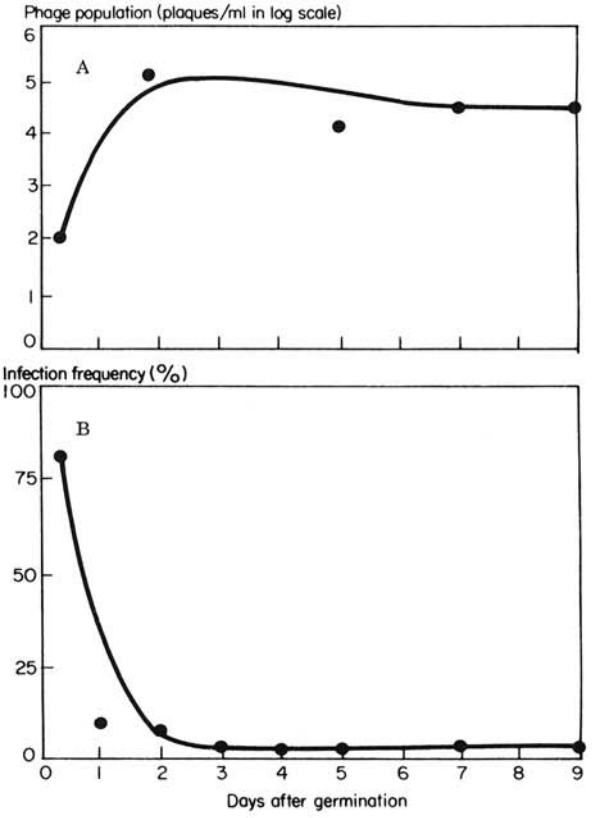

Fig. 2-(A, B). Population of **A)** *Xanthomonas oryzae* phages and **B)** *X. oryzae* in glume homogenates from infected seeds at various numbers of days after germination.

Population levels of X. oryzae and its bacteriophage in germinating seeds.—Since the bacterium was readily isolated from the husks of recently harvested seeds and yet the disease was not transmitted from these seeds, the question was asked why the bacterium could not migrate from the husks to susceptible growing tissue in the coleoptile or radicle. Was the bacterium being inactivated, or was the physical isolation of the bacterium in the glume's vascular system a barrier to its movement? What roles were bacteriophages playing in determining bacterial populations in the husks of germinating seeds?

At the time of soaking in 10 ml of water, 50 husks yielded enough bacteria to induce infection on 80% of the leaves inoculated by the concentrated-suspension method. After one day of soaking, the population declined considerably so that the resulting suspension induced infection on only 10% of TN1 leaves. By the third day, the glume homogenates caused no infection when inoculated to leaves (Fig. 2).

While the population of *X. oryzae* in the glumes rapidly declined, the population of the residual bacteriophage increased rapidly the first 2 days after seed soaking, from 2×10^3 plaques per milliliter (pl ml) at 0 time to 1×10^7 pl/ml at 2 days. From the third day to the seventh day, the phage population gradually declined to 1×10^5 pl/ml and remained at that level until the tenth day. No bacteria were detected on the brown rice, or on the coleoptile or radicle of the growing seedling. Although phages were detected on these organs, they most likely were a result of contamination.

The phage appeared both on the surface of and inside the glumes. During the monsoon season when a random sample of seeds from 25 breeding selections from the AICRIP farm were soaked in water for 30 minutes and the water was assayed for phage populations, 96% of the samples carried residual phages on their surfaces. When the same samples were assayed for phages 10 hours after soaking, 40% showed extensive increases in phage populations, indicating the presence of *X. oryzae* on or in these seeds.

DISCUSSION.—*Xanthomonas oryzae* was present in the glumes of seeds as reported by Wakimoto (11), Fang (3), and Srivastava (9), but infected seeds occurred at different frequencies. In fields where the disease was mild or disease development was delayed, a low percentage of seeds carried the bacterium. Bacterial streaming from the glumes of freshly harvested seeds appeared highly correlated with the presence of *X. oryzae*. Several months after harvest, however, streaming was not necessarily indicative of the presence of viable *X. oryzae*.

These results indicate that *X. oryzae* is not effectively isolated by direct plating of the seed because of high populations of yellow saprophytic bacteria. In a visual identification of colonies these yellow colonies can be confused with *X. oryzae*.

The bacterium in the seeds stored under natural conditions survived for only 2 months whether tested by

the direct-isolation, concentrated-suspension, or phage methods. The bacterium may have survived longer but at such a low level that they could not be detected by the techniques used in this study. Since the period of survival is dependent on temperature and humidity (6), the bacteria may survive longer under certain conditions.

In these studies, the rapid phage increase after seed soaking, and the simultaneous decline of the bacterial population, may be the reasons why even freshly harvested infected seed did not readily transmit the disease. The soaking may have broken down the physical isolation of the bacterium and allowed the bacterium and the phage to interact.

The negative results of these seed-transmission studies indicate that seeds may not be an important primary source of inoculum. But this does not preclude the possibility that seed transmission might occur when billions of rice seeds are sown by farmers.

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