Growth Dynamics of *Xanthomonas campestris* pv. *oryzae* in Leaves of Rice Differential Cultivars

P. A. Barton-Willis, P. D. Roberts, A. Guo, and J. E. Leach

Department of Plant Pathology, Throckmorton Hall, Kansas State University, Manhattan 66506.
Present address of first author: 107 Washington Crossing Road, Pennington, NJ 08534; present address of second author: Department of Plant Pathology, University of Hawaii, Honolulu 96822.
Research was supported by U.S. Department of Agriculture Competitive Grant 85-CRCR-1-1765 and the Kansas Agricultural Experiment Station, Kansas State University, Manhattan 66506.
Contribution 89-33-J from the Kansas Agricultural Experiment Station, Kansas State University, Manhattan.
We thank S. Kelemu for selection of antibiotic resistant strains and M. Rhoads for technical assistance.
Accepted for publication 28 November 1988 (submitted for electronic processing).

ABSTRACT

Barton-Willis, P. A., Roberts, P. D., Guo, A., and Leach, J. E. 1989. Growth dynamics of *Xanthomonas campestris* pv. *oryzae* in leaves of rice differential cultivars. Phytopathology 79:573-578.

Bacterial multiplication and spread were monitored in race-specific interactions of *Xanthomonas campestris* pv. *oryzae* and rice (*Oryza sativa*). Bacterial numbers in compatible and incompatible interactions increased equally until levels reached 10^7–10^8 colony-forming units/leaf. Thereafter, the bacterial growth rates in incompatible interactions slowed in comparison with those in compatible interactions. Maximum bacterial numbers per leaf were dependent on host cultivar. In both compatible and incompatible interactions, bacteria spread from the inoculation point; however, bacteria in compatible interactions spread more rapidly. Symptoms were not observed in advance of bacteria. Lesion lengths, in general, were positively correlated with bacterial numbers. When leaves were inoculated with bacterial mixtures (1:1 of race 1 or 6 [compatible] to race 2 [incompatible]), the growth rate of race 2 isolates was severely restricted compared with that of the control (race 2 alone). The same effects were observed in a host in which all races were compatible. The apparent interaction between bacterial isolates confounded analysis of the effect of mixed inoculations on race-specific resistance induction.

Pathogenic specialization of *Xanthomonas campestris* pv. *oryzae*, the causal agent of bacterial blight, has been demonstrated on rice cultivars that have specific genes for resistance to the disease. In the interaction between rice and *X. c. oryzae*, the classic hypersensitive response (8) is absent (18), and relative lesion length is the main criterion for host reaction type or resistance (17). Lesion length data are most useful for critical analysis of compatibility versus incompatibility when they are used in conjunction with bacterial multiplication data.

When multiplication of an isolate of one race of a pathogenic bacterium is compared in resistant and susceptible dicotyledonous hosts, incompatibility is generally reflected in lower pathogen numbers (1,6,23). Few studies of bacterial multiplication dynamics in race-specific interactions have been done with monocotyledonous plants, and the available published information for the interaction between rice and *X. c. oryzae* is contradictory. In one study, Mohiuddin and Kauffman (12) monitored multiplication of *X. c. oryzae* in 5-mm disks of rice leaves taken from the inoculation site and from 1 and 3 cm below the site. At the inoculation site, no difference in bacterial numbers was detected. At 1 and 3 cm below the inoculation site, however, the number of bacteria (colony-forming units [cfu]/5-mm disk) in incompatible interactions 12–14 days after inoculation was only 1% of that in the compatible interactions. These workers concluded that incompatible interactions were characterized by lower total bacterial numbers per leaf and reduced pathogen spread. In contrast, Parry and Callow (18) found little difference in multiplication or spread of the pathogen in 10-cm leaf sections during either compatible or incompatible interactions, although lesion lengths were substantially different. They concluded that expression of resistance in rice was not characterized by lower bacterial numbers but was the result of reduced symptom expression by the host.

The effects of mixed inoculations (using isolates of two different races of *X. c. oryzae*, one compatible and one incompatible) on both bacterial multiplication and the disease reaction also are not clear. The expected phenotype resulting from such inoculations is incompatible, if resistance is assumed to be physiologically predominant over susceptibility (3). For example, when soybean leaves were inoculated with a 1:1 mixture of isolates representing compatible and incompatible races of *Pseudomonas syringae* pv. *glycinea*, multiplication of bacteria from the compatible race was restricted, presumably because the expression of hypersensitivity inhibited the growth of both isolates (9). In rice leaves inoculated with isolates of compatible and incompatible races of *X. c. oryzae* (1:1 ratio), lesion lengths were equal to those of the singly inoculated compatible control (18,21) or, in one cultivar, intermediate in length to those of the compatible and incompatible controls (18). The numbers of bacteria either were not measured (18) or, when determined from 5-mm leaf disks taken immediately around the inoculation site, were not different from single-race control inoculations (21). Thus, in these race-specific interactions, physiological predominance of resistance was not apparent. However, when low virulence (weakly aggressive to the cultivars used) and high virulence (highly aggressive) isolates of *X. c. oryzae* were introduced into rice at ratios of 20 low:1 high or greater, lesion development in the host and growth of the high-virulence isolate were substantially reduced (2,21). Although the isolates were not grouped into races, their interactions with different rice cultivars suggested race specificity (2,21). Overall, the existing information is not sufficient to explain the effects of mixed inoculations on bacterial populations or host reaction in the interaction between rice and *X. c. oryzae*.

To evaluate race-specific interactions between *X. c. oryzae* and rice more critically, the effects of the host's response on bacterial multiplication in compatible and incompatible interactions and mixed inoculations were studied. Bacterial multiplication in whole-leaf samples, spread of bacteria from the inoculation point throughout the leaf, and the effect of different starting populations on the onset of resistance are described. The effects of mixed inocula (1:1 compatible to incompatible) on the multiplication of *X. c. oryzae* and lesion development in different rice cultivars also are presented.

MATERIALS AND METHODS

Bacterial isolates. Isolates PXO61 (race 1), PXO86 (race 2), and PXO99 (race 6) of Philippine *X. c. oryzae* were obtained from

T. W. Mew at the International Rice Research Institute (IRRI, Los Baños, Philippines). Bacterial cultures were grown on peptone sucrose agar (25) for immediate use. For long-term storage, bacteria were suspended in 15% glycerol (10) and frozen at −80 C or suspended in sterile skim milk (Difco Laboratories, Detroit, MI) and lyophilized. Virulence of *X. c. oryzae* to rice is lost upon repeated transfer of cultures (22). Therefore, cultures for experiments were grown fresh (one to two transfers) from glycerol or lyophilized stocks.

To select streptomycin (Sm)-resistant PXO61 or PXO99 strains ($PXO61^{Sm}$ and $PXO99^{Sm}$), 1-ml aliquots of 17-hr-old bacterial cultures (about 10^{10} cfu/ml) were inoculated into 15 ml of peptone sucrose broth containing 50 μg of streptomycin per milliliter. After incubation overnight as described above, streptomycin-resistant colonies were selected on peptone sucrose agar containing 100 μg of streptomycin per milliliter. A rifampicin (Rif)-resistant PXO86 strain ($PXO86^{Rif}$) was obtained by a gradient plate technique (24). Growth of antibiotic-resistant strains was compared to that of wild-type parents in culture (peptone sucrose broth and nutrient broth) and in plants (as described below).

Rice cultivars. Seed of rice (*Oryza sativa* L.) cultivars IR8 (*Xa-11*, gene for resistance to *X. c. oryzae*) (11), IR20 (*Xa-4*) (19), Cas 209 (*Xa-10*) (27), and IR1545-339 (*xa-5*) (16) were supplied by T. Mew (IRRI). The interactions of these cultivars with isolates of races 1, 2, and 6 of *X. c. oryzae* are shown in Table 1. Two rice seeds were planted per 8.9-cm-sq pot in Bacto potting soil (Michigan Peat Co., Houston, TX) supplemented with complete fertilizer (Peters 20-20-20, W. R. Grace, Cambridge, MA). Plants were grown in a greenhouse (28–32 C days and 22–26 C nights). After 2 wk, pots of seedlings were maintained in flats containing 5 cm of water and fertilizer.

Preparation of inocula. Suspensions for inoculations were prepared by growing bacteria in nutrient broth (Difco Laboratories) overnight on a rotary shaker (200 rpm) at 28 C. Cells were collected by centrifugation at 17,000 *g* for 10 min at 22 C. The pellet was washed twice and resuspended in distilled water. The inoculum was adjusted to desired concentrations using a Klett-Summerson meter with a red #66 filter (Klett Manufacturing Co., New York, NY).

To assess bacterial growth in rice leaves, plants were inoculated with bacterial suspensions containing 5×10^9 cfu/ml. For mixed inocula, suspensions of $PXO61^{Sm}$ and $PXO86^{Rif}$ or $PXO99^{Sm}$ and $PXO86^{Rif}$ containing 1×10^{10} cfu/ml were combined in equal amounts (final concentration: 5×10^9 cfu/ml of each isolate). A second inoculum was obtained by combining the 1:1 bacterial mixture with an equal portion of water to yield a mixture containing a total population (combined isolates) of 5×10^9 cfu/ml of bacteria.

Inoculation and sampling of rice plants. A double sewing machine needle (Schmetz, 2.0/80, Herzogenrath, West Germany) dipped into inoculum (prepared as described above) was used to stab fully expanded rice leaves (30–40 days past sowing, four- to five-leaf stage) once between the margin and midrib, perpendicular to the veins, 10 cm from the leaf tip. The double-needle inoculation technique is adapted from that of Muko and Yoshida (13). Only the second-youngest leaf per plant was inoculated. After inoculation, plants were placed in flats of water and incubated in a growth chamber (32 C days and 22 C nights, 12-hr photoperiod, 70% RH). Lesion lengths were measured on each sampling day.

TABLE 1. Interactions of isolates of Philippine races of *Xanthomonas campestris* pv. *oryzae* with rice cultivars IR8, IR20, Cas 209, and IR1545-339 (after Mew, 11)

Differential cultivar (R gene)	Isolate (race)[a]		
	$PXO61^{Sm}$ (1)	$PXO86^{Rif}$ (2)	$PXO99^{Sm}$ (6)
IR8 (*Xa-11*)	C	C	C
IR20 (*Xa-4*)	I	C	C
Cas 209 (*Xa-10*)	C	I	C
IR1545-339 (*xa-5*)	I	I	C

[a] C = compatible; I = incompatible.

For experiments in which varied bacterial concentrations were used in inocula, a Hagborg device (4) was used to infiltrate a circular area 1.5 cm in diameter in the center of the leaf blade, 10 cm from the leaf tip of 8-wk-old plants.

To assess the reproducibility of the double-needle inoculation technique, 18 plants each of Cas 209 and IR20 were inoculated with $PXO61^{Sm}$ (1×10^{10} cfu/ml). Leaves were ground immediately after inoculation, and diluted samples were plated as described below to determine the number of bacteria deposited per leaf. Reproducibility of the Hagborg inoculation technique was assessed by inoculating leaves with each of two $PXO61^{Sm}$ concentrations (1×10^{10} cfu/ml and 1×10^8 cfu/ml). Leaves were immediately sampled, and bacterial populations were determined as described below.

Estimation of bacterial growth. Unless otherwise noted, four whole, inoculated rice leaves per treatment were randomly sampled immediately after inoculation and then at days 1–6, 8, 10, and 12. Each leaf was ground individually in 2–10 ml of water with a mortar and pestle under aseptic conditions. Tenfold serial dilutions of the leaf suspensions were made by a microplating technique (6), and dilutions were plated onto casein-peptone glucose agar (7) containing cycloheximide (75 μg/ml) and either streptomycin (100 μg/ml) or rifampicin (20 μg/ml). Plates were incubated at 28 C, and colonies were counted within 3–5 days.

Single and mixed inoculations. Rice leaves were double-needle inoculated with individual bacterial suspensions or suspensions containing bacterial mixtures prepared as described above. Single and mixed inoculation experiments comparing incompatible and compatible interactions with Cas 209, IR1545-339, and IR20 were repeated five, two, and three times, respectively. The experiment comparing single and mixed inoculations of IR8 with $PXO86^{Rif}$ and $PXO61^{Sm}$ was repeated twice.

Assessment of spread of bacteria in inoculated leaves. Suspensions of $PXO61^{Sm}$ and $PXO86^{Rif}$ (5×10^9 cfu/ml) were used to inoculate leaves of rice cultivar Cas 209 with the double needle, as described above. Leaves were sampled immediately after inoculation and every 2 days through day 12. The top 1 cm of leaf tissue was discarded, and the adjacent portion of the leaf (12 cm) was cut into six 2-cm sections, such that the inoculation point was in the center of the fifth section. Four replications of each section were ground individually; dilutions and plating were as described. Lesions were measured on sampling days. The experiment was repeated twice as described and twice by grinding and plating every other section.

Effect of inoculum density on expression of resistance. A Hagborg device was used to infiltrate leaves of Cas 209 in separate inoculations with $PXO61^{Sm}$ and $PXO86^{Rif}$ at high (1×10^{10} cfu/ml) and low (1×10^8 cfu/ml) inoculum densities, as described above. Plants were infiltrated just before the beginning of the daily photoperiod. Four whole leaves per treatment were individually sampled, and bacterial numbers were estimated as described. The experiment was repeated four times.

RESULTS

Antibiotic resistant strains. Streptomycin- and rifampicin-resistant strains of *X. c. oryzae* were selected to expedite detection and allow differentiation of isolates in mixed inoculations. Antibiotic-resistant strains had growth rates similar to those of the wild types in cultivars Cas 209 (Fig. 1), IR1545-339, and IR8 (data not shown) and produced similar lesion lengths on rice leaves (data not shown). In addition, growth rates were identical in culture (data not shown).

Sampling procedures. Grinding leaves in phosphate buffer instead of water did not increase the number of bacteria recovered (data not shown). Recovery was 2.5 (± 0.4, standard error) $\times 10^4$ cfu/leaf from leaves after double-needle inoculations with 5×10^9 cfu/ml bacterial suspensions. After Hagborg infiltration of Cas 209, bacteria were recovered at 1.6 (± 0.1) $\times 10^7$ cfu/leaf when a 1×10^{10} cfu/ml suspension was used and 2.7 (± 0.6) $\times 10^4$ when a 1×10^8 cfu/ml suspension was used.

Bacterial multiplication in rice leaves. Although starting

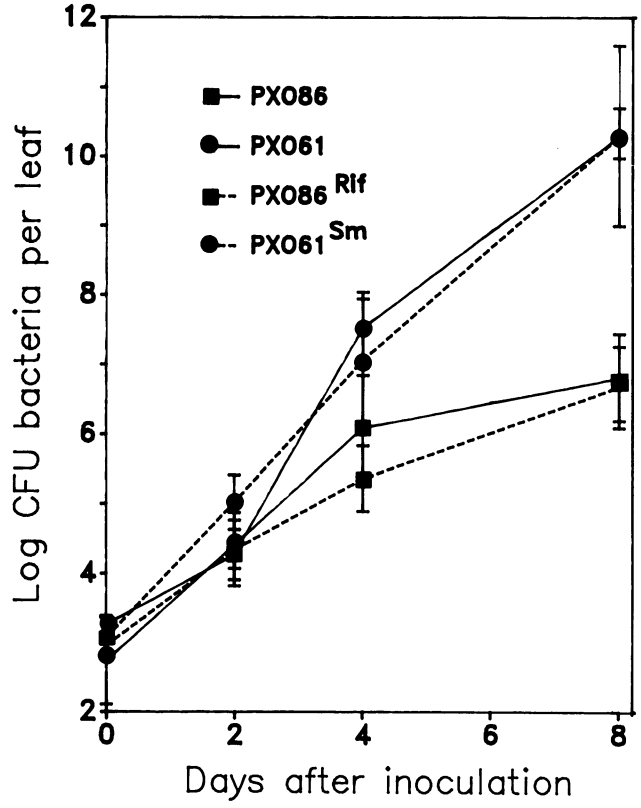

Fig. 1. Multiplication of wild-type (solid lines) (PXO86 ■ and PXO61●) and antibiotic-resistant (dashed lines) (PXO86^{Rif} ■ and PXO61^{Sm} ●) isolates of *Xanthomonas campestris* pv. *oryzae* in leaves of rice cultivar Cas 209. Data (four replications per treatment) from a representative experiment were log transformed before calculating the means and standard errors.

inoculum concentrations varied between experiments, the overall shapes of the bacterial growth curves in all interactions tested were very reproducible. The growth curves shown are from representative experiments.

In compatible and incompatible interactions with Cas 209 (Fig. 2A) and IR1545-339 (Fig. 2B), bacteria multiplied steadily and at similar rates in rice leaves for 4–6 days after inoculation, reaching 10^7–10^8 cfu/leaf. Thereafter, bacterial growth in incompatible interactions with these hosts slowed in comparison with bacterial growth in compatible interactions. Bacterial numbers in compatible interactions with Cas 209 (Fig. 2A) and IR1545-339 (Fig. 2B) reached 10^{11}–10^{13} cfu/leaf within 8–10 days after inoculation. Inoculation of Cas 209 with PXO99^{Sm} (race 6, compatible) and PXO86^{Rif} (race 2, incompatible) or with PXO99^{Sm} (compatible) and IRN793^{Rif} (race 2, incompatible) resulted in the same multiplication pattern (data not shown) as those shown in Figure 2A. In IR20, the differences in multiplication were less definitive in that bacterial numbers did not exceed 10^9 cfu/leaf in compatible interactions, which was only about 10-fold higher than in incompatible interactions (Fig. 2C).

Mixed inoculations. In mixtures of PXO61^{Sm} (compatible) and PXO86^{Rif} (incompatible) in Cas 209, the PXO61^{Sm} population grew similarly to when it was inoculated alone (Fig. 2A). However, the PXO86^{Rif} growth rate was significantly reduced in the mixture compared with its growth in single inoculations, and the final population did not exceed 10^5 cfu/leaf. Similar patterns were observed in mixed inoculations of IR1545-339 with PXO86^{Rif} (incompatible) and PXO99^{Sm} (compatible) (Fig. 2B). In cultivar IR20, both bacterial populations resulting from mixed inoculations (PXO86^{Rif}, compatible, and PXO61^{Sm}, incompatible) were about 10% lower than those of PXO86^{Rif} alone (compatible) and were similar to the levels reached by PXO61^{Sm} alone (incompatible) (Fig. 2C). In IR8, in which both race 1 and 2 isolates result in compatible interactions, populations followed a pattern similar to that observed in interactions of race 1 and 2 with Cas 209 and IR1545-339. That is, the race 1 isolate (PXO61^{Sm}) in mixed

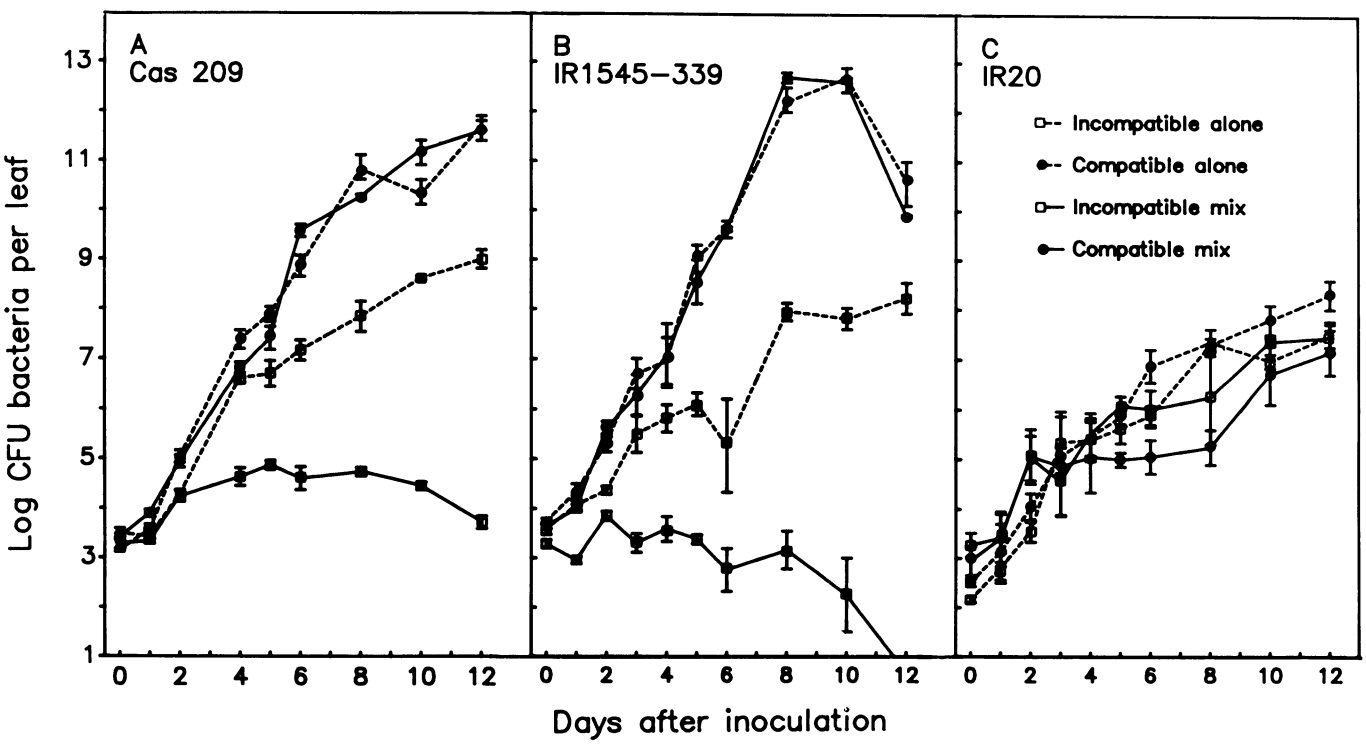

Fig. 2. Multiplication of isolates of *Xanthomonas campestris* pv. *oryzae* in leaves of rice cultivars Cas 209, IR1545-339, and IR20. Rice leaves were inoculated individually with each isolate (dashed lines) or with a mixture of the two bacteria at a 1:1 ratio (solid lines). **A,** Isolates PXO86^{Rif} (□, incompatible) and PXO61^{Sm} (•, compatible) in Cas 209. **B,** Isolates PXO99^{Sm} (•, compatible) and PXO86^{Rif} (□, incompatible) in IR1545-339. **C,** Isolates PXO86^{Rif} (•, compatible) and PXO61^{Sm} (□, incompatible) in IR20. Data (four replications per treatment) from a representative experiment were log transformed before calculating the means and standard errors.

inoculations increased at a rate equal to that when the isolate was inoculated alone (Fig. 3), but populations of the race 2 isolate (PXO86^{Rif}, compatible) did not increase above 10^4 cfu/leaf. When inoculated alone, PXO86^{Rif} reached populations of 10^8–10^9 cfu/leaf by 8 days after inoculation. The number of total bacteria in the 1:1 inoculum mixtures (5×10^9 cfu/ml of each isolate or a total of 5×10^9 cfu/ml of the combined isolates) did not affect the shape of the growth curves or the rates of growth of any isolate (data not shown).

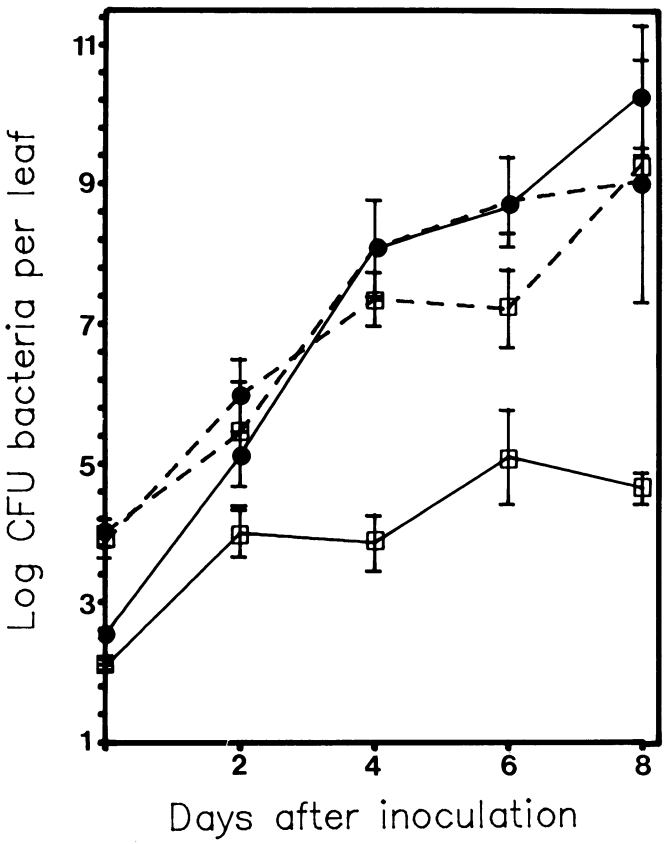

Fig. 3. Multiplication of isolates of *Xanthomonas campestris* pv. *oryzae* isolates PXO61^{Sm} (● = PXO61^{Sm} compatible) and PXO86^{Rif} (□ = PXO86^{Rif} compatible) in leaves of rice cultivar IR8. Rice leaves were inoculated individually with each isolate (dashed lines) or with a mixture of the two bacteria at a 1:1 ratio (solid lines). Data are log of the means and standard errors of four replications from each treatment of a representative experiment.

Symptom development. Lesions on all hosts began to appear 6–8 days after inoculation. Average lesion lengths at day 12 differed among cultivars (Fig. 4). Until extensive tissue necrosis occurred, lesion lengths corresponded with bacterial numbers of the compatible race in the interactions; that is, the higher the bacterial numbers were, the longer the lesions were. Lesion lengths on Cas 209 and IR1545-339 resulting from mixed inoculations were not substantially different from those resulting from inoculations only with the compatible race (Fig. 4). On IR20, mixed inoculations resulted in shorter lesion lengths than in the compatible control. Individual inoculations of IR8, a compatible host, with races 1 and 2 produced lesions of similar lengths. Mixed inoculations of IR8 also resulted in lesion lengths similar to those resulting from individual inoculations.

Spread of bacteria. Bacteria in both compatible (PXO61^{Sm}) and incompatible (PXO86^{Rif}) interactions with Cas 209 multiplied and spread outward from the inoculation point (Table 2). In the incompatible interactions, however, bacteria multiplied less rapidly and moved to adjacent sections at a much slower rate. Symptoms were never observed in advance of the bacteria; rather, bacterial numbers in a given section approached 10^8 cfu/2-cm section before symptom expression. The same trends were observed in all experiments.

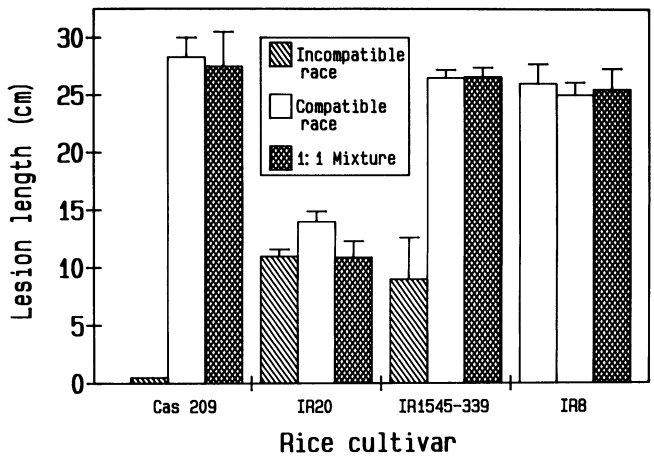

Fig. 4. Lesion lengths (cm) on four rice cultivars 12 days after inoculation with individual isolates of incompatible or compatible races of *Xanthomonas campestris* pv. *oryzae* or 1:1 mixtures. Cas 209, IR20, and IR8 were inoculated with PXO86^{Rif} (race 2) and PXO61^{Sm} (race 1). IR1545-339 was inoculated with PXO86^{Rif} (race 2) and PXO99^{Sm} (race 6). Data are the means and standard errors of four replications from each treatment of a representative experiment.

TABLE 2. Spread of isolates PXO61^{Sm} (race 1, compatible) and PXO86^{Rif} (race 2, incompatible) of *Xanthomonas campestris* pv. *oryzae* in adjacent leaf sections (A–F) of rice cultivar Cas 209. Progression from the inoculation point was monitored in the upper 12 cm of the leaf at 2-day intervals by cutting 2-cm sections above and below the point of inoculation (section E)

Day	Isolate	Race	Log colony-forming units/2-cm leaf sections (± standard error)[a]						Total lesion length (cm)
			A	B	C	D	E	F	
0	PXO61^{Sm}	1	0	0	0	0	4.2(0.1)	0	0
	PXO86^{Rif}	2	0	0	0	0	4.2(0.1)	0	0
2	PXO61^{Sm}	1	0	0	0	0	4.7(0.5)	0	0
	PXO86^{Rif}	2	0	0	0	0	4.4(0.1)	0	0
4	PXO61^{Sm}	1	0	0	5.7(0.3)	5.7(0.3)	7.4(0.1)	5.0(0)	0
	PXO86^{Rif}	2	0	0	0	4.2(0.2)	6.1(0.1)	0	0
6	PXO61^{Sm}	1	6.5(0.4)	7.0(0.7)	7.5(0.1)	7.7(0.7)	7.8(0.2)	7.7(0.2)	0
	PXO86^{Rif}	2	0	0	0	5.5(0.6)	6.8(0.7)	5.5(0.7)	0
8	PXO61^{Sm}	1	6.7(0.4)	7.7(0.1)	8.5(0.3)	8.4(0.2)	9.5(0.3)	8.7(0.4)	6.3
	PXO86^{Rif}	2	0	5.7(0.7)	7.4(0.5)	7.7(0.6)	7.8(0.4)	7.6(0.7)	0
12	PXO61^{Sm}	1	7.4(0.2)	7.4(0.4)	7.7(0.2)	8.0(0.3)	8.0(0.3)	8.0(0.2)	19.5
	PXO86^{Rif}	2	0	6.2(0.2)	7.3(0.3)	7.4(0.3)	7.8(0.7)	7.1(0.1)	3.7

[a] Data (four replications per treatment) were log transformed before calculating the means ± standard errors.

Effect of inoculum density on expression of resistance. The initial water soaking from Hagborg infiltration of Cas 209 leaves disappeared within 4 hr. At both high and low inoculum levels, PXO61^{Sm} (compatible) multiplied steadily (after a lag of 1–2 days) through days 8–10 (Fig. 5). After this time, multiplication reached a plateau. Numbers of PXO86^{Rif} (incompatible) at high inoculum did not exceed the initial 10^7–10^8 cfu/leaf. At low inoculum, the bacteria multiplied to 10^7–10^8 cfu/leaf by day 6 and did not increase beyond that level. After Hagborg inoculation with a high inoculum density, water soaking was observed in the compatible interaction at 48–72 hr. In incompatible interactions, either no symptoms appeared by day 4 or the leaf tissue that was infiltrated became dry and gray in color. The same trends were observed in all experiments.

DISCUSSION

Our investigations indicate that incompatibility in the interaction between isolates of *X. c. oryzae* and cultivars Cas 209, IR1545-339, and IR20 was reflected in lower bacterial numbers and reduced lesion lengths, although these correlations were much less pronounced in cultivar IR20. In IR20, compatible interactions resulted in lower bacterial populations (Fig. 2C) and reduced lesion development (5, Fig. 4) when compared with compatible interactions in cultivars Cas 209 and IR1545-339, suggesting that some level of resistance to the compatible race exists. It is unclear whether the reduction of final PXO86^{Rif} populations in compatible interactions is a feature of the *Xa-4* gene for resistance carried by IR20. Resistance to *X. c. oryzae* conferred by *Xa-4* is not complete; that is, clear differences between compatible and incompatible interactions are not always apparent. Horino et al (5) reported that gene *Xa-4* is influenced by temperature. At high temperatures (33 C days, 25 C nights), lesions in incompatible interactions with PXO61 were up to five times longer than lesions at low temperatures (29 C days, 21 C nights). At these high temperatures, lesion lengths did not increase in compatible interactions with PXO86. The effect of temperature on bacterial multiplication was not measured. Our conditions (32 C days, 22 C nights) approached those that would favor longer lesions in incompatible interactions and, thus, may have minimized the expected differences in compatible and incompatible interactions.

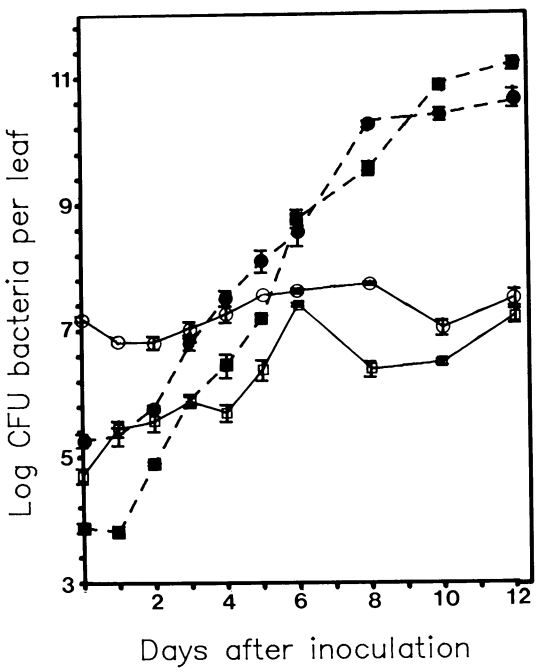

Fig. 5. Effect of inoculum density on growth of isolates PXO61^{Sm} (race 1, compatible) and PXO86^{Rif} (race 2, incompatible) of *Xanthomonas campestris* pv. *oryzae* per leaf of rice cultivar Cas 209. Rice leaves were infiltrated using a Hagborg apparatus with starting inocula of 1×10^{10} (PXO61^{Sm} = ●, PXO86^{Rif} = ○) and 1×10^8 (PXO61^{Sm} = ■, PXO86^{Rif} = □) colony-forming units per leaf. Data (four replications per treatment) from a representative experiment were log transformed before calculating the means and standard errors.

To assess whether resistance expression was dependent on time or a threshold number of bacteria, varied inoculum levels were used and bacterial multiplication was monitored. As shown in Figure 2A (initial inoculum about 10^3 cfu/leaf) and in the inoculum-density experiment (Fig. 5, initial inoculum about 10^5 and 10^7 cfu/Cas 209 leaf), bacteria in incompatible interactions multiplied to 10^7–10^8 cfu/leaf and did not increase further, regardless of the time necessary to reach that number. This suggested that bacterial concentration was critical in the activation of rice defense mechanisms. Although reduction of the growth rate was observed when bacterial numbers had reached 10^7–10^8 cfu/leaf, activation of host defenses probably took place at a lower population level.

Parry and Callow (18) measured bacterial movement basipetally in rice leaves by plating sections and scoring for presence or absence of bacterial growth from the ends of those sections. Bacteria were found in the same number of leaf sections in both compatible (PXO71) and incompatible (PXO86) interactions with Cas 209. They concluded that, because in both interactions bacteria were present throughout the leaf, the difference between compatible and incompatible interactions was in the expression of symptoms by the host; that is, longer lesions did not necessarily result from more bacterial growth and spread. However, they did not enumerate bacteria in the leaf sections. In our experiments, isolates of both compatible and incompatible races of *X. c. oryzae* colonized leaf tissues. However, in the incompatible interaction, bacteria did not invade the leaf as aggressively as did those in the compatible interaction (Table 2). Also, in the incompatible interaction, populations of bacteria if present in a section were always one or two log units lower than those in the compatible interaction. Previous reports indicated that differences in bacterial numbers between compatible and incompatible interactions were two log units or less (12,14,18,20,26). However, in those studies, leaf sections of 10 cm or less were sampled. Our experiments suggest that whole-leaf samples give a more reliable estimate of the true differences in compatible and incompatible interactions for the following reasons: 1) bacteria in incompatible interactions did grow and move in leaves to a limited extent; 2) bacterial numbers in both interactions were similar at the inoculation site (Table 2, E); and 3) compatible bacteria accumulated to fairly high concentrations in the lower portions of the leaf before symptom expression (17; our data, not shown). No symptoms were seen in sections with less than 10^7 cfu/leaf, indicating a minimum bacterial number necessary for symptom expression.

In mixed inoculation experiments of Cas 209 or IR1545-339, when the mixture contained equal numbers of bacteria from both races, bacterial multiplication of isolates representing the compatible interaction was not restricted compared with bacterial growth in leaves inoculated with only the compatible isolate (Fig. 2A and B). However, multiplication of bacteria representing incompatible races was inhibited, and bacterial numbers dropped over time. We observed the same phenomenon in IR8 (Fig. 3), which carries no known genes for resistance to these isolates, suggesting that inhibition of growth of isolates of incompatible races in Cas 209, IR1545-339, and IR8 after mixed inoculations probably was due to some interaction between the isolates. No inhibition of one isolate by another was observed in in vitro mixed cultures (nutrient broth, peptone sucrose broth), but the race 2 isolate (PXO86^{Rif}) multiplied at a slightly slower rate than did the race 1 isolate (PXO61^{Sm}, data not shown). Other isolates (IRN793^{Rif}, race 2, and PXO99^{Sm}, race 6) in Cas 209 produced growth curves similar to those of PXO86^{Rif} and PXO61^{Sm} in single and mixed inoculations. It is possible that, in the host plant, isolates such as PXO61^{Sm} or PXO99^{Sm} can outcompete other isolates for available nutrients. Restricted growth of the isolate from the incompatible race might prevent that isolate from reaching the threshold level (10^7–10^8 cfu/leaf) necessary for resistance induction. Thus, susceptibility would be the observed phenotype. Lesion-length data obtained from some cultivars

(Cas 209 and IR1545-339) were consistent with this hypothesis. When these cultivars were inoculated with 1:1 mixtures of compatible and incompatible races, the resulting lesion lengths were not significantly different from those of leaves inoculated with only bacteria from the compatible race (Fig. 4). Other researchers using different host cultivar-bacterial race interactions reported similar results (18,21).

In IR20, initial growth of the isolate PXO86^{Rif} (compatible) in mixed inoculations with PXO61^{Sm} (incompatible) was not reduced to the extent that it was in mixed inoculations of cultivars Cas 209 and IR8. However, the final PXO86^{Rif} population in IR20 was about one log unit lower in mixed inoculations than in individual control inoculations. Multiplication patterns of isolate PXO61^{Sm} (incompatible) in IR20 were similar in mixed and single inoculations. The reduction of PXO86^{Rif} populations in IR20 could be explained in two ways: Expression of specific resistance to PXO61^{Sm} reduced the populations of that isolate to the extent where its presence would not affect growth of PXO86^{Rif} as dramatically as observed in other cultivars; or the host factors induced by PXO61^{Sm} also affected growth of PXO86^{Rif}, that is, that resistance was physiologically predominant over susceptibility. Our data do not favor either scenario because measurement of resistance in IR20 by differences in bacterial growth and final populations was not definitive under our conditions (Fig. 2C). Lesion lengths resulting from mixed inoculations on IR20 were reduced in leaves in comparison with the compatible control, but in our conditions, lesion lengths between compatible and incompatible interactions were not significantly different (Fig. 4).

With the exception of IR20, the population and lesion length data collectively suggest that incompatibility is not physiologically predominant to compatibility in the interation between rice and *X. c. oryzae*. The inhibitory or competitive interaction between bacterial isolates in mixed inoculations, however, confounds this conclusion. In an attempt to avoid or mask the effects of isolate-isolate interactions, several approaches are under way. Preliminary evidence (authors, *unpublished*) indicates that, if higher ratios (incompatible to compatible) of bacteria are inoculated into rice leaves of cultivars Cas 209 and IR1545-339 or if incompatible races are inoculated before inoculation with compatible races, then lesion lengths and multiplication of bacteria from both races are reduced. In addition, we are developing isogenic mutants of *X. c. oryzae* differing only in the gene responsible for incompatibility to a particular host. These will be used in concert with newly released host isolines, which differ only in those resistance genes of interest (15), to evaluate more critically race-specific induction of resistance.

LITERATURE CITED

1. Borkar, S. G., and Verma, J. P. 1985. Population dynamics of *Xanthomonas campestris* pv. *malvacearum* (Xcm) in compatible and incompatible reaction of cotton cultivars. Acta Phytopathol. Acad. Sci. Hung. 20:31-34.
2. Devadath, S. 1970. Effect of mixture of inoculations of virulent and less virulent isolates of *Xanthomonas oryzae*. Curr. Sci. 39:420-421.
3. Ellingboe, A. H. 1976. Genetics of host-parasite interactions. Pages 761-778 in: Physiological Plant Pathology. R. Heitefuss and P. H. Williams, eds. Springer-Verlag, Berlin.
4. Hagborg, W. A. F. 1970. A device for injecting solutions and suspensions into thin leaves of plants. Can. J. Bot. 48:1135-1136.
5. Horino, O., Mew, T. W., and Yamada, T. 1982. The effect of temperature on the development of bacterial leaf blight on rice. Ann. Phytopathol. Soc. Jpn. 48:72-75.
6. Keen, N. T., Ersek, T., Long, M., Bruegger, B., and Holliday, M. 1981. Inhibition of the hypersensitive reaction of soybean leaves to incompatible *Pseudomonas* spp. by blasticidin S, streptomycin or elevated temperature. Physiol. Plant Pathol. 18:325-337.
7. Kelman, A. 1954. The relationship of pathogenicity in *Pseudomonas solanacearum* to colony appearance on a tetrazolium medium. Phytopathology 44:693-695.
8. Klement, Z., Farkas, G. L., and Lovrekovich, L. 1982. Hypersensitivity. Pages 149-177 in: Phytopathogenic Prokaryotes. M. S. Mount and G. H. Lacy, eds. Academic Press, New York.
9. Long, M., Barton-Willis, P., Staskawicz, B. J., Dahlbeck, D., and Keen, N. T. 1985. Further studies on the relationship between glyceollin accumulation and the resistance of soybean leaves to *Pseudomonas syringae* pv. *glycinea*. Phytopathology 75:235-239.
10. Maniatis, T., Fritsch, E. F., and Sambrook, J. 1982. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York. 545 pp.
11. Mew, T. W. 1987. Current status and future prospects of research on bacterial blight of rice. Annu. Rev. Phytopathol. 25:359-382.
12. Mohiuddin, M. S., and Kauffman, H. E. 1975. Multiplication studies of *Xanthomonas oryzae* isolates on differential rice varieties. Curr. Sci. 44:637-638.
13. Muko, H., and Yoshida, K. 1951. A needle inoculation method for bacterial leaf blight disease of rice. Ann. Phytopathol. Soc. Jpn. 15:179.
14. Nakanishi, K., and Watanabe, M. 1977. Studies on the mechanism of resistance of rice plants against *Xanthomonas oryzae*. III. Relationship between the rate of production of antibacterial substances and of multiplication of pathogenic bacteria in infected leaves of resistant and susceptible varieties. Ann. Phytopathol. Soc. Jpn. 4:265-269.
15. Ogawa, T., and Yamamoto, T. 1987. Selection of recurrent parents to develop near-isogenic lines resistant to bacterial leaf blight of rice. Jpn. Agric. Res. Q. 21:65-69.
16. Olufowote, J. O., Khush, G. S., and Kauffman, H. E. 1977. Inheritance of bacterial blight resistance in rice. Phytopathology 67:772-775.
17. Ou, S. H. 1985. Rice Diseases. Commonw. Mycol. Inst., Kew, Surrey, England.
18. Parry, R. W. H., and Callow, J. A. 1986. The dynamics of homologous and heterologous interactions between rice and strains of *Xanthomonas campestris*. Plant Pathol. 35:380-389.
19. Petpisit, V., Khush, G. S., and Kauffman, H. E. 1977. Inheritance of resistance to bacterial blight in rice. Crop Sci. 17:551-554.
20. Reddy, A. P. K., and Kauffman, H. E. 1973. Multiplication and movement of *Xanthomonas oryzae* in susceptible and resistant hosts. Plant Dis. Rep. 57:784-787.
21. Reddy, A. P. K., and Kauffman, H. E. 1974. Population studies of mixed inoculum of *Xanthomonas oryzae* in susceptible and resistant varieties of rice. Ann. Phytopathol. Soc. Jpn. 40:93-97.
22. Reddy, A. P. K., and Kauffman, H. E. 1977. Loss of virulence associated with ageing of *Xanthomonas oryzae* cultures. Indian Phytopathol. 30:106-111.
23. Stall, R. E., and Cook, A. A. 1966. Multiplication of *Xanthomonas vesicatoria* and lesion development in resistant and susceptible pepper. Phytopathology 56:1152-1154.
24. Szybalski, W. 1952. Microbial Selection. I. Gradient plate technique for study of bacterial resistance. Science 116:46-48.
25. Tsuchiya, K., Mew, T. W., and Wakimoto, S. 1982. Bacteriological and pathological characteristics of wild types and induced mutants of *Xanthomonas campestris* pv. *oryzae*. Phytopathology 72:43-46.
26. Watanabe, M., and Asaumi, T. 1975. Studies on the physiological changes in the rice plants infected with *Xanthomonas campestris* pv. *oryzae*. I. Relationship between the increase in respiratory rate and multiplication of the bacteria in infected leaves. Ann. Phytopathol. Soc. Jpn. 41:364-372.
27. Yoshimura, A., Mew, T. W., Khush, G. S., and Omura, T. 1983. Inheritance of resistance to bacterial blight in rice cultivar Cas 209. Phytopathology 73:1409-1412.